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ORIGINAL ARTICLE

Syringin Protects against Cerebral Ischemia and Reperfusion Injury via Suppression of Inflammatory Mediators and Toll-Like Receptor/MyD88 Signaling Pathway in Rats

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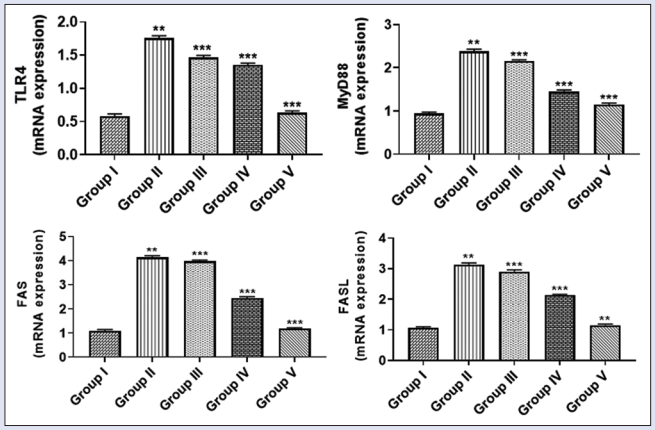
ABSTRACT

Background: The cerebral ischemic stroke befalls in response to the abrupt occlusion of blood vessels that leads to deprived oxygen and glucose in brain and causes brain injuries. Stroke is associated with higher morbidity and mortality rate globally. Syringin is a bioactive compound from *Eleutherococcus senticosus* and possesses plentiful biological actions. **Objectives:** In this research work, we envisioned to observe the neuroprotective actions of syringin against ischemic-reperfusion (I/R)-provoked ischemic stroke in rats. **Materials and Methods:** The focal cerebral I/R injuries were roused to the male Wistar rats through the middle coronary artery occlusion (MCAO) technique. The syringin (10, 25, and 50 mg/kg) was administered to the rats through intragastric route for 7 successive days prior to MCAO and another 3 days after MCAO. Sham rats were administered with usual diet. The neurological score and parameters were measured by standard methods. The status of inflammatory cytokines and mediators in both serum and brain tissues was considered by commercial assay kits. The mRNA expression of toll-like receptor (TLR) 4, MyD88, Fas, and FasL was inspected by reverse transcription–polymerase chain reaction technique. **Results:** The syringin administration to I/R rats established the lessened neurological score and parameters. Syringin supplementation was meritoriously suppressed the levels of the inflammatory cytokine, i.e., interleukin (IL)-1 β, IL-6, and tumor necrosis factor-α and enhanced the IL-10 status in I/R rats. Syringin abridged the inflammatory mediators like cyclooxygenase-2, prostaglandin-2, and nuclear factor kappa B levels in the serum and brain tissues. The mRNA expression of TLR4, MyD88, Fas, and FasL was noticeably downregulated by the syringin. **Conclusion:** Taken together, our results showed the curative role of syringin against ischemic stroke in rats. Syringin can be an auspicious anti-stroke agent in future to treat ischemic stroke.

Key words: Brain injury, inflammation, ischemic stroke, syringin, toll-like receptor/MyD88 pathway

SUMMARY

- Syringin supplementation could lessen the interleukin (IL)-6, tumor necrosis factor-α, and IL-1 β levels in both serum and brain tissues of middle coronary artery occlusion rats
- Syringin effectively blocked the inflammatory mediators' status in both serum and brain tissues of ischemic-reperfusion (I/R) rats. Furthermore, syringin substantially downregulated the mRNA expressions of toll-like receptor-4, MyD88, Fas, and FasL in I/R rats.



Abbreviations used: I/R: Ischemic reperfusion; MCAO: Middle coronary artery occlusion; CIR: Cerebral ischemic reperfusion; BBB: Blood–brain barrier

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INTRODUCTION

Stroke is an imperative cause of irreparable damage, disability, and mortality globally, while 80% of morbidity and mortality cases befall due to the cerebral ischemic-reperfusion (CIR) injury. The cerebral ischemic stroke happens in response to the instant occlusion of blood vessels via embolism of thrombus that leads to deprived oxygen and glucose in brain tissues and causes continuing brain injuries.[1] The pathological processes of CIR injury were extremely complicated and the inflammation shows a vivacious function in the CIR events. In the commencement stage, CIR pledges the inflammatory processes that implicate the stimulation of astrocytes and microglia; hematogenous cell influx via cytokines, adhesion molecules, and chemokines; and outcomes in the increased behavioral defects and brain injury. Therefore, preventing early inflammation was started as an optimistic curative

