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Research article

Scutellarin alleviates depression-like behaviors induced by LPS in mice partially through inhibition of astrocyte-mediated neuroinflammation

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ABSTRACT

Depression is a kind of common mental disorder associated with neuroinflammation, and astrocytes play a vital role in regulating and mediating neuroinflammation in central nervous system. Scutellarin has significant anti-inflammatory and neuroprotective effects. However, whether scutellarin exerts antidepressant effect remains unknown. In present study, it was found that scutellarin suppressed LPS-induced neuroinflammation in the hippocampus and alleviated depression-like behaviors in mice. In addition, scutellarin inhibited LPS-induced elevation of TNFα, IL-1β, IL-6 and iNOS, and reversed the downregulation of IL-4 and BDNF in astrocytes *in vitro*. Furthermore, the activated TLR4/NF-κB pathway in LPS-treated astrocytes was suppressed by scutellarin. Collectively, these results suggest that scutellarin ameliorates depression-like behaviors induced by neuroinflammation partially through inhibiting the TLR4/NF-κB pathway in astrocytes.

1. Introduction

Depression is one of the most common psychiatric disorders, characterized by anhedonia, depression, alteration in weight and appetite, changes in mental activity and other related symptoms such as anxiety and social avoidance. According to the World Health Organization (WHO) report in 2020, approximately 350 million people worldwide suffer from certain degree of depression, which has become one of the leading causes of death and disability. Currently, major therapeutic strategies rely on monoamine transmitter systems. But most of these antidepressants generates severe side effects, and one third of patients don't response to the drugs [1,2]. Therefore, new drugs with better efficacy and lower adverse effects need to be developed and explored.

Numerous studies have proved that depression is closely related to neuroinflammation [3,4]. The expression of pro-inflammatory cytokines is apparently increased in serum and cerebrospinal fluid of patients with depression [5,6]. Microglia and astrocytes are two kinds of glia involved in neuroinflammation in the central nervous system (CNS). Microglia are innate immune cells, which play an important role in monitoring and initiating immune responses in physiological and pathological conditions. Astrocytes, the most abundant and widely distributed glial cells in mammalian brain, are closely associated with the immune response and neuroinflammatory disorders including neurodegenerative disease, stroke, anxiety, and depression [7]. The autopsy results show that the number, density, morphology, and function of astrocytes are changed in the patients with depression [7–9], suggesting the role of astrocytes in the pathogenesis and pathophysiological process of depression.

Scutellarin (SCU) is a flavonoid extracted from *Erigeron breviscapus*, which is widely used in cardiocerebrovascular diseases. Previous studies have shown that SCU has a neuroprotective effect on neurological disorders for its anti-inflammatory effect [10], thus we speculated that SCU had a certain therapeutic effect on depression. In present study, lipopolysaccharide (LPS) was used to induce the inflammation-related depression in mice, and depression-like behaviors and inflammatory factors were determined to confirm the effects of SCU on depression.

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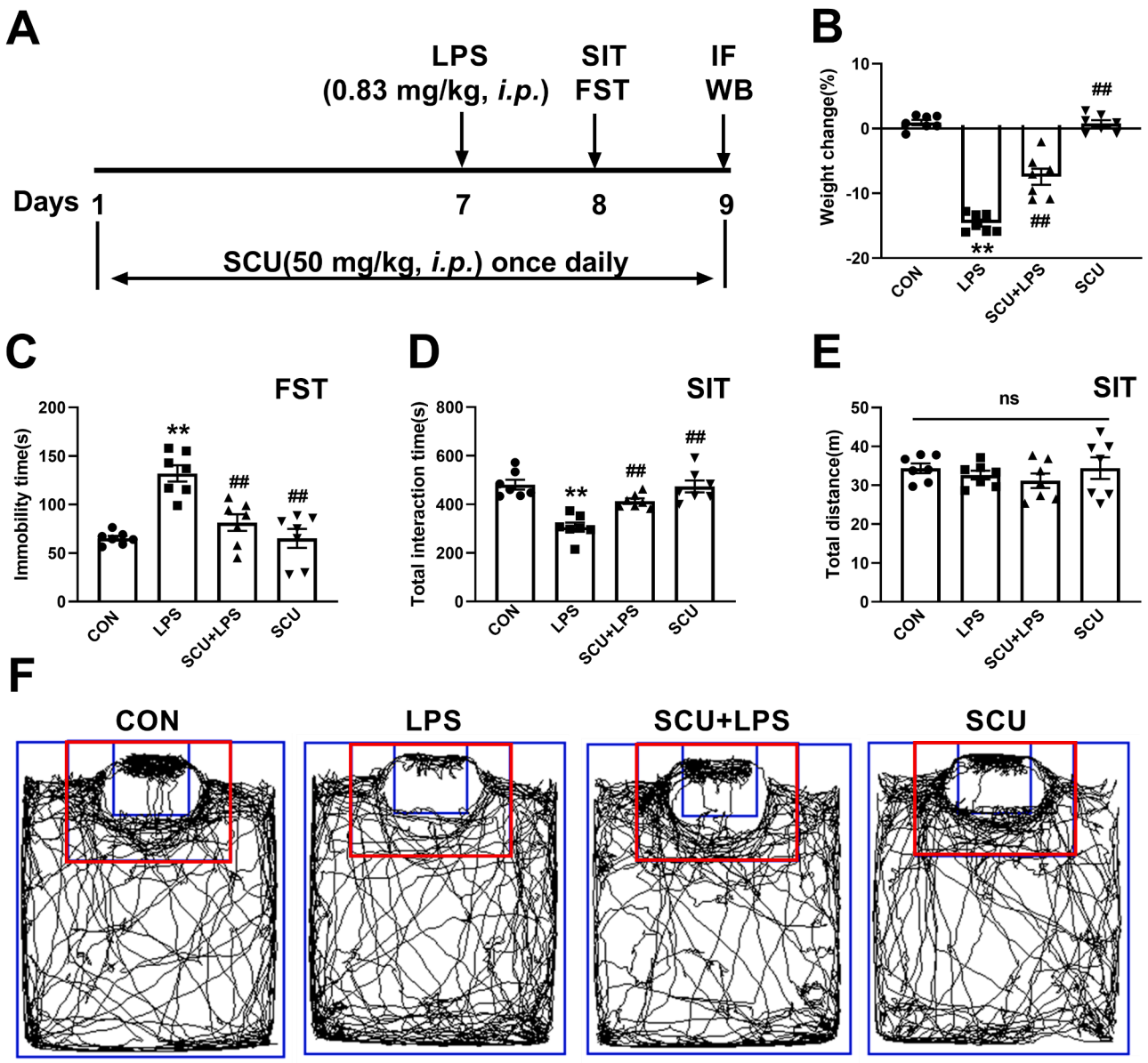

