Original Article

Spinal Serotonergic Signaling Mediates the Neuroprotective Effects of Duloxetine Following Chronic Constriction Injury in Rats

Masoud Sharifian[1], Ali Rashidian-Rashidabadi[2], Farzaneh Bagheri[3], Zohre Aghaei[4], Zahra Haghighatian[5], Elham Goodarzi[6], Atefeh Marzban[7]

[1] Department of Surgery, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran.

[2] Department of Anatomical Science, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran.

[3] Department of Pharmacology and Toxicology, Sapienza university, Rome, Italy.

[4] Department of Physiology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.

[5] Department of Pathology, School of Medicine, Lorestan University of Medical Science, Khorramabad, Iran.

[6] Department of Biostatistics and Epidemiology, School of Health and Nutrition, Lorestan University of Medical Sciences, Khorramabad, Iran.

[7] Department of Anesthesiology, Lorestan University of Medical Sciences, Khorramabad, Iran.

***Corresponding author:** Atefeh Marzban, Department of Anesthesiology, Lorestan University of Medical Sciences, Khorramabad, Iran. E-mail: atefehmarzban92@gmail.com

Received Date: 22 May 2026; **Revised Date:** 1 July 2026; **Accepted Date**: 10 July 2026; **Published Date:** 12 July 2026.
Nexus of Pathophysiology and Therapeutics (NPT). 2026;1(1): 011, https://doi.org/10.22034/npt.2026.1.011

Abstract:

Background: Duloxetine is widely prescribed for the management of neuropathic pain; however, the contribution of spinal serotonergic signaling to its neuroprotective effects following peripheral nerve injury remains incompletely understood. This study investigated the role of spinal serotonin in duloxetine-mediated functional and biochemical recovery using a chronic constriction injury (CCI) model in rats.

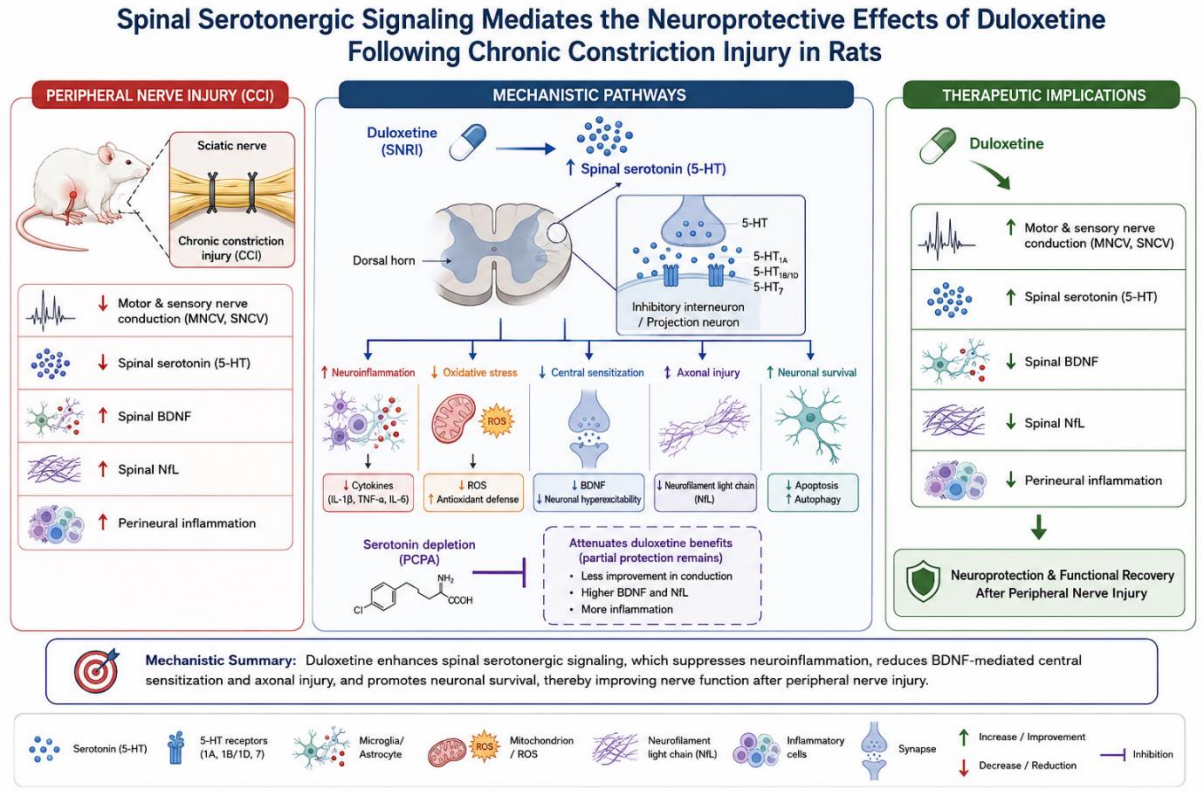
Methods: Forty adult male Wistar rats were randomly assigned to five groups (n = 8 each): sham-operated, CCI, CCI + duloxetine (10 mg/kg, i.p.), CCI + p-chlorophenylalanine (PCPA, 100 mg/kg, i.p.), and CCI + duloxetine + PCPA. Electrophysiological function (MNCV and SNCV), spinal serotonin (5-HT), brain-derived neurotrophic factor (BDNF), neurofilament light chain (NfL), and sciatic nerve histopathology were evaluated.

Results: CCI significantly impaired motor and sensory nerve conduction, reduced spinal serotonin levels, and increased spinal BDNF and NfL concentrations, accompanied by marked perineural inflammatory cell infiltration. Duloxetine significantly restored MNCV and SNCV, increased spinal serotonin levels, reduced BDNF and NfL concentrations, and attenuated histopathological inflammation. Pharmacological depletion of serotonin with PCPA substantially diminished the beneficial effects of duloxetine on electrophysiological, biochemical, and histopathological outcomes, although partial protection was retained.

Conclusion: These findings demonstrate that spinal serotonergic signaling plays a pivotal role in the neuroprotective effects of duloxetine following peripheral nerve injury. The concurrent modulation of BDNF and NfL further suggests their potential utility as complementary biomarkers of neuronal plasticity and axonal injury during neuropathic pain progression and recovery. Targeting spinal serotonergic pathways may represent a promising therapeutic strategy for enhancing functional recovery after peripheral nerve injury.

Mechanistic and Translational Relevance: Our findings identify spinal serotonergic signaling as a principal mechanism underlying duloxetine-mediated neuroprotection after peripheral nerve injury and suggest that BDNF and neurofilament light chain may serve as complementary biomarkers for evaluating treatment response and disease progression in neuropathic pain.

