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ORIGINAL ARTICLE

Investigating the possible protective effect of caffeic acid phenethyl ester on aquaporin-2 changes in renal ischemia-reperfusion injury in rats

Investigación del posible efecto protector del éster fenetílico del ácido cafeico sobre los cambios de acuaporina-2 en la lesión por isquemia-reperfusión renal en ratas

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Abstract

Objective: *We aimed to investigate the changes in renal aquaporins (AQP) of rats in renal ischemia-reperfusion (I/R) injury and the protective effects of caffeic acid phenethyl ester (CAPE) against these changes.* **Methods:** *Forty-five adult rats were divided into six groups: control, sham (right nephrectomy), I/R (right nephrectomy + left kidney I/R), I/R+CAPE (I/R procedure after i.p. CAPE), sham + CAPE, and sham + dimethyl sulfoxide. Blood urea nitrogen, Cr, and K^+ levels were measured in the sera. Tissues were stained with hematoxylin and eosin for histopathological examination. For immunohistochemical analysis, AQP2 was applied using the streptavidin/biotin/peroxidase system. AQP2 gene expression in kidney tissues was examined by polymerase chain reaction (PCR).* **Results:** *In the I/R, congestion, inflammation, and necrosis were found to increase compared to the control. In the I/R+CAPE, improvement was observed in necrosis compared to the I/R. There was a decrease in AQP2 expression in the I/R. In PCR, no significant difference was observed in AQP2 gene expression between the I/R and control and between the I/R+CAPE and I/R.* **Conclusion:** *Renal I/R inhibits the production of AQP2 in the kidney and causes histological and biochemical damage. CAPE administration before I/R has a protective effect on the kidney.*

Keywords: *Kidney. Caffeic acid. Antioxidant. Renal ischemia. Phytochemical. Aquaporin-2.*

Resumen

Objetivo: *Investigar los cambios en las acuaporinas renales (AQP) de ratas con lesión por isquemia-reperfusión renal (I/R) y los efectos protectores del éster fenetílico del ácido cafeico (CAPE) frente a estos cambios.* **Métodos:** *Se dividieron 45 ratas adultas en seis grupos: control, simulado (nefrectomía derecha), I/R (nefrectomía derecha + I/R renal izquierda), I/R + CAPE (procedimiento de I/R tras CAPE i.p.), simulado + CAPE y simulado + dimetilsulfóxido. Se midieron los niveles de BUN, Cr y K^+ en suero. Los tejidos se tiñeron con hematoxilina y eosina para el examen histopatológico. Para el análisis inmunohistoquímico se aplicó AQP2 utilizando el sistema estreptavidina/biotina/peroxidasa. La expresión del gen AQP2 en los tejidos renales se examinó mediante PCR.* **Resultados:** *En I/R se observó un aumento de la congestión, la inflamación y la necrosis en comparación con el control. En I/R + CAPE se observó una mejoría de la necrosis en comparación con I/R. Se observó*

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una disminución de la expresión de AQP2 en I/R. En la PCR no se observaron diferencias significativas en la expresión del gen AQP2 entre I/R y control ni entre I/R + CAPE e I/R. **Conclusión:** *La I/R renal inhibe la producción de AQP2 en el riñón y causa daño histológico y bioquímico. La administración de CAPE antes de la I/R tiene un efecto protector sobre el riñón.*

Palabras clave: *Riñón. Ácido cafeico. Antioxidante. Isquemia renal. Fitoquímico. Acuaporina-2.*

Introduction

Water, which is a major component of human cells and all living things, passes through membranes through membrane channel proteins called aquaporins (AQP)[1,2]. More than 10 AQPs have been identified in mammals. AQP2 is expressed in the renal collecting ducts and is localized in the apical membrane of principal cells[2,3]. AQP2, which shuttles between the plasma membrane and the intracellular stores, passes from the stores inside the cell to the plasma membrane and plays a role in the passage of water through the membrane[2]. In healthy individuals, it is released from the apical plasma membrane under the action of antidiuretic hormone (ADH) and allows the reabsorption of water from the filtrate[1]. The presence of mutations in AQP2, which plays an important role in concentrating urine, results in diabetes insipidus, characterized by polyuria and polydipsia[2,4]. It was shown that AQP2 expression decreases in a compensatory manner in experimentally induced hypertension and consequently increases urine volume[5].

Ischemic damage to the kidney occurs in cases such as kidney transplant surgery, partial nephrectomy, aortic aneurysm surgery, hydronephrosis, sepsis, and trauma[6-10]. Ischemia refers to a decrease in blood flow to an organ, thus reducing the oxygen and nutrients carried by the blood. Restored blood supply after ischemia, called reperfusion, increases tissue damage and organ dysfunction more than ischemia. Renal ischemia/reperfusion (I/R) causes acute renal failure and has a high morbidity and mortality rate[11].