Fig. 1. Scutellarin prevented LPS-induced weight loss and depression-like behaviors. A. The schedule of experimental protocol. B. SCU treatment prevented the weight loss induced by LPS. C. SCU treatment decreased the immobility time in FST. D, E. In SIT, administration of SCU increased the total interaction time (D) but did not affect the motor function in mice (E). F. Representative traces in SIT for 20 min. The interaction time was recorded when mice moved inside the red region (SIA). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. CON; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. LPS. ns, no significance. SIT, social interaction test; FST: forced swimming test; IF: immunofluorescence staining; WB: western blot; CON, control; SCU, scutellarin.

2. Materials and methods

2.1. Drugs and reagents

Scutellarin (purity > 98%) was purchased from MACKLIN (Shanghai, China). LPS, poly-L-lysine, 2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI), 3-(4,5-Dimeth-ylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Anti-β-actin (A5316) antibody was obtained from Sigma (MO, USA). Dulbecco's modified Eagle's medium (DMEM), penicillin–streptomycin, fetal bovine serum (FBS), trypsin, neurobasal and B27 were provided by Gibico (Invitrogen, Carlsbad, CA, USA). Anti-iNOS (AF0199), anti-Phospho-IκB (AF2002), anti-IκB (AF5002) and anti-TLR4 (AF7017) antibodies were offered by Affinity (Changzhou, Jiangsu, China). Anti-ionized calcium-binding adapter molecule 1 (Iba-1, ab178847) antibody was got from Abcam (Cambridge, UK). Anti-NF-κB-P65 (8242S) and Anti-glial fibrillary acidic protein (GFAP, 80788S) antibody was purchased from Cell Signaling Technology (Danvers, MA, USA). Histone H3 (17168-1-AP) was provided by Proteintech (Chicago, IL, USA). All drugs and reagents are of standard biochemical quality and available commercially.

2.2. Animals

Male C57BL/6 mice (6–8-week-old) were purchased from the Laboratory Animal Center in the Fourth Military Medical University (FMMU), and maintained under standard laboratory conditions (12:12-h light/dark cycle, temperature of 24 ± 2 °C and 60% humidity) for at least 1 week before the experiment conducted. The mice were housed in plastic cages with access to food and water freely. All experiments were under the permission of the FMMU Animal Care and Use Committee.

2.3. Drug treatments and protocol

The mice were randomly divided into four groups: the control, LPS, SCU + LPS and SCU group. SCU (50 mg/kg) was injected intraperitoneally (*i.p.*) into the mice of SCU and SCU + LPS group once daily for 9 consecutive days. SCU was dissolved in saline (10 mg/mL), and about 0.1 mL was treated per mouse. LPS (0.83 mg/kg, *i.p.*, dissolved in saline) was administrated into the mice of LPS and SCU + LPS group on day 7 (Fig. 1A). Equal volume solvent was intraperitoneally injected in control mice. The dose of SCU and LPS was selected based on previous study [11,12]. Mice were treated with gentle handling every day to eliminate their nervousness, and allowed to habituate to the testing room for 1 h before the test.

2.4. Social interaction test

Social interaction test (SIT) was performed in an open field (45 cm × 45 cm × 35 cm) for 20 min as previously reported [13]. One perforated transparent plastic box (10 cm × 8 cm × 6 cm) was installed on one side of the field, and the surrounding region (26 cm × 14 cm, Fig. 1F) was defined as the social interaction area (SIA). The mice in and outside the box were permitted to touch and smell each other through the holes. The test mouse was placed in the center of the apparatus, while another unfamiliar homogeneous mouse was put in the plastic box. Time spent in SIA and the total distance were recorded and analyzed by a tracking system (DigBehv-LR4, Shanghai Jiliang, China).

2.5. Forced swimming test

Forced swimming test (FST) was carried out as previously described [14]. The test was performed in a glass cylinder (15 cm diameter) filled with 20 cm deep water (23–25 °C). Mice were separately put in the water for 6 min, and the immobile time in last 4 min was calculated by an experienced researcher blinded to the experiment.

2.6. Immunofluorescence staining

Mice were anesthetized with isoflurance inhalation and perfused with saline followed by 4% paraformaldehyde. After gradient dehydration, coronal cryostat sections (30 μm) containing hippocampus were sliced by CM3050S freezing microtome (Leica, Germany), and immunofluorescence staining was performed. Primary astrocytes cultured on coverslips were fixed with 4% paraformaldehyde for 15 min. The brain sections and cells were blocked with 10% goat serum supplemented 0.3% Triton X-100 for 1 h at room temperature, and then incubated with primary antibodies against Iba-1 (1:200), P65 (1:200) or GFAP (1:200) at 4 °C overnight, followed by Alexa Fluor secondary antibodies for 1 h at room temperature. Nucleus was stained with DAPI. Images were photographed by confocal microscope (Olympus, Japan) and analyzed by ImageJ software (National Institutes of Health, Bethesda, MD).

