Journal of Health Sciences Institute, 8(2): 264-270, 2023
DOI: 10.51754/cusbed.1335363

Journal of Health Sciences Institute

| cusbed.cumhuriyet.edu.tr Founded: 2016 Available online, ISSN: 2587-0874 Publisher: Sivas Cumhuriyet Üniversitesi

Comparison of the Effects of Andız (Juniperus Sp) Root Extract and Vitamin B12 on Nerve Healing in Rats with Sciatic Nerve Injury

Tunahan Sancak[1,a,*], Eyüp Çetin[2,b]

[1]*Department of Surgery, Faculty of Veterinary Medicine, Sivas Cumhuriyet University, Sivas, Türkiye*
[2]*Department of Neurosurgery, Haydarpasa Training and Research Hospital, Health Sciences University, Istanbul, Türkiye*
**Corresponding author*

Research Article

History

Received: 31/07/2023
Accepted: 17/08/2023

ABSTRACT

The self-repair capacity of peripheral nerves is limited, and the repair of severely damaged or significantly flawed nerves is challenging. For preclinical investigations on peripheral nerve regeneration, the sciatic nerve injury model is the most often used experimental model. In rats, sciatic nerve injury is the most frequently used model in studies related to peripheral nerve regeneration. In this study, 32 rats were subjected to sciatic nerve injury, and applications of B12 vitamin and Andız root extract were performed. After the applications, the rats were sacrificed on the 21st day, and samples of the sciatic nerve tissue were taken. The collected samples were examined using histopathological and immunofluorescence methods. The examination revealed that B12 vitamin and Andız root extract, when applied separately to the rats, provided limited and similar benefits histopathologically, while the group that received combined treatment showed more effective positive effects compared to all other groups. Additionally, when assessing S100 and GFAP expression through immunofluorescence, it was observed that individual applications provided similar benefits, while the group that received combined treatment contributed more effectively compared to all other groups. In conclusion, in the rat model of sciatic nerve injury, it was determined that Andız root extract and B12 vitamin, when applied separately, had limited and similar effects compared to the control group in determining the levels of recovery. However, when applied together, they exhibited a synergistic effect, providing a greater contribution to nerve regeneration compared to all other groups.

Keywords: Andız root extract, B12 vitamin, injury, nerve regeneration, sciatic nerve, treatment.

[a] *tunahansancak@hotmail.com* *https://orcid.org/0000-0002-7813-1575* | [b] *eyupcet@gmail.com* *https://orcid.org/0000-0002-2045-6721*

How to Cite: Sancak T, Cetin E (2023) Comparison of the Effects of Andız (Juniperus Sp) Root Extract and Vitamin B12 on Nerve Healing in Rats with Sciatic Nerve Injury, Journal of Health Sciences Institute, 8(2): 264-270

Introduction

Motor, sensory, and autonomic functions may completely or partially loss as a result of peripheral nerve damage brought on by accidents, tumor removal, congenital abnormalities, compression, or contusion. (Li et al., 2017). In mammals, neurons generally do not divide, and losses resulting from neuron damage are usually permanent. However, if the neuron cell body remains undamaged, there are possibilities for repair. Following trauma to the axon, degeneration is initially observed, followed by regeneration. The duration and extent of regeneration in the affected area depend on the distance between that area and the neuron cell body (İshakoglu, 2019).

Peripheral nerve injuries are often treated using either surgical or non-surgical methods. While non-surgical treatments include physical therapy, electrical stimulation, hydrotherapy, and medicine, surgical techniques of treatment include primary end-to-end repair and nerve grafts. The treatment of traumatic peripheral nerve injuries has generally centered on non-surgical therapeutic methods, such as new pharmacological or physical therapies (Seo et al., 2021).

From ancient times to the present day, numerous medicinal plants have been used in traditional medicine as herbal remedies for various diseases. The use of natural products in traditional medicine is increasing nowadays because they are considered safer, have fewer side effects, and are more effective (Al-Owamri et al., 2023). One of these plants is Juniperus, whose needle-like parts and leaves are used in medicine as anthelmintic, diuretic, stimulant, and antiseptic agents, as well as for wound healing (Tumen et al., 2012).

The B-vitamin complex, which includes the vitamins B1 (thiamine), B6 (pyridoxine), and B12, is given to patients in order to treat nervous system degeneration. These B vitamins, particularly vitamin B12, play significant roles in numerous biological processes that support healthy neurological function. B-vitamin complex or B12 vitamin administration has been demonstrated to increase the number of Schwann cells, the number of myelinated nerve fibers, and the diameter of axons, thus promoting the regeneration of myelinated nerve fibers and the proliferation of Schwann cells (Altun and Kurutaş, 2016).