Various agents have been used as preventive or therapeutic agents in renal I/R[12-15]. Honey has been known to have health benefits for many years. Propolis, which is produced by honey bees using extracts collected from many plants in the environment, is a resin that protects the hives from external factors[16]. Honey contains mainly glucose and fructose along with many vitamins, minerals, flavonoids, phenolic acids, and carotenoids. Some of the phenolic and flavonoid contents found in high amounts in honey are phytochemicals called caffeic acid, caffeic acid phenethyl ester (CAPE), chrysin, and quercetin[17]. These substances have anticarcinogenic and antioxidant properties[17,18]. It was reported that CAPE, one of the important components of propolis, reduces kidney damage and serum oxidant levels[19,20]. It was also shown to be protective against renal damage due to myocardial I/R[21].

The aim of this study was to investigate the effect of renal I/R injury on renal AQP2, tissue morphology, and serum parameters in rats and the possible protective effects of CAPE against these changes by real-time polymerase chain reaction (PCR), immunohistochemical, histopathological, and biochemical methods.

Methods

Animals and experimental protocol

For the study to be conducted, the ethics committee approval numbered 2012/A-19 was obtained from the Experimental Animal Ethics Committee of İnönü University Faculty of Medicine. Our study was supported by İnönü University Scientific Research Projects Coordination Unit (Project no: 2012/77). In the study, 45 adult female rats of Sprague Dawley lineage weighing 250-300 g obtained from İnönü University Experimental Animal Production and Research Center were used. The rats were kept in an air-conditioned room with automatically controlled temperature (22 ± 1°C) and lighting (07.00-19.00 h). The animals were randomly divided into six groups. The control group (n = 7) was not given any medication, and no application was made. Right nephrectomy was performed in the Sham group (n = 8). In the I/R group (n = 8), right nephrectomy was performed, followed by 1 h of ischemia to the left kidney and a subsequent 24 h reperfusion. For the rats in the I/R+CAPE group (n = 8), 10 μmol/kg CAPE (Sigma-Aldrich, Chemie Gmbh, Steinheim, Germany) was administered intraperitoneally (i.p.) in dimethyl sulfoxide (DMSO) 30 min before the surgery. Then, a right nephrectomy was performed. The left kidney underwent 1 h of ischemia followed by 24 h of reperfusion[14]. In consistent with the literature, CAPE was given at a dose of 10 μmol/kg, which was shown to inhibit the xanthine–xanthine oxidase system and the formation

of free oxygen radicals[22]. The Sham + CAPE group (n = 7) was given 10 μmol/kg CAPE i.p. in DMSO. A right nephrectomy was then performed. The Sham + DMSO group (n = 7) was given DMSO as i.p. Then, a right nephrectomy was performed.

Surgical procedure

Anesthesia was performed using 100 mg/kg ketamine and 10 mg/kg xylazine i.p. A midline incision was made, the abdomen was opened, and right nephrectomy was performed. Then, the left renal vessels were clamped for 1-h of ischemia, followed by removal of the clamp and reperfusion for 24 h. Left kidney tissues were taken for examination and cut in half longitudinally, one half for histopathologic and the other half for immunohistochemical examination. The blood sample was taken to measure the biochemical parameters of blood urea nitrogen (BUN), creatinine (Cr), Na^+, K^+, and Cl^- levels.

Immunohistochemical and histopathological analysis

The tissue samples were first fixed in a 10% buffered formaldehyde solution for 48 h and followed up. After 5-μ thick sections were taken by embedding in paraffin, Hematoxylin and Eosin dyes were applied histochemically, and specimens were examined under a light microscope. For immunohistochemical analysis, 5-μ sections were taken from paraffin blocks, and AQP2 was applied using the streptavidin/biotin/peroxidase system. The obtained preparations were examined under a light microscope.

In histopathological evaluation, cortical, juxtacortical, and medullary areas of kidney preparations were examined for parenchymal and tubular damage. Congestion, inflammation, and necrosis were examined to determine parenchymal and interstitial damage. In addition, tubule dilatation, vacuolar degeneration of tubule epithelial cells, peritubular edema, and intratubular plug formation were examined for tubular damage, and scoring was performed on a semiquantitative scale. Accordingly, no damage was rated as 0, light and single focus damage as 1, moderately severe damage in several foci as 2, severe and widespread damage as 3. Slides that underwent immunohistochemical staining were evaluated for the intensity of chromogen staining and the distribution of stained cells.

BUN, Cr, and K□ were measured by spectrophotometric method on a Roche Cobas auto-analyzer (Roche Diagnostics, Mannheim, Germany).

