



# Neuroprotective Effects of Fe<sub>3</sub>O<sub>4</sub> Nanoparticles Coated with Omega-3 as a Novel Drug for Recovery of Sciatic Nerve Injury in Rats

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## Abstract

**Background:** Novel nanoparticle drug therapy could be an effective method to enhance peripheral nerve regeneration.

**Objectives:** The current study aimed to determine the effect of Fe<sub>3</sub>O<sub>4</sub> nanoparticles (NP) coated with omega-3 on sciatic nerve regeneration in Wistar rats.

**Methods:** The rats' left sciatic nerve was crushed with a non-serrated clamp. Animals in experimental groups were given Fe<sub>3</sub>O<sub>4</sub>@ω<sub>3</sub> NPs (10 or 30 mg/kg/d) for one week. The recovery of functions was then assessed over eight weeks using a walking track analysis and a hot plate test. In addition, histomorphometry was used to assess the histomorphometric factors.

**Results:** Results showed that Fe<sub>3</sub>O<sub>4</sub>@ω<sub>3</sub> NPs may have enhanced sciatic nerve regeneration and improved sensorimotor rehabilitation in comparison to the negative controls. These therapeutic effects were significantly greater in Fe<sub>3</sub>O<sub>4</sub>@ω<sub>3</sub> NPs at a dose of 30 mg/kg. Furthermore, Fe<sub>3</sub>O<sub>4</sub>@ω<sub>3</sub> NPs treatment, compared to negative controls, significantly reduced gastrocnemius muscle atrophy and improved histomorphometric parameters.

**Conclusions:** It was determined that Fe<sub>3</sub>O<sub>4</sub>@ω<sub>3</sub> NPs had great potential for nerve regeneration due to the ease of their manufacturing and their desired efficacy.

**Keywords:** Nanoparticles, Fe<sub>3</sub>O<sub>4</sub>, Sciatic Nerve, Crush, Rat

## 1. Background

Since full functional recovery following severe nerve injury is uncommon, nerve injuries are regarded as major clinical issues. Following nerve injuries, particularly severe injuries, a cascade of secondary processes such as oxidative stress, inflammation, and excitotoxicity occurs, resulting in expanded injury and creating an inhibitory environment for the sprouting and elongation of injured axons (1). In this regard, inflammatory responses have been shown to play an important role in Wallerian degeneration, axonal regeneration, and functional rehabilitation (2). The immune response triggers a cascade of biological processes that remove growth-inhibiting components from the lesion site and create a favorable environment for axonal regeneration (3). As a result, modulating the inflammatory response may be an effective strategy for increasing the efficacy of nerve regeneration (4).

In this regard, animal research and clinical trials have determined that omega-3, a polyunsaturated fatty acid (PUFA), has anti-inflammatory effects, implying that they may be effective in the management of inflammatory responses (5). Furthermore, omega-3 fatty acids have been reported to display neuroprotective properties when dealing with a variety of neurological diseases (6). In our study, therefore, it was attempted to employ nanotechnology in order for developing a combined therapy method for healing peripheral nerve injury through application of iron oxide (Fe<sub>3</sub>O<sub>4</sub>) and Magnetite NPs coated with omega-3. As a carrier, the drug is attached to polymeric NPs, and the NPs facilitate therapeutic agent delivery and absorption into the target tissue (7). Iron oxide NPs have received considerable attention compared to other magnetic NPs due to their unique magnetic properties, biocompatibility, and low cytotoxicity (8).

## 2. Objectives

This study aimed to investigate how Fe<sub>3</sub>O<sub>4</sub> NPs coated with omega-3 affected the sciatic nerve crush model in Wistar rats.

## 3. Methods

### 3.1. Synthesis of NPs

The Fe<sub>3</sub>O<sub>4</sub>@ω<sub>3</sub> NPs were synthesized using the co-precipitation method as follows: Fe<sup>2+</sup> (0.2 M, 30 mL) and Fe<sup>3+</sup> (0.4 M, 30 mL) ions were stirred for 10 minutes at 80°C. Then, NaOH solution (4.5 M, 30 mL) was added to the iron ions abruptly and stirred for 10 minutes. Omega-3 oil (2mL) was added to the reaction medium drop-by-drop and stirred for 60 minutes. The dark precipitation was washed and dried at 50°C.