2.7. Primary astrocyte culture and treatment

Primary astrocytes were prepared from 1 to 3 days old C57BL/6J mice as reported previously [15]. Briefly, cerebral cortex was separated and digested with 0.25% trypsin for 15 min at 37 °C. Single cells were seeded in poly-L-lysine-precoated 75 cm^2 flasks containing DMEM with 10% FBS and 1% penicillin–streptomycin. Cells were cultured in the humidified incubator with 95% air and 5% CO_2, and half medium was changed every 3 days. The astrocytes were purified by rotating at 260 rpm (12 h, 37 °C), and the purified cells were subcultured for another 1 week. Third-generation astrocytes were used in the following experiments. The purity of astrocytes was assessed by GFAP immunofluorescent staining (Supplementary Fig. 1A, B). The cells were pretreated with SCU (2, 10, 50 μM) for 2 h followed by exposure to LPS (1 μg/mL) for 24 h. SCU was dissolved in dimethyl sulfoxide (DMSO) to the concentration of 100 mM and diluted to optimal used concentration with DMEM (final DMSO concentration <0.1%). Control groups were treated with the same volume DMEM containing DMSO of the same concentration.

Table 1
Primer sequences used for q-PCR.

Gene	Primer
BDNF	Forward 5′-GGTATCCAAAGGCCAACTGA-3′ Reverse 5′-CTTATGAATCGCCAGCCAAT-3′
IL-1β	Forward 5′-GCAACTGTTCCTGAACTCAACT-3′ Reverse 5′-ATCTTTTGGGGTCCGTCAACT-3′
TNFα	Forward 5′-GTGATCGGTCCCCAAAGG-3′ Reverse 5′-GGTGTGGGCCATAGAACTGATG-3′
IL-4	Forward 5′-CTCGAATGTACCAGGAGCCA-3′ Reverse 5′-TGTGGTGTTCTTCGTTGCTG-3′
IL-6	Forward 5′-CTCGAATGTACCAGGAGCCA-3′ Reverse 5′-TGTGGTGTTCTTCGTTGCTG-3′
GAPDH	Forward 5′-CCAGCTCGTCCTGTAGACAA-3′ Reverse 5′-GCCTTGACTGTGCCGTTGA-3′

2.8. Primary neuron culture

Primary cortical neuron cultures were obtained from the cerebral cortex of E13-E15 C57BL/6J mouse embryos [16]. Briefly, single cells were counted, and cultured in 96-well plates at a density of 5 × 10^4 cells/well. The cells were cultured with neurobasal medium supplemented with 2% B27 (NB/B27), 0.5 mM glutamine and 1% penicillin–streptomycin. Half of the medium was changed every 3 days, and neurons were used on Day 7 when maturation.

2.9. Astrocyte-conditioned medium preparation and co-culture of neurons and astrocytes

The astrocytes cultured in 6-well plates at the density of 1 × 10^6 cells/well were washed with phosphate-buffered saline (PBS) for two times, and the medium was changed with 2 mL/well NB/B27 medium. The astrocytes were treated with SCU (10 μM, 2 h) and/or LPS (1 μg/mL, 24 h), then the medium was collected and centrifuged at 12,000 g for 5 min at 4 °C to remove cell debris. After that, the supernatant was recollected as the astrocyte-conditioned medium (ACM). Neurons cultured on 96-well plates were treated with 200 μL ACM per well for 24 h, and then cell viability was measured.

2.10. Cell viability measurement

MTT assay was conducted to detect cell viability as previous studies described [15]. MTT was dissolved in sterilized PBS and added into each well at final concentration of 0.5 mg/mL. After incubated at 37 °C for 4 h, the medium was completely removed, and 200 μL DMSO/well was added. Optical density at 490 nm was read on a Universal Microplate Reader (Elx 800; Bio-TEK Instruments). Cell viability was showed as percentage of control, and the experiment was repeated three times.

2.11. Quantitative PCR

Total RNA of the cells was extracted by Trizol reagent, and reverse-transcribed into cDNA with reverse transcription mixture (Sangon Biotech). Thereafter, quantitative PCR analysis was finished as the following cycling parameter: 95 °C for 30 s, 1 cycle; 95 °C for 10 s, 60 °C for 30 s, 40 cycles. The results were analyzed by comparative Ct ($2^{-\Delta\Delta CT}$) method, with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal control. All primers were listed in Table 1.

