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Carvacrol attenuated acute renal injury induced by unilateral ureteral obstruction in male rats

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ABSTRACT

Objective(s): Three-day unilateral ureteral obstruction (UUO) leads to acute renal injury. Carvacrol regulates inflammatory factors and reduces oxidative stress and apoptosis. Therefore, we are assessing the renoprotective effect of carvacrol against 3-day UUO in rats.

Materials and Methods: Thirty-six male rats were randomized into six groups (n=6): control, carvacrol (100 mg/kg, IP), UUO, and UUO+carvacrol (25, 50, or 100 mg/kg). UUO was induced by ligating the left ureter for 3 days. Carvacrol was administered intraperitoneally once daily for three consecutive days, starting one hour after UUO induction. After treatment, hemodynamic parameters including mean arterial pressure (MAP), renal perfusion pressure (RPP), renal blood flow (RBF), and renal vascular resistance (RVR) were measured. Kidney function markers such as blood urea nitrogen (BUN) and serum creatinine were assessed. Oxidative stress and anti-oxidant status were evaluated by measuring malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), and total anti-oxidant capacity (TAC) in kidney tissue. Western blot analysis determined expression levels of Bax, Bcl-2, cleaved caspase-3, tumor necrosis factor alpha (TNF-α), and nuclear transcription factor p65 (NF-κB p65). Renal damage score (RDS) was surveyed by the hematoxylin and eosin method.

Results: UUO significantly raised RVR, ROS, and MDA, serum creatinine, cleaved caspase-3, BUN, NF-κB p65, Bax, TNF-α protein levels ($P<0.05$), and also reduced RBF, TAC, GPx, SOD, and Bcl-2 protein expression ($P<0.05$) in left renal tissue, and carvacrol (100 mg/kg) could significantly mitigate the changes affected by UUO.

Conclusion: Our study revealed that carvacrol protects against UUO-induced renal injury through its potent anti-oxidant, anti-inflammatory, and anti-apoptotic properties.

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Introduction

Ureteral obstruction is a common clinical condition that can lead to renal damage (1). Unilateral ureteral obstruction (UUO) serves as a well-established experimental model for studying kidney tubulointerstitial fibrosis (2). Growing evidence suggests that UUO leads to a gradual elevation in renal tubular pressure and renal vascular resistance (RVR), accompanied by a sustained decrease in renal blood flow (RBF) due to significant vasoconstriction (3). Notably, UUO leads to elevated levels of reactive oxygen species (ROS) in the obstructed kidney (4), triggering oxidative stress and further complicating renal pathology (5-7). The progression in the obstructed kidney involves inflammation, tubular apoptosis, interstitial fibrosis, and necrosis (8, 9).

In this regard, it has been shown that following UUO, there is a progressive escalation of histopathological changes in the affected kidney, ultimately leading to obstructive nephropathy (10). Furthermore, UUO induces increased macrophage infiltration, enhanced cytokine production, and upregulation of tumor necrosis factor-α (TNF-α) expression in the obstructed kidney (11). These mediators provoke renal inflammation and fibrosis (12). Additionally, UUO exacerbates oxidative stress by increasing ROS generation (3, 4). Collectively, these factors activate apoptotic pathways by increasing the levels of Bcl2-associated X (Bax) and caspase proteins and reducing the level of antiapoptotic B-cell lymphoma 2 (Bcl-2) protein, thereby promoting renal apoptosis (13, 14). If left untreated, UUO can progress to cause significant kidney injury (14). Despite various available strategies, finding effective treatments for patients with UUO remains a major challenge for clinical urologists (4). Thus, it is crucial to identify the key factors and molecular pathways that contribute to renal injury following UUO, as these may serve as potential therapeutic targets.

There is a growing trend toward using natural products to treat various disorders (15-17). Natural resources, particularly plant-derived compounds such as carvacrol, are attracting attention for their potential to reduce kidney damage with fewer side effects (18). Carvacrol, found in plants such as Origanum vulgare and *Thymus vulgaris* (18),

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exhibits anti-oxidant, anti-inflammatory, antispasmodic, and vasodilator properties (19). Its ability to neutralize free radicals and protect against oxidative stress makes it a promising candidate for renal protection (20). In renal ischemia-reperfusion models, carvacrol demonstrates protective effects on the kidneys, primarily due to its phenolic hydroxyl group (21). Additionally, carvacrol has been reported to have anti-inflammatory effects on the kidneys in a myocardial infarction model (22).

