

Neuroprotective Effects of Sargassum horneri Polyphenols on Rotenone-Induced SH-SY5Y Cell Injury

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Abstract

Parkinson's disease (PD) is closely associated with mitochondrial dysfunction and oxidative stress. *Sargassum horneri* polyphenols (SHPP), as a marine-derived natural active substance, have potential neuroprotective effects, but their efficacy and mechanisms in PD models remain unclear. In this study, SH-SY5Y cells were first differentiated with all-trans-retinoic acid (ATRA) and then treated with rotenone to establish an *in vitro* Parkinson's disease injury model. The protective effects of SHPP were systematically evaluated, and its mechanism of action was preliminarily explored using transcriptomics. Results showed that SHPP intervention dose-dependently ameliorated rotenone-induced cell injury: cell viability in the medium- and high-dose groups was restored to 71.6% and 73.3% of the control level, ATP content to 71.2% and 74.6%, and mitochondrial complex I activity to 67.5% and 71.3%, respectively. In the high-dose group, reactive oxygen species (ROS) level decreased to 81.2% of the model group level, malondialdehyde (MDA) decreased to 68.7% of the model group level, and superoxide dismutase (SOD) activity and reduced glutathione (GSH) content was restored to 83.7% and 82.8% of the control level, respectively. In terms of apoptosis regulation, SHPP down-regulated Bax, up-regulated Bcl-2, decreased the Bax/Bcl-2 ratio, and reduced cytoplasmic cytochrome c (by 14.3%) and Cleaved Caspase-3 (by 13.6%) levels. Meanwhile, SHPP reduced the secretion of pro-inflammatory factors TNF- α , IL-6, and IL-1 β , and down-regulated *BAX* mRNA expression while up-regulating *BCL2*, *NFE2L2*, and *HMOX1* mRNA expression. Transcriptomic analysis revealed that only 28 differentially expressed genes were identified after SHPP intervention, of which 8 were common differentially expressed genes between the model and treatment groups (including *TMEM102*, *MIR34AHG*, etc.), indicating that SHPP does not function by broadly altering gene expression but rather acts precisely on core genes in the disease model. In conclusion, SHPP exerts neuroprotective effects against rotenone-induced SH-SY5Y cell injury by restoring mitochondrial function, alleviating oxidative stress, inhibiting mitochondria-mediated apoptosis, and reducing neuroinflammation, providing experimental evidence for the potential application of *S. horneri* polyphenols in the prevention and treatment of Parkinson's disease.

1. Introduction

Neurodegenerative diseases pose a growing global health challenge, characterized by neuroinflammation and oxidative stress, with Alzheimer's disease and Parkinson's disease being the two most representative types (Gonçalves et al. 2023; Carrell and Lomas 1997; Cornejo and Hetz 2013). In the pathological process, oxidative stress arises from the abnormal accumulation of reactive oxygen species (ROS) or an imbalance in the antioxidant system. Brain tissue, which has high oxygen consumption and is rich in lipids susceptible to oxidative damage, is highly sensitive to oxidative stress, leading to DNA damage, protein carbonylation, and lipid peroxidation, which in severe cases can result in neuronal death (Gonçalves et al. 2023; Aslam et al. 2021; Guo and Lee 2014; Alghazwi et al. 2016). Parkinson's disease is the second most common sporadic neurodegenerative disease after Alzheimer's disease (Su et al. 2025), primarily characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta, with an increasing incidence with age (Alghazwi et al. 2016; Silva et al. 2023; Shanks et al. 2008). In 2021, there were 13.35 million new cases of Parkinson's disease globally

and 388,200 deaths (Li et al. 2025), and the global incidence and prevalence of Parkinson's disease are projected to continue rising from 2022 to 2026 (Luo et al. 2024). The pathological features of Parkinson's disease include elevated iron levels, decreased glutathione levels, and oxidative damage to lipids, proteins, and DNA (Dexter and Jenner 2013; Ramanan and Saykin 2013; Olanow and Tatton 1999; Poewe et al. 2017), mainly caused by excessive ROS accumulation, imbalance in antioxidant function, and abnormal upregulation of metal ions or monoamine oxidase B (MAOB) (Lin and Beal 2006; Zhang et al. 2021; Wang et al. 2024). Therefore, regulating oxidative stress is a potential therapeutic strategy, but effective interventions are currently lacking (Wang et al. 2024; Silva et al. 2020).

S. horneri is a warm-temperate large brown alga widely distributed along the coast of China, with vigorous reproduction. If improperly disposed, it can easily cause marine ecological disasters, so its resource utilization has both ecological and economic value (Tian et al. 2025; Cai et al. 2019). *S. horneri* is rich in minerals, essential amino acids, and active ingredients such as polyphenols, alginate, and fucoidan, among which polyphenols are key functional factors with antioxidant, metal ion chelating, anti-tumor, and blood glucose and lipid regulating effects (Cai et al. 2019; Guo and Lee 2014; Weihao et al. 2021; Lee et al. 2020; Lee et al. 2019). Ultrasound-assisted extraction can efficiently disrupt cell structures, increase polyphenol yield, and shorten the extraction cycle, providing a superior route for the preparation of *S. horneri* polyphenols (Essa et al. 2018; Yilmaz et al. 2019).

Brown algal polyphenols are polymerized from phloroglucinol units via the acetate-malonate pathway (Fernando et al. 2016; Weihao et al. 2021; Shrestha et al. 2021). Studies have shown that they can reduce neuronal oxidative damage and inflammation by scavenging ROS, inhibiting microglial activation, and blocking inflammatory pathways such as nuclear factor- κ B (NF- κ B) (Silva et al. 2020; Barbosa et al. 2020; Kuwar and Kalia 2025; Jalouli et al. 2025; Amritpal et al. 2020). In Parkinson's disease models, polyphenols can also improve mitochondrial function, inhibit cytochrome c release, and modulate the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling pathway, thereby delaying neuronal apoptosis (Silva et al. 2023; Liu et al. 2022). Although in vitro and in vivo studies are limited by blood-brain barrier penetration and bioavailability issues (Silva et al. 2023; Liu et al. 2022), brown algal polyphenols, as natural, low-toxicity active substances, still have great potential for development in the prevention and treatment of neurodegenerative diseases.

The SH-SY5Y cell line is a widely used in vitro neuronal model, which can acquire a neuron-like phenotype after differentiation induced by all-trans-retinoic acid (ATRA), overcoming the limitations of primary neurons in terms of availability and proliferative capacity (Stepchenko et al. 2021; Sallmon et al. 2010; Neill et al. 1994). Rotenone, a specific inhibitor of mitochondrial complex I, can induce SH-SY5Y cells to produce pathological features highly similar to sporadic PD: decreased ATP levels, mitochondrial membrane potential collapse, excessive ROS production, exacerbated oxidative stress, and ultimately trigger apoptosis through increased Bax/Bcl-2 ratio, cytochrome c release, and caspase activation (Luo et al. 2019; Zhang et al. 2019; Dieter et al. 2022; Yarmohammadi et al. 2020; Sherer et al. 2001; Nakamura et al. 2004; Cheng et al. 2021; Zhou et al. 2018). This model has become an ideal platform for screening potential neuroprotective agents.

In recent years, the mechanisms of rotenone-induced SH-SY5Y cell injury have been continuously revealed, involving the Ether-à-go-go 1 (Eag1) potassium channel and microRNA-34a (*miR-34a*) (Horst et al. 2017), the mitochondrial inner membrane protein OMA1 (a metalloprotease) regulating mitochondrial dynamics (Zhou et al. 2018), changes in autophagic flux and the forkhead box protein O3a (FOXO3a) transcription factor (Yarmohammadi et al. 2020), and inactivation of the protein kinase B (Akt)/mammalian target of rapamycin (mTOR) signaling pathway (Zhang et al. 2019). This model can comprehensively simulate the pathological features of Parkinson's disease, from complex I inhibition to functional impairment, apoptosis, and autophagic dysregulation (Sallmon et al. 2010; Sherer et al. 2001), and has become an ideal model for screening neuroprotective agents. On this model, compounds such as rosmarinic acid (Han et al. 2022), ginsenoside Re (González-Burgos et al. 2017), epigallocatechin gallate (EGCG) (Chung et al. 2007), rifampicin (Deng et al. 2020), baicalein (Deng et al. 2020; Zhang et al. 2021), berberine (Deng et al. 2020), pyrroloquinoline quinone (PQQ) (Cheng et al. 2021), and melatonin (Zhou et al. 2018) have all been shown to be effective. However, the above studies mostly focus on single or a few mechanistic pathways such as antioxidant, anti-apoptotic, or anti-inflammatory effects, lacking a systematic investigation of multi-target synergistic effects. Moreover, systematic research on marine-derived *S. horneri* polyphenols in this model is still lacking.

