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Original Article

Neuroprotective Effect of *Stemona tuberose* Leaf Extract on MCAO/R-Induced Rats via P13K/Akt Signaling Pathway

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ABSTRACT

Background: Ischemic Stroke (IS) is a serious illness with great fatality and disability globally. The pathophysiological causes of IS are oxidative stress, apoptosis and neuroinflammation. **Objectives:** Cerebral Ischemia/Reperfusion Injury (CI/RI) is arecurrent pathological development resulting in IS. *Stemona tuberose* (ST), which comes under the *Stemonaceae* family, has been extensively involved in Chinese traditional medication to treat inflammatory disorders. **Materials and Methods:** The current study investigated this medicinal herbs Stemona tuberose leaf extract (STLE) for the first time concerning its neuroprotective activity inthe CI/RI ratsdeveloped by the MCAO/R technique. Rats were distributed into 5 sets; sham, MCAO/R-induced, MCAO/R+STLE (25 mg/kg bw), MCAO/R + STLE (50 mg/kg bw) and MCAO/R+STLE (100 mg/kg bw). CI/RI triggered cerebral injury was evaluated using infarction volume, oxidative stress, histopathology, microglial polarization, neuroinflammation and apoptosis. **Results:** STLE could dose-dependably alleviate infarction volume, oxidative stress, histopathological changes, neuroinflammation and apoptosis while enhancing microglia/macrophages M1/M2 polarization in CI/RI rats. STLE exerts anti-apoptotic properties by the augmented Bcl-2/Bax proportion and diminished caspase-3 levels. STLE could modulate the microglia polarization from the phenotype M1 pro-inflammatory to the M2 anti-inflammatory potential. **Conclusion:** Furthermore, STLE showed promise in reducing CI/RI-induced apoptosis by modulating the P13K/Akt signaling pathway. These results demonstrate STLE's therapeutic promise in the management of ischemic stroke and associated neuroinflammatory diseases.

Keywords: Apoptosis, Ischemia-reperfusion, Ischemic stroke, Neuroinflammation, Oxidative stress, P13K/Akt signaling, *Stemona tuberosa*.

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INTRODUCTION

Ischemic Stroke (IS) is the supreme predominant type of cerebrovascular disorder which remains an enormous risk of fatality and debility in the Universe.[1] IS is usually affected by high cholesterol levels, elevated blood pressure, diabetes mellitus, atrial fibrillation and transient ischemic attack.[2,3] Ensuring the speedy restoration of blood flow and reoxygenation and diminishing the possibility of permanent harm is the only universally accepted handling approach after IS.[4,5] Awkwardly, growing documents propose that reperfusion after IS triggers an immune cascade that results in considerable ancillary neuronal injury.[6] After an ischemic attack, cells in the ischemic portion of the brain commence to die, which leads to neurological impairment and further severe secondary damage.[7] Despite the broad investigation, couldn't find a remedy for this prevalent disease condition. Therefore, there is an insistent requirement to decrease brain damage and inflammation and to promote neurological recovery after ischemic stroke.

Stemona tuberosa (ST) is an elegant plant belonging to the family *Stemonaceae* that is intuitive to China, Southeast Asia and northeast India, which is among the 50 fundamental remedial herbs included in Chinese traditional medicine.[8] ST is mostly used for relieving lung disorders, cough and killing lice and insects in China as formally renowned in Chinese Pharmacopeia.[9] It has been isolated and structurally elucidated more than 250 Stemona alkaloids from the *Stemonaceae* family.[10] These alkaloids have exposed numerous biological properties including antitussive and anti-inflammatory activities.[10,11] The ST Root extracts have exhibited antitussive, antiviral, antibacterial, antifungal, insecticidal and neuroprotective activities.[10-12] Another study established that ST Root extracts CH_2Cl_2 fraction has expressively repressed LPS-induced NO production in murine microglial cells BV2.[12] Indeed extreme NO is described to play a contributory action in the development of inflammatory neurodegenerative ailments comprising CNS disorders, Alzheimer's,and Parkinson's

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disease.[13] In Asian traditional medicines use *Stemona* species to treat skin and respiratory illnesses and reduce pain and inflammation.[14,15] Reports indicate that *Stemona* alkaloids isolated from the *Stemona* genus possess anti-inflammatory and anti-cancer effects.[16] In *Stemona tuberosa* have stemofoline derivatives, including didehydrostemofoline and eleven synthesized derivatives with different OH- and N-substitutions in the C-3 side-chain, it's may supported for our study. However, the neuroprotective and anti-inflammatory activity of *Stemona tuberosa* Leaf Extracts (STLE) required further investigation. Herein, we evaluate the neuroprotective, anti-apoptotic and anti-inflammatory activity of STLE on the rat model of Cerebral Ischemia (CI)//Reperfusion Injury (RI), which was developed by the Middle Cerebral Artery Occlusion/Reperfusion (MCAO/R) method and elucidate the probable latent mechanisms.

