Neuroprotective Effects of Ellagic Acid in a Rat Model of Parkinson's Disease

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Abstract- Antioxidants have protective effects against free radicals-induced neural damage in Parkinson's disease (PD). We examined the effects of ellagic acid (EA) on locomotion, pallidal local EEG, and its frequency bands' power and also cerebral antioxidant contents in a rat model of PD induced by 6-hydroxidopamine (6-OHDA). 6-OHDA (16 µg/2µ l) was injected into the right medial forebrain bundle (MFB) in MFB-lesioned rat's brain. Sham group received vehicle instead of 6-OHDA. PD-model was confirmed by rotational test using apomorphine injection. EA (50 mg/kg/2 ml, by gavages) was administered in PD+EA group. One group of MFB-lesioned rats received pramipexole (PPX; 2 mg/kg/2 ml, by gavages) as a positive control group (PD+PPX group). Motor activity was assessed by stride length, rotarod, and cylinder tests. Pallidal local EEG was recorded in freely moving rats. The levels of malondialdehyde (MDA) besides Glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities were measured in both striatum and hippocampus tissues. MFB lesion caused significant reduction of stride-length (P<0.001), bar decent latency (P<0.001) and frequency bands' power of pallidal EEG (P<0.001). Use of 6-OHDA caused a reduction in the GPx (P<0.001) and SOD (P<0.001) activities while increased significantly the levels of MDA (P<0.001) in MFB-lesioned rats. EA significantly restored all above parameters. The results show that EA can improve the motor impairments and electrophysiological performance in the MFB-lesioned rats via raising the cerebral antioxidant contents. Therefore, EA can protect the brain against free radicals-induced neural damage and may be beneficial in the treatment of PD.

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Keywords: Parkinson's disease; Ellagic acid; Locomotion; EEG; Antioxidants

Introduction

Parkinson's disease (PD), an age dependent neurodegenerative disorder caused by a progressive death of striatum projecting dopaminergic neurons in the substantia nigra pars compacta (SNc), comprise a variety of movement disabilities such as resting tremor, muscle stiffness, slowed motion, impaired gait functioning and postural instability (1).

Although the etiology of the PD is not completely understood, it seems that some factors triggers oxidative imbalance such as brain aging, genetic susceptibility, mitochondrial dysfunction, free radical production and environmental toxins (2). Mostly, the oxidation of dopamine (DA) generates reactive oxygen species (ROS) and an imbalanced production of ROS, leading to oxidative stress (OS) and neuronal death. Also, these free radicals react with membrane lipids and cause lipid peroxidation (LPO) and cell death (3). Pathogenesis of PD has also been linked to dietary habits, where deficiency of antioxidant components such as vitamins (A,C,E and niacin) and selenium has been shown to increase the risk of PD (4).

Furthermore, the pathophysiological mechanisms of PD can be related to events in the substantia nigra, affecting the basal ganglia (BG). Because of a decrease in striatum's DA, activity of the globus pallidus internal segment (GPi) raises and a tonic over inhibition of the pallidal-recipient thalamic relay cells, leading to a thalamocortical dysrhythmia interaction, known by an excessive production of low threshold calcium spikes and an enhancement in low and high frequency

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electroencephalographic (EEG) power in thalamus (5). Diseases of the BG can change the motor functions. So, in motor impairments of PD, the output of GP is prominently involved (6).

Studies of the EEG activity in patients with PD can produce valued evidence of outputs that are sent from the cortex to the motor effectors. A tight thalamocortical connection provides the basis of the oscillatory cortical EEG activity, and it has been revealed that EEG oscillations are abnormal in PD patients during execution of particular tasks. Yet, a few studies have focused at the EEG power spectra in PD patients, showing changes in the power of specific frequency bands (7). Therefore, one beneficial income of evaluating variations from the normal state in PD is to study oscillatory brain processes. For example, oscillations in the beta frequency band are related to cognitive control on behavior or "executive tasks" (8).

Despite the vast efforts of researchers, there is no definite treatment for PD. Present treatments are based on pharmacological strategies that pursue DA restoration in the striatum for example; administration of DA precursors, agonists or selective inhibitors of DA uptake (9). Hence, there have been many efforts to develop valuable medical plants to achieve neuroprotection. Attention has been focused on a wide variety of natural antioxidants that can scavenge the free radicals and protect neurons from oxidative damage. Various antioxidative supplements have been suggested and have been shown to play an important role in neuroprotection (10-12).

