

Protection against Paclitaxel-Induced Hyperalgesia and Allodynia by Pomegranate Seed Oil, Nucleo-CMP Forte®, and L-Carnitine

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Abstract

Background: Hyperalgesia and allodynia are abnormal sensory signs which are usually seen along neuropathic pain (NP) in patients on paclitaxel (PT) chemotherapy. Pomegranate seed oil (PSO) has been used in the traditional medicine for the different kinds of pain. Nucleo-CMP Forte® (NCF) is a nucleotide-based drug and L-carnitine (LC) is a member of natural compounds have been shown effective in diverse peripheral neuroglial disorders. The aim of this study is to compare the anti-hyperalgesia and anti-allodynia activity of PSO, NCF, and LC in PT-induced neuropathy. **Materials and Methods:** Thirty adult male mice were divided equally into five groups of six mice in each group as follows: Control (C), PT, PSO (PT_{ps}), NCF (PT_n), and LC (PT_c) groups. NP was induced by the administration of PT (6 mg/kg, i. p., once weekly) to the mice in all groups except C group. PSO, NCF, and LC were administered orally once daily to mice with PT-induced neuropathy in their corresponding groups. Different behavior parameters were used to test the anti-hyperalgesic and anti-allodynic effect of PSO, NCF, and LC on days 0, 7, 14, 21, and 28 of PT administration. **Results:** PSO, NCF, and LC significantly attenuated NP induced by PT from day 14 up to 28 days using the different behavioral tests for thermal hyperalgesia and allodynia. **Conclusion:** PSO, NCF, and LC have significant potential anti-nociceptive and analgesic activity against PT-induced NP and PSO was the most efficacious than NCF and LC.

Keywords: Allodynia, hyperalgesia, L-carnitine, neuropathy, Nucleo-CMP Forte®, paclitaxel, pomegranate seed oil

INTRODUCTION

Paclitaxel-induced peripheral neuropathy (PIPN) is one of the major side effect experienced by patients on paclitaxel (PT) chemotherapy, characterized by the presence of spontaneous pain, hyperalgesia, and allodynia.^[1] A multitude of molecular and cellular mechanisms have been elucidated for PIPN such as targeting distal neurons, voltage gated ion channels, neurotransmitters, and glial cells, activation of proinflammatory cytokines, neuro-inflammation, and oxidative stress.^[2]

Pomegranate seed oil (PSO) represents a rich source of polyunsaturated fatty acids, especially linoleic and punicic acid, polyphenols, phytosterols, and tocopherols.^[3] It has been reported that conjugated linoleic acid (CLA) gives PSO its favorable physiological antioxidant and anti-inflammatory properties, including anti-atherosclerosis, antiobesity, anti-tumor, and antiaging, inhibiting expression of proinflammatory cytokines, and boosting immune system.^[4,5]

Nucleotides play a central role in many cellular processes, including those associated with nerve repair and drugs containing nucleotides such as Nucleo-CMP Forte® (NCF) have been prescribed to patients with peripheral neurological conditions.^[6]

L-Carnitine (LC) is a naturally occurring aminoacid derivative that plays an essential role in transporting long-chain free fatty acids into mitochondria.^[7] Several evidences suggest that mitochondrial toxicity may be a pivotal feature in chemotherapy-induced peripheral neuropathy. Thereby, drugs such as LC that protect mitochondrial function has

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demonstrated a neuroprotective effect in patients with peripheral neuropathies of different etiologies.^[8]

At present, different group of drugs had been used in alleviating neuropathic pain (NP) induced by chemotherapy, but still their efficacy is uncertain.^[2] The promising pharmacological effects of PSO are taken into consideration recently; therefore, this study was designed to evaluate the activity of PSO, NCF, and LC in the prevention of PIPN.

