# The effects of aqueous cinnamon bark extract and cinnamaldehyde on neurons of substantia nigra and behavioral impairment in a mouse model of Parkinson’s disease

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## ABSTRACT

**Background and Objective:** Parkinson's disease (PD) is characterized by a progressive loss of dopaminergic neurons in substantia nigra. In recent years, there have been interests in the role of the free radical damage in PD. Cinnamon and its derivative, cinnamaldehyde acts as powerful antioxidant and anti-inflammatory agents. This research focused on the effects of cinnamon extract and cinnamaldehyde on neurons of SNc of a mouse model of Parkinson’s disease.

**Materials and Methods:** 45 adult male mice with an average weight of 25-35 g were divided into 9 groups of 5 each: group 1: control PBS, group 2: control serum, group 3: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), group 4: MPTP + low dose of cinnamon extract pretreatment (20 mg/kg), group 5: MPTP + high dose of cinnamon extract pretreatment (40 mg/kg), group 6: MPTP + low dose of cinnamon extract treatment (20 mg/kg), group 7: MPTP + high dose of cinnamon extract treatment (40 mg/kg), group 8: MPTP + cinnamaldehyde pretreatment (30 mg/kg), group 9: MPTP+ cinnamaldehyde treatment (30 mg/kg). Rotarod test was used to assess motor and balance of the mice. After behavioral studies, all mice were anesthetized and perfused transcardially with 0.1 M PBS (PH=7.4) followed by 4% buffered paraformaldehyde fixative. The brain of the mice were removed and fixed in the paraformaldehyde and stained for Nissl and the number of Nissl-stained neurons were counted. Data was analyzed using SPSS software by one way ANOVA.

**Results:** Aqueous cinnamon extract and cinnamaldehyde improved rotarod performance of MPTP-lesioned mice and prevented loss of Nissl-stained neurons of SNc of the midbrain.

**Conclusion:** These findings suggest that cinnamaldehyde as a natural antioxidant may protect neurons of SNc neurons against Parkinson’s disease.

## 1. Introduction

Parkinson’s disease (PD) is a neurodegenerative disease that is characterized by progressive damage and destruction of neurons in substantia Nigra pars compacta (SNc) which leads to movement dysfunction and is accompanied with tremor, rigid muscles and impaired balance (1). In recent years, there have been interests in the role of the free radical damage in PD and evidences from animal models of PD showed the role of oxidative stress in PD pathogenesis (2,3).

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The neurons of SNc are at the risk from radical generation and their damages (2,3). Oxidative stress is a pathological condition that occurs when the production of free radicals exceeds the capability of the antioxidant defense system to neutralize these toxic compounds. Overabundant free radicals can cause damage of biomolecules and resulting in many diseases (4). Although until now no effective therapy is available for PD but the attentions have drawn to natural remedies (5,6). Evidences showed that cinnamon acts as a potent free radical scavenger. Cinnamon and its derivative, cinnamaldehyde act as powerful antioxidant and anti-inflammatory agents.

Cinnamon is a spice that is made from the inner bark of trees called cinnamomum. Cinnamon has been used as an ingredient throughout history, dating back as far as ancient Egypt. Cinnamon is derived from a Greek word that means sweet wood comes from the inner bark of tropical cinnamon trees (7). The smell and flavor of cinnamon is due to the oil part compound called cinnamaldehyde (8). Cinnamon inhibits the buildup of a protein called tau in the brain which is a sign of Alzheimer’s disease (49). Cinnamon is metabolized to sodium benzoate in the liver so it can pass through blood brain barrier (10). This research is focused on the effects of cinnamon extract and cinnamaldehyde on neurons of SNc of a mouse model of Parkinson’s disease

2. Materials and Methods

2.1. Chemicals

In this experimental study, cinnamon (Cinnamomum zeylanicum) sticks were purchased from medicinal plant store which is confirmed by a botanist in traditional medicine center of Iran University of Medical Sciences. The sticks were mashed into small pieces and to be ground to provide cinnamon powder, then an aqueous extract was obtained by maceration method (11). Maceration is involved soaking cinnamon powder in a container with water and allowed to stand at room temperature for 3 days, then the mixture was filtered and dried by freeze dryer. Cinnamondeyhe, the derivative of cinnamon, and MPTP (1 methyl 4-Phenyl-1,2,3-6 tetra hydro pyridine) were purchased from SigmaAldrich (USA).

