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Sanguinarine alleviates post-stroke cognitive impairment in rats by modulating the p38 MAPK signaling pathway

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Post-stroke cognitive impairment (PSCI) is common, but the understanding of its cognitive impairment mechanisms and available treatment options are limited. Sanguinarine (SG) has shown promising neuroprotective potential, but its effects and mechanism in PSCI remain unexplored. This study established a rat stroke model via middle cerebral artery occlusion (MCAO) surgery. SG was administered intraperitoneally at doses of 1.0, 2.5, or 6.25 mg/kg. Cognitive function and neuronal injury were assessed using a novel object recognition test, the Y-maze, Nissl staining, and TUNEL staining. Neuroinflammation and the activation levels of p38 mitogen-activated protein kinase (MAPK) were evaluated by ELISA, western blot, and immunofluorescence. The results showed that MCAO rats demonstrated cognitive impairments, increased neuronal apoptosis, and heightened neuroinflammation. Treatment with 6.25 mg/kg SG significantly improved cognitive function in stroke rats, as evidenced by an increased recognition index and more time spent in the novel arm of the Y-maze. SG alleviated neuronal injury and reduced neuronal apoptosis in MACO rats. Furthermore, SG treatment downregulated the pro-inflammatory cytokines tumor necrosis factor-α and interleukin (IL)-1β and upregulated the anti-inflammatory cytokine IL-10 in a dose-dependent manner, while also inhibiting the activation of p38 MAPK in MCAO rats. Notably, the co-administration of SG with anisomycin (a p38 MAPK activator) reversed these effects. Molecular docking experiments also demonstrated a strong binding affinity between SG and p38 MAPK. In conclusion, SG alleviates neuroinflammation and neuronal apoptosis by inhibiting p38 MAPK activation in PSCI, highlighting its potential as a multi-target therapeutic agent.

Keywords: Middle cerebral artery occlusion (MACO), Neuroinflammation, Apoptosis, Anisomycin

Stroke is the second leading cause of adult mortality and disability globally[1]. In addition to focal neurological deficits, post-stroke cognitive impairment (PSCI) is a common complication of stroke[2], affecting approximately 30% of patients with ischemic stroke[3]. PSCI is defined as a syndrome that meets the diagnostic criteria for cognitive impairment within 3 to 6 months after a stroke event[4]. The pathogenesis of PSCI is complex, involving factors such as neuronal damage, blood-brain barrier disruption, neuroinflammation, and neurotoxicity[5].

Studies have reported that p38 mitogen-activated protein kinase (MAPK) plays a crucial role in several neurological disorders[6,7]. Blocking p38 MAPK has been shown to protect dopaminergic neurons in Parkinson's disease models[8], as well as reduce motor neuron loss and improve function in amyotrophic lateral sclerosis animal models[9]. Additionally, down regulating the MAPK pathway is associated with mechanisms that mitigate cerebral ischemia-reperfusion injury[10]. However, the role of p38 MAPK in PSCI remains underreported.

Sanguinarine (SG), a benzophenanthridine alkaloid extracted from *Sanguinaria canadensis*[11], exhibits antitumor, anti-inflammatory and neuroprotective activities[12]. SG can modulate the nuclear factor-κB (NF-κB) pathway, thereby reducing mitochondrial dysfunction and DNA damage in acute kidney injury[13]. SG also relieves neuropathic pain by inhibiting the p38 MAPK signaling pathway[14]. However, its therapeutic effects on PSCI remain unclear.

This study established a rat stroke model via middle cerebral artery occlusion (MCAO) surgery[15]. Edaravone (Eda), approved for treating acute ischemic stroke[16], was used as a positive control drug to evaluate the efficacy of SG on PSCI. Furthermore, this study aims to identify new potential therapeutic targets and provide a theoretical basis for treating PSCI by elucidating the regulatory mechanisms of SG on the p38 MAPK signaling pathway.

Materials and Methods

MCAO model construction[17]

A total of 60 male Sprague-Dawley rats (8 weeks old, weighing 250-300 g) were purchased from Hunan

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Slack Jingda Experimental Animal Co., Ltd. The rats were housed in a controlled environment with a 12-hour light/dark cycle and free access to food and water.

After an intraperitoneal injection of sodium pentobarbital at a dose of 40 mg/kg for anaesthesia, the rats were secured in a supine position on the operating table. A mouth gag was used to pull the rat's incisors upward to straighten the neck. The surgical area was shaved and disinfected. A 1.5-cm midline incision was made along the neck, and the muscles were bluntly separated with forceps to expose the right common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA). The CCA was carefully separated from the accompanying vagus nerve, and the proximal end of the CCA was ligated. A slip knot was tied at the distal end with a 4-0 surgical suture. A small incision was made in the CCA between the two knots using ophthalmic scissors. A filament was inserted into the vessel. The slip knot was loosened to allow smooth passage of the filament, and then slightly tightened to prevent bleeding. The filament was inserted into the right ICA using forceps until resistance was felt. Then, the slip knot was secured, and the surgical site was sutured. After 90 min of occlusion, the filament was withdrawn to restore blood flow[18]. In MCAO model validation, rats exhibit ptosis of the right eyelid. When placed on the ground, they are unable to walk in a straight line and instead circle continuously toward the affected side. Additionally, when lifted by the tail, their body curves toward the affected side. These behavioural signs collectively indicate the successful establishment of the MCAO model[19].

Experimental groupings

In experiment 1, rats were randomly divided into six groups: Sham, MCAO, SG-low, SG-medium, SG-high, and Eda groups. Immediately after MCAO surgery, rats in the SG groups received intraperitoneal injections of SG (BD39893, Bide Pharmatech Ltd, Shanghai, China) at doses of 1.0, 2.5, or 6.25 mg/kg[14]. The Eda group received 4 mg/kg Eda (HY-B0099, MCE, Princeton, NJ, USA) intraperitoneally[20]. The Sham and MCAO groups received an equal volume of 0.9% saline. Drug treatments were administered once daily for 7 consecutive days.

