ONLINE FIRST

ISSN: 0015-5659

e-ISSN: 1644-3284

Anti-oxidant, anti-apoptotic and anti-inflammatory effects of geraniin in spinal cord injury in rat: role of COX-2

Authors: Yaping Wang, XinXu Chu, Guangcui Hu, Jianbo Chang

DOI: 10.5603/fm.101292

Article type: Original article

Submitted: 2024-06-24

Accepted: 2024-09-05

Published online: 2024-10-09

Anti-oxidant, anti-apoptotic and anti-inflammatory effects of geraniin in spinal cord injury in rat: role of COX-2

Yaping Wang[1], XinXu Chu[2], Guangcui Hu[3], Jianbo Chang[4]

[1]*Department of Surgical Intensive Care Unit, Yantaishan Hospital of Yantai City, Yantai Shandong, 264000 Yantai, China*

[2]*Department of Critical Care Medicine, People's Hospital Of Lixin County, BoZhou AnHui, 236700 BoZhou, China*

[3]*Department of Electrophysiology, Yantaishan Hospital of Yantai City, Yantai Shandong, 264000 Yantai, China*

[4]*Department of Emergency, Affiliated Dazu's Hospital of Chongqing Medical University, Chongqing, 402360 Chongqing, China*

Address for correspondence: Jianbo Chang, Department of Emergency, Affiliated Dazu's Hospital of Chongqing Medical University, Chongqing, 402360 Chongqing, China; e-mail: changjianbo2024@outlook.com

ABSTRACT

Background: Spinal cord injury (SCI) is devastating diseases affecting the degeneration of the spinal column, and vascular problems. However, the currently available therapeutic interventions are insufficient to address the effect of SCI which leads to significant impact on the morbidity and mortality of patients. In the present manuscript, we intend to investigate the pharmacological effect of geraniin on the SCI in Sprague-Dawley (SD) rats.

Materials and methods: The SCI in rats were induced by the conventional weight-drop method and treated with GER (2.5, 5, and 10 mg/kg). Subsequently, the locomotor activity of rats with SCI was assessed using Basso, Beattie, and Bresnahan (BBB) scores, while oxidative stress indicators and inflammatory variables were analyzed using commercially available kits. Additionally, neuronal death was quantified using TUNEL labeling. The enzymatic activity of caspase 3, 8, and 9 was also assessed. Furthermore, the expression levels of Bcl2, Bax, and COX-2 in rat spinal cords after SCI were analyzed by RT-PCR analysis.

Results: Our research indicated that therapy with GER in a manner that depends on the dosage could enhance the functional recovery, as well as reduce the occurrence of apoptosis, mitigate the inflammatory and oxidative response in rats with SCI. Furthermore, it was observed that GER increased the expression of Bcl2 and decreased the expression of Bax and COX-2. The concentration of caspase-3, -8, and -9 was observed to be decreased in SCI rats treated with GER.

Conclusions: GER might protect the spinal cord from SCI by reducing apoptosis, oxidative stress, and inflammatory response through the inhibition of COX-2.

Keywords: BBB score, oxidative stress, inflammation, apoptosis, caspase

INTRODUCTION

Devastation of the spinal cord that changes its function, either momentarily or permanently, is the hallmark of spinal cord injury (SCI) [13, 28, 32]. This condition is common, costly, and highly disabled-producing. According to mounting research, high-intensity traumas (including those suffered in falls, violent attacks, or traffic accidents) infections, tumors, diseases affecting the degeneration of the spinal column, and vascular problems are among the main causes of spinal cord injuries. Traumatic SCI affects an estimated 759,302 individuals worldwide; in China, 66,374 new cases are reported annually [18].

The impact of spinal cord injuries (SCIs) has been classified by research into primary and secondary events. The former involves changes to the motor, sensory, and autonomic nervous systems [24]. While apoptosis, tissue ischemia, an imbalance of ions homeostasis, and inflammation all result in neurological impairments belonging to the class of secondary event. The existence of axonal demyelination, neuronal death, glial scar formation, and axonal remodeling further identifies the chronic acute secondary damage. Direct and indirect methods are the two primary ones being used to fight SCI. Initially, with the direct approach, surgical procedures are used to lessen compression and relieve painful stress. Indirect interventions are mostly accomplished by pharmaceutical therapies that target different damage pathways and targets, such as the microcirculation. To sum up, a lot of studies have indicated that preventing further injury and its secondary effects is one possible treatment plan for SCI [12].

Geraniin is a polyphenol obtained from the medicinal plant *Phyllanthus amarus* (Fig. 1). Various studies demonstrated that it had antioxidant [29], antiviral [2, 19], anti-inflammatory [26], and neuroprotective properties[33], as well as antithrombotic activity. Geraniin also stimulates the process of osteogenic differentiation in bone marrow affected by osteoporosis

[22, 25]. However, despite the comprehensive pharmacological investigations, there is currently little knowledge regarding its pharmacological effects and molecular mechanism in the context of SCI. Thus, in the present study, we intend to ascertain the pharmacological effect of geraniin in SCI in rats.

