Kocatepe Tıp Dergisi
Kocatepe Medical Journal
25:525-535/Ekim 2024 Sayısı

ARAŞTIRMA YAZISI / RESEARCH ARTICLE

RATLARDA SİYATİK SİNİR YARALANMASINDA *MYRTUS COMMUNIS*'İN TERAPÖTİK ETKİNLİĞİ: BİR DENEYSEL ARAŞTIRMA

THERAPEUTIC EFFICACY OF *MYRTUS COMMUNIS* IN SCIATIC NERVE INJURY: AN EXPERIMENTAL RESEARCH IN RATS

Gökçe ZEYTİN DEMİRAL[1], Zülfikar Kadir SARITAŞ[2], Ülkü TÜRK BÖRÜ[1], Fatma GÖRÜCÜ[2], Cansu KÖSEOĞLU TOKSOY[3], Aziz BÜLBÜL[4], Hasan Hüseyin DEMİREL[5], Yusuf KOÇ[2], Zehra YAŞAR[2]

[1]Afyonkarahisar Sağlık Bilimleri Üniversitesi Tıp Fakültesi Nöroloji Ana Bilim Dalı
[2]Afyon Kocatepe Üniversitesi Veterinerlik Fakültesi Cerrahi Ana Bilim Dalı
[3]Sağlık Bilimleri Üniversitesi İstanbul Erenköy Psikiyatri ve Sinir Hastalıkları Uygulama ve Araştırma Merkezi
[4]Muğla Sıtkı Koçman Üniversitesi Milas Veteriner Fakültesi, Fizyoloji Ana Bilim Dalı
[5]Afyon Kocatepe Üniversitesi Bayat Meslek Yüksekokulu, Laboratuvar ve Veterinerlik Sağlığı Bölümü

ÖZET

AMAÇ: Sinir yaralanmaları, genellikle çeşitli nedenlerden kaynaklanan ciddi sağlık sorunları olup, genellikle uzun süreli rehabilitasyon gerektirir. Bu yaralanmaların tedavisinde kullanılan geleneksel yöntemler çoğunlukla yetersiz kalmakta ve yeni tedavi yaklaşımlarının araştırılmasını gerektirmektedir. Bitkisel kökenli aktif bileşiklerin sinir rejenerasyonu üzerindeki etkileri üzerine yapılan araştırmalar, potansiyel yeni tedavi seçenekleri sunabilir. Bu bağlamda, *Myrtus communis*'in sinir rejenerasyonu üzerindeki etkilerini araştıran bu çalışma, sinir yaralanması tedavisi alanında önemli bir boşluğu doldurmayı amaçlamaktadır.

GEREÇ VE YÖNTEM: Bu çalışmada, *Myrtus communis*'in etkinliği deneysel siyatik sinir yaralanması modelinde değerlendirildi. Otuz iki dişi Wistar Albino sıçan dört gruba ayrıldı: Kontrol, Sham, Grup I ve Grup II. Siyatik sinir yaralanması indüklendi ve Myrtus communis tedavisi gastrik lavaj yoluyla uygulandı. Hayvanların motor fonksiyonları, duyusal fonksiyonları, elektrofizyolojik ölçümleri, biyokimyasal parametreleri ve histopatolojik değerlendirmeleri incelendi.

BULGULAR: *Myrtus communis*'in siyatik fonksiyonel indeks (SFI) değerlerinde hızlı iyileşmeye katkıda bulunduğunu gösterdi. Benzer şekilde, duyusal değerlendirme ve elektrofizyolojik ölçümlerde de olumlu etkiler gözlendi. Biyokimyasal analizler, Myrtus communis'in antioksidan kapasiteyi artırdığını ve oksidatif stresi azalttığını gösterdi. Histopatolojik incelemeler, *Myrtus communis* ile tedavi edilen gruplarda daha az akson dejenerasyonu, ödem ve vakuolizasyon olduğunu ortaya koydu.

SONUÇ: Bu çalışma, *Myrtus communis*'in siyatik sinir yaralanmasının tedavisinde potansiyel bir terapötik ajan olarak kullanılabileceğini sonucuna varmıştır. Bu bulgular, *Myrtus communis*'in sinir yaralanması sonrası iyileşme sürecinde destekleyici bir rol oynayabileceğini düşündürmektedir. Bununla birlikte, bu sonuçların klinik uygulamalara dönüştürülebilmesi için daha fazla araştırmaya ihtiyaç olduğu unutulmamalıdır.

ANAHTAR KELİMELER: *Myrtus Communis*, Siyatik sinir hasarı, Terapötik etkinlik, Periferik sinir rejenerasyonu, Anti-inflamatuar etkiler.

ABSTRACT

OBJECTIVE: Nerve injuries, often resulting from various causes, pose serious health issues that typically require prolonged rehabilitation. Conventional methods used in the treatment of these injuries are often inadequate, necessitating the exploration of new treatment approaches. Research on the effects of plant-derived active compounds on nerve regeneration may offer potential new treatment options. In this context, this study investigating the effects of *Myrtus communis* on nerve regeneration aims to fill an important gap in the field of nerve injury treatment.

MATERIAL AND METHODS: In this study, the efficacy of *Myrtus communis* was evaluated in an experimental sciatic nerve injury model. Thirty-two female Wistar Albino rats were divided into four groups: Control, Sham, Group I, and Group II. Sciatic nerve injury was induced, and Myrtus communis treatment was administered via gastric lavage. The animals' motor functions, sensory functions, electrophysiological measurements, biochemical parameters, and histopathological evaluations were examined.

RESULTS: The results demonstrated that *Myrtus communis* contributed to rapid improvement in sciatic functional index (SFI) values. Similarly, positive effects were observed in sensory assessment and electrophysiological measurements. Biochemical analyses indicated that *Myrtus communis* increased antioxidant capacity and reduced oxidative stress. Histopathological examinations revealed less axon degeneration, edema, and vacuolization in the groups treated with *Myrtus communis*.

CONCLUSIONS: This study concludes that *Myrtus communis* could be used as a potential therapeutic agent in the treatment of sciatic nerve injury. These findings suggest that *Myrtus communis* may play a supportive role in post-nerve injury recovery. However, it should be noted that further research is needed before these results can be translated into clinical applications.

KEYWORDS: *Myrtus Communis,* Sciatic nerve injury, Therapeutic efficacy, Peripheral nerve regeneration, Anti-inflammatory effects.

