https://dergipark.org.tr/tr/pub/ataunivbd
2020; 15(3): 223-230

Research Article/Araştırma Makalesi
DOI: 10.17094/ataunivbd.762960

Atatürk Üniversitesi Veteriner Bilimleri Dergisi
Atatürk University Journal of Veterinary Sciences

The Effects of Ellagic Acid on Cecal Ligation and Puncture-Induced Lung Injury

Ersen ERASLAN[1a]✉, Derya GÜZEL ERDOĞAN[2b], Songül DOĞANAY[2c], Mustafa Can GÜLER[3d], Ayhan TANYELİ[3e], Selim ÇOMAKLI[4f]

1. Yozgat Bozok University, Faculty of Medicine, Department of Physiology, Yozgat, TURKEY.
2. Sakarya University, Faculty of Medicine, Department of Physiology, Sakarya, TURKEY.
3. Atatürk University, Faculty of Medicine, Department of Physiology, Erzurum, TURKEY.
4. Atatürk University, Faculty of Veterinary, Department of Pathology, Erzurum, TURKEY.

ORCID: 0000-0003-2424-2269[a], 0000-0002-0095-0917[b], 0000-0002-1730-1331[c], 0000-0001-8588-1035[d], 0000-0002-0095-0917[e], 0000-0002-8744-7686[f]

Geliş Tarihi/Received	Kabul Tarihi/Accepted	Yayın Tarihi/Published
02.07.2020	30.08.2020	31.12.2020

Bu makaleye atıfta bulunmak için/To cite this article:
Erarslan E, Guzel Erdogan D, Doganay S, Guler MC, Tanyeli A, Comaklı S: The Effects of Ellagic Acid on Cecal Ligation and Puncture-Induced Lung Injury. Atatürk University J. Vet. Sci., 15(3): 223-230, 2020. DOI: 10.17094/ataunivbd.762960

Abstract: Ellagic acid was examined on lung tissue injury caused by cecal ligation and puncture (CLP)-induced polymicrobial sepsis model in rats. 24 Wistar Albino female rats were allocated to 3 groups as: Group I (Sham), group II (CLP), and group III (CLP+ Ellagic acid 75 mg/kg). The CLP model was made by drilling the cecum. Biochemical and immunohistochemical analyzes were performed. Total oxidant status, tumor necrosis factor-α, interleukin-1β, malondialdehyde, and myeloperoxidase activity elevated, but superoxide dismutase and total antioxidant status valued declined in the CLP group compared to the sham group. On the contrary, superoxide dismutase and total antioxidant status levels increased. In contrast, myeloperoxidase activity, total oxidant status, tumor necrosis factor-α, interleukin-1β, and malondialdehyde levels decreased in the Ellagic acid treatment group. Caspase-3 and microtubule-associated protein light chain-3B immunopositivity increased significantly in the CLP group compared to the sham group while diminishing in the Ellagic acid group. In conclusion, Ellagic acid prevented CLP-induced lung injury in experimental rats. Thus, Ellagic acid may be an alternative therapeutic agent against lung tissue injury induced by sepsis.

Keywords: Autophagy, Cecal ligation and puncture, Ellagic acid, Inflammation, Lung.

