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ORIGINAL ARTICLE

Guggulipid of *Commiphora mukul*, with antiallodynic and antihyperalgesic activities in both sciatic nerve and spinal nerve ligation models of neuropathic pain

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Abstract

Context: Guggulipid is a neutral fraction of ethyl acetate extract of gum resin of the tree *Commiphora mukul* Engl. (Burseraceae) and used in Ayurvedic medicine for treatment of neurological disorders.
Objectives: The present study was undertaken to assess the antiallodynic and antihyperalgesic activities of guggulipid in rats.
Materials and methods: The screening study included the CCI and L5–L6 SNL models of neuropathic pain. Guggulipid (100 and 50 mg/kg) or saline was administered intraperitoneally in a blinded, randomized manner from postoperative day (POD) 7 to 13. Paw withdrawal duration (PWD) to spontaneous pain, chemical allodynia and mechanical hyperalgesia and paw withdrawal latency (PWL) to mechanical allodynia and thermal hyperalgesia were tested before surgery, before and after guggulipid or saline administration (from POD7 to 13) and after the withdrawal of treatment (from POD14 to 20).
Results: The activity profiles of the different doses of guggulipid were found to vary with time. In CCI rats, guggulipid (100 and 50 mg/kg) significantly ($p < 0.05$) reduced the spontaneous pain, mechanical allodynia and mechanical and thermal hyperalgesia responses and the LD_{50} of guggulipid was 1600 mg/kg. In SNL rats, both doses of guggulipid were found to be ineffective in reversing the spontaneous pain but showing antiallodynic and antihyperalgesic activity.
Discussion and conclusion: The results demonstrated that guggulipid produce antinociception in the peripheral nerve injury (CCI and SNL) models of neuropathic pain. The underlying mechanisms are expected to be modulating microglial activation occurring due to peripheral nerve injury.

Keywords

Chronic constriction injury, cytokines, L5–L6 spinal nerve ligation, microglial cell, peripheral nerve injury, pregabalin

History

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Introduction

Neuropathic pain is generally defined as a chronic pain state resulting from peripheral or central nerve injury either due to acute events (e.g., amputation, spinal cord injury) or systemic disease (e.g., diabetes, viral infection and cancer; Childers & Baudy, 2007; McCarberg & Billington, 2006). Treatment of neuropathic pain, triggered by multiple insults to the nervous system, is a clinical challenge because the underlying mechanism of neuropathic pain development remains poorly understood (Kehlet et al., 2006; Kim et al., 1997; Tsuda et al., 2005; Woolf & Mannion, 1999). Central sensitization and synaptic plasticity in the central nervous system (CNS) contribute significantly to neuropathic pain. Therefore, targeting neuronal plasticity changes in somatosensory pathways is a major direction for finding pain-relieving medications. However, it has recently been reported that neurons are not the only cell type involved in chronic pain states (Inoue & Tsuda, 2009; McMahon & Malcangio, 2009; Milligan & Watkins, 2009). Microglia, the resident macrophages and principal immune response cell in the CNS, quite evenly distributed in the brain and spinal cord, are massively activated in the dorsal horn soon after peripheral nerve injury and contribute in neuropathic pain development (Beggs & Salter, 2007; Davalos et al., 2005; Nimmerjahn et al., 2005; Saab et al., 2008; Scholz et al., 2008; Streit et al., 2004; Suter et al., 2007). Peripheral neurons transmit signals to spinal dorsal horn neurons, releasing neurotransmitters. Therefore, it is possible to suggest that these neurotransmitters may initiate microglial activation associated with neuropathic pain (Chen et al., 2010; Wu & Zhuo, 2008). Activated microglias release various cytokines and chemokines, including IL-1β, IL-6, tumor necrosis factor-α (TNFα), PGE_2, and nitric oxide. These cytokines, released by activated microglia, will amplify microglial activation and may act directly on dorsal horn neurons to cause behavioral sensitization (Beggs & Salter, 2007; Saab et al., 2008; Scholz et al., 2008; Streit et al., 2004; Suter et al., 2007; Tsuda et al., 2005). It has been shown that

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intrathecal administration of IL-1β, IL6, or TNFα can lead to symptoms of neuropathic pain in healthy rats (Krakauer, 2004; Reeve et al., 2000). Another example for neuron-glia interactions contributing to neuropathic pain are the matrix metalloproteinases (MMP2 and MMP9). They are produced by both neurons and glia and mediate pain hypersensitivity by initiating IL-1β cleavage and microglial activation. In cultured DRG neurons, TNFα and IL-1β increased both the expression and release of MMP-9. Further, intrathecal administration of MMP-9 or MMP-2 is sufficient to produce neuropathic pain symptoms (Kawasaki, 2008).

Guggulipid is a neutral fraction of ethyl acetate extract of gum resin of the tree *Commiphora mukul* Engl. (Burseraceae) and contains the active constituent known as guggulsterone [4, 17(20)-pregnadiene-3, 16-dione]. The resin of the *C. mukul* tree has been used in Ayurvedic medicine for centuries to treat such ailments as obesity, bone fractures, arthritis, inflammation, cardiovascular disease and lipid disorders (Sinal & Gonzalez, 2002; Urizar & Moore, 2003). Previous studies have shown that guggulsterone downregulates, expression of cyclooxygenase (COX-2) and matrix metalloprotease (MMP-9). Guggulsterone suppresses IL-1β and IL-6 mediated inflammatory response, TNFα and PGE_2 production, nitric oxide (NO) formation and phosphorylation of p38 (Gebhard et al., 2009; Lee et al., 2008; Lv et al., 2008; Meselhy, 2003; Niranjan et al., 2011; Sarfaraz et al., 2008; Shishodia & Aggarwal, 2004; Song et al., 2010). Collectively, these results suggest that guggulipid can be useful in neuropathic pain treatment.

Materials and methods

Plant material

The guggul resin was purchased from a local vendor of Udaipur, India in 2012. The plant material was identified as per the literature of ayurveda and further confirmed by Dr. M.S. Rathore, Professor and Head, Department of Botany, B.N. PG. College, Udaipur with BNC/2011-12/01 herbarium number.

Extraction and isolation of guggulipid

Guggulipid was isolated following the method described by Roy et al. (2009). The resin as soaked in ethyl acetate (EtOAc) at room temperature for 24 h with continuous stirring and the filtrate was then concentrated using a rotavapour. Concentrated EtOAc extract was then further washed with 3 N hydrogen chloride and 10% sodium bicarbonate to get a neutral fraction. The neutral fraction was washed three-times with brine and again concentrated on rotatory vacuum evaporator and a dark brown gummy neutral fraction was obtained. Mixture of the earlier obtained neutral fraction along with 10% semicarbazide on silica and toluene were stirred and heated at 60–65 °C for 14 h. The mixture was then cooled at room temperature and filtered. Silica was then washed with toluene thrice and refluxed with 10% oxalic acid and toluene for 2.5 h and then filtered. Silica gel was then extracted several times with EtOAc and the combined extracts were then washed with water and brine followed by removal of the solvent to collect the required neutral ketonic fraction which known as guggulipid and contains the active constituent guggulsterone. After purification, isolated compound was analyzed by UV spectroscopy, FTIR and HPLC. Data obtained were correlated with those mentioned in literature (Mesrob et al., 1998) confirmed that the isolated compound was guggulipid.

Animal and maintenance

Male Sprague-Dawley rats of body weight between 200–230 g were used for neuropathic pain models. All experiments were approved by the Institutional Animal Ethics Committee (1622/PO/a/12/CPCSEA). Each rat was housed in plastic box cage individually with well controlled supplied air, humidity (<70%) and temperature under a 12 h light/dark cycle with food and water *ad libitum*.

Drugs and chemicals

Pregabalin was obtained from Torrent Research Centre (Gandhinagar, Gujarat) and used as a positive control. Guggulipid was isolated from the guggul resin of *C. mukul* by using the procedure as described in the extraction and isolation procedure section. Pregabalin was dissolved in isotonic saline solution (0.9% NaCl, w/v) and a suspension was prepared for guggulipid with Tween 80 (4% w/v).

Induction of peripheral mononeuropathy

Chronic constriction injury model (CCI model)

Unilateral mononeuropathy was produced in rat using the CCI model performed essentially as described by Bennett and Xie (1988). The rats were anesthetized with intraperitoneal combination of ketamine and xyalazine at 60 and 6.5 mg/kg, respectively. The left hind leg was shaved, moistened with a disinfectant, and now common sciatic nerve was exposed at the level of the middle of the thigh by blunt dissection through the biceps femoris. Proximal to the sciatic trifurcation, about 7 mm of the nerve was freed of adhering tissues, and four loose ligatures were made with 4.0 braided silk suture with about 1 mm spacing. The wound was then closed by suturing the muscle using chromic catgut with a continuous suture pattern. Finally the skin was closed with 3.0 black braided silk sutures using a horizontal-mattress suture pattern. Sham surgery was performed by exposing the sciatic nerve as described above, but not damaging it. Povidone iodine ointment was applied topically to the wound and benzyl penicillin antibiotic (20 000 IU/Kg, b.i.d.) was given intramuscularly for 5 days after surgery. The animals were then transfer to their home cage and left to recover.