In contrast to a previous study (23) that focused on the protective effects of carvacrol in a chronic ureteral obstruction model, emphasizing renal fibrosis (chronic kidney disease), the present study investigates carvacrol in an acute UUO model. This acute model more closely mimics early-stage renal injury, characterized by rapid hemodynamic changes, oxidative stress, inflammation, and apoptosis, which are less well studied in the context of carvacrol treatment. Understanding carvacrol's efficacy in the acute phase of UUO-induced kidney injury is critical, as timely therapeutic interventions may prevent progression to chronic kidney damage and fibrosis. Furthermore, this study examines additional molecular mechanisms, including pro-inflammatory markers and apoptotic mediators, that have not been extensively addressed in prior chronic UUO studies. Given that UUO induces renal damage, this study aims to assess the protective effects of carvacrol on renal function, oxidative stress, inflammation, apoptosis, and histopathology in a 3-day UUO experimental model.

Materials and Methods

Animal

In this study, 36 male Wistar rats (220 ± 15 g) were obtained from the Animal Facility of Rafsanjan University of Medical Sciences, Rafsanjan, Iran. The animals were housed under standard laboratory conditions, including a 12-hour light/dark cycle, a controlled temperature of 22 ± 2 °C, and 50% relative humidity. Rats had ad libitum access to tap water and standard rodent chow. All experimental protocols were reviewed and approved by the Ethics Committee of Rafsanjan University of Medical Sciences (Ethics No. IR.RUMS.REC.1400.009), and all procedures were conducted under the guidelines for the care and use of laboratory animals as outlined in the National Institutes of Health Publication No. 85-23, revised 2010. Before the experiments began, the animals were acclimatized to the housing environment for 1 week.

Experimental groups

The animals were randomly assigned to six groups (n = 6 per group) as follows:

1. Control (Vehicle): Sham-operated rats serving as the control group received intraperitoneal (IP) injections of 1% Tween 80 in normal saline once daily for three consecutive days (16).
2. Car 100 mg/kg: Rats in this group were administered carvacrol (100 mg/kg, dissolved in 1% Tween 80 in normal saline) via IP injection once daily for three days (24).
3. UUO: Animals underwent UUO surgery model and received IP injections of 1% Tween 80 in normal saline, administered one hour post-UUO and continued once daily for three days.
4. UUO + Car 25 mg/kg: Rats in this group received carvacrol (25 mg/kg, IP) one hour after UUO surgery, administered once daily for three days (25).
5. UUO + Car 50 mg/kg: Rats in this group received carvacrol (50 mg/kg, IP) one hour after UUO surgery, administered once daily for three days.
6. UUO + Car 100 mg/kg: Rats in this group received carvacrol (100 mg/kg, IP) one hour after UUO surgery, administered once daily for three days.

Induction of the UUO model

Rats were anesthetized with an IP injection of ketamine (100 mg/kg) and xylazine (10 mg/kg) (Sigma, St. Louis, USA). Under general anesthesia, the left abdominal region was shaved and prepared for surgery. A sterile incision was made in the left abdominal quadrant. The UUO model was induced in groups 3–6 by isolating and ligating the left ureter with a 4-0 silk suture. After surgery, animals were monitored and allowed to recover from anesthesia. Sham-operated groups (groups 1 and 2) underwent identical surgical procedures, except that the ureter was left unligated. Both UUO and sham procedures were maintained for three days post-surgery. Concurrently, carvacrol (Sigma, St. Louis, USA), dissolved in 1% Tween 80 in normal saline, was administered intraperitoneally at doses of 25, 50, or 100 mg/kg, starting 1 hour after UUO and continuing once daily for 3 days (25). Animals received equivalent volumes of 1% Tween 80 in normal saline or of carvacrol, as appropriate.