### 3.2. Characterization of NPs

The synthesized NPs were analyzed by X-Ray diffraction, FT-IR spectrophotometer, VSM, and TEM.

### 3.3. Animals and Groups

A total of 40 adult male Wistar rats (240 ± 20 g) were purchased and kept in plexiglass with standard condition. They were randomly allocated into four groups. Rats in two experimental groups were given Fe<sub>3</sub>O<sub>4</sub>@ω<sub>3</sub> NPs intraperitoneally at doses of 10 or 30 mg/kg daily for one week (8). Also, animals in the negative control group underwent crushing and received deionized water as a vehicle. In the sham group, only the sciatic nerve was exposed. All animal experiments were approved by the Ethics Committee of Mohaghegh Ardabili University of Ardabil (Iran) under code number: [IR.ARUMS.REC.1400.084](https://doi.org/10.21860/IR.ARUMS.REC.1400.084).

### 3.4. Surgical Procedures

The rats' right sciatic nerves were operated under deep anesthesia. Then, sciatic nerves of the rats' right leg were operated and compressed for 30 seconds. Then, the rats' muscles and skin were sutured.

### 3.5. Walking Tract Analysis

The sciatic functional index (SFI) was measured at two, four, six, and eight weeks after surgery according to the previous method (9, 10).

### 3.6. Hot Plate Test

A hot plate latency test was performed to evaluate the sensory recovery. Tests were performed at 2, 4, 6, and 8 weeks after surgery. The temperature was set at 54°C. Then, the rats' operated legs were located on a hot plate, and their reaction was recorded as response latency.

### 3.7. Histomorphometric Analysis

Histomorphometric analysis of regenerated nerves was carried out eight weeks after surgery according to the previous method (10).

### 3.8. Histological Evaluation of Gastrocnemius Muscle

Gastrocnemius muscles from all groups were harvested, and then Masson's trichrome staining was used to assess the severity of muscle atrophy.

### 3.9. Gastrocnemius Muscle Mass Ratio

Gastrocnemius muscle samples of all groups were dissected and harvested. Then, their wet weights were calculated using a Pohl Schmitt digital scale (Switzerland).

### 3.10. Statistical Analysis

SPSS Statistics 16.0 software was used to analyze the statistical data, and all data were presented as a mean ± SEM. The P-values lower than 0.05 were considered significant. Statistical significance between groups was determined by using ANOVA test and Tukey post hoc test.