L5–L6 spinal nerve ligation model (L5–L6 SNL)

Unilateral mononeuropathy was produced in rat using the L5–L6 SNL model performed essentially as described by Kim and Chung (1992). The rats were anesthetized with intraperitoneal combination of ketamine and xyalazine at 60 and 6.5 mg/kg, respectively. The rat was placed in a prone position and the left spinal muscle was separated from the spinous process at the L4–S2 level. The L5 transverse process was carefully removed with a small rongeur to identify visually the L5–L6 spinal nerve. The left L5 and L6 spinal nerves were isolated

and tightly ligated with 6.0 silk thread. A complete homeostasis was confirmed and the wound was sutured as described in the CCI procedure. Sham surgery was performed by exposing the left L5 and L6 spinal nerve as described above, but not damaging it. Post operative care was performed as per the CCI model.

Sensory testing (Nociceptive assay)

Nociceptive assays aimed at determining the severity of behavioral neuropathic parameters, namely spontaneous pain, allodynia and hyperalgesia, were performed. The assays involved measurement of the degree of spontaneous (ongoing) pain and tests of hind limb withdrawal to cold, thermal and mechanical stimuli (dynamic mechanical allodynia, cold allodynia, mechanical hyperalgesia and thermal hyperalgesia). Separate group of animals ($n = 4$) was used for each assay.

Spontaneous pain

Spontaneous pain was assessed for a total time period of 5 min as described previously by Choi et al. (1994). The operated rats were individually placed inside an observation cage and an initial acclimatization period of 10 min was given to each of the rat. A total of 4 rats were assigned to this group. The cumulative duration that the rat holds its ipsilateral paw off the floor was noted. The paw lifts associated with locomotion or body repositioning were not counted. For each measurement, three successive readings were taken without any elapse and the mean was calculated.

Dynamic component of mechanical allodynia

Dynamic allodynic response was assessed according to the procedure described by Field et al. (1999). The operated rat was placed inside an observation cage. An initial acclimatization period of 10 min was given to each of the rat. A total of 4 rats were assigned to this group. A positive dynamic allodynia response consisted of lifting the affected paw for a finite period of time in response to mild stroking on the planter surface using a cotton bud. This stimulus is non-noxious to a normally behaving rat. The latency to paw withdrawal was then noted. If no paw withdrawal was shown within 15 sec, the test was terminated and animal was assigned non-responsive. For each measurement, three successive readings were taken with 3 min elapsed between each test and mean was calculated.

Cold allodynia

The rats demonstrating unilateral mononeuropathy were assessed for acute cold allodynia sensitivity using the acetone drop application technique, as described by Caudle et al. (2001). The operated rat was placed inside an observation cage and allowed to acclimatize for 10 min. A total of 4 rats were assigned to this group. A few drops (100–200 µl) of freshly dispend acetone were squirted as a fine mist onto the midplanter region of the affected paw. A cold allodynic response was assessed by noting the duration of the paw withdrawal response. For each measurement, the paw was sampled 3 times with 3 min elapsed between each test and the mean was calculated.

Mechanical hyperalgesia

Mononeuropathic rats were assessed for mechanical hyperalgesia sensitivity according to the procedure described by Gonzalez et al. (2000). The operated rat was placed inside an observation cage and allowed to acclimatize for 10 min. A total of 4 rats were assigned to this group. Hind paw withdrawal duration was measured after a mild pin-prick stimulus to the midplanter surface of the ipsilateral hind paw. A withdrawal was defined as being abnormally prolonged if it lasted for at least 2 s. The mean duration of withdrawal was taken from a set of three responses with 3 min elapsed between each test.

Thermal hyperalgesia

Thermal hyperalgesia response was assessed according to the procedure described by Eddy and Leimbach (1953). A total of 4 rats were assigned to this group. The temperature of the hot plate was set at 55.0 ± 0.1 °C. The operated rats was placed on the heated surface, and the time interval between placement and shaking, licking or tucking of the affected hind paws was recorded as the latency response. If no paw withdrawal was shown within 22 sec, the test was terminated and animal was assigned non-responsive. For each measurement, three successive readings were taken with 3 min elapsed between each test and mean was calculated.

Drug administration

Baseline sensory responses were measured for each group of animals ($n = 4$) preoperatively and 20 days postoperatively according to a predetermined manner. Animals showing all five neuropathic pain parameters in baseline measurements (0 h) on post-operative day 7 (POD7) were then administered the relevant drug by the intraperitoneal route according to a predetermined randomized table until POD13 and testing was performed again at 1, 2 and 3 h after drug administration along with a baseline measurement (0 h). After post-operative day 13, only baseline measurement was taken until POD20. Each group of animals was used for only one drug administration and for one parameter to ensure no 'carry-over' effects. Guggulipid (100 and 50 mg/kg) was administered at $t = 0$ after baseline measurement. Two positive control groups were run alongside drug treatment using pregabalin (50 and 30 mg/kg, respectively). A vehicle control group was also run using saline. The treatment protocol remained the same for these five groups. No drug testing was performed for sham-operated rats.

Statistical analysis

Data are presented as mean ± SEM. Statistical significance was determined for drug effects by one-way ANOVA followed by Bonferroni *post-hoc* test for multiple comparisons. Comparison results with $p < 0.05$ were considered statistically significant. The statistical software package SPSS (version 17.0) was used for the analysis.

Results

All animals included in this study exhibited characteristic neuropathic pain behavior in baseline measurement (0 h) on post-operative day 7 (POD7) after CCI and SNL surgery

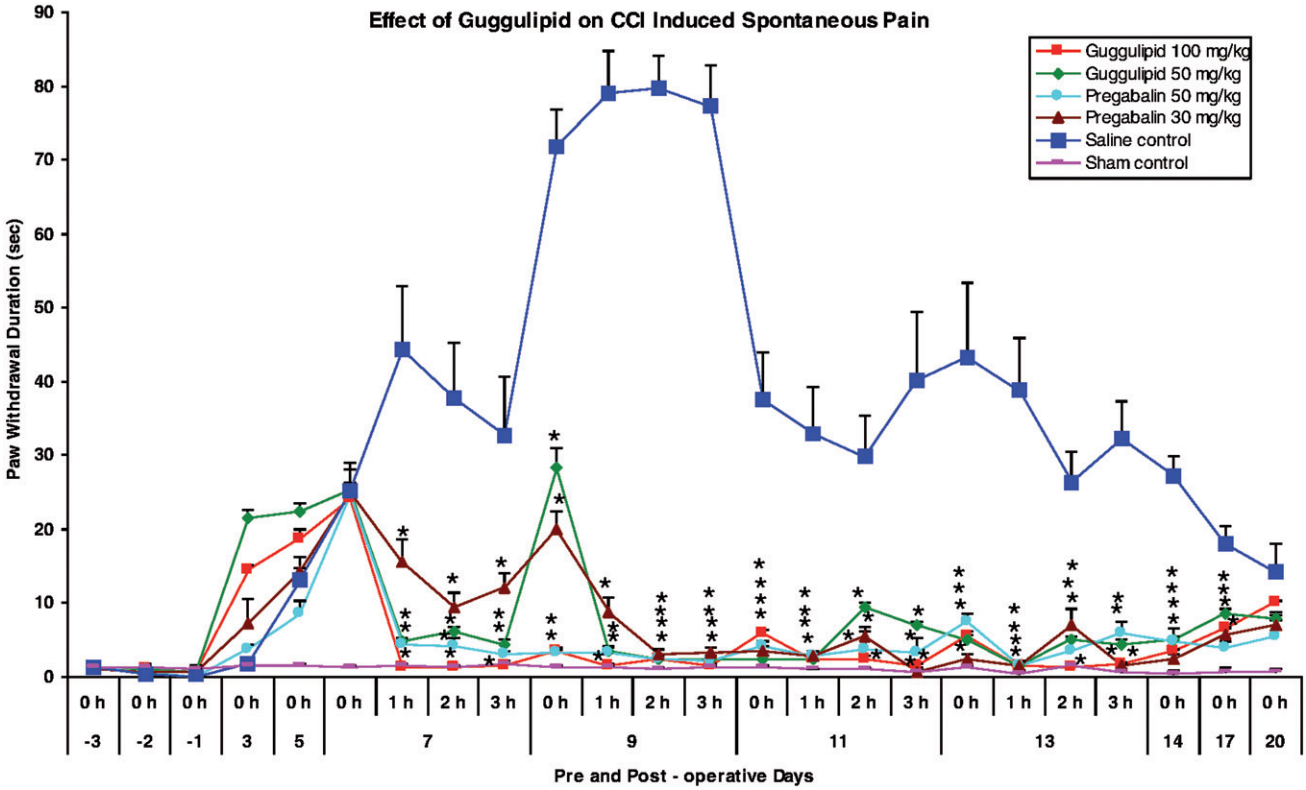

Figure 1. Effect of guggulipid in reversing the spontaneous pain response in CCI rats along with pregabalin. The results are shown as the mean paw withdrawal duration (mean PWD ± SEM) of 4 rats per group. *$p < 0.05$, in comparison with control values (one-way ANOVA followed by Bonferroni *post-hoc* test).

when compared with preoperative values except sham-operated animals.

