Original Article

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L-arginine regulates mitochondrial function and oxidative stress in the acute kidney injury model of unilateral ureter obstruction

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Abstract

Acute kidney injury (AKI) is a significant clinical problem associated with high morbidity and mortality. Unilateral ureter obstruction (UUO) is a well-established model for studying the pathophysiology of AKI. L arginine (ARG), a precursor of nitric oxide, has been shown to possess renoprotective properties. This study aims to investigate the protective effects of ARG in an animal model of UUO-induced AKI. Male BALB/c mice were randomly allotted into sham-operated, UUO, and UUO + L-Arginine. UUO was surgically induced by ligating the left ureter. The treatment group received ARG (100, 250, and 500 mg/kg/day) intraperitoneally for seven days post-UUO surgery. Renal function was assessed by measuring plasma creatinine (Cr) and blood urea nitrogen (BUN) levels. Mitochondrial function was evaluated by determining mitochondrial membrane potential, dehydrogenases activity, and mitochondrial swelling. Oxidative stress markers, including ROS formation, lipid peroxidation, protein carbonylation, and tissue antioxidant capacity, were also measured in the kidney tissue. UUO led to significant renal dysfunction, as evidenced by increased serum Cr and BUN levels ($P<0.001$). Mitochondrial dysfunction was indicated by decreased mitochondrial dehydrogenase activity, mitochondrial depolarization, and increased mitochondrial swelling ($P<0.001$). Additionally, UUO-induced oxidative stress was demonstrated. It was found that ARG treatment significantly improved renal function by modulating mitochondrial function and decreasing oxidative stress markers ($P<0.05$). These findings suggest that ARG may have therapeutic potential in managing AKI by preserving mitochondrial function and reducing oxidative damage.

Keywords: Acute kidney injury, Mitochondrial impairment, Nephrotoxicity, Renal failure.

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1. Introduction

Renal diseases encompass a wide range of disorders, from mild functional abnormalities to complete renal failure, posing a significant challenge to global health (1).

Chronic kidney disease (CKD) and end-stage renal disease are two forms of renal disease that often necessitate dialysis or kidney transplantation (2). Despite transplantation being the ultimate solution for advanced

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renal disease, early detection and understanding of the underlying mechanisms of kidney damage can provide substantial benefits. The identification of these mechanisms could lead to novel pharmacological interventions in the management of renal diseases. Pharmacological strategies could significantly blunt kidney disease progression, reduce morbidity, and improve patient outcomes.

It is well-established that oxidative stress plays a critical role in the pathogenesis of kidney damage with various etiologies. Consequently, there has been extensive research into the role of antioxidants in improving renal function. For instance, antioxidants such as silymarin and N-acetyl cysteine (NAC) have demonstrated efficacy in enhancing renal function across various experimental models and even in human cases of kidney diseases (3-5). Oxidative stress arises when the balance between producing and eliminating reactive oxygen species (ROS) in biological systems is disrupted (6). One of the most significant biological discoveries is the link between oxidative stress and damage to organelles like mitochondria (7). It is well known that mitochondria are the primary source of ROS generation within cells (7). When mitochondrial function is impaired, the level of ROS is significantly increased (7). Thus, there is a strong connection between oxidative stress and mitochondrial impairment, underscoring the importance of targeting these pathways for therapeutic intervention in various pathological conditions, including renal disease.

Kidney tissue is rich in mitochondria. These organelles are critical for generating the energy required for vital processes such as reabsorbing substances in the kidney (8, 9). Loss of mitochondrial function in renal tissue and the consequent insufficient ATP production can lead to the urinary loss of vital electrolytes. Ultimately, mitochondrial damage can result in the release of cell death mediators from these organelles, causing cytotoxicity and potentially leading to renal damage and failure (10, 11).

