Scientific Research Publishing

Yangtze Medicine, 2019, 3, 72-78
http://www.scirp.org/journal/ym
ISSN Online: 2475-7349
ISSN Print: 2475-7330

Anthocyanin-Rich Blueberry Extract Ameliorates the Behavioral Deficits of MPTP-Induced Mouse Model of Parkinson's Disease via Anti-Oxidative Mechanisms

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How to cite this paper: Qian, F., Wang, M.M., Wang, J.G. and Lu, C.B. (2019) Anthocyanin-Rich Blueberry Extract Ameliorates the Behavioral Deficits of MPTP-Induced Mouse Model of Parkinson's Disease via Anti-Oxidative Mechanisms *Yangtze Medicine*, **3**, 72-78.
https://doi.org/10.4236/ym.2019.31008

Received: September 2, 2018
Accepted: March 19, 2019
Published: March 22, 2019

Abstract

The pathological process of Parkinson's disease (PD) involves oxidative stress, mitochondrial dysfunction and dopaminergic neuronal loss. Recent studies have suggested that the fruits rich in anthocyanins (ANC) were neuroprotective, and then could reduce the risk of PD. This study was designed to investigate the effects of the ANC rich blueberry extracts (BBE) on the behavior and the oxidative stress in the mouse model of PD induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). After 3 weeks of BBE administration, we monitored the behavioral alterations and measured the level of dopamine (DA), enzymatic tyrosine hydroxylase (TH), non-enzymatic malonaldehyde (MDA), enzymatic superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) in the brain and serum. Our results demonstrated that the behavioral impairment, the increased MDA level and the decreased SOD and GSH-PX activities in the MPTP-induced PD mice, were significantly attenuated by BBE administration ($p < 0.05$). This study suggested that anthocyanin-rich BBE should have neuroprotective effects through the anti-oxidative mechanisms.

Keywords

Blueberry Extract, Parkinson's Disease, Oxidative Stress

*These two authors contributed equally to this article.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disease, characterized by bradykinesia, rigidity, resting tremor and unsteadiness due to the loss of dopaminergic cells and the reduced dopamine level in the midbrain substantia nigra [1]. Tyrosine hydroxylase (TH), an enzyme responsible for catalyzing the conversion of the L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA), the precursor of dopamine, is decreased in PD [2]. Mitochondrial failure and oxidative stress appear to be two major contributors to nigral neuronal death in PD. Deficiency in Complex I subunits of the respiratory chain in the PD patients may result in the over-production of reactive oxygen species (ROS) and the promotion of the deposition of fibril forms of α-synuclein [3] [4]. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a mitochondrial complex I inhibitor, decreased the levels of TH, depleted dopamine in nigral-striatal area and mimics symptoms of PD in the mouse model [5]. It was also reported that chronic exposure of environmental pollutants such as rotenone, another mitochondrial complex I inhibitor which was used as an insecticide and pesticide, increased the risk of PD [6].

The main treatment of PD is the application of levodopa [7]. Alternatively, the plant extracts attenuated the degeneration of dopamine neurons in PD model [8]. The blueberry extract (BBE), which contain phytochemicals including anthocyanins (ANC) and proanthocyanidins, enhanced the cognitive function in both children and older adults [9] [10]. Blueberry polyphenols also attenuated kainic acid-induced decrements in cognition in rat hippocampus [11]. Dietary supplementation with BBE improved survival of transplanted dopamine neurons [12] and alleviated neurodegeneration induced by rotenone in a cellular model [13]. These data suggested that BBE should play a beneficial role for the PD patients.

In this study, we measured behavior parameter, dopamine content, enzymatic TH activity, non-enzymatic malonaldehyde (MDA) level, and enzymatic superoxide dismutases (SOD) and glutathione peroxidase (GSH-PX) activities which are important antioxidants in nearly all cells, and investigated the effects of BBE on above indices in the MPTP induced PD model C57BL/6 mice, aimed at determining the possible role and mechanism of ANC rich BBE.

