Research Article

Momordica Charantia Polysaccharides Attenuates MPP⁺-Induced Injury in Parkinson's Disease Mice and Cell Models by Regulating TLR4/MyD88/NF- κ B Pathway

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Objective. To investigate the potential role of Momordica charantia polysaccharides (MCPs) in Parkinson's disease (PD) and reveal the molecular mechanism of its function. **Method.** 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 1-methyl-4-phenylpyridinium (1-methyl-4-phenylpyridinium, MPP⁺) were used to establish PD mice and cell models. The mice and cells were divided into 4 groups: Control group, Control+MCPs group, PD group, and PD+MCPs group. Pole climbing experiment and Rotarod experiment were used to observe the coordination ability of mice. High-performance liquid chromatography and enzyme-linked immunosorbent assay (ELISA) were used to determine neurotransmitters and metabolites, inflammatory factors TNF- α and IL-1 β , oxidative stress-related markers SOD, MDA, and GSH in striatum tissues. Western blot was used to determine the protein levels of tyrosine hydroxylase (TH), oxidative stress-related protein Cytochrome C (Cytochrome C), and apoptosis-related proteins Bcl-2, Bax, and cleaved Caspase-3 in tissues and cells. Moreover, flow cytometry, PI staining, and fluorescence were used to observe cell apoptosis. Finally, the activation effect of MCPs on TLR4/MyD88/NF- κ B signaling pathway was observed and verified. **Results.** Compared with the Control group, MPTP treatment can induce brain damage in mice (all $P < 0.05$), change the metabolic state of neurotransmitters (all $P < 0.05$), induce inflammation (all $P < 0.05$), and induce apoptosis and the occurrence of oxidation reaction (all $P < 0.05$); however, MCPs treatment can significantly reverse the above changes (all $P < 0.05$). In cell models, studies have found that MCPs can play a protective role by regulating the activation state of TLR4/MyD88/NF- κ B pathway. **Conclusion.** This study found that the application of MCPs therapy can play anti-inflammatory, antioxidative stress, and antiapoptotic effects in PD by regulating the activation of the TLR4/MyD88/NF- κ B pathway.

1. Introduction

Parkinson's disease (PD) is a common neurodegenerative disease second only to Alzheimer's disease [1, 2]. Nowadays, the incidence of PD in my country is rising rapidly with age [3], and it has become one of the main threats to the elderly. Although the etiology of PD is not yet clear, it has been confirmed that genetic factors and environmental factors (such

as poisons) are involved in the occurrence and development of PD [4]. The common cause of PD is the loss of dopaminergic neurons in the substantia nigra compact area and the accumulation of Lewy bodies in the brain [5]. A large amount of evidence indicates that inflammatory damage and oxidative damage are the main causes of PD neurodegeneration and neuroinflammation [6]. N-methyl-4-phenylpyridinium (MPP⁺) is the active metabolite of 1-methyl-4-phenyl-

1,2,3,6-tetrahydropyridine (MPTP). MPTP is a neurotoxin that selectively destroys dopaminergic neurons, and the exposure can cause PD [7]. Therefore, elucidating the mechanism of MPTP and MPP⁺ inducing PD is of great significance to elucidating the pathogenesis of PD.

In recent years, more and more scholars have begun to study the possible mechanism of traditional Chinese medicine in treating PD [8]. For example, studies have found that *Poria* can delay the progression of PD through immune regulation and other effects [9, 10]; ursolic acid can reduce oxidative stress in the substantia nigra striatum of mice and improve neurobehavior [11]; South Africa Cigarette can reduce the symptoms of Parkinson's disease by inhibiting the apoptosis of dopaminergic neurons [12]. Chinese medicine mechanisms mainly include participation in the protection of dopaminergic neurons [13], improvement of mitochondrial function [14], reduction of neuritis [15], enhancement of immune response [16], reduction of excitotoxicity [17], antiapoptosis [18], autophagy induction [19], and the inhibition of the accumulation of abnormal proteins [20]. It is widely known that a variety of natural molecules in food, such as plant polysaccharides, can protect the brain and delay aging. Studies have shown that foods rich in antioxidants, such as fruits, vegetables, and nuts, play a beneficial role in improving cognitive impairment by preventing or delaying the occurrence of cognitive decline during aging and neurodegeneration [21, 22]. *Momordica charantia* belongs to the Cucurbitaceae family. It is an important multifunctional edible and medicinal plant widely distributed throughout Asia. As a natural compound in daily food, *Momordica charantia* polysaccharides (MCPs) are famous for their antioxidant, anti-inflammatory, antitumor, hypoglycemic, and antidiabetic effects [23]; however, little is known about its role from the perspective of neurogenesis regulation. Studies have found that MCPs can protect nerve damage after stroke by scavenging free radicals [24]. The above studies suggest that MCPs can play a protective role in neurological diseases and injuries.