In experiment 2, rats were allocated into four groups: Sham, MCAO, SG, and SG+anisomycin (a p38 MAPK activator, HY-18982, MCE) groups. In the SG+anisomycin group, rats were anaesthetized 20 min before modelling and fixed in a stereotactic apparatus. The scalp was disinfected, and a 1-cm midline incision was made. The bregma was identified as the coordinate origin, and a hole was drilled using a dental drill. Anisomycin (10 μL of 5 μM) was slowly injected into the right cerebral hemisphere[21]. After a 5 min pause, the needle was withdrawn, and the scalp was sutured. Except for the sham group, all groups underwent MCAO surgery. The SG and SG+anisomycin groups received intraperitoneal injections of 6.25 mg/kg SG daily for 7 days, starting 24 h after modelling. The sham and MCAO groups received equivalent volumes of 0.9% saline.

On day 7 after modelling, behavioural tests were performed. The rats were then euthanised. Brain tissues were collected for cryosection preparation, and hippocampal tissues were dissected and stored at -80°C.

Modified neurological severity score (mNSS)[22]

mNSS was used to evaluate neurological deficits in rats, including motor function, sensory response, balance, and reflex/abnormal movements. The mNSS scores range from 0 (normal) to 18 (severe).

Novel object recognition test (NORT)[23]

In the training phase, rats were placed in an empty arena (a white box measuring 40 cm in width, depth, and height) to explore freely. They were then exposed to two identical objects for 5 min. Twenty-four hours later, the rats were tested in the same arena with one familiar object and one novel object. The recognition index (RI) was calculated as the proportion of time spent exploring the novel object relative to the total exploration time of both the novel and familiar objects.

The Y-maze test

The experimental procedures followed those described in previous literature[22]. The percentage of time spent in the novel arm was calculated using the formula: (time spent in the novel arm / total exploration time) ×100.

Nissl staining

The Nissl staining kit was obtained from Abiowell (AWI0501a, Changsha, China). Rat brain sections were dewaxed, rehydrated, and stained with Nissl stain for approximately 1 min. Differentiation was performed in 1% acetic acid until cell structures and Nissl bodies were clearly defined. Finally, the stained sections were mounted

Table 1 — The information about antibodies

Name	Dilution rate	Cat number	Company	Country
Bcl-2	1:2000	26593-1-AP	Proteintech	USA
Bax	1:5000	50599-2-Ig	Proteintech	USA
Cleaved-caspase 3	1:1000	#9661	CST	USA
p-p38	1:1000	ab4822	Abcam	UK
p38	1:1000	14064-1-AP	Proteintech	USA
β-actin	1:5000	66009-1-Ig	Proteintech	USA
HRP goat anti-mouse IgG	1:5000	SA00001-1	Proteintech	USA
HRP goat anti-rabbit IgG	1:6000	SA00001-2	Proteintech	USA

with a buffered glycerol solution and examined under an optical microscope.

Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) staining

The commercial apoptosis detection kit (40306ES50) was purchased from Yeasen Biotechnology (Shanghai) Co., Ltd. Sections were initially dewaxed and rehydrated, then treated with Proteinase K for 20 min at 37°C to enhance tissue permeability. After rinsing in phosphate-buffered saline (PBS), the sections were incubated with a TUNEL reaction mixture comprising FITC-labelled dUTP and recombinant TdT enzyme in a dark environment for 60 min at 37°C. Negative controls were established using a buffer lacking TdT enzyme, supplemented with ddH_2O. Subsequently, slides were counterstained with DAPI to delineate nuclei and mounted using a buffer glycerin solution. Fluorescence microscopy was employed to detect apoptotic cells, characterised by green fluorescence, contrasted against a blue-stained nuclear backdrop.

Western blot

Proteins were extracted from hippocampal tissues and separated by SDS-PAGE, then transferred onto nitrocellulose membranes. After blocking, membranes were incubated with primary antibodies overnight at 4°C, followed by incubation with Horseradish Peroxidase (HRP)-conjugated secondary antibodies. Table 1 provides relevant information regarding the antibodies. Protein bands were visualised using an ECL system. Band intensities were quantified with Quantity One, with β-actin levels serving as an internal reference.

Enzyme-linked immunosorbent assay (ELISA)

The concentrations of the cytokines tumor necrosis factor-α (TNF-α, CSB-E11987r, Cusabio, China), interleukin (IL)-1β (JL20884, Jonlnbio, China), and IL-10 (CSB-E04595r, Cusabio) in hippocampal tissue were quantitatively analyzed using ELISA kits following standardised protocols. The optical density was measured at 450 nm using a microplate reader, and cytokine levels were quantified based on standard curves.

Molecular docking

Molecular docking was conducted using VINA 1.1.2 software (The Scripps Research Institute, La Jolla, CA, USA) to predict the binding interactions between SG and p38 MAPK protein. The docking utilised a semi-empirical scoring function to estimate binding affinity. The results were visualised and analysed using PYMOL (Schrödinger, LLC and New York, NY, USA).

Immunofluorescence (IF) analysis

Hippocampal tissue sections were initially baked at 60°C for 12 h. The sections underwent deparaffinization by sequential incubation in xylene and a graded series of ethanol baths, concluding with a wash in distilled water. Antigen retrieval was performed by immersing the sections in 0.01M sodium citrate buffer (pH 6.0) and microwaving until boiling, followed by cooling to room temperature (23 ± 2°C) and washing three times. After blocking with a solution containing 5% bovine serum albumin, the sections were incubated with a primary antibody against phosphorylated p38 (p-p38, dilution ratio: 1:400; 28796-1-AP, Proteintech, Rosemont, IL, USA) overnight at 4°C, followed by incubation with goat anti-rabbit IgG (H+L) secondary antibody (AWS0005a, Abiowell). Nuclear counterstaining was performed using DAPI (AWC0293a, Abiowell). The slides were then mounted with a glycerin-based medium and examined under a fluorescence microscope to assess p-p38 expression.