Effect on spontaneous pain: PWD

CCI model

Administration of guggulipid after baseline measurement on POD7, completely reversed the spontaneous pain response, at the dose 100 and 50 mg/kg, after 1 h of drug administration (1.25* ± 0.25 sec and 4.75* ± 0.48 s, respectively, versus 44.25 ± 8.74 sec for control, *$p < 0.05$) and the effect continuously maintained during whole treatment period (POD7-13; Figure 1). Guggulipid at a dose of 100 mg/kg was observed to be 8-times more effective than 50 mg/kg (3.5* ± 0.29 sec versus 28.25 ± 2.78 sec, *$p < 0.05$) and 5–6-times more effective than the standard drug pregabalin (30 mg/kg; 3.5* ± 0.29 sec versus 20 ± 2.35 s, *$p < 0.05$) in baseline measurement on POD9. In this test, the standard drug pregabalin reversed the spontaneous pain behavior at both doses (50 and 30 mg/kg) during the entire treatment period. On withdrawal of treatment on post-operative day 14, both drugs showed a post-treatment effect until POD17.

SNL model

Both doses of guggulipid were observed to be active at a single time point during the entire treatment period (POD7-13). Guggulipid at a dose of 100 mg/kg was effective only at 2 h of POD9 (3.75* ± 0.25 sec versus 8.0 ± 1.47 sec for control, *$p < 0.05$) while a dose of 50 mg/kg was effective only at 1 h of POD13 (2.75* ± 0.25 sec versus 6.5 ± 1.32 sec for control, *$p < 0.05$; Figure 2). In this test, the standard drug pregabalin reversed the spontaneous pain behavior at a dose of 50 mg/kg, after 1 h of drug administration (2.0* ± 0.41 sec versus 5.0 ± 0.71 sec for control, *$p < 0.05$) and the effect was maintained until POD11 except on 3 h of POD7 and 0 h of POD11, while a dose of 30 mg/kg, showed a limited effect on reversing spontaneous pain. After withdrawal of treatment on POD14, guggulipid and pregabalin did not show any carry-over effect.

Mechanical allodynia: PWL

CCI model

Administration of guggulipid after baseline measurement on POD7 reversed the allodynic response at a dose of 100 mg/kg, after 3 h of administration (14.5* ± 0.29 sec versus 4.5 ± 0.29 sec for control, *$p < 0.05$) and the effect was continuously maintained during the entire treatment period (POD7-13), except on 2 h of POD9 and 3 h of POD11. Guggulipid was effective in continuously reversing the allodynic response at a lower dose from 2 h of POD9 (13.0* ± 0.71 sec versus 7.0 ± 0.41 sec for control, *$p < 0.05$) to POD13, except on 2 h of POD11 (Figure 3). Standard drug pregabalin showed protection at a dose of 30 mg/kg, from 3 h of POD7 (14.75* ± 0.25 sec versus 4.5 ± 0.29 sec for control, *$p < 0.05$) to POD13, except on baseline measurement of POD9 and POD13, while a dose of 50 mg/kg, showed a limited antiallodynic effect. On withdrawal of treatment on post-operative day 14, both doses of guggulipid remained effective after four days from the last dose, while pregabalin was devoid of any antiallodynic effect.

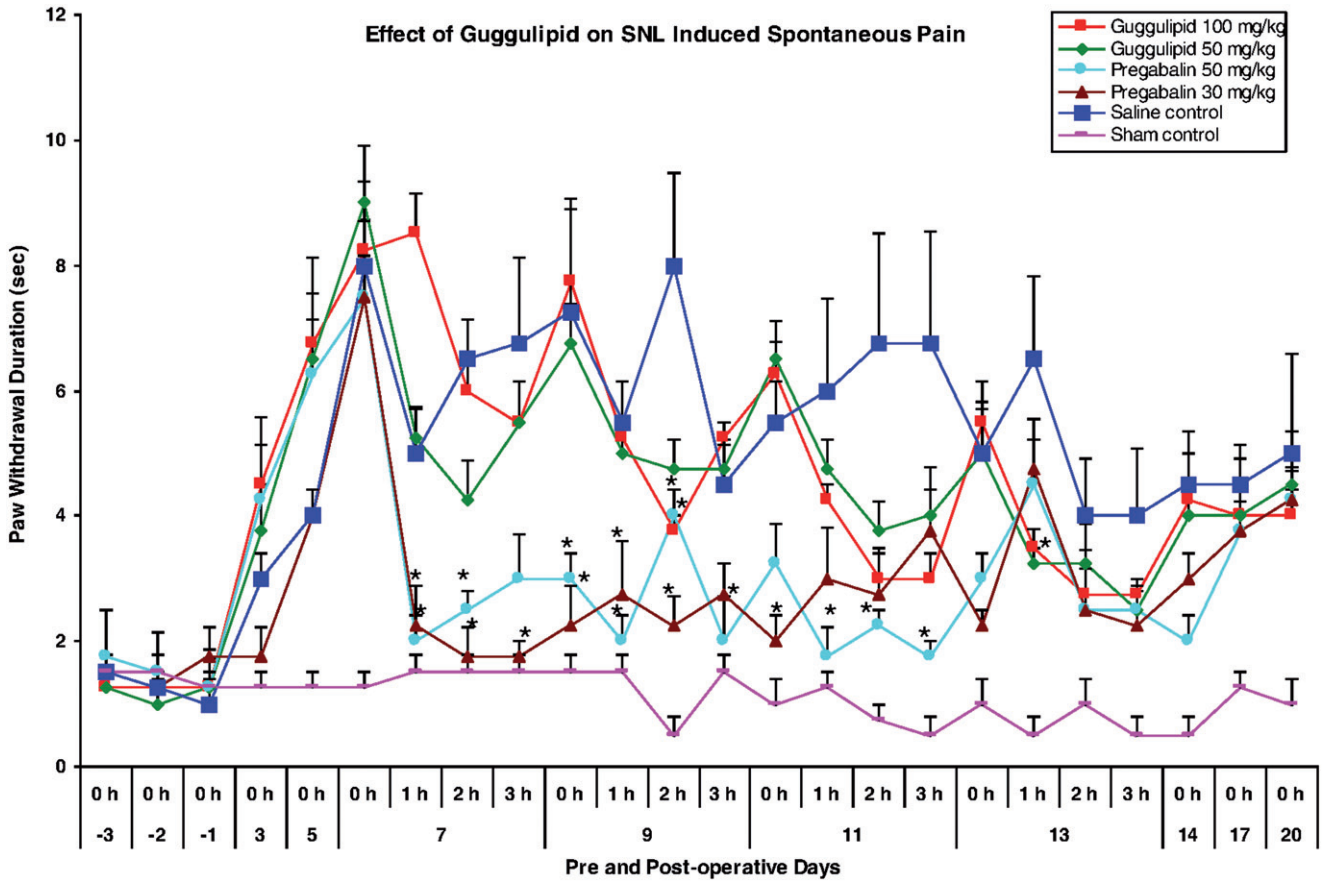

Figure 2. Effect of guggulipid in reversing the spontaneous pain response in SNL rats along with pregabalin. The results are shown as the mean paw withdrawal duration (mean PWD ± SEM) of 4 rats per group. *$p < 0.05$, in comparison with control values (one-way ANOVA followed by Bonferroni *post-hoc* test).

