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Anti-Parkinson's Disease Activity of *Sanghuangprous vaninii* Extracts in the MPTP-Induced Zebrafish Model

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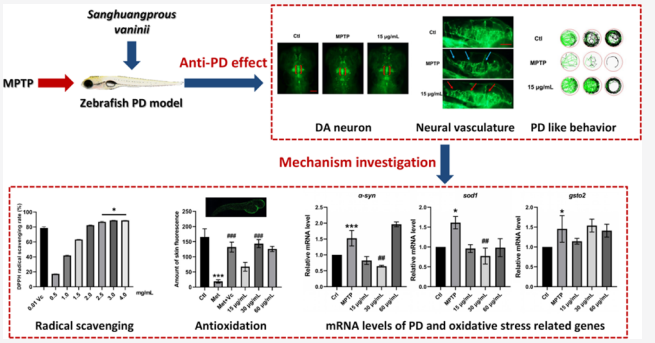
ABSTRACT: Parkinson's disease (PD) is a devastating disease of the central nervous system that occurs mainly in the elderly age group, affecting their quality of life. The PD pathogenesis is not yet fully understood and lacks the disease-modifying treatment strategies. *Sanghuangprous vaninii* (*S. vaninii*) is a perennial fungus with a plethora of pharmacological activities including anti-cancer and antioxidant activity and so on. However, no study till date has reported its neuroprotective effect against symptoms that are similar to PD in pre-clinical investigation. In the current study, we investigated anti-PD-like effects of *S. vaninii* mycelium extracts (SvMEs) on MPTP-induced PD in zebrafish. We observed that the loss of dopaminergic neurons and neurovascular reduction were reversed by using SvMEs in the zebrafish brain in a concentration-independent manner. Moreover, it also relieved locomotor impairments in MPTP-induced PD zebrafish. In addition, SvMEs exerted significant antioxidant activity *in vitro*, which was also demonstrated *in vivo* on *ktr4:NTR-hKikGR* zebrafish. Upon investigating the underlying mechanism, we found that SvMEs may alleviate oxidant stress and accelerate α-synuclein degradation and then alleviate PD-like symptoms. Antioxidant-related genes (*sod1*, *gss*, *gpx4a*, *gclm*, and *cat*) implied that the SvMEs exhibited anti-PD activity due to the antioxidation mechanism. Finally, upon analysis of chemical composition of SvMEs by liquid chromatography−mass spectrometry, we identified 10 compounds that are plausibly responsible for the anti-PD-like effect of SvMEs. On the limiting part, the finding of the study would have been more robust had we investigated the protein expression of genes related to PD and oxidative stress and compared the effects of SvMEs with any standard anti-PD therapy. Despite this, our results indicated that SvMEs possess anti-PD effects, indicating SvMEs as a potential candidate that is worth exploring further in this avenue.



KEYWORDS: *Sanghuangprous vaninii, extract, Parkinson disease, MPTP, antioxidant, α-synuclein*

1. INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disease that can lead to motion abnormality in middle-aged and elderly people.[1] Asymmetric onset of resting tremor, rigidity, postural instability, and bradykinesia are typical symptoms of PD and cognitive dysfunction, such as decreased concentration, visuospatial abilities, and verbal function, also occurs in patients with PD.[2−5] PD is characterized by the progressive loss of dopaminergic (DA) neurons.[6−10] Despite the recent advances in clinical and pre-clinical PD research, the pathogenesis of PD remains largely unknown. However, there is a growing understanding of the role of oxidative stress, neuroinflammation, senility, mitochondria dysfunction, and apoptosis as a major factor in the onset of PD.[11−18]

There is no availability of effective anti-PD therapy that could modify the disease progression. The gold standard therapy for PD is levodopa and DA medications, and they are also criticized for their side effects.[19] This reflects the pressing needs of exploring novel anti-PD therapy that could overcome the limitation of existing anti-PD medications. Several compounds from natural resources have gained increased attention in recent days in the quest of exploring novel anti-PD therapy.[20−23] Medicinal mushrooms (basidiocarps/mycelia extracts or isolated bioactive compounds) have gained attention due to their protective effects against neurodegenerative diseases.[24,25] *Ganoderma lucidum* extract protected the death of DA neurons in a dose-dependent manner in the PD model and downregulated the expression of proinflammatory cytokines like tumor necrosis factor-alpha (TNF-α) and interleckin-1β (IL-1β).[26]

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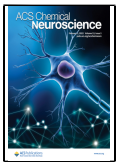