Statistical analysis

Differences among multiple groups were assessed using one-way ANOVA with GraphPad Prism

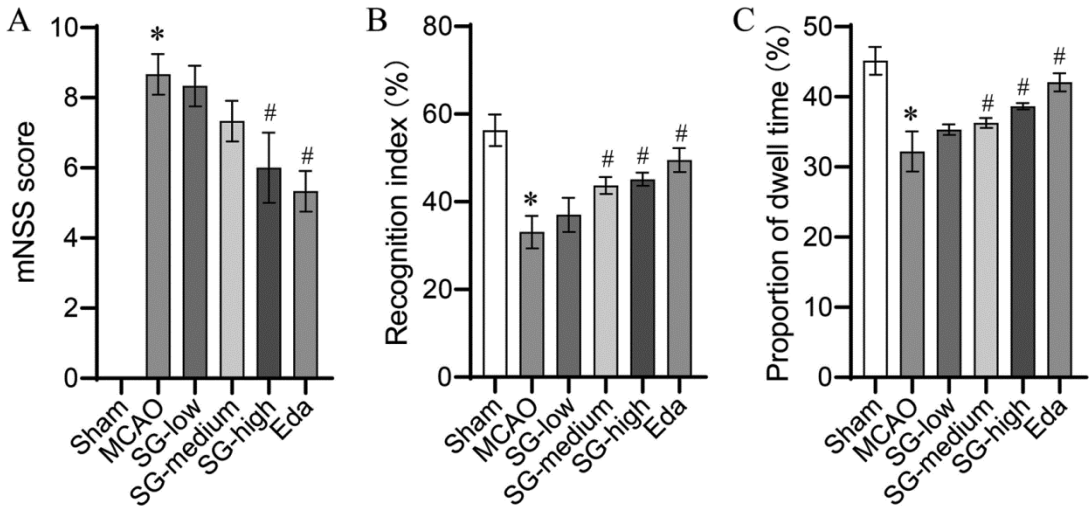

Fig. 1 — SG improved cognitive dysfunction in stroke rats. (A) mNSS assessment. (B) RI was determined by NORT. (C) Percentage of time spent in the new arm of the Y-maze. [*P < 0.05 vs. Sham, #P < 0.05 vs. MCAO. n=6. (SG, Sanguinarine. MCAO, middle cerebral artery occlusion. Eda, Edaravone. mNSS, modified neurological severity score. RI, recognition index. NORT, novel object recognition test)]

8 software, while pairwise comparisons were made using the t-test. Results are presented as mean ± standard deviation. A P-value of less than 0.05 was considered statistically significant.

Results

SG improved cognitive dysfunction in stroke rats

Eda was used as the positive control drug to evaluate the effects of SG on neurological function and cognitive function in stroke rats. The Sham group exhibited no neurological deficits, as evidenced by a mNSS of zero (Fig. 1A). In contrast, rats subjected to MCAO displayed significantly elevated mNSS, indicating severe neurological deficits after stroke (Fig. 1A). Notably, treatment with medium and high doses of SG and Eda significantly reduced the mNSS (Fig. 1A). The cognitive and spatial memory abilities of rats were subsequently evaluated using the NORT and the Y-maze test (Fig. 1B & 1C). Compared with the sham group, the RI and the proportion of time spent in the novel arm were significantly reduced in the MCAO group, confirming that cognitive and spatial memory impairments occurred in stroke rats. Treatment with SG (medium and high doses) and Eda significantly increased the RI and the time spent in the novel arm, indicating enhanced cognitive function and spatial memory. Among these treatments, the high dose of SG showed an effect closest to that of Eda. In summary, MCAO rats demonstrated cognitive impairments, confirming the successful establishment of the PSCI model. SG treatment significantly improved the neurological function and cognitive impairments in stroke-affected rats.

SG improved hippocampal neuron injury in stroke rats

Given the critical role of hippocampal neurons in cognitive function and their susceptibility to ischemic damage[24], this study aimed to investigate the protective effects of SG on hippocampal neurons in the brains of stroke rats. Nissl staining results indicated that the number of Nissl-positive neurons was significantly reduced in the MCAO group compared to the sham group, reflecting neuronal damage caused by stroke. However, treatment with different doses of SG increased the number of Nissl-positive neurons, demonstrating the protective effects of SG on hippocampal neurons (Fig. 2A). The high-dose group showed significant improvement, with effects comparable to those of the Eda group. Further analysis using TUNEL staining (Fig. 2B & 2C) revealed a significant increase in apoptotic cells in the hippocampus of stroke rats compared to the sham group. SG treatment significantly reduced the number of apoptotic cells in a dose-dependent manner. The number of TUNEL-positive cells in the high-dose SG group was similar to that in the Eda group, indicating that high-dose SG effectively inhibited apoptosis of hippocampal neurons.

Western blot analysis confirmed these findings (Fig. 2D). In the MCAO group, levels of the anti-apoptotic protein B cell lymphoma-2 (Bcl-2) were reduced, while levels of pro-apoptotic proteins

