ijlpr 2021; doi 10.22376/ijpbs/lpr.2021.11.4.P58-67

International Journal of Life science and Pharma Research

Research Article

Pharmacology

Curcumin Ameliorates Paclitaxel-Induced Pain Hypersensitivity via Alleviation of Inflammation and Oxidative Stress in Rats

Haritha Pasupulati[1,3*], Satyanarayana SV Padi[2], Bharathi Koganti[1], Prasad VSRG Koganti[1]

[1]*Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visva Vidyalayam (Women's University), Tirupati, Andhra Pradesh, India*

[2]*Jangaon Institute of Pharmaceutical Sciences, Jangaon, Telangana, India*

[3]*Bharat Institute of Technology-Pharmacy, Hyderabad, Telangana, India*

Abstract: Painful peripheral neuropathy is the main dose-limiting and long lasting side-effect of paclitaxel therapy. Despite enormous research, there is no effective treatment for paclitaxel-induced peripheral neuropathic pain owing to poor understanding of pathophysiological mechanisms. Growing evidence indicates oxidative-nitrosative stress is one of the leading factors causing chemotherapy induced peripheral neuropathy. Recently, involvement of neuroinflammation has been suggested in the development of paclitaxel-induced neuropathic pain. It is postulated that abrogating cytokine release and improving antioxidant defenses might be suitable targets in controlling neuroinflammation and oxidative-nitrosative stress mediated nociceptive hypersensitivities. Therefore, the study evaluated the effect of curcumin on paclitaxel-induced neuropathic pain in rats. Peripheral neuropathy was induced by administration of paclitaxel (2 mg/kg per injection) intraperitoneally on four alternate days (days 0, 2, 4, 6). Thermal hyperalgesia and mechanical allodynia were assessed and the markers of inflammation and oxidative-nitrosative stress were estimated. Administration of curcumin (50 and 100 mg/kg, p.o.) for 2 weeks started 14 days after paclitaxel injection significantly alleviated paclitaxel-induced nociceptive behavioural hypersensitivity observed as reduced thermal hyperalgesia and mechanical allodynia. These observed ameliorative effects of curcumin on paclitaxel-induced neuropathic pain are accompanied by reduction of tumour necrosis factor-α, a pro-inflammatory cytokine, in both spinal cord and dorsal root ganglia and oxidative-nitrosative stress in spinal cord. The results of the present study demonstrated antihyperalgesic and antiallodynic effects of curcumin. Additional clinical studies are warranted to evaluate therapeutic potential of curcumin as antinociceptive agent in the treatment of paclitaxel-induced neuropathic pain.

Keywords: Neuropathic pain, Curcumin, Inflammation, Neuropathic pain, Oxidative stress, Paclitaxel neuropathy, Pain hypersensitivity

***Corresponding Author**

Haritha Pasupulati , Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visva Vidyalayam (Women's University), Tirupati, Andhra Pradesh, India,Bharat Institute of Technology-Pharmacy, Hyderabad, Telangana, India

Received On 17 July, 2021
Revised On 25 July, 2021
Accepted On 26 July, 2021
Published On 29 July, 2021

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Haritha Pasupulati, Satyanarayana SV Padi, Bharathi Koganti, Prasad VSRG Koganti , Curcumin Ameliorates Paclitaxel-Induced Pain Hypersensitivity Via Alleviation of Inflammation and Oxidative Stress in Rats.(2021).Int. J. Life Sci. Pharma Res.11(4), P58-67 http://dx.doi.org/10.22376/ijpbs/lpr.2021.11.4.P58-67

1. INTRODUCTION

Paclitaxel is a taxane-derived chemotherapeutic agent indicated in treatment for lung, ovarian, breast, non-small cell lung and Kaposi's carcinomas. Its primary anticancer action occurs via disruption of the mitotic spindle and microtubule dynamics causing apoptosis.[1] Unfortunately, painful peripheral neuropathy is its major dose-limiting side effect. Affected patients typically report bilateral numbness, tingling, spontaneous pain and evoked pain to mechanical and cold stimuli in the hands and/or feet in a stocking and glove manner.[1,2] Neuropathic pain symptoms can persist for months or years following cessation of paclitaxel leading to the discontinuation of this highly efficacious antitumour drug.[2] The exact mechanisms underlying this dose-limiting side effect are unclear and there is no available preventive strategy or effective treatment for chemotherapy induced neurotoxicity. Understanding the underlying causative mechanisms is of paramount significance for identifying novel ways to minimize this side effect and maximize antitumor effects. Experimental evidences support the contributory role of mitochondrial dysfunction and reactive oxygen and nitrogen species in the development of paclitaxel neuropathy.[3] Arguably, oxidative stress has been considered a significant factor responsible for chemotherapy-induced peripheral neuropathy (CIPN).[4,5] Owing to this, few antioxidants, such as MitoVitE and tempol, have been studied for the prevention and treatment of CIPN.[6,7] It has been reported that cellular and neurochemical changes of peripheral nerves as well as increased activation of non-neuronal cells (microglia and astrocytes) play an important role in development and maintenance of peripheral neuropathic pain including paclitaxel-induced pain hypersensitivity.[8-10] Recent studies implicate inflammatory cascade, particularly pro-inflammatory cytokines such as interleukin-1 β (IL-1 β) and tumour necrosis factor-α (TNF- α) which through their respective receptors and signaling pathways cause neuronal hypersensitization.[8,10,11] Current drug therapy of paclitaxel-induced neuropathy is often limited and unsatisfactory due to partial effectiveness and associated side effects of possible analgesics. Thus, there is a clinical need to identify novel therapeutic agents for the treatment of paclitaxel-induced neuropathic pain to enhance quality of life in cancer survivors. Curcumin is a major bioactive component of turmeric, which has been demonstrated to have a variety of biological activities, including antitumour and antioxidant actions.[12] Emerging experimental evidence indicates that curcumin has analgesic effect plausibly by diminishing the harmful effects of oxidative stress in neuropathic pain induced by oxaliplatin and diabetes.[13,14] Further, it is also a potent inhibitor of the mitogen-activated protein kinases (MAPKs) and NF- κB, which are critical in the transcriptional regulation of pro-inflammatory cytokine gene expression.[14,15] In addition, the anti-inflammatory and neuroprotective roles of curcumin have been studied in models of peripheral and central pain hypersensitivity.[15,16] A recent study showed that systemic curcumin inhibited the activation of microglia and astrocytes in the spinal cord of neuropathic rats and attenuated pain hypersensitivity.[17,18] Therefore, agents that reduce both oxidative stress and inflammation may be an important approach in alleviating oxidative neural damage after peripheral neuropathy. Thus, the present study was designed to investigate whether curcumin decreases the development of pain hypersensitivity in experimental paclitaxel-induced neuropathy and whether the possible effects of curcumin are associated with decreased oxidative stress and inflammation.