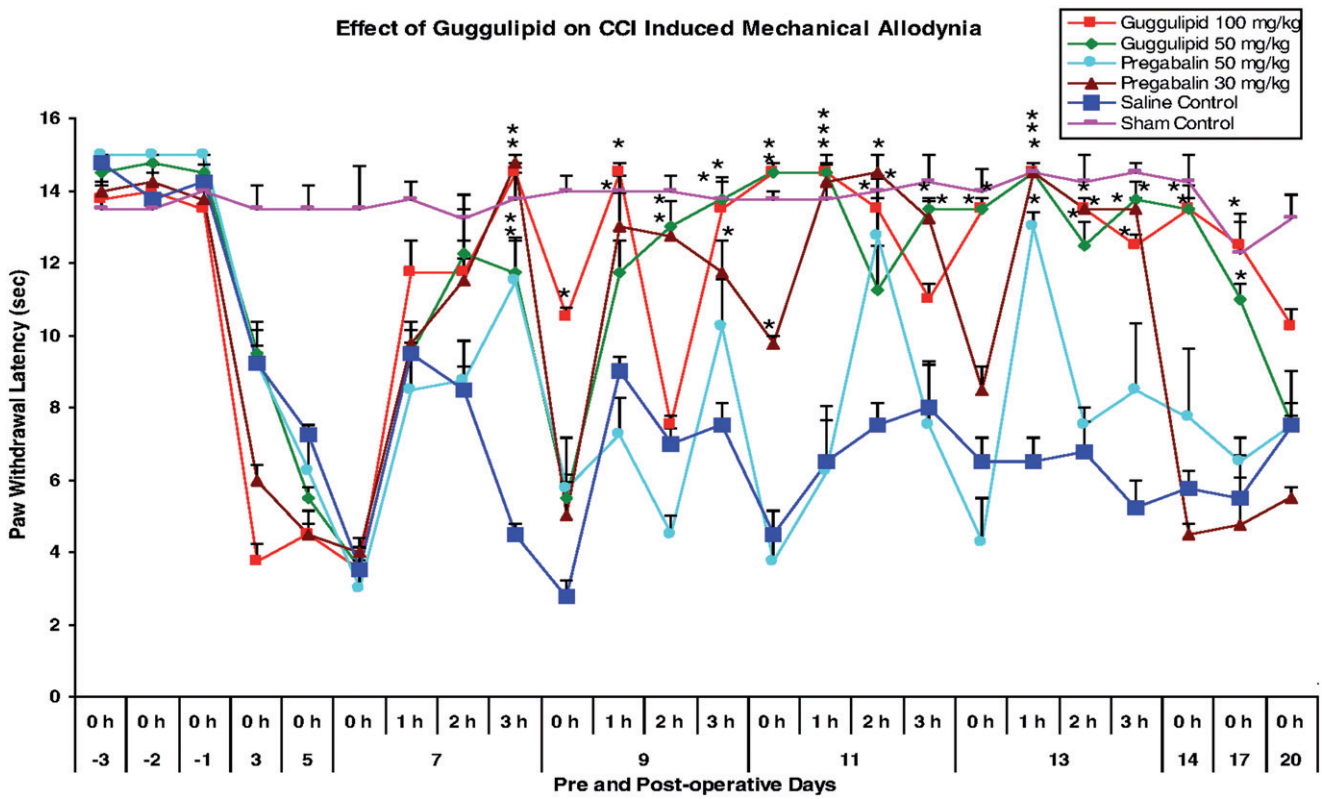

Figure 3. Effect of guggulipid in reversing the mechanical allodynia response in CCI rats along with pregabalin. The results are shown as the mean paw withdrawal latency (mean PWL ± SEM) of 4 rats per group. *$p < 0.05$, in comparison with control values (one-way ANOVA followed by Bonferroni *post-hoc* test).

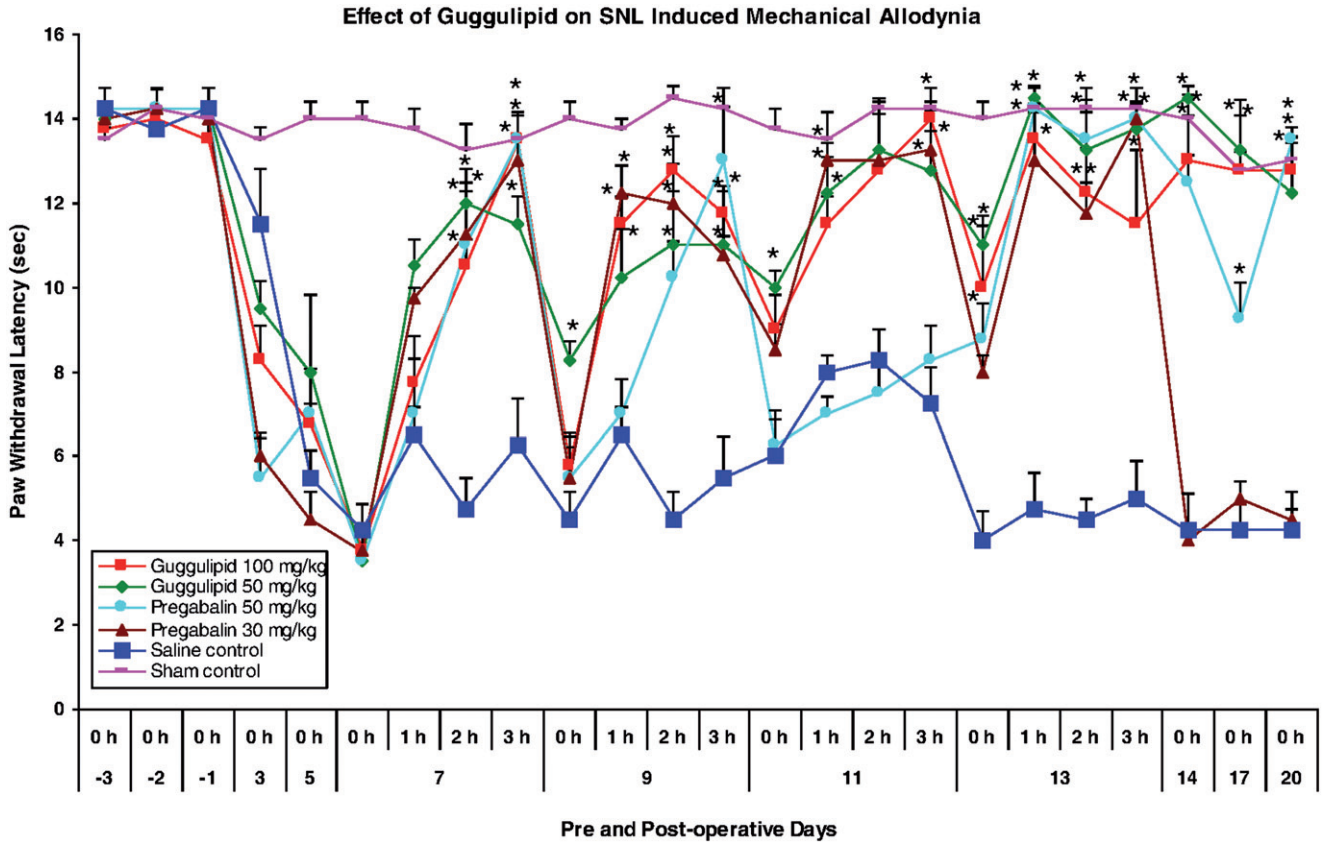

Figure 4. Effect of guggulipid in reversing the mechanical allodynia response in SNL rats along with pregabalin. The results are shown as the mean paw withdrawal latency (mean PWL ± SEM) of 4 rats per group. *$p < 0.05$, in comparison with control values (one-way ANOVA followed by Bonferroni *post-hoc* test).

SNL model

Administration of guggulipid after baseline measurement on POD7 reversed the mechanical allodynia response at a dose of 100 mg/kg, after 2 h of administration (10.5* ± 0.65 sec versus 4.75 ± 0.75 sec for control, *$p < 0.05$) and the effect continuously maintained during whole treatment period (POD7-13) except at baseline measurement on POD9, 11 and on 2 h of POD11. Guggulipid was effective in continuously reversing the allodynic response at lower dose from 2 h of POD7 (12.0* ± 0.82 sec versus 4.75 ± 0.75 sec for control, *$p < 0.05$) to POD13, except on 2 h of POD11 (Figure 4). Standard drug pregabalin showed a nonuniform trend of effects at a dose of 50 mg/kg, while a dose of 30 mg/kg, showed antiallodynic properties from 2 h of POD7 (11.25* ± 1.25 sec versus 4.75 ± 0.75 sec for control, *$p < 0.05$) to POD13 except on baseline measurement on POD9, 11 and 13 and on 2 h of POD11. On withdrawal of treatment on post-operative day 14, both doses of guggulipid and a higher dose of pregabalin showed carry-over effects until completion of the study, while pregabalin at a lower dose was devoid of any antiallodynic effect.

Chemical allodynia: PWD

CCI model

Guggulipid at both doses gave limited effects during the entire treatment period (POD7-13; Figure 5). Standard drug pregabalin at a dose of 30 mg/kg, showed complete protection until POD13, while a dose of 50 mg/kg, showed a nonuniform trend of effects with activities more pronounced at the beginning. After withdrawal of treatment, guggulipid and pregabalin did not show an antiallodynic effect, except pregabalin at 30 mg/kg, on POD14.