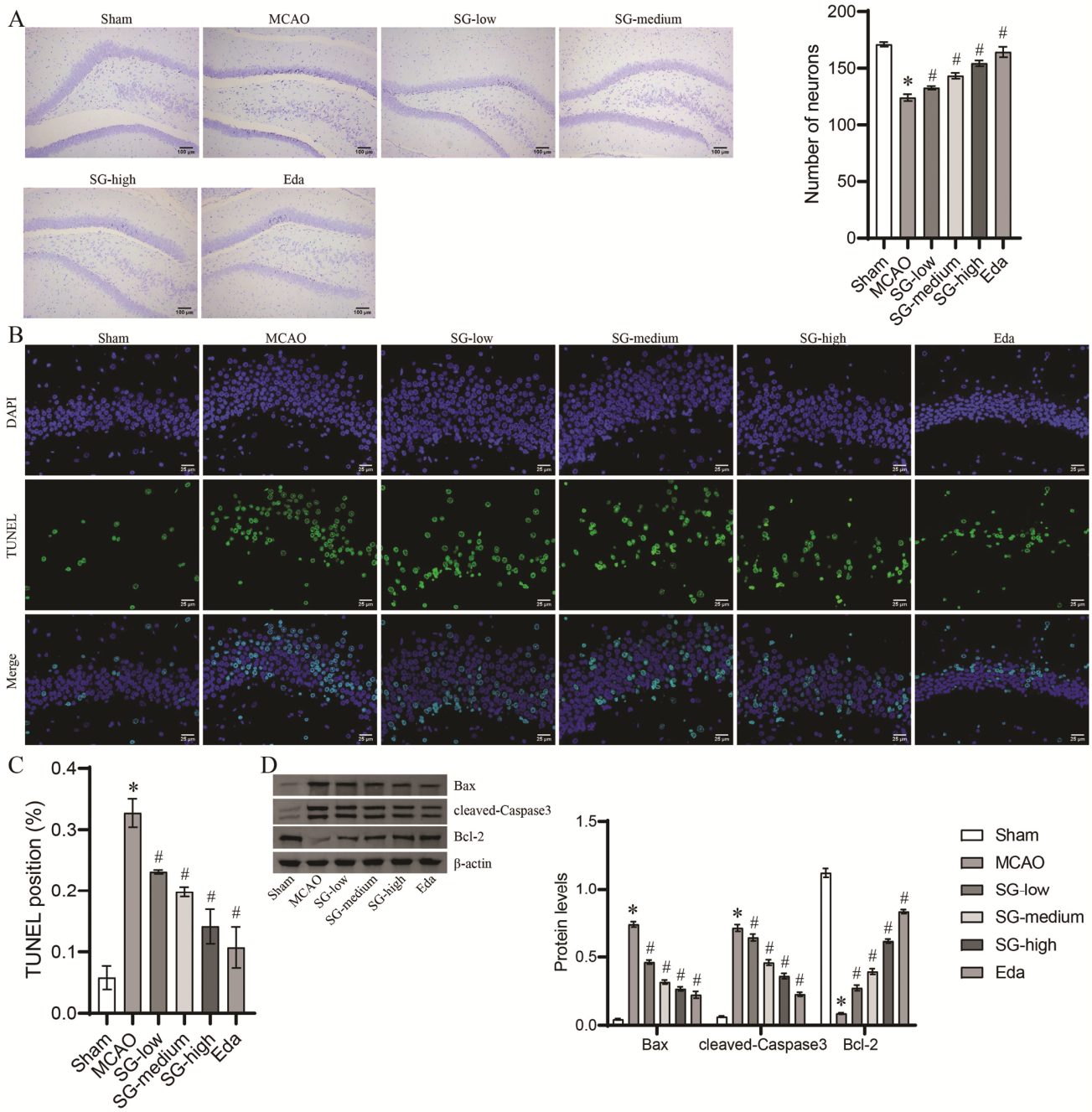

Fig. 2 — SG improved hippocampal neuron injury in stroke rats. (A) Nissl staining was used to measure the number of neurons in the hippocampus of rats. Scale bars: 100 μm. (B and C) Quantification of cell apoptosis rates was performed using TUNEL staining. Scale bars: 25 μm. (D) Levels of apoptosis-related proteins. [*P < 0.05 vs. Sham, #P < 0.05 vs. MCAO. n=6. (SG, Sanguinarine. MCAO, middle cerebral artery occlusion. Eda, Edaravone. TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling. Bcl-2, B cell lymphoma-2. Bax, Bcl2-associated X protein)]

Bcl-2-associated X protein (Bax) and cleaved caspase-3 were elevated. Treatment with SG and Eda reversed these changes, with the high-dose group showing protein levels comparable to those of the Eda group, further supporting the anti-apoptotic effects of SG. In conclusion, SG protected the hippocampal neurons of stroke-affected rats by inhibiting the apoptotic pathway.