Experimental protocol

Hemodynamic parameters measurement

Three days after UUO, all rats were anesthetized with urethane (1.7 g/kg body weight, IP) (Sigma, Louis, USA), and body temperature was maintained at 37 °C using a heated platform throughout the experiment. The trachea was intubated with a polyethylene tube (Microtube Extrusions, Australia) to ensure airway patency and facilitate respiration (26). The left carotid and femoral arteries were surgically isolated and cannulated with heparinized polyethylene tubing, which was connected via a transducer cable to a Bridge Amp (ADInstruments, Australia) (26). This setup interfaced with PowerLab hardware (ADInstruments, Australia) and LabChart software (version 6; ADInstruments, Australia) to monitor mean arterial pressure (MAP) and renal perfusion pressure (RPP), respectively. For RPP measurement in each rat, the left femoral artery was cannulated with a polyethylene catheter (Microtube Extrusions, North Rocks, NSW, Australia), and the catheter was then connected to a PowerLab system. The obstructed kidney was then surgically exposed, and RBF was assessed with a needle-type laser Doppler probe (DP4s, Moor Instruments, UK) linked to a Moor laser Doppler perfusion monitor (DRT4, Moor Instruments, UK). RBF measurements were expressed as perfusion units (PU) (27). Animals were allowed a 30-minute stabilization period before hemodynamic measurements were recorded during the final five minutes of this equilibrium phase. RVR was calculated as the ratio of RPP to RBF (mm Hg/PU) (27).

Measurement of the blood urea nitrogen (BUN) and serum creatinine (Cr) concentrations

After assessing hemodynamic parameters, blood samples were collected via cardiac puncture and then centrifuged at 3000 rpm for 20 min. BUN and serum Cr concentrations were measured using quantitative diagnostic kits (Pars Azmoon, Iran).

Kidney tissue preparation

After hemodynamic measurements, each rat's left kidney was divided into two sections. One section was rapidly frozen in liquid nitrogen for protein extraction, and the other was preserved in 10% formalin for histopathological analysis.

Protein extraction for biochemical assays and Western blotting

The freshly isolated left kidney tissue was carefully dissected and homogenized on ice in a lysis buffer containing 0.1% SDS, 1 mM EDTA, 10 mM Tris-HCl, 1% NP-40, 0.1% sodium deoxycholate, and a cocktail of protease inhibitors (aprotinin, pepstatin A, and leupeptin, each at 2 µg), as well as 0.5 µmol/l PMSF, all at pH 7.4. After homogenization, the samples were centrifuged at 6000 rpm for 20 min at 4 °C. The supernatants were transferred to new tubes. Protein content was measured by the Bradford protein assay, and the extracts were stored at −80 °C for subsequent molecular analysis.

Biochemical measurements

To assess oxidative stress, supernatants from left kidney tissue samples previously stored in liquid nitrogen were analyzed. The oxidative stress status in the left kidney was evaluated by measuring the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx), as well as the levels of malondialdehyde (MDA) and total antioxidant capacity (TAC), using various commercial assay kits (ZellBio, Germany) by a spectrophotometer (ELISA Plate Reader).

Western blot analysis

Western blotting was used to measure levels of inflammatory proteins, including TNF-α and nuclear factor kappa B p65 (NF-κB p65), as well as apoptotic markers (Bax and cleaved caspase-3) and the anti-apoptotic marker Bcl-2 in left kidney tissue. Following protocols from our previous studies (15, 17, 28), protein samples were first separated by electrophoresis and then transferred to a nitrocellulose membrane. The membrane was incubated with primary antibodies (Cell Signaling, USA, 1:1000) targeting the specific proteins for 3 hr at room temperature, followed by incubation with a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (Abcam, USA, 1:5000). Protein detection was performed using enhanced chemiluminescence. Band intensities were quantified using ImageJ software, and protein expression levels were normalized by calculating the ratio of each target protein band to the corresponding beta-actin band across all groups.

Histopathological assessment

As previously stated, half of the left kidney of rats was fixed in 10% formalin for 48 hr. After fixation, the tissues were dehydrated through a graded ethanol series, cleared with xylene, and embedded in paraffin to create paraffin blocks. The left kidney tissue was then sectioned into consecutive 5-µm-thick sections. The sections were placed on glass slides, stained with standard hematoxylin and eosin (H&E), and examined at 100× magnification. A pathologist, blinded to the study, assessed the renal damage score (RDS) using histopathological criteria, including tubular atrophy, tubular dilation, glomerular atrophy, and interstitial infiltration. The extent of renal tubulointerstitial injury was semi-quantitatively graded according to methods from previous research (29-31). The RDS ranged from 0 to 3, with 0–0.5 indicating a normal kidney without damage, 1 indicating minor damage, 2 indicating moderate damage, and 3 indicating severe damage.

Statistical analysis

All experimental results were analyzed using GraphPad Prism version 8.0 for Windows (GraphPad Software, USA) and are presented as mean ± standard deviation (SD). Comparisons among groups for each quantitative measurement were performed using one-way ANOVA with Tukey's *post hoc* test. Additionally, the Kruskal-Wallis test was used to assess differences in the RDS among groups. Statistical significance was set at the 95% confidence level.