2. MATERIALS AND METHODS

2.1. Animals

Male Wistar rats (180-200 g) were used in the present study. The animals were housed in groups of three, in polypropylene cages with husk bedding under standard conditions of light and dark cycle with standard laboratory rodent chow and water *ad libitum*. Animals were acclimatized to laboratory conditions before the test. All the behavioral assessments were carried between 9:00 AM and 11:00 AM. The experimental protocols were approved by the Institutional Animal Ethics Committee (OQNP/PCS/0813/FW/12) and study was performed in accordance with the guidelines for the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India. Each animal was used for a single treatment and each group consisted of six animals. All experiments for a given treatment were performed using age-matched animals in an attempt to avoid variability between experimental groups.[19]

2.2. Drugs and chemicals

Paclitaxel (Intaxel 100 mg Injection; Fresenius Kabi India Pvt. Ltd.) was freshly prepared by diluting in 0.9% saline to a concentration of 2 mg/ml prior to injection. Curcumin (Sigma, St. Louis, MO, USA) suspended in 0.5% carboxy methyl cellulose was orally administered through gastric lavage. Rat TNF-α ELISA kit (R&D systems, MN, USA) was used to quantify cytokine. Unless stated, all other chemicals and biochemical reagents of highest analytical grade quality were used. Treatment with either vehicle or curcumin 50 mg/kg and 100 mg/kg, per orally (p.o.) was initiated on day 14 after paclitaxel administration and was continued once daily for next 14 days.

2.3. Induction and assessment of paclitaxel-induced peripheral neuropathy in rats

Rats were intraperitoneally (i.p.) injected with paclitaxel (2 mg/kg i.p. for 4 consecutive days i.e. on day 0, 2, 4, 6) to induce neuropathic pain.[20] Age-matched control rats received, in parallel, an equal volume of normal saline. The induction of neuropathic pain was confirmed by measurement of thermal hyperalgesia and mechanical allodynia. Body weight was measured using a calibrated scale on day 0 (initial) and day 28 (final) and motor activity was systematically measured using an actophotometer on days 0, 7, 14, 21 and 28 of the experiment.

2.4. Study Design

All animals were acclimatized to the laboratory environment for at least 30 min on two or three separate days before testing. The experimental protocol comprised five groups. Following habituation and baseline testing, three groups of rats received 2 mg/kg paclitaxel intraperitoneally. Two weeks after paclitaxel administration, curcumin was administered orally at doses of 50 and 100 mg/kg and continued for further two weeks. One group of rats received a vehicle (age-matched control group) and another group of rats were administered curcumin (*per se*; 100

mg/kg, p.o.). The response to behavioral nociceptive tests was assessed on days 0 (before administration of paclitaxel), 7, 14 (on the day before initiation of the treatment), 21, and 28. All the animals were sacrificed after behavioural testing to measure markers of pro-inflammatory cytokine and oxidative stress on day 28.[9]

2.5. Behavioral Test Paradigm

2.5.1. Assessment of thermal hyperalgesia

The response to noxious thermal stimulus was determined using a plantar test apparatus (Ugo Basile, Italy) as described by Bijjem et al. (2013).[21] In brief, rats were placed individually in Plexiglas cubicles mounted on a glass surface maintained at 25 ± 0°C. After acclimatization, a thermal stimulus in the form of radiant heat emitted from a focused projection bulb, which was located under the glass floor, was focused onto the plantar surface of the left hind paw and the latency to the first sign of paw licking or withdrawal response to avoid heat pain was taken as an index of pain threshold. Paw withdrawal latencies (PWLs) were recorded at an interval of 10 min and the mean of the three values was used for analysis. The intensity of radiant heat was adjusted to give 18 – 19 sec withdrawal latency in rats. A cut-off latency of 20 sec was set to avoid tissue damage.

2.5.2. *Assessment of mechanical allodynia*

The mechanical threshold for nociceptive flexion was determined by measuring the paw withdrawal threshold (PWT) elicited by stimulation of the left hind paw using Dynamic Plantar Aesthesiometer (Ugo Basile, Italy) as described by Bijjem et al. (2013).[21] This device generates a mechanical force that increases linearly with time. The maximum force applied by a von Frey type-filament was set at 50 g. The nociceptive threshold was defined as the force at which the animal withdrew its paw. In brief, each animal was placed in a test cage with a wire mesh floor, and the tip of a von Frey-type filament was applied to the middle of the plantar surface of the hind paw. Brisk foot withdrawals in response to tactile stimulation were recorded. PWT was expressed as the threshold level in grams. Each time the test was repeated three times, and the mean values represented the threshold of the individuals. The decrease in PWT in paclitaxel treated rats indicates mechanical allodynia.

2.6. Collection of tissues samples

In this study, at the end of treatment schedule on day 28, the animals were euthanized by overdose of thiopental sodium (200 mg/kg, i.p.) immediately after behavioral assays, followed by collection of lumbar dorsal root ganglia (DRG) and spinal cord for estimation of markers of inflammation and oxidative stress. The L4-L6 DRGs were detected within their intervertebral foramina after total laminectomy and foraminotomy, carefully removed, and stored at -20°C in phosphate buffer pH 7.0. Spinal cord was collected by the excising lumbosacral region of the spinal cord with L4-L6 segments as the epicenter and immediately kept at 4°C. It was washed in normal saline and weighed. DRGs and one portion of the spinal cord were separately homogenized in a homogenization buffer containing protease inhibitors. These samples were cold centrifuged and the supernatant was used for estimation of pro-inflammatory cytokine as per manufacturer's specifications. The remaining part of spinal cord was washed with ice cold sterile normal saline, weighed separately, homogenized in ice cold phosphate buffer pH 7.0 and centrifuged for 15 min at 2000*g* to obtain the clear supernatant for the estimation of oxidative stress markers.

2.7. Pro-inflammatory cytokine

Pro-inflammatory cytokine, TNF-α, concentration was estimated using the quantitative sandwich enzyme immunoassay according to manufacturer's instructions (R&D systems, MN, USA). The cytokine level was determined by comparing samples to the standard curve generated from the kit at 450 nm and are expressed as pg per mg tissue.

2.8. Markers of oxidative stress

2.8.1. Lipid peroxidation

Lipid peroxidation in spinal cord was estimated colourimetrically by measuring thiobarbituric acid reactive substances (TBARS) by the method of Niehaus and Samuelson (1968).[22] A 0.1 ml of supernatant of spinal cord homogenate was treated with 2 ml of (1:1:1 ratio) thiobarbituric acid (0.37%)-trichloroacetic acid (15%)-hydrochloric acid (0.25 N) reagent and placed in hot water bath for 15 min, cooled and centrifuged and then clear supernatant was measured at 532 nm (UV-1700 Spectrophotometer, Shimadzu, Japan) against blank. Finally, the values are expressed as nmol per g tissue.

2.8.2. Superoxide dismutase

Superoxide dismutase (SOD) activity in spinal cord was measured according to a method described by Misra and Fridovich (1972), by following spectrophotometrically (at 480 nm) the autoxidation of epinephrine at pH 10.4.[23] In this method, supernatant of the tissue was mixed with 0.8 ml of 50 mM glycine buffer, pH 10.4 and the reaction was started by the addition of 0.02 ml (-)-epinephrine. After 5 min, the absorbance was measured at 480 nm (UV-1700 Spectrophotometer, Shimadzu, Japan). The activity of SOD was expressed as U/g tissue.

