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

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KEYWORDS

Peripheral neuropathy, α-lipoic acid, Ferulic acid, PSNL model, Gabapentin, Antioxidants, Neuropathic pain

ABSTRACT:
The goal of current preclinical study was to assess the effects of two natural antioxidants, ferulic acid and α-lipoic acid, both separately and in combination, on peripheral neuropathic pain in rats that was brought on by partial sciatic nerve ligation (PSNL). Histopathological, biochemical, and behavioral assessments were used to confirm neuropathic pain. The total of 5 groups of six adult Wistar rats were collected. Under anesthesia, a partial sciatic nerve ligation was performed to cause peripheral neuropathy. Rats were given either α-lipoic acid (25 mg/kg/day), ferulic acid (10 mg/kg/day), their combination (12 mg/kg and 5 mg/kg, respectively), or the common medication gabapentin (30 mg/kg/day, i.p.) orally for 14 days following the ligation. Cold allodynia,Mechanical allodynia, thermal allodynia andmechanical hyperalgesia were used to measure neuropathic pain. GSH, SOD, CAT, MDA, and TNF-α levels were among the biochemical estimates. Additionally, tissue from the sciatic nerve was examined histopathologically. Biochemical and behavioral characteristics were markedly changed by PSNL. These changed parameters were considerably improved by α-lipoic acid and ferulic acid treatment, with the combination group demonstrating greater efficacy than the individual treatments. The enhanced free radical scavenging, anti-inflammatory, and membrane-stabilizing capabilities of both antioxidants may be the cause of the observed effects.

Introduction

A complex and persistent pain disorder, neuropathic pain is directly caused by diseases or lesions that impact the somatosensory system in central or peripheral nervous systems. Neuropathy pain results from dysfunction in the nerves themselves, causing abnormal pain perception, including burning, shooting, or electric shock-like sensations [1]. This is in contrast to nociceptive pain, which is caused by the actual and or potential tissue damage and mediated by intact sensory pathways. Based on its aetiology, anatomical distribution, and underlying pathophysiological mechanisms, neuropathic pain is categorised by International Association for the Study of Pain (IASP) [2]. PNP has wide range of aetiologies, including number of common clinical causes. Persistent neuropathic pain can result from direct disruption of nerve integrity caused by traumatic nerve injuries like crush, stretch, or transection [3]. Nearly 50% of diabetic patients will develop diabetic peripheral neuropathy at some point in their lives, making metabolic diseases like diabetes mellitus one of the main causes of peripheral neuropathy globally [4]. Furthermore, post-herpetic neuralgia can arise from infections like herpes zoster (shingles) that harm sensory nerves. By causing axonal degeneration and mitochondrial dysfunction in peripheral nerves, chemotherapy agents—especially platinum-based medications (cisplatin, oxaliplatin),

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taxanes, and vinca alkaloids—have also been shown to cause dose-limiting peripheral neuropathy [5]. These etiological factors work together to cause inflammation, maladaptive plasticity, prolonged neuronal hyperexcitability, and impaired axonal transport, all of the contribute to persistence of the neuropathic pain symptoms.

Partial Sciatic Nerve Ligationmodel (PSNL), which firstly presented by the Seltzer et al. in 1990 [6], is one such validated model. In the model, silk or chromic gut sutures is used to create tight ligation around roughly one-third to the one-half of the sciatic nerve bundle. Localised mechanical damage brought on by this partial ligation causes Wallerian degeneration, axonal demyelination, inflammatory cell infiltration, and increase in the pro-inflammatory cytokines. The PSL model is very useful for researching the underlying mechanisms and pharmacological treatments for PNP because it accurately represents a number of characteristics of clinical neuropathic pain, likethermal hyperalgesia for the increased the sensitivity toheat,mechanical allodynia for the pain from the non-painful stimuli [6,7]. Oxidative stress, defined as imbalance between generation of the reactive oxygen species (ROS) and an antioxidant defence systems in neural tissues, is key mechanisms linked to the onset and progression of neuropathic pain [8]. The oxidative damage amplifies nociceptive signalling by activating redoxsensitive transcription factors like NF-κB and upregulating inflammatory mediators like TNF-α [10]. Excess ROS, such as hydroxyl radicals, hydrogen peroxide and superoxide anions, can damage proteins, lipids, and the DNA in peripheral nerves, worsen neuroinflammation, impair mitochondrial function, and promote neuronal apoptosis [9]. Targeting oxidative stress has therefore emerged a desirable therapeutic approach for treatment in PNP. Antioxidants are substances that can mitigate oxidative damage in neural tissues, neutralise free radicals, and restore redox balance. Antioxidants can mitigate pain behaviours, alter inflammatory pathways, and maintain nerve function in neuropathic pain models by lowering oxidative stress [11].

Human mitochondria produce trace amounts of ALA, a naturally occurring organosulfur compound. It serves as a cofactor for oxidative metabolism-related mitochondrial enzyme complexes [12]. ALA's strong anti-inflammatory and antioxidant qualities have drawn a lot of attention. It can act in several cellular compartments because it is soluble in both water and lipids. By directly scavenging ROS, restoring endogenous antioxidants such as glutathione (GSH), vitamin E, and vitamin C, and enhancing mitochondrial respiratory function, ALA demonstrates its neuroprotective effects [13]. Furthermore, inexperimental models of the neuropathic pain, ALA inhibits expression in pro-inflammatory cytokines and prevents nerve damage by modulating redox-sensitive signalling pathways like NF-κB and MAPK [14]. Clinical studies have demonstrated the translational potential of ALA by demonstrating that it can alleviate symptoms in diabetic neuropathy patients [15]. A phenolic compound called ferulic acid (FA) is widely present in plant-based foods like coffee, oats, and rice bran. It has potent anti-inflammatory, antioxidant, and free radical scavenging qualities [16]. Reactive nitrogen species (RNS) and ROS are neutralised by FA, which also inhibits lipid peroxidation and increases in activity of the endogenous antioxidant enzymes (SOD, CAT and GSH) [17]. Additionally, FA reduces activation ofNF-κB in peripheral nerve tissues and downregulates pro-inflammatory cytokines (TNF-α) to modulate inflammatory pathways [18].

MATERIAL AND METHODS

Drugs and Experimental Animals

MS University in Baroda, India, provided a gift sample of gabapentin. ALA was supplied by Sigma-Aldrich in the United States, and FA was generously supplied by Otto-Kemi in India. The remaining chemicals and reagents used in this investigation were acquired from reliable vendors and were of analytical quality.

