

Partial Sciatic Nerve Ligation – Induced Peripheral Neuropathic Pain in Rats: Neuroprotective Effects of Alpha-Lipoic Acid and Ferulic Acid

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Abstract—Background and Objective: Oxidative stress and neuroinflammation contribute directly to the development of peripheral neuropathic pain. This preclinical study evaluated the individual and combined neuroprotective and antioxidant effects of ferulic acid (FA) and α -lipoic acid (ALA) in rats subjected to partial sciatic nerve ligation (PSNL).

Methods: Thirty adult Wistar rats were assigned to five groups (n = 6 per group). Peripheral neuropathy was induced by partially ligating the sciatic nerve under anesthesia. Rats then received daily administrations of α -lipoic acid (25 mg/kg/day), ferulic acid (10 mg/kg/day), a combined low-dose regimen (12 mg/kg/day ALA plus 5 mg/kg/day FA), or gabapentin (30 mg/kg/day, i.p.) as the standard control.

Behavioral nociceptive responses including mechanical allodynia, mechanical hyperalgesia, cold allodynia, and thermal allodynia were recorded. Biochemical assays for GSH, SOD, CAT, MDA, and TNF- α , as well as histopathological evaluations, were performed on isolated sciatic nerve tissues.

Results: PSNL markedly lowered pain thresholds and disrupted biochemical and histological parameters. Treatment with ALA or FA alone significantly improved behavioral sensitivity, restored antioxidant enzyme activity (GSH, SOD, CAT), reduced lipid peroxidation (MDA) and TNF- α levels, and preserved nerve architecture. Notably, the lower-dose combination of ALA and FA outperformed the individual treatments and demonstrated efficacy comparable to gabapentin.

Conclusion: Co-administration of α -lipoic acid and ferulic acid provides enhanced neuroprotection in PSNL-induced neuropathic pain through combined free-radical scavenging, membrane-stabilizing, and anti-inflammatory actions.

Index Terms—Peripheral neuropathy, antioxidants, gabapentin, ferulic acid, α -lipoic acid, PSNL model.

I. INTRODUCTION

Neuropathic pain is a chronic debilitating condition caused by direct trauma or disease affecting either the peripheral or central somatosensory nervous system. Whereas nociceptive pain serves as a physiological warning triggered by non-neural tissue damage and carried along intact sensory pathways, neuropathic pain stems from damaged or dysfunctional nerve fibers themselves. Patients typically experience spontaneous or evoked sensory disturbances described as burning, stabbing, shooting, or electric shock-like sensations [1]. The International Association for the Study of Pain (IASP) categorizes neuropathic pain according to its underlying etiology, anatomical distribution, and pathophysiological mechanisms [2]. Peripheral neuropathic pain (PNP) arises from a wide spectrum of clinical insults. Mechanical trauma whether from nerve compression, traction, or partial transection directly compromises axonal integrity and initiates long-lasting pain signaling [3]. Metabolic disorders represent another major source of nerve damage; diabetes mellitus alone accounts for a large share of clinical cases, with roughly half of diabetic patients developing peripheral neuropathy over their lifetime [4]. Viral infections such as herpes zoster can similarly injure dorsal root ganglia and sensory fibers, leading to persistent post-herpetic neuralgia. Additionally, several classes of cancer chemotherapeutics most notably platinum derivatives (cisplatin and oxaliplatin), taxanes, and vinca alkaloids cause dose-limiting peripheral neuropathy driven by mitochondrial toxicity and progressive axonal degeneration [5]. Despite these distinct clinical

triggers, they converge on common downstream changes: ectopic neuronal firing, neuroinflammation, and maladaptive synaptic plasticity that sustain chronic pain.

Reliable animal models are essential for studying these pathological changes and screening candidate therapies. The partial sciatic nerve ligation (PSNL) model, originally introduced by Seltzer et al., is widely used to reproduce peripheral nerve injury in rodents [6]. Tightly ligating a fraction of the sciatic nerve trunk produces a focal lesion followed by Wallerian degeneration, myelin breakdown, macrophage and leukocyte infiltration, and local release of pro-inflammatory cytokines. Because PSNL consistently induces behavioral hypersensitivity that mirrors human neuropathy including thermal hyperalgesia (heightened sensitivity to noxious heat) and mechanical allodynia (pain evoked by normally innocuous touch) it provides a practical platform for preclinical drug evaluation [6,7].

