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Neuroprotective effects of voluntary wheel running and eriobotrya japonica flower extract on Parkinsonian rats

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Abstract

Backgrounds: The loss of dopamine-secreting cells and the decrease in the status of antioxidant is associated with incidents of Parkinson's disease. The purpose of the present study was to determine the protective effect of a 12- weeks voluntary wheel running (VWR) along with the injection of eriobotrya japonica flower extract (EJFE, 200 mg/kg body weight, 3 days a week) on cerebral dopamine neurotrophic factor (CDNF), superoxide dismutase (SOD), and malondialdehyde (MDA) in the cerebral cortex of a rat model of Parkinson's disease (PD).

Method: To do so, the rats were trained for 12 weeks with and without EJFE prior to the induction of Parkinson. In order to obtain the Parkinsonian model, 6-hydroxydopamine (6-OHDA) (5 µL) was injected intracerebrally. Data were statistically analyzed by one-way analysis of variance followed by LSD post-hoc test ($P < 0.05$).

Results: 6-OHDA injection significantly decreased the CDNF contents, and SOD activity while it increased MDA levels in cerebral cortex of the Parkinsonian control group. The pre-training of PD rats with and without EJFE increased the CDNF content and SOD activity and also decreased MDA levels.

Conclusion: Preconditioning by VWR and EJFE may be effective in reducing the consequences of toxins resulted in Parkinson's disease.

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Introduction

Parkinson's disease, a degenerative condition characterized by tremor and motor impairment, is caused by loss of dopamine-secreting neurons in substantia nigra (1, 2). Cerebral dopamine neurotrophic factor (CDNF) is a secretory protein with 161 amino acid residues. It protects dopaminergic nerves by attaching to their receptors, and it is widely distributed in the cerebral cortex, midbrain, cerebellum, substantia nigra and the striatum (2, 3). Superoxide dismutase (SOD), as an antioxidant enzyme, is widely distributed in mitochondria and cytosol, scavenging destructive superoxide radicals (1, 4). Studies show that CDNF content (2, 3) and

SOD expression and activity (1, 5) drop when patients are suffering from suffering from Parkinson's disease. Thus, nutritional (6) and exercise training (7, 8) approaches have been used as therapeutic models against the Parkinson's disease.

An appropriate balance between free radicals and antioxidants is necessary for the survival of dopaminergic neurons (9). As powerful antioxidant substances, phenolic compounds, which are available in vegetables and fruits, have been found to be of important contribution to the treatment of Parkinson's disorder (6). Eriobotrya japonica flower extract contains high levels of phenolic and flavonoids compounds (4, 10) and it has been shown that EJFE increases the total antioxidant capacity (TAC) (10), SOD expression and activity (4), and also reduces malondialdehyde (MDA) levels (4, 10, 11). Treatment with EJFE in adriamycin-induced nephropathy has been reported to reduce lipid peroxidation in plasma and renal tissues (11). Similarly, an increase in total antioxidant capacity and a reduction in malondialdehyde levels have been shown following 30 day EJFE injection in mercuric chloride-induced hepatotoxicity rats (10). Another study has pointed out that EJFE up-regulates SOD expression (as a dose-dependent manner), while inhibiting heme oxygenase-1 (HO-1) expression and MDA production in rats with chronic bronchitis (4). Furthermore, it has been revealed that 14 consecutive days running on treadmill (30 min per day) in parkinsonian rats reduces the rotational asymmetry, and increases the survival and the number of fibers projecting from dopaminergic neurons in the substantia nigra and striatum (8). In addition, following 14 days voluntary wheels running (VWR) motor control, asymmetry in the use of left and right forelimbs and dopamine cell loss improved in 6-OHDA-lesioned rats (7). Furthermore, both early and late exercise trainings (2 × 20 min per day, 30 days) have resulted in the attenuation of dopamine depletion in the striatum of hemi-Parkinsonian rats (12). Finally, two studies have reported that running on treadmill for 4 to 18 weeks reduces the impaired movement and balances the incoordination in chronic Parkinsonian mice through significant increase in dopamine concentration and tyrosine hydroxylase enzyme activity is observed (13, 14).

