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Original Article

Anti-nociceptive effect of black seed oil on an animal model of chronic constriction injury

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Abstract

Background and purpose: Traditionally, *Nigella sativa* L. has been known as a medical intervention to treat numerous diseases. This study aimed at investigating the antihyperalgesic effect of black seed oil (BSO) in an experimental model of neuropathic pain.
Experimental approach: Chronic constriction injury (CCI) was performed under anesthesia. The sciatic nerve was ligated with four loose ties. Two separate protocols were used to administer BSO. In chronic treatment, rats were given daily doses of BSO (250, 500, and 1000 mg/kg p.o.) from the 1st day until the 21st post-CCI day. While, in acute treatment, BSO (250, 500, and 1000 mg/kg p.o.) was administered only on the 7th, 14th, and 21st days. CCI and sham groups were given almond oil according to the same schedule. Behavioral scores were determined by evaluation of the paw withdrawal in the plantar, Von Frey, and acetone tests, on the 7th, 14th, and 21st days.
Findings/Results: Our results showed that CCI leads to significant allodynia and hyperalgesia in the ipsilateral paw after surgery. Chronic administration of BSO (500 and 1000 mg/kg) obviously attenuated heat hyperalgesia and mechanical allodynia. However, daily administration of BSO did not alter cold allodynia. Nevertheless, when BSO was administered, 30 min before the pain assessment tests, hypersensitivity was not improved in the treated animals.
Conclusion and implications: These results demonstrated BSO can inhibit neuropathic pain progression and suggests a potential use of BSO to manage hyperalgesia and allodynia. However, additional research is necessary to approve BSO effectiveness, in neuropathic pain conditions.

Keywords: Chronic constriction injury; Neuropathic pain; *Nigella sativa* L; Rat; Black seed oil.

INTRODUCTION

From ancient times, medical herbs have been considered to treat human illnesses. Recently, there has been an obvious attraction to medicinal herbs and their main components instead of the chemical drugs in neurological disorders (1).

Nigella sativa L. (*N. sativa*), also identified as the black seed, traditionally has been applied to treat many diseases, including arthritis, asthma, diabetes, and gastrointestinal disorders (2).

The effective components of *N. sativa* are primarily found in the essential or fixed oil of seeds. The black seeds consist of main active ingredients such as flavonoids, phytosterols, polyphenols, alkaloids, and saponins. Thymoquinone, thymohydroquinone, thymol, and dithymoquione are the most pharmacologically active components found in *N. sativa* seeds (3).

Recently it has been demonstrated that thymoquinone, a component of black seeds oil (BSO), has antinociceptive and anti-inflammatory properties (4).

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Previous studies have shown that oral administration of BSO attenuated the thermal and mechanical stimulus in the early phase of the formalin test. Also, in the writhing test, *N. sativa* suppressed inflammatory nociception (5). It has been demonstrated that the volatile oil of *N. sativa* seeds relieves inflammation in the carrageenan model of paw edema. Also, the anti-inflammatory effect of black cumin seed essential oil has been demonstrated in the formalin and writhing tests in mice (6,7). However, the effect of BSO on neuropathic pain has not been determined so far. Neuropathic pain is defined as a debilitating condition that results from injury or dysfunction of the nervous systems and often receives inadequate treatment from current medications (8).

Numerous etiological factors are involved in the generation and progression of peripheral neuropathy. Systemic diseases, metabolic disorders, nutritional deficiencies, alcoholism, infections, genetic disorders, and medications can lead to peripheral neuropathy (9).

The current medical options for neuropathic pain management are mainly anticonvulsants and antidepressants (10). However, in many patients, these medications are not effective. Untreated symptoms of peripheral neuropathy result in psychological conditions such as insomnia and depression (11). So, finding effective treatment with analgesic properties is crucial to achieving superior therapeutic efficacy. We hypothesized that BSO treatment would inhibit the pain-related behavior in neuropathic pain following chronic constriction injury.

MATERIAL AND METHODS

Chemicals

BSO (standardized based on at least 495-605 mg linoleic acid and 6.5 mg thymoquinone in each 1000 mg) was purchased from Barij essences pharmaceutical Co, Iran. BSO was diluted in almond oil.