Growing evidence points to oxidative stress as a primary driver of nerve injury and pain sensitization. Oxidative stress develops when the generation of reactive oxygen species (ROS) outpaces the neutralizing capacity of endogenous antioxidant defenses within neural tissue [8]. Excess superoxide anions, hydrogen peroxide, and hydroxyl radicals oxidize membrane lipids, structural proteins, and nucleic acids, which impairs mitochondrial bioenergetics and triggers neuronal apoptosis [9]. Beyond direct structural damage, elevated ROS levels amplify nociceptive transmission by activating redox-sensitive transcription factors such as nuclear factor-kappa B (NF- κ B), which in turn upregulates pro-inflammatory mediators like tumor necrosis factor-alpha (TNF- α) [10]. Directly targeting this oxidative-inflammatory cycle has therefore emerged as a promising approach to neuropathic pain relief.

Exogenous antioxidants can interrupt this cascade by scavenging free radicals, restoring cellular redox balance, and limiting secondary inflammatory injury, which helps preserve nerve conduction and reduce pain-related behaviors [11]. Alpha-lipoic acid (ALA) is a naturally occurring organosulfur compound synthesized in small amounts within mammalian mitochondria, where it acts as a cofactor for enzymatic complexes involved in energy metabolism [12]. Because its chemical structure is both water- and fat-soluble, ALA distributes readily across cytosolic and

lipid membrane compartments. It protects peripheral nerves by directly quenching free radicals, recycling endogenous antioxidants including reduced glutathione (GSH), vitamin C, and vitamin E and supporting mitochondrial ATP production [13]. In experimental neuropathy models, ALA also suppresses NF- κ B and mitogen-activated protein kinase (MAPK) signaling, blunting cytokine release and structural nerve fiber loss [14]. These preclinical findings align with clinical trials showing that ALA reduces pain and sensory deficits in patients with diabetic polyneuropathy [15].

Ferulic acid (FA), a hydroxycinnamic acid abundant in oats, rice bran, coffee, and other plant tissues, likewise possesses marked free-radical scavenging and anti-inflammatory activity [16]. FA neutralizes both reactive oxygen and nitrogen species, arrests membrane lipid peroxidation, and restores the activity of intrinsic antioxidant defenses such as superoxide dismutase (SOD), catalase (CAT), and GSH [17]. In injured peripheral nerves, FA also dampens neuroinflammation by blocking NF- κ B nuclear translocation and lowering tissue TNF- α concentrations [18].

II. MATERIALS AND METHODS

Drugs, Chemicals, and Experimental Animals

Gabapentin was generously provided as a gift sample by the Maharaja Sayajirao University of Baroda (Vadodara, India). Alpha-lipoic acid (ALA) was purchased from Sigma-Aldrich (St. Louis, MO, USA), and ferulic acid (FA) was procured from Otto Chemie Pvt. Ltd. (Mumbai, India). All remaining chemicals and analytical reagents were of analytical grade and sourced from certified commercial suppliers [19].

Adult male Wistar rats weighing 200–250 g was obtained from the Disease-Free Animal House at Lakshmi Biofarms (Pune, India). Animals were housed under controlled environmental conditions maintained at approximately 22°C on a 12-hour light/dark cycle, with free access to potable water and a standard pellet diet supplied by VRK Nutritional Solutions (Sangli, India). All experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee of Scitesla Private Limited (Approval No. SCI/IAEC/2024-25/142) and conducted in accordance with institutional and national guidelines for the care and use of laboratory

animals. For dosing, ALA, FA, and gabapentin were individually dissolved in normal saline (0.9% NaCl) immediately prior to administration [19,20].

Induction of Peripheral Neuropathy via Partial Sciatic Nerve Ligation (PSNL)

Peripheral mononeuropathy was induced using the partial sciatic nerve ligation (PSNL) procedure originally described by Seltzer et al. [6]. Rats were anesthetized via intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). Once surgical anaesthesia was confirmed, the lateral aspect of the left thigh was shaved and sterilized under aseptic conditions. A small skin incision was made, and the underlying biceps femoris muscle was bluntly separated to expose the common sciatic nerve at the mid-thigh level.

Approximately one-third to one-half of the dorsal portion of the sciatic nerve thickness was tightly ligated using a 9-0 silk suture, taking care to avoid complete nerve transection. This partial ligation creates a focal constrictive injury that reproduces key pathological features of clinical peripheral neuropathic pain. The muscle fascia and skin incisions were then closed in layers with sutures, and the rats were returned to their home cages with unrestricted access to food and water during recovery. Successful induction of neuropathic pain was verified

postoperatively by testing for mechanical allodynia and thermal hyperalgesia [7,21].

