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Carvacrol Attenuates Amikacin-Induced Nephrotoxicity in the Rats

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ABSTRACT

Objective: Amikacin (AK) is a wide-spectrum antibiotic routinely used to treat gram-negative and some gram-positive bacterial infections. However, its use is limited due to its potential to cause nephrotoxicity due to an increase in reactive oxygen radicals. The main goal of this study was to investigate the effect of carvacrol (CAR) on AK-induced nephrotoxicity in rats.
Methods: Thirty-two Sprague Dawley rats were randomly separated into four groups: the control (0.9% NaCl solution and sunflower oil), AK (400 mg/kg), CAR+AK (80 mg/kg CAR+400 mg/kg AK), and AK+CAR (400 mg/kg AK+80 mg/kg CAR) groups. AK and CAR were administered intramuscularly and orally, respectively for 7 days. Blood and kidney tissue samples were collected at the end of the experiment. The level of catalase, superoxide dismutase, malondialdehyde, and reduced glutathione, which are parameters of oxidative stress, were detected while comparing renal function and histopathological changes.
Results: Histopathological findings (necrotic changes, dilatation and inflammatory cell infiltration) were significantly greater in the AK group than in the control group. Additionally, significant weight loss was detected in the rats in the AK group. CAR treatment, both before and after AK administration, significantly improved nephrotoxicity histopathologically ($p<.05$). However, the same improvement was not identified biochemically.
Conclusion: CAR treatment significantly improved nephrotoxicity both before and after AK administration, suggesting that carvacrol has a protective effect against AK-induced kidney damage at the histopathological level.
Keywords: Antioxidant, amikacin, carvacrol, nephrotoxicity, oxidative stress, rat

Introduction

Acute kidney injury (AKI), known as acute renal failure, is a syndrome characterized by a rapid decrease in glomerular filtration (Liu et al., 2020). AKI has been reported in nearly 10-15% of all hospitalized patients, and the incidence of AKI is even greater (over 50%) among patients admitted to intensive care units (ICUs) (Ronco et al., 2019).

Aminoglycosides exhibit antibacterial effects by inhibiting bacterial protein synthesis via reversible binding to the 16S ribosomal RNA of the 30S ribosome with high affinity (Ahmed et al., 2020). Amikacin (AK) is an aminoglycoside-derived antibiotic. It is often used in intensive care units to treat life-threatening bacterial infections, especially those caused by gram-negative aerobes and gram-positive Staphylococcus aureus (Abdel-Gayoum et al., 2015, Polat et al., 2006). The essential advantages of AK are its high antibacterial activity, rapid effect, synergistic activity when combined with beta-lactam antibiotics, low-cost and low resistance (Kara et al., 2016). Despite its high efficacy, the clinical use of AK is restricted due to its ototoxicity and nephrotoxicity (Raeeszadeh et al., 2021). A well-documented side-effect of AK is nephrotoxicity, which is characterized by tubular necrosis, particularly in experimental animal studies and clinical observations in humans (Sweileh 2009, Parlakpinar et al., 2003). Most aminoglycosides are excreted in the urine without being metabolized. Some AKs also accumulate selectively in the renal cortex (Selim et al., 2017). Aminoglycosides, including amikacin, are picked up by renal proximal tubular cells through endocytosis, and their accumulation can damage various cellular structures. (McWilliam et al., 2017, Polat et al., 2006).

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Various nephrotoxicity mechanisms, including kidney tubular toxicity, glomerular injury, inflammation, crystal nephropathy, and thrombotic microangiopathy, have been explored (Al-Kuraishy et al., 2019, Ozer et al., 2020, Kang et al., 2011). Substantial evidence supports the role of reactive oxygen species (ROS) in AK-induced nephrotoxicity, where their accumulation leads to oxidative stress and disrupts the antioxidant/oxidant balance. AK induces nephrotoxicity characterized by oxidative stress through elevating the levels of malondialdehyde (MDA) and decreasing the levels of glutathione (GSH). Despite these known nephrotoxicity mechanisms, the renal toxicity of AK is not fully known. Additionally, various experimental studies have shown that nephrotoxicity can be prevented by several antioxidants (Parlakpinar et al., 2004, Parlakpinar et al., 2003, Parlakpinar et al., 2006, Abdelhamid et al., 2020).

