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Protective effects of quercetin against sepsis-induced oxidative damage on rat kidneys

Protective effect of quercetin on kidney tissue

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Abstract
Aim: Sepsis is a clinical pathology, characterized by a severe and exaggerated inflammatory response. One of the most frequently damaged organs in sepsis is the kidney. Quercetin has anti-inflammatory, anti-proliferative, and antioxidant effects. In this study, it was aimed to examine the protective effects of quercetin on the kidneys.
Material and Methods: In the scope of this study, 31 rats were planned to be used in the experiments. The groups and number of animals were as follows: Group 1: 1.5 ml saline, Group 2: 1.5 ml olive oil, Group 3: intestinal ligation and puncture procedure was used to create experimental sepsis method. Group 4: 20 mg/kg quercetin was administrated by gavage. Group 5: quercetin was administered in intragastrically at doses of 20 mg/kg. In biochemical analyzes of kidney tissue samples, BUN, creatinine, MDA and GSH values were checked. Cell damage, inflammation and fibrosis were evaluated histopathologically.
Results: As a result of this study, tissue GSH levels were significantly different between groups 3 and 4 (p= 0.001). In terms of BUN value, it was found to be significantly higher in group 3 (p= 0.002). In tissue histology, glomerulitis (p= 0.001), tubular cell necrosis (p= 0.001) and mesenchymal matrix increase (p= 0.001) were different between groups 3 and 4. Finally, no fibrosis was observed in any group (p> 0.05).
Discussion: Quercetin has protective effects on kidney tissue against organ damage caused by sepsis.

Keywords
Quercetin, Sepsis, Antioxidant, Cecal Ligation

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Introduction

Sepsis is a life-threatening organ dysfunction, resulting from an exaggerated host response to a suspected or defined infection [1]. The main reason for morbidity and mortality for septic patients is remote organ damage or multi-organ failure syndrome. The kidney is one of the most frequently damaged organs in sepsis.

Acute kidney injury (AKI) was detected in 19% of sepsis cases with mild clinical symptoms, 23% of moderately severe cases and 51% of severe sepsis cases. Sepsis constitutes 50% of the etiology of AKI for critically ill patients hospitalized in the intensive care unit. The development of AKI for patients with sepsis has been reported as an independent risk factor that increases mortality [2,3].

Quercetin is a flavonoid with anti-inflammatory and antioxidant effects, found naturally in many vegetables and fruits. Recent studies have reported that quercetin has protective effects on the kidneys [4].

In this study, it was aimed to investigate the protective effects of quercetin on the kidneys with an experimental sepsis model.

Material and Methods

It was planned to use 32 Spraque Dawley rats weighing 280-300 g in this study. Five groups were created by dividing animals randomly (Table 1). The animals were kept at room temperature 22 ± 2°C and in rooms with half-day light and dark cycles with food and water ad-libitum. One of them in the quercetin group died during the study.

Ketamine hydrochloride (70 mg/kg Ketalar; Eczacıbaşı, İstanbul, Turkey) and xylazine hydrochloride (20 mg/kg Rompun; Bayer Türk İlaç Ltd. Şti.) were administered intramuscularly to the rats under the supervision of a veterinarian for anesthesia.

Quercetin Application: 20 mg/kg quercetin was dissolved in olive oil daily for 2 weeks and given by gavage.

Sepsis Model with Cecal Ligation:

Under general anesthesia (combination of ketamine + xylazine), the rat was placed in the supine position and fixed on to the operating table. Initially, the operation area was shaved and disinfected. Later on, the abdomen was opened 2 cm from the front of the genital prominence towards the cranial. In rats, the caecum was exposed and the distal of iliocecal valve was ligated (3/0 suture). An 18G needle was used to puncture the intestinal tissue. The skin and subcutaneous tissues were covered with 3/0 silk first, and subcutaneous fluid was supplemented according to the animal's weight [5]. BUN and creatinine were planned to be checked by taking blood tissue.

Preparation of Tissue Homogenates: Sterile saline at +4 °C was used to wash the kidney tissue, cold chain principles were performed to cut small sections and placed in Eppendorf tubes and kept at -860 °C until analysis.

Analysis of MDA Level: Beuge and Aust defined the thiobarbituric acid reaction (TBARS) method that analyzes tissue MDA concentration as a lipid peroxidation marker [6]. For homogenization of the lung tissue, 10% trichloroacetic acid was used, then centrifuged. The superficial liquid parts were mixed with an equal volume of 0.67% thiobutyric acid, then incubated in boiling water at 90 °C for 15 minutes. After that, they were cooled and centrifuged. MDA concentrations that were measured under 532 nm absorbance were expressed as nmol/g.