This study aims to compare the effects of Andız (Juniperus sp) root extract and B12 vitamin application on nerve regeneration in rats with induced sciatic nerve injury using histopathological and immunofluorescence methods.

Material and Methods

The ethical committee approval for the study was obtained with the decision numbered 65202830-050.04.04-673 and dated 06.10.2022 by the Local Ethics Committee for Animal Experiments of Sivas Cumhuriyet University.

Animal Material

In the study, a total of 32 Wistar albino male rats weighing an average of 200-250 grams each were used. Xylazine hydrochloride (Xylazinbio, Bioveta, Czech Republic) at a dose of 3 mg/kg and Ketamine Hydrochloride (Ketasol, Richter Pharma, İnterhas, Ankara) at a dose of 90 mg/kg were administered intraperitoneally to induce general anesthesia in the rats. The rats were divided into 4 groups, with 8 rats in each group. Nerve injury was induced in all rats within the groups. For this purpose, a common surgical procedure was performed as described below.

Surgical Procedure

Firstly, the experimental rats were placed on the surgical station, and the incision area was shaved and cleansed with an antiseptic solution. In all groups, a skin incision was made on the right extremity, and the muscles were dissected bluntly to expose the sciatic nerve, which runs parallel to the femur. Subsequently, the nerve was transected to create sciatic nerve injury, and after bringing the nerve ends together in the incision area, an epineural nerve anastomosis technique was used to suture them with 8-0 polypropylene at two separate points. The muscles were then sutured with PGA (polyglycolic acid) (4-0), and the skin was closed with PGA (2-0) (Figure 1).

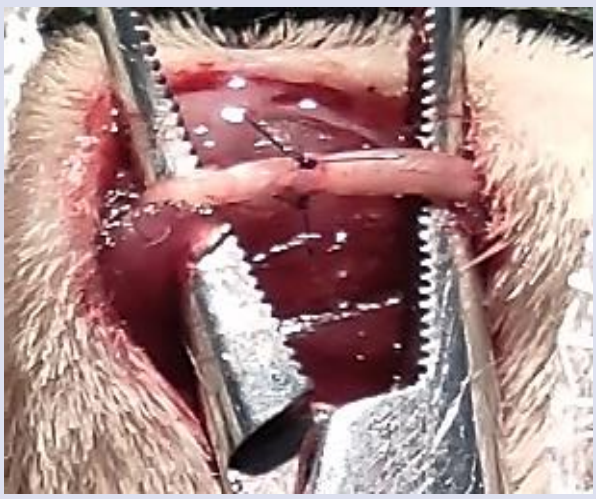

Figure 1. Epineural nerve anastomosis technique

Experimental Groups

Group 1 (Control Group): After the nerve injury was induced, the nerve ends were sutured, and only isotonic solution was applied locally to the area. Subsequently, the muscles and skin were closed, and no other intervention was performed.

Group 2 (Andız Group): After the nerve injury was induced, the nerve ends were sutured, and Andız (Juniperus sp) root extract was applied locally to the area. The muscles and skin were closed, and no other intervention was performed.

Group 3 (B12 Group): After the nerve injury was induced, the nerve ends were sutured, and the muscles and skin were closed. B12 vitamin was administered intraperitoneally for 21 days.

Group 4 (Andız + B12 Group): After the nerve injury was induced, the nerve ends were sutured, and Andız (Juniperus sp) root extract was applied locally to the area. After closing the muscles and skin, B12 vitamin was administered intraperitoneally for 21 days.

The study will last for a total of 21 days, and on the 21st day, the rats were euthanized, and the tissue with the nerve injury was collected and sent to the laboratory for histopathological and immunofluorescence examinations.

Histopathological Examinations

The sciatic nerve tissue samples were fixed in 10% buffered formaldehyde solution for 48 hours. After fixation, the tissues were processed through a series of graded alcohols and xylene and embedded in paraffin blocks. Sections with a thickness of 5 µm were obtained from the paraffin blocks at intervals of 50-100 micrometers. Hematoxylin-eosin staining was performed on the obtained sections to evaluate histopathological changes. The sections were evaluated based on the histopathological findings as follows: negative (-), mild (+), moderate (++), and severe (+++) (Güven et al., 2022).

