Pharmacogn. Mag.
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ORIGINAL ARTICLE

Attenuation of Kirenol InflammationInduced by Ischemic/ Reperfusion Cerebral Infarction Stroke via TLR4/ NLRP3 Signaling Pathway: An *in vivo* Approach

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Submitted: 27-Jul-2020 Revised: 13-Aug-2020 Accepted: 16-Feb-2021 Published: 12-Jul-2021

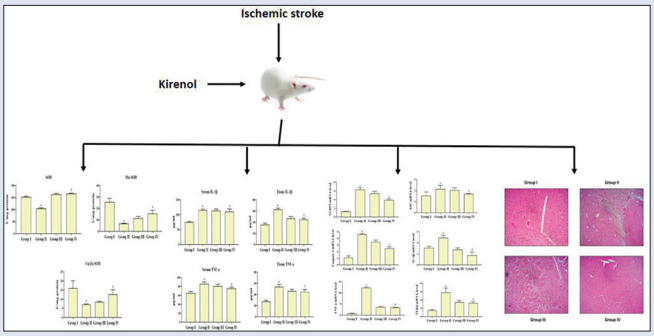
ABSTRACT

Background: Ischemia/reperfusion (I/R)-induced stroke is a long-lasting disability. Numerous reports have demonstrated that inflammation is the major cause of ischemic cerebral injury. Therefore, it is important to develop an effective anti-inflammatory agent for the attenuation of I/R-induced brain injury. **Objective:** In this study, we examined the therapeutic role of kirenol against I/R-induced neuronal damage by inhibiting inflammation in Sprague-Dawley (SD) rats. **Materials and Methods:** I/R was induced in SD rats and subsequently were administered with 10 and 20 mg/kg of kirenol. Then, we assessed the neurological score, brain water content, and infarct size. The level of antioxidant enzymes, such as superoxide dismutase, catalase, glutathione, and acetylcholinesterase, as well as the levels of malondialdehyde, was measured by using standard methods. The level of tumor necrosis factor (TNF)-α; interleukin (IL)-1 β, IL-4, IL-6, and IL-10; and vascular endothelial growth factor was measured using standard kits. The targeted messenger RNA expression (NLRP3, NLRP4, TLR-4, TNF-α, caspase-1, ASC, and IL-1β) was quantified by polymerase chain reaction technique. Histopathological analysis of the brain tissue was performed. **Results:** According to our results, kirenol decreased the neurological deficit score, ameliorated the motor function, suppressed oxidative stress, reduced inflammation, and mediated the inhibition of TLR4/NLRP3-mediated inflammatory pathway. **Conclusion:** In conclusion, these findings demonstrate the protective effects of kirenol against I/R-induced cerebral injury. The mechanism of action is associated with the inhibition of inflammation through halting the TLR4/NLRP3 signaling pathway. In summary, kirenol is a potentially new compound which can be used to improve therapeutic strategies for stroke treatments.

Key words: Antioxidants, inflammation, inflammatory cytokines, ischemia-reperfusion stroke, kirenol, TLR4/NLRP3

SUMMARY

- Kirenol is a diterpenoid found abundantly in *Siegesbeckia orientalis*, a Chinese herbal plant with numerous biological properties
- It imparts strong antioxidant and anti-inflammatory effect against ischemia/reperfusion model.



Abbreviations used: I/R: Ischemic/reperfusion; SD: Sprague Dawley; WBC: White blood cells; DMSO: Dimethyl sulfoxide; PBS: Phosphate buffer saline; TTC: 2,3,5-triphenyltetrazolium chloride; ELISA: Enzyme-linked immunosorbent assay

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DOI: 10.4103/pm.pm_261_20

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INTRODUCTION

Ischemia-reperfusion (I/R)-induced stroke is a severe neurological disorder, which causes permanent disability. In general, approximately 80% of the total cases are induced by an ischemic episode. Globally, stroke is the third leading cause of death. In fact, one in every 17 deaths is caused due to stroke. On an average, smoking and high blood pressure are the most significant contributing factors for stroke.[1] Clinically, stroke is caused due to the aggravation of blood supply to cerebral tissue which is abruptly halted and then restored. This causes tissue hypoxia which exacerbates neuro-inflammation. This condition eventually decreases blood supply to the brain leading to death of the patient.[2,3]

Microglia, the brain macrophage cells are the principle line of defense against (I/R) stroke. They are activated after stroke and remove the debris and begin repair processes.[4] Studies provide evidence that activated microglia produce excess amounts of inflammatory mediators to promote the adhesion of leukocytes to the blood vessels of the

Cite this article as: Yun D, Yang Y, Shang B, Dong H, Luo B, Attenuation of kirenol inflammation induced by ischemic/reperfusion cerebral infarction stroke via TLR4/ NLRP3 signaling pathway: An *in vivo* Approach. Phcog Mag 2021;17:268-74.

affected area. Concurrently, the accumulated white blood cells secrete inflammatory cytokines including tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1 β.[5] This response causes further damage to the brain, leading to edema and inducing microcirculatory disorders; these events continue to aggravate cerebral damage.[6] Therefore, it is important to inhibit the activation of microglia and its subsequent release of pro-inflammatory mediators in the treatment of ischemic cerebrovascular disorder.