Real-time PCR

Total RNA isolation

For the detection of AQP2 mRNA levels, kidney tissues of the study groups were cut into small pieces under sterile conditions on ice and were stored in RNA storage solution in a −35°C deep freezer until the day of analysis. Total RNA purification was performed on these tissues using the Qiagen RNeasy mini kit. Total RNAs isolated from kidney samples were run on 1% agarose gel at 100 mV in electrophoresis. RNAs samples in which 28S and 18S sharp ribosomal RNA bands were observed and no degradation was observed were used in the cDNA production.

cDNA synthesis protocol

For cDNA synthesis, the SuperScript III reverse transcriptase enzyme kit produced by Invitrogen was used as recommended by the producer. Briefly, 1 μg of total RNA, 1 μL of primer (4 pmol gene-specific primer or 100 pmol PoliT-18 primer), 1 μL of dNTP (10 mM), and ddH_2O were mixed to a total volume of 13 μL in a 100 μL PCR tube, mixed, and heated in a thermal cycler at 65°C for 15 min. To this mix was added 4 μL of ×5 First strand buffer, 2 μL of DTT, 1 μL of ddH_2O, 1 μL of SuperScript III reverse transcriptase enzyme, mixed and heated in a thermal cycler at 50°C for 60 min and at 70°C for 15 min, then stored at −20°C until analysis.

Real-time PCR protocol

Roche real-time PCR device (model LC480) and Roche PCR kit mix (Roche 04707516001) containing ROCHE real-time ×2 "syber green" dye were used. The data were analyzed according to the 2-ΔΔCT method. The reactions were performed at a total volume of 20 μL. For this, 10 μL of SYBR Green I master mix (enzyme, dNTP, Mg, buffer, and water) was prepared as 1 μL of cDNA, 1 μL of forward and 1 μL of reverse primers (10 pmol/μL) and 7 μL of double-distilled water. After optimization, the PCR conditions were as follows: initial denaturation for 10 min at 95°C, denaturation for 20 s at 95°C, annealing for 40 s at

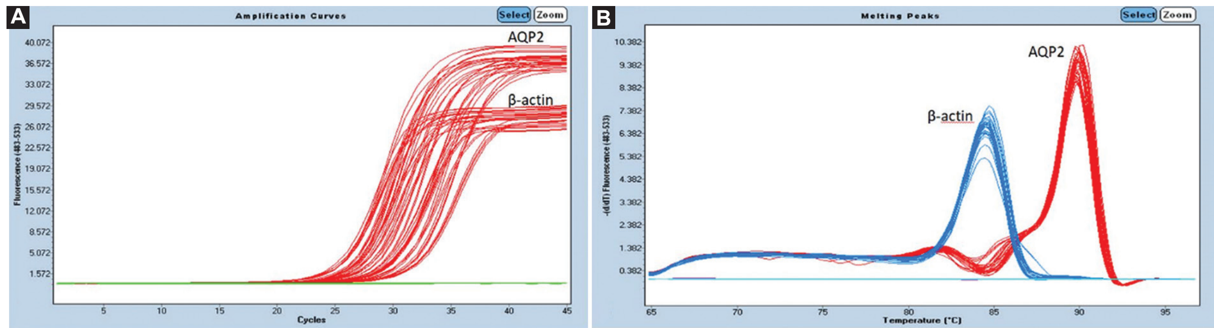

Figure 1. *Analysis of Aquaporin 2 (AQP2) gene expression by real-time PCR. Replication* **A:** *and melting* **B:** *curves of cDNAs synthesized from AQP2 and β-actin mRNAs of the experimental and control groups by real-time PCR using "SYBR Green" chemistry. PCR: polymerase chain reaction.*

60°C, and polymerization for 30 s at 72°C. A total of 45 cycles were used. All real-time PCR studies were carried out in triplicate on the same day to ensure its quantitative accuracy.

cDNA synthesis was performed in all samples using the Poly-T18 primer. In the next step, using the primers specific to the *AQP2* and β-*actin* genes, changes in *AQP* gene expression were determined in proportion to the β-*actin* gene by real-time PCR. Then, the AQP/β-*actin* gene expressions of the control group and the experimental group were compared (Fig. 1).

Primers, whose sequences and sizes are given in table 1, were used in the analysis of *AQP2* gene expression[23,24].

Statistical analyses

Data distribution models were tested using Kolmogorov-Smirnov test. Normally distributed data were expressed as average ± SD. Categorical and ordinal variables were presented as numbers and percentages. Of the quantitative data, those which did not have normal distribution were compared using Mann-Whitney U test, while analysis of variance was used for normally distributed parameters and the Tukey honestly significant difference test for multiple comparisons. The correlations between the parameters were analyzed by Spearman correlation analysis. A correlation coefficient was considered a strong correlation when r was ≥ 0.6 and a moderate correlation when r was between 0.3 and 0.6. Categorical variables were expressed as frequency and percentage, and were compared using the X^2 test. $p < 0.05$ was considered statistically significant. Statistical Package for the Social Sciences (SPSS) version 18 (SPSS Inc., Chicago, USA) software was used for statistical analyses.

Table 1. Primer sequences and product size for AQP2 and β-Actin

Genes	Primer squences	Product size (bp)
AQP2-F	5'-TTGCAGGAACCAGACACTTG-3'	174 B
AQP2-R	5'- GCGGAGACGAGCACTTTTAC-3'	
β-*Actin-F*	5'-CATCACTATCGGCAATGAGC-3'	159 A
β-*Actin-R*	5'-GACAGCACTGTGTTGGCATA-3'	

AQP2: aquaporin 2; F: forward primer; R: reverse primer.