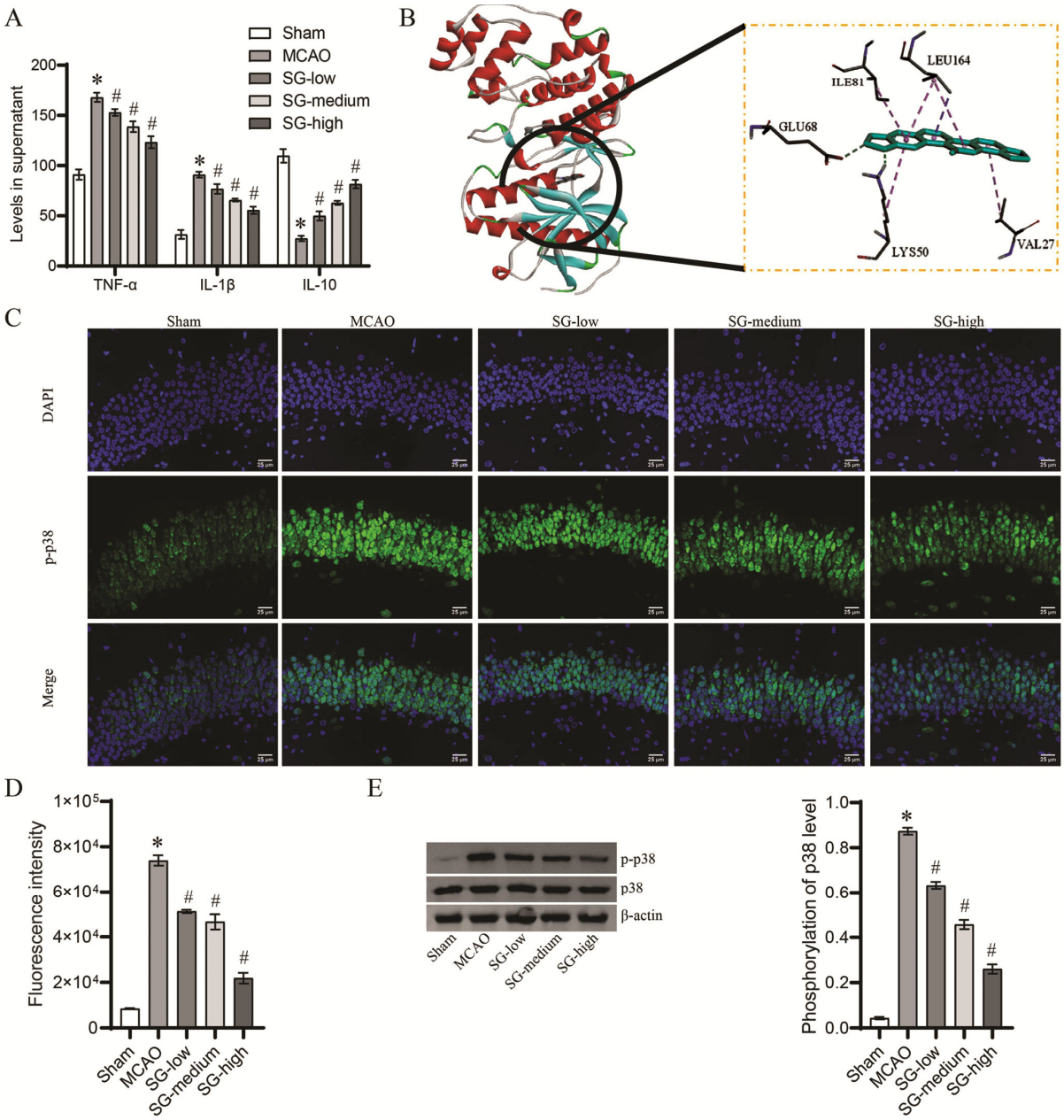

Fig. 3 — SG reduced neuroinflammation and inhibited the activation of p38 MAPK in stroke rats. (A) ELISA analysis of cytokine levels in hippocampal tissue supernatants. (B) Molecular docking analysis of SG binding to p38 MAPK. (C and D) The level of p-p38 in the hippocampus was detected by IF. Scale bars: 25 μm. (E) Western blot analysis of p38 phosphorylation levels in the hippocampus. [*P < 0.05 vs. Sham, #P < 0.05 vs. MCAO. n=6. (SG, Sanguinarine. MAPK, mitogen-activated protein kinase. MCAO, middle cerebral artery occlusion. ELISA, enzyme-linked immunosorbent assay. TNF-α, tumor necrosis factor-α. IL-1β, interleukin-1β. IL-10, interleukin-10. IF, Immunofluorescence. p-p38, phosphorylated p38)]

SG reduced neuroinflammation and inhibited the activation of p38 MAPK in stroke rats

Neuroinflammation and the activation of stress-related signaling pathways, such as the p38 MAPK pathway, are key factors in neuronal damage and cognitive impairment[6]. To verify the effects of SG on neuroinflammation in PSCI rats and its regulatory role in the p38 MAPK signaling pathway, we conducted a comprehensive evaluation using ELISA, molecular docking, IF and Western blot. Compared with the sham group, levels of the pro-inflammatory cytokines TNF-α and IL-1β were significantly elevated in the hippocampal tissue of stroke rats, while levels of the anti-inflammatory cytokine IL-10 were reduced (Fig. 3A). SG treatment

significantly decreased TNF-α and IL-1β levels in a dose-dependent manner and upregulated IL-10 levels, demonstrating its pronounced anti-inflammatory effects (Fig. 3A). Additionally, molecular docking experiments revealed that SG has a binding affinity of -9.5 kcal/mol with p38 MAPK, indicating that SG can spontaneously bind to p38 MAPK (Fig. 3B). We then assessed the activation levels of p38, a core kinase in the p38 MAPK signaling pathway, using IF and Western blot (Fig. 3C-E). Stroke rats exhibited significantly increased p-p38 expression in the hippocampal tissue, which was markedly reduced by SG treatment, with the most significant effect observed in the high-dose SG group. In summary, SG treatment significantly reduced neuroinflammation and inhibited the activation of the p38 MAPK pathway in stroke rats.

SG improved cognitive dysfunction in stroke rats by regulating the p38 MAPK pathway

To elucidate the potential therapeutic mechanisms of SG in PSCI, we investigated whether SG could improve cognitive deficits in stroke-affected rats by modulating the p38 MAPK signaling pathway. The experimental groups included the sham group, MCAO group, SG-treated group, and SG + anisomycin group. Both IF and Western blot analyses consistently demonstrated that the phosphorylation level of p38 in the hippocampal tissue of stroke rats was significantly elevated (Fig. 4A & 4B). After treatment with SG, the level of p-p38 was significantly reduced. Notably, the addition of anisomycin to SG treatment reversed this effect, leading to an increase in p-p38 levels, indicating that anisomycin counteracted the inhibitory effect of SG on the p38 MAPK pathway.