Ellagik Asidin Çekal Ligasyon ve Delmeye Bağlı Akciğer Hasarı Üzerine Etkileri

Öz: Ellagik asit, sıçanlarda çekal ligasyon ve delmeye (ÇLD) bağlı polimikrobiyal sepsis modelinin neden olduğu akciğer dokusu hasarı üzerinde incelendi. 24 Wistar Albino dişi sıçan 3 gruba ayrıldı: Grup I (sham), grup II (ÇLD) ve grup III (ÇLD + Ellagik asit 75 mg / kg). ÇLD modeli çekumun delinmesi ile gerçekleştirildi. Biyokimyasal ve immünohistokimyasal analizler yapıldı. Total oksidan kapasite, tümör nekroz faktör-α, interlökin-1β, malondialdehit ve miyeloperoksidaz aktivitesi arttı, ancak süperoksit dismütaz ve total antioksidan kapasite değerleri sham grubuyla karşılaştırıldığında ÇLD grubunda azaldı. Aksine, ellagik asit tedavi grubunda miyeloperoksidaz aktivitesi, total oksidan kapasite, tümör nekroz faktör-α, interlökin-1β, ve malondialdehit seviyeleri azalırken süperoksit dismütaz ve total antioksidan kapasite düzeyleri artmıştır. Kaspaz-3 ve LC3B immünopozitifliği ellagik asit grubunda azalırken, ÇLD grubunda sham grubuna göre anlamlı olarak artmıştır. Sonuç olarak ellagik asit, deneysel sıçanlarda ÇLD'nin neden olduğu akciğer hasarını önledi. Dolayısıyla ellagik asit, sepsisin neden olduğu akciğer dokusu hasarına karşı alternatif bir terapötik ajan olabilir.

Anahtar Kelimeler: Akciğer, Çekal ligasyon ve delme, Ellagik asit, İnflamasyon, Otofaji.

✉Ersen Eraslan
Yozgat Bozok University, Faculty of Medicine, Department of Physiology, Yozgat, TURKEY.
e-mail: ersen.eraslan@yobu.edu.tr

INTRODUCTION

Sepsis is an irregular answer to infections and causes death worldwide (1). It is strongly related to morbidity and mortality (2,3). Infections of the lower respiratory tract commonly result in death (4). Various experimental methods and several agents were examined against sepsis. As presented in previous studies, cecal ligation and puncture (CLP)-induced sepsis and acute lung injury (ALI) were carried out in this study (5-10). There are various researches about sepsis, but it still performs high morbidity and mortality rates (11). Supportive treatment forms the primary therapy for sepsis, and it is needed for specific agents (12). During sepsis, oxidative stress exists and enhances the harmful effects of oxidants (13). Reactive oxygen species (ROS) are strongly associated with hemodynamic dysfunction and organ failure during sepsis by inducing cytotoxicity in organs a changing cell signal pathways (14). During sepsis, nuclear factor-kappa B (NF-κB) is activated, and it leads to proinflammatory cytokine production, including tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), which contribute to ALI development (15).

Ellagic acid (EA) is a phenol that is found in many fruits, seeds, and vegetables (16). Its chemical name is 2,3,7,8-tetrahydroxy-chromeno [5,4,3-cde]-chromene5,10-dione (17). It performs antioxidant activity (18). EA provides scavenging ROS and reactive nitrogen species via its chemical structure (19). Various studies mentioned that EA has the potential for the treatment of several chronic inflammatory diseases (20,21). This study was performed to determine the potential protective effects of EA against lung injury caused by CLP-induced sepsis model in rats.

MATERIALS and METHODS

Ethical Approval and Animals

The current study was carried out at Atatürk University Experimental Animals Research and Application Center with the confirmation of Atatürk University Experimental Animal Ethics Committee (protocol no:02.03.2018/48). Wistar Albino female rats weighing 240-270 g, provided by the same center. They were held in cages with appropriate laboratory conditions, including 12 hours light/darkness, the humidity of 55%, and temperature of 25°C. Standard rat feed and tap water were provided for the animals. They were deprived of food 12 hours before the experiment but were allowed to drink water.

Groups and Experimental Process

The animals were immobilized in a supine position, shaved, and disinfected. 10% povidone-iodine (Baticonol, Dermosept, ALG İlaç, İstanbul) was used for disinfection. Surgical steps were performed under anesthesia with thiopental sodium (50 mg/kg). EA (Sigma-Aldrich Co, USA) was prepared in saline. Animals were classified into 3 groups;

Sham group (Group I, n=8): The abdominal midline was incised through a 1-2 cm incision and sutured. After 16-18 hours (sepsis time), lung tissues were excised.

