EXPERIMENTAL STUDY

The effects of caffeic acid phenethyl ester on inflammatory cytokines after acute spinal cord injury

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ABSTRACT

BACKGROUND: The purpose of this study was to investigate the effects of Caffeic Acid Phenethyl Ester (CAPE) on proinflammatory cytokines, IL-1β and TNF-α, and explore its healing effect after acute spinal cord injury.

METHODS: Forty-eight male Wistar-Albino rats were used in this study which was planned as three groups. All groups were divided into two sub-groups. Group 1a was the control group, in which only lower segment thoracic laminectomy was performed. In group 1b, spinal cord trauma was performed with aneurysm clip. In the second group, serum physiologic was given systemically thirty minutes after trauma, and rats were sacrificed after the first and sixth hour. In the third group, CAPE was given systemically thirty minutes after trauma, and rats were sacrificed after the first and sixth hour. Serum IL-1β and TNF-α levels were analyzed by ELISA in the serum. Histopathological analysis was performed in damaged cord tissues.

RESULTS: CAPE suppressed TNF-α and IL-1β levels in the serum. In histopathological evaluation, it was detected that CAPE decreased hemorrhage and necrosis.

CONCLUSION: CAPE suppresses the levels of proinflammatory cytokines, TNF-α and IL-1β, after acute spinal cord injury in the early phase and contributes to the healing process.

Key words: CAPE; IL-1β; inflammation; spinal cord injury; TNF-α.

INTRODUCTION

Spine injury is a serious health problem having detrimental effects on the patient, family, and economy of the country. Its general incidence is about 20-40/1.000.000 in many countries around the world.[1] In the pathophysiology of acute spinal cord injury (SCI), primary and secondary mechanisms of the injury have been proposed. There are four characteristic mechanisms in primary injury, which are impact plus persistent compression, impact alone with transient compression, distraction, and laceration/transaction. However, secondary mechanisms of injury, extending from primary injury, involve neurogenic shock, vascular insults, excitotoxicity, calcium-mediated secondary injury and fluid-electrolyte disturbances, immunologic injury, apoptosis, disturbances in mitochondrion function, and other miscellaneous processes.[2]

Caffeic acid phenethyl ester (CAPE) is one of the active components of propolis, which is a substance found in the plant extracts collected by honeybees. Antimicrobial, anti-inflam-

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matory, immunomodulatory, antimutagenic, and antioxidant effects of propolis have been revealed in several studies. CAPE, specifically by blocking NF-κB and oxygen radicals, inhibits many inflammatory agents, especially the TNF-α. It has been shown that CAPE induces apoptosis in inflammatory cells independently from glucocorticoid receptors. Protective effects of CAPE in the nervous system have been reported in cerebral ischemic reperfusion injury, ischemic damage of the spinal cord, Parkinson's disease, convulsions, multiple sclerosis, brain tumors, hepatic encephalopathy, and against toxic effects of therapeutic agents in anticancer therapy.[3-11] Moreover, studies evaluating its protective effect in spinal cord ischemia-reperfusion models and hemi-transection model have been reported; however, there is no study investigating the acute effects of CAPE in traumatic acute spinal cord injury in clip compression model. Therefore, this study was conducted to evaluate the early anti-cytotoxic effects of CAPE in acute spinal cord injury.

MATERIALS AND METHODS

After the approval of Süleyman Demirel University (SDÜ) Local Ethical Committee on Animal Experiments (121531123153855-136), surgical procedure was performed in the experimental animals' research laboratory of the medical faculty of the same university. Forty-eight Wistar albino adult male rats, weighing 250±40 gr, were used. They were divided into three main groups, and each group was divided into two subgroups including eight animals. All animals were weighed before the operation and sacrification. Biochemical and pathological examinations were performed in the laboratories of biochemistry and histology-embryology departments of SDU.

In total, rats were divided into six groups;
Group 1a (n=8) only laminectomy (1st h),
Group 1b (n=8) laminectomy + trauma (1st h),
Group 2a (n=8) laminectomy + trauma + saline (1st h)
Group 2b (n=8) laminectomy + trauma + saline (6th h)
Group 3a (n=8) Laminectomy +trauma +CAPE (1st h)
Group 3b (n=8) Laminectomy + trauma +CAPE (6th h)

Anesthesia

General anesthesia was achieved with an intraperitoneal administration of 8 mg/100 gr ketamine (Alfamine 10%, Ege Vet Hayvancılık Bornova-İzmir, Alfasan International BV Holland) and 1 mg/100 gr xylazine (Alfazyne 2%, Ege Vet Hayvancılık Bornova-İzmir, Alfasan International BV Holland).

