EXPERIMENTAL STUDY

# Efficacy of *Annona muricata* (graviola) in experimental spinal cord injury: biochemical and histopathological analysis

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## ABSTRACT

**BACKGROUND:** *Annona muricata* (AM) (graviola) is a plant that grows in tropical regions and is thought to be good for many diseases by local people. Unfortunately, there is no acceptable medical treatment for spinal cord injury (SCI) yet. In our study, we investigated the neuropeotective effects of AM leaf extract on SCI in an experimental rat model.

**METHODS:** A total of 40 Wistar albino rats were randomly divided into five equal groups (n=8). Group 1 was the control group in which only laminectomy was performed. Trauma was induced in four groups after laminectomy. Group 2 (untreated trauma group) was given no medication. In Group 3, a single intraperitoneal dose of methylprednisolone (30 mg/kg) was administered after trauma. The rats in Groups 4 received a low dose (100 mg/kg) of AM leaf extracts by oral gavage one week before trauma while the rats in Group 5 received a high-dose (300 mg/kg) of these extracts by oral gavage one week before trauma. All rats, including the control group, were sacrificed 24 h after the trauma was created.

**RESULTS:** Tissue samples taken to evaluate the neuroprotective effect were examined biochemically and histopathologically. Inflammatory findings in the trauma group were significantly better in both groups treated with AM. There was no difference between the groups in terms of clinical motor examination and inclined plane test results.

**CONCLUSION:** Our histopathological and biochemical results showed that AM is an agent with neuroprotective effects in traumatic SCI.

**Keywords:** *Annona muricata*; methylprednisolone; neuroprotection; oxidative stress; spinal cord injury.

## INTRODUCTION

Spinal cord injury (SCI) can cause the loss of neurological function depending on the severity and location of the injury.[1] SCI constitutes a medical emergency, and the time between injury and treatment is critical in reducing comorbidities and sequelae and determines the ultimate prognosis.[1] As a result, traumas that cause SCI lead to partial or complete disability depending on their extent and level. Despite long rehabilitation periods, this situation has a permanent destructive effect both emotionally and financially on patients and their families.[2,3]

According to the data of the National SCI Statistical Center, approximately 54 cases per million people or approximately 17,700 new SCI cases are seen each year in the United States of America (USA).[4] Although SCI is mostly a disease of young adults, there has been an increase in the incidence of this disease in the elderly in recent years. The average age of injury in the USA increased from 29 in 1970 to 43 in 2018, and 78% of these patients are male.[4]

Promising results have been reported in studies using stem cells, neurotrophic factors, biocompatible polymer implants, and Schwann cell transplantation to regenerate and remyelin-

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ate the spinal cord.[5] However, although current treatments partially alleviate SCI, secondary injury and additional problems still negatively affect the patient. Therefore, especially secondary injury should be further investigated to achieve optimal recovery in SCI.

*Annona muricata* (AM) is an edible tropical plant, also known as "graviola," "soursop," "guanabana," and "sapoty" (French creole).[6,7] This thorny evergreen plant produces heart-shaped fruits of green color, which can reach a length of approximately 8 m in the tropical regions of Central and South America, Africa, and Southeast Asia. Almost all parts of the plant (seeds, leaves, roots, and bark) are traditionally believed to increase the amount of breast milk, as well as curing various diseases such as cancer, malaria, diabetes, rheumatism, and cystitis, and they are widely used for treatment purposes by local people in tropical regions.[7,8] Moreau et al.[7] showed that 25% of patients treated for lung cancer in Réunion thought that AM had a positive effect on their treatment and therefore they regularly consumed this plant as medicine. Of these patients, 69.5% boiled the locally supplied AM leaves. In addition to such local uses, this botanical product has been a topic of experimental studies, with promising results having been reported in various cancer types (prostate, breast, lung, and pancreas), epilepsy, sedation, joint diseases, and diabetes.[9–13]

In this study, we investigated the therapeutic effects and antioxidant properties of AM on secondary cell damage after experimental SCI.

## MATERIALS AND METHODS

All experimental procedures were carried out in the Animal Experiments Laboratory of Zonguldak Bulent Ecevit University Faculty of Medicine after obtaining approval from the local ethics committee of the university (decree no: 2020/05).

A total of 40 Wistar albino female rats, weighing 350–450 g, were randomly divided into five groups of equal numbers (n = 8, total: 40). The animals were kept below a constant temperature (18°C–21°C), and adequate nutrition and photoperiod (12 h light/dark cycle) were provided for the duration of the experiment.

### Experimental Groups

The control group received laminectomy only. Trauma was applied to the remaining four groups after laminectomy. Group 2 was given no medication, but trauma was induced after laminectomy. In Group 3, a single intraperitoneal dose of methylprednisolone (MP) (30 mg/kg) (VEM, Istanbul, Turkey) was given after inducing trauma. The rats in Group 4 received a low dose (100 mg/kg) of AM leaf extracts (AME) by oral gavage for 1 week prior to trauma. Finally, the rats in Group 5 received a high dose (300 mg/kg) of AME by oral gavage for 1 week before trauma.