SNL model

Administration of guggulipid after baseline measurement on POD7 showed a limited effect on chemical allodynia response at a dose of 100 mg/kg, while a dose of 50 mg/kg was observed to be effective after 1 h of administration (10.25* ± 0.85 sec versus 23.75 ± 1.55 sec for control, *$p < 0.05$) and the effect was continuously maintained during the entire treatment period (POD7-13) except at 2 h of POD9 and 1 and 3 h of POD11 (Figure 6). Both doses of standard drug pregabalin (50 and 30 mg/kg) showed antiallodynic properties from 1 h of POD7 (5.0* ± 0.71 sec and 8.75* ± 0.85 sec, respectively, versus 23.75 ± 1.55 sec for control, *$p < 0.05$) to POD13 except on 3 h of POD11. On withdrawal of treatment on post-operative day 14, both doses of guggulipid and pregabalin showed a carry-over effect until POD20.

Mechanical hyperalgesia: PWD

CCI model

Hyperalgesia evoked by a mechanical pin-prick stimulus was effectively attenuated at all time-points by both doses of

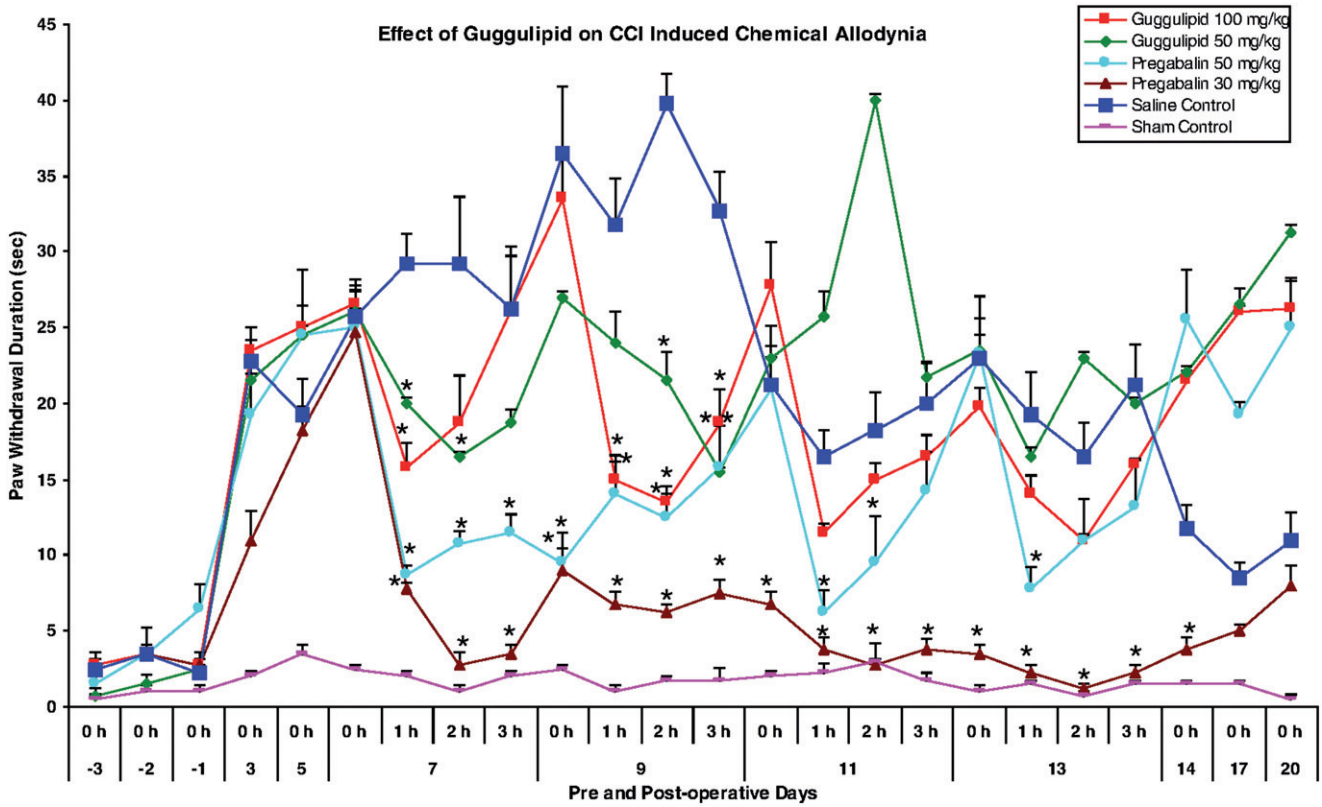

Figure 5. Effect of guggulipid in reversing the chemical allodynia response in CCI rats along with pregabalin. The results are shown as the mean paw withdrawal duration (mean PWD ± SEM) of 4 rats per group. *$p < 0.05$, in comparison with control values (one-way ANOVA followed by Bonferroni *post-hoc* test).

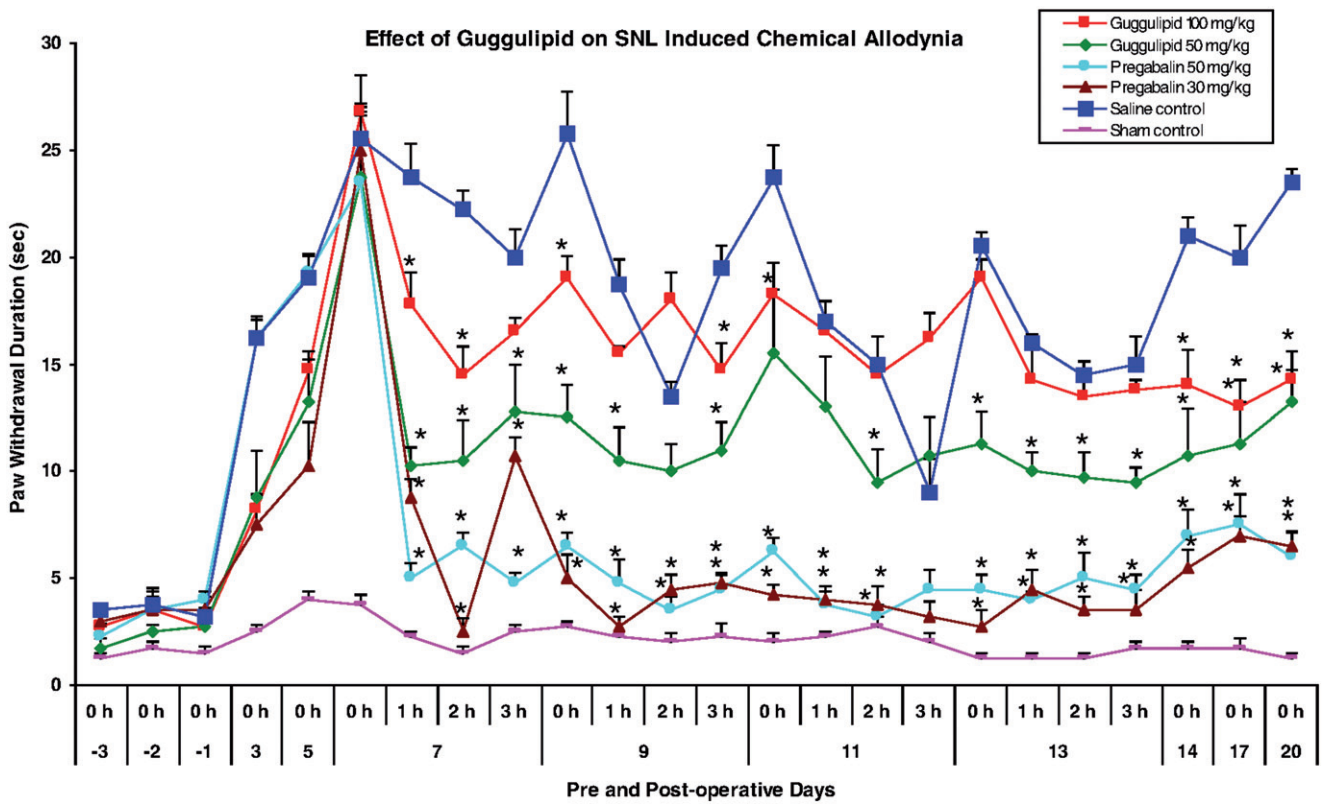

Figure 6. Effect of guggulipid in reversing the chemical allodynia response in SNL rats along with pregabalin. The results are shown as the mean paw withdrawal duration (mean PWD ± SEM) of 4 rats per group. *$p < 0.05$, in comparison with control values (one-way ANOVA followed by Bonferroni *post-hoc* test).

