Research Article / Araştırma Makalesi
doi: 10.18678/dtfd.1611897

Duzce Med J, 2025;X(X):X-X
Düzce Tıp Fak Derg, 2025;X(X):X-X

Evaluation of the Therapeutic Effects of Thymbra Spicata L. var spicata and Cyclotrichium Origanifolium in Spinal Cord Injury: An Experimental Study

Omurilik Yaralanmasında Thymbra Spicata L. var spicata ve Cyclotrichium Origanifolium'un Terapötik Etkilerinin Değerlendirilmesi: Deneysel Bir Çalışma

Figen KOÇ DİREK[1]
0000-0002-4748-2110
Mehmet Cudi Tuncer[2]
0000-0001-7317-5467
Engin DEVECİ[3]
0000-0002-2353-1184
Dilek AYGÜN KEŞİM[4]
0000-0001-5481-1130
Hüseyin ÖZEVREN[5]
0000-0002-5437-4003

[1]Department of Anatomy, Mardin Artuklu University Faculty of Medicine, Mardin, Türkiye
[2]Department of Anatomy, Dicle University Faculty of Medicine, Diyarbakır, Türkiye
[3]Department of Histology and Embryology, Dicle University Faculty of Medicine, Diyarbakır, Türkiye
[4]Department of Physical Medicine and Rehabilitation, Dicle University Faculty of Medicine, Diyarbakır, Türkiye
[5]Department of Neurosurgery, Dicle University Faculty of Medicine, Diyarbakır, Türkiye

Corresponding Author
Sorumlu Yazar
Figen KOÇ DİREK
figenkocdirek@artuklu.edu.tr

Received / Geliş Tarihi : 02.01.2025
Accepted / Kabul Tarihi : 16.05.2025
Available Online / Çevrimiçi Yayın Tarihi : 19.06.2025

ABSTRACT

Aim: Secondary tissue damage caused by oxidative stress and inflammation in spinal cord injury complicates treatment and highlights the need for novel therapeutic agents. This study aimed to evaluate the potential effects of *Thymbra spicata* L. var. *spicata* (zahter) and *Cyclotrichium origanifolium* (mountain mint) extracts in an experimental SCI model.
Material and Methods: Thirty-six male Wistar albino rats were randomly assigned to six groups: control, zahter, mountain mint, trauma, trauma+zahter, and trauma+mountain mint. Spinal cord injury was induced in the trauma groups. Blood was collected via cardiac puncture, and spinal tissues were obtained for histopathological and immunohistochemical analyses. Serum MDA, TAS, and TOS levels were measured biochemically. VEGF and GFAP expressions were assessed immunohistochemically.
Results: MDA and TOS levels decreased, while TAS levels increased in the treated groups compared to the trauma group, but these changes were not found statistically significant. Histopathological evaluation showed prominent neuronal degeneration, inflammation, and vascular dilatation in the trauma group, which were alleviated in the treatment groups. Greater histological improvement was observed in the trauma+mountain mint group compared to trauma+zahter ($p<0.001$). VEGF and GFAP expressions were elevated in the trauma group but statistically significantly reduced in the treatment groups ($p<0.001$).
Conclusion: Zahter and mountain mint extracts exhibited therapeutic potential against trauma-induced spinal cord injury by modulating inflammation and tissue damage. Mountain mint was found to be more effective in terms of histological and immunohistochemical outcomes.
Keywords: Antioxidants; spinal cord injury; oxidative stress; plant extracts; trauma.

ÖZ

Amaç: Omurilik yaralanmasında gelişen oksidatif stres ve inflamasyona bağlı ikincil doku hasarı, tedaviyi güçleştirmekte ve yeni terapötik ajanlara olan ihtiyacı artırmaktadır. Bu çalışmanın amacı, deneysel bir omurilik yaralanması modelinde *Thymbra spicata* L. var. *spicata* (zahter) ve *Cyclotrichium origanifolium* (dağ nanesi) ekstraktlarının terapötik etkilerini değerlendirmektir.
Gereç ve Yöntemler: Otuz altı erkek Wistar Albino sıçanı rastgele altı gruba ayrıldı: kontrol, zahter, dağ nanesi, travma, travma+zahter ve travma+dağ nanesi. Travma gruplarında omurilik yaralanması oluşturuldu. Kan örnekleri kardiyak ponksiyon yoluyla toplandı ve histopatolojik ve immünohistokimyasal analizler için omurilik dokuları elde edildi. Serum MDA, TAS ve TOS düzeyleri biyokimyasal yöntemlerle analiz edildi. VEGF ve GFAP ekspresyonları immünohistokimyasal yöntemlerle değerlendirildi.
Bulgular: Tedavi edilen gruplarda, travma grubuna kıyasla MDA ve TOS düzeyleri azalmış, TAS düzeyi ise artmıştır; ancak bu değişiklikler istatistiksel olarak anlamlı bulunmamıştır. Histopatolojik analizlerde, travma grubunda belirgin nöronal dejenerasyon, inflamasyon ve vasküler genişleme gözlenmiş; tedavi gruplarında ise bu bulgularda düzelme olduğu görülmüştür. Travma+dağ nanesi grubunda travma+zahter grubuna göre daha fazla histolojik iyileşme gözlenmiştir ($p<0,001$). VEGF ve GFAP ekspresyonları travma grubunda yüksek iken, tedavi gruplarında ise istatistiksel olarak anlamlı şekilde azalmıştır ($p<0,001$).
Sonuç: Zahter ve dağ nanesi ekstraktları, travmaya bağlı omurilik yaralanmasında inflamasyon ve doku hasarı mekanizmaları üzerine etki ederek terapötik potansiyel göstermiştir. Dağ nanesi, histolojik ve immünohistokimyasal parametreler açısından daha etkili bulunmuştur.
Anahtar kelimeler: Antioksidanlar; omurilik yaralanması; oksidatif stres; bitki ekstraktları; travma.

Presented partially as an oral presentation at the 4th International Başkent Congress (February 26-27, 2022; Online).