2. Materials and Methods

2.1. Reagents

MPTP and L-DOPA were purchased from Sigma Aldrich (Shanghai, China). Blueberry extract were purchased from Xi'an Qingzhi Biotech Co. Ltd (Xi'an, China) and was formulated for oral gavages. DA, TH, SOD, GSH-PX and MDA measurement kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.2. Animal Grouping and Administration

All animal studies were conducted in accordance with the Guide for the Care

and Use of Laboratory Animals as adopted and promulgated by Yangtze University, Hubei, China. C57BL/6 mice, male, 6 weeks old, weighing 18 - 22 g (purchased from the Experimental Animal Center, Institute of Military Medical Sciences, Beijing, China) were randomly divided into normal control group, MPTP + saline group, MPTP + BBE and MPTP + positive drug group (9 animals in each group). For the normal control group, animals were reared under normal circumstances and received intraperitoneal injection (i.p.) of saline. For the MPTP + saline group, each animal was received daily i.p. of MPTP 30 mg/kg for 5 days and the saline by gavages for 3 weeks [5]. For the MPTP + BBE groups, each animal was received daily i.p. of MPTP 30 mg/kg for 5 days and BBE (50 - 100 mg/kg) by gavages for 3 weeks. For the positive drug group, each animal was received daily i.p. of MPTP and fed daily with levodopa and benserazide (10 mg/kg/day). On the day 21, behavior tests were performed. After the tests, the mice were sacrificed and the whole brain of each mouse was quickly taken by cutting the skull in the middle line. Brain tissue was weighed and added in ice-cold saline (4˚C) in accordance with a ratio of quality to volume (1:10). A 10% homogenate was made followed by centrifugation for 10 min at 1000 g/min, 4˚C. The supernatant was stored at −20˚C and ready for the various biochemical testing.

2.3. Motor Coordination Measurements

Before MPTP injection, mice were trained at several different rod rotation speeds, and motor skill performance was calculated as the mean latency to fall from the rotating rod [14]. Baseline motor coordination in the rotarod test was recorded for each mouse 24 h after training was completed. 21 days after BBE treatment, hypokinesia-like symptoms were assessed by a constant speed (20 RPM) rotarod test. The tests consisted of three trials (180 s) with an inter-trial interval of 30 min. Test performance was defined as the mean of the three trials. Mice were tested starting 1.5 h after the last therapeutic treatment.

2.4. ELISA Detection of the Levels of DA and TH in Mouse Brain and Serum

The two-step double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) test was performed. Standards and test samples were added onto a microplate pre-coated with antibodies specific for dopamine (DA) and tyrosine hydroxylase (TH). After removing any unbound substances, a biotin-conjugated antibody specific for DA or TH is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) was added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate (TMB) solution was added to the wells and color develops in proportion to the amount of DA or TH in the sample (HRP catalyzed substrate and converted to the blue product and become yellow under the action of an acid). The color development was stopped and the intensity of the color was measured (The OD value was measured under 450 nm wavelength. The sample content of DA or TH was calculated according

to the standard and sample OD value).

2.5. Measurement of SOD, GSH-PX and MDA Contents in Mouse Brain and Serum

Mice were sacrificed and the whole brain removed, weighed and added in ice-cold saline (4˚C) in a mass-volume ratio of 1:10 to prepare a 10% homogenate. The homogenate was then centrifuged for 10 min at 1000 g/min, 4˚C, the supernatant was preserved at −20˚C. Measurement of MDA concentration and SOD and GSH-PX activities in mouse brain were performed according to the instruction of purchased kits from Nanjing Jiancheng Bioengineering Institute, including the MDA assay kit (2-thiobarbituric acid, TBA method), and the SOD assay kit (hydroxylamine method), GSH-PX assay kit (Colorimetric method), respectively.

2.6. Statistical Analysis

The statistical software, Sigmastat was used for the statistical analysis. The results were expressed as mean ± standard deviation (x ± s). Statistical analysis for two groups were performed using t-test analysis or a rank test and for multiple groups using ANOVA or ANOVA on ranks. *$p < 0.05$ indicates statistically significant.