CLP group (Group II, n=8): After the steps followed in group I, the cecum was reached and tied by 4.0 suture at 2 cm distal. In addition, 4 holes were drilled from this distal cecum via 18 gauges needle. And then, the intestine, including the cecum, was replaced into the abdomen. 2 ml of saline was emptied into the abdomen, and the incision line was closed. CLP model was preferred from a previous study (22).

CLP+EA 75 mg/kg group = EA 75 mg/kg group (Group III, n=8): CLP model was carried out as described in group II. 75 mg/kg EA was administered

intraperitoneally (i.p.) 30 minutes before the CLP model.

After 16-18 hours from the CLP model, these rats were sacrificed by high dose anesthesia, and lung tissues were collected.

Biochemical Examination

Lung tissues were homogenized via 10% phosphate buffer solution (PBS) and centrifuged to obtain a supernatant. The supernatants were evaluated for malondialdehyde (MDA), total antioxidant status (TAS), total oxidant status (TOS), myeloperoxidase (MPO), and superoxide dismutase (SOD) measurement. MDA level was determined as described by Ohkawa et al. (23). TAS and TOS values were measured by appropriate kits (Rel Assay Diagnostics). The oxidative stress index (OSI) ratio was gauged as follows: OSI= ([TOS, mmol H_2O_2 equivalent/L] / [TAS, mmol Trolox equivalent/L] × 10) (24). MPO level was measured as presented in a previous study (25). SOD activity determination depends on the formazan dye level (26). (TNF-α) and (IL-1β) levels were gauged via appropriate kits (Elabscience, Wuhan, China).

Immunohistochemical (IHC) Staining

Lung tissues were excised and put in 10% neutral formalin for 2-4 days. Paraffin blocks were prepared, stained, and examined as 5-µm thick sections through a microscope. Following deparaffinization, the slides were unmasked from antigens through antigen retrieval solution and placed in 3% H_2O_2 to block endogenous peroxide. Cleaved caspase-3 antibody (cat no. NB600-1235, dilution 1/200; Novus Biological, USA) and microtubule-associated protein light chain-3B (LC3B) (Cat. no.sc-376404, dilution 1/200; Santa Cruz) were used as the primary antibody, and expose mouse and rabbit specific HRP/DAB detection IHC kit was used as the second. After counterstaining with hematoxylin, immunoreactivity in samples was evaluated as 0 (none), 1 (mild), 2 (moderate), and 3 (severe).

Statistical Analysis

SPSS v20.0 was used for statistical analyses. A one-way ANOVA test was chosen for data, and Duncan test was used for multiple comparisons. Biochemical data results were presented as mean ± Standard Deviation (SD). Immunohistochemical examination data were shown in mean±Standard Error (SE). Differences were evaluated with Kruskal–Wallis test. Dual comparisons among groups were analyzed with a Mann-Whitney U test. Statistical significance level was considered when p-value below 0.05.

RESULTS

Biochemical Results

Table 1 presents the oxidant and antioxidant parameters. TAS value and SOD activity declined significantly while TOS, MDA, MPO, and OSI values elevated significantly in the CLP group compared to the sham group. In the treatment group, TOS, MDA, MPO, and OSI values diminished, while TAS and SOD levels increased significantly compared to the CLP group.

Table 1. Effects of EA treatment on biochemical parameters in CLP-induced lung injury.
Tablo 1. EA tedavisinin CLP kaynaklı akciğer hasarında biyokimyasal parametreler üzerine etkileri.

Groups (n=8)	Sham	CLP	EA 75 mg/kg
TAS (mmol/L)	0.43 ± 0.04	0.10 ± 0.02[a]	0.38 ± 0.06[b]
TOS (µmol/L)	13.79 ± 2.42	19.61 ± 1.33[a]	13.30 ± 1.82[b]
OSI (arbitrary unit)	3.22 ± 0.80	18.96 ± 4.08[a]	3.52 ± 0.87[b]
SOD (U/mg protein)	220.30 ± 52.85	100.22 ± 17.25[a]	245.24 ± 77.38[b]
MDA (µmol/g tissue)	112.54 ± 8.32	160.25 ± 23.76[a]	109.14 ± 9.03[b]
MPO (U/g protein)	316459.17 ± 108664.70	608594.22 ± 109.789[a]	307092.34 ± 127916.17[b]

All data results were presented as mean±SD. [a]P < 0.001 compared to sham group. [b]P< 0.001 compared to CLP group. TAS; Total antioxidant status, TOS; Total oxidant status, OSI; Oxidative stress index. SOD; Superoxide dismutase, MDA: Malondialdehyde MPO; Myeloperoxidase.