INTRODUCTION

Spinal cord injury (SCI) is a complex and debilitating neurological condition that leads to profound physical impairment and places substantial psychological and economic burdens on both patients and their families (1). Although considerable progress has been made in diagnostic techniques, acute management, and supportive care, the development of an effective and standardized therapeutic strategy for SCI remains a significant clinical challenge. SCI is a complex condition, typically categorized into two phases: primary and secondary injury (1). The primary injury results from direct mechanical damage to axons and neurons, while secondary injury begins within minutes of the initial trauma, gradually causing further deterioration in the surrounding spinal cord tissue (2-4). Secondary injury is characterized by processes such as vascular disruption, ion imbalance, accumulation of excitotoxic neurotransmitters, free radical generation, lipid peroxidation (LP), inflammation, edema, and necrotic cell death (5). Contemporary research is largely focused on strategies to mitigate secondary injury and prevent further tissue damage following trauma.

In light of this, we hypothesized that herbal therapies, due to their antioxidant, anti-inflammatory, and neuroprotective properties, may complement medical interventions in traumatic SCI and contribute to accelerated recovery. *Cyclotrichium origanifolium* (mountain mint), traditionally used to treat ailments such as influenza, nausea, and musculoskeletal conditions, has demonstrated significant therapeutic potential. Studies suggest that its essential oils and various extracts possess potent antioxidant and antimicrobial activities, which may protect cells from oxidative stress (6-8). Similarly, *Thymbra spicata* L. var *spicata* (zahter), a member of the Lamiaceae family, contains bioactive compounds like carvacrol and thymol, known for their robust anti-inflammatory and antioxidant effects (9-11). The pharmacological benefits of these plants, particularly their neuroprotective properties, make them promising candidates for further investigation in the context of SCI.

Mountain mint and zahter have demonstrated notable anti-inflammatory, antioxidant, and neuroprotective effects in various preclinical models. However, data regarding the effects of these herbal agents on traumatic SCI remain limited. This study aimed to evaluate the effects of both plants on biochemical, histopathological, and immunohistochemical changes in spinal cord tissue following SCI using an experimental model.

MATERIAL AND METHODS

This study was carried out following the guidelines of the Dicle University Animal Experiments Ethics Committee, which approved the study under protocol number 2019/08 dated 30/05/2019. The project received financial support from the Scientific Research Projects Coordination Unit of Dicle University, with the project number TIP.19.023.

Animals and Experimental Groups

This study utilized 36 male Wistar Albino rats, aged 8 to 10 weeks and weighing between 250 and 300 grams. The animals were housed in stainless steel cages under controlled conditions of 22±2 °C, with a 12-hour light/dark cycle. They were provided with a standard diet and tap water without restrictions.

The rats were randomly divided into six experimental groups, each consisting of six rats.

i) Control: This group was designated as the negative control, receiving neither trauma nor treatment. To ensure consistency with other groups, 4000 ppm/kg/day saline was administered intraperitoneally for one week.

ii) Mountain Mint: No trauma was induced. 4000 ppm/kg/day mountain mint, dissolved in a 1% ethanol solution, was administered intraperitoneally for one week.

iii) Zahter: No trauma was induced. 4000 ppm/kg/day zahter, dissolved in a 1% ethanol solution, was administered intraperitoneally for one week.

iv) Trauma: Trauma was induced. 4000 ppm/kg/day saline was administered intraperitoneally for one week.

v) Trauma+Mountain Mint: Trauma was induced. 4000 ppm/kg/day mountain mint, dissolved in a 1% ethanol solution, was administered intraperitoneally for one week.

vi) Trauma+Zahter: Trauma was induced. 4000 ppm/kg/day zahter, dissolved in a 1% ethanol solution, was administered intraperitoneally for one week.

Spinal Cord Injury Induction

Anesthesia was achieved through intraperitoneal injections of ketamine (90 mg/kg) and xylazine (10 mg/kg). The rats were positioned prone on mushroom blocks, and sterilization of the surgical area was performed using PVD iodine. A midline incision was made between T5 and T12, followed by lateral dissection of the paravertebral muscles to expose the laminae. A laminectomy was conducted at T7-T9, after which a 3 mm diameter, 10 g weight steel rod was dropped from a height of 10 cm onto the spinal cord using the Allen weight-drop method (12). After the trauma, the weight was removed, and the muscle and skin incisions were sutured (Figure 1).

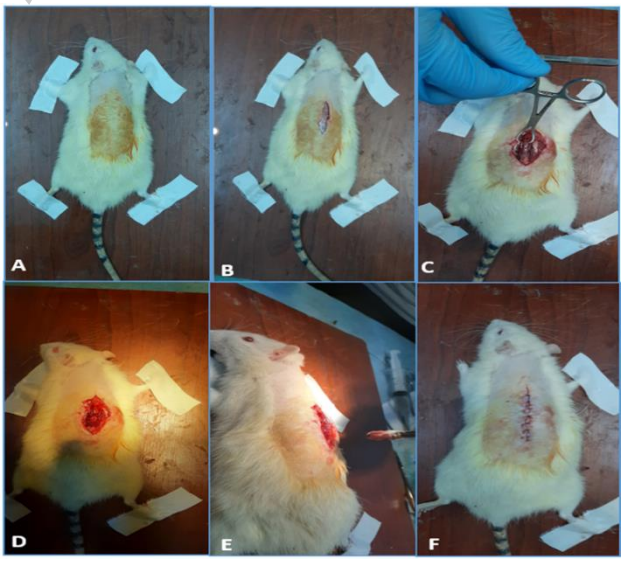

Figure 1. Steps of spinal cord trauma induction, **A)** rat was placed in the prone position, and the surgical area sterilized, **B)** midline incision was made, **C)** paravertebral muscles were dissected, and laminectomy conducted, **D)** spinal cord was exposed following laminectomy, **E)** spinal cord trauma induced using the weight-drop method (this part was oriented vertically to maintain figure consistency), **F)** muscle and skin incisions were closed with appropriate sutures

During the experiment, two rats in the trauma group did not survive due to procedure-related complications. To maintain equal numbers of rats in each group, the deceased rats were replaced with new ones subjected to the same trauma induction protocol.