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tactic in stroke treatment.[2] The inflammation, oxidative stress and apoptosis plays confirmed as the prime troupes and contributes to the brain damage associating the consolidating of pro-injury processes and promoting pro-survival processes. Inflammation, oxidative stress, and apoptosis are viewed as playing a grave function during the CIR damage.[3]

During CIR damage, the unwarranted oxidative stress and inflammatory conditions are troubled in the neural injuries.[4] After the CIR injury, the blood–brain barrier (BBB) disruption is provoked through the increased expression of matrix metalloproteinase while increasing the hemorrhage and brain edema.[5] Preceding study established that the triggering of nuclear factor kappa B (NF-κB) activates the harsh augmentation in the accretion of pro-inflammatory regulators that results in the activated inflammatory cascades and worsening the brain injury.[6] Undoubtedly, the signs from the clinical trials have shown that the ischemic stroke was enormously related to the status of pro-inflammatory regulators. The triggering of NF-κB is indispensable for the activation and accretion of plentiful pro-inflammatory regulators such as interleukin (IL)-1 β and tumor necrosis factor-α (TNF-α).[7,8] Hence, the mitigation of inflammation and also with a perfect understanding of ischemic reperfusion (I/R) injuries are an essential steps for providing the acumens of pathological progresses of ischemic brain damage.

Toll-like receptors (TLRs) execute a vigorous function in the innate immunity and are allied with the array of inflammatory ailments. The TLR4 expression is increased consequently CIR damage and the amount of neuroinflammation and CIR brain damage is particularly inferior in the TLR4-deficient animals that the wild-type.[9] In addition, NF-κB is an imperative downstream factor of the TLR4 signaling cascade, which is stimulated in response to the CIR, to stimulate inflammatory processes and secrete inflammatory mediators, which further deteriorate the CIR damage.[10] The targeting of TLR4 signaling cascade is a latent therapeutic tactic to treat ischemic stroke due to the downregulation of TLR4 expression that prevents the NF-κB and suppresses the inflammatory mediators, thereby upgrades of ischemic brain damage.[11]

Due to the contracted time gap, the choices for acute ischemic stroke still endure very deprived.[12] The prevailing treatment tactics for ischemic stroke are often associated with adverse effects and the success rate was very poor.[13] The intravenous thrombolytic method is an ideal curative choice for ischemic stroke. However, there are still plentiful difficulties to this method, such as limited time gap, risk of hemorrhagic transformation, and restricted efficiency.[14] The reflowing of blood of the ischemic region through the recombinant tissue plasminogen activator is a vital therapeutic line in clinical; however, the higher proofs exemplify that the approach of renovating the blood circulation, i.e., cerebral ischemia/reperfusion (CIR), can embellish the brain injuries, which results in the CIR injury along with numerous additional severities. Furthermore, only a few number of patients were helped from such an earlier reperfusion approach.[15] The later reperfusion following the therapy can stimulate harmful effects and fallouts in harmful effects. Hence, much courtesy should be paid in this situation to decrease the CIR-provoked secondary brain damage.[16] Therefore, the development of curative agents to treat ischemic stroke and improve the CIR damage appeared as a problem to be solved closely.

Syringin, 4-[(1E)-3-hydroxyprop-1-en-1-yl]-2,6-dimethoxyphenyl β-D-glucopyranoside], is an imperative bioactive compound, which is quarantined from the root and rhizome of *Eleutherococcus senticosus* and influenced the immense biological aids. The syringin administration through the intravenous route abridged the glucose level in the plasma of streptozotocin-provoked diabetic rats.[17,18] Syringin shows the potent hepatoprotective activity[19] and anti-osteoporotic activity[20] and improved the neurological functions in the aged rats.[21] However, the neuroprotective effects of syringin against the ischemic stroke are not appraised yet. Hence in this work, we intended to judge the neuroprotective actions of syringin against ischemic stroke in rats.

MATERIALS AND METHODS

Chemicals

Syringin (purity: ≥98.0%) and other chemicals were obtained in analytical grade from Sigma Chemicals, USA. The ELISA kits for the inflammatory cytokines were accomplished from MyBioSource, San Diego, USA. Assay kits for inflammatory mediators were assimilated from Invitrogen, USA. The reverse transcription–polymerase chain reaction (RT-PCR) kits were obtained from Thermo Fisher Scientific, USA.