MATERIALS AND METHODS

Adult male albino mice ($n = 30$) weighing 25–30 g were used in the study. The mice were housed in environmentally controlled room with temperature, humidity, and light regulated. Mice were randomly divided into five equal groups. All mice received intraperitoneal injections of 6 mg/kg PT (Taxol, Bristol-Myers Squib) diluted with saline once weekly except control group that received only saline (0.1 ml/kg/day) over 6 weeks period. The other groups were given either ([PSO, Pometone® liquicap, Vitane Pharma GmbH, Germany] at doses of 1000 mg/kg body weight) or NCF (Cytidine-5'-disodium monophosphate and Uridine-5'-trisodium triphosphate, 30 mg/kg) or LC, 100 mg/kg daily via oral gavage 30 min before PT dose over 6 weeks' period.

The effects of PSO or NCF and LC on PIPN were evaluated by different behavioral tests including thermal hyperalgesia and allodynia (heat and cold) using tail immersion, hot plate, and acetone tests, respectively.^[9,10] These behavioral testing was performed on each mouse in different groups before dosing PT and once weekly up to 4 weeks on days 7, 14, 21, and 28 day post-PT dosing. Approximately 20 min was allowed to acclimatize the mice for the assessments of pain behavior testing. Approval of Ethics was obtained from Ethics Committee of College of Medicine, Hawler Medical University before starting the experiment.

Behavioral tests

Tail immersion test for thermal hyperalgesia ($47^{\circ}\text{C} \pm 1^{\circ}\text{C}$)

The tip of the tail of the mice was immersed in the warm water bath set at $47^{\circ}\text{C} \pm 1^{\circ}\text{C}$ until tail withdrawal sign is observed with a cutoff time of 15 s. Shortening of the tail withdrawal time indicates hyperplasia.

Hot plate method (thermal hyperalgesia)

the rats were placed onto the heated plate ($50^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$) until paw withdrawal was observed (cutoff time of 10 s). The latency period was recorded in either response to thermal hyperalgesia by lifting hind-paw licking or commences jumping.

Tail immersion test for cold hyperalgesia ($4^{\circ}\text{C} \pm 1^{\circ}\text{C}$)

Tail of the mice was immersed in ice cold water set at $4^{\circ}\text{C} \pm 1^{\circ}\text{C}$ until tail withdrawal sign is observed with a cutoff time of 20 s. Shortening of the tail withdrawal time indicates cold hyperplasia.

Tail immersion for cold allodynia ($10^{\circ}\text{C} \pm 1^{\circ}\text{C}$)

the tip of the tail was immersed in cold water ($10^{\circ}\text{C} \pm 1^{\circ}\text{C}$) with a cutoff time of 15 s until tail flick from water was

observed. Shortening in tail withdrawal time indicated allodynia.

Acetone drop test for cold allodynia

A 50 μL volume of acetone was delicately sprayed onto the mid plantar surface of the left hind paw. The duration of the withdrawal response was recorded with a minimum cutoff value of 0.5 s and a maximum of 15 s.

Tail immersion test for thermal allodynia ($42^{\circ}\text{C} \pm 1^{\circ}\text{C}$)

The tip of the tail of the mice was immersed in warm water bath set at $42^{\circ}\text{C} \pm 1^{\circ}\text{C}$ until tail withdrawal sign is observed with a cutoff time of 15 s. Shortening of the tail withdrawal time indicates thermal allodynia.

Statistical analysis

The data were expressed as mean $\pm$ SEM. Data were analyzed using the SPSS software version 20 (SPSS, IBM Company, Chicago, IL 60606, US). ANOVA was used to compare between the groups, and $P \leq 0.05$ was considered statistically significant.

RESULTS

A significant ($P \leq 0.05$) reduction was seen at day 14 and persisted up to day 28 after the administration of PT in all behavioral parameters tested in the present study.

