Journal of Agriculture and Food Research 22 (2025) 101994

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.sciencedirect.com/journal/journal-of-agriculture-and-food-research

Neuroprotective effect of an unsaturated mannuronate oligosaccharide derived from enzyme-degraded polymannuronate on an *in vitro* Parkinson's disease-like model

Decheng Bi [a,1], Jinfeng Huang [a,b,1], Nanting Zhu [a], Jue Cao [a,f], Yan Wu [c], Lijun Yao [a], Xueying Ding [d], Jianyong Wu [e], Xu Cao [d,*], Xu Xu [a,**]

[a] Shenzhen Key Laboratory of Marine Bioresources and Ecology, College of Life Sciences and Oceanography, Shenzhen University, Shenzhen, 518060, PR China
[b] Digestive Diseases Center, The Seventh Affiliated Hospital, Sun Yat-sen University, Shenzhen, 518107, PR China
[c] Instrumental Analysis Center, Shenzhen University, Shenzhen, 518060, PR China
[d] Department of Neurology, Shenzhen University General Hospital, Shenzhen University Clinical Medical Academy, Shenzhen, 518055, PR China
[e] Department of Food Science and Nutrition, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong
[f] Key Laboratory Marine Biological Waste and Comprehensive Utilization of Guangdong Province, Zhanjiang, 524051, PR China

ARTICLE INFO

Keywords:
Unsaturated mannuronate oligosaccharide (MOS)
Parkinson's disease (PD)
Cell culture model
Antioxidant
Anti-apoptosis
Autophagy

ABSTRACT

This study mainly aimed to explore the intervention effect of an unsaturated mannuronate oligosaccharide (MOS) on Parkinson's disease (PD) and its potential mechanism at the cellular level. MOS, which possesses high water solubility and excellent biocompatibility, was prepared by enzyme degradation of alginate-derived polymannuronate. In 6-hydroxydopamine (6-OHDA)-induced SH-SY5Y cell culture, MOS treatment effectively augmented the expression of tyrosine hydroxylase (TH), PTEN-induced putative kinase 1 (PINK1) and parkin but suppressed the expression of α-synuclein (α-syn). Moreover, MOS exerted antioxidant activity by suppressing the excessive generation of reactive oxygen species and enhancing the activity of superoxide dismutase. MOS also inhibited 6-OHDA-induced apoptosis by enhancing mitochondrial membrane potential and mitigating the Bax/Bcl-2 pathway and improving autophagy that was blocked by 6-OHDA. In summary, these results have demonstrated the significant neuroprotective effect of MOS on SH-SY5Y cells, indicating that MOS has a certain intervention effect on the development of PD. This provides a promising foundation for developing novel therapeutic strategies targeting neurodegenerative disorders.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder with a prevalence of over 1 % among individuals aged 65 and above, and this figure is anticipated to double by 2030, imposing a heavy economic burden on society [1]. The pathological characteristics of PD include the loss of dopaminergic neurons and the Lewy bodies formed by abnormal aggregation of α-synuclein (α-syn) in the pars compacta of the substantia nigra [2]. At present, no drugs can completely cure PD and most of the existing drugs for PD have side effects. The first PD medication, levodopa, which acts as a direct dopamine precursor, relieves symptoms by promoting dopamine production in dopaminergic neurons [3]. However, this therapy is only effective in treating bradykinesia and is linked to significant side effects, including hallucinations, delusions, drowsiness, dystonia, and other adverse effects [4]. Alternative therapeutics based on the pathological mechanisms of PD have emerged to address the continuously present symptoms of the disease, including the modulation of α-syn aggregation [5], oxidative stress [6], mitochondrial dysfunction [7], and the impairment of autophagy [8]. However, the use of two non-dopaminergic medications, trihexyphenidyl and amantadine, has also been limited due to various side effects [9,10].

Therefore, the search for highly efficacious, non-toxic and neuroprotective therapeutic agents from natural products is a prospective strategy for PD treatment [11]. For example, quercetin, a dietary polyphenolic flavonoid, could alleviate 6-hydroxydopamine (6-OHDA)-induced PD-like behavior by promoting mitochondrial autophagy and

* Corresponding author.
** Corresponding author.
E-mail addresses: dr.caoxu@qq.com (X. Cao), xuxu@szu.edu.cn (X. Xu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.jafr.2025.101994
Received 24 March 2025; Received in revised form 23 April 2025; Accepted 5 May 2025
Available online 8 May 2025

α-syn clearance [12]. Another well-known dietary polyphenol with a variety of biological activities, resveratrol, could inhibit neuroinflammation to relieve PD [13]. However, a major disadvantage of these natural products is their poor water solubility and low bioavailability [14].

Active carbohydrate substances with good water solubility and diverse structural characteristics in alleviating oxidative stress, inflammation and neuronal apoptosis in *in vitro* Parkinson's disease models have been widely explored by researchers, such as trehalose, a promising candidate for PD therapy [15]. *Polygonatum sibiricum* polysaccharides also have been found to alleviate the deficiency of locomotor activity and loss of dopaminergic neurons in the PD mouse model [16]. Alginate is an acidic polysaccharide existing as a composite of β-D-mannuronate and α-L-guluronate in edible marine brown alga [17]. With its unique physicochemical properties and beneficial health effects, alginate and its derivatives have found extensive application in food and pharmaceutical industries, such as surgical dressing, gels, edible films, and thickeners[17–19]. Studies have demonstrated the potential of alginate-derived polymannuronate (PM) in strengthening motor functions and preventing dopaminergic neuronal loss by enhancing the expression of TH in the midbrain of PD mouse models [20]. However, the large PM polysaccharide molecules encounter some limitations in terms of bioavailability and blood-brain barrier crossing. Oligosaccharides produced by alginate degradation have the advantages of high water solubility, high stability and high bioavailability, and improved biological activities [17]. MOS was obtained from PM by enzymatic degradation, with the degree of polymerization varying from M2 to M11 and a double bond at the C-4 and C-5 positions of the non-reducing end (Scheme 1) [21], which could improve the pathological characteristics of Alzheimer's disease (AD) by enhancing autophagy in AD cell models [22]. Based on the above studies, we speculate that MOS might have similar effects in the alleviation of PD development.

In this study, the structural characteristics of MOS were analyzed and the potential effect of MOS on preventing the PD-like pathological process and the related molecular mechanism were investigated in 6-OHDA-triggered SH-SY5Y cell culture. The findings from this study would addresses a specific gap by investigating the neuroprotective role of MOS and could provide key insights into understanding the pathological mechanism of MOS intervention in PD, helping to establish the fundamental basis for MOS as food supplements for PD treatment.

