Neuroprotective effects of Shende'an tablet in the Parkinson's disease model

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Research

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Abstract

Background

Shende'an tablet (SDA) is a newly developed Chinese herbs formula derived from the chinese traditional medicine Zhenganxifeng Decoction, which is approved for treatment of neurasthenia and insomnia in China. The aim of this study was to investigate the in vitro and in vivo neuroprotective effects of SDA against Parkinson's disease (PD).

Methods

In the present work, PC12 cells transfected with or without A53T α-syn genes and MPTP-induced PD mice were used as models to elucidate protective effects of SDA on dopamine (DA) neurons, and the involvement of PGC-1α/Nrf2 signaling in α-syn clearance in PC12/α-syn cells stimulated with Doxycycline (Dox) and reversal of MPTP-induced toxicity in PD mice.

Results

Our results demonstrated that SDA had neuroprotection effect in dopaminergic PC12 cells with 6-OHDA. It had also displayed efficient dopaminergic neuronal protection and motor behavior alleviation properties in MPTP-induced PD mice. In the PC12/α-syn cells and MPTP-induced PD animal models, SDA was highly efficacious in α-syn clearance associated with activation of PGC-1α/Nrf2 signal pathway.

Conclusion

The results of our study suggest that SDA could be a potential therapeutic drug for PD through protecting dopamine neurons and alleviating the motor symptoms, and the mechanism might be related to the activation of PGC-1α/Nrf2 signal pathway.

1. Background

PD is the second most common neurodegenerative movement disorder, which affects 1.5% of the population above 65 years of age and markedly impact the health-related quality of life. The hallmark pathology of PD is the formation of Lewy bodies generated from accumulation and aggregation of α-synuclein (α-syn) and selective and progressive loss of dopaminergic neurons, which can consequently deplete levels of dopamine in the striatum and eventually result in motor deficits such as tremor, rigidity and bradykinesia [1].

Although extensive research has been done to elucidate mechanisms underlying the pathogenesis of PD, the exact etiology of PD are still not clear and thus the disease remains a progressive and incurable

condition [2]. Available evidence supports that the hypothesis that oxidative stress, mitochondrial dysfunction and α-syn aggregation account for most cases of PD. Oxidative stress induced by enhancing ROS is the major contributor in PD development and harmfully affects lipids, proteins and nucleic acids [3]. Inhibition of complex I in the mitochondrial electron transport chain, an important ROS generation source, can perturb cellular redox homeostasis and promote oxidative stress [2]. In addition, α-syn can misfold and aggregate into larger neurotoxic species, leading to vesicular dopamine leakage and increased oxidative stress, which in turn promote protein misfolding and α-syn aggregation [4]. Collectively, increased oxidative stress, impaired mitochondrial functions and α-syn aggregation are the mainstream predisposing factors for PD development [5].

Currently, the mainstay treatment of PD focuses on the dopamine replacement therapy [6], including Levodopa (precursor of dopamine), Apomorphine (dopamine receptor agonist) as well as Selegiline (monoamine oxidase B inhibitor) and Tolcapone (catechol-Omethyltransferase inhibitor), which can improve clinical symptoms but no potential in preventing DA neuron loss [7]. The conventional approach of using a single drug compound to act on a specific molecular target is not always effective in the trearment of PD. Therefore, preventing or slowing disease progression and alleviation of symptoms while avoiding motor complications remain a critical unmet medical needs [8].

As a complementary or alternative approach to the use of pharmacological treatments, various herbal formulations based on chinese traditional medicine or modern pharmacological theories have also been used to treat PD. Shende'an tablet (SDA), a newly developed chinese herbs formula derived from the chinese traditional medicine Zhenganxifeng Decoction, containing 10 chinese herbs, such as *radix polygoni multiflori preparata*, *prunella vulgaris*, *caulis polygoni multiflori*, *salvia miltiorrhiza*, *semen ziziphi spinosae*, *scrophularia ningpoensis*, *radix paeoniae alba*, *radix bupleuri*, *cortex albiziae* and *licorice*. In previous studies, SDA has shown a preliminary therapeutic effect on neurological disease, establishing a potential of SDA for PD treatment [9, 10]. However, to date, detailed protective effect and molecular mechanisms underlying of SDA remain to be elucidated.

In the present study, we firstly investigated the neuroprotective effects of SDA on DA neurons in PC12 cells transfected with or without A53T α-syn genes and MPTP-induced PD mice. Our results showed that SDA not only displayed potent antioxidant activity and inhibition of 6-OHDA induced neuronal cell death, but also exhibited potent in vivo activity in the MPTP induced PD animal model. We also further explored the mechanism underlying, which might be related to the activation of PGC-1α/Nrf2 signal pathway.

2. Materials And Methods

2.1 Reagents

SDA was obtained from Southern Medical University Integrated Hospital of Traditional Chinese Medicine (Guangzhou, China); 1-Methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP), G418, Doxycycline (Dox), Rapamycin (Rap), Chloroquine (CQ), MG132, Hoechst 33342, DAPI and rabbit anti-α-syn antibody were

purchased from Sigma-Aldrich (St. Louis, MO, USA.); Unless otherwise specified, all other chemicals and reagents used were of analytical grade.

2.2 Cell culture

PC12 cells were purchased from the American Type Culture Collection (ATCC, USA), were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated horse serum (HS), 5% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin (Carlsbad, CA, USA) at 37^{o}C in a 5% CO_2 atmosphere incubator. PC12 cells transfected with A53T α-syn genes (PC12/α-syn cells) were stimulated with Dox to overexpress A53T α-syn and the PC12 stable overexpressing α-syn were cultured in DMEM containing 10% HS, 5% FBS and 200 μg/mL G418 (Sigma-Aldrich, MO, USA) at 37 ^{o}C in a 5% CO_2 atmosphere incubator.

2.3 Animals

The male C57BL/6J mice (weighing 21-25 g, aged 10 weeks) and male SD rats (weighing 220-250 g), provided by Guangdong Medical Laboratory Animal Center, were used for the preparation of PD model. They were raised on a 12 h light/dark cycle with ad libitum access to food and water and were housed in clean cages under conditions of 24±1^{o}C room temperature and 55±5% relative humidity. All experimental protocols were conducted according to guidelines of the experimental animal care and use committee of Southern Medical University (Guangzhou, China). The experimental protocols were approved by the Ethics Committee for Animal Experiments of Integrated Hospital of Traditional Chinese Medicine, Southern Medical University.