For this experiment, male Wistar rats weighing between 190 and 250 gm each were selected. These animals had been brought from LacshmiBiofarms' Disease-Free Animal House in Pune, India. They received a healthy, balanced diet, a 12-hour light and dark cycle, and a temperature maintained at or close to 22°C (VRK, Nutritional Solution, Sangli, India). Every condition complied with the CPCSEA regulations established by the Indian government. The Institutional Animal Ethics Committee of Scitesla Private Limited, Navi Mumbai, 400710, India, granted prior approval for this study's conduct (Approval No. SCI/IAEC/2024-25/142). The care procedures adhered to CPCSEA

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guidelines set forth by the Ministry of Environment and Forests and Government of India. ALA, Gabapentin and FA were prepared using normal saline (NS). All the three compound was mixed separately in NS before they got administered [19,20].

Name of Animal group	No. of animals in group	Drug treatment	Route of drug	Dose of drug	Duration of drug treatment
Disease Control	6	Normal Saline Solution	P.O.	-	02 Weeks
Gabapentin	6	Gabapentin	I.P.	30 mg/kg	02 Weeks
ALA + CCI Treatment	6	ALA	P.O.	25 mg/kg	02 Weeks
FA + CCI Treatment	6	FA	P.O.	10 mg/kg	02 Weeks
FA + ALA + CCI Treatment	6	FA + ALA	P.O.	5 mg/kg + 12 mg/kg	02 Weeks

Table no. 1. Grouping of Experimental animals after confirmation of PNP.

Induction of Peripheral neuropathy by Partial Sciatic Nerve Ligation (PSL)model

Partial Sciatic Nerve Ligation (PSL) technique, first described by Seltzer [6], was used for the management of peripheral neuropathy. Xylazine and Ketamine (10 mg/kg and 80 mg/kg) were injected through intraperitoneally to anaesthetise adult male Wistar rats. A tiny incision was made on the left thigh's side, and sciatic nerve is carefully revealed by spreading muscle tissue after the surgical site had been cleaned and shaved. A 9-0 silk suture was used to ligate between one-third and half of the nerve, being careful not to cut it completely. This technique causes nerve damage that is similar to neuropathic pain in humans. The rats were given food and water to help them recover after the muscle and skin layers were sutured shut. Following surgery, behavioural indicators of pain, including allodynia and hyperalgesia, were noted to verify nerve damage [7,21].

Experimental protocol

Five experimental groups, each consisting of six rats, were randomly selected from among the animals. The Disease Control Group was used as the baseline group to track the natural progression of peripheral neuropathic pain in the absence of treatment. They were given normal saline orally for two weeks. The Gabapentin Group, which served as the standard drug-treated group, received gabapentin intraperitoneally once daily for two weeks at dose of 30 mg/kg. ALA was administered P.O. to the ALA + PSL Group at dose of 25 mg/kg day for two weeks in order to investigate any potential nerve-protective effects. Similarly, oral FA at dose of 10 mg/kg was administered for two weeks to the FA + PSL Group. Finally, to determine whether there is a combined therapeutic benefit, the FA + ALA + PSL Group received oral treatment with both FA (5 mg/kg) and ALA (12 mg/kg) once daily for the same amount of time. Using common pain testing methods, behavioural indicators such as hyperalgesia and allodynia were noted during the course of treatment.

Assessment of behavioral parameters

All behavioral tests were carried out between 08:30 a.m. to04:00 p.m., and effort was made for avoid causing stress to the rats during these procedures.

Mechanical allodynia using von Frey hair test

Von-Frey filaments testwere used to measure mechanical allodynia using up-down method outlined by Chaplan et al. [22]. Each of rat was put in the clear acrylic cage with the wire mesh floor, and it was given 15 to 20 minutes to get used to its surroundings. On plantar surface of hind paw ipsilateral to nerve injury, a series is calibrated nylon monofilaments (von Frey hairs) was applied perpendicularly. Each filament was held in place for one to two seconds after being applied with enough force to the cause the slight bend. Positive responses included licking, flinching, or a rapid paw withdrawal. The Dixon's up-down method, which offers a numerical assessment of mechanical sensitivity, was used to establish the 50% paw withdrawal threshold.

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Cold allodynia by acetone

Choi et al.'s [23] acetone drop test method was used to assess the cold allodynia. Prior to testing, rats were individually housed in clear acrylic enclosures with wire mesh floor and given 15 to 20 minutes to acclimate. Using a blunt-tipped syringe, a tiny droplet (50 µL) of acetone is carefully applied to hind paw's mid-plantar surface without coming into direct contact with the skin. Three applications were made at 5-minute intervals. During 20-second observation period, length of time and frequency of nociceptive responses—like licking, shaking, or paw withdrawal—were noted. A rise in these reactions suggested that cold allodynia was present.

Mechanical hyperalgesia by pinprick

The pinprick method was used to assess mechanical hyperalgesia in accordance with Tal and Bennett's protocol [24]. Prior to testing, each rat was given at least twenty minutes to acclimatise in its own transparent acrylic enclosure on an elevated mesh platform. Without piercing the skin, a sterile 22-gauge needle (or other comparable sharp instrument) was carefully applied perpendicularly to the hind paw's mid-plantar surface. Five applications of the stimulus were made, separated by at least five minutes. A rapid withdrawal, vocalisation, or paw licking was considered a positive response. Cutoff time of twenty sec.is employed for prevent the tissue damage and guarantee animal welfare, while a minimum response time of 1 second was deemed suggestive of hyperalgesia. Each animal's mechanical hyperalgesia was measured by counting number the positive responses.

Thermal allodynia using Eddy's hot plate method

Eddy's hot plate test, which was first presented by Eddy and Leimbach in 1953 and is a commonly used technique for assessing rodents' sensitivity to harmful heat stimuli, was used to measure thermal allodynia. A stopwatch was used to measure the latency to the first nociceptive response, such as jumping, hindpaw withdrawal, or paw licking, after rat was carefully placed on anhot plate apparatus kept at 52 ± 0.5°C. To prevent recording non-specific movements, a minimum response time of one second was deemed valid. Animals that did not react within the allotted 20 seconds were promptly taken off the plate, and a 20-second latency was noted in order to avoid tissue damage. The average latency was determined after the test was run three times at 10-minute intervals. Reduced withdrawal latency was considered a sign of elevated thermal sensitivity and thermal allodynia [25].

