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Experimental Research Article

Repair of cutaneous sciatic nerve by using bone marrow stromal cells on a chitosan-gelatin membrane with olive fruit extract in rats

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ABSTRACT

Introduction: Despite various treatment methods, the damaged peripheral nervous system has become a severe problem in today's societies. This study aimed to evaluate the effects of cultured bone marrow stromal cells (BMSCs) on the chitosan-gelatin membrane and olive fruit extract in Wistar rat cutaneous sciatic nerve repair.

Methods: The chitosan-gelatin membrane was prepared after the extraction of olive oil, the extraction of BMSCs, and cultivation in special pellets. Wistar rats were divided to 9 groups (n=5): healthy control, sciatic nerve injury, nerve injury treated with bone marrow stromal stem cells, nerve injury treated with olive fruit extract, nerve injury treated with olive fruit extract+bone marrow stromal stem cells, nerve injury treated with olive fruit extract+chitosan-gelatin, nerve injury treated with chitosan-gelatin+ bone marrow stromal stem cells, and nerve injury treated with olive fruit extract+chitosan-gelatin+ bone marrow stromal stem cells. The treatment period was 8 weeks, then the evaluation of sciatic nerve damage repair included electrophysiological, sciatic functional index, tissue sampling, immunocytochemistry, and histopathological examination.

Results: In the group treated with olive fruit extract +chitosan-gelatin+ bone marrow stromal stem cells, sciatic nerve repair was observed with an increase in nerve conduction velocity (m/s), amplitude muscle action potential (mv), number of (vessels and fiber), most significant area of vessels, myeline, axon, and fiber (1000 micro m2) and a decrease in statistic functional index (0 to -50) ($P<0.0001$).

Conclusion: The combination of olive extract, chitosan-gelatin, and bone marrow stromal stem cells (BMSCs) demonstrated a significant reparative effect on sciatic nerve injury and could serve as a promising approach for peripheral nerve repair.

Keywords:
Peripheral nervous system
Sciatic nerve
Chitosan-gelatin scaffold
Olive fruit extract
Bone marrow stromal stem cells

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Introduction

Peripheral nerve damage has become a significant concern due to various causes, including traumatic injuries, traffic accidents, natural disasters like floods and earthquakes, sports-related injuries, and diseases such as Alzheimer's and Multiple Sclerosis (Falvo et al., 2020; Navarro et al., 2007). The treatment of these conditions is costly and can lead to severe consequences, including limb amputation, permanent disabilities, and the loss of employment or social status (Varier et al., 2022). Despite the development of various surgical techniques and nerve repair methods, repairing nerve lesions continues to be a challenging task with relatively low success rates for patients (Smith et al., 2009).

The nervous system exhibits remarkable plasticity, with neurons capable of generating new dendrites and synapses. However, the regenerative capacity of the nervous system is very limited. While the peripheral nervous system demonstrates somewhat greater regenerative potential compared to the central nervous system, it remains constrained in its ability to regenerate following injury. Regeneration within the peripheral relies on the preservation and activity of both the cell body and Schwann cell. If these components are compromised, dendritic and axonal defects may fail to recover. The regeneration of the peripheral nervous system is possible thanks to the presence of the glial cells in the peripheral nervous system, the Schwann cells. Schwann cells play a pivotal role in peripheral nerve regeneration by transitioning to a proliferative phenotype, facilitating the for-

formation of basal lamina, and promoting neuronal regeneration (5).

A key challenge in nerve regeneration is understanding the primary mechanisms that regulate neurite growth to accelerate and enhance recovery (Zhelyaznik et al., 2003). Axonal repair in the peripheral nervous system involves the coordinated activity of a genetic program in dorsal root ganglion neurons, along with local intracellular reactions (Kato et al., 2010). Neurotrophic factors, such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), have been shown to promote axon growth and guide it towards the lesion site (Choi et al., 2005; Fan and Lou 2011). Recently, research has focused on cell transplantation as a method to replace lost tissue, utilizing various cell types like bone marrow stromal cells (BMSCs), fibroblasts, hair follicle stem cells, and Schwann cells (Cui et al., 2008; Ren et al., 2012).

BMSCs exhibit significant differentiation potential into various cell types, such as osteoblasts, chondrocytes, adipocytes, muscle cells, and neurons. Research has demonstrated that when BMSCs are implanted into nerve grafts, they can produce neurotrophic factors and extracellular matrix components, which promote the proliferation of axons and Schwann cells. Furthermore, BMSCs contribute to tissue repair by enhancing blood vessel formation, thereby supporting the regeneration and restoration of peripheral nerves following injury (Zarbakhsh et al., 2012).

Studies by Xin Ni et al. in 2013 showed Given a16-week recovery period for bridging of a 10-mm gap in the sciatic nerve of adult rats as experimental models, we demonstrated that the combinant nerve conduit facilitated axonal regrowth and remyelination toward functional reinnervation. The HRP-positive labeling neurons at sixteen weeks after surgery showed that the tissue-engineered nerve has an advantage of keeping injured neuronal survival and provides a connection to the transected axons at the early stage of regeneration (13)

Tissue engineering has emerged as one of the most promising alternative treatments for peripheral nerve damage. For scaffolds to perform effectively in tissue engineering, they must possess specific chemical, morphological, biological, and mechanical properties. These scaffolds not only maintain normal cellular functions but also enhance cell adhesion, serving as templates for new tissue formation (Jayaraman et al., 2004). Chitosan, a natural polymer, is highly suitable for tissue engineering due to its biocompatibility, biodegradability, and structure resembling glucosamine glycan (Cho et al., 2010; Muzzarelli et al., 1999). Additionally, gelatin, a natural collagen polymer, is commonly used in medical applications due to its excellent properties for tissue repair and regeneration (Gaspar-Pintiliescu et al., 2019).

In the 2024 study by Ahmed G. Abdulaziz et al, the cell migration-promoting effect of the flaxseed extract was attributed to the presence of anti-inflammatory phenolic compounds that promote better cell-material interactions, and larger nanofiber diameters that reinforce mechanical strength. These combined effects create a supportive environment for cell migration, indicating potential advantages for tissue regeneration in bone engineering. It is worth mentioning here that cell migration assays inherently assess both the migratory and

proliferative capabilities of cells. If gap closure were solely due to cell migration, a gap (cell-free area) would remain behind the migrating cells. Thus, the observed gap closure is a result of the combined effects of cell migration and proliferation. To accurately differentiate between these processes, future studies will incorporate additional assays, such as proliferation inhibition tests. These tests will help to isolate and verify the specific contributions of cell migration, providing a clearer understanding of the mechanisms involved in healing and cell movement(18).

The sciatic nerve, the largest and longest nerve in the body, exits the pelvic floor and descends from the pelvis and femur, providing sensation and movement to the knee and below. Scientists have expressed interest in stem cells due to their ability to regenerate and repair tissues in tissue engineering (Teixeira et al., 2003).

This study aimed to evaluate the potential of bone marrow stem cell transplantation, cultured on a chitosan-gelatin membrane and combined with olive fruit extract, in repairing the sciatic nerve in rats following the disconnection and reconnection of the proximal and distal nerve regions.

Material and Methods

This experimental research was conducted under the ethical approval of the Ethics Review Board at Islamic Azad University, Science and Research Branch (IR.IAU.SRB.REC.1400.221).

Extraction of the olive fruit extract

Olea europaea L. is an evergreen tree or shrub native to Mediterranean Europe, Asia, Africa, and Iran. Both the oil and fruit are among the main components of the Mediterranean diet. The main active constituents of olive oil include oleic acid, phenolic compounds, and squalene. The primary phenolic compounds, hydroxytyrosol and oleuropein, give extra-virgin olive oil its characteristic bitter and pungent taste. One of the potentially bioactive compounds is the secoiridoid oleuropein, which can constitute up to 6–9% of the dry matter in olive leaves. Other bioactive components found in olive leaves include related secoiridoids, flavonoids, and triterpenes. The evidence supporting the potentially beneficial effects of olive leaves on human health is presented. pharmacological activities of oleuotopein, including antioxidant, anti-inflammatory, anti-atherogenic, anti-cancer, antimicrobial, antiviral, hypolipidemic, and hypoglycemic effects. Oleuropein is a compound belonging to the secoiridoid group. Oleuropein was standardized and validated using an HPTLC-based method (Nikhal et al., 2010).

