Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2022;47(3):1137-1146
DOI: 10.17826/cumj.1124641

ARAŞTIRMA / RESEARCH

Anti-inflammatory and anti-apoptotic effects of naringin on bacterial endotoxin-induced small intestine damage in rats

Naringinin ratlarda bakteriyel endotoksin kaynaklı ince bağırsak hasarı üzerindeki anti-inflamatuvar ve anti-apoptotik etkileri

Ali Tuğrul Akın[1,2], Mohamed Lemine El Bechir[3], Emin Kaymak[4], Tayfun Ceylan[5], Meryem Sayan[6], Necla Değer[6], Derya Karabulut[7], Ayşe Toluk[1]

[1]Erciyes University, Faculty of Science, Department of Biology, Kayseri, Turkey
[2]Drug Application and Research Center, Erciyes University, Kayseri, Turkey
[3]Erciyes University, Graduate School of Natural and Applied Sciences, Biology Department, Kayseri, Turkey
[4]Yozgat Bozok University, Faculty of Medicine, Department of Histology&Embryology, Yozgat, Turkey
[5]Program of Pathology Laboratory Techniques, Kapadokya Vocational High School, Kapadokya University, Nevsehir, Turkey
[6]Erciyes University, Graduate School of Health Sciences, Histology&Embryology Department, Kayseri, Turkey
[7]Erciyes University, Faculty of Medicine, Department of Histology&Embryology, Kayseri, Turkey

Cukurova Medical Journal 2022;47(3):1137-1146

Abstract

Purpose: The aim of this study is to investigate the anti-inflammatory and anti-apoptotic effects of naringin (NRG), which has many biological properties, on bacterial endotoxin-induced small intestine damage in rats.

Materials and Methods: For this purpose, 40 female Wistar albino rats were divided into 4 groups as Control (group given no treatment), LPS (group given 10 mg/kg/i.p lipopolysaccharide), NRG (group given 100 mg/kg/i.p naringin for 14 days) and LPS + NRG (group given 100 mg/kg/i.p naringin for 14 days before 10 mg/kg/i.p lipopolysaccharide injection). After experimental procedure, small intestine tissues of animals were extracted and prepared according to tissue processing protocol. Hematoxylin and Eosin staining were performed to evaluate the histopathological changes and histological damage scoring was applied to compare experimental groups in terms of histopathological changes. Moreover, TNF-α and Caspase-3 expression levels were detected by immunohistochemical staining and the density of immunoreactivity were scored to determine the difference in the expression levels of TNF-α and Caspase-3 expressions among groups.

Results: Epithelial and Brunner's gland damage, mononuclear cell infiltration, hemorrhage, and TNF-α and Caspase-3 expressions significantly increased in the LPS group. However, NRG administrations exerted a

Öz

Amaç. Bu çalışmanın amacı, birçok biyolojik özelliği bulunan naringinin (NRG) ratlarda bakteriyel endotoksin kaynaklı ince bağırsak hasarı üzerine anti-inflamatuar ve antiapoptotik etkilerinin araştırılmasıdır.

Gereç ve Yöntem: Bu amaçla, 40 adet dişi Wistar albino ırkı rat 4 gruba ayrılmıştır: Kontrol (hiçbir uygulama yapılmayan grup), LPS (10 mg/kg/ip lipopolisakkarit uygulanan grup), NRG (14 gün boyunca 100 mg/kg/ip naringin uygulanan grup) ve LPS+NRG (10 mg/kg/ip lipopolisakkarit uygulamasından önce 14 gün boyunca naringin uygulanan grup). Deneysel prosedürün uygulanmasından sonra, deney hayvanlarının ince barsak dokuları çıkarıldı ve doku takibi protokolüne göre hazırlandı. Barsak dokusundaki histopatolojik değişiklikleri değerlendirmek amacıyla Hematoksilen-Eozin boyaması gerçekleştirildi ve histopatolojik değişiklikler açısından deney gruplarının karşılaştırılması amacıyla hasar skorlaması yapıldı. Ayrıca, immunohistokimyasal boyamalar ile TNF-α ve Kaspaz-3 ekspresyon seviyeleri belirlendi ve gruplar arasında bu proteinlerin ekspresyon seviyelerindeki değişikliklerin belirlenmesi için immunohistokimyasal boyanma yoğunluğu skorlandı.

Bulgular: LPS grubunda epitel ve Brunner bezlerinde hasar, mononüklear hücre infiltrasyonu, hemorajik alanlar belirlendi. Ayrıca TNF-α ve Kaspaz-3 ekspresyonları bu grupta anlamlı bir şekilde arttı. Ancak, NRG uygulamaları bu parametreler açısından LPS+NRG grubundaki deney

Yazışma Adresi/Address for Correspondence: Dr. Ali Tuğrul Akin, Erciyes University, Faculty of Science, Department of Biology, Kayseri, Turkey E-mail: tuqrul.akin@gmail.com
Geliş tarihi/Received: 03.06.2022 Kabul tarihi/Accepted: 03.08.2022

strong protective effect on the small intestine tissues in terms of these parameters in LPS+NRG group.
Conclusion: This study demonstrated that 100 mg/kg NRG injection can be regarded as a protective agent against negative effects of endotoxin-induced infection on the intestinal mucosa and that it should not be disregarded in further clinical trials.
Keywords:. Endotoxins, lipopolysaccharide, naringin, small intestine damage.

hayvanlarının ince barsak dokusunda güçlü bir koruyucu etki gösterdi.
Sonuç: Bu çalışma, 100 mg/kg NRG enjeksiyonunun endotoksin kaynaklı enfeksiyonun bağırsak mukozası üzerindeki olumsuz etkilerine karşı koruyucu bir ajan olarak kabul edilebileceğini ve daha ileri klinik çalışmalarda göz ardı edilmemesi gerektiğini göstermiştir.
Anahtar kelimeler: Endotoksin, ince barsak hasarı, lipopolisakkarit, naringin.