Oxidative stress assessment (endogenous antioxidant defense)

Tissue homogenization parameter

The sciatic nerve was carefully removed from each rat after the treatment period ended and placed in ice-cold Tris-HCl buffer, which was kept at a pH of 7.4. A surgical blade was then used to cut the nerve into thin slices, which were then promptly placed in a chilled of sucrose solution (0.25 M). After that, Tris-HCl buffer(10% w/v,pH 7.4, 10 mM) used forhomogenise this mixture. To ensure the sample didn't deteriorate, the homogenate then placed on ice and centrifuge for 15 min. at 0°C at 10,000 rpm. Following established procedures, top clear layer, or supernatant, was carefully collected and subsequently used to measure a variety of biochemical parameters, including cytokine levels and oxidative stress markers (26,27). Although the readings for the analysis were consistent, some samples were not entirely clear.

Estimation of reduced glutathione (GSH)

A spectrophotometric assay, as outlined by Moron et al. [28], was used to measure amount of GSH in the tissue homogenates. This technique is based on the reaction of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) with free sulfhydryl (-SH) group of the GSH, which results formation of an yellow chromophore that can be measured at 412 nm. The tissue supernatant is mixed with an equal volume the 20% trichloroacetic acid (TCA) to precipitate proteins in order to prepare the sample. The clear supernatant that resulted from centrifugation was gathered for examination. A UV-Visible spectrophotometer was use for measure of the absorbance in resultant colour complex at 412 nm after the supernatant (0.25 mL) and DTNB (2.0 mL) reagent were combined in order to estimate GSH. Microgrammes of GSH per milligramme of protein was the unit of measurement for the results.

Estimation of superoxide dismutase level (SOD)

SOD the essential antioxidant enzyme that is protects cells from oxidative stress means transforming dangerous superoxide radicals into less reactive forms such as oxygen and hydrogen peroxide. The Misra and Fridovich [29] method, which involves inhibiting epinephrine auto-oxidation, was used to measure the activity of SOD in tissue samples. First, distilled water and tissue homogenate were combined in equal amounts.

Chloroform (0.15 mL) and Icecold ethanol (0.25 mL) used to treat the mixture. After five minutes of thorough vortexing with a cyclo-mixer, this solution was centrifuged for ten minutes at 2500 rpm. 0.5 mL of the resultant supernatant was extracted and combined with carbonate buffer (1.5 mL) and EDTA(0.5 mL). Epinephrine (0.4 mL) was added to start the enzymatic reaction, and rate at which absorbance increased at 480 nm was noted. Units of SOD activity per milligramme of protein were computed and reported.

Estimation of catalase (CAT)

Based on the rate at which hydrogen peroxide ($H2O_2$) breaks down, the spectrophotometric method first reported by Aebi [30] was used to estimate the CAT activity in sciatic nerve tissue. One millilitre of tissue supernatant and one mL of the phosphate buffer with pH 7.0, 50 mmol/L were combined for assay. Two millilitres of diluted sample were mixed with one millilitre of 30 mmol/L H_2O_2 to start the reaction. The slow decline in absorbance at 240 nm over time, which indicates the catalytic activity of the enzyme, was used to track the enzymatic breakdown of $H2O_2$. According to accepted biochemical standards, catalase activity was determined by measuring the rate of $H2O_2$ decomposition, which is the amount of hydrogen peroxide broken down per minute per milligramme of protein [31].

Estimation of lipid peroxide malonaldehyde (MDA)

The thiobarbituric acid reactive substances (TBARS) method described by the Slater and Sawyer [27] was used to figure out how much MDA was in tissue samples. MDA is a sign of lipid peroxidation. To start the test, 2 mL of tissue supernatant is mixed and with equal amount of 10% (w/v) TCA to make proteins fall out of solution. Mixture puts in ice bath for 15 minutes, then it spun around in a centrifuge to get a clear supernatant. Two millilitres of TBA solution that had just been made then added the supernatant. Mixture was put inthe boiling water-bath for the 10 min. so that the pink MDA-TBA adduct could form. Then, it was quickly cooled on ice for 5 minutes. We used a spectrophotometer to measure absorbance of the coloured complex at 532 nm, using a reagent blank as a guide. We used a standard curve made with known amounts of MDA to figure out the MDA levels. The results were given in nanomoles per milligramme of protein.

Assessment of inflammatory markers

Measurement Tumor Necrosis Factor-α Level(TNF-α)

Using commercially available aELISA kit,Levelsin TNF-α on sciatic nerve tissue were measured in accordance with the protocol outlined by Muthuraman et al. [32]. To find out whether TNF-α is present in the sample, the assay uses a particular anti-TNF-α antibody. Using known concentrations of recombinant TNF-α ranging from the 0 to 20,000 pg/mL, the standard curve was created. Following the kit's instructions, tissue homogenates were processed, and microplate reader isused to measure colour development at 450 nm. Each sample's TNF-α concentration determined using standard curve and observed as picogrammes per milligramme of total protein.

Histopathology of the sciatic nerve

Both sciatic and spinal nerve tissues were meticulously removed for histopathological examination after the rats were anaesthetised and sacrificed at the conclusion of the treatment regimen. To maintain structural integrity and stop autolysis, the separated tissues were submerged right away in 10% neutral buffered formalin [33]. The fixed nerves were routinely processed, fixed in the paraffin wax, and a sectioned into the 4 mm thick slices for microscopic inspection. Sections were placed on glass slides after being cut with a microtome to a thickness of roughly 5 µm [34]. The cellular and axonal architecture was visualised using haematoxylin and eosin (H&E) staining [33]. To find neuropathological characteristics like axonal degeneration, demyelination, endoneurial oedema, and inflammatory cell infiltration, Stained sections were subsequently viewed under the light microscope [34].

Statistical assessment analysis

Results were represented and expressed as the mean ± SD (n=6). The data was observed by Two-Way ANOVA followed the Bonferroni Post Hoc analysis. * $P<0.5$ was considered as statistically significant.

RESULTS

Von-frey filaments-triggered mechanical allodynia: Effect of ALA and FA

Mechanical allodynia is assessed using von Frey filament test on Days 0, 15, and 29. In the disease control group, significant reduction in thepaw withdrawal latency (PWL) was observed on Day 15

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(5.17 ± 0.75 sec) and Day 29 (4.83 ± 0.75 sec) compared to Day 0 (12.50 ± 1.05 sec), indicating the successful induction of mechanical allodynia (###p < 0.001 vs. Day 0).

Treatment with gabapentin led to a significant increase in PWL on Day 29 (9.00 ± 0.89 sec), demonstrating effective attenuation of mechanical hypersensitivity (***p < 0.001 vs. disease control Day 15). Similarly, rats treated with α-lipoic acid (ALA) showed a significant improvement in latency (9.50 ± 1.05 sec), also indicating a protective effect against nerve injury-induced pain (***p < 0.001).