The green fruit of Olea europaea L. was collected from an olive garden around Semnan City in Semnan Province in September 2022.

Olea europaea was identified in the Medicinal Plants Herbarium at the School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran (Voucher Number: SBMU 8312).

Membrane Preparation

Thin chitosan films were fabricated by dissolving 0.25 g of chitosan and 0.08 g of polyethylene oxide in 50 mL of a 1% (v/v) acetic acid solution. The resulting mixture was stirred continuously at 40°C for 2 hours to ensure complete dissolution. To eliminate air bubbles, the solution was centrifuged at 2500 rpm for 10 minutes. The degassed solution was then poured into 75 mm diameter plastic petri dishes and left to dry at room temperature (25°C) for 24 hours. Once dried, the films were cut into 1 cm × 1 cm squares and used as scaffolds for culturing bone marrow stromal cells (BMSCs) obtained from the second passage. Following cell counting and viability assessment using trypan blue staining, a cell suspension containing 100,000 BMSCs was prepared for each well. The suspensions were seeded into 12-well plates and incubated in a Co incubator under standard culture conditions.

Extraction of Bone Marrow Stromal Cells

To isolate BMSCs from mice, anesthesia was administered via intraperitoneal injection of ketamine hydrochloride (80 mg/kg) and xylazine hydrochloride (5 mg/kg). The left femur was surgically exposed, and a 1 mm diameter hole was drilled into the outer surface of the femur. Bone marrow cells were aspirated from both the proximal and distal ends using a syringe filled with 1 mL of Alpha-MEM medium supplemented with 10% FBS. The aspirated material was transferred into a cell culture flask and incubated for 24 hours under standard conditions.

Bone Marrow Stromal Cell Culture

BMSCs were cultured at 37 °C in a humidified atmo-

sphere containing 5% CO_2. The culture medium used was Alpha-MEM supplemented with 10% FBS and 1% penicillin-streptomycin. Once the cells reached 80–95% confluency, the medium was aspirated, and the adherent cells were detached using 0.25% (w/v) trypsin-EDTA. The cells were then resuspended in complete medium, stained with trypan blue, and counted using a hemocytometer.

Immunocytochemistry

The stem cell nature of the cultured stromal cells was confirmed by immunocytochemistry using antibodies against fibronectin and CD44. First, the cells were washed with PBS and fixed with 4% paraformaldehyde for 30 minutes at room temperature. After another PBS wash, a blocking solution was applied for one hour. The cells were then incubated overnight with primary antibodies. Following additional washing steps, a biotinylated secondary antibody conjugated to peroxidase was added, and the signal was visualized using DAB substrate. Finally, the cells were washed with PBS to complete the staining process.

Labeling of BMSCs Cells

BMSCs were incubated with BrdU for 24 hours to allow incorporation into newly synthesized DNA. At the end of the experiment, BrdU-labeled cells were identified using an anti-BrdU antibody.

Experimental Animals

A total of 45 adult male Wistar rats (200–250 g) were obtained from the Pasteur Institute (Tehran, Iran). All procedures related to animal care and experimentation were conducted in accordance with the ethical guidelines approved by the Institutional Animal Care and Use Committee of Baghiyatallah University of Medical Sciences, Tehran, Iran. Efforts were made to minimize the number of animals used. Rats were housed individually in cages with ad libitum access to food and water. Environmental conditions were maintained with a 12:12-hour light/dark cycle, stable temperature (23 °C), and 55% humidity.

Determination of Phenolic and Flavonoid Compounds in Olive Fruit Extract

A total of 0.2 grams of olive fruit extract was dissolved in 1 mL of ethanol. The solution was homogenized using an ultrasonic processor and then centrifuged to separate the liquid and solid phases. A 5 µL sample from the supernatant (liquid phase) was applied onto a TLC plate. Chromatographic separation was performed using a mobile phase consisting of:

Ethyl acetate: 10 mL
Formic acid: 1.1 mL
Acetic acid: 1.1 mL
Water: 2.6 mL

After separation, the plate was treated with Natural Products reagent (NP reagent) for derivatization to visualize phenolic and flavonoid compounds. High-performance thin-layer chromatography (HPTLC) was subsequently utilized for more precise identification. The presence of phenolic and flavonoid (Boka et al., 2015).

Treatment Period and Study Design

After seven days of acclimatization, the rats were randomly divided into 9 groups (n = 5 per group), and the studied groups were treated for 8 weeks:

Group 1: Served as the healthy control (HC), healthy mice without treatment.

Group 2: Served as a sciatic nerve injury (SNI) in mice without treatment.

Group 3: Served as bone marrow stromal stem cells (BMSCs), sciatic nerve injury treated with BMSCs

Group 4: Served as an olive fruit extract (OE), sciatic nerve injury treated with OE.

Group 5: Served as OE+BMSCs, sciatic nerve injury treated with OE+BMSCs.

Group 6: Served as OE+chitosan-gelatin scaffold (C/G/S), sciatic nerve injury treated with OE+C/G/S

Group 7: Served as OE/C/G/S/BMSCs, sciatic nerve injury treated with OE and BMSCs loaded C/G/S.

Group 8: Served as C/G/S, sciatic nerve injury treated with C/G/S.

Group 9: Served as C/G/S/BMSCs, sciatic nerve injury treated with C/G/S/BMSCs.

Surgical Procedures

A surgical procedure was carried out to induce sciatic nerve injury. All operations were performed under general anesthesia, administered via intraperitoneal injection of ketamine hydrochloride (80 mg/kg) and xylazine hydrochloride (5 mg/kg). Following the induction of anesthesia, the right hind limb was shaved, and a 3

cm longitudinal skin incision was made along the posterolateral aspect of the thigh. This incision allowed for adequate exposure and isolation of the sciatic nerve for further manipulation.

Electrophysiological

At the end of the treatment period (week eight), animals were anesthetized with an intraperitoneal injection of ketamine hydrochloride (80 mg/kg) and xylazine hydrochloride (5 mg/kg). During the procedure, body temperature was maintained at approximately 30 °C using a heat lamp. Following the incision of skin and muscle layers, the sciatic nerve was exposed. Electrophysiological assessment was performed using an electromyography (EMG) recording system. A needle stimulator was positioned proximal to the lesion site on the sciatic nerve trunk. To record evoked potentials, two surface electrodes were placed—one on the gastrocnemius muscle and the other approximately 1 cm below the tibial tuberosity. Upon activation of the recording device, signals were passed through an amplifier and displayed on an oscilloscope. Baseline EMG activity was first recorded from the gastrocnemius-soleus muscle in a resting state. In a normally innervated muscle, electrical activity is absent at rest, a condition known as "electrical silence," which appears as a flat line on the oscilloscope. The presence of this pattern indicates intact neuromuscular function. Nerve conduction velocity (NCV) was also measured for further analysis of neural recovery and function.

Sciatic Functional Index

At two and eight weeks post-surgery, functional recovery of the sciatic nerve was evaluated using the Sciatic Functional Index (SFI). To perform this assessment, the plantar surfaces of the rats' hind paws were coated with ink, and the animals were allowed to walk through a corridor (dimensions: 20 × 7 × 60 cm) lined with white paper to capture their footprints.

The SFI was calculated based on the method described by Bain et al. (1989), which compares several footprint parameters between the operated and unoperated limbs. The formula used is as follows:

SFI = -38.5 × (EPL - NPL) / NPL + 109.5 × (ETS - NTS) / NTS + 13.3 × (EIT - NIT) / NIT - 8.8

Where: PL (Print Length): Distance from the heel to the third toe, TS (Toe Spread): Distance between the first and fifth toes, IT (Intermediary Toe Spread): Distance between the second and fourth toes, E: Experimental (injured) side, and N: Normal (uninjured) side

This index allows for a quantitative assessment of motor function recovery, with values approaching 0 indicating normal function, and values near -100 indicating total dysfunction.

Histopathological Examination

The protocol of previous studies was used (Yardim et al., 2020).

Statistical analysis

Tissue parameters such as nerve and blood vessel diameters and vessel count were measured using Motic Image 2000. Data were presented as mean ± SD, and graphs were generated with GraphPad Prism. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test, with $p < 0.05$ considered significant.

Results

Labeling BMSCs with BrDU

BMSCs were labeled with BrDU and observed by anti-BrDU antibody at the end of the experiment (Figure 1). Nucleus of BMSCs labeled by BrDU in the HC (Figure 1A), BMSCs (Figure 1B), and SNI (Figure 1C).