Tail immersion test for thermal hyperalgesia ($47^{\circ}\text{C} \pm 1^{\circ}\text{C}$)

The mean tail withdrawal latency in hot hyperalgesia test was reduced significantly ($P \leq 0.05$) on day 14 (9.2 ± 0.13 s) onward day 28 (5.00 ± 0.19 s) after PT administration compared to Group C (10.40 ± 0.03), as shown in Figure 1. The significant reduction in the mean tail withdrawal latency by PT (9.2 ± 0.13 s at day 14 and 5.00 ± 0.19 s at day 28) was reversed significantly ($P \leq 0.05$) by PSO, NCF, and LC administration at day 14 (10.70 ± 0.31 , 10.43 ± 0.16 , and 10.37 ± 0.21 s, respectively) up to (10.32 ± 0.09 , 9.18 ± 0.23 , and 8.63 ± 0.10 , respectively) at day 28 of the experimental period [Figure 1]. The mean tail withdrawal latency after NCF and LC administration after 28 days of PT administration was

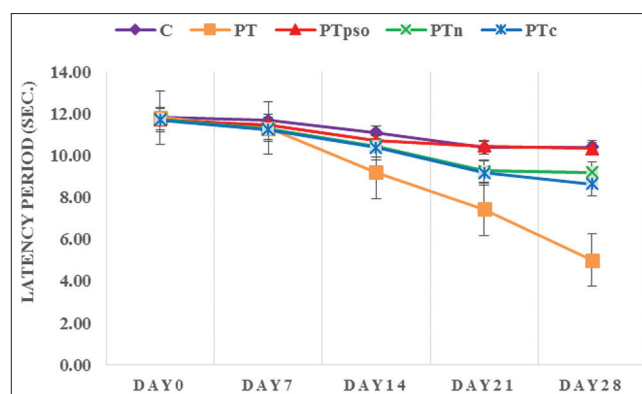


Figure 1: The mean latency period response (seconds) of mice ($n = 6$) to thermal hyperalgesia (Tail immersion test $47^{\circ}\text{C} \pm 1^{\circ}\text{C}$) of experimental groups (C: Control, PT: Paclitaxel, PTpso: Pomegranate seed oil, PTn: Nucleo CMP Forte®, PTc: L-Carnitine)

significantly ($P \leq 0.05$) shorter (9.18 ± 0.23 and 8.63 ± 0.10 s, respectively) compared to C group (10.40 ± 0.03 s) [Figure 1]. Comparison between treatment groups, the mean tail withdrawal latency at day 14 of PSO administration (10.70 ± 0.31 s) was not significantly ($P \geq 0.05$) different than that of mean latency of PTn and PTc groups (10.43 ± 0.16 and 10.37 ± 0.21 s, respectively), but at day 21 and day 28, PTps produced a significant ($P \leq 0.05$) longer mean tail flick withdrawal latency (10.43 ± 0.20 and 10.32 ± 0.09 s, respectively) compared to PTn (9.25 ± 0.19 and 9.18 ± 0.23 , respectively) and PTc group (9.17 ± 0.17 and 8.63 ± 0.10 s, respectively), as shown in Figure 1. No significant ($P \geq 0.05$) differences were observed in the mean tail withdrawal latency between PTn and PTc groups at day 14 and day 21, but PTn produced a statistically significant ($P \leq 0.05$) longer mean tail withdrawal latency (9.18 ± 0.23 s) than PTc (8.63 ± 0.10 s) at day 28 of the experimental period [Figure 1].

Hot plate method (thermal hyperalgesia)

The mean paw withdrawal latency [Figure 2] of PT was significantly ($P \leq 0.05$) reduced compared to that of C group at day 14 up to day 28 of the experimental period. The mean paw withdrawal latency after administration of PSO, NCF, and LC was statistically significantly ($P \leq 0.05$) longer than those of PT group at day 14 up to day 28 of the experimental period. The mean paw withdrawal latency after the administration of PSO was none significantly ($P \geq 0.05$) different than that of C group at day 7 onward day 28 [Figure 2]. However, the mean withdrawal latency after NCF administration was significantly ($P \leq 0.05$) shorter than that of C group at day 21 and day 28. Whereas, LC administration showed a significant ($P \leq 0.05$) shorter paw withdrawal latency from day 14 onward day 28 compared to C group but significantly ($P \leq 0.05$) longer than that of PT group [Figure 2]. Comparison between groups, PSO showed significantly ($P \leq 0.05$) longer paw withdrawal latency than PTc group at day 21 and day 28 of administration [Figure 2]. However, no statistically significant ($P \geq 0.05$) differences was observed between the mean paw withdrawal latency between PTps and PTn groups and between PTn and PTc groups throughout all the experimental periods [Figure 2].

