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RESEARCH

Effects of rosmarinic acid on cyclophosphamide-induced nephrotoxicity in rats

Rosmarinik asidin sıçanlarda siklofosfamid ile indüklenen nefrotoksisite üzerine etkileri

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Abstract

Purpose: Cyclophosphamide (CP) is an antineoplastic agent. It is used in the treatment of many types of cancer. Rosmarinic acid (RA) exhibits remarkable biological activities such as anti-inflammatory, antitumor, antibacterial, and antimicrobial effects. This study aimed to evaluate the effect of rosmarinic acid against CP-induced nephrotoxicity.

Materials and Methods: Eighteen male Sprague Dawley rats were randomly divided into 3 equal groups; Sham group (n=6): 0.9% saline solution/8 days/oral gavage + 0.9% saline solution/8th day/intraperitoneal, CP group (n=6): 0.9% saline solution/8 days/oral gavage + 200 mg/kg/8th day/intraperitoneal CP, and CP+RA group (n=6): 100 mg/kg/8 days/oral gavage RA + 200 mg/kg/8th day/intraperitoneal CP was applied. Hematoxylin and Eosin, Periodic Acid-Schiff, and Masson's Trichrome staining were performed on the collected tissues

Results: Histopathological evaluation revealed tubular atrophy, glomerular damage, vascular congestion, vacuolization, and interstitial inflammation in the CP group. Histopathological scores were significantly lower in the CP+RA group compared to the CP group. Intertubular fibrosis was observed in the CP group compared to the Sham group. Fibrosis decreased with rosmarinic acid. PAS-stained sections from the CP group showed tubular epithelial vacuolization, brush border, and basal membrane disruption. These findings decreased with rosmarinic acid. The increased blood urea nitrogen level in the CP group was lower in the CP+RA group, while the decreased SOD level in the CP group was higher in the CP+RA group.

Öz

Amaç: Siklofosfamid (CP) antineoplastik bir ajandır. Birçok kanser türünün tedavisinde kullanılmaktadır. Rosmarinik asit (RA), antiinflamatuar, antitümör, antibakteriyel ve antimikrobiyal etkiler gibi dikkate değer biyolojik aktiviteler sergiler. Bu çalışmanın amacı, rosmarinik asidin CP kaynaklı nefrotoksisiteye etkisini değerlendirmektir.

Gereç ve Yöntem: On sekiz erkek Sprague Dawley sıçanı rastgele 3 eşit gruba ayrıldı. Sham grubuna (n=6): %0,9 salin solüsyonu/8 gün boyunca/oral + %0,9 salin solüsyonu/8. gün/intraperitoneal; CP grubuna (n=6): %0,9 salin solüsyonu/8 gün boyunca/oral + 200 mg/kg/8. gün/intraperitoneal CP; CP+RA grubu (n=6): 100 mg/kg/8 gün boyunca/oral RA + 200 mg/kg/8. gün/intraperitoneal CP uygulandı. Toplanan dokularda Hematoksilen ve Eozin, Periyodik Asit-Schiff ve Masson Trikrom boyamaları yapıldı.

Bulgular: Histopatolojik değerlendirmede CP grubunda tübüler atrofi, glomerüler hasar, vasküler konjesyon, vakuolizasyon ve interstisyel inflamasyon saptandı. Histopatolojik skorlar CP+RA grubunda CP grubuna göre anlamlı derecede düşüktü. Sham grubu ile karşılaştırıldığında CP grubunda intertübüler fibrozis gözlendi. Rosmarinik asit ile fibrozis azaldı. PAS ile CP grubu kesitlerinde, tübüler epitel vakuolizasyonu, fırçamsı kenar ve bazal membran bozulması gözlendi. Bu sonuçlar rosmarinik asit ile azaldı. CP grubunda artan kan üre nitrojen değeri (BUN) CP+RA grubunda daha düşükken, CP grubunda azalan SOD değeri CP+RA grubunda daha yüksekti.

Sonuç: RA' nın böbrekte tübüler atrofi, glomerüler hasar, vasküler konjesyon, vakuolizasyon ve interstisyel

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Conclusion: RA has protective effects against CP causing tubular atrophy, glomerular damage, vascular congestion, vacuolization, and interstitial inflammation in the kidney.
Keywords: Nephrotoxicity; cyclophosphamide; rat; rosmarinic acid.

inflamasyona neden olan CP' nin etkilerine karşı koruyucu etkileri bulunmaktadır.