The mechanism underlying IS pathophysiology has exposed that energy failure, intracellular calcium overload, oxidative stress, free radical generation, neuroinflammation and atypical stimulation of immune cells play significant parts in cerebral damage after IS, which is ultimately prone to neuronal death such as necrosis and apoptosis.[17,18] The cerebral inflammation after trauma is activated by the early damage, which is regarded by microglia stimulation, leukocyte recruitment and consequent elevation of cytokines, which plays a vital role in developing ancillary damage.[19] This inflammation has also promoted edema development and neuronal death and eventually led to efficient flaws.[20] While several factors worsen brain damage, the neuroinflammatory reaction is the crucial element that facilitates cascade responses and is imperative in its pathogenesis.[21] Adequate responsiveness is essential to decrease neuroinflammation and apoptosis to lessen these harms. The general reports indicate that anti-inflammatory and anti-apoptotic elements are significant for the neuron's defense from CI.[22] Due to IS, the core cells die within minutes and the surrounding cells in that zone, namely the ischemic penumbra, remain to pass on after some hours or more than a few days after hurt. Apoptosis is the foremost process of cell demise.[23] Meanwhile, the cerebral damage in the ischemic penumbra progresses further gradually and there is sufficient time for the action of neuroprotection.[24] Hence, emerging healing agents that can avert neuronal apoptosis in the ischemic penumbra have developed a crucial task in IS treatment.

As a resident of cerebral immune cells, microglia exhibit a key action in conserving homeostasis of the Central Nervous System (CNS) in physiological environments.[25] In the CNS the first line of immune protection is microglias, which are sensors that display and react to anomalous variations in the inner and outer CNS. Microglia activation during IS development and relocation to the peri-infarct zone exacerbates the immune reaction by generating pro-inflammatory mediators, disturbing nearby vigorous neurons and producing advanced neuronal injury or demise.[26] Conversely, in pathological conditions, microglia may be promptly stimulated and phenotypes diverged into M1 or M2.[27] Principally, the M1 phenotype shows harmful properties on the brain by discharging numerous cytokines, for instance, IL-1β, IL-6 and TNF-α.[27,28] In comparison, M2 represents the protection for the brain by concealing neurotrophic elements as well as anti-inflammatory cytokines comprising IL-10, IL-4 and TGF-β.[27] Therefore, stimulating microglia phenotype conversion from M1 to M2 appears as a possible approach to prevent inflammation and, hence, to recover cerebral damage.

Apoptosis is the chief kind of cell demise in cerebral ischemia pathogenesis.[29] The pathway of PI3K/Akt is a recurrently variable signaling network that is a vital part in metabolism, cell multiplying, existence and motility in humans.[30] The PI3K/Akt pathway facilitates numerous cellular events in the body, comprising cell metabolism, autophagy, anti-apoptosis and inflammation.[31] Thus, this present research aimed to explore whether STLE treatment efficiently ameliorated MCAO/R-produced CI/RI and to examine whether the underlying mechanism was correlated to the regulation of neuroinflammation and apoptosis facilitated by the PI3K/Akt signaling in rats.

MATERIALS AND METHODS

Chemicals

Chloral hydrate, Triphenyl Tetrazolium Chloride (TTC), Dimethyl Sulfoxides (DMSO), xylazine, ketamine and Sodium Dodecyl-Sulfate (SDS) were acquired from Solar bio (Beijing, China). Biochemical assay kits were provided by Ese-Bio (Shanghai, China). The antibodies (iNOS, Bcl-2, Bax, Caspase-3, CD-86, Arg-1, CD206, p-P13K, P13K, AKT, p-AKT and β-actin) and the secondary antibody for western blot assay were attained from ZSGB-bio (Beijing, China). Biochemicals and solvents of analytical grades were used. *Stemona tuberosa* Leaves (STL) were purchased from China and the botanist identified the plant.

Preparation of STLE

STLE preparation was followed according to the previously described method.[32] Dried *Stemona tuberose* leaves (100 g) were mixed with distilled water and extracted. The resulting isolate was acquired by refluxing the sample for 90 min at room temperature. The impurities were separated from the extracted liquid was sieved over the Whatman No. 41 filter paper. The filtrate was vaporized with a rotary vacuum evaporator at 50°C under reduced pressure. When evaporation, the extracts were lyophilized by a freeze dryer. STLE was measured to prepare the required weights for each treatment group and then dissolved in distilled water at room temperature for 30 min.

Trial animals

Adult *Sprague Dawley* (SD) male rats having 200-220 g were used for the tests. These were preserved in sterile polypropylene coops under specific research environments. Rats were fed with a fixed

pellet diet with ad libitum and an unceasing 12 hr light/dark phase. Rats have adapted one week before the trials. All treatment protocols were conducted as per the Institutional Animal and Ethics Committee, Xi'an Hospital of Traditional Chinese Medicine Encephalopathy, approved (No: 2024-140).

Experimental design

Rats were separated into 5 sets containing 6 animals each. Group 1-Sham; Group 2- MCAO/R rats; Group 3-MCAO/R+STLE (25 mg/kg bw, low dose); Group 4- MCAO/R+ STLE (50 mg/kg bw, middle dose); and Group 5- MCAO/R+STLE (100 mg/kg bw, high dose). STLE was dissolved using distilled water to form the essential dose and administered intraperitoneally (i.p) injected at the onset of reperfusion.

CI/RI induction in rats

CI/RI was induced according to the MCAO and reperfusion as described previously.[33] Briefly, SD rats were starved for 10-12 hr before the surgical procedure and then sedated by using chloral hydrate (10%, 350 mg/kg) through i.p injection. The rats were static in the horizontal position and the left External Carotid Artery (ECA), the right Common Carotid Artery (CCA) and the Internal Carotid Artery (ICA) were steadily separated by the median jugular incision. Then, a slit was produced laterally on the neck median line and alienated the fascia and muscle by a blunt partition. A nylon mono-filament 2.5 was implanted in the CCA into the ICA lumen till it clogged the source of the right MCA. Sequentially detached the filament and let the reperfusion for one day after occlusion for 2 hr. The wound in the neck was then sutured and an iodophor was used as a disinfectant. Similar surgery was conducted for sham rats excluding occlusion/reperfusion. After the experimental period, animals were euthanized by using anesthesia (xylazine, 10 mg/kg and ketamine, 90 mg/kg, i.p) and the brain tissues were collected for biochemical assays and histopathological analysis.

