Article

Schisandrin A Alleviates Inflammation and Oxidative Stress in Aβ_{25-35}-Induced Alzheimer's Disease *in Vitro* Model

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Abstract

Background: Schisandra extract has therapeutic and preventive effects on Alzheimer's disease (AD). Therefore, this study evaluated the anti-AD potential of Schisandrin A (SCH A) using an *in vitro* cell model.

Methods: SH-SY5Y and SK-N-SH cells were treated with 20 µM amyloid β-protein (Aβ)$_{25-35}$. The Aβ_{25-35}-induced cells were then exposed to different concentrations of SCH A (1, 5, 10, 15 µg/mL). Moreover, to further explore the role of the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathway in the anti-AD effects of SHC A, SH-SY5Y cells were treated with SCH A following incubation with ERK activator LM22B-10. The impact of SCH A on cell viability and apoptosis was evaluated using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) and flow cytometry. Furthermore, the oxidative stress markers and inflammatory cytokine levels were also assessed. The reactive oxygen species (ROS) levels were examined using 2′,7′-Dichlorodihydrofluorescein Diacetate (DCFH-DA) method. Finally, Western blot analysis was employed to evaluate the phospho-ERK1/2 (p-ERK1/2) and ERK1/2.

Results: We observed that SCH A treatment (5, 10, 15 µg/mL) substantially increased the cell viability ($p < 0.05$), and reduced the apoptosis rate (10 and 15 µg/mL) in SH-SY5Y and SK-N-SH cells ($p < 0.05$). SCH A significantly ameliorated oxidative stress and reduced inflammatory cytokine levels in Aβ_{25-35}-induced cells ($p < 0.05$). Furthermore, SCH A up-regulated the p-ERK1/2 to ERK1/2 ratio in Aβ_{25-35}-induced cells. However, LM22B-10 treatment was found to exacerbate this effect of SCH A ($p < 0.05$).

Conclusion: SCH A reduces the Aβ_{25-35}-induced inflammatory response and oxidative stress in SH-SY5Y and SK-N-SH cells, and the activation of the ERK/MAPK signaling pathway was related to its potential mechanism.

Keywords

Alzheimer's disease; Schisandrin A; extracellular signal-regulated kinase; oxidative stress; inflammatory factors

Introduction

Alzheimer's disease (AD), a neurodegenerative disease predominantly affecting older people, is characterized by amyloid β-protein (Aβ) plaque deposition in the brain and neurofibrillary tangles (NFTs) formation due to hyperphosphorylation of Tau protein in nerve cells [1,2]. These pathological changes lead to severe cognitive impairment and mental disorders [2]. The current therapeutic and clinical approaches for AD include cholinesterase inhibitors, β-amyloid precursor protein (β-APP) secretion enzyme inhibitors, and N-methyl-D-aspartate receptor antagonists [3,4]. However, their effectiveness varies from person to person and fails to effectively stop or substantially

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delay the progression of the disease, often accompanied by low response rates and adverse reactions [3,4]. Chinese medicines have been found effective in relieving symptoms and delaying the course of AD. This treatment approach offers the advantages of minimal adverse effects and high effectiveness, which has encouraged active research into the molecular mechanism of Chinese medicines [5].

Schisandra chinensis, the dried mature fruit of *Schisandra chinensis* from the family *Magnoliaceae*, is known for its effects on tonifying the heart and kidneys, tranquillizing the heart and soothing the mind. Its chemical composition primarily comprises lignans, volatile oils, organic acids, and many other chemical constituents, with Schisandrin A (SCH A) being the main active component of *Schisandra chinensis* [6]. Research has shown that SCH A can activate neuronal autophagy, attenuate inflammatory response and oxidative stress, enhance neurotrophic activity, and provide neuroprotective effects to neuronal cells [7]. Additionally, another study has reported the neuroprotective effects of SCH A by mitigating neuronal atrophy. This study also found that SCH A substantially increases synapsin expression, while reducing alpha synuclein levels in brain tissue [8]. SCH A plays a protective role in AD by protecting nerve cells from damage [9].

The extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathway is closely related to AD. Abated levels of estradiol lead to reduced activation of key factors within the ERK/MAPK pathway, which in turn activates a cascade of downstream effectors that exacerbate the pathological changes in AD [10]. Kaempferol has been found to inhibit Aβ_{25-35}-mediated apoptosis in PC-12 cells via the estrogen receptor (ER)/ERK/MAPK signal pathway in AD-related damage [11]. Furthermore, SCH C regulates lipid metabolism and inflammation through the p38/ERK-MAPK signaling pathway, ameliorating liver fibrosis [12].