Surgical Procedure

Laminectomy was performed between the thoracic vertebrae Th8 and Th12. Aneurysm clip (Sugita no: 07-934-11, closure pressure: 1.37-1.72 N) was used to create trauma for one minute (Figs 1a, b).

In Group 1a (n=8), one hour after laminectomy, spinal cord tissues including lesion site and blood were taken for histopathological and biochemical evaluation before the rats were sacrificed with high dose of anesthesia. In Group 1b (n=8), spinal cord tissues including lesion site and blood were taken one hour after laminectomy and trauma. In Group 2a (n=8), 1cc saline was given intraperitoneally thirty minutes after trauma. One hour after trauma, blood and spinal cord tissue were taken. In Group 2b (n=8), the same steps as those of 2a were followed, but blood and tissue samples were taken six hours after trauma. In Group 3a (n=8), CAPE was given intraperitoneally (10 μg/kg) thirty minutes after trauma. One hour after trauma, blood and tissue samples were taken. In Group 3b (n=), same dosage of CAPE was given intraperitoneally thirty minutes after trauma; however, blood and tissue samples were taken six hours later.

Interleukin 1β and tumor necrosis factor-α levels were measured in the blood with ELISA kits. For light microscopy, spinal cord tissue samples were fixed with 10% formalin.

Statistical Analysis

Statistical analyses were performed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). Parametric values were given

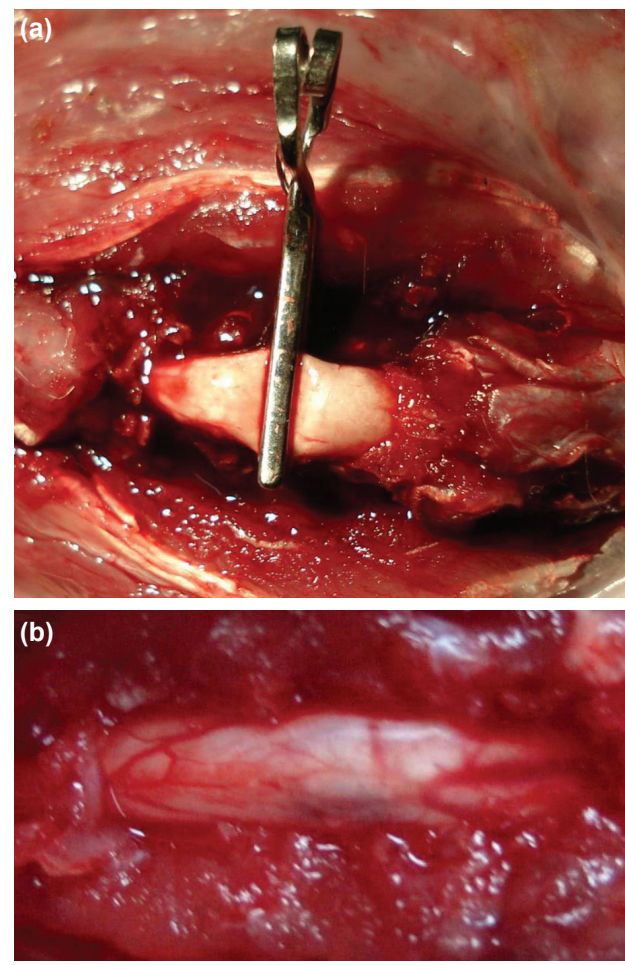

Figure 1. (a) Creating spinal cord injury with an aneurysm clip. **(b)** External view of spinal cord after creating injury.

Table 1. Mean TNF-α and IL-1β values of each group

	n	Mean
TNF-α		
1a	8	10.7798
1b	8	13.0727
2a	8	10.4561
2b	8	10.0711
3a	8	9.2311
3b	8	9.3185
IL-1β		
1a	8	32.6550
1b	8	33.8704
2a	8	25.4870
2b	8	30.7100
3a	8	23.8298
3b	8	21.1215

as mean±standard deviation and non-parametric values were given as percentage. In order to compare parametric continuous variables, Student's t-test was used, and Mann-Whitney U-test was used to compare nonparametric continuous variables. Two-tailed P-values of less than 0.05 were considered statistically significant.