Quantity of infarct volume

Following 24 hr of reperfusion, animals were anesthetized and quickly separated the brains and freezing at -20°C. These were converted into five coronal sections at 2-mm-thickness, staining with TTC (2%) at 37°C for half an hour in the dusky environments and then fixed with paraformaldehyde (4%) at 4°C for one day. These brain slices were observed, photographed and analyzed by using the ImageJ software. Determination of the infarction rate was assessed by the formulae: the left infarct volume/whole brain volume×100%.

Biochemical analysis of brain tissues

One day after reperfusion, the brain tissues were removed and homogenized with saline using a homogenizer at 4°C. Then, separate the supernatant and preserve it at 80°C. The activity of Lactate Dehydrogenase (LDH), Superoxide Dismutase (SOD) and the concentration of Malondialdehyde (MDA) were estimated by using the kits provided by Ese-Bio (Shanghai, China) following their protocols.

RT-qPCR determination of mRNA

Entire RNA was sequestered from the neuronal cells after 24 hr of reperfusion with the TRIzol® kit (Invitrogen Life Technologies) based on the manufacturer's instructions. Equivalent quantities of RNA were used for cDNA synthesis by the cDNA Synthesis kit (Abcam, USA). RT-qPCR was accomplished by using the SYBR-Green Supermix (Abcam, USA). Determined the gene expression based on the $2^{-\Delta\Delta CT}$ algorithm and standardized with internal control β-actin.

Histopathological assessment of the brain tissues

The brain tissues were removed, fixed in formalin (4%) for one day and followed by paraffin-embedded. The brain tissues were cut into slices (5 mm) and were hematoxylin and eosin (H&E) staining for microscopic study. An experienced pathologist carried out the histological examination of all experimental groups using an Olympus BX60 microscope (Olympus, Tokyo, Japan).

Western blotting analysis

The rat's brain proteins were isolated with an ice-cold solution of lysis buffer and the protein quantity was estimated by employing an assay kit (Cayman Chemical Company, MI, USA). When SDS-PAGE electrophoresis was accomplished, the gel was relocated to a PVDF film. The PVDF film was wrapped with skimmed milk (5%) and the diluents (1:1000) of the primary antibody (Bax, Bcl-2, iNOS, Caspase-3, p-PI3K, PI3K, p-Akt, Akt, CD-86, Arg-1, CD-206 and β-actin) and kept at 4°C for one day. Then, the diluents (1:5000) of the HRP-conjugated secondary antibody with skimmed milk powder (5%) and treated to the PVDF film and then incubated. Lastly, an ECL chemiluminescence device was employed for the membrane development. Quantified the protein bands through densitometry with software Image J and homogeneous to β-actin expression.

Statistical analysis

The statistical examination was conducted by the software version 9.0 GraphPad prism and expressed as mean±SD. It was working to perform an Analysis of Variance (ANOVA) and subsequently Duncan's assessment. Student *t*-test has been executed and $p<0.05$ was deliberated as significant statistically.

RESULTS

STLE alleviates MCAO/R-produced infarction volume and oxidative stress in the brain

To examine the neuroprotective efficiency of STLE on MCAO/R-developed rats, consider the results of STLE on cerebral

infarct volume and oxidative stress markers such as LDH, SOD and MDA. The cerebral infarct volume, LDH and MDA were markedly increased ($p<0.05$) in the CI/RI rats while reducing SOD activity when compared to the sham. Administration of STLE (25, 50 and 100 mg/kg bw) in CI/R rats exposed a substantial decrease ($p<0.05$) in cerebral infarct volume, LDH and MDA, whereas elevated SOD activity in a dosage-related mode (Figure 1). These findings proposed that STLE could remarkably reduce CI/RI and ameliorate neurological function.

STLE-mediated M1 to M2 microglia/macrophages phenotype transition on MCAO/R-induced rats

The RT-qPCR analysis unveiled that MCAO/R-produced CI/RI rats exhibited elevated M1 markers such as TNF-α, CD86, IL-1β and IL-6whereas mitigating the M2 indicators comprising Arg-1, CD206, TGF-β and IL-10 versus sham group (Figure 2). Treatment with STLE in MCAO-induced CI/R rats repressed the M1 marker's mRNA level and enhanced the M2 marker's mRNA expression in a dosage-related manner. These data revealed that STLE facilitates the M1 to M2 microglia/macrophage phenotype transition in MCAO/R-induced CI/RI rats.

STLE relieves brain injury in MCAO/R-induced CI/RI rats

Histopathological study of control rats exposed that the neurons present in the cerebral cortex unveil an obvious nucleus and cell membrane (Figure 3). In the MACO/R rats exhibit numerous neurotic injuries such as inflamed neurons, liquefaction, atrophy and necrosis. Treatment with STLE (25, 50 and 100 mg/kg bw) in the MCAO/R group lessened the CI injuries, liquefaction, disorganized structure and necrosis against CI/R model rats in an amount-related manner. These data evidenced that STLE could attenuate cerebral impairment developed by MCAO/R in the experimental rats.

