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Nigella sativa oil on chronic constrictive injury (CCI) induced neuropathic pain of sciatic nerve in Wistar rats

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Abstract

Background: Various adverse effects have been reported with conventional treatments of neuropathic pain. *Nigella sativa* (*N. sativa*), a medicinal herb, has been shown to possess several beneficiary effects. **Objective:** To assess the effects of *N. sativa* oil on neuropathic pain and ATP sensitive potassium channel (K_{ATP}) in Wistar rats. **Methods:** This experimental study was conducted on 120 Wistar rats (200±50gm). On the basis of treatments, all rats were grouped into Group I [normal control (NC) normal saline (NS) 5 ml/kg]; Group II [sham control (SC) {sham surgery + NS}], Group III [CCI control (CCIC) {Chronic constriction injury to sciatic nerve (CCI) + NS}]; Group IV [*N. sativa* oil experimental (NSOexp) {CCI + *N.sativa* (400 mg/kg)}], Group V [Glibenclamide experimental (Gliexp) {CCI + *N. sativa* (400 mg/kg) + glibenclamide (15 mg/kg)}] groups. Both the NSO and NS were administered orally once daily for consecutive 21 days and single dose of glibenclamide was given intraperitoneally on the day of experiment, to the respective rats. Then on the basis of neuropathic pain evaluation tests, all the groups were subdivided into 'a' [for sciatic functional index (SFI) in walking track analysis], 'b' [for tail flick latency (TFL) in cold tail immersion test], 'c' [for paw withdrawal threshold (PWT) in von Frey test], and 'd' (for rection time in hot plate test). The statistical analysis was done by one way ANOVA followed by Bonferroni post hoc test. **Results:** In this study, significantly

(pd"0.001) higher SFI, TFL, PWT and reaction time were found in NSO exp rats when compared to those of CCIC rats. In addition, there were significant (pd"0.001) differences in the above-mentioned variables between rats of NSO exp group and Gliexp group. **Conclusion:** From the present study it might be concluded that, *N. sativa* prevents worsening of neuropathic pain in Wistar rats by blocking K_{ATP} channel.

Keywords: Neuropathic pain, *Nigella sativa,* glibenclamide, walking track analysis, cold tail immersion test, Von Frey test, hot plate test.

Introduction

Neuropathic pain, most distressing human ailments can occur by any pathology of the somatosensory nervous system.[1] This chronic morbidity arises from injured axons as well as from intact nociceptors which results in abnormal sensory signs, allodynia (pain response to low threshold stimulus) and hyperalgesia (increased response to noxious stimuli). World Health Organization (WHO) refers that prevalence of chronic neuropathic pain among primary care patients worldwide is around 22%[2]. There are different probable mechanisms of neuropathic pain. It occurs by releasing inflammatory cytokines, increasing receptor signaling, accumulating intracellular Ca^{++} ion within the injured neuron, activating glial cell as well as producing ectopic discharge in injured fibers[3,4]. In addition, activation of voltage gated calcium channel[5], increased expression of voltage gated sodium channel[6] and opening of ATP sensitive potassium channel (K_{ATP})[7] in the neuronal membrane are also proposed as probable mechanisms of neuropathic pain.

There are various treatment regimens for this agonizing morbidity, such as, tricyclic antidepressants (i.e. amitriptyline, nortriptyline, imipramine) as well as anticonvulsants (i.e. phenytoin, carbamazepine, gabapentin, lamotrigine and topiramate)[8]. However, many studies have been carried out on treatment schedule of neuropathic pain with different herbal products[9-11] to limit the side effects associated with prolonged use of these drugs[12]. Among the herbal products *Nigella sativa* (*N. sativa* or black seed) has been suggested as an important medicinal herb[13].

In addition to its nutritional value, *N. sativa* showed anticancer[14], antimicrobial[15], reno-protective[16], antioxidant[17], antihypertensive[18], analgesic and antipyretic[19], antidiabetic[20], anti-inflammatory[21] as well as immunomodulatory[22] effects in different animal models. *N. sativa* also caused significant improvement in milk production in nursing mother[23], skin disorders[24] as well as bronchitis and asthma.[25] Moreover, in perspective of pain, this seeds oil was shown to affect nociceptive and inflammatory pain[26-27] as well as neuropathic pain[28] in different animal models.