Results

The effect of carvacrol on hemodynamic parameters

After three days of UUO, there were no significant differences in MAP and RPP across all groups (Table 1). However, the UUO model caused a marked decrease in RBF and a significant increase in RVR in the UUO group compared with the control (P<0.01, Table 1). Additionally, carvacrol treatment attenuated UUO-induced changes in RBF, resulting in a significant increase in RBF in the UUO + Car 100 mg/kg group compared with the UUO group (P<0.05, Table 1). Carvacrol also significantly reduced RVR in the UUO + Car 100 mg/kg group compared with the UUO group (P<0.05; Table 1).

Effect of carvacrol on BUN and serum creatinine levels

Our findings demonstrated that UUO caused a significant increase in BUN (P<0.05) and serum Cr levels (P<0.01) in the UUO group compared with the control (Figure 1). Notably, BUN and serum creatinine levels were significantly lower in UUO rats treated with carvacrol (100 mg/kg) than in untreated UUO rats at 3 days post-UUO

Table 1. Hemodynamic parameters after three days of treatment with vehicle or carvacrol in rat groups

Groups	MAP (mmHg)	RPP (mmHg)	RBF (PU)	RVR (mmHg/PU)
Control	101.5±5.40	90.5±4.15	145.3±7.39	0.69±0.04
Car 100 mg/kg	100.0±6.48	90.2±2.55	149.2±11.13	0.67±0.03
UUO	105.5±6.68	97.8±7.53	114.8±11.67**	0.92±0.13**
UUO + Car 25 mg/kg	106.3±8.29	98.1±7.60	119.5±15.96	0.90±0.11
UUO + Car 50 mg/kg	104.4±5.53	96.8±4.87	122.0±11.49	0.86±0.12
UUO + Car 100 mg/kg	104.1±4.90	96.7±5.31	137.2±12.80#	0.74±0.07#

Data are expressed as mean ± SD. **P<0.01 compared to the control. #P<0.05 compared to the UUO group
MAP: Mean arterial pressure; RVR: Renal vascular resistance; RPP: Renal perfusion pressure; Car: Carvacrol; RBF: Renal blood flow

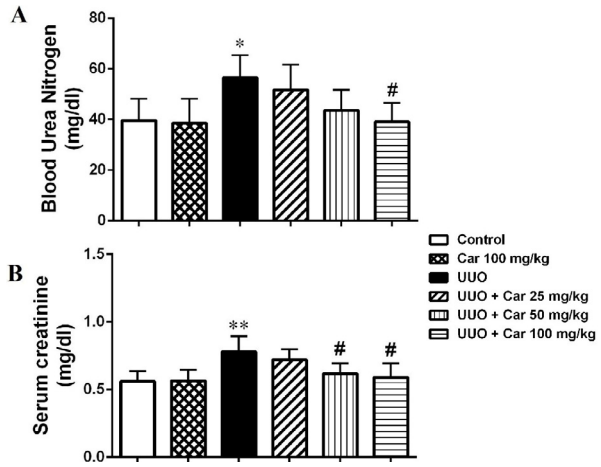

Figure 1. Serum Cr and BUN levels at three days after treatment with vehicle or carvacrol in rat groups
Data are presented as mean±SD. *P<0.05, and **P<0.01 compared to the control. #P<0.05 versus UUO group.
Cr: Creatinine; Car: Carvacrol; BUN: Blood urea nitrogen

(P<0.05, Figure 1). Furthermore, carvacrol administered at 50 mg/kg significantly reduced serum creatinine levels in the UUO + Car 50 mg/kg group compared with the UUO group (P<0.05, Figure 1).

Effect of carvacrol on renal oxidative stress

This study evaluated free radical damage after 3 days of UUO by measuring lipid peroxidation, expressed as MDA content. As shown in Table 2, UUO caused a significant increase in MDA levels in the left kidneys of UUO rats compared with controls (P<0.01). Importantly, treatment with carvacrol (100 mg/kg) significantly ameliorates the MDA concentrations in the UUO + Car 100 mg/kg group compared to the UUO group (P<0.01, Table 2).

The results indicated that UUO significantly decreased SOD and GPx activities in the left kidney of the UUO group compared with the control (P<0.01, Table 2). However, administration of carvacrol (100 mg/kg) resulted in a significant increase in SOD and GPx activities in the UUO + Car 100 mg/kg group compared with the UUO group (P<0.05, Table 2). Additionally, carvacrol at 50 mg/kg significantly increased GPx activity in UUO rats (P<0.05, Table 2).