Experimental animals

12 week-aged male Wistar rats assessing 220–250 g were attained from the Institutional Animal House (IAEC: 2020-03) and caged in clean confines. All the rats were continued beneath the organized conditions as 50% ± 10% of humidity, 25°C ± 3°C of temperature, 12 h light/dark series, and adapted to the laboratory situations for 1 week prior to beginning of work. The rats were delivered with a standard pellet diet and allowed to access the water.

Stimulation of focal cerebral ischemic-reperfusion injury

I/R injuries to the animals were stimulated via middle coronary artery occlusion (MCAO) for 2 h afterward reperfusion as stated by Bai *et al*.[22] Soon, the animals were imperiled to anesthesia through intraperitoneal administration of 10% of chloral hydrate (350 mg/kg) and located on the surgery board in a recumbent position. The left carotid portion was wide opened via an incision of midline; the external and common carotid artery was unbolted. The nontraumatic microvascular clamp was accessible to the carotid junction into the internal carotid artery, thus blocking the origin of the middle cerebral artery. After 2 h of occlusion, the reperfusion was started by withdrawing the clamp. Finally, the opening was sutured properly and then rats were allowed to recuperate from anesthesia. During this process, the body temperature was well maintained at 37°C with the aid of heating lamp. The animals in sham group recognized the identical operational procedures eliminating MCAO.

Experimental design

All rats were estranged into five groups each having six rats. The animals were assembled as stated below:

- Group I: Sham group
- Group II: MCAO control
- Group III: MCAO + 10 mg/kg of syringin
- Group IV: MCAO + 25 mg/kg of syringin
- Group V: MCAO + 50 mg/kg of syringin.

The syringin was orally administered to the rats through intragastric route for 7 successive days prior to MCAO, at the origination of reperfusion, and extended for another 3 days after MCAO.

Assessment of neurological score

The neurological scores of all animals were inspected at 24 h after MCAO surgery via the neurological scoring system given by Longa *et al*.[23] Neurological scores were demarcated as 0: no deficits, 1: difficulties in full extension of contralateral forelimb, 2: incapable of extending contralateral forelimb, 3: slight circling to the contralateral side, 4: severe circling, and 5: falling to the contralateral side. The increased neurological score specifies the serious motor motion impairments.

Measurement of infarct volume

The brains were abruptly gathered followed by the animal sacrifice under 10% of chloral hydrate anesthesia and submerged in Bouin's solution for 24 h and cleansed with 70% ethanol. Then tissues were divided into 2 mm size. The sections was then stained and preheated with 2% of 2,3,5-Triphenyltetrazolium chloride (TTC) for 30 min at 37°C and fixed in 4% of paraformaldehyde for overnight. Normal tissues were stained red and infarcted area stained in pale gray color. TTC-stained tissues were apprehended and treated with ImageJ to investigate the infarct volume. The infarct volume was examined as per the procedure recommended by Wang *et al.*[24]

Brain edema and water content analysis

Brain tissues were congregated from the experimental rats and their wet weight was enumerated. Then, the brain was positioned in an oven for 24 h at 100°C for dehydration and then weight was taken again to attain the dry weights. Water content in the brain was dogged by applying the formula: water content (%) = ([wet weight − dry weight]/wet weight) × 100%.

Evans blue assay

4% of Evans blue (1 ml/kg) was administered through the tail vein 3 h before the animal detriment. The heparinized saline was used to perfuse the animals and the ipsilateral and contralateral cortexes were assembled. The gathered tissues were homogenized with saline and precipitated with trichloroacetic acid (100%). Evans blue contents in the supernatants were observed by taking the absorbance at 620 nm, as per the formerly described technique by Chang *et al.*[25]

Detection of inflammatory cytokines level in the serum and brain tissues of ischemic-reperfusion rats

The status of TNF-α, IL-6, IL-1 β, and IL-10 was scrutinized in both serum and brain tissues of I/R rats with the aid of marker-specific commercial ELISA assay kits as per the manufacturer's protocols (MyBioSource, San Diego, USA).

