International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2025.502.509

Research Article
Lupeol Protects Neuronal Injury Against Spinal Cord Injury in Rat Models via Targeting Nf-κB/NLRP3 Activation

[1]Genbing Shi, [2]Yujie Ma, [1]Yongjia Jin, [1]Zongyi Mo, [1]Zhaogan Ren, [1]Zhanqiang Hua and [1]Luowen Wang

[1]Department of Orthopedics, Shanghai Electric Power Hospital, Shanghai 200050, China
[2]Department of Outpatient Service, Zibo Limei Plastic Surgery Hospital, Zibo, Shandong 255020, China

Abstract

Background and Objective: Spinal cord injury (SCI) is a severe traumatic disorder that threatens life and has long-term consequences, affecting a large number of people worldwide. Scientists have confirmed Lupeol's involvement in a variety of ailments. The current research examines Lupeol's efficacy in treating SCI in rats. **Materials and Methods:** This research split 30 animals into 3 groups: Sham, SCI (pentobarbital anesthesia at 35 mg/kg doses) and Lupeol (50 mg/kg p.o., for 21 days). Laminectomy was used to induce T9-T10 SCI in animals. After that, locomotor function in animals, pro-inflammatory cytokine analysis, spinal cord edema volume measurement, TUNEL labelling and western blot analysis were performed. **Results:** Lupeol was evaluated by measuring the Basso, Beattie, Bresnahan (BBB) score and spinal cord edema. The protein level of the inflammatory pathway in spinal tissue was quantified using western blot examination. Lupeol therapy substantially reduced the BBB score suppression caused by SCI ($p<0.05$). The SCI animals treated with Lupeol showed substantially reduced edema ($p<0.01$) compared to the model group. Lupeol significantly reduced cellular apoptosis in SCI animals. Lupeol therapy reduced SCI-induced IL-1β/-6/TNF-α upregulation. In Lupeol-treated animals, SCI-induced p-IκB suppression, Phosphorylated NF-κB (p-NF-κB) and Phosphorylated NLRP3 (p-NLRP3) upregulation were markedly reduced. Lupeol dramatically reduced caspase-1 and TLR4 in SCI animals. **Conclusion:** The study's results demonstrate that administering Lupeol protects against damage to neurons and inflammation in animals with spinal cord injuries via controlling the NF-κB/NLRP3 pathway.

Key words: Spinal cord injury, lupeol, neuronal apoptosis, edema volumes, neuronal injury, neuronal inflammation

Citation: Shi, G., Y. Ma, Y. Jin, Z. Mo, Z. Ren, Z. Hua and L. Wang, 2025. Lupeol protects neuronal injury against spinal cord injury in rat models via targeting NF-κB/NLRP3 activation. Int. J. Pharmacol., 21: 502-509.

Corresponding Author: Luowen Wang, Department of Orthopedics, Shanghai Electric Power Hospital, Shanghai 200050, China

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Spinal cord injury (SCI) is a commonly encountered trauma and one of the leading causes of disability in China[1]. The most prevalent causes of SCI recorded in clinical settings include traffic accidents, falls from constructed structures and encounters with tall trees[2,3]. The development of secondary neuronal injury after primary injury to the spinal cord proceeds through a complicated mechanism[3]. The initial physical injury leads to the progression of intricate pathological alterations, such as ischemia, oxidative harm, neuronal degeneration by apoptosis or necrosis and excitotoxicity[4]. There is severe irreversible damage to nerves during SCI, with very limited chances of functional recovery[2]. Although initially neurons and neuroglia are destroyed by mechanical injury, the pathological damage at the latter stage is more destructive[5]. The dysfunction of the spinal nerve is associated mainly with primary and secondary injuries to spinal cord neurons[5]. The injured neurons possess a very low/negligible tendency for regeneration and self-repair, which require the expression of multiple genes controlling apoptosis and nerve growth factor[6]. The expression of these genes is executed through multiple pathways, including signal transduction and other associated pathways[7]. The pharmacological agents for the treatment of SCI must be able to act on many dimensions of the injury, such as preventing secondary injury, reducing pain, and inhibiting inflammation, without causing side effects[7]. The initial biochemical change following SCI is the peroxidation of lipids, which is related to oxidative radical damage. The inflammatory response after SCI leads to the accumulation of inflammatory cells, which secrete toxic cytokines and oxidants at the initial stage to aggravate SCI[8,9]. The secretion of anti-inflammatory cytokines takes place only at the advanced stage of SCI[8,9].