Furthermore, the study found that UUO significantly reduced TAC content in the obstructed kidney of UUO rats compared with the control (P<0.01, Table 2). Notably, the UUO + Car 100 mg/kg group showed a significant increase in kidney TAC levels compared with UUO rats (P<0.01, Table 2).

Effect of carvacrol on TNF-α and NF-κB p65 protein levels

Western blot analysis demonstrated that UUO caused a significant increase in the expression levels of TNF-α and NF-κB p65 proteins in the left kidney of UUO rats compared to the control (P<0.01 and P<0.001, respectively; Figure 2). Furthermore, treatment with carvacrol at a dose of 100 mg/kg significantly alleviated the expression levels of TNF-α and NF-κB p65 proteins in UUO rats compared to untreated UUO rats (P<0.05, Figure 2). However, carvacrol administered at doses of 25 mg/kg and 50 mg/kg did not significantly reduce TNF-α and NF-κB p65 protein expression levels in the UUO + Car 25 mg/kg and UUO + Car 50 mg/kg groups compared with the UUO group (Figure 2).

Table 2. Oxidative stress indicators (SOD and GPx activities, MDA levels, and TAC content) three days after treatment with vehicle or carvacrol in rat groups

Groups	MDA level (µM)	SOD activity (U/ml)	GPx activity (U/ml)	TAC (µmol/l)
Control	4.12±0.82	41.8±4.70	224.5±16.01	533.8±68.97
Car 100 mg/kg	4.13±0.87	42.3±5.82	226.2±19.77	529.7±51.63
UUO	7.4±1.70**	28.9±5.53**	160.3±27.11**	366.7±67.40**
UUO + Car 25 mg/kg	7.1±1.79	33.5±5.68	169.5±36.26	399.8±75.84
UUO + Car 50 mg/kg	6.3±0.98	36.5±4.72	207.8±22.87#	460.0±78.35
UUO + Car 100 mg/kg	4.7±0.66##	39.0±6.16#	213.5±22.44#	519.3±54.35##

Data are shown as mean±SD. **P<0.01 versus control. #P<0.05, and ##P<0.01 compared to the UUO group
TAC: Total anti-oxidant capacity; GPx: Glutathione peroxidase; Car: Carvacrol; SOD: Superoxide dismutase; MDA: Malondialdehyde; UUO: Ureteral obstruction

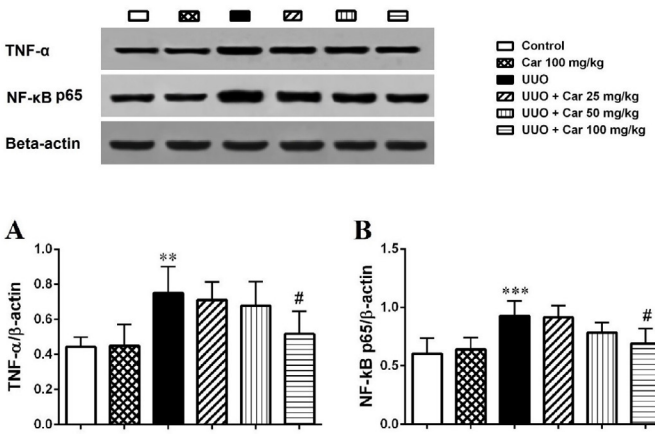

Figure 2. Immunoblotting of the TNF-α and NF-κB p65 proteins in the rat left kidney at the study's conclusion
Data are shown as mean±SD. **P<0.01, and ***P<0.001 compared to the control. #P<0.05 versus UUO group.
TNF-α: Tumor necrosis factor alpha; Car: Carvacrol; NF-κB p65: Nuclear factor kappa B p65; UUO: Ureteral obstruction

