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Oleuropein Administration Alleviates Nerve Damage Induced by Sciatic Nerve Constriction Injury in Rats

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ABSTRACT

Objective: Oleuropein has previously been reported to provide neuroprotection in ischemia/reperfusion injury and to alleviate allodynia in neuropathic pain models. This study aims to assess the therapeutic potential of oleuropein, a polyphenolic compound found in olive trees, on sciatic nerve damage induced by chronic constriction injury (CCI) in male rats by comparing its efficacy with gabapentin, a widely used antiepileptic drug for the treatment of neuropathic pain.

Materials and Methods: Adult male Wistar rats were randomly allocated into control, neuropathic pain (NP), NP treated with 15 mg/kg oleuropein, and NP treated with 100 mg/kg gabapentin groups. The neuropathic pain model was induced by CCI via ligation of the sciatic nerve at four different locations, each separated by an interval of 1 mm. Treatments were administered via oral gavage for 14 days. Nociceptive behavior in response to thermal stimuli and the sciatic functional index (SFI) were assessed weekly. Nerve conduction velocity, sciatic nerve histology, and the level of thiobarbituric acid reactive substances were evaluated at the end of treatment.

Results: Sciatic CCI led to a reduction in nerve conduction and function, increased oxidative stress, and altered neuronal integrity, accompanied by decreased myelin sheath thickness and myelinated fiber diameter. Oleuropein administration significantly increased nerve conduction velocity, improved SFI values over time, significantly reduced oxidative stress, and enhanced neuronal integrity and myelination, surpassing the effects of gabapentin administration.

Conclusion: These findings highlight the therapeutic potential of oleuropein in nerve constriction injury and nerve damage, warranting further investigation into its use for nerve injury treatment.

Keywords: Constriction, gabapentin, myelin sheath, neuropathic pain, oleuropein, sciatic nerve.

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INTRODUCTION

Chronic and recurrent pain negatively affects quality of life, leading to reduced mobility, eventual loss of strength, an increased likelihood of depression or anxiety, a weakened immune system,

and impaired ability to concentrate, sleep, or interact with others.[1] Neuropathic pain, which results from nerve damage or dysfunction, is considered one of the most challenging pain conditions to treat[2] and a serious neurological disorder.[3] The pathophysiology of neuropathic pain is highly complex, involving fluctuations in neurotransmitter release, alterations in pain pathway regulation, sensitivity of afferent nociceptor terminals, and ectopic neuron activation.[4–6] Additionally, changes in the expression of ion channels, receptors, and chemokines in the dorsal root ganglia have also been implicated in the pathophysiology of neuropathic pain.[7–9] Various categories of drugs, including non-steroidal anti-inflammatory drugs, opioids, anticonvulsants, and antidepressants, have been explored for the treatment of neuropathic pain. However, their use is limited due to dose-dependent side effects,[10] and nearly two-thirds of patients do not respond to these treatments and are considered treatment-resistant.[11] Therefore, the development of an effective alternative treatment with minimal side effects is crucial for improving patient outcomes.

The olive tree (*Olea europaea*) is an evergreen, short tree that grows naturally in the Mediterranean region. Olive leaves and olive oil contain various compounds, including sterols, triterpene alcohols, and polyphenols such as flavonoids, phenolic acids, and secoiridoids. Oleuropein, a secoiridoid, is the most active phenolic component in olive leaves and oil.[12,13] Oleuropein has been reported to provide several pharmacological benefits due to its antioxidant, antibacterial, anti-inflammatory, anti-atherogenic, antitumor, hypoglycemic, and neuroprotective properties.[12,14,15] Oleuropein injection has been shown to provide neuroprotection against focal cerebral ischemia/reperfusion injury in mice by increasing anti-apoptotic activity[15] and to attenuate oxidative stress along with neurological deficits in a rat model of intracerebral hemorrhage.[16] Additionally, pretreatment with oleuropein has been reported to improve learning and memory by suppressing oxidative stress in a rat model of colchicine-induced cognitive dysfunction.[17] A 14-day treatment with 10 and 20 mg/kg oleuropein was observed to attenuate cold and mechanical allodynia in vincristine- and chronic constriction injury (CCI)-induced neuropathic pain in rats.[18]

Although its role in alleviating allodynia has been studied, the effects of oleuropein administration on nerve conduction and histology remain unclear. This study was conducted to evaluate the therapeutic potential of oleuropein in sciatic nerve damage induced by CCI, a model of neuropathic pain. Specifically, the efficacy of oleuropein on thermal nociceptive behavior, sciatic nerve conduction, oxidative stress, and nerve histology was examined in comparison to gabapentin, a clinically used drug for neuropathic pain treatment.

KEY MESSAGES

- Oleuropein alleviates nerve damage induced by chronic constriction injury more effectively than gabapentin by:
- Managing oxidative stress, Preserving nerve conduction and improving the sciatic functional index,
- Maintaining neuronal integrity and enhancing myelination.