### Surgical Technique

General anesthesia was provided by 10 mg/kg intramuscular xylazine (5 mg/kg; Bioveta, Ankara, Turkey) and 80 mg/kg intramuscular ketamine HCl (Pfizer, Istanbul, Turkey) in each experimental animal before the surgical procedure. After the lumbar region was shaved, the operation area was sterilized with povidone. A midline skin incision was made extending between the T6 and T12 spinous processes. The lumbosacral fascia was opened longitudinally, and the paraspinal muscles were dissected bilaterally in a subperiosteal fashion to expose the T8-T10 lamina. After T8-T10 laminectomy and flavectomy, epidural adipose tissue was removed and the dura mater was exposed. No dural defects were observed during these procedures. Hemostasis was achieved using bipolar cautery. The epidural space was widened laterally to fit the standard spinal trauma model (Rivlin and Tator; spinal cord trauma model).[14] With the clipping method, the rats in Groups 2–5 were temporarily injured for 60 s under a microscope using a spinal cord aneurysm clip at the T9 level (Takagı Seıko Co, Japan), Yasargil FE 624K, 1.77 Newton, Valley, Aesculap, PA, USA) (Fig. 1). After the trauma, the layers were closed in an anatomical plan, and the surgical procedure was terminated by ensuring that the rats were awakened normally.

The rats were sacrificed with a lethal dose of pentobarbital (200 mg/kg, Bioveta, Ankara, Turkey) 24 h after surgery. Then, the surgical field was reopened, and the injured spinal cord was carefully removed. Spinal cord samples were taken and evaluated biochemically and histologically.

### Clinical Motor Examination

In our study, functional recovery in the rats was evaluated by performing the inclined plane test described by Rivlin and Tator by applying painful stimulus on the tail.[14] A periodic (every 8 h) clinical motor examination was performed.

### Chemical

Air-dried AM (Graviola) leaves were obtained from a local supplier in the Mediterranean region in Turkey. They were washed with distilled water and cut into small pieces. 25 g samples were extracted in 70:70 ethanol for seven days with occasional shaking. The extract was concentrated by removing ethanol using a rotary evaporator (Heidolph, Germany). Then, the extract was lyophilized (Telstar- LyoQuest, Spain) overnight to prepare a dry extract, which was kept at −20°C until use.

### Biochemical Analysis

All tissues were washed twice with cold saline solution, placed in glass bottles, labeled, and stored in a deep freezer (−80°C) until processing. Tissue samples were homogenized with phosphate buffered saline (pH 7.4) using a glass Teflon homogenizer (Ultra Turrax IKA T18 Basic). The homogenates were centrifuged at 5000× g at 4°C for 15 min, and the supernatants were immediately analyzed.

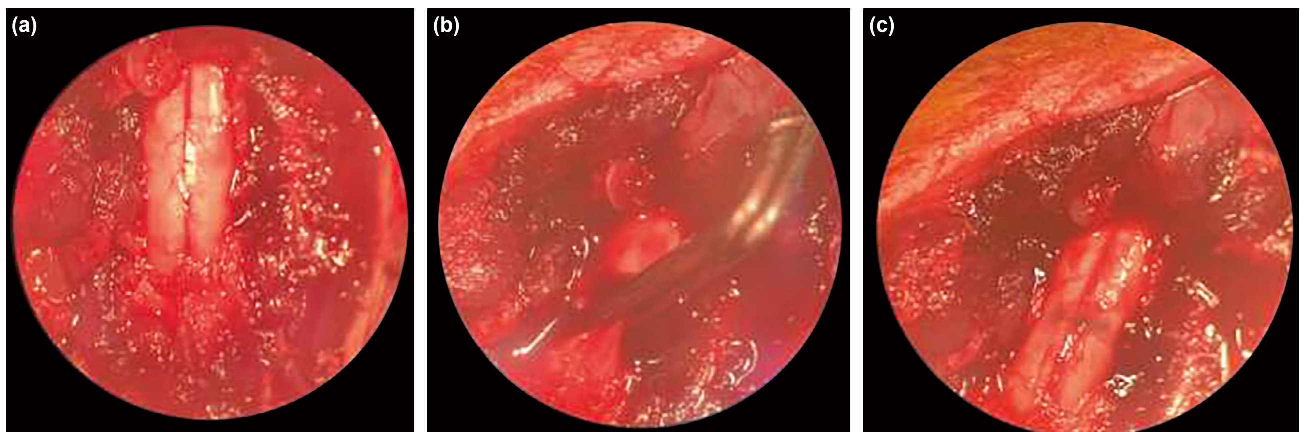


**Figure 1.** Intraoperative photographs showing spinal cord compression at the T10 level with the application of an aneurysm clip under a microscope before **(a)**, during **(b)** and after **(c)** the procedure.

Malondialdehyde (MDA) levels were measured by ELISA immunoenzymatic assays (Cloud –Clone Corp., Wuhan, China). The Trolox equivalent antioxidant capacity assay for total antioxidant status (TAS) estimation was undertaken using an ABTS-based method (Cloud-Clone Corp, Wuhan, China). The results were reported as ng/mg protein for MDA and as mmol trolox/mg protein for TAS.

## Histopathological Examination

Forty spinal cord tissue samples fixed with 10% formalin were routinely processed using automated tissue processing equipment (Leica ASP300S, Wetzlar, Germany). Then, the tissues were embedded in paraffin and sectioned to a 4-µm thickness using a Leica RM2255 rotary microtome (Wetzlar, Germany). The tissue sections were stained with hematoxylin-eosin (H&E) and examined microscopically for all samples. All the study materials were examined using a Nikon Eclipse Ni-U microscope equipped with a Nikon Digital DS-Ri2 camera and related software (Nikon, Tokyo, Japan).

