Toxicology Reports 13 (2024) 101781

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

# Protective effects of *Boswellia* and *Curcuma* extract on oxaliplatin-induced neuropathy via modulation of NF-κB signaling

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ARTICLE INFO

Handling Editor: Prof. L.H. Lash

*Keywords:*
Oxaliplatin
Neuropathy
NF-κB, Inflammation
Oxidative stress
Curcumin, Boswellia

ABSTRACT

Oxaliplatin is a third-generation anticancer agent with better efficacy, lower toxicity, and a broad spectrum of antineoplastic activity. Its use is frequently associated with chronic oxaliplatin-induced neuropathy (OIN), a cumulative phenomenon manifesting as loss of sensation, paresthesia, dysesthesia, and irresolvable fluctuations in proprioception that greatly affect the patients' quality of life. The inevitable nature and high incidence of OIN, along with the absence of efficacious preventive agents, necessitate the development of effective and reliable protective options for limiting OIN while maintaining anticancer activity. The pathogenesis of chronic OIN involves neuroinflammation and oxidative stress. This study aimed to explore the neuroprotective effects of *Boswellia serrata* and *Curcuma longa* via modulation of nuclear factor-kappa B (NF-κB) signaling. Behavioral tests were conducted to assess cold allodynia, heat hyperalgesia, mechanical allodynia, mechanical hyperalgesia, and slowed nerve conduction velocity associated with chronic oxaliplatin administration. The modulation of NF-κB signaling and the subsequent activation of cytokines were evaluated through quantitative analysis of inflammatory cytokines in sciatic nerve homogenates. Additional assessments included oxidative stress parameters, serum neuronal biomarkers, and examination of sciatic nerve cross-sections. The findings indicate improvements in behavioral and biochemical parameters, as well as nerve histology, with the combined extract of *Boswellia serrata* and *Curcuma longa* at doses of 50 mg/kg and 75 mg/kg. Thus, this study presents evidence for the protective potential of the combined extract of *Boswellia serrata* and *Curcuma longa* in OIN through modulation of NF-κB signaling.

## 1. Introduction

Cancer, characterized by abnormal, repetitive, and uninhibited cell proliferation, is a major reason for deaths globally. According to GLOBOCAN reports, in 2022 alone, 20 million new cancer patients were diagnosed, with nearly 10 million fatalities worldwide. Lung cancer was the most prevalent cause of cancer-related mortality, followed by colorectal, hepatic, female breast, and stomach cancers [1]. Oxaliplatin, a diamino cyclohexane (DACH) carrier ligand-based organoplatin, is a broad-spectrum anticancer agent approved in conjunction with 5- fluorouracil/leucovorin (FU/LV) infusion by the FDA for treating advanced colorectal cancer (CRC) [2,3,4]. However, the use of oxaliplatin is restricted due to its cumulative dose-dependent and dose-limiting peripheral neuropathy commonly observed during or immediately after infusion. Chronic oxaliplatin-induced neuropathy (OIN) is a cumulative phenomenon frequently seen in patients receiving a total of more than 540–850 mg/m$^2$ oxaliplatin. It takes 9–10 turns of 85 mg/m$^2$ and around 6 turns of 130 mg/m$^2$ of the drug for the chronic neuropathic symptoms to be seen [5,6]. Chronic OIN manifests as loss in sensation, paresthesia, dysesthesia, and irresolvable fluctuations in proprioception [7,8]. It results in a declined quality of life with a significant loss of functional abilities [9]. The alterations in proprioception may influence day-to-day functions that demand fine motor coordination, like buttoning shirts, holding objects, writing, and picking up coins. Rare incidences of central neuropathy, typified by urinary retention and Lhermitte's sign (an electrical feeling along with cervical flexion), have

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https://doi.org/10.1016/j.toxrep.2024.101781
Received 6 August 2024; Received in revised form 4 October 2024; Accepted 18 October 2024
Available online 20 October 2024

also been reported [5].

The pathogenesis of chronic OIN primarily involves neuroinflammation, oxidative stress overload, mitochondrial dysfunction, kinase pathway disruption, and nuclear and mitochondrial DNA damage [7]. Preclinical and clinical research findings demonstrate that neuropathic pain produced by chemotherapeutics involves the activation of glia followed by liberation and elevation of proinflammatory cytokines (PIC) like interleukin-1 (IL-1), interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) [10,11]. These cytokines modulate ion channels, leading to sensitization of the nociceptors [12]. Xu et al. noted that oxaliplatin triggers the activation of IL-6, TNF-α, and IL-1β and respective receptors in the periaqueductal gray matter (PAG). Occluding the PIC receptors reduced oxaliplatin-related neuropathic pain. Moreover, PIC compromised GABAergic-mediated descending inhibition, likely due to apoptosis-led damage to GABAergic neurons within the PAG. The study concluded that elevated PIC release, followed by a fall in GABA transmission in the PAG, probably contributes to cold and mechanical hypersensitivity in oxaliplatin-treated animals [13]. Nuclear factor-kappa B (NF-κB) is a transcription factor that activates signaling pathways in OIN. It induces the expression of PICs like TNF-α, IL-1β, and IL-6. These PICs cause spinal cord sensitization, leading to hyperalgesia and ultimately resulting in neuropathic pain [14]. Additionally, oxidative overload leads to the development of OIN. The association between OIN and oxidative stress is substantiated by both preclinical and clinical evidence reporting the effectiveness of antioxidative regimens in alleviating neuropathic symptoms [15]. Transient Receptor Potential Ankyrin 1 (TRPA1) is a non-selective positive ion channel acting as a sensor of oxidative stress and lower temperatures. It is pivotal in facilitating cold hypersensitivity evoked by oxaliplatin through oxidative damage [16,17]. Exposure to oxaliplatin produces oxidative stress-related by-products like nitrooleic acid, $H_2O_2$, and hypochlorite, which activate TRPA1, leading to nociceptive responses and neuroinflammation [18,19,16]. Antioxidants such as lipoic acid, acetyl-l-carnitine, or vitamin C demonstrate inhibitory effects on oxaliplatin-triggered hyperalgesia in rats, implying a crucial function of oxidative stress in OIN. Reactive oxygen species (ROS) also regulate sodium channel activity; thereby, impacting nociceptor sensitivity. Moreover, oxaliplatin can damage axonal mitochondria, causing disturbances of the electron transport chain in dorsal root ganglion (DRG) neurons [20].

Duloxetine, a potent monoamine modulator, is a commonly used antidepressant approved by the FDA for treating diabetes-related peripheral neuropathic pain. It blocks the neuronal re-uptake of norepinephrine (NE) and serotonin (5-HT) [21]. Recently, in 2020, ASCO recommended duloxetine for treating chemotherapy-induced painful neuropathy in patients who have completed the neurotoxic chemotherapeutic regimen. Duloxetine can efficiently alleviate the pain related to OIN without diminishing or interfering with the antitumor effects of oxaliplatin [22]. However, duloxetine can only be used once the chemotherapeutic regimen is over. Therefore, currently, there is a need for safe and effective preventive options for chemotherapy-induced neuropathy (CIN).