In view of the fact that there is no research report on the relationship between MCPs and PD, therefore, this study is the first to investigate whether MCPs have antineuronal apoptosis and inhibit inflammation effects in mice and cellular PD models. In addition, it is known that TLR4/MyD88/NF- κ B is an important signaling pathway involved in apoptosis and inflammatory response [26]. In addition to exploring the protective effects of MCPs on tissues and cells, this study also detected changes in the expression of the TLR4/MyD88/NF- κ B signal axis, so as to explore the potential mechanism of MCP. It provides a theoretical basis for in-depth understanding of the role of MCP in the treatment of PD and the transformation of population research.

2. Method

2.1. Preparation and Analysis of MCPs. Bitter melon purchased from the local market in our city in July 2020 was washed, dried, cut into pieces, seeded, then ground into a homogeneous powder (40-60 mesh), and stored in a dry, ventilated place for later use. The bitter melon-dried fruit

powder was extracted with 80°C water for 2 h. The extract was filtered with glass wool and centrifuged at 6,000 g for 10 min to separate the supernatant and sample residues. The Sevag method was used to remove related proteins in the solution. After removing the Sevag reagent, the aqueous phase was concentrated and precipitated with ethanol, and the polysaccharide was precipitated overnight in a refrigerator at 4°C. The precipitate was collected, redissolved in water, and then further dialyzed in distilled water (MWCO 8000) for 24 h [27]. After concentration, the precipitate was frozen and made into freeze-dried powder.

The anthrone sulfuric acid method was used as the standard, and d-glucose was used as the internal standard to determine the total sugar content of MCPs. Analyze protein content with the folic acid reagent. According to the method of Blumenkrantz and Asboe-Hansen [28], d-glucuronic acid was used as the internal standard to determine the content of uronic acid. The high-performance liquid chromatography (HPLC) was equipped with an ultra-hydrogel linear column (7.8 mm $\times$ 300 mm). 0.5% MCPs (20 μ L) was dissolved in distilled water, 0.7% NaCl was used as mobile phase, 0.5 mL/min, 35°C as HPLC conditions, and T series dextran was used as a standard for calibration. On a Shim Pack C18 column (4.6 mm $\times$ 250 mm), using an HPLC system, using 82.0% PBS (0.1 mol/L, pH 7.0) and 18.0% acetonitrile at a flow rate of 1 mL/min, dimethyl trifluoroacetic acid was used for hydrolysis. After labeling the hydrolysate with 1-phenyl-3-methyl-5-pyrazolone (PMP), the monoacid composition of MCPs was analyzed by HPLC.

2.2. The Establishment and Administration of PD Animal Model. In the study, 40 SPF grade C57B/6 mice (male, 5-8 weeks old) were purchased. Place the mice in a 12-hour light-dark cycle, 22 $\pm$ 2°C breeding cage, and drink and eat freely. All experimental protocols involving animals in this study have been reviewed and approved by the ethics committee of our hospital and comply with the requirements of national health institutions.

The mice were randomly divided into 4 groups (10 in each group). PD model group (PD, n = 10): mice were intraperitoneally injected with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) 25 mg/kg, once a day, for 7 days, to construct the PD model. Control group (Control, n = 10): the same amount of saline was given at the same time as the model group stimulated with MPTP. Control+MCP group (Control+MCPs, n = 10): after being treated with saline for 7 days, MCPs (100 mg/kg) were given for 7 days. PD model+MCP group (PD+MCPs, n = 10): after 7 days of MPTP treatment, MCPs (100 mg/kg) were given for 7 days [29, 30]. At the same time, mice in the Control group and PD model group were given the same volume of normal saline as the MCP treatment. After the motor function test was completed, all mice were weighed, and the neck was cut to death. The brain tissue (striatum) of each mouse was collected, and part of it was stored in liquid nitrogen for future analysis. Besides, the mouse venous blood was collected in the coagulation tube, and the serum was collected by centrifugation at 5,000 g for 20 min at room temperature and frozen for later use.

