

# Salidroside protects PC-12 cells against amyloid $\beta$ -induced apoptosis by activation of the ERK1/2 and AKT signaling pathways

ZHENG-LUAN LIAO<sup>1,2</sup>, HENG SU<sup>1,2</sup>, YUN-FEI TAN<sup>1,2</sup>, YA-JU QIU<sup>1,2</sup>, JUN-PENG ZHU<sup>1,2</sup>, YAN CHEN<sup>1,2</sup>, SI-SI LIN<sup>3</sup>, MING-HAO WU<sup>4</sup>, YAN-PING MAO<sup>3</sup>, JIAO-JIAO HU<sup>4</sup> and EN-YAN YU<sup>1,2,5</sup>

<sup>1</sup>Department of Psychiatry, Zhejiang Provincial People's Hospital; <sup>2</sup>Psychiatry and Mental Health, People's Hospital of Hangzhou Medical College, Hangzhou, Zhejiang 310014; <sup>3</sup>Psychiatry and Mental Health, Zhejiang Chinese Medical University, Hangzhou, Zhejiang 310053; <sup>4</sup>Psychiatry and Mental Health, Bengbu Medical College, Bengbu, Anhui 233030; <sup>5</sup>Clinical Psychology, Zhejiang Cancer Hospital, Hangzhou, Zhejiang 310022, P.R. China

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**Abstract.** Alzheimer's disease (AD) is one of the most frequent diseases in elderly people and causes high mortality. Its incidence is increasing annually and no effective therapeutic treatment currently exists. In the present study, salidroside, a major active ingredient of *Rhodiola rosea*, was able to protect PC-12 cells from the toxicity and apoptosis induced by AD inducer amyloid (A $\beta$ )<sub>1-42</sub>. Salidroside significantly protected PC-12 cells by inhibiting A $\beta$ <sub>1-42</sub>-induced cytotoxicity and mitochondria-mediated endogenous caspase apoptotic pathways. Mechanistic studies demonstrated that salidroside significantly activated the extracellular signal regulated kinase (ERK)1/2 and protein kinase B (AKT) signaling pathways. This observation was further confirmed using the ERK1/2 inhibitor PD98059 and the AKT inhibitor LY294002, which demonstrated that salidroside promoted PC-12 cell survival and proliferation by activating the ERK1/2 and AKT signaling pathways. Salidroside is a therapeutic candidate for the treatment of AD and provides a basis for further drug development.

## Introduction

Alzheimer's disease (AD) is a major neurodegenerative disease characterized by significant memory impairment in addition to other cognitive impairments and maybe associated with mental symptoms and behavioral abnormalities (1-3). AD is prevalent in the elderly of >65 years of age, with 1 newly

diagnosed case every 3 sec worldwide (4). The number of patients with AD is estimated to increase to 135.46 million by 2050, thereby leading to serious social issues in addition to the health, economic and social burden (5).

The accumulation of amyloid  $\beta$  (A $\beta$ ) and mutation of gene polymorphisms of clusterin (CLU) can be attributed to AD (6,7). A $\beta$  is cleaved from the amyloid precursor protein and a mutation in CLU causes the brain to lose its A $\beta$ -scavenging function. Of note, an excessive accumulation of A $\beta$  has been detected in the brains of patients with AD (8-10). Accumulation of A $\beta$  can eventually cause the cytotoxic death of nerve cells due to oxidative stress, decreased mitochondrial membrane potential and nuclear pyknosis (11-13). At present, modulation of A $\beta$ -induced neurotoxicity is an effective therapeutic approach for the treatment of AD; however, there are no safe and effective therapeutics for AD at present (14-16). Therefore, the identification and evaluation of potential protective candidates for AD treatment are necessary.

Salidroside is a major active ingredient extracted from *Rhodiola rosea* (17); it is widely employed in traditional Chinese medicine and has been reported to exert anti-inflammatory, anti-oxidative and anti-autophagic effects (18-20). Based on these functions, it has been speculated that salidroside may be able to treat AD. However, the molecular mechanisms underlying these effects of salidroside are currently not well understood and further research is required to clarify them. The present study aimed to confirm the anti-AD effects of salidroside and unravel its mechanism of action. The toxic effects of A $\beta$  in the PC-12 cell line were established for use as an *in vitro* AD model for drug evaluation (21). The results revealed that salidroside could effectively inhibit the toxicity and apoptosis of PC-12 cells that was induced by A $\beta$ . Furthermore, the protective effect of salidroside against A $\beta$ -induced damage in PC-12 cells was mediated by activation of the extracellular signal regulated kinase (ERK)1/2 and protein kinase B (AKT) signaling pathways. By promoting cell survival and proliferation, the toxic effects of A $\beta$  were effectively inhibited by salidroside, thereby further demonstrating that salidroside is a potential candidate for AD treatment.

**Correspondence to:** Dr En-Yan Yu, Department of Psychiatry, Zhejiang Provincial People's Hospital, 158 Shang-Tang Road, Hangzhou, Zhejiang 310014, P.R. China  
E-mail: yuenyan@aliyun.com

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## Materials and methods

**Cell viability assay.** Cell viability was evaluated using cytotoxicity assays. Briefly, PC-12 cells were seeded into 96-well plates with 5,000 cells per well and incubated with drugs or inhibitors at the indicated concentrations for 48 h. The salidroside was added at the concentrations of 12.5, 25, 50, 100 or 200  $\mu$ M;  $A\beta_{1-42}$  was added at concentrations of 0.01 to 1  $\mu$ M; while the inhibitors were added at concentrations of 5 to 20  $\mu$ M. A volume of 25  $\mu$ l MTT solution (5 mg/ml) was added to each well and incubated for additional 4 h. Then, dimethyl sulfoxide (DMSO) was added to dissolve the MTT formazan product and the absorbance was determined at 570 nm using a SpectraMax M5 device (Molecular Devices, LLC, Sunnyvale, CA, USA). The relative cell viability rates of the test group were calculated vs. the untreated controls.