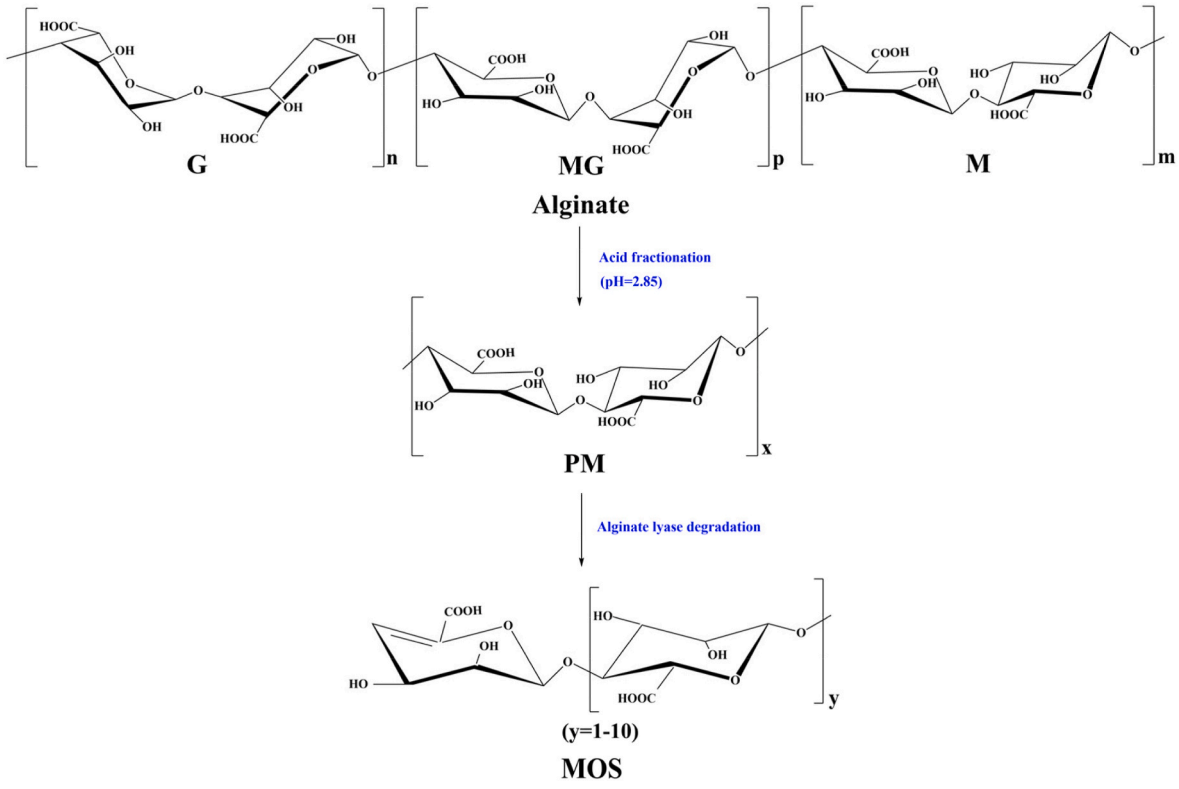
2. Materials and methods

2.1. Materials and reagents

Sodium alginate (20 cps) and 6-OHDA were provided by Sigma-Aldrich (St. Louis, MO, USA). Antibiotics, fetal bovine serum (FBS), dulbecco's modified eagle medium (DMEM), and DMEM/F-12 were provided by Gibco (Grand Island, NY, USA). All antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). The cell counting kit-8 (CCK-8), lactate dehydrogenase (LDH) cytotoxicity assay kit, 2′,7′-dichlorofluorescin diacetate (DCFH-DA) reagent, total superoxide dismutase (SOD) assay kit, JC-1 fluorescence probe and RIPA buffer were purchased from Beyotime (Jiangsu, China). The enhanced chemiluminescent kit was from Thermo Scientific (Hudson, NH, USA). Other chemicals were provided by Macklin Biochemical Technology (Shanghai, China).

2.2. Procedure for preparation of MOS from alginate

The unsaturated mannuronate oligosaccharide (MOS) was prepared as reported previously, including the acid fractionation of alginate to PM and enzymatic degradation of PM (Scheme 1) [21,23]. Firstly, 20 g of alginate was dissolved in 1000 mL of 0.5 M HCl and heated for 7 h at 90 °C. After centrifugation, the sediment was obtained and dissolved in 8 % $NaHCO_3$. The pH value of the solution was adjusted to 2.85 and the PG precipitation was removed by centrifugation (2000 g, 10 min). Then, three times the volume of anhydrous ethanol was added to make PM precipitate. Following freeze-drying by vacuum-freeze dryer (DGJ-7C,



Scheme 1. Preparation of MOS from alginate-derived PM.

BODENG, Shanghai, China), 0.5 g of PM was dissolved in 20 mL of 0.05 M phosphate buffer and then mixed with 20 μL of 0.3 mg/mL alginate lyase, which was a gift from Professor Tatsuya Oda of Nagasaki University. An equal enzyme solution was added after 2 h incubation and further incubated for 24 h. Then, the MOS was obtained after filtration and freeze-drying.

2.3. Cell culture and treatment

SH-SY5Y cells were cultured in DMEM supplemented with 50 % DMEM/F-12, 10 % FBS, and 1 % each of antibiotics, nonessential amino acids, sodium pyruvate and L-glutamine at 37 °C with 5 % CO_2. For the experiment on MOS treatment effects on 6-OHDA-stimulated responses, the cells were seeded in 96-well (5×10^4 cells/well) or 6-well (5×10^5 cells/well) plates overnight and then pretreated with 1, 10, or 100 μg/mL MOS for 4 h in the medium without FBS. Following co-stimulation with 50 μM 6-OHDA for 24 h, the cells were collected for subsequent experiments.

2.4. Cell viability assay

After various treatments, SH-SY5Y cells were incubated with 100 μL CCK-8 reagent for 1.5 h. Then, the absorbance of all samples was detected at 450 nm by a Spectra Max microplate reader (Thermo Scientific, Hudson, NH, USA).

2.5. LDH release assay

After various treatments, the cell-culture medium in 96-well plates was totally removed and 150 μL of LDH release reagent was added. After 1 h incubation in the incubator with 5 % CO_2, 100 μL of the supernatant was mixed with 50 μL of LDH detection working solution and incubated at 25 °C for 30 min in the darkness without CO_2. Subsequently, the absorbance of all samples was determined at 490 nm by a microplate reader.

2.6. Measurement of intracellular ROS and SOD activity

Intracellular ROS level was determined with a DCFH-DA probe. After the treatments, the cell-culture medium in 6-well plates was completely removed and 10 μM DCFH-DA in 2 mL of new medium was added. After 30 min incubation at 37 °C, the fluorescence of each group was analyzed using flow cytometry (FACS, BD, New Jersey, USA) with an excitation wavelength of 488 nm and an emission wavelength of 525 nm.

For SOD measurement, 100 μL of SOD preparation solution was added to 96-well plates for cell lysis. After 30 min incubation, 20 μL of supernatant was mixed with the working solution and incubated for another 30 min. Subsequently, the absorbance was detected at 450 nm by a microplate reader.

2.7. Mitochondrial membrane potential (MMP) analysis

After the treatments, SH-SY5Y cells were resuspended and probed by JC-1 at 37 °C for 20 min. After being washed, the cells were analyzed by FACS with excitation at 485 nm and emission at 590 nm.

2.8. Western blot analysis

The protein from SH-SY5Y cells was collected using RIPA buffer and quantified by the BCA Protein Quantitation kits. Then 10 μg of each protein was separated using SDS-PAGE and transferred onto PVDF membranes (100 mA, 120 min). After being blocked, the PVDF membranes were incubated with primary antibodies (Table S1) at 4 °C overnight and HRP-conjugated secondary antibody at RT for 1 h. After rinsing, the membranes were scanned by the automatic chemiluminescence imaging system (Tanon 5200, Guangdong, China) with an ECL kit, and the grayscale value of each band was analyzed using Fiji software (NIH, Bethesda, MD, USA).

2.9. Immunofluorescence analysis

Following various treatments, cells grown on confocal dishes were fixed using 4 % paraformaldehyde and permeabilized using 0.2 % Triton X-100. After blocked, the SH-SY5Y cells were administrated with anti-α-syn antibody. For the assessment of mitochondrial function, the SH-SY5Y cells were exposed to JC-1. Following rinses, the cells were incubated with DAPI or fluorescently labelled secondary antibody. Following three washes, the cells were observed by confocal microscopy (ZEISS LSM 880, Leica, Germany).

2.10. Statistical analysis

The experimental data were analyzed by GraphPad Prism 5.0 software (GraphPad Software, CA, USA) and presented as the mean ± standard deviation (SD). The significance of a treatment effect was determined by two-tailed Student's *t-test* at $p < 0.05$.