Anahtar kelimeler: Nefrotoksisite; siklofosfamid; sıçan; rosmarinik asit.

INTRODUCTION

Cyclophosphamide (CP), an alkylating agent with cytotoxic, immunosuppressive, anti-inflammatory, and antineoplastic effects, is used to treat of many types of cancer, autoimmune and rheumatological diseases. Cyclophosphamide's unique metabolism and inactivation by aldehyde dehydrogenase are responsible for its distinct cytotoxic properties. Due to its toxic metabolites, despite its widespread use, CP also causes numerous undesirable side effects including urinary, pulmonary, and cardiovascular system toxicity. Although the toxicities associated with cyclophosphamide are serious, this agent remains a highly effective drug in many situations[1,2].

Rosmarinic acid (RA) is a highly valued natural phenolic compound commonly found in plants of the Lamiaceae and Boraginaceae families, including Rosmarinus officinalis, Coleus blumei and Salvia officinalis. Rosmarinic acid (RA) exhibits remarkable biological activities such as antioxidant, antimutagenic, anti-inflammatory, antitumor, antiviral, antibacterial, and antimicrobial effects. The demand for RA in the pharmaceutical industry is very high[3–5]. Its antioxidant effects are mainly related to membrane stabilization and inhibition of free radical propagation[6].

Antioxidants are consumed by reacting with free radicals produced during oxidative stress. As a result of the shift in the antioxidant-oxidant balance towards the oxidant direction, damage occurs in the tissues. The damage caused by free radicals can be detected by measuring oxidative products malondialdehyde (MDA) in various tissues and body fluids[7]. The first defense against intracellular superoxide and hydrogen peroxide-mediated damage involves the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione reductase, and glutathione peroxidase (GSH-Px)[8].

The protective and therapeutic effects of various antioxidants on CP-associated kidney damage have been evaluated in several studies. However, the potential effects of RA on CP-induced nephrotoxicity have not been investigated. Therefore, the aim of this study is to elucidate the protective effects of RA against nephrotoxicity induced by CP, a widely used chemotherapeutic agent, using histopathological and biochemical methods.

MATERIALS AND METHODS

Experimental animals

Experiments and surgical procedures were performed at Karadeniz Technical University (KTU) Experimental Animal Surgical Research and Application Centre (Trabzon, Turkey) (KTU-EXAC) and the Department of Histology and Embryology, Faculty of Medicine, KTU. All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council (US) Committee Update of the Guide for the Care and Use of Laboratory Animals Citation 2011). Our animal study protocols and experimental procedures were approved by the KTU Animal Care and Ethics Committee, as required by law for animal experimentation in Turkey. The authors made the applications were with a laboratory animal certificate.

Eighteen male Sprague Dawley rats, weighing 250-300 g, were housed in standard Type III cages under ad libitum feeding conditions in a 12 h light and 12 h dark environment. The temperature was set at 22±2°C, and the humidity was 50±5%. Animal care and all experimental procedures were performed at the Animal Care Ethical Committee of Karadeniz Technical University (protocol number: 2018/36).

Experimental design

Rats were randomly divided into three groups consisting of six rats (n=6). The number of animals used in the experiment was determined in accordance with the Guide for the Care and Use of Laboratory Animals, local ethics committee recommendations and G-Power analysis. Power analysis was performed using G-Power 3.1.9.7. With an effect size of 0.3, an alpha margin of error of 0.05, and a power of 65%, the total number of experimental animals for the 3

groups was determined to be 18. The Sham group received a saline solution from the first day of the experiment for 8 days by oral gavage. Intraperitoneal saline was applied on the eighth day. In the CP group, saline was administered by oral gavage for 8 days from the first day of the experiment. On the eighth day, a single dose of CP (200 mg/kg) was administered intraperitoneally. In the CP+RA group, RA (100 mg/kg) was administered by oral gavage for 8 days from the first day of the experiment. On the eighth day, a single dose of CP (200 mg/kg) was administered intraperitoneally. The dosages of CP (Endoxan, 1 g vial containing IV infusion powder, Eczacıbaşı, Baxter Oncology GmbH Halle, Germany) and RA (536954-5 g, 96% Sigma-Aldrich Chemie Gmbh) used in this study were determined according to the literature review[9,10].