**Lactate dehydrogenase (LDH) assay.** The quantity of LDH that is released into the incubation medium when the cell membrane is destroyed was determined for evaluation of  $A\beta$ -induced cytotoxicity. A total of  $5 \times 10^3$  PC-12 cells were seeded into 96-well plates. After incubation with 50  $\mu$ M salidroside for 1 h, followed by incubation with  $A\beta$  for additional 24 h, the activity of the LDH that was released into the medium was determined according to the protocol of CytoTox-ONE™ Homogeneous Membrane Integrity Assay (Promega Corporation, Madison, WI, USA). The fluorescent intensity was determined using a microplate reader (Multiskan™ FC; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at an excitation wavelength of 560 nm and an emission wavelength of 590 nm. The percentage values of the released LDH were normalized to the control group.

**Nuclear staining.** PC-12 cells were fixed with 3.7% formaldehyde in PBS for 5 min at room temperature and blocked with 5% bovine serum albumin (Sangon Biotech Co., Ltd., Shanghai, China) containing 0.1% Triton X-100 (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) for 30 min at room temperature. The prepared specimens were stained with DAPI (SouthernBiotech, Birmingham, AL, USA) at a concentration of 10  $\mu$ g/ml at room temperature for 2 min and then observed under a confocal microscope (Nikon Corporation, Tokyo, Japan). Apoptotic cells exhibited nuclear condensation with intensely stained nuclei, while the normal cells did not exhibit nuclear condensation (22).

**Determination of intracellular reactive oxygen species (ROS), malondialdehyde (MDA) and superoxide dismutase (SOD) levels.** Cells were seeded in 6-well plates at a density of  $5 \times 10^4$  cells/cm<sup>2</sup>. After 24 h of incubation, cells were exposed to 50  $\mu$ M salidroside for 1 h and to 0.3  $\mu$ M  $A\beta$  for 24 h. Then, some of the cells were incubated with 5  $\mu$ M fluorescent probe of H2DCF-DA for 20 min and the fluorescence emission was visualized using a fluorescence microscope (Nikon Corporation). The other cells were analysed using the MDA and SOD detection kits (KeyGen Biotech Co., Ltd., Nanjing, China).

**Determination of mitochondrial membrane potential.** PC-12 cells were seeded into a black 96-well plate with

5,000 cells per well, then incubated with 50  $\mu$ M salidroside for 1 h, followed by incubation with  $A\beta$ . Following 24 h, cells were incubated with a fluorescent probe of JC-1 (Beyotime Institute of Biotechnology, Haimen, China) at a concentration of 5 mg/ml for 15 min at 37°C and then washed twice with PBS. The intensity of red and green fluorescence was determined using an Infinite M200 PRO Multimode Microplate (Tecan Group, Ltd., Mannedorf, Switzerland) and a fluorescence microscope (Nikon Corporation). The mitochondrial membrane potential was calculated using the ratio of JC-1 red/green fluorescence intensity, the color changed from red to green when apoptosis occurred and the value was normalized to the control group.

**Caspase-3/7 activity assay.** Once PC-12 cells had been incubated with salidroside for 1 h and with  $A\beta$  for 24 h, the cells were lysed with lysis buffer of radioimmuno-precipitation assay (RIPA; KeyGen Biotech Co., Ltd.) and centrifuged at 12,500  $\times$  g for 5 min at 4°C. Then, the cell lysate was incubated with 2X substrate working solution at room temperature for 30 min in 96-well plates. The activity of caspase-3/7 was determined using the commercially available Caspase-Glo 3/7 Assay (Invitrogen; Thermo Fisher Scientific, Inc.). The fluorescence intensity of each sample was normalized to the protein concentration of the sample. All the percentage values of caspase-3/7 activities were normalized to the control group.

**Flow cytometry assay.** PC-12 cell apoptosis was evaluated with the Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) Apoptosis Detection kit (KeyGen Biotech Co., Ltd.). Upon treatment, the adherent and non-adherent cells were harvested. The cells were then stained with Annexin V-FITC and PI in binding buffer for 15 min. Flow cytometric analysis was performed on a fluorescence-activated cell sorting flow cytometer (BD Biosciences; Franklin Lakes, NJ, USA) and the data were analyzed with Cell Quest software (version 3.4, BD Biosciences).

**Western blotting.** The cells were lysed with RIPA buffer, the concentration of proteins were determined by Bicinchoninic Acid detection kit (KeyGen Biotech Co., Ltd.). Immunoblots were performed with samples that contained total protein (30  $\mu$ g), which had been separated by 12% SDS-PAGE gel and then transferred onto polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA, USA). The membranes were incubated with primary antibodies against phosphorylated (p)-ERK1/2 (1:1,000; AF1015; Affinity Biosciences, Cincinnati, OH, USA), total (t)-ERK (1:1,000; AF6240; Affinity Biosciences), p-AKT (1:1,000; AF0016; Affinity Biosciences), t-AKT (1:1,000; AF4718; Affinity Biosciences), B-cell lymphoma (BCL)-2 (1:1,000; AF6139; Affinity Biosciences), BCL2 associated X (Bax; AF0120; 1:1,000; Affinity Biosciences) and GAPDH (1:1,000; AF7021; Affinity Biosciences), followed by incubation with a secondary antibody of goat anti-rabbit IgG-horseradish peroxidase (1:5,000; sc-2004; Santa Cruz Biotechnology, Inc., Dallas, TX, USA). Blots were developed using an enhanced chemiluminescence detection reagent (EMD Millipore). GAPDH was used as an internal loading control.