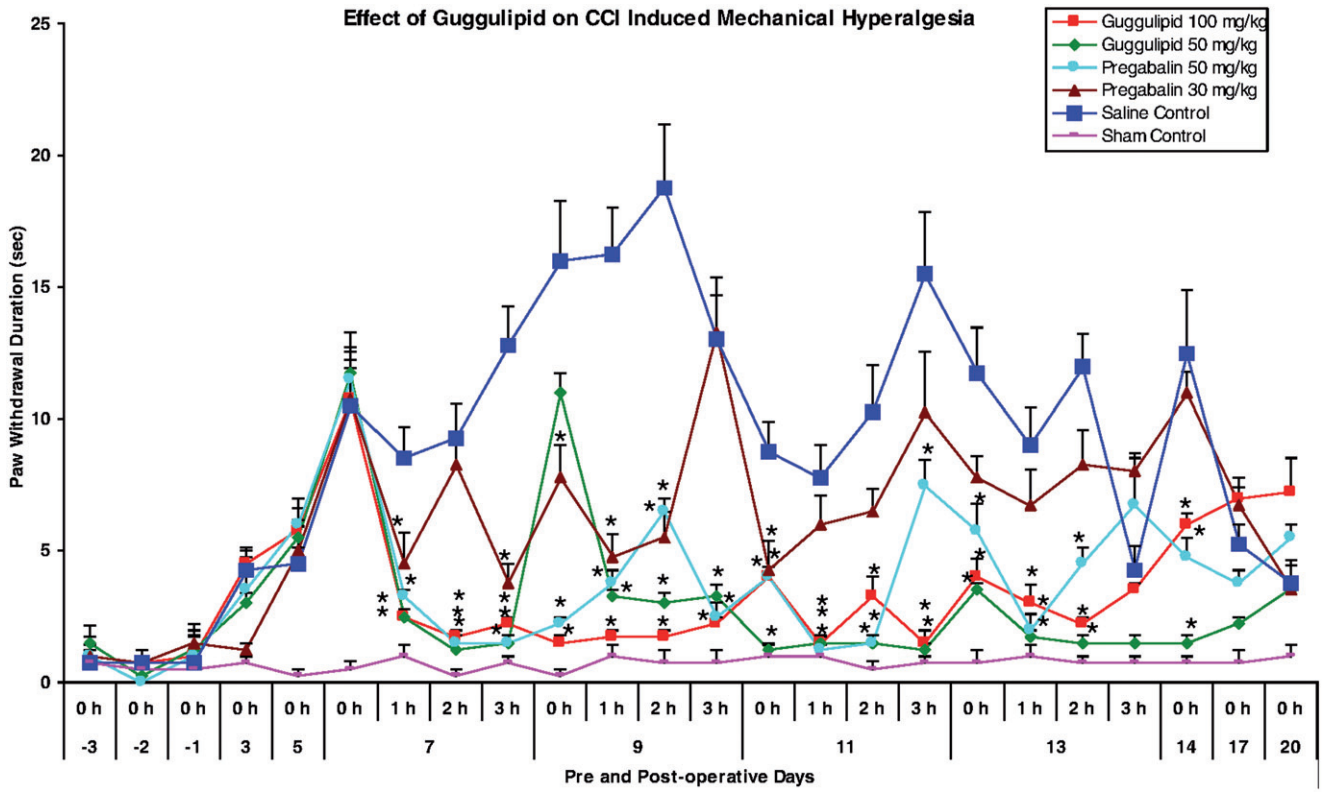

Figure 7. Effect of guggulipid in reversing the mechanical hyperalgesia response in CCI rats along with pregabalin. The results are shown as the mean paw withdrawal duration (mean PWD ± SEM) of 4 rats per group. *$p < 0.05$, in comparison with control values (one-way ANOVA followed by Bonferroni *post-hoc* test).

guggulipid (100 and 50 mg/kg) after 1 h of administration on POD7 (2.5* ± 0.29 sec and 2.5* ± 0.29 sec, respectively, versus 8.5 ± 1.19 sec for control, *$p < 0.05$) to 2 h of POD13, except on baseline measurement on POD9 in the case of guggulipid 50 mg/kg (Figure 7). Higher dose of standard drug pregabalin was observed to be effective from the beginning (3.25* ± 0.25 sec at 1 h of POD7 versus 8.5 ± 1.19 sec for control, *$p < 0.05$) to 2 h of POD13 while the lower dose possessed limited antihyperalgesic activity. On withdrawal of treatment, both doses of guggulipid the and higher dose of pregabalin (50 mg/kg) were effective until POD14.

SNL model

Hyperalgesia evoked by a mechanical pin-prick stimulus was effectively attenuated by both doses of guggulipid (100 and 50 mg/kg) after 1 h of administration on POD7 (3.25* ± 0.63 sec and 3.25* ± 0.25 sec, respectively, versus 8.5 ± 0.96 sec for control, *$p < 0.05$) and the effect was maintained on continuous dosing at all time-points until 2 h of POD13, except in baseline measurement on POD9 by guggulipid 50 mg/kg (Figure 8). Standard drug pregabalin was observed to be effective at 50 mg/kg dose from 1 h of POD7 (4.0* ± 0.41 sec versus 8.5 ± 0.96 sec for control, *$p < 0.05$) to 1 h of POD13, except on 2 h of POD9 and baseline measurement on POD11 and 13. Standard drug pregabalin at a dose of 30 mg/kg, possessed limited antihyperalgesic activity. On withdrawal of treatment guggulipid at a lower dose (50 mg/kg) was effective until POD17, while a higher dose of guggulipid (100 mg/kg) and pregabalin (50 mg/kg) showed effects only on POD14.

Thermal Hyperalgesia: PWL

CCI model

Guggulipid at a dose of 50 mg/kg significantly improved paw withdrawal latency after 1 h of administration on POD7 (18.5* ± 1.04 sec versus 10.75 ± 1.11 sec for control, *$p < 0.05$) and the effect was continuously maintained until 2 h of POD13, while 100 mg/kg reversed the thermal hyperalgesic response after 2 h of administration (16.25* ± 1.31 sec versus 11.0 ± 0.91 sec for control, *$p < 0.05$) and the effect continued until POD13, except in baseline measurement on POD11 and 13 (Figure 9). Both doses of standard drug pregabalin (50 & 30 mg/kg) showed complete protection after 2 h of administration on POD7 (18.75* ± 0.63 sec and 19.5* ± 0.65 sec, respectively, versus 11.0 ± 0.91 sec for control, *$p < 0.05$) to POD13, except on baseline measurement on POD9, 11 and 13 in the case of pregabalin 30 mg/kg. On withdrawal of treatment on POD14, post-treatment effect was not observed in any case.

SNL model

Administration of guggulipid after baseline measurement on POD7 reversed the thermal hyperalgesia response at 100 mg/kg, after 1 h of administration (13.75* ± 0.85 sec versus 9.0 ± 1.08 sec for control, *$p < 0.05$) and the effect was continuously maintained until 2 h of POD13. Guggulipid on lower dose showed a nonuniform trend of effects during the entire treatment period (Figure 10). In this test, a higher dose of standard drug pregabalin was observed to be effective after

