Scutellarein suppresses Aβ-induced memory impairment via inhibition of the NF-κB pathway in vivo and in vitro

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Abstract. The flavonoid compound scutellarin (Scu) is a traditional Chinese medicine used to treat a variety of diseases; however, the use of scutellarein (Scue), the hydrolysate of Scu, and its mechanisms of action in Alzheimer's disease (AD) have not been fully elucidated. In the present study, the effects of Scue on amyloid β (Aβ)-induced AD-like pathology were investigated. An in vitro model of inflammation and an aged rat model were used to confirm the effects of Scue. In vitro MTT assays and flow cytometry were used to assess the effects of Scue on cell viability and apoptosis, respectively. A Morris water maze was used to evaluate spatial learning and memory, and the levels of Aβ deposition, superoxide dismutase, malondialdehyde, apoptosis, neuro-inflammatory factors and nuclear factor-kB (NF-kB) activation in hippocampal tissues in vivo were measured to determine the effect of Scue in AD. Scue may be protective, as it decreased the apoptosis of hippocampal cells in vitro, inhibited Aβ-induced cognitive impairment, suppressed hippocampal neuro-inflammation and suppressed activation of NF-kB in vivo. Therefore, Scue may be a useful agent for the treatment of Aβ-associated pathology in the central nervous system through inhibition of the protein kinase B/NF-kB signaling pathway and thus, future studies are required to investigate the efficacy of Scue in patients with AD.

Introduction

Alzheimer's disease (AD) is an age-associated neurodegenerative disease that causes dementia, and severely affects the health and quality of life of elderly individuals (1). AD interrupts various brain functions, including memory, intelligence, judgment and learning (2). A previous study demonstrated that the etiology and pathogenesis of AD involves a variety of factors, including environmental elements, genetic factors and aging (3). The accumulation of pathological amyloid β (Aβ) peptide fragments serve a specific role in AD by inducing neuronal apoptosis and subsequent cognitive dysfunction (4,5). Models involving Aβ peptide deposition are widely used to simulate AD in animals (6-8). At present, modern medicine lacks an effective treatment for the core symptoms of AD, which includes progressive cognitive decline. Therefore, it is important to identify novel therapeutic approaches to counteract AD-associated cognitive impairment.

The flavonoid compound scutellarin (Scu) is ubiquitous in nature, particularly in the genus Erigeron (Asteraceae) and Scutellaria (Lamiaceae), and has been widely used in traditional Chinese medicine for a number of millennia (9-11). Recently, studies have made significant progress assessing the benefits of Scu, which exhibited neuroprotective effects, including anti-oxidant, anti-inflammatory and anti-apoptotic activities in numerous diseases (12-14). Additionally, previous studies demonstrated the beneficial effects of Scu in vascular endothelial dysfunction (15,16), myocardial infarction (17), colorectal cancer (18,19) and other types of cancer (20,21). Scutellarein (5,6,7,4'-tetrahydroxy flavone; Scue) is the hydrolyzed product of Scu in perennial herbs, including Scutellaria baicalensis, Scutellaria lateriflora and Scutellaria barbata (22,23). Previous studies demonstrated that Scue is absorbed more easily following oral administration compared with Scu, when administered in equal doses (22,24). Recent studies additionally demonstrated that Scu/Scue may prevent neuronal injury and the protective effects of Scue were determined to be superior to Scu (25-27). However, it remains to be determined whether Scue may affect Aβ-induced cell apoptosis and neurotoxicity, which are important pathogenic mechanisms of AD. Therefore, the present study aimed to examine whether Scue is able to exert therapeutic activity through protein kinase B/nuclear factor-κB (NF-κB) signaling in AD.

In the present study, a cellular model and a rodent model of Aβ-induced AD were used to evaluate the ability of Scue; the results demonstrated that Scue may inhibit neuro-inflammation, and ameliorate the cognitive and cellular effects of Aβ toxicity through inhibition of the NF-κB signaling pathway. The present data provided evidence for the use of Scue as a novel therapeutic to treat patients with AD.

