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Research Paper

# Evaluation of the Neuroprotective Effect of Eugenol on the Improvement of Sciatic Nerve Injury in Rats

Soheila Alipoor [1], Fariba Mahmoudi [1,*], Arash Abdolmaleki [1,2], Khadijeh Haghighat [1], Milad Solouki [1]

[1] *Department of Biology, Faculty of Science, University of Mohaghegh Ardabili. Ardabil, Iran.*
[2] *Department of Engineering Sciences, Faculty of Advanced Technologies, University of Mohaghegh Ardabili. Namin, Iran.*

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*** Corresponding author:**
Fariba Mahmoudi, Department of Biology, Faculty of Science, University of Mohaghegh Ardabili. Ardabil, Iran. E-mail: f.mahmoudi@uma.ac.ir

## ABSTRACT

**Background:** Sciatica is a common human disorder associated with chronic pain. To speed up the recovery of damaged sciatic nerve, using plant derivatives, such as Eugenol can be effective due to its known neuroprotective properties. This study, investigated the effects of Eugenol on the regenerative process of experimentally induced sciatic nerve injury in rats.

**Methods:** Twenty eight male Wistar rats weighing 250-300 grams were divided into four groups of seven rats each. The control and sciatica model groups received normal saline only. The other two groups of sciatica model received Eugenol intraperitoneally at either 50 or 100 mg/kg daily for one week. Behavioral tests were also performed, and samples of the gastrocnemius muscles were removed under anesthesia for histopathological examinations.

**Results:** The pace of nerve injury improvement and recovery of both sensory and motor functions increased significantly in Eugenol-treated groups compared to both the sciatica model and control groups.

**Conclusion:** Eugenol administration improved the repair and regenerative process of the induced peripheral sciatic nerve damage in rats. Therefore, this compound may be considered as a beneficial treatment option for sciatica in humans.

**Keywords:** Eugenol; Neural Regenerative Process; Neuroprotective Effect; Sciatic Nerve injury

## Introduction

Peripheral nerves consist of the axons of various cell types that are essential for optimal sensory and motor functions. The peripheral nervous system can regenerate by itself to some extents, and the successful recovery of neural tissue requires complex cellular and molecular events. In this context, the repair process begins from the distal part of the damaged nerve tissues, and is completed gradually concurrent with the secretion of various cellular mediators. Sciatica is one of the most common peripheral nerve injuries. The pain that is caused by sciatic nerve damage leads to both physical and mental disorders in humans. Pelvic bones fractures, lumbar disc herniation, tumors and infections can contribute to the sciatic nerve damages. Also, sciatic injuries are associated with arterial damage, sensory-motor dysfunction, and muscle paralysis (1, 2). Although injured peripheral nerves recover to some extents by themselves, in case of extensive damages, complementary drug therapy is also required. Patients with peripheral nerve damages are often prescribed anti-inflammatory drugs, corticosteroids, muscle relaxants, and opioid pain medications. In the absence of optimal improvement, surgery may be considered as an alternative treatment option (3).

Since ancient times, it was popular to use medicinal plants to treat various diseases. In recent years, the tendency to use herbal medicines and substances with antioxidant and anti-inflammatory properties has increased considerably. This is because these medicines are both inexpensive and easy to access, and have fewer side effects than chemical drugs (4). Also, the physiology of the human body is more compatible with herbal medicines. Eugenol is a phenylpropene compound that is found in various plants such as basil, cinnamon, bay leaves, and nutmeg. Eugenol, a methoxy-phenol with a short hydrocarbon chain, is

the main component (80-90%) of clove oil. Eugenol, as an antioxidant and anti-inflammatory agent, has promising potentials to eliminate oxidative stress and inflammatory reactions. Also, the antidepressant activity of Eugenol on the central nervous system and its effect as a local anesthetic has already been known. Eugenol has analgesic effect due to selectively binding to capsaicin receptors, and having anticonvulsant and antifungal activities (5, 6). Also, several studies have reported the neuroprotective and neuromodulating properties of Eugenol as a natural phenolic compound (7-9).