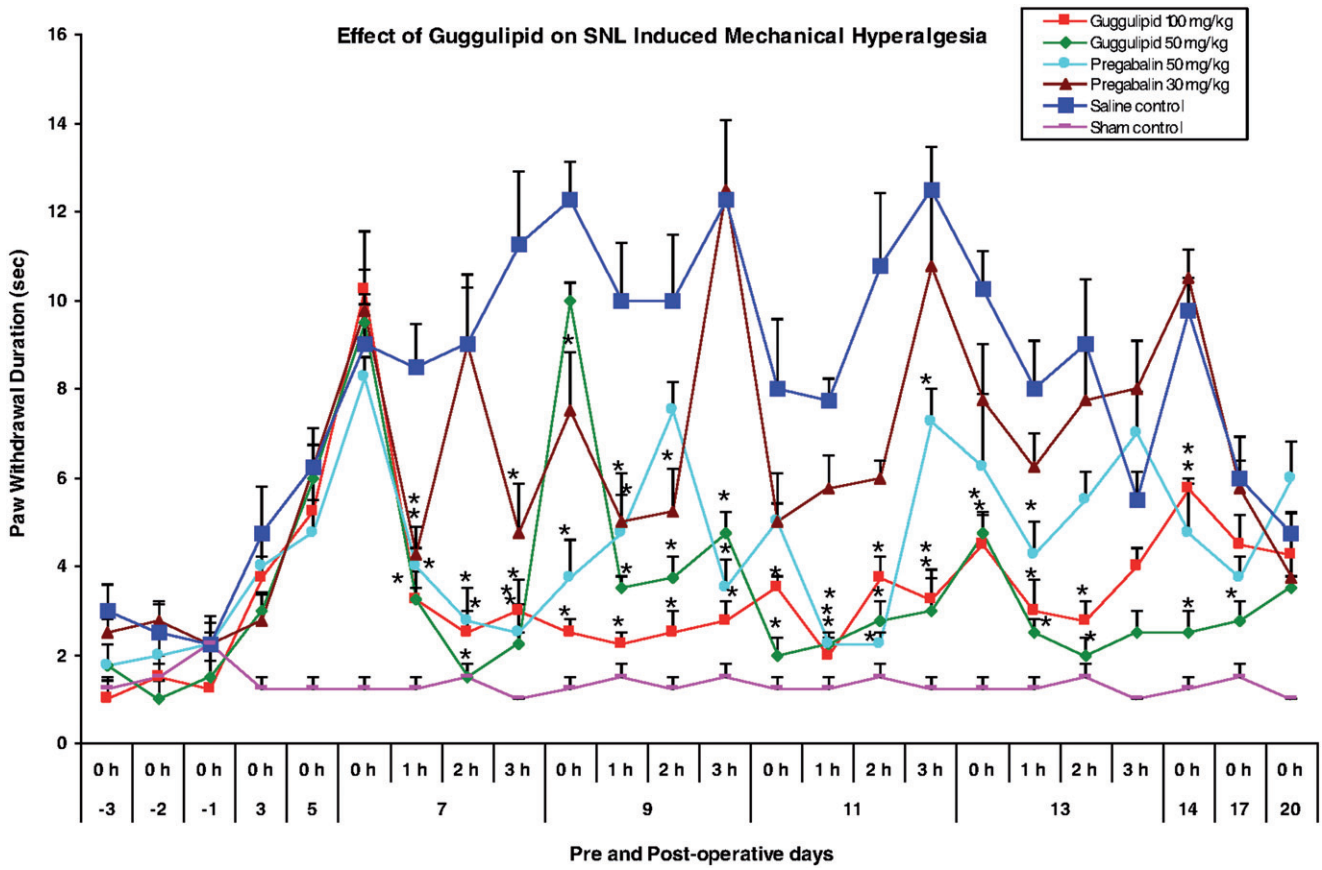

Figure 8. Effect of guggulipid in reversing the mechanical hyperalgesia response in SNL rats along with pregabalin. The results are shown as the mean paw withdrawal duration (mean PWD ± SEM) of 4 rats per group. *$p < 0.05$, in comparison with control values (one-way ANOVA followed by Bonferroni *post-hoc* test).

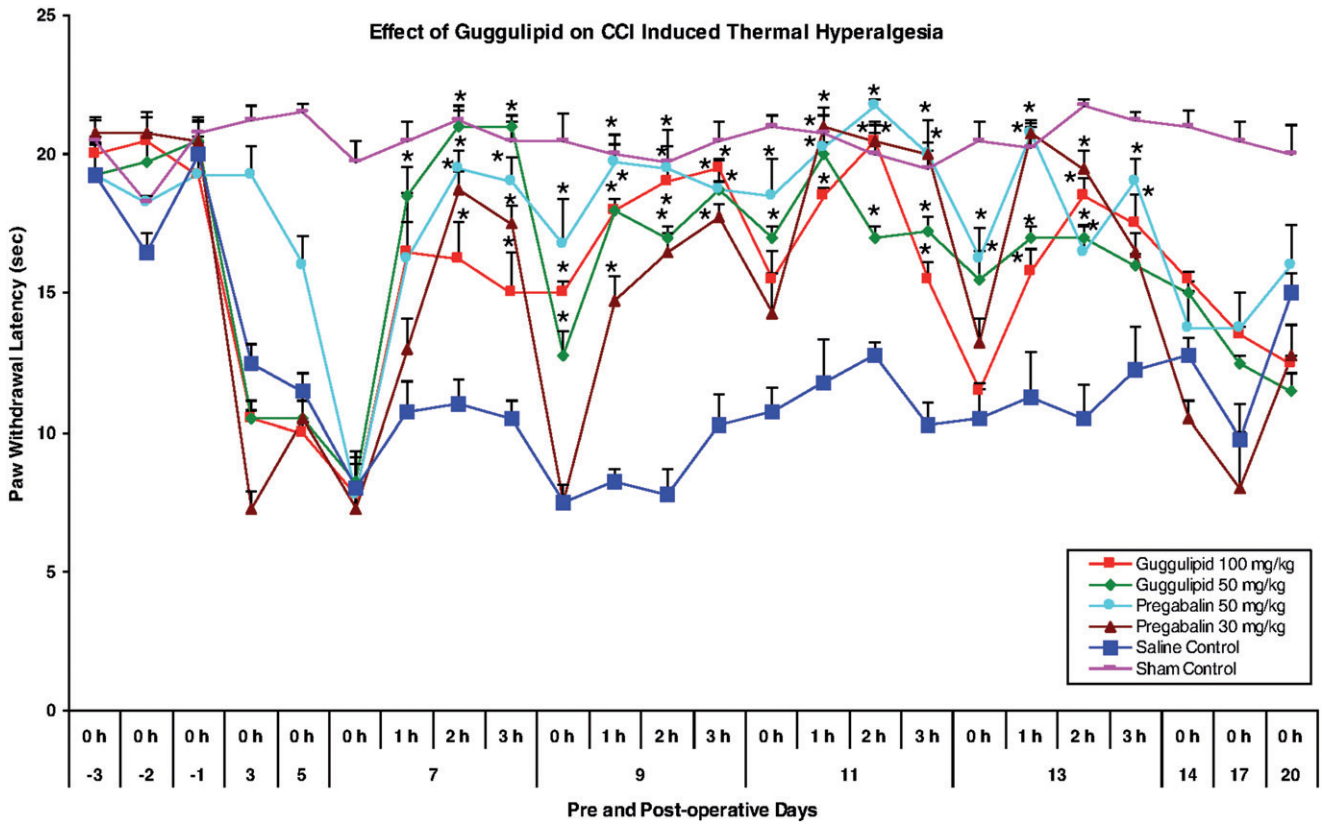

Figure 9. Effect of guggulipid in reversing the thermal hyperalgesia response in CCI rats along with pregabalin. The results are shown as the mean paw withdrawal latency (mean PWL ± SEM) of 4 rats per group. * $p < 0.05$, in comparison with control values (one-way ANOVA followed by Bonferroni *post-hoc* test).

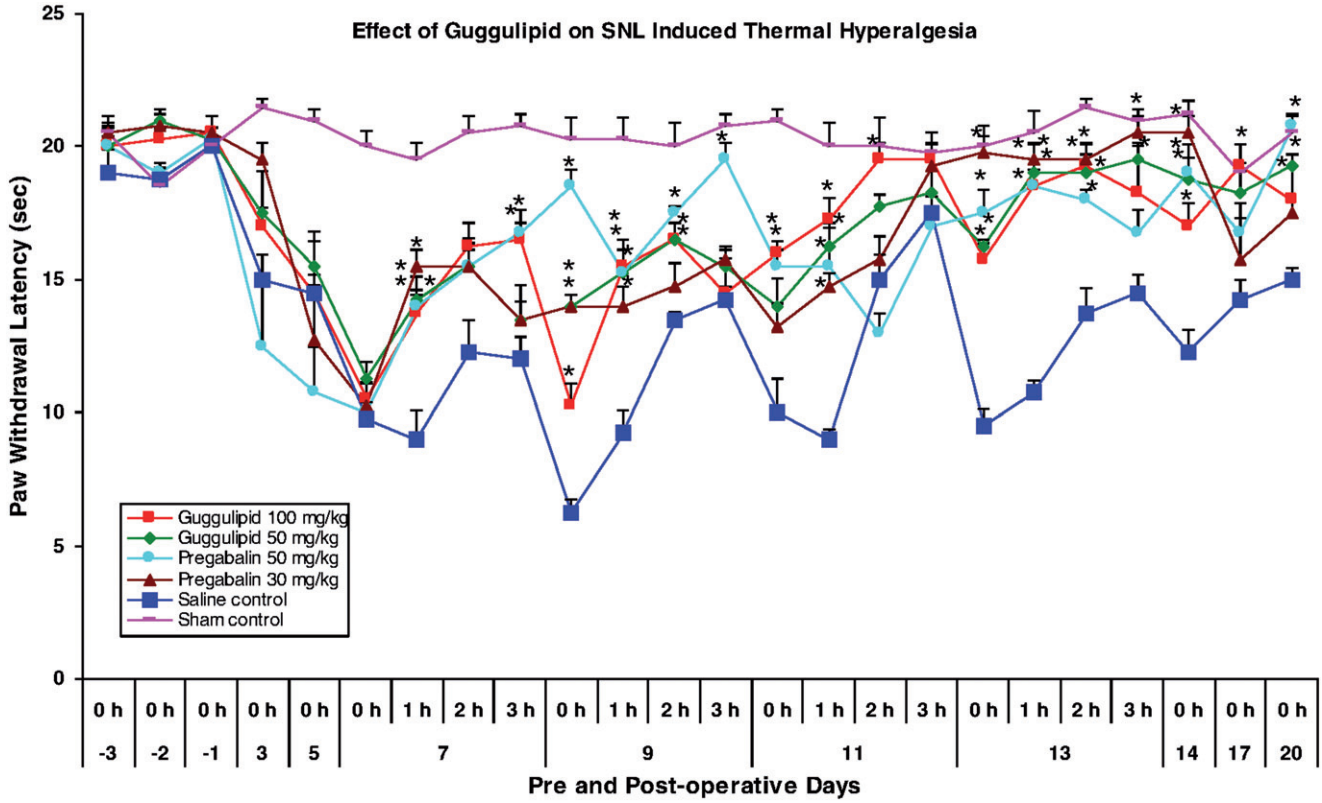

Figure 10. Effect of guggulipid in reversing the thermal hyperalgesia response in SNL rats along with pregabalin. The results are shown as the mean paw withdrawal latency (mean PWL ± SEM) of 4 rats per group. * $p < 0.05$, in comparison with control values (one-way ANOVA followed by Bonferroni *post-hoc* test).