2.4 Serum samples

Briefly, 10 male SD rats were randomly divided into two groups. Rats in SDA group were intragastric administrated 900 mg/kg SDA twice a day for 5 days. Solvent saline was used in control group. Then on the 6th day, serum samples were collected and analyzed for PC12 cells protection.

2.5 Cell viability

CCK-8 assay was performed in a 96-well plate. Briefly, PC12 cells (1×10^4 cells/well) were incubated with different concentrations of SDA serum samples for 24 h and then exposure to 60 μM 6-OHDA (Sigma-Aldrich, MO, USA) for further 24 h. The absorbance was measured at 570 nm on a microplate reader. Cell viability was expressed as a percentage of control group.

2.6 Flow cytometer analysis

Annexin V-FITC/PI double staining analysis for apoptosis. For experiment group, PC12 cells were treated with 20% SDA serum for 24 h and then exposure to 60 μM 6-OHDA for further 24 h. After treatment, the cells were collected and stained with Annexin V-FITC/PI and subjected to flow cytometry analysis (BD

Accuri C6). The positive control group was only treated with 6-OHDA and the negative control cells were cultured in complete medium.

2.7 Hoechst staining assay

PC12 cells were subcultured in a 35 mm confocal dish at a density of 5×10^4 cells/dish and allowed to adhere for 12 h. After washing with PBS for 3 times, the cells were fixed with 4% polyformaldehyde, incubated with Hoechst 33342 (5 mg/mL) for 5 min at 4^{o}C, and then observed by fluorescence microscope.

2.8 siRNA transfection

PC12 cells transfected with A53T α-syn genes were stimulated with Dox resulting in overexpressing A53T α-syn (inducible PC12/α-syn cells), which were seeded in 6-well plates at a density of 2×10^5 cells/well and allowed to adhere for 12 h. Cells were transfected with Nrf2 siRNA or scrambled siRNA (Santa Cruz, CA, USA) for 48 h using SureFECT transfection reagent according to the manufacturer's instructions. After induction by Dox (1 μg/mL) for 24 h to induce the expression of α-syn, cells were treated with SDA for another 24 h and then collected for western blot assay.

2.9 MPTP-induced mice PD model

The mice PD model was created by acute MPTP protocol [11]. Briefly, MPTP, a selective DA neurons neurotoxin, was injected (30 mg/kg/day i.p.) for 5 days to selectively destruct DA neurons and induce experimental Parkinsonism. After 3 days for a resting period, SDA treatment groups (100, 300 and 900 mg/kg), Selegiline (10 mg/kg) or equal volume of saline were administered orally once daily for 5 days for 2 consecutive weeks to PD mice.

2.10 Behavior test

At the end of treatment, mice were tested behaviorally (pole, rotarod and open field tests) to evaluate the different aspects of parkinsonism.

The pole test was used to evaluate the coordination of mice limbs [12]. The pole (length 50 cm, diameter 1 cm) was fixed one 1.5 cm-diameter ball in its upper end and wrapped in gauze to prevent slipping. The trial of mice descending to the bottom platform was performed three successive times with 1 h interval. The time was recorded and average value of three trials was calculated for statistical analysis.

Rotarod test was also used to measure the movement and coordination of PD model mice [13]. Mice were positioned on the rotarod (Anhui Zheng Hua Instrument Co., Ltd., China), and then tested on the revolving rod at the speed of 5 rpm for up to 120 s. Automatically recorded time, mice first fell off the rod, was designated as latency. Mice were tested three successive trials with 1 h interval. The trial was performed three successive times with 1 h interval and average time was calculated for statistical analysis.

The open field test was performed in an open field apparatus (length 50 cm, width 50 cm, height 40 cm), which consists of clear plexiglass walls and floor. Briefly, mice were placed individually in an acrylic apparatus with a floor divided into equal squares (length 25 cm, width 25 cm), then left to freely explore the arena for 5 min and movement distance was recorded for statistical analysis [14].

2.11 TH immunofluorescence

Briefly, the brains were collected, fixed with 4% PFA (v/w = 10:1) overnight at 4^{o}C, dehydrated with graded sucrose solution (10 to 30%), OTC-embedded and sliced to make 25 μm coronal sections encompassing the entire SNpc for TH staining. Sections were permeabilized with 0.3% Triton X-100 for 15 min, and then blocked with 5% BSA in PBS for 1 h at room temperature. After they were incubated with anti-TH antibody (Millipore, Billerica, MA, USA) overnight at 4^{o}C, the sections were incubated with anti-rabbit secondary antibody conjugated with FITC (Beverly, MA, USA) for 60 minutes at 37°C. Photographs were taken under a fluorescence microscope (BX51, Olympus Corp, Japan).

2.12 HPLC analysis

Endogenous level of DA and its metabolites (DOPAC, 3,4-dihydroxyphenylacetic acid and HVA, homovanilic acid) were measured using reverse-phase HPLC. Briefly, striatal tissues were dissected rapidly on ice, immediately frozen in liquid nitrogen and stored at -80^{o}C for further biochemical analysis. The weighed striatum was sonicated in 0.1 N perchloric acid, the homogenate was centrifuged at 12000 g for 10 min at 4^{o}C, and then 20 μL of supernatant collected was injected into an HPLC-electrochemical detection system equipped with Agilent Eclipse Plus C18 reverse phase column (4.6×150 mm). The endogenous level of DA and its main metabolites was expressed as ng/mg tissue weight.

2.13 Western blotting

Samples were homogenized with RIPA buffer containing 1 mM PMSF and 1% halt phosphatase inhibitor cocktail on ice. After centrifugation, the supernatants were retrieved and protein concentrations were measured with a BCA kit. 30 μg protein per sample was separated by 10-15% SDS-PAGE and transferred to PVDF membranes. After blocking with 5% skim milk for 1 h, the membranes were incubated with various primary antibodies overnight at 4^{o}C and then incubated with secondary antibodies at room temperature for 2 h. The protein bands were detected using an enhanced chemiluminescence plus kit (Amersham Bioscience, Aylesbury, UK). β-actin was used as the loading control and the immunoreactivity of each protein was performed using Care stream Molecular Imaging Software.

2.14 Statistical analysis

All experimental were analyzed by the GraphPad Prism software 5 (GraphPad, San Diego, CA, USA). One-way ANOVA and Tukey's multiple comparison test methods were used in the analysis method. The data were expressed as mean ± SEM. $P < 0.05$ was considered to be significant statistically. All experiments were repeated at least three independent times.