Experimental Design and Treatment Protocol

Following postoperative confirmation of peripheral neuropathic pain, thirty rats were randomly assigned to five experimental groups ($n = 6$ per group) and treated once daily for 14 consecutive days:

- Group 1 (Disease Control): PSNL-operated rats receiving oral normal saline daily for two weeks to track untreated neuropathy progression.
- Group 2 (Standard Control): PSNL-operated rats treated with gabapentin (30 mg/kg/day, i.p.) for two weeks.
- Group 3 (ALA Monotherapy): PSNL-operated rats administered α -lipoic acid (25 mg/kg/day, p.o.) for two weeks.
- Group 4 (FA Monotherapy): PSNL-operated rats administered ferulic acid (10 mg/kg/day, p.o.) for two weeks.
- Group 5 (Combined Therapy): PSNL-operated rats co-administered ferulic acid (5 mg/kg/day, p.o.) and α -lipoic acid (12 mg/kg/day, p.o.) for two weeks.

Behavioral nociceptive assessments including mechanical allodynia, cold allodynia, mechanical hyperalgesia, and thermal sensitivity were recorded at scheduled intervals throughout the study period to monitor pain progression and therapeutic response.

Table no. 1. Animal Groups after confirmation of PNP.

Grouping of Animals	Animals per group	Treatment of Drugs	Drug route	Drug dosing (mg/kg)	Drug treatment duration
Group 1: Disease Control	6	Normal Saline	P.O.	-	14 Days
Group 2: Gabapentin Standard	6	Gabapentin	I.P.	30	14 Days
Group 3: ALA with CCI	6	ALA	P.O.	25	14 Days
Group 4: FA with CCI	6	FA	P.O.	10	14 Days
Group 5: FA, ALA with CCI	6	FA + ALA	P.O.	5 + 12	14 Days

III. BEHAVIORAL ASSESSMENT OF NEUROPATHIC PAIN

Mechanical Allodynia (von Frey Filament Test)

Tactile sensitivity to non-noxious mechanical stimuli was measured using calibrated von Frey nylon monofilaments according to the up-down paradigm of Chaplan et al. [22]. Rats were placed individually in clear acrylic chambers positioned over an elevated

wire-mesh floor and allowed to acclimate for 15–20 minutes. Filaments of graded bending forces were applied perpendicularly to the mid-plantar surface of the ipsilateral (operated) hind paw until the filament bent slightly, and held in place for 1–2 seconds. Brisk paw withdrawal, flinching, or immediate licking of the stimulated paw was scored as a positive nociceptive response. The 50% paw withdrawal threshold was then calculated using Dixon's up-down formula.

Cold Allodynia (Acetone Drop Test)

Cold allodynia was evaluated by the acetone evaporation method described by Choi et al. [23]. After a 15–20-minute habituation period inside individual mesh-floored acrylic enclosures, 50 μ L of acetone was gently delivered onto the mid-plantar skin of the injured hind paw using a blunt-tipped syringe without touching the skin surface.

Each animal underwent three trials separated by 5-minute intervals. Nociceptive behaviors including brisk paw withdrawal, shaking, or prolonged licking were timed and counted over a 20-second observation window following application, with increased response frequency or duration indicating cold allodynia.

Mechanical Hyperalgesia (Pinprick Test)

Mechanical hyperalgesia was quantified via the pinprick test as outlined by Tal and Bennett [24]. Following at least 20 minutes of acclimation on an elevated wire-mesh platform, the plantar surface of the injured hind paw was briefly touched at a perpendicular angle with the point of a sterile 22-gauge needle, applying enough pressure to indent but not pierce the skin. Five trials were conducted per rat at intervals of at least 5 minutes. Rapid paw withdrawal (within 1 second), vocalization, or paw licking was recorded as a positive hyperalgesic response, whereas a 20-second cutoff was maintained to prevent tissue trauma. Results were recorded as the number of positive withdrawal responses across the five trials.

Thermal Sensitivity (Eddy's Hot Plate Test)

Thermal nociceptive thresholds were measured using Eddy's hot plate apparatus according to the method of Eddy and Leimbach [25]. Individual rats were placed onto a thermostatically controlled metal plate maintained at $52 \pm 0.5^\circ\text{C}$.

Using a stopwatch, the latency to the first nociceptive sign hind paw licking, brisk withdrawal, or jumping was recorded. Responses occurring in under 1 second were excluded to rule out non-specific locomotor movements, and a strict 20-second cutoff was enforced to avoid thermal injury to the plantar tissue. Each rat was tested three times at 10-minute intervals to obtain a mean withdrawal latency, where shorter latencies reflected heightened thermal sensitivity.