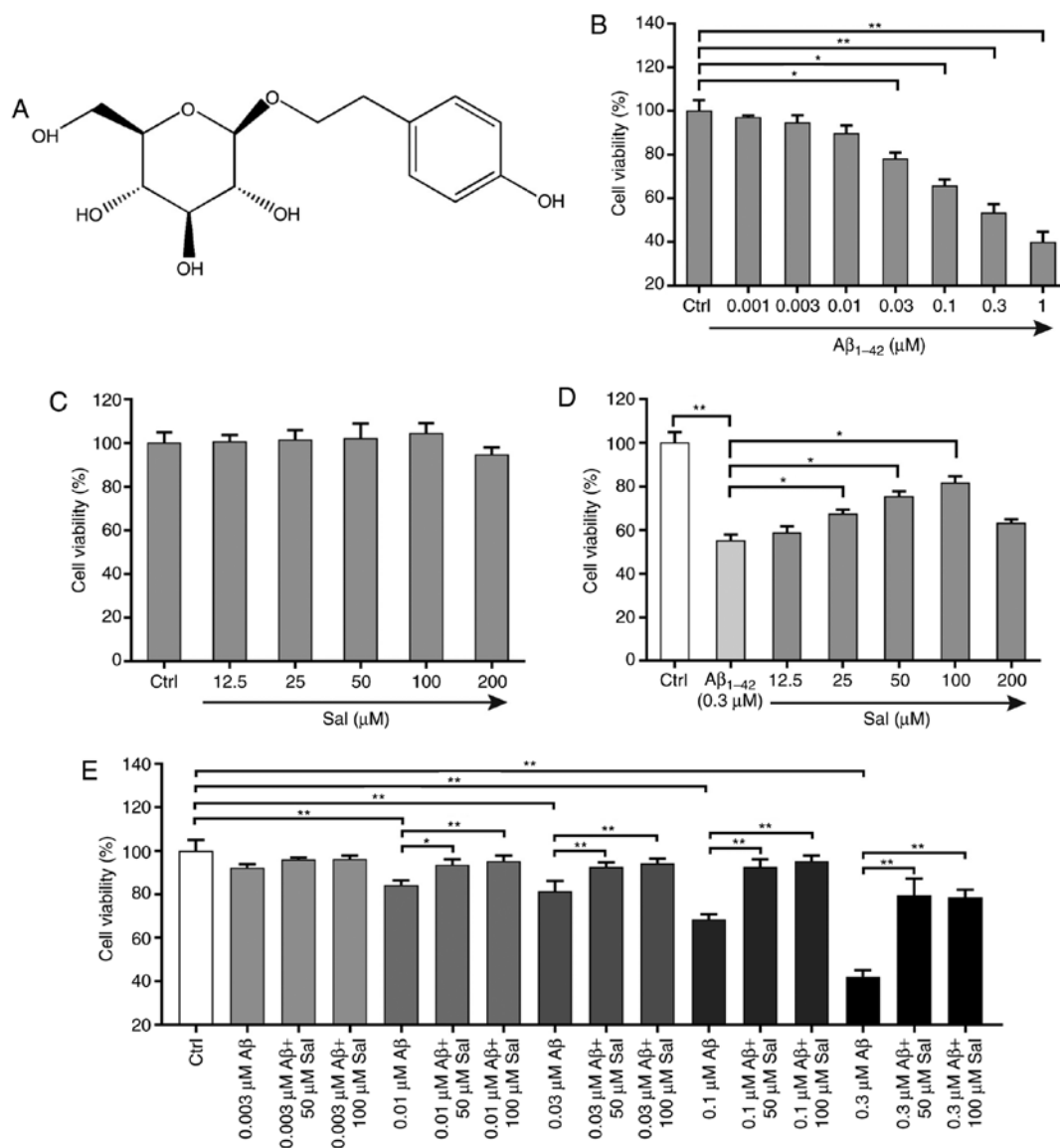


Figure 1. Protective effects of salidoside against  $A\beta_{1-42}$ -induced injury in PC-12 cells. (A) Chemical structure of salidoside. (B)  $A\beta_{1-42}$ -induced toxicity in PC-12 cells. (C) Cytotoxicity of salidoside in PC-12 cells. (D) Effects of salidoside on  $A\beta_{1-42}$ -induced loss of cell viability in PC-12 cells. (E) PC-12 cells were incubated with 0.003-0.3  $\mu\text{M}$   $A\beta_{1-42}$  for 30 min and post-treated with salidoside (50 or 100  $\mu\text{M}$ ) for an additional 24 h. Next, cell viability was measured by MTT assay. Data are presented as the mean  $\pm$  standard deviation ( $n=3$ ). \* $P<0.05$  and \*\* $P<0.01$ . Ctrl, control. A $\beta$ , amyloid  $\beta_{1-42}$ .

**Statistical analysis.** All experiments were repeated at least in triplicate, all data were evaluated using SPSS v.19.0 (IBM Corp., Armonk, NY, USA). Data are presented as the mean  $\pm$  standard deviation. One-way analysis of variance and Bonferroni's test were used to compare different treatment samples.  $P<0.05$  was considered to indicate a statistically significant difference.

## Results

**Salidoside suppresses  $A\beta_{1-42}$ -induced cytotoxicity in PC-12 cells in a concentration-dependent manner.** The biological activity of salidoside (Fig. 1A) was assessed by cell viability assays. The cytotoxicity of  $A\beta_{1-42}$  on PC-12 cells was first examined by MTT assay. As presented in Fig. 1B, exposure of PC-12 cells to  $A\beta_{1-42}$  (0.001-1  $\mu\text{M}$ ) for 24 h induced a dose-dependent decrease in cell viability, thereby demonstrating that  $A\beta_{1-42}$  could induce toxicity in PC-12 cells. The viability of

PC-12 cells was only increased or slightly decreased and the differences compared with the control group were not significant, following treatment with salidoside (12.5-200  $\mu\text{M}$ ) for 24 h, thereby indicating that salidoside was not toxic to PC-12 cells under these treatment conditions (Fig. 1C). Compared with the  $A\beta_{1-42}$  group, pre-treatment with salidoside at concentrations of 25, 50 and 100  $\mu\text{M}$  significantly improved cell viability ( $P<0.05$ ; Fig. 1D). The multi-combination test results further demonstrated that salidoside protected and rescued PC-12 cells from  $A\beta_{1-42}$ -induced cell death (Fig. 1E).