RESULTS

Biochemical Evaluation

Mean serum TNF-α levels were 10.7798 pg/ml in Group 1a, 13.0727 pg/ml in Group 1b, 10,456 pg/ml in Group 2a, 10.0711 pg/ml in Group 2b, 9.2311 pg/ml in Group 3a, and 9.3185 pg/ml in Group 3b (Table 1). TNF-α levels were increased in Group 1b when compared to Group 1a; however, no statistically significant difference was observed (p=0.070). There was not a statistically significant difference between Groups 1a, 2a, and 2b (p=0.999 and p=0.949, respectively). Similarly, there was not a significant difference between Groups 1a, 3a, and 3b (p=0.404 and p=0.469, respectively). Significant differences were detected between Groups 1b and Groups 2a or 2b, and Groups 3a and 3b (p=0.026, 0.007, 0.001, and 0.001, respectively). No difference was observed between Groups 2, 3 and their subgroups (Table 2).

Mean serum IL-1β levels were 32.6550 pg/ml in Group 1a, 33.8704 pg/ml in Group 1b, 25.4870 pg/ml in Group 2a, 30.7100 pg/ml in Group 2b, 23.8298 pg/ml in Group 3a, and 21.1215 pg/ml in Group 3b (Table 1). IL-1β level was decreased in Groups 3a and 3b when compared to Group 1b; however, no significant difference was observed between these groups (p=0.539 and 0.278, respectively). In addition, no statistically significant difference was detected between all groups and their subgroups (Table 3).

Histological evaluation

Stained tissue samples were examined under binocular microscope (Olympus BX50, NY), and microphotographs of the sections were evaluated.

For histopathological evaluation, a semi-quantitative scoring system which was previously used by Ercan et al. was selected.[12] According to this system;

(-) score (negative score): no structural change
(+) score (1 positive score): slight changes.
(++) score (2 positive score): moderate changes

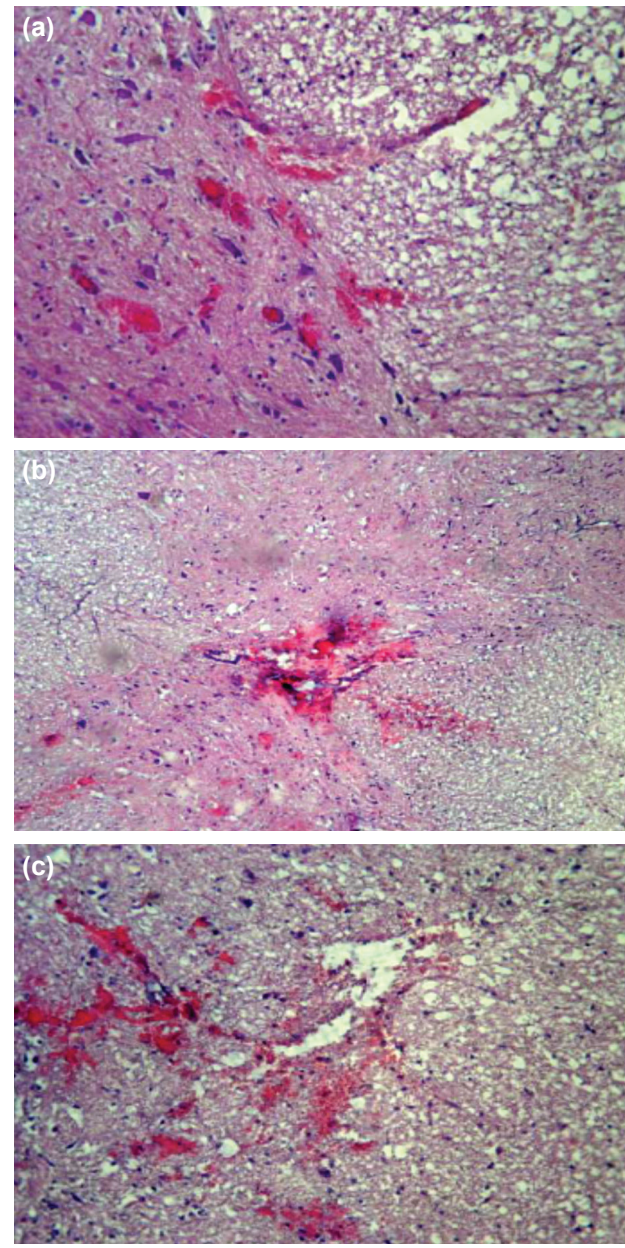

Figure 2. (a) Microscopic appearance in group 1a (H&E, x100). **(b)** Microscopic appearance in group 2a (H&E, x100). **(c)** Microscopic appearance in group 3a (H&E, x100).