Results

Congestion, inflammation, and necrosis were significantly more common in the I/R group than in the control group ($p < 0.05$). There was an improvement in necrosis in the I/R+CAPE group compared to the I/R group ($p < 0.001$) (Figs. 2 and 3; Table 2).

In immunohistochemical staining, it was determined that AQP2 expression in renal tubules in the I/R group was less than the control and sham groups (Fig. 4).

AQP2 gene expressions were lower in the I/R group than in the other groups ($p > 0.05$). *AQP2* gene expressions increased in the I/R+CAPE group compared to the I/R group ($p > 0.05$) (Fig. 5 and Table 3).

After real-time PCR using AQP 2 and β-actin cDNAs, the specificity of primer binding was checked by running the samples in an agarose gel. As a result of the analysis, it was seen that a single DNA band of the desired length was created for both genes (Fig. 5).

In terms of the biochemical parameters, BUN, Cr, and K^+ levels were significantly higher in the I/R group compared to the control group ($p < 0.05$) (Table 3).

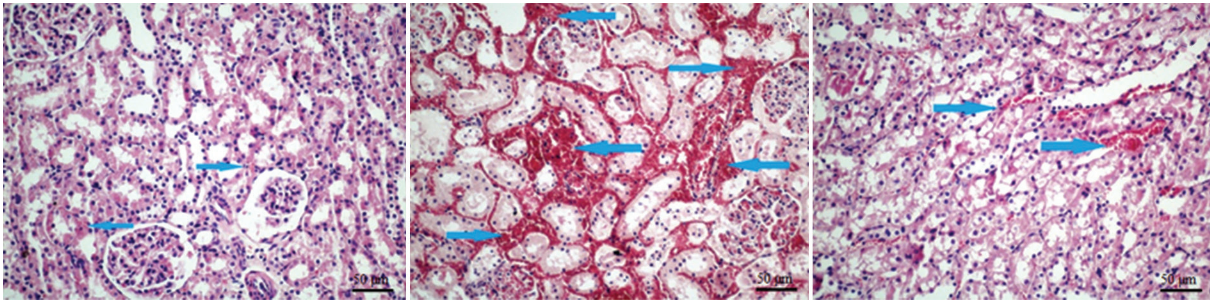

Figure 2. *Histopathologic evaluation of parenchymal and interstitial damage. Blue arrows indicate congested vascular areas.* **A:** *mild congestion (Control group).* **B:** *severe congestion (I/R group).* **C:** *moderate congestion (I/R+CAPE group). I/R: ischemia-reperfusion; CAPE: caffeic acid phenethyl ester; Mag ×20; H&E staining for all images.*