In addition, *N. sativa* was shown to prevent neuropathic pain by its antioxidant activity.[29,30] But as far as we searched, no study was found to explore the role of K_{ATP} channel in alleviation of neuropathic pain by this medicinal herb. On the basis of this background, the present study has been designed to evaluate the effect of *N. sativa* on neuropathic pain in Wistar rats and also to evaluate its role on K_{ATP} channel.

Methods

Duration and setting of study

The study was conducted in the Pain laboratory of Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, from March 2019 to July 2021 after getting approval from the Institutional Review Board of this University.

Procurement and maintenance of animals

A total of 120 rats (200±50 gm) of both sexes[31] were collected from the central animal house of BSMMU. Then under a 12/12 hour light/dark cycle[32] the rats were kept in specially built plastic

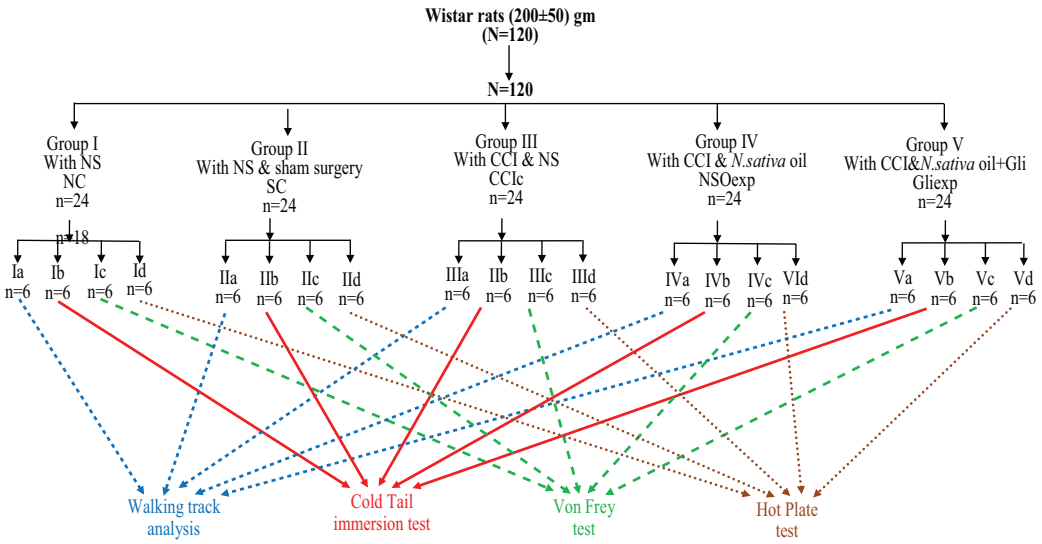
cages with 6 rats per cage. The ambient room temperature was maintained at 27°C, as the thermoneutral zone for rodents is around 27 to 28°C[33]. All rats were provided with free access to standard laboratory food[34] and cooled boiled water *ad libitum* during acclimatization. All the experiments were performed at daytime between 08:00 and 16:00 hours to avoid circadian influences[35] in accordance with the international guidelines on the use of laboratory animals of International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b).

Oil collection

According to Pighinelli and Gambetta[36], fresh *N. sativa* seeds were purchased from local market. Initially the oilseeds were weighed and cleaned by fresh water. Then the seeds were dried in sunlight. After drying the seed dehulling were done. Then, by continuous mechanical pressing (oil expeller machine/'Ghani') raw oil was collected. About 250 ml oil was collected from 1kg black seeds.

Experimental design and dose schedule

Based on treatments, all rats were randomly grouped into the following groups: Group I: normal control (NC) consists of 24 rats treated with normal saline (NS 5 ml/kg) for consecutive 21 days. Group II: Sham control (SC) consists of 24 rats with sham surgery done (open and close surgery, no sciatic nerve injury) and treated with normal saline (NS, 5ml/kg). Group III: CCI control (CCIC) also consists of 24 rats; CCI (Chronic constrictive injury) to the left sciatic nerve done, followed by treatment with NS for 21 days. Group IV: *Nigella sativa* oil experimental (NSOexp) group consists of 24 rats, CCI to the left sciatic nerve done, followed by treatment with NSO (400 mg/kg) orally for 21 days. Group V: Glibenclamide experimental (Gliexp) group also consists of 24 rats, CCI to the left sciatic nerve done, followed by treatment with NSO (400 mg/kg) for consecutive 21 days and Glibenclamide (Gli,15 mg/kg) 10 minute before the experiment. Glibenclamide was given once through an intraperitoneal route on the day of the experiment. Then on the basis of neuropathic pain evaluation tests, all the rat groups were subdivided into subgroups (n=6 rats, in each subgroup). Here, 'a' (for walking track analysis), 'b' (for cold tail immersion test), 'c' (for Von Frey test), 'd'(for hot plate test) as shown in figure 1.