Tissue collection

The rats were sacrificed 24 h after the CP application (day 9th), under ketamine anesthesia (Ketalar flk, 50 mg/ml solution, Pfizer, Kırklareli, Turkey), and the blood samples were collected in vacuum separator gel tubes. Plasma obtained from the collected blood was used for biochemical analysis. The left kidneys were removed and washed with saline, and half of each left kidney was placed in 10% neutral buffered formalin solution for histological evaluation. The remaining half of the left kidney was rinsed with saline and placed in eppendorf tubes for biochemical evaluation.

Histopathological analysis

The kidney samples were fixed in 10% neutral buffered formalin for 72 h. Tissues were washed in running water and were dehydrated through graded ethanol series. Then, specimens were placed into xylene and embedded in paraffin. Paraffin blocks were cut at 5 μm, mounted on slides and stain with Hematoxylin and Eosin (H&E), Periodic Acid-Schiff (PAS), and Masson's trichrome. The sections were examined by light microscopy (Olympus BX51, Tokyo, Japan).

The H&E stained sections were scored according to the severity of tubular atrophy, cytoplasmic vacuolization, glomerular damage, vascular congestion, and interstitial inflammation in ten different fields for each section. For this analysis, kidney damage was semi-quantitatively graded as (0) no damage, (1) mild damage (0-25%), (2) moderate damage (25-75%), (3) severe damage (75-100%) for each criterion[11,12].

Biochemical analysis

SOD activities in tissues were measured by the method described by Sun et al. (1988), based on the reduction of nitroblue tetrazolium by the xanthine-xanthine oxidase system[13]. Formazan formation was assessed spectrophotometrically at 560 nm. CAT activity was determined by the method described by Aebi (1974), based on the principle that the absorbance at 240 nm decreases due to the dismutation of H_2O_2[14]. Tissue MDA levels were determined by the method described by Uchiyama and Mihara[15]. This was based on the measurement of thiobarbituric acid-reactive substances spectrophotometrically at 532 nm. Serum blood urea nitrogen (BUN) and creatinine levels were measured using an autoanalyzer.

Statistical analysis

Statistical analysis of the data obtained was performed using SPSS 23.0 software (SPSS Inc., Chicago, IL, USA). Histopathological and biochemical data were expressed as arithmetic mean + standard error of the mean (SEM). Groups conforming to normal distribution were compared using the ANOVA test (Bonferroni test as post hoc for paired comparisons of significant groups). In contrast, groups that did not comply with normal distribution were compared using the Kruskal-Wallis test (Tamhane's test for paired comparisons of groups). P values of <0.05 were considered statistically significant.

RESULTS

The Sham group revealed a normal structure in Bowman's capsule, glomeruli, proximal tubules, and distal tubules, no pathological findings were observed (Figure 1A). Tubular atrophy, tubular epithelial vacuolization, glomerular damage, vascular congestion, and interstitial inflammation were observed in the CP group. Epithelial shedding, interstitial inflammation, hemorrhage, and hyaline material accumulation were also observed in the CP group (Figure 2A-D). Except for mild tubular atrophy, no significant pathological findings were observed in the CP+RA group (Figure 3A).

The mean histological damage score each revealed a statistically significant increase in tubular atrophy, vascular congestion, vacuolization, glomerular damage, and interstitial inflammation in the CP group compared to the Sham group ($p<0.05$). Although a statistically significant decrease ($p<0.05$) was observed in tubular cell vacuolization, glomerular damage, vascular congestion, and interstitial inflammation in the CP+RA group compared to the CP group, the decrease in tubular atrophy was not significant. Histological damage scores are shown in Table 1.

In PAS-stained sections, glomeruli, proximal, and distal tubules showed a normal structure in the Sham group (Figure 1B). However, kidney sections from the CP group showed pathological findings such as basal membrane disruption, brush border loss, and tubular desquamation (Figure 2E). Histological findings were reduced in the CP+RA group when compared to the CP group (Figure 3B). In Masson's trichrome, kidney structure was in normal appearance (Figure 1C). Intertubular fibrosis was observed in the CP group when compared to the Sham group (Figure 2F). The fibrosis was reduced in the CP+RA group (Figure 3C).