In five separate groups, 11 parameters were examined for inflammatory cells, hyperplasia of gliocytes, glial scar, neuronal swelling, necrosis, repair holes, loss of Nissl substance, cavitation around the nucleus, presence of pyknotic homogenous nuclei, vacuolization in the cytoplasm, and edema in spinal cord tissue. Histomorphological findings in tissues and cells were scored. Each criterion was scored from 0 to 3 (0 = normal, 1 = light, 2 = medium, and 3 = heavy).

## Statistical Analysis

The data were analyzed using the Statistical Package for the Social Sciences version 22.0. Descriptive statistics for quantitative variables are expressed as mean and standard deviation. For normal distribution assumptions, the Shapiro-Wilk test was used. Skewness and Kurtosis coefficients and distribution in q-q plot graphs were checked. As the data showed a normal distribution, the groups were compared using analysis of variance (ANOVA). To determine statistical significant difference in the average scores of the groups, the Bonferroni correction was used when the variances were equally assumed and the Games-Howell test when the variances were not equally assumed. The results were accepted as statistically significant based on a $p<0.05$.

# RESULTS

## Biochemical Evaluation

In the study, it was examined whether there was a significant difference between the groups in terms of MDA and TAC values. For MDA, a statistically significant difference was found between the groups according to the results of the Bonferroni test ($p=0.00 <0.05$, Table 1). Group 2 showed the highest sensitivity concerning the MDA results, while there was no significant difference between the other groups in relation to MDA. For TAC, the difference between the groups was not statistically significant ($p=0.22 >0.05$, Table 1).

## Histopathological Evaluation

Group 1 had normal histomorphological structure in the spinal cord tissues (Fig. 2). All groups were evaluated in terms of inflammatory cells, hyperplasia of gliocytes, glial

**Table 1.** Effect of *Annona muricata* on the serum MDA and TAS levels of rats after experimental spinal cord injury

| Group | n | MDA, ng/mg protein | TAS, mM Trolox/mg protein |
|---|---|---|---|
| 1 | 8 | 214.574±33.23 | 2.715±0.67 |
| 2 | 8 | 366.363±55.27 | 2.880±0.58 |
| 3 | 8 | 201.560±62.78 | 3.429±0.99 |
| 4 | 8 | 227.012±84.32 | 3.170±0.92 |
| 5 | 8 | 219.507±98.82 | 3.465±0.51 |
| P | | 0.00* | 0.22 |

*Significant at the 0.05 level. The differences in MDA and TAS between the groups were evaluated with one-way analysis of variance. G: Group; MDA: malondialdehyde; TAS: Total antioxidant status.