2.3. Cell Culture and Processing. The human neuroblastoma cell line (SK-N-SH) was purchased from Shanghai Fuheng Biotechnology Co., Ltd. DMEM (Invitrogen, USA) medium containing 10% fetal bovine serum (Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA) was used to culture cells in a humidified incubator at 37°C and 5% CO₂. The cells were divided into 4 groups: (1) PD model group (PD): SK-N-SH cells were placed at 37°C and stimulated with 1 mM MPP⁺ for 24 hours to establish a PD cell model. (2) Control group: the cells were treated with PBS for 24 h at the same time as the model group stimulated with MPP⁺. (3) Control +MCP group (Control+MCPs): after being treated with PBS for 24 hours, given MCPs (80 µg/mL) for 24 hours. (4) PD model+MCP group (PD+MCPs): after MPP⁺ treatment for 24 h, MCPs (80 µg/mL) were given for 24 h. At the same time, the Control group and PD model group were given the same volume of PBS as the MCP treatment.

2.4. Pole Climbing Experiment. Pole climbing experiment was used to assess the degree of retardation. On the 7th day of the MCP treatment, the mice were placed on the top of a rod with a length of 50 cm and a radius of 4 mm, and the following activities were recorded three times: the time it takes for the mouse to climb down the upper half, the time it takes for the mouse to climb the lower half, and the time it takes for the mouse to complete the total length of the climbing pole. If the mouse completes the above three steps in 3 s, 6 s, or more than 6 s, the motor coordination score will be 3, 2, or 1 point. The scores of the three steps were accumulated, the test was performed 3 times, and the average value was taken.

2.5. Rotarod Experiment. Rotarod experiment was used to analyze the motor function of mice. It was performed on the 5-7th day of MCPs treatment. Before using ROTA ROD (UgoBasile, Italy) for a 1-day experiment, all mice received a 2-day training (5 times/day). On day 3, the mice were tested 4 times in accelerated mode (4-40 rpm in 5 minutes). The mouse did not fall from the rod after the maximum residence time of 300 s. The average residence time before falling off was measured, and the average of the 3 longest residence times for each animal was analyzed.

2.6. The Measurement of Neurotransmitters and Metabolites by High-Performance Liquid Chromatography. Weigh the striatum tissue and homogenize it in 0.1 µM perchloric acid. After incubating for 1 h on ice, centrifuging at 12,000 g at 4°C for 20 min. Then, the supernatant was collected and filtered through a 0.22 µm filter, and 25 µL of the sample was injected into the column and mixed with the HPLC mobile phase. The HPLC mobile phase includes water, acetonitrile, and 0.01 µM phosphate buffer (adjusted to pH 4 with phosphoric acid), and gradient elution was adopted. At the same time, by diluting the stock solution in the mobile phase, freshly prepared the dopamine (DA), serotonin (5-HT), and its metabolites (including dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindole acetic acid (5-HIAA)) and hydrochloride standard solution (Sigma-Aldrich, USA). The serial concentration of the standard solu-

FIGURE 1: HPLC analysis of MCPs monosaccharide composition. Monosaccharides can be identified and quantified by comparison with standard products. Rib: ribose; Man: mannose; Rha: rhamnose; Glu: glucose; Xyl: xylose; Gal: galactose; Ara: arabinose; Fuc: fucose.

tion was used to determine the linear range of the above substances before testing.

2.7. Enzyme-Linked Immunosorbent Test (ELISA). Strictly follow the instructions, and the ELISA kit (Biotech (Shanghai) Co., Ltd., China) was used to detect the concentration of tumor necrosis factor α (TNF- α) and interleukin-1 β (interleukin-1 β , IL) in tissues, and the concentration of inflammatory factors is expressed in pg/mg protein.

2.8. The Evaluation of Oxidative Stress Markers. According to the instructions, the kit (Nanjing Jiancheng Institute of Bio-engineering, China) was used to measure the levels of GSH, SOD, and MDA in the supernatant of the striatal brain tissue, and the concentration is expressed in µg/mg protein.