Geliş Tarihi / Received: 22.07.2024
Kabul Tarihi / Accepted:16.09.2024
Yazışma Adresi / Correspondence: Dr. Öğr. Üyesi Gökçe ZEYTİN DEMİRAL
Afyonkarahisar Sağlık Bilimleri Üniversitesi Tıp Fakültesi Nöroloji Ana Bilim Dalı
E-mail: gokce_zeytin@hotmail.com
Orcid No (Sırasıyla): 0000-0002-9635-5804, 0000-0002-7659-6635, 0000-0002-0094-5624, 0000-0001-7630-0788, 0000-0002-9224-9203, 0000-0003-0995-3986, 0000-0002-4795-2266, 0000-0002-6342-5466, 0000-0002-9030-5478
Etik Kurul / Ethical Committee: Afyon Kocatepe Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu (20.01.2023- 49533702/04).

INTRODUCTION

Peripheral nerve injury (PNI) stands as a prevalent consequence of traumatic incidents (1), with an estimated incidence ranging from 13 to 23 cases per 100,000 individuals (2). These injuries can arise from various sources including mechanical, chemical, and thermal factors (3). PNI can disrupt motor and sensory functions, affecting both efferent (motor and autonomic) and afferent (sensory) pathways. This disruption can significantly impair a person's functional abilities and work capacity (4, 5). Treatment strategies for PNI focus on surgically repairing the damaged nerve or improving patients' quality of life through medical interventions (6). However, there remains a lack of consensus regarding the optimal approach for PNI treatment. Current methods for treating PNI include a range of interventions. These interventions encompass non-steroidal anti-inflammatory drugs, steroids, nerve growth factors, erythropoietin, thyroid hormone, growth hormone, adrenocorticotropic hormone, and insulin-like peptides, among others, used in experimental settings (7 – 12).

Myrtle (*Myrtus communis* L., MC) is a plant from the Myrtle family commonly found in the Mediterranean region (13). The primary compounds in MC leaves include phenolic acids (such as gallic acid, ferulic acid, caffeic acid, syringic acid, and vanillic acid), flavonoids, water-soluble tannins (gallotannins), proanthocyanidins, and essential oils (including a-pinene, myristenyl acetate, 1,8-cineole, limonene, and linalool) (14). Recently, plants with high levels of phenolic compounds, polyunsaturated fatty acids, and essential oils have gained attention for their potential health benefits, such as antioxidant, anticancer, and anti-inflammatory effects (15). MC leaves and fruits are known for their anti-inflammatory, antifungal, antibacterial, neuroprotective, hepatoprotective, anticancer, antidiabetic, and antiviral properties, as well as their ability to scavenge free radicals due to their antioxidant activity (16 – 20).

In this study, we aimed to evaluate the efficacy of Myrtus communis in sciatic nerve injury.

MATERIALS AND METHODS

Study Design

The research was conducted at the Experimental Animal Laboratory of Afyon Kocatepe University between January and March 2022. All animals involved in the study were treated with care and in accordance with the standards set forth in the National Institutes of Health's Guide for the Care and Use of Laboratory Animals.

Animals

The study used thirty-two female Wistar Albino rats, each weighing between 250-300 grams and aged 4-6 months. These rats were obtained from the Experimental Animal Production and Research Center in Afyonkarahisar, Turkey. Due to the unavailability of male rats and project timeline constraints, female rats were used. The rats received normal rat chow and unlimited water prior to the experiment. For a minimum of one week, they were kept in similar cages with regulated temperatures, 50% humidity, and a 12-hour light/dark cycle. Up to eight rats might be housed in each cage.

Anesthesia and surgical procedures

80 mg/kg of ketamine hydrochloride and 5 mg/kg of xylazine hydrochloride (both from Alfasan, The Netherlands) were injected intraperitoneally to induce anesthesia; further doses were given as needed. Throughout the procedure, electrocardiography (ECG) was monitored to keep the patient's heart rate between 190 to 260 beats per minute. An infrared lamp was used to maintain the rats' body temperature between 35.5 and 37.5°C, which was measured using a rectal probe. On a table, the rats were placed supine. Using microsurgical procedures, a single surgeon carried out each stage of the procedure.

After shaving, the right gluteal area was cleaned with a povidone-iodine antiseptic solution. The femoral biceps muscle was exposed through an oblique incision made in the right lower leg, which was made after the hip joint crease. The right sciatic nerve was exposed by blunt dissection, which released it from the tissues surrounding it from the sciatic process to the popliteal area. A bulldog clamp, measuring approximately 2 cm in length, was used to compress the nerve for 60 seconds at its midpoint. Following compression, 4-0 silk sutures were used to ligate the damaged area, and the wound was closed. Treatment for myrtus communis was started as soon as possible following nerve damage and continued for eight weeks using gastric gavage. After nerve injury, treatment for *myrtus communis* began immediately and involved once-daily gastric gavage for eight weeks.

Experimental groups

Four groups of eight rats each were randomly selected from the group of rats (n = 8).

Control: Absence of involvement.
Sham: Despite the exposed and damaged sciatic nerve, no more care was provided beyond the standard course of antibiotics.
Group I: 100 mg/kg (0.3 ml) of *Myrtus communis* was given daily along with a sciatic nerve damage.
Group II: 150 mg/kg (0.3 ml) of *Myrtus communis* was given daily along with a sciatic nerve damage.

Following the surgery, the rats received antibiotic therapy for seven days and were maintained for eight weeks. The rats were killed at the conclusion of this time by intracardiac injection of 150 mg/kg of sodium thiopental.

Evaluation Tests

Functional Gait Assessment

To ensure uniform walking direction, a 42x8.2x12 cm walking track ending in a dark room with a wooden floor was prepared. The rats' paws were dipped in ink, and paper was placed on the floor to capture their footprints. The rats were trained to walk on a track with closed sides and a dark shelter at one end, following the method described by De Medicanelli et al. (21). The footprints were used to measure the distance between the heel and toe (print length, PL), the distance between the first and fifth toes (stride width, TS), and the distance between the second and fourth toes (intermediate toe spread, IT) with a millimeter ruler. The obtained footprints were subjected to Sciatic Functional Index (SFI) evaluation, calculated using the Bain-Maccinon-Hunter Sciatic Functional Index formula (22).

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

E: experimental, N: normal, PL: print length, TS: the total spread of toes (1st to 5th), IT: the spread of intermediate toes (2nd to 4th).

SFI ranges from 0 for a normal nerve to -100 for a nerve that has lost all motor function. The range of values is contingent upon the severity of the damage. SFI was determined prior to injury, 24 hours following injury, and one, two, three, four, five, six, seven, and eight weeks following injury.