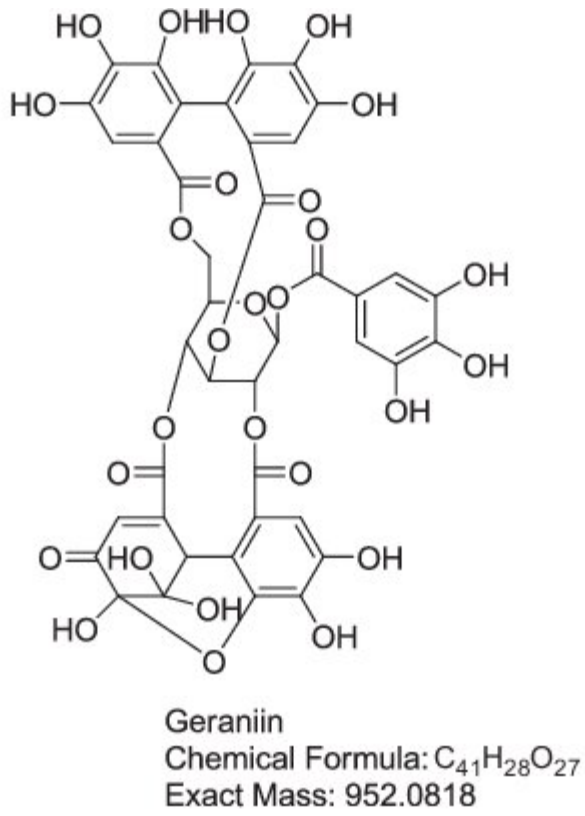

Figure 1. Structure of geraniin.

MATERIALS AND METHODS

Chemicals

Geraniin was obtained from the ChemFaces, China (> 95%; Lot No. CFS202202). All chemicals and reagents unless otherwise stated were procured from Sigma Aldrich (St. Louis, MO, USA).

Animals

Thirty-six mature male Sprague-Dawley rats, weighing between 200 and 250 grams and aged 9 to 11 weeks, were obtained from the institutional animal home. The rats were reared in an appropriate setting with a temperature of 24±3˚C and a 12-hour light/dark cycle. They were

housed in separate cages with a relative humidity of 55-60%. All rats were provided unrestricted access to food and water.

Spinal cord injury

The spinal cord injury (SCI) was deliberately caused in the rats using Allens approach with few alterations. At first, a 2 cm cut was made on the skin directly above the eighth thoracic segment (T8) along the vertebrae. The laminectomy was conducted by excising the spinal lamina of T8. A SCI was generated by dropping a 10 g rod from a distance of 5 cm into the spinal cord of rats. The rats were then allowed to rest for 3 minutes. The rats in the sham treatment group simply underwent laminectomy. Following the surgery, the surgical site was stitched closed, and a penicillin injection of 200,000 units was given intramuscularly for three consecutive days. The compound, after being mixed with a 5% CMC solution, was given to rats once a day for 14 days. The rats were divided into groups and received different doses of the GER (2.5 mg/kg, 5 mg/kg, and 10 mg/kg) through intraperitoneal injection immediately after surgery [4].

Evaluation of motor function

The impact of GER on the motor function of rats was assessed using the Basso, Beattie, and Bresnahan (BBB) locomotor rating scale. The scale ranges from 0 to 21, and measurements were taken on days 1, 4, 7, 10, and 14 after SCI. The impact was assessed by an individual who was unaware of the treatment group, and the average of the three measurements was documented [6, 7].

Biochemical determination

The spinal cord homogenates (1.5 cm length from the injury epicenter) were prepared with cold phosphate-buffered saline using tissue homogenizer and were centrifuged at 4°C and resulting supernatant was used for the measurement of malondialdehyde (MDA) and GSH (glutathione) levels and SOD (superoxide dismutase) activity *via* commercially available kits according to the given protocols.

Enzyme-linked immunosorbent assay (ELISA)

The determination of TNF-α (tumor necrosis factor-α), IL (interleukin)-1β and IL-6 were performed using commercially available ELISA kits as per manufacturer’s instructions.

Terminal dexynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining

The spinal cord sections were stained using a TUNEL apoptosis detection kit (Wuhan Boster Biological Engineering Co., Ltd., Wuhan, China) according to the manufacturer's instruction.

Caspase activity

The activity of cytosolic caspases-3, -8, and -9 was measured in a solution consisting of 50 mM TrisHCl buffer (pH 7.0), 0.5 mM Na-EDTA, 20% glycerol, 300 mg cytosolic protein, and 75 mM of a synthetic fluorogenic substrate that contains the specific sequence recognized by the caspase being tested (Upstate Biotechnology, Lake Placid, NY, USA). The caspase activity was measured using spectrofluorometry at a wavelength of 460 nm, with an excitation wavelength of 380 nm. The measurements were taken at a temperature of 37°C for a duration of 150 seconds for caspase-3 and -8, and 20 minutes for caspase-9.

RT-PCR analysis

The spinal cord homogenate was processed using the Manual TriPure technique (TriPure RNA Minikit, Penzberg, Germany) to extract total RNA. To perform reverse transcription (RT), the RNA was used to create first strand complementary DNA (cDNA) using the RevertAidTm First Strand cDNA Synthesis Kit from Fermentas, following the instructions provided by the manufacturer. Following incubation at room temperature (RT) for 60 minutes, the polymerase chain reaction (PCR) was conducted using a PCR-101 Taq Master Mix (Genet Bioscience, Rostock, Germany) as per the manufacturer’s instructions. The specific primer pairs utilized in this investigation are enumerated in Table 1.

Table 1. Details of primers used in RT-PCR analysis.

Gene	Forward primer	Reverse primer
Bcl-2	5′- GTGGATGACTGAGTACCT -3′	5′- CCAGGAGAAATCAAACAGAG -3′
bax	5′- CTACAGGGTTTCATCCAG -3′	5′- CCAGTTCATCTCCAATTCG -3′
COX-2	5′-CCCCATTAGCAGCCAGTT-3′	5′-CATTCCCCACGGTTTTGA-3′
β-actin	5′-TCGTGCGTGACATCAAAGA-3′	5′-CAT ACC CAA GAA GGA AGG CT-3′

Statistical analysis

Data are recorded as mean ± SEM of three independent experiments, and were statistically analyzed by one-way analysis followed by the by a Tukey's *post hoc* test using statistical software GraphPad Prism 5.0 (San Diego, CA, USA). The P-value < 0.05 was considered as statistically significant.