2.9. The Detection of Cell Viability. Cell counting kit (CCK-8, Dojin, Japan) was used to determine cell viability. The cells were seeded in a 96-well plate (10⁴ cells/well), and after the corresponding treatment the next day, 10 µL of CCK-8 reagent was added to each well and placed in an incubator to continue culturing for 3 hours. The absorbance was recorded with a spectrophotometer at a wavelength of 450 nm.

2.10. The Determination of Apoptosis. Flow cytometry was used to determine the rate of apoptosis. SK-N-SH cells were inoculated in a 24-well culture plate, and after corresponding treatments the next day, the cells were washed twice with pre-cooled PBS. After that, the cells were resuspended in 200 µL binding buffer (1%) by centrifugation, and 5 µL each of Annexin V/FITC and PI staining solution (Invitrogen, USA) was added, which was then incubated at room

FIGURE 2: MCPs reduce the dyskinesia of MPTP-induced PD and regulate DA and 5-HT metabolism. (a) Pole climbing test score. (b) Rotarod test retention ability. (c–g) DA, DOPAC, HVA, 5-HT, and 5-HIAA levels in striatum tissue homogenate. Compared with the Control group (Control+MCPs group), *** $P < 0.001$, and compared with the PD group, ### $P < 0.001$.

temperature for 15 min in the dark, and placed in a flow cytometer to determine the cell apoptosis rate.

PI-Hoechst staining was used to observe cell apoptosis. The apoptotic cells were stained with PI (4 mM, Sigma-

Aldrich, USA) and Hoechst 33432 (0.5 mg/mL, Sigma-Aldrich, USA) at 37°C for 10 min. PI-positive cells were counted under a fluorescence microscope at excitation and emission wavelengths of 535 nm and 615 nm, respectively.

FIGURE 3: Continued.

FIGURE 3: MCPs reduce the inflammation, oxidative stress, and apoptosis of PD induced by MPTP. (a, b) Expression of proinflammatory cytokines TNF- α and IL-1 β in striatum tissue homogenate. (c–e) Expression of oxidative stress-related factors MDA, SOD, and GSH in striatum tissue homogenate. (f) The expression levels of oxidative stress-related protein Cytochrome C and apoptosis-related proteins Bcl-2, Bax, and cleaved Caspase-3 in striatal tissue homogenate. (g) Tyrosine hydroxylase (TH) protein level in striatum tissue homogenate. Compared with Control group (Control+MCPs group), *** $P < 0.001$, compared with PD group, ** $P < 0.01$, ### $P < 0.001$.

2.11. The Determination of Protein Levels by Western Blotting Method. RIPA strong lysate (Shanghai Biyuntian Biotechnology Co., Ltd., China) was used to extract total tissue/cell protein. First, lysed on ice for 30 minutes, centrifuged at 4°C, 12,000 g for 20 minutes, and extracted the supernatant. Then, protein quantification was performed by the BCA method (Thermo Fisher Scientific, USA). An equal amount of protein (50 mg) was electrophoresed on a 10% SDS polyacrylamide gel and, then, transferred to a PVDF membrane for blotting (Millipore, USA). Blocked in 5% skimmed milk at room temperature for 1 hour, with primary antibodies Bcl-2 (ab182858, Abcam, UK), Bax (ab32503, Abcam, UK), cleaved Caspase-3 (ab32042, Abcam, UK), Cytochrome C

(ab133504, Abcam, UK), TLR4 (ab13556, Abcam, UK), MyD88 (ab133739, Abcam, UK), p-NF- κ B p65 (ab183559, Abcam, UK), Tyrosine hydroxylase (ab137869, Abcam, UK), and TH/ β -actin (ab6276, Abcam, UK) incubated the PVDF membrane overnight at 4°C. The next day, horseradish peroxidase-conjugated secondary antibody (Abcam, UK) was added and incubated for 1 h at room temperature, and then, ECL luminescent developer (Shanghai Biyuntian Biotechnology Co., Ltd., China) was used to quantify protein levels in the imaging system. According to the manufacturer's recommendations, an enhanced chemiluminescence method (Thermo Fisher Scientific) was used to develop the western blot.