Lupeol is chemically a triterpenoid isolated from several medicinal plants such as *Sebastiania adenophora, Bombax ceiba, Crataeva nurvala, Leptadenia hastata, Zanthoxylum riedelianum, Celastrus paniculatus, Himatanthus sucuuba, Allanblackia monticola and Tamarindus indica*[10,11]. Lupeol is reported to have strong anti-inflammatory activity as it suppresses inflammatory cytokine levels and modulates the phagocytic activity of macrophages[12]. It reduces collagen synthesis in arthritic animals[13]. Lupeol protects neuronal inflammation by regulating inflammatory cytokines, oxidative stress and the MAPK/JNK pathway[12]. This experiment aims to assess the impact of Lupeol on SCI.

MATERIALS AND METHODS

Study area: The current investigation was conducted at the Shanghai Electric Power Hospital, Shanghai, China from June to August, 2023.

Animals: This investigation used a group of 30 male Sprague-Dawley rats, aged 10-12 weeks and weighing 220-240 g, obtained from the animal centre affiliated with Nanchang University, China. The animals were housed in controlled environments with a temperature range of 23-25°C, a humidity level of around 60% and a light-dark cycle of 12 hrs each. The animals were provided unrestricted access to pellet animal meals and water.

The Animal Ethics Committee at Shanghai Electric Power Hospital in Shanghai, China, validated this study's protocol (CHTCMH/2020/05).

Induction of SCI animals: The current research used the previously described procedure to induce SCI in animals[14]. Animals were split into 3 groups: Sham, SCI and Lupeol-treated groups. The Lupeol-treated group got 50 mg/kg orally for 21 days. In the SCI model and SCI+Lupeol treatment groups, animals received 35 mg/kg of pentobarbital anesthesia intravenously (i.v.). The animals underwent laminectomy to produce SCI within the T9-T10 level, as stated in the research.

All the animals were anesthetized and an incision was made of 2 cm to expose T8-T9 of the spinal cord region, clam it to stabilize and drop a rod from 12.5 mm height having 10 g weight on the exposed part to produce the contusion. Later suture the exposed part of each rat. The spinal cord in the area was meticulously subjected to edema-induced injury, ensuring that the dura mater remained intact. Animals in both the sham and SCI model groups were given the same amount of phosphate-buffered saline (PBS). The animals were euthanized on the 21st day after surgery after anesthesia with pentobarbital, by the experimental procedures.

Behavioral assessments: The locomotive performance of animals after SCI was assessed utilizing the BBB scale for rating[15]. Two researchers separately inspected the animals to determine their scores. The animals with full paralysis received an assessment of 0, while those that demonstrated normal mobility received a score of 21.

Analysis of pro-inflammatory cytokines: The blood sample was taken from the retro-orbital plexus of each rat and subjected to centrifugation at 4°C for 15 min at 2,000 xg for

the collection of serum. The levels of oxidative stress factors TNF-α (Cat. number RTA00), IL-6 (Cat. number R6000B) and IL -1β (Cat. number RLB00; R&D Systems, Inc., Minneapolis, Minnesota, USA) were determined using the immunoassay kits available commercially.

Measurement of volume of spinal cord edema: The decapitation procedure after pentobarbital anesthetization and transcardiac perfusion by applying a left ventricular cannula with 250 mL of normal saline and the right atrium kept open to sacrifice the animals on day 21st of SCI. The spinal cords of animals were extracted and then washed with PBS to measure their wet weight. The tissue samples were dehydrated for 48 hrs at 72°C and their weight was assessed.

TUNEL staining: The extracted spinal cord tissues (SCTs) were subjected to fixing in paraformaldehyde (4%) for 24 hrs and the tissue was seeded in wax to prepare wax cubes. Dehydration of tissues in ethyl alcohol and washing with PBS was followed by treatment with 3% H_2O_2 solution for 20 min. The slices were incubated with proteinase K for 30 mins at 37°C and then stained with TUNEL dye (BioVision Co., USA). The tissues were incubated for 2 hrs with TdT and dUTP-digoxigenin at 37°C under a humid atmosphere. The sections after washing in PBS were treated with streptavidin-biotin complex before DAB colorization. The tissues stained with hematoxylin were seen using a microscope at a magnification of ×350.

