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Trigonelline exerts its neuroprotective effects in experimental spinal cord injury through modulation of inflammation, apoptosis, and neurotrophic factors

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ABSTRACT

Objective: To assess the protective effects of trigonelline against spinal cord injury (SCI) in rats.

Methods: Rats (Sprague-Dawley, male) were randomly assigned to seven groups (n=15 per group): normal, sham, SCI control (1% DMSO), methylprednisolone (30 mg/kg), and trigonelline (50, 100, and 200 mg/kg). Rats received respective treatment daily for 28 days. SCI was induced by using a temporary aneurysm clip. Behavioral, biochemical, and histological analyses were performed to investigate the neuroprotective effect of trigonelline.

Results: Trigonelline (100 and 200 mg/kg) treatment effectively (P<0.05) mitigated SCI-induced changes in mechano-tactile sensation, allodynia, hyperalgesia, and motor nerve conduction velocity. It notably (P<0.05) downregulated apoptotic (Bax and caspase-3) and inflammatory (COX-Ⅱ) markers, while upregulating *Bcl-2* and *BDNF* mRNA expression in the spinal cord (P<0.05). Furthermore, trigonelline effectively alleviated (P<0.05) SCI-induced alterations in mitochondrial complex levels, resulting in enhanced nicotinamide adenine dinucleotide dehydrogenase, succinate dehydrogenase, redox activity, and cytochrome-C levels. Histological examination of spinal cord tissue indicated that trigonelline significantly (P<0.05) ameliorated the histological damage caused by SCI, thereby improving neuronal degeneration, inflammatory cell infiltration, and necrosis.

Conclusions: Trigonelline shows neuroprotective properties in SCI rats by reducing allodynia, hyperalgesia, and inflammation, stabilizing mitochondrial enzyme complexes, and modulating apoptotic and neurotrophic factors. Thus, trigonelline holds promise as a potential neuroprotective agent.

KEYWORDS: Bax; Bcl-2; BDNF; COX-Ⅱ; Spinal cord injury; Trigonelline

1. Introduction

Individuals with spinal cord injury (SCI) may experience a reduced life expectancy, contributing to more than 4.5 million disability-adjusted life years globally[1]. This injury significantly reduces quality of life and imposes substantial financial burdens on healthcare systems and affected individuals[2]. During the acute and chronic phases of SCI, researchers have noted a sequence of harmful reactions, including oxidative stress, inflammatory reactions, apoptosis, ischemia, and impaired locomotor function[3]. Additionally, SCI can result in pain, loss of sensation, multiple organ dysfunction, including issues with the urinary and respiratory tracts, and neurological and skeletal problems[4]. SCI can result

Summary

Question: Does trigonelline show protective effects against spinal cord injury in rats?

Findings: Trigonelline exhibits the neuroprotective effects against spinal cord injury by alleviating inflammation and histological damages, and modulating apoptotic and neurotrophic factors.

Meaning: Trigonelline can be used as a promising therapeutic agent for spinal cord injury.

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from traumatic incidents, such as spinal fractures, and non-traumatic causes, including infections and damage to blood vessels[5].

SCI contributes significantly to global morbidity and mortality with approximately 15.4 million people living with SCI by 2021. The incidence rate of SCI is 23.77 per million people[6]. In the Chinese population, the incidence of SCI significantly increased by 138.32% between 1990 and 2019, with the number of cases increasing from 2.14 million to 5.10 million. Consequently, the age-adjusted prevalence increased from 0.20% to 0.27% over the same time frame[7]. The growing incidence and prevalence of SCI have severely influenced the socioeconomic and financial burden of patients, contributing to rehabilitation treatments, costly personal support, and diminished productivity due to impairment and social isolation. The healthcare costs for patients with SCI vary significantly across different countries. In China, the direct costs range from $1.1 million to $4.7 million per patient over their lifetime[8].

SCI pathogenesis consists of two phases. Initial mechanical trauma, such as compression, contusion, or laceration, causes immediate damage to neurons, glial cells, and blood vessels[3]. This results in axonal damage, hemorrhage, and ischemia. After the primary injury, a series of pathological processes in secondary injury and a series of pathological processes further contribute to tissue damage, including inflammatory infiltration at the injury site and the release of pro-inflammatory cytokines such as interleukins (ILs) and tumor necrosis factor-α, which exacerbate tissue damage[9]. Furthermore, the production of reactive oxygen species (ROS) leads to lipid peroxidation, protein denaturation, and DNA damage, which further injuries neurons. Thus, SCI poses significant challenges for modern healthcare because of its complex pathophysiology and lifelong consequences. SCI is also associated with long-term complications, such as neurogenic shock, respiratory complications, autonomic dysreflexia, chronic pain, and pressure ulcers[5]. Therefore, management of SCI is important for the improvement of SCI during the initial phase of injury and for improving the long-term outcomes in patients with SCI.