NS= normal saline; CCI= Chronic constrictive injury; NSO= *Nigella sativa* oil; Gli= glibenclamide.

Figure 1: Experimental design and animal grouping

Chronic Constrictive Injury of sciatic nerve (CCI)
Chronic constrictive injury of sciatic nerve (CCI) was performed according to Austin, Wu and Taylor (2012)[39] to produce neuropathic pain. Firstly, anesthesia of rat was obtained by intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg) mixture. Proper positioning was done after shaving and sterilization. On the left thigh 3 to 4 mm below the femoral head an incision was given and the sciatic nerve was exposed. At 1 (one) mm intervals four loose ligations were made around the common sciatic nerve. To prevent arresting of the epineural blood flow constriction of the nerve was minimal. Lastly, the muscle layer and the skin were closed. In sham surgery, skin and muscle were opened and sutured without giving nerve ligation.

Walking track analysis
According to published procedures[40,41] walking track analysis was performed. Six (6) rats from each group (total 30) were separated for this behavioral test. All of the rats were acclimatized individually for consecutive 7 days to walk freely on a smooth, flat surface covered by a sheet of ink absorbing paper guarded by wooden plate. After CCI induction this test was done every 4 day interval. On the day of experiment just one hour after the pre-scheduled treatment, left hind paw of each rat was dipped in blue ink. Then each rat was kept individually in above mentioned walking place on a sheet of ink absorbing paper. Every time 3 to 4 foot prints of left hind paw were marked and a measuring scale was used to take the measurements as mentioned below. Then sciatic functional index (SFI) was calculated as follows: SFI = [-38.3 X (EPL-NPL)/NPL] + [109.5 X (ETS-NTS)/NTS] + [13.3 X (EIT-NIT)/NIT] - 8.8; where NPL=normal print length, EPL=experimental print length, NTS=normal toe spread, ETS=experimental toe spread, NIT=normal intermediate toe spread, and EIT=experimental intermediate toe spread [Figure. 2(a)].

Cold tail immersion test
Cold tail immersion test procedure was performed according to the methods of previous articles[42,43]. Six (6) rats from each group were recruited for this test and instrumental acclimatization was done for consecutive 7 days. Then during experiment, each rat was kept individually in the plexiglass mechanical restrainer for 5 minutes to accommodate with the cage environment, with the tail hanging freely. Then distal 5 cm of the freely hanging tail of the rat was immersed into cold water maintained at 10°C and latency period of the tail withdrawal (tail-flick) was recorded. The mean of the measurements was obtained from three similar successive maneuvers, performed at 5 minutes intervals and was recorded as the baseline latency. One hour after the last dose of the treatment (NS/ NSO), another tail immersion test was done. Here the mean of 3 tail withdrawal latencies at 5 minutes interval was noted as test latency (TL). Maximum latency of 20 seconds was considered as cut off time to minimize tissue damage. The antinociceptive effect was expressed as percentage of maximum possible effect (% MPE) as follows: [(TL-BL) / (Cut off time-BL)] X 100 [Figure. 2(b)].

Von Frey test
According to Rafiq et al. (2009) and Detloff et al. (2010)[44,45] Von Frey test was performed. Six (6) rats from each group (Total 30) were separated for Von Frey test and for next 7 days instrumental acclimatization was done. After performing CCI, this test was done on every 4 days. On experiment day, each rat was placed individually on the wide gauge wire mesh surface just after 1 hour of treatment. Then, on planter surface of left hind paws of the rat between first and second metatarsal about 1cm proximal to the ankle joint the calibrated Von Frey filaments (VFF) was touched in an ascending order. Each VFF (of varying tensile strengths of 2 to18 gm) was applied three times at 30 seconds intervals and the strength of the VFF was recorded. Application of the next larger VFF was done unless paw withdrawal occurred in at least two of the three. No more VFF was applied, if the rat fails to withdrawal its paw at maximum force of 18 gm, to prevent tissue injury [Figure. 2(c)].