RESULTS

Effect on motor function

Initially, the impact of geraniin on the motor function of rats with SCI is assessed using the Basso, Beattie, and Bresnahan (BBB) scale at different doses. Figure 2 demonstrates that the rats with SCI experienced a notable decline in motor performance compared to the rats treated with a sham procedure. However, when geraniin was administered, a significant improvement in motor function was found. The observed results followed a dose-dependent pattern, with the rats treated with geraniin at a dosage of 10 mg/kg showing the maximum level of recovery, similar to the sham-treated group.

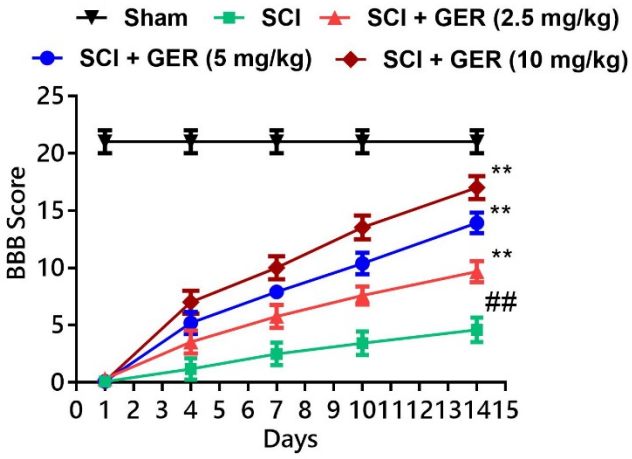

Figure 2. Effect of geraniin on the motor function of rats as illustrated by BBB score. Values represent the mean ± SEM and are representative of three independent experiments. $^{\#\#}P < 0.05$ *vs* sham; $^{**}P < 0.01$ *vs.* SCI, one-way analysis of variance followed by a Tukey's *post-hoc* test. BBB — Basso, Beattie, and Bresnahan; SCI — spinal cord injury.

Effect on oxidative stress biomarkers

The levels of MDA, GSH, and SOD were measured in both the non-SCI Sham and SCI rats, including the group treated with geraniin. Figure 3 demonstrates that the level of MDA was dramatically increased in the rats treated with SCI, whereas the levels of GSH and SOD were

reduced relative to the sham group. Nevertheless, the levels of these biomarkers were observed to be restored close to the levels of the control group after the administration of geraniin, and this restoration was dependent on the dosage.

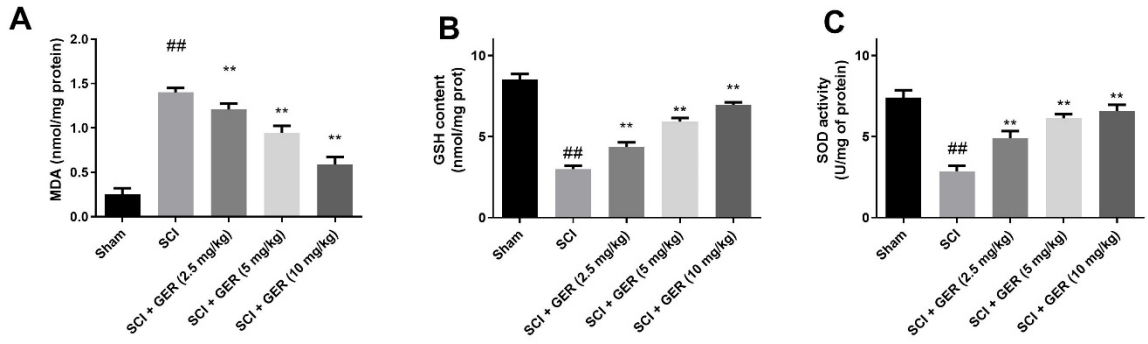

Figure 3. Effect of geraniin on the oxidative stress biomarkers, (**A**) MDA, (**B**) GSH, and (**C**) SOD. Values represent the mean ± SEM and are representative of three independent experiments. $^{\#\#}P < 0.05$ *vs* sham; $^{**}P < 0.01$ *vs.* SCI, one-way analysis of variance followed by a Tukey's *post-hoc* test. GSH — glutathione; MDA — malondialdehyde; SOD — superoxide dismutase.

Effect on pro-inflammatory cytokines

Following that, we proceeded to examine the impact that geraniin had on a number of pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, that were present in the serum of rats that had sustained spinal cord injuries (SCI). Figure 4 demonstrates that the SCI rats exhibited significantly greater levels of the cytokines in comparison to the Sham group. In contrast, the rats who were given geraniin showed a significant decrease in the serum levels of these cytokines when compared to the group that had been subjected to SCI. These results suggest the anti-inflammatory effects of geraniin on SCI rats possibly due to the reduction in the levels of cytokines in the body.