Double Immunofluorescence Examinations

Tissue sections obtained on adhesive (poly-L-Lysine) slides were deparaffinized and dehydrated for immunofluorescence examination. The tissues were incubated with primary antibodies (GFAP Cat No: sc-33673, Dilution Ratio: 1/100, US) and incubated according to the instructions for use. As a secondary labeling step, immunofluorescence secondary antibodies (FITC Cat No: ab6785, Dilution Ratio: 1/500, UK) were applied to the tissues and incubated in the dark for 45 minutes. Then, the tissues were incubated with second primary antibodies (S100 Cat No: sc-53438, Dilution Ratio: 1/100, US) according to the instructions for use. Subsequently, immunofluorescence secondary antibodies (Texas Red Cat No: ab6719, Dilution Ratio: 1/500, UK) were applied to the tissues and incubated in the dark for 45 minutes. In the next step, the sections were incubated with mounting medium containing DAPI (Cat No: D1306, Dilution Ratio: 1/200, UK) for 5 minutes in the dark, and then the tissues were covered with coverslips. The stained tissues were examined under a fluorescence attachment microscope (Zeiss AXIO, Germany).

Statistical Analysis

For the analysis of differences between groups in the semiquantitative data obtained from the histopathological examination, the nonparametric Kruskal-Wallis test was used, and the Mann-Whitney U test was used for pairwise comparisons between groups. The SPSS 13.0 software package was used for these statistical analyses.

To determine the intensity of positive staining from the images obtained through immunofluorescence staining, 5 randomly selected areas were chosen from each image, and evaluations were performed using the ZEISS Zen Imaging Software program. The data were statistically defined in terms of mean and standard deviation (mean±SD) for the percentage of area. One-way ANOVA and subsequent Tukey's test were conducted to compare the immunoreactive cells and immunopositive stained areas with healthy controls. A p-value of <0.05 was considered statistically significant, and the data were presented as mean ± SD.

Results

Histopathological Findings

When the sciatic nerves were examined histopathologically:

Group 1: Severe degeneration, focal demyelination, and edema in the interfascicular connective tissue were observed in the neurons (Figure 2).

Group 2: Mild degeneration, mild demyelination, and mild edema in the connective tissue were observed in the neurons (Figure 2).

Group 3: Mild degeneration in neurons, demyelinated areas, and edema in the blood vessels in the connective tissue were observed (Figure 2).

Group 4: Mild demyelination was observed (Figure 1). A statistically significant difference ($p<0.05$) was found compared to Group 1. The histopathological findings are summarized in Table 1.

Table 1. Scoring of histopathological findings in sciatic nerve tissues.

	Group 1 (CON)	Group 2 (AND)	Group 3 (B12)	Group 4 (AND+B12)
Degeneration in neurons	+++	+	+	-
Demyelination in axons	+++	++	++	+
Edema in interfascicular connective tissue	+++	++	++	-

Immunofluorescence Findings

When sciatic nerves were examined using the immunofluorescence method:

Group 1: Severe levels of GFAP and S100 expressions were observed in neurons (Figure 2).

Group 2: Mild levels of GFAP and S100 expressions were identified in neurons (Figure 2).

Group 3: Mild levels of GFAP and S100 expressions were detected in neurons (Figure 2).

Group 4: Very mild levels of GFAP and S100 expressions were observed in neurons (Figure 2). A statistically significant difference ($p<0.05$) was found when compared to Group 1. Immunofluorescence findings are summarized in Table 2.

Table 2. Scoring of immunofluorescence findings observed in sciatic nerve tissues.

	Group 1 (CON)	Group 2 (AND)	Group 3 (B12)	Group 4 (AND+B12)
GFAP Expression	80,35±3,17^a	40,91±2,14^b	41,51±1,74^b	21,44±0,48^c
S100 Expression	79,74±3,52^a	40,25±1,96^b	40,75±1,81^b	20,21±0,5^c

a, b, c; Different letters within the same row indicate a statistically significant difference (p<0.05).

Discussion

The term "peripheral nerve injury" describes damage to the peripheral nerve plexus, nerve trunk, or nerve branches brought on by external forces (crush injury, traction injury, and contusion) (Lv et al., 2020). Peripheral nerve injuries are common clinical injuries and have a greater capacity for regeneration compared to the central nervous system after injury, although there are still limitations (Duan et al., 2023). In cases where complete recovery is not achieved following peripheral nerve injury, serious conditions that often lead to lifelong impairment occur (Aman et al., 2023). In nerve injuries, the end-to-end anastomosis technique is commonly applied to repair nerve damage (Horasanli et al., 2017; Zhang et al., 2023). Luo et al. (2022) and Ozay et al. (2023) observed the healing process by performing suturing after making an incision for sciatic nerve injury in their studies. The method of inducing nerve injury in the present study is similar to the nerve injury method employed by Luo et al. (2022) and Ozay et al. (2023).