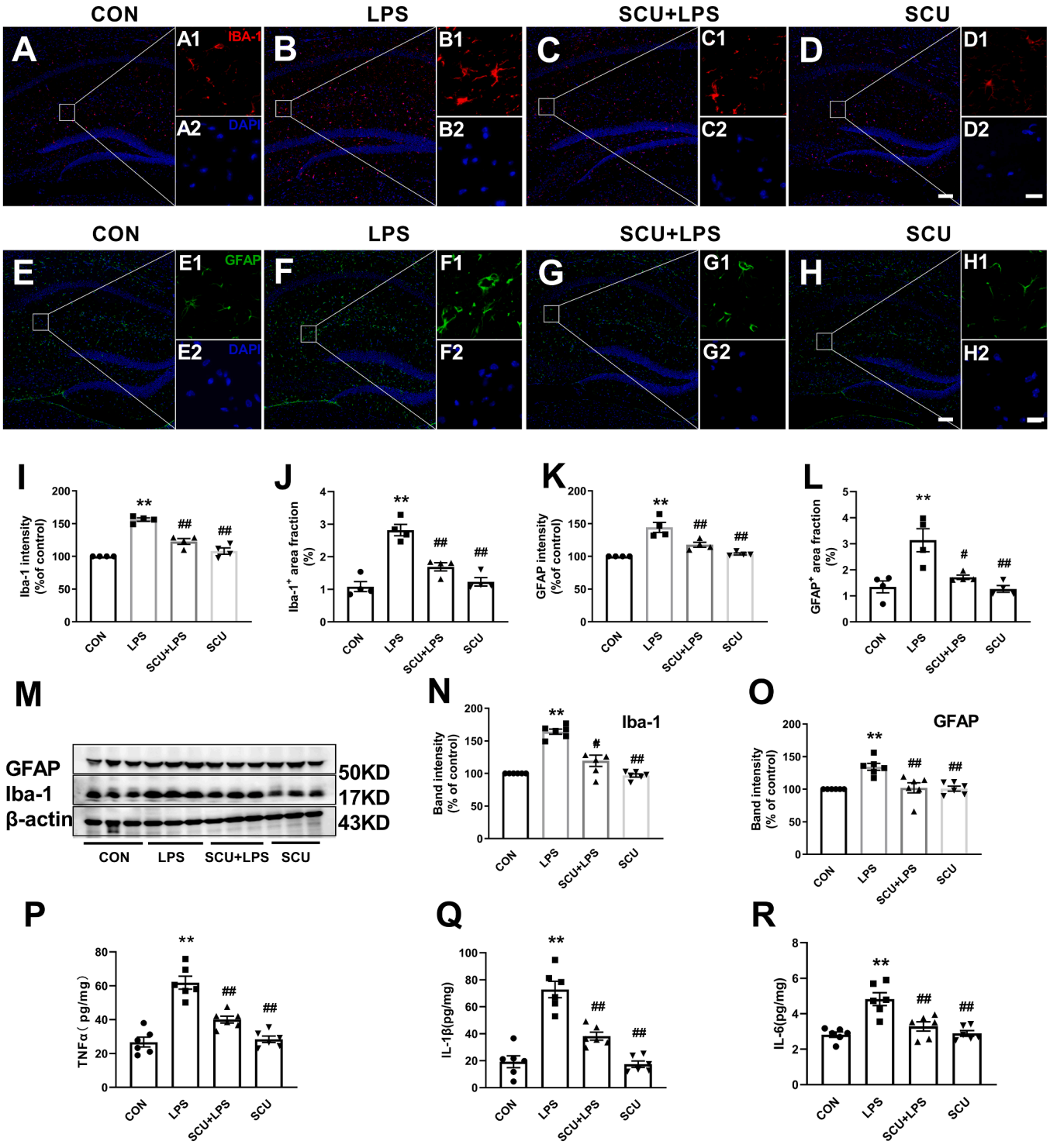

Fig. 2. Scutellarin inhibits LPS-induced glia activation and neuroinflammation in hippocampus. A-J. Immunofluorescence staining of Iba-1 (A-D) and GFAP (E-H) in the hippocampus and the statistical analysis of Iba-1 (I, J) and GFAP (K, L). M. The levels of Iba-1 and GFAP in the hippocampus was detected by western blot. The SCU administration reversed the upregulations of Iba-1 (I, J and N) and GFAP (K, L and O). P-R. The increased levels of TNFα (P), IL-1β (Q) and IL-6 (R) were inhibited by SCU. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. CON; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. LPS. Scale bar: A-H, 100 μm; A_1-H_1 and A_2-H_2, 20 μm.

2.12. Enzyme-linked immunosorbent assay (ELISA)

The hippocampus of mice was collected, and the pro-inflammatory factors were measured with ELISA kits of Tumor necrosis factor-α (TNFα, MTA00B, R&D Systems), Interleukin-1β (IL-1β, MLB00C, R&D Systems) and Interleukin-6 (IL-6, ZC-37988, ZCi Bio) following the manufacturer's instructions.

2.13. Western blot

Western blot was carried out as previous study [17]. The proteins were obtained from the hippocampal tissues or cultured astrocytes, and the protein content was quantified by the BCA Protein Assay Kit (P0914, Thermo Fisher Scientific, Rockford, IL, USA). The cytoplasmic and nuclear proteins were obtained from astrocytes by nuclear and cytoplasmic