## 4. Results

### 4.1. Characterization of NPs

#### 4.1.1. XRD Patterns

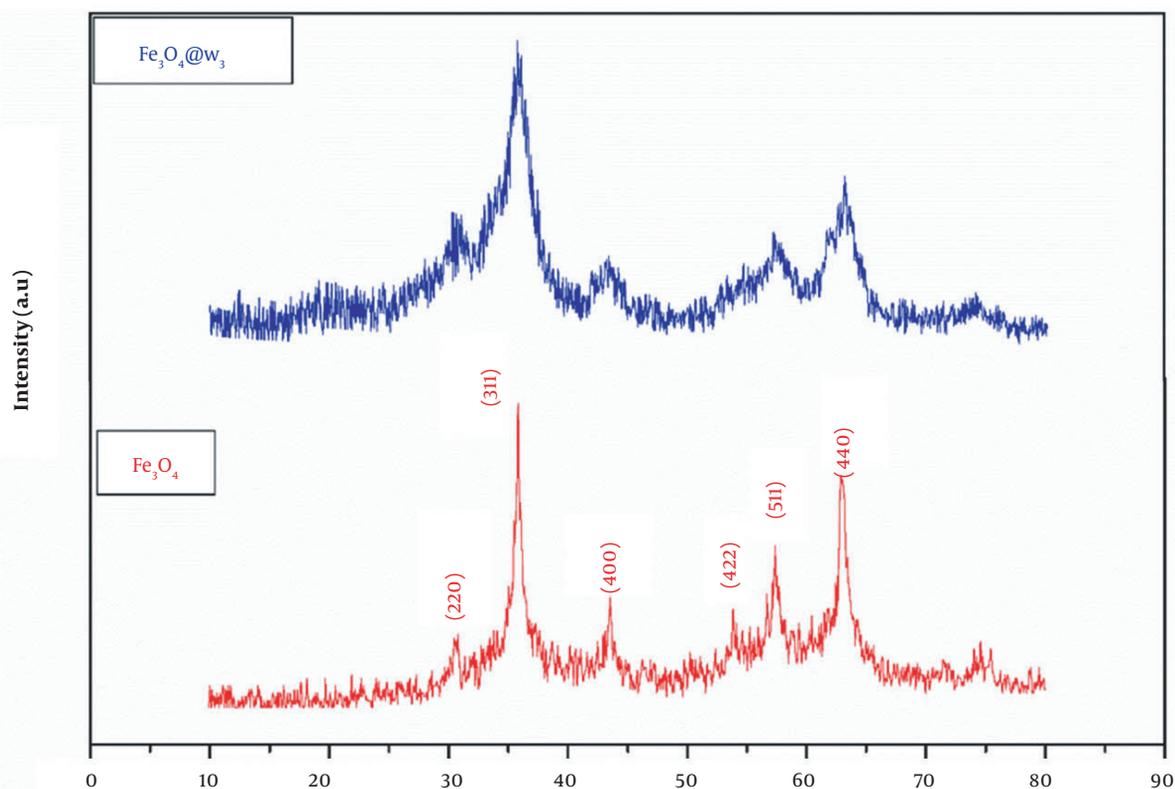
XRD patterns confirmed the cubic spinel structure of the bare and ω<sub>3</sub> coated magnetite NPs (Figure 1). The appeared peaks at 2θ ~ 30.48, 35.78, 43.53, 53.83, 57.38, and 63.03 relating to the crystal planes of (220), (311), (400), (422), (511), and (440), respectively, were consistent with JCPD card no. 19-0629. The average crystallite sizes of the Fe<sub>3</sub>O<sub>4</sub> and Fe<sub>3</sub>O<sub>4</sub>@ω<sub>3</sub> were measured by Debye-Scherrer equation:

$$d = \frac{0.9\lambda}{\beta \cos\theta}$$

And were found to be about 13 and 7 nm, respectively. In addition, the lattice constant of the coated particles was nearly 8.324 Å, which was decreased proportional to the bare one (8.376 Å).

#### 4.2. TEM and SEM Images

Figure 2A and B displays the TEM and SEM images of the Fe<sub>3</sub>O<sub>4</sub>@ω<sub>3</sub> NPs. Obviously, NPs had an approximately uniform size distribution, and their size was smaller than 10 nm, which was in close agreement with XRD data (Figure 2A). According to both the TEM and SEM images, particles appeared to be quasi-spherical, and some aggregation was observed due to their magnetic nature.



**Figure 1.** XRD Patterns of the  $\text{Fe}_3\text{O}_4$  and  $\text{Fe}_3\text{O}_4@w_3$  NPs

#### 4.3. FT-IR Characterization

FT-IR spectra of the  $w_3$  and  $\text{Fe}_3\text{O}_4@w_3$  samples are presented in Appendix 1. The spectrum of  $w_3$  exhibited absorption bands between  $1000 - 1500 \text{ cm}^{-1}$  that were related to C-O and C-H vibrations. An absorption band occurred at  $1745 \text{ cm}^{-1}$  which corresponded to the C=O vibration. In addition, the stretching vibrations of the -C-H ( $\text{CH}_2$ ) bands were observed at  $2855$  and  $2924 \text{ cm}^{-1}$ . The spectrum of  $\text{Fe}_3\text{O}_4@w_3$  presented two absorption deeps at  $445$  and  $625 \text{ cm}^{-1}$  that were in accordance with Fe-O vibrations in the spinel structure. Furthermore, the absorption band of C=O became very weak and the characteristic band of the C-O vibration shifted to the lower frequencies, which confirmed the interaction between  $w_3$  and the surface of magnetite NPs.

