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Curcumin has neuroprotective effects in rotenone-induced Parkinson disease in mice by affecting bcl-2 family gene expressions

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Abstract

Background: One of the common diseases in different societies is Parkinson's disease (PD), for which no suitable treatment has been found yet. Curcumin a natural compound has shown neuroprotective properties in many studies. Therefore, in this research, the administration of this compound to female mice was studied.
Methods: Induction of PD in mice was done by administering 2 mg rotenone, and then a Rotarod test was performed on female mice. Also, 50 mg/kg of curcumin was administered every day for 21 days and its effects were evaluated in PD mice. Finally, the expression levels of bax and bcl-2 were measured in the brain of female mice by RT-PCR technique. GraphPad Prism V.8 software was used for data analysis.
Results: Induction of PD led to a decrease in the performance of mice in the Rotarod test. However, the administration of curcumin to these mice improved their performance. Also, overexpression of bax and downregulation of bcl-2 genes were observed in the brains of PD mice, to whom the administration of curcumin downregulated bax gene and overexpressed bcl-2 gene.
Conclusion: Curcumin has neuroprotective effects in PD conditions, which can be attributed to the change in expressions of the bcl-2 family gene. More studies are needed in this field.
Keywords: Gene, Curcumin, Neuroprotection, RT-PCR.

1. Introduction

Parkinson's disease (PD) is one of the diseases caused by neurodegeneration, which has symptoms such as tremors, muscle stiffness, slowness of movements, and imbalance (1). The increasing trend of this disease is predicted until 2030 (2, 3). The most important cause of this disease is the deterioration of dopaminergic neurons in the substantia nigra and striatal cells of the hippocampus, which can be caused by oxidative stress, inflammation, the accumulation of damaged proteins, and mitochondrial dysfunction (4).

In PD studies, various models have been used to induce this disease, one of which is the systematic administration of rotenone (5). This lipophilic compound can cross the blood-brain barrier (BBB) and its mechanism of action includes the inhibition of mitochondrial complex I (6). Also, the destruction of nigrostriatal pathways in the substantia nigra and the formation of Lewy bodies are among the consequences of the systemic administration of this compound (5).

Curcumin is a compound isolated from Curcuma longa, which has wide pharmacological properties such as antioxidant, anti-inflammatory, neuron protection, antimicrobial, blood lipid-lowering, cyclooxygenase inhibition, and antitumor effects (7). This lipophilic compound has phenolic groups and conjugated links (8). The ability of this compound to inhibit cyclooxygenase 2 (COX 2), lipoxygenase (LOX) and nitric oxide synthase (NOS) enzymes inhibit the production of inflammatory factors such as cytokines (9). Many studies have shown that curcumin has neuroprotective effects in PD, and its mechanism has been attributed to antioxidant and anti-inflammatory effects (10, 11). NF-κB activation is one of the main pathways of inflammatory cytokine production (12), and it has been shown that curcumin can inhibit this inflammatory factor (13, 14). Therefore, it seems this compound has the potential for adjuvant treatment in PD conditions.

One of the reasons for the death of dopaminergic cells in Parkinson's disease is apoptosis. This process is affected by the expression of Bcl-2 family genes. Two genes, bax (pro-

bcl-2 (anti-apoptotic), play an essential role in the apoptosis process, and increasing the expression of bax and decreasing the expression of bcl-2 leads to an increase in apoptosis. Therefore, the interaction between these two genes in the process of apoptosis of dopaminergic cells of the hippocampal substantia nigra plays a central role in PD pathogenesis. For example, our research group recently showed bax gene overexpression and bcl-2 downregulation in PD mice (15). Also, Jamali et al. (2023) also found that apoptosis increases in the cells of the substantia nigra of PD rats as a result of up-regulation of bax and down-regulation of bcl-2 (16). Therefore, the role of these two genes in the process of apoptosis needs to be considered in research related to PD.

In the current study, we investigated the possible curcumin neuroprotection effects in rotenone-induced PD in mice models and its effects on bax and bcl-2 gene expressions.

2. Materials and Methods

2.1. Preparation of animals and their grouping

24 female mice were obtained from the Pasteur Institute of Iran-Tehran, and they were kept in an animal house with a room temperature of 23-25 °C and a light cycle of 12 hours (light-dark). Animal treatment was by ARRIVE guidelines. After one week, the animals were grouped as follows:

Group I: Control healthy (n=6)

Group II: healthy animals administrated by 50 mg/kg curcumin i.p. (17) (n=6)

Group III: Rotenone-induced PD mice (injected once every 2 days for 19 days) without treatment (n=6)

Group IV: Rotenone-induced PD mice (injection once every 2 days for 19 days) administrated by 50 mg/kg curcumin i.p. (17) 5 days before PD induction and 3 weeks after that (n=6)

2.2. Rotarod Test

Five days before the test, all the animals were subjected to a pre-exercise program consisting of rotation 4 times a day for 5 minutes to maintain stability during the test. On the test days (days 1 to 19), the Rotarod test was performed at a speed of 15 rpm (18).