Based on this, this study used *S. horneri* as the research object to obtain *S. horneri* polyphenols (SHPP) through the establishment of an efficient extraction and purification process, and constructed an in vitro Parkinson's disease model of rotenone-induced injury using ATRA-differentiated SH-SY5Y cells. The protective effects of SHPP against rotenone-induced SH-SY5Y cell injury were systematically verified by evaluating cell viability, mitochondrial function, oxidative stress, apoptosis, and neuroinflammation. Combined with transcriptomics, the molecular mechanism of SHPP's neuroprotective effects was preliminarily explored, and its potential key regulatory targets were identified, aiming to provide a theoretical basis for the high-value utilization of *S. horneri* resources and the development of novel marine-derived neuroprotective agents.

2. Materials and Methods

2.1 Extraction and Purification of *S. horneri* Polyphenols

Fresh *S. horneri* was collected from Shengshan Island, Zhoushan City, Zhejiang Province, China. The algae were air-dried, ground, and sieved to obtain algal powder. In this study, a method combining cellulase pretreatment with ultrasound-assisted extraction was used to extract *S. horneri* polyphenols (SHPP). Briefly, the algal powder was enzymatically hydrolyzed with 1.5% (w/w) cellulase in acetate-sodium acetate buffer (pH 4.8) at 50°C for 2 h. After enzyme inactivation, ethanol was added and the mixture was subjected to ultrasonic treatment (500 W, ≤ 40°C). To clarify the effects of various factors on polyphenol yield and optimize the extraction process, single-factor experiments were first conducted to examine the effects of cellulase concentration, ultrasonic time, ultrasonic power, ethanol concentration, and liquid-to-solid ratio. On this basis, a Plackett-Burman (PB) design was used to screen key influencing factors from the above five variables to simplify subsequent optimization. The PB results

showed that liquid-to-solid ratio, ultrasonic time, and ethanol concentration had significant effects on polyphenol yield ($P < 0.05$), while cellulase concentration and ultrasonic power had no significant effects ($P > 0.05$). Subsequently, using these three significant factors as independent variables, the extraction conditions were further optimized by Box-Behnken response surface methodology (RSM). The final optimal extraction conditions were determined as: liquid-to-solid ratio 26:1, ultrasonic time 53 min, and ethanol concentration 41%. Under these conditions, to further improve purity, the crude extract was dynamically adsorbed and purified using ADS-21 macroporous adsorption resin to obtain SHPP with higher purity for subsequent experiments.

2.2 Chemicals and Reagents

Fetal bovine serum (FBS) was purchased from Gibco (USA). DMEM (high glucose)/F12 (1:1) medium, phosphate-buffered saline (PBS), and penicillin-streptomycin were purchased from Bio-Channel (Nanjing, China). Dimethyl sulfoxide (DMSO) was purchased from Solarbio (Beijing, China). Rotenone was purchased from Aladdin (Shanghai, China). All-trans-retinoic acid (ATRA) was purchased from BIOBYING (Beijing, China).

Human Cleaved Caspase-3 rapid detection kit (ELISA), ATP content chemiluminescence assay kit, and mitochondrial respiratory chain complex I colorimetric assay kit were purchased from Elabscience (Wuhan, China). ELISA kits for IL-6, Bcl-2, IL-1 β , TNF- α , and cytochrome c (Cyt-C) were purchased from Yibaishun Biotechnology Co., Ltd. (Guangzhou, China). Reactive oxygen species (ROS) assay kit, superoxide dismutase (SOD) activity assay kit, and malondialdehyde (MDA) assay kit were purchased from Yuanye (Shanghai, China). Human BAX ELISA kit was purchased from Dogesce (Beijing, China).

Total RNA extraction kit was purchased from TIANGEN Biotech (Beijing) Co., Ltd. (TIANGEN). Reverse transcription cDNA synthesis kit and real-time quantitative PCR kit (SYBR® Premix Ex Taq™ II) were purchased from TaKaRa (Japan). Primers were synthesized by Genewiz (Suzhou, China).

2.3 Cell Culture and Differentiation

The human neuroblastoma SH-SY5Y cell line was purchased from Zhejiang Meisen Cell Technology Co., Ltd. (Zhejiang, China). Cells were cultured in DMEM/F12 (1:1) medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μ g/mL streptomycin, at 37°C in a humidified atmosphere containing 5% CO₂.

Differentiation was performed following established methods. When cell confluence reached 60%-70%, the original medium was discarded and replaced with differentiation medium containing 8 μ M ATRA. The differentiation medium was refreshed every 2 days, and cells were continuously induced for 5 days. Cell morphology was observed under an inverted microscope. Cells exhibiting dense neurite outgrowth and a typical neuron-like phenotype were considered successfully differentiated. The differentiation procedure is illustrated in Fig. 1.

2.4 Establishment of Rotenone-Induced Cell Injury Model and Drug Intervention

Differentiated SH-SY5Y cells were seeded in 96-well plates at a density of 1×10^4 cells/well or in 6-well plates at a density of 1.5×10^6 cells/well and cultured for 24 h to allow cell attachment. Cells were divided into five groups: (1) Control group: complete medium containing 0.5% DMSO; (2) Model group: complete medium containing 0.3 μ M rotenone; (3) SHPP low-dose group (SHPP-L): Model group supplemented with 25 μ g/mL SHPP; (4) SHPP medium-dose group (SHPP-M): Model group supplemented with 50 μ g/mL SHPP; (5) SHPP high-dose group (SHPP-H): Model group supplemented with 75 μ g/mL SHPP. After treatment for 24 h, cells or culture supernatants were collected for subsequent assays.

2.5 Cell Viability Assay (CCK-8 Method)

Differentiated SH-SY5Y cells were seeded in 96-well plates at a density of 1×10^4 cells/well. After treatment according to the above groups for 24 h, 10 μ L of CCK-8 reagent was added to each well and incubated at 37°C in the dark for 2 h. The absorbance (OD value) was measured at 450 nm using a microplate reader. Cell viability was expressed as a percentage relative to the control group (set as 100%).

2.6 Detection of Mitochondrial Function-Related Indicators

2.6.1 ATP Content Assay

Cells from each group were collected, lysed, and then heated in boiling water for 5 min. After cooling on ice, the lysates were centrifuged (4°C, 12,000 rpm, 5 min), and the supernatants were collected. ATP content was measured using a chemiluminescence method according to the manufacturer's instructions of the ATP assay kit. Results were expressed as relative values (control group = 100%).

2.6.2 Mitochondrial Respiratory Chain Complex I Activity Assay

Cells from each group were collected and lysed with lysis buffer from a mitochondrial extraction kit on ice for 30 min, followed by centrifugation (4°C, 12,000 rpm, 10 min). The supernatant was collected as the mitochondrial protein extract. Protein concentration was determined using the BCA method. According to the instructions of the complex I activity assay kit, using NADH as a substrate, absorbance changes were continuously measured at 340 nm to calculate enzyme activity. Results were expressed as relative values (control group = 100%).

2.7 Detection of Oxidative Stress-Related Indicators

2.7.1 Intracellular Reactive Oxygen Species (ROS) Level Detection

The DCFH-DA fluorescent probe method was used. After treatment, the culture medium was discarded from each group, and 10 μ M DCFH-DA working solution was added. The cells were incubated at 37°C in the dark for 30 min. After washing twice with PBS, fluorescence intensity was measured using a fluorescence microplate reader at an excitation wavelength of 488 nm and an emission wavelength of 525 nm. Results were expressed as relative values (control group = 100%).

2.7.2 Determination of Malondialdehyde (MDA), Superoxide Dismutase (SOD), and Reduced Glutathione (GSH)

Cells from each group were collected, fully lysed with lysis buffer, and centrifuged (4°C, 12,000 rpm, 10 min). The supernatants were collected. MDA content, SOD activity, and GSH content were measured strictly according to the instructions of the respective kits. MDA content was expressed as nmol/mg protein, SOD activity as U/mg protein, and GSH content as μ g/mg protein. Results were expressed as relative values (control group = 100%).

2.8 Detection of Apoptosis- and Inflammation-Related Proteins (ELISA Method)

Culture supernatants were collected from each group for the detection of inflammatory factors (TNF- α , IL-6, IL-1 β). Meanwhile, cells from each group were collected to extract total protein and cytoplasmic protein, respectively. Protein concentrations were determined using the BCA method. The levels of Bax, Bcl-2, and Cleaved Caspase-3 in total protein, as well as the level of cytochrome c in cytoplasmic protein, were measured according to the instructions of the respective ELISA kits. Results were expressed as ng/mg protein and also presented as relative values (control group = 100%). The Bax/Bcl-2 ratio was also calculated.

2.9 Real-Time Quantitative PCR (qPCR)

2.9.1 Total RNA Extraction and Reverse Transcription

Total RNA was extracted from cells of each group using RNAiso Plus reagent. RNA integrity was assessed by agarose gel electrophoresis, and RNA concentration and purity were measured using a microspectrophotometer (A_{260}/A_{280} ratio between 1.8 and 2.0). One microgram of total RNA was used to synthesize cDNA according to the manufacturer's instructions of the reverse transcription kit.

