Carvacrol attenuates amikacin-induced nephrotoxicity in the rat

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Abstract

Amikacin (AK) is frequently used in the treatment of gram-negative and some gram-positive infections. However, its use is limited due to nephrotoxicity due to the increase in reactive oxygen radicals. The aim of this study was to investigate the role of carvacrol (CAR) against AK-induced nephrotoxicity in rats. Thirty-two Sprague Dawley rats were randomly divided into four groups as control (Vehicle), 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 via intramuscular and per-oral for 7 days, *respectively*. Blood and kidney tissue samples were taken at the end of the experiment. Renal function and histopathological changes were compared, and the relevant parameters of oxidative stress and inflammation were detected. Histopathological findings (necrotic changes and dilatation and inflammatory cell infiltration) significantly increased in the AK group compared to the control group. Also, the rats in the AK group lost weight significantly. It was found that CAR treatment before and after AK significantly improved nephrotoxicity histopathologically ($p < 0.05$). However, this improvement was not detected biochemically. These results show that CAR treatment before and after AK improves nephrotoxicity in the histopathological level.

Introduction

Acute kidney injury (AKI) is a syndrome characterized by a sudden decrease in glomerular filtration (Hoste et al. 2018). AKI has been reported in 10–15% of hospitalized patients and more than 50% of intensive care patients (Ronco et al. 2019).

Aminoglycosides bind reversibly to the 16S ribosomal RNA of the 30S ribosome with high affinity and inhibit bacterial protein synthesis (Ahmed et al. 2020). Amikacin (AK) is an aminoglycoside-derived antibiotic. It is used in intensive care units to treat life-threatening bacterial infections, including 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 with beta-lactam antibiotics, low resistance, and low cost (Kara et al. 2016). Despite its high efficacy, the clinical use of AK is limited due to its ototoxicity and nephrotoxicity (Ozer et al. 2020). Nephrotoxic side effects of AK characterized by tubular necrosis have been demonstrated in experimental animals and humans (Parlakpinar et al. 2003; Sweileh 2009). Most aminoglycosides are excreted in the urine without being metabolized. Some of AK also accumulates selectively in the renal cortex (Selim et al. 2017). Aminoglycosides are taken up by renal proximal tubular cells and cause damage to cellular structures (McWilliam et al. 2017; Polat et al. 2006).

Various nephrotoxicity mechanisms, including renal tubular toxicity, inflammation, glomerular injury, crystal nephropathy, and thrombotic microangiopathy, have been identified due to the studies (Al-Kuraishy et al. 2019; Kang et al. 2011; Ozer et al. 2020). Substantial evidence supports the role of reactive oxygen species (ROS) and disruption of antioxidant/oxidant balance in AK-induced nephrotoxicity pathogenesis. Despite these known nephrotoxicity mechanisms, the renal toxicity of AK is not fully known. Also, various experimental studies have shown that nephrotoxicity can be prevented with several antioxidants (Abdelhamid et al. 2020; Parlakpinar et al. 2004; Parlakpinar et al. 2003; Parlakpinar et al. 2006).

In recent years, numerous studies have been conducted to find effective renoprotective compounds that can be used in clinical practice (Rehman et al. 2014). CAR is a monoterpenic phenol found in the essential oil of Labitae, including Origanum, Satureja, Thymbra, Thymus, and Corydothymus (Ili and Keskin 2013; Melo et al. 2011). It is widely used as an additive in the food industry (Ultee et al. 1999). CAR has numerous biological and pharmacological activities, including antioxidant, anticancer, antibacterial, antifungal, hepatoprotective both in vitro and in vivo (Ili and Keskin 2013; Potočnjak and Domitrović 2016; Suntres et al. 2015). CAR has an anti-inflammatory effect by increasing the synthesis of IL-10 and decreasing the production of pro-inflammatory mediators such as IL-1β (da Silva Lima et al. 2013). CAR's major biological and pharmacological activities are shown in Fig. 1.

Some studies investigate the effects of CAR on nephrotoxicity, however there are no studies on CAR's protective and therapeutic effects, a known potent antioxidant, on AK-induced nephrotoxicity. Therefore, the present study designed to investigate the possible antioxidants and anti-inflammatory activity of CAR against AK-induced nephrotoxicity in rats.

