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Neuroprotective Effects of Alpha-Lipoic Acid and Ferulic Acid on Peripheral Neuropathic Pain in Rats

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ABSTRACT

Background: Increased nociceptive sensitivity and neuroinflammation are hallmarks of peripheral neuropathic pain (PNP), a chronic and incapacitating condition brought on by nerve damage or metabolic abnormalities. Rats' chronic constriction injury (CCI) model accurately mimics human PNP. Natural antioxidants ferulic acid (FA) and alpha-lipoic acid (ALA) have strong anti-inflammatory and neuroprotective effects. In a CCI-induced PNP model in rats, this study examined and contrasted the therapeutic potential of ALA, FA, and their combination with the common medication gabapentin.

Methods: Five groups (n=6) of adult Wistar rats were created: Disease Control, Gabapentin (30 mg/kg, i.p.), alpha-lipoic acid (25 mg/kg, p.o.), ferulic acid (10 mg/kg, p.o.), and a combination of alpha-lipoic acid + ferulic acid (12 mg/kg + 5 mg/kg, p.o.). Mechanical allodynia (von Frey), cold allodynia (acetone), thermal hyperalgesia (hot plate), and mechanical hyperalgesia (pinprick) tests were among the behavioural evaluations. In sciatic nerve homogenates, biochemical analyses determined the levels of SOD, CAT, GSH, MDA, and TNF-α. Axonal integrity and inflammation were evaluated by histopathological analysis.

Results: Pro-inflammatory markers, oxidative stress, and nociceptive behaviour were all markedly elevated by CCI. ALA or FA monotherapy produced modest gains. However, their combined effects closely mirrored those of gabapentin, significantly reducing pain behaviour and normalising oxidative and inflammatory parameters. Better axonal preservation in combination-treated rats was confirmed by histology.

Conclusion: The synergistic neuroprotective and antioxidant effects of ALA and FA, particularly when combined, point to their potential as an adjuvant or alternative therapy for management of PNP.

Keywords: *Peripheral Neuropathic Pain; Chronic Constriction Injury; Alpha-Lipoic Acid; Ferulic Acid; Antioxidant Therapy; TNF-α*

1. INTRODUCTION

International Association for the Study of Pain (IASP), neuropathic pain is "pain caused by a lesion or disease of the somatosensory nervous system" [1]. Unlike nociceptive pain, which results from nociceptors activating in thr response to real and potential tissue damage [2] Central (origin into the brain and spinal cord) or peripheral (origin into the peripheral nervous system) forms can help to define neuropathic pain [3].

Particularly in diseases like diabetic neuropathy, postherpetic neuralgia, or traumatic nerve injury [4], peripheral neuropathic pain (PNP) especially describes pain resulting from a lesion or disease affecting peripheral nerves. PNP patients frequently report burning pain, electric-shock-like sensations, hyperalgesia that is, increased the response into painful stimuli and allodynia that pain response convert into nonpainful stimuli [5]. Based on epidemiological studies, neuropathic pain is thought to be rather common worldwide—between 6.9% and 10% in the general population [6]. Affected roughly 20%–30%

of people, peripheral neuropathic pain is a main cause of handicap and poor quality of life [7]. PNP has a clinically negative effect on physical functioning, emotional distress, disturbed sleep, and healthcare use [8].

Originally invented by Bennett and Xie in 1988, Chronic Constriction Injury (CCI) model is most often use and well-validated animal models for investigating peripheral neuropathic pain (PNP), since it closely reflects the clinical characteristics of prolonged nerve compression and damage in humans [9]. Furthermore important in the CCI model is oxidative stress, where higher generation of the reactive oxygen species (ROS) and matching decrease of endogenous antioxidant defences aggravate nerve damage and pain signalling [10,11]. By scavenging free radicals, restoring redox homeostasis, and so altering inflammatory responses, antioxidants both preventative and therapeutic.

By lowering oxidative damage, preserving mitochondrial integrity, and so preventing glial activation, α-lipoic acid (ALA) and ferulic acid (FA) have shown great potential to reduce neuropathic pain symptoms [12,13]. These agents improve activity in endogenous antioxidant systems including superoxide dismutase, glutathione peroxidase, catalase, so lowering oxidative stress-induced neuronal damage and neuroinflammation [14]. Targeting oxidative stress helps antioxidants not only reduce pain but also possibly stop the spread of nerve damage, so providing a disease-modifying effect in neuropathic pain treatment [15].

Made from octanoic acid, naturally occurring organosulfur compound ALA acts as a strong antioxidant and mitochondrial cofactor. Central in mitochondrial energy metabolism, ALA has shown ability to rebuild endogenous antioxidants including vitamin C, glutathione and vitamin E, so strengthening the cellular antioxidant defence system [12]. By altering important inflammatory pathways including NF-κB, so lowering in the generation of the inflammatory cytokines like TNF-α and so preventing oxidative stress-induced neuronal damage [16], it shows anti-inflammatory and neuroprotective effects. ALA can cross the blood-brain barrier because of its dual solubility in water and lipids, hence it is especially useful in treating central and peripheral nervous system diseases [15,17].

Plant-derived phenolic compounds widely found in cereals, fruits, and vegetables are FA. By neutralising reactive oxygen species (ROS) and so preventing lipid peroxidation [18], this potent free radical scavenger shows strong antioxidant and anti-inflammatory properties. FA also lowers neuroinflammation, so modulating cellular signalling pathways involved in inflammation and death including MAPKs and COX-2, and so offers a potential treatment for neurodegenerative and neuropathic pain disorders [19]. Recent preclinical research indicates that by lowering microglial activation and so preventing oxidative damage in peripheral nerves, FA can help to reduce neuropathic pain behaviours in rodent models [13]. ALA and FA's combined anti-inflammatory and antioxidant properties point to their perhaps synergistic ability to change the complicated pathophysiology of peripheral neuropathic pain.