The anti-inflammatory processes can reverse the progression of I/R-induced cerebrovascular stroke injury. Furthermore, recent studies have demonstrated that downregulation of anti-inflammatory mediators in ischemic models reduce infarction subsequently to ischemic insult via regulation of immune response.[7] Although numerous anti-inflammatory agents have been commercialized for the treatment of ischemic injury, almost all drugs show substantial side effects.[8,9] In addition, an effective clinical application has not been developed as there is lack of insight on the pathological mechanisms of (I/R) stroke. Therefore, it is important to develop a new therapeutic agent with anti-inflammatory activity for the treatment of ischemic cerebral disease.

Kirenol, a diterpenoid, is found abundantly in *Siegesbeckia orientalis*, which is a Chinese herbal plant with numerous biological properties. It shows antioxidant, anti-inflammatory, anti-tumor, and antimicrobial activities.[10] Recent studies have shown that kirenol protects the brain from damage caused due to inflammation.[11] Therefore, in this study, we explored the hypothesis that kirenol protects against I/R-induced cerebral injury by inhibiting inflammation. To achieve this goal, we developed an I/R injury model using Sprague-Dawley (SD) rats.

MATERIALS AND METHODS

Drugs and chemicals

In this study, the following drugs and reagents were purchased from Sigma-Aldrich: Phosphate-buffered saline (PBS); 2,3,5-triphenyltetrazolium chloride (TTC); enzyme-linked immunosorbent assay (ELISA) colorimetric kit; hematoxylin and eosin (H and E) staining dye, TRIzol reagent and ReverTra Ace quantitative polymerase chain reaction (qPCR) RT Kit. Ethanol, other solvents, and chemicals were of HPLC grade. Kirenol was dissolved in dimethyl sulfoxide.

Experimental model

A total of 24 male SD rats (10–14 weeks, 250–300 g) were caged under controlled laboratory conditions and were provided with tap water and rodent pellets *ad libitum*. The use of animals in this study was permitted by the Institutional Animal Ethics Committee. The animals were acclimatized to the laboratory conditions for 7 days prior to the commencement of the experiments. All study protocols were conducted according to the guidance and were approved by the ethics committee of animal care and use of laboratory animals.

Drug administration and establishment of ischemia/reperfusion injury model

There were four groups with six animals in each group ($n = 6$). After the administration of kirenol (10 and 20 mg/kg b. w.) through oral route continuously for a week, focal cerebral ischemia was established as mentioned previously.[12] All animals were anesthetized via inhalation of 3% isoflurane. Then, their skin was exposed through incision made on the midline on the neck. The right, internal, and external carotid arteries were exposed respectively. Occlusion was provoked via inserting a blunted tip with nylon filament into the internal carotid artery. After 1 h, the filament was detached, and the blood flow was restored for 24 h. The control group was sham operated. After the establishment of I/R model except in control group, all groups received an equal amount of normal saline. Finally, the animals were euthanized with an overdose of ketamine and xylene after 3 days of I/R induction. Subsequently, the brain and the blood samples were collected for pathological and biochemical analysis. Following are the assigned group for the development of experimental model:

1. Control/vehicle (0.1% NaCl) group
2. I/R group
3. I/R + Kirenol (10 mg/kg b. w.) group
4. I/R + Kirenol (20 mg/kg b. w.) group.

Assessment of neurological function in ischemia/reperfusion-induced Sprague-Dawley rats

Neurological evaluation was performed after 3 days of reperfusion. Then, the infarct volume was measured using TTC staining solution. The frozen cerebral tissues were sectioned and stained with 2% TTC before placing it in dark at room temperature for 15 min. Then, the infarct section (pale colored) was weighed again to determine the water content. The water content in the brain was examined by measuring the wet and dehydrated ischemic brain weights to achieve the dry-wet weight (W/D), which was calculated using following formula:

wet weight-dried weight/wet weight

The infarct size was analyzed by Image J software by differentiating between non-TTC-stained area (white or pale, infarct area) and TTC-stained area (red, noninfarct area) and calculated using formula below:

(infarct area/whole heart area) ×100%

Assessment of neurological score in ischemia/reperfusion-induced Sprague-Dawley rats

Neurological deficit scores were assessed in all the groups after 3 days of I/R induction based on motor test, beam balance test, and reflex test. The scoring ranged from 0 to 4 with the following characteristic:

- 0: No observable neurological deficit
- 1: Unable to fully extend motor function
- 2: Unable to circle the motor function
- 3: Unable to balance
- 4: Failure to reflex.

