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Naringin is a Possible Protective Agent in a Rat Model of Polymicrobial Sepsis

Sıçanlarda Oluşturulan Polimikrobiyal Sepsis Modelinde Potansiyel Koruyucu Bir Ajan: Naringin

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ABSTRACT

Objective: The current study aimed to investigate the potential protective effect, the levels of some inflammatory cytokines, and the activities of oxidative stress markers of naringin, which is a flavonoid, on a rat model of polymicrobial sepsis-induced kidney injury.

Method: Thirty ($n=30$) Wistar albino rats were randomly divided into five groups. The inflammatory cytokine levels were measured by enzyme-linked immunosorbent assay technique. Oxidative stress parameters were measured by tissue biochemistry.

Results: Cecal-ligation and puncture sepsis led to a dramatic increase in all pro-inflammatory cytokine levels, and naringin applications led to an important decrease in the levels of inflammatory cytokines in a dose-dependent manner. Additionally, naringin caused an increase in the enzyme activities of malondialdehyde and superoxide dismutase and a decrease in the level of glutathione.

Conclusion: Natural products especially flavonoids have the potential to be a protective agent in sepsis-induced organ injury models. In our study, we preferred to use naringin as a natural agent and detected that it affected the inflammatory cytokine levels and oxidative stress enzyme activities. Further detailed studies should be performed to investigate the molecular mechanisms of the anti-inflammatory and antioxidant effects of naringin.

Keywords: Inflammation, naringin, oxidative stress, rat, sepsis

ÖZ

Amaç: Mevcut çalışma ile sıçanlarda polimikrobiyal sepsis ile indüklenen böbrek hasarında bir flavonoid olan naringin'in koruyucu etkilerinin, proinflamatuar sitokin düzeyleri ve oksidatif stres enzimlerine ait aktivitelerin araştırılması amaçlanmıştır.

Yöntemler: Otuz dişi Wistar albino rat rastgele olarak 5 gruba ayrıldı. Proinflamatuar sitokin seviyeleri ELISA tekniği ile ölçüldü. Oksidatif stres parametreleri ise doku biyokimyası ile ölçüldü.

Bulgular: CLP-sepsis, tüm proinflamatuar sitokin düzeylerinde dramatik bir artışa neden olurken, naringin proinflamatuar sitokin düzeylerinde doza bağlı olarak önemli bir azalmaya yol açmıştır. Ayrıca naringin, MDA ve SOD enzim aktivitelerinde artışa ve GSH düzeyinde azalmaya neden olmuştur.

Sonuç: Doğal ajanlar, özellikle flavonoidler sepsis kaynaklı organ hasarı modellerinde koruyucu bir ajan olma potansiyeline sahiptirler. Çalışmamızda naringini doğal ajan olarak kullanmayı tercih ettik ve inflamatuvar sitokin düzeyleri ile oksidatif stres enzim aktiviteleri üzerine etkili olduğunu saptadık. Naringin'in, anti-inflamatuvar ve anti-oksidant etkilerinin moleküler mekanizmalarını araştırmak için daha ayrıntılı çalışmalar yapılmalıdır.

Anahtar Kelimeler: İnflamasyon, naringin, oksidatif stres, sıçan, sepsis

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Introduction

It is well-known that sepsis is a common reason for mortality and morbidity all over the world and causes more complex pathophysiological events such as systemic inflammatory response and tissue damage including acute kidney injury (AKI). The AKI, a syndrome described as the rapid loss of renal

function, is considered as an important independent risk factor for mortality (Angus & van der Poll, 2013; Cadirci et al., 2011; Ozogul et al., 2015; Uchino et al., 2006; Uyanik et al., 2012).

Inflammation and oxidative stress, two critical cellular processes, have important roles in the pathophysiological issues of sepsis. The most known pro-inflammatory cytokines are tumor necrosis factor (TNF)-alpha, interleukin (IL)-1-beta, and IL-6, and their uncontrolled and excessive secretions cause the activation of phagocytic cells and tissue infiltration during the sepsis. Furthermore, reactive oxygen species (ROS) and secretion of lipid mediators are the cause of excessive released cytokines. The uncontrolled ROS production and its degradation by cellular antioxidants including glutathione (GSH) and superoxide dismutase (SOD) may occur oxidative cellular damage in especially major organ systems in sepsis process (Aksak Karamese et al., 2015; Karamese et al., 2020; Latief et al., 2015; Parrish et al., 2008).