2. MATERIAL AND METHODS

Drugs and reagents

The following were used: gabapentin (gift sample, MS University, Baroda, India), FA (gift sample, Otto-Kemi, India), and ALA (gift sample, Sigma-Aldrich, USA). A reputable supplier provided all of the standard-grade chemicals and reagents used in the investigation.

Experimental Animals

For this investigation, Wistar strain male rats weighing 200–250 gm were used. Disease-Free Animal House Lacshmi Biofarms in Pune, India, is where these rats were bought. According to the Committee for the Purpose of Control and Supervision of Experiments on Animals, India's guidelines, a palatable food and diet (VRK, Nutritional Solution, Sangli, India), a temperature of 22°C, and a 12-hour cycle of bright and dark light were all maintained for the rats. The Institutional Animal Ethics Committee of Scitesla Private Limited, Navi Mumbai, 400710, India, granted prior approval for this research study (SCI/IAEC/2024-25/142). Guidelines for animal care were provided by the Government of India, CPCSEA, Ministry of Environment and Forests.

Preparation of drug solutions

Normal saline (NS) was used for the preparing drugs gabapentin [20], ALA and FA was prepared in NS [21].

Induction of Peripheral neuropathy by CCI

CCI was used in this study to induce neuropathy in Wistar rats, which were assigned to five groups (n = 6) (Bennett and Xie in 1988). A tiny incision was made in biceps femoris muscle is expose and treatment on sciatic nerve at the mid-thigh level while under ketamine xylazine anesthesia (Flecknell et al., 2015). Three to four loose ligatures of 4-0 silk, with spaced 1 mm apart, were tied around a 7 mm section of the nerve that was isolated proximal to the sciatic trifurcation until a momentary muscle twitch was noticed. Appropriate analgesia and two weeks of observation were part of post-operative care. Following two weeks of surgery to induce neuropathic pain, the animals received once-daily treatment for 14 days with either ferulic acid, α-lipoic acid, or regular gabapentin. On the 15th day following surgery and the 29th day following treatment, behavioral evaluations were carried out [22].

Experimental protocol

Drug treatments were given for two weeks after neuropathic pain was induced in this experimental study, for a total experimental duration of two weeks following CCI surgery. Group I rats were designated as a Disease control group and they were given a normal saline solution orally. For two weeks after neuropathy induction, rats in Group II, the standard treatment group, received intraperitoneal injections of gabapentin (30 mg/kg, I.P., once in a day). Two weeks following CCI induction, Group III rats were given α-lipoic acid (25 mg/kg, P.O., once in a day). For two weeks, Group IV received oral FA (10 mg/kg, P.O., once in a day). For two weeks following CCI induction, Group V was given combination of FA (5 mg/kg), ALA (12 mg/kg) orally once daily.

This protocol objective was to assess and contrast the neuroprotective effects of FA and ALA, both separately and together, against peripheral neuropathic pain caused by CCI in rats, using gabapentin as a common reference medication.

Name of group	No. of animals	Drug treatment	Route of drug	Dose of drug	Duration of drug treatment
Disease Control Group	6	Normal Saline Solution	P.O.	-	2 Weeks
Gabapentin Group	6	Gabapentin	I.P.	30 mg/kg	2 Weeks
ALA + CCI Group	6	ALA	P.O.	25 mg/kg	2 Weeks
FA + CCI Group	6	FA	P.O.	10 mg/kg	2 Weeks
FA + ALA + CCI Group	6	FA + ALA	P.O.	5 mg/kg + 12 mg/kg	2 Weeks

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

Assessment of behavioral parameters

Adequate measures were taken to prevent a stressful situation for the rats during the behavioral parameter testing, which took place from 08.30 am to 04.00 pm.

Mechanical allodynia using von Frey hair test

Von Frey filaments (Samitek Instruments, Lajpat Nagar, New Delhi) were used to measure mechanical allodynia in accordance with a defined protocol [23]. Rats were given 15 to 30 minutes to acclimate after being placed individually in clear enclosures on an elevated mesh floor. Plantar surface of the hind paw was covered and vertically applied calibrated monofilaments weighing between 0.4 and 15 g. Each filament was held for one to two seconds after being gently pressed until it just bent. A positive response was defined as licking, shaking, or a sharp paw withdrawal. A mid-range filament (usually 2.0 g) was used for testing, and following stimuli were chosen according to the response, shifting to a lower force if there was no reaction or to a higher force if there was. 50%-paw withdrawal threshold is established observed by up down method. To track the onset or decrease of allodynia after nerve damage or therapy, this test was conducted again on particular days.

Cold allodynia by acetone

The acetone drop method, a behavioral test frequently employed in neuropathic pain models, was used to evaluate cold allodynia [24-26]. A fine-tipped applicator was used to carefully apply a small volume (20 µL) of acetone (99.7%) to the glabrous skin area just below both ears, avoiding excessive pressure or dripping. Animals were monitored for 60 seconds after application, during which time nociceptive reactions like quick head shaking, paw withdrawal, scratching, or grooming were noted. These actions served as markers for pain or discomfort brought on by the cold. To reduce stress and guarantee consistent behavioral responses, the test was administered in a calm setting. This process aided in assessing the degree of cold sensitivity and the possible analgesic benefits of neuropathic condition treatments.