Animals with 0 point indicate absence of brain damage and those scoring 4 points indicate severe brain injury.

Assessment of SOD generation in the brain tissue homogenates of ischemia/reperfusion-induced Sprague-Dawley rats

The brain tissue section from each experimental group was homogenized, froze, and centrifuged at 800 ×g for 20 min. The recovered supernatants were transferred into fresh tubes and the activity of three types of SOD, namely, extracellular SOD (ECSOD), manganese SOD (MnSOD), and copper zinc SOD (CuZnSOD) was measured in the supernatant using RANSOD kit (Randox Labs, Crumlin, UK). The absorbance was measured at a wavelength of 420 nm SOD. The activity is expressed as units per milligram protein.

Assessment of oxidative stress generation in the brain tissue homogenates of ischemia/reperfusion-induced Sprague-Dawley rats

The supernatant of brain tissue homogenates was used to determine the activities of catalase (CAT), glutathione (GSH), and glutathione

peroxidase (GSH-Px), levels of malondialdehyde (MDA), and activities of acetylcholinesterase (AChE) as described by Ohkawa *et al.*,[13] Gross *et al.*,[14] Xu *et al.*,[15] Quistorff *et al.*,[16] respectively. The concentration of total protein in tissue homogenates was determined by Bradford method using bovine serum albumin as the standard. The activity of the antioxidant enzymes was calculated in units per milligram of protein.

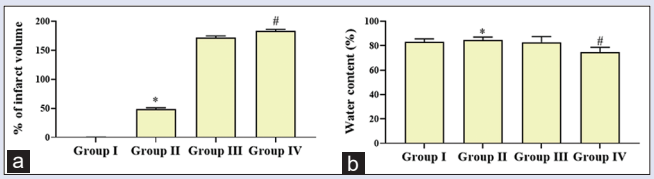

Figure 1: The neuroprotective effects of kirenol in Ischemia-Reperfusion-Induced Sprague Dawley rats (a) Quantitative analysis of the infarct volume (b) Quantification of the water content 24 h after stroke. Results articulated as mean ± standard error of the mean by ($P < 0.05$ and $P < 0.01$) from 6 individual rats. Group I: non-ischemia/reperfusion group, Group II: untreated ischemia/reperfusion group, Group III: kirenol (10 mg/kg) - ischemia/reperfusion group and Group IV: kirenol (20 mg/kg) - ischemia/reperfusion group

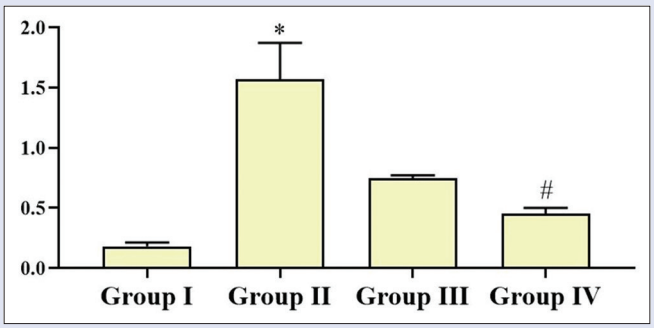

Figure 2: The neuroprotective effects of kirenol in Ischemia-Reperfusion-Induced Sprague Dawley rats. The neurological deficit scores by kirenol treatment at 10 and 20 mg/kg. Results articulated as mean ± standard error of the mean by ($P < 0.05$ and $P < 0.01$) from 6 individual rats. Group I: non-ischemia/reperfusion group, Group II: untreated ischemia/reperfusion group, Group III: kirenol (10 mg/kg)- ischemia/reperfusion group and Group IV: kirenol (20 mg/kg)- ischemia/reperfusion group

Assessment of pro-inflammatory cytokines in the serum and brain tissue homogenates of ischemia/reperfusion-induced Sprague-Dawley rats

The cerebral blood was collected after decapitation, and the serum was obtained. Then, we measured the levels of TNF-α and IL-1 βin supernatant obtained from the brain tissue homogenate and from serum. IL-4, IL-6, and IL-10 and vascular endothelial growth factor (VEGF) were measured in the samples of brain tissue homogenate. The level of cytokines was determined using ELISA kits. The absorbance was measured at 450 nm and the level of cytokines was determined by comparing to the standard graph.