Scientists have been researching a number of natural agents for their beneficial effects on AKI models (McDonough & Doucette, 2003; Yang et al., 2016). Naringin is an active component of especially grapefruits, and its chemical formula is 4′,5,7-trihydroxyfla vanone 7-rhamnoglucoside. It has numerous beneficial pharmacological features including anti-oxidant, anti-bacterial, anti-viral, anti-inflammatory, anti-tumorigenic, and apoptotic activities. Naringin has the ability to scavenge free radicals; therefore, it has been receiving remarkable attention as a natural agent to act against oxidative stress and kidney injury (Lim et al., 2018; Meng et al., 2022).

The current study aimed to investigate the potential protective effect, the levels of some inflammatory cytokines, and the activities of oxidative stress markers of naringin, which is a flavonoid, on a rat model of polymicrobial sepsis-induced kidney injury.

Methods

Thirty female Wistar rats, 10–12 weeks old, weighing 200–250 g were purchased, transferred in an appropriate condition, and kept in polycarbonate cages at room temperature (between 20°C and 22°C). Food and water were available ad libitum.

The rats were randomly divided into five groups each including six rats. Group 1 was named as "control group" and no surgical or treatment process was applied. At the end of the experiments, all healthy control groups' rats were killed, and then kidney and blood samples were collected. Group 2 was named as "Sham group" and objected to laparotomies, and cecal-ligation and puncture (CLP) procedure was not performed. Group 3 was named as "CLP sepsis." The rats were intraperitoneally anesthetized with thiopental sodium (25 mg/kg). The peritoneum was opened, and the cecum was isolated and ligated with a 4/0 silk ligature just distal to the ileocecal valve. The ligated part of the cecum was then punctured twice with a 20-gauge needle. After repositioning the cecum, the abdominal incision was closed with a 4.0 sterile absorbable suture. Group 4 and Group 5 were named as "naringin application before CLP sepsis procedure." Fifty milligram/kilogram and 100 mg/kg naringin (Sigma-Aldrich Inc., USA, CAS No: 10236-47-2) were dissolved in sterile saline and were applied 7 days by oral gavage before the CLP procedure for Group 4 and Group 5, respectively. Then, the CLP procedure was performed on 12 rats in Group 4 and Group 5.

All rats ($n = 30$) were euthanized by an overdose (50 mg/kg) of sodium thiopental anesthetic 18 h after CLP sepsis. The kidneys were removed, washed in ice-cold saline, and stored at −80°C for tissue biochemistry. Additionally, intracardiac blood samples were taken from the rats into sterile tubes with EDTA. Then, they are centrifuged at 4000 rpm for 15 minutes, and the supernatant was aliquoted into microcentrifuge tubes and stored at −80°C for enzyme-linked immunosorbent assay (ELISA) analysis.

In this study, we aimed to measure the levels of three different cytokines. For this reason, we used the ELISA technique and the following ELISA kits (Elabscience, Houston, TX, USA); TNF-alpha (Rat, E-EL-R0019), IL-1-beta (Rat, E-EL-R0012), and IL-6 (Rat, E-EL-R0015). The ELISA technique was applied according to the manufacturer's protocol. Finally, the microplate was read at a wavelength of 450 nm with an ELISA reader (Thermo, MultiSkan GO). The optical density values were noted and the calculations were performed.

Additionally, activities of some oxidant and antioxidant system enzymes including malondialdehyde (MDA), GSH, and SOD in the rat kidney tissue were determined by tissue biochemistry. For tissue biochemistry, all solutions and chemicals were freshly prepared in experiment day and purchased from Sigma-Aldrich Inc. To obtain tissue homogenates, tissues were first ground with liquid nitrogen. The ground tissues (0.5 g) were mixed with the appropriate buffer (4.5 mL), and the mixtures were homogenized on ice using an Ultra-Turrax homogenizer for 15 minutes. The homogenates were filtered and centrifuged at 15,000 rpm for 15 minutes in a refrigerated centrifuge (4°C), and the supernatants were used for analyses. All analyses were performed at room temperature in triplicate. The MDA, GSH, and SOD activities were determined according to the methods of Ohkawa, Sedlak, and Winterbourn, respectively (Ohkawa et al., 1979; Sedlak & Lindsay, 1968; Winterbourn et al., 1975).

Ethics committee approval was received for this study from the ethics committee of Kafkas University (Date: January 25, 2022, Number: KAU-HADYEK/2022–011).

Statistical Analysis

In this study, all data (numerical, independent, more than two groups) were analyzed using the one-way analysis of variance test by the Statistical Package for the Social Sciences version 21.0 statistical software (IBM, Armonk, NY, USA). The graphs were drawn by GraphPad Prism v5.0 (Graph Pad Software, San Diego, CA, USA). A p-value less than .05 was accepted as statistically significant.