Although certain treatment methods, such as cell therapy, have shown beneficial clinical effects, no definitive cure exists for SCI. The high cost of treatment, prolonged recovery periods, and loss of labor force significantly impact individuals and families, imposing a heavy burden on society[4]. The surgical treatment of SCI focuses on decompressing the spinal cord and restoring stability. However, nonsurgical treatment is equally important. Research supports the early application of steroids (methylprednisolone) within 6-8 hours of injury to reduce spinal cord edema and prevent further damage[10]. However, these treatment approaches can only alleviate the symptoms of SCI, it is important to prevent further injury and multiple side effects that occur from steroidal intervention, including immune system suppression, gastrointestinal tract ulceration, impaired wound healing, and respiratory and urinary tract problems. Therefore, exploring new therapies and interventions with complementary and alternative medicines to manage SCI is necessary to achieve promising efficacy and fewer side effects in individuals.

Trigonelline, a natural alkaloid, is extensively distributed in the seeds of *Trigonella foenum-graecum* (fenugreek)[11]. Trigonelline has demonstrated the ability to mitigate cellular damage across various organ systems, including the cardiovascular system, gastrointestinal system, kidney, liver, and skin, aiding in neurological recovery (*e.g.*, neurodegeneration, ischemia, cognitive decline), and promotion of diabetic foot ulcer healing by activating angiogenesis and growth factors[12–14]. Although trigonelline shows promise in alleviating various neurological disorders, its pharmacological effects on SCI have not yet been investigated. This study aimed to assess the neuroprotective effects of trigonelline in a surgical aneurysm clip-induced-SCI rat model through behavioral, biochemical, and histological assessments.

2. Materials and methods

2.1. Animals

Rats (male, Sprague-Dawley, 180-220 g, obtained from Jingdezhen Hospital of Traditional Chinese Medicine) were kept under the following laboratory conditions: temperature (24±1)℃, normal light and dark cycle, relative humidity (45%-55%), and free access to feed and water.

2.2. Ethical statement

The experimental protocol was approved by Kangping Experimental Animal Research Institute (approval no. 20240000474S) to ensure compliance with the ethical guidelines of the National Institute of Health for the use of experimental animals.

2.3. Experimental groups

Induction of SCI was performed according to the previously reported method using a temporary application of an aneurysm clip on the spinal cord at T10 for 60 s in anesthetized rats (thiopental sodium, 40 mg/kg, *i.p.*)[15]. After a full recovery from anesthesia, rats were randomly divided into various groups (n=15 in each group) as follows: SCI control receiving DMSO (10 g/kg, 1%), methylprednisolone (30 mg/kg) and trigonelline (50, 100 and 200 mg/kg; purity: ≥ 98%; Sigma-Aldrich, St. Louis, MO, USA). Another separate group of animals (n=15, in each group) was also maintained, including normal [did not induce spinal cord injury and received DMSO (10 g/kg, 1%)] and sham [the spinal cord was exposed but did not injure and received DMSO (10 g/kg, 1%)]. The dosages of trigonelline and methylprednisolone were selected based

on previous studies[14]. The rats received oral treatment for four weeks, and various behavioral parameters were recorded 2 d before treatment and 0, 7, 14, 21, and 28 d after treatment.

2.4. Assessment of behavioral parameters

Mechano-tactile allodynia (Von Frey hair apparatus, IITC, Woodland Hills, USA) and mechanical hyperalgesia (Randall-Selitto paw pressure apparatus) were assessed according to previously reported methods[16]. Radiant heat hyperalgesia of the left hind paw and tail was assessed using a radiant heat lamp source as described previously[16]. A rota-Rod apparatus (25 rpm) was used to evaluate motor incoordination[16]. Motor nerve conduction velocity (MNCV) was recorded by stimulating the sciatic nerves (AD Instrument Pvt. Ltd., LabChart 7.3, Australia) according to a previously reported method[16]. Body weight (using an animal-weighing balance), water intake, food intake, and urine output were determined using a metabolic cage (Techniplast, Italy). The sperm count was determined according to a previously reported method[16].