3. Results

3.1 SDA alleviates 6-OHDA-induced PC12 cell death

PC12 cells treated with SDA rat serum (< 20%) alone showed nearly the same cell viability compared to untreated controls (Fig. 1A), indicating the nontoxic profile of SDA rat serum at the doses below 20%. 6-Hydroxydopamine (6-OHDA) is a widely used parkinsonian toxin, which mimics oxidative stress generation observed in PD and induces degeneration of DA neurons. Treatment of PC12 cells with 6-OHDA, a significant neurotoxicity was observed and the cell viability was remarkably decreased to ∼55% in cells exposed to 60 μM of 6-OHDA for 24 h. In contrast, in the cells pretreated with SDA rat serum followed by exposure to 6-OHDA treatment group, 20% SDA rat serum afforded the greatest protective effect, which increased the cell survival by ∼20% compared to 6-OHDA alone (Fig. 1B). The potential neuroprotective effect of SDA on 6-OHDA-induced toxicity was further evaluated by flow cytometry assays and Hoechst staining, which indicated that SAD could significantly reverse PC12 cells death induced by 6-OHDA (Fig. 1C-D). These data strongly suggest SDA provides a solid neuroprotective effect for 6-OHDA-induced PC12 cell death and apoptosis.

3.2 SDA increases the clearance of α-syn via PGC-1α/Nrf2 signaling regulated by UPS pathway

To investigate the potential α-syn clearance of SDA and underlying mechanisms, the expression levels of PGC-1α/Nrf2 and related protein factors were observed using western blotting analysis. PC12 cells transfected with A53T α-syn genes (PC12/α-syn cells) were stimulated with doxycycline (Dox) resulting in overexpressing A53T α-syn, while the PC12 stably overexpressing α-syn decreased markedly with SDA pretreatment. However, inducible PC12/α-syn cells stimulated with Dox showed no significant change in the protein expression of PGC-1α. Compensatory increase was observed in the protein expression of Nrf2, which were increased significantly after SDA treatment (Fig. 2A). Moreover, Nrf2 knockdown abolished the increased clearance of α-syn with SDA treatment (Fig. 2B). The ubiquitin-proteasome system (UPS) and the autophagy-lysosomal pathway (ALP) are the two main routes for α-syn clearance [15, 16]. As shown in Fig. 3A, A53T α-syn expression was induced in the stable inducible PC12/α-syn cells while mTOR inhibitor rapamycin (Rap), a well-established inducer of autophagy, enhanced the clearance of A53T α-syn. SDA could reduce the expression of α-syn even under the treatment with autophasy inhibitor CQ and no significantly difference in the α-syn expression was observed between the CQ/SDA group and SDA alone. α-syn levels was largely increased by treatment with the proteasome inhibitors MG132, which showed no significantly statistical difference compared to that of MG132/SDA group. Furthermore, the immunoexpression of the autophagy-related proteins p62, LC3 showed no statistically significant differences at different time points after SDA treatment (Fig. 3B). Together, the findings illustrated that SDA increases the clearance of α-syn were associated with activation of PGC-1α/Nrf2 signal pathway, which was regulated by UPS but not ALP.

3.3 SDA improves motor functions in MPTP-lesioned mice

MPTP is a most frequently used parkinsonian neurotoxin applied in animal models. To investigate whether the impairments of motor performance induced by MPTP were reverted by SDA, we evaluated motor performance with the pole test, rotarod test and the open field test. We observed no significant changes in body weight after SDA, MPTP or anti-PD drug Selegiline administration (Fig. 4A). Statistical analysis of quantitative data showed that MPTP administration induced a significant increase in pole-climbing time as well as the decrease in rotarod time and total-travelled distance compared with vehicle, suggesting motor function impairment. Motor function improvement was observed in high-dose SDA (900 mg/kg) group compared with the MPTP group. Moreover, the observed improvement efficacy of high-dose SDA was somewhat greater than that of anti-PD drug Selegiline (10 mg/kg), as indicated in Fig. 4B-D. These data strongly suggest treatment with high-dose SDA prevented the motor function impairment induced by MPTP.

3.4 Reversal of MPTP-induced toxicity in mice by SDA

To evaluate the effect of SDA on dopaminergic neurons loss, tyrosine hydroxylase (TH) immunoreactivity was performed and these data confirmed that MPTP induced a significant loss of TH-positive cells compared to vehicle and developed a PD-like behavioral and pathological phenotypes. Combined treatment with MPTP plus SDA or anti-PD drug Selegiline prevented the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). As shown in Fig. 5A-B, SDA inhibited MPTP induced the loss of dopaminergic neurons in a dose-dependent manner and SDA at the dose of 900 mg/kg was highly efficacious in reversing neurons number in mice compared to MPTP treatment alone. Similar trend was also observed with the clinically used anti-PD drug Selegiline at the dose of 10 mg/kg, which was effective as SDA at 300 mg/kg. Western blots of TH protein confirmed its expression showed a significant decrease in SNpc challenged by MPTP, and this effect was significantly ameliorated with 900 mg/kg SDA (Fig. 5C).

To assess the effect of SDA on DA metabolism, levels of DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the striatum were measured by reverse phase HPLC-electrochemical detection (Fig. 5D). MPTP treatment significantly reduced the levels of DA, DOPAC and HVA in the striatum of mice, which were markedly increased by SDA at 900 mg/kg. Similarly, 10 mg/kg Selegiline also attenuated MPTP-induced the decrease of striatal DA and its metabolites levels, but the reversal effect had no statistical significance. The results of these evaluation indicated that SDA can effectively reverse the neurological damage in the MPTP-induced PD mice.

3.5 SDA promoted PGC-1α/Nrf2 expression in the SNpc of MPTP-induced mice

Finally, to establish a possible mechanism of SDA in reversing the MPTP-induced neurological damage, we assessed the expression of HO-1, PGC-1α and Nrf2 in the SNpc of MPTP-induced mice. As shown in

Fig. 6, MPTP-mediated decrease in the expression of HO-1, PGC-1α and Nrf2 levels was observed and this effect was dose-dependently abolished after SDA administration, indicating that neuroprotective effects of SDA was associated with activation of PGC-1α/Nrf2 signal pathway.

