Available online at http://jbcp.shahed.ac.ir/

Journal of Basic and Clinical
PATHOPHYSIOLOGY

Volume 5, Number 1, Autumn-Winter 2016-2017

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

Fereshteh Mehraein[1,2*], Maryam Zamani[1], Mehrdad Roghani[3], Feraidoon Negahdar[1], Asieh Shojaee[4]

1. *Department of Anatomy, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran*
2. *Minimally Invasive Surgery Research Center, Iran University of Medical Sciences, Tehran, Iran*
3. *Neurophysiology Research Center, Shahed University, Tehran, Iran*
4. *Traditional Medicine Faculty, Iran University of Medical Sciences, Tehran, Iran*

Article info
Received: 17 Dec 2016
Revised: 26 Jan 2017
Accepted: 04 Feb 2017

p-ISSN:2322-1895
e-ISSN: 2345-4334

Key Words:
Cinnamon
Cinnamaldehyde
Dopaminergic neurons
Parkinson's disease
MPTP

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), group7: 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 cinnamaldehye 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).

***Corresponding Author:** Fereshteh Mehraein
Department of Anatomy, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran
Email: femehra@yahoo.com

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' 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. Cinnamldeyhe, 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, group2: control serum, group3: 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 boundries 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. 1B1and 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 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).

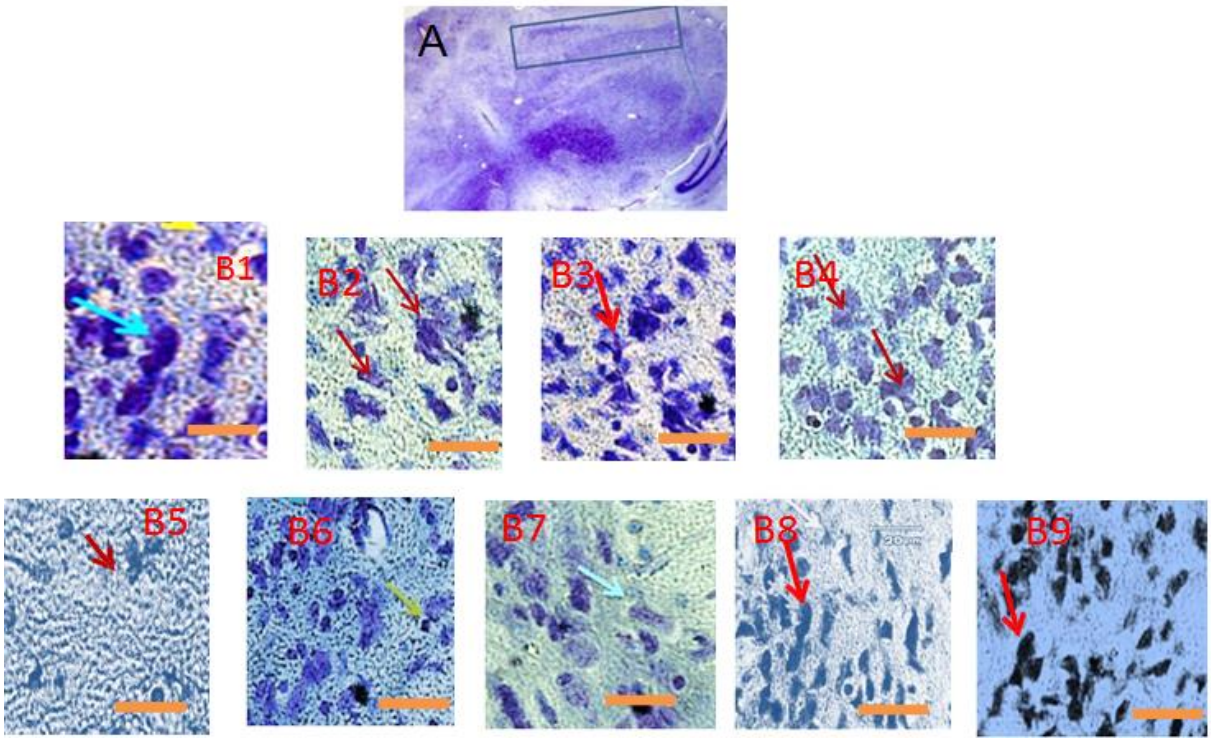

Fig. 1. Photomicrograh A shows the boundaries of the SNc. B1: MPTP + high dose of cinnamon extract treatment (40 mg/kg) B2: MPTP + low dose of cinnamon extract treatment (20 mg/kg), B3: control PBS, B4: control serum, B5: MPTP, B6: MPTP + high dose of cinnamon extract pretreatment (40 mg/kg), B7: MPTP + 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.