2.3. bax and bcl-2 genes' expression

At first, the cerebral homogenate was prepared. After the separation of cerebral tissue, they were placed in a phosphate buffer solution, and tissue lysis was performed using a sonicator. RNA extraction kit (Qiagen, Hilden, Germany) was used to extract RNA, and its quantity was measured using a picodrop spectrometer (UK). cDNA synthesis was performed from 1 μg of extracted RNA using a cDNA synthesis kit (Qiagen, Hilden, Germany).

The sequence of primers used in the present study is given in Table 1. After designing the primer, the expression of the studied genes was measured using the RT-PCR machine. RT-PCR time and temperature schedule were according to the recent study of our research group (15). Briefly, in step 1, 95 °C for 180s, in step 2, 95 °C for 30 s, 35 s at 60 °C and 40 cycles of 72 °C for 30 s, and the final step for 180s at 72 °C were applied

Table 1. The sequences of primers used in current study

Genes	Sequences [5'-3']
β- actin-F	ACCGTGGAGAAGAGCTACG
β- actin-R	GTACTTGCGCTCAGAAGGAG
bax-F	CTACAGGGTTTCATCCAG
bax-R	CCAGTTCATCTCCAATTCG
bcl-2-F	GTGGATGACTGAGTACCT
bcl-2-R	CCAGGAGAAATCAAACAGAG

2.4. Statistical analysis

Normal distribution of data was confirmed by the Kolmogorov Smirnov test and one-way analysis variance (ANOVA) was used for data analysis. The post hoc test was performed by Tukey multiple range test at a probability level of $P<0.05$. GraphPad Prism V.8 was used for data analysis.

3. Results

3.1. Rotarod Test

The results of the Rotarod test showed that induction of PD in mice by administering rotenone every other day for 19 days leads to a significant decrease in muscle strength and balance in mice because a significant difference in latency time was observed between the two groups ($P<0.0001$), which indicates decreased performance in PD mice. However, curcumin administration resulted in improved performance of PD mice compared to PD control ones on day 19, indicating improvement in muscle strength and balance ($P<0.001$, Figure 1).

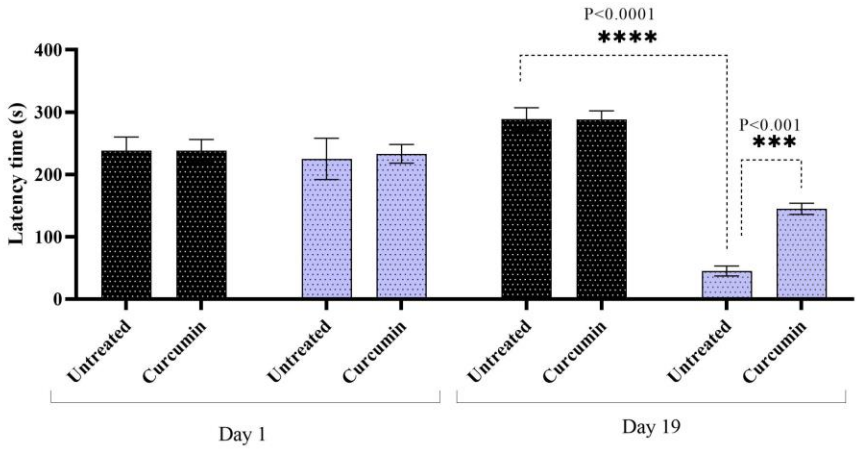

Figure 1. The latency time in the Rotarod test for the evaluation of muscle strength and balance in healthy and PD mice administrated by curcumin (n=3). 50 mg/kg curcumin i.p. was administrated 5 days before PD induction and 3 weeks after that.

3.2. bax and bcl-2

Induction of PD in mice caused significant changes in the expressions of bax and bcl-2 genes in the brain so there was a significant increase in the expression of bax and a significant decrease in the expression of bcl-2 in the PD brain mice compared to the control. However, the administration of curcumin to mice was able to partially prevent the increase in bax and decrease in bcl-2 expression, so that the average expression levels of bax significantly decreased and the level of bcl-2 significantly increased in the PD brains mice treated with curcumin in comparison with untreated PD mice (Figure2).