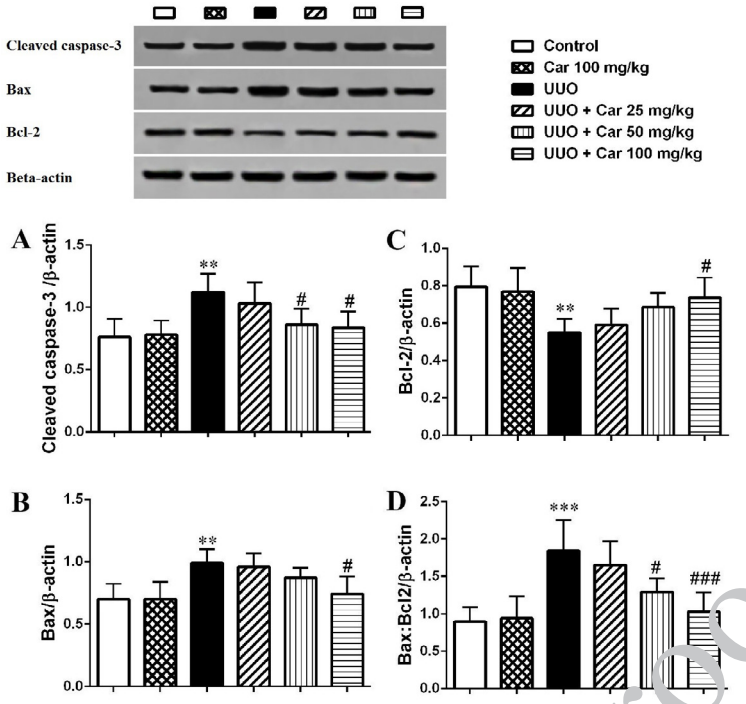

Figure 3. Immunoblotting analysis of cleaved caspase-3, Bax, Bcl-2, and the Bax:Bcl-2 ratio in the rat left kidney at the study's conclusion
Data are shown as mean±SD. **P<0.01 and ***P<0.001 versus control; #P<0.05 and ###P<0.001 compared to the UUO group.
Bax: Bcl2-associated X; Car: Carvacrol; Bcl-2: B-cell lymphoma 2 ; UUO: Ureteral obstruction

Effect of carvacrol on renal apoptosis

Molecular analysis in our study also revealed that UUO significantly upregulated cleaved caspase-3 and Bax protein expression and decreased Bcl-2 protein levels in the obstructed kidney of the UUO group compared with the control (P<0.01, Figure 3). Carvacrol administration effectively mitigated the UUO-induced increase in cleaved caspase-3 (at 50 and 100 mg/kg) and Bax (at 100 mg/kg) protein expression (P<0.05, Figures 3A-B). Additionally, treatment with carvacrol at 100 mg/kg significantly enhanced Bcl-2 protein expression in UUO + Car 100 mg/kg rats compared with the UUO group (P<0.05, Figure 3C). Furthermore, as shown in Figure 3D, carvacrol at doses of 50 mg/kg (P<0.05) and 100 mg/kg (P<0.001) significantly reduced the Bax:Bcl-2 ratio in the UUO + Car 50 mg/kg and UUO + Car 100 mg/kg groups compared with the UUO group.

Effect of carvacrol on renal tissue damage

Histopathological analysis revealed no abnormalities in the renal tissue of the control group (Figure 4A). H&E staining showed that UUO caused significant glomerular and tubulointerstitial injury in the left kidney, with renal tubular injury in the UUO group compared with the control (Figure 4A). Additionally, the RDS was markedly increased in the UUO group compared with the control (P<0.05, Figure 4B). However, treatment with carvacrol at 100 mg/kg mitigated the RDS in the UUO + Car 100 mg/kg group compared with the UUO group (P<0.05, Figure 4B).

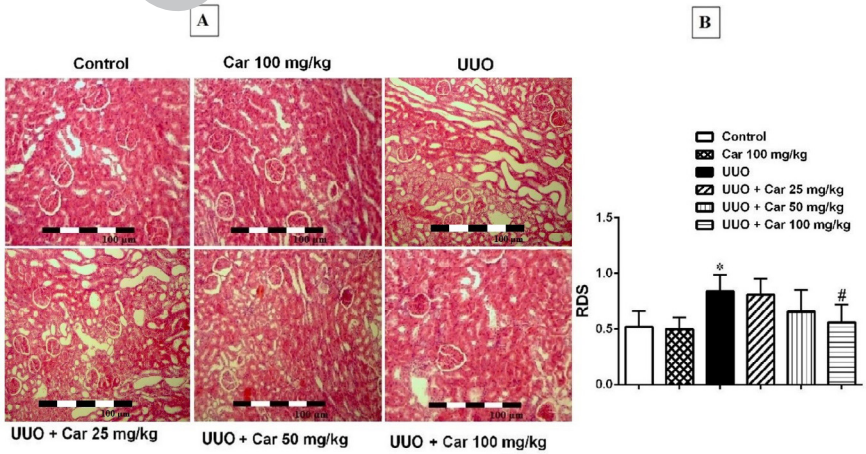

Figure 4. A. Hematoxylin and eosin-stained sections of the left kidney (magnification 100×) at the study's conclusion. B. A score ranging from 0 to 0.5 indicated normal kidney tissue, while scores from 1 to 3 reflected the presence of tubular atrophy, tubular dilation, glomerular atrophy, and interstitial infiltration
Panel B shows the renal damage score (RDS) in the left kidney across experimental groups three days post-treatment. Results are expressed as mean±SD. *P<0.05 compared to control; #P<0.05 compared to the UUO group. UUO: Ureteral obstruction