However, the role of SCH A in regulating the ERK/MAPK signaling pathway in AD injury is yet to be investigated. Therefore, this study aimed to explore the anti-AD effect of SCH A and analyze the potential signaling mechanisms. For this purpose, a cell injury model was established by stimulating SH-SY5Y and SK-N-SH cells with 20 μM Aβ_{25-35} to assess the role and mechanism of SCH A in this model.

Materials and Methods

SCH A Interventions

Human neuroblastoma cell lines, SH-SY5Y (CL-0208) and SK-N-SH (CL-0214) cells were purchased from Punosai Biology (Wuhan, China). These cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (11875119, Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (11011-8611, Tianhang Biologicals, Huzhou, China) and incubated at 37 °C in the presence of 5% CO_2. The cell cultures were authenticated using short tandem repeat (STR) profiling and underwent mycoplasma testing before experiments.

Aβ_{25-35} (S41991, source leaf organism, Shanghai, China) was prepared at a concentration value of 20 μM. The cells were treated with 20 μM Aβ_{25-35} for 24 hours [13]. Furthermore, the cell injury model was exposed to varying concentrations (1, 5, 10, 15 μg/mL) of SCH A (SS8180, Soleibao, Beijing, China). The cells were divided into a control subgroup, a model subgroup (Aβ_{25-35}), and SCH A treatment subgroups (1, 5, 10, 15 μg/mL). To further explore the role of ERK/MAPK signaling pathway in the anti-AD effects of SHC A, SH-SY5Y cells were treated with SCH A after incubation with the ERK activator LM22B-10 (HY-104047, MedChemExpress, Princeton, NJ, USA).

3-(4,5-Dimethyl-2-Thiazolyl)-2,5-Diphenyl-2-H-Tetrazolium Bromide (MTT) Assay

Cells were seeded in 96-well plates at a density of 4 × 10^3 cells per well, with five wells designated for each subgroup. After 24 hours of incubation, SCH A was added at different final concentrations and incubated for another 24 hours, followed by treatment with 20 μM Aβ_{25-35}. After this, the culture medium was eliminated, and MTT reagent (M1020, Solarbio, Beijing, China) was added. Then, the cells were incubated for four hours at 37 °C with 5% CO_2 using an HF-90 incubator (Lixin, Shanghai, China). Following incubation, the supernatant was aspirated, and the cells were incubated with dimethyl sulfoxide (DMSO; D8371, Solarbio, Beijing, China) for 10 minutes in the dark. Finally, optical density (OD) at 570 nm was assessed using a microplate reader (VL0000D2, ThermoFisher, Waltham, MA, USA).

Flow Cytometry

After a 24-hour treatment with 20 μM Aβ_{25-35}, the cells from each subgroup were collected and washed

twice with phosphate buffer saline (PBS, Solarbio, Beijing, China). After centrifugation, the cells were collected and resuspended for an apoptosis assay using Annexin V-FITC cell apoptosis detection kit (C1062L, Beyotime, Shanghai, China). The cells were incubated with Annexin V-FITC and propidium iodide for 15 minutes at room temperature in the dark and then analyzed using flow cytometry (NovoCyte, Aceabio, San Diego, CA, USA).

Detection of Oxidative Stress Factors

Malondialdehyde (MDA; 19A100) was determined using the thibabituric acid (TBA) method, superoxide dismutase (SOD, 02A070) using the hydroxylamine method, and glutathione (GSH, 06A110) utilizing the microplate method. These kits were provided by Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Cells were resuspended in PBS and lysed by sonication under ice bath conditions. After centrifugation at 1500 g for 10 minutes, the supernatant was collected for assessing MDA, SOD, and GSH levels following the kit instructions. OD was determined using a microplate reader (VL0000D2, ThermoFisher, Waltham, MA, USA) at 532 nm, 450 nm, and 405 nm for MDA, SOD, and GSH, respectively.

Reactive Oxygen Species (ROS) Detection

Each subgroup of cells was seeded in 24-well plates at a density of 2×10^4 cells per well. All following protocols required a dark environment. The cells were incubated with RPMI-1640 containing 2′,7′-Dichlorodihydrofluorescein Diacetate (DCFH-DA; 10 µmol/L, D6470, Solarbio, Beijing, China) at 37 °C for 30 minutes. After this, the cells were thoroughly washed with PBS. Fluorescence microscope (Leica, Wetzlar, Germany) was for the observation and photography (excitation wavelength: 485 nm; emission wavelength: 538 nm).