In recent years, various studies have been conducted to identify effective renoprotective compounds that could be beneficial in clinical practice (Rehman et al., 2014). CAR is indeed a monoterpene phenol found in the essential oils of different Labiatae plants, such as Satureja, Origanum, Corydothymus, Thymus, and Thymbra (Melo et al., 2011, Ili and Keskin 2013). It is widely used as an additive in the food industry (Ultee et al., 1999). CAR is known for its biological and pharmacological activities, such as anticancer, antioxidant, anti-inflammatory, antibacterial, antifungal, and hepatoprotective activities both in vitro and in vivo (Potočnjak and Domitrović 2016, Suntres et al., 2015, Ili and Keskin 2013, Ghorani et al., 2021). CAR has been shown to have anti-inflammatory effects by increasing the synthesis of IL-10 and lowering the production of proinflammatory mediators such as IL-1β (da Silva Lima et al., 2013). The major biological and pharmacological activities of CAR are shown in Figure 1.

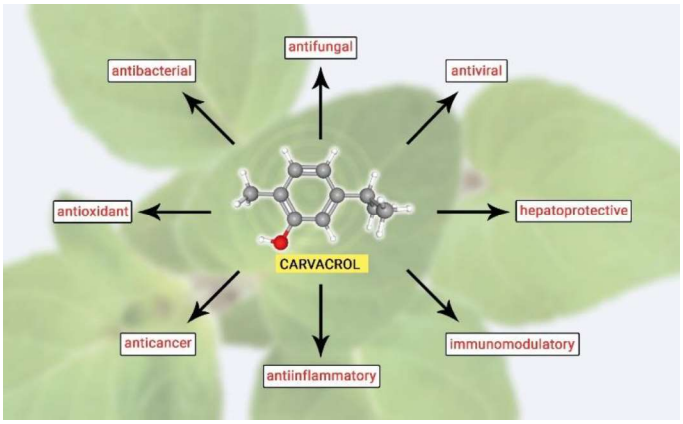

Figure 1. CAR's major biological and pharmacological activities.

Some studies have investigated the effects of CAR on nephrotoxicity; however, there are no studies on the protective and therapeutic effects of CAR, a known potent antioxidant, on AK-induced nephrotoxicity. The hypothesis of this study is that CAR, whose antioxidant and anti-inflammatory effects have been shown in previous studies, may be effective in treating nephrotoxicity, in which reactive oxygen species and inflammation play role. Therefore, this study was designed to investigate the possible antioxidant and anti-inflammatory effects of CAR on AK-induced nephrotoxicity in rats.

Methods

Animals

For the present study, 32 female 4- to 6-month-old Sprague–Dawley rats with weights of 218–320 g were obtained from the Inonu University Laboratory Animals Research Center and maintained in a controlled room with a controlled temperature (21 ± 2 °C) and humidity (60 ± 5%) and a 12:12 h light: dark cycle. Rats were fed with a standard chow pellet diet and provided with tap water ad libitum. Animals were randomly assigned to different experimental groups to assemble and process the data and to allow investigators to blindly analyze the treatment groups. Almost all of the experiments in the study were performed according to the National Institutes of Health Animal Research Guidelines and ARRIVE guidelines (Çolak % Parlakpınar, 2012). The protocol of this study was authorized by the Committee of Ethics on Animal Research (reference no: 2015/A-79) of the Faculty of Medicine, Inonu University, Malatya, Türkiye. A simple randomization technique was applied to allocate the rats to different experimental groups to minimize bias and enhance the credibility of the findings in the experiment.

Chemicals

AK (Amikozit 500 mg®, Eczacibasi Corp., İstanbul, Türkiye), CAR (CAS number: 499-75-2, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), and ethyl carbamate (urethane® CAS number: 51-79-6, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) were purchased.

Experimental design

As described in Figure 2, thirty-two female Sprague Dawley rats were randomly assigned to four groups (n=8 for each group) as listed below:

1. Control group: Rats were given 0.5 mL of 0.9% NaCl solution via intramuscular (i.m.) administration and 1 mL

of sunflower oil via a per-oral (p.o.) administration of one dose daily for 7 days.

2. AK group: Rats were given 0.5 mL of AK (400 mg/kg) via the i.m. route and 1 mL of sunflower oil via p.o. one dose daily for 7 days.