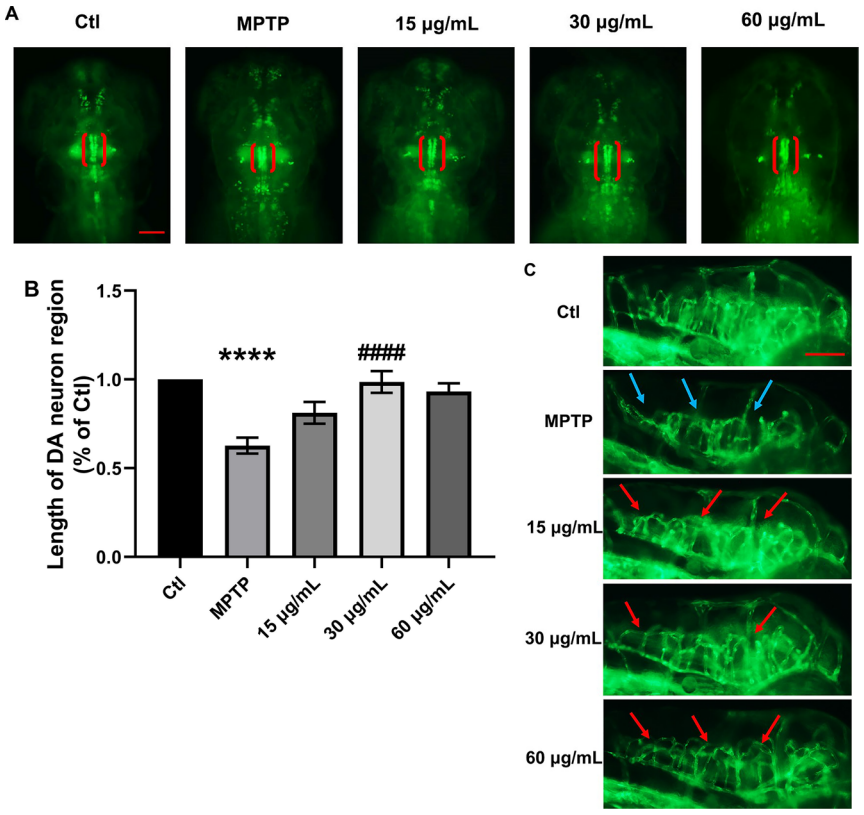

Figure 1. Alleviation of the loss of DA neurons and neural vasculature in zebrafish. (A) Fluorescent microscopy photographs of *vmat: GFP* zebrafish in the control group, model group, and treatment groups, and DA neurons are indicateded by the red brackets. (B) Cartogram length of the DA neuron region in each group, $n = 10$. (C) $^{****}P < 0.0001$ vs control group and $^{\#\#\#\#}P < 0.0001$ vs model group. (C) Fluorescent microscopy photographs of *vegf: GFP* zebrafish in the control group, model group, and treatment groups. Loss and recovery vasculature are indicated by blue arrows and red arrows. All scale bars are 100 μm.

Sanghuangprous vaninii (*S. vaninii*), also called "*sanghuang*" in China, is a well-known mushroom within the Hymenochaetaceae family. *S. vaninii* is widely distributed in several Asian regions including China, Korea, Japan, and Thailand,[27,28] where it is used as a physiologically functional food and an exemplary source of natural medicines. In China, *S. vaninii* is used as a traditional Chinese medicine for treating bloody gonorrhea and spleen-hypofunction diarrhea syndrome, as well as to nurse health, eliminate toxins, and promote blood circulation.[28,29] Previously, polysaccharides from "*sanghuang*" have been extensively demonstrated to have a variety of pharmacological activities.[30,31] However, other extracts of "*sanghuang*" including polyphenols, triterpenoids, and alkaloids, have been the compounds of interest.[32] Liu et al. reported that polyphenolic compositions of *Inonotus sanghuang* had antioxidant, anti-proliferative, and anti-microbial activities.[33] However, the neuroprotective effect of *S. vaninii* mycelium extract (SvMEs) on PD-like symptoms has not been reported yet neither on rodents nor on the zebrafish model. Hence, we have investigated the anti-PD-like effects of SvMEs against methylene, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD in zebrafish. We also identified the chemical compositions of SvMEs by liquid chromatography−mass spectrometry (LC−MS) and unraveled the underlying mechanism behind the neuroprotective effect of SvMEs.

2. RESULTS AND DISCUSSION

2.1. SvMEs Alleviated the Loss of DA Neurons and Neural Vasculature. The DA system in zebrafish is well characterized and is completely developed by 96 h post fertilization (*hpf*).[34] DA neuronal populations in zebrafish larvae that resemble the human substantia nigra have been identified, reflecting zebrafish as a promising model for induction of the PD phenotype, enabling the high-throughput analysis for screening therapeutic drugs.[35] Reduction in the length of DA neurons has been observed in the zebrafish PD model.[36] MPTP is an extensively used neurotoxin to recapitulate parkinsonism in rodents[37] and in zebrafish[36,38] mainly via damaging DA neurons. Motor dysfunction has been acknowledged to be related to the DA nerve injury in PD.[39,40]

To investigate the anti-PD effect of SvMEs, two transgenic lines *vmat: GFP* (green fluorescent protein) and *vegf: GFP* were used to evaluate PD-like symptoms, in which DA neurons and blood vessels were labeled with *GFP*, respectively.[36] Wen et al. described that an enhancer trap transgenic zebrafish, treated with MPTP, exhibited decreased length of the DA neuron region, which has been widely used in subsequent studies.[38,41] We observed significant reduction ($^{****}P < 0.0001$) in the length of DA neuron in the model group compared to the control group. However, treatment with SvMEs increased the MPTP-induced reduction of DA neurons in the midbrain of zebrafish (indicated by the red brackets); however, a significant ($^{\#\#\#\#}P < 0.0001$) elevation was only observed with 30 μg/mL SvMEs (Figure 1A,B). This reflects