Aim of the Study: Due to the unique therapeutic properties of Eugenol, this study was planned to investigate its effects on the repair process of induced sciatic nerve damage in Wistar rats. Such a study has not been undertaken previously.

## Materials and Methods

Chemicals and Experimental Animals: Eugenol was purchased from Sigma-Aldrich (Darmstadt, Germany). In this study, twenty-eight male Wistar rats, weighing 250 to 300g, were examined at Iran University of Medical Sciences (Tehran, Iran). The rats were kept at laboratory environment for one week for adaptation prior to conducting the experiments. The laboratory temperature was set at 22-25°C under 12 hours of alternating light and dark cycles.

Induction of Sciatic Nerve Injury: In order to develop the sciatic crush injury model, the rats were first anesthetized with either Ketamine or Xylazine. Next, the sciatic nerve in each rat was exposed by splitting the muscle tissue layers covering it. For the purpose of sciatic nerve injury, it was compressed above its trifurcation point. The nerve compression was induced in all rats, using the same pressure method. Finally, the muscle layers and skin were sutured at the surgical site and covered with dressing that contained 3% tetracycline antibiotic ointment to prevent infection. After surgery, all rats were injected with buprenorphine at one mg/kg for two successive days to reduce their pain (10).

Animal Groups and Treatment: Following the surgery, rats were divided into four groups of seven each. The control and sciatic model groups received normal saline (0.5 ml daily) intraperitoneally (IP) for one week. The two sciatic model groups were injected with Eugenol at either 50 or 100 mg/kg, IP. All injections were made daily between 9 and 11a.m. over a one-week period. After the completion of the treatment, samples of the gastrocnemius muscle at the surgical site were removed to examine the histological changes under light microscopy.

### Behavioral Tests

Walking Track Test: All groups were evaluated by Walking Track test twice a week over two consecutive months. For this test, each animal's hind paws were smeared with ink, and allowed to move along an experimental corridor, the dimensions of which were 25x10x100 cm. Then, the pattern of each animal's footprints on the corridor surface was documented and analyzed. Finally, the sciatic function index (SFI) was determined for each animal, using Bain's formula (11).

Hotplate Test: To evaluate the pain threshold and functional recovery rate of sensory neurons, we performed hot plate tests. The rats' injured paws were placed separately on a plate heated at 52 ±1°C. The response latencies were recorded as the hind paw jumped in each animal, and the timing of responses was manually recorded, using a stopwatch. Also, to avoid further damage, the test's cutoff time was limited to 12 seconds.

Histomorphometric Analyses: For this analysis, a piece of the damaged sciatic nerve was removed, fixed in 1% osmium tetroxide, and dehydrated in 70% ethanol. The fixed nerve samples were embedded in resin, and stained with 1% toluidine blue. Finally, the samples' blocks were thin sectioned at 10µm, and examined under light microscopy (LEO-1430VP; Germany).

Histological Evaluation of Gastrocnemius Muscles: For histological evaluations, 10µm thick sections from the dissected gastrocnemius muscle samples were prepared, and stained with Masson's trichrome according to standard methods (11). Finally, the dimensions, number of muscle fibers, and the fibrous connective tissue among the muscle fibers were measured under light microscopy.

Gastrocnemius Muscle Mass: To evaluate atrophy, samples of the gastrocnemius muscles were isolated from both rats' legs, and were immediately weighed using a digital scale. Then, the data were analyzed using the weight ratios of the muscle mass in the operated leg to that of the non-operated leg for each rat.

Statistical Analyses: The collected data in each category were analyzed by one way ANOVA using SPSS software, version 23. Also, Tukey's post hoc test was used to determine the significant differences among the various study groups at $P<0.05$. Finally, the results were expressed as means ± standard deviations of the means (±SEM).