Animals

Male Sprague-Dawley rats, weighing 200-250 g, were kept in a constant environment of temperature, humidity, 12/12-h light/dark cycles and allowed free access to food and water. This research was carried out on. All the experiments were permitted by the Ethics Committee for Animal Research of the Kashan University of Medical Sciences (Ethics No. IR.KAUMS.AEC.1400.002).

Acute treatment

In this treatment protocol, BSO (250, 500, and 1000 mg/kg p.o.) was administered only on days 7, 14, and 21, and 30 min later, behavioral tests were performed. Indeed, the effect of a single dose of BSO on pain sensitivity was tested on the 7^{th}, 14^{th}, and 21^{st} days.

Chronic treatment

It was hypothesized that neuroprotective agents should be administered promptly after nerve lesion (12). So, rats received BSO (250, 500, and 1000 mg/kg p.o.) from the 1^{st} day after nerve ligation to the 21^{st} day. Behavioral tests were evaluated on days 7, 14, and 21 after chronic constriction injury (CCI). CCI and sham groups received almond oil according to the same schedule.

CCI surgery

CCI was done in accordance with the study of Bennett and Xie (13,14). Briefly, rats were anesthetized by ketamine (50 mg/kg i.p.) and xylazine (10 mg/kg i.p.) cocktail. After a skin incision, the sciatic nerve was uncovered and dissociated from the surrounding tissue, and then four loose ligatures were tied around the (4.0 chromic gut, Harvard Apparatus Inc., Holliston, MA) sciatic nerve at 1 mm intervals. In the sham group, surgery has been done, but the sciatic nerve remains intact (15).

Behavioral tests

Heat hyperalgesia

Rats were located into the Plexiglas apparatus and permitted to acclimatize to the environment (16). The hind paw is exposed to an infrared beam from a heat source (Plantar Analgesia Meter, Ugo Basile, and Varese, Italy). The paw withdrawal latency in the injured hind paw was recorded in seconds (17).

Mechanical allodynia

To determine mechanical allodynia, the nerve-ligated hind paw was stimulated by Von Frey filaments (steeling, Wood Dale, IL, USA) (18). Mechanical allodynia is defined as the maximum (gram) force used to provoke paw withdrawal (19).

Cold allodynia

Cold allodynia was assessed, by acetone (100 μL) spraying the injured hind paw. Acetone

evaporation evoked a cooling sense, accompanied by paw withdrawal (20). This test was done five times (at 5 min intervals). Cold allodynia was reported as a percentage of paw withdrawal frequency.

Statistical analysis

The Kolmogorov-Smirnov test was used to investigate the normal distribution of data. Data were represented as the mean ± SEM. Results were analyzed by one-way repeated-measures ANOVA followed by Tukey post hoc test. The student's *t*-test was used to characterize differences between two groups, such as sham and CCI groups. Nonparametric data were subjected to Kruskal-Wallis analysis followed by the Mann-Whitney test.

RESULTS

Behavioral tests of neuropathic pain

Following CCI, paw withdrawal threshold (Fig. 1A), as well as paw withdrawal latency (Fig. 1B) in the injured paw was significantly reduced and paw withdrawal frequency (Fig. 1C) increased compared to the sham group.