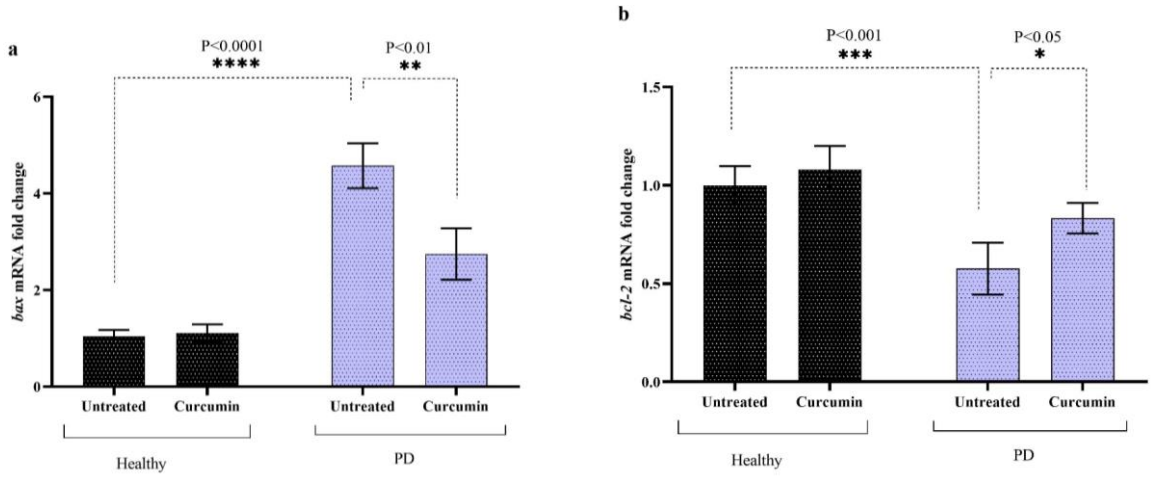

Figure 2. The changes in bax (a) and bcl-2 gene expressions in healthy and PD mice brain treated with 50 mg/kg curcumin. 50 mg/kg curcumin i.p. was administrated 5 days before PD induction and 3 weeks after that.

4. Discussion

The results of the present study indicated the neuron protection effects of curcumin in PD conditions. Improved performance of curcumin-treated PD mice in the Rotarod test and decreased expression of bax and upregulation of bacl-2 compared to control PD mice were seen, indicating the neuron-protective effects of this compound.

PD is a progressive disease of dopaminergic neurodegeneration that leads to movement disorders in patients. As a result of the breakdown of the nerve in the substantia nigra, there is a decrease in the production of dopamine, which ultimately leads to the appearance of PD symptoms such as muscle stiffness, tremors, and imbalance (19). One of the known causes of this disease is oxidative damage caused by free radicals, especially reactive oxygen species (ROS), which lead to the death of neurons (20). In this context, the consumption of natural antioxidants seems to have therapeutic effects and prevent the occurrence of this disease (21).

In recent years, a lot of research has been done in this field, and it has been found that curcumin has neuroprotective effects (22). For example, Song et al. (2016) showed that curcumin protects the neurological disorder caused by 6-OHDA administration and this was attributed to the improvement of the antioxidant capacity of the cell, such as increasing the activity of the antioxidant enzymes superoxide dismutase and glutathione peroxidase. One of the interesting results of their research was the improvement of in the substantia nigra of the brain (23). Also, in another study, it was reported that the motor performance of PD rats was improved

by administering 50 mg/kg of curcumin (17), which is in line with the findings of the present study. In a recent clinical study, it was shown that curcumin was able to cross the blood-brain barrier and led to the reduction of phosphorylated α-synuclein (p-syn) in the skin samples of PD patients (24). These findings show that curcumin has a good therapeutic potential in PD, and it is necessary to conduct more studies in this field. In the present study, it was shown that administration of curcumin to PD mice could partially prevent the increase of bax and decrease of bcl-2 in the brain. This indicates that this compound had anti-apoptotic effects and thus exerted neuroprotective effects (11, 25). Also, the improvement in the performance of PD animals receiving curcumin in the Rotarod test can be caused by the reduction of apoptosis in their brain as a result of curcumin administration and its effects on the expression changes of bax and bcl-2 genes. Many studies have shown the neuron protection effects of this compound (25, 26), and the anti-apoptotic effects of curcumin have been mentioned in various studies (27, 28), which is by the findings of the present study.

5. Conclusion

In general, we concluded that curcumin has neuroprotective effects by downregulating bax and overexpressing of bcl-2 genes. Clinical trials are needed in this regard.

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