The mechanism through which exercise prevents the loss of dopaminergic neurons in Parkinson's disease is not well documented. It is believed that some of the beneficial effects of exercise resulted from neurotrophins' up-regulation and antioxidant improvement. Although, there have been studies on the therapeutic effects of exercise training on Parkinson's disease, barely have any investigated the effect of exercise preconditioning on the consequences of Parkinson's disease induced by toxins. Furthermore, the prophylactic effect of exercise training along with antioxidant EJFE has not been studied in 6-OHDA-lesioned rats. Hence, the aim of the present study was to investigate the preconditioning effects of VWR along with EJFE on CDNF, SOD, and MDA levels of cerebral cortex in Parkinson's rats.

Materials and Methods

Animals

All animal experiments conformed to the guidelines for the use and care of laboratory animals ("Principles of laboratory animal care", NIH publication No. 86-23. Revised 1996), and the study was approved by the local ethics committee. Forty-three male Albino Wistar rats (12 weeks old, weight 215 g), were obtained from the Animal Breeding Unit, Pasteur Institute of Amol (Iran). The rats had free access to standard rat food (Behparvar Company, Iran) and tap water in controlled light/dark (12-12 hours), humidity (50%) and temperature (22°C) room. All animals were given 2 weeks to

acclimate to the running wheel and were then divided into six groups: healthy control (HC) (n=8); healthy training (HT) (n=6); Parkinsonian control (PC) (n=8); pre-trained rats for 12 weeks prior to induction of Parkinson (TP) (n=7); pre-treated rat with EJFE before inducing Parkinsonian (EP) (n=8); and a group that was exercised and injected by EJFE and then became Parkinsonian (TEP) (n=6).

EJFE preparation

To prepare the EJFE, eriobotrya japonica flower powders (100g) were added to 600 mL of 20% (V/V) ethanol/aqueous solvent. The mixture was placed in the shaker (KS500) at 325 rpm for 24 h. In the next step, the sample solution was filtered using a standard filter paper (Whatman, N.4) for two times. The filtered solution was transferred into the distillation balloon, and the solvent was evaporated by rotary vacuum for 6 hours at 40 °C (15). Finally, the soluble extract was obtained by adding normal saline to the drying product.

Parkinsonism induction

Animals were fully anesthetized with ketamine (80 mg/kg) and xylazine (8 mg/kg, IP) and placed in a stereotaxic apparatus (Stoelting, U. S. A). A small hole was made at the targeted region according to atlas of Paxinos and Watson stereotaxic coordinates: anteroposterior 0.5 mm, lateral 1 mm, and dorsoventral 1.5 mm (16). To obtain 6-hydroxydopamine (6-OHDA) (Sigma, U.S.A) solution, 16 µg 6-OHDA was dissolved in 4 µl of sterile 0.9% saline containing 0.2% ascorbic acid (17). As 6-OHDA is also toxic to norepinephrine neurons, a norepinephrine transporter blocker, desipramine (15 mg/kg, Sigma, U.S.A), was injected intraperitoneally 30 min before 6-OHDA infusion (7). 6-OHDA solution was infused into the rat's substantia nigra (18) by Hamilton syringe via microinjection pump. The injection volume of 6-OHDA for each rat was 5 µL (19). Rotation test was used immediately, 24, 48 and 72 hours after 6-OHDA administration to confirm occurrence of Parkinson in rats (20).

Exercise training protocol and EJFE injection

The rats in the exercise groups were individually placed in cages equipped with wheel running apparatus for 12 weeks (University of Mazandaran, Iran). EJFE (200 mg/kg body weight) were injected intraperitoneally to rats in EP and TEP groups for 12 weeks (3 days per week) (10).

Tissue preparation and biochemical assays

Rats were sacrificed by decapitation under deep anesthesia (Ketamine, 80 mg/kg and Xylazine, 9 mg/kg; IP) 72 hours after the last session of exercise training and extract injection. The cerebrum cortex was removed, washed by normal saline, and finally stored at -80 °C until analyses were made. The protein contents of CDNF in cerebrum cortex were measured with commercially 96-well ELISA kits (#CSB-MP002119RA, Cusabio Biotech CO., LTD. Sino-American). The sensitivity of the kit was less than 0.039 ng/ml. Also, SOD activity and MDA levels in cerebrum cortex were measured by applying the method of Kono (1978) (21) and Draper and Hadley (1990) (22), respectively.