Keywords: Duloxetine; Neuropathic pain; Chronic constriction injury; Serotonin; Brain-derived neurotrophic factor and Neurofilament light chain.



Graphical Abstract: Mechanistic and Translational Overview of Duloxetine-Mediated Neuroprotection Through Spinal Serotonergic Signaling Following Chronic Constriction Injury. Peripheral nerve injury induced by CCI impairs motor and sensory nerve conduction, decreases spinal serotonin (5-HT), increases spinal brain-derived neurotrophic factor (BDNF) and neurofilament light chain (NfL) levels, and promotes perineural inflammation. Duloxetine enhances spinal serotonergic neurotransmission, leading to attenuation of neuroinflammation, reduction of maladaptive BDNF-associated central sensitization and axonal injury, preservation of neuronal integrity, and improvement of electrophysiological function. Pharmacological depletion of serotonin with p-chlorophenylalanine (PCPA) diminishes, but does not completely abolish, the neuroprotective effects of duloxetine, indicating that spinal serotonergic signaling is a principal mediator of functional recovery following peripheral nerve injury, while additional mechanisms may also contribute.

Introduction

Neuropathic pain is a chronic and debilitating condition that arises from injury or dysfunction of the somatosensory nervous system (1, 2). It is characterized by spontaneous pain, hyperalgesia, allodynia, and persistent sensory abnormalities that markedly impair quality of life (2, 3). Despite advances in pain management, currently available therapies provide only partial symptom relief in many patients, highlighting the need for a better understanding of the mechanisms underlying neuropathic pain and for the development of more effective therapeutic strategies (2, 4). Peripheral nerve injury initiates a cascade of pathological events that extends beyond the site of damage (5). Along with axonal degeneration and neuroinflammation, sustained activation of spinal nociceptive pathways contributes to central sensitization and the maintenance of chronic pain (5, 6). These changes are accompanied by alterations in the expression of several molecular mediators involved in neuronal plasticity and nerve integrity (7, 8). Among these, brain-derived neurotrophic factor (BDNF) has been implicated in the development of central sensitization (9, 10), whereas neurofilament light chain (NfL) has

emerged as a sensitive biomarker of axonal injury (11, 12). Therefore, functional, biochemical, and histopathological assessments together provide a comprehensive evaluation of peripheral nerve recovery following injury. Descending inhibitory pathways originating from supraspinal regions play a fundamental role in regulating nociceptive transmission within the spinal cord (13, 14). Serotonin (5-hydroxytryptamine, 5-HT) is a major neurotransmitter in this system and modulates spinal pain processing through multiple receptor subtypes (15, 16). Experimental evidence has shown that peripheral nerve injury disrupts spinal serotonergic neurotransmission, thereby weakening endogenous pain inhibition and facilitating the development of neuropathic pain (17, 18). However, the contribution of spinal serotonin to neural recovery after peripheral nerve injury remains incompletely understood (19, 20). Duloxetine, a serotonin-norepinephrine reuptake inhibitor (SNRI), is widely used as a first-line pharmacological treatment for neuropathic pain (21, 22). In addition to its analgesic efficacy, accumulating evidence suggests that duloxetine may exert anti-inflammatory and neuroprotective effects that extend beyond symptom control (23, 24). Previous studies have reported that duloxetine can attenuate neuroinflammation, improve peripheral nerve function, and modulate the expression of molecules associated with neuronal survival and plasticity (25, 26). Nevertheless, it remains unclear whether these beneficial effects are primarily mediated through enhancement of spinal serotonergic signaling (15, 25). Pharmacological depletion of serotonin using p-chlorophenylalanine (PCPA), an irreversible inhibitor of tryptophan hydroxylase, provides a useful experimental approach for examining the involvement of endogenous serotonin in the actions of duloxetine (25, 27). By selectively reducing serotonin synthesis, PCPA allows direct evaluation of the contribution of serotonergic signaling to functional and biochemical recovery following peripheral nerve injury (25, 27).

Therefore, the present study was designed to investigate the role of spinal serotonergic signaling in the neuroprotective effects of duloxetine in a rat model of chronic constriction injury (CCI). Electrophysiological recovery was assessed by measuring motor and sensory nerve conduction velocities, while spinal serotonin, BDNF, and NfL levels were quantified as biochemical indicators of serotonergic activity, neuronal plasticity, and axonal injury. Histopathological examination of the sciatic nerve was also performed to evaluate perineural inflammatory changes. We hypothesized that depletion of endogenous serotonin would attenuate the beneficial effects of duloxetine, thereby demonstrating a critical role for spinal serotonergic signaling in functional recovery following peripheral nerve injury.

Methods

Animals

Forty adult male Wistar rats (8 weeks old, 200–220 g) were used in the present study. Animals were housed in standard polypropylene cages under controlled laboratory conditions, including a 12 h light/dark cycle, ambient temperature of 22 ± 2 °C, and relative humidity of 50–60%. Standard pellet diet and tap water were provided ad libitum throughout the experimental period. Animals were allowed to acclimatize to the housing environment for at least one week before the start of the experiments. Health status was monitored daily, and all efforts were made to minimize animal suffering and reduce the number of animals used. All experimental procedures were performed in accordance with the ethical principles for laboratory animal research and were approved by the Ethics Committee of the Ministry of Health and Medical Education of Iran (IR.LUMS.REC.1405.132).

Experimental Design

Animals were randomly assigned to the experimental groups (n = 8 per group) using a computer-generated randomization sequence as follows: (1) Control (sham-operated) group, (2)

chronic constriction injury (CCI) group, and (3) CCI treated with duloxetine (10 mg/kg, i.p., in a volume of 0.2 mL/rat). For mechanistic evaluation of serotonergic involvement, two additional groups were included: (4) CCI + p-chlorophenylalanine (PCPA, 100 mg/kg, i.p.) and (5) CCI + duloxetine + PCPA.

Treatment was initiated three days before CCI surgery and continued throughout the experimental period until day 14 after nerve injury. In groups receiving PCPA, the compound was administered according to the experimental protocol to deplete endogenous serotonin levels prior to duloxetine treatment. Animals were observed daily for changes in body weight, locomotor activity, grooming behavior, food intake, and signs of postoperative complications. No procedure-related mortality occurred during the study period. PCPA (Sigma No: C6506) is an irreversible inhibitor of tryptophan hydroxylase, the rate-limiting enzyme in serotonin biosynthesis, and is widely used experimentally to deplete central serotonin levels. The dose of duloxetine was selected based on a previously published study (28). PCPA (100 mg/kg, i.p.) was administered once daily for four consecutive days (days 0–3) and repeated on days 11–14 to maintain serotonin depletion throughout the experimental period (29).