Western blot assessment: The SCTs taken from animals were subjected to RIPA buffer to prepare the lysate. The sample was subjected to homogenization for 30 min sat a centrifugal force of 12,500 xg at 4°C. The protein content in the liquid portion was then assessed by a bicinchoninic acid test. The protein samples, each weighing 30 µg, were split using a 10% SDS-PAGE gel. To prevent non-specific binding, the membranes were incubated in a 5% solution of skimmed milk powder for 2 hrs. The proteins TLR4, anti-NLRP3, p-NF-κB, NF-κB p65, caspase-1 and β-actin (Cell Signaling Technology, Inc.) were examined by incubating them with primary antibodies overnight at 4°C. Subsequently, the membrane was subjected to a 2 hrs incubation at 37°C with secondary antibodies. The presence of immunopositive bands was identified using the BeyoECL Star and GeneTools software, version 4.1 (Synoptics, Ltd., Cambridge, UK) systems.

Statistical assessment: The data was analysed via one-way ANOVA in SPSS version 17.0 (SPSS, Inc., Chicago, Illinois, UA) and reported as Mean±Standard Error Mean (SEM). Statistics show a ($p<0.05$) for the outcomes.

RESULTS

Improvement of BBB score by Lupeol in SCI animals: The impact of Lupeol on the BBB score in animals with SCI was assessed on the 0th and 21st days of the experiment (Fig. 1). On the first day of the surgery, there was no significant difference between the SCI and Lupeol-treated groups and the sham group in terms of BBB scores. The BBB score on the 21st day of the treatment was considerably reduced ($p<0.01$) in the SCI group than the sham group of animals. On the 21st day of the experiment, the group treated with Lupeol showed a noteworthy ($p<0.01$) rise in BBB score than the group with SCI.

Suppression of edema volumes in the spinal cord by Lupeol in SCI animals: A percentage of edema volume was observed in Lupeol-treated SCI animals, as demonstrated in Fig. 2. The SCI group of animals exhibited a substantial ($p<0.01$) rise in the proportion of spinal cord contusion volume, specifically edema volume, than the sham group. The Lupeol-treated group of animals had a considerably ($p<0.01$) lower proportion of edema volume than the SCI group.

Preventive role of Lupeol against SCI-mediated apoptosis: The TUNEL staining revealed neuronal apoptosis in the STC of Lupeol-treated SCI animals (Fig. 3). The SCI group had considerably greater rates ($p<0.01$) of neuronal death in spinal tissue than the sham group of animals. Lupeol treatment substantially decreased ($p<0.01$) neuronal cell death in STC ($p<0.01$) than in the SCI group of animals.

Inhibition of inflammatory cytokines by Lupeol in SCI animals: The ELISA was utilised to assess inflammatory cytokines in the spinal cord tissue homogenate of a Lupeol-treated rat model with an SCI. The levels of inflammatory cytokines, namely IL-1β, IL-6 and TNF-α, were considerably greater($p<0.01$) in the spinal tissue of animals with SCI than in animals without injury (the sham group). The concentrations of IL-1β, IL-6 and TNF-α in the spinal tissue homogenate of the Lupeol-treated group showed a statistically significant decrease ($p<0.01$) compared to the negative control group of animals (Fig. 4).

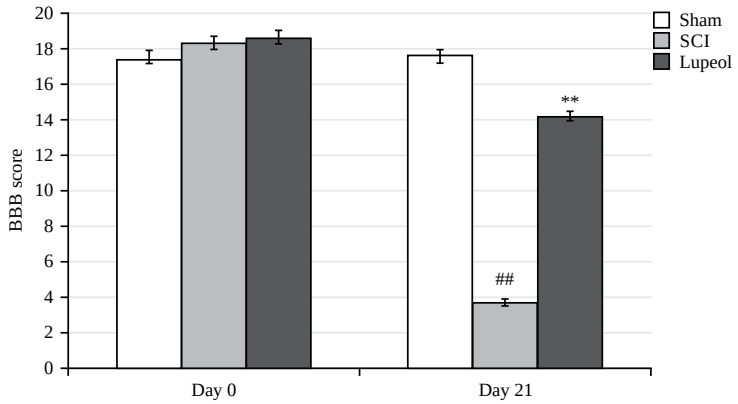

Fig. 1: Effect of Lupeol on the BBB score on 0th and 21st day of protocol in SCI rat model
Mean±SEM (n = 10); ##p<0.01 than sham group and **p<0.01 than SCI group