Statistical analysis

Data were analyzed by SPSS software (version 16.0) and presented in terms of means ± SD. Initially, Kolmogorov-Smirnov's and Levene's tests were performed on all dependent variables in all groups to test the normality and equality of variances, respectively. Then, data were statistically analyzed by one-way analysis of variance and followed by Bonferroni post-hoc comparison (conservative and to reduce the risk of a type I error) to test the differences between among groups. The significance level was set at $P < 0.05$.

Results

Exercise training with and without EJFE treatment attenuate 6-OHDA-induced decrease in CDNF

As depicted in Figure 1, the cerebral cortex of CDNF significantly was reduced in the PC (3.18 ± 0.3 ng/ml) group compared to the control (4.1 ± 0.49 ng/ml, P = 0.017) following 6-OHDA injection. In contrast, cerebral cortex of CDNF was significantly increased in HT (4.42 ± 0.66 ng/ml, P = 0.002), TP (4.4 ± 0.78 ng/ml, P = 0.002), EP (4.46 ± 0.67 ng/ml, P = 0.001), and TEP (4.04 ± 0.05 ng/ml, P = 0.025) groups compared to PC group.

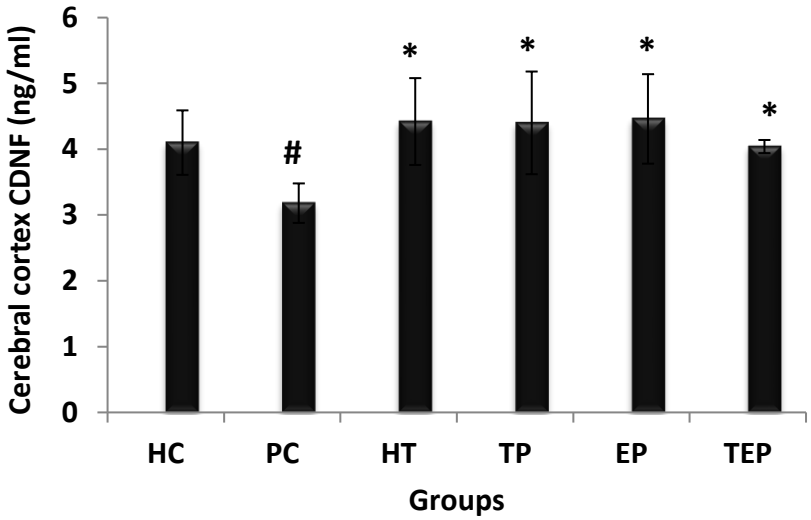

Figure 1. Effect of preconditioning with VWR and EJFE on CDNF contents in cerebral cortex of healthy and Parkinsonian rats. The asterisk (*) indicates a significant difference from PC (P<0.05). The hash sign (#) indicate sasignificant difference from C (P<0.05). Abbreviations: healthy control (HC); Parkinsonian control (PC); healthy training (HT); pre-trained rat for 12 weeks prior to induction of Parkinson (TP); pre-treated rat with EJFE before the induction of Parkinsonian (EP); and a group that was exercised and injected by EJFE which then became Parkinsonian (TEP)

Effect of exercise training with and without EJFE treatment prevent on SOD level

To elucidate the role of reactive oxygen species in the induction of Parkinson by 6-OHDA, SOD in the cerebral cortex was measured. In figure 2, it is shown that 6-OHDA resulted in significant reduction in SOD level compare to control group (0.0016 ± 0.0008 U/ml vs. 0.0027 ± 0.0004 U/ml, P = 0.005). Interestingly, exercise training (0.0024 ± 0.0001 U/ml, P = 0.0028), and EJFE (0.0027 ± 0.0008 U/ml, P = 0.003), alone or together (0.0023 ± 0.0004 U/ml,P = 0.043) attenuate the loss of SOD.

Exercise training with and without EJFE prevent 6-OHDA induced lipid peroxidation

Oxidative stress biomarker, thiobarbituric acid reactive substances (TBARS) (Figure 3), increased in the cerebral cortex following the infusion of 6-OHDA (62.56 ± 9.76 nmol/ml vs. 26.72 ± 8.66 nmol/ml of control, P = 0.001). Preconditioning by exercise and/or EJFE decreased significantly MDA levels induced by 6-OHDA (Figure. 3).