2.9.2 qPCR Reaction

PCR amplification was performed using the SYBR® Premix Ex Taq™ II kit on a real-time PCR system. The reaction system (20 μ L) consisted of 10 μ L SYBR Premix Ex Taq II, 0.8 μ L each of forward and reverse primers, 2 μ L cDNA template, and RNase-free water to make up to 20 μ L. The reaction program was: 95°C for 30 s (pre-denaturation); 95°C for 5 s and 60°C for 30 s for 40 cycles. *ACTB* (β -actin) was used as

the internal reference gene. Relative expression levels were calculated using the 2^{-ΔΔCt} method. The primer sequences used are shown in Table 1.

Table 1
Primer sequences

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>ACTB</i>	CCTTCCTTCCTGGGCATGG	GATCTTCATTGTGCTGGGTGC
<i>BAX</i>	GAGCAGCCCAGAGGCG	CGGCGGCAATCATCCTCT
<i>BCL2</i>	GGATAACGGAGGCTGGGATG	GGGCCAAACTGAGCAGAGTC
<i>NFE2L2</i>	CCAACTACTCCCAGGTTGCC	GACTGGGCTCTCGATGTGAC
<i>HMOX1</i>	AAGACTGCGTTCCTGCTCAA	GGGGGCAGAATCTTGCACT
<i>BDNF</i>	AGCCCTGGTCACAGTCTTGC	GGTCTCGCAAATGCTCTTCA

2.10 Transcriptome Sequencing Analysis

2.10.1 Sample Preparation and RNA Extraction

Four experimental groups were set up: Control group, Model group, Treatment group (75 µg/mL SHPP + 0.3 µM rotenone), and SHPP-only group (75 µg/mL SHPP). Each group had three biological replicates. After 24 h of treatment, total RNA was extracted using the Trizol method. RNA quality was assessed by agarose gel electrophoresis. Qualified samples were used for subsequent library construction.

2.10.2 Library Construction and Sequencing

Library construction and sequencing were entrusted to Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Majorbio, Shanghai, China). The raw image data generated by sequencing were converted into raw sequencing reads (Raw Reads) through base calling analysis and stored in FASTQ file format for subsequent bioinformatics analysis. A total of 12 samples were sequenced, yielding 76.33 Gb of clean data. Each sample had at least 5.87 Gb of clean data, with a Q30 base percentage above 96.36%.

Reference genome source: *Homo sapiens*; reference genome version: GRCh38.

Clean Reads from each sample were aligned to the designated reference genome, with alignment rates ranging from 97.85% to 98.13%.

2.10.3 Bioinformatics Analysis

After filtering and quality control, high-quality clean reads were obtained. Clean reads were aligned to the human reference genome (GRCh38). Differentially expressed genes (DEGs) were screened using the criteria $|\log_2FC| \geq 2$ and adjusted P value (padj) < 0.05. Gene Ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed on the identified DEGs.

2.11 Statistical Analysis

All experiments were independently repeated at least three times. Data are presented as mean $\pm$ standard error of the mean (mean $\pm$ SEM). Statistical analyses were performed using GraphPad Prism 9.0 software. Comparisons between two groups were conducted using two-tailed Student's t-test. Comparisons among multiple groups were performed using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test (with reference to the control or model group). A P value < 0.05 was considered statistically significant. Significance levels are indicated as $* P < 0.05$, $** P < 0.01$.

3. Results

In this section, all data were normalized to the control group (set as 100%) unless otherwise stated, and are presented as relative percentages to show overall trends.

3.1 Establishment of a Rotenone-Induced SH-SY5Y Cell Injury Model

To determine the optimal modeling concentration, differentiated SH-SY5Y cells were treated with various concentrations of rotenone for 24 h. CCK-8 results showed that rotenone decreased cell viability in a concentration-dependent manner (Figure 2A). At a rotenone concentration of 0.3 μ M, cell viability decreased to $62.3\% \pm 3.5\%$ of the control group, which fell within the effective modeling window of 50%-70%. Further analysis showed that, compared with the control group, intracellular ATP content in the model group was significantly reduced to $57.8\% \pm 5.1\%$ ($** P < 0.01$), mitochondrial respiratory chain complex I activity decreased to $57.4\% \pm 4.2\%$ ($** P < 0.01$), ROS levels increased by 1.8-fold ($** P < 0.01$), and MDA content increased to $167.2\% \pm 6.3\%$ ($** P < 0.01$) after 24 h of treatment (Figures 2B–2E). These results indicate that treatment with 0.3 μ M rotenone for 24 h successfully established a mitochondrial injury model in SH-SY5Y cells.

3.2 Neuroprotective Effects of SHPP on Rotenone-Induced Cell Injury

3.2.1 Effect of SHPP on Cell Viability

CCK-8 results showed that cell viability in the model group was significantly decreased compared with the control group ($** P < 0.01$). After intervention with 25, 50, and 75 μ g/mL SHPP, cell viability recovered in a dose-dependent manner. Cell viability in the medium- and high-dose groups was restored to $71.6\% \pm 4.1\%$ and $73.3\% \pm 3.5\%$ of the control value, respectively, showing statistically significant differences compared with the model group ($* P < 0.05$ or $** P < 0.01$) (Figure 3A).

3.2.2 Effect of SHPP on Mitochondrial Function

Compared with the control group, intracellular ATP content and complex I activity in the model group were significantly decreased ($** P < 0.01$). SHPP intervention dose-dependently reversed these changes: ATP content in the medium- and high-dose groups was restored to $71.2\% \pm 4.8\%$ and $74.6\% \pm 5.2\%$ of the control group, respectively, and complex I activity was restored to $67.5\% \pm 5.1\%$ and $71.3\% \pm 4.9\%$ of the

control value, respectively (Figures 3B, 3C). The differences between the high-dose group and the model group were significant (* $P < 0.05$).

3.2.3 Effect of SHPP on Oxidative Stress

Intracellular ROS levels and MDA content were significantly increased, while SOD activity and GSH content were significantly decreased in the model group (all ** $P < 0.01$). After SHPP intervention, ROS and MDA levels decreased, whereas SOD and GSH levels recovered. In the high-dose group, ROS level decreased to $81.2\% \pm 4.3\%$ of the model group (* $P < 0.05$), MDA content decreased to $68.7\% \pm 4.5\%$ of the model group level (** $P < 0.01$), and SOD activity and GSH content were restored to $83.7\% \pm 4.8\%$ and $82.8\% \pm 4.7\%$ of the control group, respectively (* $P < 0.05$) (Figures 4A–4D).

3.2.4 Effect of SHPP on Apoptosis

ELISA results showed that in the model group, the pro-apoptotic protein Bax was up-regulated, the anti-apoptotic protein Bcl-2 was down-regulated, and the Bax/Bcl-2 ratio was significantly increased. Meanwhile, the levels of cytoplasmic cytochrome c and Cleaved Caspase-3 were also significantly increased (** $P < 0.01$). After SHPP intervention, the above indicators were dose-dependently improved. In the high-dose group, the Bax/Bcl-2 ratio was decreased, and the levels of cytoplasmic cytochrome c and Cleaved Caspase-3 were reduced by $14.3\% \pm 5.1\%$ and $13.6\% \pm 5.4\%$, respectively, compared with the model group (* $P < 0.05$) (Figures 5A–5C).

3.2.5 Effect of SHPP on Neuroinflammation

The levels of pro-inflammatory factors TNF- α , IL-6, and IL-1 β in the culture supernatant of the model group were significantly higher than those in the control group (** $P < 0.01$). SHPP intervention dose-dependently inhibited the secretion of these factors. In the high-dose group, TNF- α , IL-6, and IL-1 β levels were reduced by $14.7\% \pm 1.6\%$, $18.3\% \pm 0.8\%$, and $15.4\% \pm 2.3\%$, respectively, compared with the model group (** $P < 0.01$), with the most pronounced inhibitory effect observed on IL-6 (Figures 6A–6C).

3.2.6 Effect of SHPP on mRNA Expression of Key Genes

qPCR results showed that, compared with the control group, the mRNA expression of the pro-apoptotic gene *BAX* was significantly up-regulated, while the anti-apoptotic gene *BCL2* was significantly down-regulated in the model group (** $P < 0.01$). The expression of antioxidant pathway-related genes *NFE2L2* and *HMOX1* showed a compensatory slight up-regulation, while the expression of the neurotrophic factor *BDNF* showed no significant change. After SHPP intervention, *BAX* expression was down-regulated in a dose-dependent manner, while *BCL2*, *NFE2L2*, and *HMOX1* expression showed up-regulatory trends. *BDNF* expression showed no significant change. The expression levels of *BAX*, *BCL2*, *NFE2L2*, and *HMOX1* in the high-dose group were statistically different from those in the model group (* $P < 0.05$) (Figures 7A–7E).

3.3 Transcriptomic Analysis

3.3.1 Differentially Expressed Gene Analysis

A total of 33,924 expressed genes and 190,488 expressed transcripts were detected in this analysis.