Immunocytochemistry

Fibronectin, CD44, and CD45 antibodies were used to prove the stromal nature of BMSCs and to determine their purity (Figure 2).

Analysis Method: High-Performance Thin-Layer Chromatography (HPTLC)

The use of olive fruit extract in this study is based on its anti-inflammatory properties, which promote the accelerated healing of sciatic nerve injuries. These anti-inflammatory effects are primarily attributed to the presence of different compounds. To separate and identify these bioactive compounds, high-performance thin-layer chromatography (HPTLC) was employed. Additionally, derivatization (staining) was performed to detect these compounds in olive fruit extract (Figure 3).

Identification of Terpenoid Compounds

Separation was performed based on the specified mo-

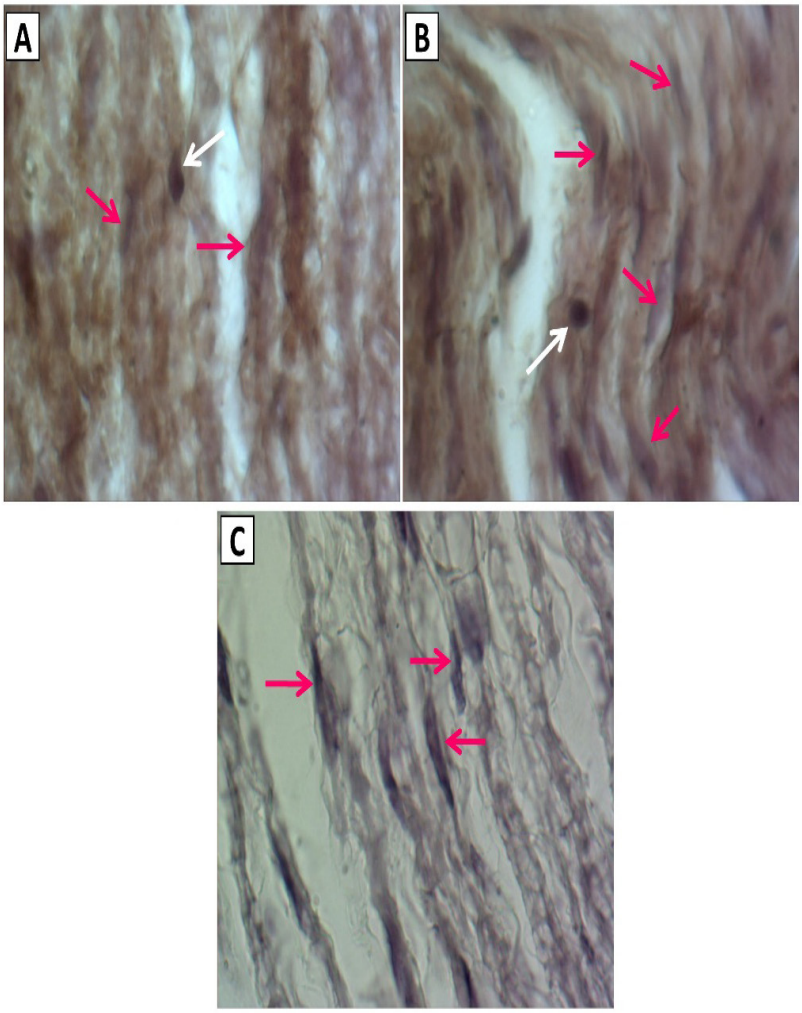

FIGURE 1. BrDU-labeled BMSCs in the injured sciatic nerve (magnification x1000). Nucleus of BMSCs labeled by BrDU in the HC (A), BMSCs (B), and SNI (C). The white arrow indicates the nucleus of BMSC, and the pink arrow indicates the fibroblast nucleus.

bile phase ratio. Subsequently, derivatization (staining) was conducted using anisaldehyde reagent, a specific indicator for terpenoid compounds. The appearance of a spot in the lower right section of the image confirms the presence of terpenoid compounds in the sample (Figure 3A).

Identification of Alkaloid Compounds

Separation was performed based on the specified mobile phase ratio. Subsequently, derivatization (staining) was carried out using Dragendorff's reagent, which is a specific reagent for the identification of alkaloid compounds. No spot appeared in the lower right section of the image, indicating the absence of alkaloid compounds in the sample (Figure 3B).

Identification of Semi-Polar Phenolic and Flavonoid Compounds

Separation was performed based on the specified mobile phase ratio. Subsequently, derivatization (staining) was carried out using Natural Products reagent, which is a specific reagent for the identification of semi-polar phenolic and flavonoid compounds. The appearance of a spot in the middle right section of the image indicates the presence of semi-polar phenolic and flavonoid compounds in the sample (Figure 3C).

Identification of Saponin Compounds

Separation was performed based on the specified mobile phase ratio. Subsequently, derivatization (staining) was carried out using FeCl3 reagent, which is a specific reagent for the identification of saponin compounds.

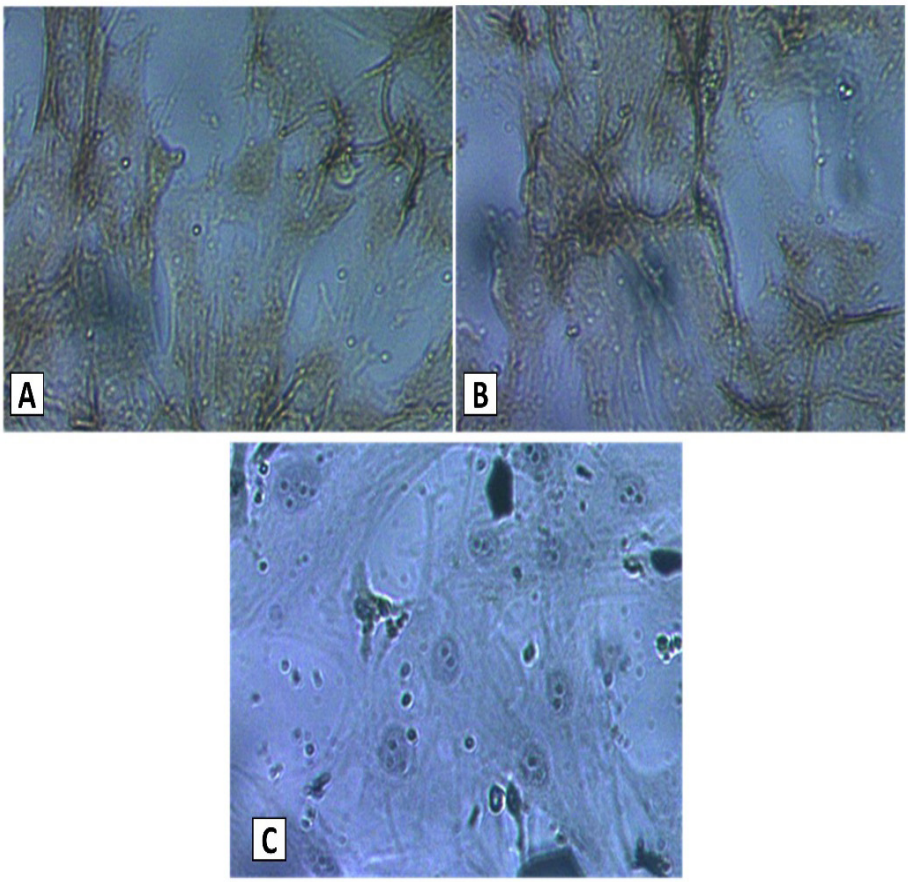

FIGURE 2. Microscopic figures of stromal cells after performing immunocytochemistry (400x magnification). Fibronectin primary antibody (A), anti-CD44 (B), and negative control of undifferentiated BMSCs at the end of the fourth passage (C).

The appearance of a spot in the lower right section of the image indicates the presence of saponin compounds in the sample (Figure 3D).

Identification of Tannin Compounds and Other Water-Soluble Compounds

Separation was performed based on the specified mobile phase ratio. Subsequently, staining was carried out using sulfuric acid reagent, which is a general reagent for the identification of various compounds. The appearance of a spot in the right section of the image indicates the presence of tannin compounds and other water-soluble compounds in the sample (Figure 3E).