INTRODUCTION

Inflammation is a complicated process that is induced by a variety of external and internal factors and situations, including infectious microorganisms, tissue damage, and aging[1,2]. Significant progresses have been achieved in gaining a new perspective for understanding of the processes of inflammation resulting in the inflammatory response to infectious microorganisms or tissue damage. Inflammation dysregulation during infection or tissue injury is a long and complicated process with no apparent cause. The main endogenous molecular component of gram-negative bacteria's cell wall is lipopolysaccharide (LPS), often known as endotoxin[3]. LPS is frequently administered as an external inflammatory trigger[4]. LPS-induced endotoxemia is a useful tool for studying the process of inflammation[5,6].

Endotoxin-induced toxicity has been referred to tissue injury in the liver, kidney, and brain, as well as infection, which can lead to sepsis and a high rate of morbidity and mortality[7]. LPS induces tissue deterioration in several tissues by stimulating inflammatory cells to produce high quantities of inflammation-related cytokines (cytokine storm) such as interleukin (IL)-1, IL-6, and Tumor Necrosis Factor-alpha (TNF-α)[8]. The cytokine storm (CS) in the inflammation is characterized by overexpressions of cytokines, triggering the innate and adaptive immune cells and induction of apoptosis[9]. Many studies have reported that LPS-induced cytokine storm drives cells to apoptosis in experimental rat models[10].

The intestine regulates water, electrolytes, and nutrient transfer, among other things. There is a very strong connection between epithelial cells of the intestinal mucosa and the lumen of the digestive tract to maintain these activities. Epithelial cells must prevent bacterial and antigen loading from the gastrointestinal lumen into other tissues of the gastrointestinal tract as a result of these interactions[11]. For the mechanical and physiological operation of the intestinal mucosal barrier, the entire intestinal epithelium must be present. By using selective permeability, intestinal epithelial cells limit the movement of foreign and harmful substances from the gastrointestinal lumen into other tissues while allowing the free passage of nutrients[12].

Many researches have reported that the immunomodulatory function of intestinal barrier is maintained by an organization of several cytokines such as ILs, IFNs and TNF-α[13]. Hence, the imbalance between pro- and anti-inflammatory cytokines can be harmful to intestinal mucosa. It is shown that LPS-induced inflammation can increase permeability of intestinal mucosa *in vivo* and it can induce the activation of inner immune cells. Activation of the immune cells leads the mucosal immune system response[14]. TNF-α, IL-6, and IFN-γ are major components of inflammation and determine the severity of inflammation[15].

Naringin (NRG, Figure 1), a flavanone glycoside consisting of the flavanone naringenin and the disaccharide neohesperidose, is one of the main biologically active components in Chinese herbal medicines like *Drynaria fortunei* (Kunze) J. Sm. (DF), *Citrus aurantium* L. (CA), and *Citrus medica* L. (CM). It's also detected in citrus fruits, giving them a bitter flavor[16]. Antioxidant, anticancer, antiulcer, antibacterial, anti-inflammatory, antiapoptotic, hepatoprotective, antidiabetic, and anti-hypolipidemic are among the active pharmacological properties of NRG[17-20].

We know that cytokines are overexpressed when inflammation is triggered by various reasons, and apoptosis is related to increased inflammatory response in many cells. Thus, it is inevitable that LPS-induced inflammation in the small intestine will not induce the increased expression levels of cytokines like TNF-α and apoptotic pathways including Caspase-3. Thus, we hypothesized that naringin

which has many biological properties such as anti-inflammatory, anti-oxidant, and anti-apoptotic exerts a protective effect against LPS-induced intestinal damage. Following a thorough study of the literature, we found out that research on intestinal damage induced by bacterial endotoxins such as LPS are insufficient. Therefore, we investigated the intestinal tissue damage by histological analysis and showed the LPS-induced inflammation and apoptosis by detecting the alterations in the immunoreactivities of TNF-a and Caspase-3. Moreover, there are a few studies demonstrating the protective effects of naringin in the LPS-induced small intestine damage. Thus, this study tried to reveal the potential protective properties of NRG on LPS-induced intestinal damage via assessment of inflammation and apoptosis.

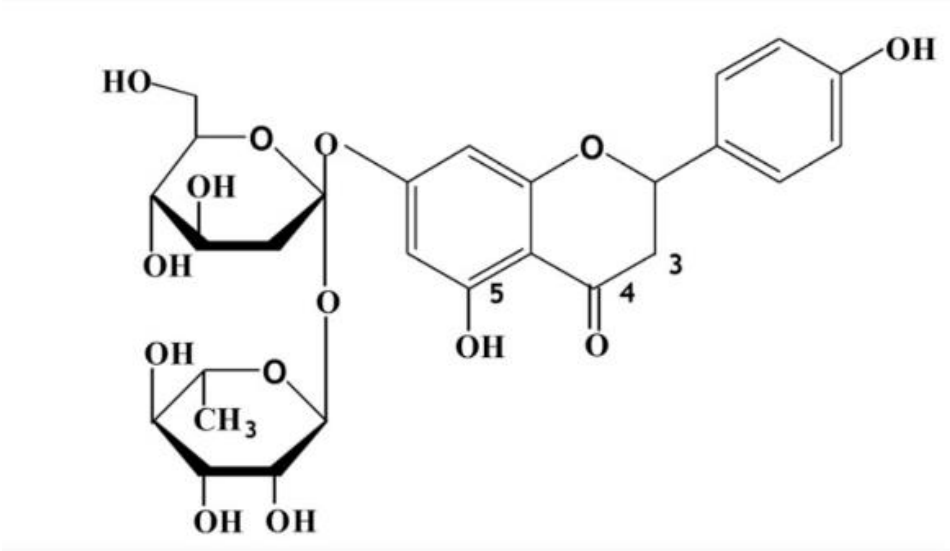

Figure 1. Chemical structure of naringin.