Table 2. Statistical comparisons of TNF-α among the groups

Dependant variable	(I) group	(J) group	p
TNF-α	1a	1b	0.070
		2a	0.999
		2b	.949
		3a	.404
		3b	.469
	1b	1a	.070
		2a	.026
		2b	.007
		3a	.001
		3b	.001
	2a	1a	.999
		1b	.026
		2b	.997
		3a	.654
		3b	.720
	2b	1a	.949
		1b	.007
		2a	.997
		3a	.901
		3b	.935
	3a	1a	.404
		1b	.000
		2a	.654
		2b	.901
		3b	1.000
	3b	1a	.469
		1b	.001
		2a	.720
		2b	.935
		3a	1.000

Table 3. Statistical comparisons of IL-1β among the groups

Dependant variable	(I) group	(J) group	p
IL-1β	1a	1b	1.000
		2a	.828
		2b	.999
		3a	.670
		3b	.386
	1b	1a	1.000
		2a	.715
		2b	.994
		3a	.539
		3b	.278
	2a	1a	.828
		1b	.715
		2b	.948
		3a	1.000
		3b	.976
	2b	1a	.999
		1b	.994
		2a	.948
		3a	.851
		3b	.588
	3a	1a	.670
		1b	.539
		2a	1.000
		2b	.851
		3b	.997
	3b	1a	.386
		1b	.278
		2a	.976
		2b	.588
		3a	.997

Table 4. Histopathological findings of each group

Group number	Degeneration or hemorrhage in central canal	Necrosis in gray and white matter	Hemorrhage in gray and white matter	Liquefaction necrosis
1a	–	+	+	–
1b	+++	+++	+++	+++
2a	+++	+++	+++	+++
2b	+++	+++	+++	+++
3a	+	+	+	+
3b	+	+	+	+

(+++) score (3 positive score): prominent changes.

Group 1a: Slight hemorrhage was detected in gray and white matter (+). Also, slight necrosis was seen in both matters (+). Degeneration, hemorrhage, and liquefaction necrosis were not observed in the central canal (Fig. 2a).
Group 1b: Prominent hemorrhage and necrosis were observed in both matters (+++). Also, degeneration, hemorrhage, and liquefaction necrosis were prominent in the central canal (+++).

Group 2a: Prominent hemorrhage and necrosis were observed in both matters (+++). Also, degeneration, hemorrhage, and liquefaction necrosis were prominent in central canal (+++) (Fig. 2b).

Group 2b: Similar findings were observed as those of Groups 1b and 2a.

Group 3a: There was slight hemorrhage and liquefaction necrosis around central canal. Moderate hemorrhage and necrosis were observed in white and gray matter less than previous groups (Fig. 2c).

Group 3b: Similar findings were observed as those of Group 3a (Table 4).

DISCUSSION

In the present study, CAPE was detected to decrease TNF-α and IL-1β levels after spinal cord injury in the early period. Furthermore, it reduces hemorrhage and necrosis in gray and white matter, as well as in the central canal.

Two known mechanisms of spinal cord injury are primary mechanical injury and secondary injury. Underlying mechanisms of pathophysiology in these injuries include acute hemorrhage, ischemia, inflammation, abnormal intracellular ion shifts (Na+, Ca +2), lipid peroxidation of cell membrane induced by free radicals, edema, leukocyte infiltration, and excitotoxic cell death.[13]

Inflammation begins immediately after spinal cord injury. Edema, hemorrhage, accumulation of neuroexcitotoxin, and biochemical changes beginning after injury create difficulties on determining the main effects of inflammation on central nervous system. Although inflammation includes vascular, neurologic, humoral, and cellular responses around the injury site, it is a process to remove harmful stimuli and "contribute to tissue repair".[14] The cellular source of IL-1 β and TNF-α after acute spinal cord injury is controversial. Some authors believe that these cytokines are the primary products of neutrophils and macrophages. However, other studies have shown that endogen central nervous system cells (microglia) secrete pro-inflammatory cytokines in many injury models.[15]

This study utilized CAPE which inhibits lipid peroxidation by suppressing protein tyrosine kinase, cyclooxygenase (non-specifically), and lipoxygenase. Anti-inflammatory activity of CAPE was found equivalent with diclofenac and hydrocortisone.[16] It has also been suggested that CAPE induces apoptosis independently from glucocorticoid receptors.[17]