IV. BIOCHEMICAL AND HISTOPATHOLOGICAL ANALYSES**Sciatic Nerve Homogenization**

Upon completion of behavioral testing, rats were euthanized and the ipsilateral sciatic nerves were rapidly dissected and rinsed in ice-cold Tris-HCl buffer (pH 7.4). Nerve segments were finely minced with a sterile scalpel blade, immersed in chilled 0.25 M sucrose, and homogenized in 10 mM Tris-HCl buffer (pH 7.4) to yield a 10% (w/v) tissue homogenate. Homogenates were kept on ice and centrifuged at 10,000 rpm for 15 minutes at 0°C to preserve enzymatic activity. The resulting clear supernatants were aliquoted for spectrophotometric and immunoassay determinations of oxidative stress markers and inflammatory cytokines [26,27]. Slight turbidity noted in a small number of supernatant fractions did not interfere with optical density readings.

Estimation of Reduced Glutathione (GSH)

Sciatic nerve reduced glutathione (GSH) content was quantified spectrophotometrically following the procedure of Moron et al. [28], which relies on the reduction of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) by sulfhydryl groups to form a yellow 2-nitro-5-mercaptobenzoic acid chromophore absorbing at 412 nm.

Briefly, an aliquot of tissue supernatant was mixed 1:1 with 20% trichloroacetic acid (TCA) to precipitate proteins and centrifuged. Next, 0.25 mL of the deproteinized supernatant was incubated with 2.0 mL of DTNB reagent, and absorbance was read at 412 nm on a UV-visible spectrophotometer. Tissue GSH levels were expressed as $\mu\text{g GSH/mg protein}$.

Estimation of Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) enzymatic activity was assayed by the epinephrine auto-oxidation inhibition method of Misra and Fridovich [29]. Tissue homogenate was diluted with distilled water and extracted with ice-cold ethanol and chloroform, followed by centrifugation. An aliquot (0.5 mL) of the upper aqueous phase was combined with carbonate buffer and EDTA, and the reaction was initiated by adding epinephrine. The rate of adrenochrome formation was monitored spectrophotometrically at 480 nm, and enzyme activity was expressed as units/mg protein.

Estimation of Catalase (CAT) Activity

Catalase (CAT) activity was determined by tracking the rate of hydrogen peroxide (H_2O_2) decomposition according to Aebi [30]. The reaction mixture consisted of tissue supernatant diluted in 50 mmol/L phosphate buffer (pH 7.0). Adding H_2O_2 initiated the reaction, and the decline in absorbance was followed at 240 nm over time. Enzyme activity was calculated from the H_2O_2 extinction coefficient and expressed as $\mu\text{mol } H_2O_2$ decomposed/min/mg protein [31].

Estimation of Lipid Peroxidation (Malondialdehyde, MDA)

Membrane lipid peroxidation was evaluated by measuring malondialdehyde (MDA) via the thiobarbituric acid reactive substances (TBARS) assay of Slater and Sawyer [27]. Equal volumes of nerve supernatant and chilled 10% TCA were mixed on ice and centrifuged to remove precipitated proteins. The clear supernatant was then combined with thiobarbituric acid (TBA) reagent and heated in a boiling water bath for 10 minutes to generate the pink MDA-TBA adduct. After cooling to room temperature, absorbance was recorded at 532 nm, and MDA concentrations were determined against a standard calibration curve and expressed as nmol/mg protein.

Quantification of Tumor Necrosis Factor-Alpha (TNF- α)

Sciatic nerve levels of the pro-inflammatory cytokine TNF- α were measured using a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions and the protocol of Muthuraman et al. [32]. Serial dilutions of recombinant rat TNF- α (0–20,000 pg/mL) were run in parallel to construct a calibration curve. Optical density was read at 450 nm on a microplate reader, and tissue cytokine concentrations were normalized to total protein content (pg TNF- α /mg protein).

Histopathological Examination of Sciatic Nerve Tissue

At study termination, rats were deeply anesthetized and euthanized, and segments of the sciatic and spinal nerves were excised and immediately fixed in 10% neutral buffered formalin to prevent autolysis [33]. Fixed tissues were dehydrated through graded alcohols, cleared in xylene, and embedded in paraffin wax.

Longitudinal and transverse sections (approximately 5 μm thick) were cut on a rotary microtome, mounted on glass slides, and stained with hematoxylin and eosin (H&E) [34]. Stained sections were examined under a light microscope to evaluate axonal degeneration, myelin sheath disruption, endoneurial edema, and leukocytic infiltration [33,34].

Statistical Analysis

All quantitative data are expressed as mean $\pm$ standard deviation (SD) for $n = 6$ animals per group. Group comparisons across time points were analyzed by two-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test for multiple comparisons. Differences were considered statistically significant at $p < 0.05$.