2.9. Nitrite levels

The spinal cord nitrite levels were measured by the Griess reaction.[24] A 0.1 ml of supernatant was mixed with 250 µl l of 1% sulfanilamide (prepared in 3N HCl) and 250 µl l of 0.1% N-naphthylenediamine with shaking. After 10 min, 1.4 ml of water was added and absorbance was measured at 545 nm (UV-1700 Spectrophotometer, Shimadzu, Japan). The results are expressed as nmol per g tissue.

3. STATISTICAL ANALYSIS

The results are presented as mean ± S.E.M. for at least six animals per group. The data were analyzed by one-way ANOVA (Sigma Stat Version 2.0, SPSS Inc., Chicago, IL, USA) and the significance of the differences between groups was analyzed by Tukey's test. $P < 0.05$ was considered as statistically significant.

4. RESULTS

4.1 Paclitaxel injection and induction of neuropathy

The baseline paw withdrawal responses (PWLs and PWTs)

in each test obtained on day 0 for each rat was relatively stable and showed no significant difference. The PWLs and PWTs to thermal and mechanical stimulation in age-matched vehicle control animals remained unchanged from baseline values, throughout the observation period. The PWLs and PWTs in paclitaxel administered rats were significantly less than that of vehicle treated age-matched control rats from day 7 onwards and reached steady-state between days 14 and 28 post-paclitaxel administration indicating the development and maintenance of stable hyperalgesia and allodynia (Fig. 1 and 2).

3.1 Effect of paclitaxel on body weight

All the groups of rats had similar body weight at the beginning of the study. Rats showed normal gain in weight and no significant difference in body weight was observed in the saline, paclitaxel treated, and curcumin *per se* groups at the end of experimentation. Chronic administration of curcumin (50 or 100 mg/kg, p.o., for two weeks) also had no effect on body weight of rats on day 28 (Table 1).

3.2 Effect of paclitaxel on locomotor activity

The motor activity scores were normal in saline, paclitaxel, and curcumin *per se* treated rats during the entire study period (on days 7, 14, 21 and 28) as compared to their respective scores observed on day 0 (basal). Also, repeated oral administration of curcumin (50 and 100 mg/kg, for 2 weeks), did not produce any significant effect on locomotor activity as compared to the paclitaxel treated group (Table 2).

Table 1: Effect of chronic oral administration of curcumin on bodyweight in paclitaxel treated rats

Treatment (mg/kg)	Body weight (g)	
	Initial	Final
Control (age-matched)	224.15 ± 12.35	260.29 ± 18.45
Paclitaxel (PAC) treated	218.19 ± 10.30	250.10 ± 19.31
CUR *per se* (100)	220.16 ± 12.72	248.11 ± 16.19
PAC + CUR 50	215.20 ± 13.39	256.16 ± 14.17
PAC + CUR 100	221.16 ± 9.34	249.14 ± 17.10

Body weights were measured on day 0 (initial) and day 28 (final). CUR: Curcumin; PAC: Paclitaxel. All values are mean ± SEM.

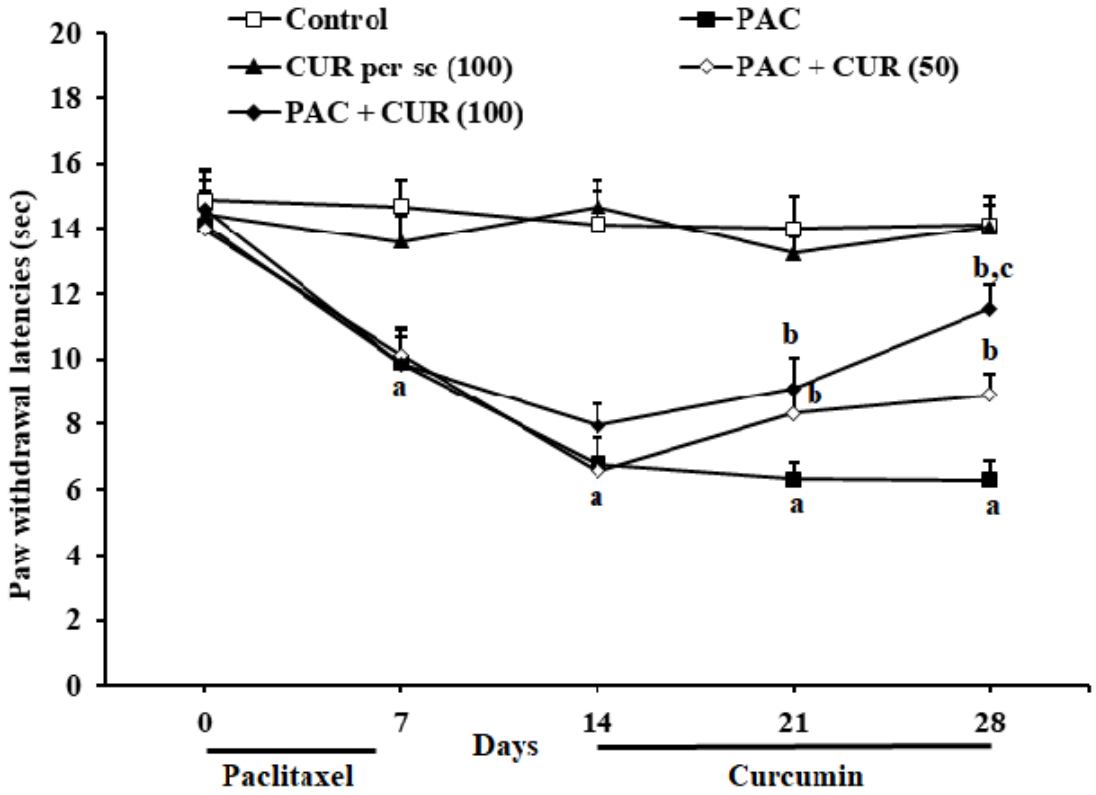
Table 2: Effect of chronic oral administration of curcumin on locomotor activity in paclitaxel treated rats

Treatment (mg/kg)	Day 0	Day 7	Day 14	Day 21	Day 28
Control	199.67 ± 9.70	209.83 ± 10.91	214.83 ± 11.61	195.83 ± 6.39	208.50 ± 9.94
PAC	205.33 ± 9.33	203.67 ± 6.18	212.33 ±12.03	202.17 ± 8.90	199.67 ± 7.88
CUR *per se* 100	204.33 ± 7.91	211.83 ± 7.10	214.83 ± 10.92	191.00 ± 7.59	189.00 ± 4.32
PAC +CUR 50	195.17 ± 8.67	205.67 ± 3.90	202.33 ± 5.86	219.50 ±8 .85	193.67 ± 8.16
PAC +CUR 100	210.83 ± 9.66	204.00 ± 3.97	208.50 ± 9.64	192.50 ± 7.14	197.50 ± 9.57

CUR: Curcumin; PAC: Paclitaxel. All values are mean ± SEM

3.4. Effect of curcumin on thermal hyperalgesia

Chronic administration of curcumin (*per se;* 100 mg/kg, p.o., for 2 weeks) was without any effect on PWLs in rats, whereas similar administration of curcumin (50 or 100 mg/kg, p.o., for 2 weeks) markedly reduced the developed hyperalgesia in paclitaxel injected rats as compared to vehicle-treated paclitaxel rats. At the end of study period, curcumin 100 mg/kg for two weeks showed significant improvement in paclitaxel induced-hyperalgesia as compared to that of curcumin 50 mg/kg in rats (Fig. 1).