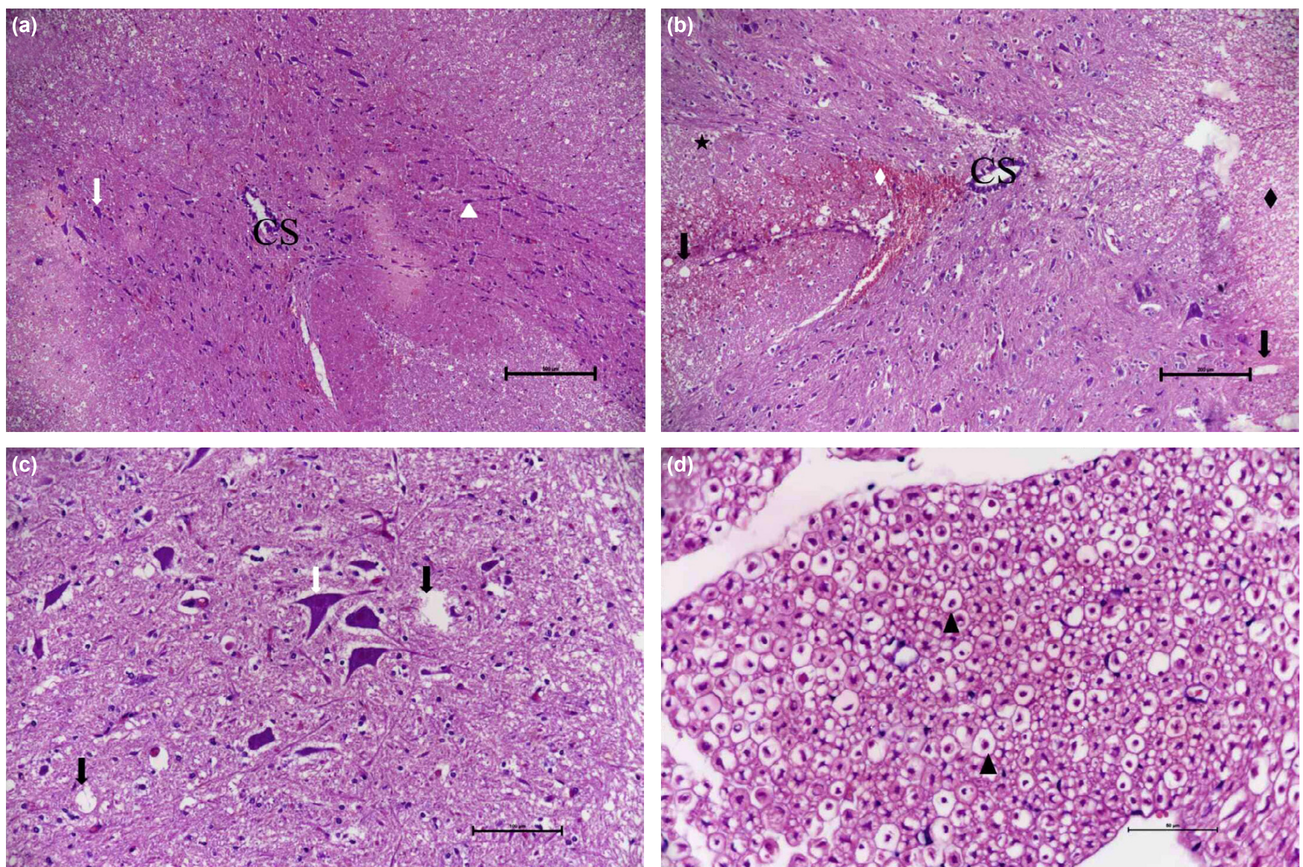


**Figure 2.** Representative photomicrographs of spinal cord sections showing **(a)** the control group (G1) with nearly normal histological features demonstrating evenly spaced, well-preserved neurons (hematoxylin and eosin [H&E], Scale Bar X500 µm). White arrow, (normal) neuron swelling and loss of Nissl substance; white triangle, hyperplasia of gliocytes (normal); CS, central channel. **(b-d)** Severe edema, necrosis and neuronal degeneration, respectively in the untreated trauma group (G2) (H&E, Scale Bar X200 µm, X100 µm, and X50 µm). White arrow, neuron swelling (heavy) and loss of Nissl substance (medium); black arrow, repair holes (heavy); black asterisk, necrosis (heavy); white diamond, inflammatory cells (medium); black diamond, edema (heavy); black triangle, picnotic nuclei (heavy) and cavitation around the nucleus (medium); CS: Central channel.

scarring, neuronal swelling, necrosis, repair holes, loss of Nissl substance, cavitation around the core, presence of pyknotic homogenous core vacuolization in the cytoplasm, and edema. There was no statistically significant difference in terms of glial scar between the groups. The findings were at the most severe level in Group 2 (Fig. 2). In the groups that received treatment, the best result was found in Group 5 (Figs. 3 and 4).

Inflammatory cells increased in Group 2 compared to the control group (Fig. 2b). However, this increase was moderate in the treatment groups, and there was a significant difference between Groups 2 and 3 (p=0.001) (Fig. 3a and b), Groups 2 and 4 (p=0.022) (Fig. 3c and d) and Groups 2 and 5 (p=0.001) (Fig. 4d) in favor of the treatment groups (Table 2).

Hyperplasia of gliocytes was seen at the lowest rate in Group 5 (Fig. 4c and d). A statistically significant difference was found between Groups 2 and 3 (p=0.034) and Groups 2 and 5 (p=0.001) (Table 2).

Neuronal swelling was observed at the lowest rate in Group 5 (Fig. 4b and d) and highest rate in Group 2 (Fig. 2c). A statistically significant difference was found between Groups 2 and 5 (p=0.000), Groups 3 and 5 (p=0.000), and Groups 4 and 5 (p=0.044) in favor of the treatment groups (Table 2).

Necrosis increased in all groups except Group 5 compared to the control group (Table 2). In Group 5, a moderate increase was observed, and this was statistically significantly less compared to Group 2 (p=0.000, Table 2) (Fig. 4b).

Repair holes were at a severe level in Group 2 (Fig. 2b and c). Among the treatment groups, they were least seen in Group 4 (Fig. 3c). When the differences between paired groups were examined, a statistically significant difference was found between Groups 2 and 3 (p=0.015), Groups 2 and 4 (p=0.000), and Groups 2 and 5 (p=0.000) (Table 2). When the treatment groups were compared, the differences between Groups 3 and 4 (p=0.000) and Group 3 and 5 (p=0.000) were statistically significant (Table 2).