Mechanical hyperalgesia by pinprick

A pinprick stimulation technique, as outlined in well-established pain models, was used to evaluate mechanical hyperalgesia [27]. Without puncturing the skin, a sterile safety pin was gently pressed against the lateral side of the injured hind paw. The stimulus was enough to produce a slight indentation, and the length of paw withdrawal was used to gauge the response. In order to prevent prolonged stress or injury, a cut-off limit of 15 seconds was established, and a minimum response time of 0.5 seconds was deemed valid. Three independent trials with adequate recovery time in between stimulations were performed on each animal. For additional analysis, the mean of these trials was computed. In neuropathic pain conditions, this method offered a consistent and accurate way to measure mechanical hyperalgesia.

Thermal allodynia using Eddy's hot plate method

The Eddy's hot plate test, a common technique for determining rodents' nociceptive thresholds, was used to evaluate thermal allodynia [28]. Every animal was put separately on a hot plate device that was kept at a steady 55 ± 0.1°C. The number of seconds that passed before the first noticeable pain response, like paw licking, flicking, or jumping, was noted. An 18-second maximum cut-off time rigorously adhered to into order avoid thermal damage. To prevent tissue damage, any animal that failed to show a nocifensive reaction within this time frame was taken out right away. Thermal hypersensitivity in response to neuropathic pain or therapeutic interventions was measured using the results of each test, which was carried out in a consistent environment.

Oxidative stress assessment (endogenous antioxidant defense)

Tissue homogenization parameter

Following the completion of treatment protocol, each rat was euthanized, and sciatic nerve is carefully excised. The tissue was dipped right away in ice-cold Tris-HCl buffer kept at pH 7.4. Nerve is finely sliced with a sterile surgical blade and placed into a cold 0.25 M sucrose solution. Under cold conditions, these tissue sections were homogenised in 10% (w/v) Tris-HCl buffer (10 mM, pH 7.4). Centrifugation at 10000 rpm for 15 minutes at 0°C turned the homogenate into Collected, utilised for the biochemical analysis of oxidative stress markers and cytokine levels [29,30], the resulting clear supernatant.

Estimation of reduced glutathione (GSH)

A spectrophotometric technique based on the protocol developed by Moron et al. [31] was used to measure the amount of GSH present in tissue samples. This technique depends on the formation of a yellow chromophore that can be detected at 412 nm as a result of reactions between sulfhydryl group in 5,5'-dithiobis-(2-nitrobenzoic acid) and GSH. Tissue supernatant is mixed with equal volume of 20% trichloroacetic acid to precipitate proteins in order to prepare the sample. The clear supernatant was separated and used for analysis following centrifugation. GSH was measured by reacting 0.25 ml of supernatant with 5,5'-dithiobis-(2-nitrobenzoic acid) reagent (2 ml), then measuring developed of color absorbance at 412 nm. Microgrammes of GSH per milligramme of protein was the unit of measurement for the results.

Estimation of superoxide dismutase level (SOD)

By preventing oxidation of adrenaline into adrenochrome by transforming harmful superoxide radicals into less reactive molecular species, SOD plays a critical role in cellular defence. The methodology outlined by Misra and Fridovich [32] was used to calculate the SOD activity. First, distilled water was used to dilute equal parts of tissue homogenate. A 0.15 ml of chloroform and 0.25 ml of cold ethanol is used to treat this mixture. A cyclo-mixer was used to vortex the resultant solution for five minutes, and it was then centrifuged at 2500 rpm. The 0.5 ml of clear supernatant was taken and mixed with 1.5 ml of carbonate buffer and 0.5 ml of EDTA. The reaction was then started by adding of epinephrine (0.4 ml). SOD activity was observed by units per milligramme of the protein, the changes into absorbance was measured at 480 nm.

Estimation of catalase (CAT)

Sciatic nerve tissue's CAT activity was measured spectrophotometrically using Aebi-developed protocol, which measures the rate at which hydrogen peroxide ($H2O_2$) breaks down [33]. The tissue supernatant and phosphate buffer (pH 7.0) 1 ml of 50 mmol/l were combined for assay. 1 ml of a 30 mmol/l $H2O_2$ solution was combined with 2 ml of diluted sample to start the reaction. As time passed, absorbance decreased, indicating that $H2O_2$ was being broken down by enzymes. The quantity of H_2O_2 broken down per minute per milligram of protein was used to express CAT activity [34].

Estimation of lipid peroxide malonaldehyde (MDA)

MDA content measured in tissue samples assess to lipid peroxidation levels using the Slater and Sawyer [30] method. In order to allow protein precipitation, 2 ml of the tissue supernatant were treated with 10% (w/v) trichloroacetic acid and placed in ice water bath for 15 minutes. Two millilitres of freshly made thiobarbituric acid solution is added in the mixture after it had been centrifuged to produce a clear supernatant. To promote colour development, the resultant mixture is heated in the boiling water bath for ten min. then quickly cooled on ice for five minutes. Using the spectrophotometer and the reagent blank used as reference, the absorbance in the coloured complex was measured at 532 nm. MDA content of the tissue is determined and assessed as nanomoles of MDA per milligramme of protein by plotting a standard curve using known concentrations of MDA.

Assessment of inflammatory markers

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

Using commercially available ELISA kit, this method outlined by Muthuraman et al. was used to observed and measure TNF-α in the sciatic nerve tissue. For detection, the assay used an anti-TNF-α antibody. To create a calibration curve, standard TNF-α solutions with concentrations ranging from 0 to 20,000 pg/ml were made. A microplate reader was used to measure in absorbance the yellow-colored that resulted in the reaction development at 450 nm. The tissue homogenate's final

values were reported in pg of TNF-α per mg of total protein [35].