Table 1. Histopathological and immunoreactivity scores among experimental groups.

Groups	Control	LPS	NRG	LPS + NRG	*p*
Score of damage	0.25±0,050	1.32±0.073*	0.25±0.052	0.57±0.066*#	0.001
TNF-α	0.14±0.034	0.88±0.051*	0.21±0.040	0.23±0.041	0.001
Caspase-3	1.21±0.046	2.58±0.057*	1.33±0.057	1.44±0.052*#	0.001

Data are expressed as mean±SEM. Significancy among groups were considered when $p<0.05$. * shows statistically significant difference between Control and other groups.; # shows statistically significant difference when compared with LPS group.; LPS: Lipopolysaccharide; NRG: Naringin; TNF-α: Tumor necrosis factor-alpha.

MATERIALS AND METHODS

Animals

This study was conceived and carried out at Erciyes University's Drug Application and Research Center in Turkey. The Experimental Animal and Local Ethics Committee of Erciyes University approved the experimental procedure used in this investigation, which was given the number 22/108/2022. Hakan Cetinsaya Experimental and Clinic Research Center, Erciyes University, provided a total of 40 female Wistar albino rats (8 weeks old, weighing 200250 g). The rats were housed in cages in the typical order of the day, at 21°C and 12 hours of light/dark, with their water and nutrient needs met ad libitum.

Chemicals

Naringin (NRG) was purchased from Sigma-Aldrich (San Louis, MO, 71162-100G). The purity of powdered NRG was approximately 90% according to

the manufacturer's statement and it was dissolved in methanol and diluted in serum physiologic solution for the intraperitoneal injections to experimental animals according to literature [21]. LPS was also purchased from Sigma-Aldrich (San Louis, MO, L4130-100G) and distilled water was used as a solvent to prepare LPS for administrations.

Experimental protocol

The sample size of this experimental study was calculated by power analysis using the G*Power v3.1 software. There was a total of 40 rats in 4 groups, 90.94% power expectation was found with 10 rats in each group. The count of animals in experimental groups was determined according to these results.

The rats were put into four groups at random, each with ten rats. The following are the groups that were formed:

1. Control group (n=10): no treatment.
2. LPS group (n=10): 10 mg/kg lipopolysaccharide was intraperitoneally administered to rats six hours before scarification[22].
3. NRG group (n=10): 100 mg/kg naringin was intraperitoneally administered to rats for 14 days[23].
4. LPS +NRG group (n=10): For 14 days, 100 mg/kg naringin was injected to rats intraperitoneally and 10 mg/kg lipopolysaccharide was administered 30 minutes after the last naringin administration.

Six hours following LPS treatment, animals were sedated with Ketamine (70 mg/bw) and Xylazine (10 mg/bw), and small intestinal tissues were removed for histological and immunohistochemical analyses.

Histological evaluation

The small intestine tissues were histologically evaluated using standard histological techniques. For 24–48 hours, tissues were fixed in 10% formaldehyde, dehydrated with an alcohol series, cleared with xylene, and embedded in paraffin blocks. They were then cut into 5- µm thick sections.

Hematoxylin-eosin (H&E) staining

The histological alterations in the intestinal tissue were determined using hematoxylin and eosin (H&E) staining[24]. Images were captured and processed using a light microscope (Leica DM IL LED; Leica Microsystems, Germany). The study group looked at the structure of the small intestinal tissue.

Histopathological score

Changes in intestinal morphology were graded on a scale of 0 to 3 based on histological findings such as intestinal epithelial degeneration, bleeding, and Brunner's gland damage. The data was evaluated to see how much damage the experimental groups had to their intestines[10].

Immunohistochemistry

The immunohistochemistry method was applied to determine the alterations in the expression levels of TNF-α and Caspase-3 antibodies as described in earlier studies of our research team[25,26]. The blocks of paraffin were cut into 5 m lengths. Xylene was used for the deparaffinization of the tissues, and they hydrated with an alcohol series. Sections were put in a sterile urine cups containing 0.01 M citrate buffer and heated in a microwave oven at 350 W for antigen retrieval. Phosphate-buffered saline (PBS) was used to wash the slices three times for five minutes each time. The slices were treated with 3 percent (w/v) H_2O_2 for 10 minutes to reduce endogenous peroxidase activity. After being rewashed three times with PBS and stored in the incubation tank for five minutes, the sections were treated with Ultra V Block solution. TNF-α (Anti TNF- antibody, E-AB-40015, Elapscience, USA) and Caspase-3 (Anti Caspase-3 antibody, E-AB-63602, Elapscience, USA) antibodies diluted in a 1:75 ratio were then applied to the tissues overnight at 4 °C.