Histopathology

Animal surgery for treatment with BMSC/C/G/S was successfully performed in several stages (Figure 4). After preparing transverse sections of the sciatic nerve in the studied groups, histopathology was performed with H and E stain (Figure 5). Motic software was used to measure axon and myelin space (Figure 6).

SFI Test

The footprints of the HC, SNI, BMSCs, OE, OE+BMSCs, OE+C/G/S, C/G/S, C/G/S/BMSCs, and OE/C/G/S/BMSCs groups were presented in Figure 7A. The footprints of the OE/C/G/S/BMSCs group after 8 weeks, compared to other groups, showed a high rate of recovery. After 2 (Figure 7B) and 8 (Figure 7C) weeks, the SFI was assessed in both control and treatment groups. The results of SFI showed that the SNI, BMSCs, OE, OE+BMSCs, OE+C/G/S, C/G/S, C/G/S/BMSCs, and OE/C/G/S/BMSCs groups had a significant increase compared to the HC group ($P<0.0001$). Conversely, the BMSCs, OE, OE+BMSCs, OE+C/G/S, C/G/S, C/G/S/BMSCs, and OE/C/G/S/BMSCs groups exhibited a significant decrease compared to the SNI group ($P<0.0001$). The OE/C/G/S/BMSCs treatment group had a lower SFI

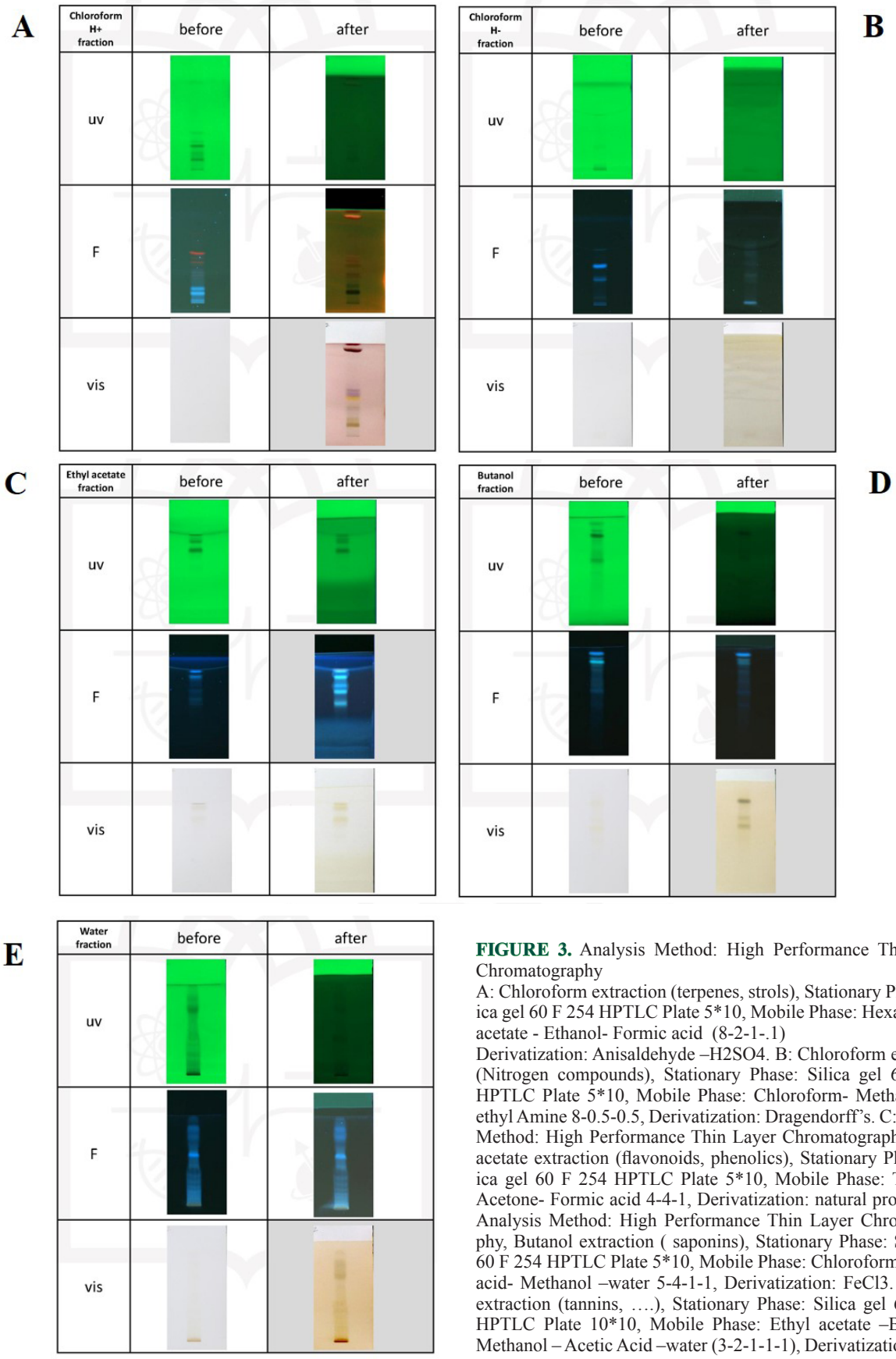

FIGURE 3. Analysis Method: High Performance Thin Layer Chromatography
A: Chloroform extraction (terpenes, strols), Stationary Phase: Silica gel 60 F 254 HPTLC Plate 5*10, Mobile Phase: Hexane-Ethyl acetate - Ethanol- Formic acid (8-2-1-.1)
Derivatization: Anisaldehyde –H2SO4. B: Chloroform extraction (Nitrogen compounds), Stationary Phase: Silica gel 60 F 254 HPTLC Plate 5*10, Mobile Phase: Chloroform- Methanol- Diethyl Amine 8-0.5-0.5, Derivatization: Dragendorff's. C: Analysis Method: High Performance Thin Layer Chromatography, Ethyl acetate extraction (flavonoids, phenolics), Stationary Phase: Silica gel 60 F 254 HPTLC Plate 5*10, Mobile Phase: Toluene - Acetone- Formic acid 4-4-1, Derivatization: natural products. D: Analysis Method: High Performance Thin Layer Chromatography, Butanol extraction (saponins), Stationary Phase: Silica gel 60 F 254 HPTLC Plate 5*10, Mobile Phase: Chloroform – Acetic acid- Methanol –water 5-4-1-1, Derivatization: FeCl3. E: water extraction (tannins, ….), Stationary Phase: Silica gel 60 F 254 HPTLC Plate 10*10, Mobile Phase: Ethyl acetate –Butanol – Methanol – Acetic Acid –water (3-2-1-1-1), Derivatization: Metanol-H2SO4 (10%).

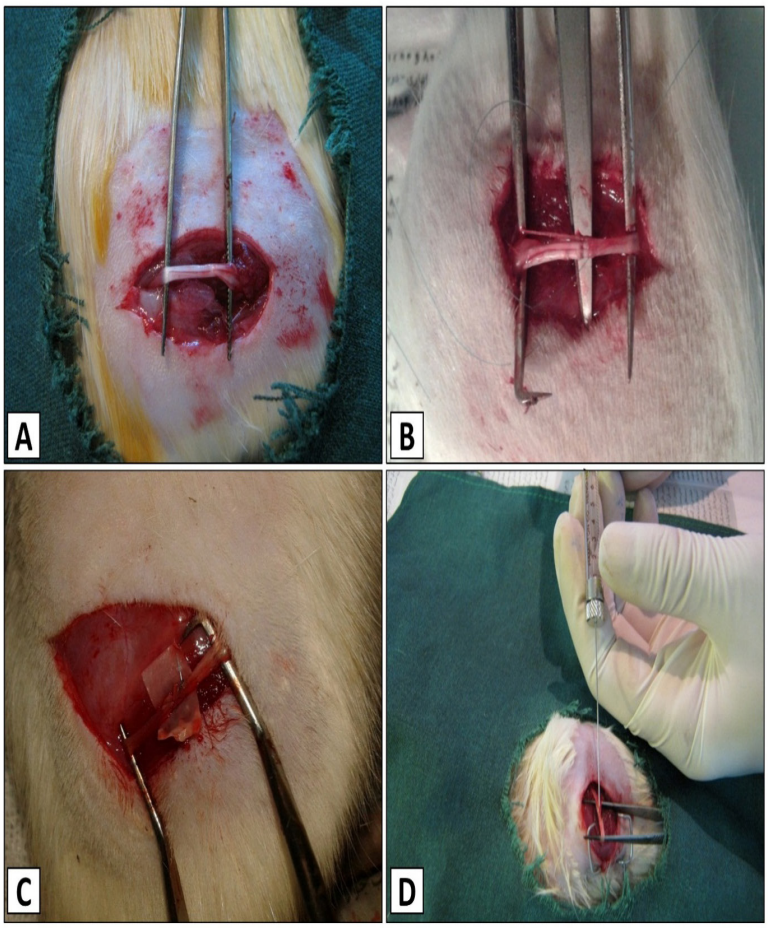

FIGURE 4. Exposure of the sciatic nerve in the rat (A), creating a lesion in the sciatic nerve and suturing the sciatic nerve with 0.6 suture thread (sutures, Iran) (B), placing the BMSC cultured cells on the C/G/S (C), in the location of the sciatic nerve lesion, injecting the culture medium Alpha Mem along with 100,000 BMSCs to the site of sciatic nerve damage using a Hamilton syringe (D).

compared to the BMSCs, OE, OE+BMSCs, OE+C/G/S, C/G/S, and C/G/S/BMSCs groups, and this increase was significant (P<0.0001).