Ferulic acid (FA) treatment produced a comparable effect, significantly increasing PWL to 8.67 ± 0.82 sec by Day 29 (***p < 0.001). However, the combination of ALA and FA resulted in a modest increase (9.17 ± 1.04 sec) without reaching statistical significance when compared to the disease control on Day 15.

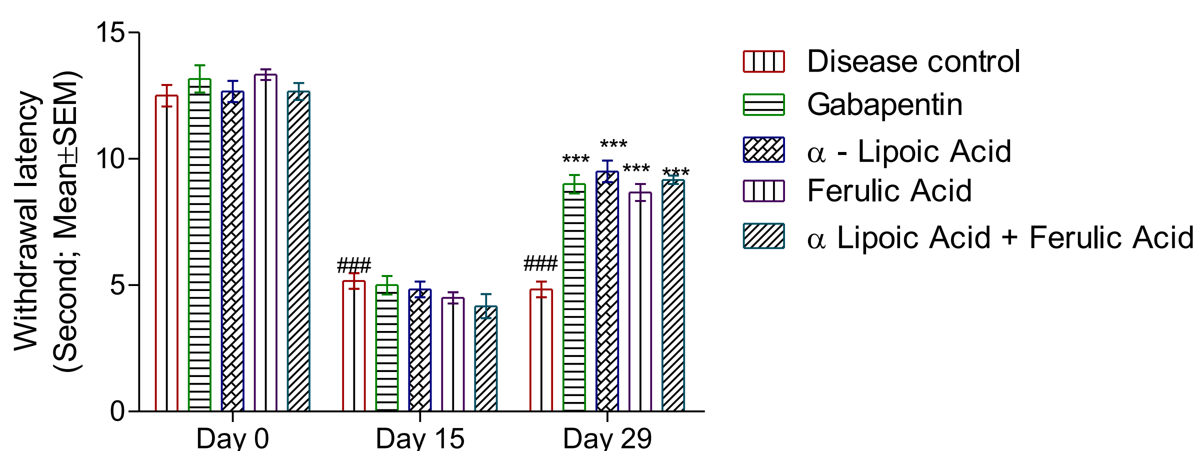

Figure 1: Withdrawal latency time (WLT) in response to anmechanical stimulation was assessed using von Frey filament test on Day 0, Day 15, and Day 29 to evaluate mechanical allodynia. Data are observed as the mean ± SD (n = 6) and theanalyzed using Two-Way ANOVA followed the Bonferroni post hoc test. *p < 0.05, **p < 0.01, ***p < 0.001 vs. disease control; ###p < 0.001 vs. Day 0.

Cold allodynia by acetone test: Effect of ALA and FA

The effect of different treatments on cold allodynia was carriedby using the acetone drop test measuring withdrawal latency time on days 0, 15, and 29. The disease control showed significant reduction onthe withdrawal latency time on day 15 (23.00 ± 6.32 sec) and a further marked decline on day 29 (8.67 ± 2.07 sec) compared to baseline (45.00 ± 6.26 sec, ###P<0.001), indicating increased sensitivity to cold stimuli due to sciatic nerve ligation. Gabapentin treatment (30 mg/kg, i.p.) significantly improved withdrawal latency on day 29 (43.50 ± 6.06 sec, ***P<0.001), almost restoring baseline values. Rats is treated with the ALA (25 mg/kg, p.o.) and FA (10 mg/kg, p.o.) also showeda improvement in withdrawal latency on day 29 (25.50 ± 4.51 sec and 23.83 ± 6.27 sec, respectively; ***P<0.001), though less effective than gabapentin. Combination of ALA (12 mg/kg) and FA (5 mg/kg) administered orally produced a superior response compared to either compound alone, with a withdrawal latency time of 35.00 ± 5.44 sec on day 29 (***P<0.001 vs disease control), suggesting a possible synergistic effect in mitigating cold allodynia.

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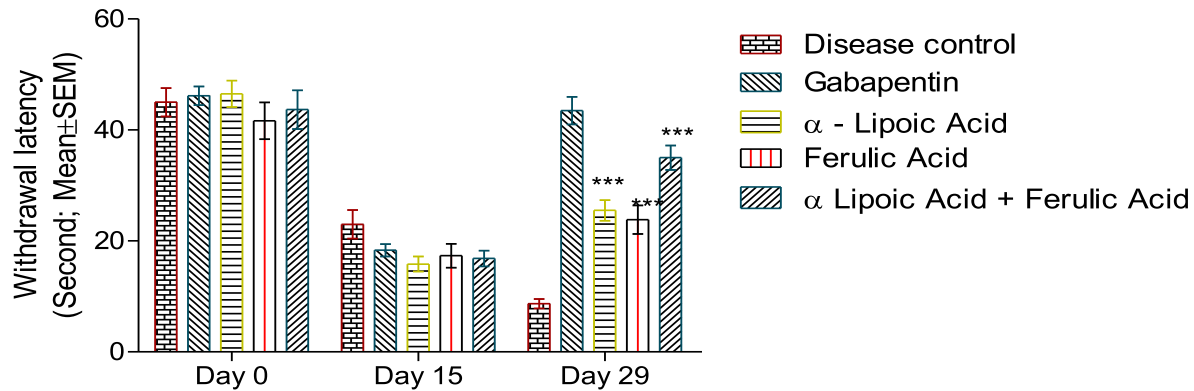

Figure 2: Withdrawal latency time (WLT) in response to cold stimuli observed using the acetone drop test on Day 0, Day 15, and Day 29 to evaluate cold allodynia. Data are observed as the mean ± SEM (n = 6) and the analyzed using Two-Way ANOVA by the Bonferroni post hoc test. *p < 0.05, **p < 0.01, ***p < 0.001 vs. disease control; ###p < 0.001 vs. Day 0. Significant reduction in withdrawal latency is observed in disease control on Day 15 and Day 29 compared the Day 0 (###p < 0.001), indicating the development of the cold allodynia.

Mechanical hyperalgesia by pinprick test: Effect of ALA and FA

Mechanical allodynia is assessed with using prick test, and withdrawal latency time was recorded on Days 0, 15, and 29. Significant decrease in withdrawal latency was observed in an disease control on Day 15 (5.87 ± 1.61 sec) and Day 29 (6.62 ± 1.42 sec) compared to Day 0 (10.53 ± 0.78 sec) (##P < 0.001), confirming the induction of mechanical allodynia following sciatic nerve ligation. Gabapentin - 30 mg/kg, i.p.treatment significantly increased withdrawal latency on Day 29 (10.78 ± 0.73 sec; ***P < 0.001), showing a strong reversal of mechanical hypersensitivity ALA (25 mg/kg, p.o.) treatment led to a modest increase in latency on Day 29 (7.83 ± 1.56 sec), though it did not reach statistical significance. FA (10 mg/kg, p.o.) treatment resulted a significant increase (9.28 ± 0.50 sec; **P < 0.01), indicating a measurable anti-allodynic effect. The combination of ALA and FA (12 mg/kg + 5 mg/kg, p.o.) showed a markedly improved response with a withdrawal latency of 10.52 ± 0.54 sec on Day 29 (***P < 0.001), nearly restoring baseline values.