3. Results

3.1. Effects of MOS on cell cytotoxicity induced by 6-OHDA

The results of cytotoxicity assay showed that MOS alone improved the cell viability of SH-SY5Y cells slightly (Fig. 1A). When the cells were treated with 10 μg/mL MOS, the viability reached a maximum of 118 % ± 2.69 %, and then decreased to 108 % ± 1.70 % at 100 μg/ml MOS concentration, but all were higher than the blank control group ($p < 0.01$) (Fig. 1A). This biphasic trend may reflect a mild cytostatic effect or cellular stress response triggered at higher concentrations, a phenomenon commonly observed with bioactive oligosaccharides and other natural compounds [24]. Treatment with 6-OHDA alone caused a reduction in the viability of SH-SY5Y cells by approximately 68 % ± 2.63 % in comparison with the control group; MOS pretreatment was found to effectively attenuate the 6-OHDA-induced cytotoxicity in a concentration-dependent manner (Fig. 1A). These results indicated that MOS's protective effects are predominant over its effects on cell viability.

From Fig. 1B, it could be found that treatment with 6-OHDA effectively increased the release of LDH from SH-SY5Y cells compared to the control group ($p < 0.01$). As expected, the 6-OHDA-triggered LDH release from SH-SY5Y cells was significantly eliminated by MOS pretreatment in a dose-dependent manner ($p < 0.01$) (Fig. 1B).

3.2. Effects of MOS on the protein expression of TH and α-syn

TH and α-syn are important markers of PD pathology [25] and the effect of MOS on their expression induced by 6-OHDA were determined by Western blotting analysis. As shown in Fig. 2A and B, stimulation with 6-OHDA alone resulted in an obvious reduction in the expression level of TH and a significant increase in the expression level of α-syn ($p < 0.05$). When pretreated with MOS, this situation was significantly reversed in contrast to the model group ($p < 0.05$), indicating that MOS could reduce the PD-related pathological characteristics induced by 6-OHDA. In addition, the effect of MOS on the expression of α-syn was further confirmed by immunofluorescence using confocal microscopy, which showed that MOS treatment could significantly reduce the α-syn expression and aggregation in 6-OHDA-treated SH-SY5Y cells (Fig. S1).

3.3. Effects of MOS on the mitochondrial homeostasis

Mitochondrial dysfunction can exacerbate the PD process [26]. It was shown that 6-OHDA notably reduced the expression of PINK1 and parkin protein in SH-SY5Y cells in comparison to the control group ($p <$

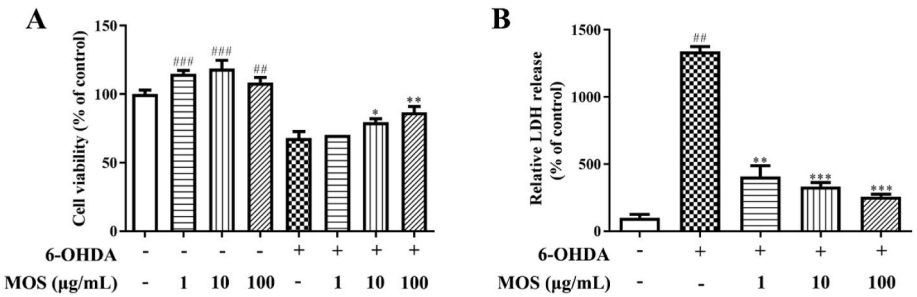


Fig. 1. Effects of MOS on cell cytotoxicity in 6-OHDA-triggered SH-SY5Y cells. SH-SY5H cells were pretreated with 1, 10, or 100 μg/mL MOS for 4 h followed by 50 μM 6-OHDA for 24 h. (A) Cell viability was tested by the CCK-8 kit. (B) LDH level in cells, assessed by LDH cytotoxicity assay kit. Data presented as the mean ± SD (n ≥ 3), $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to control group. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared to 6-OHDA group.

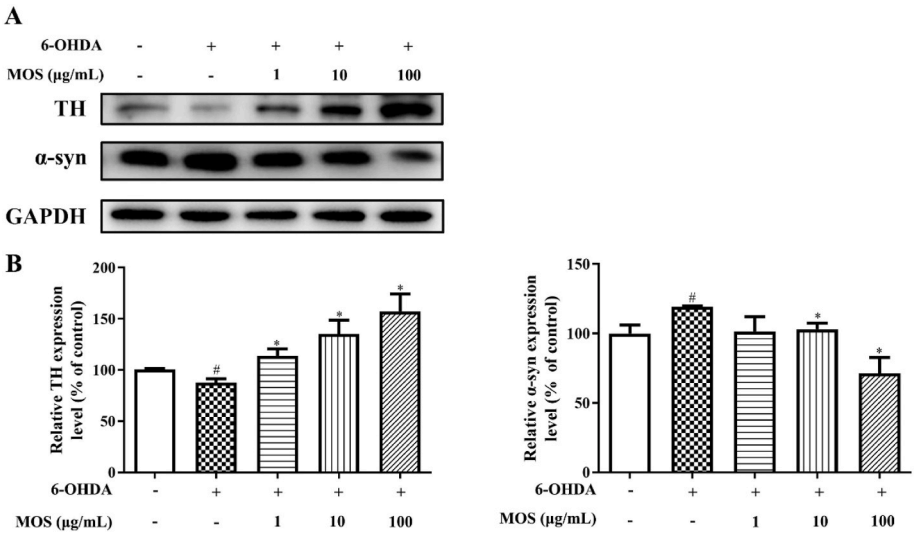


Fig. 2. Effects of MOS on TH and α-syn protein expression in SH-SY5Y cells induced with 6-OHDA. SH-SY5H cells were pretreated with 1, 10, or 100 μg/mL MOS for 4 h followed by 50 μM 6-OHDA for 24 h. (A) The expression level of TH and α-syn evaluated by Western blotting analysis and (B) normalized to GAPDH. Data presented as the mean ± SD (n = 3). $^{\#}p < 0.05$, compared to control group. $^{*}p < 0.05$ compared to 6-OHDA group.

0.05), and MOS treatment could effectively reverse the 6-OHDA-triggered reduction of these proteins ($p < 0.05$) (Fig. 3A and B). The expression of mitochondrial fission and fusion proteins in SH-SY5Y cells is also closely related to mitochondrial homeostasis. It was shown that 6-OHDA notably reduced the expression of mitofusin-2 and increased the expression of dynamin-related protein 1 (DRP1) in SH-SY5Y cells in comparison to the control group ($p < 0.05$), and MOS treatment could effectively reverse this situation ($p < 0.05$) (Fig. 3C and D). This suggests that MOS can effectively regulate mitochondrial homeostasis, thereby helping to slow down the process of PD.

3.4. Effects of MOS on ROS generation and SOD activity

As reported, 6-OHDA can cause cell damage by inducing large amounts of ROS production and reducing SOD activity [27], which was confirmed in this study. The ROS production in 6-OHDA-induced SH-SY5Y cells was 2.8 times higher than that of the control group ($p < 0.001$), and the SOD activity was reduced to 54 % of the control group ($p < 0.01$) (Fig. 4A and B). Interestingly, the MOS pretreatment significantly attenuated the excessive ROS generation and the 6-OHDA-suppressed SOD activity in SH-SY5Y cells in a dose-dependent manner (Fig. 4).