2.2. Animals

45 adult male Suri mice with an average weight of 25-35 g were purchased from animal house of Iran university of Medical Sciences and housed in a temperature controlled room at 23±2°C. All animal works were approved by the ethical guidelines for the care of laboratory of cellular and molecular research center of Iran University of Medical Sciences.

2.3. Experimental design

The animals were divided into 9 groups of 5 each as follows: group 1: control PBS, group 2: control serum, group 3: MPTP, group 4: MPTP + low dose of cinnamon extract pretreatment (20 mg/kg), group 5: MPTP + high dose of cinnamon extract pretreatment (40 mg/kg), group 6: MPTP + low dose of cinnamon extract treatment (20 mg/kg), group 7: MPTP + high dose of cinnamon extract treatment (40 mg/kg), group 8: MPTP + cinnamaldehyde pretreatment (30 mg/kg), group 9: MPTP + cinnamaldehyde treatment (30 mg/kg). Groups 3, 4, 5, 6, 7, 8, and 9 were injected intraperitoneally with a single daily dose of 20 mg/kg of MPTP for four days. Single daily doses of 20 mg/kg and 40 mg/kg of cinnamon extract dissolved in physiological serum were intraperitoneally administrated to groups 4 and 5, respectively, for two weeks prior to induction of PD. Groups 6 and 7 were intraperitoneally injected with a single low dose (20 mg/kg) and high dose (40 mg/kg) of cinnamon extract dissolved in physiological serum one hour after MPTP injection. Single daily dose of 30 mg/kg of cinnamaldehyde dissolved in PBS was injected intraperitoneally to group 8 for two weeks before the injection of MPTP. One hour after PD induction of group 9, a single dose of 30 mg/kg of cinnamaldehyde dissolved in PBS was injected. Group 1 and 2 received only PBS and physiological serum.
2.4. Measurement of motor coordination

Rotarod test was used to assess motor and balance of the mice. In this test, the mouse has to keep its balance on a rotating rod (12). After placing the mouse on the rod, each mouse was trained to stay on the rotating rod. Once the trained mice were able to stay on the rod rotating at 4 rpm for 1 minute, they were proceeded to the test. Each mouse was evaluated for three trials separated by 15 minutes intertrial intervals each day at an accelerating speed at 20 rpm in 300 seconds. The periods that each mouse was able to maintain its balance on the rod was recorded before and after MPTP administration.

2.5. Histochemical analysis

After behavioral studies, all the mice were anesthetized with ketamine (50 mg/kg) in combination with xylazine (5 mg/kg) and perfused transcardially with 0.1 M PBS (PH=7.4) followed by 4% buffered paraformaldehyde fixative. The brains of the mice were removed and fixed in the paraformaldehyde overnight, then the brains dehydrated in ascending alcohol series, cleared in xylene, infiltrated with paraffin and embedded in paraffin. The 5 µm coronal sections were collected and stained for Nissl (Cresyl violet). The number of the neurons were counted in 5 fields of each section.

2.6. Statistical analyses

Data was analyzed using SPSS software by one way analysis of variance (ANOVA) and t tests. The results are expressed as the mean ± SD.