STLE inhibits MCAO/R-induced neuronal apoptosis

It is noticeable that CI/RI creates severe pathophysiological apoptotic episodes in brain cells (Figure 4). In this experiment, the Bcl-2 protein expression was moderated, whereas Caspase-3 and Bax were intensively increased ($p<0.05$) in CI/RI rats relative to the sham. Treatment with STLE reduced the protein expression of pro-apoptotic proteins, with substantial upregulation of Bcl-2 protein. The western blot analysis data established that STLE could reduce neuronal apoptosis in MCAO/R-produced CI/RI in rats.

STLE-mediated M1 to M2 phenotype protein transition evidenced by western blot analysis

MCAO/R-produced CI/RI rats displayed elevated protein expression of M1 surface markers such as iNOS and CD86, whereas reducing the M2 surface markers comprising Arg-1 and CD206 in contrast to the group sham (Figure 5). Administration of STLE (25, 50 and 100 mg/kg bw) in MCAO-induced CI/R rats repressed the protein expression of the M1 marker's iNOS and

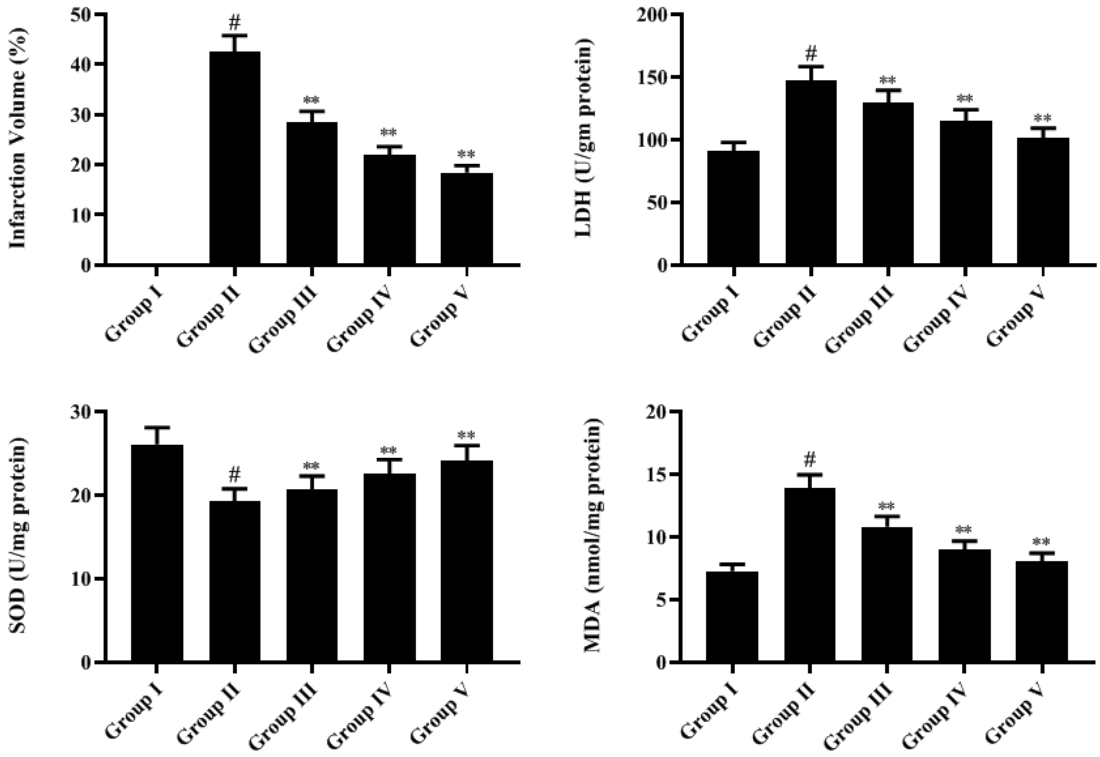

Figure 1: Neuroprotective effect of STLE on MCAO/R induced CI/RI rats. Infraction volume, LDH, SOD and MDA. Results were displayed as mean±SD of 6 observations and the statistical significance is considered as $^{*,**}p<0.05$ against the sham group.