4. Discussion

Oxidative stress has been considered as a major contributor in PD progression and PD patients have shown accumulations of oxidative damage. The mitochondrial dysfunction is a major source of ROS, which comes from the inhibition of complex I of the mitochondrial electron transport chain. Excessive ROS and increased oxidative products harmfully affects proteins, lipids, and nucleic acids [17–21]. In this study, we also observed a significant increase in the 6-OHDA-induced PC12 cells death. 6-OHDA, a hydroxylated dopamine analogue, is a commonly used neurotoxin that mimics the generation of oxidative stress via its autooxidation and subsequent hydrogen peroxide generation to study PD [22–24]. Encouragingly, SDA remarkably reversed PC12 cells loss induced by 6-OHDA. These results suggest the neuroprotective effect of SDA was achieved via reducing the oxidative stress in the 6-OHDA treated PC12 cell model.

One of the characteristic pathologies of PD is α-syn accumulation and aggregation [20, 25, 26]. A53T missense mutations in the α-syn gene has been proved to increase the α-syn expression [27]. A growing body of research suggests that a connection between α-syn, oxidative stress, and mitochondrial dysfunction in PD progression [20]. The interplay of oxidative damage and α-syn aggregation has been studied by several groups, indicating increased oxidative damage contributes to α-syn aggregation and α-syn overexpression also enhances the ROS production [28, 29]. In our in vitro model, PC12/α-syn cells were stimulated with doxycycline resulting in overexpressing A53T α-syn and SDA could promote the efficient clearance of α-syn through activation of PGC-1α/Nrf2 signaling. We further found that α-syn clearance of SDA could be significantly reversed by the pretreatment with proteasome inhibitors MG132 in the stable doxycycline-inducible PC12/α-syn cells, while not disturbed by autophasy inhibitor CQ, indicating α-syn clearance of SDA was regulated by ubiquitin-proteasome system but not the autophagy-lysosomal pathway.

The culture-based PC12 cell survival assays represent a simplified experimental systems, and they might not correctly reflect the complexity of PD. Therefore, to extend these investigations to in vivo conditions and in order to clarify their underlying mechanism of action [30], in the present study, MPTP-induced PD animal model was used for further evaluation. MPTP is a mitochondrial complex I inhibitor, which can be converted to the toxic metabolite 1-methyl-4-phenylpyridinium (MPP^+) by the monoamine oxidase (MAO) enzyme in astrocytes. Thereafter, MPP^+ is released into the extracellular space and then selectively taken up to the dopaminergic nerve terminals resulting in the oxidative damage of dopaminergic neurons. In addition, MPP^+ can accumulate within mitochondria and cause mitochondrial dysfunction by inhibiting complex I of the electron transport chain [22]. As it readily passes the blood brain barrier, exposure to MPTP can replicate the neuropathological and behavioral features of PD. In the present study, a dose of 30 mg/kg/d of MPTP for 5 days significantly impaired motor behavior, decreased nigral DA neurons and

deplete striatal DA and its metabolites (DOPAC and HVA) levels in the SNpc. Remarkably, these PD-like symptoms were reversed by SDA at a dose of 900 mg/kg/d for 5 days. Thus, SDA can protect the dopaminergic pathway and improve behavioral deficits in PD animals. In this study, SDA at 300 mg/kg was effective as Selegiline at the dose of 10 mg/kg, which is a potent irreversible monoamine oxidase B inhibitor for PD therapy.

PGC-1α plays a significant role in promoting mitochondrial biogenesis, which can promote the expression of ROS scavenging enzymes and reduce oxidative stress. Recently, it is reported that dopaminergic neurons were more sensitive to MPTP in PGC-1α knockout mice [31, 32] and a negative correlation existed between the activation of PGC-1α and α-syn accumulation [33]. What's more, PGC-1a and its downstream genes expression are reduced in the brains of PD patients [34]. Nrf2, a nuclear transcription factor, is an attractive target for anti-oxidative stress. According to previous studies, Nrf2 overexpression ameliorates neurodegeneration in PD drosophila model [35], while Nrf2 deficiency aggravates α-syn associated protein aggregation[36]. Currently, the molecular interaction between PGC-1a and Nrf2 has not yet been completely elucidated. However, PGC-1a and Nrf2 coordinate a large part of the enzymatic antioxidant defense system. More and more evidence indicates that PGC-1α/Nrf2 pathway is a promising target for drug discovery against PD [34, 37, 38]. Recently, Zhang et al. reported that fucoidan has the ability to protect the dopamine system in PD rats, which may be mediated by reserving mitochondrial function involving the PGC-1α/Nrf2 pathway [38]. In the present study, we found that SDA could significantly increase the PGC-1α and Nrf2 expression in our in vitro PC12/α-syn cells model as well as in the MPTP-induced PD animal model. These results indicate that neuroprotective effect of SDA may be partly mediated by reducing oxidative stress and enhancing mitochondrial respiratory function through the PGC-1α/Nrf2 pathway.

Based on the diversity of the pathogenesis of PD, multi-targeted drugs acting on multiple molecular targets can be employed to address more than one pathological factor [5, 39]. Traditional Chinese herbal remedies a hope in addressing these complex pathological aspects by combining drug molecules with different modes of action, acting on multiple malfunctioning targets and biological processes that cause the chronic and progressive neurodegeneration observed in PD [40, 41]. Activating PGC-1α/Nrf2 as the therapeutic target is attractive because it affects multiple processes that are implicated in the pathogenesis of PD. These findings will promote the research and development of SDA as a potential therapeutic agent in PD.

5. Conclusion

In conclusion, our findings demonstrated that SDA had neuroprotection effect in dopaminergic PC12 cells induced with 6-OHDA. It had also displayed efficient dopaminergic neuronal protection and motor behavior alleviation properties in MPTP-induced PD mice. In the PC12/α-syn cells and MPTP-induced PD animal models, SDA was highly efficacious in α-syn clearance associated with activation of PGC-1α/Nrf2 signal pathway, which means that its neuroprotective effects were mainly mediated by the activation of

PGC-1α/Nrf2 signaling. These findings suggest that SDA has potential applications as a therapeutic drug for PD.

Abbreviations

PD: Parkinson's disease; SDA: Shende'an tablet; α-syn: α-Synuclein; 6-OHDA: 6-Hydroxydopamine; MPTP: 1-Methyl-4-phenyl-1,2,3,6- tetrahydropyridine; PGC-1α: Peroxisomeproliferator-activated receptor-γ coactivator-1α; Nrf2: Nuclear factor erythroid-2-related factor 2; SNpc: Substantia nigra pars compacta; UPS: ubiquitin-proteasome system.