Enzyme-Linked Immunosorbent Assay (ELISA)

The cell culture supernatant of each subgroup was collected, and the levels of tumor necrosis factor (TNF)-α (E-EL-H0109c), interleukin-(IL)-1β (AZ-003-50ug), and IL-6 (21865-1-AP) were evaluated using corresponding ELISA kits (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions.

Western Blot

After treatment, cells were washed three times with pre-cooled PBS. In the next step, radioimmunoprecipitation assay (RIPA) lysis solution (B0013B, Yuanye Biotechnology, Shanghai, China) was added. Protease inhibitor was added at a ratio of 1:50 (A826, solarbio, Beijing, China). The mixture was shaken on ice for 20 seconds, then scraped off with a cell scraper and transferred to an Eppendorf tube. The cells were lysed using an ultrasonic homogenizer on ice followed by centrifugation. The resultant supernatant was used to assess protein concentration using a bicinchoninic acid assay kit (02A120, Thermo Fisher Scientific, Waltham, MA, USA). Protein samples (30 µg) were electrophoresed using acrylamide gel, and subsequently transferred onto 0.22 µm polyvinylidene difluoride (PVDF) membrane (IPVH00010, Millipore, Boston, MA, USA). The membrane was blocked with tris-buffered saline containing tween 20 (TBST, Solarbio, Beijing, China) and 5% skim milk for 1 hour at ambient temperature. After this, the membrane underwent overnight incubation with primary antibodies against phospho-ERK1/2 (p-ERK1/2, MA5-15174, Invitrogen, Carlsbad, CA, USA; 1:1000 dilution), ERK1/2 (MA5-15134, Invitrogen, Carlsbad, CA, USA; 1:1000 dilution) and GAPDH (MA5-35235, Invitrogen, Carlsbad, CA, USA; 1:5000) at 4 °C. The following day, the membrane was washed with TBST and incubated with a secondary antibody (ab205718, 1:5000; Abcam, Cambridge, MA, USA) for 1 hour at ambient temperature. Protein bands were developed by exposing the membrane to enhanced chemiluminescence (ECL) chromogenic agent (WB600, Thermo Fisher Scientific, Waltham, MA, USA) and photographs were captured using a LAS-4000 image analyzer (FujiFilm, Tokyo, Japan). GAPDH was used as an internal reference, and grey values of the protein bands were analyzed with Image J Software (version 1.8.0, Media Cybernetics, Silver Spring, MD, USA).

Statistical Analysis

Statistical analysis was performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). To enhance statistical significance, each experiment was repeated three times. The measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$). A *t*-test was utilized for comparison between the two groups, while a one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls (SNK)-q test was employed for comparison among multiple subgroups. A p-value < 0.05 was statistical significance.