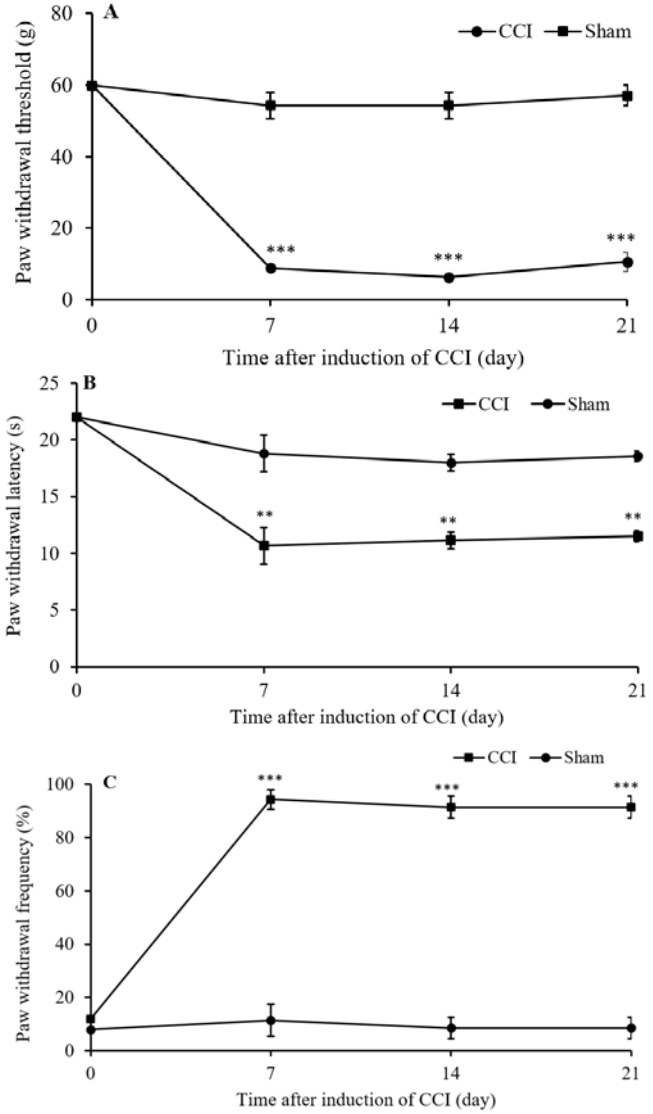

Fig. 1. The effects of CCI on (A) the mechanical allodynia; (B) the heat hyperalgesia; and (C) the cold allodynia. The CCI groups received normal saline according to the treatment program. Sham group had the same surgery, nerve ligation was not made. CCI and Sham groups were given almond oil according to the same schedule. The results are expressed as mean ± SEM, n = 8. $^{**}P < 0.01$ and $^{***}P < 0.001$ indicate significant differences compared with the sham group. CCI, chronic constriction injury.

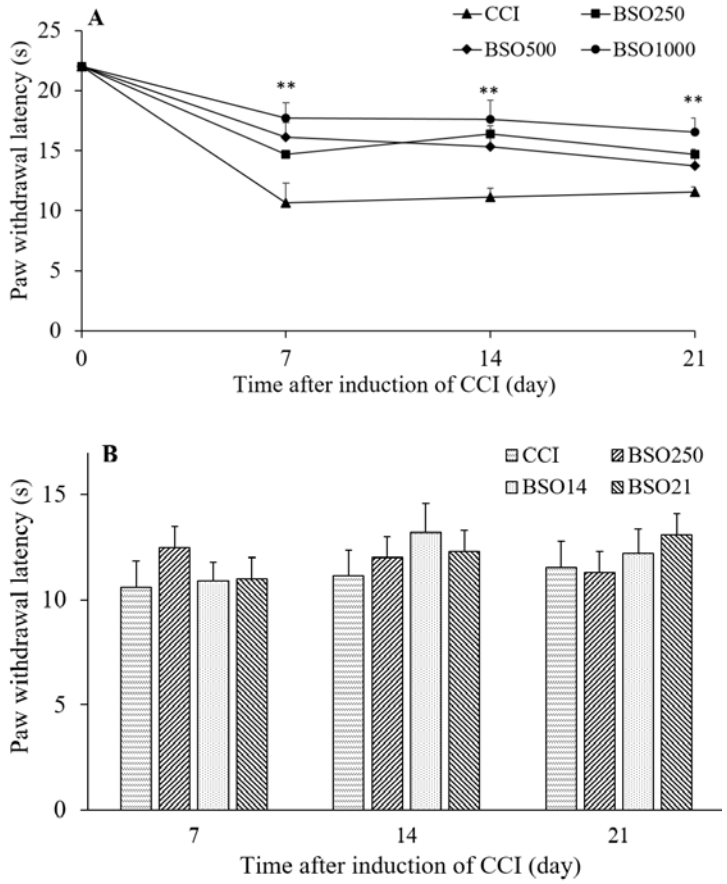

Fig. 2. The effects of BSO on the development of neuropathic pain. The effect of chronic treatment with BSO (250, 500, and 1000 mg/kg; p.o.) from the 1st day to the 21st day after surgery on the development of heat hyperalgesia; (B) the effect of acute treatment with BSO (250, 500, and 1000 mg/kg; p.o.) on the heat hyperalgesia. The CCI groups received vehicles according to the treatment schedule. CCI group was given almond oil according to the same schedule. The results are expressed as mean ± SEM, n = 8. $^{**}P < 0.01$ indicates significant differences versus the CCI group. BSO, Black seed oil; CCI, chronic constriction injury.