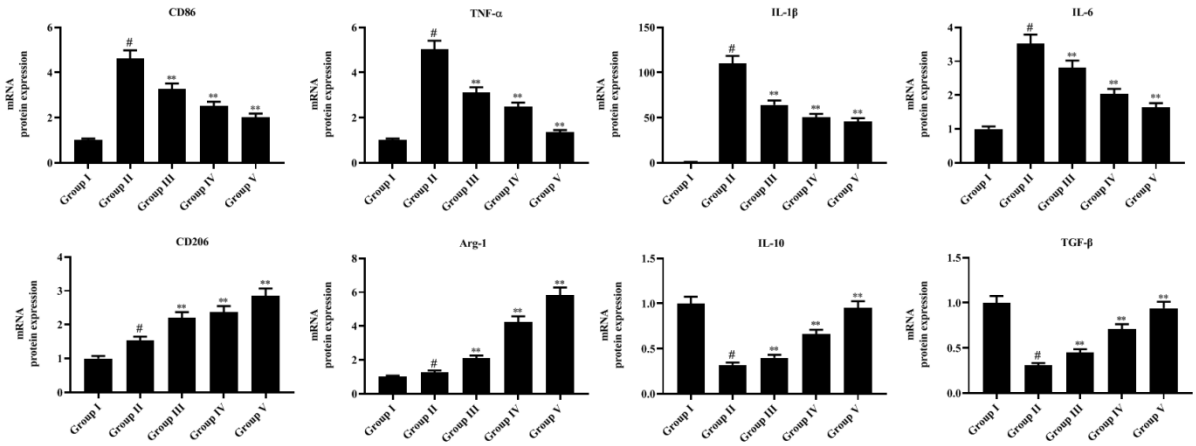

Figure 2: STLE-mediated M1 to M2 microglia/macrophages phenotype transition on MCAO/R-induced rats. The mRNA expression was evaluated by RT-qPCR analysis. Results were displayed as mean±SD of 6 observations and the statistical significance is considered as $^{*,**}p<0.05$ against the group sham.

CD86 while enhancing the M2 marker's Arg-1 and CD206 protein expression in a dosage-related way. These data uncovered that STLE facilitates the M1 to M2 microglia/macrophage phenotype shift in MCAO/R-induced CI/RI rats.

STLE triggered PI3K/AKT signaling pathway in MCAO/R-induced CI/RI rats

The PI3K/AKT pathway is crucial for brain protection from apoptosis-correlated damage (Figure 6). The MCAO/R-developed CI/RI rats considerably attenuated ($p<0.05$) phosphorylation of PI3K/AKT than the sham group. STLE (25, 50 and 100 mg/kg bw) markedly enhanced ($p<0.05$) the phosphorylation of PI3K/AKT against the I/R group in a quantity-related mode. These data established that STLE could involve a neuroprotective action on MCAO/R-induced CI/RI rats through activating P13K/AKT signalling pathway.

DISCUSSION

In this current study, we examined STLE-prompted neuroprotection, which diminishes oxidative stress, neuroinflammation and neuronal apoptosis in the MCAO/R-developed CI/RI rat model. Treatment with STLE (25, 50 and 100 mg/kg bw) revealed quantity-related enhancement of neurological tasks, diminished cerebral infarct volume, inflammation, apoptosis and histopathological alterations after CI/RI. Herein, we established the anti-apoptotic and anti-inflammatory activity of STLE on MCAO/R-produced CI/RI rats were also explored. An extensively putative perspective is that immune reactions are powerfully accountable for CI/RI and secondary impairment.[6,7] Previously, it has been reported that *Stemona tuberosa* (ST) extracts and their isolated alkaloids have demonstrated anti-inflammatory activities.[10,11] A crucial finding observed that STLE enabled the phenotype transformation of M1 to M2 in microglia of CI/RI rats. Furthermore, STLE enhanced the intensity of p-PI3K and p-Akt, which might be the principal mechanism by which STLE exerts its neuroprotective action. We found that PI3K/AKT augmented the expression of Bcl-2, however, alleviated caspase-3 and Bax protein intensities in the STLE-treated MCAO/R model. We rely on that STLE-stimulated neuroprotection can be ascribed to the increase of the signaling PI3K/AKT. Our data proposed that STLE-triggered neuroprotection was due to the inhibition of neuronal apoptosis and inflammation through the elevation of the P13K/AKT pathway. This may be the first report of the neuroprotective efficacy of ST Leaf Extracts (STLE) on the MCAO/R-produced CI/RI model in rats.

In this report, we determined the neuroprotective activity of STLE against oxidative stress for controlling the CI/RI in rats. Oxidative stress is a vital process of IS pathology that leads to neurocyte damage.[17,18] Data exhibited that administration of STLE (25, 50 and 100 mg/kg bw) decreases the brain destruction of MCAO/R-stimulated rats including reduced infarction volume, histopathological alterations of the brain, LDH and MDA while enhancing SOD activity in a concentration-related way. Oxidative free radicals correlate with cell membranes comprising unsaturated fatty acids and exposed to lipid peroxidation and subsequent lipid peroxides can break down to produce several secondary products, mainly MDA.[34,35] Following CI, extensive cell death occurs which is formed by oxidative damage to DNA and other macromolecules[36] and is not only produced by the transitory lack of oxygen and energy stream when the brain is starved of blood flow but also by ROS caused through reactions with incoming oxygen throughout reperfusion.[37] Accumulating evidence suggests that ST Root extracts have exhibited antioxidative antitussive, anti-inflammatory and neuroprotective activities.[10-12] Our outcomes are also in line with the results that STLE showed antioxidative, anti-inflammatory and neuroprotective activity.

Several researches have documented that CI/R-produced inflammation is presented by CNS-resident microglia