*Boswellia serrata*, also called Salai guggul, is a well-known ancient herb. It produces an oleogum resin exudate from its bark. The resin comprises 11-keto-β-boswellic acid (KBA) and 3-acetyl-11-keto-β-boswellic acid (AKBA), responsible for the beneficial therapeutic effects of *Boswellia* [23]. These acids inhibit 5-lipoxygenase, a pro-inflammatory enzyme, thereby producing anti-inflammatory effects. Moreover, *B. serrata* extract and 3-acetyl-11-keto-β-boswellic acid (AKBA) have exhibited neuroprotective influences on oxidative impairment in rat pheochromocytoma-derived (PC-12) cells through the decline of lipid peroxidation and ROS production [24,25,26]. AKBA also exhibited neuroprotective effects in ischemic brain, and nerve injury by promoting nerve repair and regeneration [27]. *Curcuma longa*, also known as turmeric or golden spice, is a perennial herbal medicine. It is a pleiotropic agent, traditionally used for treating several diseases owing to its well-known antimicrobial, anti-inflammatory, antioxidant, neuroprotective, and wound-healing properties. Recent studies have attributed these properties of turmeric to curcumin, a polyphenol extracted from the rhizome of *C. longa*. Turmeric contains curcuminoids, volatile oils, resins, vitamins, fats, and minerals. Curcuminoids consist mainly of curcumin (77 %), and smaller amounts of demethoxycurcumin and bis-demethoxycurcumin [28]. The anti-inflammatory property of curcumin has been demonstrated by decreased NF-κB activation, thereby leading to a diminished release of IL-1β, TNF-α, and IL-6 in the sciatic nerve, brain and spinal cord samples of CIN, sciatic nerve injury, and alcohol-induced neuropathic models. Inflammation and oxidative stress are closely linked, each capable of inducing the other. The antioxidant effect of curcumin scavenges ROS and lipoperoxidation of membrane lipids, thus preventing oxidative damage. It also upregulates Nuclear factor erythroid 2-related factor 2 (Nrf2), which in turn activates antioxidant response element (ARE), ultimately leading to the production of antioxidative enzymes: catalase, superoxide dismutase (SOD), and Glutathione peroxidase (GPx) [28]. With this background, the current study aimed to investigate the anti-inflammatory influence of *Boswellia serrata* and *Curcuma longa* on NF-κB signaling modulation for preventing OIN in rats.

The inevitable nature and high incidence of OIN lead to limited patient compliance. This, coupled with the lack of effective treatment options necessitates the development of effective and reliable preventive options for limiting OIN while maintaining anticancer activity. *Boswellia serrata* and *Curcuma longa* are well known for their antioxidant and anti-inflammatory properties. With this rationale, the current study sought to explore the neuroprotective effects of simultaneous administration of *Boswellia serrata* and *Curcuma longa* compared to duloxetine in OIN. For the study, LN20192, a patented proprietary product comprising a combined extract of *Boswellia serrata* and *Curcuma longa*, was provided by Sundyota Numandis Probioceuticals Pvt. Ltd., Ahmedabad, India. The extract contained total curcuminoids 20 %, boswellic acid 18 %, and 11-keto-β-boswellic acid (KBA) + 3-acetyl-11-keto-β-boswellic acid (AKBA) 4 %. A chronic OIN model was established in rats through repeated intraperitoneal administration of oxaliplatin. Chronic oxaliplatin administration has been associated with cold allodynia, heat hyperalgesia, mechanical allodynia, mechanical hyperalgesia, and slowed nerve conduction velocity [29]. Hence, tests like hot and cold water tail immersion, hot plate, Randall Selitto, Von Frey, and motor nerve conduction velocity were performed. The role of *Boswellia serrata* and *Curcuma longa* in the modulation of NF-κB signaling and subsequent activation of PIC was ascertained by quantitative estimation of inflammatory cytokines in sciatic nerve homogenates. Moreover, the oxidative stress parameters were also evaluated. In addition, serum neuronal biomarkers and sciatic nerve cross-sections were examined.

## 2. Materials and methods

### 2.1. Pharmacological treatments

Forty-eight, 7-week-old healthy male Sprague Dawley rats (200–220 g) were procured from National Institute of Biosciences, Pune, India. Rats were placed in polypropylene cages. Each cage contained four rats. A standard laboratory setting of 23 ± 2 °C, 60–65 % relative humidity, and a 12-h light/12-h dark cycle was maintained. The rats were supplied with potable water *ad libitum* and fed with Amrut™ Laboratory Animal Feeds from Krishna Valley Agrotech, India. The animals were acclimatized to the laboratory for 7 days and subsequently taken up for investigation. The study was undertaken post-approval by and under the superintendence of the Institutional Animal Ethics Committee (Protocol no. IAEC-BCP/2022–02/01, dated 17 December 2022). Oxaliplatin was a gift from Bruck Pharma Private Limited, Daman & Diu, India.

Clinical studies have demonstrated the anti-inflammatory effects of curcumin at doses ranging from 200 to 2000 mg/day [30]. A

randomized, double-blind placebo-controlled study reported significant improvements in osteoarthritis with 333 mg of *Boswellia serrata* extract [31]. The current study utilized LN20192, a patented proprietary product comprising a combined extract of *Boswellia serrata* and *Curcuma longa*. Since, this formulation contains both *Boswellia serrata* and *Curcuma longa*, synergistic effects were anticipated and a human therapeutic dose of 500 mg/day of LN20192 was selected for oxaliplatin-induced neuropathy. Based on this, the equivalent rat dose was derived as ~50 mg/kg/day. Further, to ascertain the protective effects of LN20192 at different doses, the preclinical study considered 0.5, 1 and 1.5 times this dose: 25 mg/kg, 50 mg/kg, and 75 mg/kg.

The study evaluated the protective effects of LN20192 at three doses: 25 mg/kg, 50 mg/kg, and 75 mg/kg in comparison to the standard treatment, duloxetine 30 mg/kg. The dose of duloxetine was selected based on previous reports from the literature [32,33]. Rats were randomized into six groups (n=8) and subjected to the following regimen for 4.5 weeks:

Vehicle control: 5 % dextrose, i.p. twice weekly + acid saline, p.o. daily

Disease control: Oxaliplatin 4 mg/kg, i.p. twice weekly

Standard treatment: Oxaliplatin 4 mg/kg, i.p. twice weekly + duloxetine 30 mg/kg, p.o.

LN20192 25 mg/kg: Oxaliplatin 4 mg/kg, i.p. twice weekly + LN20192 25 mg/kg, p.o.

LN20192 50 mg/kg: Oxaliplatin 4 mg/kg, i.p. twice weekly + LN20192 50 mg/kg, p.o.

LN20192 75 mg/kg: Oxaliplatin 4 mg/kg, i.p. twice weekly + LN20192 75 mg/kg, p.o.

### 2.2. Behavioral tests

#### 2.2.1. Cold and hot water tail immersion test

Oxaliplatin associated cold allodynia and hyperalgesia were evaluated by immersing the rat's tail in water kept at a temperature of 10 ± 0.5 °C and 4 ± 0.5 °C, respectively. Thermal allodynia and thermal hyperalgesia induced by oxaliplatin were evaluated by immersing the rat's tail in water kept at a temperature of 42 ± 0.5 °C and 46–52 °C, respectively. The time taken for flicking of the tail was recorded. To evade any tissue damage, the tail was immersed for a maximum of 15 s. The tail-flick latency for each rat was measured thrice, and the average was reported [34].

#### 2.2.2. Hot plate

An unrestrained rat was put on a hot plate apparatus (IITC Life Sciences, USA) maintained between 50 and 55 °C. The response latency is the time taken to exhibit nocifensive actions such as hind paw withdrawal, stamping, licking, or jumping, was recorded thrice, and the average was reported. The cut-off time was 15 s [34].

#### 2.2.3. Von Frey

The Von Frey test was undertaken to evaluate mechanical allodynia. Each rat was kept in a small cage (30 x 20 x 20 cm) with a mesh floor to acclimatize for 20 min. A single, un-bending filament was introduced perpendicularly to the hind paw plantar surface with intensifying force until paw withdrawal occurred. The force at which this withdrawal response occurred was recorded on an Electronic von Frey Anesthesiometer (IITC Life Science Inc, CA, USA) thrice, and the average was reported as the paw mechanical withdrawal threshold (g) [35].