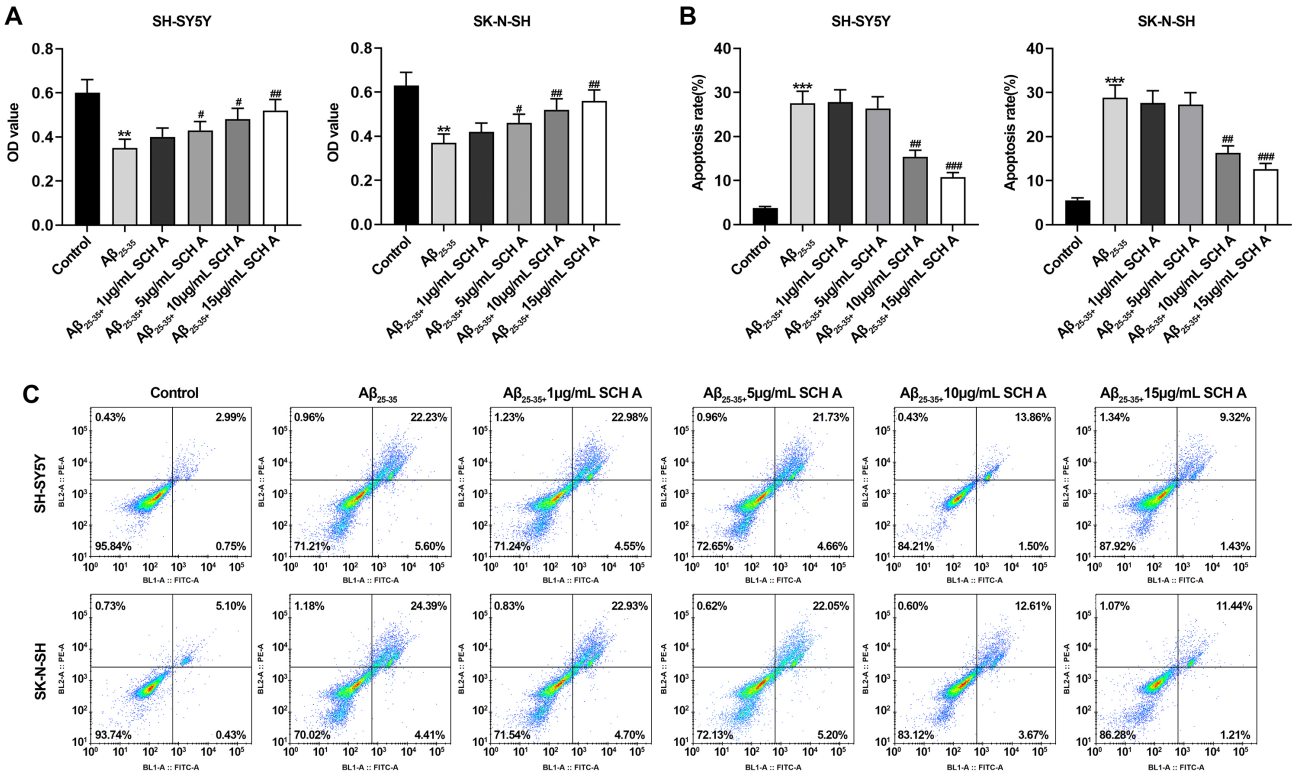

Fig. 1. Schisandrin A (SCH A) treatment increases cell viability and inhibits apoptosis in amyloid β-protein$_{25-35}$ (Aβ_{25-35}) treated SH-SY5Y and SK-N-SH cells. (A) 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay was used to evaluate the cell viability. (B,C) Flow cytometry was utilized to assess the cell apoptosis rate. n = 3, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control subgroup; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. Aβ_{25-35} subgroup.

Results

SCH A Treatment Increases Cell Viability and Inhibits Apoptosis in Aβ_{25-35}-Induced SH-SY5Y and SK-N-SH Cells

We observed that Aβ_{25-35} substantially reduced the viability of both SH-SY5Y and SK-N-SH cells compared to the control subgroup ($p < 0.05$). However, treatment with 5, 10, and 15 μg/mL SCH A significantly increased cell viability in Aβ_{25-35}-exposed SH-SY5Y and SK-N-SH cells ($p < 0.05$, Fig. 1A). Compared to the control subgroup, Aβ_{25-35} substantially increased the apoptosis rate in SH-SY5Y and SK-N-SH cells ($p < 0.05$, Fig. 1B,C). However, treatment with 10 and 15 μg/mL SCH A alleviated apoptosis in Aβ_{25-35}-treated cells ($p < 0.05$, Fig. 1B,C). Furthermore, no significant difference was found between the Aβ_{25-35} + 10 μg/mL SCH A and Aβ_{25-35} + 15 μg/mL SCH A groups ($p < 0.05$, Fig. 1B,C). Based on these findings, 10 μg/mL was selected as the optimal concentration for subsequent experiments.

SCH A Alleviates Aβ_{25-35}-Induced Oxidative Stress Injury

Analysis of oxidative stress markers revealed that Aβ_{25-35} substantially reduced SOD and GSH levels while increasing MDA levels compared to the control subgroup ($p < 0.05$, Fig. 2A,B). Conversely, except for the 1 μg/mL SCH A treatment, 5, 10, and 15 μg/mL SCH A substantially elevated SOD and GSH levels compared to the Aβ_{25-35} subgroup ($p < 0.05$, Fig. 2A,B). Furthermore, SCH A at 1, 5, 10, and 15 μg/mL decreased MDA levels in Aβ_{25-35}-exposed cells in a concentration-dependent manner ($p < 0.05$, Fig. 2A,B). The effects of SCH A on ROS were evaluated using a DCFH-DA fluorescence probe. Aβ_{25-35} substantially increased ROS levels compared to the control subgroup, while SCH A decreased ROS levels compared to the Aβ_{25-35} subgroup ($p < 0.05$, Fig. 2C). Overall, SCH A alleviated oxidative stress injury in SH-SY5Y and SK-N-SH cells treated with Aβ_{25-35}.