Histopathology of the sciatic nerve

Rats were anaesthetised and euthanized to collect the sciatic nerves once the treatment course was over. To preserve cellular and tissue architecture, the removed tissues were straightly fixed in 10% formalin. The nerves were sliced 4 mm thick following fixation. Haematoxylin and eosin staining helped these sections be handled for standard histological study. Under a light microscope [36], morphological changes and neuropathological indicators were evaluated.

Statistical analysis

Results were represented and assessed by mean ± SD (n=6). Data is assess by Two-Way ANOVA observed by the Bonferroni Post Hoc analysis. *P<0.5 was observed and considered as statistically significant.

3. RESULTS

Mechanical allodynia von-frey filaments-triggered: Effect of ALA and FA on

Using von Frey filament-induced paw withdrawal latency (PWL), mechanical allodynia is expressed after neuropathy induced by CCI. Day 15 (4.17 ± 0.75 sec) and later Day 29 (4.00 ± 0.89 sec) showed a notable decrease in PWL in the disease control group, so confirming the development of mechanical allodynia (###p < 0.001 vs. Day 0).

With a PWL of 8.00 ± 0.57 sec on Day 29 (***p < 0.001 vs. disease control), gabapentin notably inverted this effect. While FA produced a similar effect (8.50 ± 1.03 sec, ***p < 0.001), α-lipoic acid also enhanced mechanical sensitivity, so increasing the PWL to 8.50 ± 1.04 sec (***p

Significantly restoring PWL to 9.17 ± 0.99 sec by Day 29 (***p<0.001 vs disease control), the combination of ALA, FA produced the notable improvement suggesting a synergistic neuroprotective effect against von Frey-induced mechanical hypersensitivity.

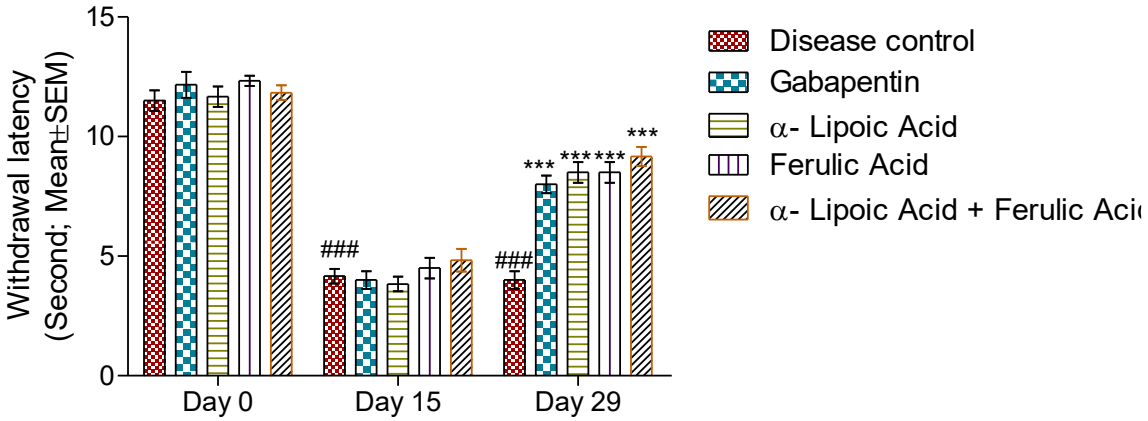

Figure 1: PWL was measured using von Frey filaments on Day 0, Day 15, and Day 29 to assess mechanical allodynia. The results are observed as mean±SEM (n = 6) and assess using the Two-Way ANOVA observed by Bonferroni post hoc test. In *p<0.05, **p<0.01, ***p < 0.001 vs. disease control. ###p < 0.001 vs. Day 0. A significant reduction in the PWL is observed in the disease control on Day 15 and 29 compared to Day 0, confirming the development of mechanical allodynia (###p<0.001). Treatment in the gabapentin significantly increased the PWL on Day 29 (***p < 0.001), indicating attenuation of mechanical hypersensitivity. Likewise, ALA (***p < 0.001), FA (***p < 0.001), and their combination (***p < 0.001) showed marked protective effect, with combination therapy producing the most pronounced reversal of von Frey-induced allodynia.

Cold allodynia by acetone test: Effect of ALA and FA

Cold allodynia after CCI-induced neuropathy was evaluated using acetone-induced PWL. By Day 15 (16.83 ± 5.38 sec) and Day 29 (11.00 ± 2.24 sec) in comparison to Day 0 (45.83 ± 8.66 sec), the disease control group's PWL had significantly decreased, indicating the onset of cold allodynia (###p < 0.001). PWL significantly increased by Day 29 (28.50 ± 5.75 sec, ***p<0.001 vs. disease control), indicating a improvement following gabapentin treatment. Likewise, cold allodynia was considerably reduced by FA (23.83 ± 4.62 sec, **p < 0.01) and ALA (26.67 ± 5.13 sec, **p < 0.01). The greatest effect was obtained when ALA and FA were administered together; this increased PWL to 42.67 ± 4.64 seconds (***p < 0.001), suggesting a synergistic improvement in cold sensitivity.