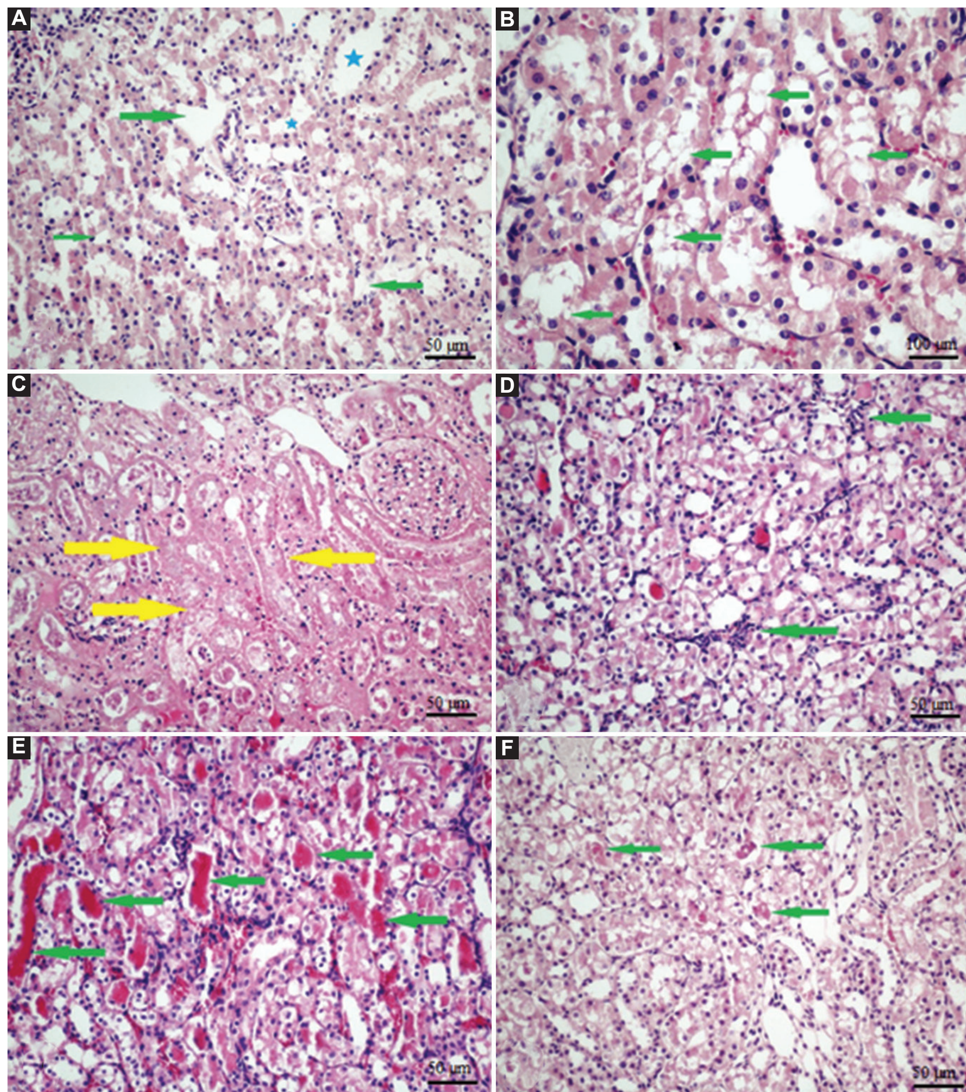

Figure 3. *Areas of edema and vacuolization* **(A and B)**. **A:** *interstitial and intertubular edematous areas (green arrows) and dilated tubules (blue stars) are seen.* **B:** *findings of vacuolar degeneration in proximal tubule cells (green arrows), this microphotograph was taken under x40 magnification to emphasise belp formation (control group). Necrosis and inflammation* **(C and D)**. **C:** *development of coagulation necrosis in a juxtacortical focus (yellow arrows).* **D:** *focal inflammation and leukocytic aggregation in medullary areas (green arrows) (I/R group). Tubular plug development* **(E and F)**. **E:** *dense eosinophilic plugs in proximal tubules (green arrows).* **F:** *development of pale eosinophilic protein plugs in proximal tubules (green arrows) (sham group). I/R: Ischemia-reperfusion. Mag x20. H&E staining for all images.*

Table 2. Comparison of the frequencies of histopathological findings between groups

Damage severity	Control n (%)	Sham n (%)	I/R n (%)	I/R+CAPE n (%)	Sham+CAPE n (%)	Sham+DMSO n (%)
Congestion*						
Absent	2 (25.0)	5 (62.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)
Mild/single focus	5 (21.7)	3 (13.0)	1 (4.3)	3 (13.0)	5 (21.7)	6 (26.1)
Moderate/a few foci	0 (0.0)	0 (0.0)	7 (53.8)	4 (30.8)	2 (15.4)	0 (0.0)
Severe, extensive	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)
Inflammation**						
Absent	3 (18.8)	1 (6.3)	2 (12.5)	1 (6.3)	5 (31.3)	4 (25.0)
Mild/single focus	4 (16.7)	5 (20.8)	3 (12.5)	7 (29.2)	2 (8.3)	3 (12.5)
Moderate/a few foci	0 (0.0)	2 (40.0)	3 (60.0)	0 (0.0)	0 (0.0)	0 (0.0)
Necrosis*						
Absent	7 (18.4)	8 (21.1)	3 (7.9)	6 (15.8)	7 (18.4)	7 (18.4)
Mild/single focus	0 (0.0)	0 (0.0)	1 (33.3)	2 (66.7)	0 (0.0)	0 (0.0)
Moderate/a few foci	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	0 (0.0
Severe, extensive	0 (0.0)	0 (0.0)	1 (100.0	0 (0.0	0 (0.0)	0 (0.0)
Dilation						
Absent	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mild/single focus	4 (18.2)	2 (9.1)	1 (4.5)	4 (18.2)	6 (27.3)	5 (22.7)
Moderate/a few foci	3 (13.6)	5 (22.7)	7 (31.8)	4 (18.2)	1 (4.5)	2 (9.1)
Edema						
Absent	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)
Mild/single focus	6 (17.6)	5 (14.7)	6 (17.6)	8 (23.5)	6 (17.6	3 (8.8)
Moderate/a few foci	1 (10.0)	3 (30.0)	2 (20.0)	0 (0.0)	1 (10.0)	3 (30.0)
Vacuolization						
Mild/single focus	4 (20.0)	4 (20.0)	1 (5.0)	3 (15.0)	3 (15.0)	5 (25.0)
Moderate/a few foci	3 (12.0)	4 (16.0)	7 (28.0)	5 (20.0)	4 (16.0)	2 (8.0)
Plug*						
Absent	5 (20.8)	2 (8.3)	2 (8.3)	2 (8.3)	7 (29.2)	6 (25.0)
Mild/single focus	2 (9.5)	6 (28.6)	6 (28.6)	6 (28.6)	0 (0.0)	1 (4.8)

*$p < 0.001$.
**$p < 0.05$ (X^2 test).
I/R: ischemia-reperfusion; DMSO: dimethyl sulfoxide.