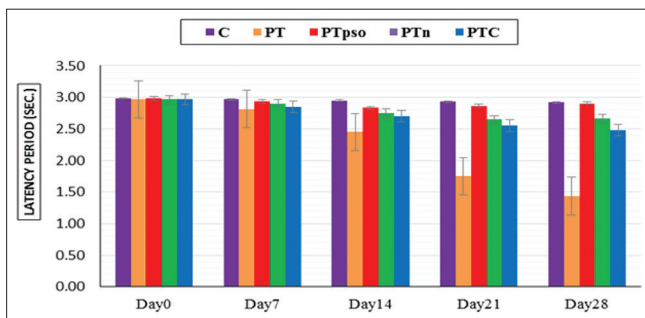


Figure 2: The mean latency period response (seconds) of mice ($n = 6$) to thermal hyperalgesia (Hot plate method $50^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$) of experimental groups (C: Control, PT: Paclitaxel, PTPSO: Pomegranate seed oil, PTn: Nucleo CMP Forte®, PTc: L-Carnitine)

Tail immersion test for cold hyperalgesia ($4^{\circ}\text{C} \pm 1^{\circ}\text{C}$)

The effect of administration of PSO, NCF, and LC on tail flick latency in cold hyperalgesia test ($4^{\circ}\text{C} \pm 1^{\circ}\text{C}$) in PT-induced neuropathic mice is represented in Figure 3. The administration of PT significantly ($P \leq 0.05$) reduced the mean withdrawal latency of tail in cold hyperalgesia test ($4^{\circ}\text{C} \pm 1^{\circ}\text{C}$) from day 14 onward to day 28 compared to C group. From day 14 onward day 28, PSO administration had a significantly ($P \leq 0.05$) longer mean tail withdrawal latency in cold hyperalgesia test compared to PT group but with no significant differences with C group [Figure 3]. Both PTn and PTc group had significantly ($P \leq 0.05$) longer mean tail withdrawal latency at day 21 and day 28 compared to PT group, but both produced a statistically significant ($P \leq 0.05$) reduction in the withdrawal latency of the tail compared to C group at days 14.21 and 28 [Figure 3]. Comparison between groups revealed a significant ($P \leq 0.05$) longer mean tail withdrawal latency in cold hyperalgesia test ($4^{\circ}\text{C} \pm 1^{\circ}\text{C}$) of PTps group compared to PTc group at day 14 up to day 28 and at day 21 and day 28 compared to PTn group, whereas PTc group showed a significant ($P \leq 0.05$) shorter mean withdrawal latency only at day 28 compared to PTc group.

Tail immersion test for cold allodynia ($10^{\circ}\text{C} \pm 2^{\circ}\text{C}$)

In tail immersion method of testing cold allodynia ($10^{\circ}\text{C} \pm 2^{\circ}\text{C}$), flicking of the tail was considered a positive response. The effect of different treatments with PSO, NCF, and LC on tail withdrawal latency of PT induced neuropathy in mice is presented in Figure 4. PT significantly ($P \leq 0.05$) decreased the mean tail flicking latency from the 2nd week and persisted up to the 4th week as compared to C group. Daily administration of PSO, NCF, and LC significantly ($P \leq 0.05$) produced longer mean tail withdrawal latency compared to PT group from day 14 up to day 28. Comparison between the treatment groups revealed that PSO administration produced a significant ($P \leq 0.05$) longer mean tail withdrawal latency of cold allodynia at day 21 onto day 28 compared to PTn group and from day 14 onto day 28 compared to PTc group. NCF administration showed a significant ($P \leq 0.05$) longer mean tail