Slices were rewashed three times with PBS the next morning before being incubated with the secondary antibody for 10 minutes (TA-125-HDX, Thermo Fisher Scientific, Waltham, MA, USA). The immunoreaction was amplified using streptavidin–avidin–peroxidase solution after rewashing with PBS, and the small intestine sections were seen with 3,3-p-diaminobenzidine tetrahydrochloride (TA-060-HDX, Thermo Fisher Scientific, Waltham, MA, USA). The photographs were taken with a light microscope. At least ten randomly chosen fields in each slide were scored at x20 magnification. Based on histological findings, the immunoreactivity was evaluated on a scale of 0 to 3 [10], with 0 denoting no staining and 1, 2, and 3 denoting less staining, moderate staining, and high staining, respectively.

Statistical analysis

All quantitative data were statistically analyzed via using GraphPad Prism v9.0 for MacOS (GraphPad Software, La Jolla, California, USA). To determine the data's normal distribution, the D'Agostino Pearson omnibus test was performed. In the situation of normal distribution, comparison of the quantitative variables was determined using one-way analysis of variance (ANOVA) and Tukey's post-hoc test. When data showed abnormal distribution, Kruskal-Wallis test were performed to analyze variables and Tukey's post-hoc test applied to determine the changes among groups. To express data with a normal distribution, the mean and standard error of the mean (SEM) were given. $p<0.05$ was used to determine statistically significant differences.

RESULTS

Intestinal mucosa presented a normal histological morphology in the Control and NRG group and there were no detected histopathological changes in the intestinal mucosa and villi. In the LPS group, it is observed several haemorrhagic regions and increased degeneration of epithelial cells and Brunner's glands and mononuclear cell infiltration, and scoring performed in terms of these criteria were importantly higher in this group than Control and NRG groups ($p<0.0001$). However, score of damage were substantially less in the LPS+NRG group than LPS group ($p<0.0001$) and relatively similar to Control and NRG groups suggesting the protective effect of NRG against LPS administrations (Figure 2).

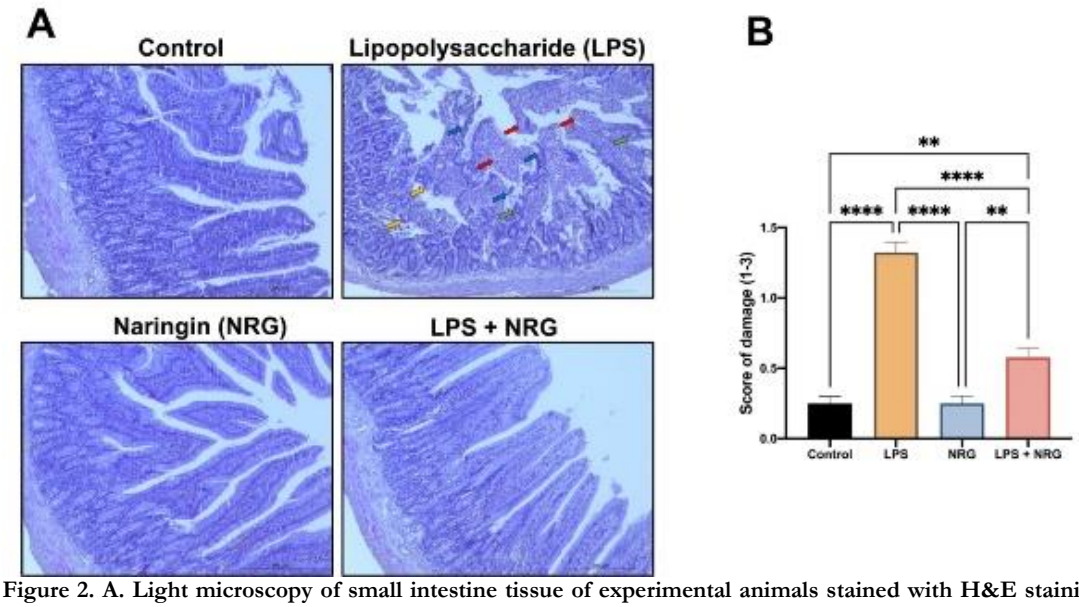

Figure 2. A. Light microscopy of small intestine tissue of experimental animals stained with H&E staining protocol. Control group and NRG group showed normal histological tissue structure. In the LPS group, haemorrhagic regions (red arrows) and increased degeneration of epithelial cells (blue arrows) and Brunner's glands (yellow arrows) and mononuclear cell infiltration (green arrows). Unlikely, in the LPS+NRG group, it is clearly seen the damage were substantially less when compared to LPS group**. B. Statistical analysis of score of damage in the small intestine tissue according to criteria mentioned above.** It is clearly seen that the score of damage were significantly higher in the LPS group when compared with the other experimental groups ($p<0.0001$). Scale bar = 200 µm. *= $p<0.05$, **= $p<0.01$, ***= $p<0.001$, ****= $p<0.0001$. Abbreviations: H&E, hematoxylin-eosin; LPS, lipopolysaccharide; NRG, naringin.

TNF-α and Caspase-3 expressions were observed in the intestinal epithelium. In the LPS group, TNF-α expressions substantially raised compared to Control and NRG groups ($p<0.0001$). However, In the LPS+NRG group, TNF-α expressions were statistically lower when compared with those in the LPS group ($p<0.0001$) and were similar to the expression levels in the Control and NRG groups (Figure 3).