**Table 2.** Histological analysis results of all groups

| Parameters | n | G1 | G2 | G3 | G4 | G5 | p |
|---|---|---|---|---|---|---|---|
| Inflammatory cells | 40 | 1.00±0.00 | 2.25±0.46 | 1.13±0.35 | 1.38±0.52 | 1.00±0.00 | 18.221 (<0.001)[a,e,f,g] |
| Hyperplasia of gliocytes | 40 | 0.00±0.00 | 1.88±0.64 | 0.75±0.71 | 1.25±0.71 | 0.38±0.52 | 12.920 (<0.001)[a,c,e,g] |
| Glial scar | 40 | 0.00±0.00 | 1.00±0.00 | 0.63±0.52 | 0.63±0.52 | 0.00±0.00 | 14.350 (<0.001)[a,b,c,g,h,l] |
| Neuronal swelling | 40 | 0.00±0.00 | 2.63±0.52 | 2.13±0.35 | 1.75±1.04 | 0.38±0.52 | 29.768 (<0.001)[a,b,c,g,k,l] |
| Necrosis | 40 | 0.00±0.00 | 2.50±0.53 | 2.25±0.46 | 1.88±0.35 | 0.88±0.35 | 57.917 (<0.001)[a,b,c,d,e,k,l] |
| Repair holes | 40 | 0.00±0.00 | 2.88±0.35 | 2.00±0.53 | 0.00±0.00 | 0.38±0.52 | 101.592 (<0.001)[a,b,e,f,g,h] |
| Loss of Nissl substance | 40 | 0.00±0.00 | 2.38±0.52 | 1.50±0.53 | 1.00±0.00 | 0.25±0.46 | 48.186 (<0.001)[a,b,e,f,g,k,l] |
| Cavitation around the nucleus | 40 | 0.00±0.00 | 2.75±0.46 | 1.88±0.83 | 1.75±0.71 | 1.13±0.35 | 27.064 (<0.001)[a,b,c,d,f,g] |
| Presence of pyknotic homogenous nuclei | 40 | 0.00±0.00 | 2.75±0.46 | 2.13±0.35 | 2.13±0.83 | 0.75±0.71 | 33.494 (<0.001)[a,b,c,g,k,l] |
| Vacuolization in the cytoplasm | 40 | 0.00±0.00 | 2.38±0.52 | 0.75±0.89 | 0.00±0.00 | 0.00±0.00 | 40.339 (<0.001)[a,e,f,g] |
| Edema | 40 | 1.00±0.00 | 2.63±0.52 | 3.00±0.00 | 2.63±0.52 | 1.88±0.35 | 38.500 (<0.001)[a,c,d,g,k,l] |

Data given as mean±standard deviation. G: Group. P significant at the 0.05 level; a: between G1 and G2; b: between G1 and G3; c: between G1 and G4; d: between G1 and G5; e: between G2 and G3; f: between G2 and G4; g: between G2 and G5; h: between G3 and G4; k: between G3 and G5; l: between G4 and G5.