GSH Analysis: It was measured by the glutathione reaction in the tube with 5,5'-dithiobis 2-nitrobenzoic acid to acquire a yellow-greenish color and to determine the amount of reduced glutathione by reading the light intensity of this color in a spectrophotometer with a wavelength of 410 nm.

Histological Evaluation:

The obtained tissues were histopathologically stained with H&E (Hematoxylin-Eosin) staining method, then cell damage was evaluated in terms of inflammation and fibrosis. Glomerular sclerosis, glomerulitis and mesangial matrix increases were evaluated. The presence of inflammatory cells in the glomeruli was defined as glomerulitis. It was graded as 0 if there was no glomerulitis, 1 if it was rare, and 2 if it was widespread. Tubulointerstitial area was evaluated for interstitial fibrosis and interstitial inflammation. Tubulitis, atrophy and tubular necrosis were evaluated. There were two patterns of acute tubular necrosis. The first one was tubular coagulation necrosis and the other was single-cell necrosis and shedding. In single-cell necrosis, the epithelium was flattened, vacuolized, pale and flattened, and its brush-like ends disappeared. Tubules were evaluated for single-cell necrosis, vacuolization, and brush border damage. It was evaluated as 0 if it was absent, 1 if it was present in 25% or less of the tubules, 2 if it was present in 26-50%, and 3 if it was present in more than 50% [7].

Statistical Analysis:

SPSS 15.0 software for Windows (SPSS Inc.) was used for statistical analysis. The Kolmogorov-Smirnov test was used to determine whether the data were normally distributed. One Way ANOVA or Kruskal-Wallis H tests were chosen according to their suitability for the comparison of the groups. The groups, found to be significant as a result, were compared using the Tukey multiple range test or the dual Mann-Whitney U test (for those with a p-value <0.005, applying Bonferroni correction (i.e., and 0.05/10 comparisons), the results were reported as mean±SD or median (min-max). P value <0.05 was considered statistically significant.

Ethical statement

The study was approved by the Adiyaman University Animal Experiments Local Ethics Committee (ADYÜ-HADYEK) (approval No. 2019/048).

Results

Glomerular sclerosis formation was not observed in any group. As a result of histological scoring for glomerulitis (P=0.001), there was a statistically significant difference between group 3 (had sepsis), group 4 (was treated with quercetin), and the other groups. No statistical differences were found between groups 3, 4 and the other groups (groups 1, 2 and 5) (P>0.05).

Tubular necrosis and tubular atrophy were not observed in any group in histological examination of the tubules. Although no acute tubular necrosis in the form of coagulation necrosis was observed, tubular single cell necrosis was present. In the comparison between groups in terms of tubular single cell necrosis, there was a statistically significant difference in groups 3 and 4 compared to the other groups (P=0.001). In addition, there was no difference between groups 1, 2 and 5 in terms of tubular single cell necrosis, vacuolization, and brush

border damage (P>0.05). Moreover, there was no statistically significant difference between groups 3 and 4 (P>0.05).

In the histological examination of the tubulointerstitial area, interstitial fibrosis and interstitial inflammation were not observed in any group. While there was a statistical difference between groups 3,4 and the other groups in histological scoring for mesenchymal matrix increase (P=0.001), there was no statistical difference among themselves and between groups 1, 2 and 5 (P>0.05). Graph of the mean values of the groups for tubular necrosis, glomerulitis, and mesenchymal matrix increase were shown in Figure3.

Besides, BUN and creatinine values in the blood were compared statistically, and no difference was observed between the groups in terms of creatinine values (P>0.05), while the mean value of the BUN values in group 3 was different from all the other groups (P=0.002) (Table 2).

Furthermore, when comparing GSH and MDA between groups, there was a statistical difference between GSH values in group 3 compared to all the other groups (P=0.001). Among MDA levels, although the highest MDA level was observed in group

Table 1. Establishment of experimental animal groups

Groups	Application	Feature
Group 1 (n=6)	-Saline was administered intragastrically at 1.5 ml doses.	Control
Group 2 (n=6)	-Olive oil was given at 1.5 ml doses	Sham surgery
Group 3 (n=7)	-A sepsis model was created using the cecal ligation and puncture method.	Sepsis
Group 4 (n=7)	-Starting 15 days before the surgical application, quercetin was administered at doses of 20mg/kg intragastrically until the end of the experiment.	Sepsis + Quercetin
Group 5 (n=5)	-Starting 15 days before the surgical application, quercetin was administered at intragastric doses of 20 mg / kg until the end of the experiment.	Quercetin