3. CAR+AK group: CAR (80 mg/kg) was applied via the p.o. route. One dose daily for 7 days before the first dose of AK (400 mg/kg) was administered via the i.m. route, and one dose daily and continued for 7 days to assess the protective effects of CAR against AK-induced nephrotoxicity.

4. AK+CAR group: After one dose of AK (400 mg/kg) daily for 7 days, one dose of CAR (80 mg/kg) was given via the p.o. route daily for 7 days.

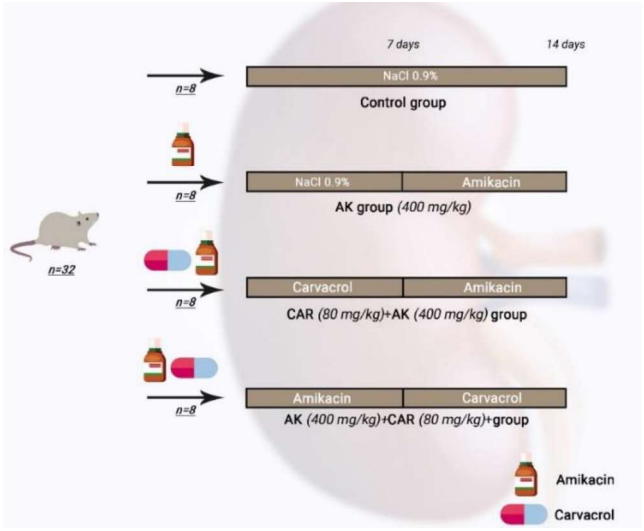

Figure 2. Schematic representation of the experimental design.

Each rat was weighted and then anesthetized via intraperitoneal (i.p.) administration of ethyl carbamate at a dose of 1.2 g/kg 24 h after the last injection. Then, blood samples and kidney tissues were obtained from anesthetized rats, and histopathological and biochemical analyses were performed. Kidneys were quickly extracted, decapsulated, and split equally into two longitudinal segments. Half of the tissue was fixed with formalin for histopathological analysis, and the remaining half of the kidney tissue was stored at -70 °C for biochemical analysis. Blood samples were obtained in tubes without anticoagulant to determine blood urea nitrogen (BUN) and creatinine (Cr) levels. Histopathological examinations (tubular dilatation, infiltration of inflammatory cells, and necrosis under a light microscope) and biochemical evaluations [catalase (CAT), superoxide dismutase (SOD), MDA, and reduced GSH] were performed at the end of the study protocol.

Biochemical analysis

After centrifuging blood samples from the rats at 3500 rpm for 10 min, the serum samples were separated in Eppendorf tubes and stored at -80 °C. One day before the biochemical analysis, the frozen samples were transferred to the +4 °C unit for thawing. Afterwards, serum parameters such as BUN and Cr were studied at Inonu University Turgut Ozal Medical Center Laboratories (Abbott Architect c16000).

The levels of CAT, MDA, SOD, and GSH in the renal tissue were measured. Protein determination in the tissue was performed by Biuret protein analysis using bovine serum albumin as a standard (Hiller et al., 1948).

The activity of MDA (an important marker of lipid peroxidation) was calculated according to Uchiyama and Mihara (Uchiyama & Mihara 1978). The kidney samples were homogenized on ice for 1 min at 15,000 rpm in a 1.15% KCl solution to form a 10% homogenate. This homogenate was directly used for analyzing MDA. The prepared solutions were added to the test tubes and vortexed, and the tubes were left in boiling water at 95 °C for 1 h. The tubes were vortexed for 5 min after adding two ml of n-butanol and then centrifuged at 3,000×g for a minute. The absorbances were measured by using a spectrophotometer at 532 nm. The value of lipid peroxides was computed as the TBARS of lipid peroxidation, and the results are presented in nmol/g tissue according to the adjusted standard graph.

The detection of GSH was performed according to the methods of Ellman (Ellman 1959). Kidney tissue samples were homogenized on ice for 1-2 min at 15,000 rpm to form a 10% homogenate, and the homogenate tissues were subsequently centrifuged at 3,000 rpm for 15 min at +4 °C. To prepare the samples for GSH analysis, trichloroacetic acid (TCA) solution was added to the resulting supernatant, which was mixed and centrifuged again. The processed solutions were placed in test tubes and vortexed, and the color intensity was measured at 410 nm in a spectrophotometer after 5 min. The results were evaluated from the standard chart of GSH and are presented as nmol/g wet tissue.