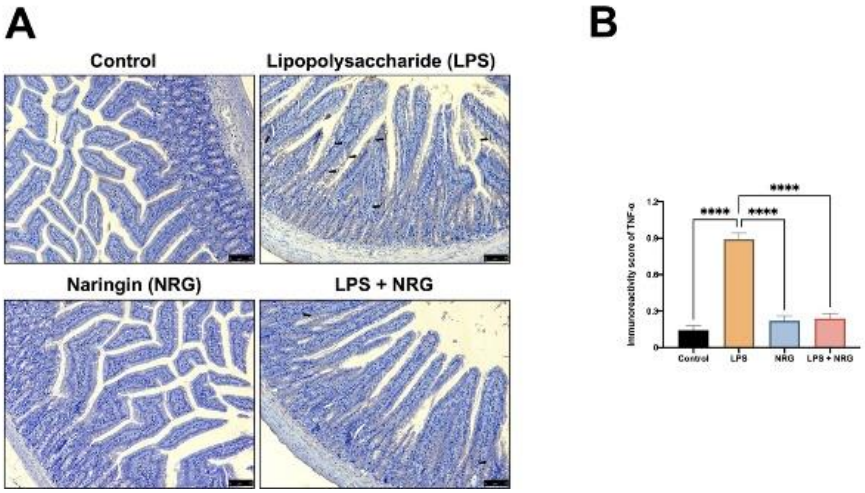

Figure 3. A. Immunohistochemical staining of TNF-α in small intestine sections. Control and NRG groups showed weak TNF-α immunostaining in mucosal and submucosal regions of small intestine tissue. In the LPS group, TNF-α expressions increased in both mucosa and submucosa. However, LPS+NRG group, showed less immunoreactivity and were similar to Control and NRG group. Black arrows show the immunohistochemically stained areas**. B. Statistical analysis of the immunoreactivity score among experimental groups.** It can be seen that the immunoreactivity score of TNF-α were significantly higher in the LPS group when compared with the other experimental groups ($p<0.0001$). Moreover, the scoring of the immunohistochemically stained areas in the LPS+NRG group were significantly less than those in the LPS group ($p<0.0001$). Scale bar: 75 µm. Abbreviations: LPS, lipopolysaccharide; NRG, naringin; TNF-α, Tumor Necrosis Factor-alpha.

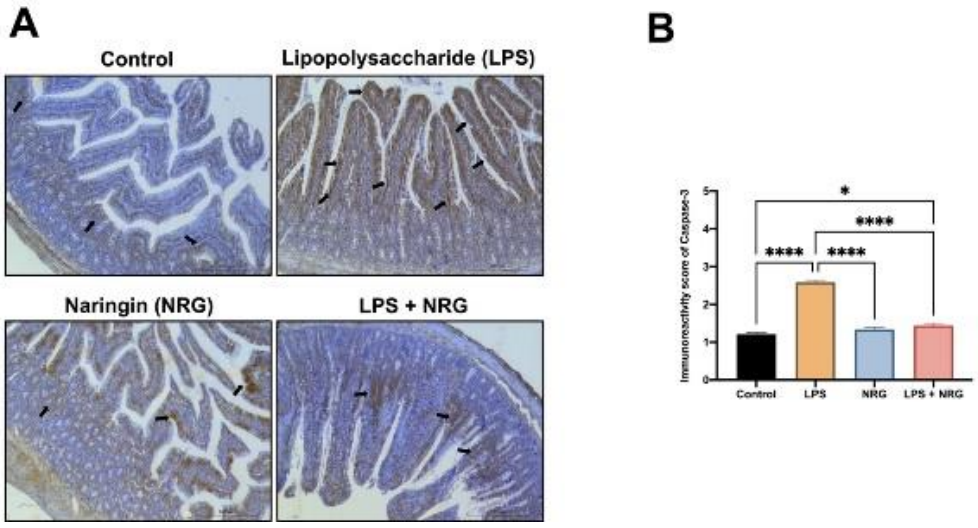

Figure 4. A. Caspase-3 immunostaining of small intestine tissues of the experimental groups. Control and NRG groups showed weak Caspase-3 immunoreactivity in both mucosa and submucosa of small intestine tissue. LPS group presented strong Caspase-3 immunoreactivity. However, in the LPS+NRG group, Caspase-3 immunoreactivity were substantially less compared to those in the LPS group. Black arrows show the immunohistochemically stained areas. **B. Statistical analysis of the immunoreactivity score of Caspase-in the intestinal tissue.** The immunoreactivity score of Caspase-3 were significantly much higher in the LPS group compared to the other groups ($p<0.0001$). Moreover, the Caspase-3 scoring of the stained areas in the LPS+NRG group were significantly less than those in the LPS group ($p<0.0001$). Scale bar: 200 µm. Abbreviations: LPS, lipopolysaccharide; NRG, naringin.

The expression levels of Caspase-3 are also significantly increased in the LPS group when compared with Control and NRG groups ($p<0.0001$). Similarly, NRG administrations were significantly preserved the intestinal tissue against increased Caspase-3 expressions in the LPS+NRG group compared to LPS group ($p<0.0001$). The differences in the expression levels of Caspase-3 and the statistical analysis of immunoreactivity score of experimental groups are presented in the Figure 4.

DISCUSSION

Sepsis is considered as an infection lead to systemic inflammatory response syndrome[27]. Severe sepsis in organisms invariably causes organ failure or tissue hypoperfusion[28]. Among this damage, intestinal damage or dysfunction is one of the most common complications of sepsis in organisms. During systemic inflammation, the intestinal tissue is both damaged and the major cause of the accelerated process of sepsis[29]. Injuries or dysfunction in the intestinal tissue can result in bacterial translocation and a large release of intestine-derived inflammatory factors into the systemic circulation via lymphatic and portal routes. These bacterial and nonbacterial mechanisms may contribute to disease, including villous epithelial lifting and enterocyte necrosis, as well as promoting the dysfunction of other organs[30].