FIGURE 4: MCPs improve the viability of glioblastoma cells. (a) The effects of different concentrations of MCPs (0 $\mu\text{g/mL}$, 30 $\mu\text{g/mL}$, 60 $\mu\text{g/mL}$, 80 $\mu\text{g/mL}$, 120 $\mu\text{g/mL}$, 150 $\mu\text{g/mL}$) on cell viability. (b) In the PD cell model, the effect of MCPs on cell viability. Compared with the Control group (Control+MCPs group), *** $P < 0.001$, and compared with the PD group, ** $P < 0.01$, *** $P < 0.001$.

3.12. Statistical Analysis. The data were expressed as mean $\pm$ standard deviation. Statistical analysis was performed using the statistical software SPSS 19.0. Student's t -test was used for comparison between different groups, and one-way analysis of variance was used for comparison between groups under the same conditions. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Analysis of MCPs. After filtering the bitter melon precipitate, it was freeze-dried to obtain a yellow powder to obtain a polysaccharide with a yield of 3.2%. The total sugar content is $71.3 \pm 1.1\%$, and the protein content is $9.1 \pm 0.3\%$. The content of uronic acid in MCPs was $20.2 \pm 0.3\%$. The molecular weight of polysaccharides was determined in the range of 85–100 kDa. In order to further study the composition of MCPs, we used different monosaccharide standards and recorded their retention time to identify the monosaccharide components of MCPs by HPLC. Analysis of monosaccharide components showed that according to HPLC retention time and peak area, MCPs contained arabinose, xylose, galactose, and rhamnose in a ratio of 1.01 : 1.13 : 4.17 : 1.67 (Figure 1).

3.2. Changes in Appearance and Weight of Mice. Throughout the experiment, the mice were visually observed and weighed daily. This study found that after administration of MPTP, the mice showed typical disease behaviors, including reduced exercise, hunchback, anorexia, and weight loss, but after treatment with MCPs, the symptoms of the mice gradually alleviated.

3.3. MCPs Reduce the Dyskinesia of MPTP-Induced PD and Regulate DA and 5-HT Metabolism. In this study, the pole climbing experiment and the Rotarod experiment were used to evaluate the exercise ability of mice. As shown in

Figures 2(a) and 2(b), in the former, compared with the Control group, the pole climbing experiment time of the PD group mice was increased ($P < 0.05$), while the Rotarod experiment stay time was shortened ($P < 0.05$). In contrast, treatment with MCPs can significantly reverse the above changes (all $P < 0.05$). The above results indicate that the administration of MCP treatment can reduce the dyskinesia caused by MPTP.

Moreover, to evaluate the potential protective effect of MCP on brain function, this study used HPLC fluorescence detection to determine the concentration of striatal neurotransmitters DA, 5-HT, and their metabolites DOPAC, HVA, and 5-HIAA. As shown in Figures 2(c)–2(g), the levels of DA, DOPAC, and HVA in the brain tissue of the PD group significantly decreased ($P < 0.05$), while the levels of 5-HT and its metabolite 5-HIAA increased significantly (all $P < 0.05$), and the administration of MCPs can significantly reverse the changes in the levels of the above substances (all $P < 0.05$). The above results suggest that MCPs can participate in the metabolism of neurotransmitters in the brain by inhibiting the MPTP-induced decrease in striatal DA, 5-HT, and their metabolites.

3.4. MCPs Reduce the Inflammation, Oxidative Stress, and Apoptosis of PD Induced by MPTP. To investigate the inflammatory state of PD mice and the effect of MCP on the inflammatory response, the expression levels of proinflammatory cytokines TNF- α and IL-1 β in the striatum were detected. As shown in Figures 3(a) and 3(b), the expression levels of TNF- α and IL-1 β in the brain tissue of mice induced by MPTP were higher than those in the Control group (both $P < 0.05$). It shows that MPTP can cause inflammation and the release of proinflammatory cytokines, and the treatment of MCPs can significantly reduce the expression levels of these factors (all $P < 0.05$), suggesting that MCP has anti-inflammatory effects.

FIGURE 5: Continued.

FIGURE 5: MCPs inhibit MPP⁺-induced apoptosis and reduce oxidative stress. (a) Flow cytometry to detect cell apoptosis rate after Annexin V-FITC/PI staining. (b) PI staining fluorescence microscope to observe cell apoptosis. (c) The expression level of an oxidative stress-related protein Cytochrome C, and the expression levels of apoptosis-related proteins Bcl-2, Bax, and cleaved Caspase-3 in cells. (d) TH protein level in the cells. Compared with Control group (Control+MCPs group), *** $P < 0.001$, compared with PD group, ## $P < 0.01$, ### $P < 0.001$.