Assessment of messenger RNA expression in the brain tissue homogenates of ischemia/reperfusion-induced v rats

The targeted mRNA expression (NLRP3, NLRP4, TLR-4, TNF-α, caspase-1, ASC, and IL-1 β) was quantified by PCR technique. The total RNA from the brain tissue homogenate was harvested using TRIzol reagent. Reverse transcription reactions were performed to obtain cDNAs using ReverTra Ace qPCR RT Kit. The quantitative RT-PCR analysis was conducted using SYBR Green Master Mix. The fold change or relative expression of target mRNA was calculated using $2^{-\Delta\Delta Ct}$ formula and gene expression levels standardized to the GAPDH.

Histopathological analysis of brain injury

The harvested brain tissue section was immersed in 10% buffered formalin for 3 days and then serially dehydrated in a series of graded ethanol (50%, 70%, 80%, 90%, 95%, and 100%). Then, the samples were embedded in liquid paraffin for 3 h from which paraffin blocks were prepared. The blocks (5 μm thick) were sliced using microtome

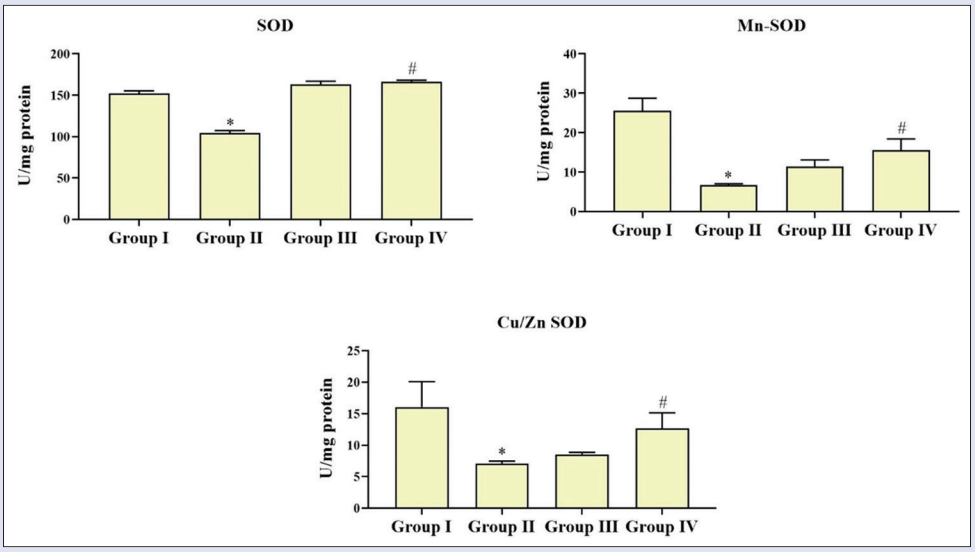

Figure 3: The neuroprotective effects of kirenol in Ischemia-Reperfusion-Induced Sprague Dawley rats. The SOD activity of kirenol treatment at 10 and 20 mg/kg. Results articulated as mean ± standard error of the mean by ($P < 0.05$ and $P < 0.01$) from 6 individual rats. Group I: non-ischemia/reperfusion group, Group II: untreated ischemia/reperfusion group, Group III: kirenol (10 mg/kg)- ischemia/reperfusion group and Group IV: kirenol (20 mg/kg)- ischemia/reperfusion group

