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Idebenone Attenuates Sciatic Nerve Ligation–Induced Neuropathic Pain Through Modulating Antioxidant and Anti-Inflammatory Mechanisms in Rats

Sinan Şafak

snnsafak@gmail.com

Ondokuz Mayıs University

Mehmet Emin Önger

Bahattin Avcı

Ondokuz Mayıs University

S. Sırrı Bilge

Ondokuz Mayıs University

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Abstract

Neuropathic pain is a complex clinical condition arising from damage to or dysfunction of the peripheral or central nervous system. This study aimed to investigate the therapeutic potential of idebenone, a compound with potent antioxidant and anti-inflammatory properties, in a rat model of neuropathic pain induced by chronic constriction injury (CCI) of the sciatic nerve, and to elucidate its underlying mechanisms through biochemical, electrophysiological, and histopathological analyses.

A total of 42 male Sprague–Dawley rats were randomly allocated into six groups: Sham, CCI, idebenone 50 mg/kg, idebenone 100 mg/kg, idebenone 200 mg/kg, and pregabalin (10 mg/kg). Following 14 days of treatment, thermal and mechanical hyperalgesia were assessed using the hot plate and paw withdrawal tests. Biochemical analyses included serum levels of superoxide dismutase (SOD), glutathione peroxidase (GPx), malondialdehyde (MDA), tumor necrosis factor-alpha (TNF-α), and interleukin-10 (IL-10), while electrophysiological, histopathological, and stereological evaluations were conducted to assess nerve tissue alterations.

Idebenone treatment significantly attenuated both thermal and mechanical hyperalgesia induced by CCI. Moreover, idebenone increased GPx, SOD, and IL-10 levels, while reducing MDA and TNF-α levels. Electrophysiologically, idebenone enhanced compound muscle action potential amplitude without significantly affecting latency. Histopathological findings indicated that idebenone did not significantly alter the number of myelinated axons but was unable to prevent the CCI-induced reduction in myelinated axon area. Overall, idebenone exhibited antioxidant, anti-inflammatory, and neuroprotective effects, as evidenced by modulation of oxidative stress markers, suppression of pro-inflammatory cytokines, enhancement of anti-inflammatory responses, and improvement in functional nerve parameters.

These findings suggest that idebenone may represent a promising therapeutic candidate for neuropathic pain; however, further clinical studies are warranted to establish its efficacy and translational potential in human subjects.

Introduction

Neuropathic pain is a chronic pain condition resulting from a lesion or dysfunction of the peripheral or central nervous system and is typically described as a burning or electric shock-like sensation*(*Colloca et al., 2017). It affects approximately 3–10% of the global population and is becoming increasingly prevalent due to population aging and the rising incidence of chronic diseases such as diabetes and cancer*(*Bouhassira, 2019*)*. The pathogenesis of neuropathic pain is complex and involves multiple interconnected mechanisms, including increased oxidative stress, neuroinflammation, endoplasmic reticulum stress, and ion channel dysregulation *(*Inceoglu et al., 2015). This condition significantly reduces patients' quality of life and imposes a substantial socioeconomic burden, highlighting the urgent need for more effective therapeutic approaches.

Idebenone, a short-chain synthetic analogue of coenzyme Q10 (CoQ10), is a quinone derivative with potent antioxidant properties and a critical role in mitochondrial energy metabolism (Gueven et al., 2021). It has been investigated as a therapeutic agent in disorders characterized by mitochondrial dysfunction and oxidative stress(Kumar & Singh, 2015). In addition to its antioxidant effects, idebenone exerts anti-inflammatory actions and contributes to maintaining cellular redox homeostasis(Yan et al., 2019). Beneficial effects have been reported in both clinical and preclinical studies of neurodegenerative conditions such as Alzheimer's disease, Friedreich's ataxia, Leber's hereditary optic neuropathy, and Duchenne muscular dystrophy(Di Prospero et al., 2007). Although idebenone has demonstrated neuroprotective effects in several neurological disorders, its potential role in neuropathic pain has not been fully elucidated.

Given the central involvement of oxidative stress and inflammation in the pathophysiology of neuropathic pain, we hypothesized that idebenone may attenuate neuropathic pain via modulating oxidative and inflammatory pathways and improving mitochondrial function. Therefore, the present study aimed to investigate the protective effects of idebenone and to elucidate its underlying molecular, cellular, and functional mechanisms in a sciatic nerve constriction-induced neuropathic pain model.

Materials and Methods

Animals

Forty-two adult male Sprague–Dawley rats (250–300 g) were obtained from the Ondokuz Mayıs University Experimental Animal Research Center. Animals were housed under controlled laboratory conditions (22 ± 2°C, 12-h light/dark cycle, 50–60% humidity) with ad libitum access to standard chow and water. A maximum of three rats were housed per cage.

All experimental procedures were approved by the Institutional Animal Care and Use Committee (HADYEK_2023/52) and conducted in accordance with the ARRIVE guidelines and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Animals were randomly assigned to experimental groups using a computer-generated randomization schedule.

Experimental Groups

Animals were divided into six groups (n = 7 per group):

Sham group – Sciatic nerve exposure without ligation; vehicle administered.

CCI group – Chronic constriction injury (CCI) + vehicle.

CCI + Idebenone 50 mg/kg

CCI + Idebenone 100 mg/kg

CCI + Idebenone 200 mg/kg

CCI + Pregabalin 10 mg/kg

Idebenone and pregabalin were administered intraperitoneally (i.p.) once daily for 14 consecutive days, starting 24 hours after surgery. The vehicle group received an equivalent volume of physiological saline intraperitoneally (i.p.).