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Materials and methods

**Extraction of Scue.** A *Scutellaria baicalensis* root was provided from Jilin Northeast Asia Pharmaceutical Co., Ltd. (Yanji, China). Extract standards were obtained from the Jilin Institutes for Food and Drug Control (China). The *Scutellaria baicalensis* root was dried and crushed, soaked for 1.5 h in a 10-fold water volume (v/w), and subsequently refluxed three times for 30 min. Polyamide column chromatography was used to separate Scue and monitored using high-performance liquid chromatography (HPLC). Measurements were performed using HPLC U3000 with a Corona® charged aerosol detector (Thermo Fisher Scientific, Inc., Waltham, MA, USA). A XB-C18 (250x4.6 mm; 5 µm) column (Welch Materials, Inc., Hurst, TX, USA) was used for sample separation at 30°C. A 5 µl injection volume with 20:80 gradient was used with acetonitrile as mobile phase A and 0.1% (v/v) formic acid in water as mobile phase B. The flow rate was set to 0.8 ml/min.

**Cell culture.** Rat pheochromocytoma (PC12) cells were purchased from The Type Collection Culture of The Chinese Academy of Medical Sciences (Shanghai, China). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Thermo Fisher Scientific, Inc.) supplemented with 8% fetal bovine serum and 8% horse serum (Gibco; Thermo Fisher Scientific, Inc.) and maintained at 37°C in a humidified atmosphere of 5% CO₂. For the experimental treatments, cells were pre-incubated with different concentrations (0, 5, 10, 15 and 30 µg/ml) of the test compound (Scue) and (0, 5, 10, 15 and 30 µg/ml) of the test compound (Scu) dissolved in 0.2% dimethyl sulfoxide (DMSO) in DMEM for 4 h and subsequently treated with 2 µg/ml Aβ for 24 h (7).

**MTT assay.** Cell viability was determined using a MTT assay. Cells were seeded in 96-well microplates at a density of 2x10⁴ cells/well and incubated for 24 h to allow the cells to adhere. Subsequently, MTT (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) at a final concentration of 0.2 mg/ml was added to each well and incubated at 37°C for 72 h. Following incubation, the cell supernatants were removed and the remaining formazan crystals were dissolved in 200 µl DMSO for 15 min. The optical density of each well was determined using an ELX-800 spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA) at 490 nm.

**Flow cytometry analysis of apoptosis.** A flow cytometer (FACSCalibur; BD Biosciences, San Jose, CA, USA) and an apoptosis detection kit (Biogot Technology Co., Ltd., Nanjing, China) were used according to the manufacturer’s protocol to detect apoptotic cells. Cells were harvested, centrifuged at 2-8°C for 10 min at 1,500 x g, washed and subsequently resuspended in 400 µl binding buffer. A total of 5 µl Annexin V-fluorescein isothiocyanate was added to each cell suspension and the suspension was incubated at 2-8°C for 15 min in the dark. Subsequently, 10 µl propidium iodide was added and the suspension was incubated at 2-8°C for 5 min in the dark. Flow cytometry was performed within 1 h of the final step. FlowJo software (version 7.6; FlowJo LLC, Ashland, OR, USA) was used for analysis.

**Immunofluorescence staining.** Cells were fixed in 4% paraformaldehyde for 15 min at room temperature and subsequently gently rinsed in PBS. To permeabilize the cells, they were incubated for 30 min in PBS containing 0.1% Triton X-100. Cells were blocked with 8% goat serum (Gibco; Thermo Fisher Scientific, Inc.) for 15 min at room temperature and each slide was incubated with NF-kB p65 primary antibody (1:100; cat. no. bs-3543R; BIOUS, Beijing, China) overnight at 4°C, and Cy3-labeled goat anti-rabbit secondary antibody (1:200; cat. no. A0516; Beyotime Institute of Biotechnology, Haimen, China) in the dark at room temperature for 60 min. The cells were counterstained with DAPI for 10 min at room temperature. Cells were viewed under a fluorescent microscope (Olympus Corporation, Tokyo, Japan; magnification, x400) subsequent to adding anti-fading reagent.