Sensory Function Test (Pinch test)

Pinch test was used to evaluate the return of sensory function. A stimulus was given by squeezing the tip of the big toe towards the knee with forceps. Foot withdrawal reflex was evaluated between 0-3 points. Above the ankle was evaluated as 1 point, metatarsal area as 2 points, and pulling reflex at the level of the big toe as 3 points. A score of 0 was given if there was no pulling reflex. Using the pinch test, functional sensory recovery was examined. The foot's skin was squeezed with forceps, and the rats were handled gently and not subjected to stress (Splinter & Potts-Smith, Leica, NuŲloch, GmbH, Germany). The response levels were assigned a grade of 0 for no response, 1 for weak response, 2 for mild response, and 3 for strong response.

Electrophysiologic Evaluation

At 0 hours, 4 weeks, and 8 weeks, Electromyography (EMG) recordings were made of all the rats in the control, sham, group 1, and group 2 in order to evaluate nerve injury. Rats were given ketamine at a dose of 140 mg/kg without muscle relaxant. Using stainless steel needle electrodes, the recording was done with the active electrode inside the tibialis anterior muscle and the reference electrode, in accordance with the muscle-tendon concept, on the tendon of the same muscle. The ground electrode was placed between the stimulator and the active electrode. A 0.2 ms square wave stimulus at the lowest stimulus intensity (1.5-8 mA) that would supramaximally activate the sciatic nerve without propagating to the surrounding area was applied to the active electrode at the level of the "sciatic bulge," proximal to the nerve injury. For the sciatic nerve (right leg), at least three repeats of the amplitude and morphology were recorded. Then, the distal latencies and compound muscle action potential (CMAP) negative peak amplitude were measured.

Histologic Evaluation

Following the EMG testing, the rats were slaughtered by administering a fatal dosage of intracardiac Thiopental sodium injection. Subsequently, 2 cm long nerve samples were collected for histological analysis. To histological analyses, tissue samples obtained from the sciatic nerve were preserved in a 10% buffe-

red formalin solution. Tissue samples that had been fixed with formalin were trimmed to a thickness of 2-3 mm and acceptable dimensions, and then inserted into tissue tracking cassettes. Following an overnight rinse in tap water, the samples were immersed in 50%, 70%, 80%, 96% absolute alcohol, xylol, xylolised paraffin, and paraffin melted at 56-58ºC for 2 hours each. They were then sealed in paraffin. A 5-micron thick portion of each paraffin block was cut using a microtome (Leica, RM 2245) and then placed on slides using a water bath (Leica, HI 1210). Following a 10-minute drying period in a Thermo Histopathological oven, they were ready for use in histological techniques. Following a 10-minute drying period in a Thermo Histopathological oven, they were ready for use in histological techniques. Following the procedure described by Luna in 1968 (23), all sections were stained with haematoxylin-eosin (HE) in absolute, 96%, 80%, 70%, 50% alcohol series, and xylol series. The stained preparations were visualised using a binocular light microscope manufactured by Nikon, Eclipse Ci, based in Tokyo, Japan. Microscopic images were captured using the required equipment (Nikon DS FI3, Mikroscopic Digital Camera Systems, Tokyo, Japan). Evaluation of axonal degeneration, vacuolization, and edema was conducted **(Figure 1)**. Objective morphometric evaluations were conducted by two separate investigators using a 10X200µm magnification. Resolution of disagreements among the investigators was achieved by team deliberation.

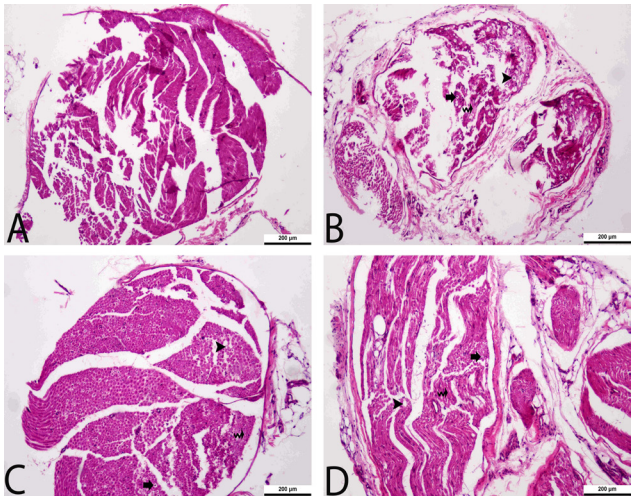


Figure 1: Histopathological findings. A, Control. B, Sham. Thick Arrow: Axonal degeneration. Arrowhead: Vacuolization. Curved Arrow: Edema. C, Group 1. Thick Arrow: Axonal degeneration. Arrowhead: Vacuolization. Curved Arrow: Edema. D, Group 2. Thick Arrow: Axonal degeneration. Arrowhead: Vacuolization. Curved Arrow: Edema.

Oxidant/Antioxidant Capacity Measurements in Blood Samples

Quantification of total antioxidant status (TAS) and total oxidant status (TOS) levels, as well as NGF and TGF-β levels, was conducted in nerve cells to examine the impact of *Myrtus communis* therapy on oxidant and antioxidant parameters. Following the procedure, venous blood was collected 8 weeks later, and the resulting serum samples were stored at a temperature of -80°C. Measurements of Nerve Growth Factor (NGF) in the serum (Bioassay Technology Laboratory, catalog No.: E0539Ra, Shanghai, China), TAS (Bioassay Technology Laboratory, catalog No.: E1710Ra, Shanghai, China), TOS (Bioassay Technology Laboratory, catalog No.: E1512Ra, Shanghai, China), and Transforming Growth Factor-Beta (TGF- β) (Bioassay Technology Laboratory, catalog No.: E1688Ra, Shanghai, China) were performed using the VGt Lambda Scan 200 ELISA device (Bio-Tech Instrument, Winooski, VT, USA).

Preparation of *Myrutus communis*

The leaves and stems of the myrtle tree are boiled at a concentration of 6 g/L in 100°C water for 15 minutes. The extracts are then subjected to a rotary evaporator to roughly remove the water, and the remaining portion is lyophilized to completely remove the water. Subsequently, solutions of 150 µg/mL are prepared from each of the samples and sterilized in an autoclave at 120°C for 1 hour. The solutions are stored in 50 mL Falcon tubes at +4°C for use in experiments. Alcohol extracts are prepared using a Soxhlet apparatus, and the alcohol is evaporated using a rotary evaporator (Aksay, 2016). The myrtle tree extract was provided by Ars Arthro Biotechnology Inc., Ankara. Note: Systemic toxicity studies of the myrtle tree extract were conducted in accordance with ISO 10993-11 standards, and no side effects were observed. Additionally, skin and eye irritation and sensitization tests showed that the plant extracts used did not cause irritation or sensitization on the skin (SANTEZ-0352.STZ.2013-2 project final report).