Results of Cytokines

In Figure 1, TNF-α and IL-1β levels were presented. TNF-α and IL-1β levels raised in the CLP group compared to the sham group. On the other side, these values decreased via EA treatment.

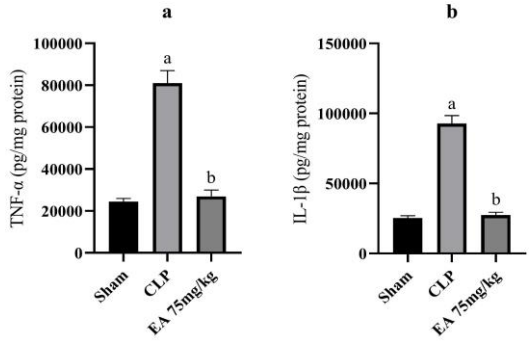

Figure 1. Effects of EA treatment on **(a)** TNF-α and **(b)** IL-1β levels in CLP-induced lung injury. All data results were presented as mean±SD. [a]P<0.001 compared to the Sham group. [b]P<0.001 compared to the CLP group.

Şekil 1. EA tedavisinin CLP ile indüklenen akciğer hasarında **(a)** TNF-α ve **(b)** IL-1β seviyeleri üzerindeki etkileri. Tüm veri sonuçları ortalama ± SD olarak sunuldu. Sham gruba kıyasla [a]P<0.001. CLP grubuna kıyasla [b]P<0.001.

Immunohistochemical Examination

Caspase-3 and LC3B immunopositivity were not observed in the sham group. On the other side, while caspase-3 and LC3B immunopositivity were severe in the CLP group, it was found to be less in EA 75 mg/kg group. The interstitial areas were immune positive (Figure 2).

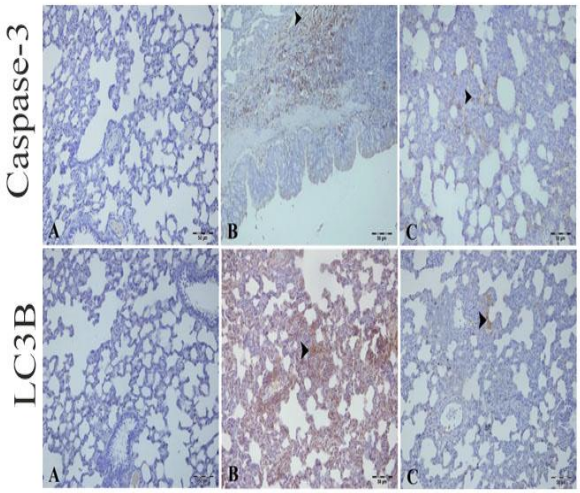

Figure 2. A) Sham group, B) CLP group, severe caspase-3 and LC3B immunopositivity in interstitial area (arrowhead) C) EA 75 mg/kg group, mild Caspase-3 and LC3B immunopositivity in interstitial area (arrowhead). IHC. 50X magnification.

Şekil 2. A) Sham grubu, B) CLP grubu, interstisyel alanda şiddetli kaspaz-3 ve LC3B immünopozitifliği (ok başı) C) EA 75 mg / kg grubu, interstisyel bölgede hafif Kaspaz-3 ve LC3B immünopozitifliği (ok başı). İHK. 50X büyütme.