V. RESULTS

Effect on von Frey Filament-Induced Mechanical Allodynia

Partial sciatic nerve ligation (PSNL) induced pronounced mechanical allodynia in the untreated disease control group, reflected by a sharp drop in paw withdrawal latency (PWL) on Days 15 and 29 relative to baseline values on Day 0 (### $p < 0.001$). Daily administration of gabapentin, α -lipoic acid (ALA), or ferulic acid (FA) individually counteracted this tactile hypersensitivity, significantly raising PWL by Day 29 compared with the disease control group ($***p < 0.001$). In contrast, the low-dose combination of ALA and FA produced only a modest, statistically non-significant increase in withdrawal latency in this test (Figure 1).

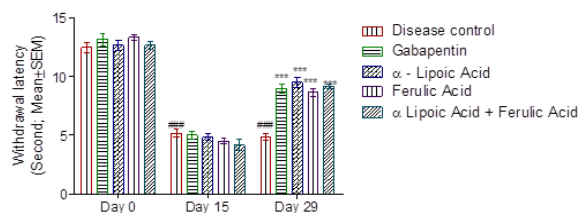


Figure 1: Effect of α -lipoic acid (ALA), ferulic acid (FA), and gabapentin on mechanical allodynia assessed by the von Frey filament test on Days 0, 15, and 29. Data are expressed as withdrawal latency time (WLT, mean $\pm$ SD, $n = 6$) and analyzed by two-way ANOVA followed by Bonferroni's post hoc test. ### $p < 0.001$ compared with Day 0 baseline; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared with the disease control group.

Effect on Acetone-Induced Cold Allodynia

Rats in the untreated disease control group developed marked cold allodynia following nerve injury, demonstrated by a significant reduction in withdrawal latency from Day 0 to Days 15 and 29 ($***p < 0.001$). Standard treatment with gabapentin returned withdrawal latencies nearly to baseline levels ($***p < 0.001$). Monotherapy with either ALA or FA also significantly prolonged withdrawal latency relative to the disease control group, although to a lesser extent than gabapentin. Co-administration of ALA and FA yielded a greater recovery in cold withdrawal latency than either antioxidant given alone, indicating a complementary protective effect against cold hypersensitivity (Figure 2).

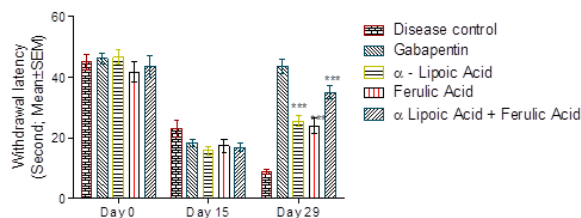


Figure 2: Effect of α -lipoic acid (ALA), ferulic acid (FA), and gabapentin on cold allodynia evaluated using the acetone drop test on Days 0, 15, and 29. Data are presented as withdrawal latency time (WLT, mean $\pm$ SD, $n = 6$). $***p < 0.001$ compared with Day 0 (confirming cold allodynia induction in the Group 1 disease control); $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ compared with the disease control group.

Effect on Pinprick-Induced Mechanical Hyperalgesia
Mechanical hyperalgesia was clearly established in the disease control group, as shown by a steep decline in withdrawal latency on Days 15 and 29 compared with Day 0 ($***p < 0.001$). Gabapentin treatment markedly reversed this hypersensitivity, restoring withdrawal latencies close to pre-surgical baseline values ($***p < 0.001$).

Among the individual antioxidant treatments, FA significantly elevated withdrawal latency ($p < 0.01$), whereas ALA alone produced a mild, non-significant improvement. Combining ALA and FA, however, restored withdrawal latency to near-normal levels ($***p < 0.001$), outperforming either single-agent regimen in attenuating mechanical hyperalgesia (Figure 3).

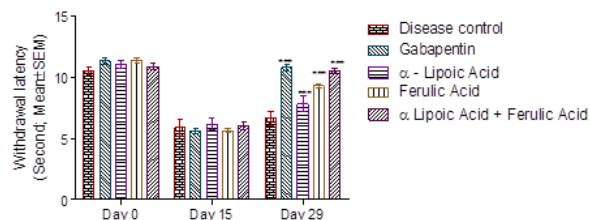


Figure 3: Effect of α -lipoic acid (ALA), ferulic acid (FA), and gabapentin on mechanical hyperalgesia measured by the pinprick test on Days 0, 15, and 29 in PSNL-injured rats. Values represent withdrawal latency time (WLT, mean $\pm$ SD, $n = 6$), analyzed using two-way ANOVA with Bonferroni's post hoc test. $***p < 0.001$ compared with Day 0; $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ compared with the disease control group.