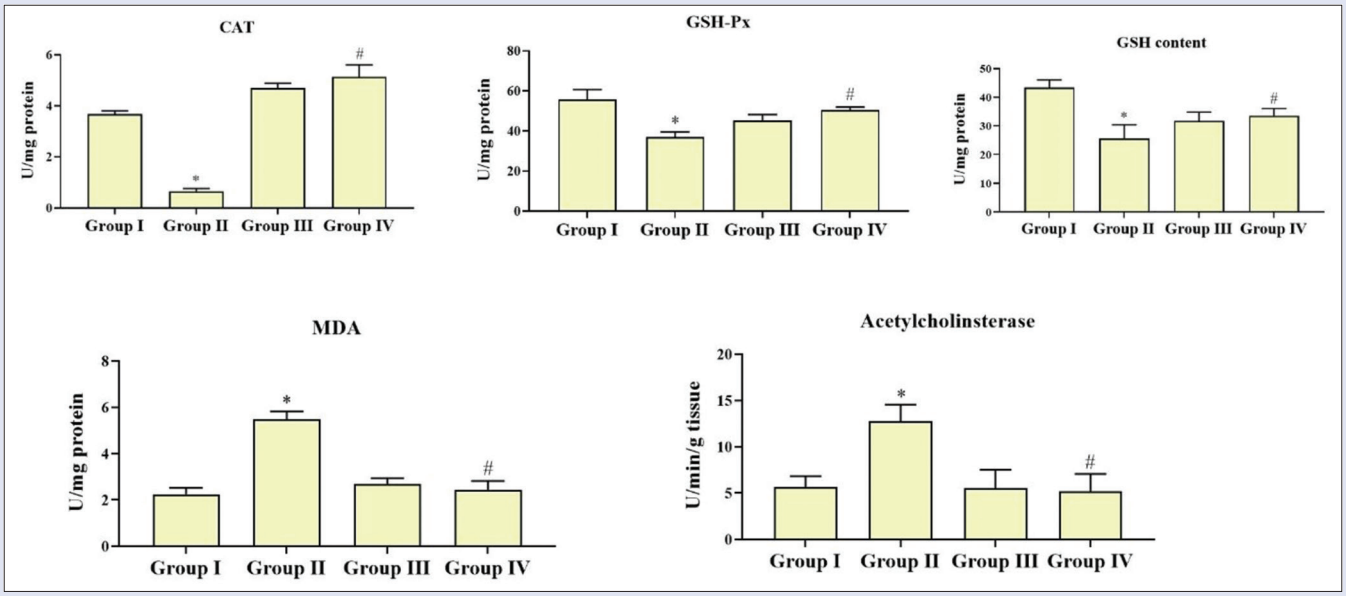

Figure 4: The neuroprotective effects of kirenol in ischemia-reperfusion-induced Sprague Dawley rats. The oxidative scavenging activity of kirenol treatment at 10 and 20 mg/kg. Results articulated as mean ± standard error of the mean by ($P < 0.05$ and $P < 0.01$) from 6 individual rats. Group I: non-ischemia/reperfusion group, Group II: untreated ischemia/reperfusion group, Group III: kirenol (10 mg/kg)-ischemia/reperfusion group and Group IV: kirenol (20 mg/kg)-ischemia/reperfusion group

and stained by H and E staining. The section was analyzed using a bright field light microscope to detect the changes in cerebrovascular tissue.

Statistical evaluation

All values are presented as mean ± standard error of at least triplicate experiments and calculated using SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA). Assessments between experimental groups were scrutinized with the Student–Newman–Keuls multiple comparison test. A $P < 0.05$ was considered as statistically significant.

RESULTS

Effect of kirenol on neuronal parameters in ischemia/reperfusion-induced injury in Sprague-Dawley rats

Water content in the brain tissue and cerebral infarct volume was measured after reperfusion. There was no infarcted area detected in sham-operated animals [Figure 1a]; however, the infarct region in the I/R group (40%) was sharply elevated when compared with the sham group. Kirenol treatment resulted in a significant increase in infarct volume (150% and 160%) with respective to its dosage as compared with I/R group. In the case of brain water content [Figure 1b], the values were significantly higher in I/R group compared with sham-operated group. Treatment with kirenol reduced the brain water content similarly in sham group and I/R group. These results suggested that kirenol inhibited the injury in the blood–brain barrier and decrease its permeability in I/R rats.

Effect of kirenol on neurobehavioral outcomes in ischemia/reperfusion-induced injury in Sprague-Dawley rats

The neurological scoring was performed 1 day after I/R induction [Figure 2]. The behavioral test resulted in dramatically increased neurological

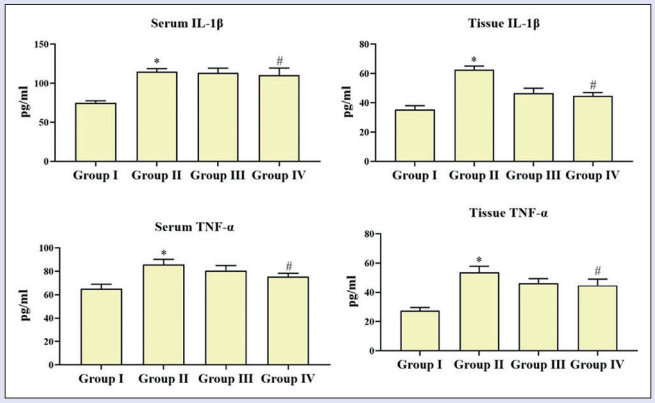

Figure 5: The neuroprotective effects of kirenol in ischemia-reperfusion-induced Sprague Dawley rats. The anti-inflammatory activity of kirenol treatment at 10 and 20 mg/kg. Results articulated as mean ± standard error of the mean by ($P < 0.05$ and $P < 0.01$) from 6 individual rats. Group I: non-ischemia/reperfusion group, Group II: untreated ischemia/reperfusion group, Group III: kirenol (10 mg/kg)-ischemia/reperfusion group and Group IV: kirenol (20 mg/kg)-ischemia/reperfusion group

deficit scores in I/R group compared to sham group. The treatment of kirenol at 10 and 20 mg/kg respectively decreased the scores of I/R group. Overall, our results show that kirenol could improve neurological function by reducing cerebral ischemic injury.