#### 4.4. VSM Characterization

The magnetic properties of the bare and coated magnetite NPs were measured using VSM at room temperature. According to Appendix 2, both samples are superparamagnetic with nearly zero coercivity. Due to the very small size of the particles and the biocompatible shell

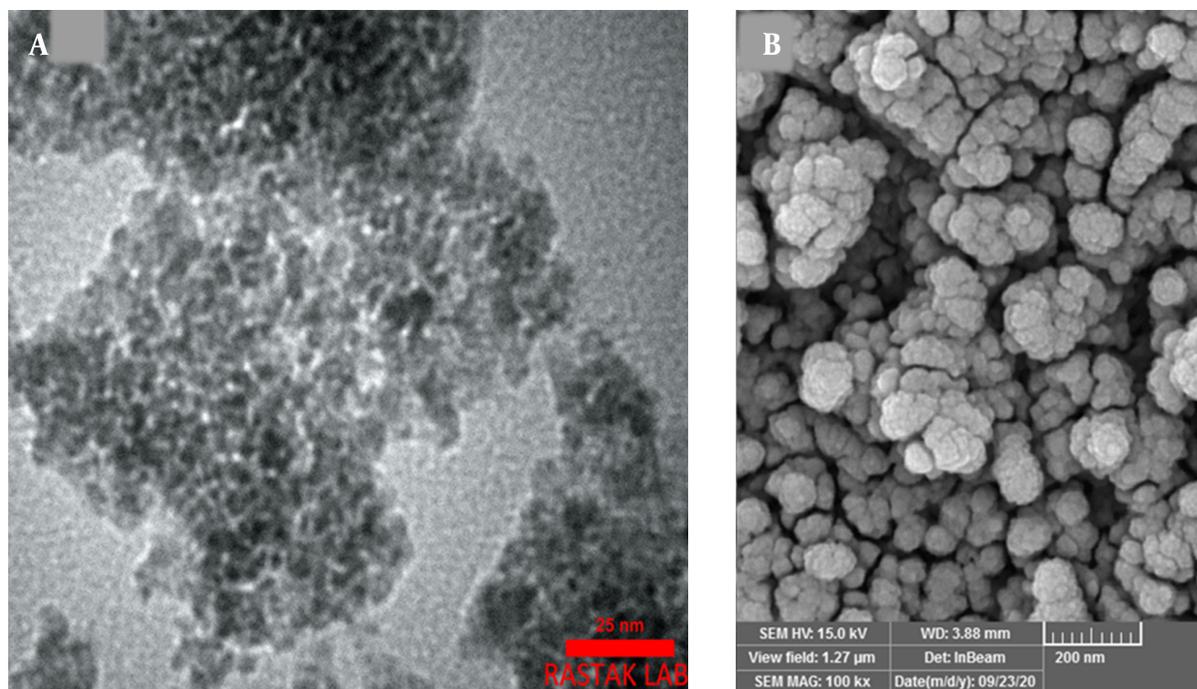
on their surface, moreover, the saturation magnetization of the omega-3 loaded NPs was decreased proportional to the bare one.

#### 4.5. Motor Functional Recovery Evaluation

Results showed that the SFI values in all groups were reduced after surgery (Figure 3). Then, SFI values in all groups were increased progressively, with a significantly higher improvement detected in  $\text{Fe}_3\text{O}_4@w_3$  NP groups ( $P < 0.05$ ; Figure 3) in increased progressively with the negative control group. Also, the best nerve recovery in comparison with the negative control group was seen in  $\text{Fe}_3\text{O}_4@w_3$  NP ( $30 \text{ mg/kg}$ ) group six weeks after surgery ( $P < 0.05$ ; Figure 3).