The activity of tissue SOD was calculated according to the methods of Sun et al (Sun et al., 1988). Kidney tissue samples were homogenized on ice for 1 min at 15,000 rpm to form a 10% homogenate, which was subsequently centrifuged at 10,000 rpm for 20 min. A prepared mixture of chloroform/ethanol at a ratio of 3 to 5 was added to

the supernatant. Then, the sample was centrifuged at 5,000 rpm for 20 min at +4 °C. Afterwards, for CuZn-SOD analysis, the top clear white chloroform phase was carefully pipetted and used. The arranged test tubes were centrifuged for 20 min at 25 °C. At the end of the process, CuCl2 was added to each tube, and the reaction was stopped (0.8 mmol/L). A spectrophotometric evaluation was performed at 560 nm. The absorbance of both the blank and the samples was recorded, and the enzyme activity was measured. SOD enzyme activity was given as U/g protein.

The activity of tissue CAT was measured based on the method of Luck (Luck 1974). Kidney tissue samples were homogenized on ice for 1 min at 15,000 rpm to form a 10% homogenate. Then, the same homogenate tissues were centrifuged at 10,000 rpm for 20 min, and CAT analysis supernatant was used. The absorbance of the spectrophotometer was adjusted to zero by a blunt and was brought to 240 nm. After the supernatant was added to the sample tubes, the absorbance at 240 nm was measured. Then, the absorbance decreased gradually, followed by repeated readings every 15th sec for 90 sec. At the end of the period, the absorbance value was recorded. The time interval of the linear absorbance reduction was evaluated. CAT activity is presented as K/g protein.

Histopathological analysis

Finally, renal tissue samples were fixed in 10% formaldehyde and embedded in paraffin. After tissue follow-up procedures, four- to five-µm-thick slices of paraffin blocks were cut, prepared and subjected to hematoxylin-eosin (H&E) staining for general histological evaluation. Kidney tissues were analyzed for tubular dilatation, inflammatory cell infiltration, and necrosis. For semiquantitative scoring of each variable, the following scale was used: 0, normal tissue; 1, <25% of the entire area was damaged; 2, 25–50% of the entire area was injured; and 3, >50% of the entire area was damaged. A Leica Q Win Image Analysis System (Leica Micro Imaging Solutions Ltd., Cambridge, UK) with a Leica DFC-280 research microscope was used to carry out histopathological analysis.

Data analysis

The required power and sample sizes used in the experiment were defined by using statistical power analysis to detect even minor effects. Power analysis using the type I error probability to detect bidirectional variance between experimental groups (α=0.05) and type II error probability (β=0.20), along with past laboratory results, showed that the minimum sample size required to detect a significant difference in the levels of Cr in the pilot study should be at least 8 per group (24 in total) according to the Web-Based Sample Size and Power Analysis Software required for each experimental group (Arslan et al., 2018). The Kolmogorov–Smirnov test was used to verify the normality of the distribution. Based on the normality tests, the measurable variabilities of all groups in the study did not show a normal distribution. Consequently, for the statistical assessment of histopathological results, Kruskal–Wallis variance analysis was used. For comparisons between paired groups, the Mann–Whitney U test was used. A p value less than 0.05 was considered to indicate statistical significance. IBM SPSS Corp., Armonk, NY for Windows package program was used for the data analysis. The data are presented as the median (minimum-maximum).

Results

Experimental toxicity and body weight

During the experiment, none of the animals died due to interventional procedures or any other cause. AK application led to significant weight loss compared to that in the control group ($p<.05$). CAR treatment before and after AK administration did not significantly improve body weight. Rat weight and serum and tissue biochemical parameters are presented in Table 1.