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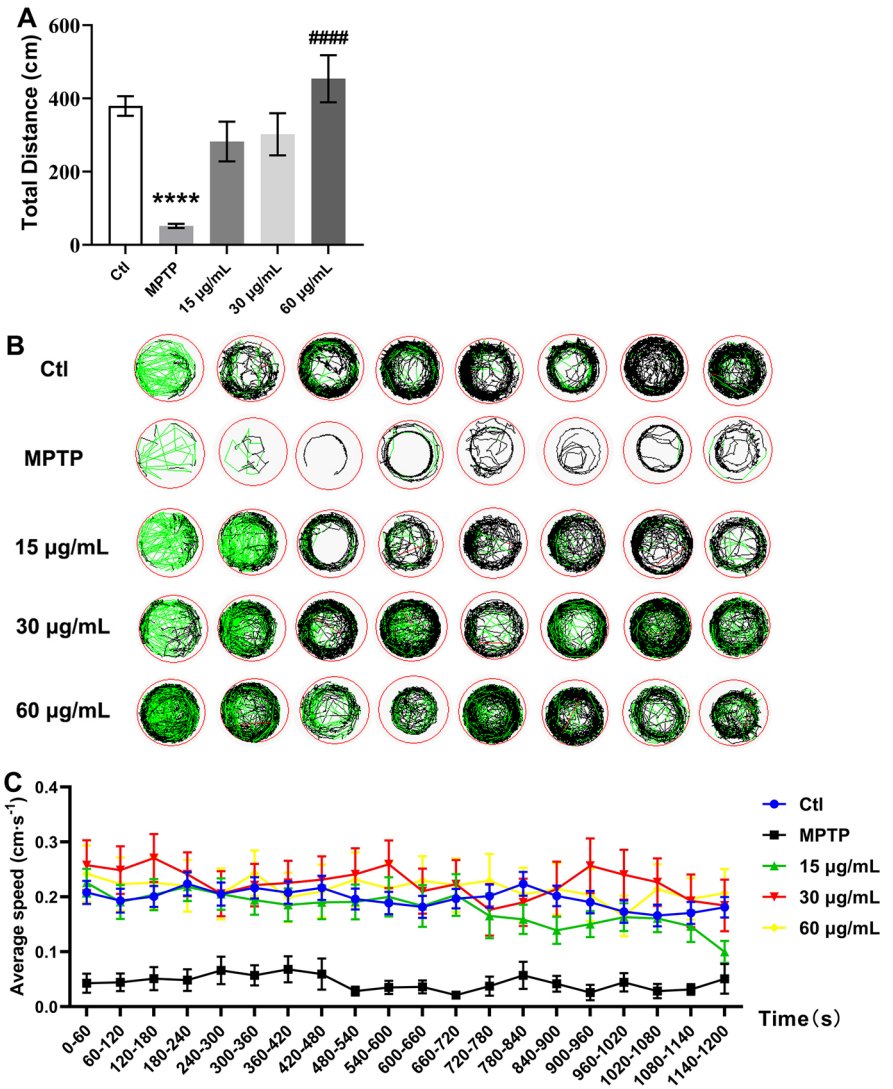

Figure 2. Effects of SvMEs on MPTP-induced locomotor impairments in zebrafish. (A) Total distance moved in the control group, model group, and treatment groups, $n = 8$ per groups. ****$P < 0.0001$ and $^{\#\#\#\#}P < 0.0001$. (B) Digital track map ($n = 8$ group). Medium and slow movement is indicated by green and black lines, respectively. (C) Average speed in the control group, model group, and treatment groups, $n = 8$ per groups.

that SvMEs reversed the MPTP-induced reduction in the length of DA neurons.

In the current study, MPTP administration resulted in the significant loss of vasculature as shown by red arrow in Figure 1C, which corroborates the earlier documented study.[36] Similarly, SvMEs reversed disorganization and loss of vasculature, and the model group had significantly more disorganized vasculature than the control group (Figure 1C). Our finding reflects that SvMEs can inhibit the loss of vasculature in zebrafish induced by MPTP.

2.2. SvMEs Inhibited Locomotor Impairments Induced by MPTP. Dysfluencies are common in a population with PD and similar to the linguistic features associated with developmental stuttering.[42] Learning and memory deficits, including incognitive impacts of PD, are typical non-motor symptoms of PD.[43,44] However, especially in animal models, these symptoms of PD are difficult to be diagnosed clinically. Thus, motor symptoms, including rest tremor, muscle rigidity, and bradykinesia, can be recognized by behavioral analysis.[45,46] The symptoms of PD in zebrafish are decreased locomotion, which has been confirmed in previous experiments.[47] 6 *dpf* is the earliest timepoint at which zebrafish exhibits maximal spontaneous locomotion.[48] Thus, to assess if SvMEs can relieve this symptom, we performed the behavioral assessment on zebrafish larvae at above time.

There was a significant reduction (****$P < 0.0001$) in the total distance travelled in the MPTP-treated group in comparison to the control group. However, treatment with SvMEs increased the MPTP-induced reduction in the total distance travelled; however, a significant ($^{\#\#\#\#}P < 0.0001$) elevation was only observed with 60 μg/mL SvMEs (Figure 2). In addition, the swimming velocity of zebrafish demostrated a similar pattern with the total distance traveled (Figure 2C). Treatment with SvMEs reversed the MPTP-induced decline in swimming speed (Figure 2C), reflecting that SvMEs possesses a protective effect on the locomotor pattern (Figure 2C). Summing up, our finding implies that SvMEs ameliorated MPTP-induced decrease in the locomotor activity in zebrafish.

2.3. Anti-Oxidant Capacity of SvMEs *in Vitro* and *in Vivo*. According to the previous reports, many edible fungal extracts have antioxidant properties.[49,50] Thus, we examined

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two antioxidant activities *in vitro*, and *ktr4:NTR-hKikGR:GFP* transgentic zebrafish was used for antioxidant test *in vivo*.