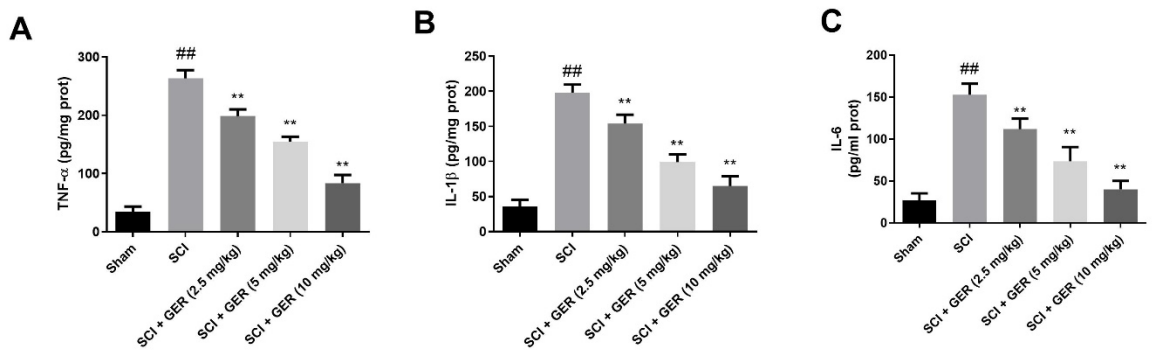

Figure 4. Effect of geraniin on the level of various pro-inflammatory cytokines, (A) TNF-α, (B) IL-1β, and (C) IL-6. Values represent the mean ± SEM and are representative of three independent experiments. $^{\#\#}P < 0.05$ *vs* sham; $^{**}P < 0.01$ *vs.* SCI, one-way analysis of variance followed by a Tukey's *post-hoc* test. SCI — spinal cord injury.

Effect on the neuronal apoptosis

To have a better understanding of the impact that geraniin has on the neuronal apoptosis of SCI rats, flow-cytometry analysis was carried out. A considerable increase in the number of apoptotic cells was seen in the rats that had sustained a SCI, as depicted in Figure 5. However, the administration of geraniin has a significant impact on the number of apoptotic cells, causing a reduction in the number of apoptotic cells that is dose-dependent. After performing a comparative dose analysis, among the treated group, it was shown that the highest activity of geraniin was observed in a dose of 10 mg/kg, followed by a dose of 5 mg/kg, and the group that was treated with 2.5 mg/kg exhibited the least impact on the apoptotic activity.

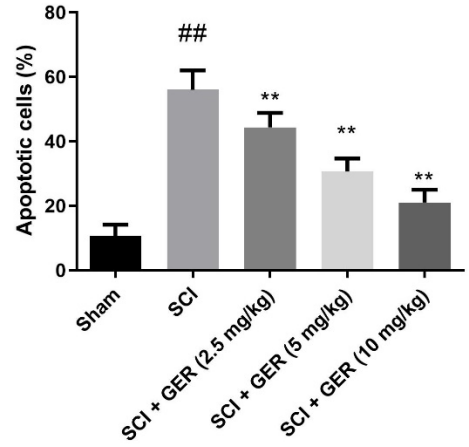

Figure 5. Effect of geraniin on percentage of apoptotic cells. Values represent the mean ± SEM and are representative of three independent experiments. $^{\#\#}P < 0.05$ *vs* sham; $^{**}P < 0.01$ *vs.* SCI, one-way analysis of variance followed by a Tukey's *post-hoc* test. SCI — spinal cord injury.

Effect on the Bcl2 proteins

Figure 6 displays the findings of the RT-PCR investigation that was carried out to examine the impact of geraniin on the genes associated with the Bcl2 family protein. The rats with SCI exhibited increased levels of Bax and lower levels of bcl2 in comparison to the rats in the sham group. Nonetheless, these Bcl2 family proteins were restored to levels comparable to a sham in a dose-dependent manner. The findings imply that *via* regulating the amounts of Bcl2 family proteins, geraniin may have functioned as an antiapoptotic agent.

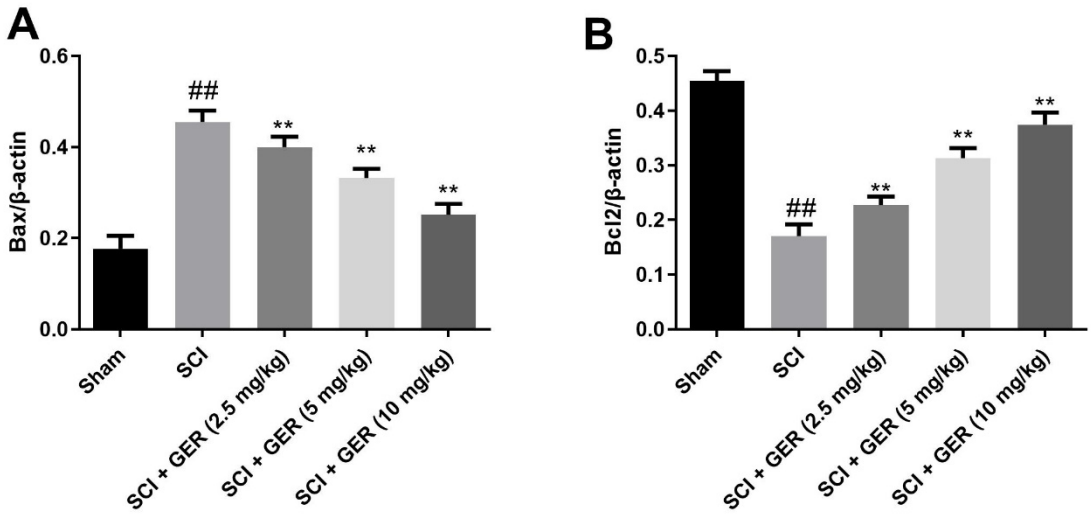

Figure 6. Effect of geraniin on the (**A**) Bax and (**B**) Bcl2. Values represent the mean ± SEM and are representative of three independent experiments. $^{\#\#}P < 0.05$ *vs* sham; $^{**}P < 0.01$ *vs.* SCI, one-way analysis of variance followed by a Tukey's *post-hoc* test. SCI — spinal cord injury.