2.5. Assessment of biochemical parameters

Urine protein levels were measured with a spectrophotometer (UV-visible spectrophotometer, Jasco V-530, Japan) using reagent kits according to the procedure provided by the manufacturer's instructions (Accurex Biomedical Pvt. Ltd., Mumbai, India). On the last day (29th day), all animals were sacrificed, and isolated spinal cord dorsal horn was maintained at –80 ℃. Other organs, including the seminal vesicle, testis, urinary bladder, prostate gland, epididymis, and kidney, were also isolated and weighed using a balance. One part of the spinal cord (n=6) was homogenized to estimate the activities of nicotinamide adenine dinucleotide dehydrogenase (NADH; mitochondrial Complex Ⅰ), succinate dehydrogenase (SDH; Complex Ⅱ), mitochondrial redox activity (MTT assay), and cytochrome oxidase assay (Complex Ⅳ) as per method reported previously[17]. Another portion of the spinal cord tissue (n=6) was used to estimate mRNA expressions of *Bax* (forward: 3'-GGGAATTCTGGAGCTGCAGAGGATGATT-5', reverse: 3'-GCGGATCCAAGTTGCCATCAGCAAACAT-5', base pair: 96), *Bcl-2* (forward: 3'-CTGTACGGCCCCAGCATGGCG-5', reverse: 3'-GCTTTGTTTCATGGTACATC-5', base pair: 213), *caspase-3* (forward: 3'-CTCGGTCTGGTACAGATGTCGATG-5', reverse: 3'-GGTTAACCCGGGTAAGAATGTGCA-5', base pair: 238), *COX-Ⅱ* (forward: 3'-ACAACATTCCTTCCTTC-5', reverse: 3'-CCTTATTTCCTTTCACACC-5', base pair: 253), and *BDNF* (forward: 3'-TCCCTGGCTGACACTTTTGAG-5', reverse: 3'-ATTGGGTAGTTCGGCATTGCG-5', base pair: 348) in spinal cord using quantitative reverse transcription-polymerase chain reaction (MP Biomedicals India Private Limited, India) according to the method described elsewhere[15]. *β-actin* was used as a reference standard (forward: 3'-GTCACCCACACTGTGCCCATCT-5', reverse: 3'-ACAGAGTACTTGCGCTCAGGAG-5', base pair: 764).

2.6. Assessment of histological parameters

Spinal cord tissue samples (n=3 from each group) were examined microscopically by H&E staining. The analysis was conducted using a light microscope (Nikon E200, Japan) at 40× magnification to evaluate histopathological changes. Histological observations (such as necrosis, neuronal degeneration, and inflammatory infiltration) were scored (0-4) as previously reported[14].

2.7. Statistical analysis

Results are presented as mean±standard error of the mean (SEM). Statistical analyses were conducted using the GraphPad Prism software (San Diego, CA, USA). For behavioral test data, two-way repeated analysis of variance (ANOVA) with Bonferroni's *post-hoc* test was employed. Other data were evaluated using one-way ANOVA followed by Tukey's multiple range test or Kruskal-Wallis test for *post-hoc* comparisons. Statistical significance was established at P<0.05.

3. Results

3.1. Body weight, intake, urinary volume, and protein

Compared with the normal and sham groups, body weight, food, and water intake were decreased (P<0.05) while urinary volume and protein levels were increased (P<0.05) in the SCI control group. Rats treated with trigonelline (100 and 200 mg/kg) showed significant improvements in body weight, food and water intake, urinary volume, and protein levels (P<0.05) (Table 1).

3.2. Sperm count and weight of seminal vesicle, testis, urinary bladder, prostate gland, epididymis and kidney

SCI control rats showed significant decreases in the weights of the seminal vesicle, testis, prostate gland, epididymis, and kidney and sperm counts, along with a significant increase in the weight of the urinary bladder, compared with sham and normal rats. Treatment with trigonelline (100 and 200 mg/kg) significantly (P<0.05) restored the weights of the seminal vesicle, testis, urinary bladder, prostate gland, epididymis, and kidney, as well as sperm counts (Table 1).

3.3. Mechano-tactile allodynia and thermal hyperalgesia

SCI control rats showed reduced allodynia and hyperalgesia,

Table 1. Effects of trigonelline on body weight, food and water intake, urine output, testis, seminal vesicle, prostate gland, urinary bladder, epididymis, kidney weight, sperm count, and urine protein levels in rats (mean±SEM, n= 6).