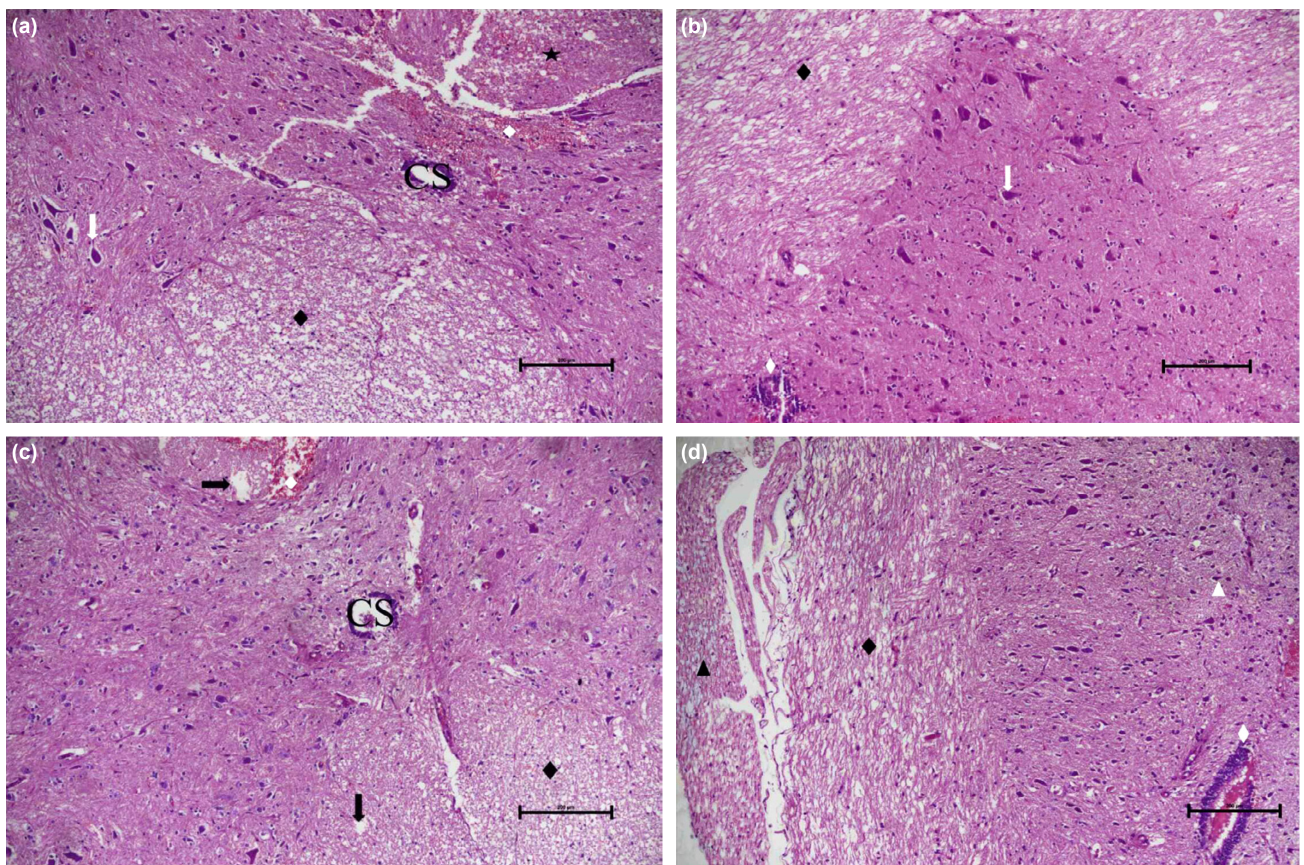


**Figure 3.** Spinal cord tissue morphology staining in the methylprednisolone group (G3) and the low-dose *Annona muricata* group (G4). **(a)** Normal neurons and glial cells in G3 **(b)** inflammation significantly less in G3 compared to G2 that had the most prominent inflammation (hematoxylin-eosin [H&E], Scale Bar X200 µm). White arrow, neuron swelling and loss of Nissl substance (medium); black asterisk, necrosis (medium); white diamond, inflammatory cells (light); black diamond, edema (heavy); CS, central channel. **(c and d)** degenerative changes in G4 (less compared to G3 and more compared to G5) (H&E, Scale Bar X200 µm). Black arrow, repair holes (normal); white diamond, inflammatory cells (light); black diamond, edema (heavy); black triangle, pycnotic nuclei and cavitation around the nucleus (medium); white triangle, hyperplasia of gliocytes (light); CS: Central channel.

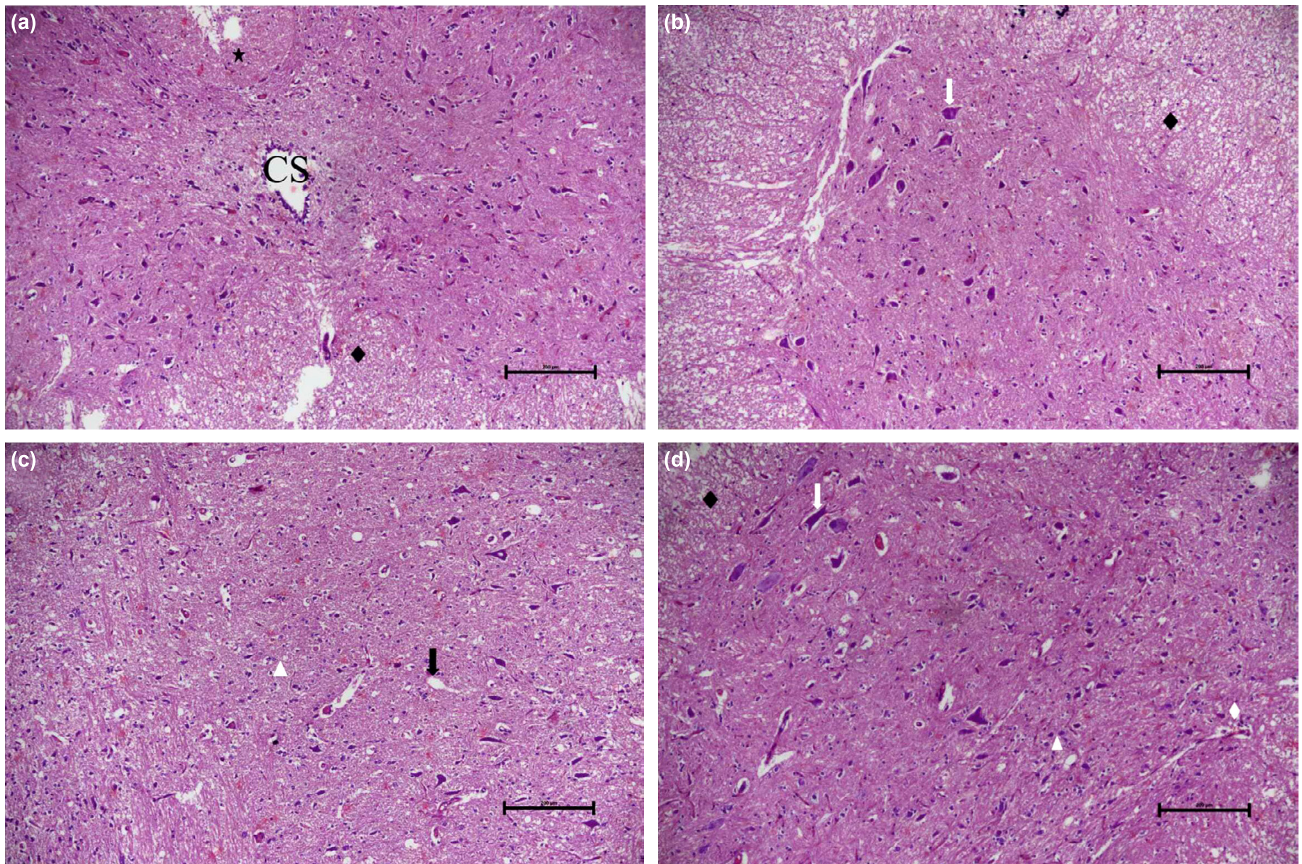


**Figure 4.** Spinal cord tissue morphology in the high-dose *Annona muricata* group (G5). **(a-d)** degenerative changes least seen in G5 compared to the other groups (hematoxylin-eosin, Scale Bar X200 μm). White arrow, neuronal swelling and loss of Nissl substance (normal); black arrow, repair holes (normal); black asterisk, necrosis (light); white diamond, inflammatory cells (light); black diamond, edema (medium); white triangle, hyperplasia of gliocytes (normal); CS: Central channel.