Transcriptome sequencing was performed on the control, model, treatment (SHPP 75 µg/mL + rotenone), and SHPP-only groups. Compared with the control group, 1,083 differentially expressed genes (DEGs) were identified in the model group (Model_vs_Control), including 366 up-regulated and 717 down-regulated genes. Compared with the model group, only 28 DEGs were identified in the treatment group (Treatment_vs_Model), including 16 up-regulated and 12 down-regulated genes. Venn diagram analysis revealed 8 common DEGs between the model and treatment groups (Figures 8A, 8B), including *TMEM102* involved in mitochondrial apoptosis regulation, non-coding RNA-related genes *MIR34AHG* and *MIR22HG*, and histone H2B family members *H2BC11*, *H2BC21*, etc.

3.3.2 GO Functional and KEGG Pathway Enrichment Analysis

GO functional classification analysis showed that DEGs in the Treatment_vs_Model group were mainly enriched in terms such as "biological regulation", "cellular process", "protein-containing complex", and "binding" (Figure 8C). KEGG enrichment analysis revealed that the DEGs were significantly enriched in pathways such as Systemic lupus erythematosus and the Fc epsilon RI signaling pathway (Figure 8D).

The core of the SLE pathway is immune regulation and inflammatory responses (Singer et al. 2022; Mamais et al. 2022). The pathogenesis of central nervous system involvement in SLE (NP-SLE) is closely related to the abnormal activation of microglia (Lin and Carroll 2025), and NP-SLE microglia express gene signatures highly similar to disease-associated microglia (DAM) in PD, which also play a core role in neurodegenerative diseases such as Parkinson's disease (Makinde et al. 2020). This finding suggests that although SLE and PD are clinically different, they may share molecular intersections in microglia-mediated neuroinflammatory mechanisms, and SHPP may play a role in regulating related pathways, thereby affecting the expression of differential genes.

FcεRI is a high-affinity IgE receptor. Studies have shown that Fc receptors (FcRs) are widely expressed on microglia, astrocytes, and neurons (Okun et al. 2010). During chronic neurodegeneration, systemic inflammation can up-regulate the expression of activating FcγRs on microglia, thereby lowering the threshold for antibody-mediated cell activation, leading to excessive microglial activation and accelerating neuronal damage (Lunnon et al. 2011). Our results show enrichment of differential genes in the FcεRI signaling pathway. Combined with the inhibitory effect of SHPP on TNF-α, IL-6, and IL-1β, it is plausible that SHPP may inhibit microglia-like inflammatory responses by regulating Fc receptor-mediated inflammatory signaling pathways.

In summary, the enrichment of these two pathways is not accidental but reveals the potential molecular basis of SHPP's neuroprotective effect from an immuno-inflammatory perspective—namely, alleviating neuroinflammation by regulating microglia-related inflammatory signaling pathways, including Fc receptor-mediated activation pathways. Of course, this study used the SH-SY5Y neuronal cell line rather

than primary microglia, so the enrichment of the above pathways primarily reflects the regulation of immune-related gene expression in neurons by SHPP. Further studies are needed to verify this mechanism in primary microglia or animal models.

4. Discussion

Parkinson's disease, as the second most common neurodegenerative disease, has a complex pathogenesis involving multiple pathological processes such as mitochondrial dysfunction, oxidative stress, neuroinflammation, and apoptosis (Lunnon et al. 2011). In recent years, marine-derived natural products have gained extensive attention in the field of neuroprotection due to their multi-target properties and low toxicity (Liu et al. 2022; Pereira and Valado 2026). *S. horneri* is a brown alga with abundant biomass along the coast of China, and the neuroprotective effects of its polyphenolic compounds have not been systematically studied. In this study, we extracted and purified polyphenolic compounds (SHPP) from *S. horneri* and systematically investigated their protective effects and molecular mechanisms against rotenone-induced SH-SY5Y cell injury. The main findings are as follows: (1) SHPP ameliorated rotenone-induced decrease in cell viability, restored mitochondrial dysfunction, and alleviated oxidative stress injury; (2) SHPP inhibited mitochondria-mediated apoptosis and reduced neuroinflammation; (3) SHPP regulated the mRNA expression of genes related to apoptosis, antioxidant defense, and neurotrophic support; (4) Transcriptomic analysis revealed that SHPP exerted multi-target synergistic protection by precisely regulating core genes such as *TMEM102* and *MIR34AHG*.

4.1 Regulation of Mitochondrial Function and Oxidative Stress by SHPP

Mitochondrial dysfunction is a key pathological feature of Parkinson's disease. Rotenone, as a specific inhibitor of mitochondrial respiratory chain complex I, can block the electron transport chain and reduce ATP synthesis, accompanied by excessive production of reactive oxygen species (Lin and Beal 2006; Chen et al. 2020; Horst et al. 2017). In this study, after treatment with 0.3 μ M rotenone for 24 h, complex I activity in SH-SY5Y cells was significantly decreased, ATP content was reduced accordingly, and ROS levels and MDA content were increased, which is consistent with the criteria for establishing an injury model (Ganguly et al. 2022; Manigandan et al. 2019; Villeneuve et al. 2013; Çelik et al. 2025), confirming the successful establishment of a mitochondrial damage model.

SHPP intervention dose-dependently improved the above indicators: complex I activity in the medium- and high-dose groups was restored to 67.5% and 71.3% of the control level, respectively, and ATP content was restored to 71.2% and 74.6%, respectively. This suggests that SHPP may act directly on the mitochondrial respiratory chain or indirectly maintain its function by protecting complex I from oxidative damage. Furthermore, after SHPP treatment, ROS levels decreased to 81.2% of the model group level, MDA content decreased to 68.7% of the model group level, while SOD activity and GSH content were restored to 83.7% and 82.8% of the control level, respectively. SOD and GSH are important antioxidant defense systems within cells, and the restoration of their activity indicates that SHPP not only directly

scavenges free radicals but also activates the endogenous antioxidant enzyme system. This result is consistent with previous studies on the antioxidant activity of brown algal polyphenols (such as dieckol and eckol) (Li et al. 2009). This study is the first to demonstrate this effect of *S. horneri* polyphenols in a PD cell model.

4.2 Inhibition of the Mitochondria-Mediated Apoptosis Pathway by SHPP

Persistent mitochondrial dysfunction and oxidative stress can ultimately trigger mitochondria-mediated apoptosis. When the mitochondrial membrane potential collapses, the mitochondrial permeability transition pore opens, promoting the release of cytochrome c into the cytoplasm, which subsequently activates Caspase-9 and Caspase-3, leading to cell apoptosis (Newhouse et al. 2004; Luo et al. 2019). In this study, the model group showed a significantly increased Bax/Bcl-2 ratio and elevated levels of cytoplasmic cytochrome c and Cleaved Caspase-3, indicating activation of the apoptotic pathway.

After SHPP intervention, Bax expression was down-regulated, Bcl-2 expression was up-regulated, the Bax/Bcl-2 ratio was decreased, and the levels of cytoplasmic cytochrome c and Cleaved Caspase-3 were reduced. qPCR results further confirmed that SHPP down-regulated the mRNA expression of the pro-apoptotic gene *BAX* and up-regulated the anti-apoptotic gene *BCL2*. These results indicate that SHPP maintains mitochondrial membrane stability by regulating the expression of Bcl-2 family proteins, thereby blocking cytochrome c release and the downstream caspase cascade, exerting an anti-apoptotic effect. Similar mechanisms have been reported in neuroprotective studies of natural products such as the green tea polyphenol EGCG (Chung et al. 2007). This study is the first to incorporate *S. horneri* polyphenols into this mechanistic framework, demonstrating that SHPP can block the mitochondria-mediated apoptotic pathway by reducing the ratio of pro-apoptotic to anti-apoptotic proteins and decreasing the activation of key apoptotic proteins in a rotenone-induced SH-SY5Y cell model of Parkinson's disease, providing evidence for the anti-apoptotic mechanism of *S. horneri* polyphenols.

4.3 Regulation of Neuroinflammation by SHPP

Neuroinflammation is a key pathological feature of Parkinson's disease, and pro-inflammatory cytokines released by activated microglia can exacerbate neuronal damage (Kim et al. 2025; Herath et al. 2021). In this study, rotenone treatment significantly increased the levels of TNF- α , IL-6, and IL-1 β in the culture supernatant of SH-SY5Y cells, indicating a marked inflammatory response in the model group. SHPP intervention dose-dependently inhibited the secretion of these inflammatory factors, with the most significant inhibitory effect observed on IL-6. This finding is consistent with reports that brown algal polyphenols exert anti-inflammatory effects by inhibiting the NF- κ B signaling pathway (Catarino et al. 2020; Catarino et al. 2017). Notably, SH-SY5Y cells themselves are not typical immune cells and have a limited capacity to secrete inflammatory factors. Nevertheless, this study still detected significant changes in these factors, indicating that rotenone can directly induce inflammatory responses in neurons, and SHPP can inhibit this process.