DISCUSSION and CONCLUSION

Sepsis is a life-critical condition that causes shock and organ failure (27). Bacterial infections lead to sepsis, which is an uncontrolled inflammatory response leading to organ dysfunction, morbidity, and mortality (28). Although antimicrobial treatments, sepsis is a major death cause in intensive care units (29). Early diagnosis prevents septic shock that enhances mortality rate (30).

Infections are among the factors leading to ALI, and they also cause sepsis (31). CLP model is an up to date and common method for sepsis studies (32). Lungs are among the organs which are vulnerable to inflammation induced by sepsis (33). Oxidative stress damages the lungs during sepsis (34).

Myeloperoxidase activity is a biomarker for infiltration of neutrophils in lung tissue (35). In the current study, myeloperoxidase activity elevated in the CLP group, and EA treatment diminished this parameter. Sepsis is associated with high ROS and low antioxidant levels (36). An antioxidant system, including SOD, scavenge ROS (37) protects tissues against oxidative damage (38). In the current study, SOD activity decreased in the CLP group, but EA treatment elevated the SOD activity again. OSI, the ratio of TOS to TAS (39), decreased with EA treatment. MDA levels elevated in pulmonary tissues in previous CLP studies (40). Here, MDA levels increased in the CLP group, but EA treatment reversed this condition.

Systemic infections are associated with various inflammatory cytokines like TNF-α and IL-1β. High levels of these mediators play a role in tissue injury (41). TNF-α induces sepsis (42) and prompts the

release of various proinflammatory cytokines (43). Here, TNF-α and IL-1β levels significantly declined with EA treatment.

Caspase-3 plays a key role in the pathway of apoptotic cell death. In addition to this, recent studies have shown that caspase-3 also gets involved in autophagic processes (44). It has been shown that upregulation of autophagic activity may provide great benefits through affecting inflammatory response and recovering impaired organ function (45). LC3B, one of the key molecules for autophagy, has been reported to increase 6 hours after CLP and eliminate some pathogens (46). It has been claimed that caspase-3 may help the export of autophagic vesicles containing maturing LC3B through the plasma membrane in human endothelial cells which lack nutrition which in turn promotes cell volume shrinkage, a decisive apoptotic marker (47). Although there is a complex relationship between autophagy and caspase-3, cell death and elimination of dead cells may contribute to maintaining tissue integrity. In this study, caspase-3 and LC3B were suppressed with EA therapy in lung immunohistochemical examination.

In conclusion, EA declined CLP-induced inflammation, oxidative stress, and apoptosis. Therefore, EA may be a new agent against diseases related to increased cell death and inflammation.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgement

This paper was presented as an abstract in the conference "International Conference on Multidisciplinary, Engineering, Science, Education and Technology (IMESET'18 Dubai, UAE) October 25-27, 2018, Dubai, UAE".