The protective effects of CAPE have been practiced in various studies dealing with cerebral ischemia-reperfusion injury, spinal cord ischemia-reperfusion injury, Parkinson's disease, hypoxic ischemic brain damage in newborns, multiple sclerosis, convulsions, brain tumors, toxic effects of therapeutic agent in anticancer therapies, and hepatic encephalopathy.[3-11]

There are only five studies in the literature evaluating the effects of CAPE in the spinal cord.[5,8,11,18,19] Two of these studies have been performed to evaluate the protective effects of CAPE against experimental allergic encephalomyelitis-induced oxidative stress, and in methotrexate administered rats.[8,11] Kasai et al. have reported that CAPE might be a promising therapeutic agent for reducing secondary neural damage in their hemi-transection model.[18] In the remaining two studies, researchers have evaluated the effects of CAPE in ischemia-reperfusion injury model. Authors have reported that CAPE decreases injury more than methylprednisolone with its antioxidant and anti-inflammatory effects.[5,19] In the present study, clip compression model, which suits more to the trauma model in humans, was used. This is the first study evaluating the effects of CAPE in this model. Same dosage of CAPE (10 μg/kg) with the previous reports was administered. It was found that CAPE decreased the levels of proinflammatory cytokines but not in significant levels, which may be due to the fact that the evaluation process was conducted in serum, not in tissue. This may be a limitation of our study.

Conclusion

CAPE decreases inflammation, necrosis and hemorrhage in the injured spinal cord tissue. It may become a promising agent in the management of spinal cord injury with further studies.

Conflict of interest: None declared.

REFERENCES

1. Selvarajah S, Hammond ER, Haider AH, Abularrage CJ, Becker D, Dhiman N, et al. The burden of acute traumatic spinal cord injury among adults in the united states: an update. J Neurotrauma 2014;31:228-38.
2. Dumont RJ, Okonkwo DO, Verma S, Hurlbert RJ, Boulos PT, Ellegala DB, et al. Acute spinal cord injury, part I: pathophysiologic mechanisms. Clin Neuropharmacol 2001;24:254-64. CrossRef
3. Khan M, Elango C, Ansari MA, Singh I, Singh AK. Caffeic acid phenethyl ester reduces neurovascular inflammation and protects rat brain following transient focal cerebral ischemia. J Neurochem 2007;102:365-77.
4. Tsai SK, Lin MJ, Liao PH, Yang CY, Lin SM, Liu SM, et al. Caffeic acid phenethyl ester ameliorates cerebral infarction in rats subjected to focal