Variables

The main dependent variable of the research was the levels of SFI. Secondary outcome factors in the study included biochemical markers rela-

ted to oxidants and antioxidants, as well as histologic and neurophysiologic metrics. The groups of rats served as the independent study variable.

Ethical Committee

The study protocol received approval from the Local Ethics Committee for Animal Experiments at Afyon Kocatepe University in Afyonkarahisar, Turkey (Approval No: 49533702/04, Date: January 20, 2023).

Statistical Analysis

Computerized data were inputted and analysed using SPSS 25.0 software developed by SPSS Inc., based in Chicago, IL, USA. Quantitative data were displayed using frequencies, percentages, mean, and standard deviation (SD). An analysis of the normal distribution of numerical data was conducted using the Shapiro-Wilks test. An Independent Samples Analysis of Variance (One Way ANOVA) was employed to compare the groups based on each parameter. The Duncan multiple comparison test was employed to compare each group pairwise. Furthermore, a repeated measures ANOVA was used to compare each group based on time (Pre-op, Post-op 24 hours, 1. week, 2. week, 3. week, 4. week, 5. week, 6. week, 7. week, 8. week). The standard statistical significance level was set at 0.05..

RESULTS

Motor Function Evaluation

According to the results of this test, the SFI was between (0 and -10) in healthy rats, indicating normal motor function. When the sciatic nerve functional index results were analyzed, when the differences between all groups were examined, differences were found between the groups at 24th hour and 1st week. At 24 hours, the control group was significantly higher than groups 1 and 2 and similar to the sham group. The sham group was similar to all groups (p=0.028). At the 1st week evaluation, SFI values of the control and sham groups were similar. Group 1 and group 2 were similar to each other and had significantly lower SFI values than the other groups (p=0.024).

The SFI values of group 1 at 0th hour, 24th hour and 1st week were significantly lower and similar at 2nd week and later (p<0.001). SFI values of group 1 at 24 hours were significantly lower than SFI values measured at all other times (p<0.001). Group 1; 1st week SFI value was significantly higher than 24th hour and significantly lower than 0th hour, 7th and 8th week SFI values. Group 1; 1st week SFI value is similar to the SFI measurements at weeks 2-6.

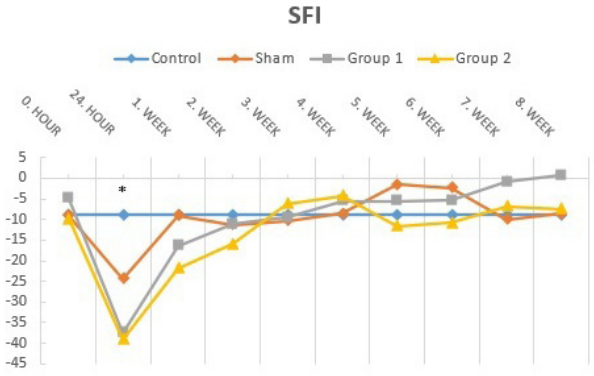
Group 2; SFI value at hour 0 is similar to the measurements at week 3 and later. The 24th hour SFI value is the lowest SFI values measured. Week 1 and week 2 SFI measurements are similar and significantly lower than all other times except 24 hours (p<0.001). **Table 1** and **Graph 1** show the evaluation of SFI values over time and between groups.

Table 1: Comparison of SFI measurements by time and groups

Groups	0th hour	24th hour	1th week	2nd week	3th week	4th week	5th week	6th week	7th week	8th week	F	p
Control	-8,90±0,28	-8,90±0,28	-8,90±0,28	-8,90±0,28	-8,90±0,28	-8,90±0,28	-8,90±0,28	-8,90±0,28	-8,90±0,28	-8,90±0,28	-	-
Sham	-9,10±9,19	-24,31^{AB}±9,10	-9,00^{A}±5,68	-11,36±5,37	-10,33±4,77	-8,51±16,58	-1,53±13,38	-2,36±18,42	-10,01±12,45	-8,53±15,09	1,426	0,197
Group 1	-4,88^{Ba}±5,99	-37,49^{Bc}±4,25	-16,46^{Bb}±2,53	-11,11^{ab}±14,83	-9,46^{ab}±8,60	-5,57^{ab}±10,9	-5,48^{ab}±13,16	-5,31^{ab}±9,38	-0,88±13,46	-0,70^{a}±12,82	6,268	**<0,001***
Group 2	-9,83^{a}±4,13	-39,13^{Bc}±8,79	-21,75^{Bb}±2,25	-15,90^{b}±13,97	-6,19^{a}±9,78	-4,19^{a}±5,57	-11,67^{a}±6,49	-10,83^{a}±5,00	-6,83^{a}±6,62	-7,42^{a}±6,86	9,022	**<0,001***
F	1,163	3,529	3,682	0,622	0,529	0,393	1,558	1,003	1,395	1,163		
p	0,342	**0,028***	**0,024***	0,607	0,666	0,759	0,222	0,406	0,265	0,236		

*: p<0.05 A, B: Differences between means with different letters in the same column are significant (p<0.05).

a, b, c: Differences between means with different letters in the same row are significant (p<0,05).



Graph 1: Evaluation of Sciatic Functional Index (SFI

Sensory Evaluation

At 24 hours, group 1 and group 2 are similar and significantly lower than the others, all other groups are different from each other (p<0.001). At 1-4 weeks, the control group is significantly higher than the others, all other groups are similar (p<0,001). At week 5, control group and group 2 are similar and higher than the others, all other groups are different from each other (p<0,001). At week 6, the sham group was significantly lower than the others and the other groups were similar (p<0,001).

The results of pinch test in the sham group were similar to hour 0 and higher than all other times at week 7 and week 8. The 24th hour pinch value was lower than all other times. Between 1st and 6th week, pinch tests were similar (p<0.001).

Group 1; pinch test results were similar and higher than other times at 0th hour and 4th week and all times thereafter. 24th hour was lower than all other times. Between 1st and 3rd week, pinch tests were similar (p<0.001).