REFERENCES

1. Bhan C., Dipankar P., Chakraborty P., Sarangi PP., 2016. Role of cellular events in the pathophysiology of sepsis. Inflamm Res, 65, 853-68.
2. Mayr FB., Yende S., Angus DC., 2014. Epidemiology of severe sepsis. Virulence, 5, 4-11.
3. Angus DC., Wax RS., 2001. Epidemiology of sepsis: an update. Crit Care Med, 29, 109-116.
4. Troeger C., Blacker B., Khalil IA., Rao PC., Cao J., Zimsen SRM., Albertson SB., Deshpande A., Farag T., Abebe Z., Adetifa IMO., Adhikari TB., Akibu M., Al Lami FH., Al-Eyadhy A., Alvis-Guzman N., Amare AT., Amoako YA., Antonio CAT., Aremu O., Asfaw ET., Asgedom SW., Atey TM., Attia EF., Avokpaho EFGA., Ayele HT., Ayuk TB., Balakrishnan K., Barac A., Bassat Q., Behzadifar M., Behzadifar M., Bhaumik S., Bhutta ZA., Bijani A., Brauer M., Brown A., Camargos PAM., Castañeda-Orjuela CA., Colombara D., Conti S., Dadi AF., Dandona L., Dandona R., Phuc Do H., Dubljanin E., Edessa D., Elkout H., Endries AY., Fijabi DO., Foreman KJ., Forouzanfar MH., Fullman N., Garcia-Basteiro AL., Gessner BD., Gething PW., Gupta R., Gupta T., Hailu GB., Hassen HY., Hedayati MT., Heidari M., Hibstu DT., Horita N., Ilesanmi OS., Jakovljevic MB., Jamal AA., Kahsay A., Kasaeian A., Kassa DH., Khader YS., Khan EA., Khan MN., Khang YH, Kim YJ., Kissoon N., Knibbs LD, Kochhar S., Koul PA., Kumar GA, Lodha R., El Razek HMA., Malta DC., Mathew JL., Mengistu TD., Mezgebe HB., Mohammad KA., Mohammed MA., Momeniha F., Murthy S., Nguyen CT., Nielsen KR., Ningrum DNA., Nirayo YL., Oren E., Ortiz JR., Pa M., Postma MJ., Qorbani M., Quansah R., Rai RK., Rana SM., Ranabhat CL., Ray SE., Rezai MS., Ruhago GM., Safiri., Salomon JA., Sartorius B., Savic M., Sawhney M., She J., Sheikh A., Shiferaw MS., Shigematsu M., Singh JA., Somayaji R., Stanaway JD., Sufiyan MB., Taffere GR.,

Temsah MH., Thompson MJ., Tobe-Gai R., Topor-Madry R., Tran BX., Tran TT., Tuem KB., Ukwaja KN., Vollset SE., Walson JL, Weldegebreal F., Werdecker A., West TE., Yonemoto N., Zaki MS., Zhou L., Zodpey S., Vos W., Naghavi M, Lim SS., Mokdad AL., Murray CJL., Hay SI., Reiner Jr RC., 2016. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study. Lancet Infect Dis, 18, 1191-210.

5. Chen X., Wang T., Song L., Liu X., 2019. Activation of multiple Toll-like receptors serves different roles in sepsis-induced acute lung injury. Exp Ther Med, 18, 443-450.
6. Zhou J., Fu Y., Liu K., Hou L., Zhang W., 2019. miR-206 regulates alveolar type II epithelial cell Cx43 expression in sepsis-induced acute lung injury. Exp Ther Med, 18, 296-304.
7. Störmann P., Becker N., Vollrath JT., Köhler K., Janicova A., Wutzler S., Hildebrand F., Marzi I., Relja B., 2019. Early local inhibition of Club Cell Protein 16 Following Chest Trauma Reduces Late Sepsis-Induced Acute Lung Injury. J Clin Med, 8, 896-901.
8. Güzel D., Tanyeli A., 2018. Inhibition of NADPH oxidase attenuates sepsis induced acute lung oxidative damage in rats. J Cell Neurosci Oxid Stress, 10, 714.
9. Tanyeli A., Güzel D., 2019. Alliin mitigates Cecal Ligation Puncture (CLP)-induced lung injury through antioxidant and antiinflammatory effects. TJOS, 4, 46-59.
10. Tanyeli A., Guzel D., 2019. Investigation into the Biochemical Effects of Barbaloin on Renal Tissue in Cecal Ligation and Puncture-Induced Polymicrobial Sepsis Model in Rats. South Clin Ist Euras, 30, 285-9.
11. Lelubre C., Vincent JL., 2018. Mechanisms and treatment of organ failure in sepsis. Nat Rev Nephrol, 14, 417-27.
12. Gotts JE., Matthay MA., 2016. Sepsis: pathophysiology and clinical management. BMJ, 353, 1585-2005.
13. Kaymak C., Basar H., Sardas S., 2011. Reactive oxygen species (ROS) generation in sepsis. FABAD J Pharm Sci, 36, 41-47.
14. De Jong HK., Van Der Poll T., Wiersinga WJ., 2010. The systemic pro-inflammatory response in sepsis. J Innate Immun, 2, 422-430.
15. Li K., Yang J., Han X., 2016. Ketamine attenuates sepsis-induced acute lung injury via regulationof HMGB1-RAGE pathways. Int Immunopharmacol, 34, 114-128.
16. Derosa G., Maffioli P., Sahebkar A., 2016. Ellagic acid and its role in chronic diseases. Adv Exp Med Biol, 928, 473-479.
17. Zafrilla P., Ferreres F., Tomas-Barberan FA., 2001. Effect of processing and storage on the antioxidant ellagic acid derivatives and flavonoids of red raspberry (Rubus idaeus) jams. J Agric Food Chem, 49, 3651-3655.
18. Seeram NP., Zhang Y., McKeever R., Henning SM., Lee RP., Suchard M., Li Z., Chen S., Thames G., Zerlin A., Nguyen M., Wang D., Dreher M., Heber D., 2008. Pomegranate juice and extracts provide similar levels of plasma and urinary ellagitannin metabolites in human subjects. J Med Food, 11, 390-394.
19. Salem AM., Mohammaden TF., Ali MAM., Mohamed EA., Hasan HF., 2016. Ellagic and ferulic acids alleviate gamma radiation and aluminium chloride-induced oxidative damage. Life Sci, 160, 2-11.
20. Masamune A., Satoh M., Kikuta K., Suzuki N., Satoh K., Shimosegawa T., 2005. Ellagic acid blocks activation of pancreatic stellate cells. Biochem Pharmacol, 70, 869-878.