Induction of Chronic Constriction Injury

Experimental neuropathy was established using the chronic constriction injury (CCI) model of the sciatic nerve (30). Animals were anesthetized with pentobarbital sodium (60 mg/kg, i.p.) and positioned under sterile surgical conditions. Following skin preparation, an incision was made over the lateral aspect of the thigh, and the underlying muscle layers were gently separated to expose the sciatic nerve. Once identified, the nerve was carefully isolated from the surrounding connective tissue. Three loose ligatures were then placed around the sciatic nerve using 4-0 silk sutures, leaving approximately 1 mm between adjacent ligatures. The sutures were tightened sufficiently to induce a mild and reproducible constriction while preserving nerve continuity and local blood supply. After completion of the procedure, the muscle and skin layers were sutured separately, and animals were returned to their home cages for recovery. Rats in the sham group underwent identical surgical exposure of the sciatic nerve without placement of ligatures.

Electrophysiological Assessment

Electrophysiological studies were performed on day 14 following CCI induction to assess functional integrity of the sciatic nerve. Animals were anesthetized with pentobarbital sodium (60 mg/kg, i.p.), and body temperature was maintained within the physiological range throughout the recording session. After skin disinfection, a longitudinal incision was made along the hind limb. The overlying muscle layers were carefully separated to expose the sciatic nerve while minimizing mechanical manipulation. Once the nerve was identified, stimulating electrodes were positioned at proximal and distal sites along the nerve pathway. Electrophysiological recordings were obtained using a PowerLab data acquisition system. Compound action potentials were evoked by electrical stimulation and recorded under standardized conditions. Motor nerve conduction velocity (MNCV) and sensory nerve conduction velocity (SNCV) were determined by calculating the ratio of the distance between stimulation sites to the corresponding latency differences. All electrophysiological measurements were performed by the same investigator, who was blinded to treatment allocation, to reduce inter-observer variability and ensure consistency among experimental groups (31).

Biochemical Analysis

Following completion of the electrophysiological assessments, rats were deeply anesthetized and euthanized. The lumbar spinal cord (L4–L6 segments) was rapidly exposed by laminectomy, carefully dissected, rinsed with ice-cold phosphate-

buffered saline (PBS) to remove residual blood, snap-frozen in liquid nitrogen, and stored at −80 °C until analysis. Frozen spinal cord tissues were weighed and homogenized in ice-cold PBS supplemented with a protease inhibitor cocktail (1:9, w/v). The homogenates were centrifuged at 12,000 × g for 15 min at 4 °C, and the resulting supernatants were collected for biochemical analyses. Total protein concentration was determined using a bicinchoninic acid (BCA) protein assay. Spinal serotonin (5-hydroxytryptamine, 5-HT) levels were measured using a commercially available competitive ELISA kit (Cat. No. E02H0106; BlueGene Biotech Co., Ltd., Shanghai, China) according to the manufacturer's instructions. Brain-derived neurotrophic factor (BDNF) concentrations were determined using a commercially available Rat BDNF ELISA kit (Cat. No. E02B0042; BlueGene Biotech Co., Ltd., Shanghai, China) following the manufacturer's protocol. Neurofilament light chain (NfL) concentrations were quantified using a Rat Neurofilament Light Polypeptide (NEFL) ELISA kit (Cat. No. MBS913456; MyBioSource, Inc., San Diego, CA, USA) according to the manufacturer's instructions. All samples and standards were assayed in duplicate. Absorbance was measured at 450 nm using a microplate reader. Biomarker concentrations were calculated from the corresponding standard curves. The concentrations of 5-HT, BDNF, and NfL were normalized to the total protein content of each sample and expressed as ng/mg protein or pg/mg protein, as appropriate (32).

Histopathological Evaluation

At the end of the experimental period, the sciatic nerves were carefully harvested and immediately fixed in 10% neutral-buffered formalin for histological examination. Following fixation, tissue specimens were dehydrated through graded ethanol solutions, embedded in paraffin, and sectioned into 5-µm-thick slices using a rotary microtome. The prepared sections were stained with hematoxylin and eosin (H&E) to assess general tissue morphology. Microscopic evaluation was performed using a light microscope by an investigator blinded to the experimental groups. Histopathological analyses focused on structural alterations associated with peripheral nerve injury, including axonal degeneration, myelin sheath disorganization, inflammatory cell infiltration, and changes in overall nerve architecture. Perineural inflammatory cell infiltration was assessed using a semi-quantitative histopathological scoring system and graded on a four-point scale as follows: 0 = no inflammatory cell infiltration; 1 = mild infiltration; 2 = moderate infiltration; and 3 = severe inflammatory cell infiltration. Scores were assigned based on the overall extent of inflammatory changes observed in each tissue section (33).

Statistical Analysis

Statistical analyses were performed using GraphPad Prism software (version 8.0). Data are presented as mean ± standard error of the mean (SEM). The normality of data distribution was assessed using the Shapiro–Wilk test before statistical analysis. Comparisons among experimental groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple pairwise comparisons when a significant overall effect was detected. A p value < 0.05 was considered statistically significant.

Results

Effects of Duloxetine and PCPA on Motor and Sensory Nerve Conduction Velocity Following CCI

The MNCV results demonstrated that induction of chronic constriction injury (CCI) in the second group significantly reduced motor nerve conduction velocity in the sciatic nerve compared with the control group ($p < 0.001$). Treatment with duloxetine in the third group significantly improved MNCV compared with the CCI group ($p < 0.01$). However, co-administration of PCPA with duloxetine attenuated the beneficial effects of

duloxetine on MNCV (Figure 1). Similarly, the SNCV results showed that CCI-induced neuropathic pain significantly decreased sensory nerve conduction velocity compared with the control group ($p < 0.001$). Duloxetine treatment significantly improved SNCV relative to the CCI group ($p < 0.01$). However, co-treatment with PCPA attenuated the beneficial effect of duloxetine on SNCV ($p < 0.05$, Figure 2).

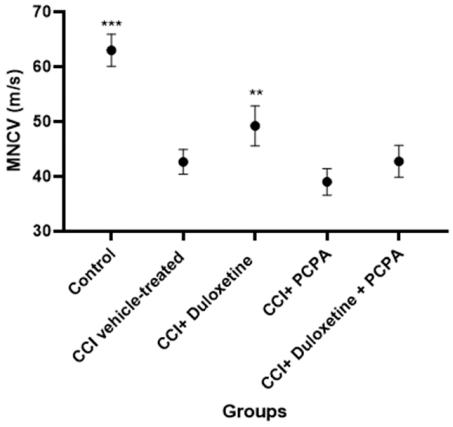


Figure 1. Effects of Duloxetine and PCPA on MNCV following CCI. Data are presented as mean ± SEM (n = 8 per group). Statistical significance was analyzed using one-way ANOVA followed by Tukey's post hoc test. *p < 0.05, **p < 0.01, ***p < 0.001 versus the CCI group.