Biochemical findings

Kidney function tests

AK application caused a significant increase in the BUN level in all groups compared to that in the control group ($p<.05$). However, CAR treatment before and after AK administration did not significantly increase the BUN level

Tissue biochemical findings

The application of AK did not significantly change the level of MDA compared to that in the control group. Additionally, CAR administration before and after AK treatment resulted in a statistically significant decrease in MDA levels (P>0.05). The application of AK caused a significant increase in the GSH level compared to that in the control group. However, CAR administration before and after AK treatment did not cause significant changes in GSH levels (P>0.05). Administration of AK did not cause any significant changes in the level of SOD compared with

that in the control group. However, CAR administration both before and after AK treatment resulted in a statistically significant increase in the level of SOD compared to that in the control group. Administration of AK caused an insignificant decrease in the level of CAT compared to that in the control group. There was also a significant increase in the level of CAT in the AK-CAR group compared to that in the AK group.

Table 1. Rat weight, serum and tissue biochemical parameters.

Parameters*	Groups*				*p*
	Control	AK	CAR+AK	AK+CAR	
Rat weight (g)	279[a,b] (254-307)	241 (235-320)	251 (218-272)	262 (237-284)	.0117
BUN (mg/dL)	24.57[a,b,c] (21.35-26)	31.27 (20.37-74.31)	33.7 (25.21-95.77)	34.87 (31.47-80.37)	.0027
Creatinine (mg/dL)	0.6[c] (0.58-0.68)	0.66[c] (0.5-1.38)	0.72[c] (0.57-1.88)	0.9 (0.82-1.53)	.0034
MDA (nmol/g wet tissue)	175.78[a,b,c] (154.36-204.68)	149.6 (107.44-190.4)	115.6 (104.04-169.32)	129.54 (114.92-162.52)	.0097
GSH (nmol/g wet tissue)	589.13[a,b,c] (488.4-779.4)	855.72 (563.7-1056.16)	828.25 (543.35-954.41)	768.21 (667.48-1058.2)	.0198
SOD (U/g protein)	573.99[b,c] (511.67-708.48)	637.33[b] (224.14-851.39)	726.38 (679.93-822.68)	701.95 (585.85-808.13)	.0092
CAT (K/g protein)	2479.18[c] (1667.6-5012.1)	2467.8[c] (1294.4-3930.81)	2404.12 (1333.41-4259.17)	3912.29 (2614.4-4331.32)	.045

[a]Significant compared to AK group (p<.05).
[b]Significant compared to CAR+AK group (p<.05).
[c]Significant compared to AK+CAR group (p<.05).
*Data are expressed as median (min-max).
MDA, malondialdehyde; GSH, reduced glutathione (GSH); SOD, superoxide dismutase; CAT, catalase;

Histopathological findings

Kidney tissues in the control group exhibited a normal histological structure (Figure 3A). Nonetheless, necrotic changes and dilatation were detected in the tubules in the cortical area of the AK group (Figure 3B). Infiltration of inflammatory cells in the interstitial tissue was another important finding detected in the AK group (Figure 3C). In terms of these changes, the difference between the AK and control groups was statistically significant (p<.05). CAR administration before AK significantly reduced all histopathological changes observed in the AK group (p<.05) (Figure 3D and 3E). Tubular dilatation and necrosis were reduced considerably in the CAR group after AK (p<.05), while infiltration remained similar to that in the AK group (Figure 3F and 3G). Moreover, the administration of CAR before and after AK treatment did not cause any changes in tubular dilatation or inflammatory cell infiltration. However, tubular necrosis was significantly less common in the CAR group before AK surgery (p<.05). The histopathological evaluation results of the groups are given in Table 2.