L-arginine (ARG) is a semi-essential amino acid pivotal in various physiological processes. ARG has been extensively studied for its therapeutic potential in protecting various organs (12-15). One of the most significant benefits of ARG is its ability to modulate oxidative stress and enhance mitochondrial function (13, 16, 17). In this regard, the beneficial role of ARG supplementation in cardiovascular disease, renal injuries, neuronal system disorders, and hepatic impairment has been widely investigated (12-15). In the context of renal diseases, ARG has demonstrated significant nephroprotective effects. Animal studies have shown that ARG supplementation could decrease oxidative stress biomarkers and enhance mitochondrial function in kidney tissue (12, 18-20). Through these mechanisms, ARG significantly improved renal outcomes (19, 20).

The current study aimed to evaluate the role of oxidative stress and mitochondrial impairment in the pathogenesis of renal injury in a UUO model of AKI and examine the beneficial effects of ARG supplementation. The data obtained from this study could help develop novel therapeutic options against renal disease with different etiologies.

2. Material and Methods

2.1. Reagents

L-arginine, 2′,7′ Dichlorofluorescein diacetate (DCFH-DA), trichloroacetic acid, malondialdehyde, reduced glutathione (GSH), 3-[4,5dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, sucrose, 3 (N-morpholino) propane sulfonic acid, ferric chloride hexahydrate ($FeCl_3.6H_2O$), D-mannitol, rhodamine123 (Rh 123), coomassie brilliant blue, 2, 4, 6-tripyridyl-s-triazine, dithiothreitol, fatty acid-free bovine serum albumin fraction V, sodium citrate, chloramine-T, 2,4-dinitrophenyl hydrazine, P-dimethylaminobenzaldehyde, and thiobarbituric acid were obtained from Sigma (Sigma-Aldrich, St. Louis, MO). Kits for measuring serum biomarkers of renal injury were purchased from Pars Azmoon® (Tehran, Iran). Ethylenediaminetetraacetic acid, n-propanol, perchloric acid, n-Butanol, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 5, 5-bis-dithio-nitro benzoic acid, meta-phosphoric acid, and 2 amino 2-hydroxymethyl-propane-1, 3-diol-hydrochloride

(Tris-HCl) were obtained from Merck (Darmstadt, Germany).

2.2. Animals

Male BALB/c mice (n=30; 23±2 g) were obtained from Shiraz University of Medical Sciences, Shiraz, Iran. Animals were maintained at an environmental temperature of 23±1 ºC with 42±3% relative humidity and adequate ventilation. During experiments, mice had free access to tap water and a standard pellet chow. The institutional laboratory animal care and use committee at Shiraz University of Medical Sciences approved all animal experiments (Code: IR.SUMS.REC.1398.1220).

2.3. Experimental setup

Animals were randomly allotted into the UUO group (model group, n=24) and sham-operated (control group, n=6). The UUO model was induced based on a previously reported protocol (21). Briefly, mice were anesthetized (a mixture of xylazine: acepromazine: ketamine; 8: 2: 70 mg/kg, i.p). The left ureter was identified, isolated, and doubly ligated (No. 04 un-absorbable sterile silk suture). The sham-operated mice underwent an identical surgical intervention for ureter identification and manipulation without ligation (21). The treatments were as follows: 1) Sham-operated; 2) UUO; 3) UUO + L-arginine (100 mg/kg, i.p); 4) UUO + L-arginine (250 mg/kg, i.p); and 5) UUO + L-arginine (500 mg/kg, i.p). L-arginine treatment was continued for seven consecutive days after UUO induction. L-arginine dose was selected based on previous studies (22). On day 8, animals were deeply anesthetized (thiopental 100 mg/kg, i.p). Blood samples (1 mL from the inferior vena cava) were transferred to sodium citrate-coated tubes, and the plasma was prepared (3000 g, 15 min, 4 °C). Kidneys were also excised and used to assess histopathological alterations and markers of oxidative stress. Kidney mitochondria were also isolated and evaluated.