Hot plate test

According to Maurya (2017) and Austin, Wu and Taylor (2012)[31,39] hot plate test was performed. Six rats from each group (total 30) rats were recruited for hot plate test. These rats were also acclimatized in the instrument for 1 (one) hour daily for 7 consecutive days along with laboratory acclimatization. Then, the hot plate was heated at a temperature of 54±0.5º C and the rat was placed on heated surface on the day of experiment. A stop watch was started as the rat placed on the hot plate till the first paw licking or trying to jump out. To avoid any damage to the paw, cut off period of 20 seconds was set. After *N. sativa* oil administration, test was done at interval of 30, 60, 90 minutes. The analgesia was expressed as percentage of maximal possible effect (%MPE) as follows: [(Test Latency – Basal Latency) / (Cut off time - Basal Latency)] X 100 [Figure. 2(d)].

Anesthesia and sacrifice

Each rat was placed in a large glass desiccator and 3 to 4 ml of di-ethyl ether (99%) was poured into it[46]. For the following 5 to 10 minutes the rat was observed closely and the ventilation was maintained by intermittent opening and closing of the desiccator lid. The painless death of the deeply anesthetized rat was ensured by decapitation.

Statistical analysis

Results were expressed as mean±SEM. Data were analyzed using ANOVA followed by Bonferroni post hoc test in SPSS (version 23.0), where $p \leq 0.05$ was considered as statistically significant.

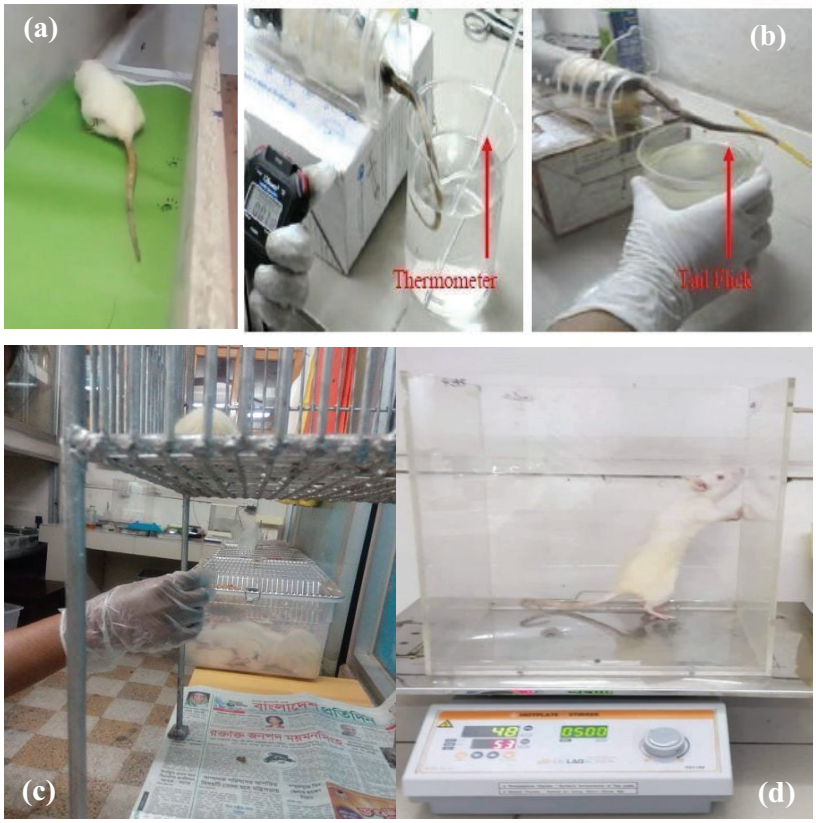


Figure 2: Procedure of the tests done in our study: (a) walking track analysis, (b) cold tail immersion test, (c) Von Frey test, (d) hot plate test.