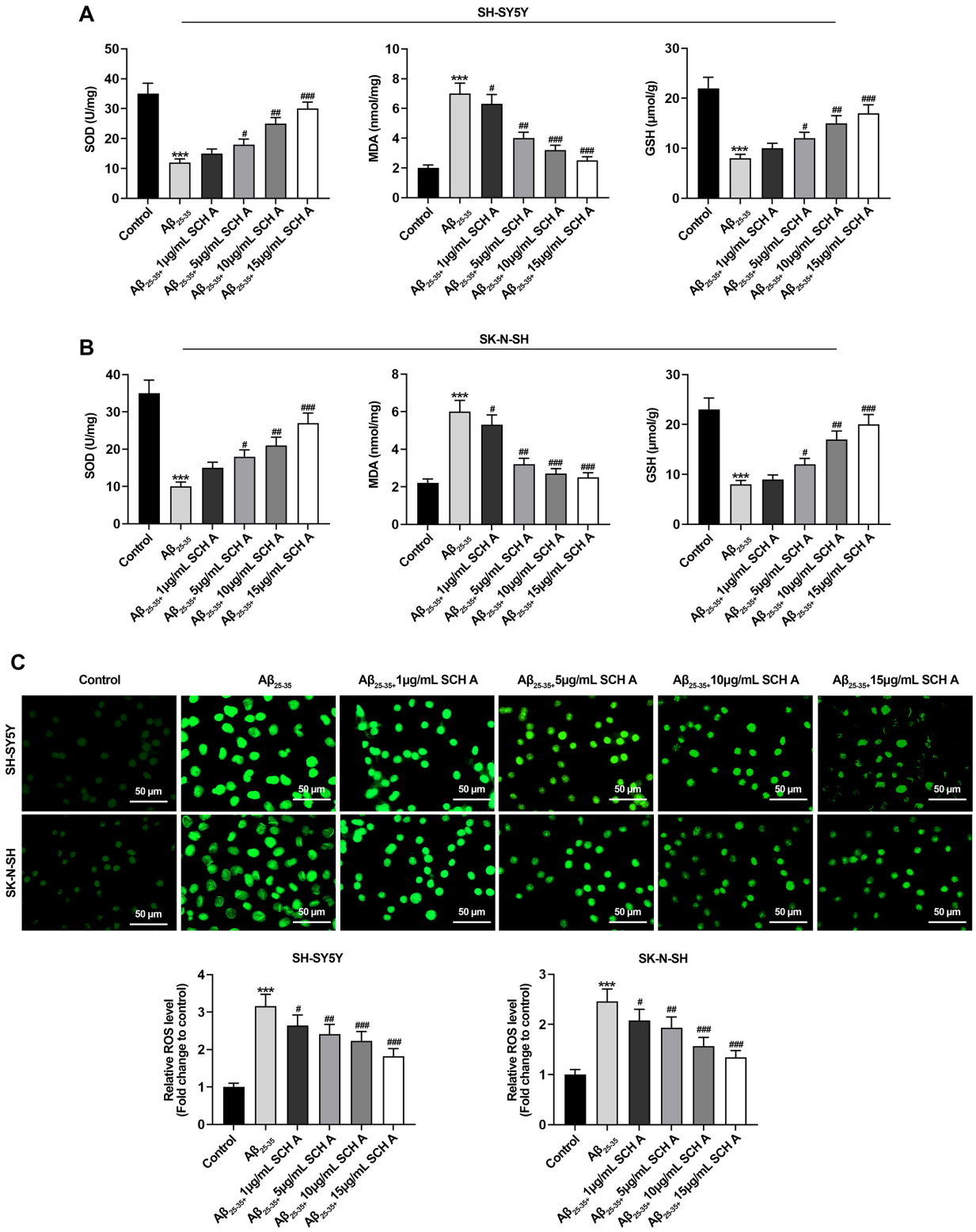

Fig. 2. SCH A treatment alleviates Aβ_{25-35}-induced oxidative stress injury. (A,B) The superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione (GSH) levels were assessed using corresponding kits. (C) 2′,7′-Dichlorodihydrofluorescein Diacetate (DCFH-DA) fluorescent probe was used to determine reactive oxygen species (ROS) contents in the cells. n = 3, $^{***}p < 0.001$ vs. control subgroup; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. Aβ_{25-35} subgroup.