Effect on various caspases

A study was conducted to determine the impact that geraniin has on the activity of caspase-3, -8, and -9, and the findings are now displayed in Figure 7. The rats in the SCI group had much higher levels of these tested caspases; yet, after geraniin was administered, these levels

were much recovered to those of the sham treated group. The dose-dependent decrease of these caspases activity by geraniin was observed; the 10 mg/kg treated group showed the highest activity.

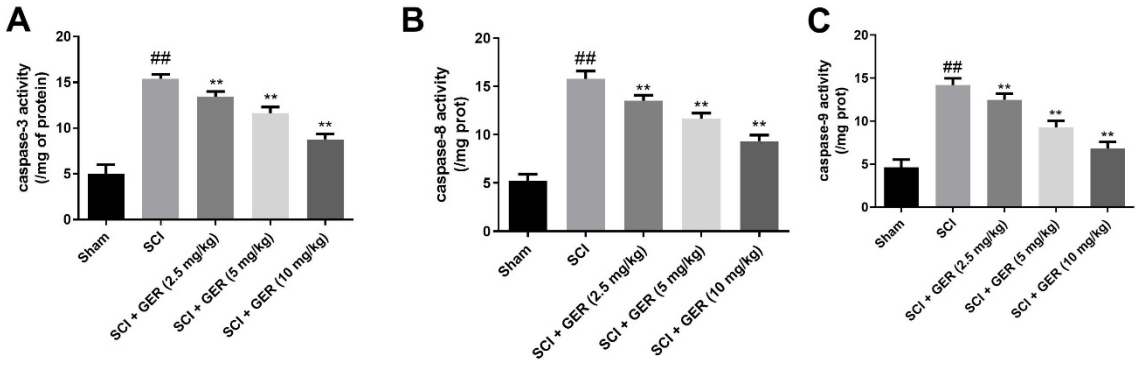

Figure 6. Effect of geraniin on (**A**) caspase-3, (**B**) caspase-8, and (**C**) caspase-9 activity. Values represent the mean ± SEM and are representative of three independent experiments. $^{\#\#}P < 0.05$ *vs* sham; $^{**}P < 0.01$ *vs.* SCI, one-way analysis of variance followed by a Tukey's *post-hoc* test. SCI — spinal cord injury.

Effect on COX-2 expression

A western blot study was conducted to elucidate the effect of geraniin on the COX-2 expression of both treated and non-treated rats. As shown in Fig. 8 geraniin showed marked reduction in the expression of COX-2 which was found elevated in the SCI treated rats. It has been suggested that geraniin might exert anti-inflammatory effect in SCI rats probably via inhibition of COX-2.

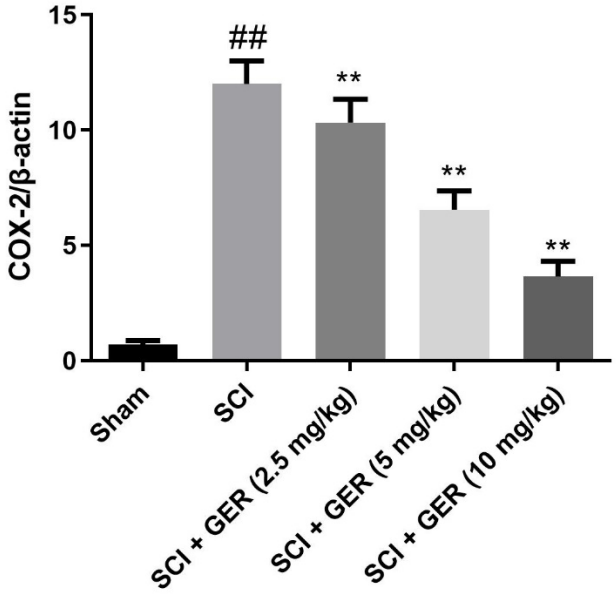

Figure 8. Effect of geraniin on the expression of COX-2. Values represent the mean ± SEM and are representative of three independent experiments. $^{\#\#}P < 0.05$ *vs* sham; $^{**}P < 0.01$ *vs.* SCI, one-way analysis of variance followed by a Tukey's *post-hoc* test. COX-2 — Cyclooxygenase-2; SCI — spinal cord injury.

DISCUSSION

The term "spinal cord injury" describes damage or injury to the spinal cord that results in paralysis and the loss of sensation. Furthermore, the location of the injury may affect the symptoms of a SCI, its etiology is characterized by a primary insult that occurs first, and then a secondary phase of injury in which apoptosis, inflammation, and oxidative stress play major roles [28]. Primary injury, which includes a wound, damage to the blood vessels, and axonal shearing, happens soon after the first impact. On the contrary, secondary injury is a trauma-related indirect result of primary injury. After the initial injury, the secondary injury appears hours, days, or weeks later. This results in harm to neighboring tissue which had not been initially impacted in addition to the site of the original the primary injury. Since secondary injury plays a major role in the development of spinal cord disease, medications mainly prevent SCI by limiting the repercussions of secondary damage. Many naturally occurring compounds have been experimentally shown to offer possible protection against SCI within the last few decades. These medications haven't, however, shown to be totally successful in lessening the effects of SCI [12, 20]. Thus, the purpose of this study was to describe the

pharmacological benefits of geranin for the treatment of SCI and to clarify the underlying mechanism underlying these benefits.

The SCI in rats were established using the previously described method [3], where authors have firstly used a weight drop to injure the animal dorsal spinal cord. This was later classified as widely accepted classical model of experimental spinal cord contusion injury because it greatly mimics the human SCI pathology.