Drug doses were selected based on previously published studies demonstrating pharmacological efficacy in neuroprotective models(Karakus et al., 2025).

Induction of Chronic Constriction Injury (CCI)

Neuropathic pain was induced using the chronic constriction injury model. Rats were anesthetized with ketamine/xylazine (80/10 mg/kg, i.p.). The right sciatic nerve was exposed at the mid-thigh level under sterile conditions. Four loose ligatures (4/0 chromic gut) were placed around the nerve at 1-mm intervals until a brief twitch in the corresponding hind limb was observed. In the sham group, the sciatic nerve was exposed but not ligated. Muscle and skin layers were sutured separately. Animals were monitored daily for signs of distress or wound complications.

Locomotor Activity Assessment

Spontaneous locomotor activity was assessed using an automated activity monitoring system (Ugo Basile, Italy). Rats were placed individually in the activity chamber, and horizontal movements were recorded for 5 minutes. Measurements were performed on Day 0 (baseline) and Day 15.

Thermal Nociception

Thermal hyperalgesia was evaluated using a hot plate apparatus maintained at 55 ± 2°C (Ugo Basile, Italy). The latency to hind paw withdrawal or jumping was recorded. A cut-off time of 20 seconds was established to prevent tissue damage. Tests were conducted on Days 0, 8, and 15.

Mechanical Hyperalgesia

Mechanical hyperalgesia was assessed using a Randall–Selitto analgesimeter (Ugo Basile, Italy). A gradually increasing mechanical force was applied to the plantar surface of the hind paw until a withdrawal response occurred. The mean of three measurements was recorded. Assessments were performed on Days 0, 8, and 15.

Electrophysiological Analysis

Electromyographic (EMG) recordings were performed prior to sacrifice using a PowerLab 4SP system (AD Instruments, Australia). Under anesthesia, the right sciatic nerve (injured side) was re-exposed. The stimulating electrode was placed 10 mm distal to the sciatic notch, and recording electrodes were positioned over the gastrocnemius muscle. Compound muscle action potentials (CMAPs) were recorded by delivering supramaximal electrical stimulation (0.01–10 mV). CMAP amplitude and latency were calculated as the mean of three consecutive recordings.

Histopathological Analysis

The harvested sciatic nerve tissue was fixed in 4% glutaraldehyde solution, processed for tissue embedding, and ultimately embedded in resin blocks. Semi-thin sections (0.5 mm thickness) were prepared using an ultramicrotome and stained with toluidine blue. Sections were examined under a light microscope (Olympus, BX43, Center Valley, PA, USA) equipped with a camera attachment and analyzed using dedicated microscopy software (cellSens Entry). Sections for stereological analysis were imaged at x40 magnification, while images for detailed histopathological examination of cellular components were captured at x100 magnification.

Stereological Assessment

The quantification of myelinated axons in the sciatic nerve cross-sections was performed using the 2D dissector method. For myelinated axon counting, a step size of 57506.08 μm² and an unbiased counting frame (8929.03 μm²) were used. Axon area and myelin sheath thickness were measured using the planimetric method with the specialized microscopy software (CellSens Entry).

Biochemical Analysis

Following anesthesia, blood samples were collected from the left ventricle and centrifuged at 3000 × g for 10 minutes. Serum was separated, aliquoted into Eppendorf tubes, and stored at − 80°C until analysis. Serum levels of TNF-α, IL-10, glutathione peroxidase (GPx), superoxide dismutase (SOD), and malondialdehyde (MDA) were quantified using commercially available rat-specific ELISA kits (SunLong Biotech Co., Zhejiang, China) according to the manufacturer’s instructions. Absorbance was measured at 450 nm.

Statistical Analysis

The Shapiro-Wilk test was used with GraphPad Prism (trial version) to assess the normality of the data distribution. Normally distributed data across the groups were compared using one-way Analysis of Variance (ANOVA), followed by post-hoc analyses using Duncan’s and Tamhane's T2 tests for multiple comparisons. Non-normally distributed data were analyzed using the Kruskal-Wallis test, with Dunn's test utilized for post-hoc multiple comparisons. A significance level of $p < 0.05$ was established.

Results

Locomotor Activity

Neither idebenone (50, 100, or 200 mg/kg) nor pregabalin (10 mg/kg) altered spontaneous locomotor activity compared to the CCI or sham groups at baseline (Day 0) or on Day 15 ($p > 0.05$ for all comparisons). Locomotor activity levels remained comparable across all experimental groups throughout the study period **(**Fig. 1**).**

Thermal Nociception

At Day 8, no treatment group differed significantly from the CCI group ($p > 0.05$), indicating a lack of early antinociceptive effect. By Day 15, CCI animals exhibited significantly reduced paw withdrawal latency compared with the sham group ($p < 0.05$), confirming the development of thermal hyperalgesia. Idebenone at 100 and 200 mg/kg significantly attenuated thermal hyperalgesia relative to the CCI group ($p < 0.01$), whereas the 50 mg/kg dose remained ineffective. Pregabalin (10 mg/kg) produced a robust antihyperalgesic effect ($p < 0.001$) **(**Fig. 2**).**

Mechanical Hyperalgesia

On Day 8, CCI significantly reduced paw withdrawal thresholds compared with the sham group ($p < 0.001$). At this time point, neither the idebenone doses nor pregabalin significantly reversed mechanical hypersensitivity relative to the CCI group ($p > 0.05$).