4.4 Regulation of Key Gene Transcription by SHPP

To further verify the neuroprotective mechanism of SHPP at the transcriptional level, this study examined the mRNA expression of the antioxidant pathway-related genes *NFE2L2* and *HMOX1*, as well as the neurotrophic factor-related gene *BDNF*. The results showed that SHPP up-regulated the expression of *NFE2L2* and its downstream target gene *HMOX1*. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a key transcription factor that regulates the antioxidant response element (ARE). Its activation induces the expression of a series of antioxidant enzymes and phase II detoxifying enzymes, including heme oxygenase-1 (HO-1), SOD, and GSH (Kumar and Kalia 2025). In this study, SHPP simultaneously increased SOD activity and GSH content, which was consistent with the up-regulation of *NFE2L2/HMOX1* expression, suggesting that SHPP may enhance cellular antioxidant defense capacity by activating the Nrf2 signaling pathway. In addition, SHPP did not significantly alter *BDNF* expression in this model, suggesting that its neuroprotective effects are not primarily mediated through neurotrophic support, but rather through other pathways such as antioxidant and anti-apoptotic regulation.

4.5 Precision Regulation Characteristics Revealed by Transcriptomics

This study further explored the molecular basis of the neuroprotective effect of SHPP through transcriptome sequencing. Compared with the model group, only 28 differentially expressed genes were identified in the treatment group, far fewer than the 1,083 differentially expressed genes identified between the model group and the control group. This result has important implications: SHPP does not function by broadly altering gene expression, but rather acts in a targeted manner on a small subset of core genes in the disease model. Venn diagram analysis revealed eight common differentially expressed genes between the model and treatment groups, which may represent the core targets through which SHPP exerts its protective effects.

Notably, these eight genes include *TMEM102*, which is involved in the regulation of mitochondrial apoptosis; the non-coding RNA-related genes *MIR34AHG* and *MIR22HG*; and several members of the histone H2B family. *TMEM102* encodes a transmembrane protein that has been shown to participate in the regulation of mitochondrial outer membrane permeability and apoptotic signal transduction (Tai et al. 2022). Its expression was abnormally up-regulated in the model group and down-regulated after SHPP treatment, consistent with the observed anti-apoptotic phenotype of SHPP in this study. *MIR34AHG* is the host gene of *miR-34a*, and *miR-34a* is an important effector molecule downstream of the p53 pathway, involved in cell cycle arrest and apoptosis induction (Chen et al. 2021; Bai and Sun 2022; Rokavec et al. 2014). The regulation of *MIR34AHG* by SHPP suggests that it may exert anti-apoptotic effects by influencing the p53/*miR-34a* pathway. The changes in the expression of histone H2B family members suggest that SHPP may influence the gene expression network through epigenetic regulation, such as chromatin state remodeling (Jos et al. 2025; Feerman et al. 2025; Klokharis and Migdalska-Richards 2024). Although the specific functions of these core genes require further validation, this study provides the first transcriptome-level evidence revealing the precise regulatory molecular characteristics of *S. horneri* polyphenols.

4.6 Research Innovations and Limitations

The innovations of this study are mainly reflected in the following aspects. First, unlike previous studies that have mostly focused on a few brown algae such as *Ecklonia cava* and *Fucus vesiculosus*, this study focuses on *S. horneri*, which has a large biomass but whose neuroprotective effects have not been studied, thus expanding the source spectrum of marine-derived active substances (Yasuda et al. 2024; Cha et al. 2016; Meshalkina et al. 2023; Barbosa et al. 2020). Second, this study used all-trans-retinoic acid-differentiated SH-SY5Y cells to construct a rotenone-induced injury model, which is closer to the mature neuronal phenotype than undifferentiated cells, enhancing the clinical translational value of the *in vitro* screening results (Edina et al. 2025; Sallmon et al. 2010; Neill et al. 1994). Third, this study systematically evaluated the protective effects from four dimensions: mitochondrial function, oxidative stress, apoptosis, and neuroinflammation. Transcriptomics revealed for the first time that *S. horneri* polyphenols can precisely regulate non-coding RNAs (such as *MIR34AHG*) and histone-related genes, suggesting that they may exert multi-target synergistic effects through mild epigenetic interventions, complementing the single or few pathway reports in existing studies (Tai et al. 2022; Chen et al. 2021; Bai and Sun 2022; Jos et al. 2025; Feerman et al. 2025). In summary, this study demonstrates innovation in species selection, model optimization, multi-dimensional phenotypic validation, and mechanistic depth, providing an experimental basis for the application of *S. horneri* polyphenols in the prevention and treatment of Parkinson's disease.

This study also has certain limitations. First, the *S. horneri* polyphenols used in this study were a crude purified product, and the specific active components are still unclear. Future studies can use chromatographic separation techniques combined with activity tracking to screen and identify the monomeric compounds with neuroprotective activity. Second, the functions of the core genes screened by transcriptomics (such as *TMEM102* and *MIR34AHG*) have not yet been verified by siRNA interference or overexpression techniques. Subsequent experiments can interfere with the expression of these genes in the rotenone injury model to clarify their specific functions in the neuroprotective effect of SHPP. In addition, it should be acknowledged that the qPCR validation in this study focused on well-established PD-related genes (*BAX*, *BCL2*, *NFE2L2*, *HMOX1*, and *BDNF*), whereas the 8 common differentially expressed genes identified by transcriptomic analysis—including *TMEM102* and *MIR34AHG*—were not included in the qPCR panel. This gap limits the direct cross-validation between the transcriptomic findings and the qPCR results. Future studies should prioritize qPCR validation of these candidate core genes to confirm their expression changes and to better integrate the transcriptomic and phenotypic data. Third, this study was only conducted at the *in vitro* cellular level; the *in vivo* neuroprotective effects of SHPP and its pharmacokinetic characteristics are still unclear. Future studies can establish an animal model of Parkinson's disease for verification. Finally, the small number of differentially expressed genes in the treatment group limited the depth of conventional KEGG enrichment analysis. Subsequent studies can use gene set enrichment analysis (GSEA) to further explore the synergistic regulatory effects at the pathway level.

5. Conclusion

In summary, this study demonstrated that *S. horneri* polyphenols (SHPP) exert clear neuroprotective effects against rotenone-induced injury in SH-SY5Y cells. The mechanisms involve improving mitochondrial function, alleviating oxidative stress, inhibiting mitochondria-mediated apoptosis, and reducing neuroinflammation. Transcriptomic analysis further revealed that SHPP precisely regulates the expression of core genes such as *TMEM102* and *MIR34AHG*. This study provides a theoretical basis for the potential application of *S. horneri* polyphenols in the prevention and treatment of Parkinson's disease, and also lays a scientific foundation for the deep development and high-value utilization of marine-derived brown algal polyphenols. Future research should focus on the isolation and identification of active monomers, functional validation of core genes, and *in vivo* pharmacodynamic evaluation, in order to provide more substantial evidence for the clinical translation of *S. horneri* polyphenols.

Declarations

Competing interests

This study has no conflicts of interest.

Financial interests

The authors have no financial interests to declare.

Ethics approval

Not applicable. This study used the commercially available human neuroblastoma SH-SY5Y cell line and did not involve animal or human subjects.

Authors' contributions

Qing-Yu Xu: Formal analysis, Methodology, Visualization, Writing original draft. Mo Chen: Methodology. Chun-Er Cai: Writing - review & editing. Pei-Min He: Writing - review & editing. Ya Zhao: Conceptualization, Methodology. Rui Jia: Conceptualization, Supervision, Funding acquisition, Writing - review & editing.

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Data availability

Data are available from the corresponding author upon reasonable request.

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Figures

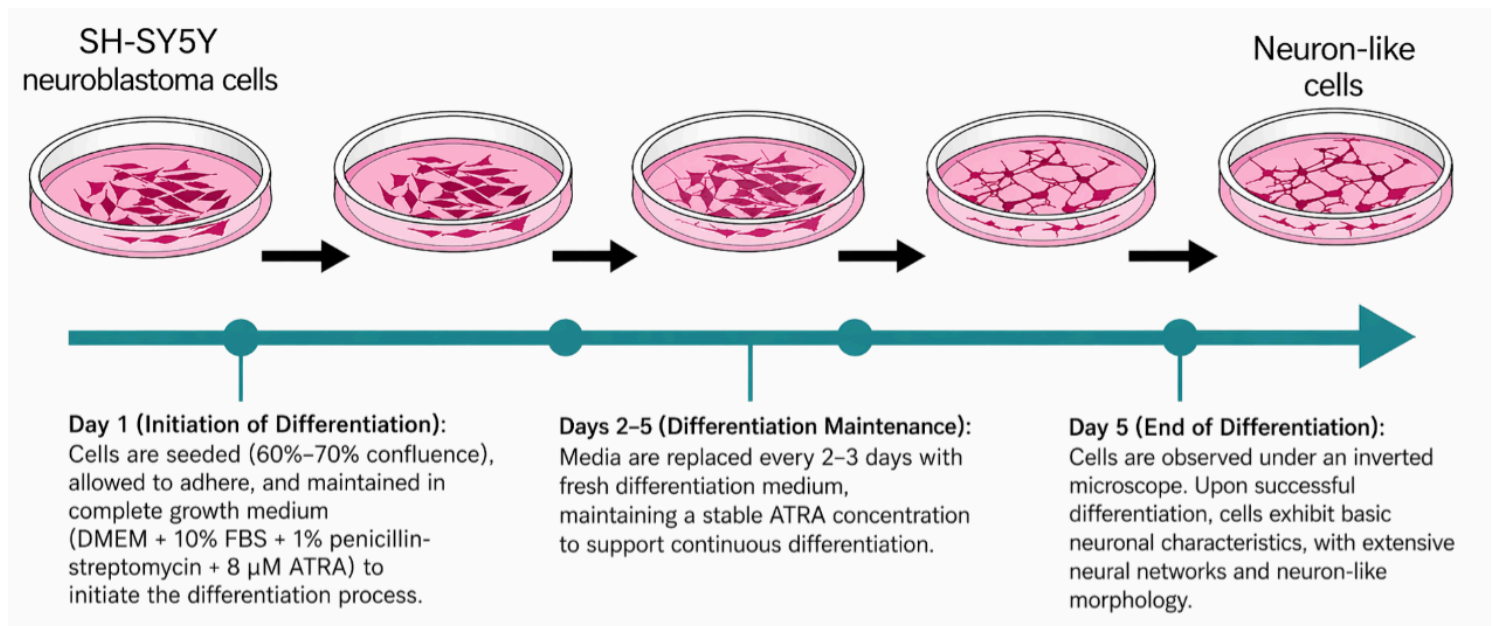


Figure 1

Schematic Diagram of the Establishment of an ATRA-Induced Differentiated SH-SY5Y Cell Model