The loss of neurological function is the characteristic feature of SCI injury and therefore, the BBB score was used to examine how geraniin affected the motor function of SCI rats. The locomotor rating scale, or BBB score, is a 21-point metric used to assess the behavioural effects of rat SCI. The scale, which goes from 0 (total paralysis) to 21 (normal mobility), is used to assess how a rat responds to SCI in terms of movement. The measure is capable of distinguishing between hind limb locomotor skills across a broad spectrum of lesion severity [7]. In this work, we discovered that immediately after the surgery, the BBB score of each rat was nearly 0, indicating the successful development of the SCI model. However, after 4 days, the BBB score of every rat was observed to have dramatically increased till the completion of the trial, providing unequivocal evidence of the protective effect of geraniin against SCI.

Over the last two decades, a great deal of research has demonstrated that increased formation of reactive oxygen species (ROS) and the oxidative stress plays an important role in SCI [28]. The central nervous system, which includes the spinal cord, contains neurons and glia that are particularly vulnerable to oxidative and electrophilic stress. This is due to various factors, such as elevated level of polyunsaturated fatty acids, excessive oxidative metabolic activity, persistent production of reactive oxygen metabolites, and a relatively low antioxidant capacity [15]. Particularly in SCI, the oxidative stress induces cytotoxicity via enhancing lipid peroxidation in the injured neuronal tissues. Thus, it is widely recognized as an important hallmark of the secondary injury of SCI, and several empirical studies have shown that compounds with antioxidant properties provide a significant protective effect in animal models by slowing the progression of spinal cord damage [17]. In the current investigation, we quantified the concentrations of MDA, SOD, and GSH in the neural tissues to ascertain the impact of geraniin on oxidative stress. The findings demonstrated that geraniin increases SOD and GSH activity while concurrently lowering MDA levels. It implies that geraniin has strong antioxidant properties, which may be the cause of the rats' protection against SCI. These results were found in accordance with the previous studies where geraniin shows excellent antioxidant activity, such as, against high-fat diet-induced oxidative stress [10], cerebral ischemia/reperfusion injury [33], and carbon tetrachloride induced acute

hepatotoxicity [1]. A series of inflammatory reactions, including microglia activation and peripheral immune cell infiltration, begins after the primary injury. These immune cells release pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, which accelerate injury to tissues and deaths of neurons [34]. It ends up abandoning individuals who have an impairment of both sensory and motor function beyond the degree of injury. Multiple research investigations have revealed a rise in pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNFα) and several of its downstream signaling markers in the lesioned spinal cord after SCI [5, 20]. So as to safeguard the spinal cord against the repercussions of the secondary injury, regulation of these inflammatory cytokines is essential. In the present study, we have shown the geraniin restored the level of TNF-α, IL-1β, and IL-6 in the spinal cord tissues of rats near to non-treated rats.

Apoptosis, also known as programmed cell death, is a crucial step in the secondary injury phase of SCI. After the initial physical injury, apoptosis plays a crucial role in causing additional death of neurons and glial cells, which worsens tissue damage and hinders the process of healing. The regulation of this apoptotic cascade is closely controlled by the equilibrium between pro-apoptotic and anti-apoptotic proteins, with Bcl-2 and Bax playing a crucial role in this regulation. Bcl-2, also known as B-cell lymphoma 2, is a protein that prevents cell death by blocking the release of cytochrome c from the mitochondria. This is an important step in the activation of caspases, which are enzymes responsible for carrying out apoptosis. Bcl-2 preserves cell survival after SCI by maintaining the integrity of mitochondria and blocking the activation of the caspase cascade [23]. On the other hand, Bax (Bcl-2-associated X protein) is a protein that induces apoptosis, which is the process of programmed cell death. After SCI, Bax undergoes a change in its shape and moves to the mitochondria, where it helps in the release of cytochrome c. This release initiates the activation of caspases, resulting in the methodical breakdown of cellular components and ultimately, the demise of the cell [14]. Following SCI, there is typically an increase in the expression of Bax and a decrease in the expression of Bcl-2, which promotes apoptosis. This disparity enhances the process of cellular demise, so amplifying the size of the injury and exacerbating any additional impairments in functionality [30]. The heightened apoptotic activity not only results in the destruction of neurons but also impacts oligodendrocytes, resulting in demyelination and compromised signal conduction. The comprehension of the functions of Bcl-2 and Bax in SCI has substantial therapeutic implications. Potentially, employing strategies to enhance Bcl-2 expression or impede Bax function could decrease apoptosis, safeguard neuronal and glial cell populations, and enhance recovery outcomes.

Manipulating this programmed cell death pathway is a hopeful approach for creating therapies to reduce the harmful consequences of SCI and improve neurological healing [8, 23]. In this investigation, we observed a decrease in the expression of Bcl2 in SCI rats, accompanied by an increase in Bax levels, when compared to non-injured rats. However, the treatment of geraniin resulted in a dramatic restoration of Bcl2 gene levels, bringing them close to those observed in sham rats.

Caspase-3, caspase-8, and caspase-9 play critical roles in apoptosis following SCI. In response to extrinsic cues, caspase-8 can either directly activate caspase-3 or augment the intrinsic pathway by cleaving Bid to tBid. Both of these capabilities are possible. Through the activation of caspase-9 by the release of mitochondrial cytochrome c, the intrinsic route is initiated, which in turn activates caspase-3 [9, 27, 31]. Caspase-3, often known as the executioner caspase, fragments cellular components, which ultimately results in apoptosis. The cascade exacerbates secondary injury, leading to a considerable loss of neuronal and glial cells, which in turn makes the damage to the tissue even more severe and hinders the recovery process. Caspases could be targeted to minimize apoptosis and enhance outcomes following spinal cord injuries [11]. In our present study, we have found aberrantly high concentration of these caspase in SCI rats, which were significantly controlled by the administration of geraniin in a dose-dependent manner. These above results clearly signify the antiapoptotic effect of geraniin against the SCI.