## Results

Effect of Eugenol on Sensory and Motor Recovery: Starting the second week after crushing the nerve, the sciatic functional index (SFI) in all of the tested groups significantly reached the lowest value in rats. In weeks 4, 6 and 8, the SFI increased in the groups that had received 50 or 100 mg/kg Eugenol compared to the sciatic control model. However, the improvement in SFI was only significant in rats that received 100 mg/kg Eugenol for week 8 ($P<0.05$; Figure 1).

As shown in Figure 2, starting from the second week onward, the groups that were treated with Eugenol had better sensory performance than those of the sciatic model group, and the improvement in recovery was statistically significant. However, the highest recovery in sensory function was achieved in rats that received Eugenol at 100 mg/kg for eight weeks (P<0.05).

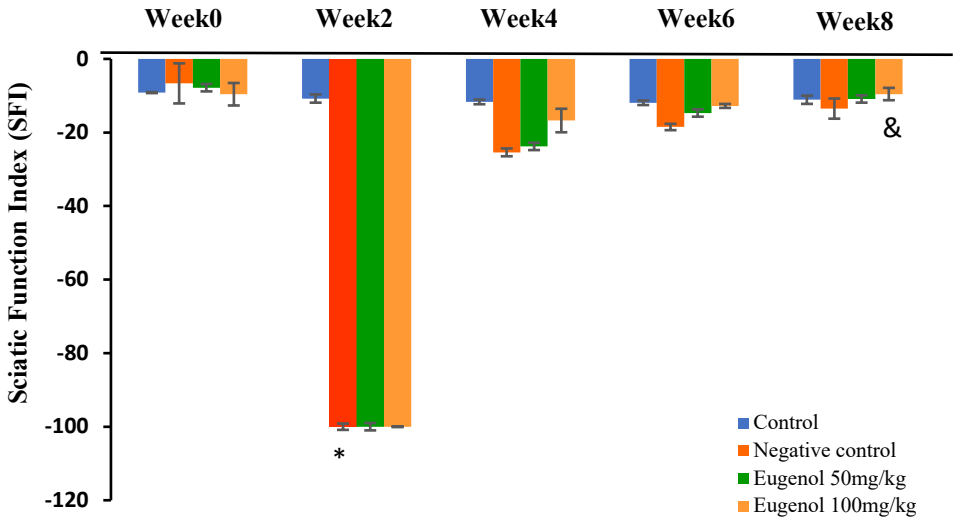


**Figure 1.** Assessment of the sciatic nerve recovery post-injury based on sciatic function index (SFI). Analyses were made at 2, 4, 6, and 8 weeks after surgery. Data are shown as means ± standard error of the means (SEM). * = Compared to the controls, & = Compared to the negative group (*P*<0.05).