3.5. Effects of MOS on mitochondrial membrane potential (MMP)

To further evaluate the effect of MOS on 6-OHDA-induced MMP, the JC-1 probe was used. JC-1 probe can selectively enter mitochondria, and when the mitochondrial membrane potential is reduced, JC-1 changes from red fluorescence to green fluorescence. It could be seen that JC-1 red fluorescence was dominant in untreated SH-SY5Y cells, while JC-1 green fluorescence was dominant in 6-OHDA-treated SH-SY5Y cells. When pre-treated with MOS, the red fluorescence of JC-1 was enhanced and superimposed with the green fluorescence, presenting a certain yellow color (Fig. 5A), which was also confirmed by flow cytometry analysis. As expected, in SH-SY5Y cells treated with 6-OHDA alone, the MMP decreased by 43.31 % in comparison to the control group ($p < 0.001$) and MOS pretreatment significantly reversed the 6-OHDA-induced reduction in MMP ($p < 0.01$) (Fig. 5B). These results indicate that MOS could effectively relieve mitochondrial damage induced by 6-OHDA.

3.6. Effects of MOS on the mitochondrial apoptosis pathway

The reduction of MMP will trigger a series of signaling cascades leading to apoptosis, which was further confirmed in this study. After 6-OHDA treatment, the levels of Bax and cleaved caspase 3 were increased in the SH-SY5Y cells, whereas the level of Bcl-2 was decreased compared to the control group (Fig. 6). As expected, MOS pretreatment can reverse these proteins' expression (Fig. 6A). From the band density analysis, it can be found that the 6-OHDA-triggered augmentation in cleaved caspase 3 expression ($p < 0.01$) and Bax/Bcl-2 ratio ($p < 0.05$) was significantly decreased by MOS pretreatment ($p < 0.01$) (Fig. 6B). This

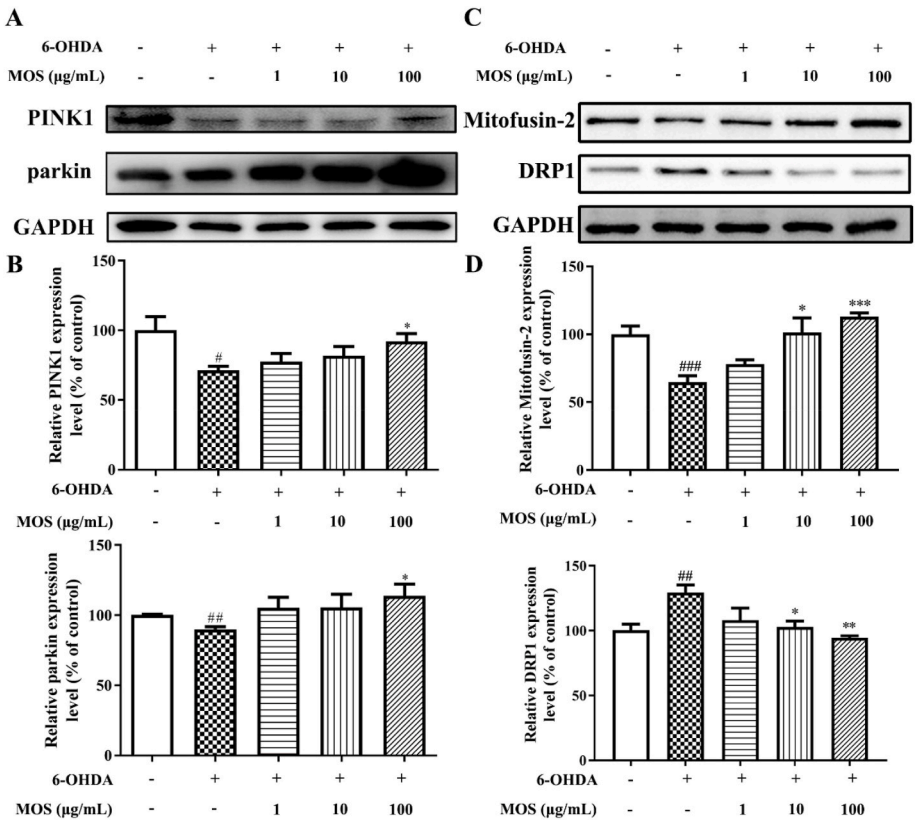


Fig. 3. Effects of MOS on mitochondrial homeostasis in SH-SY5Y cells induced with 6-OHDA. SH-SY5H cells were pretreated with 1, 10, or 100 μg/mL MOS for 4 h followed by 50 μM 6-OHDA for 24 h. (A) The expression level of PINK1 and parkin evaluated by Western blotting analysis and (B) normalized to GAPDH. (C) The expression level of mitofusin-2 and DRP1 evaluated by Western blotting analysis and (D) normalized to GAPDH. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ compared to control group. Data presented as the mean ± SD (n = 3). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{*}p < 0.001$ compared to 6-OHDA group.

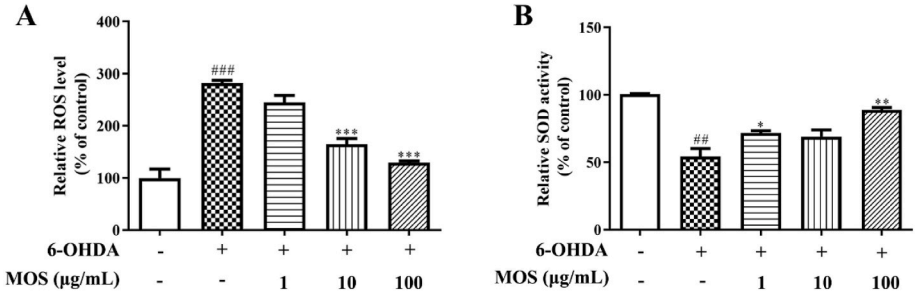


Fig. 4. Effects of MOS on oxidative stress in SH-SY5Y cells induced with 6-OHDA. SH-SY5H cells were pretreated with 1, 10, or 100 μg/mL MOS for 4 h followed by 50 μM 6-OHDA for 24 h. (A) ROS production was tested by DCFH-DA staining. (B) SOD activity was assessed using a commercial kit. Data presented as the mean ± SD (n = 3). $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to control group. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared to 6-OHDA group.

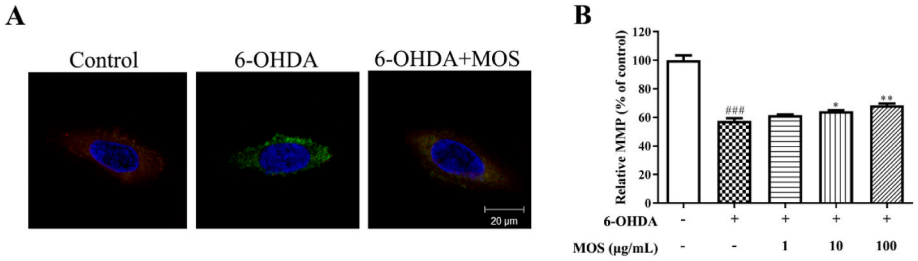


Fig. 5. The effect of MOS on MMP in 6-OHDA-induced SH-SY5Y cells. SH-SY5H cells were pretreated with 1, 10, or 100 μg/mL MOS for 4 h followed by 50 μM 6-OHDA for 24 h, and then the MMP was determined using JC-1 fluorescence probe. (A) Immunofluorescence analysis. (B) Flow cytometry analysis. Data presented as the mean ± SD (n ≥ 3). $^{\#\#\#}p < 0.001$ compared to control group. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to 6-OHDA group.