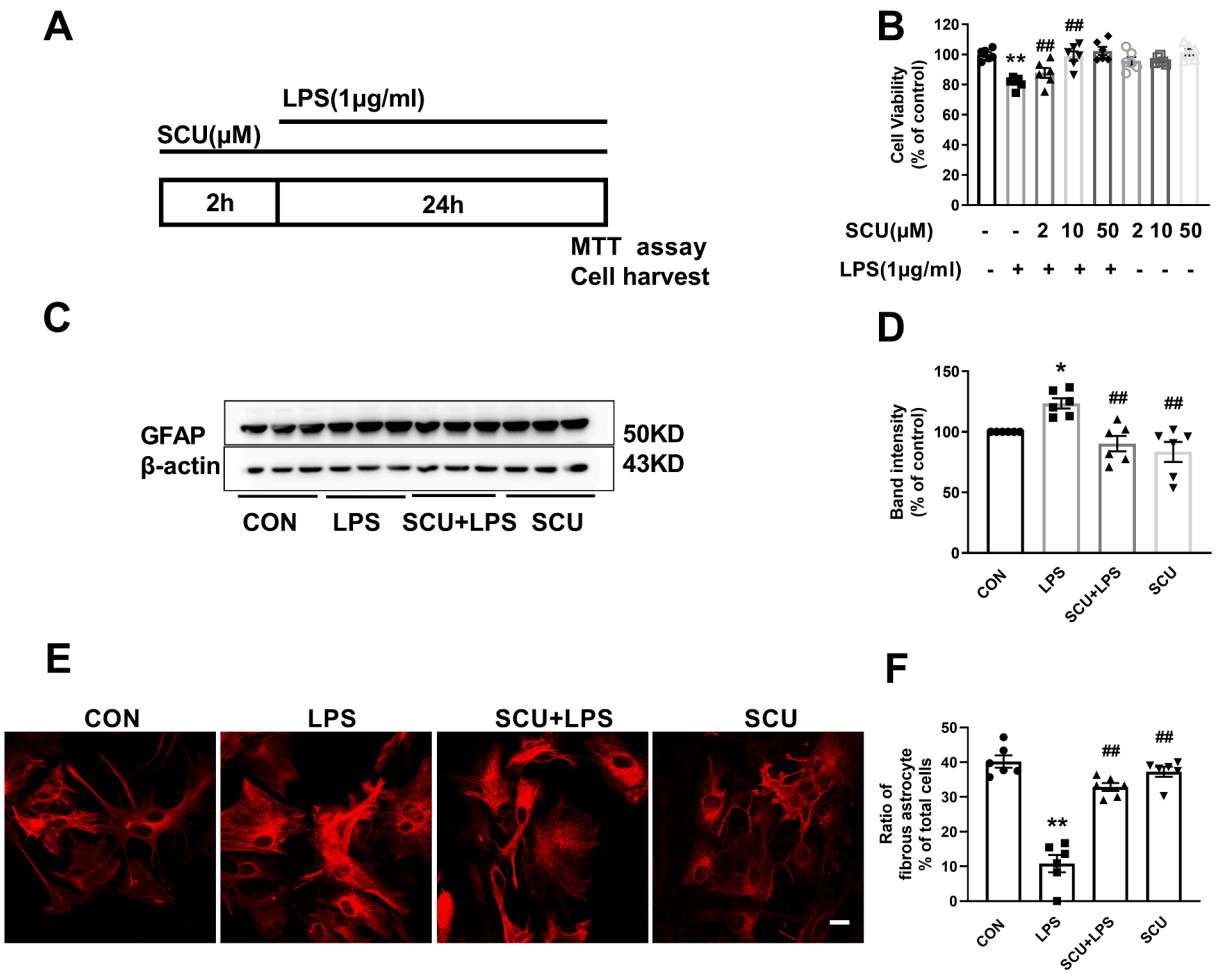

Fig. 3. Scutellarin suppressed the activation of astrocytes exposure to LPS. A. The schedule of experimental protocol. B. SCU enhanced LPS-induced decrease of cell viability in dose-dependent manner, and SCU alone did not affect cell viability in cultured astrocytes. C, D. GFAP level was measured by western blot in astrocytes (C), and pretreatment of SCU inhibited the increased GFAP expression induced by LPS exposure (D). E. Immunofluorescence staining of GFAP showed that SCU suppressed the structural remodeling of astrocytes exposed to LPS. Scale bar = 20 μm. F. The ratio of fibrous astrocytes was presented as the percentage of fibrous astrocytes in total astrocytes. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. CON; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. LPS.

protein extraction kits (P0028, Beyotime Institute of Biotechnology, Shanghai, China), according to the manufacturer's instructions. Equal amounts of protein were separated with 9% or 15% sodium dodecyl sulfate polyacrylamide gels and then electro-transferred to polyvinylidene difluoride (PVDF) membranes (Invitrogen). Then the membrane was blocked with 5% non-fat milk and incubated with the primary antibodies at 4 °C overnight as follows: anti-TLR4 (1:1000), anti-NF-κB P65 (1:1000), anti-p-IκB (1:1000), anti-IκB (1:1000), anti-iNOS (1:1000), anti-GFAP (1:1000), anti-Iba-1 (1:1000), anti-β-actin (1:10000), anti-Histone H3 (1:1000). After incubation with secondary antibodies for 1 h at room temperature, the membranes were scanned by an ECL system (PerkinElmer, Zaventem, Belgium) and then quantified through ImageJ software. The data analysis was expressed by the relative band intensity of β-actin or Histone H3.

2.14. Statistical analysis

Data were represented as the mean ± SEM. The statistical analysis was carried out by GraphPad Prism 8.30 software. One-way ANOVA with Tukey's multiple comparisons test was adopted to compare multiple groups. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Scutellarin ameliorates LPS-induced weight loss and depression-like behaviors in mice

Firstly, the body weight was measured after 24 h exposure to LPS (Fig. 1A). Body weight was significantly reduced in LPS-treated mice as compared to that in the control, and SCU administration markedly prevented the weight loss (Fig. 1B). Secondly, the depression-like behaviors were assessed by FST and SIT. In FST, LPS injection notably enhanced immobility time of mice (Fig. 1C), which was remarkably reduced by administration of SCU. LPS-treated mice spent much less time in SIA (Fig. 1D, F), and SCU treatment greatly increased the social interaction time. However, the total distance mice moved was no difference between the four groups (Fig. 1E, F). Overall, these results indicate that SCU treatment ameliorated LPS-induced weight loss and depression-like behaviors.

3.2. Scutellarin inhibits LPS-induced glia activation and neuroinflammation in hippocampus

As depression is closely related to neuroinflammation, Western blot and immunofluorescence staining were next performed to examine neuroinflammatory responses. LPS treatment greatly increased the