#### 4.6. Sensory Neuron Recovery Evaluation

Rats in all groups showed no reaction to the hot plate two weeks after surgery, except for the sham group which reacted after  $0.97$  seconds. Sensory recovery after nerve surgery (4, 6, and 8 weeks) improved gradually in all groups, with a noticeably faster recovery detected in the  $\text{Fe}_3\text{O}_4@w_3$  NPs groups ( $P < 0.05$ ; Figure 4).



**Figure 2.** A, TEM; and B, SEM Images of the  $\text{Fe}_3\text{O}_4@ \omega_3$  NPs.

#### 4.7. Histomorphometric Analysis

Table 1 shows the histomorphometric parameters. The results showed that the  $\text{Fe}_3\text{O}_4@ \omega_3$  NPs treatment groups had significantly higher fiber numbers than the negative control and sham surgery groups. This increase, however, was significantly lower in the sham group. On the other hand,  $\text{Fe}_3\text{O}_4@ \omega_3$  NPs (30 mg/kg/day) treated rats had considerably higher fiber number, fiber diameter, and axon diameters than  $\text{Fe}_3\text{O}_4@ \omega_3$  NPs (10 mg/kg/day) treated animals (Appendix 3).

#### 4.8. Histological Evaluation of Gastrocnemius Muscle

As shown in appendix 4, the muscle fibers were stained in red, whereas the collagen fibers showed the blue staining. Denervated muscles in nerve crush groups displayed varying degrees of atrophy compared to those in the sham surgery group. However, the atrophy in the  $\text{Fe}_3\text{O}_4@ \omega_3$  NPs-treated groups was significantly lower in the negative control group.

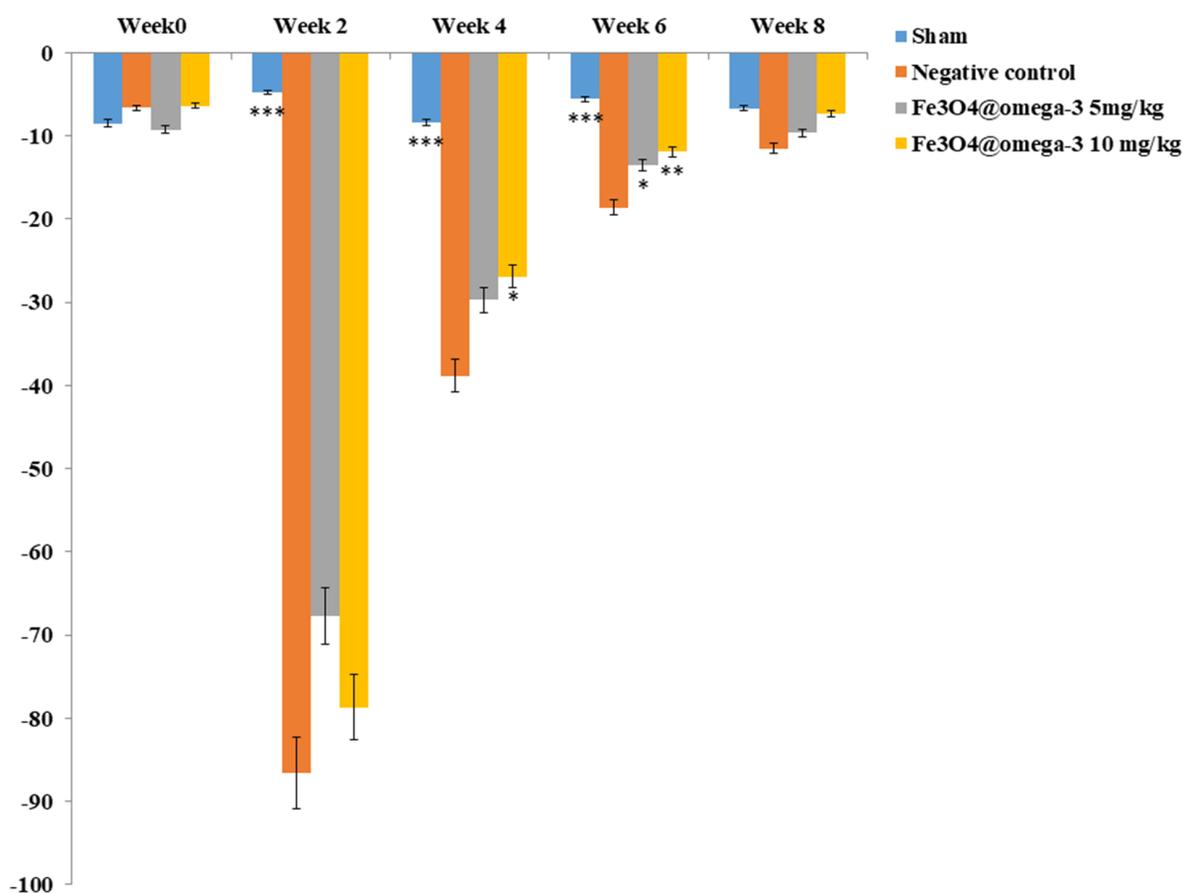
#### 4.9. Gastrocnemius Muscle Mass Ratio