2.4. Reactive oxygen species (ROS) formation

Reactive oxygen species (ROS) were estimated in the kidneys of UUO animals using 2′, 7′ dichlorofluorescein diacetate (DCF-DA) (23). Briefly, 200 mg of tissue samples were homogenized in 5 mL of ice-cooled Tris-HCl buffer (40 mM, pH = 7.4). Then, 100 µL of the resulting tissue homogenate was mixed with Tris-HCl buffer (1 mL) and DCF-DA (10 µM final concentration). Samples were incubated in the dark for 10 minutes (37ºC). Finally, the fluorescence intensity was assessed using a FLUOstar Omega® multifunctional fluorimeter (λ excit = 485 nm and λ emiss = 525 nm) (23, 24).

2.5. Lipid peroxidation

The thiobarbituric acid reactive substances (TBARS) test was used to assess lipid peroxidation in the kidneys of UUO animals (23). For this purpose, 500 µL of tissue homogenate (10% w: v in 40 mM Tris-HCl buffer) was treated with 4 mL of TBARS assay reagent (a mixture of 1 mL of thiobarbituric acid 0.375% w: v, 1 mL of 50% w: v of trichloroacetic acid, and 3 mL of meta-phosphoric acid 1% w: v, pH=2). Samples were vortexed (1 min) and heated (100 °C water bath) for 45 minutes. Afterward, 2 mL of n-butanol was added. Samples were mixed well and centrifuged (10000 g, 20 min, 4 °C). Finally, the n-butanol phase's absorbance was measured at 532 nm (23, 25).

2.6. Total antioxidant capacity of the kidney tissue

The ferric-reducing antioxidant power (FRAP) assay measured the kidney's total antioxidant capacity in UUO animals (23). Briefly, the FRAP assay reagent was prepared by mixing ten volumes of 300 mM acetate buffer (pH=3.6) with one volume of TPTZ (10 mM in 40 mM HCl) and one volume of 20 mM ferric chloride hexahydrate ($FeCl_3.6H_2O$). The tissue homogenate (100 µL) was added to 900 µL of the FRAP reagent and incubated at 37

ºC (5 min, in the dark). Finally, the absorbance was measured at 595 nm (23).

2.7. Protein carbonylation

Renal tissue protein carbonylation was evaluated using the dinitrophenylhydrazine (DNPH) test (26, 27). Briefly, 200 mg of kidney tissue was homogenized in 5 mL phosphate buffer (pH=7.5, with 0.1% v: v of triton X-100). Samples were centrifuged (700 g, 10 min, 4 ºC), and the resultant supernatant was treated with 1500 µL of DNPH solution (10 mM DNPH dissolved in HCl). Subsequently, the samples were incubated at room temperature for one hour with vortexing every 10 minutes. Then, 500 µL of trichloroacetic acid (20% w: v) was added and centrifuged (17000 g, 5 min, 4 ºC). The supernatant was discarded, and the pellet was washed five times with ethanol: ethyl acetate (1 mL of 1:1 v: v). The final pellet was re-dissolved in a 6 M guanidine chloride solution (pH=2.3) at 60 ºC. Finally, the samples were centrifuged (17000 g, 5 min, 4 ºC), and the absorbance of the supernatant was measured at 370 nm (26).

2.8. Renal glutathione content

Kidney tissue and isolated mitochondria GSH levels were determined by utilizing Ellman's reagent (DTNB) (23). Briefly, 1 mL of the tissue homogenate was treated with 200 µL of trichloroacetic acid (50% w-v; 4 °C). After thorough mixing and centrifugation (16000 g, 4 °C, 10 min), the supernatant was then mixed with 1 mL of Tris-HCl buffer (pH=8.9; 4 °C) and 100 µL of 40 mM DTNB (dissolved in pure methanol). Subsequently, the absorbance of the resulting color was measured at λ=412 nm using an EPOCH® plate reader (23, 28).