By Day 15, paw withdrawal thresholds in the CCI group remained markedly lower than those in the sham group ($p < 0.001$). Treatment with idebenone at 100 and 200 mg/kg significantly increased withdrawal thresholds compared with the CCI group ($p < 0.001$), whereas the 50 mg/kg dose remained ineffective ($p > 0.05$). Pregabalin (10 mg/kg) also significantly attenuated mechanical allodynia ($p < 0.001$).

These findings indicate a dose-dependent antiallodynic effect of idebenone that becomes evident after prolonged administration **(**Fig. 3**).**

Electrophysiological Analysis

Compound muscle action potential (CMAP) amplitude values were significantly reduced in the CCI group compared to the sham group ($p < 0.001$). Idebenone at doses of 100 and 200 mg/kg, as well as pregabalin (10 mg/kg), significantly increased CMAP amplitudes relative to the CCI group ($p < 0.01$ and $p < 0.001$, respectively; Table 1). No statistically significant differences were observed among the groups with respect to CMAP latency durations ($p > 0.05$) **(**Table 1**).**

Table 1
Effect of idebenone (50, 100, and 200 mg/kg) on compound muscle action potential (CMAP) parameters. ***p < 0.001 compared to the Sham group; ##p < 0.01, ###p < 0.001 compared to the CCI group.

Groups	CMAP Latency (ms)	CMAP Amplitude (mV)
Sham	0.212 ± 0.0153	77.2 ± 5.02
CCI	0.225 ± 0.00653	65.1 ± 5.67***
Idebenone 50 mg/kg	0.217 ± 0.0232	60.5 ± 4.62
Idebenone 100 mg/kg	0.211 ± 0.0133	75.4 ± 1.74##
Idebenone 200 mg/kg	0.207 ± 0.0157	74.4 ± 5.43##
Pregabalin 10 mg/kg	0.205 ± 0.0100	76.6 ± 2.43###

Stereological Findings

Histopathological examination of sciatic nerve sections revealed normal axonal morphology, large-diameter myelin sheaths, and intact Schmidt–Lanterman incisures (*) in the sham group. In contrast, the CCI group exhibited degenerative alterations in myelin sheath structure (Dax) and clusters of regenerating myelinated axons (dashed circles). In the idebenone 50 mg/kg group, axonal morphology appeared largely preserved, similar to the sham group, with maintained myelin integrity and Schmidt–Lanterman incisures (*). The idebenone 100 mg/kg group showed regenerating axon clusters alongside areas of tissue reorganization. However, in the idebenone 200 mg/kg group, a marked number of degenerated axons (Dax) and prominent myelin vacuolization (white arrow) were observed. Similarly, in the pregabalin-treated group, increased inter-axonal spacing and myelin sheath vacuolization (white arrow) were evident in some axons **(**Fig. 4**).**

The number of myelinated axons was significantly increased in the CCI group compared to the sham group ($p < 0.01$). None of the idebenone treatment doses resulted in a significant change in axon number relative to the CCI group ($p > 0.05$). In contrast, the pregabalin-treated group exhibited a significantly lower number of myelinated axons compared to the CCI group ($p < 0.001$; Table 2).

Myelinated axon area was significantly reduced in the CCI group compared to the sham group ($p < 0.001$). Neither idebenone nor pregabalin treatments produced a statistically significant improvement in axon area ($p > 0.05$). Additionally, no significant differences were observed among the groups with respect to myelin sheath thickness ($p > 0.05$) **(**Table 2 **).**

Table 2
Effect of idebenone (50, 100, and 200 mg/kg) on axon count, axon area, and myelin sheath thickness. **p < 0.01, ***p < 0.001 compared to the Sham group; ###p < 0.001 compared to the CCI group.

Groups	Axon Count	Axon Area (μm^2)	Myelin Sheath Thickness (μm)
	Mean ± SD	Mean ± SD	Mean ± SD
Sham	6472 ± 90.6	73.5 ± 9.38	1.62 ± 0.246
CCI	7891 ± 122**	38.6 ± 10.7***	1.53 ± 0.212
Idebenone 50 mg/kg	7641 ± 176	39.2 ± 7.82***	1.51 ± 0.425
Idebenone 100 mg/kg	7434 ± 129	39.5 ± 3.37***	1.47 ± 0.163
Idebenone 200 mg/kg	7619 ± 90.8	41.9 ± 6.71***	1.53 ± 0.399
Pregabalin 10 mg/kg	6025 ± 195###	33.9 ± 4.00***	1.42 ± 0.373

Biochemical Findings

Blood glutathione peroxidase (GPx) levels were significantly reduced in the CCI group compared with the sham group ($p < 0.05$). Treatment with idebenone at 100 and 200 mg/kg significantly restored GPx levels ($p < 0.01$ and $p < 0.001$, respectively), comparable to the effect observed with pregabalin ($p < 0.01$). Similarly, Superoxide dismutase (SOD) levels were markedly decreased following CCI ($p < 0.001$). This reduction was significantly reversed by idebenone at 200 mg/kg and by pregabalin ($p < 0.001$). In contrast, Malondialdehyde (MDA) levels, a marker of lipid peroxidation, were significantly elevated in the CCI group ($p < 0.01$). All doses of idebenone, as well as pregabalin, significantly attenuated this increase ($p < 0.01$), indicating reduced oxidative damage. Regarding inflammatory mediators, TNF-α levels were significantly increased in the CCI group ($p < 0.05$), whereas IL-10 levels were significantly decreased ($p < 0.01$). Idebenone at 200 mg/kg significantly suppressed TNF-α ($p < 0.001$) and enhanced IL-10 levels ($p < 0.01$). Pregabalin exerted similar modulatory effects ($p < 0.01$ and $p < 0.05$, respectively). Collectively, these findings indicate that idebenone mitigates CCI-induced oxidative stress and inflammatory imbalance in a dose-dependent manner **(**Table 3**).**