Motor Function Assessment and Biomaterial Collection

One day after the trauma, motor function was assessed using the scale described by Rivlin and Tator (13), where the scores ranged from 0 (complete paralysis) to 4 (normal movement). Paraplegia was observed in all rats subjected to SCI following the trauma. On the eighth day, blood samples were collected via cardiac puncture under ketamine anesthesia, and the rats were subsequently euthanized. Spinal cord tissues were harvested for histopathological and immunohistochemical analyses. The immunohistochemical examination focused on the expression of vascular endothelial growth factor (VEGF) and glial fibrillary acidic protein (GFAP). To measure malondialdehyde (MDA), total antioxidant status (TAS), and total oxidant status (TOS) levels, biochemical analyses were conducted.

Obtaining Plant Samples and Essential Oils

Zahter and mountain mint species were collected from nature and dried in the shade. The above-ground parts of the plants dried in the shade were cut into small particles and boiled with hot water in a distillation device, the Clevenger apparatus. The essential oil entrained by the resulting steam was condensed in the cooler and collected in a container. The essential oils obtained were kept in sodium sulfate (Na_2SO_4) at +4 °C and saved from the water it contained (14). Stock solutions at a concentration of 4000 ppm (µg/ml) were prepared with ethanol solvent for application.

Biochemical Analysis

Blood samples were centrifuged at 3500 rpm for 20 minutes to separate the serum, which was then stored at -80 °C until further biochemical analyses. Serum levels of TAS, TOS, and MDA were measured. Clinical chemistry tests were conducted using the Architect C1600 Auto analyzer (Abbott Laboratories). TAS and TOS levels, along with photometric measurements, were determined in the serum using commercially available kits from Rel Assay.

Tissue Tracking

Spinal cord tissues were processed for routine paraffin embedding. After 24 hours of fixation, the tissues were washed overnight and subjected to a series of increasing alcohol concentrations, followed by clearing with xylene (3 cycles of 30 minutes each). Paraffin infiltration was performed at 58 °C. Once embedded in paraffin blocks, sections of 4-6 µm thickness were cut using a microtome (Leica RM2265, Wetzlar, Germany) for hematoxylin-eosin staining.

Hematoxylin-Eosin Staining

Paraffin-embedded spinal cord sections were incubated in a 37 °C water bath, followed by heating at 58-62 °C for 6 hours to remove excess paraffin. The sections were deparaffinized using xylene (3 cycles, 15 minutes each) and passed through a graded ethanol series (100%, 96%, 90%, 70%, and 50%). After a 5-minute rinse in distilled water, Harris Hematoxylin was applied for 8 minutes, followed by a 5-minute wash. Eosin staining was performed for 6 minutes, and the sections were dehydrated with ethanol (80%, 90%, and 96%). Finally, sections were cleared in xylene (3 cycles, 15 minutes each) and mounted using Entellan. Stained sections were evaluated under a Zeiss A2 imager light microscope.

Immunohistochemical Staining

Paraffin-embedded sections were incubated in a 37 °C bain-marie, then transferred to poly-l-lysine-coated slides. Excess paraffin was removed by placing the sections in a 58-62 °C oven for 6 hours. Deparaffinization was achieved using xylene (3 cycles of 15 minutes), followed by a graded ethanol series. After washing in distilled water and phosphate buffer solution (PBS) for 3 cycles of 5 minutes, heat-induced antigen retrieval was performed using EDTA buffer (pH 8.0). The sections were incubated with hydrogen peroxide for 20 minutes to block endogenous peroxidase activity, followed by Ultra V Block incubation for 7 minutes. Anti-GFAP and anti-VEGF primary antibodies were applied at +4 °C overnight. The next day, sections were incubated with biotinylated secondary antibodies for 14 minutes, followed by streptavidin-peroxidase for 15 minutes. Diaminobenzidine (DAB) was used for chromogenic detection, and the reaction was stopped with PBS. After counterstaining with Harris hematoxylin, the sections were mounted using Entellan and visualized with a Zeiss Imager A2 microscope.

Statistical Analysis

Statistical analyses were conducted using the R Studio IDE, v.3.6.3 software. Normality of the data was assessed using the Shapiro-Wilk test. As the data did not meet the assumptions for parametric testing, the non-parametric Kruskal-Wallis test was employed to evaluate differences across groups. Given the limited sample size and the appropriateness of the data for non-parametric methods, the Dunn post-hoc test with Bonferroni correction was utilized to reduce the likelihood of Type I errors and improve the power of the analysis. Mahalanobis distance was used to detect potential outliers before conducting the analysis; however, no significant outliers were identified. Statistical significance was established at $p<0.05$.

RESULTS

Serum Biochemical Parameters

Serum MDA levels were found significantly higher in the trauma group compared to the control (p=0.001), mountain mint (p=0.008), and zahter (p=0.002) groups. Although MDA levels were lower in the trauma+mountain mint and trauma+zahter groups compared to the trauma group, these differences were not statistically significant (p=0.137 and p=0.118, respectively, Table 1, Figure 2A).

Serum TAS levels showed a statistically significant increase in the zahter group compared to the trauma group (p=0.002). Although the trauma+mountain mint and trauma+zahter groups demonstrated slight increases in TAS levels relative to the trauma group, these differences did not reach a statistical significance level (p=0.365 and p=0.540, respectively, Table 1, Figure 2B).

Serum TOS levels in the trauma group showed a statistically significant increase compared to the mountain mint (p=0.029) and zahter (p<0.001) groups. TOS levels in the trauma+mountain mint and trauma+zahter groups were found to be higher than in the control group, however, a non-significant decrease was observed in both groups compared to the trauma group (p=0.081 and p=0.248, respectively, Table 1, Figure 2C).