Effect on Thermal Sensitivity (Eddy's Hot Plate Test)
PSNL surgery significantly lowered thermal withdrawal latency in the disease control group on Days 15 and 29 relative to Day 0 ($***p < 0.001$), confirming heightened sensitivity to noxious heat. Both gabapentin ($***p < 0.001$) and FA monotherapy ($***p < 0.001$) strongly prolonged thermal response latencies, while ALA monotherapy offered a smaller but statistically significant improvement ($*p < 0.05$). Notably, rats receiving the combined ALA and FA regimen displayed the most pronounced increase in thermal withdrawal latency ($***p < 0.001$), demonstrating enhanced relief of thermal allodynia (Figure 4).

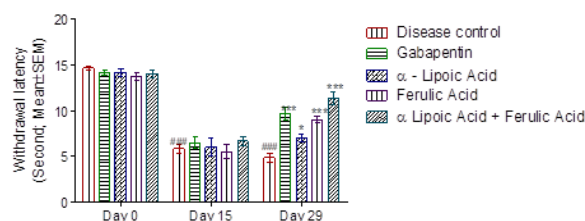


Figure 4: Thermal withdrawal latencies recorded using Eddy's hot plate test on Days 0, 15, and 29. Data are presented as mean $\pm$ SD ($n = 6$). $***p < 0.001$ compared with Day 0; $*p < 0.05$ and $***p < 0.001$ compared with the disease control group.

Effect on Sciatic Nerve Oxidative Stress and Endogenous Antioxidant Defense

Sciatic nerve homogenates from the untreated disease control group exhibited depleted endogenous antioxidant defenses specifically reduced glutathione

(GSH), superoxide dismutase (SOD), and catalase (CAT) accompanied by elevated malondialdehyde (MDA) concentrations, reflecting severe lipid peroxidation and oxidative nerve injury. Gabapentin administration produced only slight, statistically non-significant shifts in these redox markers.

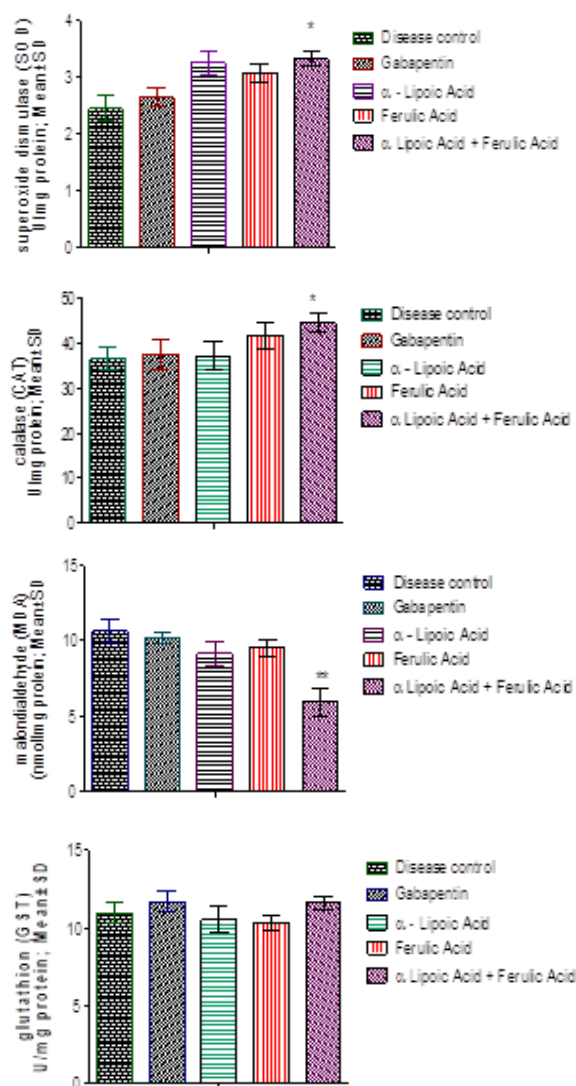


Figure 5: Effect of α -lipoic acid (ALA), ferulic acid (FA), and gabapentin on sciatic nerve oxidative stress markers superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and reduced glutathione (GSH) in PSNL-injured Wistar rats. Data are expressed as mean $\pm$ SD ($n = 6$). * $p < 0.05$ and ** $p < 0.01$ compared with the disease control group.

In contrast, individual treatment with ALA or FA strengthened intrinsic antioxidant capacity by elevating SOD activity (as well as CAT activity in the

FA group) and lowering tissue MDA levels. Co-administration of ALA and FA produced the strongest recovery across all oxidative parameters, markedly restoring SOD, CAT, and GSH levels while suppressing MDA accumulation (Figure 5).