**Animal model construction.** Healthy male Wistar rats (n=24; age, 6-8 weeks; weight, 200 g) were purchased from the Laboratory Animal Center of Jilin University (Changchun, China). Animals were housed in laboratory facilities under the following conditions: Temperature, 21.0-25.0°C; relative humidity, 40.0-70.0%; complete air replacement 15 times/h; and 12-h light/dark cycle. Prior to the experiments, the rats were group-housed in a pathogen-free grade animal room with ad libitum access to food and water and allowed to acclimate for 1 week. The Animal Ethics Committee of Jilin University approved the experimental protocol.

Each rat was randomly assigned to one of four groups: Control, Aβ, Scue (50 mg/kg; intragastrically) or Scu (50 mg/kg; intraperitoneally). Non-control rats were anesthetized with sodium pentobarbital (60 mg/kg; intraperitoneally) and injected in the deep frontal cortex (3.2 mm anteroposterior, 2 mm doroventral relative to bregma; depth 3 mm) with 3 µl Aβ (10 ng/µl) (28), whereas, control rats received an injection of 3 µl saline. Following these injections, rats were subjected to treatment and testing over a period of 4 weeks.

All the mice were sacrificed with CO₂ (the displacement rate of CO₂ was 10% chamber volume/min) for 0.5 h subsequent to behavioral testing (7 days), and hippocampal tissues were dissected. Subsequently, one-half of the hippocampal tissues were frozen in liquid nitrogen and stored at -70°C for future protein analyses. The other tissues were fixed for 12 h in 4% paraformaldehyde at room temperature, and embedded in paraffin for sectioning and staining.

**Morris water maze (MWM) testing.** The MWM test includes an acquisition period and a special probe trial, and is a well-established method for the evaluation of learning and memory in animals (29). In the present study, the acquisition period lasted for 6 days, with two training sessions per day and a 15 min interval between sessions. Each animal was placed at a designated starting point in a different quadrant of the maze, facing the tank wall. In one quadrant, a platform was hidden just below the surface of the water. If a rat identified and climbed onto the platform, and remained there for >3 sec, the swimming time was documented as the latency to locate the platform. If a rat failed to locate the platform within 120 sec, it was manually placed onto the platform for 30 sec and the latency was recorded as 120 sec.
The spatial probe trial was only performed once. The platform was removed from the pool and the rat was placed at any starting point that was not adjacent to the platform. The number of times that each rat swam across the original location of the platform within 120 sec was documented.

**Western blotting.** Cells [cytoplasmic and nuclear NF-κB were separated by the membrane protein separation method (30)] were lysed in nonyl-phenoxoypolyethoxyethanol-40 lysate (Beyotime Institute of Biotechnology), and total protein and nucleoproteins from the hippocampal tissues were extracted using radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology). Protein concentrations were determined using the bicinchoninic acid method (Beyotime Institute of Biotechnology). For each sample, 40 µg total protein was run on an SDS-PAGE gel (15%), followed by transfer to a polyvinylidene fluoride membrane (EMD Millipore, Billerica, MA, USA). Membranes were blocked for 1.5 h in 5% BSA (cat. no. A8806; Sigma-Aldrich; Merck KGaA) at 2-8°C. The membranes were incubated with primary antibodies against nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor α (IkBα; cat. no. YS-kt-0097), phospho-p-IκBα (cat. no. YS-KT1551) (Shanghai Yansheng Biochemical Reagent Co., Ltd., Shanghai, China), NF-κB, cleaved caspase-3 (cat. no. bs-0081R), B cell lymphoma-2 (Bcl-2; cat. no. bs-20351R), apoptosis regulator BAX (Bax; cat. no. bs-4564R), β-actin (cat. no. bs-0061R) and lamin-A (cat. no. bs-1839R) (1:10000; BIODSS) overnight at 4°C. Subsequently, the membranes were incubated with goat anti-rabbit immunoglobulin G-horseradish peroxidase antibodies (1:5,000; cat. no. A0208; Beyotime Institute of Biotechnology) at room temperature for 1 h, followed by chromogenic detection using an enhanced chemiluminescence reagent (cat. no. WP20005; Thermo Fisher Scientific, Inc.). Films were scanned and analyzed using Gel-Pro-Analyzer 4.0 software (Media Cybernetics, Inc., Rockville, MD, USA), and the optical densities of the target bands were quantified relative to β-actin.