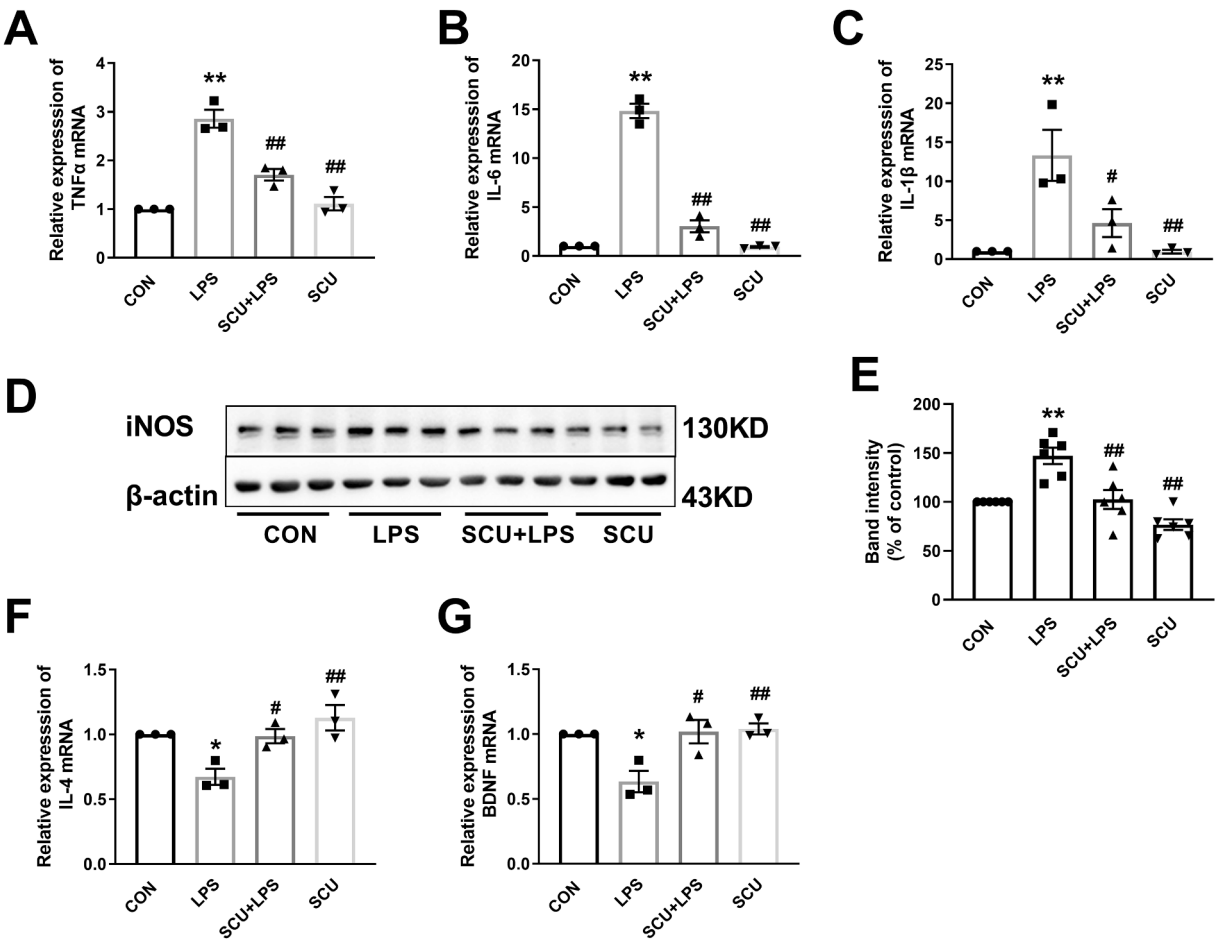

Fig. 4. Scutellarin inhibited pro-inflammatory factors and promoted anti-inflammatory factors release in astrocytes. Quantitative PCR and western blot were performed to measure the levels of pro/anti-inflammatory factors in cultured astrocytes. The levels of TNFα mRNA (A), IL-6 mRNA (B), IL-1β mRNA (C) and iNOS expression (D, E) were significantly increased in the presence of LPS, but decreased to basic levels in the pretreatment of SCU. F, G. LPS treatment declined the mRNA levels of IL-4 (F) and BDNF (G), whereas SCU elevated their levels. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. CON; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. LPS. BDNF: Brain derived neurotrophic factor.

levels of Iba-1 (microglia marker, Fig. 2A, B, C, D, I, J) and GFAP (astrocyte marker, Fig. 2E, F, G, H, K, L) in the hippocampus, which were inhibited by administration of SCU. The results were further confirmed by western blot (Iba-1: Fig. 2M, N. GFAP: Fig. 2M, O). LPS-induced upregulations of Iba-1 and GFAP indicate microglia and astrocyte activation. Then, ELISA was employed to detect the levels of pro-inflammatory factors in the hippocampus since activated microglia and astrocytes could secrete inflammatory factors. The results showed that LPS treatment markedly increased the pro-inflammatory factors (Fig. 2P, Q, R), whereas SCU administration significantly reduced their levels. These data suggest that scutellarin inhibits LPS-induced microglia and astrocytes activation and attenuates the inflammatory responses in the hippocampus.

3.3. Scutellarin suppresses the activation of astrocytes exposure to LPS

Previous studies have shown that SCU could inhibit microglia-mediated neuroinflammation [18]. However, the role of astrocytes in antidepressant effect of SCU remains unknown. In order to explore whether the antidepressant mechanism of SCU was through suppressing the inflammation of astrocytes, the primary astrocytes were cultured, and SCU and/or LPS were treated in astrocytes (Fig. 3A). MTT results showed that LPS treatment decreased the cell viability (Fig. 3B), and SCU pretreatment notably repaired astrocyte viability in dose-dependent manner. In the following experiments, 10 μM SCU was used because of its optimal effective concentration. LPS exposure could activate astrocytes characterized by increased expression of GFAP and somatic hypertrophy [19]. Therefore, GFAP was detected by western blot and immunofluorescence staining. LPS exposure greatly enhanced the level of GFAP (Fig. 3C, D) and significantly decreased the ratio of fibrous astrocytes (Fig. 3E, F). SCU pretreatment reversed these changes. This result was consistent with above data *in vivo*. Collectively, these findings provide evidence that LPS-induced cytotoxicity and activation can be reduced by SCU in astrocytes.

3.4. Scutellarin inhibits pro-inflammatory factors and promotes anti-inflammatory factors released from astrocytes

To further verify the anti-inflammatory effect of SCU on astrocytes, quantitative PCR and western blot was conducted to measure the levels of pro/anti-inflammatory factors in cultured astrocytes. As compared to the control, the mRNA levels of TNFα, IL-6 and IL-1β were significantly increased in the presence of LPS (Fig. 4A, B, C). The protein level of iNOS, a promoter of nitric oxide (NO), was also elevated (Fig. 4D, E). Pretreatment with SCU abolished their enhancements. Meanwhile, LPS exposure decreased interleukin-4 (IL-4) and brain derived neurotrophic factor (BDNF) mRNA levels (Fig. 4F, G), which was blocked by SCU pretreatment. The results indicate that SCU could inhibit pro-inflammatory factors and promote anti-inflammatory factors released from astrocytes.