#### 2.2.4. Randall Selitto

Mechanical hyperalgesia was evaluated by determining the paw-withdrawal thresholds during the Randall Selitto test. The rat was restrained, and the hind paw was rested between a pointed probe tip and a flat surface. The pressure on the dorsal paw surface was increased until vocalization or withdrawal occurred. The force at which withdrawal or vocalization occurred was recorded using a digital paw pressure Randall Selitto instrument meter (IITC Life Science Inc., CA, USA) thrice, and the average was reported as the paw mechanical withdrawal threshold (g). The cut-off pressure was 450 g [35].

### 2.3. Electrophysiological examination: Motor nerve conduction velocity (MNCV)

Rats were anesthetized with ketamine (60 mg/kg) and xylazine (10 mg/kg) i.p. injection. The receiving electrodes were attached percutaneously at a distance of 1 cm at the dorsal hind paw muscles of the animal. The sciatic nerve was stimulated at the sciatic nerve proximal to the sciatic notch with a single stimulus (5 V) using the MLA0320 animal nerve stimulating electrode. The recordings due to motor fibers' stimulation were logged by the physiograph software (AD Instruments, Australia). The M-wave and H-wave reflexes were digitally documented using a data acquisition system. The M-wave latency, used for calculating MNCV, is measured in seconds. The distance between the recording electrode and the stimulation point was measured manually using a measuring tape. A heating pad maintained the rat's body temperature at 37 ± 0.5 °C throughout the experiment [36].

$$\textit{Motor Nerve Conduction Velocity}\ \ (\textit{MNCV}) = \frac{\textit{Distance between the nerve stimulation points in metre}(m)}{\textit{Latency in seconds}(s)}$$

### 2.4. Tissue preparation

Sciatic nerves were isolated and homogenized in ice-cold 0.1 M tris-HCL buffer (pH 7.4) in a 1:10 proportion with ten bursts of 10 s on-and-off using a Polytron (Kinematica, Switzerland) homogenizer [37]. The homogenates were centrifuged in a cooling centrifuge (Eppendorf, Germany) at 2000*g* for 10 min. A temperature of 4 °C was maintained throughout the procedure. The supernatant was further evaluated [38].

### 2.5. Biochemical estimations

#### 2.5.1. Oxidative stress markers

Catalase activity in the sciatic nerve homogenate was ascertained by the method of Aebi, 1984 [39]. The results were stated as U/mg tissue protein, where one unit of catalase activity is the enzyme amount that decomposes 1 μmol of $H_2O_2$ per min at 25 °C and pH 7.3. SOD activity was determined by the method described by Misra and Fridovich., 1972. The assay was principled on the inability of SOD to inhibit auto-oxidation of epinephrine at pH 10.2 to form adrenochrome [40]. Results were stated as U/mg tissue protein, where one unit of SOD activity is the enzyme amount required to prevent the epinephrine auto-oxidation rate by 50 %. Reduced GSH levels were ascertained based on the method of Sedlak et al., using 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB/Ellman's reagent). Reduced GSH levels were expressed as μg/mg tissue protein [41].

#### 2.5.2. Inflammatory cytokines

The quantitative estimation of inflammatory cytokines: NF-κB, IL-6, TNF-α, and IL-1β was performed employing a sandwich ELISA-based GENLISA™ kit (Krishgen Biosystems, India) following the manufacturer's instructions.

#### 2.5.3. Serum biomarkers

The quantitative estimation of neuronal serum biomarkers: neurotensin, BDNF, and NGF was performed using sandwich ELISA kits from Krishgen Biosystems, India, and Invitrogen, Thermo Fisher Scientific, USA, following the manufacturer's instructions.

### 2.6. Sciatic nerve histology

Sciatic nerves were isolated from rats of all the groups. The sciatic

nerve was immediately stored in 10 % buffered formalin. This was followed by dehydration in increasing ethanol concentrations, clearing with xylene, and embedding into paraffin. 5 μm sections were made from paraffin blocks and stained with hematoxylin and eosin (H&E). The stained sections were examined under a microscope at 400X.

### 2.7. Statistical analysis

Statistical analyses were carried out using GraphPad Prism 10 (GraphPad Software, Inc., Boston, MA 02110, USA). A comparison was made by one-way ANOVA followed by *post hoc* Tukey's test. Values are reported as mean ± S.E.M.

i) ####, ###, ## and # denote statistical significance at $p < 0.0001$, $p < 0.001$, $p < 0.01$ and $p < 0.05$ respectively, in comparison to vehicle control group.
ii) ****, ***, ** and * denote statistical significance at $p < 0.0001$, $p < 0.001$, $p < 0.01$ and $p < 0.05$ respectively, in comparison to disease control group.
iii) & & & &, & & &, & & and & denote statistical significance at $p < 0.0001$, $p < 0.001$, $p < 0.01$ and $p < 0.05$ respectively, in comparison to duloxetine 30 mg/kg.

## 3. Results

### 3.1. LN20192 improves escape latency in cold and hot water tail immersion test

The disease control group displayed a significant decline in escape latency compared to the vehicle control group in cold and hot water tail immersion tests ($p < 0.0001$). In cold water tail immersion test at 4 °C, treatment with LN20192 significantly improved the escape latency at doses 50 mg/kg ($p < 0.01$) and 75 mg/kg ($p < 0.001$) compared to duloxetine 30 mg/kg (Fig. 1. A). However, no remarkable improvement in escape latency was seen at 10 °C (Fig. 1. B). In hot water tail immersion test at 42 °C, treatment with LN20192 significantly improved the escape latency at doses 50 mg/kg ($p < 0.0001$) and 75 mg/kg ($p < 0.0001$) than duloxetine (Fig. 1. C). At 55 °C, rats administered with doses 25 mg/kg and 50 mg/kg of LN20192 showed escape latency comparable to that of duloxetine. While 75 mg/kg LN20192 exhibited a higher escape latency which was significantly better than duloxetine ($p < 0.01$) (Fig. 1. D) (Table I).

### 3.2. LN20192 ameliorates the escape latency in the hot plate test

During the hot plate test performed between 50 and 55 °C, the disease control group unveiled a significant decrease in escape latency compared to the vehicle control group ($p < 0.0001$). The escape latency exhibited by rats treated with 75 mg/kg LN20192 was comparable to that of duloxetine 30 mg/kg (Fig. 1. E) (Table I).

### 3.3. LN20192 improves mechanical allodynia

The disease control group revealed a significant decrease in Von Frey's mechanical withdrawal threshold compared to the vehicle control group ($p < 0.0001$). Rats administered with doses 50 mg/kg and 75 mg/kg of LN20192 presented a mechanical withdrawal threshold comparable to duloxetine 30 mg/kg (Fig. 1. F) (Table I).

### 3.4. LN20192 ameliorates mechanical hyperalgesia

In the Randall Selitto test, the disease control group exhibited a significant decrease in the mechanical withdrawal threshold compared to the vehicle control group ($p < 0.0001$). The mechanical withdrawal threshold seen at doses 25 mg/kg and 50 mg/kg of LN20192 was comparable to duloxetine 30 mg/kg, while a remarkable improvement was seen at 75 mg/kg ($p < 0.001$). (Fig. 1. G) (Table I).