NCV (Nerve conduction velocity)

After 8 weeks, the NCV rate (m/s) was assessed in both control and treatment groups. The results showed that the SNI, BMSCs, OE, OE+BMSCs, OE+C/G/S, C/G/S, C/G/S/BMSCs, and OE/C/G/S/BMSCs groups had a significant decrease compared to the HC group (P<0.0001). Conversely, the BMSCs, OE, OE+BMSCs, OE+C/G/S, C/G/S, C/G/S/BMSCs, and OE/C/G/S/BMSCs groups exhibited a significant increase compared to the SNI group (P<0.0001). The OE/C/G/S/BMSCs treatment group had a higher NCV rate compared to the BMSCs, OE, OE+BMSCs, OE+C/G/S, C/G/S, and C/G/S/BMSCs groups, and this increase was significant (Figure 8).

AMP (Amplitude muscle action potenitial)

According to the the AMP rate (mv) of the studied group, the results showed that the SNI (P<0.0001), BMSCs (P<0.0001), OE (P<0.0001), OE+BMSCs (P<0.001), OE+C/G/S (P<0.0001), C/G/S (P<0.0001), C/G/S/BMSCs (P<0.0001), and OE/C/G/S/BMSCs (P<0.001) groups had a significant decrease compared to the HC group (P<0.0001). The BMSCs, OE, OE+BMSCs, OE+C/G/S, C/G/S, C/G/S/BMSCs, and OE/C/G/S/BMSCs groups exhibited a significant increase compared to the SNI group (P<0.0001). The OE/C/G/S/BMSCs treatment group had a higher NCV rate compared to the OE, OE+C/G/S, and groups (P<0.0001) (Figure 9).

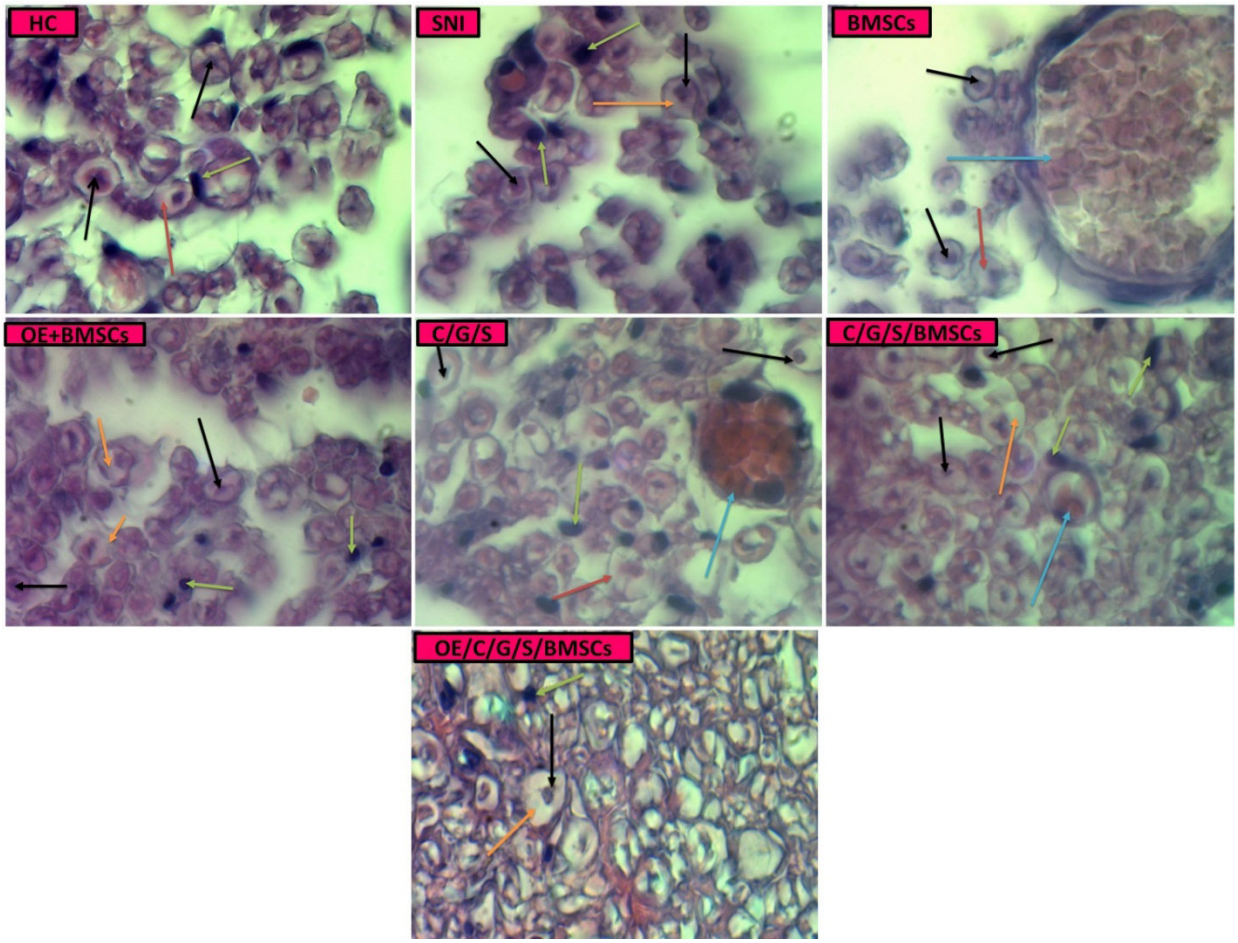

FIGURE 5. Light microscopy images of sciatic nerve hematoxylin and eosin (H&E) staining of: HC, SNI, BMSCs, OE, OE+BMSCs, C/G/S, C/G/S/BMSCs, and OE/C/G/S/BMSCs groups. Black arrow: axon, green arrow: Schwann nucleus, orange arrow: myelin; blue arrow: blood vessels.

Number of Vessels and Fibers

After finishing the treatment period, the number of vessels was analysed in the study groups. The results showed that the SNI, BMSCs, OE, OE+BMSCs, OE+C/G/S, C/G/S, C/G/S/BMSCs, and OE/C/G/S/BMSCs groups had a significant decrease compared to the HC group (P<0.0001). On the other hand, the BMSCs, OE, OE+BMSCs, OE+C/G/S, C/G/S/BMSCs, and OE/C/G/S/BMSCs groups exhibited a significant increase compared to the SNI group (P<0.0001). The OE/C/G/S/BMSCs treatment group had a higher NCV rate compared to the BMSCs, OE, OE+BMSCs, OE+C/G/S, and C/G/S groups (P<0.0001) (Figure 10A).

After 8 weeks of treatment, the number of fibers was studied in all groups. The results showed that the SNI, BMSCs, OE, OE+BMSCs, OE+C/G/S, C/G/S, C/G/S/BMSCs, and OE/C/G/S/BMSCs groups had a significant decrease compared to the HC group (P<0.0001). The BMSCs, OE, OE+BMSCs, OE+C/G/S, C/G/S/BMSCs, and OE/C/G/S/BMSCs groups exhibited a significant increase compared to the SNI group (P<0.0001). The OE/C/G/S/BMSCs treatment group had a higher NCV rate compared to the BMSCs, OE, OE+BMSCs, OE+C/G/S, C/G/S, and C/G/S/BMSCs groups (P<0.0001) (Figure 10B).