Table 3
Effect of idebenone (50, 100, and 200 mg/kg) on serum biochemical parameters (GPx, SOD, MDA, TNF-α, and IL-10). *p < 0.05, **p < 0.01, ***p < 0.001 compared to the Sham group; #p < 0.05, ##p < 0.01, ###p < 0.001 compared to the CCI group. **GPx**: Glutathione peroxidase; **SOD**: Superoxide dismutase; **MDA**: Malondialdehyde; **TNF-α**: Tumor necrosis factor-alpha; **IL-10**: Interleukin-10.

Groups	GPx (pg/mL)	SOD (pg/mL)	MDA (ng/mL)	TNF-α (ng/mL)	IL-10 (pg/mL)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Sham	1575 ± 71.6	19015 ± 2532	161 ± 22.7	153 ± 38.0	220 ± 90.0
CCI	365 ± 119*	2392 ± 250***	278 ± 66.1**	352 ± 98.8*	69.1 ± 33.8**
Idebenone 50 mg/kg	666 ± 462	2177 ± 236	164 ± 10.0##	211 ± 42.9	94.2 ± 35.2
Idebenone 100 mg/kg	1916 ± 1496##	5146 ± 6398	164 ± 6.94##	212 ± 80.5	168 ± 57.0
Idebenone 200 mg/kg	3850 ± 1611###	17610 ± 7677###	159 ± 11.2##	102 ± 27.0###	201 ± 69.6##
Pregabalin 10 mg/kg	2153 ± 1571##	22809 ± 6208###	165 ± 11.5##	130 ± 32.6##	192 ± 68.6#

Discussion

In the present study, we investigated the therapeutic effects of idebenone in a rat model of neuropathic pain induced by chronic constriction injury (CCI) of the sciatic nerve. Our findings demonstrate that idebenone significantly attenuated thermal hyperalgesia and mechanical allodynia—hallmark behavioral features of CCI-induced neuropathic pain. These behavioral improvements were accompanied by modulation of oxidative stress markers, regulation of inflammatory cytokines, partial restoration of electrophysiological function, in addition to histological improvement.

Idebenone, a short-chain analog of coenzyme Q10 with a hydroxydecyl side chain, is known to activate mitochondrial function by transferring electrons to the electron transport chain complex III, thereby promoting adenosine triphosphate (ATP) production(Yi et al., 2025). Idebenone is a multifaceted therapeutic agent, extending its utility beyond a simple mitochondrial electron carrier to a potent modulator of oxidative stress and systemic inflammation. According to Choi et al., idebenone significantly attenuates systemic inflammatory responses by suppressing the NF-κB signaling pathway and inhibiting lipid peroxidation, thereby improving survival rates in models of acute sepsis(Choi et al., 2024). In the realm of neuroprotection, Chen et al. demonstrated that idebenone orchestrates a dual defense mechanism following intracerebral hemorrhage: it activates the Nrf2 pathway to bolster endogenous antioxidant defenses while simultaneously reducing pro-inflammatory cytokines such as TNF-α and IL-1β(Chen et al., 2025). Furthermore, research by Li et al. underscores its cardioprotective

potential, revealing that idebenone preserves cardiac function after myocardial infarction by inhibiting ferroptosis and excessive autophagy via the ROS-AMPK-mTOR axis(Li et al., 2023). Collectively, these findings suggest that idebenone's ability to neutralize reactive oxygen species and quench inflammatory cascades positions it as a versatile candidate for treating a wide spectrum of oxidative stress-related disorders.

Recent experimental evidence suggests that idebenone may exert beneficial effects in neuropathic pain conditions primarily through its antioxidant and mitochondrial-targeting properties. In preclinical models of neural injury, idebenone has been shown to attenuate oxidative stress–induced neuronal damage, inhibit apoptosis and ferroptosis, and promote functional recovery, mechanisms that are highly relevant to the pathophysiology of neuropathic pain. Given that mitochondrial dysfunction, excessive reactive oxygen species (ROS) production, and neuroinflammation are key drivers of neuropathic pain, idebenone's ability to restore mitochondrial electron transport and reduce oxidative damage may indirectly alleviate pain hypersensitivity. Although direct clinical studies on neuropathic pain are still limited, recent reviews emphasize idebenone as a promising neuroprotective agent with potential applications beyond classical indications, warranting further investigation in chronic pain models and clinical settings(Yi et al., 2025).

In our study, Idebenone (50 mg/kg) did not significantly alter mechanical allodynia or thermal hyperalgesia. In contrast, moderate and high doses (100 and 200 mg/kg) significantly attenuated mechanical allodynia and prevented CCI-induced thermal hyperalgesia. Consistent with these findings, CoQ10 has been reported to reduce mechanical allodynia and reverse thermal hyperalgesia in a streptozotocin-induced diabetic neuropathy model(Zhang et al., 2013). Collectively, these observations suggest that the neuroprotective effects of idebenone may extend beyond symptomatic pain relief to functional improvement of injured peripheral nerves.