1 h of administration on POD7 (14.0* ± 0.41 sec versus 9.0 ± 1.08 sec for control, *$p < 0.05$) to 2 h of POD13, except on 2 h of POD7 and 11 and 3 h of POD11, while a lower dose showed irregular effects during the entire treatment period. On withdrawal of treatment, a lower dose of guggulipid and pregabalin showed effects until POD14 while a higher dose of guggulipid was observed to be effective until POD17 and pregabalin until POD20.

Discussion

This study examined the potential therapeutic value of ''guggulipid'' in the treatment of neuropathic pain using the CCI and SNL models of neuropathic pain. The results of this study indicated that guggulipid possessed antiallodynic and antihyperalgesic action in both the CCI and SNL models and also showed property to relieve spontaneous pain only in the CCI model.

There is considerable evidence supporting a palliative role for microglial activation in neuropathic pain conditions (Beggs & Salter, 2007; Saab et al., 2008; Scholz et al., 2008; Streit et al., 2004; Suter et al., 2007; Tsuda et al., 2005). Microglia, the resident macrophages and principal immune response cell in the CNS, quite evenly distributed in the brain and spinal cord, are massively activated in the dorsal horn soon after peripheral nerve injury and their activation leads to pro-inflammatory responses with pathological effects, such as neuronal hyperexcitability, neurotoxcity and chronic inflammation, which contribute in neuropathic pain development (Davalos et al., 2005; Nimmerjahn et al., 2005; Streit et al., 2004). Microglial activation occurs in a topographically organized fashion close to the central terminals of injured afferents and release many immune modulators (cytokines and chemokines) that contribute to the induction and maintenance of neuropathic pain by altering neuronal function (Beggs & Salter, 2007; Saab et al., 2008; Scholz et al., 2008; Streit et al., 2004; Suter et al., 2007; Tsuda et al., 2005). Peripheral neurons transmit signals to spinal dorsal horn neurons, releasing neurotransmitters such as calcitonin gene-related protein (CGRP), substance P, glutamate, and ATP. Locally in the dorsal horn, there are also other neurotransmitters involved, such as GABA, glycine, serotonin. Therefore, it is possible to suggest that these neurotransmitters may initiate microglial activation associated with neuropathic pain (Chen et al., 2010; Wu & Zhuo, 2008). Activated microglias release various cytokines and chemokines, including IL-1β, IL-6, tumor necrosis factor-α (TNFα), PGE_2, and nitric oxide. It has been shown that TNFα activates p38 mitogen-activated kinase (MAPK) in DRG, which leads to the expression of IL-1β, IL6, and COX-2. These cytokines, released by activated microglia, will amplify microglial activation in an autocrine manner and may act directly on dorsal horn neurons to cause behavioral sensitization. It has been shown that intrathecal administration of IL-1β, IL-6 or TNFα can lead to symptoms of neuropathic pain in healthy rats (Krakauer, 2004; Reeve et al., 2000).

Another example for neuron-glia interactions contributing to neuropathic pain are the matrix metalloproteinases (MMP-2 and MMP-9). They are produced by both neurons and glia and mediate pain hypersensitivity by initiating IL-1β cleavage

and microglial activation. In cultured DRG neurons, TNFα and IL-1β increased both the expression and release of MMP-9. Further, intrathecal administration of MMP-9 or MMP-2 is sufficient to produce neuropathic pain symptoms (Kawasaki, 2008).

Previous studies have shown that guggulsterone, which is an active constitute of guggulipid, down-regulates expression of matrix metalloprotease (MMP-9). Guggulsterone suppresses IL-1β and IL-6 mediated inflammatory response, TNFα and PGE_2 production, nitric oxide formation (NO) and phosphorylation of p38 (Gebhard et al., 2009; Lee et al., 2008; Lv et al., 2008; Meselhy, 2003; Niranjan et al., 2011; Sarfaraz et al., 2008; Shishodia & Aggarwal, 2004; Song et al., 2010).

Hence, in the present work, the hypothesis that administration of guggulipid relives the pain associated with neuropathy was tested. The data demonstrated that guggulipid exhibited antiallodynic and antihyperalgesic activities in one or more of the sensory nociceptive assays. In CCI rats, both doses of guggulipid (100 and 50 mg/kg) were found to be effective in reversing spontaneous pain, mechanical allodynia and mechanical and thermal hyperalgesia responses. The activity profiles of the different doses of guggulipid were found to vary with time. Guggulipid (100 mg/kg) was found more effective in reversing spontaneous pain, mechanical allodynia and mechanical hyperalgesia responses. Guggulipid (50 mg/kg) was more effective in reversing completely the thermal hyperalgesia response. Both doses of guggulipid showed limited effectiveness on chemical allodynia response. In the case of standard drug, pregabalin, both doses (50 and 30 mg/kg) were effective in completely reversing spontaneous pain response. Pragabalin (50 mg/kg) was found more effective in reversing mechanical and thermal hyperalgesia responses, while pregabalin 30 mg/kg, showed effectiveness in reversing mechanical and chemical allodynia.

In the case of SNL rats, both doses of guggulipid (100 and 50 mg/kg) were found to be ineffective in reversing spontaneous pain. Guggulipid (100 and 50 mg/kg) showed antiallodynic and antihyperalgesic activity but the activity profile of different doses were found to vary with time as seen in CCI. Guggulipid (100 mg/kg) afforded the maximum protection against mechanical and thermal hyperalgesia, while guggulipid (50 mg/kg) was more effective in attenuating mechanical and chemical allodynia. In case of standard drug, pregabalin, both doses (50 and 30 mg/kg) showed similar activity in reversing the chemical allodynia response, while possessing limited effectiveness on spontaneous pain but a higher dose showed better results than lower. Pragabalin (50 mg/kg) was found more effective in reversing mechanical and thermal hyperalgesia responses, while pregabalin 30 mg/kg, showed effectiveness in reversing mechanical allodynia.

In the CCI model, guggulipid showed a carry-over effect on spontaneous pain and mechanical allodynia seen at 4 days after the final drug administration. In the SNL model, guggulipid showed prolonged carry-over effects on mechanical and chemical allodynia seen at 7 days, and mechanical and thermal hyperalgesia seen at 4 days after the final drug administration. These results are important because guggulipid is the first drug tested in our laboratory that had such prolonged effects. The reason for this prolonged carry-over effect is not clear but may be related to the long half-life of guggulipid in rats.

As seen with standard drug data, the different doses of guggulipid also exhibited differential responses in the CCI and SNL models. This could be due to the difference in severity in both the models. Chronic pain syndromes consist of a wide range of symptoms likely to be mediated by multiple mechanisms. Therefore, blockade of few mechanisms may not provide full pain relief in every patient. A better approach may be, as in this case, designing and developing novel compounds with more mechanisms of action.

Overall, from this study, guggulipid emerged as the active compound with activities against spontaneous pain, mechanical allodynia and mechanical and thermal hyperalgesia responses in the CCI model and against mechanical and chemical allodynia and mechanical and thermal hyperalgesia in SNL rats.

Conclusion

From experimental data collected it is concluded that guggulipid produced antinociception in the peripheral nerve injury (CCI and SNL) models of neuropathic pain. The underlying mechanisms are expected to be modulating microglial activation occurring due to peripheral nerve injury. This study presents the first preliminary report of guggulipid on neuropathic pain. Further research is required to confirm the effectiveness in the large group of animals with rational use of male rats and to prove the hypothesized molecular mechanisms of action of the reported compound.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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