MATERIALS AND METHODS

Animals

All experimental procedures in this study were approved by the Aydın Adnan Menderes University Animal Experiments Local Ethics Committee (Ethics Committee No: 64583101/2018/070). Thirty-six male Wistar rats (250–350 g) were obtained from the Aydın Adnan Menderes University Medical Faculty Experimental Animal Production Center and maintained in a controlled ambient environment with standard rat chow provided ad libitum. The sample size was determined using power analysis. The animals were randomly allocated into four groups: control (n=8), neuropathic pain (NP, n=10), NP treated with oleuropein (NP+OLE, n=9), and NP treated with gabapentin (NP+GP, n=9). The neuropathic pain model was established using sciatic nerve CCI.

Establishment of the Rat Model of Neuropathic Pain and Treatments

On the first day of the study, all animals were anesthetized via intraperitoneal injection of 60 mg/kg ketamine (Alfamine 10%, Alfasan, Netherlands) and 10 mg/kg xylazine (Alfazyme 2%, Alfasan, Netherlands). CCI was applied to the left sciatic nerve. In the experimental groups, an incision was made in the femoral skin on the dorsolateral part of the left hind limb. The muscles were separated using blunt dissection to expose the trunk of the sciatic nerve, which was loosely ligated with a 4–0 silk thread at four different locations, each with a 1 mm interval.[19] This procedure created a constriction that reduced circulation in the superficial epineural vasculature without completely stopping it. The facia, muscle, and skin surrounding the exposed sciatic nerve were then sutured in layers. In the control group, the left sciatic nerves were exposed, but no further ligation procedure was applied. The animals were left untreated for 24 hours, and treatments commenced the following day. A decline in nerve conduction and sciatic functional index, along with alterations in nerve histology, was observed in all animals subjected to CCI; therefore, no animals were excluded from the study.

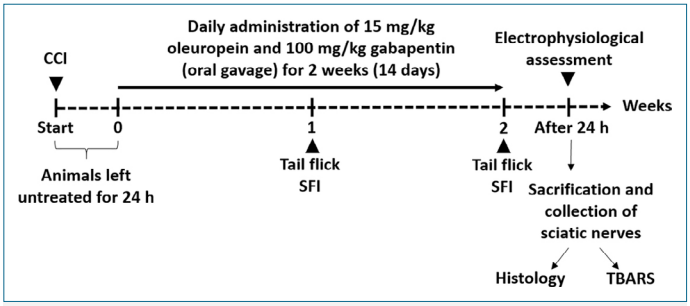

Figure 1. Schematic diagram illustrating the timing of experimental procedures applied to rats.

During the treatment period, physiological saline was orally administered to the rats in the control and NP groups for 14 consecutive days. The rats in the NP+OLE group received a daily oral dose of 15 mg/kg of oleuropein (Catalog# 21220, Cayman Chemical, Michigan, USA), while the animals in the NP+GP group received a daily oral dose of 100 mg/kg gabapentin (Neurontin, Pfizer, NY, USA). Both compounds were dissolved in physiological saline and administered for 14 days. The selected doses of oleuropein[17,20] and gabapentin,[21] as well as the administration schedule,[18] were based on previous studies. The body weights of the animals were recorded daily to ensure accurate dosing, and weekly changes in body weight were compared between groups. The timing of the procedures is graphically illustrated in Figure 1.

Sciatic Functional Index (SFI)

SFI calculation, a widely accepted method for evaluating functional recovery following nerve injury, was performed once a week on the 7th and 14th days of treatment to assess hind limb locomotor activity and sciatic nerve function following oleuropein and gabapentin administration. For SFI measurement, the hind paws of the animals were coated with blue ink, and the animals were allowed to move freely on blotting paper. Comparative measurements were made between the normal (N) and experimental (E) sides, with at least three paw prints evaluated per animal in a single measurement. From the paw prints, the following parameters were measured:

- Toe spread (TS): The distance between the first and fifth toes.
- Intermediate toe spread (ITS): The distance between the second and fourth toes.
- Print length (PL): The distance between the heel and the third toe.

SFI was then calculated for all experimental groups using the following formula:[22] SFI = -38.3 [(EPL - NPL) / NPL] + 109.5 [(ETS - NTS) / NTS] + 13.3 [(EITS - NITS) / NITS] - 8.8. An SFI score around 0 represents normal function, whereas an SFI score of -100 indicates complete dysfunction.[23]

Nociceptive Test

The tail-flick test was performed on the 7th and 14th days of treatment. Prior to the experiment, rats were habituated to restraint. A beam of radiant heat was focused on the lower one-third portion of the tail using an automated tail-flick device (May TF211-01 Tail Flick, Commat Ltd., Ankara, Türkiye), and the latency for tail withdrawal upon thermal stimulation was recorded in seconds. To prevent irreversible damage, the cut-off time was set to 10 seconds.