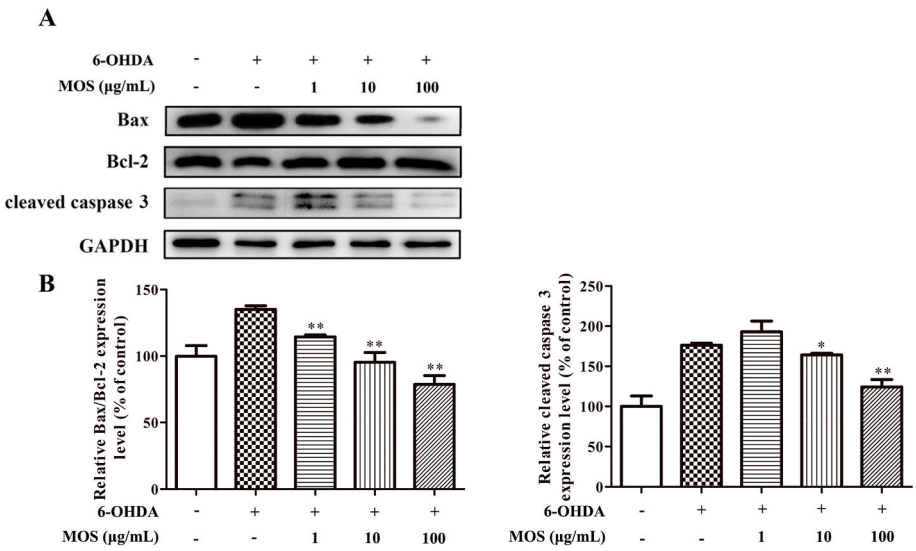


Fig. 6. The effects of MOS on apoptosis in 6-OHDA-induced SH-SY5Y cells. SH-SY5H cells were pretreated with 1, 10, or 100 μg/mL MOS for 4 h followed by 50 μM 6-OHDA for 24 h. (A) The expression levels of Bax, Bcl-2 and cleaved caspase 3, evaluated by Western blot analysis (B) normalized to GAPDH. Data presented as the mean ± SD (n = 3). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to control group. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to 6-OHDA group.

suggests that MOS could attenuate 6-OHDA-induced activation of mitochondria-mediated endogenous apoptosis signaling pathway and antagonize cell apoptosis.

3.7. Activation effects of MOS on the autophagy pathway

As illustrated in Fig. 7, treatment with 6-OHDA alone resulted in an increased expression of phosphorylated mammalian target of rapamycin (p-mTOR) and a higher LC3-II/LC3-I ratio compared to the control group ($p < 0.05$). As expected, pre-treatment with MOS could effectively attenuate this trend ($p < 0.05$). To confirm the relationship between the inhibition of apoptosis and autophagy augmented by MOS, the expression level of cleaved caspase 3 in 6-OHDA-triggered SH-SY5Y cells supplemented with autophagy inhibitors (3-methyladenine, 3-MA, 0.35 mM) was detected. From Fig. 8, it was observed that the level of cleaved caspase 3 was obviously augmented in the presence of autophagy inhibitors compared to treatment with 6-OHDA alone ($p < 0.05$). In addition, the decreased production of cleaved caspase 3 by MOS was effectively restored by co-incubation with 3-MA ($p < 0.01$) (Fig. 8).

4. Discussion

Recent investigations have demonstrated that some saccharides extracted from seaweeds have neuroprotective activity. A fucoidan extracted from *Laminaria japonica* has been found to ameliorate motor disorders and dopaminergic neurodegeneration [28] and another isolated from *Fucus vesiculosus* can prevent apoptosis and oxidative stress in PD-like cell models [29]. Trehalose has been documented to enhance autophagy and prevent MPTP-induced dopaminergic neuron damage [30]. Our previous studies have shown that alginate-derived saccharides can inhibit lipopolysaccharide (LPS)-induced neuroinflammation and facilitate the phagocytosis of BV-2 cells to β-amyloid [31]. In the current study, MOS, an oligosaccharide derived from enzymatic depolymerization of PM, was found to enhance cell viability and decrease cytotoxicity

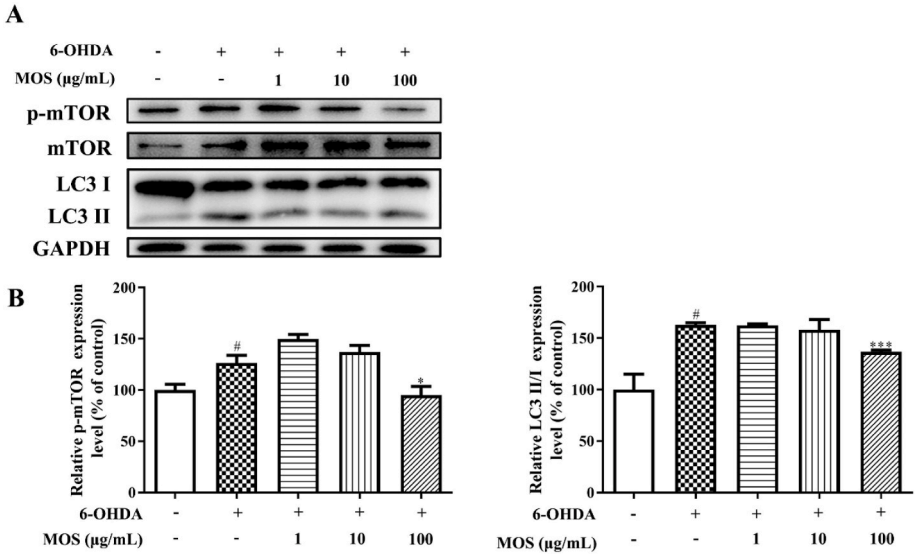


Fig. 7. The effects of MOS on the autophagy pathway in 6-OHDA-induced SH-SY5Y cells. SH-SY5H cells were pretreated with 1, 10, or 100 μg/mL MOS for 4 h followed by 50 μM 6-OHDA for 24 h. (A) The expression levels of p-mTOR, mTOR and LC3 were evaluated by Western blot analysis, (B) normalized to GAPDH. Data presented as the mean ± SD (n = 3). $^{\#}p < 0.05$ compared to control group. $^{*}p < 0.05$, $^{***}p < 0.001$.

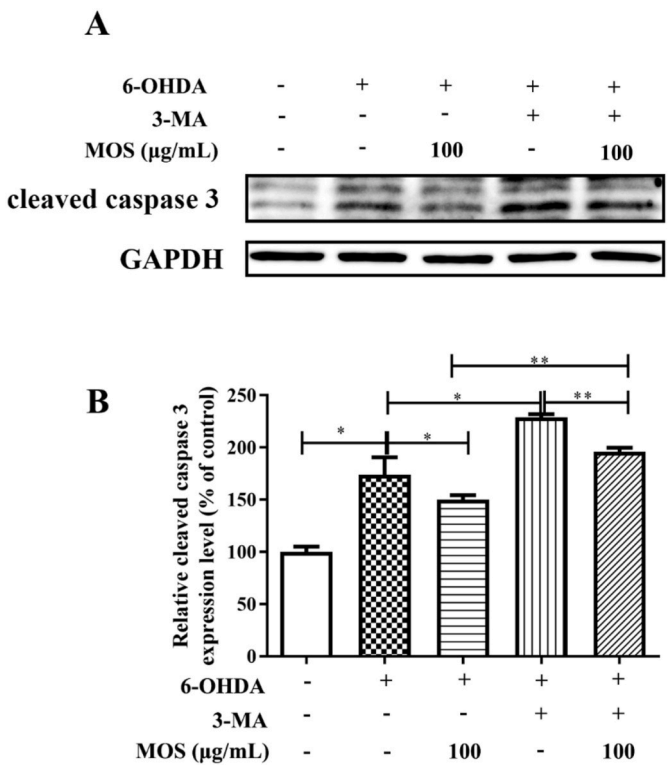


Fig. 8. Effect of autophagy inhibitor on MOS-reduced the expression of cleaved caspase 3 levels via autophagy. SH-SY5H cells were pretreated with 1, 10, or 100 μg/mL MOS for 4 h followed by 50 μM 6-OHDA and 3-MA (0.35 mM) for 24 h. (A) The expression level of cleaved caspase 3 was evaluated by Western blot analysis, (B) normalized to GAPDH. Data presented as the mean ± SD (n = 3). $^{*}p < 0.05$, $^{**}p < 0.01$.

in 6-OHDA-stimulated SH-SY5Y cells (Fig. 1), suggesting that MOS has neuroprotective activity.