Values are expressed as mean ± S.E.M. [a]P < 0.05 vs Control; [b]P < 0.05 vs PAC group; [c]P < 0.05 vs PAC + CUR (50) group.

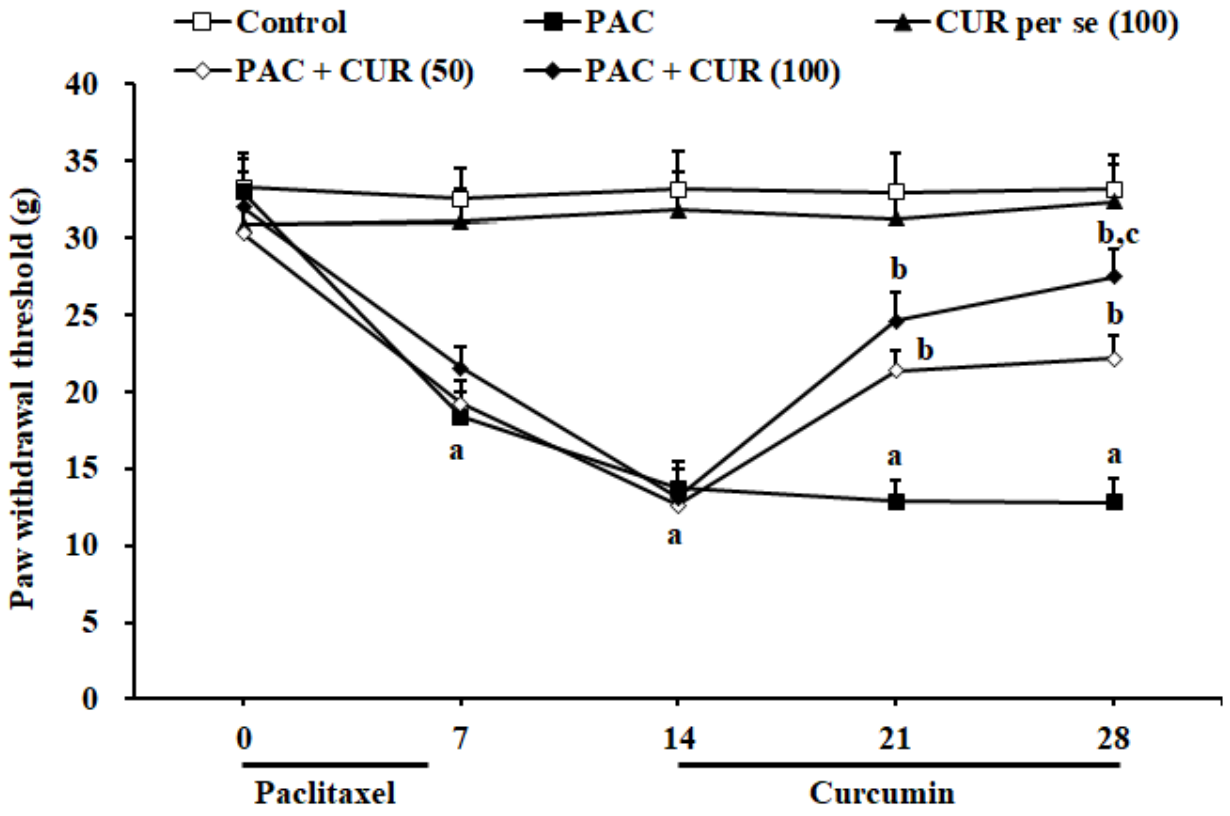
Fig. 1: Effect of repeated oral administration of curcumin (CUR; 50 and 100 mg/kg) on thermal paw withdrawal latencies in paclitaxel (PAC) treated rats.

3.5 Effect of curcumin on mechanical allodynia

A two-week administration of curcumin (*per se;* 100 mg/kg, p.o.) did not alter paw withdrawal responses to mechanical stimulation as compared to basal PWTs at the specified time points during the entire observation period in rats. However, administration of curcumin (50 or 100 mg/kg, p.o., for 2 weeks) had shown significant increase in PWTs in paclitaxel treated rats indicates reversal of established mechanical allodynia. Furthermore, on day 28, repeated administration of high dose curcumin (100 mg/kg, p.o., for two weeks) showed marked reversal of paclitaxel-induced allodynia as compared to that of low dose (50 mg/kg, p.o., for two weeks) in rats (Fig. 2).

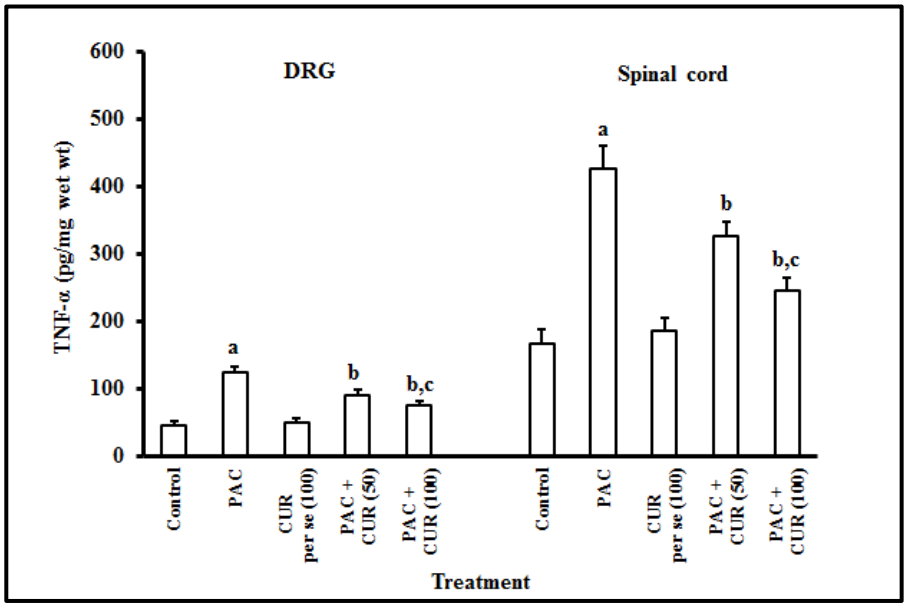
3.6 Effect of curcumin on pro-inflammatory cytokine in DRG and spinal cord

The concentration of lumbar DRG and spinal TNF-α were significantly elevated in paclitaxel rats when compared to that of vehicle-treated age-matched control rats on day 28. Repeated oral administration of curcumin (*per se*; 100 mg/kg, p.o., for 2 weeks) did not affect DRG and spinal TNF-α levels in rats. Nonetheless, the levels of this cytokine in both DRG and spinal cord were significantly lower in paclitaxel administered rats that had been treated with curcumin (50 or 100 mg/kg, p.o., for 2 weeks) with more prominent effect was noticed with the high dose (Fig. 3).