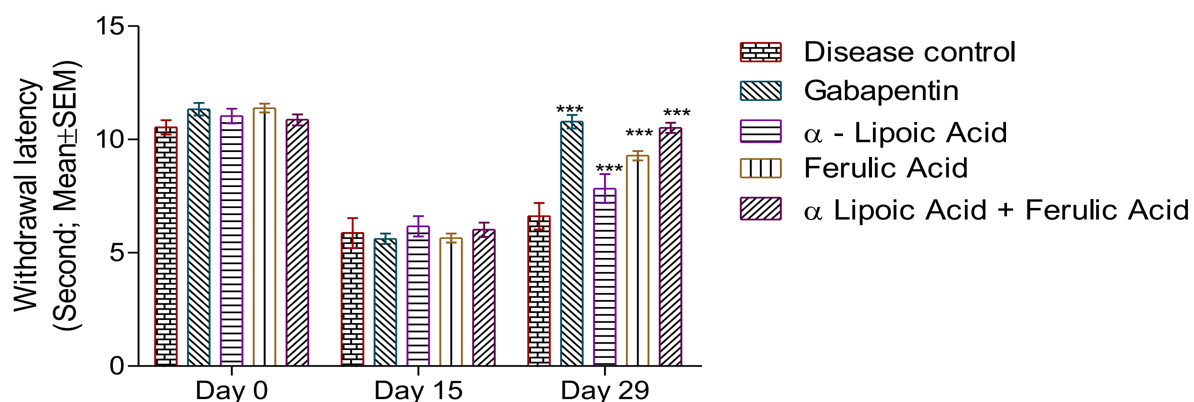

Figure 3: Withdrawal latency time (WLT) was measured using the prick test on Day 0, Day 15, and Day 29 to evaluate mechanical allodynia in the sciatic nerve-injured rats. Data are observed as the mean ± SEM (n = 6) and were statistically analysed using Two-Way ANOVA by Bonferroni post hoc test. *p < 0.05, **p < 0.01, ***p < 0.001 vs. disease control;

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###p < 0.001 vs. Day 0. A significant reduction in WLT was observed in disease control on Day 15 and Day 29 (###p < 0.001), confirming the development of mechanical allodynia.

Thermal allodynia using Eddy's hot plate method: Effect of ALA and FA

Disease control showed significant reduction in anWithdrawal latency time (WLT) on Day 15 (5.83 ± 1.17 sec) and Day 29 (4.82 ± 1.17 sec) compared to Day 0 (14.62 ± 0.52 sec) (###p < 0.001), confirming the development of the thermal allodynia following sciatic nerve injury.Gabapentin treatment significantly increased the latency time to 9.67 ± 1.63 sec on Day 29 (***p < 0.001), indicating its effectiveness in attenuating thermal hyperalgesia. α-Lipoic acid (ALA) treatment moderately improved WLT on Day 29 (7.00 ± 1.10 sec; *p < 0.05), suggesting a partial neuroprotective effect.Ferulic acid (FA) administration led to a significant increase in WLT to 9.00 ± 0.89 sec (***p < 0.001), showing notable anti-allodynic activity. Notably, the combination of ALA and FA produced the highest improvement in WLT on Day 29 (11.33 ± 1.28 sec; ***p < 0.001), suggesting a synergistic effect in reversing thermal hypersensitivity.

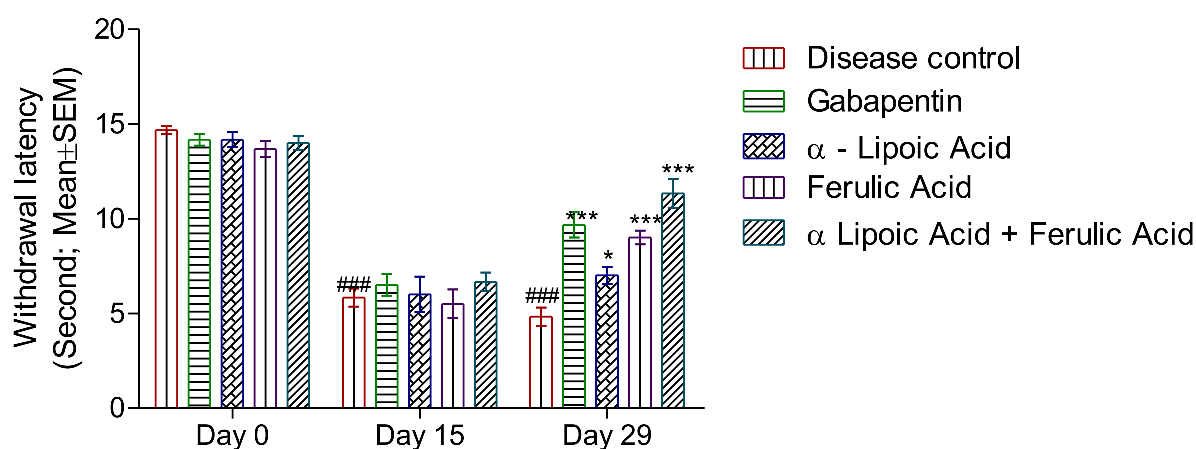

Figure 4: Withdrawal latency time (WLT) in response to a thermal stimulus was assessed using Eddy's Hot Plate Test on Day 0, Day 15, and Day 29 to evaluate thermal allodynia in rats. Data are presented as the mean ± SD (n = 6) and analysed using Two-Way ANOVA by Bonferroni post hoc test. *p < 0.05, ***p < 0.001 vs. disease control Day 15; ###p < 0.001 vs. Day 0.