The loss of dopaminergic neurons is a widely recognized hallmark of PD, with TH serving as a crucial marker for the viability of dopaminergic neurons [2]. Our findings revealed that MOS effectively increased TH expression in SH-SY5Y cells (Fig. 2), indicating MOS could inhibit the reduction in the number of dopaminergic neurons. This observation further confirmed the neuroprotective potential of MOS. The abnormal expression and aggregation of α-syn represents another significant pathological feature of PD. Notably, our investigation demonstrated that MOS treatment significantly reduced α-syn protein expression in 6-OHDA-mediated neuronal cell death model (Fig. 2). The finding indicated that MOS treatment might also protect the function of the mitochondria function of SH-SY5Y cells. As expected, MOS treatment maintained mitochondrial homeostasis by enhancing the levels of PINK1 and parkin (Fig. 3A and B), which could collaborate to ubiquitinate the damaged mitochondria and remove the damaged organelles through mitochondrial autophagy [32]. Mitochondria regulate their structural integrity and functionality through continuous cycles of fission and fusion, ensuring the dynamic equilibrium of the mitochondrial network. Disruptions in this balance can lead to structural abnormalities and functional impairments. Excessive mitochondrial fission results in fragmentation, which can compromise mitochondrial function. Maintaining this dynamic interplay is crucial for cellular homeostasis [33]. Western blot analysis revealed that exposure to 6-OHDA significantly reduced mitofusin-2 expression while increasing DRP1 levels, indicating dysregulated mitochondrial dynamics. Notably, MOS post-treatment reversed these changes by upregulating mitofusin-2 and downregulating DRP1, suggesting its role in restoring mitochondrial network balance (Fig. 3C and D). These findings imply that MOS mitigates the abnormal mitochondrial fission and fusion induced by 6-OHDA, thereby preserving mitochondrial integrity and function.

ROS is reported to act a critical role in cell homeostasis. However, when its production is unlimited, ROS can, in turn, damage cells [34]. MOS pretreatment significantly reduced the ROS level in 6-OHDA-triggered SH-SY5Y cells (Fig. 4A) and enhanced the activity of SOD (Fig. 4B). These results indicated that MOS possesses significant antioxidant activity, which might be due to the unsaturated double bonds present at the C-4 and C-5 positions of MOS [35,36]. However, the potential involvement of receptor-mediated or signaling pathway-based mechanisms cannot be excluded and further studies are needed to clarify whether MOS interacts with specific cellular receptors or redox-sensitive transcription factors. The previous study has reported that the MMP of PD patients in peripheral blood mononuclear cells was much lower than that of healthy controls [37]. Interestingly, our study demonstrated that MOS effectively prevented the reduction of MMP induced by 6-OHDA in SH-SY5Y cells (Fig. 5). Given the lack of evidence for direct mitochondrial targeting, we speculate that MOS may regulate mitochondrial dynamics indirectly by alleviating oxidative stress and restoring mitochondrial membrane potential, two critical upstream signals known to influence mitochondrial dynamics proteins [32].

Furthermore, MOS significantly increased the levels of anti-apoptotic Bcl-2 protein while decreasing the levels of pro-apoptotic proteins in 6-OHDA-treated SH-SY5Y cells (Fig. 6), indicating MOS can effectively inhibit cell apoptosis and exert a neuroprotective role in neuro cells. This finding parallels the effects of the polysaccharide prepared from *Antrodia camphorate*, which played a neuroprotective role by reducing the production of ROS and inhibiting neuronal apoptosis in 6-OHDA-mediated neuronal cell death models [38].

Evidence has demonstrated that autophagy defects play a significant role in the pathophysiology of PD [39], which was also confirmed in this study. In 6-OHDA-treated SH-SY5Y cells, the increase of the level of p-mTOR and the ratio LC3-II to LC3-I indicated the initiation of autophagy and the fusion of autophagosomes and lysosomes were inhibited, respectively [40]. Notably, MOS treatment could effectively reduce the level of p-mTOR and LC3-II/LC3-I ratio (Fig. 7A and B), suggesting that MOS could effectively regulate autophagy in nerve cells, consistent with our previous reports [21]. To confirm the relationship between increased autophagy and inhibited apoptosis, autophagy inhibitor 3-MA was used. As anticipated, the cleaved caspase 3 level in the presence of 3-MA was significantly elevated compared to those treated with MOS alone (Fig. 8). This finding demonstrated that the anti-apoptotic effects of MOS can be disrupted by using autophagy inhibitors, which confirm that autophagy is related to the neuroprotective effects of MOS on nerve cells. Similarly, astragalus polysaccharide also has been shown to exert neuroprotective effects by activating autophagy through the mTOR pathway in 6-OHDA-induced PC12 cells [41].

The antioxidant and anti-inflammatory effects of MOS likely contribute to the beneficial effects observed in this study, with these biological activities being closely linked to the molecular structure of MOS. Specifically, MOS possess an unsaturated double bond at the C-4 and C-5 positions, forming conjugated structures with carboxyl groups. According to resonance hybrid theory, the parent radicals of MOS undergo delocalization through allylic rearrangement, enabling the conjugated structure to effectively neutralize oxygen free radicals when encountering radical oxidants [36]. Furthermore, the conjugated alkene acid structure renders MOS an acidic saccharide, potentially allowing MOS to modulate the functions of certain organelles upon cellular entry, such as maintaining the acidic environment of lysosomes to enhance autophagy. Compared to conventional neuroprotective agents, MOS demonstrates a unique profile. Levodopa is a dopamine precursor widely used in Parkinson's disease to alleviate motor symptoms, but it neither addresses underlying neuroinflammation nor prevents neuronal loss, and long-term use can lead to motor complications [42]. In contrast, quercetin and resveratrol are polyphenols that exert neuroprotective effects by scavenging ROS, inhibiting pro-inflammatory cytokines, and modulating pathways such as Nrf2 and NF-κB [43]. In addition,

compared to other algal-derived polysaccharides, MOS features a lower molecular weight and an unsaturated structure, which may enhance its cell permeability and antioxidant potency. Furthermore, MOS appears to exert a multifaceted protective effect, simultaneously modulating oxidative stress, mitochondrial integrity, and autophagy. This integrated mechanism may distinguish it from other algal polysaccharides [44].

Currently, studies on MOS have concentrated on their potential therapeutic effects. However, the current limitations in the alginate lyase industry hinder the large-scale production of MOS. As advancements in the alginate lyase sector progress, it is anticipated that the production of enzymatically derived alginate oligosaccharides will significantly increase. Consequently, MOS could become more widely available as a nutritional supplement, potentially at a much lower cost.

In summary, at the cellular level, MOS can effectively ameliorate PD-related pathological features, while simultaneously reducing oxidative stress and apoptosis in 6-OHDA-mediated neuronal cell death. Moreover, the enhancement of autophagy by MOS likely plays a pivotal role in these protective effects. Of course, these findings are based on undifferentiated SH-SY5Y cells, which presents certain limitations. Although undifferentiated SH-SY5Y cells are commonly used as a rapid and cost-effective *in vitro* model for neurotoxicity screening, they lack the mature neuronal characteristics of differentiated cells, such as extended neurite outgrowth, synaptic activity, and specific neurotransmitter profiles. These limitations restrict the translational relevance of the current findings. In future work, we plan to utilize retinoic acid-differentiated SH-SY5Y cells, which more closely resemble mature dopaminergic neurons, to assess whether MOS confers similar protective effects in a more neuron-like environment. Furthermore, *in vivo* validation will be performed using a well-established PD animal model to evaluate the therapeutic efficacy of MOS in a complex biological system, including its impact on motor function, dopaminergic neuron survival, and neuroinflammation in the substantia nigra and striatum.