Tissue MDA values were significantly higher in the CP+RA group compared to the Sham and CP groups ($p<0.05$). No significant difference in SOD and CAT values was observed among the three groups. BUN values were significantly higher in the CP and CP+RA groups than in the Sham group ($p<0.05$). Although BUN values were lower in the CP+RA group than in the CP group, the difference was not statistically significant. Creatinine values were significantly higher in the CP and CP+RA groups compared to the Sham group ($p<0.05$). However, the increase in creatinine observed in the CP+RA group compared to the CP group was not statistically significant. Data for tissue SOD, CAT, and MDA, and serum BUN and creatinine are shown in Table 2.

Table 1. Histopathological scores of all groups.

	Sham	CP	CP+RA
Tubular atrophy	0.33±0.51	1.83±0.98 *	0.83±0.4
Vacuolization	0.16±0.4	1.83±0.75 *	0.66±0.51 **
Glomerular damage	0.16±0.4	2.33±0.81 *	0.5±0.83 **
Vascular congestion	0.16±0.4	2.16±0.98 *	0.33±0.81 **
Interstitial inflammation	0.5±0.83	2.66±0.51 *	1.16±0.4 **

CP: Cyclophosphamide, RA: Rosmarinic Acid.; Data are expressed as the arithmetic mean ± SEM (n = 6).
* $p < 0.05$ when compared to the Sham group; ** $p < 0.05$ when compared to the CP group.

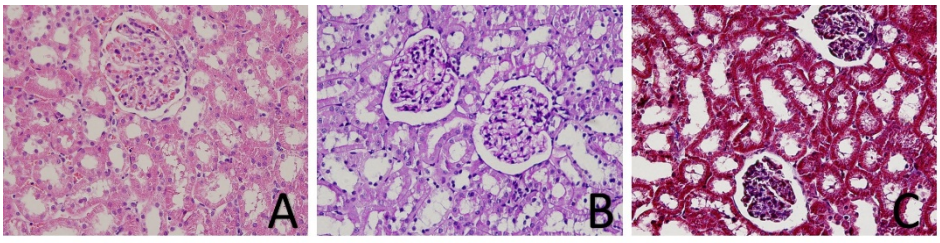

Figure 1. Photomicrograph of renal tissue from the Sham group (A-C). (A: H&E, X40, B: PAS, X40, C: Masson's Trichrome, X40).