Materials And Methods

Animals

For this study, 32 female Sprague Dawley rats with age 4–6 months and weight 218–320 g, were obtained from the Inonu University Laboratory Animals Research Center and placed in a temperature (21 ± 2 C) and humidity (60 ± 5%) controlled room in which a 12:12 h light:dark cycle was maintained. Rats were fed a standard chow pellet diet with tap water ad libitum. Randomization was used to assign animals to different experimental groups and to collect and process data, with analysis performed by investigators blinded to the treatment groups. All experiments in this study were performed by the National Institutes of Health Animal Research Guidelines and ARRIVE guidelines (Çolak and Parlakpınar 2012).

The study protocol was approved by the Ethics Committee on Animal Research (reference no: 2015/A-79) under the Faculty of Medicine, Inonu University, Malatya, Turkey.
A simple randomization technique was used to allocate the rats to the groups to avoid bias in the way the experiment was carried out.

Chemicals

AK (Amikozit 500 mg®, Eczacibasi Corp., Istanbul, Turkey), 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 Fig. 2, thirty-two female Sprague Dawley rats were randomly set into four groups (n = 8 for each group) as follows:

1. Control group: Rats were given 0.5 ml of NaCl 0.9% solution via intramuscular (i.m.) one dose daily for 14 days.

2. AK group: Rats were given 0.5 ml of AK (400 mg/kg) via i.m. one dose daily for 7 days.
3. CAR + AK group: CAR (80 mg/kg) was applied via per-oral (p.o.) one dose daily for 7 days before the first AK (400 mg/kg) administration via i.m. one dose daily and continued for 7 days to test the protective effect of CAR against AK-induced nephrotoxicity.
4. AK + CAR group: After the AK (400 mg/kg) treatment via i.m. one dose daily for 7 days, CAR (80 mg/kg) was given via p.o. one dose daily for 7 days.

All rats were weighted and then anesthetized with administering i.p. ethyl carbamate (1.2 g/kg) after 24 h of the last injection. Then, kidney tissue and blood samples were taken from anesthetized rats, and histopathological and biochemical analyses were performed. Kidneys were quickly removed, decapsulated, and divided equally into two longitudinal segments. One half was fixated with formalin for histopathological examination, and the remaining tissues were stored at -70˚C for biochemical analysis. Blood samples were collected in tubes without anticoagulant to determine blood urea nitrogen (BUN) and creatinine (Cr). Histopathological examinations (inflammatory cell infiltration, tubular dilatation, and necrosis under a light microscope) and biochemical evaluations [malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH)] were performed at the end of the study protocol.

Biochemical analysis

After the rats' blood samples were centrifuged at 3500 rpm for 10 min, serum samples were taken into eppendorf tubes and stored in a refrigerator (-80°C). The day before the biochemical analysis, frozen samples were moved to the + 4°C unit for thawing. Subsequently, serum BUN, Cr parameters were studied at Inonu University Turgut Ozal Medical Center Laboratories (Abbott Architect c16000).

Levels of MDA, SOD, CAT, and GSH content in the kidney tissue were determined. Protein determination in the tissue was made by Biuret protein analysis using bovine serum albumin as a standard (Hiller et al. 1948).

The determination of the MDA (one of the important end product of lipid peroxidation) activity was studied according to Uchiyama and Mihara (Uchiyama and Mihara 1978). The rat kidney sample was homogenized on ice for 1 min at 15000 rpm in a 1.15% KCl solution to form 10% homogenate. This homogenate was used directly in the MDA analysis. The prepared solutions were added to the test tubes, vortexed, and the tubes were left in boiling water at 95°C for 1 h. Two ml of n-butanol was added to the tubes vortexed for 5 min. Then the samples were centrifuged at 3000xg for 10 min. The absorbance was measured using a spectrophotometer at 532 nm. The amount of lipid peroxides was calculated as TBARSs of lipid peroxidation, and the results were given in nmol per g tissue (nmol/g tissue) according to a prepared standard graph.

GSH was determined according to the method of Ellman (Ellman 1959). The kidney sample was homogenized on ice for 1–2 min at 15.000 rpm to form 10% homogenate. The homogenate was then centrifuged at 3.000 rpm for 15 min at + 4°C. Trichloroacetic acid (TCA) solution was added to the supernatant obtained, mixed, and centrifuged again to make the sample ready for GSH analysis. The prepared solutions were added to test tubes, vortexed, and the intensity of the colour formed after 5 min was read at 410 nm in the spectrophotometer. The results were evaluated from the GSH standard chart and given as nmol/g wet tissue.