5. Conclusions

This study suggests MOS has the potential for reducing abnormal expression of α-syn, promoting dopaminergic neuron repair, and enhancing mitochondrial quality control by increasing the expression of PINK1 and parkin. Moreover, MOS may exert a neuroprotective effect by inhibiting oxidative stress and apoptosis and enhancing autophagy. These findings indicate that MOS has a protective effect on SH-SY5Y cells to against 6-OHDA, laying a foundation for future exploration of candidate compounds or functional food ingredients for PD treatment.

CRediT authorship contribution statement

Decheng Bi: Writing – original draft, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Jinfeng Huang:** Project administration, Methodology, Data curation, Conceptualization. **Nanting Zhu:** Project administration, Methodology. **Jue Cao:** Project administration, Methodology. **Yan Wu:** Methodology. **Lijun Yao:** Project administration. **Xueying Ding:** Project administration. **Jianyong Wu:** Writing – review & editing. **Xu Cao:** Writing – review & editing, Supervision, Resources. **Xu Xu:** Writing – review & editing, Visualization, Validation, Supervision, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported financially by the National Natural Science Foundation of China (32172193 and 32302058), the National Key Research and Development Program of China (2024YFD2401605 and 2023YFF1001200), the Shenzhen Science and Technology Program (JCYJ20240813142721028), the Project of DEGP (2023ZDZX4047), Guangdong Science and Technology Planning Project (2020B121202014), and Natural Science Foundation of Shenzhen University General Hospital (SUGH2018QD005).

The authors would like to thank the Instrumental Analysis Center of Shenzhen University and the Central Research Facilities of the College of Life Sciences and Oceanography in Shenzhen University for their assistance in our experiments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jafr.2025.101994.

Data availability

Data will be made available on request.

References

[1] D. Aarsland, L. Batzu, G.M. Halliday, G.J. Geurtsen, C. Ballard, K. Ray Chaudhuri, D. Weintraub, Parkinson disease-associated cognitive impairment, Nat. Rev. Dis. Primers 7 (2021) 1–21.
[2] T.N. Taylor, S.P. Alter, M. Wang, D.S. Goldstein, G.W. Miller, Reduced vesicular storage of catecholamines causes progressive degeneration in the locus ceruleus, Neuropharmacology 76 (2014) 97–105.
[3] M. Hayes, Parkinson's disease and parkinsonism, Am. J. Med. 132 (2019) 802–807.
[4] M. Hutny, J. Hofman, A. Klimkowicz-Mrowiec, A. Gorzkowska, Current knowledge on the background, pathophysiology and treatment of levodopa-induced dyskinesia: literature review, J. Clin. Med. 10 (2021) 4377.
[5] H.A. Lashuel, C.R. Overk, A. Oueslati, E. Masliah, The many faces of alpha-synuclein: from structure and toxicity to therapeutic target, Nat. Rev. Neurosci. 14 (2013) 38–48.
[6] D.J. Surmeier, J.N. Guzman, J. Sanchez-Padilla, P.T. Schumacker, The role of calcium and mitochondrial oxidant stress in the loss of substantia nigra pars compacta dopaminergic neurons in Parkinson's disease, Neuroscience 198 (2011) 221–231.
[7] J.S. Park, R.L. Davis, C.M. Sue, Mitochondrial dysfunction in Parkinson's disease: new mechanistic insights and therapeutic perspectives, Curr. Neurol. Neurosci. Rep. 18 (2018) 21.
[8] G. Minakaki, S. Menges, A. Kittel, E. Emmanouilidou, I. Schaeffner, K. Barkovits, A. Bergmann, E. Rockenstein, A. Adame, F. Marxreiter, B. Mollenhauer, D. Galasko, E.I. Buzas, U. Schlotzer-Schrehardt, K. Marcus, W. Xiang, D.C. Lie, K. Vekrellis, E. Masliah, J. Winkler, J. Klucken, Autophagy inhibition promotes SNCA/alpha-synuclein release and transfer via extracellular vesicles with a hybrid autophagosome-exosome-like phenotype, Autophagy 14 (2018) 98–119.
[9] V.D. Sharma, K.E. Lyons, R. Pahwa, Amantadine extended-release capsules for levodopa-induced dyskinesia in patients with Parkinson's disease, Therapeut. Clin. Risk Manag. 14 (2018) 665–673.
[10] A.J. Jagadeesan, R. Murugesan, S. Vimala Devi, M. Meera, G. Madhumala, M. Vishwanathan Padmaja, A. Ramesh, A. Banerjee, S. Sushmitha, A.N. Khokhlov, F. Marotta, S. Pathak, Current trends in etiology, prognosis and therapeutic aspects of Parkinson's disease: a review, Acta Biomed.: Atenei Parmensis 88 (2017) 249–262.
[11] M.U. Rehman, A.F. Wali, A. Ahmad, S. Shakeel, S. Rasool, R. Ali, S.M. Rashid, H. Madkhali, M.A. Ganaie, R. Khan, Neuroprotective strategies for neurological disorders by natural products: an update, Curr. Neuropharmacol. 17 (2019) 247–267.
[12] W. Wang, R. Han, H. He, J. Li, S. Chen, Y. Gu, C. Xie, Administration of quercetin improves mitochondria quality control and protects the neurons in 6-OHDA-lesioned Parkinson's disease models, Aging 13 (2021) 11738–11751.
[13] H. Li, Y. Shen, H. Xiao, W. Sun, Resveratrol attenuates rotenone-induced inflammation and oxidative stress via STAT1 and Nrf2/Keap1/SLC7A11 pathway in a microglia cell line, Pathol. Res. Pract. 225 (2021) 153576.
[14] A. Singh, P. Tripathi, A.K. Yadawa, S. Singh, Promising polyphenols in Parkinson's disease therapeutics, Neurochem. Res. 45 (2020) 1731–1745.
[15] M. Khalifeh, G.E. Barreto, A. Sahebkar, Trehalose as a promising therapeutic candidate for the treatment of Parkinson's disease, Br. J. Pharmacol. 176 (2019) 1173–1189.
[16] S. Huang, H. Yuan, W. Li, X. Liu, X. Zhang, D. Xiang, S. Luo, Polygonatum sibiricum polysaccharides protect against MPP-induced neurotoxicity via the Akt/mTOR and Nrf2 pathways, Oxid. Med. Cell. Longev. 2021 (2021) 8843899.
[17] D. Bi, X. Yang, J. Lu, X. Xu, Preparation and potential applications of alginate oligosaccharides, Crit. Rev. Food Sci. Nutr. 63 (2023) 10130–10147.
[18] T. Senturk Parreidt, K. Müller, M. Schmid, Alginate-based edible films and coatings for food packaging applications, Foods 7 (2018).