Discussion

Our study found that although 3-day UUO did not significantly alter MAP or RPP, it markedly reduced RBF and increased RVR, changes indicative of renal hemodynamic compromise. Importantly, carvacrol at 100 mg/kg mitigated these alterations, improving RBF and decreasing RVR in UUO rats. Notably, these improvements in RBF and RVR occurred without significant changes in MAP, indicating that carvacrol's renoprotective hemodynamic effects are likely mediated primarily through local renal vasodilation rather than systemic blood pressure changes. This aligns with evidence that carvacrol can modulate vascular tone and endothelial function, thereby improving renal circulation under stress or injury (32, 33). Notably, Ünlü *et al.* (2025) (22) demonstrated a similar protective hemodynamic effect of carvacrol in a kidney injury model, highlighting its ability to decrease vascular resistance. The observed improvement in RBF and reduction in RVR following carvacrol treatment in UUO rats may be attributed to several interconnected mechanisms. First, carvacrol possesses potent anti-oxidant properties, as evidenced by reduced MDA levels and restored SOD and GPx activities in our study. Oxidative stress is known to impair endothelial function and promote vasoconstriction by reducing nitric oxide bioavailability (34). By scavenging free radicals and preserving endogenous anti-oxidant defenses, carvacrol may enhance NO-mediated vasodilation, thereby reducing RVR and improving RBF. Second, carvacrol exhibits anti-inflammatory effects, including suppression of NF-κB p65 and TNF-α expression, as demonstrated in our study. Inflammatory cytokines can induce endothelial dysfunction and vasoconstriction; thus, their downregulation may improve renal hemodynamics (23). Third, previous studies have reported direct vasorelaxant effects of carvacrol on vascular smooth muscle. Carvacrol has been shown to block L-type calcium channels in smooth muscle cells, which is the most probable mechanism of its vasorelaxant action (35). Collectively, these anti-oxidant, anti-inflammatory, and direct vasodilatory actions, mediated through calcium channel blockade, likely work synergistically to preserve renal perfusion in the setting of acute ureteral obstruction.

The observed rise in BUN and serum creatinine after UUO in our study reflects impaired renal function, a hallmark of obstructive nephropathy. Carvacrol at 100 mg/kg significantly reduced these biomarkers, suggesting an ameliorative effect on renal dysfunction. Previous investigations corroborate this, reporting that carvacrol confers renoprotection by attenuating elevations in BUN and creatinine in various nephrotoxic models (16, 36) and in ischemic injury (37). The nephroprotective effect of carvacrol is attributed to its potent anti-oxidant properties, reduction in oxidative stress, and inhibition of inflammatory pathways that contribute to renal tissue injury (38).

Our results also demonstrated that UUO elevates renal MDA levels, an indicator of lipid peroxidation and oxidative damage. Simultaneously, TAC, SOD, and GPx activities were reduced, underscoring compromised anti-oxidant defense. Treatment with carvacrol restored anti-oxidant enzyme activities and reduced MDA concentration, highlighting its robust anti-oxidant action. Similar observations were reported by Samarghandian *et al.* (2016), who demonstrated carvacrol's efficacy in mitigating oxidative stress across multiple organs (39). Carvacrol's anti-oxidant mechanisms have been further elucidated in recent studies (37, 40), which report upregulation of endogenous anti-oxidant enzymes and direct scavenging of free radicals.

Moreover, we observed significant upregulation of TNF-α and NF-κB p65 in the kidneys following UUO, indicating a robust inflammatory response. Carvacrol administration effectively suppressed expression of these pro-inflammatory markers. This aligns with mounting evidence that carvacrol modulates key inflammatory pathways, particularly NF-κB and cytokine production, in renal and hepatic injury (36). The anti-inflammatory actions of carvacrol are linked to inhibition of TNF-α and NF-κB in kidney tissue, corroborating our findings (41, 42).