Loss of Nissl substance was seen at the lowest rate in Group 5 (Fig. 4b and d) among the treatment groups. A statistically significant difference was found between Groups 2 and 3 (p=0.034), Groups 2 and 4 (p=0.001), and Groups 2 and G5 (p=0.000) (Table 2). When the treatment groups were compared, the differences between Groups 3 and G5 (p=0.002) and Groups 4 and 5 (p=0.015) were statistically significant (Table 2).

The presence of pyknotic homogenous nuclei and cavitation around the nucleus were most seen in Group 2 (Fig. 2d) and least in Group 5. For cavitation around the nucleus, the differences between Groups 2 and 4 (p=0.038) and Groups 2 and 5 (p=0.000) were statistically significant (Table 2). For the presence of pyknotic homogenous nuclei, statistically significant differences were found between Groups 2 and 5 (p=0.000), Groups 3 and 5 (p=0.004), and Groups 4 and 5 (p=0.023) (Table 2).

Concerning vacuolization in the cytoplasm, a statistically significant difference was found between Group 2 (untreated trauma group) and Groups 3 (p=0.006), 4 (p=0.000) and 5 (p=0.000) (Table 2).

There was a significant increase in edema between the untreated trauma group (Group 2) compared to the control group (Fig. 2a). In particular, a significant reduction in edema was observed after SCI in Group 5 compared to Group 2 (p=0.035, Table 2) (Fig. 4a, b, d).

## Clinical Motor Evaluation

In our study, a periodical clinical motor examination was undertaken with the inclined plane test by applying painful stimulus on the tail. Paraplegia was observed in the untreated trauma and treatment groups in both evaluations.

## DISCUSSION

There is still no effective medical treatment for SCI in routine neurosurgery practice. The main purpose of this study was to evaluate whether AME showed a neuroprotective effect on rats after experimental SCI. The results showed that the high-dose AME group had better biochemical and histopathological data compared to other treatment groups.

To discuss an effective treatment method for SCI, all aspects of the pathophysiological process should be clearly understood. In this context, the pathophysiology of SCI has been

most commonly described as biphasic (primary and secondary injury).[15] Primary injury refers to a compressive-contusion type of injury involving initial mechanical trauma (partially or completely) that disrupts the anatomical continuity of the cord by applying direct force to the spinal cord. This is followed by secondary injury triggered by primary injury, including edema, ischemia, and glutamate-mediated excitotoxicity in addition to axonal degradation due to lipid peroxidation caused by free radical production, as well as apoptotic death of both neuron and glial cells. While neurological symptoms appear immediately in primary injury, the secondary injury phase with immediate, acute, intermediate and chronic stages involves a long, destructive and irreversible process.[15,16]

The positive results of the third and last National Acute SCI Study on MP have often been questioned as a data analysis and statistical method.[3,17,18] There is no Class I or Class II medical evidence indicating the benefit of MP in SCI, and its prescription is no longer approved by the Food and Drug Administration; however, it is still widely used by clinicians.[3,17] A study conducted by Falavigna et al.[18] revealed that some clinicians did not believe that MP was effective in SCI patients while others prescribed MP for medicolegal problems. The modest neuroprotective effect of MP, which has been mostly investigated in SCI, is due to the prevention of intracellular accumulation and lipid peroxidation of Ca+2 in the secondary injury phase.[19–21] This is why current studies on SCI treatment focus on secondary injury, and the antioxidant effects of neuroprotective agents play an important role in this area.[22–24] The negative results obtained from many clinical and experimental studies have shown that there is an urgent need for an effective, non-toxic neuroprotective agent with a low side effect profile in the treatment of SCI.

MDA is an end product produced by the peroxidation of fatty acids containing three or more double bonds.[25] MDA damages cells by affecting ion exchange in the cell membrane, and being the most important product of lipid peroxidation; it is a sensitive indicator of oxidative stress in experimental SCI.[22,25] As an antioxidant parameter, TAS is used to monitor the antioxidant defense system in experimental SCI models.[26]

In the present study, we investigated the neuroprotective effect of AME on traumatic SCI, as an agent that has been shown to have anticarcinogenic, anticonvulsant, antiarthritic, and antidiabetic properties.[9–13] The literature contains only few studies on the neuroprotective effect of AM, and the neuroprotective mechanism of this plant remains unknown.[27,28] Kim et al.[27] showed that AM reduced Ca+2 loads, free oxygen radicals, and MDA production in the oxidative stress model applied to the hippocampal neuronal cells. The authors stated that AM was a promising new agent for the treatment of neurodegenerative diseases.[26] Souza et al.[28] performed a phytochemical analysis of the hydroalcoholic extract obtained from AM leaves using the HPLC method for the first time and reported that it contained flavonoids (quercetin, catechin, epicatechin, and kaempferol) and phenolic compounds (gallic acid and caffeic acid) with strong antioxidant activities. There are also studies showing that phenolic compounds have potent antioxidant effects, as well as antimutagenic and anticancer activities.[29,30] In a study conducted with water and methanol extracts of AM leaves, George et al.[31] found that methanol extracts had higher free radical scavenging and DNA protective effects in a dose-dependent manner. Jiménez et al.[32] referred to the presence of phytochemical studies on nutrient content and alkaloids and determined cinnamic acid and p-coumaric acid with a high antioxidant effect in fruit pulp using the HPLC-DAD-MS method.