The results showed that the muscle mass ratios in  $\text{Fe}_3\text{O}_4@ \omega_3$  NPs (30 mg/kg/day) treated rats were substantially greater in comparison to those in the negative group ( $P < 0.05$ ). However, no discernible change

was observed between the  $\text{Fe}_3\text{O}_4@ \omega_3$  NPs (10 mg/kg/day) treated with the negative control and the  $\text{Fe}_3\text{O}_4@ \omega_3$  NPs (30 mg/kg/day) groups (Appendix 5).

## 5. Discussion

The administration of neuroprotective medications after nerve injuries is an effective method for limiting damage and enhancing nerve regeneration. Utilizing nanotechnology to synthesize neuroprotective agents can improve pharmacotherapy efficacy (7, 11). In this regard, magnetic NPs such as  $\text{Fe}_3\text{O}_4$  are effective nano systems for managing drug delivery (12, 13). In addition, magnetic NPs have a synergistic impact on the target site, improve therapeutic outcomes, and prevent drug degradation (14, 15). Our findings revealed that one-week treatment with  $\text{Fe}_3\text{O}_4@ \omega_3$  NPs (10 or 30 mg/kg) after a sciatic nerve injury had the potential to increase the nerve regeneration. Walking track analysis demonstrated that  $\text{Fe}_3\text{O}_4@ \omega_3$  NPs at 10 or 30 mg/kg doses were effective in enhancing motor recovery. However, only  $\text{Fe}_3\text{O}_4@ \omega_3$  NPs at a dose of 30 mg/kg were capable of increasing SFI values significantly compared to the negative control group. Moreover, muscle mass ratio and histomorphometric parameters were also considerably improved in  $\text{Fe}_3\text{O}_4@ \omega_3$  NPs at 30 mg/kg compared to negative control, confirming the results from



**Figure 3.** The motor functional recovery evaluation using SFI value [SFI was evaluated in all experimental groups after crush. Values are presented as mean ± standard error of the mean (n=10); \*P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001, respectively].

**Table 1.** Morphometric Parameters in All Experimental Groups 8 Weeks After Surgery<sup>a, b</sup>

Groups	Fiber Count	Fiber Diameter	Axon Diameter	Myelin Thickness
Sham surgery	5241 ± 24	5.41 ± 0.31	4.16 ± 0.12	1.25 ± 0.11
Negative control	6321 ± 26**	2.61 ± 0.41**	1.88 ± 0.41**	0.73 ± 0.62**
Fe <sub>3</sub> O <sub>4</sub> @ω <sub>3</sub> NPs (10 mg/kg)	7121 ± 38**	4.23 ± 0.31*	3.22 ± 0.22*	1.01 ± 0.06**
Fe <sub>3</sub> O <sub>4</sub> @ω <sub>3</sub> NPs (30 mg/kg)	8951 ± 44***	4.91 ± 0.11*	3.82 ± 0.13	1.09 ± 0.21**