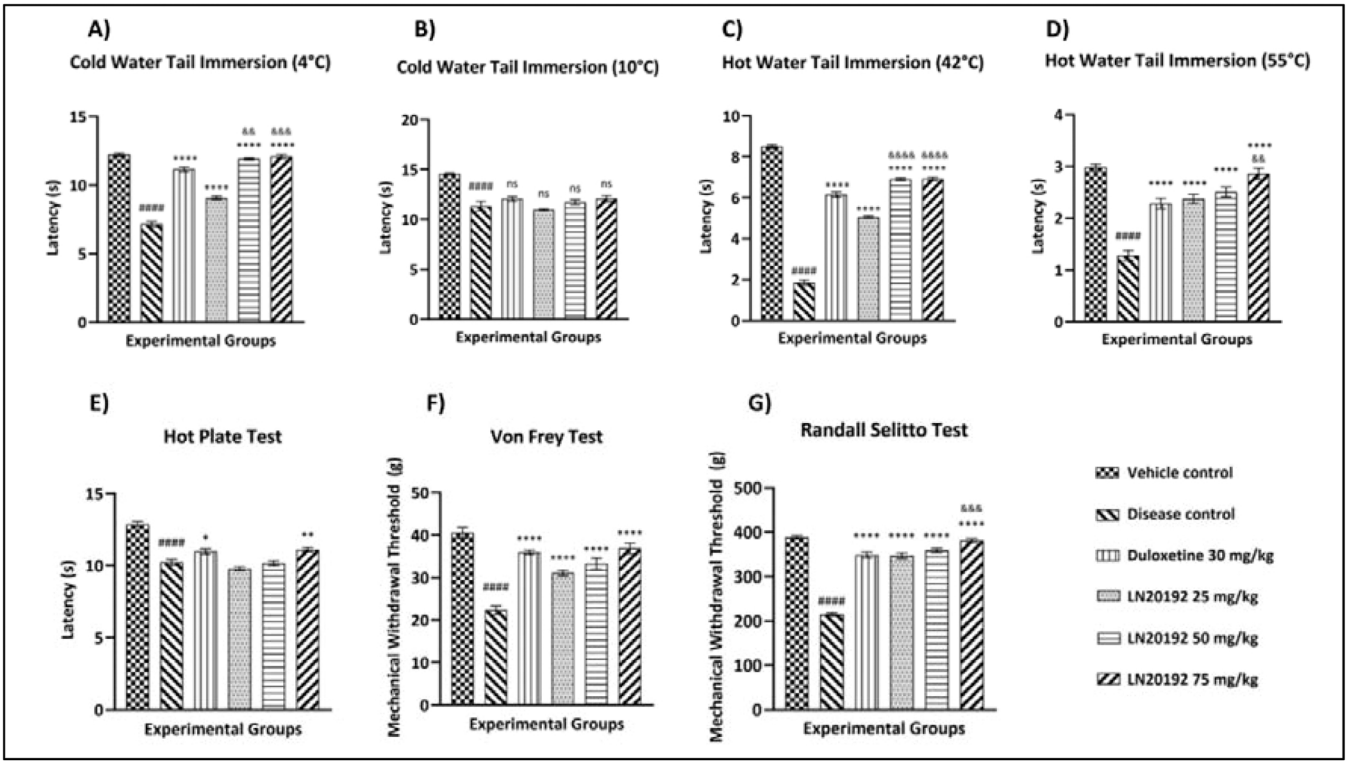


**Fig. 1. Effect of LN20192 on behavioral parameters.** LN20192 attenuates cold hyperalgesia, thermal allodynia, thermal hyperalgesia, mechanical allodynia and mechanical hyperalgesia. Values are expressed as mean ± S.E.M. (n=8).

**Table I**
Behavioral parameters.

| Behavioral Parameters | Vehicle Control | Disease Control | Duloxetine 30 mg/kg | LN20192 25 mg/kg | LN20192 50 mg/kg | LN20192 75 mg/kg |
|---|---|---|---|---|---|---|
| Cold water tail immersion latency at 4 °C (s) | 12.22 ± 0.1 | 7.15 ± 0.18 #### | 11.14 ± 0.17 **** | 9.06 ± 0.13 **** | 11.9 ± 0.08 ****, & & | 12.08 ± 0.14 ****, & & & |
| Cold water tail immersion latency at 10 °C (s) | 14.56 ± 0.1 | 11.32 ± 0.48 #### | 12.06 ± 0.22 | 10.96 ± 0.1 | 11.72 ± 0.23 | 12.08 ± 0.29 |
| Hot water tail immersion latency at 42 °C (s) | 8.50 ± 0.07 | 1.86 ± 0.13#### | 6.16 ± 0.14 **** | 5.06 ± 0.06 **** | 6.91 ± 0.06 ****, & & & & | 6.93 ± 0.08****, & & & & |
| Hot water tail immersion latency at 55 °C (s) | 2.98 ± 0.06 | 1.28 ± 0.1#### | 2.28 ± 0.1 **** | 2.37 ± 0.09 **** | 2.50 ± 0.09 **** | 2.85 ± 0.1****, & & |
| Hot plate latency 50–55 °C (s) | 12.87 ± 0.1 | 10.23 ± 0.19 #### | 10.98 ± 0.18 * | 9.79 ± 0.14 | 10.16 ± 0.16 | 11.1 ± 0.15** |
| Von Frey mechanical withdrawal threshold (g) | 40.5 ± 1.29 | 22.3 ± 1.05 #### | 35.97 ± 0.53 **** | 31.09 ± 0.65 **** | 33.29 ± 1.32 **** | 36.85 ± 1.26**** |
| Randall Selitto mechanical withdrawal threshold (g) | 388.5 ± 3.74 | 214.8 ± 3.1#### | 349.2 ± 6.56 **** | 347.5 ± 6.31 **** | 359.2 ± 4.98 **** | 381.6 ± 4.18****, & & & |

### 3.5. LN20192 improves MNCV

The disease control group exhibited a significant decrease in MNCV than the vehicle control group ($p < 0.001$). 75 mg/kg of LN20192 demonstrated MNCV comparable to that of duloxetine 30 mg/kg (Fig. 2.) (Table II).

### 3.6. LN20192 abrogates oxaliplatin-induced oxidative stress

The catalase activity was significantly reduced in the disease group than the vehicle control group ($p < 0.01$). 75 mg/kg of LN20192 unveiled a substantial elevation in catalase activity compared to the disease group ($p < 0.05$) (Fig. 3. A). The SOD activity was remarkably reduced in the disease group compared to the vehicle control group ($p < 0.001$). At doses of 50 mg/kg ($p < 0.01$) and 75 mg/kg ($p < 0.001$), LN20192 significantly improved the SOD activity compared to the disease group. (Fig. 3. B). The GSH levels were markedly reduced in the disease group than the vehicle control group ($p < 0.001$). The GSH levels produced by doses 50 mg/kg ($p < 0.05$) and 75 mg/kg ($p < 0.001$) of LN20192 were considerably better than the disease group (Fig. 3. C) (Table III).

### 3.7. LN20192 attenuates the rise in oxaliplatin-induced inflammatory cytokines

The disease control group exhibited a significant rise in the levels of NF-κB, IL-1β, IL-6, and TNF-α than the vehicle control group ($p < 0.0001$). Treatment with LN20192 lowered this rise at all three doses. The 75 mg/kg dose of LN20192 significantly blunted the rise in NF-κB levels compared to duloxetine 30 mg/kg ($p < 0.05$) (Fig. 4. A). The IL-1β levels exhibited by rats administered with doses 50 mg/kg and 75 mg/kg of LN20192 were comparable to that of duloxetine (Fig. 4. B). All three doses of LN20192 significantly lowered the elevated IL-6 levels ($p < 0.01$) (Fig. 4. C). The TNF-α levels produced by 25 mg/kg LN20192 were comparable to that of duloxetine. While doses 50 mg/kg ($p < 0.01$) and 75 mg/kg ($p < 0.05$) of LN20192 significantly lowered TNF-α than duloxetine (Fig. 4. D) (Table IV).