[19] A. Balde, S.K. Kim, S. Benjakul, R.A. Nazeer, Pulmonary drug delivery applications of natural polysaccharide polymer derived nano/micro-carrier systems: a review, Int. J. Biol. Macromol. 220 (2022) 1464–1479.
[20] Z. Du, X. Wang, X. Cao, X. Liu, S. Zhou, H. Zhang, R. Yang, K. Wong, Q. Tang, X. Dong, Alginate and its two components acted differently against dopaminergic neuronal loss in Parkinson's disease mice model, Mol. Nutr. Food Res. 66 (2022) e2100739.
[21] D. Bi, L. Yao, Z. Lin, L. Chi, H. Li, H. Xu, X. Du, Q. Liu, Z. Hu, J. Lu, X. Xu, Unsaturated mannuronate oligosaccharide ameliorates β-amyloid pathology through autophagy in Alzheimer's disease cell models, Carbohydr. Polym. 251 (2021) 117124.
[22] D. Bi, S. Xiao, Z. Lin, L. Yao, W. Fang, Y. Wu, H. Xu, J. Lu, X. Xu, Alginate-derived mannuronate oligosaccharide attenuates tauopathy through enhancing autophagy, J. Agric. Food Chem. 69 (2021) 4438–4445.
[23] X. Ji, J. Guo, J. Tian, K. Ma, Y. Liu, Research progress on degradation methods and product properties of plant polysaccharides, Journal of Light Industry 38 (2023) 55–62.
[24] E.J. Calabrese, Hormesis and bone marrow stem cells: enhancing cell proliferation, differentiation and resilience to inflammatory stress, Chem. Biol. Interact. 351 (2022) 109730.
[25] R. Que, J. Zheng, Z. Chang, W. Zhang, H. Li, Z. Xie, Z. Huang, H. Wang, J. Xu, D. Jin, W. Yang, E. Tan, Q. Wang, Dl-3-n-butylphthalide rescues dopaminergic neurons in Parkinson's disease models by inhibiting the NLRP3 inflammasome and ameliorating mitochondrial impairment, Front. Immunol. 12 (2021) 794770.
[26] P. González-Rodríguez, E. Zampese, K.A. Stout, J.N. Guzman, E. Ilijic, B. Yang, T. Tkatch, M.A. Stavarache, D.L. Wokosin, L. Gao, M.G. Kaplitt, J. López-Barneo, P. T. Schumacker, D.J. Surmeier, Disruption of mitochondrial complex I induces progressive parkinsonism, Nature 599 (2021) 650–656.
[27] J. Shao, X. Liu, M. Lian, Y. Mao, Citronellol prevents 6-OHDA-induced oxidative stress, mitochondrial dysfunction, and apoptosis in Parkinson disease model of SH-SY5Y cells via modulating ROS-NO, MAPK/ERK, and PI3K/Akt signaling pathways, Neurotox. Res. 40 (2022) 2221–2237.
[28] L. Zhang, J. Hao, Y. Zheng, R. Su, Y. Liao, X. Gong, L. Liu, X. Wang, Fucoidan protects dopaminergic neurons by enhancing the mitochondrial function in a rotenone-induced rat model of Parkinson's disease, Aging And Disease 9 (2018) 590–604.
[29] Y. Han, J. Lee, S. Lee, Fucoidan suppresses mitochondrial dysfunction and cell death against 1-Methyl-4-Phenylpyridinum-induced neuronal cytotoxicity via regulation of PGC-1α expression, Mar. Drugs 17 (2019) 518.
[30] A.B. Pupyshev, M.A. Tikhonova, A.A. Akopyan, M.V. Tenditnik, N.I. Dubrovina, T. A. Korolenko, Therapeutic activation of autophagy by combined treatment with rapamycin and trehalose in a mouse MPTP-induced model of Parkinson's disease, Pharmacol. Biochem. Behav. 177 (2019) 1–11.
[31] R. Zhou, X. Shi, D. Bi, W. Fang, G. Wei, X. Xu, Alginate-derived oligosaccharide inhibits neuroinflammation and promotes microglial phagocytosis of β-amyloid, Mar. Drugs 13 (2015) 5828–5846.
[32] A.M. Pickrell, R.J. Youle, The roles of PINK1, parkin, and mitochondrial fidelity in Parkinson's disease, Neuron 85 (2015) 257–273.
[33] H. Tang, Z. Zheng, H. Wang, L. Wang, G. Zhao, P. Wang, Vitamin K2 modulates mitochondrial dysfunction induced by 6-hydroxydopamine in SH-SY5Y cells via mitochondrial quality-control loop, Nutrients 14 (2022) 1504.
[34] Y. Jiang, S. Krantz, X. Qin, S. Li, H. Gunasekara, Y.M. Kim, A. Zimnicka, M. Bae, K. Ma, P.T. Toth, Y. Hu, A.N. Shajahan-Haq, H.H. Patel, S. Gentile, M.G. Bonini, J. Rehman, Y. Liu, R.D. Minshall, Caveolin-1 controls mitochondrial damage and ROS production by regulating fission - fusion dynamics and mitophagy, Redox Biol. 52 (2022) 102304.
[35] M. Li, D. Bi, L. Yao, J. Yi, W. Fang, Y. Wu, H. Xu, Z. Hu, X. Xu, Optimization of preparation conditions and in vitro sustained-release evaluation of a novel nanoemulsion encapsulating unsaturated guluronate oligosaccharide, Carbohydr. Polym. 264 (2021) 118047.
[36] M. Falkeborg, L.Z. Cheong, C. Gianfico, K.M. Sztukiel, K. Kristensen, M. Glasius, X. Xu, Z. Guo, Alginate oligosaccharides: enzymatic preparation and antioxidant property evaluation, Food Chem. 164 (2014) 185–194.
[37] R. Qadri, M. Namdeo, M. Behari, V. Goyal, S. Sharma, A.K. Mukhopadhyay, Alterations in mitochondrial membrane potential in peripheral blood mononuclear cells in Parkinson's disease: potential for a novel biomarker, Restor. Neurol. Neurosci. 36 (2018) 719–727.
[38] C. Han, H. Shen, Y. Yang, Y. Sheng, J. Wang, W. Li, X. Zhou, L. Guo, L. Zhai, Q. Guan, Antrodia camphorata polysaccharide resists 6-OHDA-induced dopaminergic neuronal damage by inhibiting ROS-NLRP3 activation, Brain and Behavior 10 (2020) e01824.
[39] X. Hou, J.O. Watzlawik, F.C. Fiesel, W. Springer, Autophagy in Parkinson's disease, J. Mol. Biol. 432 (2020) 2651–2672.
[40] J. Zhu, S. Dou, Y. Jiang, B. Bai, J. Chen, C. Wang, B. Cheng, Apelin-36 exerts the cytoprotective effect against MPP(+)-induced cytotoxicity in SH-SY5Y cells through PI3K/Akt/mTOR autophagy pathway, Life Sci. 224 (2019) 95–108.
[41] Y. Tan, L. Yin, Z. Sun, S. Shao, W. Chen, X. Man, Y. Du, Y. Chen, Astragalus polysaccharide exerts anti-Parkinson via activating the PI3K/AKT/mTOR pathway to increase cellular autophagy level in vitro, Int. J. Biol. Macromol. 153 (2020) 349–356.
[42] H. Qiu, C. Liu, Z. Wang, Levodopa-induced motor complications associated with benserazide and carbidopa in Parkinson's disease: a disproportionality analysis of the FAERS database, Front. Pharmacol. 16 (2025) 1529932.
[43] M. Grabarczyk, W. Justyńska, J. Czpakowska, E. Smolińska, A. Bielenin, A. Glabinski, P. Szpakowski, Role of plant phytochemicals: resveratrol, curcumin, luteolin and quercetin in demyelination, neurodegeneration, and epilepsy, Antioxidants 13 (2024) 1364.
[44] A.M.M. Lucena, E.E. de Souza Lucena, S.P.D. Neto, L.T.D.B. Nobre, H.A.O. Rocha, R.B.G. Câmara, Algal polysaccharides: new perspectives for the treatment of basal ganglia neurodegenerative diseases, Front. Neuroanat. 18 (2024) 1465421.