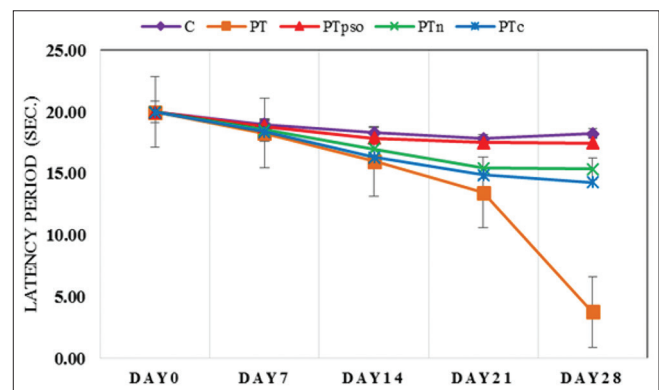


Figure 3: The mean withdrawal latency period (seconds) of mice ($n = 6$) to cold hyperalgesia (Tail immersion test $4^{\circ}\text{C} \pm 1^{\circ}\text{C}$) of experimental groups (C: Control, PT: Paclitaxel, PTPSO: Pomegranate seed oil, PTn: Nucleo CMP Forte®, PTc: L-Carnitine)

withdrawal latency of cold allodynia compared to PTc group only at day 28 [Figure 4].

Acetone drop method

In this method, hind paw licking, shaking, or rubbing was considered as a positive withdrawal response. Before administration of any treatment, each group had equivalent withdrawal latencies in the acetone test. At day 14 onward day 28, PT significantly ($P \leq 0.05$) shortened the mean withdrawal latency compared with C group in the acetone drop test [Figure 5]. PSO, NCF, and LC administration significantly ($P \leq 0.05$) reversed the PT-induced shortening of the withdrawal latency at day 14 onward day 28.

The comparison between treatment groups revealed no significant ($P \geq 0.05$) differences in the mean withdrawal latency of acetone drop test between C, PTpso, and PTn groups at day 14 onward day 28. However, PTc group produced significantly ($P \leq 0.05$) shorter mean withdrawal latency compared to C group at days 21 and 28 and significantly ($P \leq 0.05$) shorter mean withdrawal latency than PTpso group at day 28. While a none significant ($P \geq 0.05$) differences were observed between PTn and PTc groups throughout the experimental period [Figure 5].

Hot allodynia (tail immersion test $42^{\circ}\text{C} \pm 2^{\circ}\text{C}$)

The mean withdrawal latency response to PT was significantly ($P \leq 0.05$) reduced compared to that of C group at day 14 onto day 28 of the experimental period [Figure 6]. The mean withdrawal latency response after the administration of PSO, NCF, and LC was significantly ($P \leq 0.05$) longer than those of PT group at day 14 and onto day 28 of the experimental period [Figure 6]. The mean withdrawal latency response after the administration of PSO, NCF, and LC produced nonsignificant ($P \geq 0.05$) differences compared to that of C group at day 14 [Figure 6]. However, NCF, and LC administration produced a significant ($P \leq 0.05$) shorter mean withdrawal latency than C group at days 21 and 28 [Figure 6]. Comparison between groups, PTpso group has a significant ($P \leq 0.05$) longer latency compared to PTc group at days 21 and 28, whereas no significant ($P \geq 0.05$) differences were shown between PTpso and PTn at day 21 and none significant ($P \geq 0.05$) differences found between PTn and PTc groups throughout the experimental period [Figure 6].

DISCUSSION

The significant ($P \leq 0.05$) reduction in the mean withdrawal latency after PT administration in all sensory tests performed in this study starting from day 14 onward day 28 compared to the control and treatment groups indicates that PT induced neuropathy, dysfunction of sensory neurons, increased the excitability of nociceptors and enhanced pain impulses.^[11] Similar finding has also been shown in the neuropathic models induced by this chemotherapeutic agent in laboratory animals.^[12]

The administration of PSO that significantly attenuated the reduction in the mean latency period of all behavioral tests