**Salidoside suppresses  $A\beta_{1-42}$ -induced LDH release and apoptosis in PC-12 cells.** LDH assay was used for evaluation of the protective activity of salidoside. When pre-treated with 50  $\mu\text{M}$  salidoside for 1 h, PC-12 cells exhibited a significantly reduced  $A\beta_{1-42}$ -induced LDH leakage (from 135-110% relative to the control group;  $P<0.01$ ; Fig. 2A). Nuclei condensation was detected in PC-12 cells upon exposure to  $A\beta_{1-42}$  by DAPI

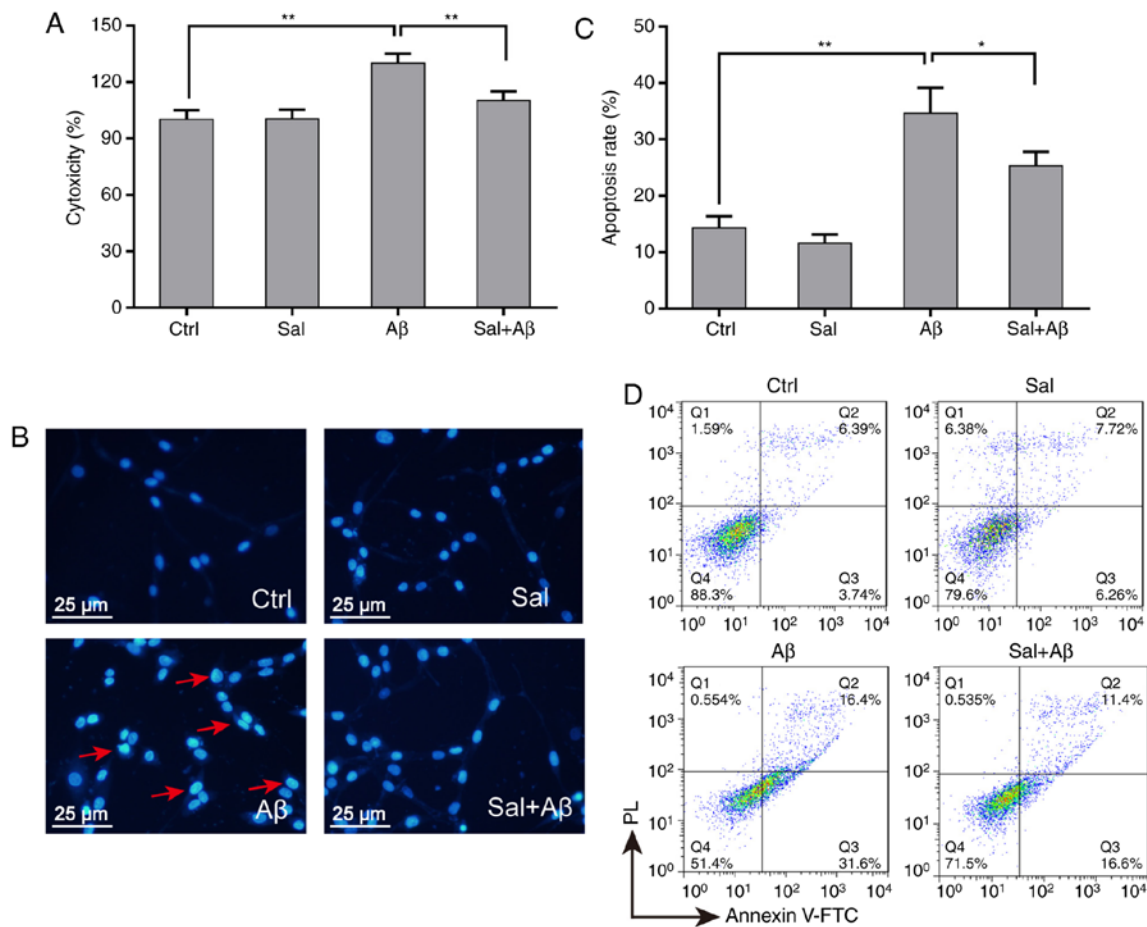


Figure 2. Sal suppresses A $\beta_{1-42}$ -induced LDH release and apoptosis in PC-12 cells. Cells were treated with 50  $\mu$ M Sal for 1 h prior to exposure to 0.3  $\mu$ M A $\beta_{1-42}$  for 24 h. (A) Release of LDH was determined by LDH assay. (B) Apoptotic cells were detected by staining with 4',6-diamidino-2-phenylindole. The typical apoptotic cells are marked by arrows. (C) The number of apoptotic nuclei with condensed chromatin was counted from the photomicrographs and presented as a percentage of the total number of nuclei. (D) Representative flow cytometric analysis of apoptosis revealed that salidroside protected PC-12 cells from A $\beta_{1-42}$ -induced apoptosis. Data are presented as the mean  $\pm$  standard deviation (n=3). \*P<0.05 and \*\*P<0.01. A $\beta$ , amyloid  $\beta_{1-42}$ ; LDH, lactate dehydrogenase; Sal, salidroside; FITC, fluorescein isothiocyanate; PI, propidium iodide; Ctrl, control.

staining (Fig. 2B). Pre-treatment with 50  $\mu$ M salidroside significantly inhibited A $\beta_{1-42}$ -induced apoptosis compared with A $\beta_{1-42}$  alone (from 35-23%; P<0.05 Fig. 2C). To further evaluate the influence of salidroside in response to oxidative stress in PC-12 cells, the cells were treated with A $\beta_{1-42}$  for 24 h following being incubated with salidroside for 1 h. Cell apoptosis rates were detected by flow cytometry (Fig. 2D). The apoptosis rate of PC-12 cells increased following 24 h of A $\beta_{1-42}$  treatment, while salidroside treatment could protect PC-12 cells from A $\beta_{1-42}$ -induced apoptosis.

*Salidroside affects A $\beta_{1-42}$  induced ROS, MDA and SOD productions in PC-12 cells.* Since the toxicity of A $\beta_{1-42}$  may be affected by the generation of ROS, MDA and SOD (23), the inhibitory/promotional effect of salidroside on ROS, MDA, and SOD production was examined. PC-12 cells were pre-treated with or without 50  $\mu$ M salidroside for 1 h and then treated with A $\beta_{1-42}$  for 24 h. Differences in fluorescence intensity were observed among the different groups (Fig. 3A). The results revealed that A $\beta_{1-42}$  could effectively induce intracellular ROS generation, whereas salidroside could significantly inhibit the generation of ROS induced by A $\beta_{1-42}$  (from 145 to 125% relative to control group; P<0.05; Fig. 3B). While the MDA detection

results demonstrated that the MDA levels were significantly increased due to oxidative stress injury but significantly decreased by salidroside pre-treatment (P<0.01; Fig. 3C). By contrast, the SOD activity in salidroside-pre-treated cells increased compared with A $\beta$ -treated cells (Fig. 3D).