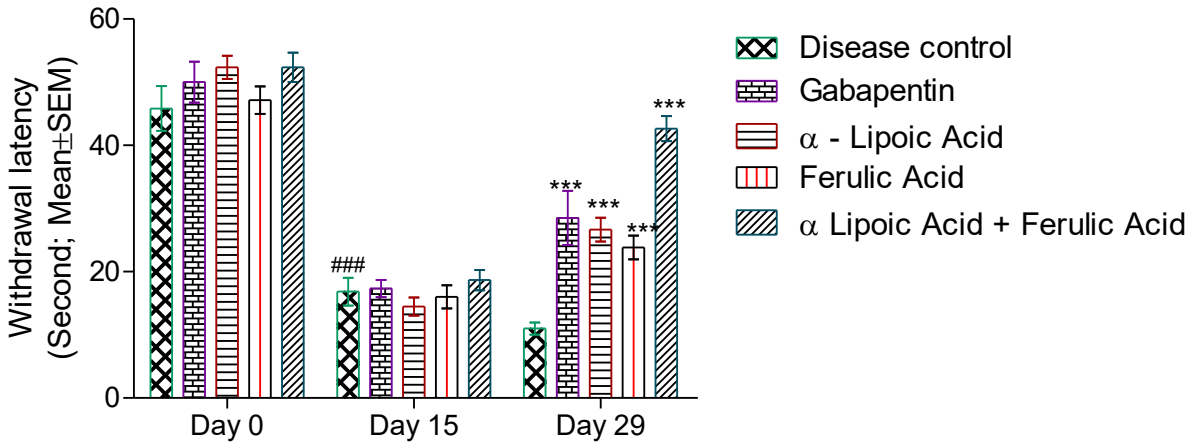

Figure 2: PWL was measured on Day 0, 15, and 29 to assess cold sensitivity. Results are Observed as mean ± SD (n = 6) and assess using the Two-Way ANOVA observed by the Bonferroni post hoc test. In *p<0.05, **p<0.01, ***p<0.001 vs. disease control. The significant reduction in PWL was observed into disease control group on Days 15, 29 for confirming the development of cold allodynia (###p < 0.001 vs. Day 0). Treatment with gabapentin, ALA, and FA significantly reversed this effect, with the combination therapy showing the most pronounced improvement.

Mechanical hyperalgesia by pinprick test: Effect of ALA and FA

PWL in response to the prick test was measured in order to evaluate mechanical allodynia. On Day 15 (5.27 ± 0.78 sec) and Day 29 (4.82 ± 0.80 sec), the disease control group's PWL significantly decreased in comparison to Day 0 (11.20 ± 0.72 sec), suggesting the onset of mechanical allodynia (###p < 0.001). Withdrawal latency was significantly improved by gabapentin treatment (9.72 ± 1.10 sec, ***p < 0.001 vs. disease control), ALA (9.60 ± 0.82 sec, ***p < 0.001), and FA (9.65 ± 0.92 sec, ***p < 0.001). Interestingly, PWL was further increased by the combination of FA and ALA (10.03 ± 0.53 sec, ***p < 0.001), indicating a synergistic effect in reducing mechanical allodynia.

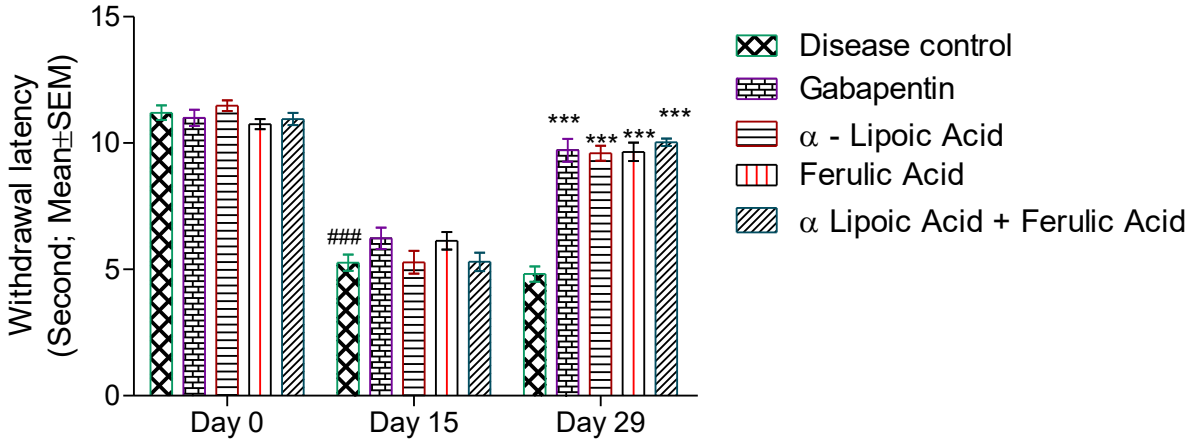

Figure 3: PWL in response to the pin prick test was recorded on Day 0, 15, and 29 to assess mechanical allodynia into CCI-induced neuropathic rats. The results are observed as mean ± SD (n = 6) and assess using the Two-Way ANOVA observed by the Bonferroni post hoc test. In *p< 0.05, **p< 0.01, ***p< 0.001 vs. disease control. The significant decline in withdrawal latency is noted in disease control on Days 15, 29 (###p< 0.001 vs. Day 0), indicating the progression of mechanical allodynia. Treatment with gabapentin, ALA, FA, and their combination significantly increased latency values on Day 29, with the combination group showing the highest protective effect.

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

PWL was measured on Days 0, 15, and 29 in order to evaluate thermal allodynia using the Eddy's hot plate. Induction in thermal allodynia after CCI was confirmed by the significant decrease in PWL in the disease control group on Day 15 (4.00 ± 0.64 sec) and Day 29 (2.33 ± 1.21 sec) in comparison to baseline (13.67 ± 1.23 sec) (###$p < 0.001$ vs. Day 0). PWL increased significantly with gabapentin treatment, reaching 8.33 ± 1.11 sec by Day 29 (***$p < 0.001$ vs. disease control). Significant improvements were also observed with ALA and FA treatments (4.67 ± 1.09 sec and 6.17 ± 1.72 sec, respectively; ***$p < 0.001$). The most noticeable effect was produced by the combination of FA and ALA, which restored PWL to 9.67 ± 1.51 sec on Day 29 (***$p < 0.001$), suggesting a synergistic analgesic action.