Idebenone appears to exhibit a clear dose-dependent pharmacological profile, which may explain the lack of efficacy observed at low doses in neuropathic pain models. Although direct evidence in neuropathic pain is limited, findings from other neurological conditions support the presence of a therapeutic threshold. For instance, in a randomized clinical study on Friedreich's ataxia, low-dose idebenone (10–30 mg/kg/day) failed to demonstrate significant clinical benefit, whereas higher doses (up to 90 mg/kg/day) significantly slowed disease progression, indicating that subtherapeutic dosing may be insufficient to elicit mitochondrial and antioxidant effects(Meier & Buyse, 2009). Given that neuropathic pain is strongly associated with mitochondrial dysfunction, oxidative stress, and neuroinflammation, insufficient dosing may fail to adequately modulate these mechanisms. Therefore, the apparent lack of effect at low doses in neuropathic pain models likely reflects an inability to reach the critical pharmacodynamic threshold required for neuroprotection and analgesia. Our findings, which manifest the insufficient role of idebenone (50 mg/kg), may be attributed to the mechanisms indicated above.

The underlying mechanism of this antinociceptive effect appears to be multifactorial. Idebenone modulated the systemic immune response by suppressing pro-inflammatory cytokines and restoring anti-inflammatory cytokine levels, while concurrently reducing oxidative stress markers. These biochemical effects were supported by electrophysiological findings demonstrating that idebenone restored compound muscle action potential (CMAP) amplitudes diminished by nerve injury. Notably, although idebenone did not significantly alter the total number of myelinated axons or prevent the reduction in myelinated axon area, its functional benefits were clearly preserved. Collectively, these findings suggest that idebenone may represent a promising therapeutic candidate for neuropathic pain following peripheral nerve trauma.

In our study, idebenone significantly reduced malondialdehyde (MDA) levels while increasing superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities, indicating restoration of redox balance. These biochemical changes parallel the observed behavioral improvements and support the hypothesis that antioxidant reinforcement plays a central role in idebenone's antinociceptive effects. Oxidative stress plays a central role in the pathogenesis of neuropathic pain. Excessive production of reactive oxygen and nitrogen species (ROS/RNS) contributes to neuronal damage, pain hypersensitivity, and the amplification of inflammatory responses(Uttara et al., 2009). Antioxidant interventions have demonstrated therapeutic benefits in various neuropathic pain models. Free radical scavengers and antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) reduce oxidative damage and alleviate hyperalgesia(Kim et al., 2010). Superoxide radicals, key mediators of inflammatory pain, can react with nitric oxide to form peroxynitrite, which impairs SOD activity and further disrupts redox balance(Wang et al., 2004). GPx plays a critical role in detoxifying hydrogen peroxide and limiting peroxynitrite-mediated oxidative injury. Reduced GPx activity and elevated malondialdehyde (MDA)—a marker of lipid peroxidation—have been consistently reported in neuropathic pain models(Hendrix et al., 2020). Notably, antioxidant compounds such as Tanshinone IIA, diosgenin, and mitoquinone have been shown to decrease MDA levels and restore antioxidant enzyme activity in experimental neuropathic pain(Cao et al., 2015; Kiasalari et al., 2017; Chen et al., 2020).

Inflammation is another major contributor to neuropathic pain chronification. Following nerve injury, pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 increase in peripheral nerves, dorsal root ganglia, and the central nervous system(Sommer et al., 2018). These mediators are counterbalanced by anti-inflammatory cytokines, particularly IL-10, which suppresses pro-inflammatory signaling at multiple regulatory levels. Modulating inflammatory pathways has been shown to reduce nociceptive responses in CCI models. Idebenone has demonstrated anti-inflammatory properties across several experimental conditions(Jiang et al., 2021). In our study, idebenone significantly reduced TNF-α levels and increased IL-10 levels in CCI rats, indicating a shift toward an anti-inflammatory profile. These findings support the hypothesis that idebenone mitigates neuropathic pain not only through antioxidant mechanisms but also by modulating inflammatory pathways.

To further characterize nerve function, we evaluated the effects of idebenone on CMAP amplitude and latency in the sciatic nerve. CMAP amplitude correlates with axonal area in peripheral nerves, whereas

latency reflects conduction velocity and myelin integrity(Wolthers et al., 2005). Indeed, decreased myelin thickness is strongly associated with prolonged latency(Zhou et al., 1995). In our study, the reduction in CMAP amplitude in the CCI group confirms that chronic constriction induces substantial axonal damage. This finding is noteworthy given the limited number of electrophysiological studies reporting CCI-induced reductions in CMAP amplitude. The persistently low amplitude observed in the low-dose idebenone group (50 mg/kg) suggests insufficient functional recovery, whereas the higher amplitudes detected in the 100 and 200 mg/kg idebenone groups and the pregabalin group (10 mg/kg) indicate marked restoration of axonal function. The absence of significant latency differences among groups suggests that the severity of CCI in our model was insufficient to compromise myelin sheath integrity, consistent with preserved mean myelin thickness. Although CMAP and latency measurements are useful indicators of functional recovery, they do not necessarily reflect the exact number of regenerated axons. Regenerated axons can compensate for fiber loss by reinnervating multiple muscle fibers, thereby restoring CMAP amplitude despite incomplete structural regeneration(Rafuse & Gordon, 1996). For this reason, histological evaluation of the sciatic nerve was necessary to more accurately assess the extent of axonal regeneration. In our study, histopathological examination of the idebenone-treated groups (100 and 200 mg/kg) and the pregabalin group (10 mg/kg) revealed empty myelinated axonal spaces filled with phagocytosed connective tissue, a limited number of intact myelinated axons, and residual myelin debris. These findings suggest that the macrophage infiltration required for effective nerve regeneration may have been attenuated by the anti-inflammatory properties of idebenone and pregabalin, potentially delaying regeneration and impairing the formation of an optimal microenvironment for nerve repair. This interpretation is supported by our findings regarding myelinated axon counts and axonal areas, as none of the treatment groups demonstrated a significant improvement in myelinated axon regeneration or prevention of axonal area reduction during the 14-day acute phase.