Tissue SOD activity was measured according to Sun et al (Sun et al. 1988). The kidney sample was homogenized on ice for 1 min at 15.000 rpm to form 10% homogenate. This homogenate was centrifuged for 20 min at 10.000 rpm. A mixture of chloroform/ethanol (3 units of chloroform/5 units of ethanol) prepared at a ratio of 3 to 5 was added to this supernatant. Then the samples were centrifuged for 20 min at 5.000 rpm at + 4°C. The top clear white chloroform phase was carefully pipetted and used for CuZn-SOD analysis. The prepared test tubes were incubated at 25°C for 20 min. At the end of the period, 1 ml of CuCl2 was added in both tubes, and the reaction was stopped (0.8 mmol/L). A spectrophotometric evaluation was performed at 560 nm. The absorbance of the blank and the samples were recorded, and the enzyme activity was calculated. SOD enzyme activity was given as U/g protein.

Tissue CAT activity was measured according to the method of Luck (Luck 1974). The kidney sample was homogenized on ice for 1 min at 15.000 rpm to form 10% homogenate. This homogenate was centrifuged at 10.000 rpm for 20 min, and the supernatant was used in CAT analysis. The spectrophotometer was brought to 240 nm and adjusted to zero absorbance by a blunt. Absorbance was read at 240 nm immediately after the supernatant addition to the sample tubes. Then, the absorbance decrease was followed for 90 sec by doing a reading every 15 sec. The absorbance value was recorded at the end of the period. The time interval of the linear absorbance decrease was evaluated. CAT enzyme activity was given as K/g protein.

Histopathological analysis

At the end of the experiment, kidney tissues were fixed in 10% formaldehyde and embedded in paraffin. Four-five µm thick sections were taken from the paraffin blocks prepared after tissue follow-up procedures. The hematoxylin-eosin (H-E) staining method was applied to the sections for general histological evaluations. Kidney sections were evaluated in terms of inflammatory cell infiltration, tubular dilatation, and necrosis. For semiquantitative scoring of each variable was used the following scale: 0, normal tissue; 1, damage involving < 25% of the total area; 2, an injury involving 25–50% of the total area; 3, damage involving > 50% of the total area. The analysis is carried out using Leica Q Win Image Analysis System (Leica Micros Imaging Solutions Ltd., Cambridge, UK) with a Leica DFC-280 research microscope.

Data analysis

The required power and sample sizes used in this experiment were defined using statistical power analysis to detect even minor effects. Power analysis using the type I error probability to detect a bidirectional variance between experimental groups $\alpha = 0.05$; type II error probability $\beta = 0.20$ together with previous laboratory results showed that the minimum sample size required to detect a significant difference in the Cr levels of the pilot study should be at least $n = 8$ per group (24 in total) using Web-Based Sample Size and Power Analysis Software required in every experimental group (Arslan et al. 2018). The normality of the distribution was verified using the Kolmogorov-Smirnov test. It was found that all study groups' measurable variables did not show normal distribution according to normality tests. Consequently, Kruskal Wallis variance analysis was used for the statistical assessment of histopathological results. The Mann-Whitney-U test was used for comparisons between paired groups. The values of "p" which are less than 0.05 were considered statistically significant. IBM SPSS Statistics v. 25 SPSS Inc., Chicago, IL for Windows package program was used for the data analysis. Data were presented as median (minimum-maximum).

Results

Experimental toxicity and body weight

No animals deceased due to interventional procedures or any other cause during the experiment. AK application caused significant body weight loss when compared with the control group ($P < 0.05$). Administration of CAR-treatment before and after AK administration did not significantly improve body weight. Rat weight, serum and tissue biochemical parameters were presented in the Table 1.

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)	**0.0117**
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)	**0.0027**
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)	**0.0034**
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)	**0.0097**
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)	**0.0198**
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)	**0.0092**
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)	**0.045**

[a]Significant compared to AK group ($p < 0.05$).

[b]Significant compared to CAR + AK group ($p < 0.05$).

[c]Significant compared to AK + CAR group ($p < 0.05$).

*Data are expressed as median (min-max).

MDA, malondialdehyde; GSH, reduced glutathione (GSH); SOD, superoxide dismutase; CAT, catalase;

Biochemical findings

Kidney function tests

AK application caused a significant increase in BUN level in the all groups when compared to the control group ($P < 0.05$). However, CAR-treatment before and after AK administration did not cause a significant advance in the BUN level.