21. Favarin CD., Teixeira MM., Lemos de Andrade E., de Freitas Alves C., Lazo Chica JE., Sorgi CA., Faccioli LH., Rogerio AP., 2013. Anti-inflammatory effects of ellagic acid on acute lung injury induced by acid in mice. Mediators Inflamm, 2013, 1-13.
22. Ekinci AFN., Tanyeli A., 2019. The antioxidant effect of fraxin against acute organ damage in polymicrobial sepsis model induced cecal ligation and puncture. TJOS, 4, 22-29.
23. Ohkawa H., Ohishi N., Yagi K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 95, 351-358.
24. Erel O., 2005. A new automated colorimetric method for measuring total oxidant status. Clin Biochem, 38, 1103-1111.
25. Bradley PP., Priebat DA., Christensen RD., Rothstein G., 1982. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol, 78, 206-209.
26. Sun Y., Oberley LW., Li Y., 1988. A simple method for clinical assay of superoxide dismutase. Clin Chem, 34, 497-500.
27. Lo S., Yuan SS., Hsu C., Cheng YJ., Chang YF., Hsueh HW., Lee PH., Hsieh YC., 2013. Lc3 over-expression improves survival and attenuates lung injury through increasing autophagosomal clearance in septic mice. Ann Surg, 257, 352-363.
28. Rittirsch D., Flierl MA., Ward PA., 2008. Harmful molecular mechanisms in sepsis. Nat Rev Immunol, 8, 776-787.
29. Deutschman CS., Tracey KJ., 2014. Sepsis: current dogma and new perspectives. Immunity, 40, 463-475.
30. Napolitano LM., 2018. Sepsis 2018: Definitions and guideline changes. Surg Infect (Larchmt), 19, 117-125.
31. Ware LB., 2006. Pathophysiology of acute lung injury and the acute respiratory distress syndrome. Semin Respir Crit Care Med, 27, 337-349.
32. Polat B., Cadirci E., Halici Z., Bayir Y., Unal D., Bilgin BC., Yuksel TN., Vancelik S., 2013. The protective effect of amiodarone in lung tissue of cecal ligation and puncture-induced septic rats: a perspective from inflammatory cytokine release and oxidative stress. Naunyn Schmiedebergs Arch Pharmacol, 386, 635-643.
33. Butt Y., Kurdowska A., Allen TC., 2016. Acute lung injury: A clinical and molecular review. Arch Pathol Lab Med, 140, 345-350.
34. Akpinar E., Halici Z., Cadirci E., Bayir Y., Karakus E., Calik M., Topcu A., Polat B., 2014. What is the role of renin inhibition during rat septic conditions: preventive effect of aliskiren on sepsis-induced lung injury. Naunyn Schmiedebergs Arch Pharmacol, 387, 969-978.
35. Guo RF., Ward PA., 2007. Role of oxidants in lung injury during sepsis. Antioxid Redox Signal, 9, 1991-2002.
36. Macdonald J., Galley HF., Webster NR., 2003. Oxidative stress and gene expression in sepsis. Br J Anaesth, 90, 221-232.
37. Gough DR., Cotter TG., 2011. Hydrogen peroxide: a Jekyll and Hyde signalling molecule. Cell Death Dis, 2, 213-221.
38. Halliwell B., Aeschbach R., Löliger J., Aruoma OI., 1995. The characterization of antioxidants. Food Chem Toxicol, 33, 601-617.