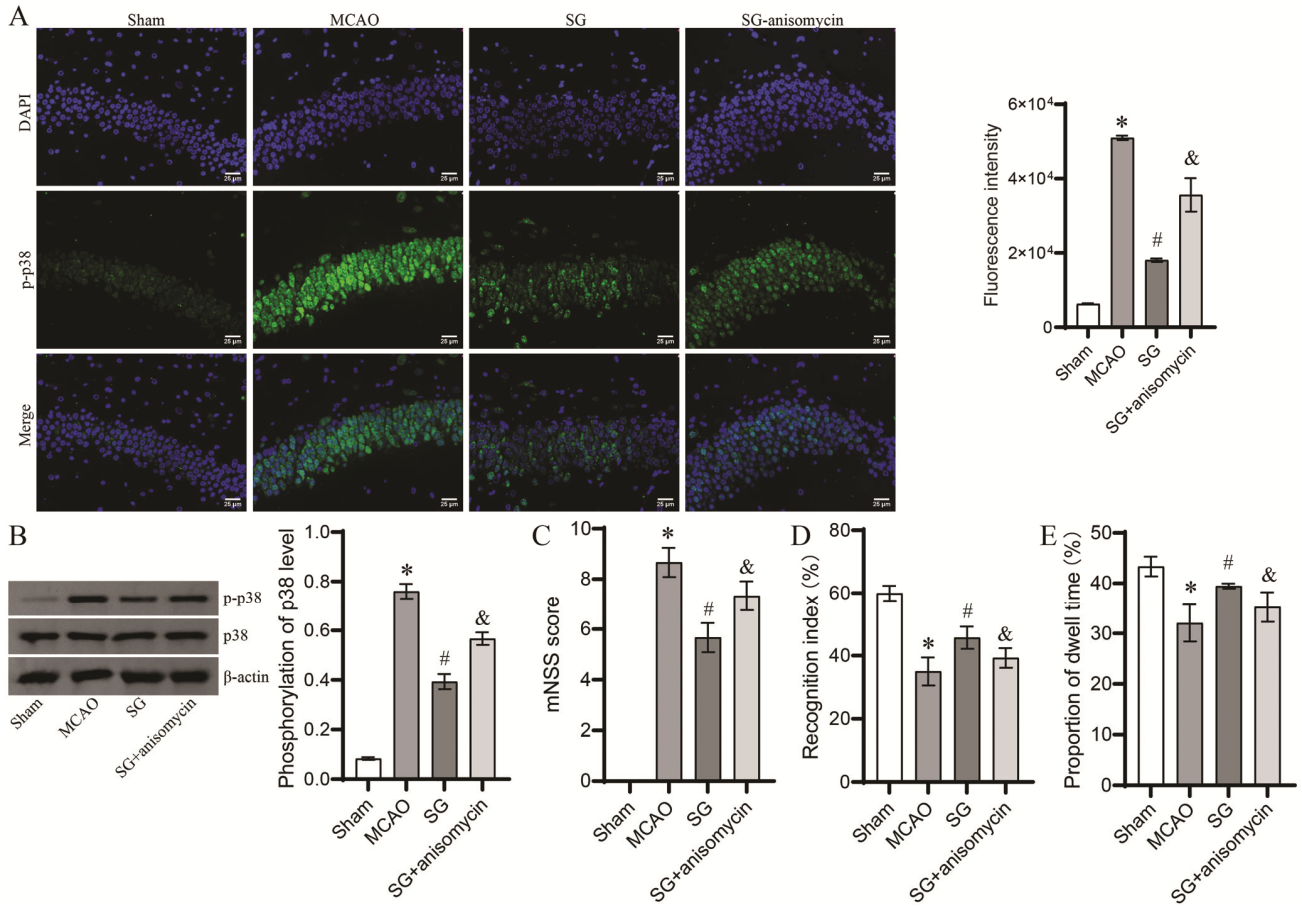

Fig. 4 — SG improved cognitive dysfunction in stroke rats by regulating the p38 MAPK pathway. (A) The level of p-p38 in the hippocampus was detected by IF. Scale bars: 25 μm. (B) Western blot analysis of p38 phosphorylation levels in the hippocampus. (C) mNSS assessment. (D) RI was determined by NORT. (E) Percentage of time spent in the new arm of the Y-maze. [*P < 0.05 vs. Sham, #P < 0.05 vs. MCAO, &P < 0.05 vs. SG. n=6. (SG, Sanguinarine. MAPK, mitogen-activated protein kinase. MCAO, middle cerebral artery occlusion. IF, Immunofluorescence. p-p38, phosphorylated p38. mNSS, modified neurological severity score. RI, recognition index. NORT, novel object recognition test)]

Subsequently, the neurological and cognitive functions of each group were evaluated (Fig. 4C-E). The mNSS was significantly higher in the MCAO group than in the sham group, but it was reduced following SG treatment, indicating that SG improved neurological function. However, the addition of anisomycin to SG treatment resulted in a higher mNSS score compared to the SG group, suggesting that activation of the p38 MAPK pathway counteracted the neuroprotective effects of SG. Additionally, compared to the sham group, the RI and the proportion of time spent in the novel arm were significantly lower in the MCAO group, while SG treatment significantly increased these indices. Conversely, the combination of SG and anisomycin decreased the RI and the time spent in the novel arm, indicating that the p38 MAPK pathway played a crucial role in the cognitive-enhancing effects of SG. In summary, these results demonstrated that SG improved cognitive deficits in stroke-affected rats by inhibiting the p38 MAPK pathway.

SG inhibited neuronal damage and reduced inflammatory response in stroke rats by regulating the p38 MAPK pathway

To further elucidate the neuroprotective and anti-inflammatory mechanisms of SG in PSCI, we investigated whether SG could inhibit neuronal damage and inflammatory responses in stroke-affected rats by modulating the p38 MAPK pathway. Nissl staining revealed the fewest Nissl-positive neurons in the MCAO group (Fig. 5A). Rats treated with SG had a significantly higher number of Nissl-positive neurons in the hippocampus compared to the MCAO group, indicating reduced neuronal loss (Fig. 5A). However, the number of Nissl-positive neurons was significantly reduced after combined treatment with SG and anisomycin, indicating that the neuroprotective effects of SG were associated with the p38 MAPK pathway (Fig. 5A). This observation was further confirmed by TUNEL staining (Fig. 5B). Compared to the MCAO group, rats treated with SG had significantly fewer apoptotic cells in the hippocampus, a number that increased significantly when anisomycin was added to the treatment. Western blot analysis also showed that SG treatment upregulated the expression of the anti-apoptotic protein Bcl-2 while downregulating the expression of pro-apoptotic proteins Bax and cleaved Caspase-3 (Fig. 5C). The addition of anisomycin to SG treatment reversed these changes, indicating that SG inhibited neuronal apoptosis by downregulating the p38 MAPK pathway (Fig. 5C). In addition to its neuroprotective effects, SG exhibited significant anti-inflammatory properties (Fig. 5D). ELISA analysis revealed that SG treatment reduced the levels of pro-inflammatory cytokines TNF-α and IL-1β, while increasing the level of the anti-inflammatory cytokine IL-10 in the hippocampus. The combination of SG and anisomycin treatment reversed these cytokine expression levels, indicating that the p38 MAPK pathway mediated the anti-inflammatory effects of SG. Overall, SG inhibited neuronal damage and reduced inflammatory responses in stroke rats by inhibiting the p38 MAPK pathway.