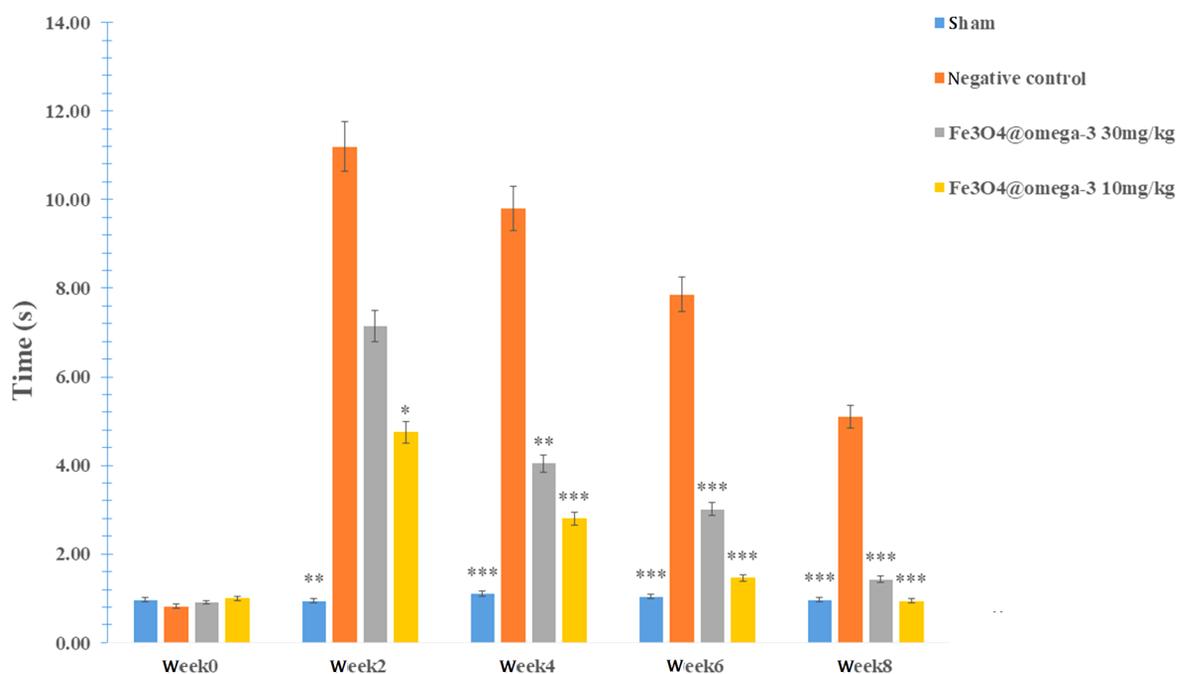
<sup>a</sup> Values are expressed as mean ± SEM.

<sup>b</sup> \*P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001, respectively.

the walking track analysis. Furthermore, the hot plate test results suggested that Fe<sub>3</sub>O<sub>4</sub>@ω<sub>3</sub> NPs-treated (10 or 30 mg/kg) therapy reduced the sensory threshold.

According to our findings, increased tissue levels of omega-3 using Fe<sub>3</sub>O<sub>4</sub> as a carrier improved sciatic nerve regeneration in a rat crush model. Omega-3 fatty acids cannot be produced in the body and must therefore be obtained from diets or supplements (16).

Omega-3 has been proven to have many biological effects, including boosting the synthesis of anti-inflammatory and neuroprotective metabolites via prostaglandin metabolism (17). On the other hand, animal research have indicated that omega-3 fatty acids may contribute to neuronal survival and axonal healing following peripheral nerve injury (18). In an in vitro study, Robson et al. reported that omega-3 PUFA had a significant



**Figure 4.** Sensory recovery test. The hot plate test was performed in each experimental group after crush. All data are presented as mean  $\pm$  standard error of the mean (n = 10) (\*P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001, respectively).

neurite-promoting capacity in neurons from adult and aged animals (19). In a study by Baazm et al. to evaluate the protective effects of parenteral administration of omega-3 on spinal cord injury in rats, it was discovered that omega-3 treatment increased motor function recovery and reduced inflammation (20). Pu et al investigated the neuroprotective effects of a 2-month omega-3 polyunsaturated fatty acid (PUFA)-enriched diet against controlled cortical impact (CCI) in mice, and found that an omega-3-rich diet reduced behavioral deficits (21). Furthermore, several clinical trials on diabetic patients have shown that long-term omega-3 diets can improve diabetic neuropathy, promote nerve regeneration, and restore sensory function (22). Omega-3 appears to prevent oxidative stress-induced apoptosis by decreasing pro-apoptotic signaling and suppressing pro-inflammatory responses (23).