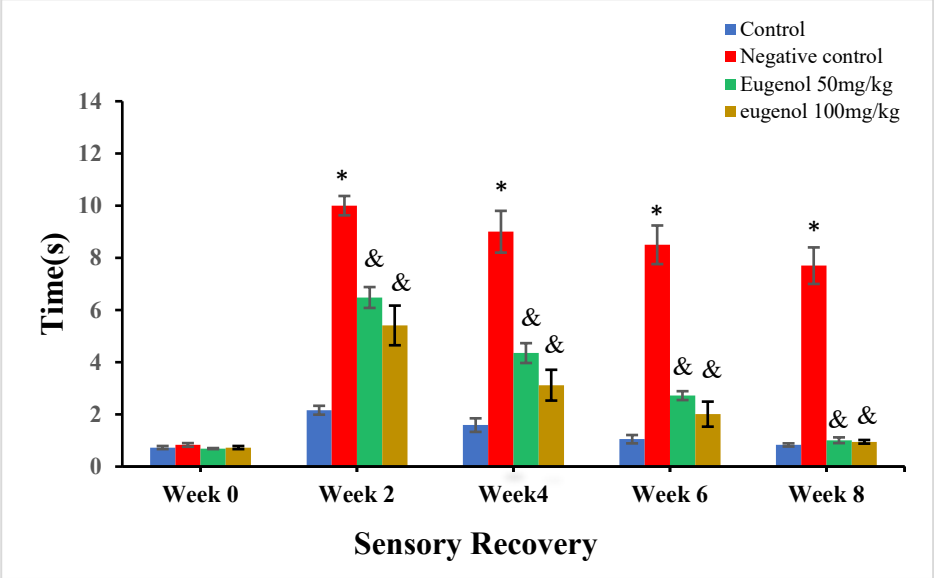


**Figure 2.** Assessment of sensory recovery of the rat's injured sciatic nerve. Analyses were made at 2, 4, 6, and 8 weeks after surgery. Data are shown as means ± standard error of the means (SEM). * = Compared to the controls, & = Compared to the negative group (*P*<0.05).

Effect of Eugenol on Gastrocnemius Muscle Mass: Based on the collected data, muscle atrophy was evident in all groups that underwent surgery on their sciatic nerve. The extent of muscle atrophy in both groups treated with Eugenol at 50 or 100 mg/kg declined insignificantly compared to that in the sciatic model group (Figure 3).

As shown in Figure 4, a decline occurred in the cross-sectional areas of the atrophied muscles. The results demonstrated that in the rat groups treated with Eugenol, the extent of muscle atrophy was lower than that of the sciatic model group. Also, the highest level of muscle fibrosis was observed in the sciatic model group. In the rat group that received

Eugenol at 100 mg/kg the recovery process improved significantly.

Histomorphometric Analyses: The analyzed data indicated that at eight weeks after surgery, the morphometric values in the group treated with Eugenol increased significantly compared to those of the sciatic model group. In addition, the number of myelinated nerve fibers increased in all treated groups compared to that of the sciatic model group. However, the increase in the number of myelinated nerve fibers was significant only in the group that received 100 mg/kg Eugenol ($P<0.05$). The myelin sheath thickness increased in the groups that were treated with Eugenol at either 50 or 100 mg/kg compared to those of the sciatic model group. However, this increase was only statistically significant in rats that received 100 mg/kg Eugenol ($P<0.05$). See Table 1 and Figure 5.