3. Results and Discussion

Given the data listed in Table 1, we demonstrated that the continuous MPTP treatment for 5 days (30 mg/kg/day) significantly reduced the ability of mice to balance on a rotating rod by measuring the mean latency to fall in rotarod test ($p < 0.01$), which meant the MPTP treatment successfully induced motor coordination impairment of the PD model mice. The mouse brain DA, TH, SOD, and GSH-PX contents in the MPTP group were significantly lower than the control group ($p < 0.01$), while the MDA content in the MPTP group were significantly

Table 1. Effects of BBE treatment on MPTP-induced PD mice.

Mice group (n = 9)	Mean latency to fall (s)	DA (U/mg protein)	TH (nmol/mg protein)	MDA (nmol/mg protein)	SOD (U/mg protein)	GSH-PX (nmol/mg protein)
Control	174.1 ± 6.6	465.1 ± 33.2	522.8 ± 56.0	2.34 ± 0.23	217.3 ± 14.6	42.1 ± 3.5
MPTP	62.0 ± 7.0$^{\#}$	273.3 ± 13.2$^{\#}$	215.2 ± 29.6$^{\#}$	3.96 ± 0.34$^{\#}$	182.1 ± 17.6$^{\#}$	24.3 ± 3.8$^{\#}$
BBE 50 mg/kg	87.6 ± 9.5*	359.5 ± 24.5*	386.8 ± 22.9*	2.88 ± 0.51*	231.4 ± 29.3*	36.0 ± 3.2*
BBE 100 mg/kg	90.0 ± 6.8*	378.0 ± 25.5*	403.9 ± 28.4*	2.61 ± 0.49*	263.5 ± 40.2*	35.9 ± 4.8*
Madopar	95.0 ± 10.3*	385.3 ± 22.5*	477.1 ± 35.2*	2.50 ± 0.53*	248.0 ± 26.4*	39.2 ± 5.8*

Abbreviations and symbols: MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, 30 mg/kg/day for 5 days; BBE, blueberry extract, 50 or 100 mg/kg/day for 21 days; Madopar, Levodopa and benserazide, 10 mg/kg/day for 21 days; DA, dopamine; TH, tyrosine hydroxylase; MDA, malonaldehyde; SOD, superoxide dismutase; GSH-PX, glutathione peroxidase; PD, Parkinson's disease. *$p < 0.05$, compared with MPTP induced PD mice; $^{\#}p < 0.01$, compared with control mice (male C57BL/6 mice, 9 per group).

higher than that ($p < 0.01$). These data indicated that the MPTP treatment induced significant oxidative stress to the model mice and decreased the content of DA in the mouse brain which finally lead to the motor coordination impairment. In this study, we demonstrated that both low (50 mg/kg) and high (100 mg/kg) BBE treatments improved motor coordination and increased DA and TH contents in PD mice ($p < 0.05$). Furthermore, as a possible mechanism of its neuroprotective effect, BBE increased SOD and GSH-PX activity and decreased MDA level in the brains of MPTP-treated mice ($p < 0.05$). Compared with the group treated with Levodopa and benserazide (10 mg/kg), the BBE treatment achieved similar therapeutic efficacy to the induced motor coordination impairment by MPTP treatment. However, different from the previously reported data which showed that the Levodopa and benserazide treatment could significantly increase the oxidative stress [15], our results suggested the opposite effect of it. The only possibility was that the Levodopa and benserazide treatment had reversed the oxidative stress induced by MPTP treatment by their neuroprotective effects [16].

TH has been used as a marker for dopaminergic neurons [17]. BBE significantly increased TH contents in MPTP-treated mice ($p < 0.05$), which suggested that BBE could increase the survival of dopamine neurons in PD mice. Increased oxidative stress could lead to neurodegeneration in the PD via protein and/or lipid peroxidation and disruption of mitochondrial function [18]. Our results demonstrated the anti-oxidative effect of BBE, which should contribute to its neuroprotective role in PD mice, as reported previously [19] [20].

4. Conclusion

In conclusion, our data provided evidence that BBE improved motor function in MPTP-induced PD mice via a possible mechanism of its anti-oxidative capacity to scavenge free radicals and reduced oxidative damage to the neurons. The detailed mechanism remained to be further studied.

Acknowledgements

This work was supported by The National Natural Science Foundation of China (grant numbers: 31070938, 81271422, 81771517), the Applied Basic Research Plan Key Program of Hebei Province of China (grant number: 12966119D).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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