Values are expressed as mean ± S.E.M. [a]P < 0.05 vs Control; [b]P < 0.05 vs PAC group; [c]P < 0.05 vs PAC + CUR (50) group.

Fig. 2: Effect of repeated oral administration of curcumin (CUR; 50 and 100 mg/kg) on mechanical paw withdrawal threshold in paclitaxel (PAC) treated rats.



All values are expressed as mean ± S.E.M. [a]P < 0.05 vs Control; [b]P < 0.05 vs PAC group; [c]P < 0.05 vs PAC + CUR (50) group.

Fig. 3: Effect of repeated oral administration of curcumin (CUR; 50 and 100 mg/kg) on tumour necrosis factor-alpha (TNF-α) in lumbar dorsal root ganglia (DRG) and spinal cord.

3.7 Effect of curcumin on the markers of oxidative and nitrosative stress

Free radicals generated by mitochondrial dysfunction and activated microglia in response to administration of paclitaxel lead to oxidative and nitrosative stress that play an important role in central sensitization. Thus, we also evaluated the effect of curcumin on the markers of oxidative and nitrosative stress. Administration of paclitaxel induced a marked increase in TBARS and nitrite levels, and decrease in the activity of SOD in the spinal cord. A two-week administration of curcumin at both the doses significantly

attenuated paclitaxel-induced oxidative and nitrosative stress. On the contrary, treatment with curcumin (*per se;* 100 mg/kg, p.o., for 2 weeks) had no effects on the markers of oxidative and nitrosative stress as compared to age-matched control groups. Notably, high dose curcumin (100 mg/kg) had shown significant reduction in oxidative stress as compared to low dose (50 mg/kg) (Table 3). Furthermore, the activity of SOD in the spinal cord was significantly decreased by paclitaxel suggesting that paclitaxel could disrupt the antioxidant defense systems in the spinal cord. However, activity of SOD in spinal cord tissue markedly restored by curcumin (50 and 100 mg/kg) administration without significant differences in the activity at both the doses (Table 3).

Table 3: Effect of chronic oral administration of curcumin on the markers of oxidative and nitrosative stress in spinal cord of paclitaxel treated rats

Treatment (mg/kg)	TBARS (nmol/g tissue)	SOD (U/g tissue)	Nitrite (nmol/g tissue)
Control (age-matched)	59.12 ± 6.71	30.8 ±1.34	95.77 ± 5.87
Paclitaxel treated	164.15 ± 13.57[a]	7.32 ± 0.91[a]	199.51 ± 12.16[a]
CUR *per se* (100)	53.29 ± 8.97	29.8 ± 1.09	98.06 ± 6.91
PAC + CUR 50	111.28 ± 10.27[b]	19.91 ± 1.35[b]	141.42 ± 13.46[b]
PAC + CUR 100	69.18 ± 6.14[b,c]	22.17 ± 1.07[b]	121.72 ± 10.12[b,c]

CUR: Curcumin; PAC: Paclitaxel; TBARS: Thiobarbituric acid reactive substances. SOD: Superoxide dismutase. All values are mean ± SEM; [a]P < 0.05 vs Control; [b]P < 0.05 vs PAC group; [c]P < 0.05 vs PAC + CUR 50 group.

4. DISCUSSION

The present study revealed that four doses of paclitaxel (2 mg/kg, i.p.) on every alternate day during 6 days treatment schedule induced neuropathic pain symptoms such as thermal hyperalgesia and mechanical allodynia as previously described in humans and experimental models.[2,20] In particular, we observed that paclitaxel-induced behavioral changes are accompanied by inflammation and oxidative stress. Indeed, the results of the present study demonstrate that (1) administration of curcumin ameliorated paclitaxel-induced neuropathic pain, (2) that is accompanied by decreased level of pro-inflammatory cytokine in DRG and spinal cord and oxidative stress in the spinal cord, and supports that (3) inflammatory and oxidative stress pathways become activated after paclitaxel administration leading to elevation of pro-inflammatory cytokine and oxidative stress markers. In the present study, paclitaxel administered rats showed normal body weight gain and spontaneous motor activity indicating that paclitaxel evoked time-dependent development and maintenance of mechanical allodynia and thermal hyperalgesia. Although we had not measured IENF degeneration, we found that administration of curcumin (50 and 100 mg/kg, p.o.) had antiallodynic and antihyperalgesic effects on maintenance of behavioral hypersensitivity in paclitaxel administered rats. Moreover, curcumin at the same doses attenuated established paclitaxel-induced neuropathic pain. It is well reported that curcumin cross blood-brain-barrier readily and provides significant neuroprotection and anti-inflammatory effects after systemic administration.[15,25,26] Furthermore, the observed results are consistent with previous reports, which showed antinociceptive effects of curcumin in animal models of peripheral nerve injury, diabetes, oxaliplatin and cisplatin-induced neuropathic pain.[13-16,27,28] Indeed, curcumin had not affected basal responses in vehicle-treated animals indicating that the beneficial antinociceptive effects of curcumin in paclitaxel administered rats are not due to hypoalgesic effects. These results indicate that the improvement in paclitaxel-induced neuropathic pain is the clearest evidence of an ameliorative effect of curcumin when administered during existing behavioral hypersensitivity. Recent studies have emphasized on the role of inflammatory and immune reactions in the nervous system and emerging evidence reveals that pro-inflammatory cytokines play an essential role in experimental neuropathic pain. Therefore, in the present study, we have estimated the levels of TNF- α in lumbar DRG and spinal cord in order to gain an insight into antihyperalgesic and antiallodynic effects of curcumin. In the present study, we found paclitaxel treatment showed an increase in TNF- α in lumbar DRG and spinal cord supporting role of involvement of both peripheral and spinal inflammation. It has been reported that paclitaxel treatment induces mRNA expression of pro-inflammatory cytokines such as IL-1 β, IL-20, TNF-α, immune cell markers and increased levels of phosphorylated NF-κB in sciatic nerve, and lumbar DRG and spinal cord.[11,28-30] Further, monocyte chemoattractant protein 1 (MCP-1) and circulating cytokines impairs the blood spinal cord permeability and triggers the influx of inflammatory mediators into spinal cord leading to activation of spinal immune cells.[10,29,32-34] Moreover, repeated administration of paclitaxel has been found to activate non-neuronal immune cells, microglia and astrocytes, and CC-chemokine ligand 3 (CCL3), IL-1β, IL-20, and TNF-α release in DRGs and spinal cord.[8,29-31] We observed that chronic administration of curcumin in post-treatment paradigm reduced elevated level of pro-inflammatory cytokine TNF-α in DRG and spinal cord, which was parallel with the reduced allodynia and hyperalgesia. Several lines of evidence proved that curcumin attenuated the nerve injury and diabetes-induced allodynia and hyperalgesia and reduced the spinal expression of TNF-α, TNF- α receptor 1 and IL-1β.[28,30,35] In addition, curcumin suppressed activation of ERK and JNK and expressions of NF-κB p65 and CX3CR1 in the DRG and spinal cord as well as activation of non-neuronal cells, microglia and astrocytes following nerve injury and prevented the development of spinal neuroinflammation and chronic neuropathic pain.[16-18,35] It seems curcumin relieved neuropathic pain state, possibly through an inhibitory action on TNF-α release in spinal cord. Since systemically administered curcumin cross blood-brain-barrier and provides beneficial protective effects and its effects on peripheral nerves and DRG cannot be excluded. We also observed that curcumin reduced the level of TNF-α in the periphery in lumbar DRG. Accumulating data indicates that