Table 1. Comparison of serum MDA, TAS, and TOS levels in experimental groups

		Control	Mountain Mint	Zahter	Trauma	Trauma+ Mountain Mint	Trauma+ Zahter	p
MDA (µmol/L)	Median (Q_1-Q_3) [min-max]	0.92 (0.74-1.07) [0.12-1.31]	1.04 (0.94-1.19) [0.91-1.90]	1.04 (0.86-1.09) [0.65-1.12]	4.28 (3.39-4.85) [3.11-7.49]	2.87 (2.28-3.16) [1.90-4.12]	1.94 (1.56-2.39) [1.27-2.49]	**<0.001**
TAS (mmol/L)	Median (Q_1-Q_3) [min-max]	1.17 (1.06-1.26) [0.86-1.39]	1.50 (1.31-1.66) [1.02-2.11]	2.16 (1.75-2.48) [1.36-2.55]	1.03 (0.84-1.05) [0.45-1.35]	1.18 (1.03-1.30) [0.89-1.67]	1.14 (1.08-1.27) [1.05-1.47]	**0.003**
TOS (µmol/L)	Median (Q_1-Q_3) [min-max]	69.46 (65-79) [61-80]	70.43 (66-75) [63-77]	46.69 (42-50) [40-52]	130.10 (122-138) [120-142]	92.67 (86-98) [82-102]	73.15 (68-79) [64-83]	**<0.001**

MDA: malondialdehyde, TAS: total antioxidant status, TOS: total oxidant status, Q_1-Q_3: interquartile range, 25th-75th percentile

Histopathological Scoring

Histopathological scoring assessed neuronal degeneration, inflammation, and vascular dilation. Measurements were obtained from 10 different regions per section in each group. Neuronal degeneration was quantified by calculating the ratio of degenerated neurons to the total neuron count, while other parameters were similarly quantified. Scores for neuronal degeneration, inflammation, and vascular dilation were assigned as follows: 0= no change, 1= mild, 2= intermediate, 3= high, and 4= very intense. Statistically significant differences were observed across the groups for neuronal degeneration, inflammation, and dilatation ($p<0.001$, Table 2).

Histopathological Analysis

Hematoxylin-eosin staining revealed normal histological architecture in the control, mountain mint, and zahter groups, with no pathological alterations. In contrast, the trauma group displayed significant vascular dilation, neuronal degeneration, central canal disruption, as well as notable cell infiltration and neuroglial changes. Treatment with mountain mint and zahter ameliorated trauma-induced damage, showing improved tissue architecture (Figure 3).

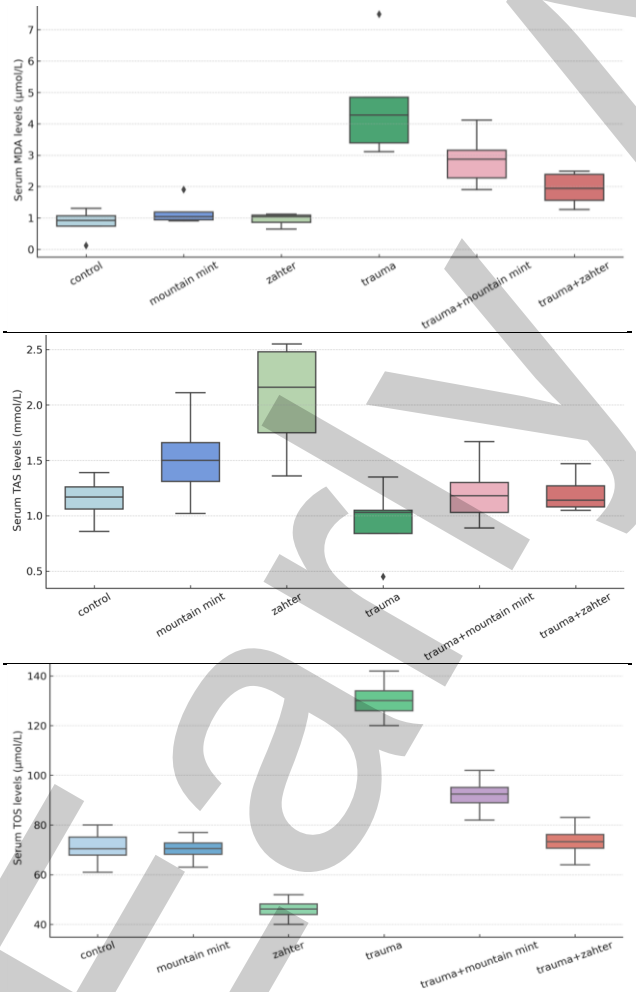

Figure 2. A) MDA, **B)** TAS, and **C)** TOS levels in groups
MDA: malondialdehyde, TAS: total antioxidant status, TOS: total oxidant status

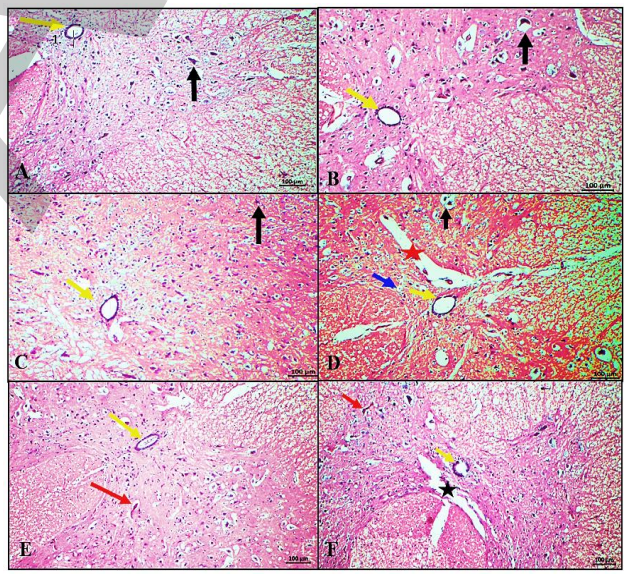

Figure 3. Hematoxylin-eosin staining of rat spinal cord sections (scale bar=100 µm), **A)** control group showed no pathology, with normal neurons (black arrow) and ependymal cells (yellow arrow), **B)** mountain mint group resembled the control, **C)** zahter group also showed similar histology, **D)** trauma group exhibited vascular dilatation (red star), neuronal degeneration (black arrow), and central canal disruption (yellow arrow), with significant cell infiltration and neuroglial changes (blue arrow), **E)** trauma+mountain mint group had mild trauma-related damage with vascular dilatation (red arrow), and central canal regeneration (yellow arrow), **F)** trauma+zahter group showed reduced spinal damage with vascular dilatation (red arrow), central canal cell degeneration (yellow arrow), tissue integrity disruptions, and inflammation (black star)