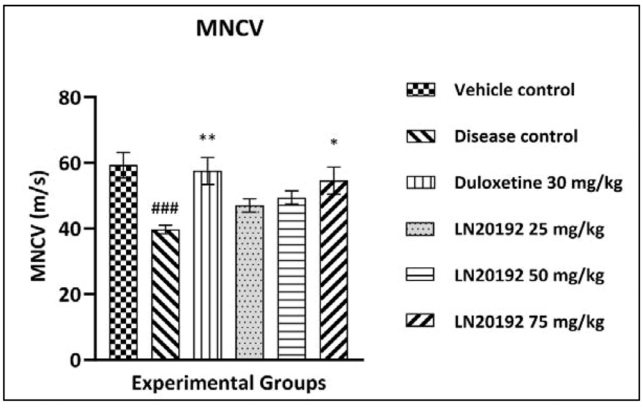


**Fig. 2. Effect of LN20192 on MNCV.** LN20192 improves MNCV comparable to duloxetine. Values are expressed as mean ± S.E.M. (n=8).

### 3.8. LN20192 improves neuronal serum biomarkers

The disease control group exhibited a significant reduction in serum BDNF levels compared to the vehicle control group ($p < 0.001$). 30 mg/kg duloxetine failed to improve the serum BDNF level, while LN20192 significantly elevated the serum BDNF levels at all three doses ($p < 0.05$, $p < 0.001$, $p < 0.0001$) (Fig. 5. A). An increase in neurotensin levels in the disease control group than the vehicle control group was seen. LN20192 blunted this rise in neurotensin levels at all three doses (Fig. 5. B). The disease control group demonstrated a significant decline in NGF levels in comparison to the vehicle control group ($p < 0.0001$). All three doses of LN20192 raised the serum NGF levels. The rise in serum NGF levels produced by LN20192 at a dose of 75 mg/kg was significantly higher than that of duloxetine 30 mg/kg ($p < 0.05$) (Fig. 5. C) (Table V).

### 3.9. Improved sciatic nerve histology with LN20192

The H&E stained sciatic nerve cross-section of the control group rats showed normal histological morphology. Individual nerve fibers were held together by a delicate connective tissue endoneurium. Each nerve fiber was composed of a faint acidophilic axon surrounded by a clear myelin area; outside which the eosinophilic neurilemma was detected (Fig. 6. A). In the disease control group, oxaliplatin administration disrupted the nerve histology. Wide spaces between nerve fibers were detected (Fig. 6. B). Treatment with duloxetine exhibited scattered individual nerve fibers as well as disrupted nerve fibers (Fig. 6. C). Treatment with LN20192 attenuated the oxaliplatin-induced neuronal damage in a dose-dependent manner (Fig. 6. D, E, F). A significant improvement in the sciatic nerve cross-section morphology was seen at a dose of 75 mg/kg (Fig. 6. F).

## 4. Discussion

Oxaliplatin is a commonly used first-line chemotherapy agent for advanced CRC. However, it adversely affects the quality of patients' lives due to chronic OIN. This limits the duration of treatment and its widespread use. Currently, there is no preventive agent for chronic neuropathic symptoms associated with oxaliplatin. The present study examined the neuroprotective effects of LN20192, a patented proprietary product comprising a combined extract of *Boswellia serrata* and

**Table II**
MNCV.

| | Vehicle Control | Disease Control | Duloxetine 30 mg/kg | LN20192 25 mg/kg | LN20192 50 mg/kg | LN20192 75 mg/kg |
|---|---|---|---|---|---|---|
| MNCV (m/s) | 59.29 ± 3.88 | 39.68 ± 1.34$^{\#\#\#}$ | 57.57 ± 4.11$^{**}$ | 47 ± 1.98 | 49.43 ± 1.99 | 54.62 ± 4.12* |

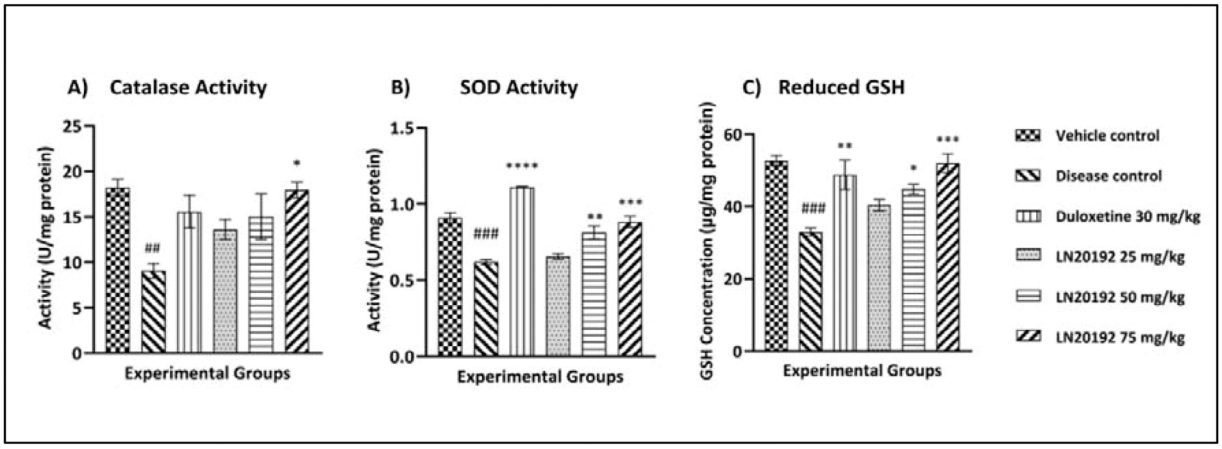


**Fig. 3. Effect of LN20192 on oxidative stress parameters in sciatic nerve homogenate.** LN20192 abrogates oxaliplatin-induced oxidative stress. Values are expressed as mean ± S.E.M. (n=8).

**Table III**
Oxidative stress parameters in sciatic nerve homogenate.

| | Vehicle Control | Disease Control | Duloxetine 30 mg/kg | LN20192 25 mg/kg | LN20192 50 mg/kg | LN20192 75 mg/kg |
|---|---|---|---|---|---|---|
| Catalase Activity (U/mg tissue protein) | 18.22 ± 0.89 | 9.07 ± 0.8$^{\#\#}$ | 15.58 ± 1.81 | 13.62 ± 1.09 | 15.02 ± 2.5 | 17.95 ± 0.86* |
| SOD Activity (U/mg tissue protein) | 0.9 ± 0.03 | 0.61 ± 0.01 $^{\#\#\#}$ | 1.11 ± 0.004 $^{****}$ | 0.65 ± 0.01 | 0.81 ± 0.04 $^{**}$ | 0.88 ± 0.03 $^{***}$ |
| Reduced GSH (µg/mg tissue protein) | 52.62 ± 1.46 | 32.94 ± 1.1$^{\#\#\#}$ | 48.75 ± 4.05$^{**}$ | 40.43 ± 1.55 | 44.76 ± 1.36* | 51.98 ± 2.64$^{***}$ |

*Curcuma longa*, in OIN compared to duloxetine.