Measurement of inflammatory mediators' level in the serum and brain tissues of ischemic-reperfusion rats

The approximation of inflammatory mediator levels like cyclooxygenase-2 (COX-2), prostaglandin (PGE) 2, and NF-κB was completed with the help of marker-specific ELISA assay kits by following the directives of manufacturer (Invitrogen, USA). The status of inflammatory markers was determined and portrayed as pg/mg for brain tissues and pg/mL for serum. All the assays were showed in a triplicate manner.

Reverse transcription–polymerase chain reaction analysis

Total RNA was quarantined from the brain tissues of both control and experimental rats with the assistance of RNeasy kit (Qiagen CA, USA). The isolated total RNA was used to the reverse transcription and reversed into cDNA by using the commercial PCR kit (Thermo Fisher Scientific, USA). The primers applied in this work are as recorded below: TLR4 upstream: 5'-TGGAAGTTGAACGAATGGAATGTG-3' and downstream: 5'-ACCAGAACTGCTACAACAGATACT-3'; MyD88 upstream: 5'-GACCCCTGGTGCAAGTACC-3' and downstream: 5'-AGTAGCTTACAACGCATGACAG-3'; Fas upstream: 5'-ATGCACACTCTGCGATGAAG-3' and downstream: 5'-CAGTGTTCACAGCCAGGAGA-3'; FasL upstream: 5'-GCAGAAGGAACTGGCAGAAC-3' and downstream: 5'-TTAAATGGGCCACACTCCTC-3'. Each sample was examined in a triplicate manner. The mRNA expression was standardized with the β-actin and the fallouts were portrayed as fold induction.

Statistical analysis

The results were statistically examined by using GraphPad Prism software version 5.0. (GraphPad, San Diego, CA, USA). One-way ANOVA then Tukey's *post hoc* calculation was implemented to study the results. Results were exemplified as mean ± standard deviation of triplicates and $P < 0.05$ was significant.

RESULTS

Syringin attenuated the neurological scores

Figure 1 exposes that the neurological deficit score was extremely increased in MCAO rats, which is in contrast to the control. The syringin (10, 25, and 50 mg/kg)-supplemented rats established the considerable reduction ($P < 0.05$) in the neurological scores. It was clear that the syringin prevented the MCAO-provoked neurological scores in rats. The 50 mg/kg of syringin showed the alike outcomes with the control group.

Syringin prevented the neurological parameters

MCAO rats unveiled the severely raised status of brain edema, infarct volume, brain water content, and Evans blue leakage when related to the control [Figure 2]. It designates that the MCAO was caused severe neurological complications. Interestingly, the syringin (10, 25, and 50 mg/kg) supplementation was effectively ($P < 0.05$) downregulated the neurological complications such as brain edema, infarct volume, brain water content, and Evans blue leakage, which is in contrast to the MCAO group. This outcome demonstrated that the syringin substantially amended the neurological parameters.

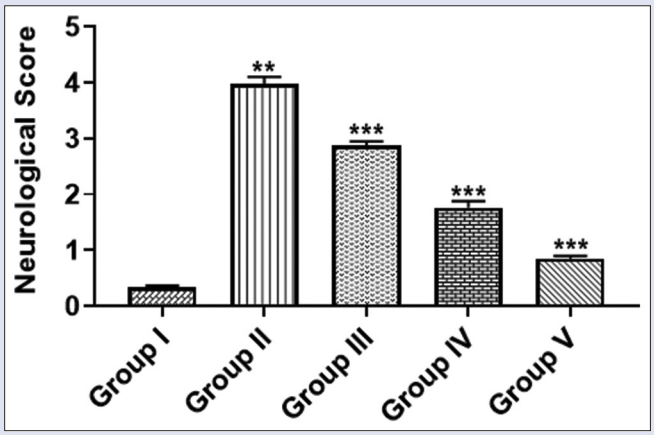

Figure 1: Effect of syringin on the ischemic-reperfusion provoked neurological deficit scores in experimental rats. Results were portrayed as mean ± standard deviation of triplicates. Significance was assessed through one-way ANOVA; subsequently, Tukey's *post hoc* test was executed to examine the data. $P < 0.05$ was regarded as significant. $^{**}P < 0.05$ when related with control and $^{***}P < 0.05$ when related with ischemic-reperfusion group

Syringin assuaged the inflammatory cytokines' level in the serum of ischemic-reperfusion rats

The status of TNF-α, IL-6, IL-1 β, and IL-10 was examined in the serum of control and experimental rats and the consequences are illustrated in Figure 3. The status of TNF-α, IL-6, and IL-1 β was drastically raised ($P < 0.05$) and IL-10 level was reduced in the serum of MCAO rats. The administration of 10, 25, and 50 mg/kg of syringin was strangely lessened ($P < 0.05$) the TNF-α, IL-6, and IL-1 β levels and improved the IL-10 in the serum of MCAO rats. It was exhibited the anti-inflammatory potential of syringin against the I/R-provoked neuroinflammation in rats.