cerebral ischemia. Life Sci 2006;78:2758-62. CrossRef
5. Ilhan A, Koltuksuz U, Ozen S, Uz E, Ciralik H, Akyol O. The effects of caffeic acid phenethyl ester (CAPE) on spinal cord ischemia/reperfusion injury in rabbits. Eur J Cardiothorac Surg 1999;16:458-63. CrossRef
6. Noelker C, Bacher M, Gocke P, Wei X, Klockgether T, Du Y, et al. The flavanoide caffeic acid phenethyl ester blocks 6-hydroxydopamine-induced neurotoxicity. Neurosci Lett 2005;383:39-43. CrossRef
7. Wei X, Zhao L, Ma Z, Holtzman DM, Yan C, Dodel RC, et al. Caffeic acid phenethyl ester prevents neonatal hypoxic-ischaemic brain injury. Brain 2004;127(Pt 12):2629-35. CrossRef
8. Ilhan A, Akyol O, Gurel A, Armutcu F, Iraz M, Oztas E. Protective effects of caffeic acid phenethyl ester against experimental allergic encephalomyelitis-induced oxidative stress in rats. Free Radic Biol Med 2004;37:386-94. CrossRef
9. Ilhan A, Iraz M, Gurel A, Armutcu F, Akyol O. Caffeic acid phenethyl ester exerts a neuroprotective effect on CNS against pentylenetetrazol-induced seizures in mice. Neurochem Res 2004;29:2287-92. CrossRef
10. Lin YH, Chiu JH, Tseng WS, Wong TT, Chiou SH, Yen SH. Antiproliferation and radiosensitization of caffeic acid phenethyl ester on human medulloblastoma cells. Cancer Chemother Pharmacol 2006;57:525-32.
11. Uzar E, Sahin O, Koyuncuoglu HR, Uz E, Bas O, Kilbas S, et al. The activity of adenosine deaminase and the level of nitric oxide in spinal cord of methotrexate administered rats: protective effect of caffeic acid phenethyl ester. Toxicology 2006;218:125-33. CrossRef
12. Ercan I, Cakir BO, Başak T, Ozbal EA, Sahin A, Balci G, et al. Effects of topical application of methotrexate on nasal mucosa in rats: a preclinical assessment study. Otolaryngol Head Neck Surg 2006;134:751-5. CrossRef
13. Zhang N, Yin Y, Xu SJ, Wu YP, Chen WS. Inflammation & apoptosis in spinal cord injury. Indian J Med Res 2012;135:287-96.
14. Beck KD, Nguyen HX, Galvan MD, Salazar DL, Woodruff TM, Anderson AJ. Quantitative analysis of cellular inflammation after traumatic spinal cord injury: evidence for a multiphasic inflammatory response in the acute to chronic environment. Brain 2010;133:433-47. CrossRef
15. Herx LM, Rivest S, Yong VW. Central nervous system-initiated inflammation and neurotrophism in trauma: IL-1 beta is required for the production of ciliary neurotrophic factor. J Immunol 2000;165:2232-9. CrossRef
16. Koksel O, Ozdulger A, Tamer L, Cinel L, Ercil M, Degirmenci U, et al. Effects of caffeic acid phenethyl ester on lipopolysaccharide-induced lung injury in rats. Pulm Pharmacol Ther 2006;19:90-5. CrossRef
17. Zaeemzadeh N, Hemmati A, Arzi A, Jalali M, Rashidi I. Protective Effect of Caffeic Acid Phenethyl Ester (CAPE) on Amiodarone-Induced Pulmonary Fibrosisin Rat. Iran J Pharm Res 2011;10:321-8.
18. Kasai M, Fukumitsu H, Soumiya H, Furukawa S. Caffeic acid phenethyl ester reduces spinal cord injury-evoked locomotor dysfunction. Biomed Res 2011;32:1-7. CrossRef
19. Sahin S, Sogut S, Ozyurt H, Uz E, Ilhan A, Akyol O. Tissue xanthine oxidase activity and nitric oxide levels after spinal cord ischemia/reperfusion injury in rabbits: comparison of caffeic acid phenethyl ester (CAPE) and methylprednisolone. Neuroscience Research Communications 2002;31:111-21. CrossRef

DENEYSEL ÇALIŞMA - *ÖZET*

Kafeik asit fenetil esterin akut spinal kord hasarı sonrasında enflamatuvar sitokinler üzerine etkisi

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AMAÇ: Bu çalışmada, akut spinal kord hasarı sonrası erken dönemde kafeik asit fenetil esterin (KAFE) enflamatuvar sitokinlerden interlökin 1 beta (IL-1β) ve tümör nekrotizan faktor alfa (TNF-α) üzerine etkisini ve histopatolojik olarak KAFE'nin olası iyileştirici etkisini araştırmak amaçlandı.
GEREÇ VE YÖNTEM: Çalışmada ağırlıkları 250-300 gram arasında değişen 48 Wistar-Albino cinsi sıçan kullanıldı. Denekler üç gruba ayrıldı. Her grup kendi altında iki alt gruba ayrıldı. 1a grubu kontrol grubu olup bu grupta yalnızca laminektomi yapıldı. Grup 1b'de laminektomi sonrası anevrizma klibi ile travma oluşturuldu. İkinci gruptaki deneklerde travma oluşturulduktan yarım saat sonra serum fizyolojik sistemik olarak verilip birinci ve altıncı saatte denekler sakrifiye edildi. Üçüncü grupta travma oluşturulmuş deneklere yarım saat sonra sistemik yoldan KAFE verildi ve bu denekler birinci ve altıncı saatte sakrifiye edildi. Sakrifikasyon öncesi kalpten alınan kanda ELİSA kitleri ile serum IL-1β ve TNF-α düzeyleri ölçüldü. Hasarlanmış kordan alınan doku örneklerinde histopatolojik değerlendirme yapıldı.
BULGULAR: Kafeik asit fenetil esterin verilen grupta TNF-α ve IL-1β düzeylerinin azaldığı tespit edildi. Histopatolojik değerlendirmede KAFE verilen grupta hemoraji ve nekroz oranında azalma tespit edildi.
TARTIŞMA: Akut spinal kord hasarı sonrası erken dönemde KAFE enflamatuvar sitokinlerden TNF-α ve IL-1β düzeylerini baskılamaktadır ve hasar sonrası iyileşmeye katkıda bulunmaktadır.
Anahtar sözcükler: Enflamasyon; interlökin-1β; kafeik asit fenetil ester; spinal kord hasarı; tümör nekrotizan faktör-α.

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