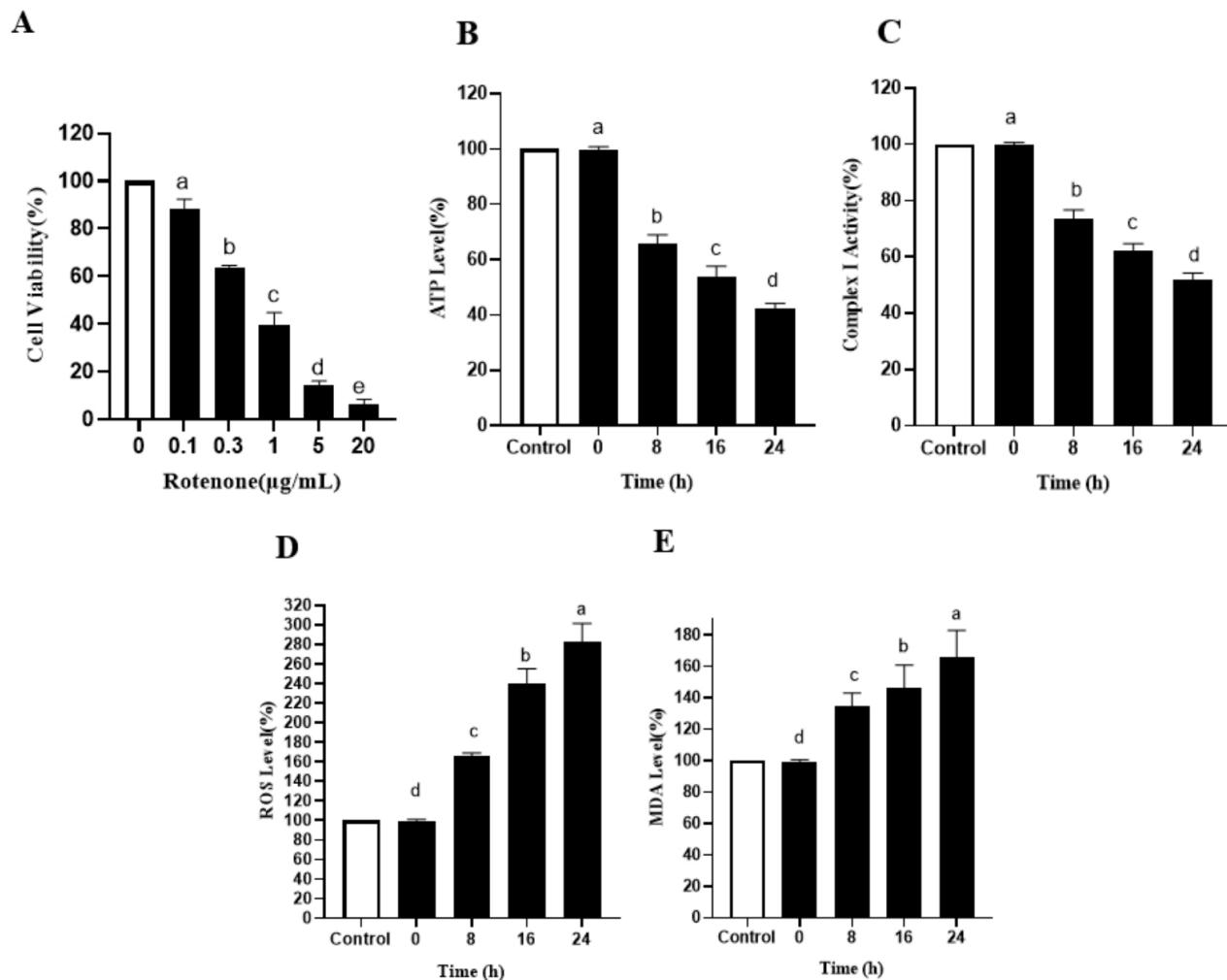


Figure 2

Effects of rotenone on SH-SY5Y cell viability (A), and time-dependent changes of ATP content (B), mitochondrial respiratory chain complex I activity (C), ROS level (D), and MDA content (E) in the rotenone-induced mitochondrial injury model. Data are presented as mean $\pm$ SEM ($n = 3-5$). ** $P < 0.01$ vs. control group.

Values are expressed as mean $\pm$ standard error of the mean (SEM). Bars marked with different lowercase letters indicate statistically significant differences between groups at $P < 0.05$, while bars sharing identical lowercase letters show no significant difference ($P > 0.05$).

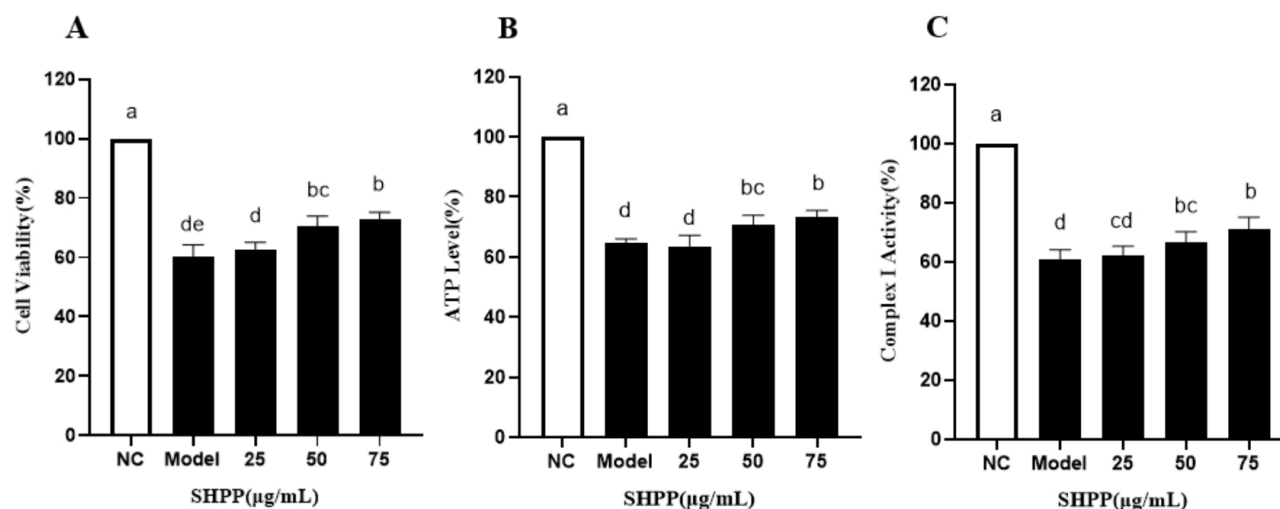


Figure 3

Effects of different concentrations of SHPP on cell viability (A), ATP content (B), and mitochondrial respiratory chain complex I activity (C) in rotenone-induced mitochondrial injury. Data are presented as mean $\pm$ SEM ($n = 3-5$). ** $P < 0.01$ vs. control group; * $P < 0.05$, ** $P < 0.01$ vs. model group.

Values are expressed as mean $\pm$ standard error of the mean (SEM). Bars marked with different lowercase letters indicate statistically significant differences between groups at $P < 0.05$, while bars sharing identical lowercase letters show no significant difference ($P > 0.05$).