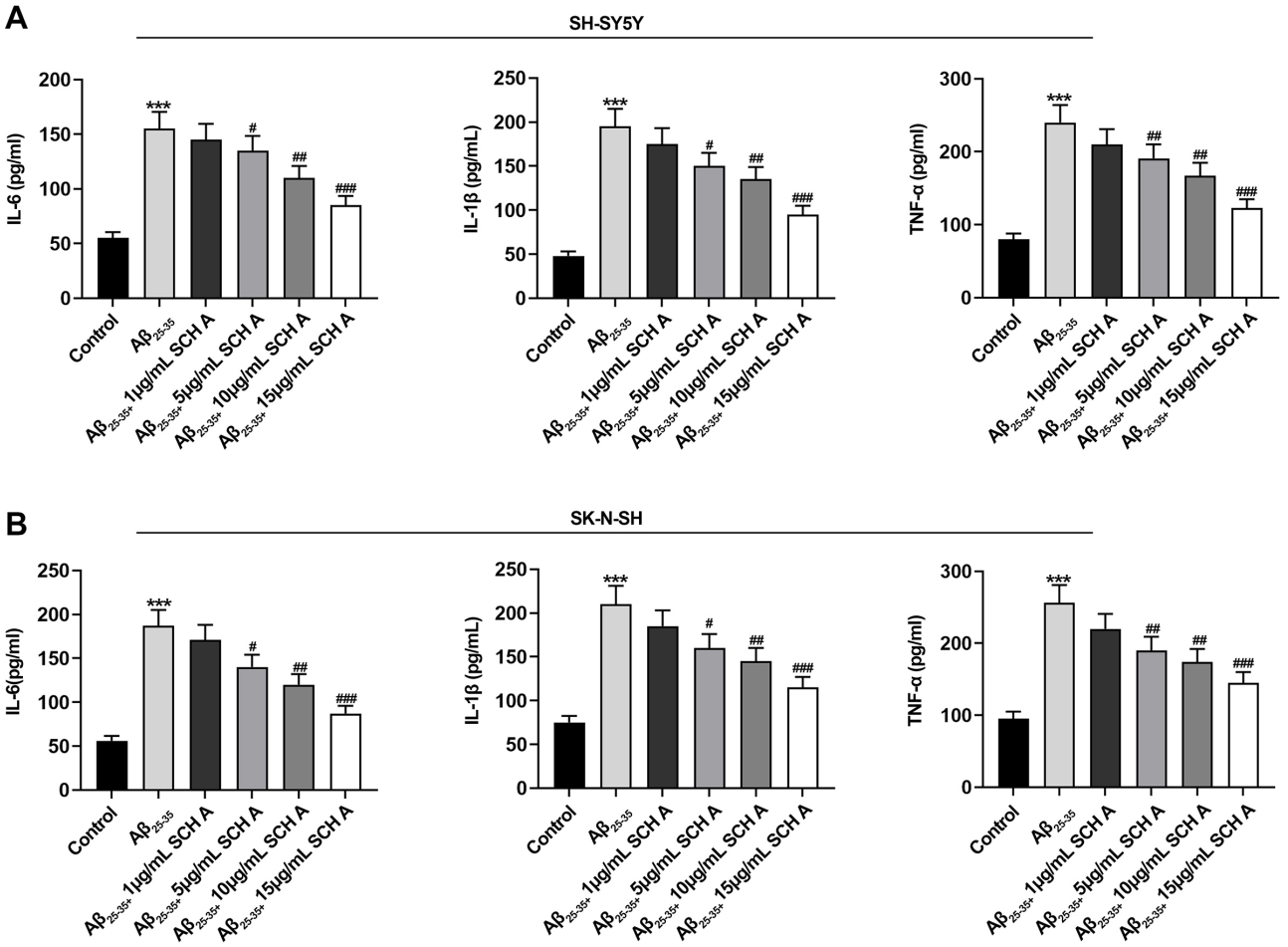

Fig. 3. SCH A treatment inhibits Aβ_{25-35}-induced inflammatory injury. (A,B) Enzyme-linked immunosorbent assay (ELISA) was used to assess interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF)-α levels. n = 3, $^{***}p < 0.001$ vs. control subgroup; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. Aβ_{25-35} subgroup.

SCH A Treatment Inhibits Aβ_{25-35}-Induced Inflammatory Injury

Compared to the control subgroup, Aβ_{25-35} substantially increased the cellular levels of IL-6, IL-1β, and TNF-α. Except for the 1 μg/mL SCH A treatment, 5, 10, and 15 μg/mL SCH A substantially reduced the levels of IL-6, IL-1β, and TNF-α in a concentration-dependent manner compared to the Aβ_{25-35} subgroup ($p < 0.05$, Fig. 3A,B).

ERK/MAPK Pathway was Associated with the Mechanism by which SCH A Improves the Damage Caused by Aβ_{25-35}

We observed that Aβ_{25-35} substantially down-regulated the expression levels of phospho-ERK1/2 (p-ERK1/2) protein compared to the control subgroup. However, compared to the Aβ_{25-35} subgroup, SCH A treatment significantly up-regulated the p-ERK1/2 to ERK1/2 ratio in a concentration-dependent manner ($p < 0.05$, Fig. 4A).