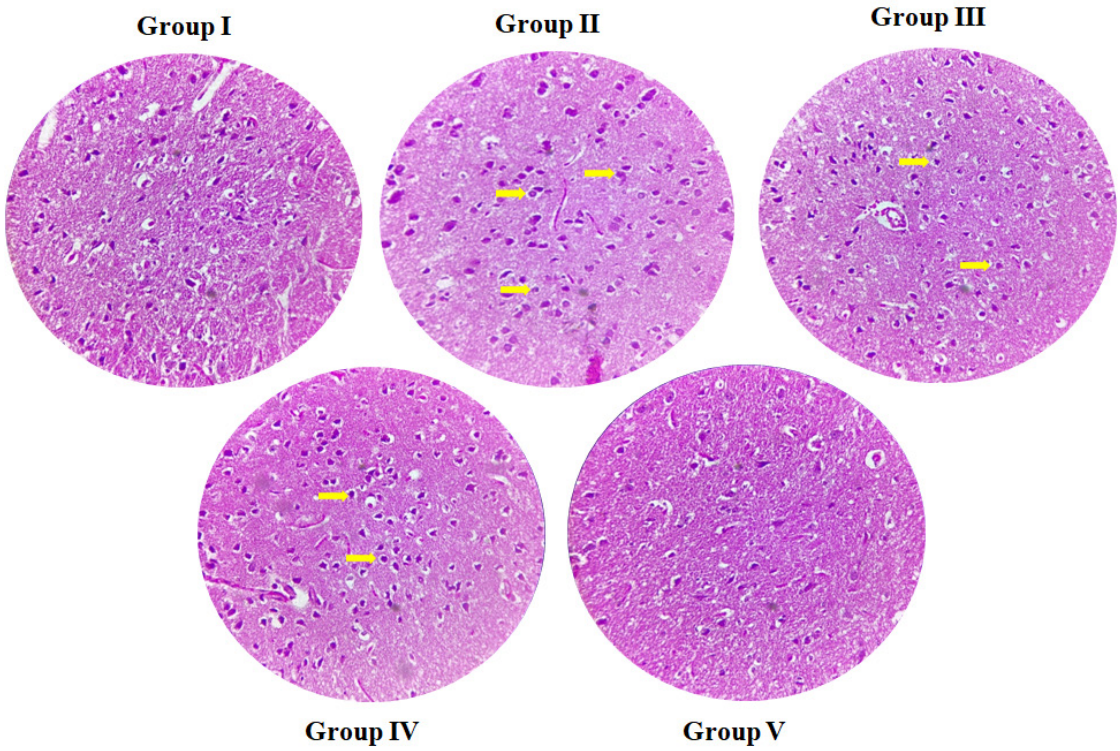

Figure 3: Impact of STLE on the brain histopathology of MCAO/R-induced rats (Scale bar=50 µm). Group-I: Sham rats demonstrating the neurons present in the cerebral cortex unveil an obvious nucleus, cell membrane and normal histology of the brain; Group-II: MCAO/R-produced CI/RI rats display many neurotic injuries including inflamed neurons, liquefaction, atrophy and necrosis; Group III-V: In CI/RI rats administered with STLE (25, 50 and 100 mg/kgbw) decreased the CI injuries, liquefaction, disorganized structure and necrosis against CI/R alone rats in a dosage-related manner.

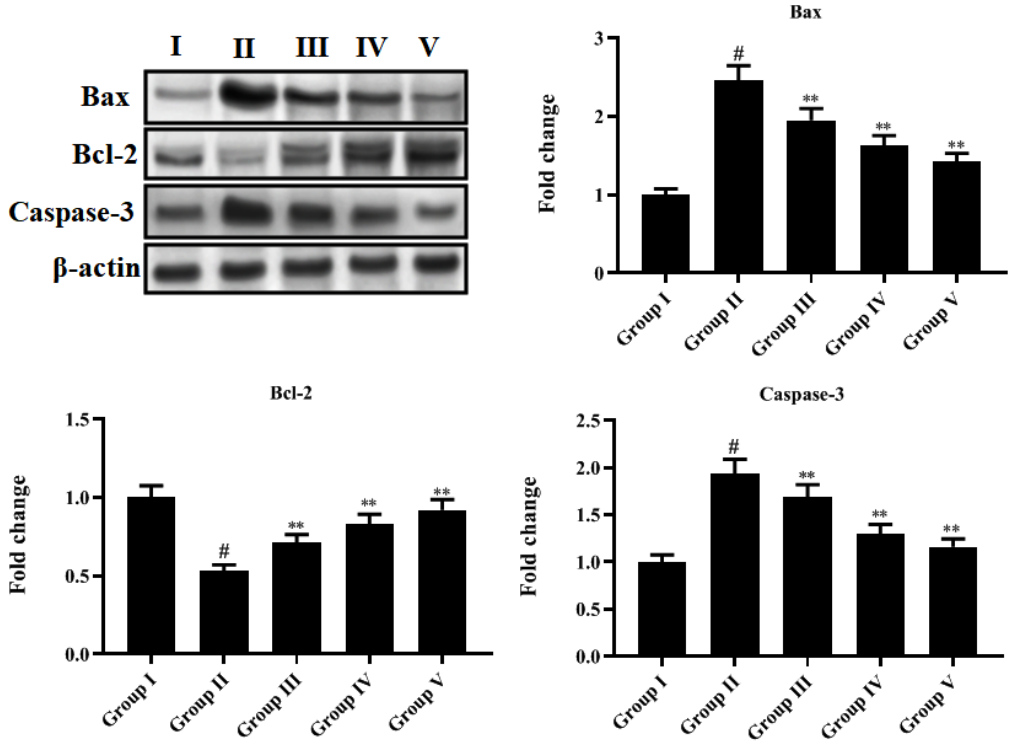

Figure 4: STLE moderates neuronal cell apoptosis in MCAO/R-induced CI/RI rats. The intensities of Bax, Bcl-2 and caspase-3 protein were assessed by western blot. Findings were displayed as mean±SD of 6 observations and the statistical significance is considered as $^{*,**}p<0.05$ against the sham group.