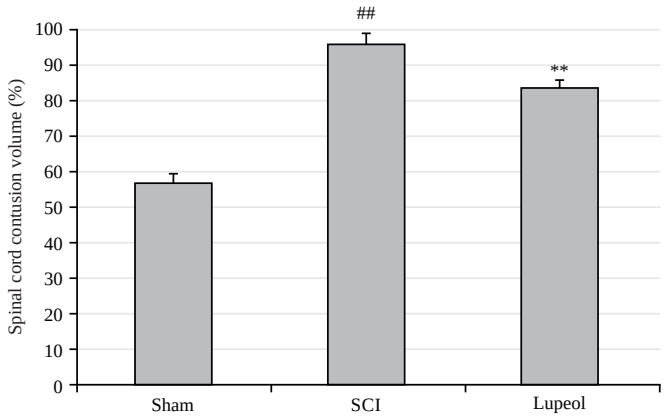

Fig. 2: Effect of Lupeol on the percentage of edema volume in SCI rat model
Mean±SEM (n=10); ##p<0.01 than sham group and **p<0.01 than SCI group

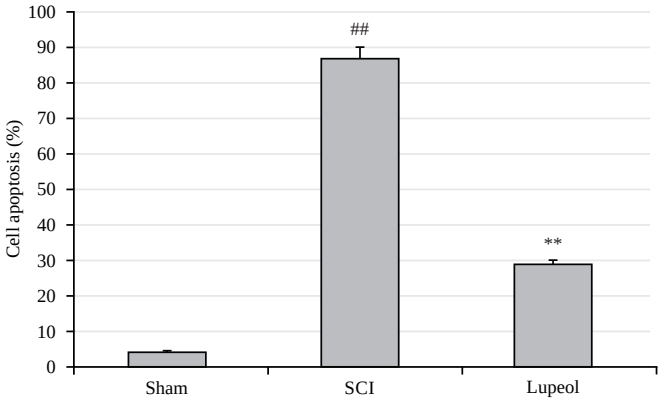

Fig. 3: Effect of Lupeol on the percentage of apoptosis of neuronal cells in the STC of SCI rat model
Mean±SEM (n=10); ##p<0.01 than sham group and **p<0.01 than SCI group

Lupeol suppresses NF-κB/NLRP3 expression and activates IκB in SCI animals: The western blot evaluation was utilized to assess the concentrations of p-NF-κB/NF-κB, p-NLRP3/NLRP3 and p-IκB/IκB proteins in the STC of animals with SCI that were treated with Lupeol. Animals with SCI showed substantially higher concentrations of p-NF-κB, NF-κB, p-NLRP3 and NLRP3 proteins in their spinal tissue than in the sham group ($p<0.01$). Conversely, the expression of phosphorylated IκB (p-IκB) and IκB proteins was seen to be decreased in the SCI group (Fig. 5).

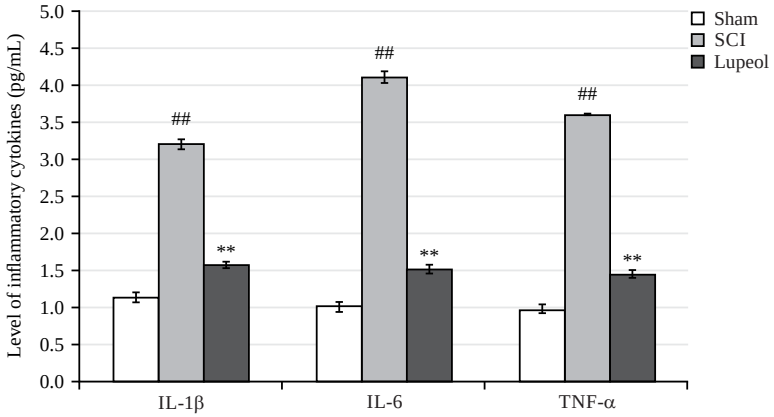

Fig. 4: Effect of Lupeol on the level of inflammatory cytokines (IL-1β, IL-6 and TNF-α) in the STC of SCI rat model
Mean±SEM (n=10); ##p<0.01 than sham group and **p<0.01 than SCI group