**Nissl staining.** Paraffin-embedded hippocampal tissues were cut into 5 µm sections, processed with conventional Nissl staining (31), and examined under a light microscope (magnification, x400).

**ELISA.** Commercially available ELISA kits were used to detect interleukin-6 (IL-6; cat. no. ml02293), tumor necrosis factor-α (TNF-α; cat. no. ml002095), interferon-γ (IFN-γ; cat. no. ml027464), superoxide dismutase (SOD; cat. no. ml643059), malondialdehyde (MDA; cat. no. ml077384), acetylcarnol (Ach; cat. no. ml401805) (Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China) and Aβ (cat. no. JL14446-48T; Shanghai Jianglai Industrial Ltd., Shanghai, China) expression levels in the hippocampal tissues, according to the manufacturer’s protocols.

**Statistical analysis.** Data are presented as the mean ± standard deviation. In vitro experiments were repeated three times, whereas in vivo experiments were conducted in six rats. Comparisons between groups were performed using one-way analysis of variance and Bonferroni post hoc tests. GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA) was used to analyze all the data and images. P<0.05 was considered to indicate a statistically significant difference.

**Results**

**Extraction, separation and purification of Scue.** Scue was obtained as described above. The results demonstrated that a single symmetrical peak was identified by HPLC; the shape of the peak was sharp and steep, and the retention time was the same as the reference substance (Fig. 1).

**Scue attenuates Aβ-induced cell death in PC12 cells.** Cell viability was assessed by an MTT assay in order to determine the effects of Scue on Aβ-induced cell death. To determine the proper concentration ranges for the study of neuroprotective effects, the cytotoxicity of each test compound was evaluated using PC12 cells. Scue did not demonstrate any detectable toxicity at any of the concentrations tested (Fig. 2A). The protective effects of Scue on Aβ-induced cell death were measured. Treatment with 2 µg/ml Aβ for 24 h decreased cell viability by 31%, whereas, pretreatment with Scue (30 µg/ml) significantly attenuated Aβ-induced cell death (Fig.2B; P<0.01). Aβ-induced cell death was further investigated by flow cytometry. Treatment with Aβ (2 µg/ml) significantly increased apoptosis compared with the control group; however, pretreatment with Scue (30 µg/ml) significantly decreased the number of apoptotic cells compared with treatment with Aβ alone (P<0.001). Apoptotic ratios with respect to the control group were as follows: 24.0% in the Aβ (2 µg/ml) treatment group; 13.4% in the Scue (30 µg/ml) treatment group; 10.8% in the Scu (30 µg/ml) treatment group. Therefore, Scue and Scu decreased the apoptotic ratios (Fig. 2C and D).

**Scue suppresses NF-κB activation in PC12 cells.** Western blotting was used to evaluate the expression of NF-κB p65 in cells. Compared with the control group, cytoplasmic NF-κB was significantly decreased by 54% (P<0.01; Fig. 3A) and nuclear NF-κB was significantly increased by 1.549-fold (P<0.001; Fig. 3B) in the Aβ group. Scue and Scu increased the expression level of cytoplasmic NF-κB compared with the Aβ group (P<0.01 and P<0.05, respectively; Fig. 3A), and decreased the expression level of nuclear NF-κB compared with the Aβ group (P<0.01 and P<0.05, respectively; Fig. 3B). This suggested that Scue and Scu inhibited the NF-κB signaling pathway.

Immunofluorescence staining demonstrated that NF-κB p65 was primarily expressed in the cytoplasm of the cells, and the NF-κB p65 expression in the nucleus was increased in the Aβ group compared with the control group (Fig. 3C). Treatment with Scue and Scu reversed the Aβ-induced alterations in NF-κB p65 localization. These results suggested that Scue may inhibit activation of the NF-κB signaling pathway in Aβ-induced injury of PC12 cells.