Declarations

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Authors' contributions

SL, EMD and KP designed the experiments; XYS, SYY, XMW, XZ, YFY, PZ and LMZ performed the experiments and analyzed the data; SL and EMD drafted and revised the paper.

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Availability of data and materials

Please contact corresponding authors for data requests.

Ethics approval and consent to participate

All procedures in this study were approved and supervised by the animal research ethical committee of Southern Medical University, and strictly obeyed the rules of animal experiment ethic to reduced number as well as suffering of animals.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

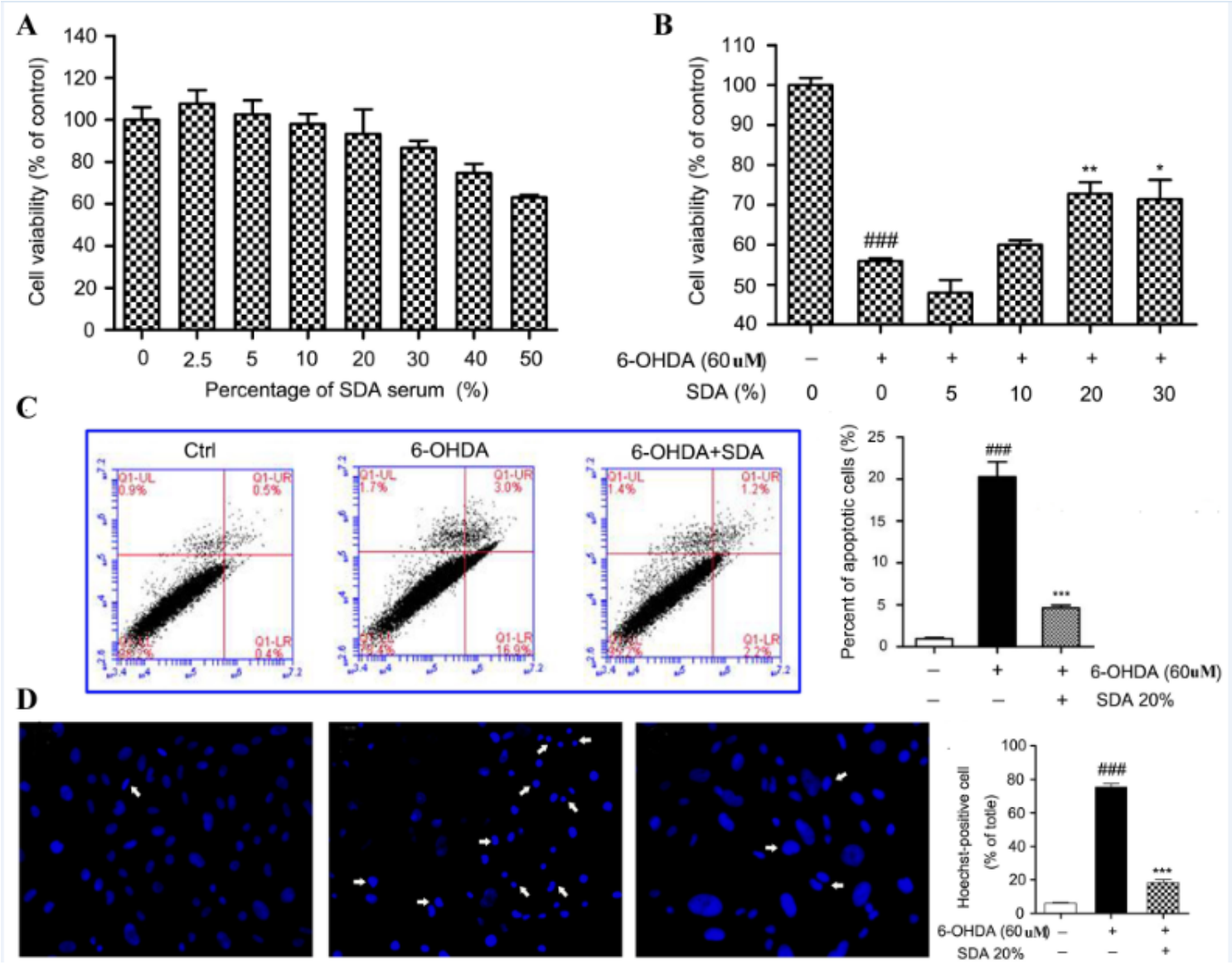

Figure 1

Neuroprotection of SDA rat serum against 6-OHDA-induced toxicity in PC12 cells. (A) The dose-dependent effect of SDA rat serum on the viability of PC12 cells; Neuroprotection of SDA rat serum against 6-OHDA-induced toxicity in PC12 cells was measured by CCK-8 assay (B), flow cytometry assay (C), and Hoechst staining (D). The values are presented as the mean ± SEM from three independent experiments (### P < 0.001 compared to control group; * P<0.05, ** P < 0.01 and *** P < 0.001 compared to 6-OHDA group).