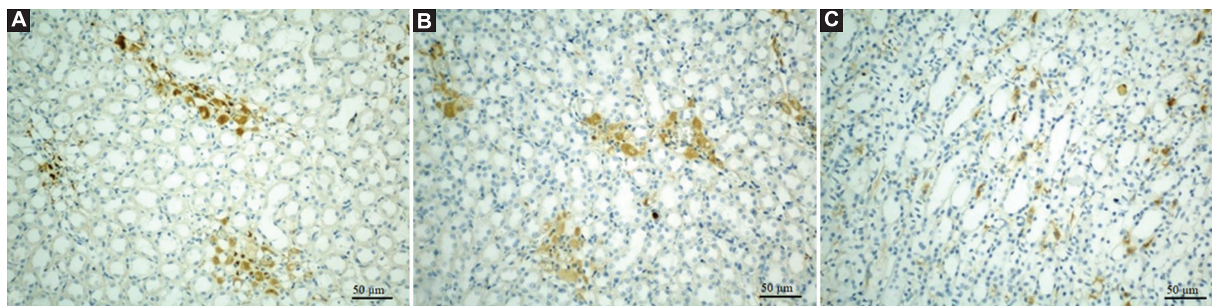

Figure 4. *Aquaporin 2 (AQP2) expression was performed immunohistochemically.* **A** *and* **B:** *regional cytoplasmic and membranous AQP2 expression in tubules in Control and Sham groups, respectively.* **C:** *a decrease in AQP2 expression was observed in the distal collector tubules in the I/R group. Mag x20.*

Discussion

Renal I/R injury is associated with impaired renal blood flow. This damage can occur due to direct kidney-related causes, such as kidney transplant surgery, as well as non-renal causes, such as trauma or aortic aneurysm rupture[12]. Our study was carried out to investigate the tissue damage and AQP2 expression changes in the kidney due to I/R in rats, as well as the possible protective effects of CAPE against these changes.

Table 3. Comparison of biochemical and RT-PCR values of the groups

Parameters	Control	Sham	I/R	I/R+CAPE	Sham+CAPE	Sham+DMSO	p
BUN (mg/dL)	19.28 ± 03.60[a,b]	22.63 ± 04.50[a,b]	127.13 ± 22.79[c,d]	102.00 ± 42.12[c,d]	41,57 ± 25.57	40.14 ± 23.21	< 0.001
Cr (mmol/L)	0.58 ± 0.03[a,b]	0.67 ± 0.04[a,b]	2.15 ± 0.63[c,d]	1.53 ± 0.72[c,d]	0.67 ± 0.06	0.66 ± 0.04	< 0.001
Na^+ (mg/dL)	142.29 ± 2.69	140.25 ± 1.38	137.00 ± 5.55	138.00 ± 7.25	140.57 ± 1.62	138.71 ± 1.38	0.178
Cl^- (mmol/L)	99.14 ± 1.68[b,c]	101.25 ± 0.89	101.00 ± 4.87	104.50 ± 3.50	104.00 ± 2.00	102.57 ± 0.53	0.006
K^+(mmol/L)	4.66 ± 0.24[a,b]	4.99 ± 0.27[a,b]	6.85 ± 1.55[c,d]	6.93 ± 1.76[c,d]	5.06 ± 0.45	4.77 ± 0.40	< 0.001
RT-PCR	0.72 (0.25-1.69)	0.63 (0.30-3.71)	0.11 (0.02-1.77)	0.77 (0.26-8.75)	2.45 (0.06-2.95)	0.71 (0.01-2.79)	0.182

[a]$p < 0.05$, different compared to I/R.
[b]$p < 0.05$, different compared to I/R+CAPE.
[c]$p < 0.05$, different compared to Sham+CAPE.
[d]$p < 0.05$, different compared to Sham+DMSO.
I/R: ischemia-reperfusion. Tukey honestly significant difference, ANOVA Multiple Comparisons (mean ± SD). ANOVA: analysis of variance; RT-PCR: real-time polymerase chain reaction; DMSO: dimethyl sulfoxide.

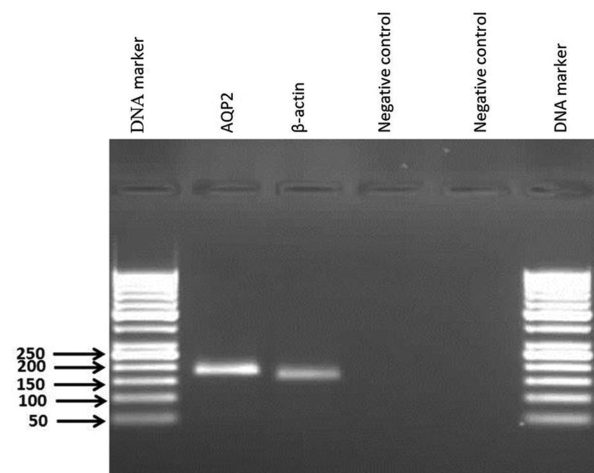

Figure 5. *Agarose gel (2%) electrophoresis of the PCR amplification of β-Actin and Aquaporin 2 (AQP2) cDNAs. The DNA size marker used was a 50 bp DNA ladder (Fermentas). PCR: polymerase chain reaction.*