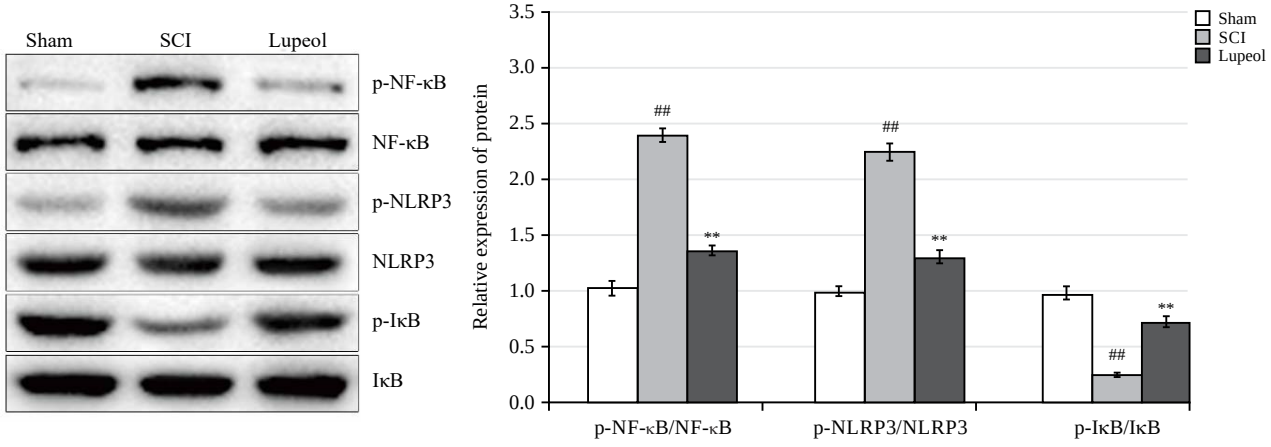

Fig. 5: Effect of Lupeol on the expression of p-NF-κB/NF-κB, p-NLRP3/NLRP3 and p-IκB/IκB in the STC of SCI rat model
Mean±SEM (n=10); ##p<0.01 than sham group and **p<0.01 than SCI group

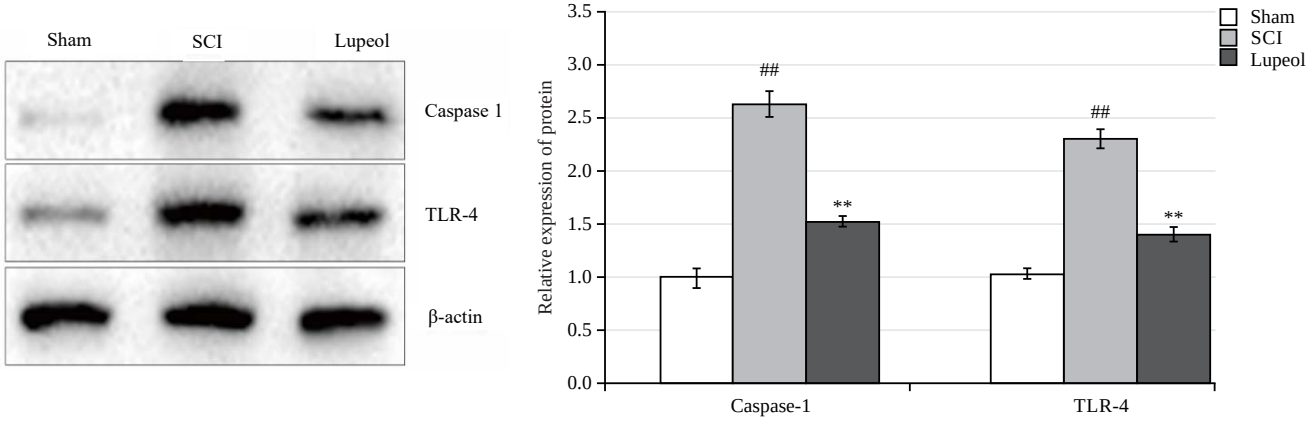

Fig. 6: Effect of Lupeol on the expression of caspase-1 and TLR-4 protein in the STC of SCI rat model
Mean±SEM (n=10); ##p<0.01 than sham group and **p<0.01 than SCI group

Lupeol inhibits caspase 1/TLR4 expression in SCI animals: The SCI group had a substantial rise ($p<0.01$) in the expression of caspase 1 and TLR-4 protein in their STC homogenate ($p<0.01$) than the sham-operated group of animals. The levels of TLR-4 and caspase-1 proteins were markedly decreased ($p<0.01$) in the STC homogenate of the group treated with Lupeol than the group of animals with SCI (Fig. 6).