In conclusion, idebenone attenuated behavioral manifestations of neuropathic pain and improved electrophysiological function in a CCI model, primarily through modulation of oxidative stress and inflammatory pathways. Although structural regeneration of myelinated fibers was not significantly enhanced during the acute phase, the functional benefits observed support further investigation of idebenone as a potential adjunctive strategy in neuropathic pain management. Long-term studies are warranted to clarify its effects on nerve regeneration and chronic pain progression.

Declarations

Conflict of interest

The authors have no conflicts of interest to declare.

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Author Contribution

All authors reviewed the manuscript

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Figures

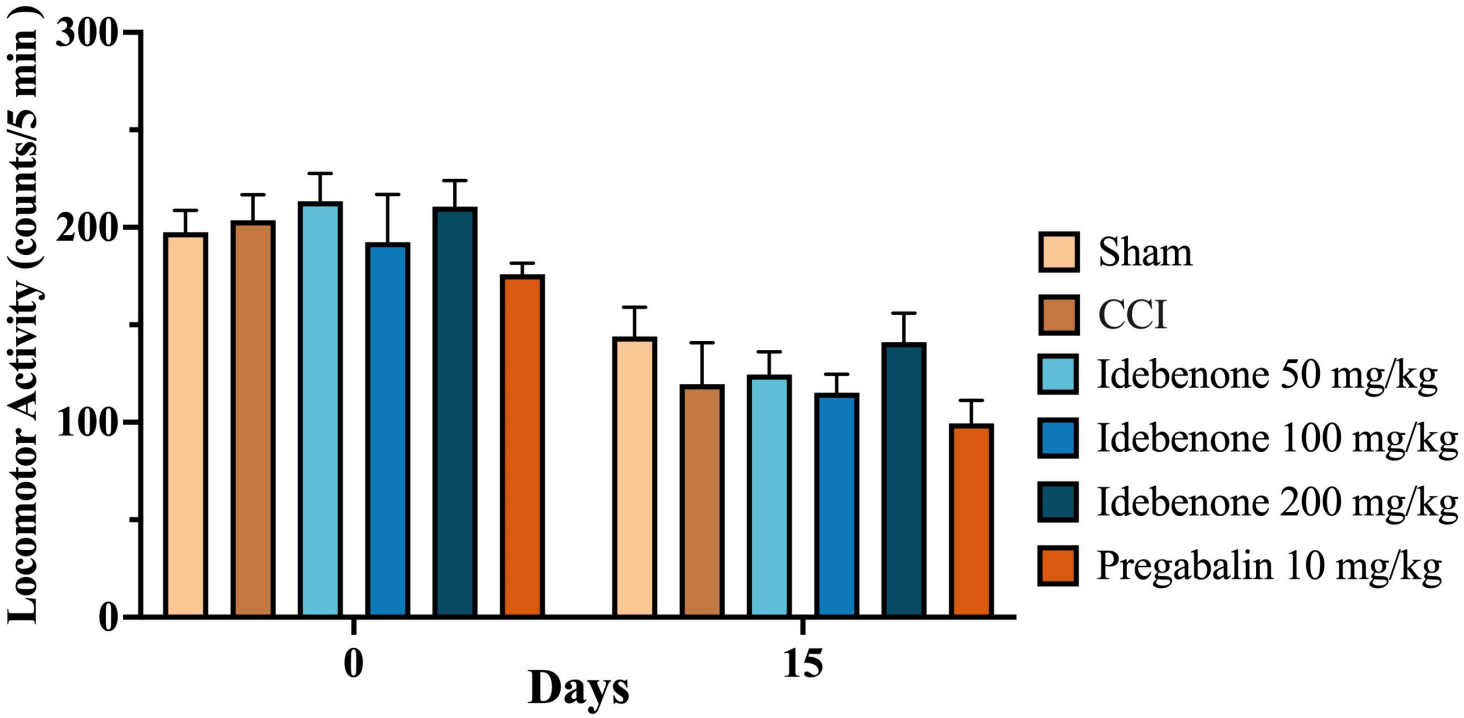


Figure 1

Effect of idebenone (50, 100, and 200 mg/kg) and pregabalin (10 mg/kg) on locomotor activity. Data are presented as mean ± SEM (n = 7 per group). No significant differences were observed between groups at any time point.