Electrophysiological Measurements

The rats were anesthetized via intraperitoneal injection of 60 mg/kg ketamine and 10 mg/kg xylazine. The trunk of the sciatic nerve was exposed through an approximately 3 cm incision at the mid-thigh level. The sciatic nerve was then placed onto *in vivo* electrodes, which were separated by a fixed distance of 1.1 cm and connected to the Biopac MP100 system (Biopac Systems Inc., USA). The sciatic nerve was stimulated with two supramaximal (0.1 ms, 1 Hz, 7 mV) electrical stimulations at proximal and distal positions. The resulting compound muscle action potentials were recorded from the gastrocnemius muscle, amplified, transferred to a computer, and analyzed using AcqKnowledge data acquisition and analysis software (Version 3.7.2, Biopac Systems Inc., USA). From the recordings, latencies were measured, and motor nerve conduction velocity (MNCV, m/s) was calculated by dividing the distance between the electrodes (1.1 cm) by the difference between proximal and distal latencies.[24,25]

Examination of Oxidative Stress

Prior to sacrifice, the sciatic nerves of the rats were dissected under anesthesia and stored at -80°C until use. The nerves were homogenized in radioimmunoprecipitation assay (RIPA) buffer (pH7.4, Merck, Germany) and centrifuged at 2,000 g for 15 minutes. Oxidative stress levels were assessed by measuring the malondialdehyde (MDA) concentration in the supernatants of the homogenates using a thiobarbituric acid reactive substances (TBARS) assay kit (Catalog No. 10009055, Cayman Chemical, USA) according to the manufacturer's protocol.

Examination of Nerve Histology

Histological analysis was performed as previously described.[24,26] Briefly, after fixation in 10% formaldehyde (Tekkim, Türkiye), the sciatic nerves were embedded in paraffin (Merck, Germany), and 5 µm-thick tissue sections were obtained using a microtome (Leica, RM 2265, Germany). The sections were stained using a Luxol Fast Blue (LFB) staining kit (LBC-1-IFU, ScyTek Laboratories Inc., USA) following the manufacturer's instructions. The stained sections were then examined, and images were captured using a light microscope (Olympus CX23, Japan) at 10x and

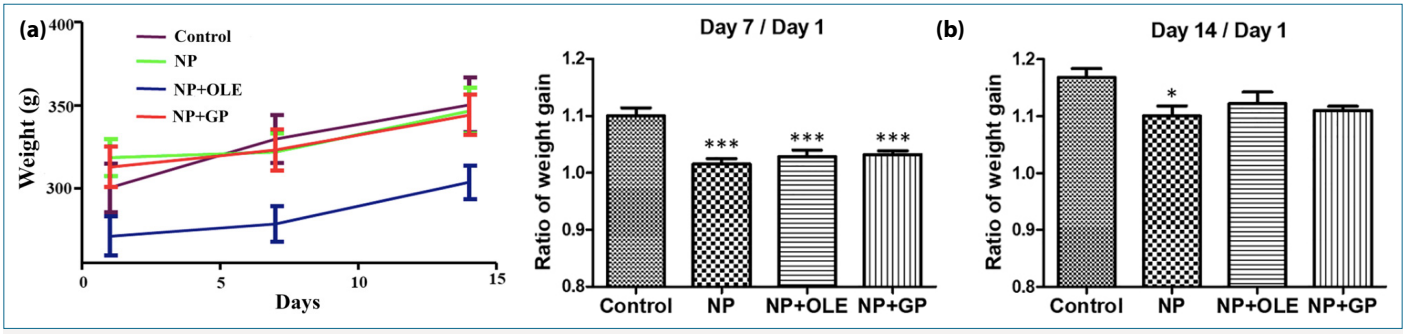

Figure 2. Weekly changes in body weights (g) of experimental groups **(a)**, and the ratios of weight gain on the 7th and 14th days compared to the 1st day of the experiment **(b)**. NP: Neuropathic pain, NP+GP: NP treated with gabapentin, NP+OLE: NP treated with oleuropein.

40x magnifications. The LFB-stained images were analyzed using ImageJ software (NIH, USA) to measure axon diameter, myelinated fiber diameter, and myelin thickness.[24]

Statistical Analysis

Power analysis was used to determine the sample size for the study, with the error rate, study power, and standardized effect size set at 0.05, 0.80, and 1.614, respectively, based on previously published data.[26] The obtained data were expressed as mean±standard deviation. To select the appropriate statistical tests, the Kolmogorov-Smirnov test was performed using the Statistical Package for the Social Sciences (SPSS) software (SPSS Version 16, IBM, USA), confirming that the data followed a normal distribution. Intergroup comparisons were conducted using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test to determine significance when variances showed significant differences among experimental groups. For the intergroup comparisons of nociceptive tail-flick test results, two-way ANOVA was performed, followed by Sidak's multiple comparison test to determine significance. In the figures, significance levels were denoted as follows: an asterisk (*) indicates a comparison with the control group, a dagger (†) indicates a comparison with the NP group, and a double dagger (‡) indicates a comparison with the NP+OLE group. A statistical difference was considered significant at $p<0.05$.