Effect of kirenol on SOD activity in ischemia/reperfusion-induced injury in Sprague-Dawley rats

SOD is involved in the prevention of brain injury. Hence, the activity of SOD reflects the extent of brain damage recovery in the animals. SOD is an antioxidative enzyme that effectively scavenges free-radicals after cerebral ischemic events. As shown in Figure 3, the activity of ECSOD,

MnSOD, and CuZnSOD was elevated statistically in I/R group when compared to sham group. However, kirenol treatment that SOD activity elevated in all the groups with I/R group. The results reflect the protective nature of kirenol against damage induced by I/R.

Effect of kirenol on oxidative stress in ischemia/reperfusion-induced injury in Sprague-Dawley rats

Excessive production of intracellular ROS is responsible for the development of cerebrovascular I/R stroke. Figure 4 shows the effect of kirenol on intracellular ROS production. The result shows the changes in the markers of antioxidant enzymes (CAT, GSH-Px, and GSH content) and parameters of oxidative damage (MDA and AChE). The content of antioxidant enzymes decreased significantly 1 day after the induction of I/R when compared with sham group. However, compared with I/R, kirenol restored antioxidant enzymes content significantly. The expression of markers of oxidative stress (MDA and AChE) was reduced to the level of sham group after treatment with kirenol in I/R group. This result shows that the treatment with kirenol improved the redox state against damage induced by I/R by markedly suppressing the oxidative stress following treatment with kirenol antioxidant capacity.

Effect of kirenol on inflammatory responses in ischemia/reperfusion-induced injury in Sprague-Dawley rats

Inflammatory cytokines is the principle marker of I/R damage. As shown in Figure 5, there were visible increase in both serum tissue levels of TNF-α and IL-1 β in the I/R group compared to the Sham group. Importantly, these increases were dramatically inhibited in the kirenol treatment (10 and 20 mg/kg) against damage induced I/R in a dose-dependent fashion indicating the potential anti-inflammatory properties of kirenol treatment. By improving cerebral injury through the inhibition of inflammatory cytokines release.

Effect of kirenol on interleukins in ischemia/reperfusion-induced injury in Sprague-Dawley rats

The modulation of neuro-inflammatory processes, changes in pro-inflammatory indicators (IL-6 and VEGF) and anti-inflammatory indicators (IL-4 and IL-10) were assessed. Both pro-inflammatory and anti-inflammatory markers level in the I/R group were significantly increased when associated with sham group. Upon treatment with kirenol, the pro-inflammatory markers were significantly decreased. By contrast, the expression of anti-inflammatory markers exhibited elevated changes in comparison to I/R group [Figure 6]. These results prove that kirenol can exercise neuroprotective effects by down regulating inflammatory factors in I/R injury.

Effect of kirenol on NLRP3 inflammasome activation in ischemia/reperfusion-induced injury in Sprague-Dawley rats

The effects of kirenol on NLRP3 inflammasome activated, the mRNA expression of NLRP3, caspase-1, TNF-α, ASC, IL-1 β, and TLR4 in brain tissues was measured by RT-PCR [Figure 7]. Levels of these expression in the I/R group were statistically higher when associated with the sham group. However, the levels were reduced abruptly in the kirenol treated group comparatively. Results illustrated kirenol may improve the cerebral I/R injury by halting the NLRP3 inflammasome activation.

Effect of kirenol on histopathology features in ischemia/reperfusion-induced injury in Sprague-Dawley rats

A histopathological examination in sham-operated group displayed a normal cell structures with constant staining of the cell nucleus [Figure 8]. However, in I/R group, the analysis showed a bare and disoriented cell organization, vacuolar deterioration, cell-gap expansion, cell membrane distortion, nuclear pyknosis, constriction of cell bodies, necrotic cell death [Figure 8]. Injury to the brain tissue in kirenol-treated group was not visible and the level of injury in group treated with a highest dose of kirenol was remarkably abridged [Figure 8]. This shows that kirenol plays a crucial part in the protection of brain tissue from injuries.