To validate whether ERK signaling is involved in the effect of SCH A on AD, SH-SY5Y cells were treated with the ERK activator LM22B-10. The ratio of p-ERK1/2 to ERK1/2 was substantially elevated in the Aβ_{25-35} + 10 μg/mL SCH A + LM22B-10 subgroup compared to the Aβ_{25-35} + 10 μg/mL SCH A subgroup ($p < 0.05$, Fig. 4B). Additionally, compared to the Aβ_{25-35} + 10 μg/mL SCH A subgroup, the Aβ_{25-35} + 10 μg/mL SCH A+LM22B-10 subgroup showed decreased apoptosis rates, alleviated levels of MDA, IL-6, and TNF-α, and elevated levels of SOD ($p < 0.05$, Fig. 4C–E). These findings indicate that the ERK signaling pathway plays a crucial role in the protective effect of SCH A on Aβ_{25-35}-exposed SH-SY5Y cells.

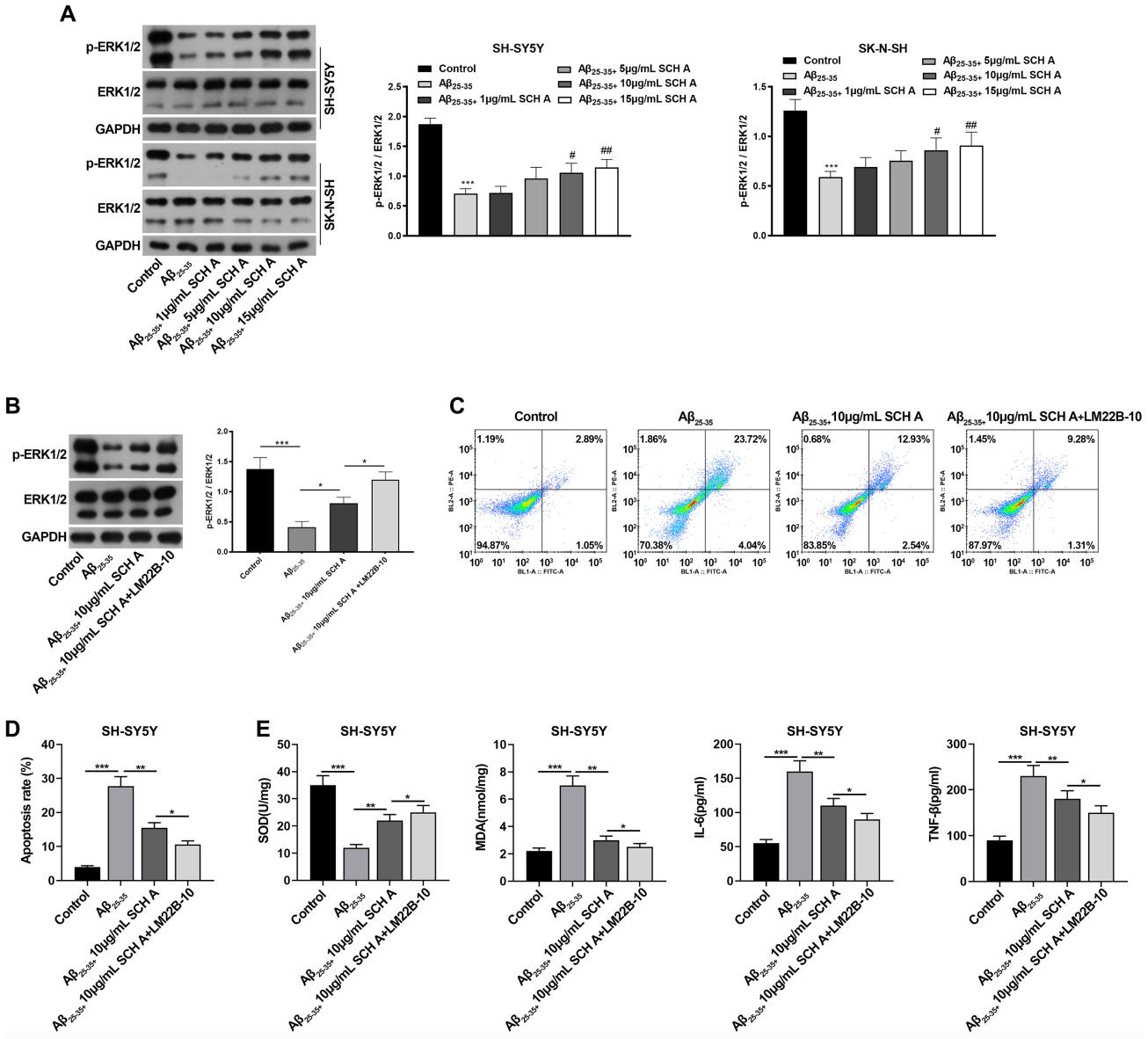

Fig. 4. Extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathway was associated with the mechanism by which SCH A improves the damage caused by Aβ_{25-35}. (A) Western blot analysis was used to determine the levels of ERK1/2 and p-ERK1/2 proteins. n = 3, $^{***}p < 0.001$ vs. control subgroup; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. Aβ_{25-35} subgroup. (B) SH-SY5Y cells were treated with ERK activator LM22B-10, and the levels of p-ERK1/2 and ERK1/2 proteins were assessed using Western blot analysis. (C,D) The apoptosis rate of each group was determined using flow cytometry. (E) SOD, MDA, IL-6, and TNF-α levels were examined using corresponding ELISA kits. n = 3, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Discussion

Oxidative stress plays a crucial role in the pathological process of AD. Normally, antioxidants, including SOD, Glutathione Peroxidase (GPX), and glutathione oxidoreduction, contribute to the treatment of AD [14]. In this study, we observed that compared to the control subgroup, Aβ_{25-35} substantially reduced SOD and GSH levels while increasing MDA and ROS levels in SH-SY5Y and SK-N-SH cells. Similarly, the inflammatory response is strongly linked to the progression of AD. During the pathogenesis of AD, over-activation of microglia results in the release of large amounts of inflammatory factors, disrupting the balance between pro- and anti-inflammatory cytokines. This process triggers a chronic and persistent inflammatory response, leading to neuronal damage and brain dysfunction

[15]. TNF-α plays a crucial role in regulating the cytokine cascade during inflammation, enhancing the inflammatory response, and ultimately leading to neuronal damage in AD. TNF-α works in synergy with IL-1β and IL-6 to promote the inflammatory response in the brain [16]. In our study, SH-SY5Y and SK-N-SH suffered inflammatory damage after exposure to Aβ_{25-35} and exhibited high levels of IL-6, IL-1β and TNF-α. This observation suggests that AD-related injury is accompanied by significant oxidative stress and inflammatory damage.