Table 2. BUN, Creatinine and MDA GSH mean ± SD values

	Groups (n)	Mean ± SD
BUN (mg/dL)	Group 1 (6)	43 ±5,1
	Group 2 (6)	37,3 ±2,7
	Group 3 (7)	81,3 ±47,6*
	Group 4 (7)	42 ±10,1
	Group 5 (5)	33,2±10,6
Creatinine (mg/dL)	Group 1 (6)	0,48 ± 0,08
	Group 2 (6)	0,57 ± 0,03
	Group 3 (7)	0,59 ± 0,09
	Group 4 (7)	0,52 ± 0,14
	Group 5 (5)	0,55 ± 0,05
MDA (nmol/g)	Group 1 (6)	1420,4 ± 227,6
	Group 2 (6)	1899,4 ± 390,8
	Group 3 (7)	2035 ± 914,9
	Group 4 (7)	1775 ± 406,8
	Group 5 (5)	1534,9 ± 108,3
GSH (nmol/g)	Group 1 (6)	1749,9 ± 78,9
	Group 2 (6)	1858,9 ± 47,3
	Group 3 (7)	1925 ± 103,2*
	Group 4 (7)	1805,8 ± 60,9
	Group 5 (5)	1792,2 ± 108,2

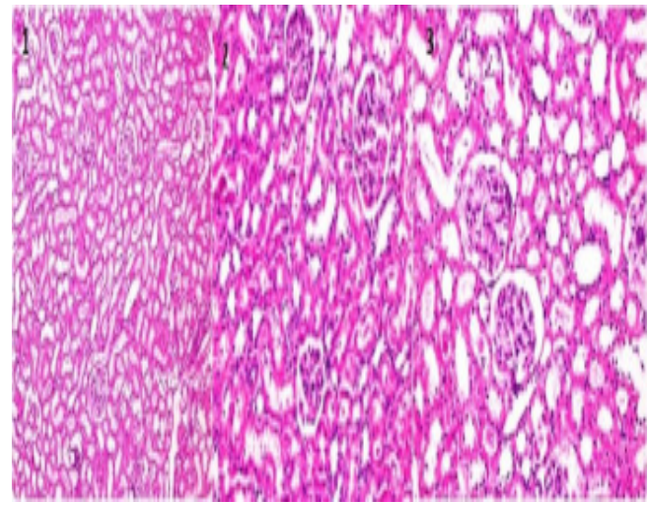

Figure 1. Histopathologic image of control[1], sham[2] and quercetin[3] groups

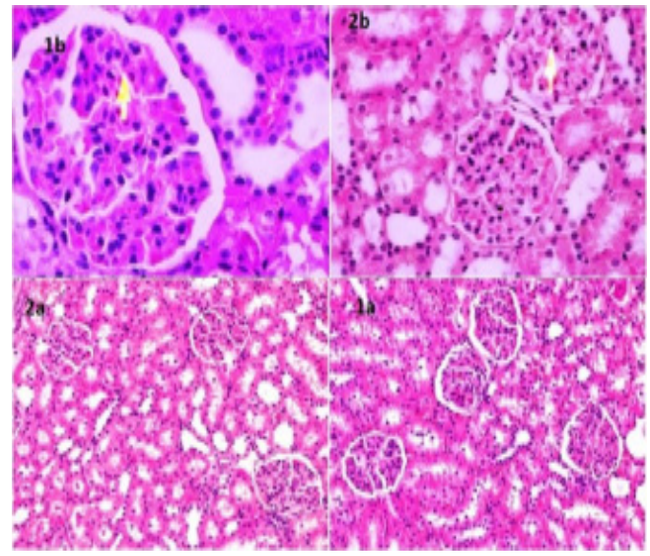

Figure 2. Histopathologic image of sepsis and sepsis[1a,1b]+ quercetin groups [2a,2b]. Arrow: Neutrophil in the glomerulus

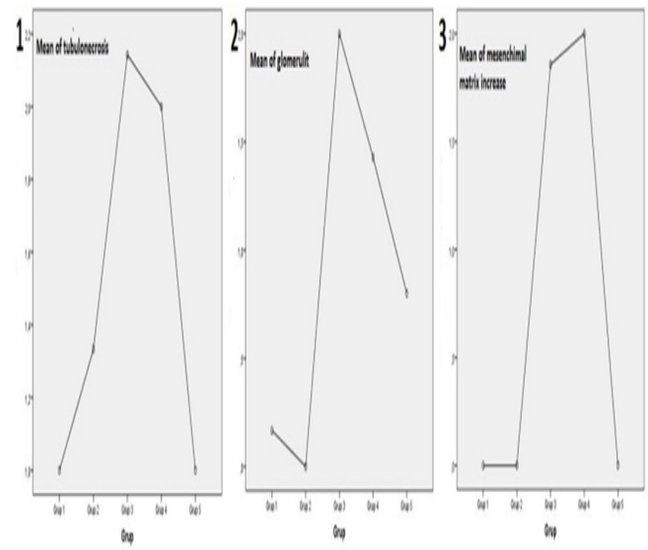

Figure 3. The graphics of histopathologic scores between groups.