Biggest Area of Vessels, Myelin, Axon, and Fiber

According to the significant decrease in the vessels area (1000 micro m2) in the groups of SNI (P<0.0001), BMSCs (P<0.0001), OE (P<0.0001), OE+BMSCs (P<0.0001), OE+C/G/S (P<0.0001), C/G/S (P<0.0001), C/G/S/BMSCs (P<0.0001), and OE/C/G/S/BMSCs (P<0.01) compared to the HC group. The significant increase of the groups of BMSCs (P<0.0001),

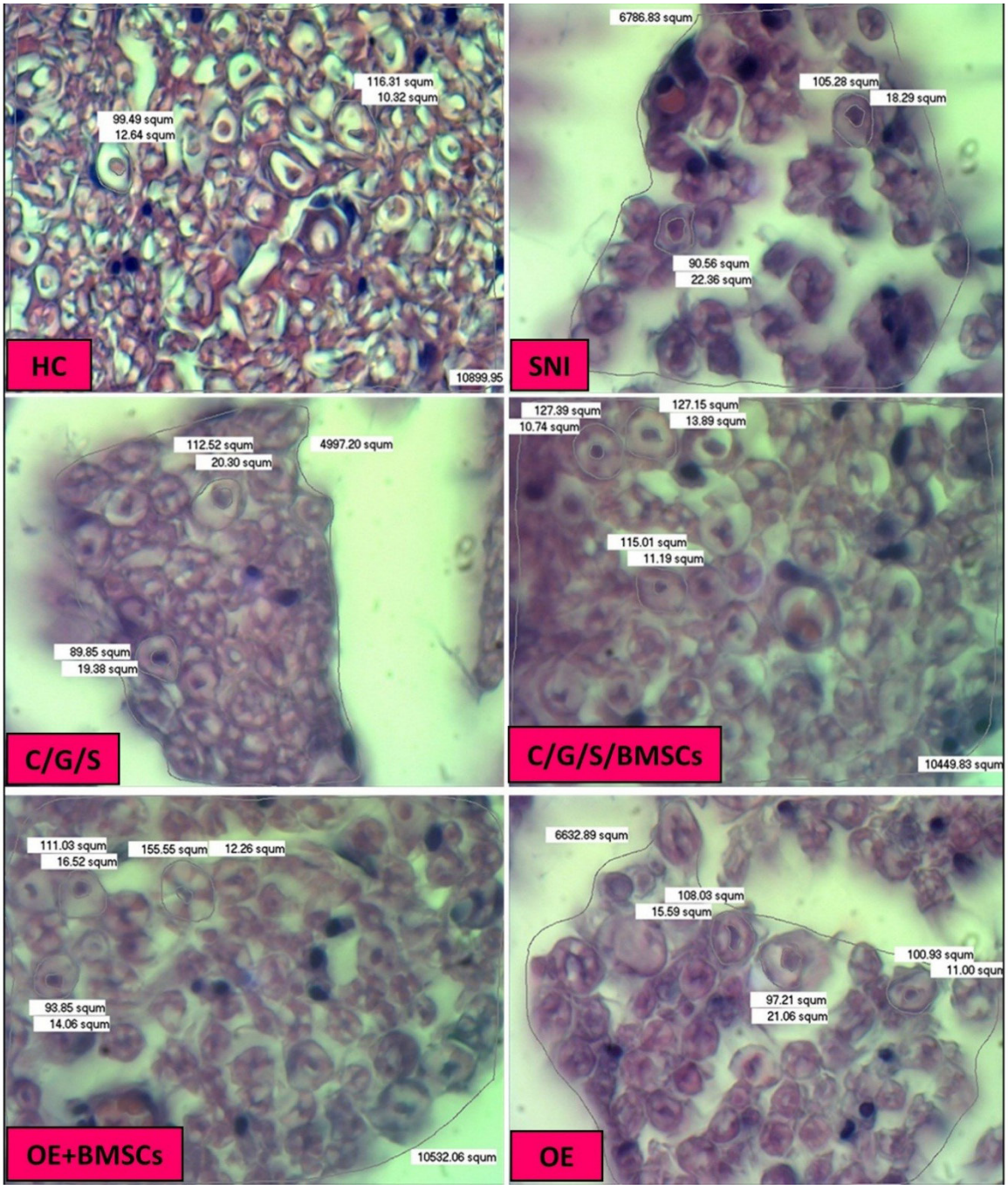

FIGURE 6. Light microscopy images of transverse sections of the sciatic nerve in HC, SNI, OE, OE+BMSCs, C/G/S, and C/G/S/BMSCs groups.

OE (P<0.0001), OE+BMSCs (P<0.0001), OE+C/G/S (P<0.0001), C/G/S (P<0.01), C/G/S/BMSCs (P<0.0001), and OE/C/G/S/BMSCs (P<0.0001) in comparison to SNI. The results showed that the vessel area (1000 micro m2) with OE/C/G/S/BMSCs after 8 weeks was higher than the BMSCs (P<0.0001), OE (P<0.0001), OE+BMSCs (P<0.0001), OE+C/G/S (P<0.0001), C/G/S (P<0.0001), C/G/S/BMSCs (P<0.01) groups (Figure 11A). Also, the vessel area (1000 micro m2) with OE/C/G/S/BMSCs after 8 weeks was higher than the BMSCs, OE, OE+BMSCs, OE+C/G/S, C/G/S, C/G/S/BMSCs (P<0.0001) groups (Figure 11B). The biggest area of axon (1000 micro m2) with OE/C/G/S/BMSCs after 8 weeks was higher than the BMSCs, OE, OE+BMSCs,

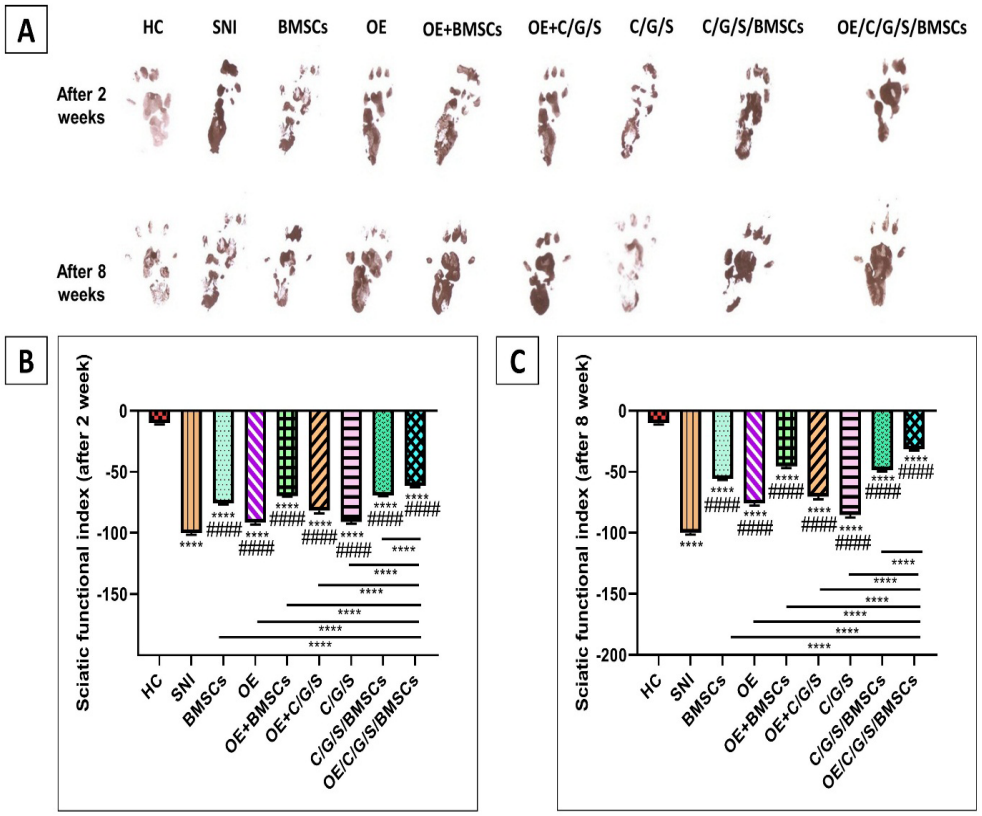

FIGURE 7. Footprints change at 2 and 8 weeks after surgery (A). Comparison of the SFI index functional recovery in HC, SNI, BMSCs, OE, OE+BMSCS, OE+C/G/S, C/G/S/BMSCs, and OE/C/G/S/BMSCs groups after 2 (B) and 8 (A) weeks. Comparison of treated groups with HC ****P<0.0001; Comparison of groups treated with SNI ####P<0.0001.