Syringin reduced the inflammatory cytokines' level in the brain tissues of ischemic-reperfusion rats

As seen in the serum level, Figure 4 exposes that the status of TNF-α, IL-6, and IL-1 β was harshly augmented and IL-10 was weakened ($P < 0.05$) in the MCAO rats, which is in contrast to the control. The syringin (10, 25, and 50 mg/kg)-supplemented rats confirmed the diminished status of TNF-α, IL-6, and IL-1 β and improved ($P < 0.05$) IL-10 level in the brain tissues. The 50 mg/kg of syringin-administered rats and control rats were revealed the similar kind of outcomes.

Syringin decreased inflammatory mediator levels in the serum of ischemic-reperfusion rats

Figure 5 exemplifies the status of inflammatory mediators, i.e., COX-2, PGE-2, and NF-κB in the serum of control and experimental rats. The status of these markers was enlarged significantly ($P < 0.05$) in the serum of MCAO rats than the control. Captivatingly, the 10, 25, and 50 mg/kg of syringin-supplemented rats exhibited marked attenuation ($P < 0.05$) in the levels of inflammatory regulators COX-2, PGE-2, and NF-κB in the serum. This outcome also showed that the syringin obsessed potent

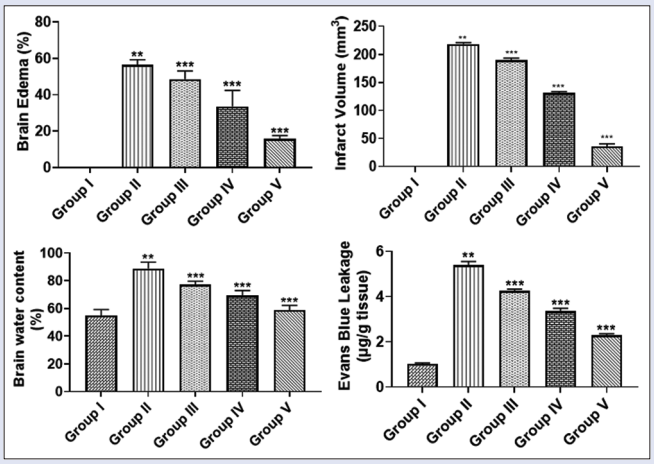

Figure 2: Effect of syringin on the neurological parameters in experimental rats. Results were portrayed as mean ± standard deviation of triplicates. Significance was assessed through one-way ANOVA; subsequently, Tukey's *post hoc* test was executed to examine the data. $P < 0.05$ was regarded as significant. $^{**}P < 0.05$ when related with control and $^{***}P < 0.05$ when related with ischemic-reperfusion group

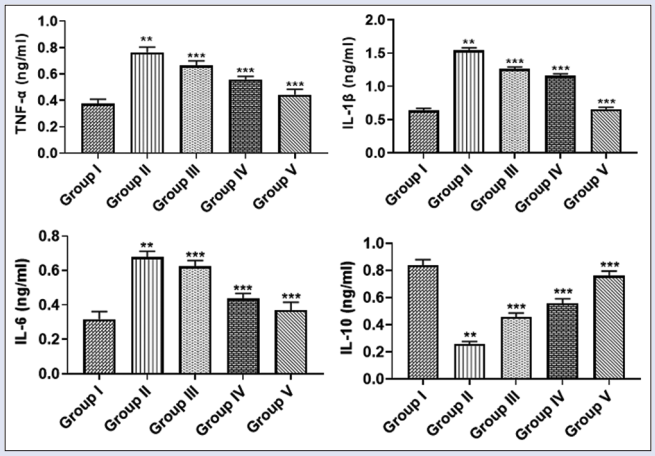

Figure 3: Effect of syringin on the inflammatory markers status in the serum of experimental rats. Results were portrayed as mean ± standard deviation of triplicates. Significance was assessed through one-way ANOVA; subsequently, Tukey's *post hoc* test was executed to examine the data. $P < 0.05$ was regarded as significant. $^{**}P < 0.05$ when related with control and $^{***}P < 0.05$ when related with ischemic-reperfusion group