Chronic OIN was successfully established in Sprague Dawley rats via repeated i.p. oxaliplatin administration and confirmed by intensified sensitivity to noxious and non-noxious temperatures and mechanical stimulus. The increased sensitivity to various stimuli was inferred from the time taken for exhibiting nocifensive behaviors such as tail flick, paw licking, jumping, or withdrawal of body parts. Cold allodynia and cold hyperalgesia induced by oxaliplatin were evaluated by immersing the rat's tail in cold water maintained at 10 °C and 4 °C, respectively. 10 °C is a normally non-noxious cold temperature, while 4 °C is a noxious cold temperature for healthy rodents. Similarly, 42 °C is a normally non-noxious hot temperature while 46–52 °C is noxious. Thermal allodynia and thermal hyperalgesia were assessed by immersing the rat's tail in hot water maintained at 42 °C and 46–52 °C, respectively. In addition, thermal hyperalgesia was also interpreted using a hot plate apparatus maintained between the noxious range of 50–55 °C. Von Frey and Randall Selitto's test denoted mechanical allodynia and hyperalgesia, respectively. The development of cold allodynia, cold hyperalgesia, thermal allodynia, thermal hyperalgesia, mechano-allodynia, and hyperalgesia in rats seen after oxaliplatin administration was consistent with previous reports [42,43,44].

Preclinical investigations by Kim et al. in rodents revealed that 30 mg/kg of duloxetine successfully attenuates oxaliplatin-induced mechanical and cold allodynia [32]. In a study by Zhang et al., curcumin improved mechanical and cold allodynia [45]. The proprietary product failed to improve cold allodynia at all three doses. Zhang et al. employed the acetone drop method for evaluating cold allodynia, while our study relied on a cold water tail immersion test at 10 °C [45,35]. The improvement in mechanical allodynia at doses 50 mg/kg and 75 mg/kg of LN20192, inferred by the Von Frey test, was comparable to the standard treatment, duloxetine 30 mg/kg. Doses of 50 mg/kg and 75 mg/kg LN20192 were significantly more effective in improving thermal allodynia and cold hyperalgesia than duloxetine. The 75 mg/kg dose of LN20192 significantly improved thermal hyperalgesia than duloxetine. Mechanical hyperalgesia, deduced from Randall Selitto, was significantly improved at 75 mg/kg dose of LN20192 compared to duloxetine. Additional studies evaluating the effects of curcumin on other pain-related parameters in chronic OIN are not available. However, the therapeutic impact of LN20192 on behavioral nocifensive symptoms of chronic OIN is evident in this study. MNCV is an electrophysiological test that measures the speed of conduction of an electrical impulse. A drop in MNCV is indicative of neuropathy. Zhang et al. demonstrated that oxaliplatin significantly reduces MNCV in chronic OIN rats, while curcumin improves it [45]. Similarly, in the current study, oxaliplatin displayed a reduced MNCV, while a 75 mg/kg dose of LN20192 corrected MNCV equivalent to duloxetine.

The molecular mechanism of OIN is not yet fully established; however, inflammation and oxidative stress are major known contributors to oxaliplatin-associated neurotoxicity. Nerves are highly susceptible to oxidative damage due to their mitochondria-loaded axoplasm, excessive phospholipid content, and weak antioxidant systems. Moreover, the absence of BBB at the periphery makes them more susceptible to injury due to the accumulation of chemotherapeutic agents. In the present study antioxidant enzyme assays were performed on sciatic nerve

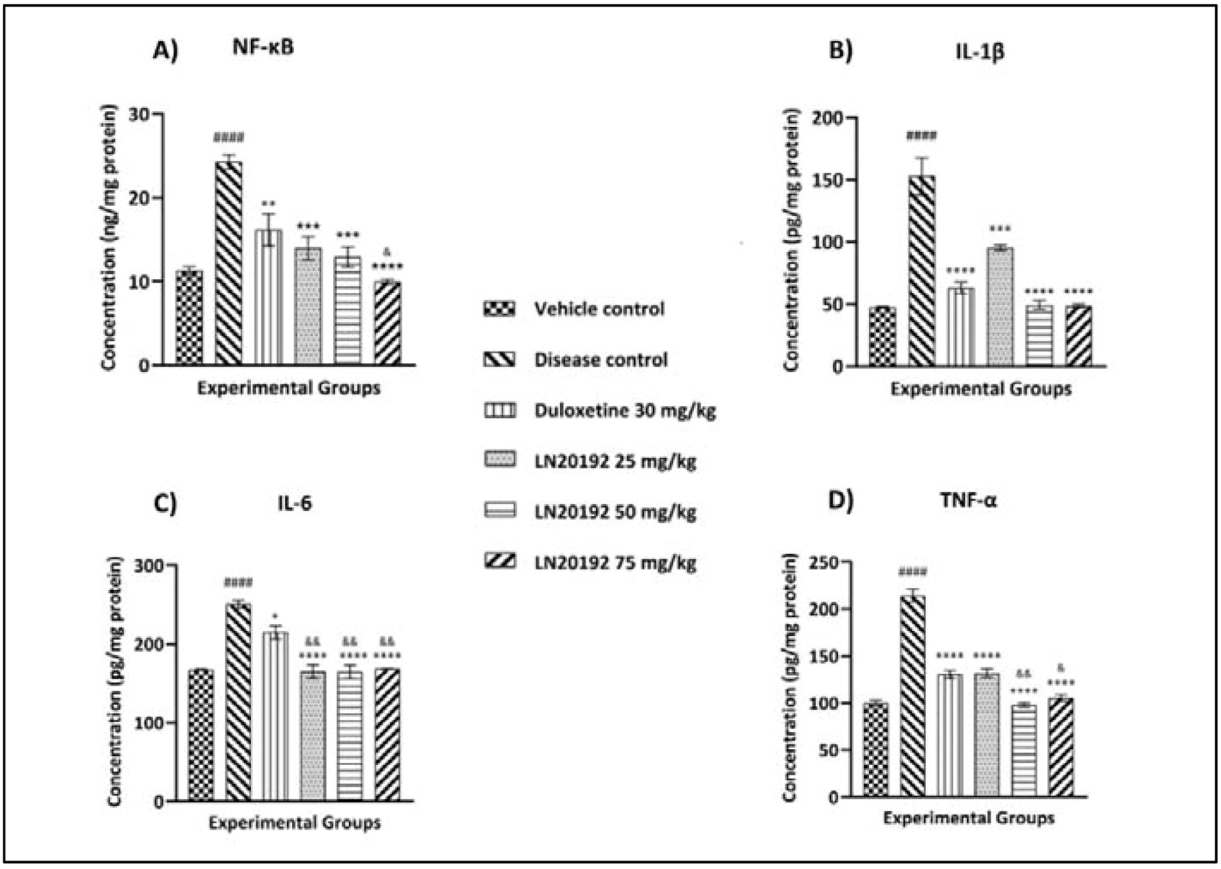


**Fig. 4. Effect of LN20192 on inflammatory cytokines in sciatic nerve homogenate.** LN20192 attenuates the rise in oxaliplatin-induced inflammatory cytokines. Values are expressed as mean ± S.E.M. (n=8).