**Scue attenuates Aβ-induced cognitive impairment and hippocampal alterations in rats.** The MWM task was used to investigate the effects of Scue on Aβ-induced spatial memory impairments. Rats in the Aβ group at day 6 demonstrated significantly longer latencies to locate the platform (68.25±8.67 sec)
Figure 1. Extraction, separation and purification of Scue from scutellarin. (A) High-performance liquid chromatography chromatograms of herbal extract Scue. (B) Chemical reference and (C) final extract of Scue. Scue, scutellarein; mAU, milli-arbitrary units; UV_VIS, ultraviolet-visible spectroscopy; 1WVL, wavelength.

Figure 2. Scue alleviates Aβ-induced PC12 cell death. (A) Cell viability following treatment with Scue at a range of concentrations, P<0.05 vs. 0 µg/ml. (B) Cell viability following treatment with Aβ and pretreatment with 30 µg/ml Scu/Scue. (C) Cultured cells were double-stained with FITC-conjugated anti-Annexin V antibody and PI, and subjected to flow cytometry analysis of apoptotic cells. (D) Representative flow cytometry plots are presented. Data are presented as the mean ± standard deviation. n=3, *P<0.05, **P<0.01, ***P<0.001 vs. control group; #P<0.05, ##P<0.01, ###P<0.001 vs. Aβ group. Aβ, amyloid β; PI, propidium iodide; Scu, scutellarin; Scue, scutellarein; FITC, fluorescein isothiocyanate; UL, upper left; UR, upper right; LL, lower left; LR, lower right; FL1-A/FL2-A, area of fluorescence; Q, quadrant.
compared with the control group (31.90±5.68 sec; P<0.001; Fig. 4A). Treatment with Scue significantly attenuated the effects of Aβ-induced latency to locate the platform during the acquisition period and increased the number of platform crossings in the spatial probe trial (Fig. 4B; P<0.001).

ELISA was used for the analysis of hippocampal tissues from each of the groups. The Aβ content was increased 1.45-fold in the Aβ group relative to the control group (Fig. 4C; P<0.01), and decreased 0.05-fold in the Scue group compared with in the Scu group (P<0.01). SOD expression was decreased 0.35-fold in the Aβ group (Fig. 4D; P<0.01), and increased 0.04-fold in the Scue group compared with in the Scu group (P<0.05).

The MDA content in the hippocampal tissues was significantly increased in the Aβ group by 118% relative to the control (P<0.01; Fig. 4E), and decreased 0.05-fold in the Scue group compared with in the Scu group (P<0.05). Hippocampal

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**Figure 3.** Scue inhibits the NF-κB signaling pathway. Cytoplasmic expression of (A) NF-κB p65 and (B) nuclear NF-κB p65. Gray scale analyses were conducted using either (A) β-actin or (B) lamin A as an internal reference. Data are presented as the mean ± standard deviation. n=6. (C) Distribution of NF-κB p65 in the cytoplasm and the nucleus were detected by immunofluorescence. NF-κB p65 expression appeared green under a fluorescent microscope and the nucleus was stained blue. Scale bar, 20 µm. Images are representative of repeated experiments. **P<0.01, ***P<0.001 vs. the control group; #P<0.05, **P<0.01 vs. the Aβ group. NF-κB p65, nuclear factor κ-light-chain-enhancer of activated B cells p65; Scu, scutellarin; Scue, scutellarein; Aβ, amyloid β.
Ach content was significantly decreased by 79% relative to the control (P<0.01; Fig. 4F), and increased 0.06-fold in the Scue group compared with in the Scu group (P<0.05). These observations were significantly reversed by treatment with Scu/Scue, with Scue presenting more prominent effects (P<0.05).

Nissl staining was used to assess neurons in the hippocampus. Fewer neurons were labeled in the Aβ group compared with the control group. Furthermore, numerous cells demonstrated an irregular arrangement and loss of cytomorphology in samples from the Aβ group. Treatment with Scu/Scue improved the cytomorphology of the hippocampal neurons (Fig. 4G).