Tissue biochemical findings

The application of AK did not cause a significant change in the MDA level compared to the control group. Also, CAR administration before and after AK treatment resulted in a statistically insignificant decrease in MDA level ($p > 0.05$). Application of AK caused a significant increase in GSH level when compared to the control group. However, CAR administration before and after AK treatment did not cause a statistical change in GSH level ($p > 0.05$). The administration of AK did not cause a significant change in the SOD level when compared to the control group. However, CAR administration before and after AK treatment resulted in a statistically significant increase in the SOD level when compared to the control group. Administration of AK caused a non-significant decrease in CAT level when compared to the control group. It also caused a statistically significant increase in CAT level in the AK-CAR group when compared to the AK group.

Histopathological findings

The kidney tissue had a normal histological structure in the control group (Fig. 3A). Despite this, necrotic changes and dilatation were observed in the tubules in the AK group's cortical area (Fig. 3B). Inflammatory cell infiltration in the interstitial tissue is another important finding observed in the AK group (Fig. 3C). In terms of these changes, the difference between AK and control groups was statistically significant ($p < 0.05$). CAR administration before AK significantly reduced all histopathological changes observed in the AK group ($p < 0.05$) (Fig. 3D and 3E). Tubular dilatation and necrosis were reduced considerably in the CAR group after AK ($p < 0.05$), while infiltration continued similar to the AK group (Fig. 3F and 3G). Moreover, administration of CAR before and after AK treatment did not cause any change tubular dilatation and inflammatory cell infiltration. However, tubular necrosis was significantly less in the CAR group before AK ($p < 0.05$). The histopathological evaluation results of the groups were given in Table 2.

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 ($p < 0.05$).

[b] Significant decrease compared to the AK group ($p < 0.05$).

[c] Significant decrease compared to AK + CAR group ($p < 0.05$).

*Data are expressed as median (min-max).

Discussion

This study aims to reveal the protective and therapeutic role of CAR treatment on oxidative damage and disruption of the antioxidant defense system in the light of biochemical and histopathological analyzes. According to our results, histopathological changes (necrotic changes and dilatation and inflammatory cell infiltration) were significantly increased in the AK group when compared to the control group. It was found that CAR administration before AK significantly reduced all nephrotoxicity findings observed in the AK group ($p < 0.05$). However, tubular dilatation and necrosis decreased significantly in the CAR group after AK treatment ($p < 0.05$) whereas infiltration was not changed. It was also found that tubular necrosis was significantly reduced in the CAR + AK group than the AK group.

Oxidant products such as 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 and Parlakpinar 2020). However, the shift of this physiological balance in favor of oxidant 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 (Abdel-Daim et al. 2019; Gunata and Parlakpinar 2020; Gunata et al. 2020; Kapucu 2021). ROS damages many structures such as cell membrane and nucleus (Gunata and Parlakpinar 2020; Zare Mehrjerdi et al. 2020).

Nephrotoxicity is an essential clinical complication of aminoglycoside antibiotics widely used to treat gram-negative infectious diseases (Parlakpinar et al. 2006). The kidneys are an organ that maintains body homeostasis and removes metabolism products from the body. The kidney functions include maintaining water and electrolyte balance, producing various hormones, removing bioactive substances that affect body functions, regulating blood pressure, synthesis of erythropoietin, regulation of vitamin D production, and

regulation of 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 a relatively common cause of AKI. Drug-induced nephrotoxicity in adults accounts for approximately 14–26% of AKI in prospective cohort studies, while 16% of hospitalized AKI is due to drugs in the pediatric population. Drug-induced nephrotoxicity is more common in hospitalized patients, especially in intensive care patients (Hoste et al. 2015; Perazella 2018). It is estimated that approximately 25% of patients receiving aminoglycoside therapy develop nephrotoxicity (Lopez-Novoa et al. 2011). Various risk factors have been identified that facilitate the development of aminoglycoside-associated nephrotoxicity. These risk factors include patient-specific factors such as advanced age, impaired renal function, dehydration, hypothyroidism, hepatic dysfunction, metabolic acidosis, and sodium depletion. In addition, long treatment duration, higher doses and taking the drug in divided doses, and its use with various drugs that are eliminated by the renal route are also important risk factors (Wargo and Edwards 2014).

Aminoglycosides show concentration-dependent, bactericidal activity (Wargo and Edwards 2014). Due to the widespread use of aminoglycosides, side effects such as ototoxicity and nephrotoxicity became 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, synergistic activity with beta-lactam antibiotics, low resistance, and low cost (Kara et al. 2016). AK is a broad spectrum aminoglycoside derivative drug that causes nephrotoxicity by 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 angionthecin 2 may cause the decrease in the blood supply of the renal tissue (Abdel-Daim et al. 2019; Krause et al. 2016).