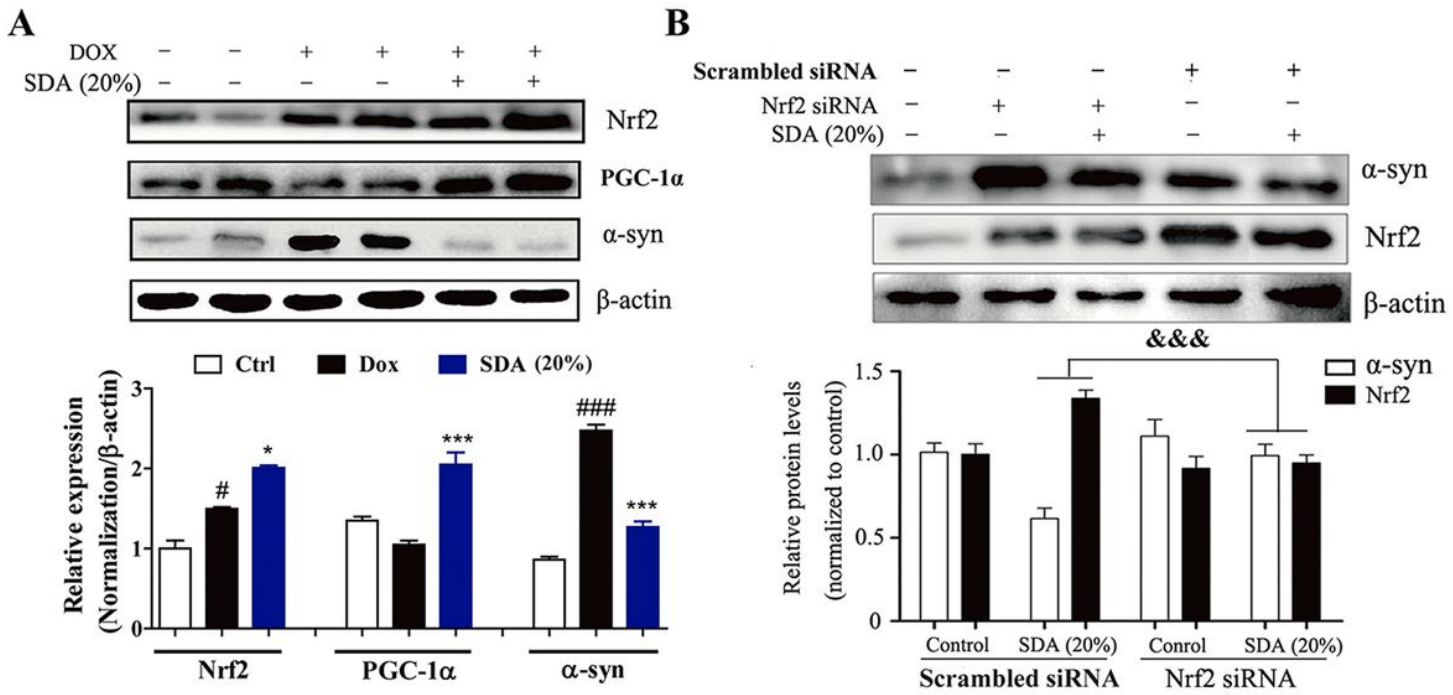

Figure 2

SDA promotes the α-syn clearance through activation of PGC-1α/Nrf2 signaling. (A) Representative immunoblots and densitometry data for α-syn, Nrf2 and PGC-1α in the inducible PC12/α-syn cells treated with doxycycline (Dox) followed by SDA; (B) Representative immunoblots and densitometry data for Nrf2 and α-syn levels in the inducible PC12/α-syn cells transfected Nrf2 siRNA or scrambled siRNA. Data from three independent experiments were expressed as mean ± SEM (# P < 0.05, ### P < 0.001 compared to control group; * P < 0.05, *** P < 0.001 compared to Dox-treated group; &&& P < 0.001).

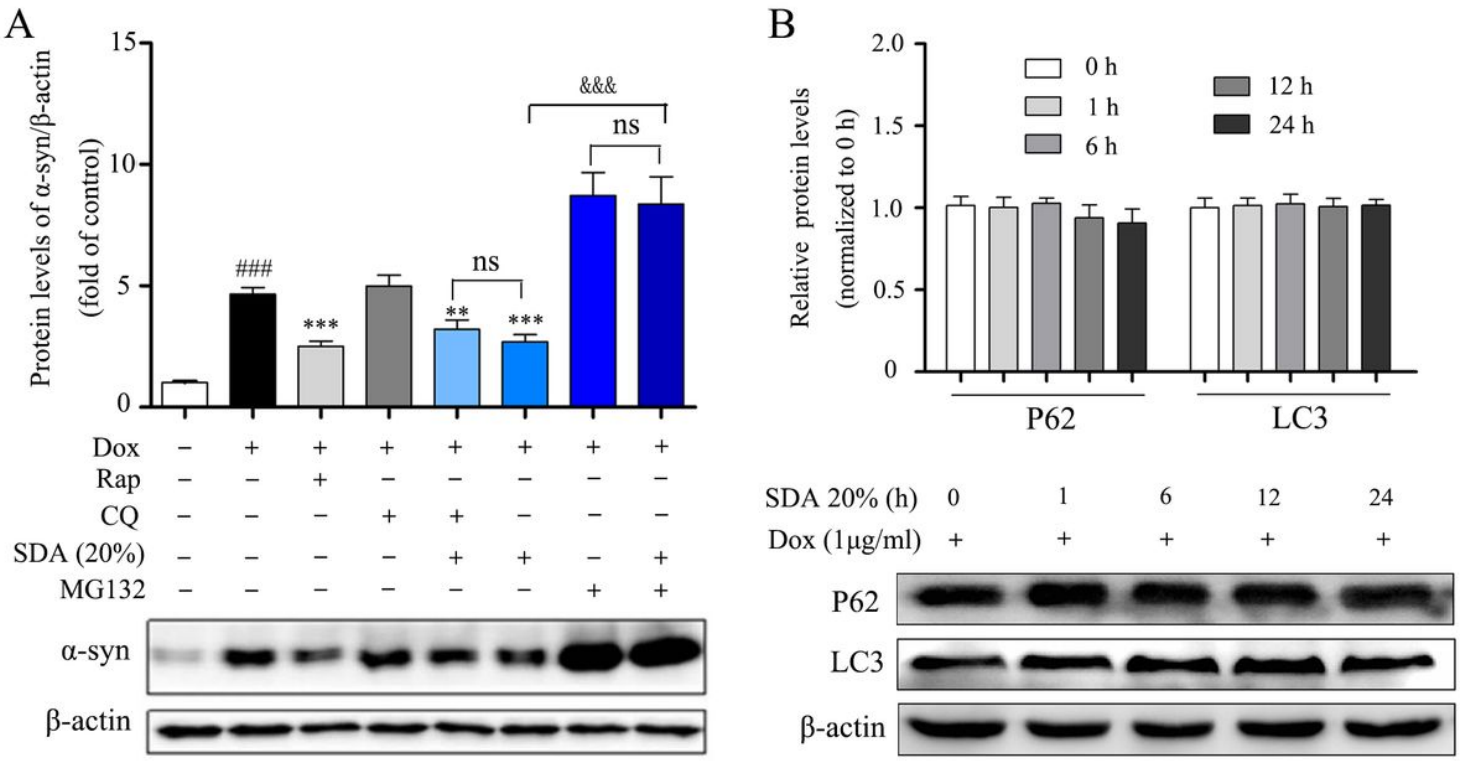

Figure 3

SDA promotes the α-syn clearance regulated by UPS pathway and independent of ALP pathway. (A) Representative immunoblot and quantification of α-syn levels in the inducible PC12/α-syn cells treated with Dox followed by 20% SDA, 20 μM autophagy inhibitor CQ, 0.7 μM proteasome inhibitor MG132 or 0.2 μM mTOR inhibitor Rap for another 24 h; (B) Representative immunoblots and quantification of p62 and LC3 levels in the inducible PC12/α-syn cells treated with Dox followed by SDA. Data from three independent experiments were expressed as mean ± SEM (### P < 0.001 compared to control group; ** P < 0.01, *** P < 0.001 compared to Dox-treated group; & P < 0.05, &&& P < 0.001).