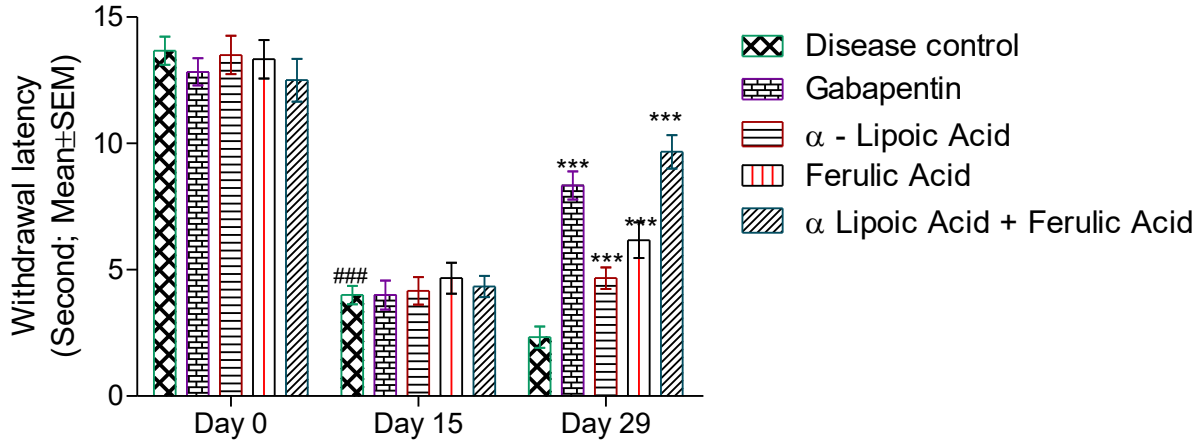

Figure 4: PWL was measured on Day 0, 15, and 29 using Eddy's hot plate into assess thermal allodynia. Results are observed in mean ± SD (n = 6) and assess using the Two-Way ANOVA observed by the Bonferroni post hoc test method. In *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. disease control.

A significant reduction in PWL was observed in the disease control on Days 15, 29, confirming development of thermal allodynia (###$p < 0.001$ vs. Day 0). Treatment with gabapentin (***$p< 0.001$), ALA (***$p< 0.001$), and for FA (***$p< 0.001$) is significantly reversed the CCI-induced decrease in PWL. Notably, the combination of ALA and FA exhibited the greatest improvement in thermal sensitivity by Day 29, indicating a potential synergistic therapeutic effect.

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

Superoxide dismutase (SOD: 2.52 ± 0.59 U/mg protein), catalase (CAT: 42.75 ± 7.91 U/mg protein), and glutathione (GSH: 10.99 ± 1.47 U/mg protein) levels were significantly reduced and malondialdehyde (MDA: 9.96 ± 2.99 nmol/mg protein) was elevated, indicating oxidative stress in sciatic nerve tissue, reflecting the CCI's significant disruption of the antioxidant defence system.

In contrast to the disease control, the antioxidant enzyme levels in SOD (3.13 ± 0.70), CAT (56.89 ± 8.25, *$p < 0.05$), GSH (14.30 ± 2.37), and MDA (7.50 ± 1.72) were significantly improved after two weeks of intraperitoneal gabapentin administration following surgery.

Likewise, administration of FA (SOD: 3.81 ± 0.99; CAT: 57.94 ± 7.41, *$p < 0.05$; GSH: 14.82 ± 3.14) and ALA (SOD: 3.45 ± 0.79; CAT: 59.39 ± 7.17, **$p < 0.01$; GSH: 15.53 ± 2.93) markedly decreased MDA and increased antioxidant levels (7.34 ± 3.03 and 5.08 ± 1.50, respectively; **$p < 0.01$ for FA).

With significantly higher levels of SOD (4.10 ± 0.82, *$p < 0.05$), CAT (61.30 ± 7.49, **$p < 0.01$), and GSH (18.29 ± 4.41, **$p < 0.01$), as well as the lowest MDA levels (4.84 ± 3.45, **$p < 0.01$), the combination of ALA and FA produced the most pronounced protective effect.