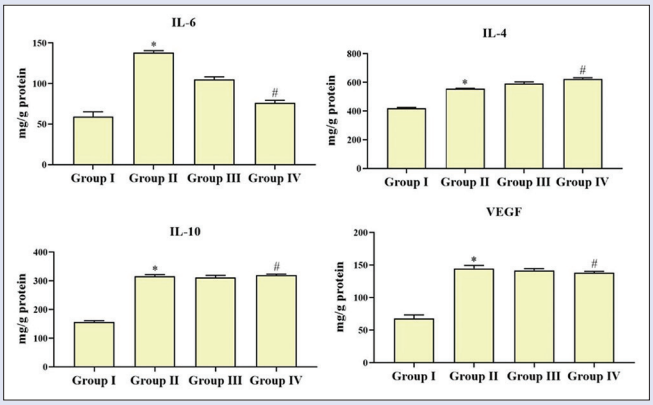

Figure 6: The neuroprotective effects of kirenol in ischemia-reperfusion-induced Sprague Dawley rats. The effect on interleukin by kirenol treatment at 10 and 20 mg/kg. Results articulated as mean ± standard error of the mean by ($P < 0.05$, $P < 0.01$ and $P < 0.0.001$) from 6 individual rats. Group I: non-ischemia/reperfusion group, Group II: untreated ischemia/reperfusion group, Group III: kirenol (10 mg/kg)-ischemia/reperfusion group and Group IV: kirenol (20 mg/kg)-ischemia/reperfusion group

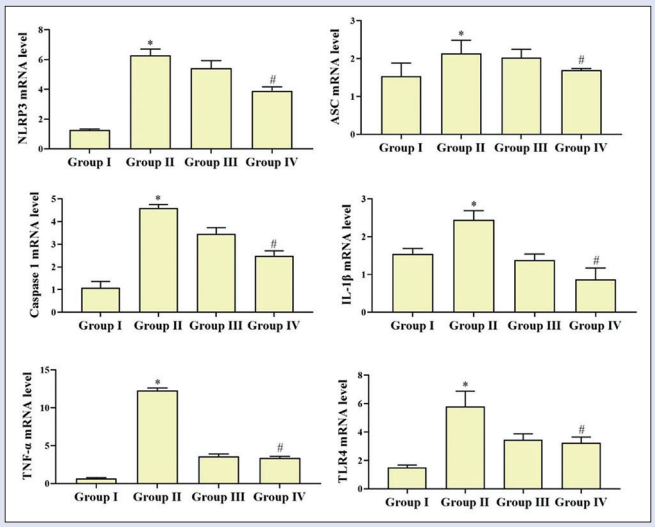

Figure 7: The neuroprotective effects of kirenol in ischemia-reperfusion-induced Sprague Dawley rats. The effect on NLRP3 inflammasome activation by kirenol treatment at 10 and 20 mg/kg. Results articulated as mean ± standard error of the mean by ($P < 0.05$, $P < 0.01$ and $P < 0.0.001$) from 6 individual rats. Group I: non-ischemia/reperfusion group, Group II: untreated ischemia/reperfusion group, Group III: kirenol (10 mg/kg)-ischemia/reperfusion group and Group IV: kirenol (20 mg/kg)-ischemia/reperfusion group

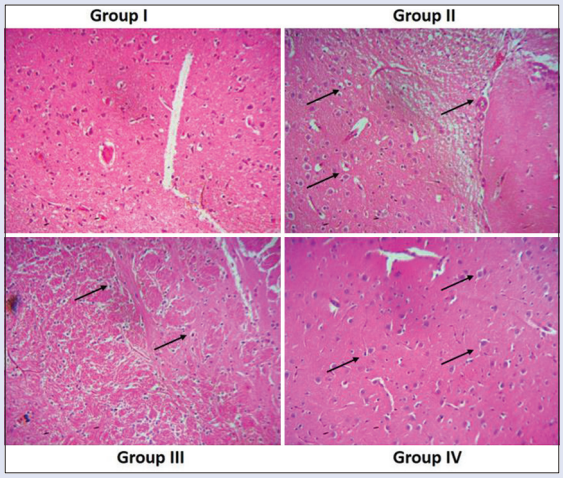

Figure 8: The neuroprotective effects of kirenol in ischemia-reperfusion-induced Sprague Dawley rats. The effect on histopathological features by kirenol treatment at 10 and 20 mg/kg. Results articulated as mean ± standard error of the mean by ($P < 0.05$, $P < 0.01$ and $P < 0.0.001$) from 6 individual rats. Group I: non-ischemia/reperfusion group, Group II: untreated ischemia/reperfusiongroup,GroupIII:kirenol(10mg/kg)-ischemia/reperfusion group and Group IV: kirenol (20 mg/kg)-ischemia/reperfusion group. The histological alterations were pointed by using the black arrow marks