Table 2. Histopathological scoring results of the groups

		Control	Mountain Mint	Zahter	Trauma	Trauma+ Mountain Mint	Trauma+ Zahter	p
Neuronal Degeneration	Median [min-max]	0 [0-1]	0 [0-1]	0 [0-1]	3.5 [3-4]	2.5 [1-4]	2.5 [0-3]	**<0.001**
Inflammation	Median [min-max]	0 [0-1]	0 [0-1]	0 [0-1]	4 [3-4]	2 [0-4]	2.5 [0-4]	**<0.001**
Dilatation	Median [min-max]	0 [0-1]	0 [0-1]	0 [0-1]	4 [3-4]	2 [0-4]	2 [0-4]	**<0.001**

Immunohistochemical Findings

VEGF immunohistochemical staining revealed slight expression in the control group, with a marginal increase observed in the mountain mint and zahter groups. The trauma group showed intense VEGF expression, which was markedly reduced in the trauma+mountain mint and trauma+zahter groups, approaching levels similar to those in the control group (Figure 4). GFAP expression was mild in the control group, and was also similar in both the mountain mint and zahter groups. The trauma group displayed strong GFAP expression, which was notably reduced in the trauma+mountain mint and trauma+zahter groups when compared to the trauma group (Figure 5).

Statistically significant differences were observed in VEGF and GFAP expressions between the control, trauma, and treatment groups ($p<0.001$, Table 3).

Table 3. VEGF and GFAP expression scoring results of the groups

		Control	Mountain Mint	Zahter	Trauma	Trauma+ Mountain Mint	Trauma+ Zahter	p
VEGF	Median [min-max]	1.5 [0-3]	1 [0-3]	0.5 [0-3]	3 [2-4]	2 [1-3]	2 [0-4]	**<0.001**
GFAP	Median [min-max]	1 [0-2]	1 [0-3]	1 [0-3]	3.5 [2-4]	2.5 [1-4]	2 [1-4]	**<0.001**

VEGF: vascular endothelial growth factor, GFAP: glial fibrillary acidic protein

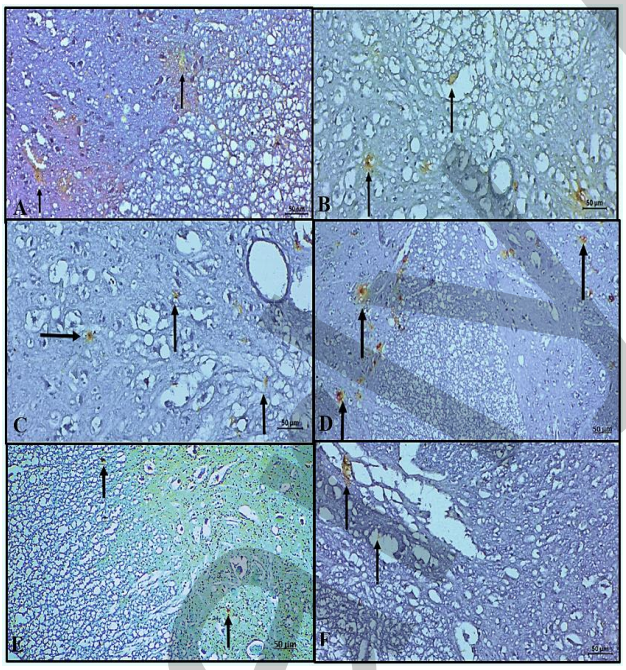

Figure 4. VEGF immunohistochemistry staining of rat spinal cord sections (scale bar=50 µm), **A)** control group, low VEGF expression, **B)** mountain mint group, mild VEGF expression, **C)** zahter group, comparable to control, showing slight VEGF expression, **D)** trauma group, increased VEGF expression, **E)** trauma+mountain mint group, reduced VEGF expression compared to trauma group, **F)** trauma+zahter group, decreased VEGF expression

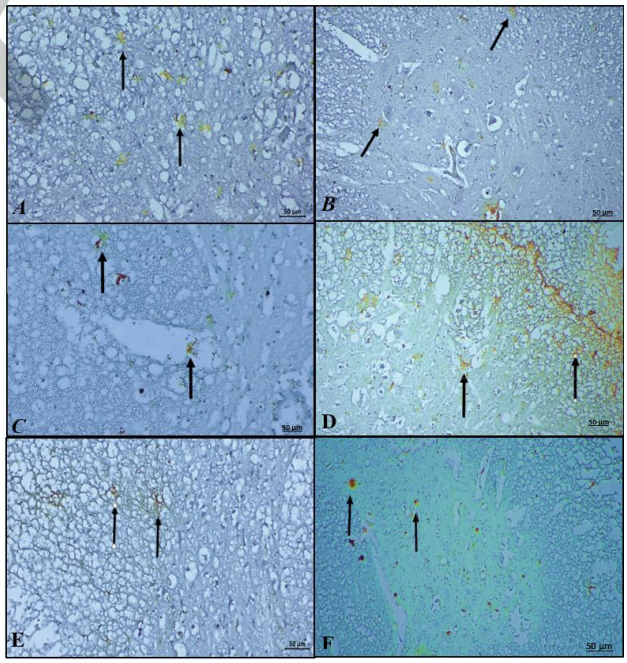

Figure 5. GFAP immunohistochemistry staining in rat spinal cord sections (scale bar=50 µm), **A)** control group, low GFAP expression, **B)** mountain mint group, similar GFAP expression to control, **C)** zahter group, showing comparable GFAP expression to control, **D)** trauma group, increased GFAP expression, **E)** trauma+mountain mint group, reduced GFAP expression compared to trauma group, **F)** trauma zahter group, decreased GFAP expression compared to trauma group

DISCUSSION

SCI, resulting from damage to the spinal cord due to causes such as trauma, is a devastating condition characterized by the loss of motor, sensory, and autonomic functions (15). Following trauma, a series of inflammatory events occur as an acute physiological response to hemorrhage, ischemia, and tissue damage. The acute inflammatory response is necessary to protect cells and tissues from damage and initiate healing, forming a protective inflammatory response. However, excessive inflammation or delayed suppression of inflammation can lead to the accumulation of pro-inflammatory mediators and increased tissue damage.