Results

Three different pro-inflammatory cytokine levels were measured in collected serum samples as seen in Figure 1. When the data were evaluated, it was determined that CLP sepsis caused a dramatic increase in all pro-inflammatory cytokine levels ($p = .000$ between groups). The application of 50 mg/kg and 100 mg/kg doses of naringin for 7 days before CLP procedure led to an important decrease in the levels of pro-inflammatory cytokines in a dose-dependent manner. These decreases were statistically significant ($p = .000$), when the data of treatment groups were compared to CLP group data. An interesting finding about the pro-inflammatory cytokines is that there was no significant difference between 50 mg/kg (Group 4) and 100 mg/kg doses (Group 5) of naringin applications ($p = .075$).

On the other hand, MDA is a crucial biological marker of tissue damage and the current data reported that CLP sepsis caused a serious increase in the activity of the MDA enzyme

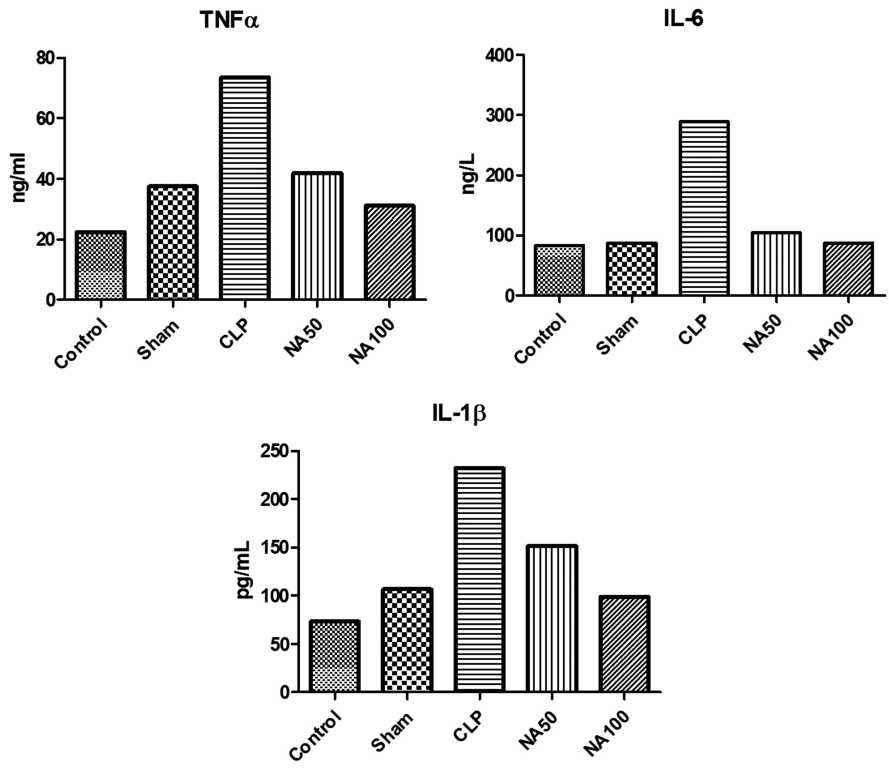

Figure 1.
The Levels of Three Different Cytokines in Experimental Groups.

(606.22 nmol/mg protein). About 50 mg/kg and 100 mg/kg doses of naringin cause a decrease in MDA levels (316.06 and 386.23 nmol/mg protein, respectively) when compared to the CLP group as expected ($p = .000$). Cecal-ligation and puncture sepsis caused an important decrease in the levels of GSH (3.65 nmol/mg protein) when compared to control (6.14 nmol/mg protein),