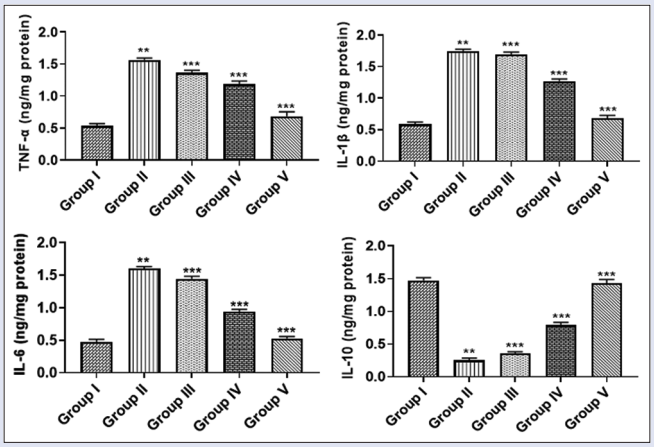

Figure 4: Effect of syringin on the inflammatory markers' status in the brain tissues of experimental rats. Results were portrayed as mean ± standard deviation of triplicates. Significance was assessed through one-way ANOVA; subsequently; Tukey's *post hoc* test was executed to examine the data. $P < 0.05$ was regarded as significant. $^{**}P < 0.05$ when related with control and $^{***}P < 0.05$ when related with ischemic-reperfusion group

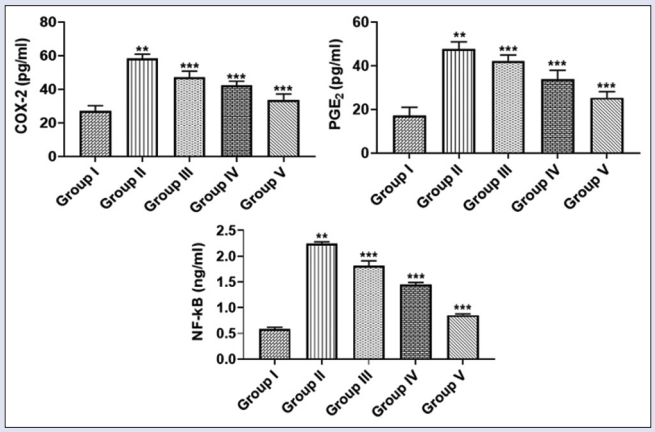

Figure 5: Effect of syringin on the inflammatory mediators status in the serum of experimental rats. Results were portrayed as mean ± standard deviation of triplicates. Significance was assessed through one-way ANOVA; subsequently, Tukey's *post hoc* test was executed to examine the data. $P < 0.05$ was regarded as significant. $^{**}P < 0.05$ when related with control and $^{***}P < 0.05$ when related with ischemic-reperfusion group

anti-inflammatory activity. Similar kinds of outcomes were distinguished in control and 50 mg/kg of syringin-supplemented rats.

Syringin decreased inflammatory mediator status in the brain tissues of ischemic-reperfusion rats

The inflammatory mediators COX-2, PGE-2, and NF-κB status in the brain tissues of normal and experimental animals were measured and the results are represented in Figure 6. MCAO rats discovered the raised levels ($P < 0.05$) of COX-2, PGE-2, and NF-κB in the brain tissues than the control. Stimulatingly, the syringin (10, 25, and 50 mg/kg)-supplemented rats established the amazing shrinking in the MCAO rats. The control and 50 mg/kg of syringin-supplemented rats presented the analogous outcomes.

Syringin downregulated the mRNA expression of TLR-4, MyD88, Fas, and FasL

The mRNA expression of TLR-4, MyD88, Fas, and FasL in the brain tissues of normal and experimental animals was scrutinized by RT-PCR analysis and the outcome is proved in Figure 7. MCAO-provoked I/R rats displayed the upregulated ($P < 0.05$) mRNA expression of TLR-4, MyD88, Fas, and FasL in the brain tissues than the control. Particularly, the treatment with the 10, 25, and 50 mg/kg of syringin was decidedly ($P < 0.05$) suppressed the mRNA expression of TLR-4, MyD88, Fas, and FasL in the MCAO-provoked I/R rats. Similar kinds of outcomes were distinguished in control and 50 mg/kg of syringin-supplemented rats.