2.3.1. DPPH Radical Scavenging Activity Assay. DPPH is a typical radical well known in biochemistry, which has been widely used to evaluate the free radical scavenging capacity of natural extracts.[51,52] Nowadays, many natural product extracts have antioxidant activity. According to previous studies, polysaccharides from sanghuang have antioxidant activity in general.[53] However, earlier reports suggested that sanghuang polyphenols also have an antioxidant effect.[54−56] Therefore, we evaluated the DPPH radical scavenging activity of SvMEs. SvMEs was shown to scavenge directly the stable DPPH radical over a concentration range of 0.5 mg/mL (17.31 ± 0.05%) to 4.0 mg/mL (88.86 ± 0.04%) (Figure 3). It

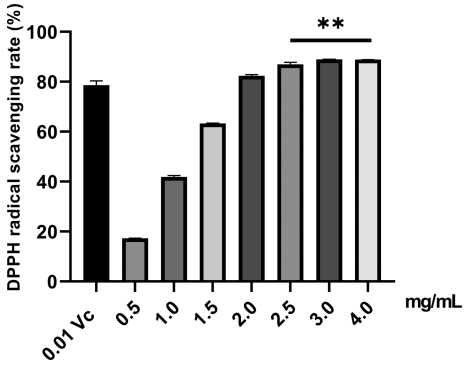

Figure 3. DPPH radical scavenging activity of SvMEs. 0.1 mg/mL Vc was used as a control, and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 4.0 mg/mL represent the concentration of SvMEs; $^{**}P < 0.01$ vs other groups; and free radical clearance is calculated as a percentage.

scavenged the stable radical DPPH in a concentration-dependent manner. The DPPH radical scavenging activity of 2 mg/mL (82.38 ± 0.48%) SvMEs was similar to that of the 0.01 mg/mL (78.63 ± 1.78%) vitamin C positive control group. These values suggest that active component(s) contained in SvMEs should have strong free radical scavenging activity.

2.3.2. Hydroxyl Radical Scavenging Assary. Hydroxyl radicals are the most aggressive free radicals; according to the study, MPTP can induce DA neuronal toxicity and hydroxyl radical production.[57,58] In the above study, SvMEs effectively alleviated MPTP-induced DA toxicity in zebrafish. Thus, we futher studied the hydroxyl radical scavenging ability of SvMEs *in vitro*. Scavenging activity of SvMEs against hydroxyl radicals was significantly less than that of 0.01 mg/mL Vc in the range of 0.5−1.5 mg/mL ($P < 0.05$), while it was more than that of 0.01 mg/mL Vc in the range of 2.0−4.0 mg/mL ($P < 0.05$) (Figure 4). The scavenging rate of SvMEs against hydroxyl radicals at 3.0 mg/mL and 4.0 mg/mL was 87.95 ± 0.56% and 91.92 ± 2.32%, respectively (Figure 4).

2.3.3. In Vivo Antioxidant Capacity. *Ktr4:NTR-hKikGR:GFP* is a zebrafish with fluorescent spots on the skin, and the antioxidant capacity of a substance can be judged from the number of fluorescent spots on the skin surface. In other words, the fluorescence value is positively correlated with the antioxidant capacity. After metronidazole administration to the zebrafish embryos, weak fluorescence signals were observed on the skin surface at 72 h, while intense fluorescence signals were observed for the control group (Figure 5A). The skin fluorescence signal was recovered to various degrees after Vc

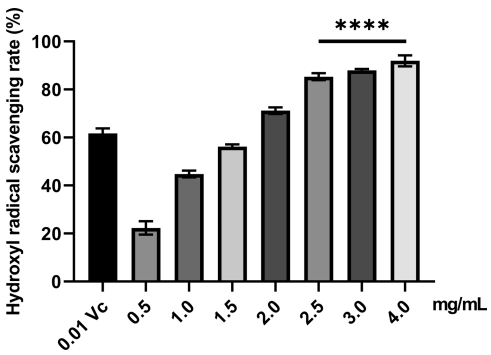

Figure 4. Hydroxyl radical scavenging rate of SvMEs. 0.1 mg/mL Vc was used as a control, and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 4.0 mg/mL represent the concentration of SvMEs; $^{****}P < 0.0001$ vs other groups; and free radical clearance is calculated as a percentage.

(200 μg/mL) and SvMEs (15, 30, 60 μg/mL) administration, and the antioxidant effect of 30 μg/mL SvMEs was equivalent to that of Vc (Figure 5A,B).

2.4. Effects of SvME on the Oxidative Stress and Expression of PD-Related Genes. In the preceding part of the article, we evaluated the antioxidant activity of SvMEs *in vitro* and *in vivo*, and they all showed great antioxidant capacity. Other studies have shown that the development of PD may be strongly related to human aging and oxidative stress.[59] To investigate whether SvMEs are active against PD-like symptoms through oxidative stress, we assayed the expression of oxidation-related genes.