Effect of BSO on heat hyperalgesia

Chronic constriction injury, an animal model of hyperalgesia, mimics human clinical pain conditions. Thermal hyperalgesia is defined as a thermal stimulus that at normal temperature induces paw withdrawal. Daily administration of BSO (1000 mg/kg p.o.) inhibited heat hyperalgesia at the 7th, 14th, and 21st post-CCI days (Fig. 2A). However, acute BSO administration at the 7th, 14th, and 21st days did not modify paw withdrawal latency (Fig. 2B).

Effect of BSO on mechanical allodynia

Allodynia is described as a response to pressure that does not generally elicit pain. Identification of allodynia is a very principal feature in pain-related behavior detection (9). In comparison to the sham group, the pain threshold was increased in the injured hind paw. Paw withdrawal threshold was attenuated when rats received daily BSO (500 and 1000 mg/kg p.o.) and the response was measured at days 7, 14, and 21 post-surgery (Fig. 3A). On the other hand, acute BSO administration did not alter pain sensitivity (Fig. 3B).

Effect of BSO on cold allodynia

Long-term administration of BSO had no significant effect on the response to acetone spraying. In these groups, the number of responses did not change significantly. Also, short-term administration of BSO did not change the paw withdrawal frequency (Fig. 4A and 4B).

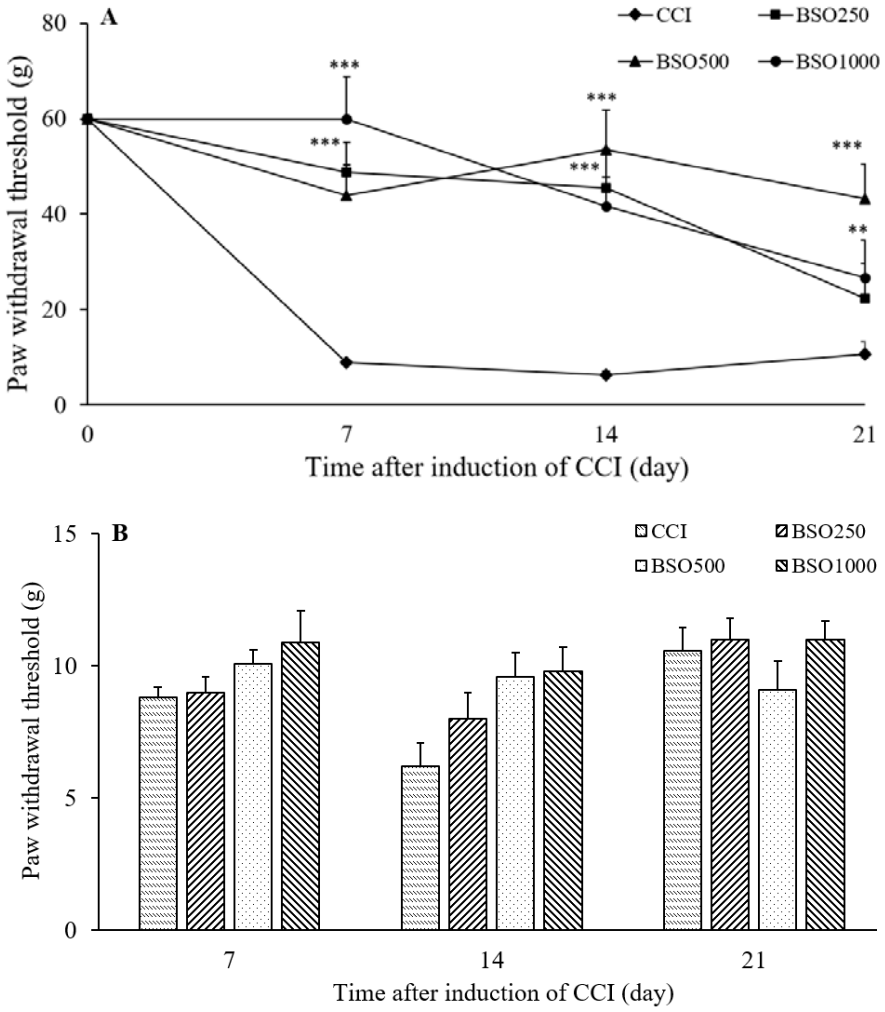

Fig. 3. (A) The effect of chronic treatment with BSO (250, 500, and 1000 mg/kg; p.o.) from the 1st day to the 21st day after surgery on the development of the mechanical allodynia; (B) the effect of acute treatment with BSO (250, 500, and 1000 mg/kg; p.o.) on the mechanical allodynia. The CCI groups received vehicles according to the treatment schedule. CCI group was given almond oil according to the same schedule. The results are expressed as mean ± SEM, n = 8 $^{**}P < 0.01$, $^{***}P < 0.01$ indicate significant differences versus the CCI group. BSO, Black seed oil; CCI, chronic constriction injury.