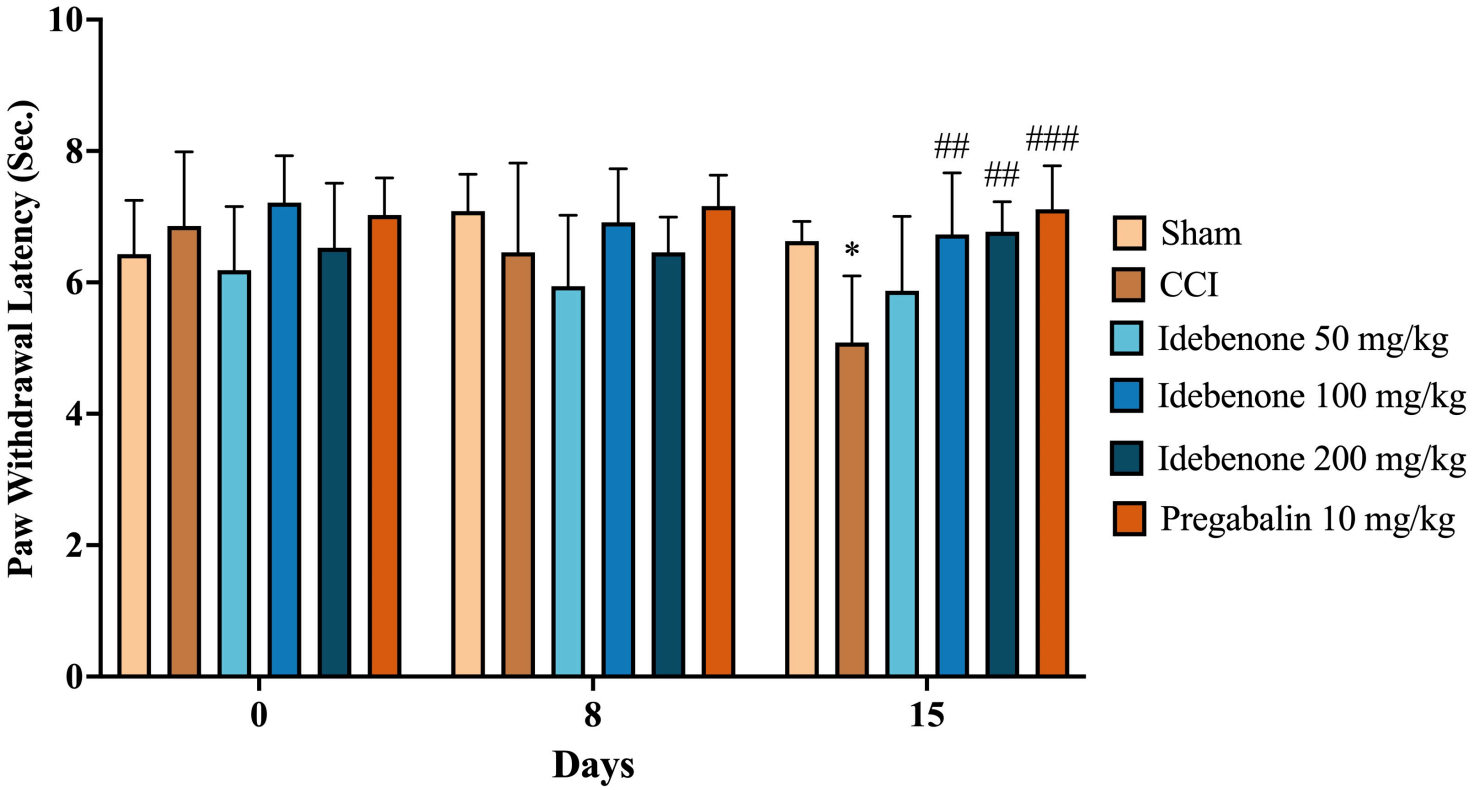


Figure 2

Effect of idebenone (50, 100, and 200 mg/kg) and pregabalin (10 mg/kg) on hot plate latency (*p<0.05 compared to the Sham group; ##p<0.01, ###p<0.001 compared to the CCI group). Data are presented as mean ± SEM (n = 7 per group)