RESULTS

Administered Agents Did Not Significantly Affect Body Weight

The weekly changes in body weight gain among the experimental groups are shown in Figure 2a. A continuous increase in body weight was observed in all groups, with the highest increase recorded in the control group. Figure 2b presents the ratios of weight gain on the 7th and 14th days relative to the first day of the experiment. As shown, weight gain was considerably higher in the control group compared to the other groups on the 7th day. By the 14th day, weight gain in the NP group was significantly lower than in the control group ($p<0.05$), while body weight gain followed a similar trend in the treatment groups.

Oleuropein Significantly Improved Sciatic Nerve Conduction

Representative compound muscle action potentials recorded from the gastrocnemius muscles after distal and proximal stimulation are shown in Figure 3. Sciatic MNCVs of the operated left limb and unoperated contralateral limb are presented in Figure 4. MNCV values were significantly reduced in the NP group (34.69±2.63 m/s) compared to the control group (57.06±1.27 m/s) ($p<0.001$), indicating a marked decline in nerve conduction following CCI. However, MNCV values significantly increased in the oleuropein-treated (40.00±2.63 m/s, $p<0.01$) and gabapentin-treated (43.90±2.25 m/s, $p<0.001$) groups compared to the NP group. Gabapentin administration was observed to be more effective than oleuropein in enhancing nerve conduction velocity ($p<0.05$). Sciatic MNCV values in the unoperated limb showed a tendency to increase in the oleuropein-treated group (59.39±2.39 m/s) and were significantly increased ($p<0.01$) in the gabapentin-treated group (61.16±2.92 m/s) compared to the control (56.07±1.85 m/s) and NP (56.57±2.56 m/s) groups. This finding suggests that gabapentin enhances nerve conduction even in the absence of nerve damage.

Oleuropein Administration Led to a Comparatively Greater Improvement in SFI

Sciatic nerve function was assessed through weekly SFI analysis. Sample paw prints of healthy and nerve-constricted groups on the 14th day are shown in Figure 5a, while the changes in SFI values on the 7th and 14th days of treatment are presented in Figure 5b. A significant decrease