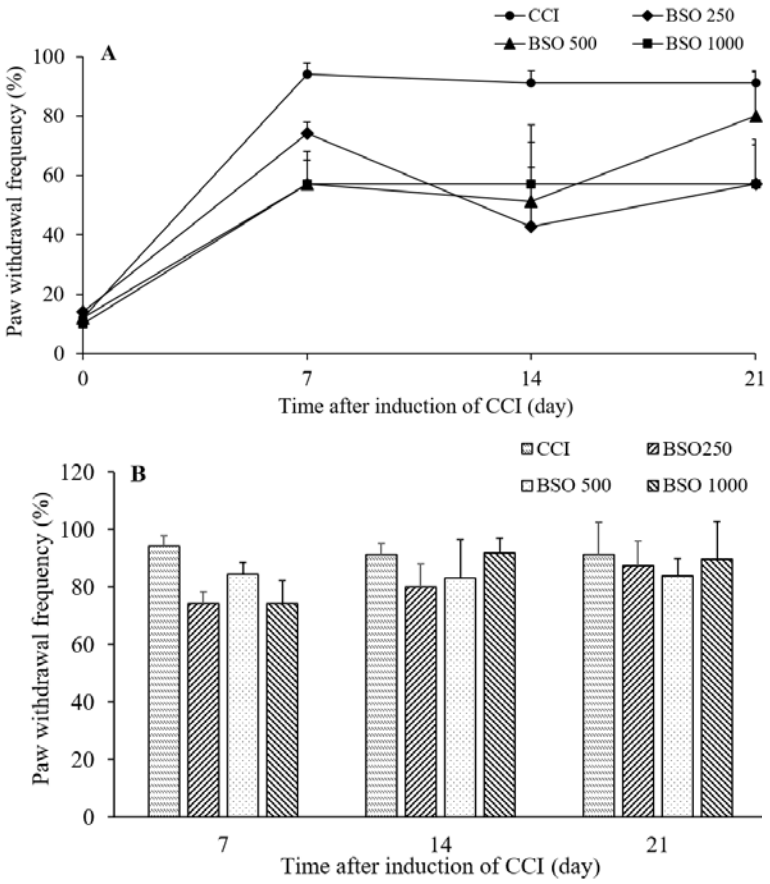

Fig. 4. (A) The effect of chronic treatment with BSO (250, 500, and 1000 mg/kg; p.o.) from the 1st day to the 21st day after surgery on the development of the cold allodynia; (B) the effect of acute treatment with BSO (250, 500, and 1000 mg/kg; p.o.) on the cold allodynia. The CCI groups received vehicles according to the treatment schedule. CCI group was given almond oil according to the same schedule. The results are expressed as mean ± SEM, n = 8.

DISCUSSION

The peripheral nerve injury model is an experimental model, which induces persistent hyperalgesia and allodynia. CCI combined with pain hypersensitivity testing is usually used to explore new interventions for neuropathic pain. Like previous studies, our result proved that CCI resulted in hyperalgesia and allodynia, particularly seven days after surgery (Fig. 1) (21,22). This study used an animal model of CCI to explore the anti-hyperalgesic effects of BSO in rats. After surgery, daily administration of BSO (500 and 1000 mg/kg p.o.) obviously attenuated pain sensitivity in the plantar and Von Frey tests. The underlying mechanisms could be different despite similar anti-nociceptive effects shown by BSO in the plantar and Von Frey tests. To study the underlying mechanisms of neuropathic pain, experimental models of hyperalgesia that resemble human clinical pain conditions have been used. The paw withdrawal to an innocuous thermal stimulus after nerve injury defines as thermal hyperalgesia because withdrawal occurs at a normal temperature. However, allodynia defines as a reaction due to a non-noxious stimulus. Hyperalgesia and allodynia are critical signs of neuropathic pain. Low-threshold, large-diameter, myelinated Aβ nerves transmit mechanical stimulus, while thin unmyelinated primary C-fiber chiefly transmit cold stimulus. Thermal stimulation mainly activates transient receptor potential channels (23,24). These results demonstrate that BSO, when administered immediately after CCI, can modify the progression of neuropathic pain and suggested the probable neuroprotective effects of the oil.