In experimental SCI models related to quercetin, a flavonoid, the neuroprotective effect of this agent was shown based on reduced MDA and increased TAS levels.[33] In an experimental SCI study, Gocmez et al.[34] performed an intrathecal injection of caffeic acid, a phenol component, and showed that it inhibited lipid peroxidation and the increase of oxidants. In light of these studies, we expected that AME containing high antioxidant compounds would have similar results in SCI in our study.[35] We found that the MDA levels were significantly lower in Groups 3 to 5 compared to Group 2. Both AME (at high and low doses) and MP decreased MDA levels, which was more pronounced and significant in the MP group compared to the high-dose AME group. Concerning TAS levels, although there was no significant difference between the treatment groups and the remaining groups, they tended to be higher in Groups 3 and 5 compared to Group 2 group.

In a study conducted in 2014, Ishola et al.[36] showed that in rats, the extract of the AM fruit had a better anti-inflammatory effect on ear edema than prednisolone. In another study, the ethanolic extract of AM leaves was shown to be effective in acute inflammation and chronic inflammation in a dose-dependent manner.[37] In our study, the histopathological results were in favor of the treatment groups: Neural degeneration was significantly reduced in the group treated with high-dose AME (Group 5) compared to the untreated trauma group (Group 2). However, despite the improvements in the biochemical and histopathological results in the treatment groups, there was no difference between the groups in terms of the inclined plane test results. Obtaining results similar to this paradox, Ocal et al.[33] attributed this to the use of the upper extremities of rats in the inclined plane test.

In contrast to the traditional use of AM for therapeutic purposes in neuropsychiatric disorders, especially in tropical regions, there are also studies showing that this plant has neurotoxic effects.[38] However, in recent years, studies have provided evidence of this traditional use. It has been observed that this tropical plant, which is used from root to bark, has neuroprotective effects, such as sedative, anxiolytic and anticonvulsant properties due to the flavonoid compounds contained.[27,28] Our results showing the antioxidant activities of

AM and absence of toxic effects suggest that this plant is promising in terms of its efficacy in the treatment of SCI.

## Conclusion

This study is the first to examine the effect of AM on experimental SCI. The results of our study showed that histopathologically and biochemically, po 300 mg/kg AM had a significant positive effect on SCI in rats. Consequently, it may be important to conduct clinical or experimental studies using different parameters to reveal the effect of AM on the primary mechanism in the future.

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**Ethics Committee Approval:** This study was approved by the Zonguldak Bulent Ecevit University Animal Experiments Local Ethics Committee (Date: 04.06.2020, Decision No: 2020/05).

**Peer-review:** Internally peer-reviewed.

**Authorship Contributions:** Concept: E.K., Ö.E.; Design: E.K., H.H.K.K.; Supervision: B.G.; Materials: E.K., Ö.E.; Data: E.K.; Analysis: B.G., H.H.K.K.; Literature search: E.K.; Writing: E.K.; Critical revision: E.K., B.G.

**Conflict of Interest:** None declared.

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DENEYSEL ÇALIŞMA - *ÖZ*

## *Annona muricata*'nın (graviola) deneysel spinal kord hasarı modelinde biyokimyasal ve histopatolojik analizi


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AMAÇ: *Annona muricata* (graviola) tropikal bölgelerde yetişen bir bitki olup; yerel halk tarafından pek çok hastalığa iyi geldiği düşünülmektedir. Spinal kord yaralanmasının ne yazık ki henüz kabul edilebilir bir medikal tedavi yöntemi yoktur. Bu çalışmada, *Annona muricata* yaprak ekstraktının deneysel sıçan modelinde omurilik yaralanması üzerindeki nöropeotektif etkilerini araştırdık.

GEREÇ VE YÖNTEM: Toplam 40 Wistar albino sıçan rastgele beş eşit gruba (n=8) ayrıldı. Grup 1, sadece laminektomi yapılan kontrol grubuydu. Laminektomi sonrası dört grupta travma oluşturuldu. Grup 2'ye (tedavi edilmemiş travma grubu) hiç ilaç verilmedi. Grup 3'e travma sonrası periton içine tek doz metilprednizolon (30 mg/kg) uygulandı. Grup 4'teki sıçanlar, travmadan bir hafta önce oral gavaj yoluyla düşük dozda (100 mg/kg) *Annona muricata* yaprağı ekstresi alırken, Grup 5'teki sıçanlar ise travmadan bir hafta önce bu ekstrakttan yüksek dozda (300 mg/kg) oral gavaj yoluyla aldılar. Travma oluşturulduktan 24 saat sonra kontrol grubuda dahil tüm sıçanlar sakrifiye edildi.

BULGULAR: Nöroprotektif etkiyi değerlendirmek için alınan doku örnekleri biyokimyasal ve histopatolojik olarak incelendi. Travma grubundaki inflamatuvar bulgular *Annona muricata* ile tedavi edilen her iki grupta da anlamlı olarak daha iyiydi. Klinik motor muayene ve eğik düzlem testi sonuçları açısından gruplar arasında fark yoktu.

TARTIŞMA: Histopatolojik ve biyokimyasal sonuçlarımız *Annona muricata*'nın travmatik omurilik yaralanmasında nöroprotektif etkiye sahip bir ajan olduğunu göstermiştir.



Anahtar sözcükler: *Annona muricata*; metilprednizolon; nöroprotektif; oksidatif stress; spinal kord hasarı.

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