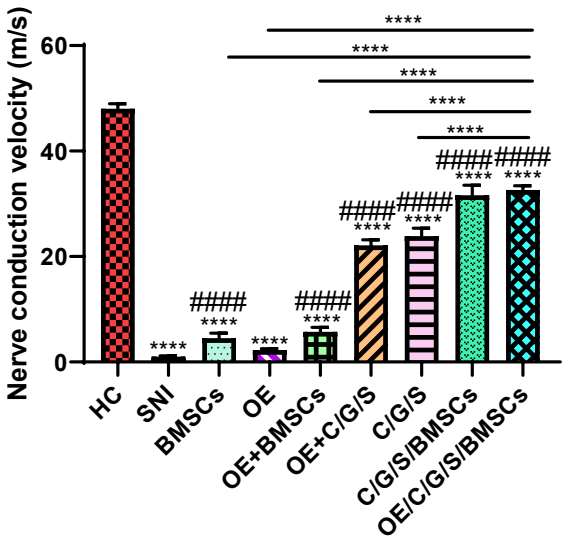

FIGURE 8. Comparison of the nerve conduction velocity (m/s) in HC, SNI, BMSCs, OE, OE+BMSCS, OE+C/G/S, C/G/S/BMSCs, and OE/C/G/S/BMSCs groups after 8 weeks. Comparison of treated groups with HC ****P<0.0001; Comparison of groups treated with SNI ####P<0.0001.

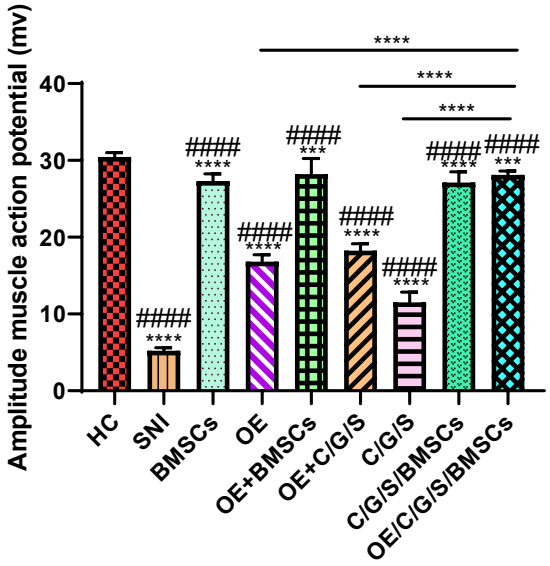

FIGURE 9. Comparison of the amplitude of muscle action potential (mv) in HC, SNI, BMSCs, OE, OE+BMSCS, OE+C/G/S, C/G/S/BMSCs, and OE/C/G/S/BMSCs groups after 8 weeks. Comparison of treated groups with HC ***P<0.001, ****P<0.0001; Comparison of groups treated with SNI ####P<0.0001.

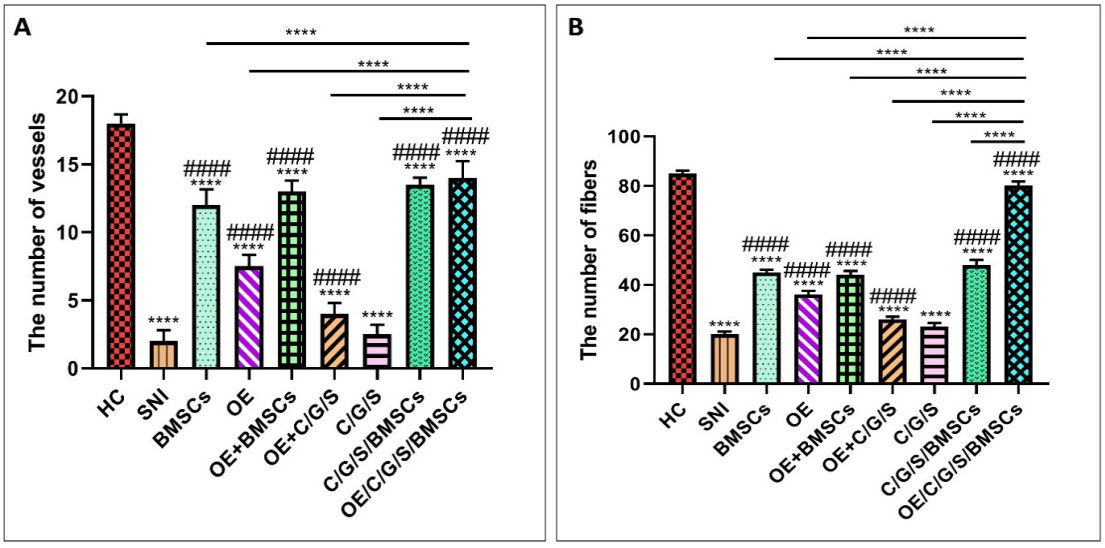

FIGURE 10. The number of fibers (A) and vessels in HC, SNI, BMSCs, OE, OE+BMSCS, OE+C/G/S, C/G/S/BMSCs, and OE/C/G/S/BMSCs groups after 8 weeks. Comparison of treated groups with HC ****P<0.0001; Comparison of groups treated with SNI ####P<0.0001.

OE+C/G/S, C/G/S, C/G/S/BMSCs (P<0.0001) groups (Figure 11C). The results showed that the biggest area of fiber (1000 micro m2) with OE/C/G/S/BMSCs after 8 weeks was higher than the BMSCs, OE, OE+BMSCs, OE+C/G/S, C/G/S, C/G/S/BMSCs groups (P<0.0001) (Figure 11D).

Discussion

The primary goal of researching BMSCs is to harness their potential for clinical applications in the treatment of various diseases and injuries affecting multiple organ systems. For cell transplantation to be effective, the selected cells must meet several essential criteria. These