*Salidroside improves A $\beta_{1-42}$ -induced alterations in the mitochondrial membrane potential and caspase-3/7 activity.* Previous studies reported that the loss of the mitochondrial membrane potential was involved in the progression of neuron apoptosis caused by A $\beta_{1-42}$  during AD (24,25). In the present study, mitochondrial membrane permeability was detected using the JC-1 probe in order to evaluate the anti-apoptotic effects of salidroside. Red and green fluorescence represented high mitochondrial membrane permeability in viable cells and low mitochondrial membrane permeability in apoptotic cells, respectively (Fig. 4A). When incubated with A $\beta_{1-42}$  for 24 h, the mitochondrial membrane permeability was significantly depolarized in A $\beta_{1-42}$ -treated PC-12 cells compared with the control (P<0.01), whereas pre-treatment with salidroside effectively prevented the loss of mitochondrial membrane permeability (Fig. 4B). Treatment of PC-12 cells with 0.3  $\mu$ M A $\beta_{1-42}$  for 24 h triggered a significant increase in caspase-3/7

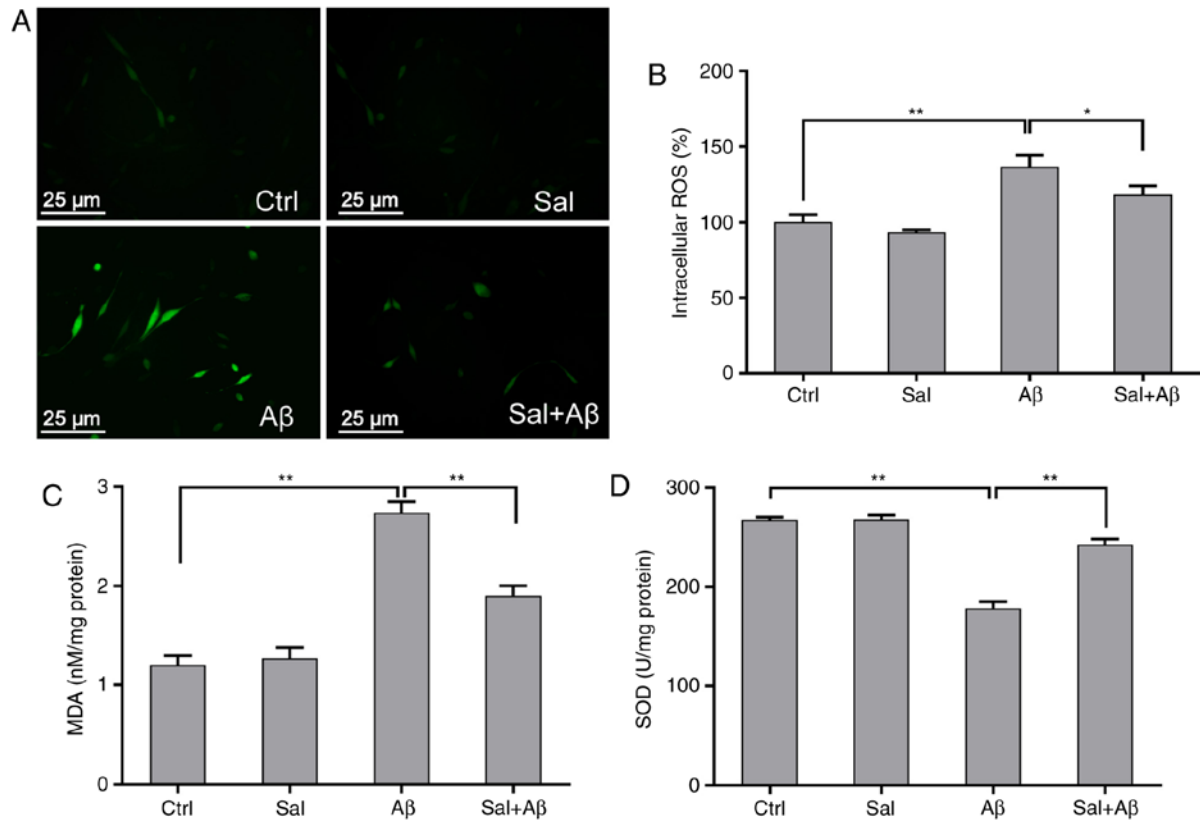


Figure 3. Effects of salidroside on A $\beta_{1-42}$ -induced ROS, MDA and SOD production in PC-12 cells. Cells were treated with 50  $\mu$ M salidroside for 1 h prior to exposure to 0.3  $\mu$ M A $\beta_{1-42}$  for 24 h. (A) Intracellular ROS levels were determined by fluorescence microscopy. (B) Sal could inhibit the generation of ROS induced by A $\beta_{1-42}$ . (C) Sal inhibited MDA content in A $\beta$ -treated PC-12 cells. (D) Sal restored SOD activity in A $\beta$ -treated PC-12 cells. Data are presented as the mean  $\pm$  standard deviation (n=3). \*P<0.05 and \*\*P<0.01. A $\beta$ , amyloid  $\beta_{1-42}$ ; ROS, reactive oxygen species; MDA, malondialdehyde; SOD, superoxide dismutase; Sal, salidroside; Ctrl, control.