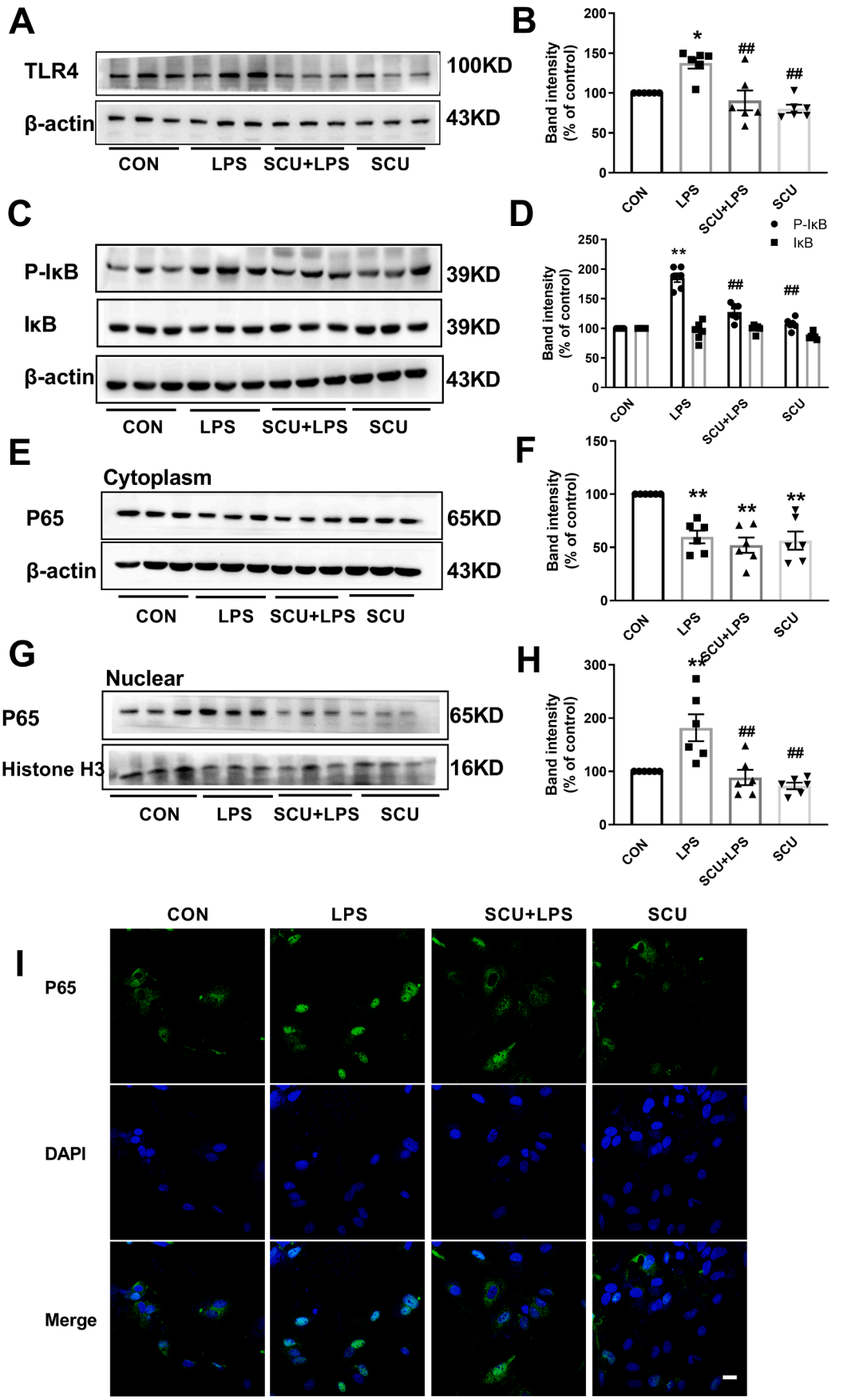

Fig. 5. Scutellarin suppressed neuroinflammation through TLR4/ NF-κB pathway in astrocytes. Western blot was performed to detected TLR4 (A), P-IκB/IκB (C), P65 in cytoplasm (E) and nuclear (G) in cultured astrocytes. LPS treatment increased TLR4 (B) and IκB phosphorylation levels (D), decreased cytoplasm P65 (F) and increased the nuclear P65 level (H). Pretreatment of SCU reversed these changes. I. Representative images of immunofluorescence staining of P65. LPS led to enhanced green fluorescence in the nuclear, which was reversed by SCU pretreatment. Scale bar = 20 μm. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. CON; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. LPS.

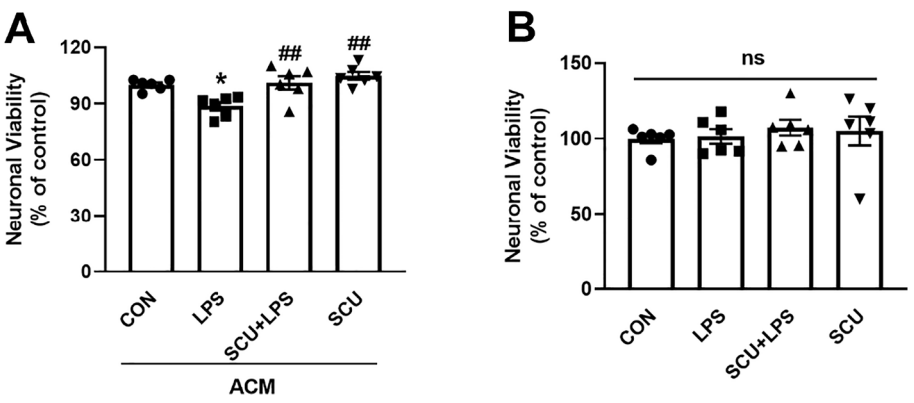

Fig. 6. Scutellarin prevented neuronal loss induced by LPS-treated astrocyte-conditioned medium. The astrocyte medium treated with SCU (50 μM, 2 h) and/or LPS (1 μg/mL, 24 h) was collected, which was defined as ACM. A. LPS-treated ACM caused neuronal death, which was blocked by SCU-pretreated ACM. ACM treated with SCU alone did not induce neuronal loss. B. Neither LPS nor SCU affected neuronal viability. $^{*}p < 0.05$, $^{**}P < 0$ 0.01 vs. CON; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. LPS. ACM, astrocyte-conditioned medium.