**Table IV**
Inflammatory cytokines in sciatic nerve homogenate.

| | Vehicle Control | Disease Control | Duloxetine 30 mg/kg | LN20192 25 mg/kg | LN20192 50 mg/kg | LN20192 75 mg/kg |
|---|---|---|---|---|---|---|
| NF-κB (ng/mg protein) | 11.29 ± 0.45 | 24.28 ± 0.85 #### | 16.16 ± 1.93 ** | 13.97 ± 1.37 *** | 12.91 ± 1.23 *** | 9.95 ± 0.27 ****, & |
| IL−1β (pg/mg protein) | 47.76 ± 0.5 | 152.9 ± 15.06 #### | 63.1 ± 4.41 **** | 95.37 ± 2.39 *** | 49.39 ± 3.45 **** | 48.69 ± 1.31 **** |
| IL−6 (pg/mg protein) | 167.4 ± 0.62 | 250.7 ± 5.28 #### | 214.5 ± 8.29 * | 164.9 ± 8.75 ****, & & | 164.6 ± 8.6 ****, & & | 168.3 ± 0.25 ****, & & |
| TNF-α (pg/mg protein) | 99.8 ± 3.06 | 214.9 ± 6.86 #### | 130.3 ± 4.44 **** | 131.7 ± 4.42 **** | 97.8 ± 2.26 ****, & & | 105.3 ± 3.49 ****, & |

homogenate. SOD is a metalloenzyme that catalyzes the dismutation of superoxide anions into hydrogen peroxide and water. Catalase, a ubiquitous antioxidant enzyme, mitigates oxidative damage by further breaking down the hydrogen peroxide into inactive components, water and oxygen [46,47]. Oxaliplatin led to a marked decline in catalase activity, SOD activity, and reduced GSH levels. A decrease in these antioxidant parameters is suggestive of an imbalance between ROS generation and degradation. LN20192 significantly alleviated SOD activity at doses of 50 mg/kg and 75 mg/kg than the disease control group. Only 75 mg/kg of LN20192 significantly improved catalase activity than the disease group. In addition, the reduced GSH levels produced by LN20192 at doses 50 mg/kg and 75 mg/kg were markedly greater than the disease control group. A study by Zhang et al. reported similar improvements in the spinal cord [45]. Thus, LN20192 exerts an overall antioxidant effect in chronic OIN. NF-κB, a transcriptional regulator, is the key mediator of inflammation, immunity, and cell survival. Oxidative damage triggers the activation and phosphorylation of NF-κB. The phosphorylated NF-κB enters the nucleus and upregulates the PICs: TNF-α, IL-1β, and IL-6. These PICs trigger the initiation and maintenance of spinal cord sensitization, leading to hyperalgesia. A study by Meng et al. revealed that duloxetine inhibits the activation of p38 phosphorylation, thereby averting the activation and nuclear translocation of NF-κB [33]. *Boswellia* and *Curcuma* are antioxidants and anti-inflammatory agents that may influence OIN by acting on the inflammatory and oxidative stress pathway. The present study mainly focused on these pathways, specifically on the role of NF-κB and other PIC in OIN. At the end of the regimen, oxaliplatin considerably raised NF-κB, TNF-α, IL-6, and IL-1β levels in the disease control group. All doses of LN20192 significantly blunted the rise in IL-6 levels than duloxetine. 50 mg/kg and 75 mg/kg of LN20192 markedly lowered IL-1β levels than the disease group. At the same doses, LN20192 attenuated the rise in TNF-α significantly than duloxetine. However, only 75 mg/kg of LN20192 significantly blunted the rise in NF-κB. The overall decrease in levels of NF-κB and PICs on treatment with LN20192 aligns with previous studies and is suggestive of the anti-inflammatory properties of the proprietary product.

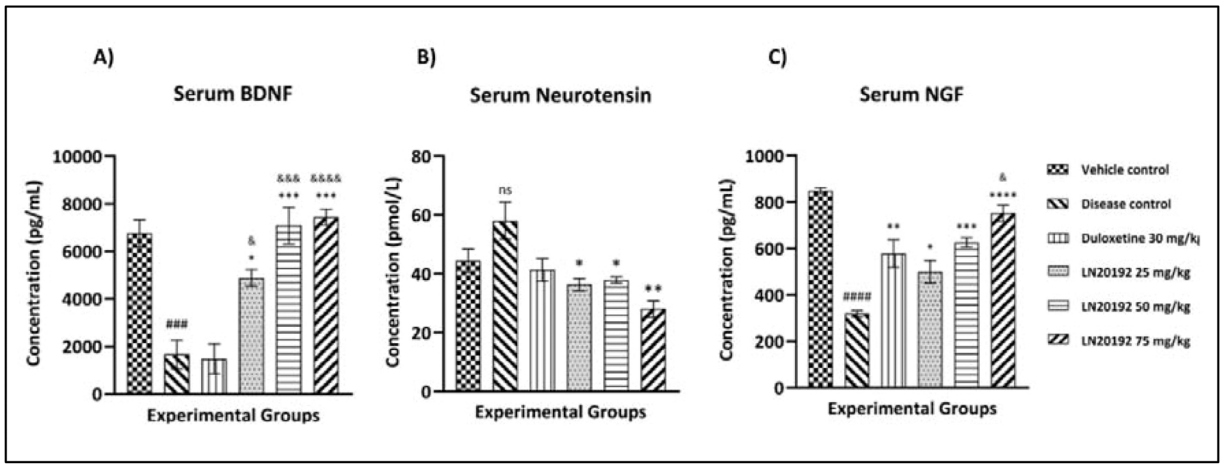


**Fig. 5. Effect of LN20192 on neuronal biomarkers in serum.** LN20192 improves neuronal serum biomarkers. Values are expressed as mean ± S.E.M. (n=8).

**Table V**
Neuronal biomarkers in serum.

| | Vehicle Control | Disease Control | Duloxetine 30 mg/kg | LN20192 25 mg/kg | LN20192 50 mg/kg | LN20192 75 mg/kg |
|---|---|---|---|---|---|---|
| BDNF (pg/mL) | 6764 ± 563.8 | 1662 ± 603.4$^{\#\#\#}$ | 1488 ± 626.8 | 4883 ± 357.1 $^{*, \&}$ | 7091 ± 766.8 $^{***, \& \& \&}$ | 7434 ± 337.9 $^{***, \& \& \& \&}$ |
| Neurotensin (pmol/L) | 44.44 ± 3.9 | 58.04 ± 6.24 | 41.32 ± 3.8 | 36.44 ± 2.07 $^{*}$ | 37.89 ± 1.07 $^{*}$ | 28.07 ± 2.81 $^{**}$ |
| NGF (pg/mL) | 847.6 ± 13.42 | 319.3 ± 13.63 $^{\#\#\#\#}$ | 578 ± 58.91 $^{**}$ | 499.4 ± 48.81 $^{*}$ | 625.5 ± 20.34 $^{***}$ | 752.4 ± 35 $^{****, \&}$ |