While the initial injury occurs in the spinal cord, neurogenic damage in many patients leads to oxidative stress and activation of inflammatory pathways in other body systems. Functional impairments in various systems, particularly musculoskeletal disorders, but also cardiovascular, respiratory, and urogenital system disorders, are frequently observed.

Researchers are investigating cellular and molecular mechanisms involved in SCI while also exploring numerous studies in hopes of identifying new and effective therapeutic targets. Early therapeutic intervention in SCI is crucial for achieving the maximum possible recovery. Medicinal aromatic plants play a significant role in drug development and refinement processes (16). Considering secondary damage mechanisms in SCI, medical treatments with high antioxidant and anti-inflammatory potential can enhance successful treatment options (17).

Traditional medicinal plants, historically used for treatment purposes by communities for centuries, are undergoing extensive research to uncover their effects within modern medicine. Various substances and active ingredients derived from herbal extracts known for their antioxidant and anti-inflammatory activities have been utilized in the treatment of SCI. Plants such as curcumin, resveratrol, selenium, ginseng, and quercitrin have shown promise as candidates for treatment in various experimental studies (18-20). Zahter and mountain mint extracts are known for their natural antioxidant potential and are widely used in the treatment of inflammation and pain associated with various diseases. Due to their potent effects in preventing both acute and chronic inflammation, they are commonly utilized for medicinal purposes.

Carvacrol, the most potent component of the zahter, exhibits neuroprotective effects on SCI. In a rat trauma model induced by the weight-drop method, it has been demonstrated that administering various doses of Carvacrol improves neurological functions and prevents SCI by modulating oxidative stress and the eNOS signaling pathway (21). Free radical formation and LP an important mechanisms in the development and progression of various pathologies. The increase in free oxygen radicals causes oxidative stress by oxidizing lipids, proteins, carbohydrates, and nucleic acids in the cell membrane. Secondary damage that begins after mechanical destruction of tissues is a major cause of LP (22,23). LP causes changes in membrane structure and loss of membrane function. After the breakdown of per-oxidized lipids, a wide variety of end products are released. MDA is a secondary product formed as a result of the LP of spinal myelin, glial, and neuronal nerve membranes (24). MDA level is an indicator of the degree of LP in injured cells. Measurement of MDA levels in serum is used as an indicator of tissue damage and oxidative stress, in which free oxygen radicals play a role (25). As a result of the increase in oxidative stress, MDA indicates LP, which indicates SCI, and agents with antioxidant properties are used to reduce the effect of this increase. It has been shown that the effect of oxidative stress and LP mechanisms is reduced with these antioxidant treatments (26,27).

Sahin Kavaklı et al. (28), in their experimental study in which they investigated the antioxidant effect of Curcumin in SCI in rats, found that the serum MDA level increased due to trauma, and the MDA level decreased in the Curcumin-treated group compared to the control group. Accordingly, it has been stated that Curcumin effectively protects spinal cord tissues against oxidative damage.

In the present study, it was thought that MDA levels increased after SCI by the current experiments in trauma groups, thus significantly affecting LP, tissue damage, and cellular degeneration, while affecting cell signaling in the apoptotic aspect. Even though the MDA level decreased in the trauma+zahter and trauma+mountain mint groups compared to the trauma group, this difference was not found to be statistically significant in the treatment groups. This may be related to the dose and duration of the extracts administered.

Oxidative stress is defined as an imbalance between oxidants and antioxidants. TAS and TOS analyses are frequently used parameters to investigate oxidant and antioxidant levels, providing reliable results through an economical method (29). TAS represents the total antioxidant capacity in body fluids and plasma, while TOS indicates the total effect of all oxidants. The TAS level is often considered a well-maintained and powerful oxidative stress marker in organisms (30).

In the study by Aras et al. (31), the dose-dependent antioxidant activity of Minocycline, an antibiotic used in SCI, was demonstrated, showing that TAS and TOS levels increased significantly in the trauma group receiving high doses of Minocycline compared to the low-dose groups. This highlights the importance of optimized dosing to achieve desired outcomes in antioxidant response.

In the present study, the increase in LP and oxidative stress in the trauma groups was associated with changes in TAS and TOS values, which were also linked to the rate of degeneration observed in histopathological examination. The statistical difference observed between the control and trauma groups indicates an increase in TOS levels in traumatized rats, suggesting elevated oxidative stress as a result of SCI. Although the TAS levels in the trauma+zahter and trauma+mountain mint groups were not statistically significant, it is worth noting that the absence of statistical significance could be related to the duration of treatment and dosage of the plant extracts used. Oxidative stress is a dynamic process, and the limited duration of the experiment may have restricted the potential of these extracts to induce a measurable systemic effect in the serum. Additionally, physiological buffering mechanisms and the localized nature of SCI may have limited the reflection of antioxidant changes in systemic circulation.

Histopathological examination indicates that the apoptotic process is accelerated by significant degeneration in neurons and pyknotic changes in the nuclei of neuroglia cells in the trauma group. In the trauma group, it was predicted that the disruption of the integrity of the epithelial cells in the canalis centralis would cause an increase in inflammation in the substantia grisea and a change in the metabolism of the cerebrospinal fluid.

It was observed that there were degenerative changes and cellular atrophy in the neurons and neuroglia cells in the trauma+mountain mint group, but the structural integrity of the canalis centralis continued as a result of regeneration. Consistently, in a comparable experimental study, isotretinoin administration was found to significantly reduce neuronal apoptosis, alleviate histopathological tissue damage, and promote structural recovery in the spinal cord, highlighting its neuroprotective potential in traumatic SCI (32).