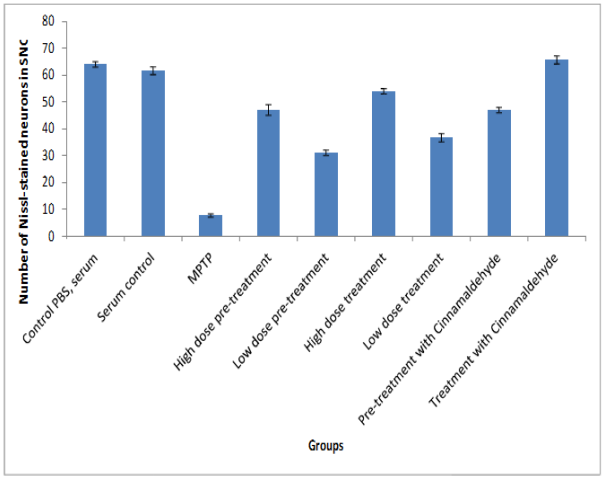

Fig. 2. The histogram shows the difference of the number of Nissl-stained neurons between control and experimental groups.

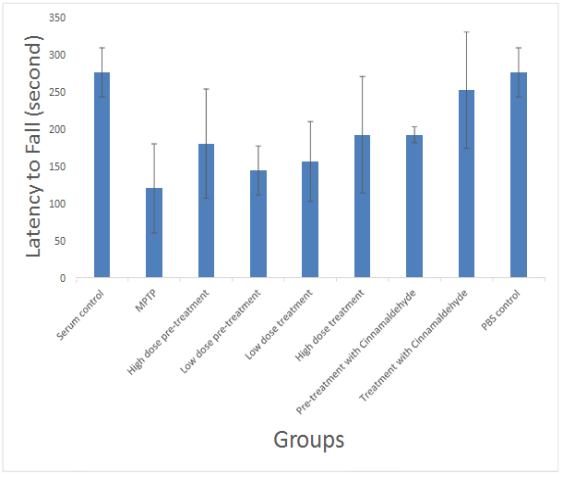

Fig 3. Effect of cinnamon extract and cinnamaldehyde on the impairment locmotion of the MPTP-induced mice. As shown in the histogram, there was no significant difference between MPTP-induced mice that treated with cinnamaldehyde in comparison with the control, but the impairment of behavioral activities 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 nitricoxide 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 neurodegenrative 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 neurodegenrative diseases using animal models. These findings suggest that cinnamaldehye as a natural antioxidant may protect dopaminergic neurons against Parkinson’s disease.

Acknowledgements

This research was supported by Iran University of Medical Sciences.

References

1. Yacoubian TA, Standaret DG. Targets for neuroprotection in Parkinson’s disease. Biochemica et Biophysica Acta 2009:1792(7):676-687.
2. Munch G, Gerlach M, Sian J, Wong A, Riederer P. Advanced glycation end products in neurodegeneration: more than early markers of oxidative stress? Annals of Neurology 1998;44:S85-8.
3. Serra JA, Dominguez RO, de Lustig ES, Guareschi EM, Famulari AL, Bartolome EL, et al. Parkinson's disease is associated with oxidative stress: Comparison of peripheral antioxidant profiles in living Parkinson's Alzheimer's and vascular dementia patients. Journal of Neural Transmission 2001;108:1135-48.
4. Yan MH,Wang X,Zhu X. Mitochondria defectsand oxidative stress in Alzheimer disease and Parkinson disease. Free Radical Biology and Medicine 2013: 62; 90-101.
5. Mehraein F, Sarbishegi M, Aslani, A. Evaluation of effect of oleuropein on skin wound healing in aged male Balb/c mice. Cell Journal 2014: 16(1): 25-30.