Effect on Sciatic Nerve Pro-Inflammatory Cytokine (TNF- α) Levels

Peripheral nerve injury triggered a sharp rise in sciatic nerve tumor necrosis factor- α (TNF- α) concentrations in the disease control group, pointing to sustained local neuroinflammation. Gabapentin treatment significantly blunted this cytokine surge (** $p < 0.001$). Both ALA and FA monotherapies also significantly lowered neural TNF- α levels compared with untreated controls, with FA achieving reductions comparable to gabapentin. Furthermore, the combined ALA and FA group exhibited the greatest suppression of tissue TNF- α levels (** $p < 0.001$), pointing to an enhanced anti-inflammatory effect when both compounds were given together (Figure 6).

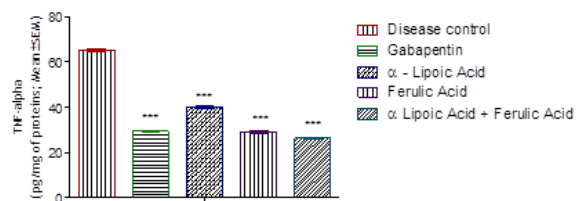


Figure 6: Effect of α -lipoic acid (ALA), ferulic acid (FA), and gabapentin on sciatic nerve TNF- α concentrations in PSNL-injured Wistar rats. Data are expressed as mean $\pm$ SD ($n = 6$). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared with the Group 1 disease control.

Histopathological Evaluation of the Sciatic Nerve

Light microscopic examination of H&E-stained sciatic nerve sections from the disease control group revealed widespread structural disruption, characterized by axonal swelling and degeneration, myelin sheath fragmentation, and dense infiltration of inflammatory cells. By contrast, sciatic nerve sections from gabapentin-treated rats showed well-preserved neural architecture with intact myelin sheaths and uniformly arranged nerve fibers.

Individual administration of either ALA or FA partially protected the injured nerve, lessening axonal degeneration and preserving myelin organization relative to untreated PSNL controls. Rats treated with

the combination of ALA and FA displayed the highest degree of histological preservation among the antioxidant groups, featuring compact myelin sheaths, organized axon bundles, and minimal leukocytic infiltration comparable to the gabapentin standard group.

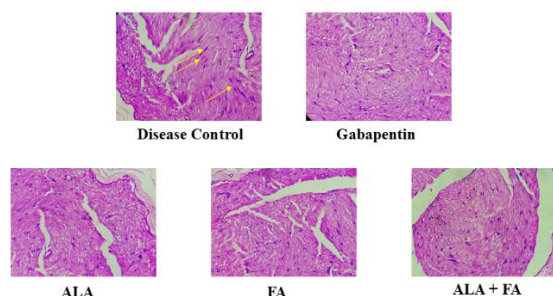


Figure 5: Histopathology seen the sciatic nerve in different groups after completion of studies induced by PSNL Model.

VI. DISCUSSION

Peripheral neuropathic pain (PNP) is a persistent clinical disorder marked by abnormal sensory processing, including mechanical allodynia, cold and thermal allodynia, and mechanical hyperalgesia. These sensory disturbances develop from maladaptive changes across both peripheral nerves and central pain pathways, driven by nociceptor sensitization, glial cell activation, oxidative stress, and sustained neuroinflammation [1,3]. The partial sciatic nerve ligation (PSNL) model reliably reproduces the behavioral and cellular hallmarks of human traumatic neuropathy and serves as a well-validated platform for screening candidate analgesics [6,7]. In the present study, sequential behavioral testing confirmed the induction of chronic neuropathic pain following PSNL and tracked the time-dependent recovery achieved by each treatment regimen [22].

This study evaluated the neuroprotective and antinociceptive potential of α -lipoic acid (ALA) and ferulic acid (FA), administered either individually or as a low-dose combination, alongside the standard reference drug gabapentin [35]. As an endogenous mitochondrial cofactor, ALA directly scavenges reactive oxygen species (ROS), recycles intracellular antioxidants such as reduced glutathione (GSH), and supports mitochondrial bioenergetics [12,36]. Ferulic acid, a plant-derived hydroxycinnamic acid, protects neural membranes from lipid peroxidation and

dampens inflammatory signaling cascades, including mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF- κ B) pathways [14,37]. Administering both agents together pairs ALA's intracellular redox-restoring activity with FA's membrane-stabilizing and cytokine-suppressing effects.

Across the 29-day observation period, untreated PSNL control rats displayed progressive behavioral hypersensitivity. Daily treatment with gabapentin markedly reversed these nociceptive deficits, consistent with its established clinical and preclinical efficacy [39]. In the von Frey filament assay, PSNL surgery caused a steep drop in paw withdrawal thresholds indicative of tactile allodynia. While gabapentin strongly restored withdrawal thresholds and single-agent ALA or FA produced moderate relief, co-administration of ALA and FA yielded a pronounced recovery, pointing to complementary modulation of mechanosensitive pain pathways [40].