Effects of Duloxetine and PCPA on Spinal Serotonin Levels

As shown in Figure 3, spinal serotonin (5-HT) levels were significantly reduced in the CCI vehicle-treated group compared with the control group. Treatment with duloxetine significantly increased spinal serotonin levels relative to the CCI vehicle-treated group ($p < 0.001$). Administration of PCPA alone did not produce a significant change in spinal serotonin levels compared with the CCI vehicle-treated group. However, co-administration of PCPA with duloxetine resulted in a smaller, yet still significant, increase in spinal serotonin levels compared with the CCI vehicle-treated group ($p < 0.05$), indicating that PCPA attenuated the serotonin-enhancing effect of duloxetine (Figure 3).

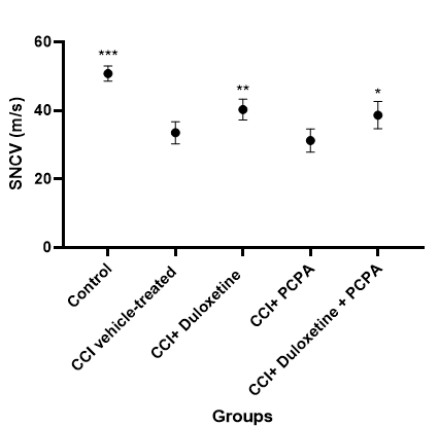


Figure 2. Effects of Duloxetine and PCPA on SNCV following CCI. Data are presented as mean ± SEM (n = 8 per group). Statistical significance was analyzed using one-way ANOVA followed by Tukey's post hoc test. *p < 0.05, **p < 0.01, ***p < 0.001 versus the CCI group.

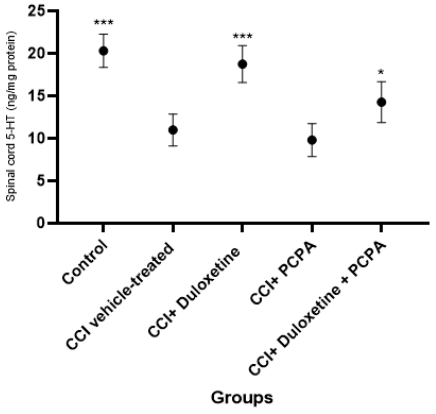


Figure 3. Effects of Duloxetine and PCPA on spinal serotonin (5-HT) levels following CCI. Data are presented as mean ± SEM (n = 8 per group). Statistical significance was analyzed using one-way ANOVA followed by Tukey's post hoc test. *p < 0.05, **p < 0.01, ***p < 0.001 versus the CCI group.

Effects of Duloxetine and PCPA on Spinal BDNF Levels

Analysis of spinal BDNF levels revealed a significant increase in the CCI group compared with

the control group ($p < 0.001$). Treatment with duloxetine significantly reduced spinal BDNF levels compared with the CCI group ($p < 0.001$). In contrast, administration of PCPA alone significantly increased spinal BDNF levels relative to the CCI group ($p < 0.01$). Co-administration of PCPA with duloxetine significantly decreased spinal BDNF levels compared with the CCI group ($p < 0.01$, Figure 4).

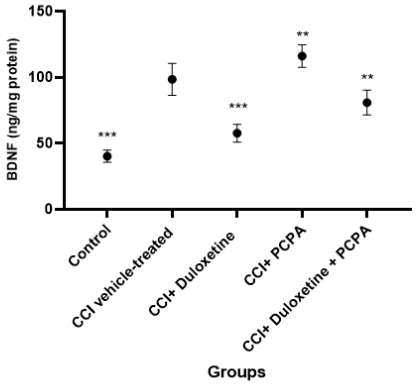


Figure 4. Effects of Duloxetine and PCPA on Spinal BDNF Levels. Data are presented as mean ± SEM (n = 8 per group). Statistical significance was analyzed using one-way ANOVA followed by Tukey's post hoc test. *p < 0.05, **p < 0.01, ***p < 0.001 versus the CCI group.

Effects of Duloxetine and PCPA on Spinal Neurofilament Light Chain (NfL) Levels

Analysis of spinal neurofilament light chain (NfL) levels revealed a significant increase in the CCI vehicle-treated group compared with the control group ($p < 0.001$). Duloxetine treatment significantly reduced spinal NfL levels relative to the CCI vehicle-treated group ($p < 0.001$). Conversely, treatment with PCPA alone significantly increased spinal NfL levels compared with the CCI vehicle-treated group ($p < 0.001$). Likewise, co-administration of duloxetine and PCPA resulted in significantly higher spinal NfL levels than those observed in the CCI vehicle-treated group ($p < 0.05$, Figure 5).

Effects of Duloxetine and PCPA on Perineural Inflammation Following CCI

Histopathological examination of the sciatic nerve demonstrated a marked increase in perineural inflammatory cell infiltration in the CCI group compared with the control group ($p < 0.001$). Treatment with duloxetine significantly reduced inflammatory cell infiltration compared with the CCI group ($p < 0.01$). In contrast, co-administration of PCPA attenuated the anti-inflammatory effect of duloxetine, resulting in increased perineural inflammatory cell infiltration. No significant difference was observed between the CCI + PCPA group and the CCI group (Figure 6).

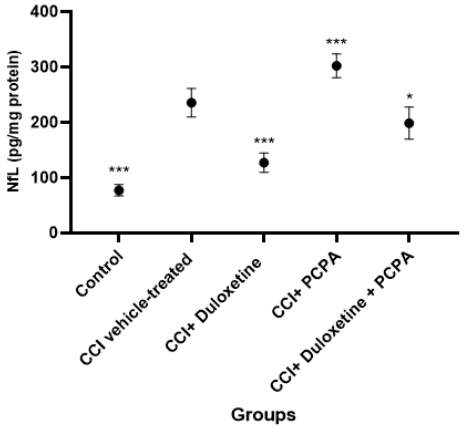


Figure 5. Effects of Duloxetine and PCPA on Spinal NfL Levels. Data are presented as mean ± SEM (n = 8 per group). Statistical significance was analyzed using one-way ANOVA followed by Tukey's post hoc test. *p < 0.05, **p < 0.01, ***p < 0.001 versus the CCI group.

Discussion

The present study demonstrates that duloxetine markedly improved functional recovery following chronic constriction injury, as evidenced by the restoration of motor and sensory nerve conduction velocities, increased spinal serotonin levels, reduced spinal BDNF and NfL concentrations, and attenuation of perineural inflammatory cell infiltration. Pharmacological depletion of serotonin with PCPA substantially diminished these beneficial effects, indicating that intact spinal serotonergic signaling is required for the full neuroprotective

action of duloxetine. Collectively, these findings suggest that enhancement of spinal serotonin not only contributes to pain modulation but also plays an important role in preserving peripheral nerve function and limiting pathological changes following nerve injury.