Parameters	Normal	Sham	SCI control	MP30	T50	T100	T200
Body weight (g)	228.20±0.48	226.50±1.93	166.20±2.60$^{\#,\&}$	218.30±1.28*	175.80±1.83	191.20±1.96*	212.30±1.76*
Food intake (g)	24.17±0.54	21.83±0.70	11.33±0.61$^{\#,\&}$	20.33±0.92*	12.33±0.67	17.33±0.56*	20.17±0.60*
Water intake (mL)	42.33±0.88	39.00±1.00	23.33±1.12$^{\#,\&}$	38.67±1.12*	23.00±0.82	30.33±0.99*	34.83±0.87*
Volume of urine expressed in 24 h (mL)	1.50±0.22	1.33±0.21	5.67±0.21$^{\#,\&}$	2.83±0.17*	5.00±0.26	3.50±0.22*	2.50±0.22*
Testis weight (g)	2.70±0.05	2.40±0.09	1.04±0.13$^{\#,\&}$	2.07±0.09*	1.15±0.09	1.48±0.06*	1.76±0.08*
Seminal vesicle weight (g)	2.71±0.09	2.28±0.12	0.91±0.11$^{\#,\&}$	2.18±0.10*	1.29±0.12	1.68±0.11*	1.97±0.13*
Prostate gland weight (g)	0.51±0.01	0.47±0.01	0.25±0.01$^{\#,\&}$	0.41±0.01*	0.29±0.01	0.34±0.01*	0.41±0.01*
Urinary bladder weight (g)	0.39±0.01	0.48±0.02	1.22±0.01$^{\#,\&}$	0.58±0.03*	1.10±0.02	0.90±0.02*	0.67±0.02*
Epididymis weight (g)	2.66±0.08	2.53±0.07	1.24±0.05$^{\#,\&}$	2.28±0.06*	1.37±0.05	1.93±0.08*	2.30±0.07*
Kidney weight (g)	133.00±3.77	121.20±4.81	99.00±3.31$^{\#,\&}$	117.70±3.04*	102.20±2.40	116.50±4.14*	122.30±4.36*
Sperm count (million/mL)	54.00±2.81	47.00±3.52	24.83±3.08$^{\#,\&}$	46.00±2.49*	29.83±2.34	39.17±2.63*	41.67±1.20*
Urine protein (mg/mL)	1.55±0.24	2.21±0.15	7.09±0.26$^{\#,\&}$	3.20±0.16*	6.34±0.25	4.56±0.23*	3.43±0.10*

One-way ANOVA was used, followed by Tukey's multiple range test. $^{\#}P$<0.05 compared to the normal group, $^{\&}P$<0.05 compared to the sham group, $^{*}P$<0.05 compared to the SCI group. MP30: methylprednisolone 30 mg/kg; N: normal; S: sham; SCI: spinal cord injury; T50: trigonelline 50 mg/kg, T100: trigonelline 100 mg/kg, T200: trigonelline 200 mg/kg.

Table 2. Effect of trigonelline on mitochondrial complex levels in rats (mean±SEM, n= 6).

Parameters	Normal	Sham	SCI Control	MP30	T50	T100	T200
Complex Ⅰ (nmole of NADH oxidized/min/mg protein)	28.40±1.55	22.66±1.19	10.12±0.81$^{\#,\&}$	22.36±1.54*	9.30±1.16	14.55±1.31*	24.43±1.47*
Complex Ⅱ (nmole of SDH/mg protein)	61.69±2.48	58.23±2.08	23.69±3.11$^{\#,\&}$	54.32±1.72*	26.81±2.22	38.88±2.06*	55.86±2.37*
MTT assay (OD at 540 nm)	0.49±0.02	0.36±0.02	0.16±0.03$^{\#,\&}$	0.42±0.03*	0.22±0.02	0.28±0.02*	0.37±0.02*
Complex-Ⅳ (nmol cyto-C oxidized/min/mg protein)	5850.0±293.6	5400.0±258.2	2942.0±287.8$^{\#,\&}$	4731.0±168.6*	3300.0±111.5	4052.0±255.2*	4678.0±160.1*

One-way ANOVA was used, followed by Tukey's multiple range test. $^{\#}P$<0.05 compared to the normal group, $^{\&}P$<0.05 compared to the sham group, $^{*}P$<0.05 compared to the SCI group. SDH: succinate dehydrogenase; NADH: reduced form of nicotinamide-adenine dinucleotid; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; OD: optical density.

as evidenced by a significant (P<0.05) reduction in paw and tail withdrawal latency and threshold compared with sham and normal rats. Trigonelline treatment (100 and 200 mg/kg) significantly (P<0.05) elevated withdrawal latency and threshold in both paw and tail (Figure 1A-D).

3.4. Motor coordination

SCI induction resulted in a significant (P<0.05) decrease in the duration of the fall-off time compared with the sham and normal rats. Rats treated with trigonelline (100 and 200 mg/kg) showed significant (P<0.05) improvement in motor coordination (Figure 1E).

3.5. Nerve conduction velocities

SCI control rats exhibited a significant (P<0.05) reduction in MNCV compared with sham and normal rats. A significant improvement in MNCV was noted in rats treated with trigonelline (100 and 200 mg/kg) (P<0.05) (Figure 1F).

3.6. Mitochondrial complex levels

Table 2 indicates that SCI control rats exhibited significant reductions in mitochondrial enzyme complexes (NADH, SDH, redox, and oxidized cytochrome-C) activities (P<0.05) compared with sham and normal rats. Trigonelline (100 and 200 mg/kg) significantly increased the levels of mitochondrial enzyme complexes (P<0.05).