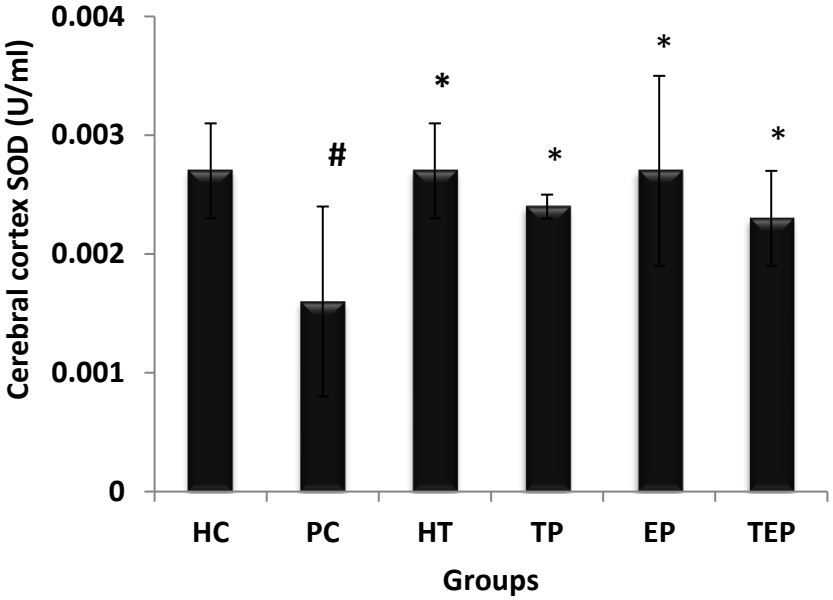

Figure 2. the effect of preconditioning with VWR and EJFE on SOD activity in cerebral cortex of healthy and parkinsonian rats. The asterisk (*) indicates a significant difference from PC (P<0.05). The hash sign (#) indicates a significant difference from C (P<0.05). Abbreviations: healthy control (HC); Parkinsonian control (PC); healthy training (HT); pre-trained rat for 12 weeks prior to the induction of Parkinson (TP); pre-treated rat with EJFE before the induction of Parkinsonian (EP); and a group that was exercised and injected by EJFE which then became Parkinsonian (TEP).

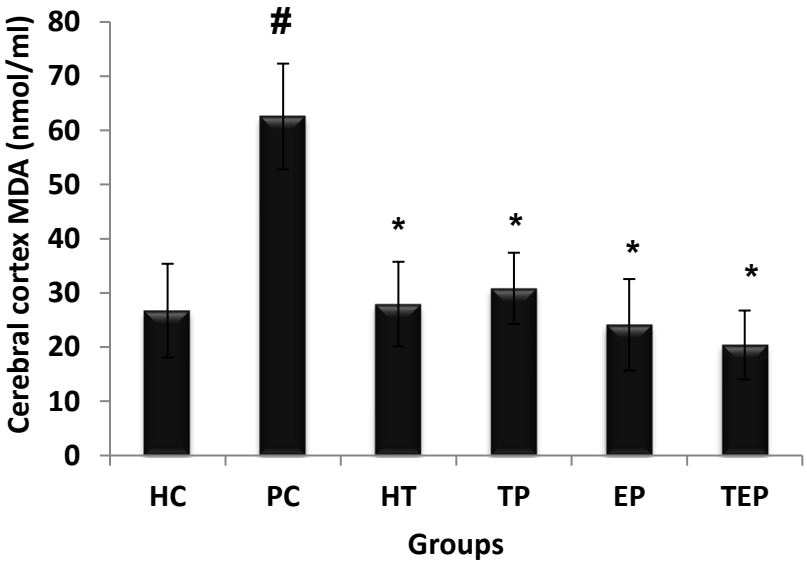

Figure 3. The efect of preconditioning with VWR and EJFE on MDA levels in cerebral cortex of healthy and Parkinsonian rats. The asterisk (*) indicates a significant difference from PC (P<0.05). The hash sign (#) indicates a significant difference from C (P<0.05). Abbreviations: healthy control (HC); Parkinsonian control (PC); healthy training (HT); pre-trained rat for 12 weeks prior to induction of Parkinson (TP); pre-treated rat with EJFE before the induction of Parkinsonian (EP); and a group that was exercised and injected by EJFE which then became Parkinsonian (TEP).