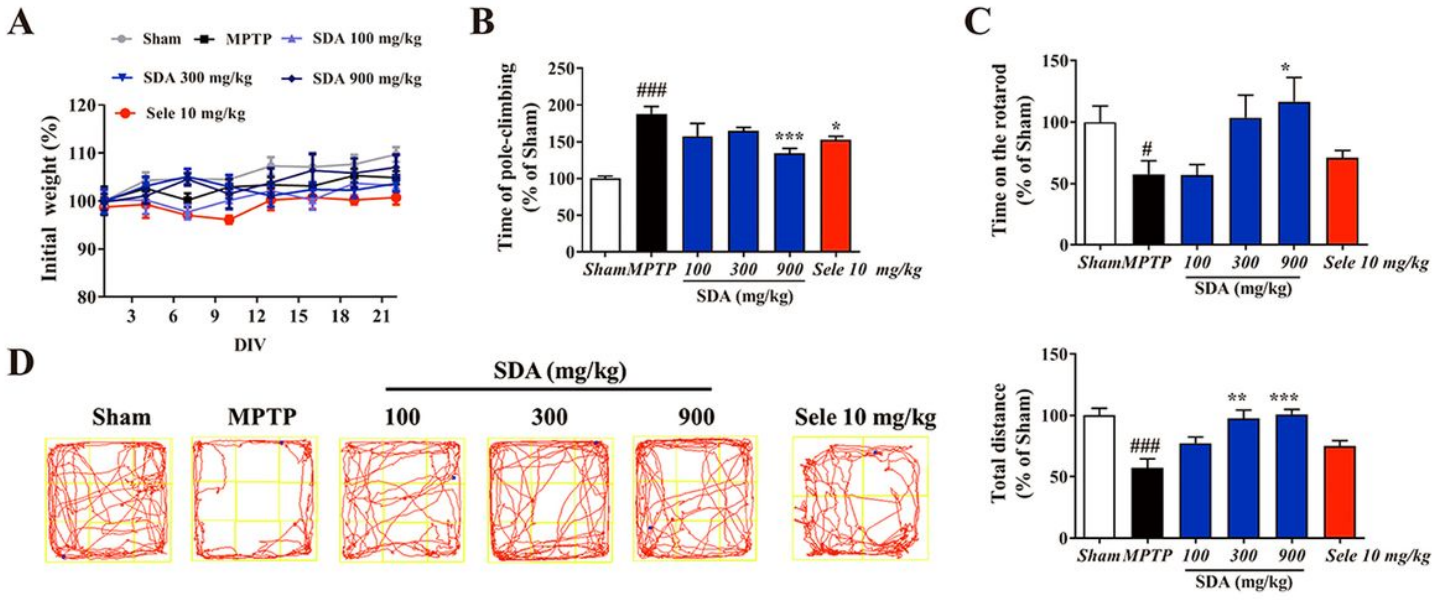

Figure 4

SDA improves motor behavior in MPTP-induced PD mice. (A) Body weight (g) after administration; (B) Climbing time spent on the pole (% of sham); (C) Time on the rotarod (% of sham); (D) Quantification the total distance in the open field test (% of sham). Data were expressed as mean ± SEM (# P < 0.05 and ### P < 0.001 compared to sham group; * P < 0.05, ** P < 0.01 and *** P < 0.001 compared to MPTP group. n=6/group).