Pomegranate (*Punica granatum L.*) extracts can protect neurons against the neurotoxic effects of 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). This effect could be associated with their phenolic constituents and antioxidant activities. Pomegranates enriched by phytochemicals such as polyphenols (including phenolic acids and flavonoids), have shown antioxidant properties and can inhibit inflammation and other harmful processes involved in neurodegenerative diseases. Moreover, pericarp of pomegranate is enriched with tannins such as gallic acid and ellagic acid, other potent antioxidants (13). Ellagic acid (2,3,7,8-tetrahydroxybenzopyrano [5,4,3-cde] benzopyran-5-10-dione) is an excretion product of many plant species (14) such as strawberries, cranberries, walnuts, pecans and red raspberry seeds (15). It has been reported that they have some different pharmacological anti-inflammatory and antioxidant properties (16). Therefore, it can be a valuable approach in new researches and PD management. In this study, we have investigated the effects of ellagic acid on motor disturbances, pallidal local EEG and its frequency bands' power besides cerebral antioxidant contents in a rat model of PD induced by 6-OHDA.

Materials and Methods

Drugs and assay kits

6-hydroxidopamine (6-OHDA), desipramine, apomorphine, ellagic acid (EA) and pramipexole (PPX) were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Ketamine and xylazine were obtained from Alfasan Company (Woerden, Holland). TBA (2-thiobarbituric acid), n-butanol, tris base, sodium acetate, phosphoric acid, potassium chloride, tetramethoxypropane were obtained from Merck Company (Darmstadt, Germany). Glutathione peroxidase activity colorimetric assay and Superoxide dismutase (SOD), activity assay kits, were purchased from BioVision Company (BioVision Incorporated, Milpitas, CA, USA).

Experimental design

Forty adult male Wistar rats (250-300 g) were obtained from central animal Lab of Ahvaz Jundishapur University of Medical Sciences (AJUMS), Ahvaz, Iran. Rats were kept in the animal house under constant temperature (22±2°C) and humidity (55-60%) on a 12:12-h light-dark cycle with food pellets and water, ad libitum. All experiments were done during the light phase of the cycle (between 8:00 am and 5:00 pm) and were directed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and with approval from the AJUMS Animal Care and Use Committee (AJACUC). We tried to minimize animals stress and to reduce the number of rats used in this study. All animals were handled for 5 days (daily 5 min) before the tests. The animals were divided randomly into five groups (n=8):

1) Sham operated; rats received a vehicle of 6-OHDA (2 µl normal saline containing 0.01% ascorbic acid) into right MFB.
2) Sham+EA; sham-operated rats, received EA.
3) PD+Veh; rats injected 6-OHDA (16 µg/2 µl normal saline containing 0.01% ascorbic acid) into right MFB and vehicle of EA (normal saline containing DMSO).
4) PD+PPX (Positive control); MFB lesioned rats were treated with PPX (2 mg/kg/2 ml, by gavages for 10 consecutive days).
5) PD+EA; MFB lesioned rats were treated with

EA.

In groups 2 and 5, EA (50 mg/kg, by gavages for 10 consecutive days) was administered 14 days after PD induction (17). The treatment schedule and the intervals for estimation of various parameters have been presented in Figure 1.

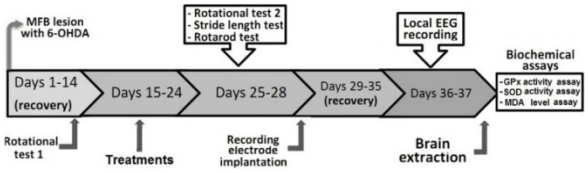

Figure 1. The design of research schedule and intervals to measure of various parameters. MFB= medial forebrain bundle, 6-OHDA= 6-hydroxy dopamine, EA= ellagic acid

Making the animal model of PD (MFB lesion)

The brain's right hemisphere MFB was lesioned by using neurotoxin 6-OHDA according to Tadaiesky's method (2008) with some modification to inducing an animal model of PD (12,18). Briefly, rats, were deeply anesthetized with a combination of ketamine/xylazine (90/10 mg/kg, i.p.), and were placed in a stereotaxic apparatus (Narishige, Japan). 6-OHDA (16 µg/2 µl normal saline containing 0.01% ascorbic acid) or its vehicle (in sham groups) was infused into the right MFB using a 5 µl Hamilton syringe according to the coordinates in Paxinos and Watson atlas: AP: -4.4 mm, ML: ±1.3 mm from bregma and DV: -8.4 mm from skull surface (19). Following injection, the cannula was left in the site for 5 min to allow complete diffusion of the drug. All animals received desipramine (25 mg/kg i.p., 30 min before surgery) to protect noradrenergic terminals depletion and consecutive depression by 6-OHDA. Sham-operated rats underwent the same protocol, but the vehicle was injected instead of 6-OHDA (20).