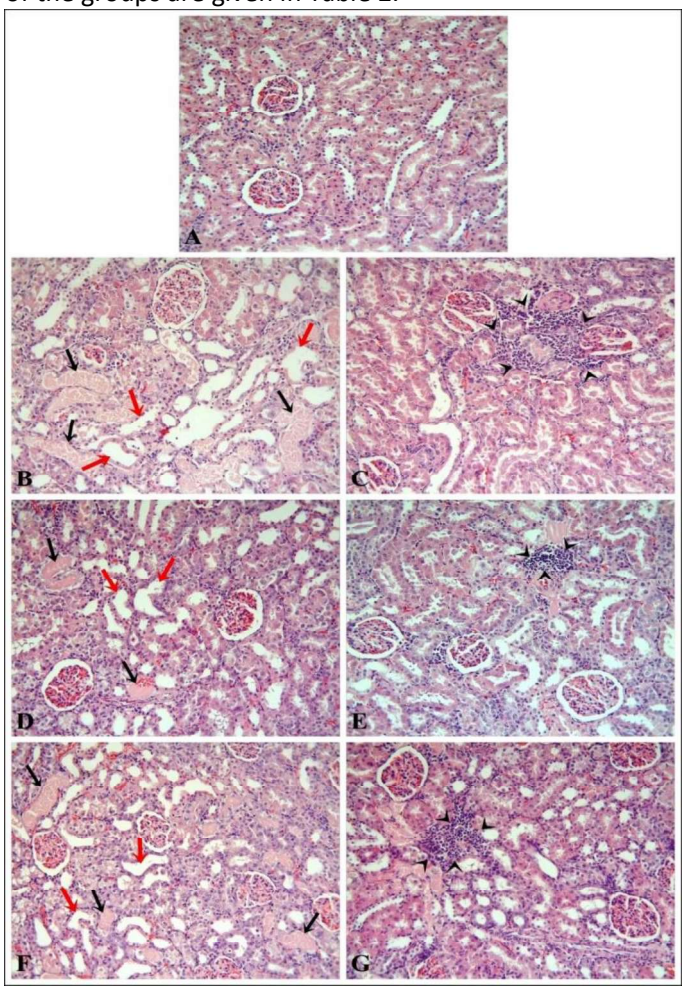

Figure 3. The renal cortical tissue has a normal histological appearance in the control group (A). Tubular necrosis, dilatation and infiltration in the interstitial space are seen in the AK group (B and C). There was a significant improvement in histopathological changes in the CAR+AK group (D and E). Tubular changes decreased significantly in the AK+CAR (F and G) group, but infiltration continued similar to the AK group. Black arrows indicate tubular necrosis, red arrows indicate tubular dilatation, arrowheads indicate infiltration. H-E; x20.

Discussion

The goal of this study was to reveal the protective and therapeutic effects of CAR treatment on oxidative casualties and disruption of the antioxidant defense system via biochemical and histopathological analysis. According to our results, histopathological changes (necrotic changes, dilatation and inflammatory cell infiltration) were significantly greater in the AK group than in the control group. All nephrotoxicity findings were significantly reduced by the administration of CAR before AK in the AK group. However, tubular dilatation and

necrosis were reduced significantly in the CAR group after AK treatment, whereas infiltration was not changed. Tubular necrosis was significantly lower in the CAR+AK group than in the AK group.

Oxidant products, such as reactive oxygen species (ROS) and nitrogen species are physiologically released as a result of cellular activities but remain in a physiological balance (Baltaci et al., 2019). Cellular antioxidant mechanisms provide this balance (Gunata & Parlakpinar, 2020). However, a shift in this physiological balance in favor of oxidants due to endogenous or exogenous causes plays a role in the pathogenesis of many diseases, such as diabetes mellitus, atherosclerosis, myocardial infarction, renal failure, rheumatoid arthritis, and nephrotoxicity (Gunata & Parlakpinar, 2020, Abdel-Daim et al., 2019, Kapucu 2021). ROS damage many structures, such as the cell membrane and nucleus (Gunata & Parlakpinar, 2020, Zare Mehrjerdi et al., 2020).

Table 2. Histopathological evaluation results

Groups	Parameters*		
	Tubular dilatation	Tubular necrosis	Inflammatory cell infiltration
Control	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
AK	1.0 (0.0-3.0)[a]	1.0 (0.0-2.0)[a]	1.0 (0.0-0.3)[a]
CAR+AK	0.0 (0.0-2.0)[b]	0.0 (0.0-1.0)[b,c]	0.0 (0.0-2.0)[b]
AK+CAR	0.0 (0.0-2.0)[b]	0.0 (0.0-2.0)[b]	0.0 (0.0-2.0)

[a] Significant increase compared to control group ($p<.05$).
[b] Significant decrease compared to the AK group ($p<.05$).
[c] Significant decrease compared to AK+CAR group ($p<.05$).
*Data are expressed as median (min-max).