stimulation.[25] Triggered microglia can diverge into the phenotypes either pro-inflammatory M1 or anti-inflammatory M2. We established the prompt instigation and microglia/macrophage polarization into M1 subsequent CI/RI, together with the discharge of inflammatory intermediaries, which was related to the prior experiments.[38,39] Thus, the involvement of M1 to M2 phenotype conversion in the primary phase of microglia/macrophage divergence may be a possible method for handling IS.[40,41] Additionally, STLE (25, 50 and 100 mg/kg bw) markedly repressed M1 markers, whereas increasing M2 markers mRNA expression in a quantity-related manner. Western blot data further supported these outcomes, representing alleviation of M1 indicators, including CD86 and iNOS, while enhanced M2 markers contain CD206 and Arg-1 after STLE administration. It has been recognized that ST root extracts CH2Cl2 fraction has suppressed LPS-induced NO production in murine microglial cells BV2.[12] Indeed extreme NO has been designated to involve a contributory part in the development of inflammatory neurodegenerative illnesses comprising Alzheimer's and Parkinson's disease and other CNS disorders.[13] Together, our findings emphasized that STLE enabled the M2 polarity of microglia/macrophages in CI/RI in rats developed by MCAO/R.

Apoptosis is a vigorous programmed cell demise that is related to the pro-apoptotic to anti-apoptotic molecules proportion.[29] The Bcl-2 family members contain Bax and Bcl-2; Bax is a pro-apoptotic protein, however, Bcl-2 is an anti-apoptotic protein. The central predictor of apoptosis is thought to be the proportion of Bax/Bcl-2.[42] When the attenuation of the Bcl-2/Bax proportion, initiated the caspases-3 cascade, causing the PARP cleavage and, ultimately leading to cell apoptosis.[43] Numerous documents have unveiled that the augmentation of caspase-3 protein expression on the MCAO/R model can accelerate brain injury.[44] The current findings indicate that MCAO/R-developed CI/RI rats increased the Bax and caspase-3 protein expression, however, STLE (25, 50 and 100 mg/kg bw) quantity-dependently decreased apoptosis-correlated protein expression, although enhancing Bcl-2 expression. Therefore, STLE protects MCAO/R-induced CI/RI in rat brain cells.

The PI3K/Akt pathway is among the greatest vital signal transduction paths and participates in cell development by controlling cell metabolism, inflammation and apoptosis in a sequence of molecules.[30,31] An earlier investigation recognized that the pathway PI3K/Akt is involved in alzheimer's and other neurodegenerative disorders.[45] PI3K is extensively found in many cells and is stimulated by numerous cytokines. Akt is a significant downstream PI3K molecule and aids as a vital center of many pathways. Nowadays, the function of the PI3K/AKT signaling in the nervous system has gained considerable attention. Activating this pathway is beneficial for protection against MCAO/R.[46,47] To evaluate the molecular mechanisms by which STLE guards against CI/RI, the PI3K/Akt protein expression and their phosphorylated forms in MCAO/R-induced rats were analyzed by western blotting. These findings exposed that p-AKT and p-PI3K expression were elevated in a concentration-related way after treatment with STLE (25, 50 and 100 mg/kg bw), which designated that STLE could trigger the PI3K/Akt signaling. The PI3K/Akt phosphorylation network protects cells by stimulating the Bcl-2 family and averting caspase-3. In this current work, the