3.7. Bcl–2, Bax, and caspase–3 mRNA expressions

SCI control rats showed significant downregulation of *Bcl–2* mRNA and (P<0.05) upregulation of *caspase–3* and *Bax* mRNA expression (P<0.05) compared with sham and normal rats. Trigonelline treatment (100 and 200 mg/kg) significantly downregulated spinal *caspase–3* and *Bax* mRNA expression and upregulated *Bcl–2* mRNA expression(P<0.05) (Figure 2A-D).

3.8. COX–Ⅱ and BDNF mRNA expressions

SCI control rats showed a significant upregulation of spinal *COX–Ⅱ* and downregulation of spinal *BDNF* mRNA expression compared with sham and normal rats (P<0.05). Administration of trigonelline (100 and 200 mg/kg) significantly downregulated *COX–Ⅱ* and upregulated *BDNF* mRNA expression compared with SCI control (P<0.05) (Figure 2E and F).

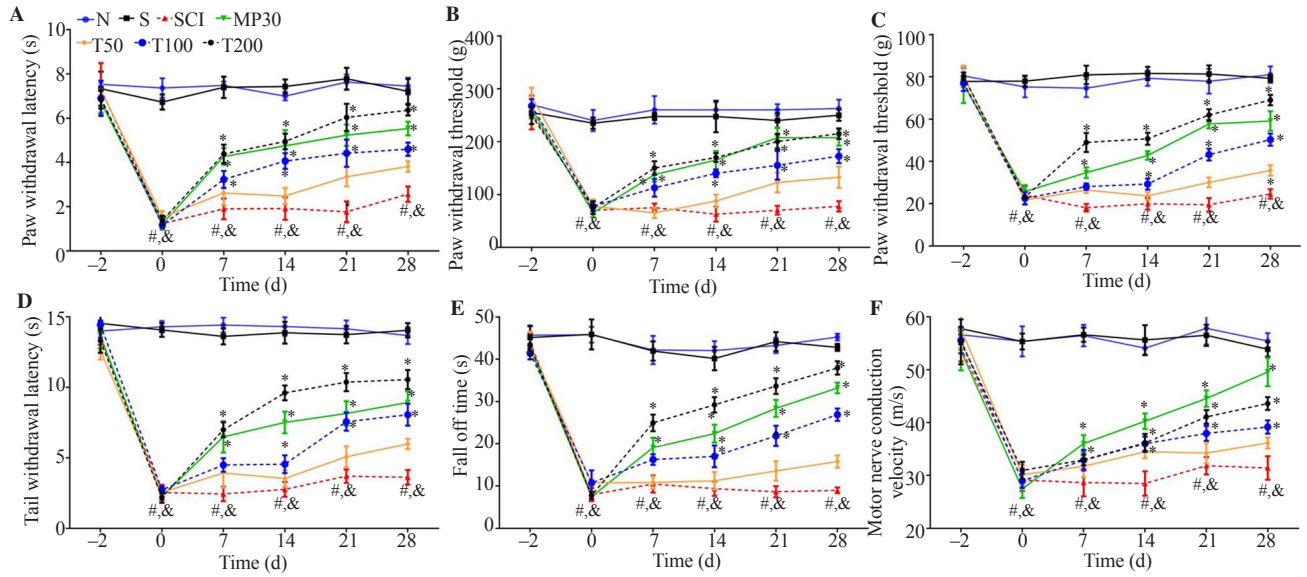


Figure 1. Effect of trigonelline on the plantar test (A), paw pressure test (B), von-Frey hair test (C), tail immersion test (D), grip strength test (E) and motor nerve conduction velocity (F). Data are expressed as mean±SEM (n=6) and were analyzed using two-way ANOVA, followed by Bonferroni's multiple range test for *post-hoc* comparisons. $^{\#}P$<0.05 compared to the normal group, $^{\&}P$<0.05 compared to the sham group, $^{*}P$<0.05 compared to the SCI group. MP30: methylprednisolone 30 mg/kg; N: normal; S: sham; SCI: Spinal cord injury; T50: trigonelline 50 mg/kg, T100: trigonelline 100 mg/kg, T200: trigonelline 200 mg/kg.