In the current study, AK caused significant renal dysfunction, as demonstrated by the substantial increase in serum urea level. 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 of the drugs accumulate in the proximal renal tubules, and the drug concentration in the proximal tubule is higher than in plasma (Bulut et al. 2016). This accumulation is associated with the nephrotoxic effect. Accumulating 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 a role in the pathogenesis of AK-induced nephrotoxicity (Yang et al. 2017). Therefore, antioxidants are valuable in controlling AK-induced nephrotoxicity. It is also known that TNF and Nrf-2 expression in the renal tissue is increased in AK-induced nephrotoxicity (Abd El-Kader and Taha 2020; El-Kashef et al. 2015; Selim et al. 2017).

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 formation of highly reactive radicals due to oxidative stress caused by AK. CAR administration before AK significantly reduced all histopathological changes observed in the AK group ($p < 0.05$) (Fig. 3D and 3E). Tubular dilatation and necrosis were reduced considerably in the CAR group after AK ($p < 0.05$) (Fig. 3F and 3G). Tubular necrosis was significantly less in the CAR group before AK ($p < 0.05$).

These features show that CAR treatment has both protective and therapeutic effects histopathologically against oxidative kidney damage.

Although other studies have shown that AK reduces the activities of SOD and CAT enzymes in kidney tissue, we could not find any significant changes in levels of antioxidant enzymes in our study protocol (Abdel-Daim et al. 2019). In accordance with our result, there are also studies showing that the levels of antioxidant enzymes such as SOD and CAT may not change due to increased oxidation and antioxidant use (Ohta and Nishida 2003; Yılmaz et al. 2018) It has also been shown that Cu (II)-aminoglycoside complexes are formed by holding the copper ions of aminoglycosides in another study (Szczepanik et al. 2004). Ulusoy et al. shown that MDA, total oxidative status, and oxidative stress indices increased in the experimental AK-induced nephrotoxicity model (Ahmed et al. 2021; Ulusoy et al. 2012). Likewise, Kose et al. found that MDA level increased significantly in the AK-induced nephrotoxicity model in rats (Kose et al. 2012). However, there was no significant increase in MDA level in the AK-induced nephrotoxicity group when compare to the control group in our study. In contrast, some studies have shown that there is no change or may decrease in MDA level in oxidant exposure (Garcia et al. 2020; Lima et al. 2019; Ubani-Rex et al. 2017). In addition, not all lipid peroxidation process produces MDA and MDA is produced by reactions other than lipid peroxidation (Jenkins 2000). There are also animal and human studies showed that the MDA value does not change despite the increased oxidation (Kamendulis et al. 1999; Ma'rifah et al. 2019; McGrath et al. 2001). A significant increase in BUN level in the AK-induced nephrotoxicity group when compared to the control group was demonstrated in the AK-induced nephrotoxicity model (Abdel-Daim et al. 2019; Parlakpinar et al. 2006). Similar to this finding, we have shown that the BUN level was higher in the AK-induced nephrotoxicity group when compared to the control group in this study.

CAR is a monoterpenic phenol found in the essential oil of Labitae, including Origanum, Satureja, Thymbra, Thymus, and Corydothymus (Ili and Keskin 2013; Shahrokhi Raeini et al. 2020). CAR has lipophilic properties and numerous biological and pharmacological activities, including antioxidant, anticancer, antibacterial, antifungal, hepatoprotective both in vitro and in vivo (Fig. 1) (Oliveira et al. 2012; Shahrokhi Raeini et al. 2020; Suntres et al. 2015). It has been proposed as a natural food preservative for the food industry, mainly due to its flavoring and antimicrobial activities (Mishra et al. 2018).

A recent study in rats revealed that CAR treatment ameliorated oxidative stress damage in the brain, liver, and kidneys (Samarghandian et al. 2016). The antioxidant and anti-inflammatory properties of CAR have been demonstrated in experimental models based on various inflammation, including arthritis, asthma, colitis, ischemia/reperfusion injury, and sepsis (Arigesavan and Sudhandiran 2015; Banji et al. 2014; Khosravi and Erle 2016; Suo et al. 2014). In a study on rats, in an experimental renal ischemia/reperfusion injury study, CAR treatment improved tubular atrophy, dilatation, loss of brush border, and hydropic epithelial cell degenerations (Ozturk et al. 2018).