Parameters of oxidative stress (endogenous antioxidant defense): Effect of ALA and FA

Parameters measured included MDA, CAT, GSH and SOD. Data are presented as the mean ± SD (n = 6). In the disease control group, levels of SOD (2.45 ± 0.55 U/mg protein), CAT (36.48 ± 6.09 U/mg protein), and GSH (10.98 ± 1.73 U/mg protein) were reduced, while MDA levels (10.63 ± 2.05 nmol/mg protein) were elevated, indicating enhanced oxidative stress due to nerve injury. Gabapentin treatment showed modest improvement in antioxidant markers, with slight increases in SOD (2.65 ± 0.42) and GSH (11.70 ± 1.69), and a slight reduction in MDA (9.15 ± 0.91), though these changes were not markedly different from the disease control group. Treatment with α-lipoic acid significantly improved antioxidant status, reflected by elevated SOD (3.27 ± 0.38) and slightly reduced MDA (9.13 ± 1.03). Ferulic acid alone also increased SOD (3.08 ± 0.45) and CAT (41.55 ± 2.57) and showed reduction in lipid peroxidation. Notably, the combination of ALA and FA exhibited the most pronounced effects. SOD (3.33 ± 0.29) and CAT (44.47 ± 5.10) levels were the highest among all groups, and GSH levels (11.62 ± 0.99) were also improved. Importantly, MDA levels showed a marked decline (5.93 ± 2.25), indicating reduced lipid peroxidation and oxidative stress.

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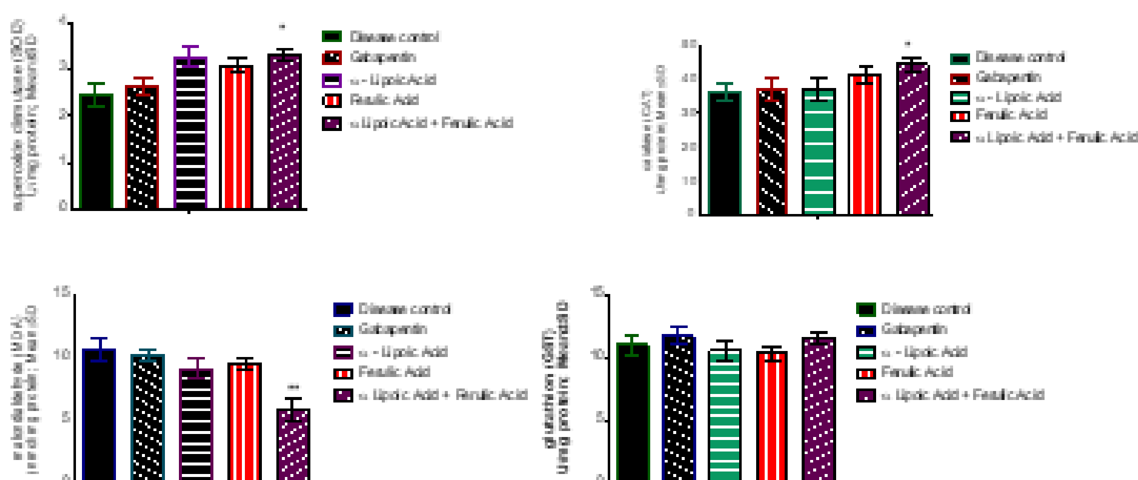

Figure 5: Effects of ALA and FA in Antioxidant Biomarkers— 5-I) SOD, 5-II) CAT, 5-III) MDA and 5-IV) GSH in Sciatic Nerve Injury-Induced Wistar Rats. The results are expressed as the Mean ± SD (n = 6) and were assess One-Way ANOVA observed by Bonferroni Post Hoc analysis. A statistically significant difference was observed, indicated by **P<0.01 and *P < 0.05 in comparison to disease control.

Inflammatory marker of the sciatic nerve: Effect of ALA and FA

The concentration of TNF-α, cytokine, was significantly elevated in disease control group (65.15 ± 0.26 pg/mg protein), confirming the presence of inflammation associated with sciatic nerve injury. Treatment with gabapentin markedly reduced TNF-α levels to 29.49 ± 0.17 pg/mg protein ($p < 0.001$ *vs. disease control*), indicating its potent anti-inflammatory action.Similarly, administration of α-lipoic acid significantly decreased TNF-α expression to 39.92 ± 0.32 pg/mg protein ($p < 0.001$ *vs. disease control*), demonstrating moderate anti-inflammatory efficacy. Treatment with ferulic acid led to a more pronounced reduction (29.24 ± 0.18 pg/mg protein), closely comparable to gabapentin ($p < 0.001$). Notably, the combination therapy of ALA and FA further reduced TNF-α levels to 26.98 ± 0.42 pg/mg protein, showing the most significant anti-inflammatory effect (**$p < 0.001$), suggesting a synergistic action between both antioxidants.

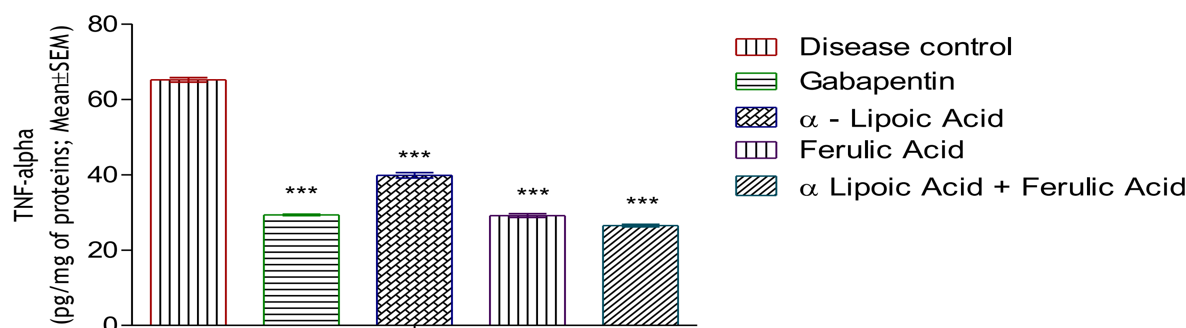

Figure 6: Effects of ALA and FA in Inflammatory markerTNF-α in Sciatic Nerve Injury-Induced Wistar Rats. The results are expressed as the Mean ± SD (n = 6) and were assess One-Way ANOVA observed by Bonferroni Post Hoc analysis. A statistically significant difference was observed, indicated by **P<0.01 and *P < 0.05 in comparison to disease control.

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Histopathology of the sciatic nerve: effects of ALA and FA

Histological analysis of sciatic nerve was carried out to assess structural changes following peripheral nerve injury and treatment. In the disease control group, mild inflammatory cell infiltration was observed, indicating ongoing neural inflammation and axonal damage. In contrast, the gabapentin-treated group showed restoration of normal nerve morphology, with clearly defined axon filaments, a well-preserved myelin sheath, and maintained nerve thickness. Rats treated with α-lipoic acid exhibited histological profiles similar to those of the standard group, including intact myelin structure and visible axon filaments, suggesting significant neuroprotective effects. Likewise, the ferulic acid-treated group showed normal nerve thickness and structural preservation of myelin and axons, indicating protection against demyelination and degeneration. Notably, the combination treatment group receiving both α-lipoic acid and ferulic acid demonstrated marked preservation of nerve architecture, comparable to that of the gabapentin group, with clearly outlined myelin sheaths and prominent axon filaments. These findings support the synergistic potential of ALA and FA in attenuating histopathological damage caused by peripheral nerve injury.