DISCUSSION

In the present study, oxidative stress mediated by SCI in animals was analysed by measuring IL-1β/TNF-α expression in spinal cords. According to the previous reports, IL-1β, 6β and TNF-α levels in SCI rat spinal cords were markedly promoted. However, in Lupeol-treated animals, SCI-mediated up-regulation of IL-1β, 6β and TNF-α levels was alleviated significantly. The present study found that SCI induction markedly promoted NF-κB expression in the rat spinal cords. Unlike animals without SCI, SCI caused an increase in IkB degradation in their STCs. Additionally, in SCI animals, NLRP3 expression was markedly promoted compared to the control group. However, Lupeol treatment alleviated SCI-mediated NF-κB up-regulation, NLRP3 over-expression and IκB degradation in the rat's STCs. The current research demonstrated elevated caspase-1 and TLR4 expression levels in the spinal cords of animals with SCI. The administration of Lupeol effectively reduced the increased levels of caspase-1 and TLR4 in animals with SCI.

The SCI, which is accompanied by motor function loss, is among the most common causes of movement impairment[16-18]. The major causes of SCI include vehicular accidents, falls of workers during construction and accidents of professionals like engineers[19]. During the last decade, the death rate due to SCI has increased markedly in China and many other countries[20]. Paralysis of the patients caused by SCI leads to physiological as well as psychological trauma and affects the economy of societies[19]. The oxidative stress associated with over-expressed pro-inflammatory cytokines causes inflammation and consequently damages the tissues[21]. The common inflammatory molecules up-regulated during oxidative stress are IL-1β/-2/-6/-12 and TNF-α[22]. Inflammatory cytokines also stimulate NF-κB by inducing oxidative stress[23].

The transcriptional factors such as NF-κB and activator protein 1 are activated by the interaction of lipopolysaccharides with TLR4, which subsequently stimulates inflammatory molecules[24]. The NF-κB exhibits multiple functions as a transcription factor and is involved in inflammatory responses induced by LPS[23]. Under normal physiological conditions, IκB maintains NF-κB in an inactive form and thereby prevents its nuclear translocation[25]. The IκB degradation and subsequent nuclear translocation of NF-κB are catalysed by IκB kinase via 42' serine phosphorylation of IκB[26]. Thus, IκB degradation stimulates NF-κB translocation by dissolving its nuclear localization pathway[27]. The cellular IL-1β/-18 and -33 were secreted at elevated levels by over-expression of the NLRP3 inflammasome[28]. Consequently, the management of the NLRP3 inflammasome is crucial in managing the development of disease caused by oxidative stress and in maintaining a stable internal environment[29]. Suppressing the activity of NF-κB and NLRP3 has been discovered to inhibit the development of colitis in mice that were induced with dextran sulfate sodium[30]. The chemotherapeutic agents alleviate acute lung injury caused by LPS in animals via NF-κB signaling inhibition mediated by TLR4[31]. The current research demonstrated elevated caspase-1 and TLR4 expression levelsin the spinal cords of animals with SCI. The administration of Lupeol effectively reduced the increased levels of caspase-1 and TLR4 in animals with SCI.

CONCLUSION

Lupeol treatment significantly improved the BBB score in the SCI animals and prevented edema formation. Treatment with Lupeol significantly reduced neuronal apoptosis in SCI animals. Moreover, Lupeol suppressed the inflammatory response in SCI animals, which was evident by a marked reduction in IL-1β, IL-6 and TNF-α levels. Lupeol decreased the expression of NF-κB and NLRP3 and enhanced the expression of IκB in animals with SCI. Lupeol treatment also inhibited the expression levels of caspase-1 and TLR4 in SCI animals. Therefore, Lupeol has the potential to be a new and powerful treatment for SCI.