2.9. Tissue hydroxyproline levels

Renal hydroxyproline content was assessed as an index of tissue fibrosis. Briefly, 500 µl of tissue homogenate (10% w: v in 40 mM Tris-HCl buffer) was digested in 1 ml of 6 N hydrochloric acid (120 °C, 12 h). Afterward, an aliquot of digested homogenate (250 µL) was added to 250 µL of citrate–acetate buffer (pH = 6) and 500 µL of 56 mM chloramines-T. Samples were incubated at room temperature (20 min). Then, 500 µl Ehrlich's reagent (15 g of p-Dimethyl amino benzaldehyde in n-propanol/perchloric acid; 2:1 v: v) was added and incubated at 65°C (15 min). Finally, the absorbance of the developed color was measured at 550 nm (29, 30).

2.10. Kidney mitochondria isolation

Mice kidneys were washed and minced in an ice-cold buffer medium (220 mM sucrose, 2 mM HEPES, 0.5 mM EGTA, 70 mM mannitol, and 0.1 % BSA, pH=7.4). Then, the minced tissue was transported into a fresh buffer (5 mL buffer: 1 g of the kidney) and homogenized. Renal mitochondria were isolated by differential centrifugation of the tissue homogenate as previously described (23). For this purpose, unbroken cells and nuclei were first pelleted by centrifugation (1000 g, 20 min, 4 ºC). Afterward, the supernatant was centrifuged at 10000 g for 20 minutes at 4 ºC to pellet the mitochondria fraction. This step was repeated three times using a fresh buffer medium. The final brown-colored mitochondrial pellets were suspended in a buffer containing 70 mM mannitol, 220 mM sucrose, 2 mM HEPES, and 0.5 mM EGTA, pH=7.4, except for the mitochondria used to assess mitochondrial depolarization and mitochondrial swelling, which were suspended in MMP assay buffer (220 mM Sucrose, 68 mM Mannitol, 10 mM KCl, 5 mM KH_2PO_4, 10 mM HEPES, 2 mM $MgCl_2$, 50 µM EGTA, and pH=7.2) and swelling buffer (125 mM Sucrose, 65 mM KCl, 10 mM HEPES, pH=7.2) (23).

2.11. Mitochondrial dehydrogenases activity

The 3-(4, 5-dimethylthiazol-2-yl)-2, the 5-diphenyltetrazolium bromide (MTT) assay was applied as a colorimetric method for the determination of renal mitochondrial de-

hydrogenases activity (31). For this purpose, a mitochondrial suspension (0.5 mg protein/ml) was incubated with 0.4% of MTT (37°C, 30 min, in the dark). Then, the product of formazan crystals was dissolved in dimethyl sulfoxide (1000 µL). Samples were centrifuged (1 min, 1000 g), and the absorbance was measured at 570 nm (31).

2.12. Mitochondrial depolarization

Mitochondrial uptake of the rhodamine 123 has been used to estimate mitochondrial depolarization (23). Briefly, the mitochondrial fractions (0.5 mg protein/mL) were incubated with 10 µM of rhodamine 123 in the MMP assay buffer (15 minutes in the dark). Afterward, samples were centrifuged (16000 g, 1 min, 4ºC), and the fluorescence intensity of the supernatant was assessed at λ excitation=485 nm and λ emission=525 nm using a fluorometer (23).

2.13. Mitochondrial swelling

TAnalysis of mitochondrial swelling was estimated through changes in light scattering as monitored spectrophotometrically at λ=540 nm (25 °C) (23). Briefly, isolated mitochondria samples (0.5 mg protein/ml) were added to the swelling buffer, and the absorbance was monitored (λ=540 nm, 30 min, EPOCH® plate reader, BioTek®, USA). The primary and final absorbance difference was calculated (23).

2.14. Tissue histopathology and organ weight index

Kidney samples underwent fixation in a buffered formalin solution composed of 10% formaldehyde in phosphate buffer saline. Then, kidney sections embedded in paraffin (5 µm) were stained using hematoxylin and eosin (H&E) (32). Renal histopathological changes in the UUO model were assessed through a scoring system ranging from 0 to 4, indicating the severity of changes (33). Fibrotic kidney alterations in UUO were identified using Masson's trichrome staining. The kidney weight index was determined as Organ weight index = [Wet organ weight (g)/Whole body weight (g)] × 100.