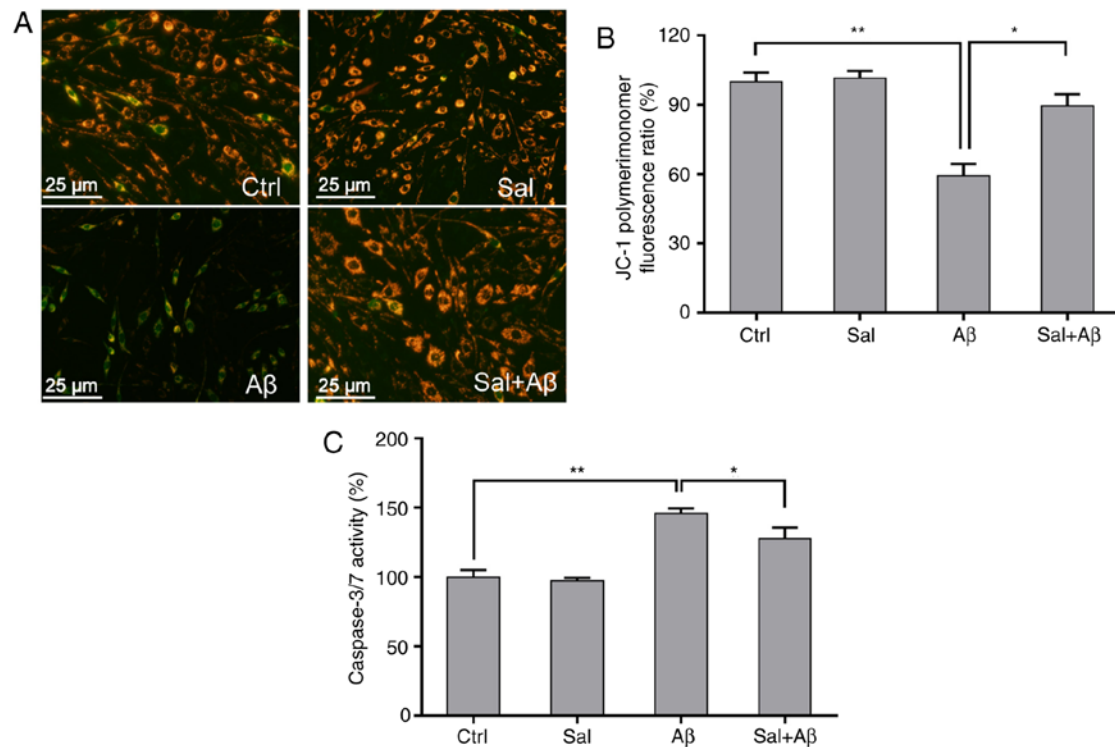


Figure 4. Protective effects of Sal against A $\beta_{1-42}$ -induced mitochondrial dysfunction in PC-12 cells. Cells were treated with 50  $\mu$ M salidroside for 1 h prior to exposure to 0.3  $\mu$ M A $\beta_{1-42}$  for 24 h. (A) Effect of Sal on mitochondrial membrane potential. (B) The mitochondrial membrane potential in each group was calculated as the ratio of red to green fluorescence of JC-1 staining. (C) Quantification of caspase-3/7 activity was determined by caspase-3/7 activity assay. Data are presented as the mean  $\pm$  standard deviation (n=3). \*P<0.05 and \*\*P<0.01. A $\beta$ , amyloid  $\beta_{1-42}$ ; Sal, salidroside; Ctrl, control.