Discussion

PSCI is a common and severe complication of stroke. Current treatment options for PSCI are limited. SG, a benzophenanthridine alkaloid extracted from *Sanguinaria canadensis*, has shown promising neuroprotective potential in various preclinical studies. This study employed an MCAO rat model to investigate the therapeutic effects of SG on PSCI and its underlying mechanisms, with a particular emphasis on the neuroprotective role of SG mediated through the p38 MAPK signaling pathway.

The hippocampus plays a pivotal role in the pathogenesis of PSCI, given its heightened vulnerability to ischemic and reperfusion injury[25]. Dysfunction of hippocampal interneurons is closely related to cognitive deficits in neurological diseases such as cerebral hypoxia and ischemia[26]. Studies have shown that following ischemic injury, apoptotic signaling pathways are activated in neurons, leading to neuronal death[27]. The ability of neurons to recover from apoptotic signals depends on anti-apoptotic Bcl-2 family proteins[27]. Additionally, the inflammatory response characteristic of PSCI involves the activation of microglia and astrocytes, as well as the release of pro-inflammatory cytokines, which further exacerbates neuronal damage and impacts cognitive function recovery[28]. Our research indicated that SG improved neurological function, spatial memory, and cognitive abilities in stroke rats. The therapeutic potential of SG for PSCI was attributed to its neuroprotective effects, including the reduction of hippocampal neuron apoptosis.

Recent studies have reported that the activation of the Nod-like receptor family pyrin domain containing 3 (NLRP3) inflammasome plays a role in driving neuroinflammation and cognitive decline following stroke[29]. A novel NLRP3 inhibitor, Britannin, has