3. Results

Fig. 1A shows the boundaries of the SNc which we measured cell number. Statistical and comparative light microscope analyses showed that the cell number of Nissl stained neurons in group 3 significantly decreased as compared to the other groups (P<0.05) (Fig. 1B and Fig. 2). The cell number in group 9 was similar to the control PBS group and there was no significant difference. On the other hand, this group had the most neurons compared to the other groups (Fig. 1B9 and Fig. 2). Population of the neurons in group 7 was more than group 3 but it was less than control serum group (P<0.05) (Fig. 1B1 and Fig. 2). The number of neurons in groups 8 and 5 was similar but there was a significant decrease of neurons in these groups when compared to group 3 but these groups had less neurons compared to groups 1 and 2 (P<0.05) (Fig. 1B8 and B6 and Fig. 2). As shown in Fig. 1, groups 4 and 6 had an apparent more neurons as compared to group 3 but these groups had less neurons compared to groups 1 and 2 (P<0.05) (Fig. 1B2 and B7 and Fig. 2). MPTP toxicity significantly impaired balance of the mice in group 3 compared to control group (P<0.05) (Fig. 3). Group 9 significantly maintained their balance on the rotating rod as compared to the other groups but there was a significant difference between this group and control (P<0.05) (Fig. 3). Groups 7, 8, 5, 6 and 4 had better locomotion as compared to group 3, but they had hypolocomotion as compared to control (P<0.05) (Fig. 3).
Fig. 1. Photomicrograph A shows the boundaries of the SNc. B1: MPTP + high dose of cinnamon extract treatment (40 mg/kg), B2: MPTP + low dose of cinnamon extract treatment (20 mg/kg), B3: control PBS, B4: control serum, B5: MPTP, B6: MPTP + high dose of cinnamon extract pretreatment (40 mg/kg), B7: MPTP + low dose of cinnamon extract pretreatment (20 mg/kg), B8: MPTP + cinnamaldehyde pretreatment (30 mg/kg), B9: MPTP + cinnamaldehyde treatment (30 mg/kg). Arrows show neurons, Bar: 30 nm.

Fig. 2. The histogram shows the difference of the number of Nissl-stained neurons between control and experimental groups. Fig. 3. Effect of cinnamon extract and cinnamaldehyde on the impairment locomotion of the MPTP-induced mice. As shown in the histogram, there was no significant difference between MPTP-induced mice that treated with cinnamaldehyde in comparison with the control, but the impairment of behavioral activities among MPTP-induced mice was significant as compared to control (P<0.05).
4. Discussion

PD is characterized by a progressive loss of neurons in substantia nigra and it is hypothesized that PD may be the result of excess production of free radicals, therefore the efforts are focused on the natural antioxidants for protection of neurons against free radicals. Since cinnamon and its derivative, cinnamaldehyde are strong antioxidant agents (13, 14), the current study was carried out to investigate their effects on neurons of SNc in PD mouse model. According to the results of this study, cinnamaldehyde had the strongest effect on protection of neurons in SNc of MPTP-induced mice as compared to cinnamon extract administration. Cinnamaldehyde is chemically more stronger than cinnamon (13, 14). Neuroprotective effects of cinnamaldehyde is due to inhibition of up-regulation of inducible nitric oxide synthase and cyclooxygenase-2 which maintain the number of neurons of SN region (13). Cinnamaldehyde has a potential neuroprotective effect against the ischemic stroke, which may be via inhibition of neuroinflammation through attenuating iNos, Cox2 expression and NF-k B signaling (14).

Several studies have shown that expression of BDNF is significantly reduced in the SNc of patients with PD and that BDNF protects DA neurons from 6-hydroxydopamine induced toxicity (15). Cinnamon increases the production of BDNF in cultured astrocytes and neurons (16) and also can up-regulate DJ-1 and parkin that are known to support the survival of DA neurons (17). Oral administration of cinnamon powder protected TH positive neurons from MPTP toxicity (17). MPTP administration caused hypolocomotion in mice while high doses of cinnamon extract and cinnamaldehyde treatment improved behavioral activities of the mice. In this study, evaluation of locomotor activities of the mice by rotarod showed that the latency to fall off significantly increased in MPTP-induced mice which treated with cinnamaldehyde. This result is in agreement with Khasnavis et al, whom reported that cinnamon treatment decreases functional impairment in MPTP intoxication in mice (17). The results of a study indicated that the rats which were treated with cinnamaldehyde of the dose level of 73.5 mg/kg showed imbalance in the antioxidant status and this may affect the neuromuscular coordination in the rats which results in changes of behavioral parameters, therefore the effects of cinnamaldehyde is time and dose dependent (18, 19). Glial activation play an important role in the pathogenesis of neurodegenerative diseases (20). It has shown that cinnamon metabolite can inhibit the expression of proinflammatory molecules in cultured astrocytes and microglia (21).

Conclusion: There are evidences that the natural antioxidant compounds prevent neurodegenerative diseases using animal models. These findings suggest that cinnamaldehyde as a natural antioxidant may protect dopaminergic neurons against Parkinson’s disease.

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