Apomorphine-induced rotational behavior

All rats were tested for rotational behavior 2 weeks after MFB lesion (before treatment) and 4 weeks after lesion (after treatment). Contralateral rotations of each animal were recorded after subcutaneous injection of apomorphine (0.5 mg/kg in normal saline containing 0.01% ascorbic acid) to confirm the dopamine depletion in nigrostriatal system (21). The rotations were counted over a period of 30 min.

Stride length test

Stride-length (walk-length) was measured in all animals using a wood box (20×17×10 cm), in which a runway (4.5 cm wide, 42 cm long with walls of 10 cm height) was designed to lead out into the dark wooden box. Stride-length was measured by moistening the forepaws of rats with ink and letting them run on a paper strip (4.5 cm wide, 40 cm long). The mean distance between two forepaw prints was measured manually, as the forelimb stride-lengths. The three stride-lengths were selected in order to perform statistical analysis, from each run. The paw prints made at the beginning (7 cm) and the end (7 cm) of the each run were excluded because of variations in velocity (20,22).

Rotarod test

The rotarod apparatus (M.T 6800, Borj Sanat Co., Tehran, Iran) was used to assess motor performance and coordination in all groups. The apparatus automatically recorded the time that each rat remained on the rotating rod (75 mm diameter, 40 cm height). This procedure was performed for two days. At the first day, the rats were located on a rod (with persistent 5 rpm for 3 min) to adapt with apparatus. Next day, the animals were placed on the rod with an initial constant rod speed of five rotations per minute (5 rpm) for 3 min, after then speed was increased to 40 rpm automatically (5-10 rpm/next 3 min, 10-20 rpm /next 3 min, 20-30 rpm/ next 3 min and 30-40 rpm/ end 3 min). Cut off time was 15 min. The test session consisted of three trials during one day. Inter trial interval was 45 min. Data were presented as mean retaining time on the rotating bar over the three trials (20)

Implantation of the electrode in globus pallidus

Four weeks after MFB lesion, in order to record the local electroencephalogram (EEG) of globus pallidus (GP) nucleus, a stainless steel bipolar metal wire electrode (stainless steel Teflon coated, 0.005" bare, 0.008" coated, A-M systems, Inc. WA, USA) was implanted in the right GP at AP: -1.3 mm (from bregma), ML: 3.2 mm, DV: -6.5 mm (from skull

surface) under ketamine/xylazine anesthesia and stereotaxic surgery (19). All implants were fixed to the skull by acrylic cement and two stainless steel small screws. After recovery period (7 days), the local EEG recording was done in freely moving rats (23).

Local EEG recording

Right GP nucleus local field potentials (LFPs) was recorded in freely moving rats using 4-Channels Powerlab, LabChart software version 7 and ML135 bio-amplifier (AD Instruments, Australia) with 1 mV amplification, sample recording 400 Hz, and 0.3-70 Hz band pass filtration for 5 sec. The crude EEG and its gamma, alpha, beta, theta, and delta bands amplitude changes during 3 periods of 5 sec were compared in all groups. Electrical power of frequency bands was measured as µV2/Hz. Finally, to confirming the location of electrode tip in GP, rats were anesthetized deeply by injection of an overdose of Ketamine HCL; the direct current (DC, 0.5 µA, 3 sec) was delivered via electrodes tip into GP. Then, rats sacrificed and brains removed from the skull and immersed in 10% formalin-normal saline solution for at least 5 days. The brain was frozen and cut into coronal sections (50 µM) using a freezing microtome. These sections were compared to the Paxinos and Watson atlas, and only the matched items were used to the statistical analysis (23,24).

Brain sample collection

The rats were deeply anesthetized and then were decapitated. The striatum and hippocampus were removed quickly, rinsed with normal saline and frozen. All samples were kept at -80 ℃ until further processing (25).