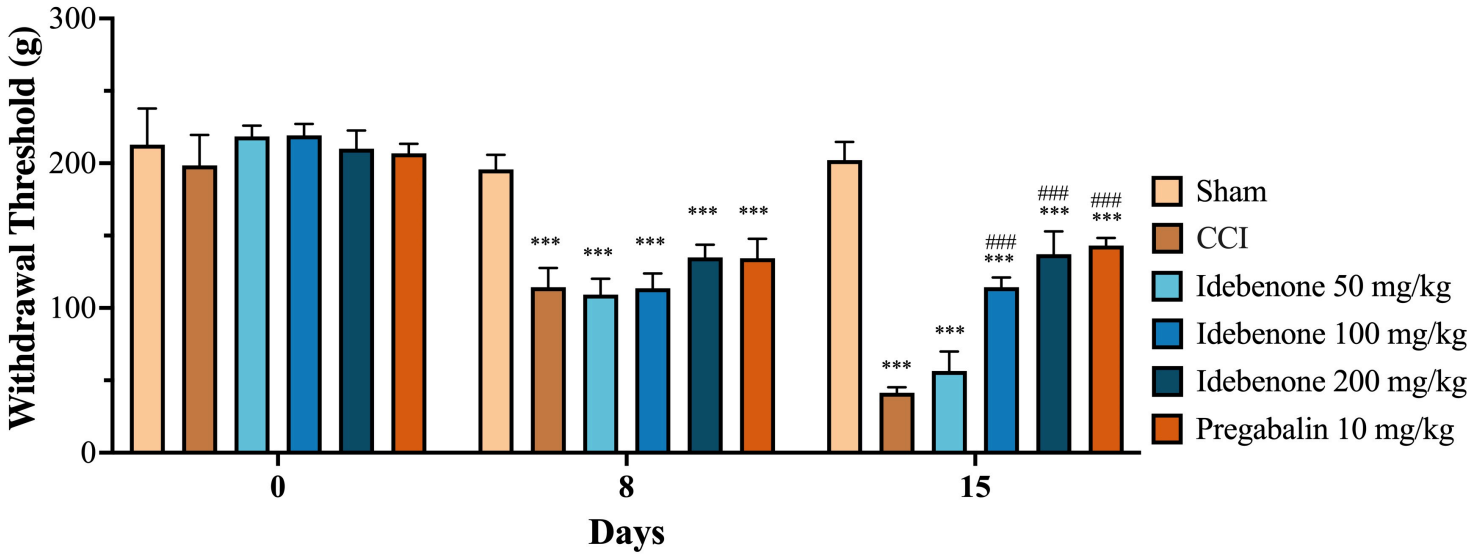


Figure 3

Effect of idebenone (50, 100, and 200 mg/kg) and pregabalin (10 mg/kg) on mechanical allodynia. (***p<0.001, compared to the sham group), (### p<0.001, compared to the CCI group).

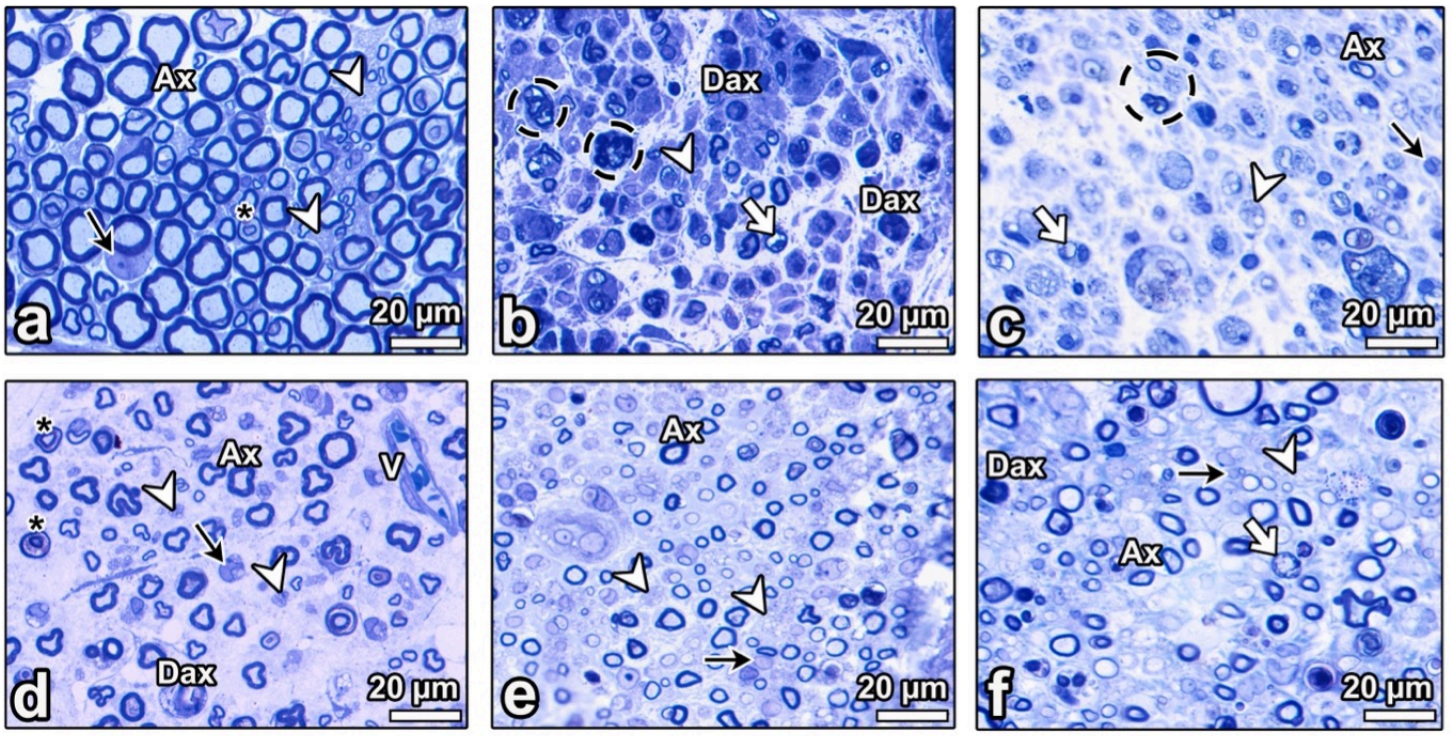


Figure 4

Histopathological examination of the sciatic nerve. **(a)** Sham group showing healthy axons (**Ax**) and Schmidt-Lanterman incisures (*). **(b)** CCI group showing degenerative changes in myelinated axons and myelin sheath structure (**Dax**). **(c)** Pregabalin (10 mg/kg) group showing vacuolization (**white arrow**). **(d)** Idebenone (50 mg/kg) group showing axons (**Ax**) and Schmidt-Lanterman incisures (*). **(e)** Idebenone (100 mg/kg) group. **(f)** Idebenone (200 mg/kg) group showing degenerated axon (**Dax**) and vacuolizations (**white arrow**). **Staining:** Toluidine blue. **Dashed circle:** Clusters of regenerating myelinated axons. **Arrowhead:** Unmyelinated nerve fibers. **Black arrow:** Schwann cell. **V:** Blood vessel. **Scale bar:** 20 µm.