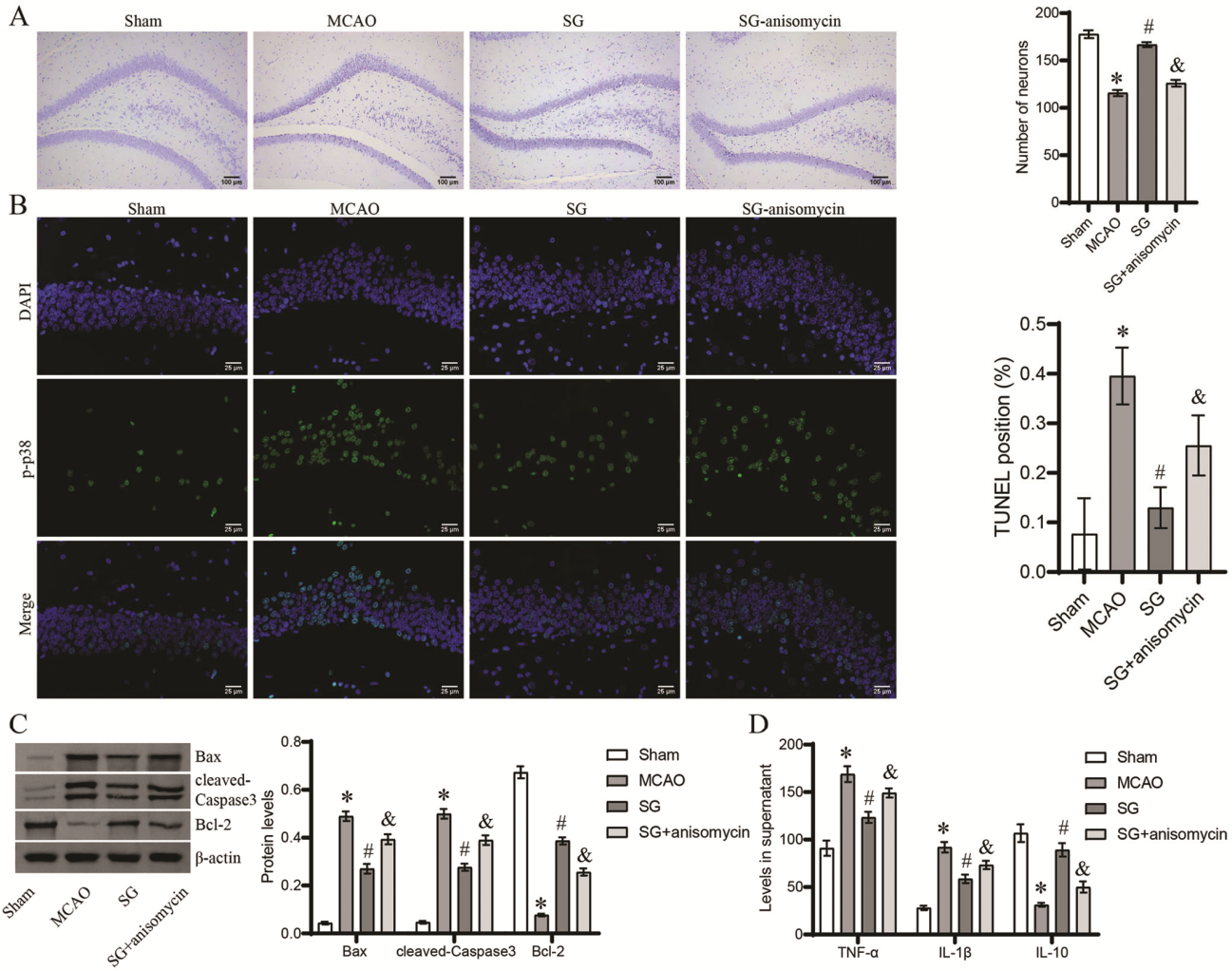

Fig. 5 — SG inhibited neuronal damage and reduced inflammatory response in stroke rats by regulating the p38 MAPK pathway. (A) Nissl staining was used to measure the number of neurons in the hippocampus of rats. Scale bars: 100 μm. (B) Quantification of cell apoptosis rates was performed using TUNEL staining. Scale bars: 25 μm. (C) Levels of apoptosis-related proteins. (D) ELISA analysis of cytokine levels in hippocampal tissue supernatants. [*P < 0.05 vs. Sham, #P < 0.05 vs. MCAO, &P < 0.05 vs. SG. n=6. (SG, Sanguinarine. MAPK, mitogen-activated protein kinase. MCAO, middle cerebral artery occlusion. p-p38, phosphorylated p38. TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling. Bcl-2, B cell lymphoma-2. Bax, Bcl2-associated X protein. ELISA, enzyme-linked immunosorbent assay. TNF-α, tumor necrosis factor-α. IL-1β, interleukin-1β. IL-10, interleukin-10)]

been shown to suppress inflammasome activation by disrupting the NLRP3-NIMA-related kinase 7 interaction, further supporting the therapeutic potential of NLRP3 modulation in neuroinflammatory diseases[30]. Notably, electroacupuncture (EA) has been demonstrated to alleviate PSCI by modulating the mTOR/NLRP3-mediated autophagy-inflammatory pathway, indicating that targeting NLRP3 and inhibiting apoptosis may be a viable therapeutic strategy[31]. Our study demonstrated that SG attenuated neuroinflammation and apoptosis by inhibiting the p38 MAPK pathway, thereby expanding on these findings. The activation of p38 MAPK can regulate a variety of transcription factors, resulting in diverse biological effects, including cell proliferation, differentiation, and apoptosis[32]. It plays a significant role in neuroinflammation and tau protein phosphorylation, promoting the formation of neurofibrillary tangles and neuronal loss[33]. Research has indicated that the p38 MAPK signaling pathway mediates reactive oxygen species-induced activation of the NLRP3 inflammasome, leading to the upregulation of IL-1β, which in turn exacerbates neuronal damage and cognitive decline[34]. Moreover, molecular docking analysis in our study further supported the direct interaction of SG with p38

MAPK. This interaction positions SG as a potential lead compound for targeting the p38 MAPK/NLRP3 pathway.

An increasing body of evidence supports the notion that cognitive recovery following stroke is closely related to synaptic plasticity[35,36]. For instance, optogenetic stimulation-induced gamma oscillations have been shown to enhance synaptic plasticity and motor function in stroke mice, thereby improving cerebral blood flow and motor function[37]. Brain-derived neurotrophic factor (BDNF) can activate a variety of downstream signaling pathways via its receptor Tropomyosin receptor kinase B, promoting neuronal survival, differentiation, and synaptic plasticity[38]. The activation of p38 MAPK can modulate the expression and secretion of BDNF[39]. Moreover, BDNF can also regulate the activity of p38 MAPK through its signaling pathways, thereby influencing neuroinflammation and neuroprotection[40]. Therefore, we speculate that the improvement of cognitive deficits in stroke rats by SG is associated with the modulation of BDNF through the p38 MAPK pathway to enhance synaptic remodelling. In addition, other signaling pathways, such as the Hippo pathway, which regulates cell division, survival, and differentiation through interactions among mammalian sterile twenty 1/2, large tumor suppressor 1/2, and yes-associated protein/transcriptional coactivator with PDZ-binding motif proteins in ischemic stroke[41]. These pathways may also be involved in the neuroprotective effects of SG, and further research is needed to elucidate the specific molecular mechanisms and potential therapeutic targets.

Current treatments for PSCI, such as cholinesterase inhibitors and memantine, are limited in efficacy and expensive[42]. This aligns with recent advancements in NLRP3-targeted therapies, such as MCC950 and Britannin, which have shown promise in preclinical models but face challenges in clinical translation due to toxicity issues[43]. In contrast, the natural origin and favourable safety profile of SG may circumvent these limitations, making it a viable candidate for further development[44]. SG offers a cost-effective, multi-targeted approach by addressing neuroinflammation, apoptosis, and synaptic dysfunction simultaneously. Although our study provides compelling evidence for the efficacy of SG, several limitations are worth considering. First, the short observation period (7 days) does not demonstrate the long-term neuroprotective effects of SG. Second, the use of only male rats limits generalizability, considering the known sex differences in stroke pathophysiology. Future studies should explore the pharmacokinetics of SG, optimal dosing regimens, and potential synergistic effects with existing therapies, such as EA or cognitive training.

Conclusion

SG has shown significant efficacy in improving PSCI. It enhances cognitive function by reducing neuronal damage and apoptosis in the hippocampus. Additionally, SG inhibits the p38 MAPK signaling pathway, resulting in decreased levels of TNF-α and IL-1β, which in turn alleviates inflammatory responses. Molecular docking experiments further support the direct interaction between SG and p38 MAPK. The protective effects of SG were reversed when it was co-administered with anisomycin, highlighting the importance of the p38 MAPK signaling pathway in the neuroprotective effects of SG. These findings identify new potential therapeutic targets and strategies for treating PSCI.

Ethical statement

The study was approved by the Ethics Committee of The First Hospital of Changsha (2023 No. 32). The study was performed in accordance with the ARRIVE guidelines and related regulations.

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Conflict of interest

The author has no conflict of interest to declare.

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