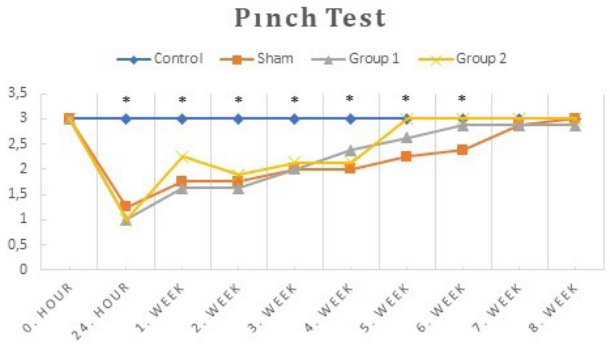
Group 2; pinch test results were similar and higher than all other times at 0th hour and 5th week and all times thereafter. 24th hour was lower than all other times. Between 1st and 4th week, pinch tests were similar (p<0.001). **Table 2** and **Graph 2** illustrate the comparison of pinch test measurements across time and groups. Comparison of CMAP Amplitude measurements

Table 2: Comparison of Pinch test measurements according to time and groups

Groups	0th hour	24th hour	1st week	2nd week	3th week	4th week	5th week	6th week	7th week	8th week	F	p
Control	3,00±0,00	3,00^{A}±0,00	3,00^{A}±0,00	3,00^{A}±0,00	3,00^{A}±0,00	3,00^{A}±0,00	3,00^{A}±0,00	3,00^{A}±0,00	3,00±0,00	3,00±0,00	-	-
Sham	3,00±0,00	1,25^{Bc}±0,46	1,75^{Bb}±0,46	1,75^{Bb}±0,46	2,00^{Bb}±0,00	2,00^{Bb}±0,00	2,25^{Cb}±0,46	2,38^{Bb}±0,52	2,88±0,35	3,00±0,00	**27,563**	**<0,001***
Group 1	3,00±0,00	1,00^{Cc}±0,00	1,63^{Bb}±0,52	1,63^{Bb}±0,52	2,00^{Bb}±0,53	2,38^{Ba}±0,74	2,63^{Ba}±0,52	2,88^{Aa}±0,35	2,88±0,35	2,88±0,35	**34,080**	**<0,001***
Group 2	3,00^{A}±0,00	1,00^{Cc}±0,00	2,25^{Ba}±0,71	1,88^{Bb}±0,35	2,13^{Ba}±0,35	2,13^{Ba}±0,35	3,00^{Aa}±0,00	3,00^{Aa}±0,00	3,00±0,00	3,00±0,00	**40,948**	**<0,001***
F	-	139,222	12,685	21,137	18,159	9,333	8,556	7,212	0,667	1,000		
p	-	**<0,001***	**<0,001***	**<0,001***	**<0,001***	**<0,001***	**<0,001***	**0,001***	0,580	0,407		

*: p<0,05 A, B, C: Differences between means with different letters in the same column are significant (p<0,05).

a, b, c: Differences between means with different letters in the same row are significant (p<0,05).



Graph 2: Evaluation of the Pinch Test

Comparison of CMAP Amplitude Measurements

In 24-hour measurements, the control group was similar to group 2 and significantly higher than the other groups. Sham group was similar to group 1 and significantly lower than all other groups and group 1 and group 2 were similar to each other (p=0.006). No difference was found between the groups in the 1st and 2nd month measurements (p=0.127, p=0.324, respectively). CMAP values measured at 24th hour and 1st month were similar in the sham group. The 2nd month CMAP value was significantly higher than the 24th hour (p=0.038).

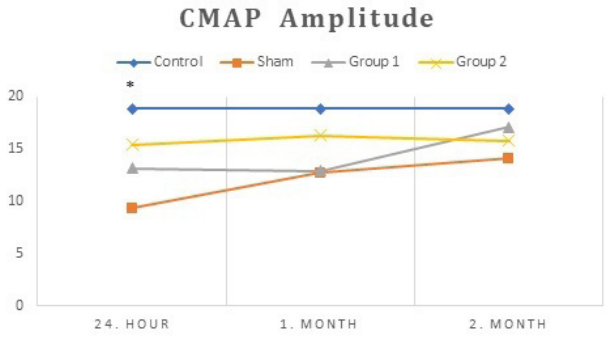
No difference was found in the measurements of the control group according to time. No significant difference was found in the CMAP values of group 1 and group 2 according to time (p=0.335, p=0.929, respectively). **Table 3** and **Graph 3** illustrate the comparison of CMAP amplitude measurements across time and groups.

Table 3: Comparison of CMAP Amplitude measurements according to time and groups

Groups	24th hour	1st month	2nd month	F	p
Control	*18,84^{A}±6,27 mV*	*18,84±6,27 mV*	*18,84±6,27 mV*	-	-
Sham	*9,33^{Cb}±1,95 mV*	*12,7^{ab}±3,95 mV*	*14,09^{a}±4,33 mV*	***4,151***	***0,038****
Gorup 1	*13,14^{BC}±5,24 mV*	*12,89±7,18 mV*	*17,11±5,93 mV*	*1,822*	*0,335*
Gorup 2	*15,43^{AB}±5,53 mV*	*16,31±5,26 mV*	*15,79±3,70 mV*	*0,074*	*0,929*
F	*5,051*	*2,072*	*1,212*		
p	***0,006****	*0,127*	*0,324*		

*: p<0,05 A, B, C: Differences between means with different letters in the same column are significant (p<0,05).

a, b: Differences between means with different letters in the same row are significant (p<0,05).



Graph 3: Evaluation of Compound Muscle Action Potential (CMAP)

Comparison of Latency Measurements

In 24-hour measurements, the control group was similar to group 2, and the latency value of the control group was significantly shorter than sham and group 1. Sham group was similar to group 1 and the latency value was significantly longer than group 2. Group 1 and group 2 were similar (p=0.002).

No difference was found in the measurements of the control group according to time. In the sham group, the 24 hour latency value was significantly longer than the 1st and 2nd month (p=0.001). Group 1; 24 hour latency value is significantly longer than the 1st and 2nd month (p<0.001). Group 2; 24 hour latency value is significantly longer than the 2nd month. Group 2 1st month latency value

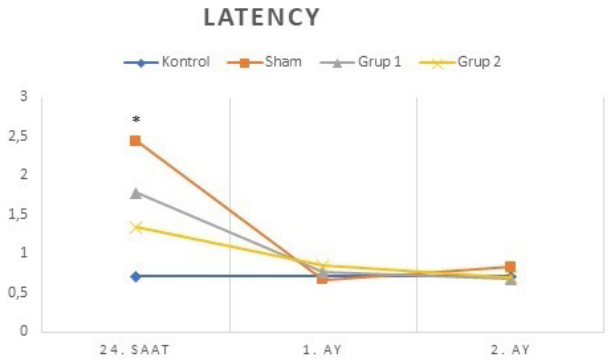
is similar to all times (p=0,003). **Table 4** and **Graph 4** illustrate the comparison of latency measurements according to time and groups.