This study also detected the levels of GSH, SOD, and MDA in the striatum. As shown in Figures 3(c)–3(e), MPTP-induced MDA content in mice was significantly higher than that in the Control group ($P < 0.05$), while GSH and SOD were significantly lower ($P < 0.05$). MCPs treatment can significantly reverse the changes in the above factors (all $P < 0.05$), suggesting that MCPs have antioxidant effects.

Furthermore, this study also detected the expression of oxidation and apoptosis marker proteins in the striatum. As shown in Figure 3(f), the expression levels of oxidative factor Cytochrome C, proapoptotic proteins Bax, and cleaved Caspase-3 in the brain tissue of PD mice were significantly increased (all $P < 0.05$), while Bcl-2 protein was significantly reduced ($P < 0.05$), and MCPs treatment can significantly

reverse the expression changes of the abovementioned proteins (all $P < 0.05$), suggesting that MCPs have antioxidant and anti-apoptotic effects. In addition, as shown in Figure 3(g), in order to study the effect of MCPs on dopaminergic neurons, we also detected the expression of TH in the striatum and found that the level of TH in the brain tissue of the PD model decreased ($P < 0.05$), while MCPs treatment can eliminate this effect ($P < 0.05$).

3.5. MCPs Improve the Activity of Glioblastoma Cells. This study first observed the effect of MCPs on the activity of glioblastoma cells. As shown in Figure 4(a), cells were given different concentrations of MCPs (0 $\mu\text{g/mL}$, 30 $\mu\text{g/mL}$, 60 $\mu\text{g/mL}$, 80 $\mu\text{g/mL}$, 120 $\mu\text{g/mL}$, 150 $\mu\text{g/mL}$) to culture for 24 h, and CCK-8 results showed that when the concentration

FIGURE 6: MCPs regulate the activation state of TLR4/MyD88/NF- κ B signaling pathway. The expression level of TLR4, MyD88, p-NF- κ B p65 protein in the striatum tissue homogenate. Compared with the Control group (Control+MCPs group), *** $P < 0.001$, and compared with the PD group, ### $P < 0.001$.

of MCPs was in the range of 0–80 μ g/mL, it can promote the increase of SK-N-SH cell activity in a dose-dependent manner ($P < 0.05$). Therefore, 80 μ g/mL was selected as the treatment concentration of MCPs in subsequent experiments.

Subsequently, to observe the effect of MCPs on the activity of MPP⁺-induced injured cells, the changes of cell activity in the Control group, PD group, Control+MCPs group, and PD+MCPs group were observed by the CCK-8 method. As shown in Figure 4(b), compared with the Control group, SK-N-SH cell viability was significantly reduced after MPP⁺ treatment ($P < 0.05$), and then, the cell viability of MCPs treatment increased ($P < 0.05$), suggesting that MCPs can reduce the damage induced by MPP⁺ and improve cell viability.

3.6. MCPs Inhibit MPP⁺-Induced Apoptosis and Reduce Oxidative Stress. As shown in Figures 5(a) and 5(b), compared with the Control group, MPP⁺ can induce an apoptotic response in glioblastoma cells, with a higher apoptotic rate ($P < 0.05$), and a significant increase in apoptotic cells ($P < 0.05$). After cell injury, treatment with MCPs can significantly inhibit the occurrence of apoptosis, reduce the rate of apoptosis ($P < 0.05$), and reduce the number of apoptotic cells ($P < 0.05$), suggesting that MCPs have anti-apoptotic effects.

This study also detected changes in the expression of apoptosis and oxidative stress-related proteins. As shown in Figure 5(c), after treatment with MPP⁺, SK-N-SH cells expressed a significant decrease in the level of antiapoptotic protein Bcl-2 ($P < 0.05$), while the levels of Bax and cleaved Caspase-3 increased significantly ($P < 0.05$). In addition, the expression of Cytochrome C, an oxidative stress index, increased in MPP⁺-induced injury ($P < 0.05$), and MCPs treatment could reverse its change. It is suggested that in addition to antiapoptotic effects, MCPs also have antioxidant stress functions. As shown in Figure 5(d), the expression of TH in the PD cell model was reduced ($P < 0.05$), and treatment with MCPs could reverse this effect ($P < 0.05$).