Conclusion

We investigated for the first time that the effects of CAR in this experimental AK-induced nephrotoxicity model in the rat. According to the results of this study, it was shown that the administration of CAR significantly improves histopathological injury in AK-induced nephrotoxicity in rats. Also, consumption of CAR improved

BUN values in the AK-induced nephrotoxicity group. However, CAR did not considerably improve biochemical markers of 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 applicability in the clinically and effectiveness of CAR.

Declarations

Ethical Approval

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

Consent to publish

All authors have read and approved the final version of the manuscript.

Author's contributions

AMD, ASD, OO, and HP are the coordinators of this study, and they planned the study protocol design. AMD, ASD, MG, OO, and HP made the mandatory requirements for the study. OO was responsible for drug administration. AMD, MG, OO, and HP were responsible for literature research. AMD, OO, and MG performed the surgical procedures. ST and CC conducted the biochemical analyses. The histopathological evaluations were carried out by AY, and NV whereas CC performed the statistical analysis. AMD, MG, OO, and HP were responsible for the interpretation of the results. MG was responsible for the design of figures and tables. This manuscript was written by AMD, MG, OO, and HP. The final manuscript is revised collaboratively by MG, OO, and HP. The authors declare that all data were generated in-house and that no paper mill was used.

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Conflict of Interest

The authors declare no competing interests.

Data availability

Data available on request from the authors.