Lipid peroxidation (LPO) assay

The tissues were homogenized in cold KCl solution (1.5%) to give a 10% homogenate suspension used for measuring thiobarbituric acid reactive substances (TBARS) value, expressed as malondialdehyde (MDA) equivalents. TBARS levels, an index of LPO, produced by free radicals were measured. MDA reacts with TBA to produce a red colored complex that has the highest absorbance at 532 nm. Briefly, 3 ml phosphoric acid (1%) and 1 ml TBA (0.6%) were added to 0.5 ml of homogenate in a centrifuge tube, and the mixture was warmed for 45 min in a boiling water bath. After cooling, 4 ml n-butanol was added to the mixture and vortex-mixed for 1 min followed by centrifugation at 2000×g for 20 min. The colored supernatant was transmitted to a new tube, and its absorbance was read at 532 nm. TBARS levels were detected by using 1,1,3,3-tetramethoxypropane as a standard. The standard curve of MDA was plotted at the concentration range of 0–20 µM (25).

Glutathione peroxidase (GPx) activity assay

Measurement of GPx activity was done with the BioVision's Glutathione peroxidase activity colorimetric assay kit (BioVision Incorporated, Milpitas, CA, USA). One unit is defined as the amount of enzyme that will cause the oxidation of 1 µmol of NADPH to NADP+ under the assay kit condition per minute at 25℃.

Superoxide dismutase (SOD) activity assay

Measurement of SOD activity was done by the BioVision's Superoxide dismutase (SOD) activity assay kit (BioVision Incorporated, Milpitas, CA, USA).

Statistics

Results were expressed as means±SEM. The statistical analysis was performed by SPSS version 22 and one-way ANOVA followed by Tukey's post hoc test for inter groups comparisons. A *P*.value less than 0.05 were considered as a significant difference.

Results

Apomorphine-induced circling behavior

As shown in Figure 2, two weeks after surgery the number of apomorphine-induced contralateral rotations increased significantly in MFB-lesioned groups as compared to sham group ($P<0.001$). Four weeks after surgery, the number of contralateral rotations in the treated groups (PD+PPX and PD+EA groups) decreased significantly versus PD+Veh group ($P< 0.001$).

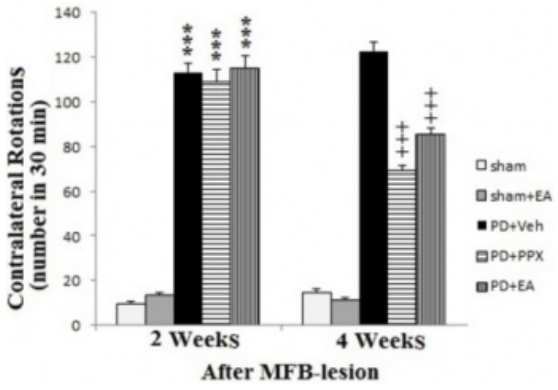

Figure 2. Effect of ellagic acid on the apomorphine-induced rotational test. Values are as mean±SEM (n=16). Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. ***$P<0.001$ vs. sham group 2 weeks after surgery, $^{+++}P<0.001$ vs. PD+veh group 4 weeks after surgery and treatment.

Stride-Length

As shown in Figure. 3, forepaws stride-length significantly decreased in PD+Veh group as compared to sham-operated rats (P<0.001). It significantly increased in PD+PPX and PD+EA groups versus PD+Veh (P<0.001).

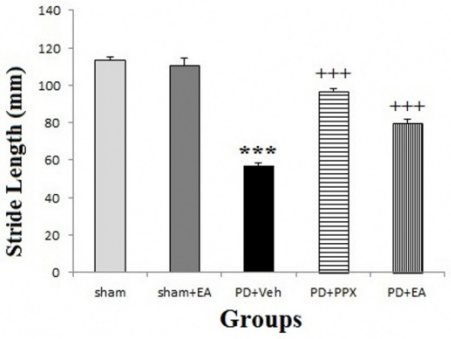

Figure 3. Effect of ellagic acid on stride-length. Values are as mean±SEM (n=16). Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. ***P<0.001 vs. sham group and ^{+++}P<0.001 vs. PD+Veh group.

Motor coordination

As shown in Figure 4, bar descending latency significantly decreased in PD+Veh group as compared to sham group (P<0.001), while it significantly increased in PD+PPX and PD+EA groups versus PD+Veh (P<0.001 and P<0.01, respectively).