Discussion

In recent years, there has been a growing interest in evaluating the effects of nutritional and exercise training approaches on the improvement of neurological factors involved in motor function (23, 24). Studies have claimed that exercise improves motor function by improving the energy metabolism, altered synaptic plasticity, and increasing thre mitochondrial functions (23). Here, in a Parkinsonian animal model, it was shown that CDNF content and SOD activity of the cerebral cortex attenuated in rats preconditioned by VWR and EJFE. Moreover, VWR and EJFE prevented the increasing of the MDA levels induced by 6-OHDA. These findings suggest exercising and EJFR have a protective role against oxidative stress induced by 6-OHDA.

Neurotrophines are secretory proteins that promote the survival, differentiation and maintenance of neurons (2, 25, 26). CDNF is a novel neurotrophic factor with the molecular weight of 18 kDa that has a strong trophic activity on dopaminergic neurons comparable to glial cell line-derived neurotrophic factor (3, 26). It is reported that injecting CDNF before and after 6-OHDA delivery into striatum would reduce ipsilateral turning behavior, rescue dopaminergic tyrosine-hydroxylase-positive cells in the substantia nigra, and restore the dopaminergic function in substantia nigra (2). In addition, Nadella and colleague have reported a reduction in 6-hydroxydopamine-induced neuroinflammation in the rat substantia nigra following transient transfection of human CDNF gene (3). Collectively, these findings suggest a critical roles of CDNF on dopaminergic neurons that degenerate during Parkinson's disease. However, the role of physiological stimuli and pathological damage on endogenous regulation of CDNF in the nervous system is not fully elucidated.

To our knowledge, there is no study that has investigated the effects of exercise training on CDNF concentration in the brain. Among neurotrophines, most scholars have focused on the effect of exercise training on brain-derived neurotrophic factor (BDNF). Studies have shown that exercise improves the cognitive processes and increases resistance to brain injury by increasing the BDNF content (23, 24). Generally, the conversion of pro-BDNF to the mature form, insulin growth factor-1, tumor necrosis factor alpha, and estrogen (24), as well as the reduction in corticosterone concentrations (13, 14) has been considered as possible mechanisms to increased BDNF following exercise. Studies have demonstrated that early and late exercise training (12) on treadmill (8) and wheel running (7) increase the dopamine concentration, survival and fibers projecting of dopaminergic neurons in the substantia nigra and striatum. It is thought that the increase in CDNF content following VWR in Parkinson's rats increases the dopamine concentration and eventually the resistance of dopaminergic neurons against to toxin insults (2, 3).

Polyphenole compounds such as flavonoids which can be found in vegetables and fruits, reduce the occurrence of several diseases due to their antioxidant properties (4, 10, 11). Data suggest that oxidative stress play a pivotal role in the pathogenesis of PD. Radical production by 6-OHDA induces neuronal death which leads to neurodegenerative disorders like PD. Therefore, any approaches to alleviate radical production or increase the antioxidant defense mechanism might be supportive in prolonging survival of dopaminergic neuron. Results of the present study demonstrate that EJFE reduces MDA levels, and increases SOD activity in cerebral cortex of Parkinson's rats. Our findings are consistent with other studies that have reported increased expression and

activity of SOD and decreased MDA levels following EJFE in nephropathy (11), hepatotoxicity (10), and chronic bronchitis (4) in rats. Yokota and colleague (2006) have pointed out scavenging of reactive oxygen species by EJFE (27) which could be attributed to their phenolic compounds (gallic acid). Also, Huang and colleagues (2006) have reported a reduction in HO-1 expression and its mRNA as oxidant's producing resource (4). In addition, studies have shown that EJFE increases TAC (10) and there is a positive correlation between phenolic compounds and TAC (28). Furthermore, the results of this study showed that EJFE prevents from CDNF reduction induced by 6-OHDA. By increasing antioxidant enzyme and neurotrophic factor, EJFE seems to reduce cell apoptosis and increase resistance of dopaminergic neurons to stress oxidative induced by 6-OHDA.

Conclusion

Although numerous studies investigated the antioxidant effects of EJFE in various pathological models, our study showed promising effects of EJFE on Parkinson's rats. In addition, although other studies have shown that exercise training leads to the survival and projection of dopaminergic neurons, the exact mechanism is not clear. However, it appears that some of the VWR and EJFE effects on Parkinson's disease may be attributed to increase in SOD and CDNF.

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