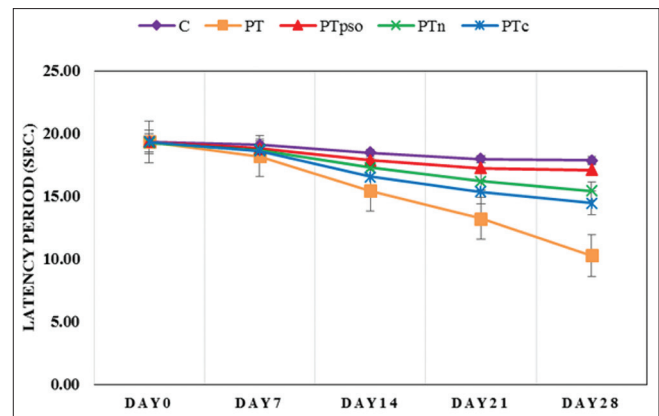


Figure 4: The mean withdrawal latency period (seconds) of mice ($n = 6$) to cold allodynia (Tail immersion test $10^{\circ}\text{C} \pm 1^{\circ}\text{C}$) of experimental groups (C: Control, PT: Paclitaxel, PTpso: Pomegranate seed oil, PTn: Nucleo CMP Forte®, PTc: L-Carnitine)

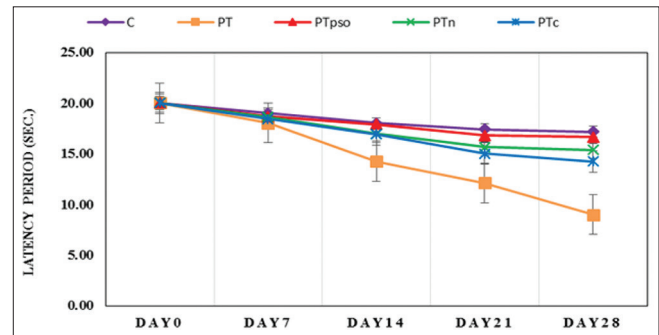


Figure 5: The mean withdrawal latency period (seconds) of mice ($n = 6$) to cold allodynia (Acetone drop test) of experimental groups (C: Control, PT: Paclitaxel, PTpso: Pomegranate seed oil, PTn: Nucleo CMP Forte®, PTc: L-Carnitine)

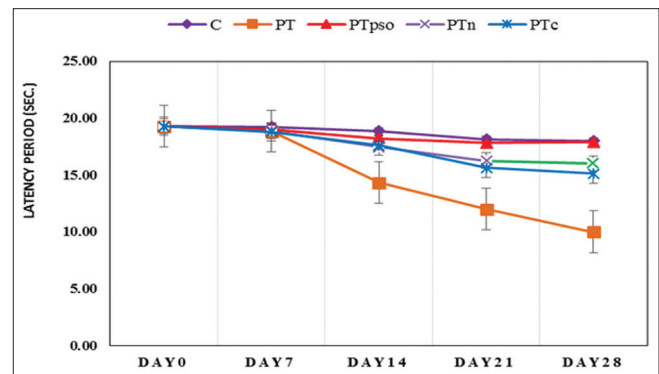


Figure 6: The mean withdrawal latency period (seconds) of mice ($n = 6$) to hot allodynia (Tail immersion test $42^{\circ}\text{C} \pm 1^{\circ}\text{C}$) of experimental groups (C: Control, PT: Paclitaxel, PTpso: Pomegranate seed oil, PTn: Nucleo CMP Forte®, PTc: L-Carnitine)

induced by PT administration in the present study revealed that PSO alleviated pain, hyperalgesia, and allodynia produced by PT administration. This anti-nociceptive effect of PSO in this study is most probably related to its constituents of polyunsaturated fatty acid (especially linoleic and punic acid),

phenolic compounds, and tocopherols that is considered as a sturdiest of a biological antioxidant and anti-inflammatory.^[3,13]

CLA has been effectively shown to attenuates NP, promote the reestablishment of motor and sensory functions, inhibits neuro-inflammation, pro-inflammatory cytokines and oxidative stress significantly,^[14,15] protects against stroke^[16] and against organophosphate nerve agent induced neuropathology.^[17] One of the recently elucidated mechanisms of PIPN is mainly through targeting linoleic acid (LA) metabolism pathway in the brain,^[18] so the CLA constituent of PSO which is essential for the development and functions of the neuronal membrane most probably would had a beneficial role in ameliorating PIPN in the present study.