Table 4: Comparison of Latency measurements according to time and groups

Groups	24th hour	1st month	2nd month	F	p
Control	0,71^{C}±0,29 ms	0,71±0,29 ms	0,71±0,29 ms	-	-
Sham	2,44^{Aa}±1,46 ms	0,67^{b}±0,2 ms	0,83^{b}±0,15 ms	11,371	0,001*
Group 1	1,78^{ABa}±0,58 ms	0,76^{b}±0,12 ms	0,68^{b}±0,12 ms	23,041	<0,001*
Group 2	1,34^{BCa}±0,41 ms	0,85^{ab}±0,26 ms	0,70^{b}±0,12 ms	8,735	0,003*
F	6,198	0,938	1,077		
p	0,002*	0,435	0,375		

*: p<0,05 A, B, C: Differences between means with different letters in the same column are significant (p<0,05).

a, b: Differences between means with different letters in the same row are significant (p<0,05).



Graph 4: Evaluation of Latency

Comparison of Biochemical Parameters According to Groups

TAS value in the control group was similar to sham and group 1 and significantly lower than group 2. Sham group TAS value was significantly lower than group 1 and group 2. Group 1 and group 2 were similar (p=0.002).

Control group TOS value was lower than sham group, group 1 and group 2. Sham group TOS value is higher than group 1 and group 2. Group 1 and group 2 are similar (p<0.001). Control group NGF value was similar to Sham and significantly lower than group 1 and group 2. Sham group; similar to all groups. Group 1 and group 2 are similar (p=0,058).

Control group TGF- β value; similar to Sham, significantly lower than group 1 and group 2. Sham group is similar to all groups. Group 1 and group 2 are similar (p=0,024). **Table 5** illustrate the comparison of biochemical parameters according to groups.

Comparison of Histopathological Parameters

All groups differ from each other in terms of axon degeneration. Axon degeneration was evaluated as Sham group, group 1 and group 2 in descending order. Axonal degeneration was not detected in the control group (p<0.001). In terms of edema, the control group was significantly lower than all other groups and no edema was detected. Sham and group 1 were similar to each other and significantly higher than group 2 (p<0,001). In terms of vacuolization, the control group was significantly lower than all other groups and no vacuolization was observed, Sham and Group 1 were similar and significantly higher than Group 2 (p<0,001). **Table 6** and **Graph 5** illustrate the comparison of histopathological parameters according to groups. In Figure 1, histopathological findings are shown.

Table 5: Comparison of biochemical parameters according to groups

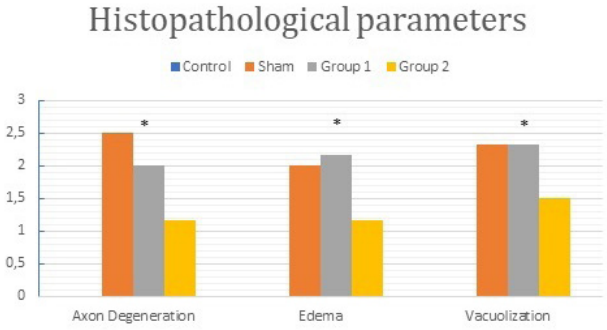
	Control	Sham	Group 1	Group 2	F	p
TAS (U/ml)	1,98±0,06^{bc}	1,82±0,04^{c}	2,12±0,11^{ab}	2,24±0,05^{a}		0,002*
TOS (U/ml)	7,10±0,32^{c}	11,09±0,46^{a}	9,70±0,25^{b}	9,33±0,30^{b}		0,001*
NGF (ng/L)	117,22±7,71^{b}	131,32±6,18^{ab}	137,29±5,74^{a}	140,94±4,91^{a}		0,058*
TGF- β (ng/L)	100,55±4,62^{b}	112,79±5,90^{ab}	116,66±4,88^{a}	123,28±4,37^{a}		0,024*

*: p<0.05 a, b, c: differences between means with different letters in the same row are significant (p<0.05).

Table 6: Comparison of histopathological parameters according to groups

Groups	Axonal Degeneration	Edema	Vacuolization
Control	0,00^{d}±0,00	0,00^{c}±0,00	0,00^{c}±0,00
Sham	2,50^{a}±0,55	2,00^{a}±0,00	2,33^{a}±0,52
Group1	2,00^{b}±0,00	2,17^{a}±0,41	2,33^{a}±0,52
Group 2	1,17^{c}±0,41	1,17^{b}±0,41	1,50^{b}±0,55
F	61,429	70,667	34,867
p	<0,001*	<0,001*	<0,001*

*: p<0.05 a, b, c: differences between means with different letters in the same column are significant (p<0.05).



Graph 5: Evaluation of Histopathological Parameters

DISCUSSION

In this study, different doses of *Myrtus communis* in sciatic nerve injury showed earlier improvement in SFI values, whereas no significant difference in this improvement was observed in those who did not receive treatment. In the SFI index evaluation, the sciatic nerve indices in group 1 and group 2 were similar to the sham but significantly worse than

the control group at 24 hours post-op. These results were similar in all groups on average at week 2. When the internal analysis of the treated groups was conducted, the indices were similar to baseline at week 2 in group 1 and similar to baseline at week 3 in group 2.

In the sensory evaluation, post-op, all groups were similarly worse than the control group; however, in group 1 at week 4, in group 2 at week 5, and in the sham group at week 7, sensory levels returned to values similar to baseline.

In the CMAP evaluation, sham had the lowest postoperative nerve conduction, followed by group 1 and, to a lesser extent, group 2. However, no significant difference was observed between the treated and untreated groups. When the latency values were analyzed, the post-op. latency was the longest in sham and no significant difference was observed between the treated and untreated groups.

In biochemical examination, TAS value was higher in treated than untreated patients. In group 1, it was similar to the control group. It was lower in the Sham group than the control group. TOS value was highest in Sham, similar and lower in groups 1 and 2, and lowest in the control group. NGF value was lower in the control group and similar in all other groups, but the highest NGF value was found in group 2. TGF-β values were also higher in groups 1 and 2 compared to the control but similar to sham.

In the histopathologic evaluation of our study, no damage was observed in the control group, whereas histopathologic changes were observed in sham and other treatment groups. Axon degeneration was the lowest in group 2 compared to the other groups, then in group 1 and the highest in sham. Edema was lowest in Group 2 and similar in Group 1 and sham group. Vacuolization was lowest in group 2 and similar in group 1 and sham group. When these results were taken into consideration, it was observed that 150 mg/kg dose provided histopathologically significant changes in the healing of nerve injury.