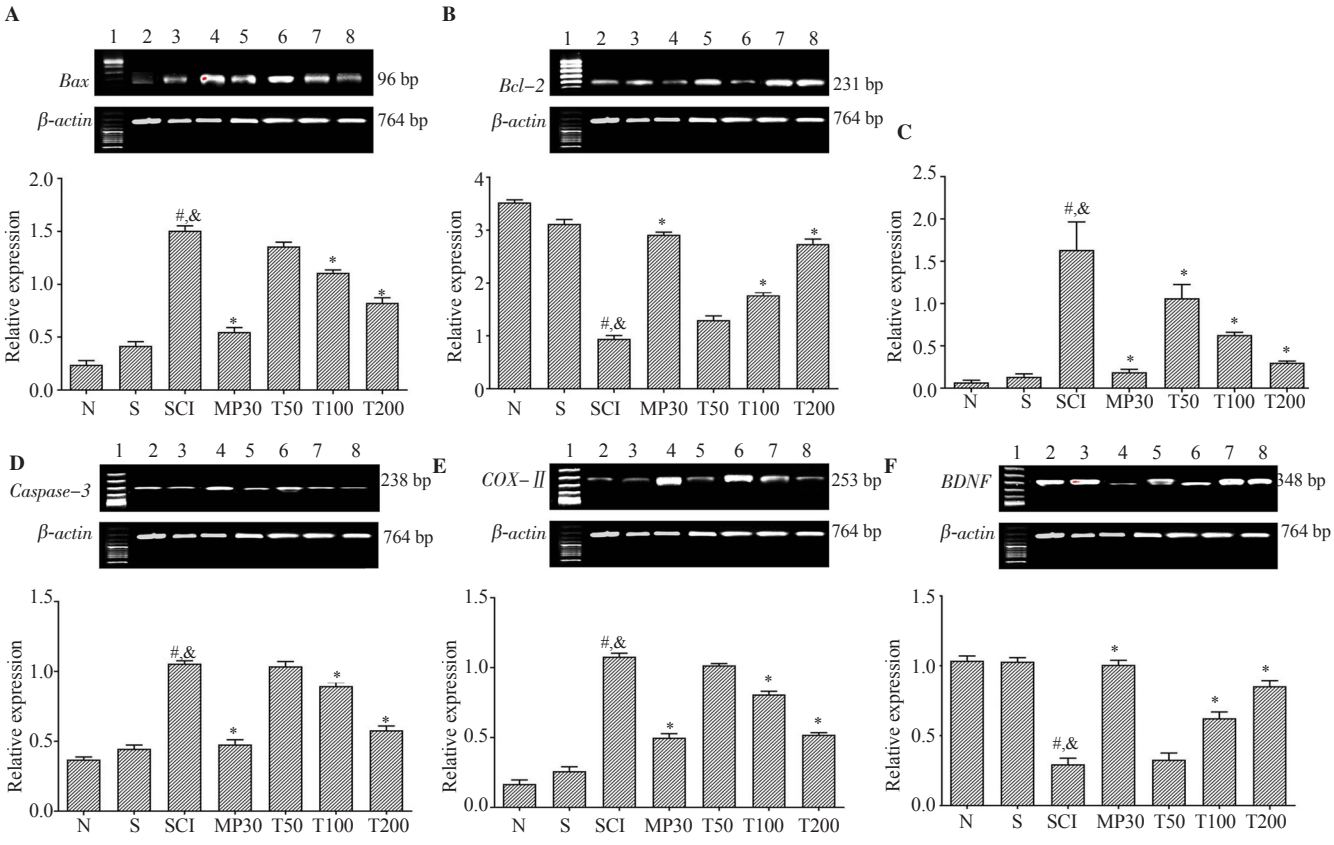


Figure 2. Effect of trigonelline on SCI-induced alterations in mRNA expression of *Bax* (A), *Bcl-2* (B), *Bax*: *Bcl-2* ratio (C), *caspase-3* (D), *COX-Ⅱ* (E), and *BDNF* (F) in the spinal cord of rats. Data are expressed as mean±SEM (n=6) and were analyzed using one-way ANOVA, followed by Tukey's multiple range test for *post-hoc* comparisons. $^{\#}P$<0.05 compared to the normal group, $^{\&}P$<0.05 compared to the sham group, $^{*}P$<0.05 compared to the SCI group. Bax: Bcl2 associated X; Bcl2: B-cell lymphoma-2; BDNF: brain-derived neurotrophic factor. 1, marker; 2, N; 3, S; 4, SCI; 5, MP30; 6, T50; 7, T100; 8, T200.