Western blotting was used to evaluate the hippocampal expression of proteins associated with apoptosis, including Bcl-2, Bax and cleaved caspase-3. The expression level of Bcl-2 in the Aβ group was reduced to 57% of that in the control group (P<0.01; Fig. 4H), whereas, expression levels of Bax (1.34-fold; P<0.01; Fig. 4H) and cleaved caspase-3 (2.60-fold; P<0.001; Fig. 4H) were increased. In the Scue 50 mg/kg group, expression levels of Bcl-2, Bax and cleaved caspase-3 were similar to those observed in the control group (Fig. 4H). In general, treatment with Scu/Scue attenuated the Aβ-induced alterations in the hippocampus, with Scue presenting more prominent effects compared with Scu.

Scue suppresses Aβ-induced hippocampal neuroinflammation and NF-κB activation. To assess the mechanism of the effects of Scue on Aβ-induced alterations in the hippocampus,
the expression levels of TNF-α, IFN-γ and IL-6 were determined by ELISA. Compared with the control group, the expression levels of TNF-α (P<0.01; Fig. 5A), IFN-γ (P<0.05; Fig. 5B) and IL-6 (P<0.01; Fig. 5C) in the hippocampi of Aβ-treated rats were significantly increased; however, these increases were significantly decreased by treatment with Scue (all P<0.05; Fig. 5A-C) compared with treatment with Aβ alone. Subsequently, the NF-κB signaling pathway was investigated: Hippocampal expressions levels of IκBα and p-IκBα in addition to expression levels of NF-κB in the cytoplasm and the nucleus were determined by western blotting. Compared with the control group, in the Aβ group, the phosphorylation of IκBα level was significantly increased and the expression of hippocampal IκBα was significantly decreased (P<0.001; Fig. 5D and E). The ratio of p-IκBα/IκBα was 83.3% in the control group and 300% in the Aβ group (P<0.001; Fig. 5F), and cytoplasmic NF-κB was reduced to 64% of control (P<0.01; Fig. 5G). Nuclear NF-κB was significantly increased by 1.54-fold (P<0.01; Fig. 5H). Scue significantly increased the expression of IκBα (P<0.05) and decreased the phosphorylation of IκBα (P<0.01; Fig. 5D and E). Scue additionally increased the cytoplasmic NF-κB (P<0.05; Fig. 5G) and decreased the nuclear NF-κB (P<0.05; Fig. 5H) compared with the Aβ group. Aβ-associated alterations in NF-κB were significantly attenuated by treatment Scue, suggesting that Scue decreased the phosphorylation of IκBα and subsequently inhibited activation of NF-κB signaling in the hippocampus.

Discussion

The diverse biological effects of traditional Chinese medicines are attracting an increasing amount of attention in modern therapeutics. In previous studies, Scu exhibited therapeutic effects in a variety of diseases (32-34). In the present study, the possibility for the use of Scue in the treatment of AD was investigated using in vitro and in vivo models of cell death and Aβ-associated pathology. The rat PC12 cell line has been widely used to evaluate the neuroprotective effects of bioactive reagents. Furthermore, this cell line is particularly sensitive to Aβ-induced injury (35-37). Therefore, the PC12 cell line was used for the therapeutic evaluation of Scue. Similar to previous studies, 2 µg/ml Aβ induced ~32% cell death, whereas, pretreatment with Scue inhibited Aβ-induced apoptosis (9,38), thus, demonstrating that Scue may protect against the pro-apoptotic cellular effects of Aβ in vitro. The MWM test is commonly used to study spatial learning and memory in animals, and is considered to be a reliable preclinical tool (39,40). In the present study, group differences in the escape latencies in the training trials and in the number of passes over the original platform location in the probe trial demonstrated that Aβ compromised rodent learning and memory performance, and treatment with Scu/Scue protected against the Aβ-induced cognitive impairments. It was previously demonstrated that the deposition of Aβ in the brain represents an important event in the etiology and pathology of AD (41,42). According to the
free radical theory, oxidative stress additionally contributes to the pathogenesis of AD (43); furthermore, decreases in Ach are associated with AD (44). In the present study, Scue decreased hippocampal Aβ and MDA content whilst increasing SOD and Ach expression, suggesting that Scutellaria baicalensis and its constituent Scue exhibit significant therapeutic potential for the treatment of AD-associated pathology.