3.7. MCPs Regulate the Activation State of TLR4/MyD88/NF- κ B Signaling Pathway. As shown in Figure 6, compared with Control, MPP⁺ treatment can significantly promote the expression of TLR4, MyD88, and p-p65 proteins (all $P < 0.05$), while MCPs can inhibit the expression of these proteins (all $P < 0.05$).

3.8. The Verification of the Role of MCPs by Using TLR4 Inhibitors. In this study, TAK-242, a small molecule inhibitor of TLR4, was used to verify the protective effect of MCPs on MPP⁺-induced damaged cells. As shown in Figure 7, this study found that the application of TLR4 inhibitors can significantly reverse the protective effect of MCPs (all $P < 0.05$).

4. Discussion

This study confirmed that MCPs have protective effects on MPTP- and MPP⁺-induced PD models in mice and cells. In terms of motor function, we found that MCPs can reduce the damage of MPTP to mice's coordination and exercise ability and can inhibit the production of inflammatory factors and oxidative stress products in the brain, thereby increasing the level of dopamine. In terms of cell function, MCPs can inhibit MPP⁺-induced apoptosis and oxidative stress, and we found that MCP exerts a protective effect by inhibiting the activation of the TLR4/MyD88/NF- κ B pathway.

PD is a common age-related neurodegenerative disease, which seriously affects the quality of life [31]. Due to insufficient knowledge of PD pathology, current treatment focuses on symptom relief, rather than PD prevention and basic treatment [32]. Previous studies have found that plant ingredients such as ursolic acid [33], maidenhair fern [34], and *Poria cocos* [35] have protective effects in delaying the progression of PD and reducing symptoms of PD. MCPs account for about 6% of bitter melon powder, which are heteropolysaccharides, composed of galactose (Gal), glucose (Glu), arabinose (Ara), rhamnose (Rha), and mannose (Man) [36]. Tan and Gan [37] have reported that an acidic

FIGURE 7: Continued.

FIGURE 7: Application of TLR4 inhibitor to verify the effect of MCPs. (a) Validation of TAK-242 inhibitor. (b) Detect the effect of MCPs on cell viability after applying TAK-22. (c, d) Detect the effect of MCPs on cell apoptosis after applying TAK-22. Compared with the Control group, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, compared with the PD group, $##P < 0.01$, $###P < 0.001$, similar to PD+TAK-242 Ratio, $\&\&P < 0.01$.

branched heteropolysaccharide isolated from bitter melon is mainly composed of Man, galacturonic acid (GalA), Rha, Glu, Gal, xylose (Xyl), and Ara, which has antioxidant and inhibitory properties. α -Amylase and inhibition of the angiotensin-converting enzyme. Recently, a water-soluble polysaccharide (MBP) was isolated from the fruit of the bitter gourd. Its main components are Ara, Xyl, Gal, and Rha, which have a significant hypoglycemic effect [38]. Raish proved that MCPs can improve oxidative stress, hyperlipidemia, inflammation, and apoptosis during myocardial infarction by inhibiting the NF- κ B signaling pathway [39]. In addition, MCPs also have the ability to increase total volatile fatty acid production, regulate rumen fermentation pathways, and affect the number of cellulose-decomposing bacteria [40]. However, there is no relevant report on the effect of MCPs on PD.

The gradual decrease of striatal DA in PD patients is the cause of motor and nonmotor symptoms [41]. The MPTP-induced PD mouse model is similar to the symptoms of PD patients with abnormal muscle tone, posture, and physical decline. The pole-climbing experiment is considered to be a way to measure MPTP-induced movement changes in PD mice. We have found through research that MCPs treatment is effective for the climbing time of PD mice, reversing the negative effects of MPTP treatment. The Rotarod test also has a similar finding that MCPs treatment can significantly reverse the decrease in balance and coordination caused by MPTP. Furthermore, in this study, it was detected by high-performance liquid chromatography that the DA content in the striatum of MPTP-treated mice was decreased, while the levels of 5-HT and its metabolites were increased, and MCPs treatment can alleviate the above changes. The above results indicate that MCPs treatment can effectively improve the motor coordination ability of PD mice and promote the recovery of nerve function.