Vitamin B12 plays an important role in alleviating degenerative processes in the nervous system (Arıkan et al., 2016). Therefore, research is being conducted on the potential regenerative effects of certain drugs like vitamin B12 on peripheral nerves following injury. Additionally, B12 vitamin contributes to enhanced motor and functional recovery of injured nerves by supporting positive regeneration through neurotrophic factors that promote both myelin and axon regeneration (Albay and Akkalp, 2021; Abushukur and Knackstedt, 2022).

Juniperus spp. is a plant species used in many scientific studies to observe various effects. Studies have been

conducted on the analgesic, anti-inflammatory, sedative, bronchodilator, antimicrobial, antimetastatic, antioxidant, apoptosis, allergic rhinitis, and antibiotic effects of Juniperus spp. (Kapdan et al., 2019). Furthermore, studies conducted on experimental wound models in fish and rats have shown its contribution to healing (Tumen et al., 2012; Avcı, 2020).

In experimental studies, various non-nerve tissues, nerve allografts, and biological materials have been used for the reconstruction of nerve defects, but their clinical applications are limited (Rustamov, 2020). However, topical applications alone or in combination to the nerve vicinity after nerve injury (such as hyaluronic acid, human amniotic fluid, 5-fluorouracil, platelet-rich plasma, platelet-rich fibrin, adipose graft, stem cells, asiatic acid) have been observed to contribute to healing, reduce axonal degeneration and degeneration in neurons, and alleviate edema histopathologically (Ozgenel and Filiz, 2004; Liu et al., 2011; Metineren et al., 2017; Lopez et al., 2018; Özkan et al., 2018; Kastamoni, 2019; Buhşem, 2021; Çağlaroğlu, 2021).

S100 proteins are a family of low molecular weight proteins found in vertebrates. S100 proteins are released by glial cells in the central nervous system such as the brain and spinal cord (Steiner et al., 2011; Salman et al., 2020; Goswami et al., 2023). After peripheral nerve repair, S100 expression increases in Schwann cells, supporting myelination (Yang et al., 2023). Additionally, S100 immunofluorescent staining is performed to evaluate axonal regeneration (Chen et al., 2022). S100 proteins play a role in regulating intracellular or extracellular functions in different cell types, as well as in many areas such as cell apoptosis, proliferation, and inflammation. After cell damage, S100 proteins are released into the extracellular space, where they have a critical role in various immune and inflammatory processes. The released S100 may help the healing of nerve damage or may cause tissue destruction due to excessive release (Steiner et al., 2011). It has also been found that the serum S100 level is closely associated with the severity of neurodegenerative diseases (Çağlaroğlu, 2021). In this study, it was found that S100 expression levels, which is an important marker of nerve damage, were severe due to neuronal damage observed in group 1, while significant damage was prevented in the other groups (groups 2 and 3) and statistically significant protection was observed in group 4, which was the combined treatment group.

Glial fibrillary acidic protein (GFAP) is released by different glial cell types in the central nervous system, including astrocytes, oligodendrocytes, microglia and ependymal cells. It was determined that GFAP expression levels increased in order to protect neurodegeneration and prevent brain damage (Otani et al., 2006; Middeldorp and Hol, 2011). Widely used indicators of active astrocytes and microglial cells include increased expression and distribution of the GFAP immunostaining. In order to identify the activation of astrocytes and microglial cells after sciatic nerve injury, indirect immunofluorescent staining for GFAP is routinely utilized (Dubovy et al., 2018). An intermediate filament protein called GFAP is mainly expressed in astrocytes. In the acute phase of stroke, elevated serum levels of GFAP are utilized as a biomarker to identify bleeding in the brain, traumatic brain damage, and traumatic spinal cord injury (Yardım et al., 2021). In research on sciatic nerve injury, GFAP is also used to follow the progress of nerve recovery (Bai et al., 2018; Yardım et al., 2021; Bretova et al., 2023). In this study, while severe GFAP expression was observed in astrocytes in group 1 due to pathological findings such as severe degeneration and local demyelination occurring in the sciatic nerves, it was determined that nerve tissue damage was prevented in group 4 (combined application of Andiz root extract and vitamin B12) and GFAP expression levels decreased at a statistically significant level.