39. Keith ES., Powers JJ., 1965. Effect of phenolic acids and esters on respiration and reproduction of bacteria in urine. Appl Microbiol, 13, 308-313.
40. Cadirci E., Altunkaynak BZ., Halici Z., Odabasoglu F., Uyanik MH., Gundogdu C., Suleyman G., Halici M., Albayrak M., Unal B., 2010. Alpha-lipoic acid as a potential target for the treatment of lung injury caused by cecal ligation and puncture-induced sepsis model in rats. Shock, 33, 479-484.
41. Akdis M., Aab A., Altunbulakli C., Azkur K., Costa RA., Crameri R., Duan S., Eiwegger T., Eljaszewicz A., Ferstl R., Frei R., Garbani M., Globinska A., Hess L., Huitema C., Kubo T., Komlosi Z., Konieczna P., Kovacs N., Kucuksezer UC., Meyer N., Morita H., Olzhausen J., O'Mahony L., Pezer M., Prati M., Rebane A., Rhyner C., Rinaldi A., Sokolowska M., Stanic B., Sugita K., Treis A., Veen W., Wanke K., Wawrzyniak M., Wawrzyniak P., Wirz OF., Zakzuk JS., Akdis CA., 2016. Interleukins (from IL-1 to IL-38), interferons, transforming growth factor β, and TNF-α: Receptors, functions, and roles in diseases. J Allergy Clin Immunol, 138, 984-1010.
42. Li JY., Wu HX., Yang G., 2017. Pachymic acid improves survival and attenuates acute lung injury in septic rats induced by cecal ligation and puncture. Eur Rev Med Pharmacol Sci, 21, 1904-1910.
43. Schulte W., Bernhagen J., Bucala R., 2013. Cytokines in sepsis: potent immunoregulators and potential therapeutic targets-an updated view. Mediators Inflamm, 2013, 1-16.
44. Sadasivan S., Waghray A., Larner SF., Dunn WA., Hayes RL., Wang KK., 2006. Amino acid starvation induced autophagic cell death in PC-12 cells: evidence for activation of caspase-3 but not calpain-1. Apoptosis, 11, 1573-1582.
45. Jiang Y., Gao M., Wang W., Lang Y., Tong Z, Kangkai Wang K., Zhang H., Chen G., Liu M., Yao Y., Xiao X., 2015. Sinomenine hydrochloride protects against polymicrobial sepsis via autophagy. Int J Mol Sci, 16, 2559-2573.
46. Wan SX., Shi B., Lou XL., Liu JQ., Ma GG., Liang DY., Ma S., 2016. Ghrelin protects small intestinal epithelium against sepsis-induced injury by enhancing the autophagy of intestinal epithelial cells. Biomed Pharmacother, 83, 1315-1320.
47. Sirois I., Groleau J., Pallet N., Brassard N., Hamelin K., Londono I., Pshezhetsky AV., Bendayan M., Hébert MJ., 2012. Caspase activation regulates the extracellular export of autophagic vacuoles. Autophagy, 8, 927-37.