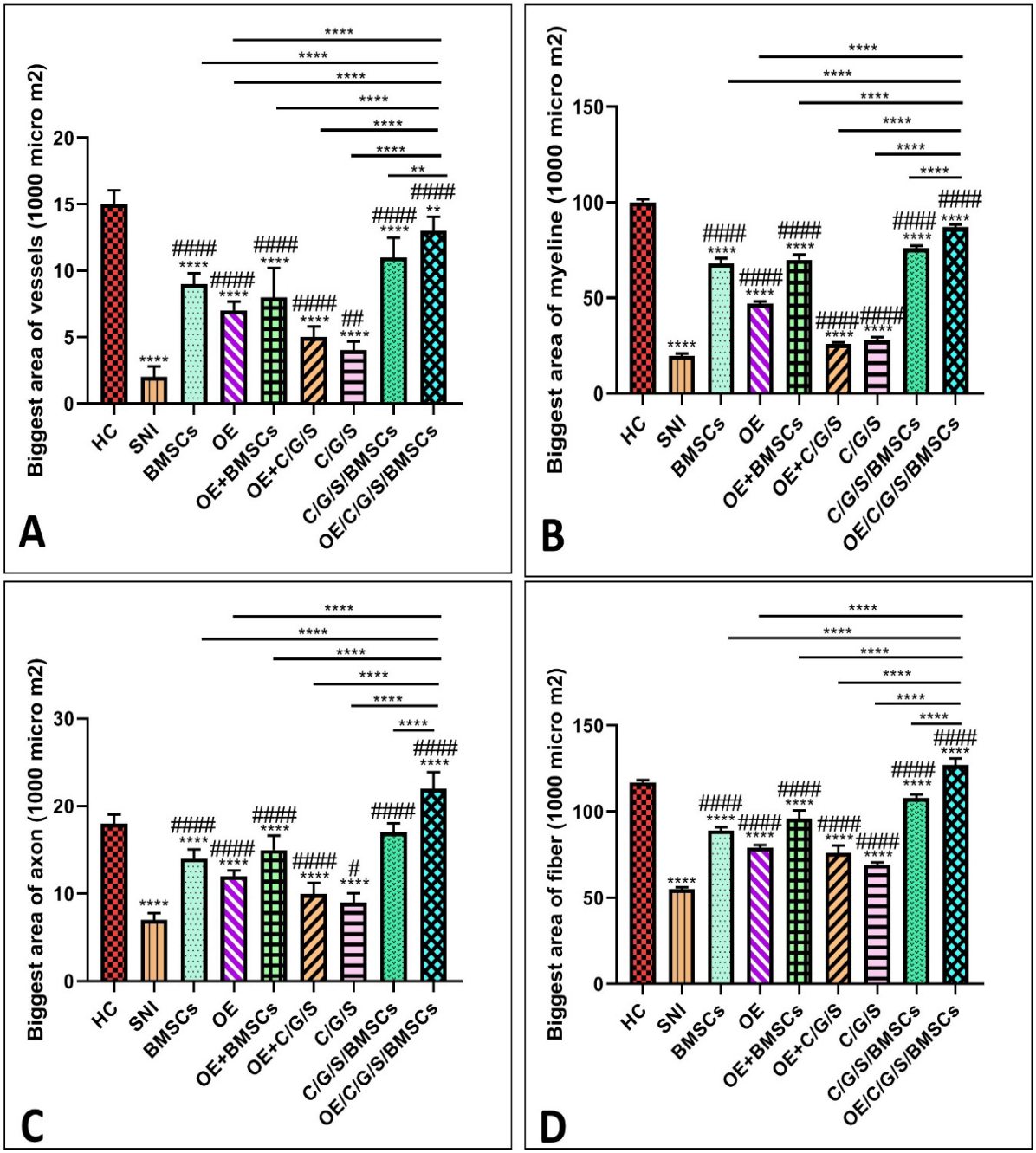

FIGURE 11. Biggest area of vessels (A), myelin (B), axon (C), and fiber (D) (1000 micro m2) in HC, SNI, BMSCs, OE, OE+BMSCS, OE+C/G/S, C/G/S/BMSCs, and OE/C/G/S/BMSCs groups after 8 weeks. Comparison of treated groups with HC **P<0.01, ****P<0.0001; Comparison of groups treated with SNI ####P<0.0001.

include ease of isolation, a high proliferative capacity in culture, immunological compatibility or neutrality with the host, and the ability to survive and integrate into the target tissue over an extended period. Given these attributes, BMSCs present a promising candidate for therapeutic transplantation (Swett et al., 1991; Temporin et al., 2008).

Moreover, due to the ease of access to BMSCs, their autologous transplantation is feasible, eliminating ethical concerns associated with the use of neural or embryonic stem cells. Research indicates that BMSCs respond to microenvironmental cues by secreting a variety of growth factors. However, other studies suggest that these cells may directly differentiate into specific

cell types required by the host tissue after migration to the injury site, guided by local environmental signals. Peripheral nerve injuries remain a significant clinical challenge, often resulting in long-term functional impairment. In this context, the present study aimed to evaluate the therapeutic potential of olive fruit extract combined with BMSCs seeded on a chitosan-gelatin scaffold for the regeneration of transected sciatic nerves (Swett et al., 1991; Temporin et al., 2008).

A study demonstrated that damaged peripheral nerves can undergo repair when exposed to Schwann cells derived from gingiva-derived mesenchymal stem cells (GMSCs). Specifically, when GMSCs are directly differentiated into Schwann cell precursors (GiSCs), there is a notable increase in the expression of Schwann cell-specific markers such as S-100β and p75NTR. Furthermore, research on sciatic nerve crush injuries in rats has shown that Schwann cells derived from GMSCs play a crucial modulatory role under in vitro conditions. These Schwann-like cells influence pro-inflammatory macrophages to significantly upregulate the expression of key neurotrophic factors, including brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF), both of which are essential for promoting nerve regeneration (Eckersley et al., 2001).

In a study by Hiroaki et al. (2002), dexamethasone was employed to mitigate inflammation resulting from nerve injury. The findings indicated an absence of edema and axonal myelin degeneration, highlighting the anti-inflammatory effectiveness of the treatment (Shishido et al., 2002). Similarly, research conducted by Becker et al. demonstrated that the topical application of glucocorticoids significantly reduced scar tissue formation and neuroma development, thereby facilitating the nerve healing process (Becker et al., 1987). Additionally, glucocorticoids were found to accelerate myelin degradation and axonal degeneration, which in turn activated Schwann cells and promoted the regeneration of myelin sheaths (Becker et al., 1987).

In this study, HPTLC and derivatization techniques confirmed the presence of terpenoids, phenolics, flavonoids, saponins, and tannins in olive fruit extract, while no alkaloid compounds were detected. These results are consistent with previous studies that have reported a similar phytochemical profile in olive leaves and extracts. For example, analyses using HPTLC and Fourier-transform infrared (FTIR) spectroscopy have demonstrated that fermentation enhances the yield of phenolic and flavonoid compounds from olive leaves (Agatonovic-Kustrin and Gegechkori 2021). The absence of alkaloids, although notable, aligns with prior phytochemical assessments indicating their limited or undetectable presence in olive-derived products (Agatonovic-Kustrin and Gegechkori 2021).

In this study, complete transection of the sciatic nerve led to the formation of collagen fibers around the injury site, which impeded nerve regeneration. Under normal physiological conditions, the resolution of inflammation is marked by a decline in neutrophil and macrophage populations at the wound site, signaling the transition from the inflammatory to the proliferative phase of healing. While inflammation is critical for infection control, debris clearance, and initiation of tissue repair, its prolonged presence can contribute to further tissue damage and delay healing. Research indicates that sustained macrophage activity may hinder wound contraction, suggesting that their timely reduction is necessary for the progression to later stages of repair. Consequently, controlling inflammation is a key therapeutic strategy, as the persistence of cellular waste and inflammatory signals in the wound environment can result in chronic, non-healing injuries.

In a study by Suk Lee (2007), the sciatic nerves of rats were transected and divided into four treatment groups. Group one received 0.3 mL of saline, group two received 0.3 mL of dexamethasone, group three again received 0.3 mL of saline, and group four was treated with 0.3 mL of amniotic fluid containing hyaluronic acid. Electromyographic and histological assessments were conducted at 4 and 12 weeks post-surgery to evaluate perineural scar thickness, myelinated nerve fiber count, and axonal regeneration at the repair site. After four weeks, the dexamethasone group showed significantly reduced epineural scar formation compared to the other groups. Overall, the findings indicated that local administration of dexamethasone, hyaluronic acid, or amniotic fluid was more effective than saline in minimizing perineural scarring and promoting nerve regeneration in rat hind limb injuries. (Lee et al., 2007).

A study by Diegelmann et al. investigated the effects of chitosan on inflammation, angiogenesis, and other wound healing factors. Using a polyvinyl alcohol sponge implant model, chitosan was applied to a standard sub-

cutaneous wound on the back of adult Sprague-Dawley rats. On days 8 and 14, the chitosan-treated group showed multinucleated leukocytes, while the control group primarily contained macrophages, fibroblasts, collagen, and newly formed blood vessels. The results indicated that chitosan promotes wound healing by reducing the influx of tissue-activated macrophages, thereby decreasing angiogenesis and connective tissue formation around the wound (Diegelmann et al., 1996).

This study investigated the effects of olive fruit extract and a chitosan-gelatin scaffold loaded with BMSCs on neurodegeneration caused by sciatic nerve injury in rats. Behavioral, electrophysiological, and histological evaluations were conducted. The results showed that this treatment effectively repaired sciatic nerve damage, with the olive fruit extract + chitosan-gelatin + BMSC group demonstrating significant improvements. These included increased nerve conduction velocity (m/s), amplitude of muscle action potential (mV), and enhancements in the number of vessels and fibers, as well as the largest vessel area, myelin, axon, and fiber area (1000 μm^2). Additionally, a significant reduction in the functional index (from 0 to -50) was observed ($P < 0.0001$).

Conclusion

The combination of olive extract, chitosan-gelatin, and BMSCs showed significant reparative effects on sciatic nerve injury, presenting a promising approach for peripheral nerve repair.

Acknowledgments

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Conflict of interest

The authors declare that there are no conflicts of interest.

Authors' contribution

All authors contributed to the design, execution, and writing of all sections of this research.

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