3.5. Scutellarin alleviates neuroinflammation through inhibiting TLR4/NF-κB pathway in astrocytes

TLR4/NF-κB pathway plays a key role in neuroinflammatory regulation, thus the relative protein expression was examined. LPS significantly increased TLR4 expression (Fig. 5A, B), upregulated the phosphorylated level of IκB (Fig. 5C, D), and promoted P65 translocation to nucleus as evidenced by the decreased P65 cytoplasm level (Fig. 5E, F) and the increased P65 nuclear level (Fig. 5G, H). However, these were abolished by SCU pretreatment. SCU alone decreased level of P65 both in cytoplasm and nucleus. The effect was verified by immunofluorescence staining (Fig. 5I). These data suggest that the anti-inflammatory effect of SCU is partially through inhibiting TLR4/ NF-κB pathway in astrocytes.

3.6. Scutellarin reduces neuronal loss induced by LPS-treated astrocyte conditional medium

Lastly, to investigate if the protective effect of SCU on astrocytes could help neuron survival, the ACM was collected and used to culture primary cortical neurons. The viability of neurons was detected in the presence of ACM derived from different group. As showed in Fig. 6A, LPS-treated ACM impaired the neuronal viability, and SCU-pretreated ACM prevent neurons from inflammatory injury. However, LPS and SCU had no significant effect on the neuronal viability either alone or together in neurons without ACM (Fig. 6B). The results show that the anti-inflammatory effect of SCU on astrocytes could protect neuronal survival.

4. Discussion

The present study showed that scutellarin could alleviate the depression-like behaviors in mice induced by LPS. The underlying mechanism was closely related to the inhibition of neuroinflammation through TLR4/NF-κB pathway in astrocytes.

The close relationship between neuroinflammation and depression has been widely confirmed. The activation of glial cells causes massive release of pro-inflammatory factors, which drives the development and progress of depressive-like phenotypes [20]. LPS is commonly used to establish animal model of depression since it can elevate pro-inflammatory factors in the brain [21]. It has been reported that the locomotor activity of mice was restored 24 h after LPS systematic administration [11]. Accordingly, behaviors of mice were detected at this time point in this study. The data demonstrated that LPS injection induced obvious depression-like behaviors in mice as shown by increased immobility time and social avoidance, which were reversed by SCU administration. Symptoms of depression include anhedonia, depressed mood, volitional activity decline, cognitive impairment and so on. In this study, we only detected the depressed mood-like and volitional activity behaviors in mice. The effect of SCU on cognitive impairment and anhedonia in mice needs be further explored.

There is no doubt that Microglia play an essential role in neuroinflammation. However, as the most abundant glia cells in the CNS, the role of astrocytes in mediating and regulating neuroinflammation could not be neglected [22]. Reactive astrocytes induced by neuroinflammation, named “A1”, could increase the production of pro-inflammatory cytokines such as TNFα, IL-6 and IL-1β. These factors would recruit leukocytes and activate neighboring astrocytes and microglia, resulting in inflammatory amplification. Suppression of astrocytes activation has become one promising target to treat neuroinflammation-related diseases. Besides, astrocytes are one of the main sources of neurotrophic factor synthesis and secretion such as BDNF, which is involved in neuronal survival and pathophysiology of depression [23]. Several studies revealed that LPS or pro-inflammatory cytokines-induced depression-like behaviors are negatively correlated with BDNF gene and protein levels [24,25], which is consistent with our result. In this study, SCU treatment reversed the decline of BDNF mRNA in cultured astrocytes exposure to LPS. It has been reported that different from mature BDNF, pro-BDNF positively modulates depression [26,27], suggesting that the imbalance of these two factors may play key role in depression. It is very meaningful to distinguish pro-BDNF and mature BDNF in future research.

TLR4 is widely expressed in both microglia and astrocytes in CNS [28,29], and it was closely related to stress and inflammation-induced psychiatric disorders [30]. LPS can activate TLR4 and downstream pathways such as NF-κB, which is a key regulator of neuroinflammation in astrocytes [31]. In this study, the inhibitory effect of SCU on TLR4/NF-κB pathways suppressed release of pro-inflammatory factors and produced protective effect on co-cultured neurons. However, we did not explore the effect of SCU with the intervention of TLR4/NF-κB pathway as the absence of the TLR4 or NF-κB agonist. It is a limitation of our study. We could not exclude the effect of SCU on other signaling pathway.

In conclusion, this study demonstrated that SCU alleviated LPS-induced depression-like behaviors and suppressed the activation of astrocytes in the hippocampus. The potential mechanism was partially due to the anti-neuroinflammatory effect through inhibiting TLR4/NF-κB pathway in astrocytes. These results indicate that SCU may be a potential therapeutic drug to treat depression.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

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Author contributions

Liang Lu, Liu-kun Yang, Jiao Yue performed the experiments. Xin-shang Wang, Jing-yu Qi, Fan Yang and Ban Feng analyzed the data. Shui-bing Liu conceived and designed experiments and finalized manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neulet.2021.136284.

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