Our molecular data also showed that UUO markedly increases cleaved caspase-3 and Bax while suppressing Bcl-2, indicating increased apoptosis in obstructed kidneys. Notably, carvacrol reversed these effects, suggesting anti-apoptotic activity. Previous studies report that carvacrol can regulate apoptosis by modulating the Bax:Bcl-2 ratio and inhibiting caspase activation (16, 43). In this regard, these protective effects may be synergistic with carvacrol's anti-oxidant and anti-inflammatory activities, reducing cellular stress and mitochondrial-mediated apoptosis (42-44).

In addition, histopathological assessment in our study revealed that UUO induces significant glomerular and tubulointerstitial injury, as reflected by an elevated RDS, while carvacrol treatment notably alleviates these lesions. Consistent with our study, Hassanshahi *et al.* (2024) (45) and Ünlü *et al.* (2025) (22) reported similar histological protection with carvacrol in models of nephrotoxicity and ischemia. The mechanism is likely multifactorial, involving attenuation of oxidative and inflammatory injury and inhibition of apoptosis, thereby preserving tissue architecture and renal function.

Unlike a prior study investigating the long-term effect of carvacrol on renal fibrosis via modulation of the TGF-β1/Smad pathway (23), our findings uniquely demonstrate that carvacrol administration in an acute 3-day UUO model improves renal hemodynamics, reduces oxidative stress, and mitigates inflammation and apoptosis during the early phase of kidney injury. The observed improvements in mean arterial pressure-independent parameters, such as RBF and RVR, highlight carvacrol's potential role in preserving renal perfusion early after ureteral obstruction. Our study further elucidates anti-inflammatory and anti-apoptotic mechanisms by showing reductions in TNF-α and NF-κB p65 expression, along with modulation of Bax, Bcl-2, and caspase-3 levels, suggesting carvacrol's multi-target action in early-stage kidney injury. Investigating carvacrol's protective effects at this acute stage is clinically relevant, as early intervention may prevent the progression from reversible injury to irreversible fibrosis and chronic kidney disease (1).

Several strengths of this study should be acknowledged. First, the 3-day UUO model enabled us to evaluate the early-phase renoprotective effects of carvacrol, including hemodynamic, oxidative, inflammatory, and apoptotic parameters, which have been less extensively studied than chronic fibrotic changes. Second, the multi-parametric approach—combining functional, biochemical, molecular, and histopathological assessments—provides a comprehensive understanding of carvacrol's mechanisms of action. However, some limitations warrant consideration. The study was conducted exclusively in male rats, limiting

the generalizability of the findings to females. Additionally, only three doses of carvacrol were tested, and the optimal therapeutic dose may vary across clinical conditions. We did not measure the direct vasodilatory effect of carvacrol on isolated renal vessels. Future studies using longer recovery periods, female animals, and selective pharmacological inhibitors are needed to validate further and expand our findings.

Conclusion

Our findings indicate that carvacrol has the potential to reduce renal injury and apoptosis induced by a 3-day UUO. In addition, treatment with carvacrol at 100 mg/kg improved RBF and mitigated oxidative stress in the left kidney within this UUO model. It also lowered inflammation by decreasing TNF-α and NF-κB p65 expression and modulated apoptosis by up-regulating Bax and cleaved caspase-3 while down-regulating Bcl-2 protein in the damaged kidney, thereby mitigating renal damage caused by UUO. Moreover, carvacrol at this dosage mitigated BUN and serum creatinine levels, suggesting its role in counteracting UUO-associated renal dysfunction. Collectively, the results propose that carvacrol could be a potential treatment for kidney injury related to UUO, although additional research is warranted.

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Availability of Data and Materials

Data could be obtained upon request from the corresponding author.

Ethical Approval and Consent to Participation

All experimental protocols were reviewed and approved by the Ethics Committee of Rafsanjan University of Medical Sciences (Ethics No. IR.RUMS.REC.1400.099). All procedures were conducted in accordance with the guidelines for the care and use of laboratory animals as outlined in the National Institutes of Health Publication No. 85-23, revised 2010.

Authors' Contributions

J H conceived and designed the experiments; E H, A K, H J, and J H performed the experiments; A K and J H analyzed the data; A K and J H contributed reagents/materials/analysis tools; and E H, A K, and J H wrote the article. All authors agree to be accountable for all aspects of work, ensuring integrity and accuracy.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Declaration

The authors declare that they have not used any AI tools or technologies to prepare this manuscript. All data were generated in-house, and no paper mill was used.

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