Modulation of PPAR γ receptor has currently been focused in inflammatory and NP^[19,20] and studies found that PSO activates PPAR γ receptors through its constituent of punic acid (CLA), and hence, improvement of peripheral neuropathy sensitivity was obtained in diabetic mice given PSO,^[21] thus the proposed effect of PSO on PPAR γ receptors may provide another possible explanation for the anti-nociceptive effects of PSO in the present study.

In addition to the analgesic and anti-nociceptive effects of PSO, various studies have shown that PSO exert anti-inflammatory, anti-proliferative, and anti-tumorigenic properties against different types of cancer, such by modulating multiple signaling pathways;^[5,22] therefore, the anti-nociceptive effect of PSO shown in this study most probably encourage the strategy of using PSO in cancer patients as a chemopreventive agent against cancer proliferation, chronic inflammation, and neuropathy since it is considered a safe natural agent that has not shown any interference with the antitumor activity of PT^[23] rather than exposing the patients to more drugs for relieving NP which did not proved complete cure and may induce significant side effects^[5] or it may be given as adjunct to NSAIDs as PSO shown to augment the antinociceptive effects of ketoprofen.^[24]

The significant improvements in withdrawal latency obtained with NCF administration in PIPN compared to PTc group shown in this study indicate it possess neuroprotective and anti-nociceptive activity, which have also been demonstrated in different types of neurological conditions that elucidate its role in the structural neuroregeneration since nucleotides serve as a potential source of extracellular ATP and other purines that regulate the actions of many processes in the body including neurons.^[25,26] The analgesic effect of LC had gained much interest in different forms of neuropathy conditions, and the significant anti-nociceptive effect of LC shown in the present study is in agreement with the findings of other clinical studies and experimental models of NP including chemotherapy-induced NP that confirmed the important role of LC in maintaining mitochondrial energy homeostasis and detoxification, nerve growth factor, and promoting peripheral nerve regeneration.^[7,8]

The less significant anti-nociceptive effect of LC than PSO and NCF shown in the present study could be related to that

it has protective effect on C-fiber mitochondria rather than the A-fiber mitochondria which is evoked in PT neuropathy.^[27] Although studies found that ALC had a no positive impact on the prevention of CIPN^[28] and diabetic neuropathy,^[29] recent systematic review data concluded that ALC can be considered both an etiological and symptomatic treatment in patients with peripheral neuropathy with a good safety profile.^[7]

The anti-nociceptive effect of PSO shown in the present study which significantly exceeded that of LC indicates that PSO may have probably a greater role in attenuating the series of events that led to the experience of neuropathic sensation and denotes the effectiveness of the biological constituents of PSO in meliorating NP in the present study which comes in accordance with previous reports that confirmed the strong antioxidant and anti-inflammatory activity of pomegranate which exceeds that of green tea and other fruit.^[30]

In addition, studies reported that patients treated with PT shown a decrease in the density of nerve fibers that innervate the epidermis of the hands and feet,^[2,31] and as the use of PSO shown to promote regeneration of epidermis;^[32] thus, this proposed property of PSO might possibly be added to the anti-nociceptive effect of PSO seen in this study.

One of the recently proposed mechanisms of neuroprotective activity of pomegranate is related to the activation of the L-arginine/NO pathway, members of the TRP superfamily (TRPA1 or TRPV1), and the opioid system which acts as a modulator in the spinal cord and dorsal root ganglia and mediates NP and protect NO from free radical destruction;^[33] this mechanism may also be involved in the significant anti-nociceptive effect of PSO shown in this study.

CONCLUSION

The greater activity of PSO than NCF and LC in attenuating PIPN shown in the present study; most probably indicates that PSO through its various constituents may have more than one role in protecting neuronal activity (central and peripheral) in comparable to NCF and LC. There was a significant reduction in mean latency period induced by PT administration in hot hyperalgesia test, hot plate method, cold hyperalgesia, cold allodynia, acetone drop test, and hot hyperalgesia tests, respectively.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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