2.15. Statistical analysis

Data are given as the mean ± SD. The Smirnove-Kolmogrove normality test checked the normal distribution of data sets. Data comparison was conducted using the one-way analysis of variance (ANOVA) with Tukey's multiple comparison test as the post hoc. $P < 0.05$ was considered significant. Scores of renal tissue histopathological changes are represented as median and quartiles for five random pictures. The analysis of non-parametric data (e.g., tissue histopathological alterations) was performed by the Kruskal–Wallis, followed by the Mann-Whitney U test.

3. Results

Organ weight index was significantly lower in animals that underwent UUO operation than in the control group (Figure 1). It was found that ARG treatment (100, 250, and 500 mg/kg) significantly improved organ weight index in UUO mice (Figure 1). BUN and Cr plasma levels as biomarkers of renal damage were also considerably high in the UUO animals (Figure 1). Meanwhile, ARG administration (100, 250, and 500 mg/kg) significantly decreased plasma BUN and Cr (Figure 1). The effects of ARG on organ weight index and plasma biomarkers of renal injury were not dose-dependent in the current study (Figure 1).

A significant increase in the biomarkers of oxidative stress, including ROS formation, lipid peroxidation, and protein carbonylation, along with decreased renal tissue antioxidant capacity and GSH level, was evident in the kidney of the UUO group (Figure 2). It was found that ARG (100, 250, and 500 mg/kg) augmented biomarkers of oxidative stress in the renal tissue of UUO mice (Figure 2). The effect of ARG on renal tissue biomarkers of

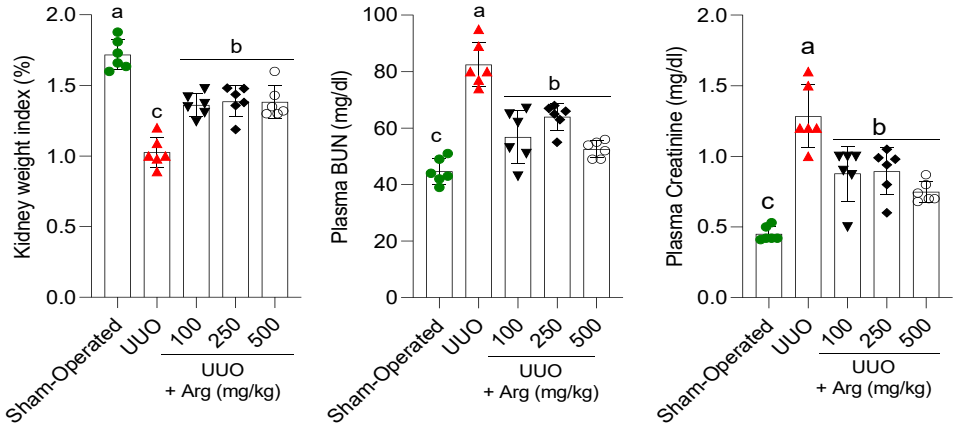

Figure 1. Renal weight index and serum biochemical measurements in unilateral ureter obstruction (UUO) model of acute kidney injury. Arg: l-arginine.
Data are represented as mean ± SD (n = 6). Data sets with different alphabetical superscripts are statistically different (P < 0.05).

oxidative stress was not dose-dependent in the current investigation.

UUO surgery caused a significant decrease in renal mitochondrial function, as revealed by decreased mitochondrial dehydrogenase activity, mitochondrial depolarization, and depletion of ATP content (Figure 3). It was found that ARG (100, 250, and 500 mg/kg) significantly improved renal mitochondrial function in UUO animals (Figure 3). The effect of ARG on renal mitochondrial function was not dose-dependent (Figure 3).