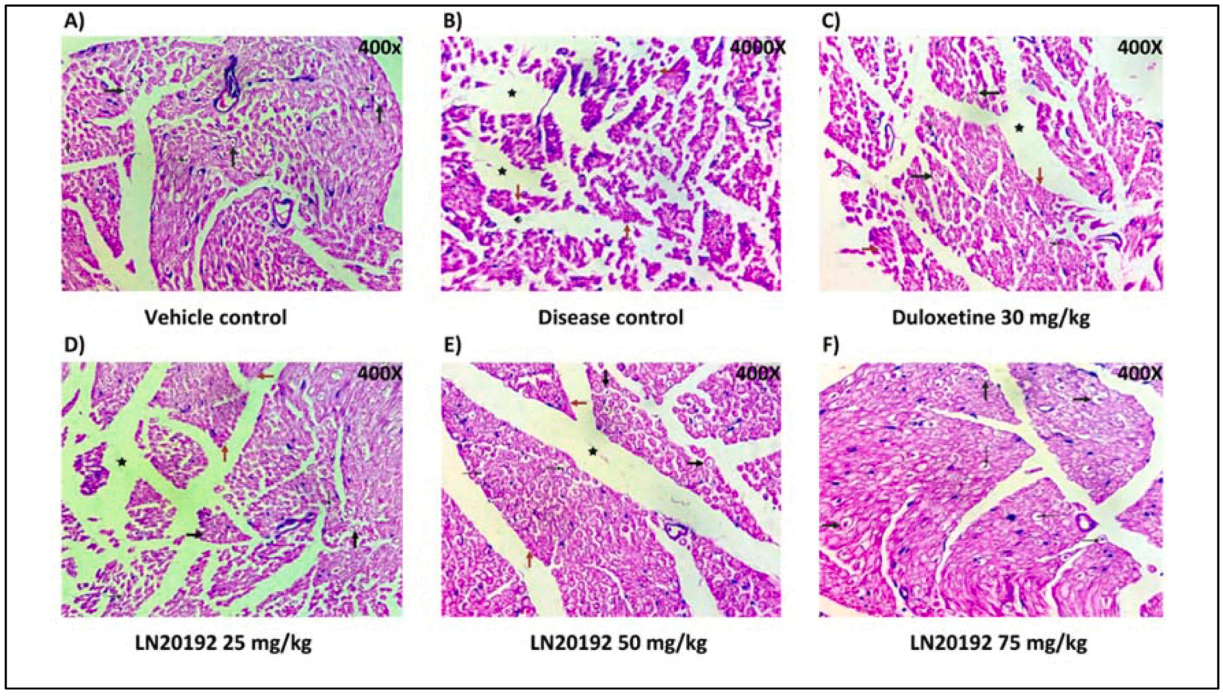


**Fig. 6. Histological analysis of sciatic nerve cross-section.** A) Sciatic nerve cross-section of rats from the control group exhibits a normal histological architecture of sciatic nerve. Each nerve fiber (thick arrows) is composed of faint acidophilic axon (thin arrows) surrounded by clear myelin area; outside the myelin area the eosinophilic neurilemma can be detected. B) Disease control group demonstrates oxaliplatin-induced disruption in nerve fibers (colored arrows) and wide neuronal spaces between the fibers (stars). C) Rats treated with duloxetine 30 mg/kg show a normal histological morphology of sciatic nerve (thick and thin arrows) as well as scattered disrupted nerve fibers (colored arrows) and wide neuronal spaces between the fibers (stars). D) In rats treated with LN20192 25 mg/kg marked disorganization of nerve fibers with disruption is seen (colored arrows). Spaces between the nerve fibers (stars) can be detected. E) At a dose of 50 mg/kg LN20192 few normal nerve fibers are noted (thick and thin arrows) along with disrupted nerve fibers (colored arrows). F) Marked improvement in the sciatic nerve cross-section is seen upon treatment with 75 mg/kg LN20192. Normal histological architecture of sciatic nerve consisting of nerve fiber (thick arrows) and faint acidophilic axon (thin arrows) can be seen.

Neurotensin is a 13 amino acid neurotransmitter involved in the modulation of pain signal transmission and perception. Inflammatory pain may elevate neurotensin. Neurotensin is affected by alcohol-induced neuropathy [48]. A phase III clinical study [Identifier: NCT05624138] listed serum neurotensin as a biomarker for OIN. In a preclinical study by Moundhri et al., oxaliplatin raised neurotensin, while curcumin blunted the rise [49]. However, the precise relation between neurotensin and OIN is not certain. In the current study, all doses of LN20192 considerably blunted the rise in neurotensin levels than the disease control group. NGF is a growth factor that regulates neuronal growth, maintenance, and survival. This factor is trophic to small fiber neurons that control temperature, pain, and autonomic functions. The role of NGF in the pathogenesis of CIN has been postulated in multiple experimental investigations, establishing its neuroprotective qualities both *in-vitro* and *in-vivo*. OIN rats presented a decline in circulating levels of NGF [50]. Meng et al. reported that oxaliplatin significantly lessens the NGF levels. However, co-administration with duloxetine elevated the levels of NGF [33]. In the present study, oxaliplatin significantly reduced serum NGF levels and LN20192 successfully restored the NGF levels. BDNF is a growth factor concerned with neuronal development, differentiation, maturation, and repair. Celik and co-workers revealed diminished BDNF in the sciatic nerve of rats administered with oxaliplatin [50]. The current study noted a marked reduction in serum BDNF levels with oxaliplatin administration. The standard treatment, duloxetine, failed to improve the BDNF levels; however, LN20192 markedly restored the BDNF levels.

H&E staining of the sciatic nerve cross-section is performed to observe the histological changes and infer the damages, if any. Previous studies on OIN and sciatic nerve injury-related neuropathy have revealed wide spaces and demyelination and degeneration of sciatic nerve fibers. Treatment with curcumin demonstrated a normal appearance of myelinated nerve fibers [49,51]. Curcumin, a well-known polyphenolic compound with antioxidant and anti-inflammatory properties, improved nerve conduction velocity and myelin thickness in rats administered with cisplatin [26]. There is increasing evidence of neuroprotective effects of curcumin through nerve regeneration and anti-apoptotic effects in models of sciatic nerve crush injury in diabetic and non-diabetic rats. Curcumin promotes axonal regeneration and enhances myelination. It also increases the number and size of myelinated axons and enhances myelination [52,53]. Schwann cells repair damaged peripheral nerves by encouraging myelination axonal regeneration and elongation. Curcumin can accelerate the repair of damaged sciatic nerve by stimulating Schwann cell proliferation and reducing their apoptosis in rats [54]. Curcumin is also known to induce NGF release, leading to cell survival signalling, thereby exerting its neuroprotective effects against impaired neurons [55]. In line with the previous evidence, we noted a dose-dependent improvement in the sciatic nerve histology on administration of LN20192.

## 5. Conclusion

In conclusion, the study highlights the protective preclinical potential of *Boswellia* and *Curcuma* extract in OIN through NF-κB signaling modulation. The results note improvements in behavioral parameters, biochemical estimations, and nerve histology after administration of the combined extract at doses of 50 mg/kg and 75 mg/kg. The combined extract attenuates cold hyperalgesia, thermal allodynia, thermal hyperalgesia, mechanical allodynia, and mechanical hyperalgesia. It also improves the electrophysiology of the sciatic nerve. The improvement in oxidative stress and inflammatory factors indicates that the combined extract abrogates oxaliplatin-related oxidative damage and inflammation through NF-κB signaling modulation. However, the results observed in the animal model may not reflect human response. Also, the behavioral results may not directly translate to a clinical setting. Hence, to establish a reliable clinical dose, additional studies on LN20192 in chemotherapeutic combination therapies of oxaliplatin are needed.

## CRediT authorship contribution statement

**Sakshi Mahajan:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Software, Writing – original draft, Writing – review & editing. **Varun Sureja:** Conceptualization, Resources, Writing – review & editing. **Dharmeshkumar Kheni:** Conceptualization, Resources, Writing – review & editing. **Vishal Dubey:** Conceptualization, Resources, Writing – review & editing. **Kiran Bhupathiraju:** Resources, Writing – review & editing. **Venkata KrishnaRaju Alluri:** Resources, Writing – review & editing. **Anuradha Majumdar:** Conceptualization, Data curation, Validation, Visualization, Supervision, Writing – review & editing.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr Anuradha Majumdar reports that the investigational product, LN20192 used in the study was provided by Sundyota Numandis Probioceuticals Pvt Ltd. Laila Nutraceuticals has patented the proprietary product LN20192. All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

We would like to thank Sundyota Numandis Probioceuticals Pvt. Ltd., (Ahmedabad, Gujarat, India) and Laila Nutraceuticals (Vijayawada, Andhra Pradesh, India) for providing us with the investigational product, LN20192. We would also like to thank Bruck Pharma Private Limited (Daman & Diu, India) for providing us with the gift sample of Oxaliplatin API for the research work.

## Data availability

Data will be made available on request.

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