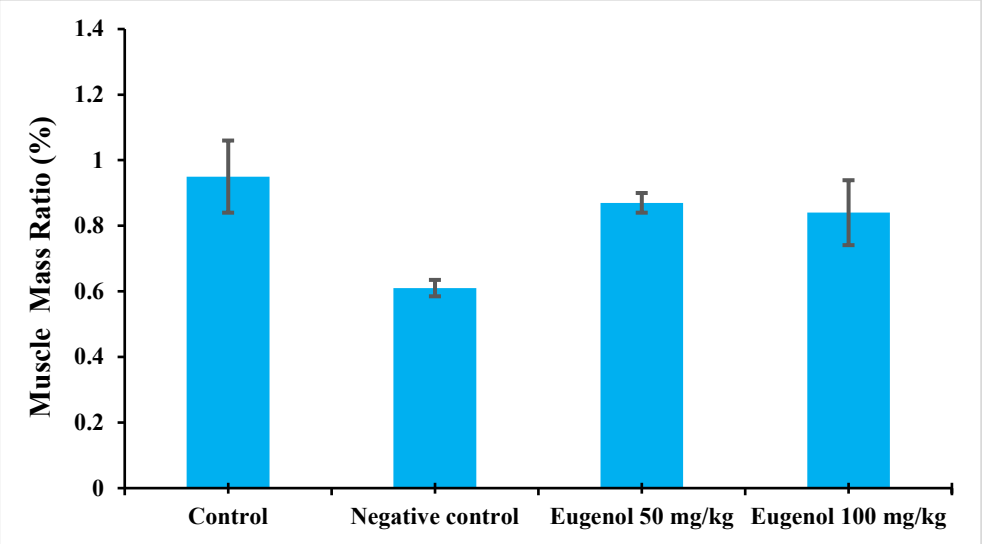


**Figure 3.** Assessment of muscle atrophy in various rat groups based on the muscle mass ratio at 8 weeks post-surgery.

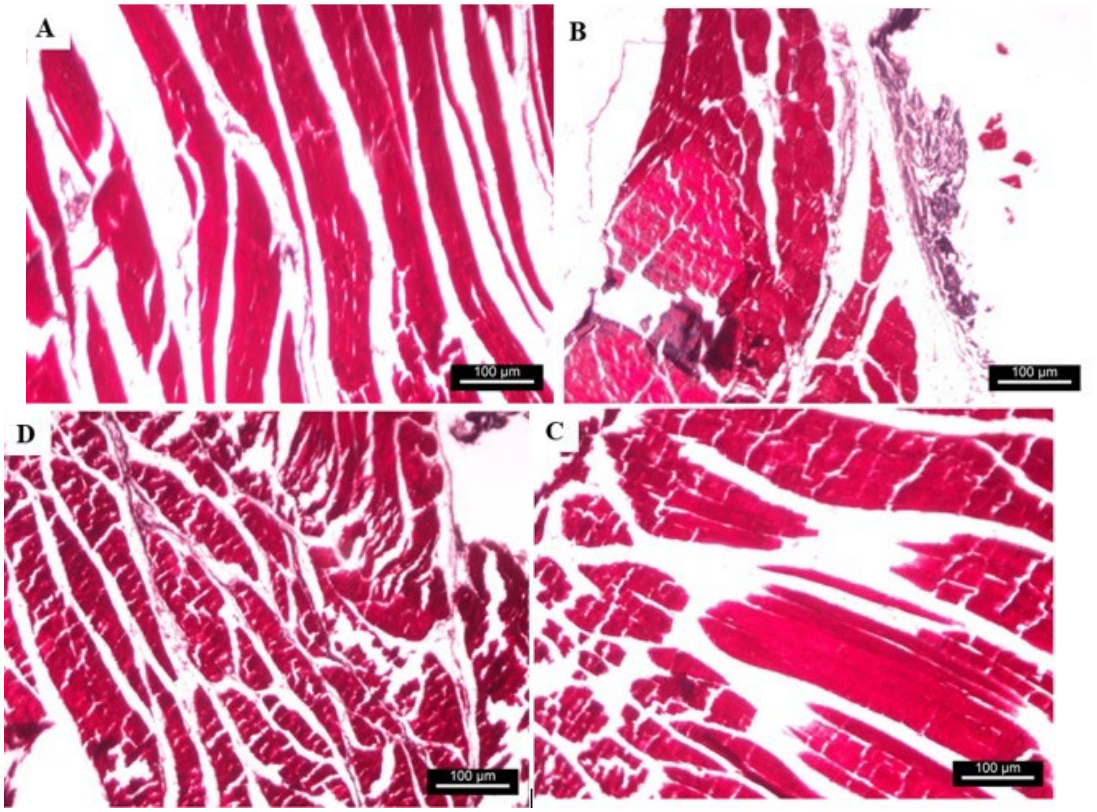


**Figure 4.** Histological assessment of the rats' gastrocnemius muscle atrophy.

Histological images of the gastrocnemius muscle samples stained with Masson's trichrome at eight weeks after surgery. The histological images are: Panel A = Control; Panel B = Negative control; Panel C = Eugenol, at 50 mg/kg, and Panel D = Eugenol at 100 mg/kg. Images were taken with a light microscope, at 400x magnification.