VEGF is an important protein involved in the regulation of endothelial cells, which is effective in vasculogenesis and angiogenesis (33). In their study, in which Cheng et al. (34) examined the role of Notch-1 activation and the physiopathological changes seen after decompression in compressive SCI, they determined that VEGF expression, which is a marker of angiogenesis, increased in the substantia grisea and substantia alba regions after decompression.

In the trauma group, VEGF expression was found to be positive in endothelial cells in the capillary vessels in the substantia grisea. Although it was less common in the trauma+zahter and trauma+mountain mint groups than in the trauma group, positive expressions continued. It was thought that the alteration of VEGF expression did not significantly affect angiogenesis in the trauma+zahter and trauma+mountain mint groups, but it may be the beginning of low-level restructuring of endothelial cells.

GFAP is a protein that is usually expressed in glial cells, which has important functions in the connection complex in the structure of glial membranes, in maintaining the integrity of the blood-brain barrier. It is involved in the fluency of conduction in the synaptic regions of some neurons (35). GFAP is thought to be important in modulating astrocyte mobility and shape by providing structural stability to astrocytic processes.

GFAP is hypersensitive to SCI. In the study investigating the therapeutic effect of trehalose, a natural disaccharide, on SCI with its anti-inflammatory and antioxidant effects, they determined that it showed a significant decrease in the early period of post-injury inflammatory and oxidative stress in the group treated with trehalose, and decreased GFAP expression 1 day after trauma. They found that GFAP expression in the trauma group was substantially elevated after injury, peaking every 3 days and remaining high 7 days after SCI (36). In the trauma group of this study, GFAP expression was impaired, whereas the structural integrity of nerve extensions and glial cells was impaired due to the degenerative effect. In the trauma+zahter group, GFAP expression was observed in the substantia alba nerve extensions and the apical surfaces of some neurons, canalis centralis, and basement membrane compared to the trauma group. In the trauma+mountain mint group, GFAP expression was more pronounced in myelinated nerve extensions, which was observed to be regular in nerve extensions and glial cells due to the reorganization of GFAP expression. In terms of GFAP, it was observed that it was more effective in the trauma+mountain mint group than in the trauma+zahter group; however, mountain mint was more effective in protecting glial cells and nerve extensions than in the zahter. When the control group and the zahter and mountain mint groups were compared in terms of GFAP expression, it was seen that GFAP expression was more pronounced in the area of nerve extensions in the mountain mint group, with GFAP expression in glial cells and neurons than in the control and zahter groups. It has been predicted that this is a sign of protein production and a structuring effect in terms of GFAP expression.

CONCLUSION

To the best of our knowledge, this is the first study to investigate the potential effects of zahter and mountain mint extracts on tissue damage resulting from traumatic SCI.

Biochemical findings of this study indicate that oxidative stress increases and antioxidant capacity decreases following SCI, which is consistent with the pathophysiology of spinal cord damage.

Histopathological and immunohistochemical analyses revealed that zahter and mountain mint extracts provide partial protective effects by reducing inflammation and oxidative stress. While mountain mint showed more pronounced effects in some parameters, both extracts demonstrated positive outcomes at the tissue level.

These findings suggest that these herbal extracts may exhibit neuroprotective effects in SCI, albeit in a limited capacity. To fully understand the therapeutic potential of these extracts, further studies involving longer treatment durations, optimized doses, and alternative administration methods are needed.

Ethics Committee Approval: The study was approved by the Animal Experiments Local Ethics Committee of Dicle University (30.05.2019, 2019/08).

Conflict of Interest: This manuscript was derived from the doctoral thesis of the first author, Figen Koç Direk, and all authors declare that there is no conflict of interest.

Financial Disclosure: This study was supported by the Scientific Research Projects Coordination Unit of Dicle University (Project Number: TIP.19.023).

Acknowledgments: None declared by the authors.

Author Contributions: Idea/Concept: FKD, MCT, ED; Design: FKD, MCT, ED; Data Collection/Processing: FKD, DAK, HÖ; Analysis/Interpretation: FKD, MCT, ED; Literature Review: FKD, DAK, HÖ; Drafting/Writing: FKD, DAK, HÖ; Critical Review: FKD, MCT, ED.