3, this difference was not statistically significant (P>0.05). Mean values of MDA and GSH of the groups and their standard deviations were given in Table 2.

Discussion

The pathophysiology of sepsis-induced AKI development is complex and multifactorial. Changes in renal hemodynamics, endothelial damage, tissue infiltration of inflammatory cells, intraglomerular thrombosis, cell necrosis, and tubular occlusion contribute to the pathophysiology [8]. Understanding the pathophysiology of a disease is always the cornerstone for the development of new diagnostic and therapeutic strategies. Although quercetin is not in a position to treat or prevent the AKI that will develop, it can alleviate the effects of acute kidney injury caused by sepsis.

In the literature, the protective effects of quercetin on AKI that develop for various reasons have been mentioned [9-11]. Wang et al. [12] showed that quercetin has a kidney protective effect by inhibiting the ferroptosis pathway in their study. In the study by Yagmurca et al. [9], quercetin reduced the kidney toxicity caused by doxorubicin. In addition, there are many studies showing that other antioxidant substances except quercetin reduce kidney toxicity that develops for various reasons. Also, literature studies show that various antioxidant substances, except for quercetin, are effective in kidney damage caused by sepsis. Tasanarong et al. [13] showed in their study that the antioxidant effect of Phyllanthus emblica extract is effective in contrast-induced AKI. In studies with ferulic acid, which is a phenolic compound with antioxidant activity, Mir et al. [14] found that ferulic acid was effective against AKI caused by LPS, Bacanlı et al. [15] stated that it was effective against oxidative damage caused by sepsis. Zang et al. [16] reported that coumarins obtained from Hydrangea paniculata were effective against sepsis-induced AKI by antioxidant and anti-inflammatory ways. In this study, it was observed that tissue GSH levels that reflecting the antioxidant activity was the highest in the sepsis group and significantly higher than the sepsis+quercetin group. Antioxidant activity was lower in the group of rats with sepsis given quercetin. Likewise, tissue MDA activity was lower in the sepsis-induced group than in the quercetin treated rats with sepsis-induced AKI. This was thought to be due to the antioxidant effect of quercetin.

Although serum creatinine is used in the diagnosis and staging of acute kidney injury, acute changes in creatinine value lag behind both kidney injury and recovery. In addition, despite kidney damage in sepsis, an increase and then a decrease are observed in creatinine and BUN values [17]. In the study by Doi et al., they found that sepsis caused a decrease in creatinine values. In the study they found that, while the creatinine values were slightly increased in mice with sepsis with CLP method, the highest increase was observed in mice with nephrectomy. However, creatinine level was lower in mice with sepsis with nephrectomy and CLP. As a result, sepsis reduces creatinine production, which masks the rise in serum creatinine after sepsis, potentially complicating early detection of acute kidney injury [18]. In this study, a slight increase in creatinine level was found in the group with sepsis, but this increase was not statistically significant. However, there was a significant increase in the BUN value of the same group. In this study, BUN and creatinine values in a single section at the 24th hour of CLP application were also studied. It has been thought that a significant increase in BUN values and a slight increase in creatinine values are compatible with the literature.

Sepsis causes various pathological changes in the kidney histologically. There are many mechanisms for these changes. Sun et al. [19] showed in their study that, autophagy can be reduced by deacetylation of P53 in kidney damage caused by sepsis. Zheng et al. [20] observed a decrease in the histopathological changes in sepsis in their study with Rhizoma Coptidis extract. In this histological examination, it was observed that tubular damage was present in groups with sepsis. Although the histological changes in the group with sepsis given quercetin were less severe than the group with sepsis alone, they were not different enough to create a significant difference between the scoring results. Although quercetin alleviated the histological changes, it did not cause a sufficient change to correct the pathology caused by sepsis.

The lack of serial measurements was the weak point of this study. This may prevent us from seeing an increase or decrease in some parameters. However, it has been believed that the time chosen for the sampling is sufficient to show the effectiveness of sepsis and quercetin among the groups.

In conclusion, quercetin is a flavonoid whose antioxidant effect is well-known. The authors believe that quercetin, which has antioxidant and anti-inflammatory effects, has protective effects on kidney tissue against organ damage caused by sepsis.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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