**Table 1.** Morphometric analyses of the sciatic nerve fibers in various rat groups.

| Groups | Fiber Number | Fiber Diameter (µm) | Axon Diameter (µm) | Myelin Sheath Thickness (µm) |
|---|---|---|---|---|
| **Control** | 24±5251 | 0.07±5.24 | 0.08±3.32 | 0.09±1.92 |
| **Negative control** | 11±5720* | 0.14±3.22* | 0.16±2.79* | 0.04±0.43* |
| **Eugenol, 50mg/kg** | 17±5310 | 0.22±3.01 | 0.09±2.4 | 0.11±0.61 |
| **Eugenol, 100mg/kg** | 22±6124& | 0.3±3.92& | 0.24±2.95 | 0.1±0.97& |

Each sciatic nerve was examined transversally distal to the injury point for the above variables after eight weeks post-surgery.

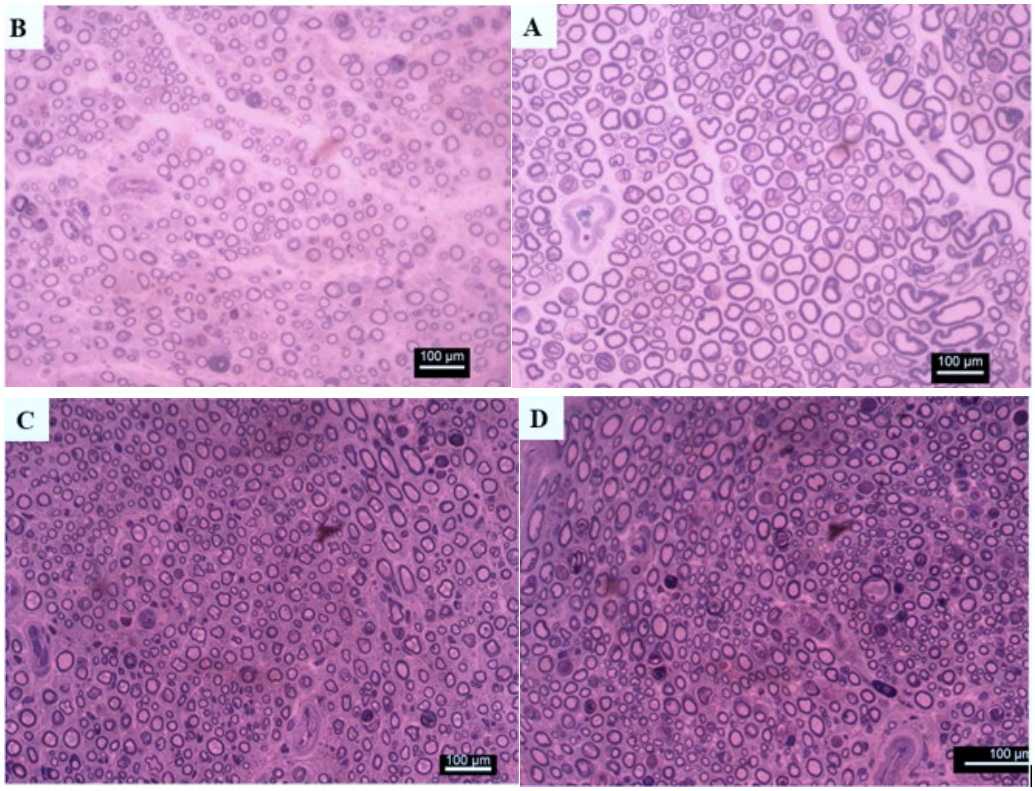


**Figure 5.** Images of transverse sections of the sciatic nerve distal to the injury.

Tissue samples were stained with toluidine blue. Images were taken on a light microscope at 400x magnification. The images are: Panel A = Control; Panel B = Negative control; Panel C = Eugenol at 50 mg/kg; and Panel D = Eugenol at 100 mg/kg.