The expression of α-synuclein (*α-Syn*) (Figure 6A) ($^{***}P < 0.001$), copper/zinc superoxide dismutase (*Sod1*) (Figure 6B) ($^{*}P < 0.5$), glutathione s-transferase omega 2 (*Gsto2*) (Figure 6C) ($^{*}P < 0.5$), glutathione synthesis enzymes (*Gss*) (Figure 4d) ($^{***}P < 0.001$), glutathione peroxidase 1a (*Gpx1a*) (Figure 6E) ($^{*}P < 0.5$), glutathione peroxidase 4 a (*Gpx4a*) (Figure 6F) ($^{*}P < 0.5$), glutamate cysteine ligase modifier subunit (*Gclm*) (Figure 6G) ($^{*}P < 0.5$), and catalase (*Cat*) (Figure 6H) ($^{***}P < 0.001$) dramatically increased with MPTP exposure, which indicated that MPTP had an effect on these protein pathways. When treated with several concentrations of SvMEs, the mRNA expression of *α-syn* ($^{\#\#}P < 0.01$), *sod1* ($^{\#\#}P < 0.01$), *gss* ($^{\#\#\#}P < 0.001$), *gpx4a* ($^{\#\#}P < 0.01$), *gclm* ($^{\#\#}P < 0.01$) and *cat* ($^{\#\#\#}P < 0.001$) in the MPTP-induced animal was completely restored to a near-normal level. These results indicate that SvMEs alleviated oxidant stress, contributing to *α-Syn* degradation and therefore relieving PD-like symptoms.

2.5. Compounds of SvME. The edible and medicinal value of *S. vaninii* largely depends on its bioactive compounds, such as polysaccharides, triterpenoids, and steroids. In this study, we have demonstrated that the SvMEs have antioxidant and anti-PD activities. However, it is not clear what compounds in the SvMEs are responsible. Thus, the compounds in SvMEs were detected by LC−MS. Figure 7 shows total ion chromatograms (TICs) of SvMEs, having carboxylic acid derivatives, cinnamic acid and its derivatives, and fatty acyl lipid compounds. SvMEs mainly contain the following compounds, as shown in Table 1.

3. MATERIALS AND METHODS

3.1. Sample. *S. vaninii* mycelium was obtained by an optimized submerged fermentation process by response surface methodology. The optimum fermentation conditions include 24.00 g/L millet flour,

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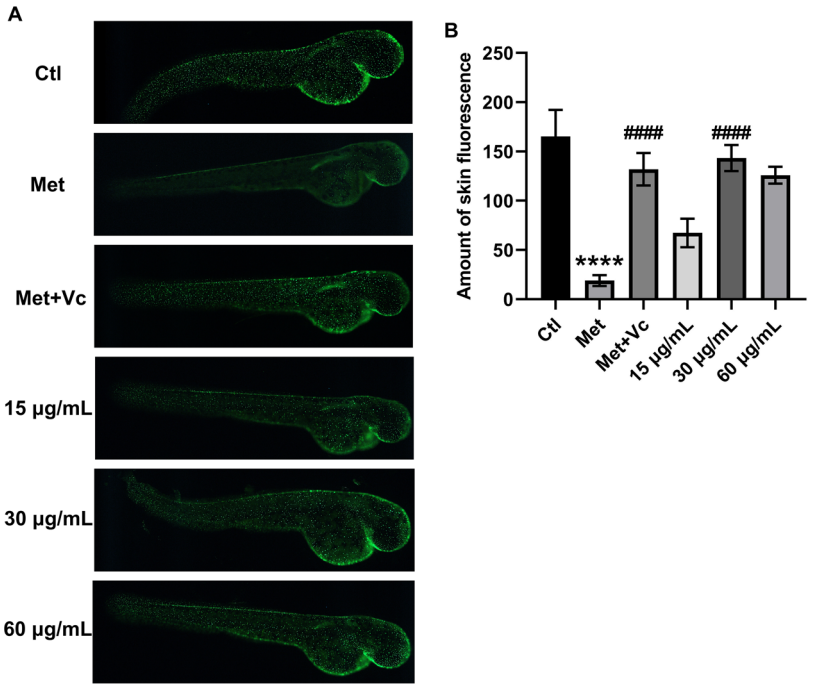

Figure 5. *In vivo* antioxidant diagram. (A) Fluorescent image of zebrafish skin; (B) statistical image of zebrafish skin fluorescence, 15, 30, and 60 μg/mL: Met + 15 μg/mL, Met + 30 μg/mL, and Met + 60 μg/mL, respectively. $^{****}p < 0.0001$ vs Ctl and $^{\#\#\#\#}p < 0.0001$ vs Met.