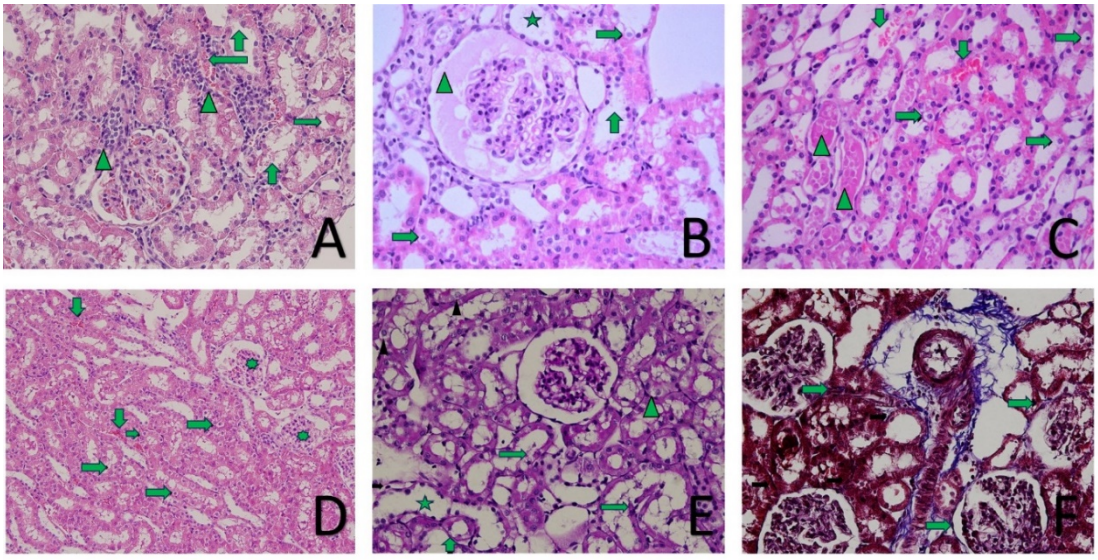

Figure 2. Photomicrograph of renal tissue from the CP group (A-F). Interstitial inflammation (arrowhead), hemorrhage (left arrow), epithelial shedding (up arrow), hyaline material accumulation (right arrow) (A: H&E, X40). Hyaline material accumulation (arrowhead), vacuolization (right arrow), tubular dilatation (star), epithelial shedding (up arrow) (B: H&E, X40). Hyaline material accumulation (arrowhead), vacuolization (right arrow), intertubular vascular congestion (down arrow) (C: H&E, X40). Vacuolization (right arrow), intertubular vascular congestion (down arrow), glomerular degeneration (star) (D: H&E, X20). Vacuolization (arrowhead), epithelial shedding (right arrow), tubular dilatation (star) (E: PAS, X40). Intertubular fibrosis (F: Masson's Trichrome, X40).

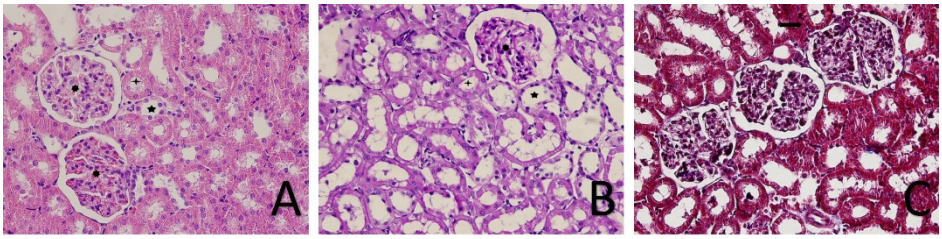

Figure 3. Photomicrograph of renal tissue from the CP+RA group (A-C). Kidney tissues in normal histological appearance (A: H&E, X40). Kidney tissues in normal histological appearance (B: PAS, X40). Intertubular fibrosis (C: Masson's Trichrome, X40).

Table 2. Biochemical parameters of all groups.

	Sham group	CP group	CP+RA group
Tissue MDA (nmol/g)	322.04±14.01	331.21±36.55	408.30±31.27 *,**
Tissue SOD (U/mg)	40.15±3.86	38.17±4.39	41.74±1.41
Tissue CAT (k/g)	1.84±1.19	2.29±1.67	0.51±0.35
Serum BUN (mg/dL)	17.17±2.13	45.67±13.86 *	35±12.13 *
Serum Creatinine (mg/dL)	0.21±0.03	0.32±0.05 *	0.33±0.08 *

CP: Cyclophosphamide, RA: Rosmarinic Acid.; Data are expressed as the arithmetic mean ± SEM (n = 6). ; * $p < 0.05$ when compared to the Sham group, ; ** $p < 0.05$ when compared to the CP group.

DISCUSSION

Cyclophosphamide (CP) is a cytotoxic alkylating agent that has been widely used for over 50 years in the treatment of neoplastic diseases, including solid tumors and lymphomas, and non-neoplastic diseases such as rheumatoid arthritis and systemic lupus erythematosus, with broad spectrum clinical uses. The principal factor limiting the high therapeutic efficacy of CP is the side effects of metabolizing CP[1,8]. Organ-specific side effects include toxicity of urinary, pulmonary, and cardiovascular systems[1,2].

CP, a cytotoxic drug, is hydroxylated in the liver and turns into its metabolites phosphoramide mustard (phosphoramide mustard=PAM=FAM) and acrolein (acrolein=ACR). The antineoplastic effects of CP are related to PAM, and it is thought that PAM binds to DNA, suppresses cell division, and mediates CP's immunosuppressive and antitumor effects. The toxic effect of CP is thought to be related to its active metabolite, ACR. ACR interferes with the tissue antioxidant defense system, causing a high rate of free radical formation. Oxidative stress is a condition with an increase in the amount of free oxygen radicals, and this increase causes lipid peroxidation in the membranes. Overproduction of free oxygen radicals in the inflammatory process causes oxidative stress, leading to necrosis through cell and DNA damage, protein denaturation, and peroxidation of membrane lipids. Overproduction of ROS causes peroxidative DNA fragmentation, damage to the plasma membrane, and toxicity[9].