## Discussion

Peripheral nerve damages with extensive pathological alterations in neurons and cells can lead to disability in a person (12). Sciatic nerve crush is usually used as an injury model for laboratory studies on animals (13). Based on this experimental model, a relatively mild damage is done to the nerve under study. In this context, a valid and quantitative method of data analysis to assess the nerve and motor functions post-injury and nerve repair process is sciatic functional index (SFI) (14, 15). Based on the data obtained in the current study, administration of Eugenol into the rats caused gradual improvement in the sciatic nerve function and structural integrity.

Nuclear factor kappa B (NF-κB) is an important transcription protein in all bodily tissues, and is involved in regulating cellular growth, proliferation and survival (16). In response to cellular damages, the NF-κB pathway is activated and releases its transcription protein, known as NF-κB (17). Studies have shown that inhibiting NF-κB leads to a reduction in the level cellular damages post injuries (18, 19). Eugenol is a flavonoid compound with antioxidant, anti-inflammatory and neuroprotective properties. Several earlier research articles have shown that Eugenol improves some disease conditions by regulating the NF-κB pathway (20-22). In the present study, it was observed that Eugenol resulted in healing of the damaged sciatic nerves in experimental rats. Therefore, it can be assumed that Eugenol prevents or minimizes cell death, and promotes cell proliferation likely by inhibiting the NF-κB pathway. On this basis, our findings suggest that treatment with Eugenol promotes the recovery of neural sensory and motor fibers in the injured sciatic nerve of experimental rats.

Increased inflammatory factors and oxidative stress are the primary causes of pathology in various diseases. Following peripheral nerve injuries, the levels of local interleukins 1 and 6, (IL-1 & IL-6),

and tumor necrosis factor alpha (TNFα) normally increase. The long-term presence of inflammatory factors inhibits the recovery of Schwann cells, which are responsible for the repair of injured axons and neural fibers. Therefore, the inhibition of pro-inflammatory factors is essential for the injured nerve fibers to regenerate. In previous studies, the antioxidant and anti-inflammatory effects of Eugenol have been established (23, 24). These studies have shown that administering Eugenol to rats decreases inflammatory markers while improving the antioxidant enzymes in the injured tissues.

In the current study, it was shown that nerve regeneration occurred more rapidly in rats treated with Eugenol compared to the untreated ones. Due to the anti-inflammatory and antioxidant properties of Eugenol, it is reasonable to believe that this compound can promote the recovery of neural fibers post injury by inhibiting the inflammatory mediators that prevent the healing processes. Indeed, one of the plausible mechanisms is Eugenol's ability to promote cell repair by reducing inflammatory mediators (25, 26). Although injured peripheral nerves may regenerate gradually by themselves; however, the longer the recovery process, the more likely is the potential for nerve atrophy (27). For this reason, a rapid and healthy rate of nerve regeneration is essential to achieve satisfactory functional outcomes; hence the reason for conducting this research. Specifically, we aimed at examining the effect of Eugenol on accelerating the process of sciatic nerve healing post experimental injury. Based on the data obtained, we suggest that Eugenol may be prescribed alone or concurrently with other treatments as an effective therapeutic supplement to accelerate the healing process of injured peripheral nerve fibers, such as sciatic.

## Conclusions

This study provided experimental evidence that administering Eugenol at doses of 50 or 100 mg/kg accelerates the recovery of both sensory and motor functions in rats after experimental sciatic nerve injury. Also, our results showed that the quality of neural fiber recovery was more pronounced with Eugenol at 100 mg/kg than the lower dose of 50 mg/kg. Therefore, considering the antioxidant and anti-inflammatory properties of Eugenol, and the neuroprotective effects of this compound should be investigated further in future studies on animals and humans.

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### Compliance with Ethical Guidelines

All of the testing processes were reviewed and approved by the Ethics Committee of Mohaghegh Ardabili University (*Registration Code:* IR.UMA.REC.1400.033).

### Conflict of Interests

The authors declared no conflict of interests with any internal or external entities in conducting this research project.

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### Authors' Contributions

All authors contributed fairly equally to the conduction of this study, preparation of the manuscript, and approved it prior to submission for publication.

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