The Nissl body is a structural characteristic of neurons that is present in neuronal cell bodies and dendrites. Nissl staining is a widely-used method for observing the cytomorphology of neurons (45). Consistent with the results of the present study, it was previously demonstrated that neuronal damage is a principle cause of AD (46). Nissl staining of the hippocampus demonstrated that neurons were arranged in disorderly patterns in the Aβ group animals, indicative of severe neuronal damage. Furthermore, it was hypothesized that hippocampal apoptosis underlies learning and memory deficits in AD (47). The apoptotic suppressor Bcl-2 serves an important role in apoptosis together with the pro-apoptotic protein Bax. Specifically, the ratio of Bax to Bcl-2 determines whether apoptosis occurs (48). Caspase-3 serves a similarly pivotal role in the apoptosis pathway (49). The present study demonstrated that Scue not only protected against apoptosis associated with Aβ exposure in vitro; however, additionally counteracted Aβ-induced decreases in the anti-apoptotic protein Bcl-2 expression level and suppressed the expression of pro-apoptotic proteins Bax and cleaved caspase-3 in vivo, suggesting that Scue exhibits neuroprotective and anti-apoptotic properties in a rodent model of AD.

NF-κB is a dimeric signaling complex that exists stably in the cytoplasm when bound to IκBα. In response to external stimuli, IκBα may be phosphorylated, resulting in the dissociation of IκBα from NF-κB and the nuclear translocation of NF-κB to initiate the transcription of certain genes that lead to inflammation and apoptosis (50,51). The present study demonstrated that Scue inhibited Aβ-induced phosphorylation of IκBα, thereby restricting the activation and nuclear translocation of NF-κB. Future studies are required to determine whether the inhibition of NF-κB activation underlies the beneficial effects of treatment with Scue in rodent AD, or instead, whether decreases in NF-κB activation occur as a consequence of the observed anti-inflammatory and antioxidative effects.

In summary, Scue inhibited Aβ-induced PC12 cell apoptosis, and protected against AD-associated pathology and cognitive impairment in rats possibly by decreasing apoptosis in the hippocampus. These results demonstrated a potential therapeutic use for Scue in AD.

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Availability of data and materials
The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors’ contributions
XBQ and MHD designed the study. XWH, YX, XS, HL, JMX, DH, DDY were responsible for experiments. GFL and YXL conducted experiments and analyzed data.

Ethics approval and consent to participate
The animal care and treatment protocols were approved by the Experimental Animal Ethics Committee of Jilin University (Changchun, China).

Patient consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

References
1. Duits FH, Teunissen CE, Bouwman FH, Visser PJ, Mattsson N, Zetterberg H, Blennow K, Hansson O, Minthon L, Andreasen N, et al: The cerebrospinal fluid ‘Alzheimer profile’: Easily said, but what does it mean? Alzheimers Dement 10: 713-723.e2, 2014.
2. Wortmann M: Dementia: A global health priority—highlights from an ADI and World Health Organization report. Alzheimers Res Ther 4: 40, 2012.
3. Blennow K, de Leon MJ and Zetterberg H: Alzheimer's disease. Lancet 368: 387-403, 2006.
4. Bagheri M, Joghataei MT, Mohseni S and Roghani M: Genistein ameliorates learning and memory deficits in amyloid β(1-40) rat model of Alzheimer's disease. Neurobiol Learn Mem 95: 270-276, 2011.
5. He FQ, Qiu BY, Zhang XH, Li TK, Xie Q, Cui DJ, Huang XL and Gan HY: Tetrandrine attenuates spatial memory impairment and hippocampal neuroinflammation via inhibiting NF-kB activation in a rat model of Alzheimer's disease induced by amyloid-β(1-42). Brain Res 1384: 89-96, 2011.
6. Zhou J, Zhou L, Hou D, Tang J, Sun J and Bondy SC: Paeonol increases levels of cortical cytochrome oxidase and vascular actin and improves behavior in a rat model of Alzheimer’s disease. Brain Res 1388: 141-147, 2011.
7. Song JX, Lin X, Wong RN, Sze SC, Tong Y, Shaw PC and Zhang YB: Protective effects of dibenzocyclooctadiene lignans from Schisandra chinensis against beta-amyloid and homocysteine toxicity in PC12 cells. Phytother Res 25: 435-443, 2011.
8. Dargahi L, Nasiraei-Moghadam S, Abdí A, Khalal L, Moradi F and Ahmadiani A: Cyclooxygenase (COX)-1 activity precedes the COX-2 induction in Aβ-induced neuroinflammation. J Mol Neurosci 45: 10-21, 2011.
9. Zeng YQ, Cui YB, Gu JH, Liang C and Zhou XF: Scutellariin mitigates Aβ-induced neurotoxicity and improves behavior impairments in AD mice. Molecules 23: E869, 2018.
29. Yamaguchi Y, Higashi M, Matsuno T and Kawashima S: Synthesis of scutellarein.