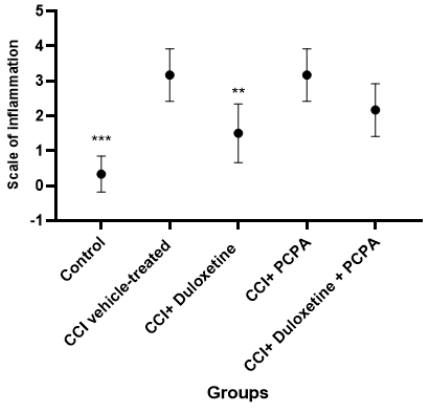


Figure 6. Semi-quantitative histopathological inflammation scores of the sciatic nerve in the experimental groups. Data are presented as mean ± SEM (n = 8 per group). Statistical significance was analyzed using one-way ANOVA followed by Tukey's post hoc test. *p < 0.05, **p < 0.01, ***p < 0.001 versus the CCI group.

Electrophysiological assessment provides an objective measure of functional integrity after peripheral nerve injury. In the present study, CCI markedly reduced both motor and sensory nerve conduction velocities, confirming successful induction of neuropathic injury. These findings are consistent with previous reports demonstrating impaired nerve conduction following chronic sciatic nerve constriction due to axonal dysfunction, demyelination, and inflammatory changes surrounding the injured nerve (34-36). Duloxetine significantly improved both MNCV and SNCV, suggesting preservation of nerve function in addition to its established analgesic activity. Similar improvements in electrophysiological parameters have been reported following duloxetine treatment in experimental models of peripheral neuropathy (36, 37). Restoration of nerve conduction may result from reduced neuroinflammation, preservation of axonal integrity, and improved neuronal excitability, all of which contribute to functional recovery after nerve injury (38, 39).

One of the principal findings of this study is the involvement of spinal serotonergic signaling in the beneficial effects of duloxetine. CCI significantly reduced spinal serotonin levels, whereas duloxetine restored serotonin concentrations toward normal values. This observation agrees with previous studies showing that peripheral nerve injury disrupts descending monoaminergic inhibitory pathways and weakens endogenous pain inhibition (40, 41). Duloxetine enhances synaptic serotonin availability by inhibiting its reuptake, thereby strengthening descending inhibitory control at the spinal level (42, 43). Importantly, depletion of endogenous serotonin with PCPA markedly attenuated the functional and biochemical effects of duloxetine. Although duloxetine retained partial efficacy after serotonin depletion, its protective effects were substantially reduced. These findings indicate that spinal serotonin is a major, but probably not the sole, mediator of duloxetine-induced neuroprotection. The remaining protective effects may be explained by increased noradrenergic transmission, suppression of neuroinflammatory signaling, or direct modulation of glial cell activity, all of which have been reported to contribute to the pharmacological profile of duloxetine (24, 25). Brain-derived neurotrophic factor (BDNF) has emerged as a critical mediator of central sensitization in neuropathic pain. Although BDNF is generally recognized for its neurotrophic and neuroprotective properties, its role within the spinal cord differs substantially under pathological conditions. Following peripheral nerve injury, activated microglia release BDNF in the dorsal horn, leading to abnormal neuronal excitability and amplification of nociceptive transmission (9). Increased spinal BDNF has therefore been consistently associated with the initiation and maintenance of neuropathic pain rather than neuronal protection (44, 45). In agreement with these observations, CCI markedly increased spinal

BDNF levels in the present study, whereas duloxetine significantly reduced BDNF expression. This reduction was accompanied by improved electrophysiological function and decreased inflammatory changes, suggesting attenuation of central sensitization. Interestingly, serotonin depletion with PCPA prevented the full suppressive effect of duloxetine on spinal BDNF. This finding indicates that serotonergic signaling contributes to the regulation of spinal BDNF following peripheral nerve injury. However, because BDNF levels in the duloxetine plus PCPA group remained below those observed in untreated CCI animals, it is likely that mechanisms other than serotonin also participate in the modulation of BDNF. Previous studies have suggested that suppression of microglial activation, inhibition of pro-inflammatory cytokine production, and enhancement of noradrenergic neurotransmission may all contribute to the ability of duloxetine to regulate BDNF expression (9, 46, 47). Neurofilament light chain (NfL) is increasingly recognized as a sensitive biomarker of neuroaxonal injury. Damage to peripheral axons results in disruption of the neuronal cytoskeleton and subsequent release of NfL into surrounding tissues and biological fluids (48). Elevated NfL concentrations have been reported in several neurological disorders characterized by axonal degeneration, including peripheral neuropathies, traumatic nerve injury, and neurodegenerative diseases (49, 50). Consistent with these observations, spinal NfL levels were significantly increased following CCI, indicating ongoing axonal damage after sciatic nerve constriction. Duloxetine markedly reduced spinal NfL levels, suggesting preservation of axonal integrity in addition to functional improvement. This finding is particularly noteworthy because most experimental studies evaluating duloxetine have focused primarily on behavioral outcomes, whereas relatively few have examined biomarkers directly reflecting structural neuronal injury (51-52). Interestingly, depletion of serotonin markedly attenuated the NfL-lowering effect of duloxetine, further supporting the involvement of spinal serotonergic signaling in maintaining axonal integrity following peripheral nerve injury. The parallel improvement in nerve conduction velocity and reduction in NfL observed in the present study further strengthens the relationship between structural preservation and functional recovery.

Histopathological findings further supported the biochemical and electrophysiological results. CCI produced marked perineural inflammatory cell infiltration around the sciatic nerve, which is a characteristic pathological feature of peripheral neuropathy. Local inflammatory responses contribute to persistent nociceptor activation through the release of cytokines, chemokines, and other inflammatory mediators, thereby sustaining neuropathic pain (6, 53). Duloxetine markedly reduced inflammatory cell infiltration, suggesting that its beneficial effects extend beyond modulation of neurotransmitter reuptake. Previous studies have similarly demonstrated that duloxetine suppresses neuroinflammatory responses by reducing glial activation and limiting the production of inflammatory mediators (24, 37). In contrast, serotonin depletion diminished this anti-inflammatory effect, and inflammatory changes became comparable to those observed in untreated CCI animals. These findings suggest that preservation of spinal serotonergic neurotransmission contributes, at least in part, to the anti-inflammatory actions of duloxetine following peripheral nerve injury.