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Figures

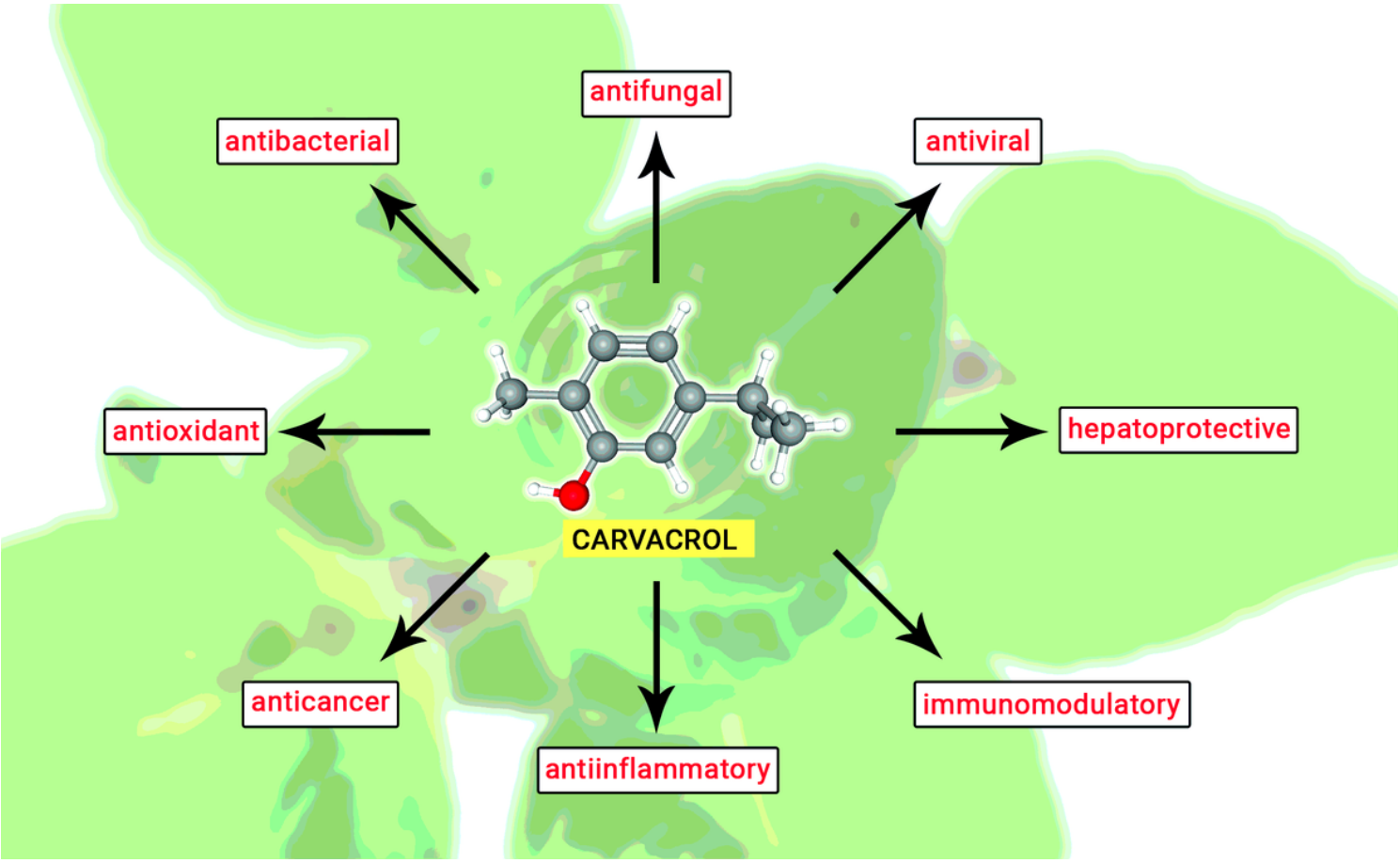

Figure 1

CAR's major biological and pharmacological activities.

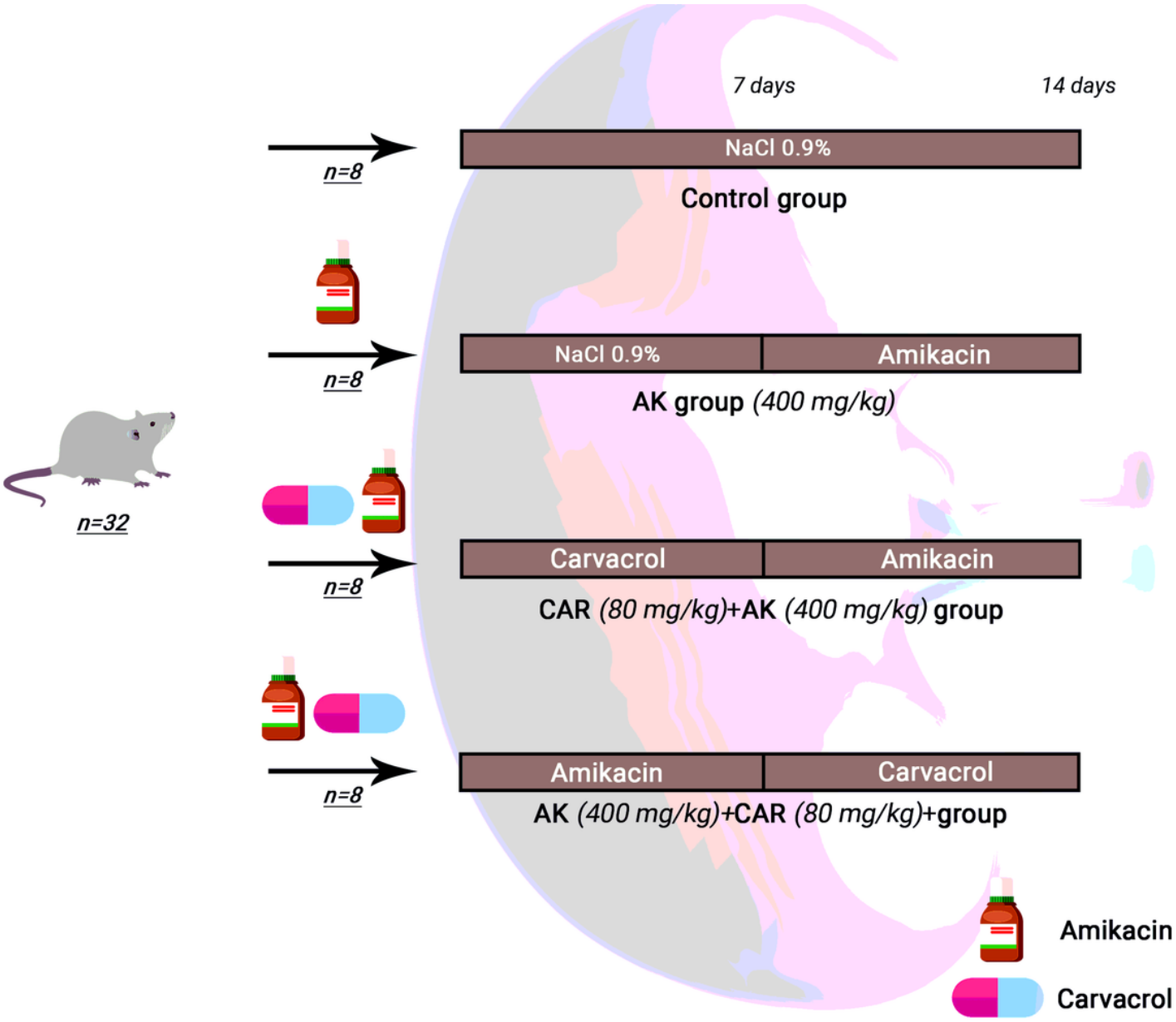

Figure 2

Schematic representation of the experimental design.