paclitaxel administration results in the activation of Toll-like receptor 4 (TLR4), fractalkine and its receptors (CX3CR1) and increased expression of MCP-1 in DRG leading to infiltration of macrophages.[8,31,36] Further, upregulation of chemokines leading to activation of p38 MAPK in macrophages has been critical for development of mechanical allodynia. Intriguingly, the time course of DRG infiltration of macrophages matches the onset of IENF loss and development of behavioral signs of neuropathic pain, which is modulated by pharmacological intervention of depleting macrophages.[33,37] Compelling evidence exist that anti-inflammatory and neuroprotective effects of curcumin mediated by inhibiting degradation of IκBα and NF-κB activity, which is useful to reduce macrophage infiltration around DRG and prevent release of pro-inflammatory cytokines (TNF-α and IL-1β).[14] Together, our findings suggest that curcumin by its peripheral and central anti-inflammatory effects decreased pro-inflammatory cytokine, particularly TNF-α, thereby alleviating paclitaxel-induced behavioral hypersensitivity. Growing body of evidence implicates overproduction of free radicals and subsequent oxidative and nitrosative stress as plausible mechanisms underlying the sensitization of nociceptors and their fibers and biochemical changes in spinal cord in neuropathic pain states.[4,6,38,39] It has been reported that paclitaxel induces biochemical changes in mitochondria of sensory axons of DRG and disrupt their functions leading to the increased generation of reactive oxygen species (ROS), reactive nitrogen species, decreased antioxidant defenses, mitochondrial energy failure and degeneration.[30,38,40-42] Indeed, paclitaxel induced increased free radical formation and reduced antioxidant defenses in cancer cell lines.[43] Consistent to *in vitro* studies, it is possible that imbalance between free radicals and antioxidant defense enzymes might cause neuropathological changes in spinal cord and DRG in neuropathic pain. Pharmacological intervention by using natural and synthetic antioxidants alleviated paclitaxel-induced neuropathic pain behaviours.[4,5,38,40] In our study, we found that paclitaxel treatment showed increased lipid peroxidation, nitrite levels, and decreased SOD, an endogenous antioxidant enzyme parallel to the maximum pain hypersensitivity. In addition, curcumin reduces oxidative stress, nitrosative stress, and has been shown to activate antioxidant defenses like SOD in the spinal cord of paclitaxel administered rats. Curcumin, a known antioxidant, has been reported to inhibit lipid peroxidation and to scavenge superoxide anions and hydroxyl radicals effectively.[13,18,30,44] Taken together, it is possible that curcumin by its antioxidant effect showed reduction in oxidative-nitrosative stress and restored antioxidant defenses, and thereby ameliorated paclitaxel-induced allodynia and hyperalgesia. In addition to its capacity for direct scavenging of free radicals it is plausible that pleiotropic effects of curcumin might also be involved in reducing the development of CIPN. It has been shown that curcumin upregulates endogenous cellular antioxidant pathways which include increase in expression of cytoprotective quinone oxidoreductase 1 (NQO1), nuclear factor erythroid 2-related factor 2 (Nrf2), heme oxygenase-1(HO-1), Nrf2-induced target genes in primary spinal cord astrocytes, decreases the level of intracellular ROS, and attenuates oxidative damage and mitochondrial dysfunction.[12,30,44,45] Recent studies on curcumin as possible antinociceptive agent along with the results of the present study further supports the therapeutic potential of curcumin in alleviating neuropathic pain.

5. CONCLUSION

The present data provided evidence that behavioral hypersensitivity evoked by paclitaxel is associated with the release of pro-inflammatory cytokine and generation of free radicals and the subsequent neuroinflammation and oxidative-nitrosative stress, respectively. Further, the results demonstrate that curcumin ameliorated existing behavioral hypersensitivity in paclitaxel-induced neuropathic pain partly by inhibiting release of pro-inflammatory cytokine in DRG and spinal cord and in part by reducing oxidative-nitrosative stress in the spinal cord. Owing to its potent antioxidant and anti-inflammatory effects and with growing interests in clinical studies over its possible usefulness in neuroprotection and antinociception, our data suggests that curcumin warrants additional studies as a therapeutic agent and/or combination adjuvant for the treatment of paclitaxel-induced neuropathic pain.

6. AUTHOR CONTRIBUTION STATEMENT

Conceptualization, Haritha Pasupulati, Prasad VSRG Koganti; Methodology, Haritha Pasupulati, Satyanarayana SV Padi, Prasad VSRG Koganti; Investigation, Haritha Pasupulati; Data analysis, Haritha Pasupulati; Validation, Haritha Pasupulati, Prasad VSRG Koganti; Writing-original draft, Haritha Pasupulati; Writing-review and editing, Haritha Pasupulati, Satyanarayana SV Padi, Bharathi Koganti, Prasad VSRG Koganti. All authors take responsibility for appropriate content, critically revise the manuscript, and approve the version of the manuscript to be published. All authors have read and approved the final version of the manuscript.

7. ACKNOWLEDGEMENT

We thank the management of Nizam Institute of Pharmacy, Deshmukhi, Nalgonda and Bharat Institute of Technology-Pharmacy, Hyderabad for providing the necessary facilities for completion of the study.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

9. REFERENCES

1. Flatters SJL, Dougherty PM, Colvin LA. Clinical and preclinical perspectives on Chemotherapy-induced peripheral neuropathy (CIPN): a narrative review. Br J Anaesth. 2017;119(4):737-49. doi: 10.1093/bja/aex229, PMID 29121279.
2. Dougherty PM, Cata JP, Cordella JV, Burton A, Weng HR. Taxol-induced sensory disturbance is characterized by preferential impairment of myelinated fiber function in cancer patients. Pain. 2004;109(1-2):132-42. doi: 10.1016/j.pain.2004.01.021, PMID 15082135.
3. Griffiths LA, Flatters SJ. Pharmacological modulation of the mitochondrial electron transport chain in paclitaxel-induced painful peripheral neuropathy. J Pain. 2015;16(10):981-94. doi: 10.1016/j.jpain.2015.06.008, PMID 26142652.