DISCUSSION

Ischemic stroke is related with the greater mortality and morbidity rate worldwide and established by manifold pathological events, which needs to find out the novel neuroprotective agent. The previous reports tinted that the diverse factors are concerned in the ischemic stroke involving oxidative stress, apoptosis, excitotoxicity, nutritive stress, and inflammation.[26] I/R injury is an imperative pathological event of ischemic stroke and it may embellish the injury to neuronal cells via reperfusion because of the occlusion of artery. The end result is the neuronal cell death due to apoptosis.[27] There are plentiful manifold interconnected procedures that lead to the progressive postischemic brain damage. Inflammation, apoptosis, and oxidative stress contribute to the indispensable functions in the pathological progressions of cerebral ischemia and also started as an activating factor of ischemic stroke. The abundant earlier reports decorated that the prevention of inflammation, apoptosis, and oxidative stress may give the auspicious curative tactic to ameliorate the I/R brain damage.[28]

The reflowing of blood is the perfect option for confining the brain damage after stroke, while reperfusion is powerfully linked with the embellishment of neuronal cell damage and inflammatory reactions, called reperfusion damage. The swelling of brain was illustrious at the time of ischemic stroke that was prompted by reflowing of blood.[29] Not only the fluid parts of brain, tissue mass also raise the extracellular space of brain area and cause the swelling of brain.[30] In this work, we observed that the syringin substantially declined the infarct area and suppressed the swelling of brain in the MCAO rats. The measurement of neurological deficit score is a decisive indicator of the initial charge of the level of brain injury and it was used to examine the level brain injury at the initial examination of MCAO rats.[31] We found that the MCAO-operated rats verified the augment neurological score and it was palpably ameliorated by the syringin treatment. Primarily, the recognition of infarct volume in the brain tissues via TTC staining to sign the preventive effects of syringin.[12] Our findings exposed that the brain infarct volume was sternly elevated in the MCAO rats; however, the syringin-treated rats discovered a considerable reduction in the infarct volume.

Inflammatory response is an imperative pathological event of I/R brain impairment, involving the instigation and progression of stroke. The inflammatory regulators' expression was strangely increased in I/R brain injury and this pathological event rouses many processes, which further exaggerates the brain injury. These events contain vasomotor contraction changes, microvessel obstructions, and accretion of free radicals.[32] The inflammatory reactions aroused within a few hours after I/R damage is mostly due to the stimulation of pro-inflammatory mediators and neutrophil penetrations.[33] The inflammatory reactions worsen the neurological damage and improve the neuronal cell apoptosis.[34] IL-6, TNF-α, and IL-1 β are regarded as imperative regulators in numerous illness of central nervous systems and raised status of these regulators

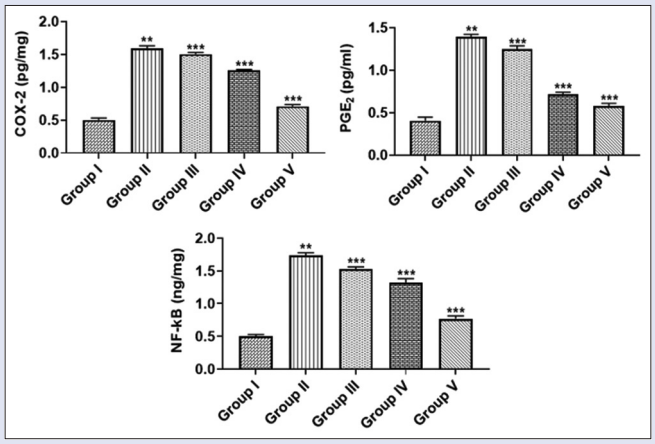

Figure 6: Effect of syringin on the inflammatory mediators status in the brain tissues of experimental rats. Results were portrayed as mean ± standard deviation of triplicates. Significance was assessed through one-way ANOVA; subsequently, Tukey's *post hoc* test was executed to examine the data. $P < 0.05$ was regarded as significant. $^{**}P < 0.05$ when related with control and $^{***}P < 0.05$ when related with ischemic-reperfusion group