In a study by Albay et al. (2020), B12 and D3 vitamin supplementation was administered after nerve injury, and they reported that both B12 and D3 vitamins were similarly effective in nerve injury recovery. Furthermore, in the group where both vitamins were administered together, a more significant reduction in damage was observed due to a synergistic effect. In a study conducted by Albay and Akkalp (2021) in rats, they found that B12 vitamin was more effective than E vitamin in nerve recovery after nerve injury when administered separately. However, when both vitamins were administered together, the recovery was better than in all other groups. In this study as well, although the groups treated with B12 vitamin and Andız extract separately showed similar levels of histopathological and immunofluorescent recovery, the group where they were applied together exhibited better recovery compared to all other groups.

In a study conducted by Yu et al. (2022) in rats, the local application of zein, a plant-derived protein, was shown to enhance nerve regeneration following sciatic nerve injury. Similarly, in a study by Shin et al. (2021), local application of glutaraldehyde-crosslinked acellular matrix (CX-CAM) from cartilage was performed on the nerve in rats with sciatic nerve injury, and it was histopathologically demonstrated to contribute to nerve healing. Furthermore, in this study, the use of Andız root extract was histopathologically shown to contribute to nerve healing (Figure 2).

In this study, the rats were randomly divided into four groups. Nerve transection injuries were created in all groups, and after injury, epineural nerve anastomosis technique was applied. Histopathological and immunofluorescent evaluations performed as a result of the postoperative applications indicated that B12 vitamin (Group 3) and Andız root extract (Group 2) had a positive effect on degeneration in neurons, demyelination in axons, and edema in interfascicular connective tissue in rats compared to the control group (Table 1). This finding was also supported by S100 and GFAP expression (Table 2). Additionally, in rats where Andız root extract was topically applied and B12 vitamin was intraperitoneally administered together for 21 days (Group 4), it was observed that the combination had a better reduction in degeneration in neurons, demyelination in axons, and edema in interfascicular connective tissue compared to all other groups (Table 1). This was further supported by the immunoreactive area percentage of S100 and GFAP expression and histopathological data (Figure 2).