Several studies have reported that endotoxin-induced sepsis in experimental animals caused a significant damage in the intestinal tissue. Some studies have showed that sepsis induced by LPS administrations causes a lot of histopathological changes such as subepithelial space development, congestion, and inflammatory cells in the lamina propria in the small intestine tissues of experimental animals[31]. There are other studies reporting that LPS-induced infection significantly decreased the villus-to-crypt ratio in the duodenum and ileum in rats[32].

When the literature is carefully examined, very few studies have been found showing that LPS induces serious injury in the small intestine tissue. In addition, there are very few studies investigating the ameliorative effects of NRG, which has a lot of biological activity such as anti-inflammatory, antioxidant and anti-apoptotic, in intestinal tissue through inflammation and apoptotic pathways. Therefore, the presented study tried to determine the potential protective effect of NRG on LPS-induced small intestine damage. Consistent with the literature, LPS-induced infection caused a significant damage including haemorrhage and degeneration of epithelial cells and Brunner's glands in the LPS administered experimental animals. On the other hand, we observed that NRG administrations had a significant protective effect against LPS-induced infection in the intestinal tissues of the LPS+NRG group. According to these results, we suggest that NRG has a strong protective effect against the intestinal damage caused by endotoxin-induced infection and no side effect on healthy intestinal tissue at the dose of 100 mg/kg.

By generating pro-inflammatory and chemotactic cytokines, which act as tissue resident watchdogs, intestinal epithelial cells play a key role in triggering of inflammatory response against endotoxins. TNF-α regulates intracellular signalling pathways involved in cell survival, death, and proliferation, as well as mucus secretion and paracellular flow via tight junction control. TNF-α is therefore important not only for maintaining and repairing the intestinal mucosa, but also for guiding cell recruitment[33]. *In vitro* studies have reported that TNF-α expressions substantially increase in the epithelial cells of intestinal mucosa in a model of LPS-induced infection[14,34]. In addition, there are other *in vivo* studies showing that TNF-α expressions significantly increase in both plasma[35] and the small intestine tissue[36] in the experimental sepsis model induced by LPS administrations. In our study, the increased TNF-α immunoreactivity in the intestinal tissues of the animals in the LPS group shows that LPS administrations induced TNF-α overexpression by causing a serious inflammation in the intestinal tissue. However, our hypothesis suggesting that NRG may exert a potential protective effect against LPS-induced intestinal injury in the experimental rat model was confirmed by the clearly seen lower expression levels of TNF-α in the intestinal tissue in the LPS+NRG group. Therefore, we think that the biologically protective property of NRG at the dose of 100 mg/kg includes a strong anti-inflammatory effect against LPS-induced intestinal damage in the experimental sepsis model. These results suggest that NRG can be considered as a protective agent to prevent intestinal tissue from LPS-induced intestinal damage.

Caspase-3 is one of the executioner caspases, and it can be activated by initiator caspases (Caspase-8, Caspase-9, or Caspase-10). It also causes the cytoskeleton to reorganize and the cell to disintegrate into apoptotic bodies [37]. While some studies have

demonstrated that Caspase-3 expressions significantly raised in the small intestine tissues of LPS-administered animals[38], there are a few studies showing that LPS-induced infection increases the expression levels of Caspase-3 in the intestinal mucosa of experimental animals, that's why we wanted to contribute to literature by demonstrating the Caspase-3 immunoreactivity in the LPS-induced experimental intestinal injury. In addition, *In vivo* studies have also showed that LPS administrations caused a serious increase in the Caspase-3 expressions in the cultured small intestine epithelial cell line used as a model for induction epithelial cell injury[39,40]. In our study, we determined that Caspase-3 were overexpressed in the small intestine tissues of animals in the LPS group. Since increased Caspase-3 expression in healthy tissues indicates that a severe apoptosis is induced due to damage in this tissue, we think that small intestinal epithelial cells are severely damaged due to increased apoptosis during LPS-induced infection. On the contrary, we think that the significant decrease in Caspase-3 immunoreactivity in the NRG-treated group when compared with the LPS group is because NRG suppresses the inflammatory response by preventing the increase in TNF-α expressions during LPS-induced infection, thus preventing TNF-α -induced Caspase-3 overexpression. Therefore, we suggest that suppressed overexpression of Caspase-3 lead to decreased apoptosis in the intestinal tissue and NRG exerted a promising protective effect at the dose of 100 mg/kg via suppressing apoptotic pathways in the intestinal tissues of the animals in the LPS+NRG group.

In conclusion, LPS-induced sepsis triggered several histopathological changes in the small intestine tissues of animals in this study. Overexpression of TNF-α and Caspase-3 in the small intestinal tissues of LPS treated rats indicate an inflammatory response against infection, and activation of apoptotic pathways. According to our histopathological and immunohistochemical analysis, NRG exerted a protective effect at the dose of 100 mg/kg against LPS-induced intestinal damage via reducing the inflammatory response and suppressing apoptosis in the intestinal tissue. But still, there are some limitations of this study. For example, we believe that sepsis-induced inflammation is a rapidly progressive process and that the level of damage caused by infection may vary depending on individual differences in experimental animals. In addition, in future studies, changes in the expression levels of other markers that play important roles in inflammation and apoptotic pathways should be examined to reveal the anti-inflammatory and anti-apoptotic effects of naringin in a more comprehensive manner. In the presented study, it has been showed that 100 mg/kg NRG injection can be considered as a protective treatment for the elimination of the negative effects of endotoxin-induced infection on the intestinal mucosa and it should not be disregarded in the further clinical studies.