It is known that PD is related to oxidative stress, inflammation, and apoptosis [42]. Many studies have shown that mitochondrial dysfunction, oxidative stress, caspase release, and electron transport chain are the main features of PD neuron death [43–45], which is consistent with our research results. MPTP can induce the decrease of GSH and SOD levels in the mouse striatum and the increase of MDA levels. The treatment of MCPs can reverse the changes of these factors, suggesting that MCPs have antioxidant effects. It is also known that Cytochrome C is a signal molecule necessary for the death of apoptotic cells. It is released from the mitochondria into the cytoplasm and can act as an apoptotic protease activator to initiate an apoptotic response. Moreover, Cytochrome C plays an important role in oxidative stress and inflammation [46]. Therefore, this study detected the expression level of Cytochrome C in mouse striatum tissues and in glioblastoma cells and found that MCPs treatment can significantly reverse the increase in PD-induced Cytochrome C expression, further verifying the antioxidant capacity of MCPs. It also suggests that MCPs have antiapoptotic and anti-inflammatory effects. In addition, this study also detected the expression level of TH in mouse striatum and glioblastoma. It is known that TH is a monooxygenase that can affect the changes in the synthesis rate and release of catecholamines during nerve stimulation. Adaptive response to maintain an appropriate supply of neurotransmitters in nerve endings [47]. This study found that MCPs treatment can significantly reverse the decrease in TH in the PD model and exert neuroprotection.

Mohammad et al. once found in alcoholic gastritis that MCPs can improve mucosal oxidative stress, inflammation, and apoptosis by inhibiting the activation of the NF- κ B signaling pathway [48]. In this study, it was found for the first time in animal and cell models of PD that MCPs have antioxidant, anti-inflammatory, and antiapoptotic effects. In terms

of anti-inflammatory response, this study detected the expression levels of inflammatory factors TNF- α and IL-1 β in brain striatum tissue. In terms of apoptosis, this study observed cell apoptosis by flow cytometry and fluorescence staining and further verified by detecting the expression level of antiapoptotic protein Bcl-2, the proapoptotic proteins Bax, and cleaved Caspase-3. Experiments have found that MCPs treatment can significantly reverse the increased levels of TNF- α and IL-1 β induced by MPTP or MPP⁺, the decrease of Bcl-2 expression, and the increase of Bax and cleaved Caspase-3 expression. This is consistent with the results of Mohammad et al. and provides a more favorable experimental basis for the protective biological effects of MCPs. In addition to animal and cell function studies, this study also deeply explored the potential mechanisms of MCPs' protective effects. The TLR4/MyD88/NF- κ B pathway is a key regulator involved in the inflammatory process [42, 49]. In this study, by observing the changes in this signaling pathway, it was found that MCPs can regulate the activation state of the TLR4/MyD88/NF- κ B signaling pathway, and we also applied TLR4 inhibitors to verify the protective effects of MCPs. At present, there are a variety of selective inhibitors with TLR4 inhibitory function, among which TAK-242 is a bioavailable TLR4 inhibitor with extensive anti-inflammatory effects [50]. In this study, the combined use of MCPs and TAK-242 in the PD state found that the use of TAK-242 could reverse the protective effect of MCPs, thus verifying the effect of MCPs on the TLR4/MyD88/NF- κ B signaling pathway.

In this study, MCPs were used to treat Parkinson's for the first time, using mouse animal models and human neuroblastoma cell models, starting from in vivo and in vitro experiments to study the protective effects of MCPs. It is worth noting that the dose concentrations in animal and cell models are similar, suggesting that the dose stability of MCPs, that is, the drug concentration plays a role in animals and cells when the drug concentration reaches 80–100 μ g/mL. This result also provides information for population studies. The theoretical basis and reference. Although this study has confirmed the protection and resistance of MCPs in terms of motor function, oxidative stress, inflammation, and apoptosis in animal and cell models and found that MCPs interfere with TLR4/The activation state of MyD88/NF- κ B pathway. However, this study also has shortcomings. In view of the diversity of mechanism studies, the protective effect of MCPs is not limited to the above studies, and mice and animal models cannot completely replace population studies, so follow-up studies are still needed to clarify the role and application prospects of MCPs.

In summary, this study found that in the PD model, MCPs can regulate the activation state of the TLR4/MyD88/NF- κ B pathway, exert protective effects such as anti-inflammatory, antioxidative stress, and antiapoptosis, improve brain function, and provide a new method for the treatment of PD.

Data Availability

The data are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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