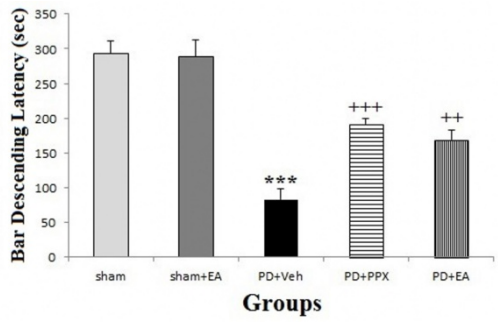

Figure 4. Effect of ellagic acid on bar descent latency in rotarod test. Values are as mean±SEM (n=8). Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. ***P<0.001 vs. sham group and ^{+++}P<0.001, ^{++}P<0.01 vs. PD+veh group.

Pallidal local EEG and its frequency bands' power

MFB lesion significantly decreased the EEG power of PD+Veh group as compared to sham-operated rats (P<0.001). Treatment of MFB-lesioned rats with PPX and EA significantly increased EEG power (P<0.01 and P<0.05, respectively) versus PD+veh (Figure 5A). Similarly, gamma power significantly decreased in PD+Veh group versus sham-operated rats (P<0.001). Treatment the MFB-lesioned rats with PPX or EA significantly increased gamma power (P<0.01) versus PD+Veh (Figure 5B). Also, beta power significantly decreased in PD+Veh group versus sham group (P<0.01) and treatment the MFB-lesioned rats with PPX or EA significantly increased beta power (P<0.05) versus PD+Veh group (Figure 5C). Although, alpha power significantly decreased in PD+Veh group versus sham group (P<0.01), the treatment could not increase the power of alpha band versus PD+Veh in the MFB-lesioned rats with PPX and EA significantly (Figure 5D). As shown in Figure 5E-F, there were no significant differences among groups in theta and delta powers.

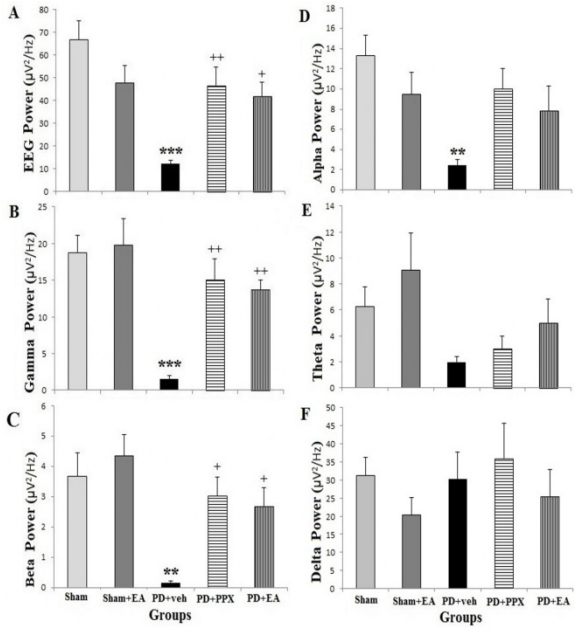

Figure 5. Effect of ellagic acid on GP local EEG and its frequency bands' powers (μV2/Hz). Values are as mean±SEM (n=8). Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. A) EEG power. ***P<0.001 vs. sham group. ++P<0.01 and +P<0.05 vs. PD+Veh group B) Gamma power. ***P<0.001 vs. sham-operated rats. ++P<0.01) vs. PD+Veh group C) Beta power. **P<0.01 vs. sham group and +P<0.05 vs. PD+veh group D) Alpha power. **P<0.01 vs. sham-operated rats.

Lipid peroxidation assay (MDA levels)

The degree of free radical induced neural damage following MFB lesion was assessed by LPO, which was measured as TBARS levels. There was an increase in TBARS levels of PD+Veh group as compared to sham-operated rats in the striatum (Figure 6A, P<0.001) and hippocampus (Figure 6B, P<0.001). Oral administration of EA caused a significant decrease of TBARS levels, in both striatum (P<0.05) and hippocampus (P<0.01) versus PD+Veh.