4. Kim HK, Zhang YP, Gwak YS, Abdi S. Phenyl N-tert-butylnitrone, a free radical scavenger, reduces mechanical allodynia in chemotherapy-induced neuropathic pain in rats. Anesthesiology. 2010;112(2):432-9. doi: 10.1097/ALN.0b013e3181ca31bd, PMID 20068451.
5. Fidanboylu M, Griffiths LA, Flatters SJ. Global inhibition of reactive oxygen species (ROS) inhibits paclitaxel-induced painful peripheral neuropathy. PLOS ONE. 2011;6(9):e25212. doi: 10.1371/journal.pone.0025212, PMID 21966458.
6. McCormick B, Lowes DA, Colvin L, Torsney C, Galley HF. MitoVitE, a mitochondria-targeted antioxidant, limits paclitaxel-induced oxidative stress and mitochondrial damage in vitro, and paclitaxel-induced mechanical hypersensitivity in a rat pain model. Br J Anaesth. 2016;117(5):659-66. doi: 10.1093/bja/aew309, PMID 27799181.
7. Kim HK, Hwang SH, Abdi S. Tempol ameliorates and prevents mechanical hyperalgesia in a rat model of chemotherapy-induced neuropathic pain. Front Pharmacol. 2016;7:532. doi: 10.3389/fphar.2016.00532, PMID 28138318.
8. Peters CM, Jimenez-Andrade JM, Kuskowski MA, Ghilardi JR, Mantyh PW. An evolving cellular pathology occurs in dorsal root ganglia, peripheral nerve and spinal cord following intravenous administration of paclitaxel in the rat. Brain Res. 2007;1168:46-59. doi: 10.1016/j.brainres.2007.06.066, PMID 17698044.
9. Padi SS, Kulkarni SK. Minocycline prevents the development of neuropathic pain, but not acute pain: possible anti-inflammatory and antioxidant mechanisms. Eur J Pharmacol. 2008 Dec 28;601(1-3):79-87. doi: 10.1016/j.ejphar.2008.10.018, PMID 18952075.
10. Padi SSV, Shi XQ, Zhao YQ, Ruff MR, Baichoo N, Pert CB, Zhang J. Attenuation of rodent neuropathic pain by an orally active peptide, RAP-103, which potently blocks CCR2- and CCR5-mediated monocyte chemotaxis and inflammation. Pain. 2012;153(1):95-106. doi: 10.1016/j.pain.2011.09.022, PMID 22033364.
11. Yan X, Li F, Maixner DW, Yadav R, Gao M, Ali MW, Hooks SB, Weng HR. Interleukin-1beta released by microglia initiates the enhanced glutamatergic activity in the spinal dorsal horn during paclitaxel-associated acute pain syndrome. Glia. 2019;67(3):482-97. doi: 10.1002/glia.23557, PMID 30578561.
12. Basu P, Maier C, Basu A. Effects of curcumin and its different formulations in preclinical and clinical studies of peripheral neuropathic and postoperative pain: A comprehensive review. Int J Mol Sci. 2021;22(9):4666. doi: 10.3390/ijms22094666, PMID 33925121.
13. Zhao WC, Zhang B, Liao MJ, Zhang WX, He WY, Wang HB, Yang CX. Curcumin ameliorated diabetic neuropathy partially by inhibition of NADPH oxidase mediating oxidative stress in the spinal cord. Neurosci Lett. 2014;560:81-5. doi: 10.1016/j.neulet.2013.12.019, PMID 24370596.
14. Zhang X, Guan Z, Wang X, Sun D, Wang D, Li Y, Pei B, Ye M, Xu J, Yue X. Curcumin alleviates oxaliplatin-induced peripheral neuropathic pain through inhibiting oxidative stress-mediated activation of NF-kB and mitigating inflammation. Biol Pharm Bull. 2020;43(2):348-55. doi: 10.1248/bpb.b19-00862, PMID 31776306.
15. Ni H, Jin W, Zhu T, Wang J, Yuan B, Jiang J, Liang W, Ma Z. Curcumin modulates TLR4/NF-κ B inflammatory signaling pathway following traumatic spinal cord injury in rats. J Spinal Cord Med. 2015;38(2):199-206. doi: 10.1179/2045772313Y.0000000179, PMID 24621048.
16. Jeon Y, Kim CE, Jung D, Kwak K, Park S, Lim D, Kim S, Baek W. Curcumin could prevent the development of chronic neuropathic pain in rats with peripheral nerve injury. Curr Ther Res Clin Exp. 2013 Jun;74:1-4. doi: 10.1016/j.curtheres.2012.10.001, PMID 24385078.
17. Ji FT, Liang JJ, Liu L, Cao MH, Li F. Curcumin exerts antinociceptive effects by inhibiting the activation of astrocytes in spinal dorsal horn and the intracellular extracellular signal-regulated kinase signaling pathway in rat model of chronic constriction injury. Chin Med J (Engl). 2013;126(6):1125-31. PMID 23506591.
18. Xiao L, Ding M, Fernandez A, Zhao P, Jin L, Li X. Curcumin alleviates lumbar radiculopathy by reducing neuroinflammation, oxidative stress and nociceptive factors. Eur Cell Mater. 2017;33:279-93. doi: 10.22203/eCM.v033a21, PMID 28485773.
19. Yezierski RP. The effects of age on pain sensitivity: preclinical studies. Pain Med. 2012;13(Suppl 2):S27-36. doi: 10.1111/j.1526-4637.2011.01311.x. PMID: 22497745
20. Polomano RC, Mannes AJ, Clark US, Bennett GJ. A painful peripheral neuropathy in the rat produced by the chemotherapeutic drug, paclitaxel. Pain. 2001;94(3):293-304. doi: 10.1016/S0304-3959(01)00363-3, PMID 11731066.
21. Bijjem KR, Padi SS, lal Sharma P. Pharmacological activation of heme oxygenase (HO)-1/carbon monoxide pathway prevents the development of peripheral neuropathic pain in Wistar rats. Naunyn Schmiedebergs Arch Pharmacol. 2013;386(1):79-90. doi: 10.1007/s00210-012-0816-1, PMID 23224421.
22. Niehaus WG Jr, Samuelsson B. Formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur J Biochem. 1968;6(1):126-30. doi: 10.1111/j.1432-1033.1968.tb00428.x, PMID 4387188.
23. Misra HP, Fridovich I. The generation of superoxide radical during the autoxidation of hemoglobin. J Biol Chem. 1972;247(21):6960-2. doi: 10.1016/S0021-9258(19)44679-6, PMID 4673289.
24. Sastry KV, Moudgal RP, Mohan J, Tyagi JS, Rao GS. Spectrophotometric determination of serum nitrite and nitrate by copper–cadmium alloy. Anal Biochem. 2002;306(1):79-82. doi: 10.1006/abio.2002.5676, PMID 12069417.
25. Jiang J, Wang W, Sun YJ, Hu M, Li F, Zhu DY. Neuroprotective effect of curcumin on focal cerebral ischemic rats by preventing blood–brain barrier damage. Eur J Pharmacol. 2007;561(1-3):54-62. doi: 10.1016/j.ejphar.2006.12.028, PMID 17303117.
26. Wang YF, Gu YT, Qin GH, Zhong L, Meng YN. Curcumin ameliorates the permeability of the blood–brain barrier during hypoxia by upregulating heme oxygenase-1 expression in brain microvascular endothelial cells. J Mol Neurosci. 2013;51(2):344-51. doi: 10.1007/s12031-013-9989-4, PMID 23494637.