Limitations

This study has several limitations. First, only male rats were included; therefore, potential sex-dependent differences could not be evaluated. Second, serotonin depletion was achieved pharmacologically using PCPA rather than through receptor-specific approaches, which may not completely distinguish serotonergic from other monoaminergic mechanisms. Third, only spinal serotonin concentrations were measured, whereas receptor subtype activation and downstream

intracellular signaling pathways were not investigated. Future studies should incorporate receptor-specific antagonists, molecular analyses, and longer follow-up periods to further clarify the mechanisms underlying duloxetine-induced neuroprotection.

Future Directions

Future investigations should identify the specific serotonergic receptor subtypes and downstream signaling pathways mediating duloxetine-induced neuroprotection. Integrating molecular analyses of neuroinflammation, glial activation, axonal regeneration, and receptor-specific pharmacological approaches, together with long-term functional and translational studies, will provide a more comprehensive understanding of the mechanisms underlying serotonergic modulation in peripheral nerve repair and may facilitate the development of targeted therapeutic strategies for neuropathic pain.

Conclusion

The present study demonstrates that duloxetine promotes functional recovery following chronic constriction injury by improving motor and sensory nerve conduction, restoring spinal serotonin levels, reducing spinal BDNF and neurofilament light chain (NfL) concentrations, and attenuating perineural inflammation. Importantly, pharmacological depletion of serotonin with p-chlorophenylalanine (PCPA) markedly diminished these beneficial effects, highlighting the essential contribution of spinal serotonergic signaling to the neuroprotective actions of duloxetine. The partial preservation of duloxetine efficacy despite serotonin depletion further suggests that additional mechanisms, including noradrenergic signaling and modulation of neuroinflammatory pathways, may also contribute to its therapeutic effects. Collectively, these findings provide mechanistic evidence that enhancement of spinal serotonergic neurotransmission is a key component of duloxetine-induced neuroprotection following peripheral nerve injury. Moreover, the concurrent changes in BDNF and NfL support their potential utility as complementary biomarkers of neuronal plasticity and axonal injury during neuropathic pain progression and recovery. Further studies are warranted to clarify the downstream molecular pathways linking serotonergic signaling to neuroinflammation, axonal preservation, and functional nerve regeneration.

Mechanistic and Translational Relevance

Duloxetine-mediated neuroprotection following chronic constriction injury appears to extend beyond symptomatic analgesia by engaging spinal serotonergic signaling pathways that influence neuroinflammation, neuronal plasticity, and axonal preservation. The marked attenuation of duloxetine efficacy after pharmacological serotonin depletion supports a mechanistic role for endogenous spinal 5-hydroxytryptamine (5-HT) in maintaining descending inhibitory control while indirectly regulating molecular processes associated with peripheral nerve repair. Restoration of spinal serotonin may reduce pathological nociceptive transmission through activation of inhibitory serotonergic receptor networks and modulation of neuron–glia interactions, thereby limiting inflammatory signaling and secondary neuronal injury. The concomitant reduction in spinal BDNF observed after duloxetine treatment is consistent with attenuation of maladaptive central sensitization rather than suppression of physiological neurotrophic activity, whereas decreased neurofilament light chain (NfL) levels suggest preservation of axonal cytoskeletal integrity. Together with improved nerve conduction and reduced perineural inflammation, these findings support a biologically coherent framework linking serotonergic modulation to structural and functional neural recovery. From a translational perspective, the data reinforce spinal serotonergic signaling as a potential therapeutic target for enhancing recovery after peripheral nerve injury and identify BDNF and NfL as complementary mechanistic biomarkers that may facilitate monitoring of neuronal plasticity and

axonal injury during treatment. Although these observations derive from a preclinical model, they provide a rationale for future investigations integrating receptor subtype-specific pharmacology, glial and intracellular signaling analyses, and biomarker-guided clinical studies to determine whether modulation of serotonergic pathways can improve patient stratification, optimize therapeutic response, and accelerate translation of neuroprotective strategies for neuropathic pain.

Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

Masoud Sharifian contributed to investigation, data curation, formal analysis, and writing the original draft. Ali Rashidian-Rashidabadi contributed to investigation, data curation, and methodology. Farzaneh Bagheri contributed to conceptualization, methodology, study design, and supervision. Zohre Aghaei contributed to conceptualization, methodology, and study design. Zahra Haghighatian contributed to laboratory experiments, histopathological analysis, and investigation. Elham Goodarzi contributed to formal analysis, statistical analysis, and data interpretation. Atefeh Marzban contributed to conceptualization, methodology, investigation, project administration, funding acquisition, supervision, and writing—review and editing. All authors read and approved the final version of the manuscript.

Ethics Statement

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgments

The research team would like to thank the esteemed Vice President for Research and Technology of Lorestan University of Medical Sciences.

Funding Statement

This study was financially supported by Lorestan University of Medical Sciences, Khorramabad, Iran.

Publisher's Note

The graphical abstract was prepared by the journal's editorial team using artificial intelligence-assisted design tools and was reviewed and approved by the authors prior to publication.

Reference

1. Finnerup NB, Haroutounian S, Kamerman P, Baron R, Bennett DL, Bouhassira D, et al. Neuropathic pain: an updated grading system for research and clinical practice. Pain. 2016;157(8):1599-606. https://doi.org/10.1097/j.pain.0000000000000492.

2. Rosner J, Shirvalkar P, de Andrade DC. Neuropathic pain–A clinical primer. International Review of Neurobiology. 179: Elsevier; 2024. p. 41-65. https://doi.org/10.1016/bs.irn.2024.10.014.

3. Leone CM, Truini A. Understanding neuropathic pain: the role of neurophysiological tests in unveiling underlying mechanisms. Journal of Anesthesia, Analgesia and Critical Care. 2024;4(1):77. https://doi.org/10.1186/s44158-024-00212-z.

4. Colloca L, Ludman T, Bouhassira D, Baron R, Dickenson AH, Yarnitsky D, et al. Neuropathic pain. Nature reviews Disease primers. 2017;3(1):17002. https://doi.org/10.1038/nrdp.2017.2.

5. Gu D, Xia Y, Ding Z, Qian J, Gu X, Bai H, et al. Inflammation in the peripheral nervous system after injury. Biomedicines. 2024;12(6):1256. https://doi.org/10.3390/biomedicines12061256.

6. Ji R-R, Donnelly CR, Nedergaard M. Astrocytes in chronic pain and itch. Nature Reviews Neuroscience. 2019;20(11):667-85. https://doi.org/10.1038/s41583-019-0218-1.