SIGNIFICANCE STATEMENT

Lupeol's effectiveness in treating SCI in rats is being studied. Overall, Lupeol therapy improved SCI animals' BBB scores and avoided edema. Lupeol dramatically decreased neuronal apoptosis in SCI mice. Lupeol significantly reduced IL-1β, IL-6 and TNF-α levels in SCI rats, indicating a suppression of the inflammatory response. In SCI animals, Lupeol reduced NF-κB and NLRP3 expression and increased IκB expression. Lupeol decreased caspase-1 and TLR4 expression in SCI mice. Thus, Lupeol may be a novel and effective SCI therapy.

ACKNOWLEDGMENT

The authors would like to express their gratitude to Shanghai Electric Power Hospital, Shanghai, China.

REFERENCES

1. Yalçın, S. and M. Ersöz, 2015. Urodynamic findings, bladder emptying methods and therapeutic approaches in patients with upper lumbar and lower lumbar-sacral spinal cord injury. Neurol. Sci., 36: 2061-2065.
2. Alizadeh, A., S.M. Dyck and S. Karimi-Abdolrezaee, 2019. Traumatic spinal cord injury: An overview of pathophysiology, models and acute injury mechanisms. Front. Neurol., Vol. 10. 10.3389/fneur.2019.00282.
3. Pirouzmand, F., 2010. Epidemiological trends of spine and spinal cord injuries in the largest Canadian adult trauma center from 1986 to 2006. J. Neurosurg., 12: 131-140.
4. Jiang, W., Y. Huang, F. He, J. Liu and M. Li *et al*., 2016. Dopamine D1 receptor agonist A-68930 inhibits NLRP3 inflammasome activation, controls inflammation, and alleviates histopathology in a rat model of spinal cord injury. SPINE, 41: E330-E334.
5. Rosety-Rodriguez, M., A. Camacho, I. Rosety, G. Fornieles and M.A. Rosety *et al*., 2014. Low-grade systemic inflammation and leptin levels were improved by arm cranking exercise in adults with chronic spinal cord injury. Arch. Phys. Med. Rehabil., 95: 297-302.
6. Yuan, Y.M. and C. He, 2013. The glial scar in spinal cord injury and repair. Neurosci. Bull., 29: 421-435.
7. Werndle, M.C., A. Zoumprouli, P. Sedgwick and M.C. Papadopoulos, 2012. Variability in the treatment of acute spinal cord injury in the United Kingdom: Results of a National Survey. J. Neurotrauma, 29: 880-888.
8. Hossain, M.S., L.A. Harvey, M. Akhlasur Rahman, S. Muldoon and J.L. Bowden *et al*., 2016. Community-based interventions to prevent serious complications (CIVIC) following spinal cord injury in Bangladesh: Protocol of a randomised controlled trial. BMJ Open, Vol. 6. 10.1136/bmjopen-2015-010350.
9. Jan, Y.K. and B.A. Crane, 2013. Wheelchair tilt-in-space and recline does not reduce sacral skin perfusion as changing from the upright to the tilted and reclined position in people with spinal cord injury. Arch. Phys. Med. Rehabil., 94: 1207-1210.
10. Park, J.S., Inayat Ur Rehman, K. Choe, R. Ahmad, H.J. Lee and M.O. Kim, 2023. A triterpenoid lupeol as an antioxidant and anti-neuroinflammatory agent: Impacts on oxidative stress in Alzheimer's disease. Nutrients, Vol. 15. 10.3390/nu15133059.
11. Beveridge, T.H.J., T.S.C. Li and J.C.G. Drover, 2002. Phytosterol content in American ginseng seed oil. J. Agric. Food Chem., 50: 744-750.
12. Saleem, M., 2009. Lupeol, novel anti-inflammatory and anti-cancer dietary triterpene. Cancer Lett., 285: 109-115.
13. Wang, W.H., H.Y. Chuang, C.H. Chen, W.K. Chen and J.J. Hwang, 2016. Lupeol acetate ameliorates collagen-induced arthritis and osteoclastogenesis of mice through improvement of microenvironment. Biomed. Pharmacother., 79: 231-240.
14. Ravikumar, R., I. Fugaccia, S.W. Scheff, J.W. Geddes, C. Srinivasan and M. Toborek, 2005. Nicotine attenuates morphological deficits in a contusion model of spinal cord injury. J. Neurotrauma, 22: 240-251.
15. Basso, D.M., M.S. Beattie, J.C. Bresnahan, D.K. Anderson and A.I. Faden *et al*., 2009. MASCIS evaluation of open field locomotor scores: Effects of experience and teamwork on reliability. J. Neurotrauma, 13: 343-359.
16. Zhang, T., H. Liu, Z. Liu and L. Wang, 2014. Acupuncture for neurogenic bladder due to spinal cord injury: A systematic review protocol. BMJ Open, Vol. 4. 10.1136/bmjopen-2014- 006249.
17. Yang, R., X. Cai, J. Li, F. Liu and T. Sun, 2019. Protective effects of MiR-129-5p on acute spinal cord injury rats. Med. Sci. Monit., 25: 8281-8288.
18. Cao, B.H., Z.M. Wu and J.W. Liang, 2019. Risk factors for poor prognosis of cervical spinal cord injury with subaxial cervical spine fracture-dislocation after surgical treatment: A CONSORT study. Med. Sci. Monit., 25: 1970-1975.
19. Neirinckx, V., D. Cantinieaux, C. Coste, B. Rogister, R. Franzen and S. Wislet-Gendebien, 2014. Concise review: Spinal cord injuries: How could adult mesenchymal and neural crest stem cells take up the challenge? Stem Cells, 32: 829-843.
20. Curt, A. and P.H. Ellaway, 2012. Clinical Neurophysiology in the Prognosis and Monitoring of Traumatic Spinal Cord Injury. In: Handbook of Clinical Neurology, Verhaagen, J. and J.W. McDonald III (Eds.), Elsevier, Amsterdam, Netherlands, ISBN: 9780444521378, pp: 63-75.
21. Evans, C.T., R.C. Hershow, A. Chin, P.R. Foulis, S.P. Burns and F.M. Weaver, 2009. Bloodstream infections and setting of onset in persons with spinal cord injury and disorder. Spinal Cord, 47: 610-615.
22. Heyninck, K., M. Lahtela-Kakkonen, P. van der Veken, G. Haegeman and W.V. Berghe, 2014. Withaferin A inhibits NF-kappaB activation by targeting cysteine 179 in IKKβ. Biochem. Pharmacol., 91: 501-509.