Results

Sciatic functional index (SFI) in walking track analysis

Here, the SFI in walking track analysis was used to determine the sensorimotor impairment of the sciatic nerve. *N. sativa* oil increased the SFI significantly (p≤0.001) in NSOexp rats in comparison to that of CCI control rats on day 9, 14, 19 and 24 of experiment. Moreover, there was significant (p≤0.001) decrement of SFI in Gliexp rats in comparison to that of NSOexp rats on day 9, 14, 19 and day 24 [Figure. 3].

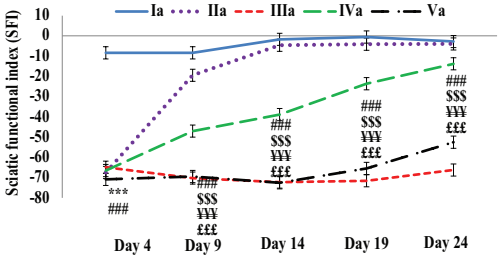


Figure 3. Sciatic functional index in rats with walking track analysis in different groups of rats. Each line symbolizes mean±SEM for 6 rats. Ia= rats treated with oral normal saline (5 ml/kg) for consecutive 21 days; IIa= rats treated with open and close surgery followed by oral normal saline (5 ml/kg); IIIa= rats treated with chronic constrictive injury to sciatic nerve (CCI) followed by oral normal saline (5 ml/kg) for consecutive 21 days; IVa= rats treated with CCI followed by oral *N. sativa* oil (400 mg/kg) for consecutive 21 days; Va= rats treated with CCI followed by oral *N. sativa* oil (400 mg/kg) for consecutive 21 days and also with single dose of intraperitoneal glibenclamide (15 mg/kg) 10 minutes before *N.sativa* oil on the day of experiment. *= Ia vs IIa, #= Ia vs IIIa, $= IIa vs IIIa, ¥= IIIa vs IVa, •= IIIa vs Va, and £= IVa vs Va. Single symbol= significant at p≤0.05, double symbol= significant at p≤0.01, triple symbol= significant at p≤0.001.

Tail flick latency in cold tail immersion test

In cold tail immersion test, *N. sativa* oil increased the tail flick latency significantly (pd"0.001) in NSOexp rats in comparison to that of CCI control rats. Moreover, there was significant (p≤0.001) decrement of tail flick latency in Gliexp rats in comparison to that of *N. sativa* exp rats [Figure. 4].

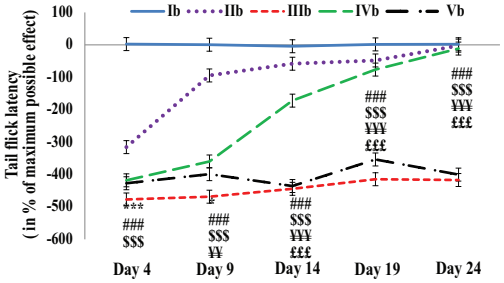


Figure 4. Tail flick latency in rats with cold tail immersion test in different groups of rats. Each line symbolizes mean±SEM for 6 rats. Ib= rats treated with oral normal saline (5 ml/kg) for consecutive 21 days; IIb= rats treated with open and close surgery followed by oral normal saline (5 ml/kg); IIIb= rats treated with chronic constrictive injury to sciatic nerve (CCI) followed by oral normal saline (5 ml/kg) for consecutive 21 days; IVb= rats treated with CCI followed by oral *N. sativa* oil (400 mg/kg) for consecutive 21 days; Vb= rats treated with CCI followed by oral *N. sativa* oil (400 mg/kg) for consecutive 21 days and also with single dose of intraperitoneal glibenclamide (15 mg/kg) 10 minutes before *N.sativa* oil on the day of experiment. *= Ib vs IIb, #= Ib vs IIIb, $= IIb vs IIIb, ¥= IIIb vs IVb, •= IIIb vs Vb, and £= IVb vs Vb. Single symbol= significant at p≤0.05, double symbol= significant at p≤0.01, triple symbol= significant at p≤0.001

Paw withdrawal threshold in Von Frey test

As shown in Figure 3, the paw withdrawal threshold increased significantly by *N. sativa* in NSOexp rats in comparison to that of CCI control rats on day 9 (p≤0.05), 14 (p≤0.001) and 19 (p≤0.001) and day 24 (p≤0.001) of experiment. Moreover, there was significant (p≤0.001) decrement of paw withdrawal threshold in Gliexp rats in comparison to that of NSOexp rats [Figure. 5].