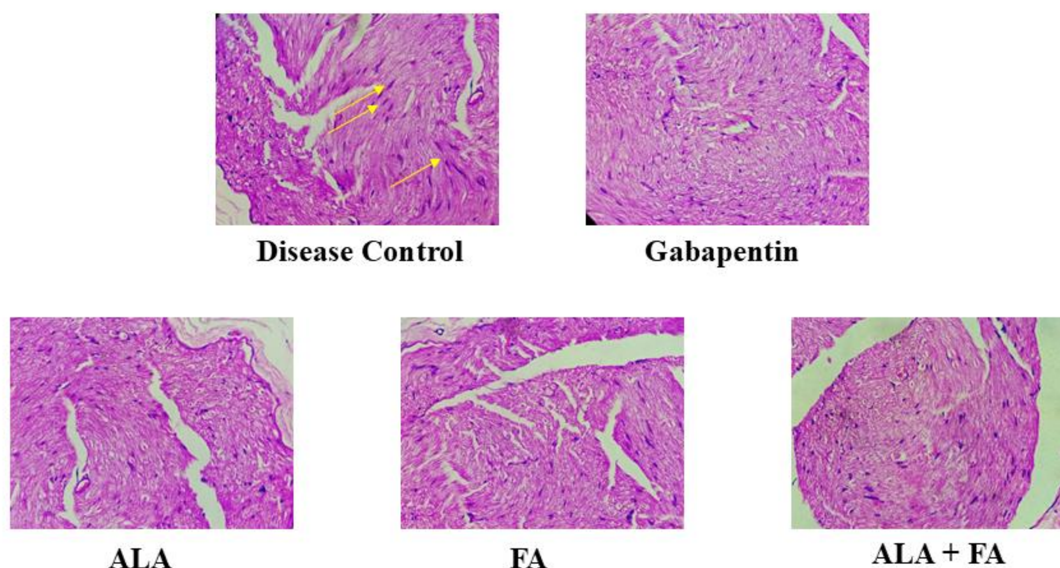

Figure 5: Changes in histopathology seen in the sciatic nerve after peripheral neuropathy caused by CCI.

Discussion

PNP is a chronic and the debilitating disorder resulting from damage to or dysfunction of peripheral nerves, characterized by altered sensory responses including mechanical hyperalgesia, cold and the thermal allodynia, andmechanical allodynia. These sensory abnormalities arise due maladaptive changes in or both peripheral and central nervous systems, including sensitization of nociceptive pathways, activation of glial cells,neuroinflammation andoxidative stress [1,3]. PSNL model is well-established and the widely accepted animal model that mimics in the clinical manifestations of human PNP and is utilized to evaluate the efficacy of analgesic and neuroprotective agents [6,7]. This model induces reproducible behavioral changes in rats which can be assessed using tests such as von Frey filaments for mechanical allodynia, pinprick for the mechanical hyperalgesia,acetone for the cold allodynia and the hot plate for the thermal hyperalgesia [22].In study, therapeutic potential of the ALA and FA, both known for their antioxidant properties and anti-inflammatory properties, were evaluated either alone or in combination in the PSNL-induced model of PNP, and their effects were compared with the standard neuropathic analgesic, gabapentin [35]. ALA is a naturally occurring organosulfur compound and a cofactor in mitochondrial oxidative metabolism, renowned for its capacity to neutralize

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reactive oxygen species (ROS), regenerate endogenous antioxidants like glutathione, and improve mitochondrial function [12,36]. FA, the phenolic compound is found in the plant cell walls, exhibits stronga antioxidant activity, stabilizes cellular membranes, and downregulates inflammatory mediators such as NF-κB and MAPK, which are implicated in neuropathic pain signaling [14,37]. The rationale for combining ALA and FA lies in their complementary mechanisms of action—ALA primarily scavenges intracellular ROS and restores redox balance, while FA fortifies membrane integrity and modulates inflammation. Gabapentin, first-line drug treatment using in neuropathic pain, acts through binding to α2δ subunit the voltage-gated calcium channels and reducing excitatory neurotransmitter is release; however, its limited efficacy and potential side effects underscore the need for alternative or adjunctive therapies [38].In this experimental setting, PSNL reliably induced peripheral neuropathy in male Wistar rats, as evidenced by significant behavioral deficits. The untreated disease control group showed progressive development of neuropathic symptoms over the 29-day study period, confirming successful model establishment. Gabapentin administration significantly improved behavioral responses, validating its role as a positive control [39].The von Frey test revealed substantial mechanical allodynia in PSNL rats, manifested by reduced paw withdrawal thresholds. Gabapentin effectively ameliorated these responses, consistent with its known analgesic profile [40]. ALA and FA, when administered separately, resulted in moderate recovery of withdrawal thresholds, whereas their combination produced a more pronounced effect, indicating potential synergy in modulating mechanosensitive nociceptive pathways. Similar trends were observed in the acetone test, which assessed cold allodynia. PSNL rats exhibited increased sensitivity to cold stimuli, while ALA and FA treatment individually provided partial relief. The combination therapy significantly restored cold sensitivity towards baseline levels, suggesting improved ion channel stabilization and suppression of peripheral cold nociceptor hyperactivity [41].Pinprick-induced mechanical hyperalgesia was also evident in disease control rats, with exaggerated withdrawal reflexes. Gabapentin normalized this hyper-responsiveness, while ALA and FA showed partial effects, which were more effective when used in combination. The results of the hot plate test, evaluating thermal allodynia, further corroborated the above findings. PSNL injury significantly reduced latency to nociceptive behavior on the hot plate, while combination treatment with ALA and FA considerably extended withdrawal latency, implying restoration of descending inhibitory pain modulation and reduced peripheral sensitization [42].Biochemically, PSNL-induced neuropathy was associated with the increased oxidative stress in sciatic nerve tissue, as indicated by elevatedMDA levels, marker of lipid peroxidationand depleted levels of the endogenous antioxidants (CAT, SOD and GSH)[33]. These findings reflect a disrupted redox homeostasis and oxidative nerve damage contributing to neuropathic pathology. Gabapentin mildly improved these oxidative markers. However, ALA significantly elevated GSH, SOD, and CAT levels due to its role in promoting antioxidant recycling, while FA further reduced MDA levels by inhibiting lipid peroxidation. The combination of ALA and FA resulted in the most notable restoration of oxidative balance, indicating a potentiated antioxidant defense response [28,30].Inflammation, crucial component in progression of neuropathic pain, was assessed by measuring levels of TNF-α. TNF-α plays a key role in sensitizing nociceptive neurons, inducing hyperexcitability, and propagating oxidative stress [43]. The PSNL group exhibited elevated TNF-α levels in sciatic nerve. Gabapentin moderately suppressed this inflammatory response. Both ALA and FA significantly reduced TNF-α, with the combination treatment demonstrating the most pronounced anti-inflammatory effect. This outcome can be attributed to parallel inhibition of pro-inflammatory transcription factors and oxidative mediators by both compounds [44].Histopathological analysis of the sciatic nerve provided further support for the biochemical and behavioral findings. Nerve sections from the disease control group exhibited classical signs of neuropathy, including axonal degeneration, demyelination, and inflammatory cell infiltration [8]. Gabapentin-treated rats showed modest improvement in nerve structure. Treatment with ALA and FA led to partial restoration of nerve integrity, with the combination group demonstrating near-normal nerve architecture, reduced inflammatory infiltration, and preserved myelin sheaths, supporting their neuroprotective and synergistic effects [45].All data were observed statistically using the two-way ANOVAby Bonferroni post hoc tests and were