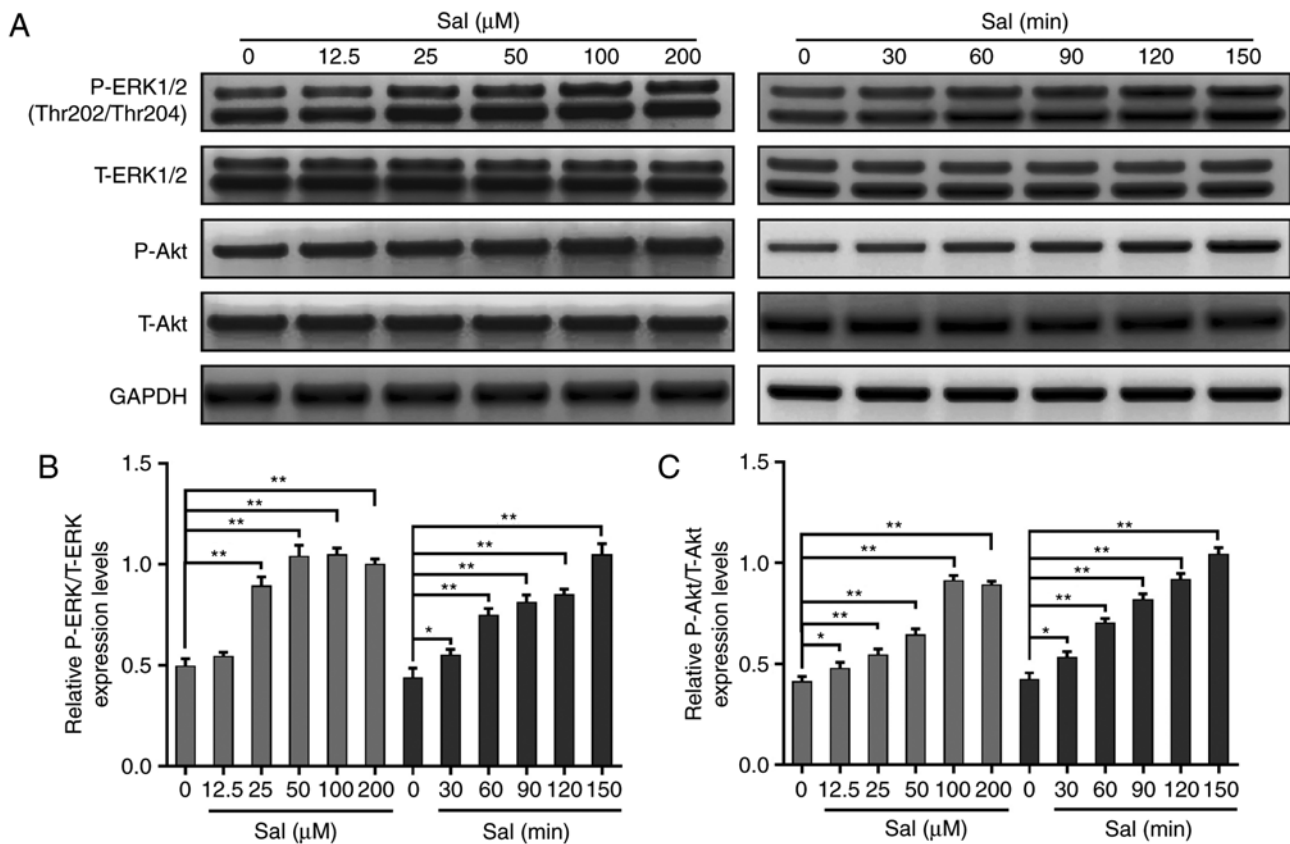


Figure 5. Effects of Sal on the ERK1/2 and AKT signaling pathways. (A) PC-12 cells were collected upon Sal treatment at different concentrations for 1 h or for different times at 50  $\mu$ M. Effects of Sal on the protein expression levels of P-ERK1/2, T-ERK1/2, P-AKT and T-AKT. (B) Quantification of P-ERK/T-ERK expression analysis by western blotting. (C) Quantification of P-AKT/T-AKT expression analysis by western blotting. Data are presented as the mean  $\pm$  standard deviation (n=3). \*P<0.05 and \*\*P<0.01. ERK1/2, extracellular signal-regulated kinases 1/2; AKT, protein kinase B; T, total; P, phosphorylated; Sal, salidroside.

activity compared with the control (P<0.01), whereas pre-treatment with 50  $\mu$ M salidroside for 1 h significantly inhibited caspase-3/7 activity (P<0.05; Fig. 4C).

*Salidroside stimulates the phosphorylation of ERK1/2 and AKT in a time and concentration-dependent manner in PC-12 cells.* Since the ERK1/2 and AKT signaling pathways are classical apoptosis-associated pathways, the present study evaluated whether the anti-apoptotic effect of salidroside was mediated by the ERK1/2 and AKT signaling pathways. The present study examined the phosphorylated and total expression levels of ERK1/2 and AKT in PC-12 cells treated with salidroside by western blotting. As presented in Fig. 5A-C, the phosphorylation levels of ERK1/2 and AKT gradually increased upon the addition of salidroside in a time and dose-dependent manner.

*Activation of the ERK1/2 and AKT signaling pathways mediates the protective effects of salidroside in  $A\beta_{1-42}$ -induced PC-12 cells.* To confirm the roles of the ERK1/2 and AKT signaling pathways in the inhibitory effect of salidroside on  $A\beta_{1-42}$ -induced apoptosis in PC-12 cells, the specific inhibitors of the ERK1/2 and AKT signaling pathways, PD98059 and LY294002, respectively, were used. The two pathway inhibitors blocked the protective effects of salidroside in cells treated with  $A\beta_{1-42}$  (Fig. 6A). PC-12 cells were pre-treated with

PD98059 or LY294002 (5, 10 and 20  $\mu$ M) for 30 min and then treated with 50  $\mu$ M salidroside for 1 h, and the viability of cells was determined by MTT assay 24 h later. The protective effect of salidroside was blocked in the presence of an all concentrations of PD98059 and LY294002 (Fig. 6B and C). Upon staining with DAPI, pyknosis was detected in treated PC-12 cells (Fig. 6D). Pre-treatment with 50  $\mu$ M salidroside reversed the effect of  $A\beta_{1-42}$  on PC-12 cells, whereas incubation with PD98059 or LY294002 abolished the protective effect of salidroside (Fig. 6E). The western blot results further demonstrated that upon treatment of cells with  $A\beta_{1-42}$ , the incubation with PD98059 or LY294002 for 24 h inhibited the phosphorylation of ERK1/2 and AKT, respectively. Salidroside reversed the decrease in the phosphorylation levels of ERK1/2 and AKT induced by  $A\beta_{1-42}$ , whereas PD98059 and LY294002 pre-treatment blocked the reversing effects of salidroside (Fig. 6F). The anti  $A\beta$ -induced apoptotic effect of salidroside may be mediated by other signaling pathways, such as BCL-2/Bax (Fig. S1); however, further investigation is required.

## Discussion

AD is a multigenetic neurodegenerative disease caused by genetic and environmental factors (26). It is associated with a series of physiological and pathological mechanisms, including