Yazar Katkıları: Çalışma konsepti/Tasarımı: ATA, EK; Veri toplama: ATA, MLEB; Veri analizi ve yorumlama: ATA, TC; Yazı taslağı: ATA, MS; İçeriğin eleştirel incelenmesi: DK; Son onay ve sorumluluk: ATA, MLEB, EK, TC, MS, ND, DK, AT; Teknik ve malzeme desteği: AT; Süpervizyon: DK, AT; Fon sağlama (mevcut ise): yok.
Etik Onay: Bu çalışma için Erciyes Üniversitesi Hayvan Deneyleri Yerel Etik Kurulundan 05.05.2021 tarih ve 21/02-05 sayılı kararı ile etik onay alınmıştır.
Hakem Değerlendirmesi: Dış bağımsız.
Çıkar Çatışması: Yazarlar çıkar çatışması olmadığını beyan etmişlerdir.
Finansal Destek: Yazar(lar), bu makalenin araştırılması, yazarlığı ve/veya yayınlanması için aşağıdaki maddi desteğin alındığını açıklamıştır: Bu deneysel çalışmada kullanılan deney hayvanlarının maliyeti, Erciyes Üniversitesi araştırma ve teknoloji bölümü'nden alınan hibe ile karşılanmıştır.
Yazarın Notu: Hayvanları içeren araştırmalar Bu çalışma, Wistar Albino sıçanları üzerindeki uygulamaları içermektedir. Deneysel bir çalışmaydı.

Author Contributions: Concept/Design : ATA, EK; Data acquisition: ATA, MLEB; ATA, MLEB; Data analysis and interpretation: ATA, TC; Drafting manuscript: ATA, MS; Critical revision of manuscript: DK; Final approval and accountability: ATA, MLEB, EK, TC, MS, ND, DK, AT; Technical or material support: AT; Supervision: DK, AT; Securing funding (if available): n/a.
Ethical Approval: Ethical approval was obtained from Çukurova University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee for this study. (With the date 6.3.2020 and the number 97/1).
Peer-review: Externally peer-reviewed.
Conflict of Interest: Authors declared no conflict of interest.
Financial Disclosure: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The cost of the experimental animals used in this experimental study has been covered by a grant from the research and technology department of Erciyes University.
Acknowledgement: Research involving animals This study includes applications on Wistar Albino rats. It was an experimental study.

REFERENCES

1. Zhang X, Meng X, Chen Y, Leng SX, Zhang H. The biology of aging and cancer: Frailty, inflammation, and immunity. Cancer J. 2017;23:201-5.
2. Bektas A, Schurman SH, Sen R, Ferrucci L. Aging, inflammation and the environment. Exp Gerontol. 2018;105:10-18.
3. Ciesielska A, Matyjek M, Kwiatkowska K. TLR4 and CD14 trafficking and its influence on LPS-induced pro-inflammatory signaling. Cell Mol Life Sci. 2021;78:1233-61.
4. Fritsche KL. The science of fatty acids and inflammation. Adv Nutr. 2015;6:293-301.