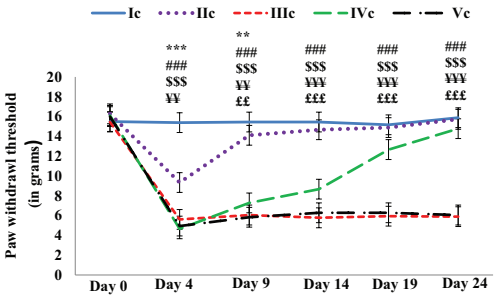


Figure 5. Paw withdrawal threshold in rats with Von Frey test analysis in different groups of rats. Each line symbolizes mean±SEM for 6 rats. Ic= rats treated with oral normal saline (5 ml/kg) for consecutive 21 days; IIc= rats treated with open and close surgery followed by oral normal saline (5 ml/kg); IIIc= rats treated with chronic constrictive injury to sciatic nerve (CCI) followed by oral normal saline (5 ml/kg) for consecutive 21 days; IVc= rats treated with CCI followed by oral *N. sativa* oil (400 mg/kg) for consecutive 21 days; Vc= rats treated with CCI followed by oral *N. sativa* oil (400 mg/kg) for consecutive 21 days and also with single dose of intraperitoneal glibenclamide (15 mg/kg) 10 minutes before *N.sativa* oil on the day of experiment. *= Ic vs IIc, #= Ic vs IIIc, $= IIc vs IIIc, ¥= IIIc vs IVc, •= IIIc vs Vc, and £= IVc vs Vc. Single symbol= significant at p≤0.05, double symbol= significant at p≤0.01, triple symbol= significant at p≤0.001.

Reaction time in hot plate test

Here, we assessed thermal allodynia by the latency of reaction time using hot plate test. The reaction times increased significantly by *N. sativa* in NSOexp rats in comparison to that of CCI control rats on day 14 (p≤0.001), day 19 (p≤0.001) and day 24 (p≤0.001) of experiment. Moreover, there was significant (p≤0.001) decrement of reaction time in Gliexp rats in comparison to that of NSOexp rats on day 9 (p≤0.05), day 14 (p≤0.001), day 19 (p≤0.001) and day 24 (p≤0.001) of experiment (Figure 6).

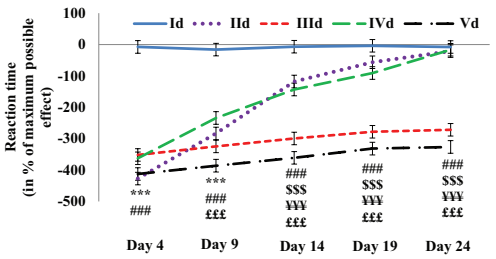


Figure 6. Reaction time in rats with hot plate test in different groups of rats. Each line symbolizes mean±SEM for 6 rats. Id= rats treated with oral normal saline (5 ml/kg) for consecutive 21 days; IId= rats treated with open and close surgery followed by oral normal saline (5 ml/kg); IIId= rats treated with chronic constrictive injury to sciatic nerve (CCI) followed by oral normal saline (5 ml/kg) for consecutive 21 days; IVd= rats treated with CCI followed by oral *N. sativa* oil (400 mg/kg) for consecutive 21 days; Vd= rats treated with CCI followed by oral *N. sativa* oil (400 mg/kg) for consecutive 21 days and also with single dose of intraperitoneal glibenclamide (15 mg/kg) 10 minutes before *N.sativa* oil on the day of experiment. *= Id vs IId, #= Id vs IIId, $= IId vs IIId, ¥= IIId vs IVd, •= IIId vs Vd, and £= IVd vs Vd. Single symbol= significant at p≤0.05, double symbol= significant at p≤0.01, triple symbol= significant at p≤0.001.