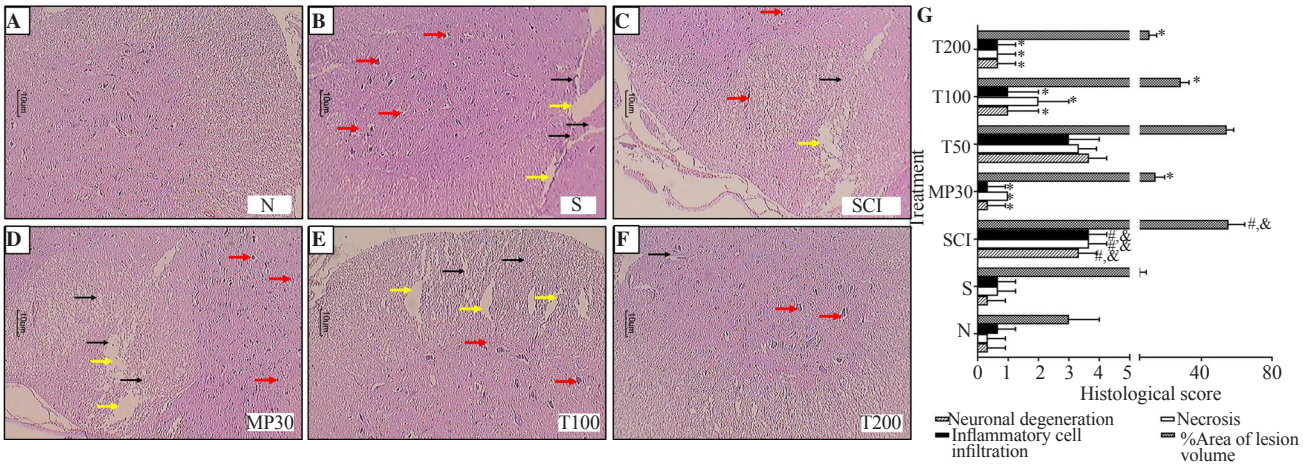


Figure 3. Effect of trigonelline on SCI-induced changes in spinal cord histology. Spinal cord microscopic imaging of normal (A), sham (B), SCI control (C), methylprednisolone (30 mg/kg) (D), trigonelline (100 mg/kg) (E), and trigonelline (200 mg/kg) (F)-treated rats stained with H&E (40×). Quantitative representation of spinal cord histology (G). Data are expressed as mean±SEM (n=3), and one-way ANOVA followed by the Kruskal-Wallis test was applied for for *post–hoc* comparisons. $^{\#}P$<0.05 compared to the normal group, $^{\&}P$<0.05 compared to the sham group, $^{*}P$<0.05 compared to the SCI group. Neuronal loss or degeneration (black arrow), inflammatory cell infiltration (red arrow) and prominent necrosis (yellow arrow).

3.9. Histology of spinal cord

SCI control rats showed significant neuronal loss, inflammatory cell infiltration, and prominent necrosis with a significant increase in lesion volume compared with sham and normal rats (P<0.05). Treatment with trigonelline (100 and 200 mg/kg) effectively (P<0.05) suppressed the neurodegeneration process involving necrosis, inflammatory cell infiltration, and percentage of lesions induced by SCI (Figure 3).

4. Discussion

Damage to the spinal cord is a common form of central nervous system trauma that significantly disrupts motor, sensory, and autonomic function. In SCI, the pathophysiology involves two phases: the primary phase, caused by direct mechanical trauma, and the secondary phase, caused by further damage to neuronal tissue. The destructive course of SCI is characterized by inflammation and neurological impairment. Oxidative stress mediated by higher levels of ROS and an imbalance in their clearance contribute considerably to secondary damage following SCI[18]. Preventing extensive cellular death resulting from secondary injury is crucial for maintaining neurological function in the damaged spinal cord[18]. In the present study, trigonelline exerted neuroprotective effects by ameliorating painful mechanical allodynia and hyperalgesia and enhancing motor nerve conduction velocity in experimental SCI rats. Furthermore, trigonelline improved spinal mitochondrial enzyme activity and neurotrophic factor (BDNF), and attenuated apoptosis (Bax and caspase-3) and inflammation (COX-Ⅱ).

SCI can cause central neuropathic pain, which is usually localized at or below the damaged site and manifests as allodynia, hyperalgesia, and motor and sensory impairments[19]. Laboratory experiments frequently use behavioral tests to assess neurological function, making behavioral examinations fundamental after any therapeutic intervention for SCI[20]. Allodynia and hyperalgesia, the key symptoms of neuropathic pain, are classified by sensory modalities, such as touch, pressure, and heat, resulting from peripheral sensitization and maladaptive central changes, each with distinct mechanisms[21]. Patients often report touch- or cold-evoked pain; upon examination, they may exhibit allodynia and hyperalgesia, alongside sensory loss and motor coordination[22]. Accurate diagnosis and treatment require careful history of pain and neurological and clinical examinations. Furthermore, nerve conduction studies, which assess signal conduction through nerves, are crucial for evaluating the condition of surviving nerve fibers[23]. Previous studies have reported that trigonelline exerts neuroprotective effects by reducing hyperalgesia and allodynia in paclitaxel-induced neuropathic pain and improving the recovery of MNCV in streptozotocin-induced diabetic rats[24]. These results are in accordance with the findings of the present study, which indicate that trigonelline significantly ameliorated the altered behavioral responses linked to impaired neurological functions by mitigating hyperalgesia, allodynia, and deficits in motor activity in SCI-induced rats.