Nephrotoxicity is a significant clinical concern associated with the use of aminoglycoside antibiotics, which are widely used to treat gram-negative infectious diseases (Parlakpinar et al., 2006). The kidney is an organ that maintains homeostasis and removes metabolic products from the body. Kidney functions include maintaining water and electrolyte balance, producing various hormones, removing bioactive substances that affect body functions, regulating blood pressure, synthesizing erythropoietin, regulating vitamin D production, and regulating calcium metabolism (Sahay et al., 2012). Approximately 25% of cardiac output passes through the kidneys. Therefore, drugs can damage the kidneys and have potentially toxic effects (Peasley et al., 2021). Medications are indeed a relatively common cause of AKI. Drug-induced nephrotoxicity is a significant contributor to AKI in both adult and pediatric populations. Prospective cohort studies have reported that approximately 14- 26% of AKI cases in adults and 16% of AKI cases in hospitalized children are due to drug-induced nephrotoxicity. Drug-induced nephrotoxicity is more common in hospitalized patients, especially patients in intensive care units (Perazella 2018, Hoste et al., 2015). It is predicted that approximately 25% of patients receiving aminoglycoside therapy may develop nephrotoxicity (Lopez-Novoa et al., 2011). Various risk factors that facilitate the development of aminoglycoside-associated nephrotoxicity have been identified. These risk factors include patient-specific factors such as advanced age, impaired renal function, dehydration, hepatic dysfunction, hypothyroidism, metabolic acidosis, and sodium depletion. In addition, long treatment duration, higher doses of the drug, the use of divided doses of the drug, and the use of various drugs that are eliminated via the renal route are also important risk factors (Wargo and Edwards 2014).

Aminoglycosides show concentration dependent, bactericidal activity (Mirazi et al., 2021). Due to the widespread use of aminoglycosides, side effects such as ototoxicity and nephrotoxicity have become more pronounced. Despite its side effects, it continues to be widely used due to its various advantages. The essential advantages of AK are its high antibacterial activity, rapid effect, low resistance, synergistic activity with beta-lactam antibiotics, and low cost (Kara et al., 2016). AK is a broad-spectrum aminoglycoside derivative drug that causes nephrotoxicity via many mechanisms. These mechanisms include decreased blood supply to the renal tissue, decreased glomerular filtration rate, and tubular cytotoxicity. The contraction of mesangial cells and the release of vasoconstrictor hormones such as angiotensin 2 may cause a decrease in the blood supply of renal tissue (Krause et al., 2016, Abdel-Daim et al., 2019).

In the present study, AK led to significant renal dysfunction, as demonstrated by the substantial increase in the serum urea concentration. Nephrotoxicity caused by aminoglycoside is an important and common cause of morbidity, especially in hospitalized patients. This causes a significant additional treatment cost (Bulut et al., 2016). Most aminoglycosides are eliminated by the renal route, usually without being metabolized. Some drugs accumulate in the proximal segments of renal tubules, and the drug concentration in the proximal segments of renal tubules is greater than that in plasma (Bulut et al., 2016). This accumulation is associated with nephrotoxic effects. The accumulation of AK increases oxidative stress by generating free radicals (Bulut et al., 2016). Oxidative stress caused by AK leads to cellular dysfunction and DNA damage (Xiong et al., 2015). Oxygen radicals are thought to play important roles in the pathogenesis of AK-induced nephrotoxicity (Yang et al., 2017). Therefore, antioxidants

are valuable for controlling AK-induced nephrotoxicity. It is also known that TNF and Nrf-2 expression in renal tissue is increased in AK-induced nephrotoxicity (Selim et al., 2017, Abd El-Kader and Taha 2020, El-Kashef et al., 2015).

Histopathological studies have shown that tubular necrosis is the main cause of nephrotoxicity (Asci et al., 2015, Parlakpinar et al., 2004). Glomerulus obstruction, proinflammatory cell migration, tubule dilatation, bleeding, and tubule degeneration were observed in the AK-induced nephrotoxicity group (Asci et al., 2015). This may be due to the generation of highly reactive radicals due to oxidative stress caused by AK. CAR administration before AK significantly decreased all histopathological changes observed in the AK group (Figure 3D and 3E). Tubular dilatation and necrosis were reduced considerably in the CAR group after AK surgery (Figure 3F and 3G). Tubular necrosis was significantly less common in the CAR group before AK resection. These findings show that CAR-T-cell therapy has both histopathalogically protective and therapeutic effects against oxidative kidney damage.