6. Ravindran P BK. Shylaja M,ed Cinnamon and Cassia: The genus Cinnamomum. Danvers, Ma: CRC;2005.
7. Patra K, Bose S, Sarkar S, Rakshit J, Jana S, Mukherjee A, et al. Amelioration of cyclophosphamide induced myelosuppression and oxidative stress by cinnamic acid. Chemico-biological Interactions 2012:195(3):231-239.
8. LI Y-q, Kong D-x, Wa H. Analysis and evaluation of essential oil components of cinnamon barks using GC-MS and FTIR spectroscopy. Industrial Crops and Products 2013:41;269-278.
9. Chauhan V, Chauhan A. Oxidative stress in Alzheimer's disease. Pathophysiology 2006;13(3):195-208.
10. Jana A, Modi KK, Roy A, Anderson JA, van Breemen RB, Pahan K. Up-regulation of neurotrophic factors by cinnamon and its metabolite sodium benzoate: therapeutic implications for neurodegenerative disorders. The Journal of Neuroimmune Pharmacology 2013; 8:739-755.
11. Azwanida NN. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Medicinal and Aromatic Plants 2015;4(3);1-6.
12. Ogura T, Ogata M, Akita H, Jitsuki S, Akiba L, Noda K, et al. Impaired acquisition of skilled behavior in rotarod task by moderate depletion of striatal dopamine in a pre-symptomatic stage model of Parkinson's disease. Neuroscience Research 2005; 51(3): 299-308.
13. Pyo JH, Jeong YK, Yeo S, Lee JH, Jeong MY, Kim SH, et al. Neuroprotective effect of trans-cinnamaldehyde on the 6-hydroxydopamine-induced dopaminergic injury. Biological and Pharmaceutical Bulletin 2013;36(12):1928-35.
14. Chen YF, Wang YW, Huang WS, Lee MM, Wood WG, Leung YM, et al. Trans-cinnamaldehyde, an essential oil in cinnamon powder, ameliorates cerebral ischemia-induced brain injury via inhibition of neuroinflammation through attenuation of iNOS, COX-2 expression and NFκ-B signaling pathway. Neuromolecular Medicine 2016;18(3):322-33.
15. Klein RL, Lewis MH, Muzyczka N, Meyer EM. Prevention of 6-hydroxydopamine-induced rotational behavior by BDNF somatic gene transfer. Brain Research 1999; 847(2):314-20.
16. Jana A, Modi KK, Roy A, Anderson JA, van Breemen RB, and Pahan K. Up-regulation of neurotrophic factors by cinnamon and its metabolite sodium benzoate: Therapeutic implications for neurodegenerative disorders. Journal of Neuroimmune Pharmacology 2013;8(3):739-55.
17. Khasnavis S, Pahan K. Cinnamon treatment upregulates neuroprotective proteins Parkin and DJ-1 and protects dopaminergic neurons in a mouse model of Parkinson's disease. Journal of Neuroimmune Pharmacology 2014;9(4):569-81.
18. Davies KJ, Shringarpure R. Preferential degradation of oxidized proteins by the 20S proteasome may be inhibited in aging and in inflammatory neuromuscular diseases. Neurology 2006;66:S93-6.
19. Gowder SJT, Halagowder D. Cinnamaldehyde induces behavioral and biochemical changes in the male albino Wistar rat. Journal of Medical Sciences 2010;3:101-109.
20. Ghosh A, Roy A, Liu X, Kordower JH, Mufson EJ, Hartley DM, et al. Selective inhibition of NF-κB activation prevents dopaminergic neuronal loss in a mouse model of Parkinson's disease. Proceedings of the National Academy of Sciences of the United States of America 2007:104:18754-18759.
21. Brahmachari S, Jana A, Pahan K. Sodium benzoate, a metabolite of cinnamon and a food additive, reduces microglial and astroglial inflammatory responses. Journal of Immunology 2009; 183: 5917-5927.