7. Pacifico P, Menichella DM. Molecular mechanisms of neuropathic pain. International Review of Neurobiology. 2024;179:279-309. https://doi.org/10.1016/bs.irn.2024.10.002.
8. Jang K, Garraway SM. A review of dorsal root ganglia and primary sensory neuron plasticity mediating inflammatory and chronic neuropathic pain. Neurobiology of Pain. 2024; 15:100151. https://doi.org/10.1016/j.ynpai.2024.100151.
9. Smith PA. BDNF in neuropathic pain; the culprit that cannot be apprehended. Neuroscience. 2024; 543:49-64. https://doi.org/10.1016/j.neuroscience.2024.02.020.
10. Xiong H-Y, Hendrix J, Schabrun S, Wyns A, Campenhout JV, Nijs J, et al. The role of the brain-derived neurotrophic factor in chronic pain: links to central sensitization and neuroinflammation. Biomolecules. 2024;14(1):71. https://doi.org/10.3390/biom14010071.
11. Gaetani L, Blennow K, Calabresi P, Di Filippo M, Parnetti L, Zetterberg H. Neurofilament light chain as a biomarker in neurological disorders. Journal of Neurology, Neurosurgery & Psychiatry. 2019;90(8):870-81. https://doi.org/10.1136/jnnp-2018-320106.
12. Coppens S, Lehmann S, Hopley C, Hirtz C. Neurofilament-light, a promising biomarker: analytical, metrological and clinical challenges. International Journal of Molecular Sciences. 2023;24(14):11624. https://doi.org/10.3390/ijms241411624.
13. Lv Q, Wu F, Gan X, Yang X, Zhou L, Chen J, He Y, Zhang R, Zhu B, Liu L. The involvement of descending pain inhibitory system in electroacupuncture-induced analgesia. Frontiers in integrative neuroscience. 2019 Aug 21; 13:38. https://doi.org/10.3389/fnint.2019.00038.
14. De Preter CC, Heinricher MM. The 'in's and out's' of descending pain modulation from the rostral ventromedial medulla. Trends in neurosciences. 2024;47(6):447-60. https://doi.org/10.1016/j.tins.2024.04.006.
15. Heijmans L, Mons MR, Joosten EA. A systematic review on descending serotonergic projections and modulation of spinal nociception in chronic neuropathic pain and after spinal cord stimulation. Molecular pain. 2021; 17:17448069211043965. https://doi.org/10.1177/17448069211043965.
16. Bannister K, Dickenson AH. What the brain tells the spinal cord. Pain. 2016;157(10):2148-51. https://doi.org/10.1097/j.pain.0000000000000568.
17. Aira Z, Buesa I, Salgueiro M, Bilbao J, Aguilera L, Zimmermann M, et al. Subtype-specific changes in 5-HT receptor-mediated modulation of C fibre-evoked spinal field potentials are triggered by peripheral nerve injury. Neuroscience. 2010;168(3):831-41. https://doi.org/10.1016/j.neuroscience.2010.04.032.
18. Liu F-Y, Qu X-X, Ding X, Cai J, Jiang H, Wan Y, et al. Decrease in the descending inhibitory 5-HT system in rats with spinal nerve ligation. Brain research. 2010; 1330:45-60. https://doi.org/10.1016/j.brainres.2010.03.010.
19. Perrin FE, Noristani HN. Serotonergic mechanisms in spinal cord injury. Experimental neurology. 2019; 318:174-91. https://doi.org/10.1016/j.expneurol.2019.05.007.
20. Choo S, Phillips R, White J, Nuelle JA. Neuromodulators can promote nerve regeneration and accelerate functional recovery after peripheral nerve injury: A systematic review. Journal of Orthopaedics. 2024; 47:122-47. https://doi.org/10.1016/j.jor.2023.11.018.
21. Finnerup NB, Kuner R, Jensen TS. Neuropathic pain: from mechanisms to treatment. Physiological reviews. 2021;101(1):259-301. https://doi.org/10.1152/physrev.00045.2019.
22. Moisset X. Neuropathic pain: Evidence based recommendations. La Presse Médicale. 2024;53(2):104232. https://doi.org/10.1016/j.lpm.2024.104232.
23. Catalisano G, Campione GM, Spurio G, Galvano AN, di Villalba CP, Giarratano A, et al. Neuropathic pain, antidepressant drugs, and inflammation: A narrative review. Journal of Anesthesia, Analgesia and Critical Care. 2024;4(1):67. https://doi.org/10.1186/s44158-024-00204-z.

24. Tawfik MK, Helmy SA, Badran DI, Zaitone SA. Neuroprotective effect of duloxetine in a mouse model of diabetic neuropathy: role of glia suppressing mechanisms. Life sciences. 2018; 205:113-24. https://doi.org/10.1016/j.lfs.2018.05.025.

25. Yamashita T, Yamamoto S, Zhang J, Kometani M, Tomiyama D, Kohno K, et al. Duloxetine inhibits microglial P2X4 receptor function and alleviates neuropathic pain after peripheral nerve injury. PloS one. 2016;11(10): e0165189. https://doi.org/10.1371/journal.pone.0165189.

26. Lu Y, Zhang P, Zhang Q, Yang C, Qian Y, Suo J, et al. Duloxetine attenuates paclitaxel-induced peripheral nerve injury by inhibiting p53-related pathways. The Journal of Pharmacology and Experimental Therapeutics. 2020;373(3):453-62. https://doi.org/10.1124/jpet.120.265082.

27. Bader M. Inhibition of serotonin synthesis: A novel therapeutic paradigm. Pharmacology & therapeutics. 2020; 205:107423. https://doi.org/10.1016/j.pharmthera.2019.107423.

28. Mohammadi N, Taheri P, Shahmoradi E, Motaghinejad M, Gholami M, Motevalian M. Preventive Effects of Duloxetine Against Methamphetamine Induced Neurodegeneration and Motor Activity Disorder in Rat: Possible Role of CREB/BDNF Signaling Pathway. Int J Prev Med. 2019; 10:195. https://doi.org/10.4103/ijpvm.IJPVM_53_18.

29. Bobinski F, Ferreira TAA, Córdova MM, Dombrowski PA, da Cunha C, Santo C, et al. Role of brainstem serotonin in analgesia produced by low-intensity exercise on neuropathic pain after sciatic nerve injury in mice. Pain. 2015;156(12):2595-606. https://doi.org/10.1097/j.pain.0000000000000372.

30. Bennett GJ, Xie Y-K. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain. 1988;33(1):87-107. https://doi.org/10.1016/0304-3959(88)90209-6.

31. Wang H, Li X, Shan L, Zhu J, Chen R, Li Y, Yuan W, Yang L, Huang J. Recombinant hNeuritin promotes structural and functional recovery of sciatic nerve injury in rats. Frontiers in neuroscience. 2016 Dec 22;10:589. https://doi.org/10.3389/fnins.2016.00589.