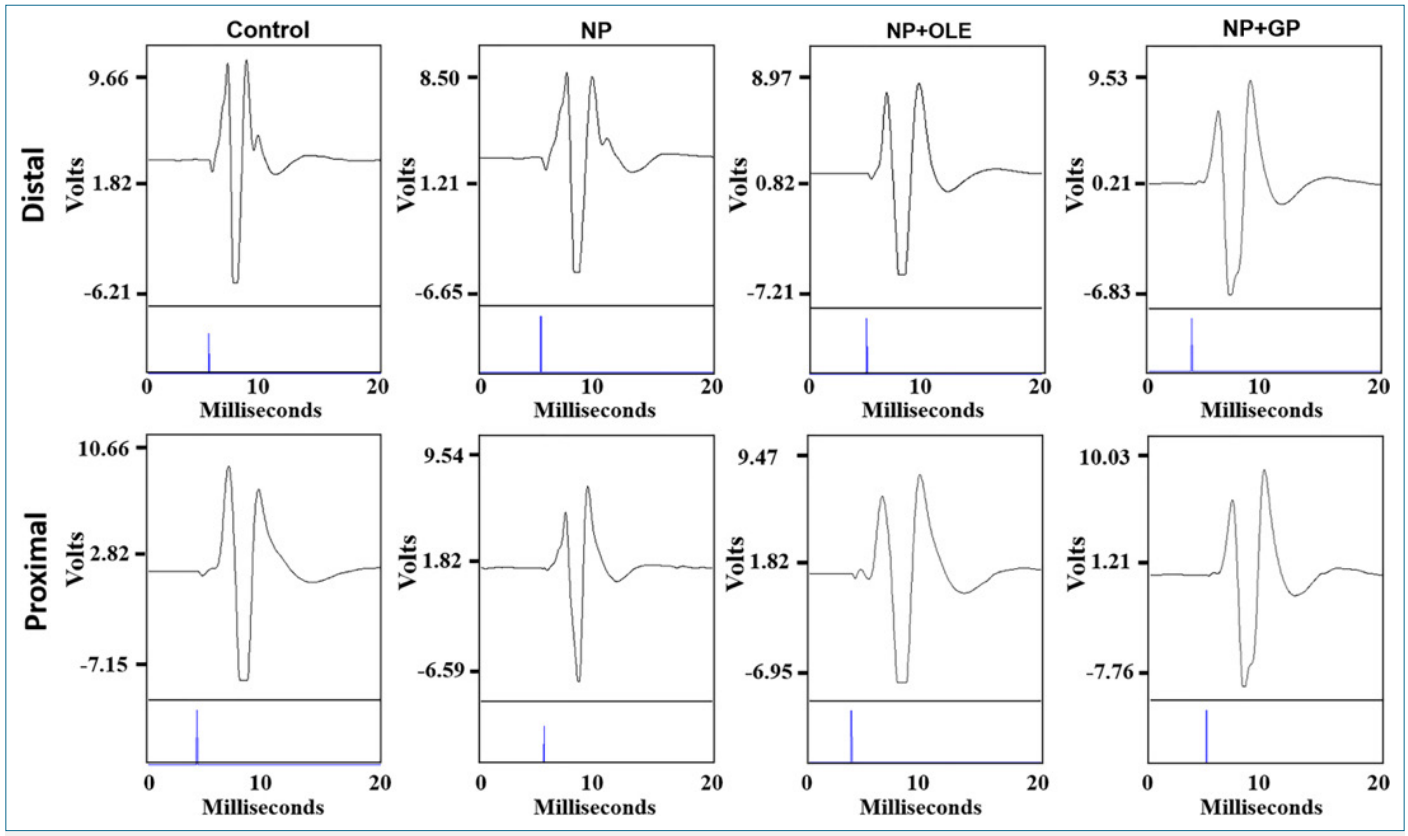

Figure 3. Representative compound muscle action potentials recorded from the gastrocnemius muscles of control, NP, NP+OLE, and NP+GP groups after distal and proximal stimulation. The blue lines below the recordings indicate electrical stimulation. NP: Neuropathic pain, NP+GP: NP treated with gabapentin, NP+OLE: NP treated with oleuropein.

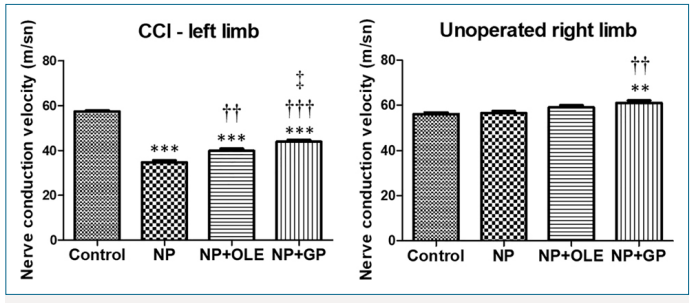

Figure 4. Sciatic motor nerve conduction velocities (MNCVs) (m/s) of the operated left limb and the unoperated contralateral limb. NP: Neuropathic pain, NP+GP: NP treated with gabapentin, NP+OLE: NP treated with oleuropein.

($p<0.05$) in SFI was observed in all groups subjected to CCI compared to the control group. Throughout the measured time intervals, SFI values increased more slowly in the NP and gabapentin-treated groups. However, a comparatively steeper increase in SFI was noted in the oleuropein-treated group between days 7 and 14.

Oleuropein Administration Significantly Reduced Oxidative Stress

Oxidative stress in the sciatic nerves was analyzed by measuring TBARS levels, specifically malondialdehyde (MDA), a marker of lipid peroxidation and oxidative damage (Fig. 5c). A significant increase in sciatic nerve TBARS levels was observed in the NP ($p<0.001$) and gabapentin-treated ($p<0.01$) groups compared to the control group. However, a significant decrease was observed in the oleuropein-treated group ($p<0.01$) compared to the NP and gabapentin-treated groups. This finding indicates that oleuropein is a potent agent in reducing oxidative stress induced by CCI.

No Significant Differences Were Observed in Nociceptive Pain Behavior

Nociceptive pain behavior was assessed on the 7th and 14th days after sciatic nerve constriction, and no significant differences were detected among the groups in the tail-flick test (Table 1). The response to thermal stimuli was comparatively shorter in the NP group, whereas response times tended to be slightly longer in the oleuropein- and gabapentin-treated groups.