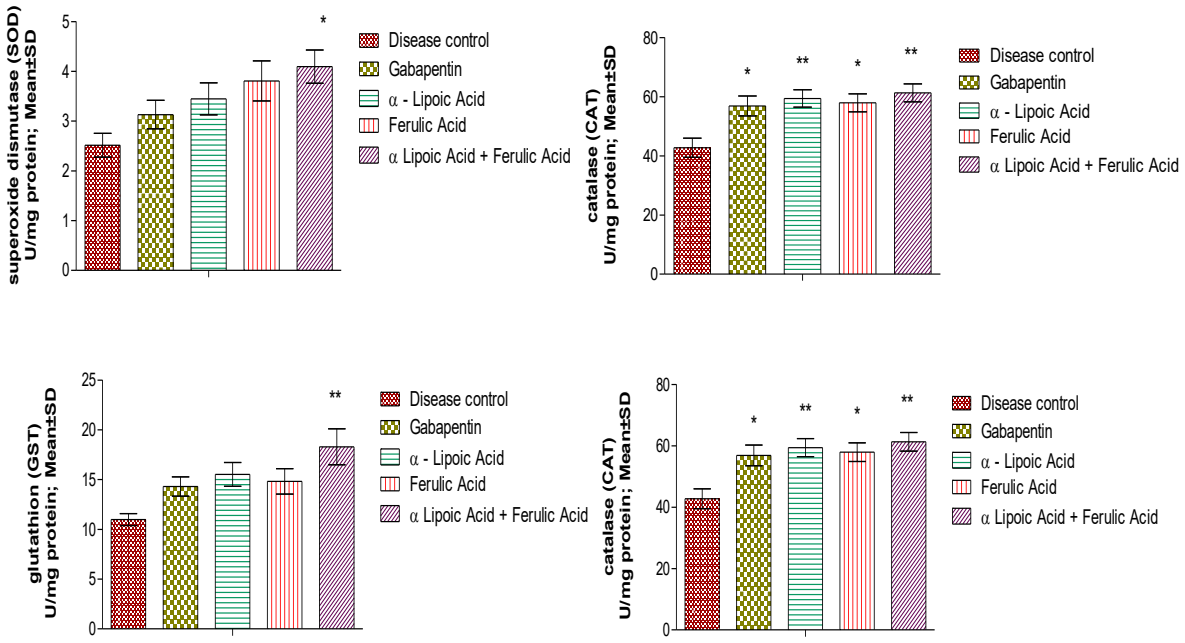

Figure 5: Effects of ALA and FA in Antioxidant Biomarkers— 5-I) SOD, 5-B) CAT, 5-C) GSH and 5-D) MDA in Sciatic Nerve Injury-Induced Wistar Rats. The results are observed in Mean ± SD (n = 6) and assess by the One-Way ANOVA observed by Bonferroni Post Hoc analysis. A statistically significant difference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

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

The inflammatory marker cytokine TNF-α, which is induced by nerve injury, was measured in sciatic nerve homogenates. In earlier research, the CCI group's TNF-α levels were significantly higher (68.12 ± 0.36 pg/mg protein) than baseline, suggesting a strong inflammatory response. Gabapentin treatment after surgery significantly reduced this elevation, bringing TNF-α levels down to 32.49 ± 0.27 pg/mg protein. Similarly, FA treatment (32.24 ± 0.28 pg/mg, *p < 0.05), α-lipoic acid treatment (42.94 ± 0.42 pg/mg, *p < 0.05) considerably reduced TNF-α levels in comparison with disease control. The most effective anti-inflammatory treatment was a combination of FA and ALA, which reduced TNF-α to 29.98 ± 0.52 pg/mg protein (*P < 0.05 in the comparison to disease control), indicating a synergistic effect in reducing CCI-induced neuroinflammation.

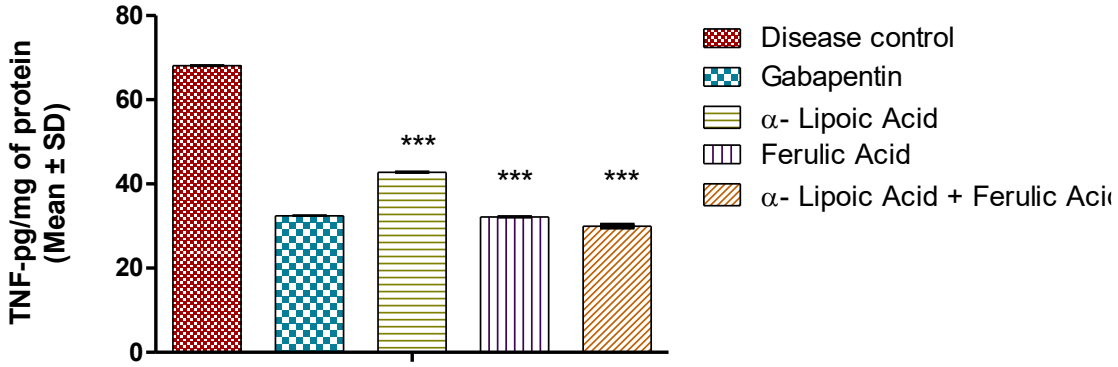

Figure 6: Effects of ALA and FA in cytokine TNF-α on Sciatic Nerve Injury-Induced Wistar Rats. The results is observed in Mean ± SD (n = 6) and assess by the One-Way ANOVA observed by Bonferroni Post Hoc analysis. Statistically significant data difference was observed, indicated by the *P < 0.05 in comparison to disease control.

Histopathology of the sciatic nerve: effects of ALA and FA

In the disease control group exhibited significant pathological changes, such as mild to moderate mononuclear cell infiltration, cystic dilatation of the myelin sheath, and axonal loss, according to histological examination of sciatic nerve sections. Conversely, no discernible abnormalities were observed in gabapentin, ALA, or the combination of ALA and FA groups, suggesting effective neuroprotection. Mild to moderate mononuclear infiltration was seen in the FA-treated group, indicating partial protective activity. These results lend credence to combination therapy's superior ability to maintain nerve integrity after CCI.