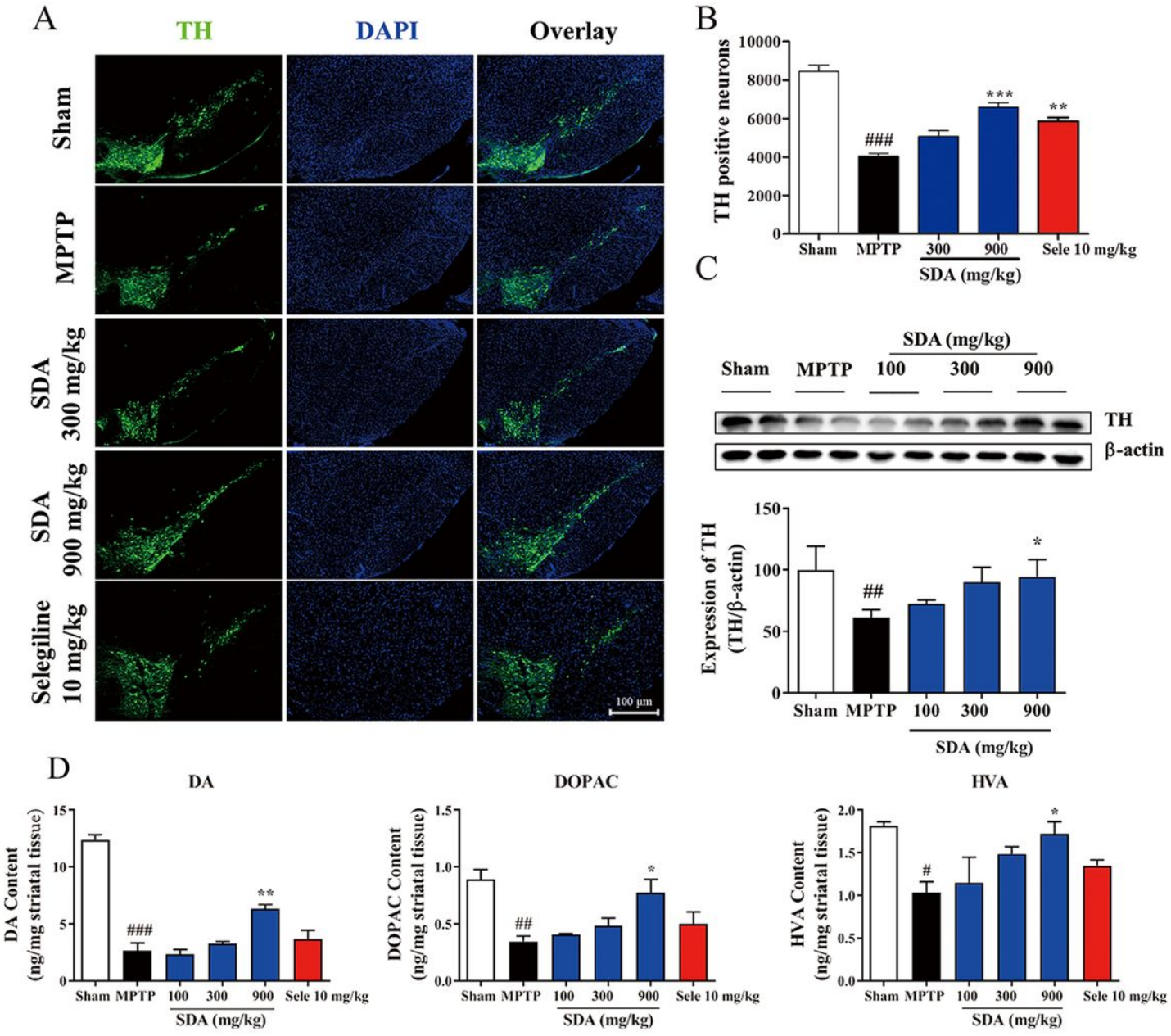

Figure 5

SDA Attenuated the Loss of Dopaminergic Neurons and its effect on Striatal DA, DOPAC, and HVA in MPTP-Induced Mice. (A) Representative photomicrographs of TH immuno-staining in the SNpc. Scale bar, 100 μm. (B) The mean number of TH-positive neurons. (C) Western blot assay of TH expression in SNpc. (D) Quantification of DA (ng/mg), DOPAC (ng/mg) and HVA (ng/mg) by HPLC in the striatum. Data were

expressed as mean ± SEM. ## P < 0.01 and ### P < 0.001 compared to Sham group; * P < 0.05, ** P < 0.01 and *** P < 0.001 compared to MPTP group. n=3/group.

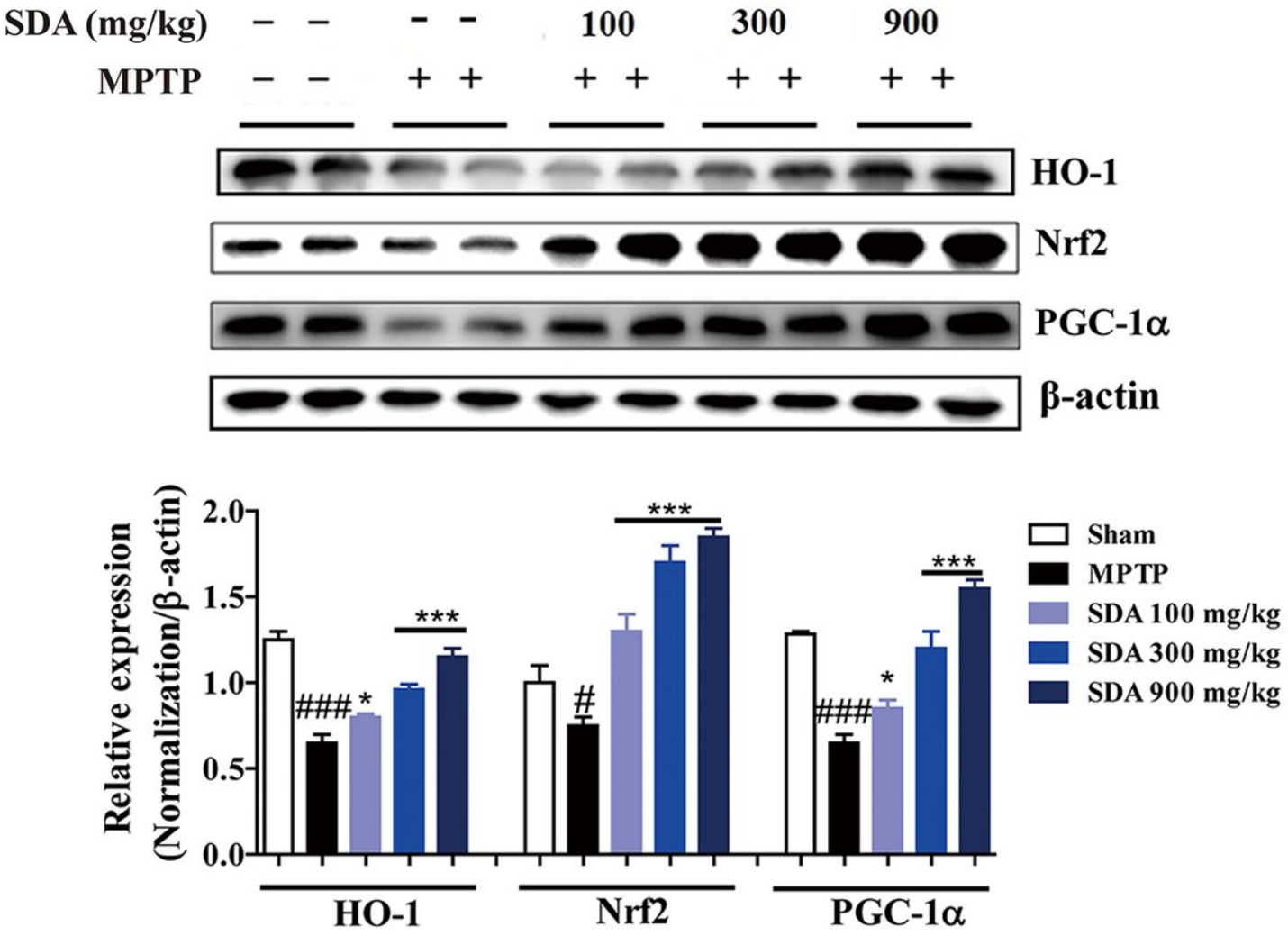

Figure 6

SDA activates PGC-1α/Nrf2 pathway to prevent neurodegeneration in MPTP-induced mice. Representative immunoblots and quantification of HO-1, Nrf2 and PGC-1α in the SNpc of MPTP-induced mice. Data were expressed as mean ± SEM. # P < 0.05 and ### P < 0.001 compared to sham group; * P < 0.05 and *** P < 0.001 compared to MPTP group. n=3/group.