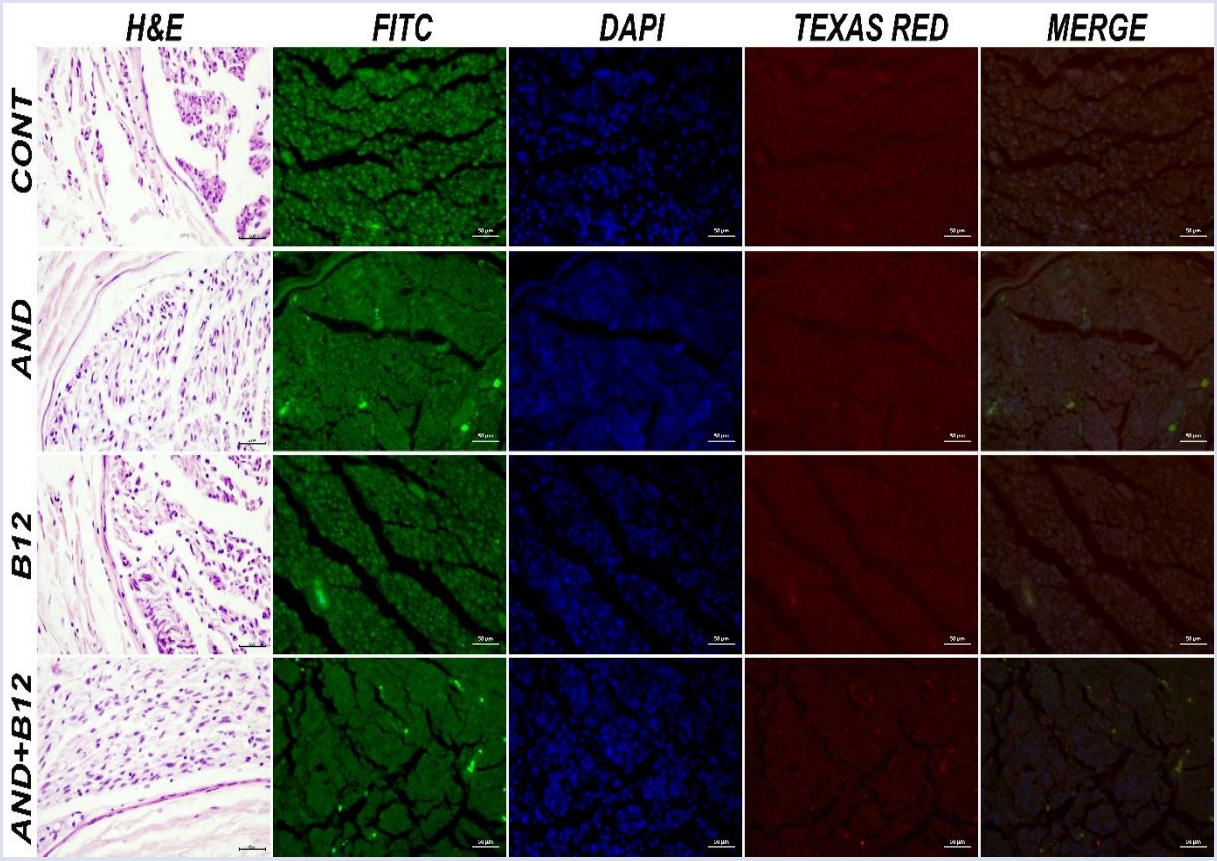

Figure 2. Sciatic nerve tissue, neuronal degeneration, demyelination, edema in connective tissue, H&E, Scale Bar: 40µm, GFAP expression in neurons (FITC), S100 expression in neurons (Texas Red), IF, Scale Bar: 50µm.

Conclusion

In conclusion, in the rat model of sciatic nerve injury applied in this study, individually administered Andız root extract and B12 vitamin showed a certain level of similar effects compared to the control group in terms of determining the levels of recovery. However, when administered together, they provided a more effective contribution to nerve healing compared to all other groups. The decrease in GFAP and S100 expression levels is thought to prevent nerve damage by synergistic effect of Andız root extract application with vitamin B12 application. Although the neuron-protective and therapeutic effect of vitamin B12 application is known, the therapeutic and supportive effect of Andiz root extract on nerve healing was also demonstrated by immunofluorescence and histopathological examinations in this study. It is thought that further studies are needed to better understand the mechanism of action and the contribution of Andız root extract used in this study to nerve healing.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

Abushukur, Y., Knackstedt, R. (2022). The Impact of Supplements on Recovery After Peripheral Nerve Injury: A Review of the Literature. Cureus, 14(5).

Albay, C., Adanir, O., Kahraman Akkalp, A., Dogan, V.B., Gulec, M.A., Beytemur, O. (2020). Cyanocobalamin and cholecalciferol synergistically improve functional and histopathological nerve healing in experimental rat model. Clin Neurosci, 73.

Albay, C., Akkalp, A.K. (2021). Alpha-Tocopherol and Cyanocobalamin Combination Accelerates Peripheral Nerve Healing: An Experimental Animal Study. Turk Neurosurg, 31(2):247-253.

Al-Owamri, F.S.N., Al Sibay, L.S.A., Reddy, S.H., Hussain, S.A., Gangireddygari, V.S.R. (2023). Phytochemical, Antioxidant, hair growth and wound healing property of *Juniperus* excelsa, Olea oleaster and Olea europaea. J King Saud Univ Sci, 35(2):102446.

Altun, I., Kurutaş, E.B. (2016). Vitamin B complex and vitamin B12 levels after peripheral nerve injury. Neural Regen Res, 11(5):842-845.

Aman, M., Zimmermann, K.S., Boecker, A.H., Thielen, M., Falkner, F., Daeschler, S., et al. (2023). Peripheral nerve injuries in children—prevalence, mechanisms and concomitant injuries: a major trauma center's experience. Eur J Med Res, 28(1): 1-7.

Arikan, M., Togral, G., Hastürk, A.E., Horasanli, B., Helvacioglu, F., Dagdeviren, A., et al. (2016). Histomorphometric and ultrastructural evaluation of long-term alpha lipoic acid

and vitamin B12 use after experimental sciatic nerve injury in rats. Turk Neurosurg, 26(6):944-952.

Avcı, E. (2020). Determination of Antimicrobial Properties of Andiz (*Juniperus Sp*) Root Extract and Its Effects on Wound Development in Rainbow Treats (*Oncorhynchus Mykiss*). MSc. Thesis, Department of Fisheries Engineering, Institute of Science and Technology, Van Yüzüncü Yıl University, Van, Türkiye.