Numerous pharmacological studies have investigated *N. sativa* and its effective component, particularly in pain and inflammation (25). *N. sativa* extract considerably improved the hot plate response time (26). In an investigation, it has been shown that thymoquinone significantly decreases the paw licking time in the formalin test (27). The ethanolic fraction of *N. sativa* demonstrated anti-nociceptive effects in the writhing test (28). Also, *N. sativa* showed considerable anti-inflammatory as well as anti-nociceptive effects in an animal model of paw edema. The inhibitory effect of thymoquinone is less than fixed oil on lipid peroxidation. Neurons are particularly susceptible to oxidative stress not only because of neuronal membrane injury but also due to the inactivation of glutamine synthetase (29). Following glutamine synthetase inhibition, glutamate uptake decreases by glial cells and glutamate availability is increased at the synapse. Increased glutamate concentration at the synapse resulted in neurotoxicity (30). Also, the increased release of glutamate into the spinal dorsal horn activates the NMDA receptor. NMDA receptor hyperactivity stimulates downstream signaling cascades and neuronal excitability (31).

It should be noted that both oxidative stress and inflammation have a critical role in neuropathic pain development (32). Inflammation and nerve injury result in the persistent activation of peripheral nociceptors and the development of central sensitization. Also, the augmented production of reactive oxygen species leads to central sensitization and is involved in the nociceptive process.

Previously, antidiabetic, anti-inflammatory, and antioxidants effects of *N. sativa* ethanolic extract have been demonstrated (33). Also, *N. sativa* has protective effects on learning and memory impairments in the seizures model (34). The oil extracts of *N. sativa* protect neurons against oxidative stress induced by encephalomyelitis (35). Probably, BSO by inhibiting reactive oxygen species improves neuropathic pain. Moreover, other components, like unsaturated fatty acids, essential oil, and oleoresins may also contribute to these effects (36,37). Quinolones and thymoquinone from *N. sativa* seeds are cyclooxygenase inhibitors (38). Prostaglandins decrease the pain threshold and contribute to neuropathic pain development.

In another study, thymoquinone significantly showed antioxidant and anti-inflammatory effects by decreasing pro-inflammatory cytokines and increasing glutathione-S-transferase, and glutathione peroxidase activity (39). Hydro-alcoholic extract of *N. sativa* improved learning and memory damages in rats by modifying hippocampal cytokine levels and brain tissue oxidative damage (40). Recent studies have shown the anxiolytic and anticonvulsant activities of *N. sativa* and these effects mediate by increasing GABAergic activity (41,42). In the dorsal horn of the spinal cord, GABAergic neurons transmit pain impulses to the brain. At the supraspinal level, GABA neurons and their receptors manage the perception and response to painful stimuli. Increased evidences have shown that GABA receptor agonists have anti-nociceptive effects in different animal models. Both GABA receptor agonists and GABA reuptake inhibitors are effective in treating pain (43).

Descending pain inhibitory system arises in the brainstem. Alteration in the periaqueductal gray and rostroventromedial medulla resulted in the activation of descending pain inhibition. Serotonin (5-HT) and norepinephrine are the most important neurotransmitters released by descending inhibitory pathways (44). *N. sativa* L. oil augmented brain 5-HT levels and reduced 5-HT turnover. Also, the tryptophan level significantly was increased following the administration of *N. sativa* L. oil (45).

CONCLUSION

Our results provided evidence to confirm the effectiveness of BSO in CCI-induced neuropathic pain. These findings clearly demonstrated the anti-hyperalgesic activity of *N. sativa*. Therefore, this medicinal plant can be noticed as a medical intervention to treat peripheral neuropathy. However, further studies are needed to confirm the underlying mechanisms of *N. sativa* in chronic pain management.

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Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contributions

A. Abed and S.A. Talaei contributed to the conception, design, statistical analysis, and drafting of the manuscript. H.R. Banafshe, A. Moravveji, M. Shabani, and Sh. Shirazi contributed to the conception, data collection, and manuscript drafting. The final version of the article was confirmed by all authors.

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