Injuries to peripheral nerves can arise from multiple causes (3). Particularly in cases of crush injuries, many complications may arise, including remyelination and demyelination, axonal regeneration and degeneration, focal, multifocal or diffuse nerve fiber loss, and endoneural edema (24). Loss of sensory and motor function after severe PNI severely affects patients' lives (4). As a result, it is critical to accelerate and improve the restoration of injured axons (25). To date, many surgical and non-surgical treatment approaches have been proposed for anatomical and functional recovery of injured peripheral nerves (5). The gold standard treatment of PNI is still widely accepted as axon-to-axon anastomosis (26), but factors such as the quality of axonal outgrowth, the number of viable neurons, the orientation of the regenerating axon and the condition of the axon are crucial for optimal nerve repair (27, 28). In addition, a number of pathophysiological events, including apoptosis (29), oxidative stress (30), inflammation (31), degradation of the extracellular matrix (27) and many more, can compromise the healing process in PNI. Microenvironmental conditions, cellular and molecular activation have proven to be really important in the nerve healing process (32). After nerve injury, ischemia-reperfusion injury due to dysfunction, microenvironmental macrophage activation via neutrophils and increased mitochondrial oxidative stress; the nerve healing process is negatively affected (33). Numerous experimental investigations have documented the efficacy of several pharmacological medications in the management of nerve damage (34 – 36). Alternative therapeutic approaches with phytochemicals including quercetin, ursocolic acid, curcumin and others have been suggested to be beneficial with experimental evidence, but most of them cause side effects at human doses (5). Therefore, newer approaches are needed for the treatment of peripheral nerve injury that manage the optimal properties for a drug, such as causing negligible side effects.

MC is a traditional medicinal plant used for treating stomach ulcers, inflammation, diarrhea, hemorrhoids, lung and skin disorders. Numerous research have shown the anti-diabetic, antioxidant, hepatoprotective, neuroprotective, anticancer, antiviral, antibacterial, and antimycotic properties of MC (37 – 41). The observed effects of MC can be attributed to biochemically active components, including phenolic chemicals, flavonoids, hydrolyzed tannins (galotanins), proanthocyanidins, and volatile oil (37).

The efficacy of *Myrtus communis* in peripheral nerve damage has not been previously assessed.

This work presents evidence of the antioxidant properties of oral MC administration through biochemical and histological responses of high-dose *Myrtus communis* treatment in peripheral nerve recovery. Specifically, the group with higher treatment dose showed increased levels of TAS, NGF, and TGF-β, and reduced vacuolization, edema, and axon degeneration in histological evaluations. The antioxidant activity of MC on the peripheral nerve may facilitate the early restoration of peripheral nerve healing following sciatic nerve injury. The responses of MC administration were recorded with two different drug doses and it was observed that both antioxidant response and histopathologic results were better in the group receiving the higher dose (MC 150 mg/kg).

NGF is one of the members of the neurotrophin family of neurotrophic factors (42). Upregulation of NGF synthesis during normal Wallerian degeneration enhances neuronal survival and axon growth in specific subgroups of sympathetic and sensory dorsal root neurons (43). In addition, many studies have shown that NGF induces the phagocytosis ability of Schwann cells. Thus, removal of damaged myelin sheaths is accelerated and the rate of regeneration is increased (44). In our study, similar to the literature, NGF value was expressed at a higher level in MC-treated groups compared to the control group and it was observed that it was expressed at a higher level in the group with higher MC dose than all other groups.

The transforming growth factor (TGF-β) is another group of neurotrophic factors that contributes to the process of regeneration. Transforming growth factor-β (TGF-β) is a cytokine that regulates cell growth, specialization, and programmed cell death. In the aftermath of neurological system damage, TGF-β controls the activity of neurons and glial cells, therefore facilitating the process of regeneration. Heightened production of TGF-β controls nerve regeneration by inhibiting the immune response, altering cellular activity, controlling the growth of nerve fibers, and facilitating the development of glial wounds (45).

One should evaluate this work considering the merits and drawbacks of experimental animal studies. A further constraint of this study is the limited sample size of the experimental animals. Given that experimental animal research involve live animals, we have included the number of experimental animals within relevant statistical boundaries. The generalizability of the results can only be achieved by clinical validation using suitable research.

There is still insufficient information about medical treatment options in the treatment of PNI. However, the role of Myrtus communis in early recovery has been demonstrated by the results of both biochemical and histopathological changes in the evaluation of functional recovery. Further studies on this subject will be guiding in the treatment of PNI.

This study was supported by Afyonkarahisar Health Sciences University Scientific Research Projects Coordination Unit with the project number 21.GENEL.016.

REFERENCES

1. Hewson DW, Bedforth NM, Hardman JG. Peripheral nerve injury arising in anaesthesia practice. Anaesthesia 2018;73:51–60.

2. Li R, Liu Z, Pan Y, et al. Peripheral Nerve Injuries Treatment: a Systematic Review. Cell Biochem Biophys. 2014;68:449–54.

3. Evans GR. Challenges to nerve regeneration. Semin Surg Oncol. 2000;19(3):312-8.

4. Benga A, Zor F, Korkmaz A, et al. The neurochemistry of peripheral nerve regeneration. Indian Journal of Plastic Surgery. 2017;50:5–15.

5. Hussain G, Wang J, Rasul A, et al. Current Status of Therapeutic Approaches against Peripheral Nerve Injuries: A Detailed Story from Injury to Recovery. Int J Biol Sci. 2020;16:116–34.

6. Carvalho CR, Oliveira JM, Reis RL. Modern Trends for Peripheral Nerve Repair and Regeneration: Beyond the Hollow Nerve Guidance Conduit. Front Bioeng Biotechnol. 2019;7:337.

7. Martinez de Albornoz P, Delgado PJ, et al. Non-surgical therapies for peripheral nerve injury. Br Med Bull. 2011;100:73–100.

8. Novak CB, von der Heyde RL. Evidence and Techniques in Rehabilitation Following Nerve Injuries. Hand Clin. 2013;29:383–92.

9. Pabari A, Lloyd-Hughes H, Seifalian AM, et al. Nerve Conduits for Peripheral Nerve Surgery. Plast Reconstr Surg. 2014;133:1420–30.

10. Chan KM, Gordon T, Zochodne DW, et al. Improving peripheral nerve regeneration: From molecular mechanisms to potential therapeutic targets. Exp Neurol. 2014;261:826–35.

11. Snyder AK, Fox IK, Nichols CM, et al. Neuroregenerative Effects of Preinjury FK-506 Administration. Plast Reconstr Surg. 2006;118:360–7.

12. Gordon T. Electrical Stimulation to Enhance Axon Regeneration After Peripheral Nerve Injuries in Animal Models and Humans. Neurotherapeutics. 2016;13:295–310.

13. Şahin G, Altuntaş E, Polatcı H. Mersin (Myrtuscommunis L.) Mevyesinin Fiziksel, Mekanik, Renk ve Kimyasal Özellikleri. Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi. 2020;23:59–68.