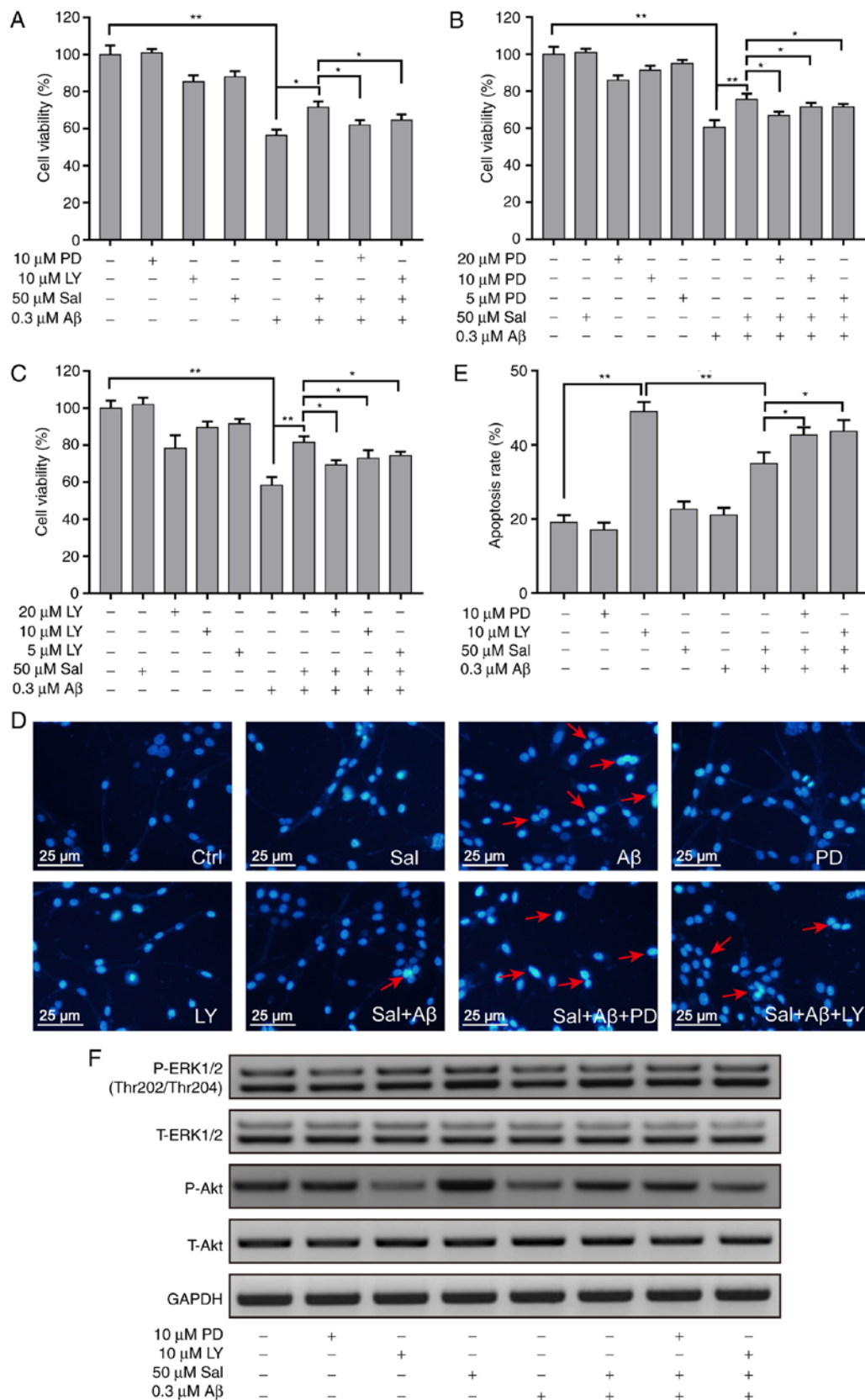


Figure 6. The ERK1/2 and AKT signaling pathways mediate the protective effects of salidroside in PC-12 cells. PC-12 cells were exposed to 50 μM Sal for 1 h with or without pre-treatment with (A) 10 μM PD98059 and 10 μM LY294002 for 1 h, or (B) 5, 10 and 20 μM PD98059, or (C) 5, 10 and 20 μM LY294002, prior to being stimulated with 0.8 mM Aβ<sub>1-42</sub> for 24 h. Next, cell viability was determined by MTT assay. (D) Apoptotic cells were detected by staining with 4',6-diamidino-2-phenylindole and visualized by fluorescence microscopy. The typical apoptotic cells were marked by arrows. (E) The number of apoptotic nuclei with condensed chromatin was counted from the photomicrographs and presented as a percentage of the total number of nuclei. (F) PC-12 cells were pre-treated with the ERK1/2 inhibitor PD98059 (10 μM) or the AKT inhibitor LY294002 (10 μM) for 30 min and then treated with 50 μM Sal for 1 h. Subsequently, the cells were incubated with or without 0.3 μM Aβ<sub>1-42</sub>. The expression levels of P-ERK1/2, ERK1/2, P-AKT, AKT and GAPDH were detected by western blotting. Data are presented as the mean ± standard deviation (n=3). \*P<0.05 and \*\*P<0.01. ERK1/2, extracellular signal-regulated kinases 1/2; AKT, protein kinase B; Aβ, amyloid β<sub>1-42</sub>; P, phosphorylated; T, total; phosphorylated; Sal, salidroside; Ctrl, control.

A $\beta$  accumulation, abnormal phosphorylation of Tau protein, lipid metabolism, inflammatory reaction and oxidative stress (6,10,11,27). Although hypotheses have been proposed for its pathogenesis, no theory has been completely validated thus far (28). The above physiological and pathological causes eventually lead to a neuroinflammatory reaction at the disease site, thereby resulting in nerve cell damage or apoptosis (29). Therefore, compounds that can effectively inhibit the nerve cell apoptosis caused by cytotoxicity-like oxidative stress may be potential therapeutic candidates for AD.

Salidroside has been reported to have therapeutic effects on various diseases including cancer, pulmonary fibrosis and cerebrovascular disease, mainly due to its pharmacological activities, including anti-fatigue, anti-aging, anti-apoptosis, immune regulation and free-radical scavenging (19,30,31). Based on these characteristics, salidroside was selected in the present study to detect its effects on AD treatment. The toxic effects of A $\beta$  on the PC-12 cell line were used in the present study for the *in vitro* screening model. A $\beta$  is a small peptide that consists of 42 amino acids and is cleaved from its precursor protein. The full length and fragments of A $\beta$  include A $\beta$ <sub>1-42</sub>, A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>25-35</sub>, which can be used as an inducer (32). Among these fragments, A $\beta$ <sub>1-42</sub> has the best induction effect of cell apoptosis (33). Therefore, A $\beta$ <sub>1-42</sub> was used in the present study to establish an *in vitro* AD model and to conduct pharmacodynamic tests. Salidroside effectively improved cell apoptosis induced by cell pyknosis, oxidative stress and mitochondrial membrane potential decrease in A $\beta$ -induced PC-12 cells. Therefore, salidroside was also most likely to exhibit activity for treating AD *in vivo* systems, which needs further evaluation.

Apoptosis involves multiple signaling pathways, including ERK1/2 and AKT (34,35). Therefore, upon confirmation of the anti-apoptotic effect of salidroside, the effect of salidroside on these two signaling pathways was examined. Salidroside significantly activated the ERK1/2 and AKT signaling pathways. To further confirm the effect exerted by the ERK1/2 and AKT signaling pathways, the ERK1/2 inhibitor PD98059 and the AKT inhibitor LY294002 were used (36,37). The results were consistent with those from previous experiments.

In conclusion, salidroside effectively inhibited the apoptosis of A $\beta$ -induced PC-12 cells by activating the ERK1/2 and AKT signaling pathways, thereby indicating that salidroside is a potential candidate for the treatment of AD. The present study provides a basis for further drug development.

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## Availability of data and materials

The data used and analyzed in this study are available from the corresponding author on reasonable request.

## Authors' contributions

EYY and ZLL made substantial contributions to the design of the present study. HS, YFT, YJQ, and JPZ performed the cell viability and apoptosis-associated experiments. YC, SSL and MHW performed all other experiments. ZLL, YPM, and JJH analyzed data. EYY and ZLL wrote the manuscript. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

No human trials were involved in this study.

## Competing interests

The authors declare that they have no competing interests.

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