Bai, J., Liu, F., Wu, L.F., Wang, Y.F., Li, X.Q. (2018). Attenuation of TRPV1 by AMG-517 after nerve injury promotes peripheral axonal regeneration in rats. Mol Pain, 14: 1744806918777614.

Bretová, K., Svobodová, V., Dubový, P. (2023). Astrocyte reactivity in the glia limitans superficialis of the rat medial prefrontal cortex following sciatic nerve injury. Histochem Cell Biol, 159(2):185-198.

Buhşem, Ö. (2021) Investigation of the Effects of the 5-Fluorouracil Applied After Rat Sciatic Nerve Repair on the Epineural and Extraneural Scar Tissue Formation and Nerve Regeneration. International Anatolia Academic Online Journal Health Sciences, 7(1);81-100.

Chen, Y., Pan, Z., Meng, F., Yu, X., Xu, Q., Huang, L., et al. (2022). Magnetic resonance imaging assessment of the therapeutic effect of combined electroacupuncture and stem cells in acute peripheral nerve injury. Front Cell Neurosci, 16:1065557.

Çağlaroğlu. Y. (2021). Investigation of the Role of Asiatic Acid in Experimentally Created Peripheral Nerve Injuries in Rats. Master Thesis, Atatürk University Faculty of Medicine, Department of Neurosurgery, Erzurum, Türkiye.

Duan, G., Li, C., Yan, X., Yang, S., Wang, S., Sun, X., et al. (2023). Construction of a mineralized collagen nerve conduit for peripheral nerve injury repair. Regen Biomater, 10.

Dubový, P., Klusáková, I., Hradilová-Svíženská, I., Joukal, M., Boadas-Vaello, P. (2018). Activation of astrocytes and microglial cells and CCL2/CCR2 upregulation in the dorsolateral and ventrolateral nuclei of periaqueductal gray and rostral ventromedial medulla following different types of sciatic nerve injury. Front Cell Neurosci, 30:12;40.

Goswami, D., Anuradha, U., Angati, A., Kumari, N., & Singh, R. K. (2023). Pharmacological and pathological relevance of S100 proteins in neurological disorders. CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders).

Güven N, Özkan S, Türközü T, Koç S, Keleş ÖF, Yener Z, Karasu A. (2022). The effect of Theranekron on femur fracture healing in an experimental rat model. Jt Dis Relat Surg, 33(2):374-384.

Horasanli, B., Hasturk, A.E., Arikan, M., Togral, G., Helvacioglu, F., Mut, S., et al. (2017). Comparative evaluation of the electrophysiological, functional and ultrastructural effects of alpha lipoic acid and cyanocobalamin administration in a rat model of sciatic nerve injury. J Back Musculoskelet Rehabil, 30(5):967-974.

İshakoğlu, Ö.K. (2019). Effects of Caparine in Experimental Sciatic Nerve Damage-induced Rats. PhD Thesis, Hatay Mustafa Kemal University, Institute of Health Sciences, Department of Physiology (Medicine). Hatay, Türkiye.

Kapdan, E., Sezgin, M., Kahya, M. (2019). The use of Juniper (*Juniperus L.*) species in the treatment of diseases among people and in Modern Medicine. Conference: Internatıonal Eurasian Conference on Biologıcal and Chemical Science. June, 2019. Ankara/ Türkiye.

Kastamoni, M. (2019). Combined effect of fat graft and platelet-rich fibrin on perineural adhesion and scar formation in a rat model. Master Thesis, Bursa Uludağ University, Faculty of Medicine, Department of Plastic, Reconstructive and Aesthetic Surgery. Bursa, Türkiye.

Li, R., Wu, J., Lin, Z., Nangle, M.R., Li, Y., Cai, P., et al. (2017). Single injection of a novel nerve growth factor coacervate improves structural and functional regeneration after sciatic nerve injury in adult rats. Exp Neurol, 288:1-10.

Liu, G.B., Cheng, Y.X., Feng, Y.K., Pang, C.J., Li, Q., Wang, Y., et al. (2011). Adipose-derived stem cells promote peripheral nerve repair. Arch Med Sci, 7(4):592-596.

Lopez, J.F., Sarkanen, J.R., Huttala, O. (2018). Adipose tissue extract shows potential for wound healing: in vitro proliferation and migration of cell types contributing to wound healing in the presence of adipose tissue preparation and platelet rich plasma. Cytotechnology, 70(4):1193-1204.