24. Mamadalieva NZ, Herrmann F, El-Readi MZ, Tahrani A, Estevez-Garcia IO, Gallegos-Nava S, Vera-Pérez E, Ni G, Tang Y, Li M, He Y and Rao G: Synthesis of scutellarein.

22. Tang H, Tang Y, Li NG, Lin H, Li W, Shi Q, Zhang W, Zhang P, Liu W, Liu ZY, Qi HW, et al.: Comparative metabolomic analysis of Lamiaceae and their biological activity. J Pharm Pharmacol 63: 637-644, 2011.

20. Erbele ID, Lin FR, Agrawal Y, Francis HW, Carey JP and Sun CY, Zhu Y, Li XF, Wang XQ, Tang LP, Su ZQ, Li CY, et al.: Comparative metabolomic analysis of Lamiaceae and their biological activity. J Pharm Pharmacol 63: 637-644, 2011.

19. Yang H, Du Y, Wan S, Trahan GD, Jin Y and Zhang W: Comparative metabolomic analysis of Lamiaceae and their biological activity. J Pharm Pharmacol 63: 637-644, 2011.

18. Sun CY, Zhu Y, Li XF, Wang XQ, Tang LP, Su ZQ, Li CY, Zheng GJ and Feng B: Scutellarein increases cisplatin-induced apoptosis and autophagy to overcome cisplatin resistance in non-small cell lung cancer via ERK/p35 and c-met/ AKT signaling pathways. Front Pharmacol 9: 92, 2018.

17. Huang H, Geng Q, Yao H, Shen Z, Wu Z, Miao X and Shi P: Protective effect of scutellarein on myocardial infarction induced by isoproterenol in rats. Iran J Basic Med Sci 21: 267-276, 2018.

16. Yuan H, Zhao Y, Wang Z, Liu Y and Zhang Y: Scutellarein suppresses growth and causes apoptosis of human colorectal cancer cells by regulating the p53 pathway. Mol Med Rep 15: 929-935, 2017.

15. Yang H, Du Y, Wan S, Trahan GD, Jin Y and Zhang W: Comparative metabolomic analysis of Lamiaceae and their biological activity. J Pharm Pharmacol 63: 637-644, 2011.

14. Liu W, Liu ZY, Qi HW, et al.: Enhancement role of baicalin used as adjuvant on the immune-responsiveness of -Tcells in vivo of mice with melanoma. Prac J Med Pharm 35: 1114-1118, 2018.

13. Yan WJ and Zhang SQ: Inhibition Mechanism of Baicalein on learning in the rat. Jpn J Pharmacol 87: 240-244, 2001.

12. Sun CY, Zhu Y, Li XF, Wang XQ, Tang LP, Su ZQ, Li CY, Zheng GJ and Feng B: Scutellarein increases cisplatin-induced apoptosis and autophagy to overcome cisplatin resistance in non-small cell lung cancer via ERK/p35 and c-met/AKT signaling pathways. Front Pharmacol 9: 92, 2018.

11. Yang H, Du Y, Wan S, Trahan GD, Jin Y and Zhang W: Comparative metabolomic analysis of Lamiaceae and their biological activity. J Pharm Pharmacol 63: 637-644, 2011.

10. Baluchnejadmojarad T, Zeinali H and Roghani M: Scutellaria alleviates lipopolysaccharide-induced cognitive deficits in the rat: Insights into underlying mechanisms. Int Immunopharmacol 54: 632-640, 2018.