5. Płóciennikowska A, Hromada-Judycka A, Borzęcka K, Kwiatkowska K. Co-operation of TLR4 and raft proteins in LPS-induced pro-inflammatory signaling. Cell Mol Life Sci. 2015;72:557-81.
6. Kuzmich NN, Sivak KV, Chubarev VN, Porozov YB, Savateeva-Lyubimova TN, Peri F. TLR4 signaling pathway modulators as potential therapeutics in inflammation and sepsis. Vaccines (Basel). 2017;5:34.
7. Anderson ST, Commins S, Moynagh PN, Coogan AN. Lipopolysaccharide-induced sepsis induces long-lasting affective changes in the mouse. Brain Behav Immun. 2015;43:98-109.
8. Bae JY, Lee DS, Cho YK, Lee JY, Park JH, Lee SH. Daphne jejudoensis Attenuates LPS-induced inflammation by inhibiting TNF-α, IL-1β, IL-6, iNOS, and COX-2 expression in periodontal ligament cells. Pharmaceuticals (Basel). 2022;15:387.
9. Kuypers FA. Hyperinflammation, apoptosis, and organ damage. Exp Biol Med (Maywood). 2022;247:1112-23.
10. Akin AT, Kaymak E, Öztürk E, Ceylan T, Yalçın B, Başaran KE et al. Mitigative effects of chloroquine treatment against hypoxia-induced intestinal injury: A histological and immunohistochemical study. Turk Hij Den Biyol Derg. 2022;79:59-70.
11. Shen L. Functional morphology of the gastrointestinal tract. Curr Top Microbiol Immunol. 2009;337:1-35.
12. Tsukita S, Furuse M, Itoh M. Multifunctional strands in tight junctions. Nat Rev Mol Cell Biol. 2001;2:285-93.
13. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature. 2007;448:427-34.
14. Li L, Wan G, Han B, Zhang Z. Echinacoside alleviated LPS-induced cell apoptosis and inflammation in rat intestine epithelial cells by inhibiting the mTOR/STAT3 pathway. Biomed Pharmacother. 2018;104:622-8.
15. Van Deventer SJ. Tumour necrosis factor and Crohn's disease. Gut. 1997;40:443-8.
16. Chen R, Qi Q-L, Wang M-T, Li Q-Y. Therapeutic potential of naringin: an overview. Pharmaceutical Biology. 2016;54:3203-10.
17. Khodayar MJ, Kalantari H, Mahdavinia M, Khorsandi L, Alboghobeish S, Samimi A et al. Protective effect of naringin against BPA-induced cardiotoxicity through prevention of oxidative stress in male Wistar rats. Drug Chem Toxicol. 2020;43:85-95.
18. Caglayan C, Temel Y, Kandemir FM, Yildirim S, Kucukler S. Naringin protects against cyclophosphamide-induced hepatotoxicity and nephrotoxicity through modulation of oxidative stress, inflammation, apoptosis, autophagy, and DNA damage. Environ Sci Pollut Res. 2018;25:20968-84.
19. Cerkezkayabekir A, Sanal F, Bakar E, Ulucam E, Inan M. Naringin protects viscera from ischemia/reperfusion injury by regulating the nitric oxide level in a rat model. Biotechnic & Histochemistry. 2017;92:252-63.
20. Yilmaz D, Teksoy O, Bilaloglu R, Çinkilic N. Anti-genotoxic effect of naringin against bleomycin-induced genomic damage in human lymphocytes in vitro. Drug Chem Toxicol. 2016;39:119-23.
21. Kim HJ, Yong HI, Park S, Kim K, Kim TH, Choe W et al. Effect of atmospheric pressure dielectric barrier discharge plasma on the biological activity of naringin. Food Chem. 2014;160:241-45.
22. Cheng PY, Lee YM, Wu YS, Chang TW, Jin JS, Yen MH. Protective effect of baicalein against endotoxic shock in rats in vivo and in vitro. Biochem Pharmacol. 2007;73:793-804.
23. Koroglu OF, Gunata M, Vardi N, Yildiz A, Ates B, Colak C et al. Protective effects of naringin on valproic acid-induced hepatotoxicity in rats. Tissue Cell. 2021;72:101526.
24. Karabulut D, Akin AT, Unsal M, Lekesizcan A, Ozyazgan TM, Keti DB et al. L-Carnitine ameliorates the liver by regulating alpha-SMA, iNOS, HSP90, HIF-1alpha, and RIP1 expressions of CCL4-toxic rats. Iran J Basic Med Sci. 2021;24:184-90.
25. Kaymak E, Akin AT, Tufan E, Başaran KE, Taheri S, Özdamar S et al. The effect of chloroquine on the TRPC1, TRPC6, and CaSR in the pulmonary artery smooth muscle cells in hypoxia-induced experimental pulmonary artery hypertension. J Biochem Mol Toxicol. 2021;35:e22636.
26. Sönmez MF, Ozdemir Ş, Guzel M, Kaymak E. The ameliorative effects of vinpocetine on apoptosis and HSP-70 expression in testicular torsion in rats. Biotech Histochem. 2017;92:92-9.
27. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Chest. 1992;101:1644-55.
28. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock, 2012. Intensive Care Med. 2013;39:165-228.
29. Zanello M, Vincenzi M, Di Mauro L, Gualdani S. Gut and sepsis: Victim of circumstance or prime mover. Trends Anaesth. Crit. Care. 2013;3:130-4.
30. Deitch EA. Gut-origin sepsis: Evolution of a concept. Surgeon. 2012;10:350-6.
31. Gao X, Hao S, Yan H, Ding W, Li K, Li J. Neutrophil extracellular traps contribute to the intestine damage in endotoxemic rats. J Surg Res. 2015;195:211-8.
32. Deng B, Wu J, Li X, Men X, Xu Z. Probiotics and probiotic metabolic product improved intestinal function and ameliorated LPS-induced injury in rats. Curr Microbiol. 2017;74:1306-15.
33. Stephens M, von der Weid PY. Lipopolysaccharides modulate intestinal epithelial permeability and inflammation in a species-specific manner. Gut Microbes. 2020;11:421-32.

34. Zhou M, Xu W, Wang J, Yan J, Shi Y, Zhang C et al. Boosting mTOR-dependent autophagy via upstream TLR4-MyD88-MAPK signalling and downstream NF-κB pathway quenches intestinal inflammation and oxidative stress injury. EBioMedicine. 2018;35:345-60.
35. Shen L, Zhou Y, Wu X, Sun Y, Xiao T, Gao Y et al. TREM1 Blockade ameliorates lipopolysaccharide-induced acute intestinal dysfunction through inhibiting intestinal apoptosis and inflammation response. Biomed Res Int. 2021;2021:6635452.
36. Liu Y, Meng F, Wang S, Xia S, Wang R. Vitamin D(3) mitigates lipopolysaccharide-induced oxidative stress, tight junction damage and intestinal inflammatory response in yellow catfish, Pelteobagrus fulvidraco. Comp Biochem Physiol C Toxicol Pharmacol. 2021;243:108982.
37. Öztürk E, Kaymak E, Akin AT, Karabulut D, Ünsal HM, Yakan B. Thymoquinone is a protective agent that reduces the negative effects of doxorubicin in rat testis. Hum Exp Toxicol. 2020;39:1364-73.
38. Zhou Y, Yuan HR, Cui L, Ansari AR, Xiao K, Luo Y et al. Effects of visfatin on the apoptosis of intestinal mucosal cells in immunological stressed rats. Acta Histochem. 2017;119:26-31.
39. Cong Z, Ye G, Bian Z, Yu M, Zhong M. Jagged-1 attenuates LPS-induced apoptosis and ROS in rat intestinal epithelial cells. Int J Clin Exp Pathol. 2018;11:3994-4003.
40. Zhang J, Wan J, Chen D, Yu B, He J. Low-molecular-weight chitosan attenuates lipopolysaccharide-induced inflammation in IPEC-J2 cells by inhibiting the Nuclear Factor-κB signalling pathway. Molecules. 2021;26:569.