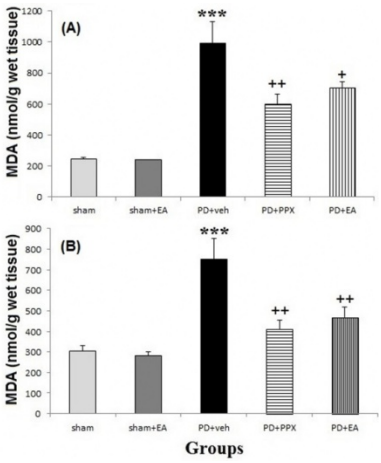

Figure 6. Effects of ellagic acid on MDA levels in the striatum (A) and hippocampus (B). Values are as mean±SEM (n=8). Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. ***P<0.001 vs. sham group, ^{++}P<0.01, ^{+}P<0.05 vs. PD+Veh group.

Glutathione peroxidase (GPx) activity assay

GPx activity (mU/ml) was measured to assess the enzymatic defense potential of the neurons against the cerebral oxidative stress. In the striatum (Figure 7A) and hippocampus (Figure 7B), the GPx activity decreased significantly in PD+Veh group as compared to sham group (P<0.001). However, the reduction of the GPx activity significantly restored in the groups which treated with EA versus PD+Veh group in both striatum and hippocampus (P<0.05).

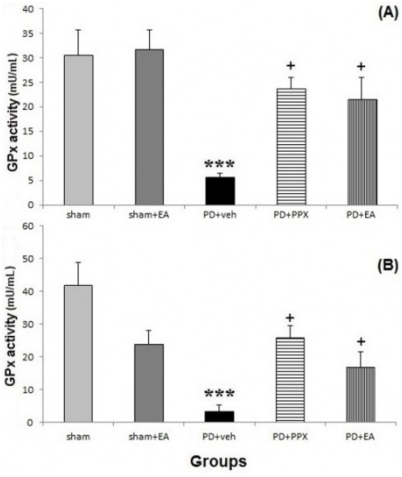

Figure 7. Effects of ellagic acid on glutathione peroxidase (GPx) activity in the striatum (A) and hippocampus (B). Values are as mean±SEM (n=8). Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. ***P<0.001 vs. sham group, ^{+}P<0.05 vs. PD+Veh group.

Superoxide dismutase (SOD) activity assay

In the striatum (Figure 8A) and hippocampus (Figure 8B), the SOD activity (U/ml) significantly decreased in PD+Veh group versus sham-operated rats (P<0.001). But, this reduction significantly restored in the groups which treated with EA versus PD+Veh group in both striatum and hippocampus (P<0.001 and P<0.01, respectively).

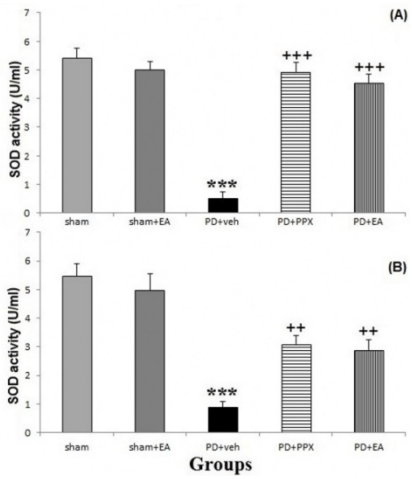

Figure 8. Effects of ellagic acid on superoxide dismutase (SOD) activity in the striatum (A) and hippocampus (B). Values are as mean±SEM (n=8). Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. ***P<0.001 vs. sham group, ^{+++}P<0.001 and ^{++}P<0.01 vs. PD+Veh group.

Discussion

Several studies have shown that injection of 6-OHDA into the MFB enhanced contralateral apomorphine-induced rotation, suggesting 6-OHDA can exert a neurotoxic effect on nigrostriatal pathway leads to enhance contralateral rotations (26). Similarly, our results have shown that apomorphine-induced contralateral rotation increased significantly after MFB lesioning and confirm the PD-model preparation. Since the EA could restore this increased rotation (Figure 2), it may have a possible neuroprotective effect on the nigrostriatal pathway.