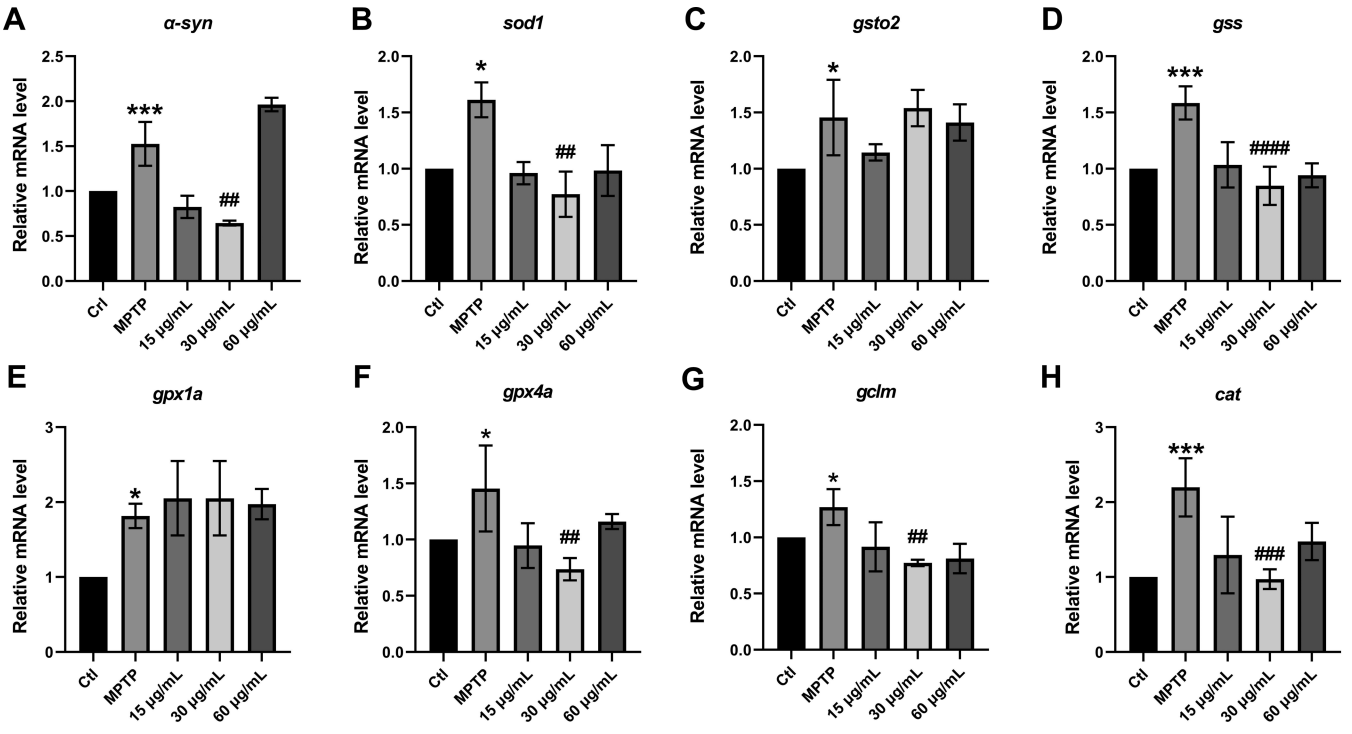

Figure 6. Expression of PD-related genes and oxidative stress. (A) Expression levels of α-syn. (B−H) Expression of genes involved in oxidative stress. The amount of gene expression is exhibited as the relative expression compared with the Ctl. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs Ctl; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, and $^{\#\#\#\#}p < 0.0001$.

10 g/L yeast power, 10 g/L mulberry leaf power, 1.08 g/L KH_2PO_4, 1 g/L $MgSO4{\cdot}7H_2O$, 1 g/L $CaCl_2$, pH 6.5, and an inoculum amount of 11.60%. After the mycelium was obtained, it was pre-kept in a microwave (1000 W) for 3 min, and the mycelium extracts were extracted with 50% ethanol by 35 kHz ultrasonic −assistance; extraction was performed at a temperature of 70 °C and a solid−liquid ratio of 1:55 g/mL for 30 min. The centrifuge separated supernatant from suspension at 5000 rpm for 10 min. The total volume of extracting solution was concentrated by rotary evaporation under negative pressure. After being concentrated to one-third of its original volume, freezing, and drying, we got a powdered sample.

3.2. Reagent and Chemicals. Anhydrous ethanol is an analytical reagent. The basic reagents used for LC−MS were methanol, distilled water, acetonitrile, and formic acid, all of which were HPLC grade. MPTP, 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole, and 1-phenyl-2-thiourea were purchased from Sigma (St. Louis, USA).

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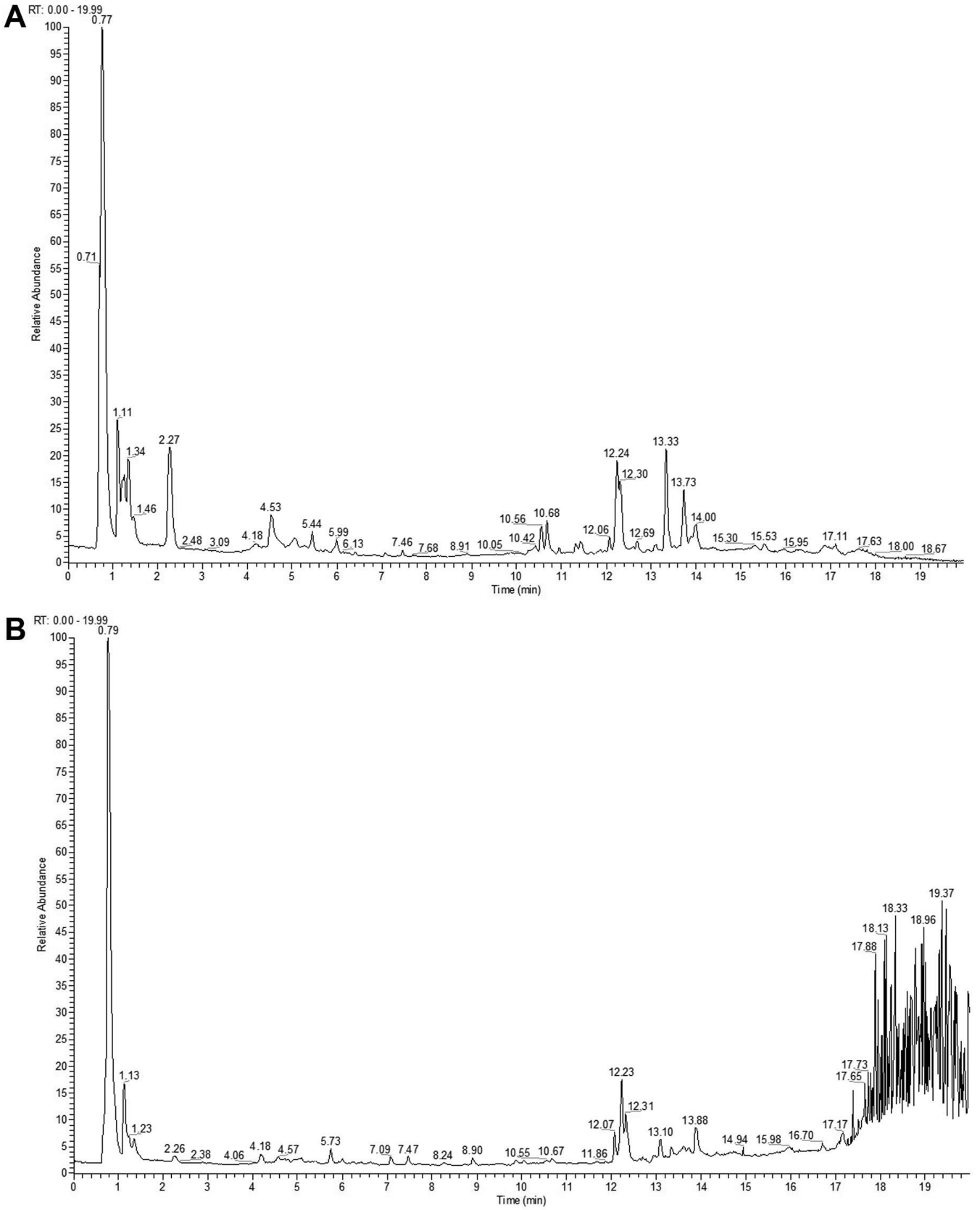