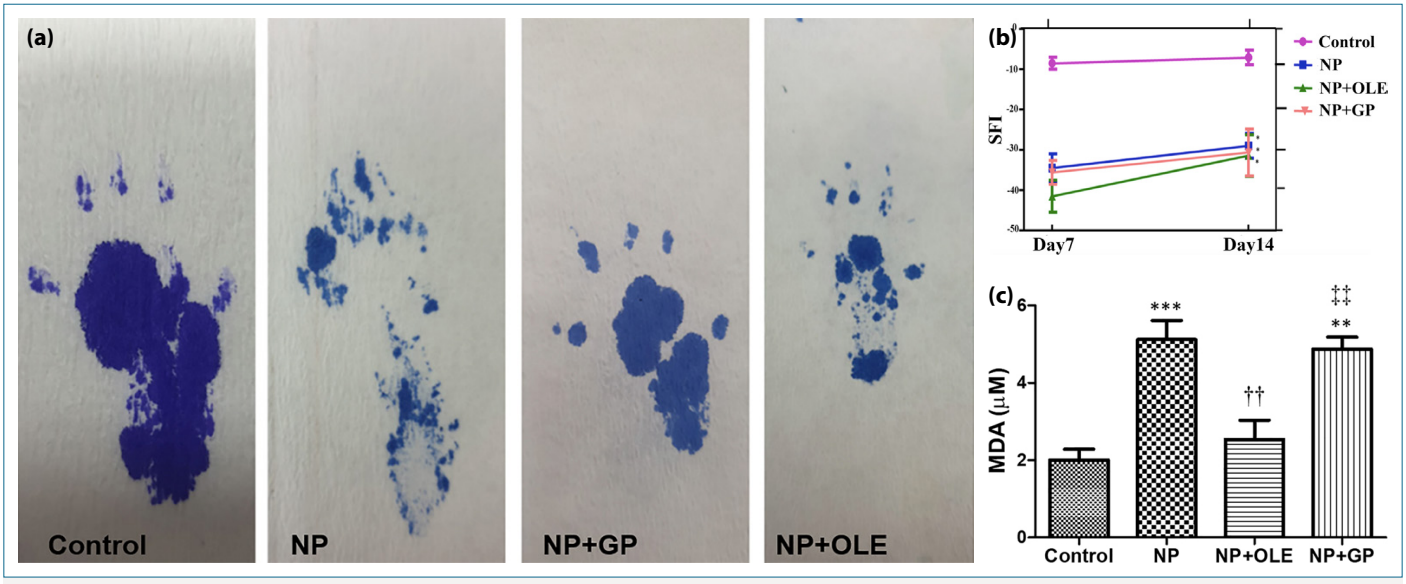

Figure 5. Sample paw prints of healthy and nerve-constricted groups on the 14th day **(a)**, changes in sciatic functional index (SFI) values on the 7th and 14th days of treatment **(b)**, and alterations in sciatic nerve malondialdehyde (MDA) levels (μM) in the experimental groups **(c)**. NP: Neuropathic pain, NP+GP: NP treated with gabapentin, NP+OLE: NP treated with oleuropein.

Table 1. Nociceptive tail-flick test results recorded as latency (seconds) of the response to thermal stimuli

Time	Control group (n=8)	NP group (n=10)	NP+OLE group (n=9)	NP+GP group (n=9)	p
7th day	5.11±1.61	4.70±1.11	5.02±0.98	4.60±1.11	0.77
14th day	5.31±1.07	4.67±0.75	4.96±1.15	4.96±1.45	0.90
p	0.99	0.99	0.99	0.95	
Time effect: p=0.69					
Column effect: p=0.57					
Column x time interaction effect: p=0.94					

NP: Neuropathic pain; NP+GP: NP treated with gabapentin; NP+OLE: NP treated with oleuropein. Data are expressed as mean ± standard deviation. Intergroup comparisons were performed using two-way analysis of variance (ANOVA) followed by Sidak's multiple comparison test to determine the significance level. The analysis revealed no significant differences between the groups.

Oleuropein Administration Led to an Improvement in Axonal Regeneration, Neuronal Integrity, and Myelin Thickness

LFB staining was used to examine sciatic nerve histology and myelination (Fig. 6), and the measurements from LFB-stained sections are presented in Table 2. In contrast to the normal perineurium layer with smooth boundaries and an even axonal distribution observed in the control group (Fig. 6a, b), the NP group exhibited regional irregularities and fragmentation in the perineurium layer at the site of compression. Additionally, dense axonal atrophy, irregular axonal arrangement, and uneven axonal distribution within the nerve fiber bundle were observed (Fig. 6c, d). There was also an occasional increase in Schwann cells in the myelinated axons in the NP group. In the gabapentin-treated group, the perineurium layer appeared more regular compared to the NP group but remained irregular compared to the control group (Fig. 6e,f). The perineurium layer was significantly dilated towards the endoneurium at the site of compression, with noticeable axonal atrophy and a decrease in the density of myelinated axons. However, axonal density appeared to be more evenly distributed in this group. In the oleuropein-treated group, the perineurium layer appeared more regular compared to the NP and gabapentin-treated groups, with a reduced degree of dilation towards the endoneurium at the site of compression (Fig. 6g, h). Axonal density within the nerve fiber bundle was more evenly distributed, resembling that of the control group. Although some areas of axonal atrophy and swelling were observed, an increase in Schwann cell numbers