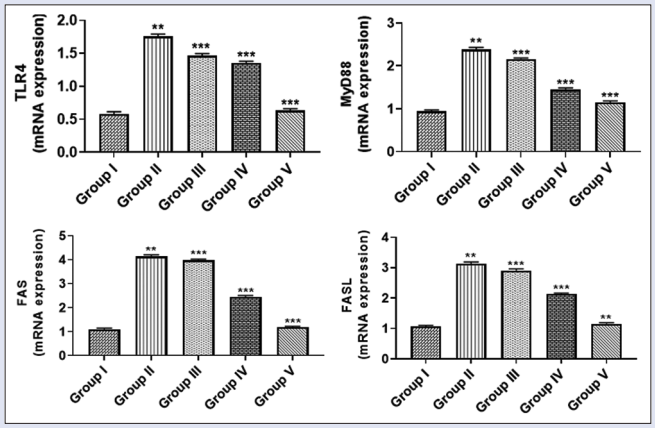

Figure 7: Effect of syringin on the mRNA expression of toll-like receptor-4, MyD88, Fas, and FasL. Results were portrayed as mean ± standard deviation of triplicates. Significance was assessed through one-way ANOVA; subsequently; Tukey's *post hoc* test was executed to examine the data. $P < 0.05$ was regarded as significant. $^{**}P < 0.05$ when related with control and $^{***}P < 0.05$ when related with ischemic-reperfusion group

connected to pathological processes of secondary injury like neural cell apoptosis and death.[35]

The NF-κB is firmly related with the beginning of inflammatory reactions. I/R injury frequently activates the generation and accretion of pro-inflammatory regulators such as IL-6, TNF-α, and IL-1 β.[36] Triggering of NF-κB and other inflammatory regulators finally results in neural cell death and irreparable brain injury. It was stated that the status of TNF-α was found to increase in the hippocampus during I/R damage.[37] In agreement with that report, in this study, we also observed that the TNF-α status was severely raised in both serum and brain tissues of MCAO rats. TNF-α was stated to possess neurotoxicity via stimulating the neural cell death by apoptosis and uplifting the expression of intercellular adhesion molecules in astrocytes, thereby results in leukocyte penetration and collapsing of BBB. The obstruction of TNF-α receptors was previously tinted to reduce the edema and infarct volume of brain followed via ischemia in animals.[38]

IL-6 is a largely imperative mediator as an IL which acts as pro-inflammatory in one hand and as an anti-inflammatory myokine in another hand.[39] The lessening in the IL-6 status was distinguished in the existing study, which concurs with the earlier report.[40] IL-10 is a multifactorial marker, which averts the generation of pro-inflammatory mediators, inhibits the inflammatory responses, and suppresses the injuries. The likely protective properties of IL-10 on ischemic stroke and inhibition of pro-inflammatory cytokines generation are at the transcriptional level.[41] IL-10 also downregulates the NF-κB and other inflammatory signaling cascades, thereby preventing the accretion of diverse inflammatory regulators to reduce the inflammation after ischemia.[42] I/R injury could be accompanied with inflammation that was aroused from a distracted equilibrium between pro and anti-inflammatory regulators. The reduction in IL-10 status in the MCAO rats was renowned in this study, which coincides with the preceding report by Yang *et al.*[43] The anti-inflammatory possessions of IL-10 could be regulated through preventing the accretion of frequent inflammatory mediators.[44] In this work, we observed that syringin supplementation can reduce the status of IL-6, TNF-α, and IL-1 β in both serum and brain tissues of MCAO rats. The above findings showed that the syringin applied the anti-inflammatory possessions against I/R damage.

The stimulation of TLR-4 may activate the adaptive immune reactions via plentiful factors.[45] The preceding study emphasized that TLR-4-like receptors play a serious function at the time of I/R injury. TLR4 also boosts up the frequent adverse reactions during I/R damage.[46] During the stimulation of TLR4, the status of TNF-α and NF-κB increased that suggests the triggering of TLR4 facilitated MyD88 signaling cascade.[47] In this work, we observed that the syringin supplementation decidedly suppressed the mRNA expression of TLR-4, MyD88, Fas, and FasL in the brain tissues of MCAO rats.

CONCLUSION

The results of this research work showed the therapeutic benefits of syringin against I/R injury in rats. The syringin-administered animals established the reduced neurological parameters. Syringin effectively suppressed the inflammatory mediators' status in both serum and brain tissues of I/R rats. Furthermore, syringin significantly downregulated the mRNA expressions of TLR-4, MyD88, Fas, and FasL in I/R rats. Taken together, our findings showed the curative actions of syringin against ischemic stroke in rats. Nonetheless, there are still wanted further trials to make clear the curative actions of syringin against stroke.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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