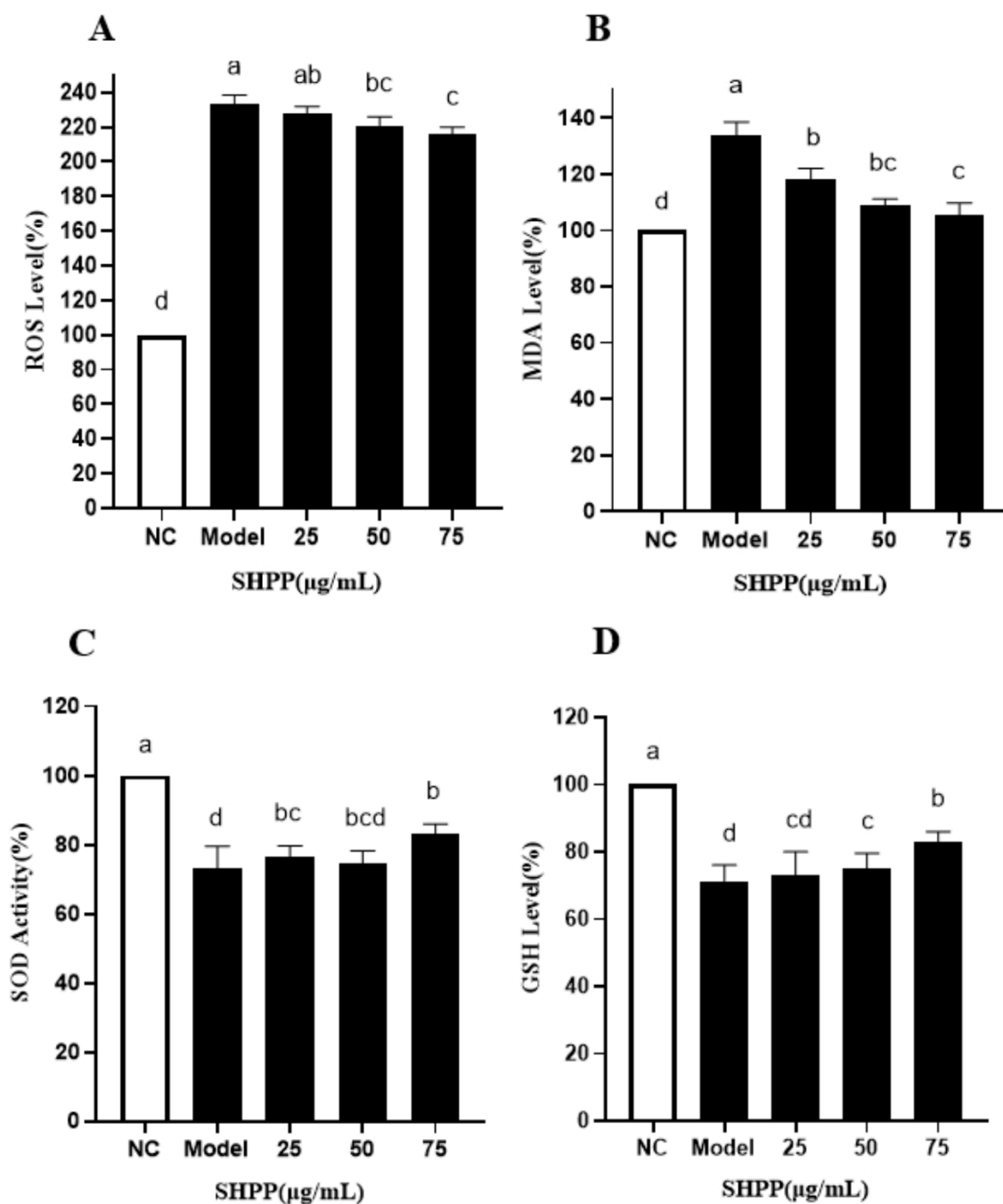


Figure 4

Effects of different concentrations of SHPP on intracellular ROS level (A), MDA content (B), SOD activity (C), and GSH content (D). Data are presented as mean $\pm$ SEM (n = 3–5). ** $P < 0.01$ vs. control group; * $P < 0.05$, ** $P < 0.01$ vs. model group.

Values are expressed as mean $\pm$ standard error of the mean (SEM). Bars marked with different lowercase letters indicate statistically significant differences between groups at $P < 0.05$, while bars sharing identical lowercase letters show no significant difference ($P > 0.05$).

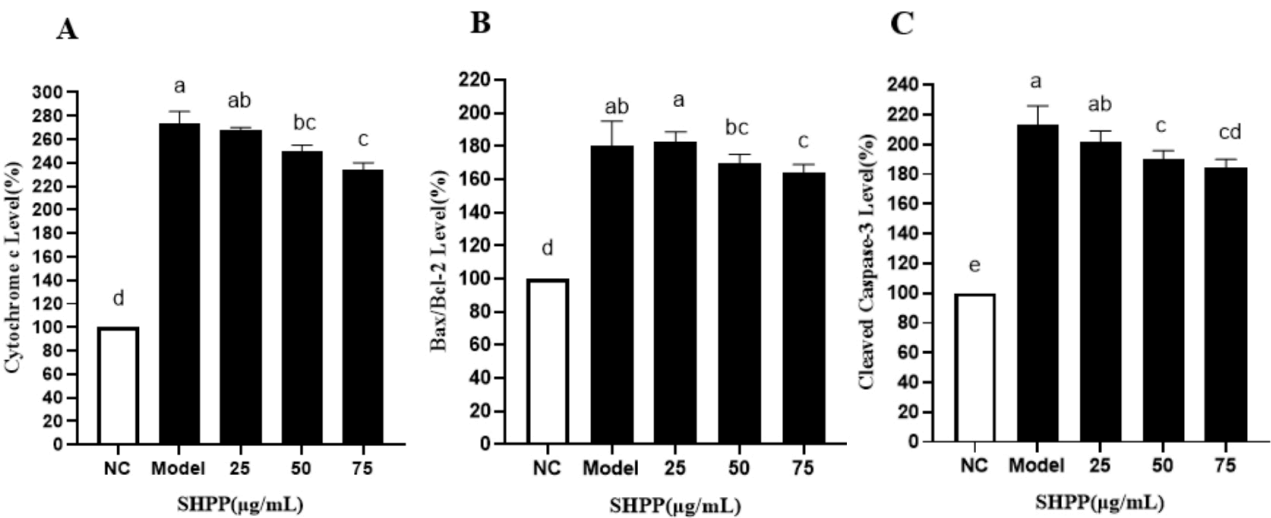


Figure 5

Effects of different concentrations of SHPP on the levels of intracellular apoptosis-related proteins: cytochrome c (A), Bax/Bcl-2 ratio (B), and Cleaved Caspase-3 (C). Data are presented as mean $\pm$ SEM (n = 3–5). ** $P < 0.01$ vs. control group; * $P < 0.05$, ** $P < 0.01$ vs. model group.

Values are expressed as mean $\pm$ standard error of the mean (SEM). Bars marked with different lowercase letters indicate statistically significant differences between groups at $P < 0.05$, while bars sharing identical lowercase letters show no significant difference ($P > 0.05$).

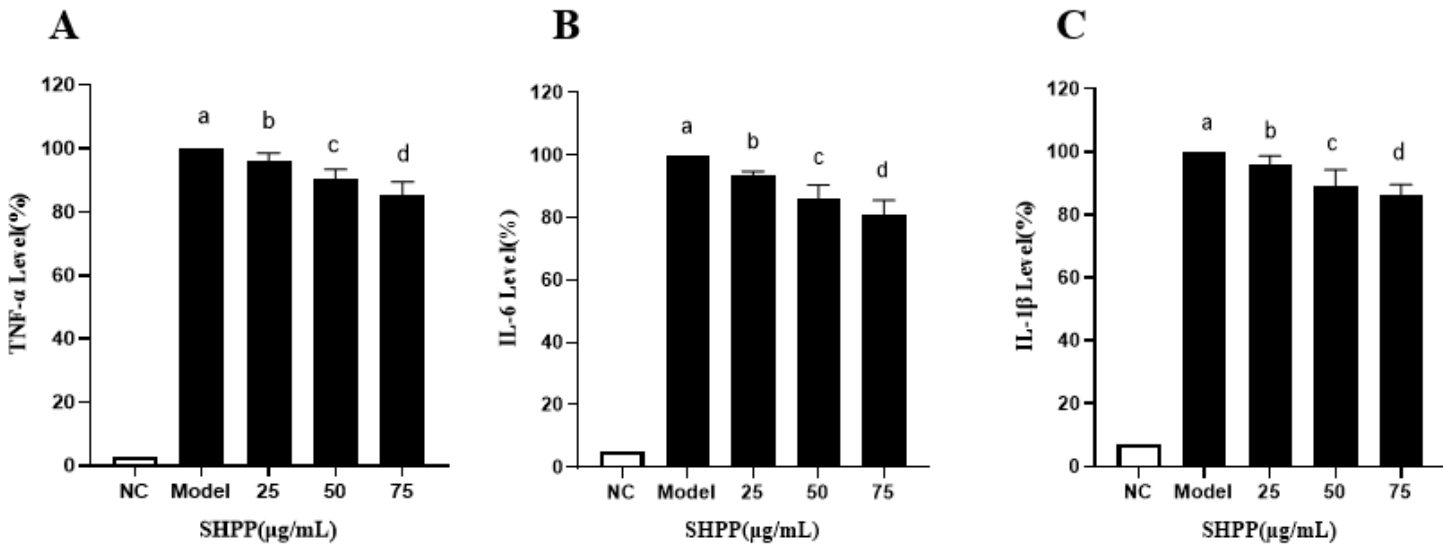


Figure 6

Effects of different concentrations of SHPP on neuroinflammatory factors TNF- α (A), IL-6 (B), and IL-1 β (C). Data are presented as mean $\pm$ SEM (n = 3–5). ** $P < 0.01$ vs. control group; * $P < 0.05$, ** $P < 0.01$ vs. model group.