Tasdemir et al.[14] observed that renal I/R induced tubular cell swelling, tubular dilatation, cellular vacuolation, medullary congestion, and necrosis in rats. Chen et al.[25] evaluated the kidneys in terms of tubular and glomerular anomalies as well as interstitial inflammation in acute tissue rejection after kidney transplantation in rats. They found an increase in histopathological score in rats with tissue rejection compared to the control group. Ozer et al.[21] observed tubular damage and periglomerular dilation along with dilatation, luminal cast formation, and involvement in some glomeruli in the renal tubules of rats with myocardial I/R. It was revealed that the histopathological damage was very mild in the group that was given CAPE before the procedure. In another experimental study, CAPE administration was found to prevent histopathological damage in rats with renal damage due to toluene[26]. It was reported that CAPE plays a protective role against tissue damage by alleviating oxidative stress and increasing antioxidant activity[27].

Yilmaz et al.[22] reported that 10 μmol/kg CAPE inhibits the formation of free oxygen radicals. It was reported in previous studies that histopathological damage occurs in rat kidneys with the administration of gentamicin, and administration of 10 μmol/kg CAPE for 2 days before gentamicin prevents tubular damage[28]. In the present study, CAPE was applied at a dose of 10 μmol/kg in accordance with the literature.

Various studies in the literature using the same[13] or similar[7] methodology to ours reported that serum BUN and Cr levels increased in rats treated with I/R protocols. However, it was shown that there was an improvement in BUN and Cr when different agents with antioxidant properties were given. It was determined that the deterioration of I/R was caused by the increase in oxidative stress. On the other hand, the administration of substances such as ivabradine and nesfatin-1, which are thought to be protective in renal I/R damage, reduced oxidative stress measured by the tissue oxidant (MDA), and antioxidant (SOD and catalase [CAT]) parameters[7,13].

In the literature, polyuria and an increase in serum BUN and Cr levels were reported to develop due to renal I/R[29-31]. This situation is explained by the deterioration of tubular functions in acute renal failure due to I/R. Reabsorption of water from the renal tubules is mediated by various sodium transporters. The decrease in sodium reabsorption in ischemic injury is due to the impairment in sodium transporters[30]. Low oxygen level and high oxygen demand in the renal tubules make the kidney vulnerable to hypoxi[29]. In the ischemic kidney, the ability to concentrate the urine is significantly reduced, and tubular reabsorption of

sodium is impaired. Leukocyte adhesion plays an important role in renal ischemia. Inflammatory cascades, which are activated during the period of reperfusion, cause the accumulation of neutrophils in the medullary vessels. Leukocyte-mediated increases in endothelial permeability lead to erythrocyte aggregation and medullary congestion. In addition, ischemic damage to the medulla continues even if kidney blood flow is restored[32]. Prevention of I/R-induced damage by antioxidant agents was shown by improvement in kidney function tests[29,30]. It was reported that BUN and Cr levels increased in rats given Gentamycin, while BUN and Cr levels returned to normal levels when CAPE was administered[33]. In line with the literature, BUN, Cr, and K^+ levels in the present study were high in I/R. It was observed that CAPE led to a slight improvement in kidney function tests. It was reported that the change in renal function tests in I/R occurs after histopathological change[12]. In the light of this finding, histopathological improvement must occur first to improve the biochemical values. Although the administered CAPE provided a slight improvement histopathologically, it may not have achieved a complete improvement in biochemical parameters. If the time between application and sampling increases in the experimental protocol, biochemical parameters could be restored.