14. Mahboubi M. Myrtus communis L. and its application in treatment of Recurrent Aphthous Stomatitis. J Ethnopharmacol. 2016;193:481–9.

15. Messaoud C, Boussaid M. Myrtus communis Berry Color Morphs: A Comparative Analysis of Essential Oils, Fatty Acids, Phenolic Compounds, and Antioxidant Activities. Chem Biodivers. 2011;8:300–10.

16. Hayder N, Abdelwahed A, Kilani S, et al. Anti-genotoxic and free-radical scavenging activities of extracts from (Tunisian) Myrtus communis. Mutation Research/ Genetic Toxicology and Environmental Mutagenesis. 2004;564:89–95.

17. Sepici-Dincel A, Açıkgöz Ş, Çevik C, et al. Effects of in vivo antioxidant enzyme activities of myrtle oil in normoglycaemic and alloxan diabetic rabbits. J Ethnopharmacol. 2007;110:498–503.

18. Miguel MG. Antioxidant and Anti-Inflammatory Activities of Essential Oils: A Short Review. Molecules. 2010;15:9252–87.

19. Mimica-Dukić N, Bugarin D, Grbović S, et al. Essential Oil of Myrtus communis L. as a Potential Antioxidant and Antimutagenic Agents. Molecules. 2010;15:2759–70.

20. Tumen I, Senol FS, Orhan IE. Inhibitory potential of the leaves and berries of Myrtus communis L. (myrtle) against enzymes linked to neurodegenerative diseases and their antioxidant actions. Int J Food Sci Nutr. 2012;63:387–92.

21. de Medinaceli L, Freed WJ, Wyatt RJ. An index of the functional condition of rat sciatic nerve based on measurements made from walking tracks. Exp Neurol. 1982;77:634–43.

22. Bain JR, Mackinnon SE, Hunter DA. Functional Evaluation of Complete Sciatic, Peroneal, and Posterior Tibial Nerve Lesions in the Rat. Plast Reconstr Surg. 1989;83:129–36.

23. Luna LG. Manual of histologic staining methods of the armed forces institute of pathology. McGrow-Hill book company, 1968.

24. Bagdatoglu C, Saray A, Surucu HS, et al. Effect of trapidil in ischemia/reperfusion injury of peripheral nerves. Neurosurgery. 2002 ;51(1):212-9; discussion 219-20.

25. Gordon T, Chan KM, Sulaiman OAR, et al. Acceleratıng Axon Growth to Overcome Lımıtatıons ın Functıonal Recovery After Perıpheral Nerve Injury. Neurosurgery. 2009;65:A132–44.

26. Griffin JW, Hogan M V., Chhabra AB, et al. Peripheral Nerve Repair and Reconstruction. J Bone Joint Surg. 2013;95:2144–51.

27. Nagappan PG, Chen H, Wang D-Y. Neuroregeneration and plasticity: a review of the physiological mechanisms for achieving functional recovery postinjury. Mil Med Res.2020;7:30.

28. Lundborg G. A 25-year perspective of peripheral nerve surgery: Evolving neuroscientific concepts and clinical significance. J Hand Surg Am.2000;25:391–414.

29. Oliveira ALR. Apoptosis of sensory neurons and satellite cells after sciatic nerve transection in C57BL/6J mice. Brazilian Journal of Medical and Biological Research. 2001;34:375–80.

30. Naik AK, Tandan SK, Dudhgaonkar SP, et al. Role of oxidative stress in pathophysiology of peripheral neuropathy and modulation by N-acetyl-L-cysteine in rats. European Journal of Pain. 2006;10:573–79.

31. Nadeau S, Filali M, Zhang J, et al. Functional recovery after peripheral nerve injury is dependent on the pro-inflammatory cytokines IL-1β and TNF: implications for neuropathic pain. The Journal of Neuroscience. 2011;31:12533–42.

32. Rotshenker S. Wallerian degeneration: the innate-immune response to traumatic nerve injury. J Neuroinflammation. 2011;8:109.

33. Coban YK, Ciralik H, Kurutas EB. Ischemic preconditioning reduces the severity of ischemia-reperfusion injury of peripheral nerve in rats. J Brachial Plex Peripher Nerve Inj. 2006;1:2.

34. Al-Bishri A, Dahlin L, Sunzel B, et al. Systemic Betamethasone Accelerates Functional Recovery After a Crush Injury to Rat Sciatic Nerve. Journal of Oral and Maxillofacial Surgery. 2005;63:973–7.

35. Lee M, Doolabh VB, Mackinnon SE, et al. FK506 promotes functional recovery in crushed rat sciatic nerve. Muscle Nerve. 2000;23:633–40.

36. Subbanna P, Prasanna C, Gunale B, et al. Acetyl salicylic acid augments functional recovery following sciatic nerve crush in mice. J Brachial Plex Peripher Nerve Inj. 2014;02:e91–4.

37. Alipour G, Dashti S, Hosseinzadeh H. Review of Pharmacological Effects of Myrtus communis L. and its Active Constituents. Phytotherapy Research. 2014;28:1125–36.

38. Özcan MM, Uyar B, Ünver A. Antibacterial effect of myrtle (Myrtus communis L.) leaves extract on microorganisms. Archiv Für Lebensmittelhygiene. 2015;66:18–21.

39. Chalchat JC, Fıgueredo G, Özcan MM, et al. Effect of Hydrodistillation and Microwave Distillation Extraction Methods on Chemical Compositions of Essential Oil of Pickling Herb And Myrtle Plants. South Western Journal of Horticulture, Biology and Environment. 2010;1:133–41.

40. Viana AFSC, Lopes MTP, Oliveira FTB, et al. (−)-Myrtenol accelerates healing of acetic acid-induced gastric ulcers in rats and in human gastric adenocarcinoma cells. Eur J Pharmacol. 2019;854:139–48.

41. Ogur R. Studies with Myrtus communis L.: anticancer properties. J Intercult Ethnopharmacol. 2014;3:135.

42. Mok S-A, Lund K, Campenot RB. A retrograde apoptotic signal originating in NGF-deprived distal axons of rat sympathetic neurons in compartmented cultures. Cell Res. 2009;19:546–60.

43. Heumann R, Korsching S, Bandtlow C, et al. Changes of nerve growth factor synthesis in nonneuronal cells in response to sciatic nerve transection. J Cell Biol. 1987;104:1623–31.

44. Li R, Li D, Wu C, et al. Nerve growth factor activates autophagy in Schwann cells to enhance myelin debris clearance and to expedite nerve regeneration. Theranostics. 2020;10:1649–77.

45. Kubiczkova L, Sedlarikova L, Hajek R, et al. TGF-β – an excellent servant but a bad master. J Transl Med. 2012;10:183.