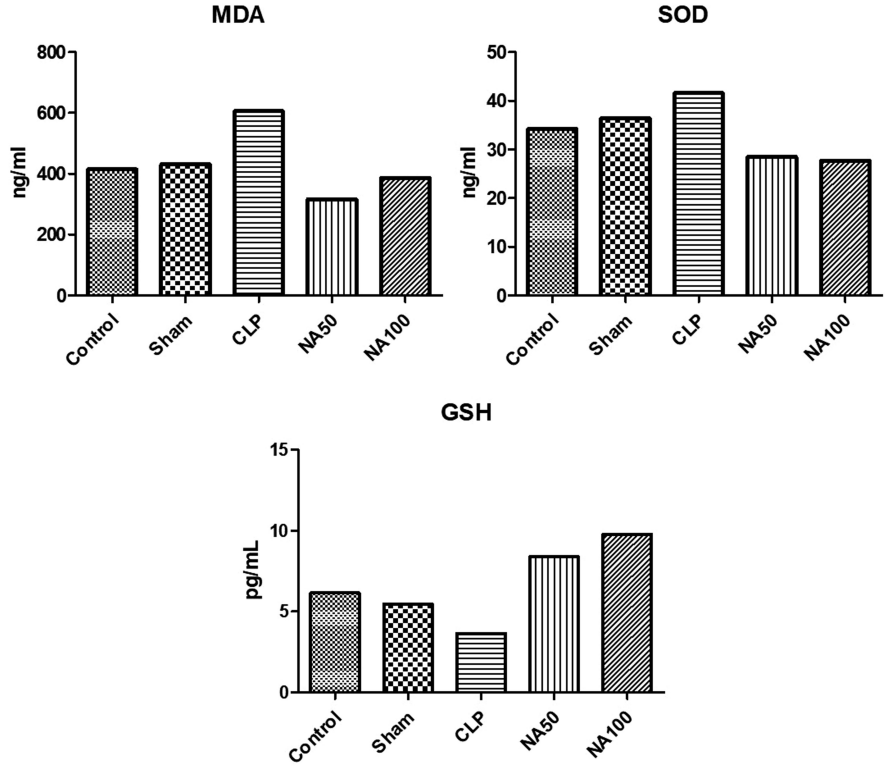

Figure 2.
The Oxidative Stress Enzyme Activities of Experimental Groups.

whereas it caused an increase in the activity of SOD (41.58 IU/mg protein vs. 34.21 IU/mg protein). There was a statistically significant difference between the CLP sepsis group and the two treatment groups in terms of GSH level and SOD activity ($p = .000$ for both). All enzyme levels are seen in Figure 2.

Discussion

In recent years, natural products are quite popular for some beneficial effects on most of diseases (Berg & Scherer, 2005; Doi et al., 2009; Zhao et al., 2015). They have both protective and therapeutic effects. The current study aimed to show the potential protective effects of naringin, a natural flavonoid, on inflammatory and oxidative stress processes in CLP-induced sepsis models. The mortality of sepsis is increasing day by day and the mentioned cellular processes such as inflammation and oxidative stress are one of the most important contributors (Lee et al., 2015). If a natural product has able to effect and normalize the crucial parameters of those processes, it may has a potential to be a protective or therapeutic agent. Our results supported that naringin has a potential to be a protective agent by affecting inflammatory cytokine levels such as TNF-alpha, IL-1-beta, IL-6, and oxidative stress parameters such as MDA, SOD, and GSH.

In our study, it has been clearly pointed out that the levels of TNF-alpha, IL-1-beta, and IL-6 cytokines increased because of CLP sepsis, while naringin was quite effective in decreasing these cytokine levels in a dose-dependent manner. The application of 100 mg/kg naringin normalized the pro-inflammatory cytokine levels. In the current literature, there are similar studies that support our findings. Karamese et al. (2016) reported that apigenin application normalized the pro-inflammatory cytokine levels in a rat model of sepsis. Additionally, Xianchu et al. (2016) reported that naringin had the potential to attenuate organ injury after the LPS challenge. Naringin inhibited LPS-induced increase of TNF-alpha, IL-1β, and IL-6 activities to alleviate inflammatory response. Similar to other studies, Sun et al. (2019) pointed out that naringin significantly decreased the inflammation response that occurred in LPS-induced sepsis-induced myocardial dysfunction.

On the other hand, oxidative stress is one of the other reasons for sepsis-originated organ damage with excessive release of ROS (Liu et al., 2014). In this cellular process, SOD, GSH, and MDA are the most important biochemical parameters. The SOD and GSH are one of the important ROS scavengers, which detoxifies hydrogen peroxidase by reducing it to water in the cell and prevent ROS attack to important organs (Chandra et al., 2000; Memos et al., 2009). The present study showed that naringin caused an increase in the enzyme activities of MDA and SOD and a decrease in the enzyme activity of GSH level. Cadirci et al. (2013) performed a study and reported that the CLP procedure caused a dramatic increase in the activity of some oxidative stress parameters such as LPO, GSH, and SOD, and agonist administration to 5-HT7 receptors reduced the levels of oxidative stress parameters. Similarly, another study demonstrated that sepsis led to an increase in SOD activity (Prauchner et al., 2011). Meng et al. (2022) also showed that one of the natural agents which is also a flavonoid suppressed oxidative stress and improved antioxidant enzyme levels in LPS-induced organ injury by significantly eliciting the activities of the antioxidant enzymes SOD and CAT and decreasing the concentration of MDA. Li et al. (2009) determined that pretreatment with a flavonoid, baicalein, significantly decreased MDA levels in rat models.

Conclusion and Recommendations

As a conclusion, natural products especially flavonoids have the potential to be a protective agent in sepsis-induced organ injury models. In our study, we preferred to use naringin as a natural agent and detected that it affected the inflammatory cytokine levels and oxidative stress enzyme activities. Further detailed studies should be performed to investigate the molecular mechanisms of the anti-inflammatory and antioxidant effects of naringin.

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