Figure 7. Total ion chromatogram of SvMEs acquired in a positive-ion mode (A) and in a negative-ion mode (B).

3.3. Animals. Three types of zebrafish were maintained according to standard procedures,[60] namely, AB wild-type zebrafish and transgenic *vmat* zebrafish and *vegf* zebrafish and *ktr4:NTR-hKikGR* zebrafish. Briefly, during a 12-h light/12 h dark cycle photoperiod, zebrafish were reared at 28 °C. Zebrafish mated naturally to obtain embryos and then cleaned three times and kept in farming water

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Table 1. Main Compounds and Relative Contents of SvMEs

compounds	relative concentration (μg/mL)	category
bardoxolone methyl	352.088	organic oxide
citric acid	312.978	carbonyl compounds and derivatives
D-(−)-mannitol	304.128	organic oxide
α,α-trehalose	236.723	organic oxide
DL-arginine	277.161	carbonyl compounds and derivatives
isocitric acid	182.522	carbonyl compounds and derivatives
salannin	124.976	isopentenol ester compounds
caffeic acid	93.925	cinnamic acid and its derivatives
p-co umaraldehyde	88.873	cinnamaldehyde
betaine	87.632	carbonyl compounds and derivatives

containing 3 mg/L methylene blue.[61] Embryos were selected under a dissecting microscope at 6 *hpf*, and those developed normally to the blastula stage fish were used for subsequent experiments.

3.4. MPTP and SvMEs Treatment. According to Zhao et al., the zebrafish PD models were generated by embryos being exposed to 50 μM MPTP.[39] First, the safety concentrations of SvMEs were determined at different concentrations (5, 10, 20, 30, 60, 100, 200, 400, 600, 800, and 1000 μg/mL). Then, LC1 of SvMEs were determined at 60 μg/mL. Thus, at the concentrations of 15, 30, and 60 μg/mL, we tested anti-PD activity of SvMEs. After release agent treatment, embryos were randomly transferred to six-well cell culture plates (35−45 embryos per well with 5 mL of bathing medium) at 1day(s) post fertilization (*dpf*). Second, zebrafish were co-treated with MPTP and three different concentrations of SvMEs from 1 *dpf* to 4 *dpf* or 6 *dpf* to investigate anti-PD activity of SvMEs. After treatment, zebrafish at 4 *dpf* were anesthetized, and 15 individuals from each group were randomly selected for visual observation and image acquisition. The transgenic zebrafish *vmat: GFP* and *vegf: GFP* were used to evaluate the development of DA neurons and neural vasculature and *in vivo* antioxidant effects, respectively.[62] Zebrafish without any treatment (control group), MPTP-treated zebrafish (model group), and zebrafish co-treated with MPTP and SvMEs of various concentrations (treatment group).

3.5. Zebrafish Behavioral Test. The zebrafish larvae from each group were collected, cleaned, and placed in 48-well plates (1 per well, with 1 mL of fish framing water). After a 10 min acclimation period, the locomotion of each larva was recorded using Zebrabox Revolution (ViewPoint, Lyon, France). Zeblab software was used to analyze the digital tracks, and the average speed was analyzed every 60 s. A total of eight zebrafish larvae (n = 8) were used for each group.

3.6. Real-Time Quantitative PCR. Total RNA was extracted from 6 *dpf* larvae (n = 30) using an EASY spin Plus RNA mini kit (Rn2802; Aidlab Biotechnologies; Beijing, China) following the manufacturer's protocol. According to the specification's protocols, reverse transcription and real-time quantitative PCR (qPCR) were performed. Briefly, cDNA was generated using PrimeScript RT Master Mix (Takara, Tokyo, Japan). qPCR was performed using SYBR Premix DimerEraser (Takara, Tokyo, Japan) and a Light Cycler 96 system (Light Cycler Instrument; Roche; Switzerland). Run were carried out in triplicate using a housekeeping gene *rpl13a* to normalize the mRNA level of target genes.