A similar pattern emerged in the acetone evaporation test for cold allodynia, where untreated PSNL rats exhibited heightened nociceptive responses to cooling stimuli. Whereas ALA or FA monotherapy offered partial protection, combining the two antioxidants returned withdrawal latencies closer to baseline values, likely by stabilizing cold-sensitive transient receptor potential channels and peripheral sensory axons [41]. Likewise, in the pinprick assay for mechanical hyperalgesia and Eddy's hot plate test for thermal sensitivity, gabapentin restored response latencies toward normal levels, individual antioxidants provided partial to substantial relief, and the combined ALA plus FA regimen consistently produced strong antinociceptive recovery.

Biochemical assays of excised sciatic nerves explained the cellular basis for these behavioral improvements. Nerve homogenates from untreated PSNL rats showed severe redox imbalance, characterized by depleted SOD, CAT, and GSH activities alongside elevated malondialdehyde (MDA) concentrations [8]. While monotherapy with ALA or FA partially restored endogenous antioxidant enzymes and curbed lipid peroxidation, the combined regimen achieved the most complete normalization of tissue redox status. This enhanced biochemical recovery reflects the pairing of ALA's mitochondrial free-radical scavenging with FA's inhibition of membrane lipid peroxidation and downstream inflammatory signaling [46,47].

Histopathological examination of H&E-stained sciatic nerve sections corroborated the behavioral and biochemical findings. Untreated PSNL nerves displayed classic features of Wallerian-like degeneration, including axonal swelling, myelin breakdown, and dense leukocytic infiltration [8]. Gabapentin-treated rats showed well-preserved nerve morphology, whereas individual ALA and FA regimens provided partial structural protection. Notably, rats receiving combined ALA and FA therapy retained near-normal sciatic nerve architecture, with intact myelin sheaths, organized axon fibers, and minimal inflammatory cell infiltration [45].

Taken together, the superior tissue protection observed with combined ALA and FA therapy stems from their dual action against oxidative damage and neuroinflammation. Gabapentin relieves neuropathic pain primarily by binding to the $\alpha_2\delta$ subunit of voltage-gated calcium channels to curb excitatory neurotransmitter release, but it does not directly neutralize free radicals or repair oxidative tissue damage [38]. By simultaneously restoring endogenous antioxidant defenses, lowering TNF- α levels, and preserving myelin integrity, the combination of α -lipoic acid and ferulic acid addresses the underlying pathophysiological drivers of peripheral nerve injury and represents a promising adjunct or alternative approach to neuropathic pain management.

VII. CONCLUSION

Peripheral neuropathic pain (PNP) remains difficult to manage clinically because its pathogenesis involves interconnected processes of oxidative stress, neuroinflammation, and peripheral and central sensitization. Conventional pharmacotherapies such as gabapentin provide effective symptomatic pain relief but rarely halt underlying oxidative nerve damage and can carry dose-limiting side effects, creating a clear need for disease-modifying or adjunct treatments.

In this study, partial sciatic nerve ligation (PSNL) in Wistar rats reliably produced chronic mechanical allodynia, cold allodynia, mechanical hyperalgesia, and thermal hypersensitivity, accompanied by sciatic nerve lipid peroxidation, antioxidant enzyme depletion, elevated TNF- α levels, and axonal degeneration. While gabapentin effectively alleviated behavioral hypersensitivity, α -lipoic acid (ALA) and ferulic acid

(FA) improved both behavioral pain thresholds and the underlying biochemical and structural nerve pathology. Co-administering ALA (12 mg/kg) and FA (5 mg/kg) delivered greater overall benefit than either antioxidant given alone at higher doses. The combination regimen markedly restored sciatic nerve GSH, SOD, and CAT levels, suppressed MDA accumulation, and blunted local TNF- α production likely through coordinated inhibition of redox-sensitive NF- κ B and MAPK pathways. Histological evaluation confirmed that combined ALA and FA therapy preserved axonal organization and myelin sheath integrity to a degree comparable to gabapentin. Because α -lipoic acid and ferulic acid are naturally derived compounds with well-established safety profiles, their low-dose combination offers a practical candidate for managing peripheral neuropathic pain. Further preclinical and clinical studies examining detailed pharmacokinetics, dose-response relationships, and long-term safety will help guide the translation of this combined antioxidant regimen into clinical practice.

CONTRIBUTIONS BY AUTHORS:

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Institutional Animal Ethics Committee (IAEC) of Scitesla Private Limited, Navi Mumbai, India, 400710, approved animal-based experimental procedures, investigation was conducted in accordance with the authorised protocol number SCI/IAEC/2024-25/142.

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This published article has all the information that was gathered or looked at during this study.

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