27. Jia T, Rao J, Zou L, Zhao S, Yi Z, Wu B, Li L, Yuan H, Shi L, Zhang C, Gao Y, Liu S, Xu H, Liu H, Liang S, Li G. Nanoparticle-encapsulated curcumin inhibits diabetic neuropathic pain involving the P2Y12 receptor in the dorsal root ganglia. Front Neurosci. 2017;11:755. doi: 10.3389/fnins.2017.00755, PMID 29422835.
28. Limcharoen T, Muangnoi C, Dasuni Wasana PW, Hasriadi, Vajragupta O, Rojsitthisak P, Towiwat P. Improved antiallodynic,antihyperalgesic and anti-inflammatory response achieved through potential prodrug of curcumin, curcumin diethyl diglutarate in a mouse model of neuropathic pain. Eur J Pharmacol. 2021;899:174008. doi: 10.1016/j.ejphar.2021.174008.
29. Ledeboer A, Jekich BM, Sloane EM, Mahoney JH, Langer SJ, Milligan ED, Martin D, Maier SF, Johnson KW, Leinwand LA, Chavez RA, Watkins LR. Intrathecal interleukin-10 gene therapy attenuates paclitaxel-induced mechanical allodynia and proinflammatory cytokine expression in dorsal root ganglia in rats. Brain Behav Immun. 2007;21(5):686-98. doi: 10.1016/j.bbi.2006.10.012, PMID 17174526.
30. Yardım A, Kandemir FM, Çomaklı S, Özdemir S, Caglayan C, Kucukler S, Çelik H. Protective effects of curcumin against paclitaxel-induced spinal cord and sciatic nerve injuries in rats. Neurochem Res. 2021;46(2):379-95. doi: 10.1007/s11064-020-03174-0, PMID 33201400.
31. Chen LH, Yeh YM, Chen YF, Hsu YH, Wang HH, Lin PC, Chang LY, Lin CK, Chang MS, Shen MR. Targeting interleukin-20 alleviates paclitaxel-induced peripheral neuropathy. Pain. 2020;161(6):1237-54. doi: 10.1097/j.pain.0000000000001831, PMID 32068666.
32. Zhao YX, Yao MJ, Liu Q, Xin JJ, Gao JH, Yu XC. Electroacupuncture Treatment Attenuates paclitaxel-induced neuropathic pain in rats via inhibiting spinal glia and the TLR4/NF-kB pathway. J Pain Res. 2020;13:239-50. doi: 10.2147/JPR.S241101, PMID 32099448.
33. Zhang H, Li Y, de Carvalho-Barbosa M, Kavelaars A, Heijnen CJ, Albrecht PJ, Dougherty PM. Dorsal root ganglion infiltration by macrophages contributes to paclitaxel chemotherapy-induced peripheral neuropathy. J Pain. 2016;17(7):775-86. doi: 10.1016/j.jpain.2016.02.011, PMID 26979998.
34. Echeverry S, Shi XQ, Rivest S, Zhang J. Peripheral nerve injury alters blood-spinal cord barrier functional and molecular integrity through a selective inflammatory pathway. J Neurosci. 2011;31(30):10819-28. doi: 10.1523/JNEUROSCI.1642-11.2011, PMID 21795534.
35. Liu S, Li Q, Zhang MT, Mao-Ying QL, Hu LY, Wu GC, Mi WL, Wang YQ. Curcumin ameliorates neuropathic pain by down-regulating spinal IL-1β via suppressing astroglial NALP1 inflammasome and JAK2-STAT3 signalling [sci rep:2016];6. Vol. 28956.
36. Li Y, Yin C, Li X, Liu B, Wang J, Zheng X, Shao X, Liang Y, Du J, Fang J, Liu B. Electroacupuncture alleviates paclitaxel-induced peripheral neuropathic pain in rats via suppressing TLR4 signaling and TRPV1 upregulation in sensory neurons. Int J Mol Sci. 2019;20(23):5917. doi: 10.3390/ijms20235917, PMID 31775332.
37. Peters CM, Jimenez-Andrade JM, Jonas BM, Sevcik MA, Koewler NJ, Ghilardi JR, Wong GY, Mantyh PW. Intravenous paclitaxel administration in the rat induces a peripheral sensory neuropathy characterized by macrophage infiltration and injury to sensory neurons and their supporting cells. Exp Neurol. 2007;203(1):42-54. doi: 10.1016/j.expneurol.2006.07.022, PMID 17005179.
38. Doyle T, Chen Z, Muscoli C, Bryant L, Esposito E, Cuzzocrea S, Dagostino C, Ryerse J, Rausaria S, Kamadulski A, Neumann WL, Salvemini D. Targeting the overproduction of peroxynitrite for the prevention and reversal of paclitaxel-induced neuropathic pain. J Neurosci. 2012;32(18):6149-60. doi: 10.1523/JNEUROSCI.6343-11.2012, PMID 22553021.
39. Shim HS, Bae C, Wang J, Lee KH, Hankerd KM, Kim HK, Chung JM, La JH. Peripheral and central oxidative stress in chemotherapy-induced neuropathic pain. Mol Pain. 2019;15:1744806919840098. doi: 10.1177/1744806919840098, PMID 30857460.
40. Duggett NA, Griffiths LA, McKenna OE, de Santis V, Yongsanguanchai N, Mokori EB, Flatters SJ. Oxidative stress in the development, maintenance and resolution of paclitaxel-induced painful neuropathy. Neuroscience. 2016;333:13-26. doi: 10.1016/j.neuroscience.2016.06.050, PMID 27393249.
41. Xiao WH, Zheng H, Zheng FY, Nuydens R, Meert TF, Bennett GJ. Mitochondrial abnormality in sensory, but not motor, axons in paclitaxel-evoked painful peripheral neuropathy in the rat. Neuroscience. 2011;199:461-9. doi: 10.1016/j.neuroscience.2011.10.010, PMID 22037390.
42. Duggett NA, Griffiths LA, Flatters SJL. Paclitaxel-induced painful neuropathy is associated with changes in mitochondrial bioenergetics, glycolysis, and an energy deficit in dorsal root ganglia neurons. Pain. 2017;158(8):1499-508. doi: 10.1097/j.pain.0000000000000939, PMID 28541258.
43. Ramanathan B, Jan KY, Chen CH, Hour TC, Yu HJ, Pu YS. Resistance to paclitaxel is proportional to cellular total antioxidant capacity. Cancer Res. 2005;65(18):8455-60. doi: 10.1158/0008-5472.CAN-05-1162, PMID 16166325.
44. Dai C, Xiao X, Zhang Y, Xiang B, Hoyer D, Shen J, Velkov T, Tang S. Curcumin attenuates colistin-induced peripheral neurotoxicity in mice. ACS Infect Dis. 2020 Apr 10;6(4):715-24. doi: 10.1021/acsinfecdis.9b00341, PMID 32037797.
45. Asadi S, Gholami MS, Siassi F, Qorbani M, Khamoshian K, Sotoudeh G. Nano curcumin supplementation reduced the severity of diabetic sensorimotor polyneuropathy in patients with type 2 diabetes mellitus: A randomized double-blind placebo- controlled clinical trial. Complement Ther. Med. 2019;43:253-60.