Values are expressed as mean $\pm$ standard error of the mean (SEM). Bars marked with different lowercase letters indicate statistically significant differences between groups at $P < 0.05$, while bars sharing identical lowercase letters show no significant difference ($P > 0.05$).

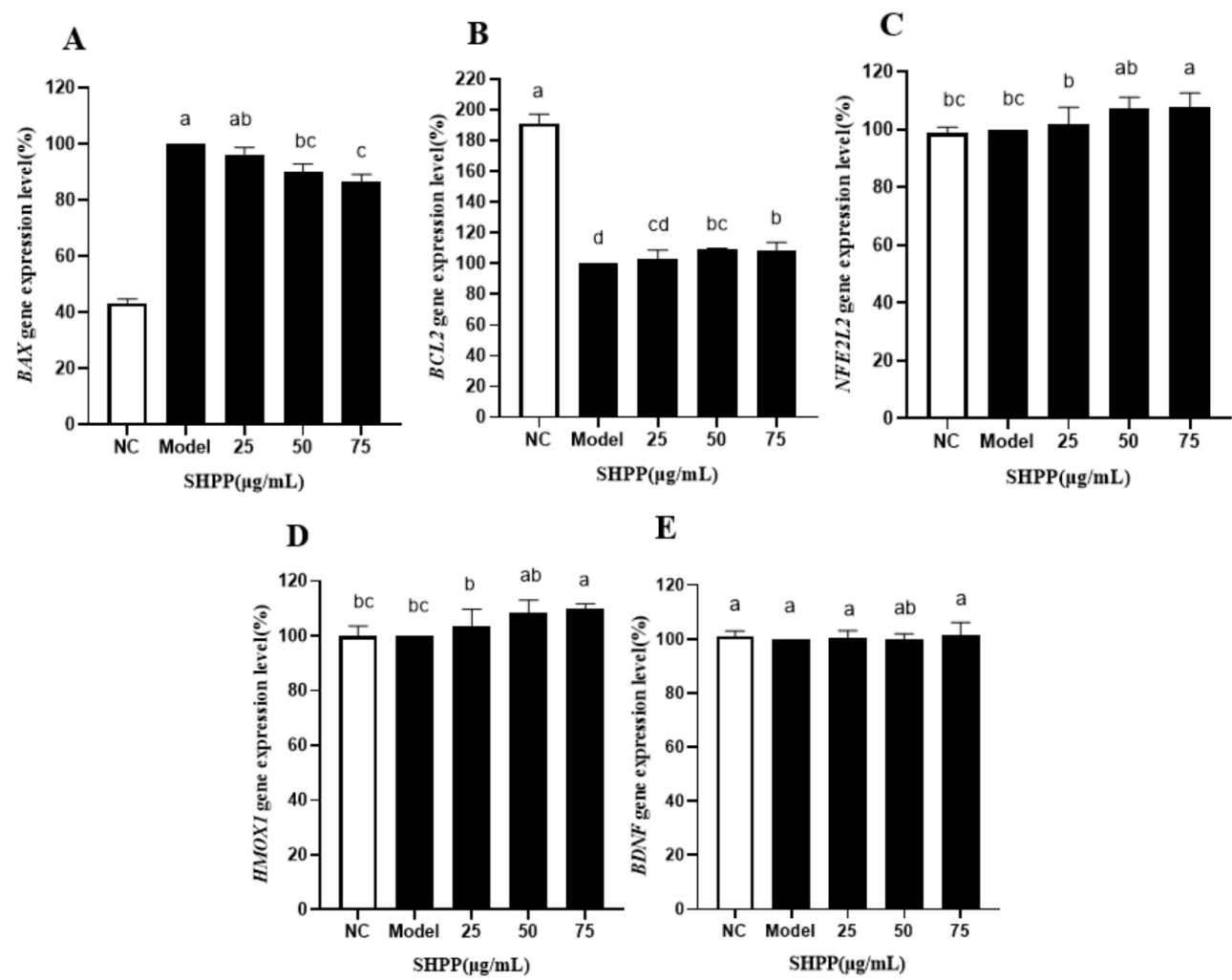


Figure 7

mRNA expression levels of *BAX* (A), *BCL2* (B), *NFE2L2* (C), *HMOX1* (D), and *BDNF* (E). Data are presented as mean $\pm$ SEM (n = 3–5). ** $P < 0.01$ vs. control group; * $P < 0.05$ vs. model group.

Values are expressed as mean ± standard error of the mean (SEM). Bars marked with different lowercase letters indicate statistically significant differences between groups at P < 0.05, while bars sharing identical lowercase letters show no significant difference (P > 0.05).

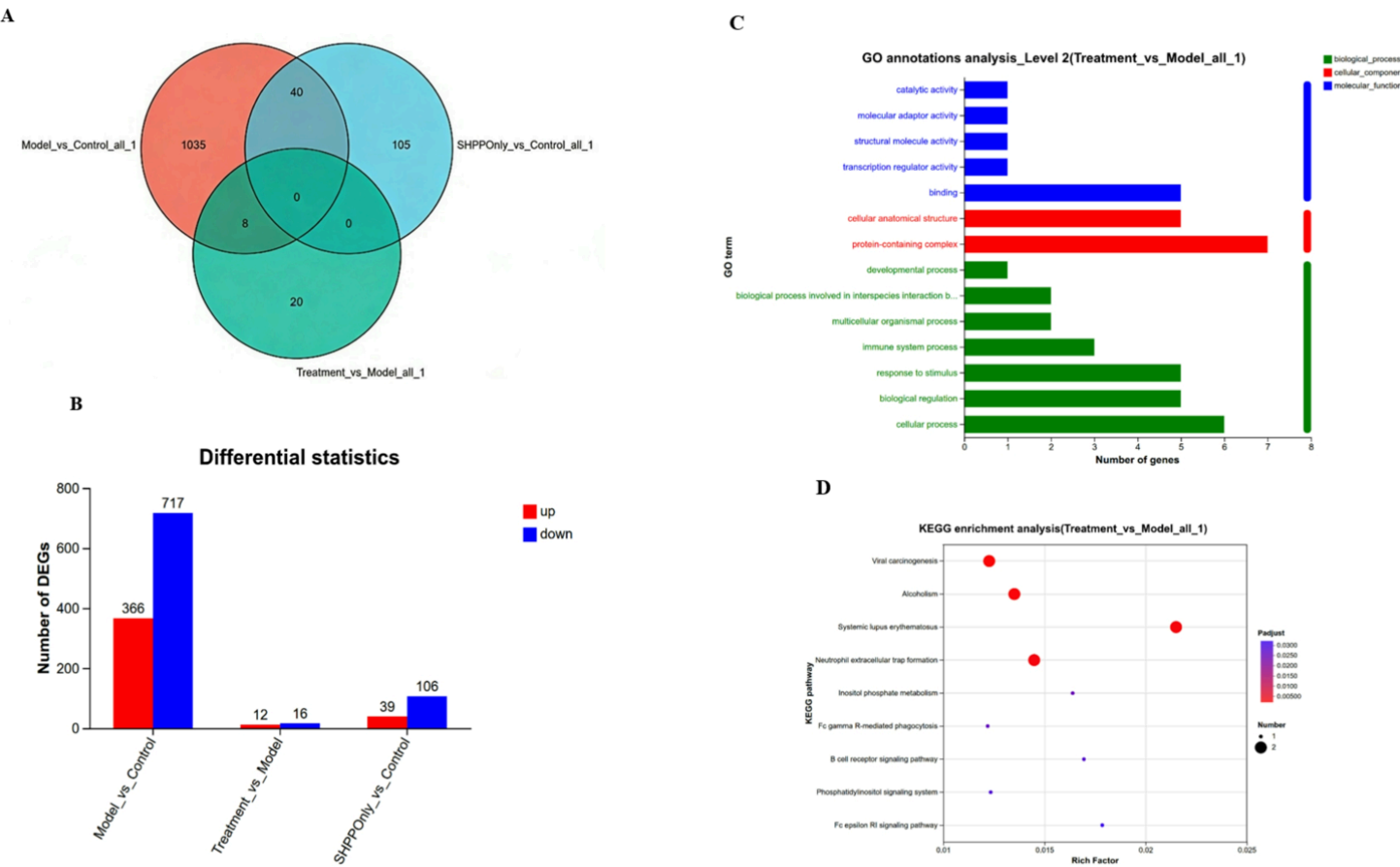


Figure 8

Venn diagram of differentially expressed genes (A), bar chart of DEG counts (B), GO classification statistical chart (Treatment_vs_Model) (C), and KEGG enrichment analysis (Treatment_vs_Model) (D). DEGs were identified using $|\log_2FC| \geq 2$ and adjusted P value (padj) < 0.05.