DISCUSSION

There are multiple pathways associated with I/R-induced cerebral stroke. In the cascade of injury and recovery, nucleotide-binding oligomerization domain-like receptors (NLRs) inflammasome may be a chief mediator in controlling the free radical formation which end in inflammatory response and apoptotic gene activation.[17] Among the NLRs, NLRP3 is broadly expressed in the glial cells of the brain. A pattern-recognition receptor that identifies microbial contamination, TLR, is involved in the initiation of immune reaction. The transcription of NLR3 activates TLR4 to release pro-inflammatory mediators such as TNF-α and IL-1 β and IL-6 to regulate damage in brain cells.[18] Hence, NLRP3/TLR4 signaling pathway is responsible for the regulation of inflammatory responses in cerebral (I/R) stroke through the production of pro-inflammatory cytokines. This led to the investigation of neuroprotective effect of kirenol by inhibition of NLR3 inflammasome activation via TLR4 signaling pathway. Patients with I/R-induced cerebral injury, the fraction of damage to the focal point of cerebral region is about 80%. Hence, the development of model focusing on I/R-induced focal cerebral region that are comparable to the clinical model.[19] The assessment of data about the neurological and motor function in SD rats revealed that the damage in the cerebral region in I/R group was greater than that of the sham group. Kirenol treatment improved the overall neuropsychological conditions especially dose groups dependently. This result is partly dependable with a previous study which evidenced paeoniflorin displayed neuroprotection by reducing the neurological impairment in mice.[20] This suggests that kirenol treatment enhanced the neuropsychological damage after cerebral I/R injury.

Numerous pathways are responsible for the cascade of chronic injury during cerebral I/R stroke such as over formation of free-radicals. Free radicals can damage lipid molecules, nucleic acids, and proteins which subsequently lead to irreversible damage to the integrity of cell membrane and even cell death. Such pathological changes lead to neuronal dysfunction.[21] Hence, given the brain's increased requirement for oxygen, it requires SOD, CAT, GSH, and GPx to keep MDA and AChE levels under control.[22,23] Our results show that kirenol statistically averts the reduction in antioxidant enzyme content and increase in oxidative stress marker in both brain tissue and serum of rats. Thus, kirenol plays a substantial role in the oxidation and the antioxidant balance of cerebral I/R model.

The inflammatory response is upregulated in the brain following the ischemic insult. Some of the important cytokines that are associated with the inflammation cerebral I/R injury are TNF-α, IL-1 β, and VEGF. Previous studies have demonstrated the anti-inflammatory effects (e.g., IL-4 and IL-10) induced by antioxidant agents can initiate repair processes and control unwarranted inflammation.[24,25] A previous study has shown that the pathway underlying the neuroprotective mechanism is related to the antioxidative effect.[7] An upregulation of anti-inflammatory and down-regulation of pro-inflammatory mediators after the administration of kirenol in I/R group comparatively, proposed that the effect of kirenol improved angiogenesis and neurogenesis in the I-R-induced brain injury.

The activation of NLRP3 in the cerebral I/R injury has expanded its prominence. A signaling cascade upregulates the expression of NLRP3, caspase-1, and pro-IL-1 for the effective development of inflammasome. The NLRP3 inflammasome transcription activates the TLR4 signaling pathway.[7] A previous study has demonstrated that NLRP3 inflammasome, caspase-1, TNF-α, and IL-1 β levels were elevated in the brain tissue of mice induced with stroke.[26] In this study, the analysis showed NLRP3, ASC, and cleaved caspase-1, TNF-α, and TLR4 mRNA transcription were substantially blocked when exposed to kirenol treatment in I/R model. We found that the activation NLRP3 inflammasome is injurious in cerebral ischemic stroke. These data showed kirenol protects the neuronal cells and improves the brain injury from inflammation by inhibition of NLRP3 inflammasome partly. Histopathological evidence has confirmed the neuroprotective role of kirenol by the attenuation of degenerated neurons from the brain damage caused in the I/R model.

CONCLUSION

Overall, kirenol demonstrated the protective role by inhibition of the TLR4/NLRP3-mediated inflammatory pathway. In fact, kirenol imparts strong antioxidative and anti-inflammatory effect against I/R model. Thus, we can say that the therapeutic activity of kirenol is encouraging, which should further be explored to confirm the mechanism of action.

Acknowledgements

Nanchong City School Science and Technology Strategic Cooperation Project, No. 18SXHZ0331. This project was supported by Researchers Supporting Project number (RSP-2020/230) King Saud University, Riyadh, Saudi Arabia

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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