Bax, Bcl-2, and caspases are important regulators of apoptosis, which is a critical step in the pathophysiology of SCI and a crucial step in the pathophysiology of SCI, leading to neurological impairment[25]. The pro-apoptotic protein Bax, a member of the Bcl-2 family, promotes cellular death by initiating the release of cytochrome-C from mitochondria and stimulating the caspase cascade[26]. Following SCI, elevated Bax expression can cause greater neuronal apoptosis and exacerbate tissue damage and functional impairment[27]. Caspases, a family of proteases crucial

for apoptosis, are particularly significant in SCI, with caspase-3 activation leading to the cleavage of key cellular components, resulting in cell death, and affecting the white and gray matter of the spinal cord. Inhibition of caspase-3 signaling can reduce spinal neuronal apoptosis and decrease nociceptor hyper-responsiveness, offering a potential strategy for treating neuropathic pain[28]. Plant phytoconstituents such as mangiferin[29], quercetin[30], and fisetin[31] have shown promising effects in regulating the Bax/Bcl-2 pathways in SCI, which are crucial mechanisms in the control of apoptosis. In contrast, trigonelline exhibits multifaceted therapeutic effects by modulating the nuclear factor erythroid 2-related factor 2, cyclin D1, Bax/Bcl-2 axis, and nuclear factor kappa B pathways, thereby significantly regulating cell cycle arrest and apoptosis[32]. In the present study, trigonelline demonstrated anti-apoptotic ability by downregulating *Bax* and *caspase-3* and upregulating *Bcl-2* mRNA in SCI-induced neuronal injury.

Mitochondrial enzymes regulate cellular energy production, apoptosis, and oxidative stress in SCI. Following SCI, mitochondrial damage often occurs following SCI, leading to dysfunction in these processes, which can aggravate cell death and tissue damage[33]. Mitochondrial oxidative stress impairs function and disrupts cellular signaling by damaging mitochondrial DNA, proteins, and lipids through excessive ROS production[34]. Overproduction of ROS can damage cytochrome-C, nicotinamide adenine dinucleotide dehydrogenase, and adenosine triphosphate synthase, resulting in the breakdown of energy production from the mitochondria[35]. Consequently, mitochondrial damage from ROS is crucial for neurodegenerative disease pathogenesis[36]. Research has demonstrated that trigonelline possesses notable antioxidant properties, offering protection against mitochondrial oxidative stress in various goat organs, including the heart, liver, brain, kidney, and aorta, under copper ascorbate-induced copper-ascorbate-induced oxidative stress[37]. Consistent with these findings, the current study further supports the protective efficacy of trigonelline against SCI-induced mitochondrial damage in the rat spinal cord.

Neurotrophic factors, including BDNF, have been linked to functional recovery following SCI[38]. BDNF, a member of the neurotrophin family, promotes axonal development, synaptic plasticity, and neuronal survival in the central nervous system. Furthermore, BDNF has anti-inflammatory and neuroprotective effects, making it a suitable mediator for promoting recovery following SCI[39]. Extensive preclinical and clinical research supports the role of BDNF in promoting functional recovery following SCI[39]. Increased BDNF levels have been linked to enhanced motor function in patients with incomplete SCI[40]. Furthermore, research indicates that increased BDNF expression is related to decreased tactile allodynia and enhanced motor performance in SCI models[41]. In the current study, rats with SCI showed lower BDNF expression in the spinal cord, which was associated with worsened allodynia and motor dysfunction, emphasizing the importance of BDNF in neuronal impairment. In contrast, trigonelline treatment effectively boosted BDNF expression in the spinal cord, leading to an improved response to allodynia. Furthermore, our findings are reinforced by research demonstrating that trigonelline may restore BDNF levels in adult mice with lipopolysaccharide-induced cognitive impairment[42].

Trigonelline is a safe compound with an LD_{50} of 5 000 mg/kg, *p.o.* in mice[43]. Clinical trials of trigonelline are limited; recent studies have indicated that it acts as an NAD^+ precursor, improving mitochondrial function, muscle health, mobility, and sarcopenia in aging individuals[44]. NAD^+ enhancement by trigonelline is consistent across various model organisms and in primary human muscle cells from healthy individuals and those with sarcopenia. In humans, reduced serum trigonelline levels are associated with sarcopenia and are positively linked to skeletal muscle strength and mitochondrial oxidative phosphorylation[44]. Despite the complex and unclear relationship between sarcopenia and SCI, a clinical study demonstrated that sarcopenia is more prevalent in SCI[45]. Our findings suggest that trigonelline has the potential to improve muscle strength, as evidenced by the delayed fall-off duration in SCI-induced rats. Additionally, the imbalance in mitochondrial enzyme complexes triggered by SCI induction was enhanced by treatment with trigonelline. Therefore, based on our findings and reported clinical investigations, trigonelline shows promise as a therapeutic molecule for SCI treatment.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

ZY conceptualized the work and performed data analysis and interpretation. YC collected data and drafted the manuscript. Both authors critically revised and approved the final version of the manuscript.

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