Also, we have shown that MFB lesion can induce motor deficiencies such as a significant decrease in the rats' forepaws stride-length (Figure 3) and motor coordination (Figure 4). Oral administration of EA could restore these motor deficiencies in MFB-lesioned rats. This confirms that EA can exert neuroprotective activity against 6-OHDA-induced damage in the MFB. Brain activity is controlled by synchronized oscillations among neurons; appear as rhythmical fluctuations in EEG and local field potentials (LFPs). Depth recordings

from the BG of patients with PD have shown synchronized oscillations of the LFPs at different frequencies (27). These recordings allow studying the power and frequency bands of the EEG during various types of movements to clarify the role of the BG in specific motor performances. For example, it was reported that fast movements induced low frequency LFPs in the BG (27). Similarly, it was shown that synchronized gamma oscillations are related to motor tasks in PD (28).

In this study MFB lesion significantly decreased the EEG power in MFB-lesioned rats and treatment with PPX and EA significantly increased EEG power (Figure 5A). In addition, there are alterations in frequency bands' power of EEG in BG. It was reported that oscillations in the beta band are noticeable in the human motor system and can be recorded in BG. A study showed that beta activity is exaggerated in the BG of patients with PD and may contribute to their motor deficiency (29). Our results showed that gamma and beta powers significantly decreased in MFB-lesioned rats and administration of PPX or EA significantly increased their frequency band powers (Figure 5B-C), while, alpha power significantly decreased in PD+Veh group and PPX or EA could not increase its power (Figure 5D). As shown in Figure 5E-F, there was no significant difference between groups in theta and delta power. Similar reports have shown that synchronized neuronal oscillations at the beta frequencies are common in the human motor system, but their functions are uncertain. Oscillatory beta activity is in turn moderated by the net dopamine levels at sites of cortical input to the basal ganglia, facilitating the appropriate production of action potentials. Therefore, loss of dopamine disrupts this function (30). Also, beta frequency oscillations are associated with the slowness of movement in PD (31). In PD, gamma activity is similarly enlarged with dopaminergic prescription accompanied by improvement of motor function, so it was proposed that coordinated activity of the gamma band in BG can flat the progress of motor tasks (32). It was reported that impaired movements are associated with the reduced cortical activity (33). Similarly, our results showed an association between motor insufficiencies and EEG disturbances in PD-model.

There are many pieces of evidence for the involvement of oxidative stress (OS) in 6-OHDA-induced neuronal damage. The neurotoxicity of 6-OHDA is due to its oxidation by molecular oxygen or monoamine oxidase in the brain, leads to production of intracellular H_2O_2 which can be transformed into highly reactive hydroxyl radicals and generation of hydrogen peroxide, reduction in glutathione (GSH) and SOD activity, increase in MDA levels and production of superoxide free radicals cause cell damage. (34). OS contributes to the injury of lipids, proteins, DNA and the cascade of events leads to the death of dopaminergic neurons in PD (35). The brain shows a high vulnerability to reactive oxygen species (ROS). Free radicals and OS can play an important role in the PD (36). In many of these processes, oxidative stress is a hallmark factor where the oxidation of dopamine (DA) produces ROS and an unbalanced production of ROS cause neural damage and death (37). Our results showed that MFB lesion significantly reduced the GPx and SOD activity and increased the levels of MDA in the striatum and hippocampus. Indicating, MFB lesion can increase the brain free radicals and cause motor and electrophysiological impairments. On the other hand, the administration of oral EA significantly increased the GPx and SOD activity and decreased the levels of MDA in MFB-lesioned rats. It can be concluded that EA can enhance the cerebral antioxidant defense leads to a reduction of oxidative stress. Thus, EA can show a neuroprotective effect against 6-OHDA induced neural oxidative damage.

Polyphenols and flavonoids prevent oxidative stress and are valuable for the prevention of cardiovascular, inflammatory and other diseases (38,39). Dietary supplements such as grape seed extract (GSE) enriched in proanthocyanidin (PA) have been proposed to have several health profits, because of antioxidant and other useful properties of the PA (40).

In this study, we investigated the possible effects of ellagic acid on motor disturbances, pallidal local EEG and its frequency bands' power and cerebral oxidative stress in a rat model of PD induced by 6-OHDA. Our results confirm that MFB lesion can impair the motor performances, pallidal local EEG and its frequency bands' power which are associated with a reduction in GPx and SOD activity besides the increase in the levels of MDA. On the other hand, EA can restore these impairments and increase the cerebral antioxidant defense leads to improving motor disturbances, pallidal local EEG, and its frequency bands' power. Taken together, these findings may provide an experimental basis for the use of EA in the treatment and prevention of free radicals induced neural damage in PD. Further studies are necessary to clarify neuroprotective mechanisms of EA.

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