A study conducted in mammalian heart tissue cells reported that excessive production of free oxygen radicals during CP therapy caused lipid peroxidation, and the resulting membrane damage disrupted the integrity of the myocardial membrane and caused dysfunction[10]. In his study, Shanholtz[11] emphasized that high-dose CP administration can cause fatal cardiotoxicity. In another study, the cardiotoxic effects of CP were reported to be dose-related cardiac damage, morphologically determined necrosis, hemorrhage, and subsequent fibrosis[12].

Saqib et al. (2019) examined the restorative effects of N-acetylcysteine following a single intraperitoneal injection of 200 mg/kg CP. The authors reported that CP caused hemorrhages and leukocyte infiltration in the rat kidney[13]. The present study observed interstitial inflammation and hemorrhagic foci in kidney sections from rats administered 200 mg/kg CP. Sharma et al. (2017) reported that intraperitoneal administration of 200 mg/kg CP for two consecutive days caused dilatation in the proximal tubules, increased cytoplasmic granulations in tubular epithelial cells, and hyaline substance accumulation in the tubular lumen, and caused narrowing in Bowman's space and glomerular degeneration[14]. In our study, we frequently encountered narrowing of Bowman's space, hyaline substance deposition, and tubular dilatation with the same dose of CP. It is quite impressive that this dose gives similar histopathological results.

BUN and creatinine are metabolic waste products excreted by the kidneys and are the simplest means of monitoring renal functions. Levels of blood urea, an indicator of protein breakdown, and creatinine, an indicator of muscle breakdown, increase in kidney diseases[15]. Liu et al. (2016) observed necrosis and leukocyte infiltration in the kidneys of mice administered a single 200 mg/kg intraperitoneal dose of CP and reported that serum uric acid and creatinine levels both increased[16]. In the present research, we observed an increase in BUN and creatinine in addition to widespread inflammatory cell migration in the CP group, which was given the same dose of CP as in that study. Abraham et al. (2007) found an increase in creatinine levels with glomerular nephritis, cortical tubular vacuolization, and interstitial edema in the histopathological and biochemical examination of the kidneys of rats administered 150 mg/kg intraperitoneal single dose CP[17]. Similarly, in our study, we found tubular dilatation and tubular vacuolization with an increase in creatinine levels in the CP group, proving the devastating effects of CP on the kidney.

MDA is an important biochemical marker of oxidative stress, while CAT and SOD are important markers of the antioxidant defense system[7]. Adikwu et al. (2019) compared the protective effects of various antioxidants against CP damage. Rats received 150 mg/kg CP, and tubular necrosis and inflammatory cell migration were observed as a result. BUN, creatinine, and MDA levels increased, while SOD decreased[18]. In our study, inflammatory cells were observed in the CP group. In addition, MDA increased, and SOD decreased. A recent study investigated the protective effect of boric acid against CP damage, administering 200 mg/kg CP to male Sprague Dawley rats. BUN, creatinine, and tissue MDA increased while CAT decreased, and histopathological examination revealed bleeding foci, vascular congestion, and constriction in Bowman's

capsule[19]. In our study, there was also an increase in MDA activity in the CP group. In addition, a statistically insignificant increase in CAT level was found. We think that the high CAT level may be due to the increase in the activity of glutathione peroxidase enzyme (GSH-Px), another important antioxidant enzyme. Although CAT and GSH-Px fulfill the same function by degrading H_2O_2 formed by SOD, their affinities are different. The affinity and rate of GSH-Px for H_2O_2 are much higher than CAT[20]. This increase in CAT activity in the CP group may be due to the activation of GSH-Px.

Rosmarinic acid (RA) exhibits remarkable biological activities such as antioxidant, antimutagenic, anti-inflammatory, antitumor, antiviral, antibacterial, and antimicrobial effects. Its antioxidant effects are mainly related to membrane stabilization and inhibition of free radical propagation[3].

Rosmarinic acid has a number of biological activities such as antioxidant, anti-inflammatory, antimutagenic, antiangiogenic, anti-apoptotic, antifibrotic, chemoprotective, neuroprotective, reduction of atopic dermatitis, photoprotection of keratinocytes and prevention of Alzheimer's disease[3-5]. There is a growing interest in using natural antimicrobials and antioxidants in foods[6].