23. Sakthivel, K.M. and C. Guruvayoorappan, 2013. *Acacia ferruginea* inhibits tumor progression by regulating inflammatory mediators-(TNF-α, iNOS, COX-2, IL-1β, IL-6, IFN-γ, IL-2, GM-CSF) and pro-angiogenic growth factor-VEGF. Asian Pac. J. Cancer Prev., 14: 3909-3919.
24. Yunusova, T., M. Akhtar and V. Poltoratsky, 2014. Analysis of LPS-Induced, NFκB-Dependent Interleukin-8 Transcription in Kidney Embryonic Cell Line Expressing TLR4 Using Luciferase Assay. In: Cytokine Bioassays: Methods and Protocols, Vancurova, I. (Ed.), Humana Press, New York, ISBN: 978-1-4939-0928-5, pp: 305-314.
25. Shifera, A.S., 2010. Protein-protein interactions involving IKKγ (NEMO) that promote the activation of NF-κB. J. Cell. Physiol., 223: 558-561.
26. Oeckinghaus, A. and S. Ghosh, 2009. The NF-κB family of transcription factors and its regulation. Cold Spring Harbor Perspect. Biol., Vol. 1. 10.1101/cshperspect.a000034.
27. Schuliga, M., 2015. NF-kappaB signaling in chronic inflammatory airway disease. Biomolecules, 5: 1266-1283.
28. Cassel, S.L., S. Joly and F.S. Sutterwala, 2009. The NLRP3 inflammasome: A sensor of immune danger signals. Semin. Immunol., 21: 194-198.
29. Butts, B., R.A. Gary, S.B. Dunbar and J. Butler, 2015. The importance of NLRP3 inflammasome in heart failure. J. Card. Fail., 21: 586-593.
30. Sun, Y., Y. Zhao, J. Yao, L. Zhao and Z. Wu *et al*., 2015. Wogonoside protects against dextran sulfate sodium-induced experimental colitis in mice by inhibiting NF-κB and NLRP3 inflammasome activation. Biochem. Pharmacol., 94: 142-154.
31. Zhang, L., Y. Ren, C. Yang, Y. Guo and X. Zhang *et al*., 2014. Wogonoside ameliorates lipopolysaccharide-induced acute lung injury in mice. Inflammation, 37: 2006-2012.