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expressed as the mean ± SD for the each group. The statistically differences were considered at $p < 0.05$, ensuring the validity and reproducibility of the results.The synergistic action observed between ALA and FA is likely due to their complementary mechanisms targeting multiple facets of neuropathic pain, including oxidative damage, mitochondrial dysfunction, and pro-inflammatory signaling. ALA restores intracellular redox capacity by enhancing mitochondrial enzyme function and regenerating antioxidants, while FA stabilizes membrane lipids and modulates inflammatory cascades [46,47]. This dual action yields enhanced neuroprotection, compared to monotherapy or gabapentin.

Conclusion

Peripheral neuropathic pain (PNP) remains a significant therapeutic challenge due to its complex pathophysiology involving neuronal sensitization, oxidative damage, and inflammatory processes. Conventional drugs such as gabapentin, though widely used, often provide only partial relief and associated with undesirable effects, necessitating the exploration of alternative or complementary strategies. In this current study explored efficacy in two naturally derived compounds—FA and ALAin well-established preclinical model in PNP induced by the PSNL. Their effects are evaluated individually and in combination, with results compared to standard treatment with gabapentin.

The study confirmed the successful establishment of a chronic pain model using PSNL in Wistar rats, as evident from consistent behavioral signs of the thermal hyperalgesia, the cold allodynia,the mechanicalhyperalgesia,and themechanical allodynia. These behavioral symptoms mimic clinical features of human neuropathic pain and provide a reliable platform for assessing analgesic efficacy. The untreated disease control group exhibited persistent nociceptive behaviors and marked alterations in biochemical and histological parameters, further validating the robustness of the PSNL model.

Gabapentin, used as a positive control, demonstrated significant efficacy in improving behavioral outcomes and provided partial correction of oxidative and inflammatory imbalances. However, its effects were predominantly symptomatic, with limited restoration of underlying cellular health. In contrast, both ALA and FA exhibited notable neuroprotective and anti-nociceptive effects across multiple domains. When administered individually, ALA and FA significantly alleviated neuropathic behaviors and improved redox status in nerve tissues. Their efficacy was markedly enhanced when used in combination, suggesting a synergistic interaction that addresses multiple aspects of neuropathic pathophysiology.

The behavioral outcomes revealed that the combination of ALA and FA was superior in reversing PSNL-induced deficits in pain thresholds. Von Frey filament testing showed significant recovery from mechanical allodynia, while the acetone and pinprick tests confirmed attenuation of cold and mechanical hyperalgesia, respectively. Moreover, the hot plate test results pointed to improved thermal nociception, indicative of restored central and peripheral sensory processing. These improvements were more substantial with combination therapy than with either agent alone or gabapentin, implying that targeting oxidative stress and inflammation simultaneously offers better symptomatic control and tissue recovery.

Biochemical assays further supported the behavioral findings. PSNL injury was associated with elevated malondialdehyde (MDA) levels withsignificant depletion of antioxidant defenses (CAT, GSH and SOD). While gabapentin modestly corrected some of these imbalances, ALA and FA provided more pronounced improvements, particularly when administered together. This combination significantly enhanced antioxidant enzyme activities and reduced lipid peroxidation, reflecting effective scavenging of reactive oxygen species and restoration of redox equilibrium.

Anti-inflammatory effects in the combination therapy were equally promising. TNF-α, a key of cytokine implicated the neuropathic pain progression, was significantly elevated in the PSNL group. ALA and FA, both known to suppress inflammatory signaling pathway such asMAPKand NF-κB, effectively reduced TNF-α levels. The reduction was most notable with the combination therapy, reinforcing the concept of a synergistic anti-inflammatory mechanism contributing to pain relief and nerve protection.

Histopathological observations corroborated the biochemical and behavioral data. Nerve sections from the disease control group showed structural degeneration, demyelination, and inflammatory infiltration. Gabapentin treatment provided moderate

histological improvement, while ALA and FA treatment preserved nerve structure to a greater extent. The most profound neuroprotection was observed in the combination-treated group, where nearly normal nerve architecture was maintained. This finding highlights the potential of the combining antioxidants and the anti-inflammatory agents for preventing or reversing nerve damage at the structural level.

The use of two-way ANOVA with Bonferroni post-hoc analysis ensured that reliability to observed differences across treatment groups. This statistical rigor adds to the validity of the findings and supports the conclusion that ALA and FA, especially in combination, are effective therapeutic agents in mitigating neuropathic pain and its underlying biochemical and structural causes.

The results suggest that the combination of ALA and FA offers a multifaceted therapeutic approach. ALA primarily exerts intracellular antioxidant effects and enhances mitochondrial function, while FA contributes to membrane stabilization and inhibition of inflammatory pathways. Together, they target critical aspects of neuropathic pain pathology—including oxidative imbalance, inflammation, and neuronal sensitization—thus providing a comprehensive strategy that surpasses the effects of conventional agents like gabapentin.Given their safety profiles and natural origins, ALA and FA hold strong translational potential for use in clinical settings. The findings encourage further investigation into their pharmacokinetics, optimal dosing, and long-term safety in humans. Moreover, their integration into adjunctive regimens with existing neuropathic pain medications may enhance overall efficacy while reducing side effect burdens.

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