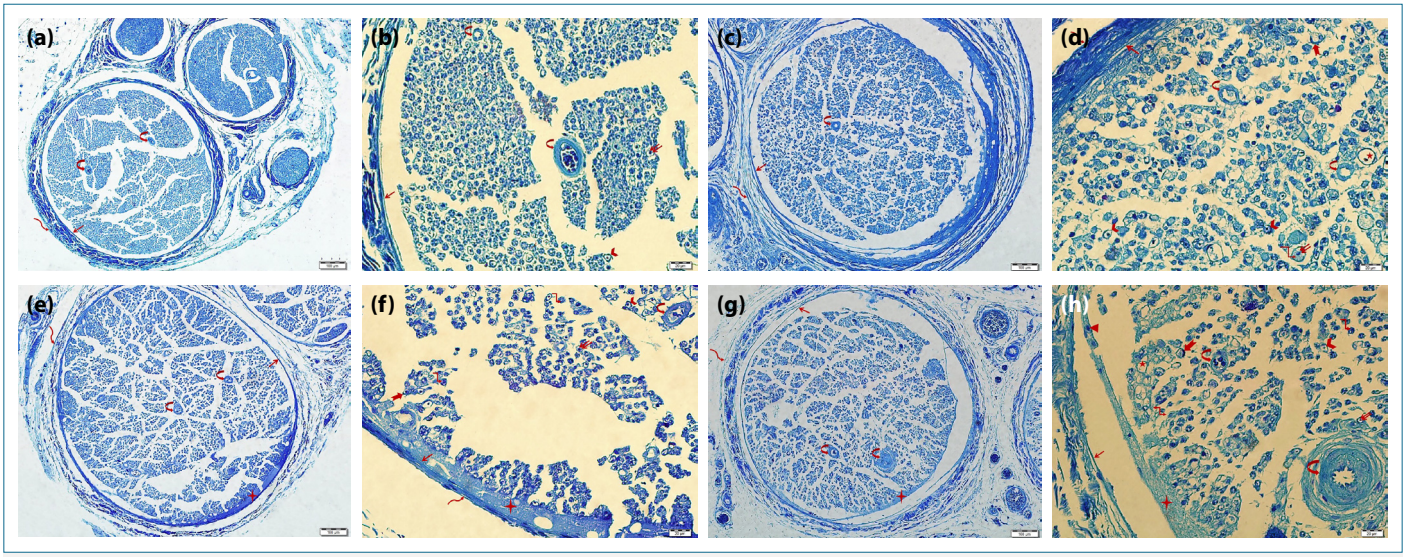

Figure 6. Representative images of Luxol Fast Blue (LFB)-stained sciatic nerve tissue sections at 10x and 40x magnification, respectively, in the control **(a, b)**, NP **(c, d)**, NP+GP **(e, f)**, and NP+OLE **(g, h)** groups. NP: Neuropathic pain, NP+GP: NP treated with gabapentin, NP+OLE: NP treated with oleuropein. A, C, E, and G show the nerve fiber bundles, while B, D, F, and H display axon and myelin structures. In the control group (A, B), the layers are well-organized, axons are evenly and regularly distributed, and the myelin sheath thickness is normal. The NP group (C, D) exhibits significant axonal atrophy, irregular axonal arrangement, a marked reduction in myelin sheath thickness, and pronounced perineural dilatation extending toward the endoneurium. In the NP+GP group (E, F), the perineurium remains irregular compared to the control, and while axons appear more organized, they remain irregular in comparison to the control. In the NP+OLE group (G, H), axon density is evenly distributed, similar to the control, with reduced perineural dilatation and increased myelin sheath thickness. The magnification scale bar is 100 µm for 10x magnification and 20 µm for 40x magnification. Discriminated features are indicated in the images as follows: Perineurium (↘); epineurium (↘); vascular structures (⊆); perineurial dilatation (✦); axonal swelling (★); Schwann cell (➡); axon (↘); myelin (↘); myelin sheath (➡).

Table 2. Changes in axon diameter (µm), myelin sheath thickness (µm), and myelinated fiber diameter (µm) calculated from Luxol fast blue (LFB)-stained section images in the experimental groups

Measurement (µm)	Control group (n=8)	NP group (n=10)	NP+OLE group (n=9)	NP+GP group (n=9)	p
Axon diameter	6.42±0.85[a]	3.92±0.72[b]	5.43±0.95[c]	4.43±0.72[d]	<0.001
Myelin sheath thickness	4.00±0.54[a]	3.13±0.54[b]	4.42±0.79[c]	3.29±0.66[b]	<0.001
Myelinated fiber diameter	13.44±1.61[a]	10.77±1.39[b]	13.09±1.97[a]	11.68±1.37[b]	<0.001

NP: Neuropathic pain; NP+GP: NP treated with gabapentin; NP+OLE: NP treated with oleuropein. Data are expressed as mean±standard deviation. Intergroup comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test to determine the significance level. Similar superscript letters in the same row indicate no significant difference, while different letters indicate statistical differences.

around the myelin sheath was noted, along with enhanced axonal regeneration. The myelin sheath thickness, axon diameter, and myelinated fiber diameter were all significantly reduced ($p<0.001$) in the NP group. Oleuropein administration resulted in a significant increase ($p<0.001$) in these parameters compared to the NP group (Table 2). Gabapentin treatment also led to a slight increase in these measurements, but the improvement was not as pronounced as in the oleuropein-treated group.