Cyclooxygenase-2 (COX-2) plays a significant role in the inflammatory response following SCI. COX-2 expression is upregulated in response to SCI, leading to increased production of pro-inflammatory prostaglandins [16]. This exacerbates inflammation, contributes to secondary injury, and promotes neuronal and glial cell death. The elevated COX-2 activity also enhances oxidative stress and disrupts the blood-spinal cord barrier, further aggravating tissue damage. Inhibiting COX-2 can reduce inflammation, mitigate secondary damage, and potentially improve functional recovery after SCI [4, 21]. Thus, we have identified the effect of geraniin on the expression of COX-2 using western blot analysis. It has been found that geraniin causes significant downregulation of COX-2 expression in a dose-dependent manner with a maximum activity was achieved in the case of 10 mg/kg treated group.

CONCLUSIONS

The results of our research showed that geraniin has a preventative effect against SCIin rats by acting on multiple targets simultaneously. Moreover, it demonstrated a considerable reduction of apoptosis and oxidative stress. The expression of COX-2 was also shown to be

downregulated, which provided evidence of the inhibition of inflammation. As a result of these findings, the clinical value of geraniin for reducing the effects of secondary injuries caused by spinal cord injuries has been demonstrated.

ARTICLE INFORMATION AND DECLARATIONS

Data availability statement

The datasets used and analyzed during the current study are all provided in the manuscript.

Ethics statement

The study has been approved the Animal Ethical Committee of Affiliated Dazu's Hospital of Chongqing Medical University (Approval No. CMU/2024/Jan/004)

Author contributions

Y.W. performed the experiment, analyzed the data, validation, wrote the first draft of the manuscript; X.C. analyzed the data, wrote the first draft of the manuscript; G. H. analyzed the data, wrote the first draft of the manuscript; J. C.: conceptualization, supervision, methodology, writing and final editing. All author approves the final version of the manuscript.

Funding

None.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Aayadi H, Mittal SPK, Deshpande A, et al. Protective effect of geraniin against carbon tetrachloride induced acute hepatotoxicity in Swiss albino mice. Biochem Biophys Res Commun. 2017; 487(1): 62–67, doi: 10.1016/j.bbrc.2017.04.013, indexed in Pubmed: 28396147.

2. Ahmad SAA, Palanisamy UD, Khoo JJ, et al. Efficacy of geraniin on dengue virus type-2 infected BALB/c mice. Virol J. 2019; 16(1): 26, doi: 10.1186/s12985-019-1127-7, indexed in Pubmed: 30813954.

3. Allen AR. Surgery of experimental lesion of spinal cord equivalent to crush injury of fracture dislocation of spinal column. J Am Med Assoc. 1911; LVII(11): 878–880, doi: 10.1001/jama.1911.04260090100008.

4. An Y, Li J, Liu Y, et al. Neuroprotective effect of novel celecoxib derivatives against spinal cord injury via attenuation of COX-2, oxidative stress, apoptosisand inflammation. Bioorg Chem. 2020; 101: 104044, doi: 10.1016/j.bioorg.2020.104044, indexed in Pubmed: 32629287.

5. Anwar MA, Al Shehabi TS, Eid AH. Inflammogenesis of secondary spinal cord injury. Front Cell Neurosci. 2016; 10: 98, doi: 10.3389/fncel.2016.00098, indexed in Pubmed: 27147970.

6. Barros Filho TE, Molina AE. Analysis of the sensitivity and reproducibility of the Basso, Beattie, Bresnahan (BBB) scale in Wistar rats. Clinics (Sao Paulo). 2008; 63(1): 103–108, doi: 10.1590/s1807-59322008000100018, indexed in Pubmed: 18305873.

7. Basso DM, Fisher LC, Anderson AJ, et al. Basso Mouse Scale for locomotion detects differences in recovery after spinal cord injury in five common mouse strains. J Neurotrauma. 2006; 23(5): 635–659, doi: 10.1089/neu.2006.23.635, indexed in Pubmed: 16689667.

8. Beattie M, FAROOQUI A, BRESNAHAN J. Review of current evidence for apoptosis after spinal cord injury. J Neurotrauma. 2000; 17(10): 915–925, doi: 10.1089/neu.2000.17.915.

9. Brentnall M, Rodriguez-Menocal L, De Guevara RL, et al. Caspase-9, caspase-3 and caspase-7 have distinct roles during intrinsic apoptosis. BMC Cell Biol. 2013; 14: 32, doi: 10.1186/1471-2121-14-32, indexed in Pubmed: 23834359.

10. Chung AP, Gurtu S, Chakravarthi S, et al. Geraniin protects high-fat diet-induced oxidative stress in sprague dawley rats. Front Nutr. 2018; 5: 17, doi: 10.3389/fnut.2018.00017, indexed in Pubmed: 29616223.

11. Cohen G. Caspases: the executioners of apoptosis. Biochem J. 1997; 326(1): 1–16, doi: 10.1042/bj3260001.

12. Delamarter R, Coyle J. Acute management of spinal cord injury. J Am Acad Orthop Surg. 1999; 7(3): 166–175, doi: 10.5435/00124635-199905000-00003.

13. Devivo MJ. Epidemiology of traumatic spinal cord injury: trends and future implications. Spinal Cord. 2012; 50(5): 365–372, doi: 10.1038/sc.2011.178, indexed in Pubmed: 22270188.