Although other studies have shown that AK decreases the activities of CAT and SOD enzymes in kidney tissue, we did not observe any significant changes in the levels of these antioxidant enzymes in our study protocol (Abdel-Daim et al., 2019). In accordance with our study results, other studies have shown that the levels of antioxidant enzymes such as CAT and SOD may not change due to increased oxidation and antioxidant usage (Yılmaz et al., 2018, Ohta and Nishida 2003). In another study, Cu(II)-aminoglycoside complexes were shown to be formed by holding the copper ions of aminoglycosides (Szczepanik et al., 2004). Ulusoy et al., showed that MDA, total oxidative status, and oxidative stress indices increased in an experimental AK-induced nephrotoxicity model (Ulusoy et al., 2012, Ahmed et al., 2021). Similarly, Kose et al., reported that MDA levels increased significantly in an AK-induced nephrotoxicity model in rats (Kose et al., 2012). However, no significant increase in the level of MDA was detected between the AK-induced nephrotoxicity group and the control group in our study. In contrast to the expected increase in MDA levels under conditions of oxidative stress or oxidant exposure, some studies have reported no change or even a decrease in MDA levels in certain contexts (Garcia et al., 2020, Lima et al., 2019, Ubani-Rex et al., 2017). Importantly, not all lipid peroxidation processes result in the production of MDA. Additionally, MDA can be formed through reactions other than lipid peroxidation (Jenkins 2000, Sharma et al., 2021). Animal and human studies have shown that MDA levels remain unchanged despite the expectation that increased oxidation would lead to elevated levels of MDA, a marker of lipid peroxidation (Ma'rifah et al., 2019, McGrath et al., 2001, Kamendulis et al., 1999). A significant increase in the level of BUN was detected by comparing outcomes between the AK-induced nephrotoxicity group and the control group in the AK-induced nephrotoxicity model (Abdel-Daim et al., 2019, Parlakpinar et al., 2006). Similarly, in the present study, we showed that the BUN level was greater in the AK-induced nephrotoxicity group than in the control group. The fact that the BUN and Cr parameters were studied without using a rat specific kit can be considered among the limitations of this study.

CAR is a monoterpene phenol found in the essential oils of Labiatae, such as Satureja, Origanum, Coridothymus, Thymus, and Thymbra (Ili & Keskin, 2013, Shahrokhi Raeini et al., 2020). CAR has lipophilic properties and various biological and pharmacological activities, including anticancer, antioxidant, antibacterial, antifungal, and hepatoprotective activities both in vitro and in vivo (Figure 1) (Suntres et al., 2015, Shahrokhi Raeini et al., 2020, Oliveira et al., 2012). It has been suggested as a natural food preservative for the food industry, mainly due to its safety, flavoring, quality and antimicrobial activities (Mishra et al., 2018).

Recent studies in rats revealed that CAR treatment has beneficial effects on ameliorating oxidative stress-induced damage in multiple organs, such as the brain, liver, and kidneys (Samarghandian et al., 2016). The anti-inflammatory and antioxidant properties of CAR have been demonstrated in experimental models of various inflammatory conditions, including arthritis, colitis, asthma, ischemia/reperfusion injury, and sepsis (Banji et al., 2014, Khosravi and Erle 2016, Arigesavan and Sudhandiran 2015, Suo et al., 2014). A study of experimental renal ischemia/reperfusion injury in rats showed that CAR treatment has beneficial effects on tubular atrophy, dilatation, brush border loss, and hydropic epithelial cell degeneration (Ozturk et al., 2018).

Conclusion

We investigated for the first time the effects of CAR in this experimental AK-induced nephrotoxicity model in rats. According to the results of the present study, CAR administration significantly improved histopathological injury in AK-induced nephrotoxicity rats. Additionally, the consumption of CAR improved the BUN concentration in the AK-induced nephrotoxicity group. However, CAR did

not considerably improve biochemical markers in kidney tissue. However, we share some of our biochemical results, which we cannot fully explain, to shed light on future studies. Furthermore, we recommend further research to determine the clinical applicability and effectiveness of the CAR. Plant extracts standardized by further analysis of medicinal plants containing CARs can be used in complementary treatments in the clinic.

Ethics Committee Approval: This study protocol was approved by the Ethics Committee on Animal Research (reference no: 2015/A-79) of the Faculty of Medicine, Inonu University, Malatya, Türkiye.
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