Luo, X., Li, B., Zhang, D., Chen, H., Zhou, X., Yao, C., et al. (2022). A new insight on peripheral nerve repair: the technique of internal nerve splinting. J Neurosurg, 1:1-12.

Lv, T., Mo, Y., Yu, T., Shao, S., Lu, M., Luo, Y., et al. (2020). Using RNA-Seq to Explore the Repair Mechanism of the Three Methods and Three-Acupoint Technique on DRGs in Sciatic Nerve Injured Rats. Pain Res Manag, 1-12.

Metineren, H., Dülgeroğlu, T.C., Metineren, M.H. (2017). Histopathological effects of Platelet rich fibrin on peripheral nerve healing. Dicle Medical Journal, 44(3): 243-250.

Middeldorp, J., Hol, E.M. (2011). GFAP in health and disease. Prog Neurobiol, 93(3): 421-443.

Otani, N., Nawashiro, H., Fukui, S., Ooigawa, H., Ohsumi, A., Toyooka, T., et al. (2006). Enhanced hippocampal neurodegeneration after traumatic or kainate excitotoxicity in GFAP-null mice. J Clin Neurosci, 13, 934-938.

Ozay, R., Keskin, E., Balkan, M.S., Aktas, A., Akturk, U.D., Esener, O.B.B., et al. (2023). Effects of Topically Applied Contractubex® on Epidural Fibrosis and Axonal Regeneration in Injured Rat Sciatic Nerve. Turk Neurosurg, 33(3):437-446.

Ozgenel, G.Y., Filiz, G. (2004). Combined application of human amniotic membrane wrapping and hyaluronic acid injection in epineurectomized rat sciatic nerve. J Reconstr Microsurg, 20:153-7.

Özkan, M.Ç., Tuncel, B., Ozgenel, G.Y. (2018). The effect of topical 5-fluorouracil application on epineural scar tissue in epineurectomized rat sciatic nerve. Marmara Med J, 31:1-10.

Rustamov, N. (2020). A New Method in Nerve Defect Reconstruction: Silk Road Technique. Kocaeli University, Faculty of Medicine, Department of Plastic, Reconstructive and Aesthetic Surgery, Master thesis, Kocaeli.

Salman, E., Salman, R.B., Tufan, A. (2020). S100 Proteins as an Important Regulator Of Inflammation. Ankara Eğt Arş Hast Derg, 53(1): 41-45.

Seo, M., Lim, D., Kim, S., Kim, T., Kwon, B.S., Nam, K. (2021). Effect of Botulinum Toxin Injection and Extracorporeal Shock Wave Therapy on Nerve Regeneration in Rats with Experimentally Induced Sciatic Nerve Injury. Toxins, 13(12):879.

Shin, Y.H., Yun, H.W., Park, S.Y., Choi, S.J., Park, I.S., Min, B.H., et al. (2020). Effect of glutaraldehyde-crosslinked cartilage acellular matrix film on antiadhesion and nerve regeneration in a rat sciatic nerve injury model. J Tissue Eng Regen Med, 15:1023–1036.

Steiner, J., Bogerts, B., Schroeter, M.L., Bernstein, H.G. (2011). S100B protein in neurodegenerative disorders. CCLM, 49(3): 409-424.

Tumen, I., Süntar, I., Keleş, H., Küpeli Akkol, E. (2012). A therapeutic approach for wound healing by using essential oils of Cupressus and *Juniperus* species growing in Turkey. eCAM, 1-7.

Yang, J., Hsu, C.C., Cao, T.T., Ye, H., Chen, J., Li, Y.Q. (2023). A hyaluronic acid granular hydrogel nerve guidance conduit promotes regeneration and functional recovery of injured sciatic nerves in rats. Neural Regen Res, 18(3):657-663.

Yardım, A., Kandemir, F.M., Çomaklı, S., Özdemir, S., Caglayan, C., Kucukler, S., et al. (2021). Protective effects of curcumin against paclitaxel-induced spinal cord and sciatic nerve injuries in rats. Neurochem Res, 46:379-395.

Yu J, Lin Y, Wang G, Song J, Hayat U, Liu C, et al. (2022). Zein-induced immune response and modulation by size, pore structure and drug-loading: application for sciatic nerve regeneration. Acta Biomater, 140:289-301.

Zhang, Z., Zhang, M., Sun, Y., Li, M., Chang, C., Liu, W., et al. (2023). Effects of adipose derived stem cells pretreated with resveratrol on sciatic nerve regeneration in rats. Sci Rep, 13(1):5812.