32. Mohammadi MT, Amini R, Jahanbakhsh Z, Shekarforoush S. Effects of atorvastatin on the hypertension-induced oxidative stress in the rat brain. Iranian biomedical journal. 2013 Jul;17(3):152. https://doi.org/10.6091/ibj.1189.2013.

33. Brummett CM, Padda AK, Amodeo FS, Welch KB, Lydic R. Perineural dexmedetomidine added to ropivacaine causes a dose-dependent increase in the duration of thermal antinociception in sciatic nerve block in rat. Anesthesiology. 2009;111(5):1111. https://doi.org/10.1097/ALN.0b013e3181bbcc26.

34. Makker PG, Keating BA, Lees JG, Burke D, Howells J, Moalem-Taylor G. Electrophysiological investigation of motor axonal excitability in a mouse model of nerve constriction injury. Journal of the Peripheral Nervous System. 2021;26(1):99-112. https://doi.org/10.1111/jns.12430.

35. Zhou P, Zhang R, Xian L, Ning L, Lu P, Liu Q, et al. Selection of sciatic nerve injury models: implications for pathogenesis and treatment. Frontiers in Neurology. 2025; 16:1521941. https://doi.org/10.3389/fneur.2025.1521941.

36. Chen S-H, Wu C-C, Lin S-C, Tseng W-L, Huang T-C, Yadav A, et al. Investigation of neuropathology after nerve release in chronic constriction injury of rat sciatic nerve. International journal of molecular sciences. 2021;22(9):4746. https://doi.org/10.3390/ijms22094746.

37. Meng J, Zhang Q, Yang C, Xiao L, Xue Z, Zhu J. Duloxetine, a balanced serotonin-norepinephrine reuptake inhibitor, improves painful chemotherapy-induced peripheral neuropathy by inhibiting activation of p38 MAPK and NF-κB. Frontiers in Pharmacology. 2019; 10:365. https://doi.org/10.3389/fphar.2019.00365.

38. Chen S-H, Wu C-C, Tseng W-L, Lu F-I, Liu Y-H, Lin S-P, et al. Adipose-derived stem cells modulate neuroinflammation and improve functional recovery in chronic constriction injury of the rat sciatic nerve. Frontiers in Neuroscience. 2023; 17:1172740.

https://doi.org/10.3389/fnins.2023.1172740.
39. Yalçın MB, Bora ES, Erbaş O. The effect of liraglutide on axon regeneration and functional recovery after peripheral nerve lesion. Current Issues in Molecular Biology. 2024;46(1):327-39. https://doi.org/10.3390/cimb46010021.
40. Ossipov MH, Morimura K, Porreca F. Descending pain modulation and chronification of pain. Current opinion in supportive and palliative care. 2014;8(2):143-51. https://doi.org/10.1097/SPC.0000000000000055.
41. Aby F, Lorenzo L-E, Grivet Z, Bouali-Benazzouz R, Martin H, Valerio S, et al. Switch of serotonergic descending inhibition into facilitation by a spinal chloride imbalance in neuropathic pain. Science Advances. 2022;8(30): eabo0689. https://doi.org/10.1126/sciadv.abo0689.
42. Enomoto H, Fujikoshi S, Funai J, Sasaki N, Ossipov MH, Tsuji T, Alev L, Ushida T. Assessment of direct analgesic effect of duloxetine for chronic low back pain: post hoc path analysis of double-blind, placebo-controlled studies. Journal of pain research. 2017 Jun 1:1357-68. https://doi.org/10.2147/JPR.S133396.
43. Hoshino H, Obata H, Saito S. Antihyperalgesic effect of duloxetine and amitriptyline in rats after peripheral nerve injury: Influence of descending noradrenergic plasticity. Neuroscience letters. 2015; 602:62-7. https://doi.org/10.1016/j.neulet.2015.06.041.
44. Smith PA. The known biology of neuropathic pain and its relevance to pain management. Canadian Journal of Neurological Sciences. 2024;51(1):32-9. https://doi.org/10.1017/cjn.2023.10.
45. Zhou W, Xie Z, Li C, Xing Z, Xie S, Li M, et al. Driving effect of BDNF in the spinal dorsal horn on neuropathic pain. Neuroscience Letters. 2021; 756:135965. https://doi.org/10.1016/j.neulet.2021.135965.
46. Wang J, Zhou F, Zhang S, Mao M, Feng S, Wang X. Participation of transient receptor potential vanilloid 1 in the analgesic effect of duloxetine for paclitaxel induced peripheral neuropathic pain. Neuroscience Letters. 2022; 773:136512. https://doi.org/10.1016/j.neulet.2022.136512.
47. Zhang T-T, Xue R, Fan S-Y, Fan Q-Y, An L, Li J, et al. Ammoxetine attenuates diabetic neuropathic pain through inhibiting microglial activation and neuroinflammation in the spinal cord. Journal of Neuroinflammation. 2018;15(1):176. https://doi.org/10.1186/s12974-018-1216-3.
48. Khalil M, Teunissen CE, Lehmann S, Otto M, Piehl F, Ziemssen T, et al. Neurofilaments as biomarkers in neurological disorders—towards clinical application. Nature Reviews Neurology. 2024;20(5):269-87. https://doi.org/10.1038/s41582-024-00955-x.
49. Khalil M, Teunissen CE, Otto M, Piehl F, Sormani MP, Gattringer T, et al. Neurofilaments as biomarkers in neurological disorders. Nature Reviews Neurology. 2018;14(10):577-89. https://doi.org/10.1038/s41582-018-0058-z.
50. Andersen NE, Boehmerle W, Huehnchen P, Stage TB. Neurofilament light chain as a biomarker of chemotherapy-induced peripheral neuropathy. Trends in Pharmacological Sciences. 2024;45(10):872-9. https://doi.org/10.1016/j.tips.2024.08.001.
51. Okcay Y, Aykaç Ö, Arslan R, Bektas N. Aripiprazole: The antiallodynic and antihyperalgesic effects in chronic constriction injury-induced neuropathic pain and reserpine-induced fibromyalgia with possible mechanisms. Neuropharmacology. 2025; 273:110454. https://doi.org/10.1016/j.neuropharm.2025.110454.
52. Wagner MA, Smith EML, Ayyash N, Toledo J, Rasheed Z, Holden JE. Effectiveness of duloxetine on oxaliplatin-induced allodynia and hyperalgesia in rats. Biological Research for Nursing. 2024;26(2):248-56. https://doi.org/10.1177/10998004231209444.
53. Fiore NT, Debs SR, Hayes JP, Duffy SS, Moalem-Taylor G. Pain-resolving immune mechanisms in neuropathic pain. Nature Reviews Neurology. 2023;19(4):199-220. https://doi.org/10.1038/s41582-023-00777-3.