On the other hand, some researchers have found that NPs can effectively promote the growth of injured axons (24, 25). Liu et al., for instance, investigated the role of nano-hydroxyapatite in regeneration in cultured brain cells of rats. According to their findings, nano-hydroxyapatite induced axonal guidance growth by regulating netrin-1 expression as an axonal guidance cue during nerve regeneration. Furthermore, some studies

have indicated that a single application of Fe<sub>3</sub>O<sub>4</sub> can cause significant axonal guidance, but it has a cytotoxic effect on cell viability. When nanohydroxyapatite-coated magnetic Fe<sub>3</sub>O<sub>4</sub> (Fe<sub>3</sub>O<sub>4</sub>n-HA) particles were added to primary cultured DRG neurons in our study, however, the cytotoxicity of single magnetic NPs Fe<sub>3</sub>O<sub>4</sub> was reduced. Moreover, Fe<sub>3</sub>O<sub>4</sub>n-HA was able to efficiently direct axonal guidance and greatly promote axonal elongation (26). Chen et al. also investigated electrospinning multilayered scaffolds loaded with melatonin and Fe<sub>3</sub>O<sub>4</sub> magnetic NPs in order for repairing sciatic nerve defects in rats, and found that scaffolds had the adequate biocompatibility and potential to improve morphological, functional, and electrophysiological parameters. Other studies have determined that NPs are capable of improving the mechanical properties of neural conduits (27). In some experiments, Fe<sub>3</sub>O<sub>4</sub> NPs have also been shown to increase neurite outgrowth by stimulating the mitogen-activated protein kinase signaling pathway (28).

### 5.1. Conclusion

In sum, it was found that a one-week treatment of Fe<sub>3</sub>O<sub>4</sub>@ $\omega_3$  NPs rats with sciatic nerve defects may have improved the regeneration process and rehabilitation. Our study results confirmed the neuroprotective effects of

Fe<sub>3</sub>O<sub>4</sub>@ω<sub>3</sub> NPs as new drug. It was also demonstrated that Fe<sub>3</sub>O<sub>4</sub>@ω<sub>3</sub> NPs had great potential for nerve regeneration due to the ease of their manufacturing and their desired efficacy. However, it was recommended that further studies should be conducted in order to confirm our findings.

### 5.2. Limitation

This study faced some limitations, one of which was the lack of molecular data about the expression of neurotrophic factors in drug-treated groups.

### Footnotes

**Authors' Contribution:** A.A. conceived and designed the evaluation and drafted the manuscript. F.M. participated in designing the evaluation, performed parts of the statistical analysis, and helped to draft the manuscript. M.T. re-evaluated the clinical data, revised the manuscript, performed the statistical analysis, and revised the manuscript. S.M. re-analyzed the clinical and statistical data and revised the manuscript. All authors read and approved the final manuscript.

**Conflict of Interests:** The authors declare that Arash Abdolmaleki, one of the authors of this study and reviewer in journal, is an editorial board member. The journal confirmed that the mentioned author with CoI was completely excluded from all review processes. The authors also introduced this author with CoI during the submission as an opposed reviewer.

**Data Reproducibility:** The dataset presented in the study is available upon the request from the corresponding author during submission or after its publication. The data are not publicly available due to the secrecy about our research.

**Ethical Approval:** This study was approved by the Ethics Committee of Mohaghegh Ardabili University of Ardabil (Iran) under code number IR.ARUMS.REC.1400.084.

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