3.7. Antioxidant Capacity. *3.7.1. DPPH (1,1-Diphenyl-2-picrylhydrazyl) Assay.* *In vitro* antioxidant activity was evaluated using the DPPH radical scavenging assay according to the earlier reported study with slight modification.[63] Briefly, 1 mL of SvMEs solution were mixed with 3 mL of 0.02 mg/mL DPPH ethanol solution, blended with a scroll tester, and incubated in a dark room at 25°C for 30 min. The absorbance at 517 nm was measured using a UV-1000 UV−visible spectrophotometer (Techcomp, Shanghai, China).

$$\text{Free radical scavenging rate (\%)} = [1 - (A_1 - A_2)/A_0] \times 100\% \quad (1)$$

where A_1 represents the absorption value of the sample reaction system, A_2 represents the absorption value of DPPH solution replaced with an anhydrous ethanol reaction system, and A_0 represents the absorption value of the anhydrous ethanol-substituted sample reaction system.

3.7.2. Hydroxyl Radical Scavenging Activity Assay. *In vitro* hydroxyl radical scavenging ability was evaluated according to the Fenton reaction principle.[64] The reaction mixture containing 8 mmol/L $FeSO_4$, 8 mmol/L salicylic acid, and 3% (v/v) H_2O_2 was added to various concentrations of test extracts. The reaction mixture was kept at 37 °C for 30 min, and the absorption value was measured at 510 nm.

$$\text{Free radical scavenging rate (\%)} = [1 - (A_1 - A_2)/A_0] \times 100\% \quad (2)$$

where A_1 represents the absorption value of the sample reaction system, A_2 represents the absorption value of the H_2O_2 replaced with deionized water, and A_0 represents the absorption value of deionized water-substituted sample reaction system.

3.7.3. Antioxidant Activity in ktr4:NTR-hKikGR: GFP Zebrafish Embryos. Twenty-four hour *ktr4:NTR-hKikGR: GFP* zebrafish embryos were placed in 1 mg/mL streptomycin protease solution for 1 min, and the outer egg membrane of skin fluorescent zebrafish embryos was removed. *ktr4:NTR-hKikGR:GFP* zebrafish embryos with removed egg membranes were randomly divided into the control group, metronidazole group (Met), metronidazole + 200 μg/mL Vc group (Met + Vc), and metronidazole + test group (Met + 5 μg/mL SvMEs, Met + 30 μg/mL SvMEs, and Met + 60 μg/mL SvMEs). There were seven embryos in each group, and three duplicate groups were set at the same time and added into 24-well plates. After being incubated in a constant-temperature incubator at 28 °C for 24 h, the fluorescent zebrafish was anesthetized with methylaminobenzoate mesesulfate (MS222) and photographed. The growth of skin-fluorescent zebrafish and the number of skin keratinocytes were observed under a fluorescence microscope. The number of fluorescent spots on the skin of each fluorescent zebrafish was counted using Image Pro-Plus software.

3.8. Identification of Compounds by HPLC-MS. Identification was made according to the method of Chen et al. and by an improved laboratory method,[65] with the instrument analysis platform: LC−MS (Thermo, Ultimate 3000LC, Q Exactive HF) and chromatographic column: C18 [Zorbax Eclipse C18 (1.8 μm × 2.1 × 100 mm)]. 0.1% formic acid was used as solvent A, and acetonitrile was used as solvent B. The column temperature was set to 30 °C, and the flow rate was 0.3 mL/min. The injection volume of sample was 2 μL. Q Exactive HF mass spectrometry was performed using both the positive- and negative-ion mode. The MS parameters were as follows :a heater temperature of 325 °C, a sheath gas flow rate of 45 arb, an auxiliary gas flow rate of 15 arb, a blow scanning rate of 1 arb, a capillary voltage of 3.5 KV, and the mass range of 100−1500 *m*/*z*. Compound Discoverer 3.1 was used for time correction, peak recognition, and peak value extraction. The Thermo mzCloud online database and Thermo mzValut local database were used for compounds identification.

3.9. Statistical Analysis. Data were analyzed using IBM SPSS Statistics 22 by one way ANOVA followed by uncorrected Fisher's LSD test and were presented as mean ± SEM. $P < 0.05$ was considered as significant. All graphics and bar charts were produced using Adobe Photo CC 2019 and Graph Pad Prism 8.0, respectively.

4. CONCLUSIONS

Concluding up, our investigation provided phytochemical and pharmacological evidence that SvMEs possess anti-PD-like activity. In line with the general goals in the treatment and

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prevention of PD, SvMEs inhibited the loss of DA neurons and improved the PD-like symptoms, indicating SvMEs as a candidate against symptoms similar to PD. Hence, SvMEs might be a promising therapeutic candidate against PD, paving the way forward in developing disease-modifying therapeutic strategies and overcoming the limitation of currently available PD therapy.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acschemneuro.1c00656.

Primers used for the real-time qPCR (PDF)

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Author Contributions

#X.L. and D.G. contributed equally to this work. M.J. conceptualized the idea. M.J. and F.H. supervised the entire study. X.L., D.G., and M.Z. performed the entire study and analyzed the results. M.J., X.L., D.G., Y.N.P., X.L., Y.M., L.C., and G.L. analyzed the results. X.L., D.G., and Y.N.P. wrote the article, and M.J. contributed to the final form. All authors have read and provided their consent to the final version of the article.

Notes

The authors declare no competing financial interest.

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