Chu et al. demonstrated in vivo the anti-inflammatory activity of RA in acute lung injuries induced by lipopolysaccharides in mice[21]. Sanchez-Campillo et al. reported that RA can be a protective agent from UV rays[22]. Many studies also show RA's antioxidant properties[23]. It shows that RA can be used to protect against DNA damage, especially within the framework of chemical inhibition strategy, due to its antigenotoxic activity.

RA has been found to exert an antioxidant effect and is a protective and therapeutic agent in clinical and experimental studies. Tavafi et al. (2011) applied 100 mg/kg and 200 mg/kg RA in the treatment of an experimental animal nephropathy model. Glomerulosclerosis and leukocyte infiltration improved in parallel with the RA doses administered in their diabetic nephropathy model. The authors reported that glomerulosclerosis and leukocyte infiltration occurring in diabetic nephropathy models improved in line with increasing dosages of RA. In the present study, while glomerular damage and interstitial inflammation were frequently encountered in the CP group, the decrease in these findings and a near-normal histopathological structure in the CP+RA group suggest that RA may be protective in CP renal toxicity[24]. Bayomy et al. (2017) investigated the protective effects of various antioxidants against the damage caused by gentamicin in the rat kidney. Tubular dilatation, desquamation of tubular lining cells, tubular dilatation, vacuolar degeneration, pyknotic nuclei, interstitial cellular infiltration, interstitial hemorrhage, edema, and glomerular damage characterized by shrinkage of the glomeruli were observed in kidneys from the gentamicin group. In addition, serum creatinine, BUN, and MDA values increased, while SOD values decreased in the gentamicin group. Oral administration of 50 mg/kg RA 1 hour before each gentamicin administration; in addition to protecting the kidneys from histopathological damage, it decreased the increases in BUN, creatinine and tissue MDA values, and it caused an increase in SOD values[25]. In the present study, a significant increase in tubular atrophy, tubular vacuolization, glomerular damage, vascular congestion, and interstitial inflammation was observed in kidney sections from the CP group compared to the Sham group. Interstitial inflammation and hyaline material accumulation were also observed. No pathological findings were observed in the CP+RA group, except for mild tubular atrophy. While the BUN values increased significantly in the CP group compared to the Sham group, a decrease occurred in the CP+RA group compared to the CP group. Creatinine values increased significantly in the CP group compared to the Sham group. No statistical difference was observed between the CP+RA group and the CP group in terms of creatinine levels. MDA increased in the CP+RA group compared to the Sham and CP groups. The increase in MDA in RA-treated groups has been reported to be due to the prooxidant effect caused by H_2O_2 and free radical formation with a peroxidase effect[26,27]. Although we think that MDA values may change with using RA at different doses, we think the present results will contribute to the literature.

Although the current study has provided valuable results, it has some limitations. The most important limitation is that more biochemical data (especially parameters such as total antioxidant level (TAS), total oxidant level (TOS), and GSH-Px) are needed, as rosemary acid is an antioxidant substance. In addition, verification of the data obtained from the studies with electron microscopy can make the results even more reliable.

We recommend that researchers carrying out new studies on this subject obtain more detailed data on the study using techniques such as immunohistochemistry, electron microscopy, flow cytometry, ELISA, and Western blot analysis to enrich the results. An RA group can be established to observe the effects of RA on tissues alone. Disease modeling with CP can be used to observe the effects of RA on diseases treated with CP. The use of CP and RA at different doses and for different durations may allow new studies to determine the most effective treatment dose and recovery period.

In conclusion, CP causes tubular atrophy, glomerular damage, vascular congestion and interstitial inflammation in the kidney. RA exerts protective effects against CP-induced nephrotoxicity. The oxidant-antioxidant balance is a delicate equilibrium that can be affected by various parameters.

Author Contributions: Concept/Design : DÇ, EY; Data acquisition: DÇ, AFB, NS; Data analysis and interpretation: DÇ, EY; Drafting manuscript: DÇ, EY; Critical revision of manuscript: DÇ, EY; Final approval and accountability: DÇ, EY, AFB, AA, NS; Technical or material support: -; Supervision: DÇ, EY, AFB; Securing funding (if available): n/a.

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