The collector ducts that express AQP2 are located mainly in the renal medulla[2,3]. Cha et al.[31] observed a significant decrease of AQP2 expression in RT-PCR in the renal cortex and medulla in rats undergoing renal I/R. They reported that 1 week after surgery, AQP2 approached control group levels in the cortex, but there was no improvement in the medulla. Similarly, based on RT-PCR, Han et al.[29] detected decreases in AQP2 and vasopressin 2 receptor mRNA levels in the renal internal medulla of rats undergoing I/R. Besides, immunohistochemically, they also observed a significant decrease in AQP2 expression. In other studies, it was shown immunohistochemically that AQP2 expression was reduced in the collecting ducts of rats in renal I/R[30,32,34]. It was reported that there was a decrease in AQP2 mRNA and AQP2 protein expression levels in acute tissue rejection after the kidney transplant model in rats, and in another study, a decrease in vasopressin receptor mRNA levels was observed in addition to the decrease in AQP2 upon the administration of diuretic drugs[24,25]. Substances such as melanocyte-stimulating hormone, erythropoietin, and lithocholic acid, which have antioxidant or anti-inflammatory properties, were found to be protecting against AQP2 changes in renal I/R[29,30,32]. In our study, there was no significant difference in *AQP2* gene expression. We are of the opinion that a change in AQP2 could be observed after a complete histopathological change takes place. Increased ADH binds to receptors on the basolateral membrane of the principal cells in the collecting ducts, which in turn initiates a pathway that causes AQP2 to cross into the apical plasma membrane. AQP2, located in the intracellular vesicle, is phosphorylated by protein kinase A. The vesicles move toward and fuse with the plasma membrane, and AQP2 passes into the membrane, increasing the permeability of the membrane to water. It was reported that in acute tissue rejection after kidney transplantation, AQP2 passes into intracellular vesicles due to the decrease in ADH, thus reducing the permeability of the membranes to water. ADH maintains the water balance of the body by acting on the reabsorption of water from collecting channels by two different mechanisms, both of which involve AQP2. In the short-term mechanism, ADH-regulated AQP2 trafficking between intracellular vesicles and the apical membrane leads to an increase in acute ADH-induced water reabsorption from the collecting channel. In the long-term mechanism, the total amount of AQP2 is regulated depending on the change in the number of water channels in the cell[35]. In a case report in the literature, two patients with congestive heart failure were given ADH antagonists, and their kidney biopsy specimens were examined. In one of these patients, who had been newly diagnosed with diabetes mellitus, it was observed that the renal medulla was intact, with evidence of severe atrophy in the renal cortical tubules. In this patient, despite severe insufficiency in renal function tests, it was reported that the amount of urine excretion increased, and AQP2 expression was observed in the renal collecting duct. On the other hand, in the other patient who had diabetes mellitus and diabetic nephropathy for 8 years, mild atrophy in the renal cortex but inflammatory cell infiltration with severe atrophy in the renal medulla was detected. Absence of AQP2 was reported in immunohistochemical examination in this patient with low renal function tests and urine excretion[36].

Free oxygen radicals are blamed for tissue damage caused by reperfusion[11]. Reperfusion, which triggers energy-deprived and metabolically restless cells to produce reactive oxygen species (ROS), increases tissue damage and organ dysfunction more than ischemia. Some of the ROS are normally formed by mitochondria via xanthine oxidase and cyclooxygenase.

In ischemia, ATP is reduced to hypoxanthine, and xanthine dehydrogenase is converted to xanthine oxidase. When oxygen reenters the ischemic tissue by reperfusion, superoxide radicals are released as xanthine oxidase converts hypoxanthine to uric acid. The production of toxic agents such as superoxide, hydroxyl radical, and hydrogen peroxide exceeds the capacity of endogenous free radical scavengers and causes significant damage to ischemic tissues[37]. Overproduction of reactive oxygen products and lack of antioxidants are the main factors in the formation of I/R damage[7]. CAPE, which has antioxidant properties, suppresses lipid peroxidation. It inhibits the activities of nitric oxide synthase and xanthine oxidase. Thanks to its lipophilic properties, it interacts with mitochondrial membranes and reduces the formation of reactive oxygen derivatives[38]. It was reported that CAPE inhibits the production of free oxygen radicals by inhibiting xanthine oxidase, alleviating nephrotoxicity[28]. Ozer et al.[21] examined the renal damage caused by myocardial IR in rats and reported that preoperative administration of 50 μmol/kg (i.p.) CAPE corrected renal damage by scavenging free radicals and exerting antioxidant activity.

Unlike these studies, in the isoproterenol-induced myocardial infarction study of Oktar et al.[39], it was observed that MPO and MDA increased, and SOD and CAT activities decreased. However, no increase was observed in SOD and CAT activities while decreases were evident in MPO and MDA in rats given 10 μmol/kg (i.p.) CAPE, along with isoproterenol, for 7 days. It was argued that the increase in SOD and CAT activities should have been prevented by CAPE, albeit indirectly.

The strength of our study is that renal function tests, histopathological and immunohistochemical evaluations, and *AQP2* gene expression were performed in the same study, thus, our results were obtained with strong evidence. In addition, since the evaluations were made by creating different groups in our study, it was seen that the results obtained were clearly due to I/R and CAPE and were not changed by any other factor.

Conclusion

It was determined that AQP2 expression, kidney morphology, and kidney function were impaired in renal I/R, and CAPE was protective against the effects of I/R.

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Conflicts of interest

The authors declare no conflicts of interest.

Ethical considerations

Protection of humans and animals. The authors declare that the procedures followed complied with the ethical standards of the responsible human experimentation committee and adhered to the World Medical Association and the Declaration of Helsinki. The procedures were approved by the institutional Ethics Committee.

Confidentiality, informed consent, and ethical approval. The authors declare that no patient data appear in this article. The authors have followed their institution's confidentiality protocols and received approval from the Ethics Committee. The SAGER guidelines were followed according to the nature of the study.

Declaration on the use of artificial intelligence. The authors declare that no generative artificial intelligence was used in the writing of this manuscript.

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