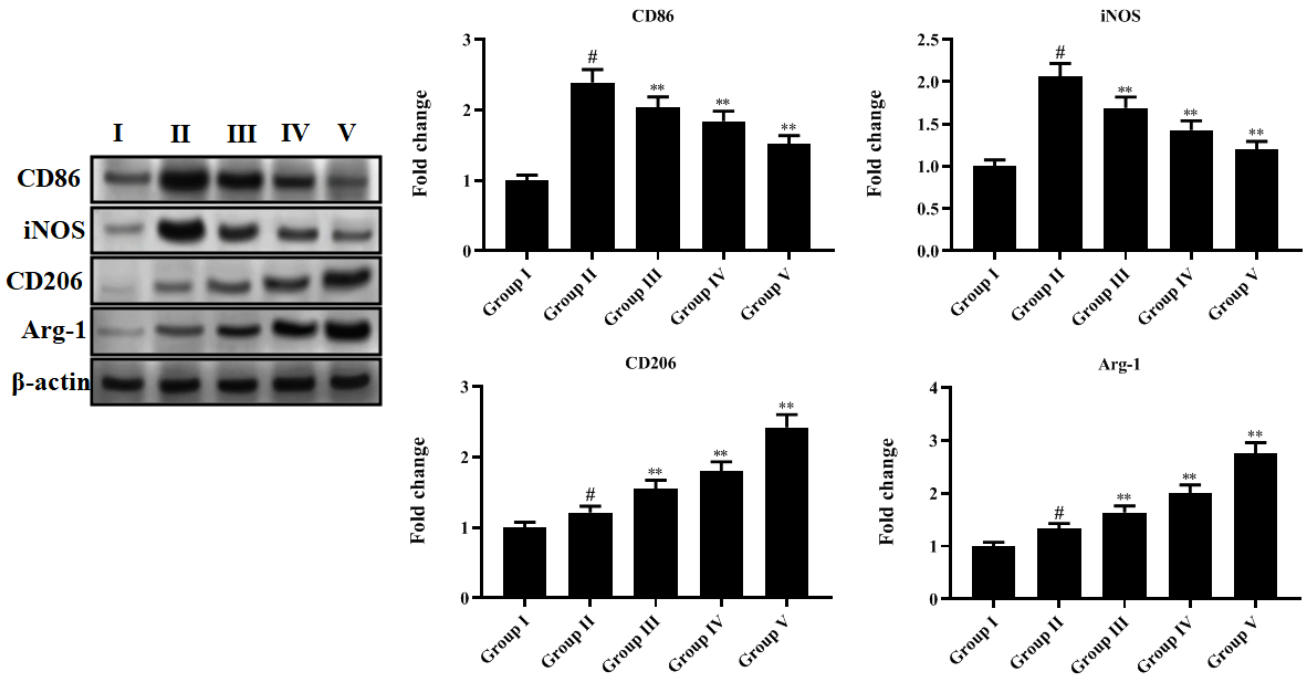

Figure 5: Influence of STLE on the M1 to M2 microglia/macrophages phenotype transition proteins in MCAO/R-induced CI/RI rats. The protein level of CD86, iNOS, CD206 and Arg-1 was evaluated by western blot. Results were displayed as mean±SD of 6 observations and the statistical significance is considered as $^{*,**}p<0.05$ against the group sham.

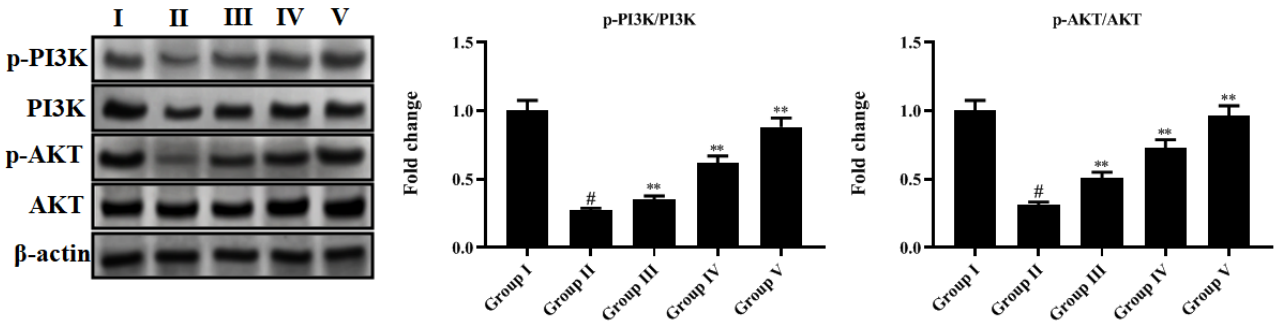

Figure 6: Result of STLE on the protein expression of P13K/AKT signaling pathways in MCAO/R-induced CI/RI rats. The intensities of p-P13K, P13K, p-Akt and Akt protein expression were evaluated by western blot. Findings were displayed as mean±SD of 6 observations and the statistical significance is considered as $^{*,**}p<0.05$ against the sham group.

intensities of inflammatory markers and apoptosis-interrelated protein levels were prominently augmented after MCAO/R, however, administration of STLE led to an intense reduction in these factors. This reveals the protecting effect of STLE is facilitated via the stimulation of the PI3K/AKT signaling.

CONCLUSION

In summary, our report established that STLE exerted protective effects against MCAO/R-produced CI/RI by inhibiting brain damage, histopathological variations, oxidative stress, neuroinflammation and apoptosis in a concentration-related manner. STLE enabled microglia/macrophage polarity from the M1 pro-inflammatory to the phenotype M2 anti-inflammatory in MCAO/R-produced CI/RI in rats. The neuroprotective mechanism of STLE may be correlated to the triggering of the PI3K/Akt signaling. Therefore, STLE may signify a possible remedial approach to CI/RI, which may offer new insight into IS clinical management.

ABBREVIATIONS

IS: Ischemic stroke; **ST:** *Stemona tuberose*; **STLE:** *Stemona tuberosa* leaf extracts; **CI:** Cerebral ischemia; **RI:** Reperfusion injury; **MCAO/R:** Middle cerebral artery occlusion/reperfusion; **CNS:** Central nervous system; **TTC:** Triphenyl tetrazolium chloride; **DMSO:** Dimethyl sulfoxides; **SDS:** Sodium dodecyl-sulfate; **SD:** Sprague Dawley; **ECA:** External carotid artery; **CCA:** Common carotid artery; **ICA:** Internal carotid artery; **LDH:** Lactate dehydrogenase; **SOD:** Superoxide dismutase; **MDA:** Malondialdehyde; **H&E:** Hematoxylin and eosin; **ANOVA:** Analysis of variance; **PI3K:** Phosphatidylinositol 3-kinase; **AKT:** Protein kinase B.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL APPROVAL

The Institutional Animal and Ethics Committee, Xi'an Hospital of Traditional Chinese Medicine Encephalopathy, approved (No: 2024-140).

SUMMARY

In summary, the study demonstrated that *Stemona tuberose* Leaf Extract (STLE) exhibits neuroprotective properties in Cerebral Ischemia/Reperfusion Injury (CI/RI) rats. STLE effectively reduced infarction volume, oxidative stress, histopathological changes, neuroinflammation and apoptosis while promoting microglia/macrophages M2 anti-inflammatory polarization. The extract also modulated the P13K/Akt signaling pathway, indicating its potential in alleviating CI/RI-induced apoptosis. These findings highlight the therapeutic potential of STLE in the treatment of ischemic stroke and related neuroinflammatory conditions.

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