Discussion

Pain management is still a challenge in clinical practice, moreover management of chronic neuropathic pain is a difficult task, as traditional analgesics are less effective to cure it.[44] However, the use of traditional analgesics is limited for their various adverse effects and several interactions with other drugs. On the basis of these rationale, we are influenced to find an effective preventive regime for this morbid condition. Various methods have been used in animal models to measure the severity of neuropathic pain. Among them the walking track analysis is a quantitative method

to analyze the functional impairment of sciatic nerve[40,41]. It provides a noninvasive method of assessing the functional status of the sciatic nerve during the regeneration process. In addition, the cold tail immersion test is one of the most common thermal method[42] where tail withdrawal latency is measured to assess cold allodynia.[43] Von Frey test is a standard method by which mechanical allodynia can be measured in neuropathic pain in rats.[44] Here the measuring variable is paw withdrawal threshold.[44,45] In addition, hot plate test is a common method for measurement of heat hyperalgesia in neuropathic pain in rats.[31,39] It is related to higher brain function and is contemplated to be a supraspinally organized response. The hot plate procedure accounts for a more global estimate of nociceptive reactivity because it depicts a complex willed behavior rather than a simple reflex.

In our study, chronic constrictive injury (CCI) of sciatic nerve produced neuropathic pain in CCI control rats as evidenced by significant difference of SFI in walking track analysis, tail flick latency in cold tail immersion test, paw withdrawal threshold in Von Frey test and reaction time in hot plate test, in comparison to those of the rats with normal saline (NS) as well as sham surgery. Here, loose ligation around sciatic nerve might compress the nerve fiber causing nerve damage in the rats with CCI[47]. From the damaged cell, various inflammatory mediators are released including interleukine-1β (IL-1β), tumor necrosis factor-á (TNF-α), Interleukin 6 (IL-6), prostaglandin E_2 (PGE_2), bradykinin, substance P, nerve growth factor[4,46]; promoting chemotaxis of inflammatory cells (macrophage, microglia, monocyte, helper T cell)[48]. These mediators activate various signaling pathways resulting in changes in different ion channels (e.g Na^+, Ca^{++}, K^+ channels), causing release of glutamate, ATP and other mediators from activated central terminal of nociceptor resulting in prolonged post synaptic depolarization of dorsal root ganglia (DRG).[4] In addition, aggregation of toxic reactive oxygen species (ROS) might cause cell membrane damage, glial activation and central censitization[49] producing stepping up of pain transmission. However, all or any of the above mentioned mechanisms might cause increment of pain transmission along with sensorimotor dysfunction of sciatic nerve, cold allodynia, mechanical allodynia and heat hyperalgesia in the experimental rats with CCI induced neuropathic pain.

We also found that, *N. sativa* prevented the worsening of all these four aspects of neuropathic pain in rats with CCI plus *N. sativa,* as evidenced by significant difference of SFI in walking track analysis, tail flick latency in cold tail immersion test, paw withdrawal threshold in von Frey test and reaction time in hot plate test, in comparison to those of rats with only CCI. However, many mechanisms might be involved in this prevention of neuropathic pain by *N. sativa* as evidenced by prevention of sensorimotor dysfunction of sciatic nerve, cold allodynia, mechanical allodynia and heat hyperalgesia in the experimental rats with *N. sativa*. Such as, increment of total antioxidant status, reduction of inflammatory mediators (IL-1β, TNF-α, IL-6, PG)[29,30] and downregulation of microglial activation in different animal models of neuropathic pain.

Furthermore, we have explored the role of K_{ATP} channel in alleviation of neuropathic pain by *N. sativa* oil. Here, in rats of Gliexp group, *N. sativa* could not prevent the development of sensorimotor dysfunction, cold allodynia, mechanical allodynia and heat hyperalgesia, when glibenclamide was administered in rats with CCI plus *N. sativa*. As glibenclamide is a well-known K_{ATP} channel[50] blocker, we can propose from this finding that the analgesic mechanism of *N. sativa* might occur through opening of K_{ATP} channel. As far as we reviewed literatures, this is the 1st data showing involvement of K_{ATP} channel in prevention of neuropathic pain by *N. sativa* oil.

Conclusion

From the results of present study, it might be concluded that *Nigella sativa* prevents worsening of neuropathic pain in Wistar rats by involving K_{ATP} channel. Although we had limitations as this study was done with only one dose and duration schedule of *Nigella sativa* oil in rats of both sexes. We recommend further researches with different doses and duration schedules of *Nigella sativa* oil in either male or female rat should be done. In addition, application of this herb on different neuropathic pain models should be evaluated further to ascertain our findings.

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Conflict of interest None

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