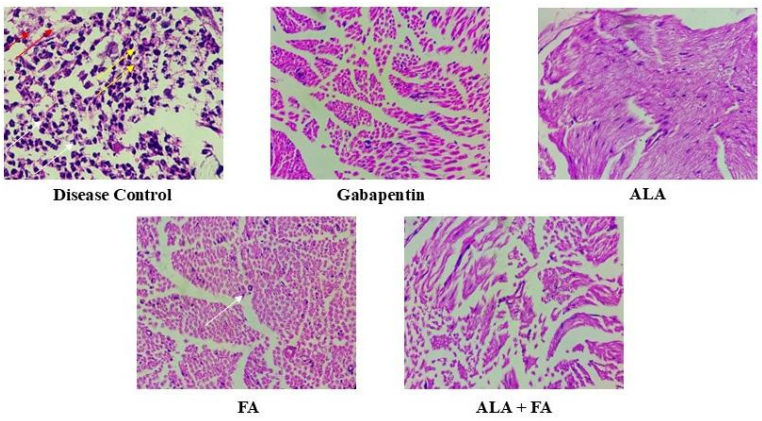

Figure 7: Changes in histopathology seen in the sciatic nerve after peripheral neuropathy caused by CCI.

4. DISCUSSION

Particularly within the model of the CCI, peripheral neuropathic pain (PNP) reflects a well-established preclinical framework simulating clinical neuropathic symptoms including mechanical allodynia, thermal hyperalgesia, and oxidative stress-induced nerve degeneration [37]. In the present work, gabapentin was compared against pharmacological interventions of ALA, FA, and their combination for their neuroprotective, antioxidant, and anti-inflammatory potential.

In peripheral nerves aggravating the pain experience, CCI triggers neuroinflammation, neuronal hyperexcitability, and immune infiltration [38]. Post-CCI behavioural testing showed notable increases in nociceptive sensitivity, validated by von Frey filaments inducing mechanical allodynia, so indicating tactile hypersensitivity in disease control rats [39]. Treatments including ALA, FA, and especially their combination greatly reduced the mechanical allodynia threshold, consistent with earlier studies on antioxidant-driven neuropathic modulation [40].

In CCI rats, cold allodynia assessed by the acetone test also showed increased pain responses suggestive of changed thermosensory perception [41]. While their combination produced similar results to gabapentin, ALA and FA treatments offered modest relief; hence, they reinforce synergistic neuroprotection by redox stabilisation and glial inhibition [42]. Reduced withdrawal responses mechanical hyperalgesia tested by pinprick supported the antihyperalgesic effects of the ALA+FA combination [43]. Moreover, the evaluation of thermal allodynia using Eddy's hot plate test showed much reduced latency in treated groups, so verifying restoration of pain thresholds [44].

Considered basic in the pathogenesis of CCI-induced neuropathy is oxidative stress. Damage-mediated mitochondrial dysfunction and immune cell invasion help to generate ROS, so upsetting redox equilibrium [38]. Reduced endogenous antioxidant defence, reflected by lower GSH, CAT, and SOD levels, with increased MDA, a lipid peroxidation, was found by analysis of antioxidant biomarkers to be CCI [45]. With even more efficacy in the ALA+FA group, ALA—known for its direct ROS scavenging and metal chelation ability—markedly restored SOD, CAT, and GSH levels, so lowering MDA concentration [40].

This redox restoration supports the theory that neuropathic pain results from mitochondrial ROS overproduction, so inducing $Ca2+$ influx, peroxynitrite synthesis, and neuronal death [44]. Both ALA and FA have shown capacity to downregulate Nrf2 expression and block NF-κB signalling, so supporting endogenous antioxidant defence [46]. Thus, the synergistic effect of ALA+FA can be ascribed to multi-pathway antioxidant mechanisms, validated by rather normalised oxidative stress biomarkers in this work.

Another pathogenic actor causing neuropathic pain is neuroinflammation. Key cytokine TNF-α upregulates ion channels, alters axonal transport, and activates glial cells to modulate nociception [47]. Confirmed its pro-nociceptive function, elevated TNF-α in sciatic nerve of disease control animal rats [48]. With the combination therapy producing better anti-

inflammatory effects, maybe through synergistic effect of inhibition microglial activation and cytokine transcription, ALA and FA treatment dramatically lowered TNF-α levels [41].

Further supporting biochemical and behavioural observations was histopathological study. Consistent with Wallerian degeneration and structural nerve damage post-CCI, sciatic nerve sections in the disease control group showed mononuclear infiltration, cystic dilation of the myelin sheath, and axonal lysis [43]. By contrast, FA showed only mild infiltration; ALA and combination-treated groups showed maintained nerve architecture. Histologically, gabapentin-treated tissues looked normal. These results confirm the test compound neuroprotective and regeneration action.

Taken together, our findings show in a CCI-induced neuropathic model strong analgesic, anti-inflammatory and antioxidants from ALA, FA especially in the combination. In treated groups mechanical allodynia, thermal hyperalgesia, and cold-induced pain responses were much reduced. Normalising redox biomarkers (SOD, CAT, GSH, and MDA) suggests strengthened antioxidant defences. TNF-α reduction verified even more the anti-inflammatory power of the treatments. At last, histomorphology confirmed functional restoration of sciatic nerve architecture.

5. CONCLUSION

The present study demonstrates that ALA and FA, individually and more effectively in combination, exhibit significant therapeutic potential in mitigating PNP induced by CCI. Behavioral assessments confirmed that combination therapy markedly reduced cold allodynia, mechanical allodynia and thermal hyperalgesia approximating efficacy of gabapentin. Biochemical analysis revealed that the combined administration of ALA and FA significantly restored redox homeostasis by enhancing antioxidant levels (CAT, GSH, SOD), reducing lipid peroxidation (MDA). Additionally, suppression of pro-inflammatory cytokine TNF-α and preservation of nerve structure in histopathological analysis corroborate the anti-inflammatory and neuroprotective effects. Collectively, these findings support the synergistic utility of ALA and FA as a promising adjunct or alternative therapeutic strategy in the management of peripheral neuropathic pain.

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