REFERENCES

1. Yu M, Wang Z, Wang D, Aierxi M, Ma Z, Wang Y. Oxidative stress following spinal cord injury: from molecular mechanisms to therapeutic targets. J Neurosci Res. 2023;101(10):1538-54.
2. Islam F, Bepary S, Nafady MH, Islam MR, Emran TB, Sultana S, et al. Polyphenols targeting oxidative stress in spinal cord injury: current status and future vision. Oxid Med Cell Longev. 2022;2022:8741787.
3. Anjum A, Yazid MD, Fauzi Daud M, Idris J, Ng AMH, Selvi Naicker A, et al. Spinal cord injury: pathophysiology, multimolecular interactions, and underlying recovery mechanisms. Int J Mol Sci. 2020;13;21(20):7533.
4. Oyinbo CA. Secondary injury mechanisms in traumatic spinal cord injury: a nugget of this multiply cascade. Acta Neurobiol Exp (Wars). 2011;71(2):281-99.
5. von Leden RE, Yauger YJ, Khayrullina G, Byrnes KR. Central nervous system injury and nicotinamide adenine dinucleotide phosphate oxidase: oxidative stress and therapeutic targets. J Neurotrauma. 2017;34(4):755-64.
6. Öz M. Chemical content and antimicrobial activity of cyclotrichium origanifolium (Labill.) manden. & scheng. plant essential oil obtained from Adana region. J Bartın Fac For. 2022;24(3):465-75. Turkish.
7. Koç Direk F, Tuncer MC, Deveci E, Yokuş B, Nas C, Aygün Keşim D. Investigation of the protective effect of thymbra spicata L. var spicata and cyclotrichium origanifolium on kidney in experimental traumatic spinal cord injury. Dicle Med J. 2021;48(2):299-308. Turkish.
8. Tepe B, Sökmen M, Sökmen A, Daferera D, Polissiou M. Antimicrobial and antioxidative activity of the essential oil and various extracts of cyclotrichium origanifolium (Labill.) manden & scheng. J Food Eng. 2005;69(3):335-42.
9. Karakaş Ö, Bekler FM. Essential oil compositions and antimicrobial activities of thymbra spicata L. var. spicata L., lavandula x intermedia emeric ex loisel., satureja macrantha C. A. meyer and rosmarinus officinalis L. Braz Arch Biol Technol. 2022;65:e22210297.
10. Kirkan B, Sarıkurkcu C, Amarowicz R. Composition and antioxidant and enzyme-inhibition activities, of essential oils from satureja thymbra and thymbra spicata var. spicata. Flavour Fragr J. 2019;34(6):436-42.
11. Kılıç T. Analysis of essential oil composition of thymbra spicata L var. spicata: antifungal antibacterial and antimycobacterial activities. Z Naturforsch C J Biosci. 2006;61(5-6):324-8.
12. Allen AR. Surgery of experimental lesion of spinal cord equivalent to crush injury of fracture dislocation of spinal column: a preliminary report. JAMA. 1911;57(11):878-80.
13. Rivlin AS, Tator CH. Objective clinical assessment of motor function after experimental spinal cord injury in the rat. J Neurosurg. 1977;47(4):577-81.
14. Gedikoğlu A, Sökmen M, Çivit A. Evaluation of thymus vulgaris and thymbra spicata essential oils and plant extracts for chemical composition, antioxidant, and antimicrobial properties. Food Sci Nutr. 2019;7(5):1704-14.
15. Lin A, Shaaya E, Calvert JS, Parker SR, Borton DA, Fridley JS. A review of functional restoration from spinal cord stimulation in patients with spinal cord injury. Neurospine. 2022;19(3):703-34.
16. Guzel A, Aksit H, Elmastas M, Erenler R. Bioassay-guided isolation and identification of antioxidant flavonoids from cyclotrichium origanifolium (Labill.) manden and scheng. Pharmacogn Mag. 2017;13(50):316-20.
17. Zhang Q, Yang H, An J, Zhang R, Chen B, Hao DJ. Therapeutic effects of traditional Chinese medicine on spinal cord injury: a promising supplementary treatment in future. Evid Based Complement Alternat Med. 2016;2016:8958721.
18. Javdani M, Barzegar A. Application of chitosan hydrogels in traumatic spinal cord injury; a therapeutic approach based on the anti-inflammatory and antioxidant properties of selenium nanoparticles. Frontiers Biomed Technol. 2023;10(3):349-69.
19. Lee YS, Cho DC, Kim CH, Han I, Gil EY, Kim KT. Effect of curcumin on the inflammatory reaction and functional recovery after spinal cord injury in a hyperglycemic rat model. Spine J. 2019;19(12):2025-39.
20. Sanli AM, Turkoglu E, Serbes G, Sargon MF, Besalti O, Kilinc K, et al. Effect of curcumin on lipid peroxidation, early ultrastructural findings and neurological recovery after experimental spinal cord contusion injury in rats. Turk Neurosur. 2012;22(2):189-195.
21. Jiang ZS, Pu ZC, Hao ZH. Carvacrol protects against spinal cord injury in rats via suppressing oxidative stress and the endothelial nitric oxide synthase pathway. Mol Med Rep. 2015;12(4):5349-54.
22. Michael-Titus AT. Omega-3 fatty acids and neurological injury. Prostaglandins Leukot Essent Fatty Acids. 2007;77(5-6):295-300.
23. Golding JD, Rigley MacDonald ST, Juurlink BH, Rosser BW. The effect of glutamine on locomotor performance and skeletal muscle myosins following spinal cord injury in rats. J Appl Physiol (1985). 2006;101(4):1045-52.
24. Rahman A, Üstündağ B, Burma O, Özercan İH, Erol FS. Neuroprotective effect of regional carnitine on spinal cord ischemia--reperfusion injury. Eur J Cardiothorac Surg. 2001;20(1):65-70.
25. Draper HH, McGirr LG, Hadley M. The metabolism of malondialdehyde. Lipids. 1986;21(4):305-7.
26. Zhang C, Zhai T, Zhu J, Wei D, Ren S, Yang Y, et al. Research progress of antioxidants in oxidative stress therapy after spinal cord injury. Neurochem Res. 2023;48(12):3473-84.
27. Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: A review. Eur J Med Chem. 2015;97:55-74.
28. Sahin Kavakli H, Koca C, Alıcı O. Antioxidant effects of curcumin in spinal cord injury in rats. Ulus Travma Acil Cerrahi Derg. 2011;17(1):14-8.
29. Birer S, Arda H. Kilic D. Baskol G. Systemic oxidative stress in non-arteritic anterior ischemic optic neuropathy. Eye (Lond). 2019;33(7):1140-4.
30. Gariballa SE, Hutchin TP, Sinclair AJ. Antioxidant capacity after acute ischaemic stroke. QJM. 2002;95(10):685-90.

31. Aras M, Altas M, Motor S, Dokuyucu R, Yilmaz A, Özgiray E, et al. Protective effects of minocycline on experimental spinal cord injury in rats. Injury. 2015;46(8):1471-4.
32. Şensoy D, Polat Ö, Kılıç G, Yakupoğlu M, Karaçor K. Clinical and histopathological effects of isotretinoin on neuroregeneration in experimental spinal cord injury. Duzce Med J. 2024;26(3):248-54.
33. Long HQ, Li GS, Cheng X, Xu JH, Li FB. Role of hypoxia-induced VEGF in blood-spinal cord barrier disruption in chronic spinal cord injury. Chin J Traumatol. 2015;18(5):293-5.
34. Cheng X, Yu Z, Xu J, Quan D, Long H. Pathophysiological changes and the role of notch-1 activation after decompression in a compressive spinal cord injury rat model. Front Neurosci. 2021;15:579431.
35. Eng LF, Ghirnikar RS, Lee YL. Glial fibrillary acidic protein: GFAP-thirty-one years (1969-2000). Neurochem Res. 2000;25(9-10):1439-51.
36. Nazari-Robati M, Akbari M, Khaksari M, Mirzaee M. Trehalose attenuates spinal cord injury through the regulation of oxidative stress, inflammation and GFAP expression in rats. J Spinal Cord Med. 2019;42(3):387-94.