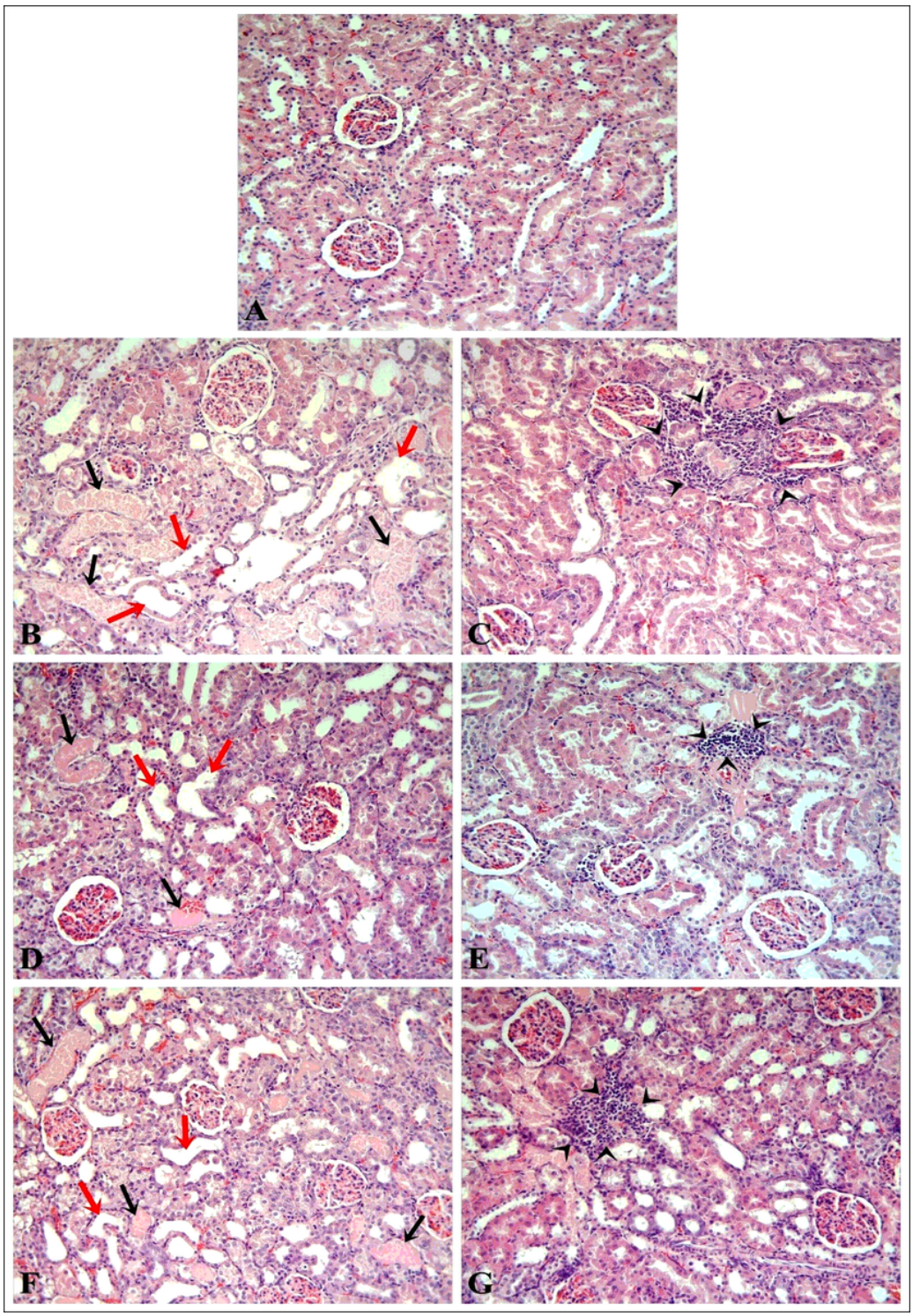

Figure 3

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.

Supplementary Files

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