DISCUSSION

Epidemiological studies have revealed a strong link between disease prevention and treatment and diets rich in polyphenols, as individuals who consume such diets tend to have better overall health and lower mortality rates than those who do not.[27,28] Oleuropein, a phenolic compound found in olive leaves and olive oil, has been demonstrated to possesses unique properties that enable it to combat oxidative stress

and inflammation, modulate the autophagy pathway, and intervene in neurodegenerative processes by preventing the onset of degeneration.[12,13,29,30] Consequently, this study was conducted to evaluate the therapeutic potential of oleuropein in the treatment of CCI of the sciatic nerve in rats, comparing its effectiveness with gabapentin, a clinically used drug for neuropathic pain treatment. The data demonstrated that oleuropein was effective in alleviating nerve damage by enhancing sciatic nerve conduction and function. Oleuropein administration was observed to be more effective than gabapentin in reducing oxidative stress, improving the sciatic functional index, promoting axonal regeneration, and preserving myelination and neuronal integrity.

Neuropathic pain can develop as a result of lesions, compression, or underlying conditions such as diabetes or cancer. The increase in reactive oxygen species, inflammation, and organelle dysfunction has been implicated in the pathophysiology of nerve damage associated with neuropathies. Previous studies have reported that among the biological activities of oleuropein, its most notable properties are antioxidant,[31,32] anti-inflammatory,[30,33] and anticancer effects.[34] In the present study, oleuropein administration was observed to produce a more effective response than gabapentin in reducing TBARS levels and preventing lipid peroxidation, which is directly correlated with oxidative damage.[35]

Another proposed mechanism of neuropathic pain involves pathways activated through the inhibition and dysfunction of voltage-gated calcium channels.[36] *Ex vivo* application of oleuropein has previously been reported to enhance intracellular calcium levels and facilitate N-methyl-D-aspartate receptor-independent long-term potentiation in the mouse hippocampal CA1 region.[37] In the present study, oleuropein administration significantly increased ($p<0.01$) sciatic MNCV, which had been reduced by CCI in the operated limb. Gabapentin administration resulted in a more pronounced increase in MNCV in both the operated and unoperated limbs. The increase in MNCV indicated an improvement in sciatic nerve function, which was further assessed through the SFI. However, the efficacy of both oleuropein and gabapentin in restoring nerve function remained limited, as SFI values in the experimental groups were still similar to those of the NP group on the 14th day of treatment. This finding suggests that the treated animals in the experimental groups were still unable to use their paws as effectively as the control animals. Nonetheless, considering the measurements taken between the 7th and 14th days of treatment, there was a noticeable increase in SFI values in the oleuropein-treated group, indicating a beneficial role of oleuropein administration following CCI. Conversely, no significant differences in nociceptive pain perception were observed among the experimental groups in the automated tail-flick test, although a slight increase in latency was noted in the oleuropein-treated group compared to the NP group. The effects of oleuropein administration on thermal nociceptive pain perception should be further investigated in future studies.

Previously, oleuropein has been reported to exert protective effects against acute and chronic neurodegeneration,[13] inhibit the impact of toxic substances on the nervous system,[38] and reduce pain associated with diabetes.[39] Given its previously documented role in preventing neuronal apoptosis[39] and promoting the protection and formation of the myelin sheath,[40] it is likely that oleuropein administration elicited a similar effect in improving sciatic nerve conduction and function following CCI in the present study. Furthermore, histological examination of the sciatic nerves revealed that oleuropein administration led to an increase in myelin sheath thickness and stimulated axonal regeneration compared to the NP and gabapentin-treated groups. Additionally, the irregular axonal arrangement and uneven axonal distribution within fibers observed in the NP group were restored following oleuropein administration.

In summary, the therapeutic effects observed following 15 mg/kg oleuropein treatment in CCI are attributable to its ability to effectively control oxidative stress, enhance axonal regeneration, and maintain myelination and neuronal integrity. These effects collectively contribute to the improvement of sciatic function and conduction. Our findings further highlight the beneficial role of oleuropein in preserving neuronal integrity and mitigating CCI-induced dysfunction in the sciatic nerve. This contribution is crucial for further elucidating the mechanisms of action of oleuropein in nerve conduction in future studies. However, certain limitations of this study should be noted. This preliminary study included a relatively small sample size, which may not be sufficient to fully characterize the therapeutic potential of oleuropein. Nevertheless, further research is necessary to determine the exact molecular mechanisms underlying oleuropein's effects, particularly its potential role in regulating pathways related to apoptosis, neuronal survival, inflammation, and myelination. Additionally, this study focused only on the short-term effects of a single dose (15 mg/kg) of oleuropein. Future studies exploring different doses and extended administration periods may provide further insights into the potential for enhanced functional recovery and pain relief following sciatic nerve constriction.

CONCLUSION

The findings suggest that 15 mg/kg oleuropein administration effectively improves nerve conduction and function, as well as supports myelination and neuronal integrity in chronic nerve constriction, demonstrating greater efficacy than gabapentin. Therefore, oleuropein supplementation may be considered as a potential treatment strategy for nerve constriction injury and neuropathic pain. Further research is required to clarify the underlying mechanisms responsible for the observed beneficial effects of oleuropein.

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