SCH A, the main active monomer component of *Schisandra chinensis*, possesses a wide range of pharmacological effects. It has shown potential in preventing and treating neurodegenerative diseases (AD and Parkinson's disease), with advantages such as regulating and improving sleep, providing antidepressant effects, and protecting against cerebral ischemia [17]. Our study revealed that different concentrations of SCH A increased the SOD and GSH levels while alleviating MDA and ROS levels. SCH A also decreased inflammatory factor levels compared to the model subgroup. These observations indicate that SCH A reduces the inflammatory and oxidative stress damages induced by Aβ_{25-35}.

The ERK/MAPK signaling pathway is associated with cell growth, division, differentiation, death, and functional synchronization between cells [18]. The activated ERK/MAPK signaling pathway can further activate a variety of downstream effectors, such as protein kinases and transcription factors, and regulate the expression of genes to act on the nervous system, thereby exerting a protective effect on slowing down AD-related pathological changes and inhibiting apoptosis [19]. In the present study, Aβ_{25-35} significantly reduced the ratio of p-ERK1/2 to ERK1/2 in SH-SY5Y and SK-N-SH cells compared to the control subgroup. Treatment with the ERK activator LM22B-10 substantially increased this ratio of p-ERK1/2 to ERK1/2 in SH-SY5Y cells and enhanced the effects of SCH A on reducing apoptosis and improving oxidative stress and inflammatory injury. These findings indicate that ERK/MAPK signaling pathway was associated with the anti-AD effect in SCH A, which may help reduce AD-related pathological damage and apoptosis by regulating a series of downstream effectors [11]. Despite several promising results, this study has the limitation of not having *in vivo* experimental support. Additionally, the role of the ERK/MAPK signaling pathway can be further validated using siRNA or pathway inhibitors. Furthermore, the mechanism by which SCH A activates the ERK/MAPK signaling pathway to regulate downstream effectors in AD needs further investigation. Additionally, future studies should address these limitations through relevant experiments.

Conclusion

In summary, SCH A exerts a protective effect on the damage of AD cell model induced by Aβ_{25-35}, substantially improving oxidative stress and inflammatory damage while reducing apoptosis. The activation of the ERK/MAPK signaling pathway may play a crucial role in the neuroprotective mechanism of SCH A.

Availability of Data and Materials

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

Author Contributions

STJ and HBG designed the research. HBG and SJZ performed the experiments. STJ and QL analyzed the data. STJ and QL jointly drafted the manuscript. All authors contributed to important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

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Conflict of Interest

The authors declare no conflict of interest.

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