14. Emery E, Aldana P, Bunge MB, et al. Apoptosis after traumatic human spinal cord injury. J Neurosurg. 1998; 89(6): 911–920, doi: 10.3171/jns.1998.89.6.0911, indexed in Pubmed: 9833815.

15. Fatima G, Sharma VP, Das SK, et al. Oxidative stress and antioxidative parameters in patients with spinal cord injury: implications in the pathogenesis of disease. Spinal Cord. 2015; 53(1): 3–6, doi: 10.1038/sc.2014.178, indexed in Pubmed: 25366528.

16. Hoffmann C. COX-2 in brain and spinal cord — implications for therapeutic use. Curr Med Chem. 2000; 7(11): 1113–1120, doi: 10.2174/0929867003374282.

17. Jia Z, Zhu H, Li J, et al. Oxidative stress in spinal cord injury and antioxidant-based intervention. Spinal Cord. 2012; 50(4): 264–274, doi: 10.1038/sc.2011.111, indexed in Pubmed: 21987065.

18. Khadour FA, Khadour YA, Meng L, et al. Epidemiological features of traumatic spinal cord injury in Wuhan, China. J Orthop Surg Res. 2023; 18(1): 72, doi: 10.1186/s13018-023-03554-6, indexed in Pubmed: 36717867.

19. Kim YS, Chung HS, Noh SG, et al. Geraniin inhibits the entry of SARS-CoV-2 by blocking the interaction between spike protein RBD and human ACE2 receptor. Int J Mol Sci. 2021; 22(16), doi: 10.3390/ijms22168604, indexed in Pubmed: 34445310.

20. Leal-Filho M. Spinal cord injury: from inflammation to glial scar. Surg Neurol Int. 2011; 2(1): 112, doi: 10.4103/2152-7806.83732.

21. Lee KM, Kang BS, Lee HL, et al. Spinal NF-kB activation induces COX-2 upregulation and contributes to inflammatory pain hypersensitivity. Eur J Neurosci.

2004; 19(12): 3375–3381, doi: 10.1111/j.0953-816X.2004.03441.x, indexed in Pubmed: 15217394.

22. Li K, Zhang X, He Bo, et al. Geraniin promotes osteoblast proliferation and differentiation via the activation of Wnt/β-catenin pathway. Biomed Pharmacother. 2018; 99: 319–324, doi: 10.1016/j.biopha.2018.01.040, indexed in Pubmed: 29353207.

23. Liu XZ, Xu XM, Hu R, et al. Neuronal and glial apoptosis after traumatic spinal cord injury. J Neurosci. 1997; 17(14): 5395–5406, doi: 10.1523/JNEUROSCI.17-14-05395.1997, indexed in Pubmed: 9204923.

24. McDonald JW, Sadowsky C. Spinal-cord injury. Lancet. 2002; 359(9304): 417–425, doi: 10.1016/S0140-6736(02)07603-1, indexed in Pubmed: 11844532.

25. Mo J, Yang R, Li F, et al. Geraniin promotes osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) via activating β-catenin: a comparative study between BMSCs from normal and osteoporotic rats. J Nat Med. 2018; 73(1): 262–272, doi: 10.1007/s11418-018-1242-6.

26. Nam H, Nan Li, Park J, et al. Geraniin ameliorate experimental acute reflux esophagitis via NF-κB regulated anti-inflammatory activities in rats. Appl Biol Chem. 2019; 62(1), doi: 10.1186/s13765-019-0412-x.

27. Porter AG, Jänicke R. Emerging roles of caspase-3 in apoptosis. Cell Death Differ. 1999; 6(2): 99–104, doi: 10.1038/sj.cdd.4400476.

28. Rossignol S, Schwab M, Schwartz M, et al. Spinal cord injury: time to move? J Neurosci. 2007; 27(44): 11782–11792, doi: 10.1523/JNEUROSCI.3444-07.2007, indexed in Pubmed: 17978014.

29. Thitilertdecha N, Chaiwut P, Saewan N. In vitro antioxidant potential of Nephelium lappaceum L. rind extracts and geraniin on human epidermal keratinocytes. Biocatalysis and Agricultural Biotechnology. 2020; 23: 101482, doi: 10.1016/j.bcab.2019.101482.

30. Wang S, Cheng L. The role of apoptosis in spinal cord injury: a bibliometric analysis from 1994 to 2023. Front Cell Neurosci. 2023; 17: 1334092, doi: 10.3389/fncel.2023.1334092, indexed in Pubmed: 38293650.

31. Wolf BB, Schuler M, Echeverri F, et al. Caspase-3 is the primary activator of apoptotic DNA fragmentation via DNA fragmentation factor-45/inhibitor of caspase-activated DNase inactivation. J Biol Chem. 1999; 274(43): 30651–30656, doi: 10.1074/jbc.274.43.30651, indexed in Pubmed: 10521451.

32. Wulf MJ, Tom V. Consequences of spinal cord injury on the sympathetic nervous system. Front Cell Neurosci. 2023; 17: 999253, doi: 10.3389/fncel.2023.999253.

33. Yang Y, He Bo, Zhang X, et al. Geraniin protects against cerebral ischemia/reperfusion injury by suppressing oxidative stress and neuronal apoptosis via regulation of the nrf2/ho-1 pathway. Oxid Med Cell Longev. 2022; 2022: 2152746, doi: 10.1155/2022/2152746, indexed in Pubmed: 35222793.

34. Zhang YK, Liu JT, Peng ZW, et al. Different TLR4 expression and microglia/macrophage activation induced by hemorrhage in the rat spinal cord after compressive injury. J Neuroinflammation. 2013; 10: 112, doi: 10.1186/1742-2094-10-112, indexed in Pubmed: 24015844.