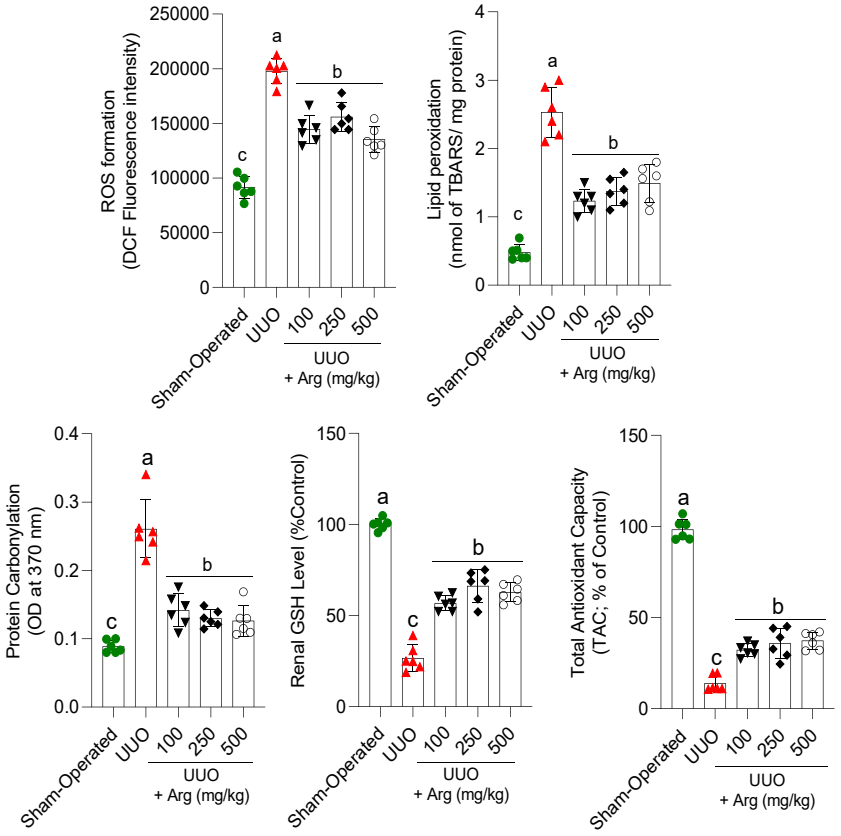

Figure 2. The role of l-arginine (Arg) in blunting biomarkers of oxidative stress in the renal tissue of unilateral ureter obstruction (UUO) mouse model of acute kidney injury.
Data are represented as mean ± SD (n = 6).Data sets with different alphabetical superscripts are statistically different (P < 0.05).

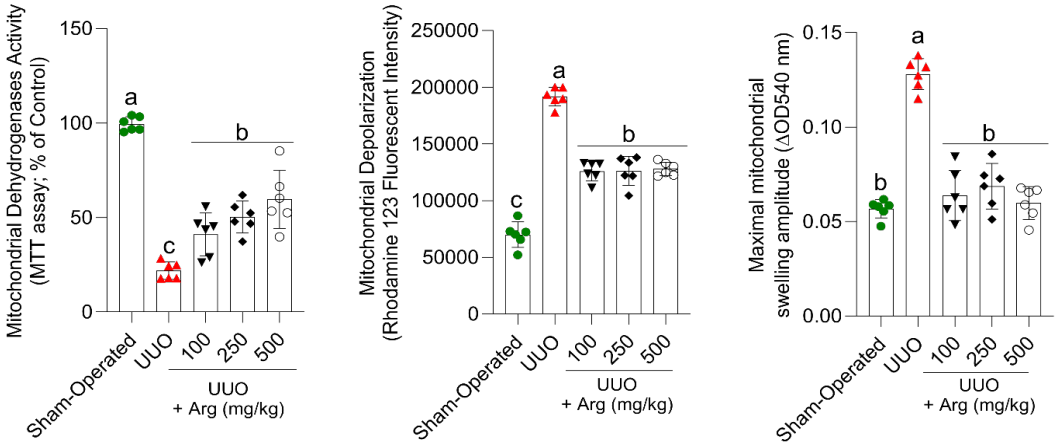

Figure 3. Effects of l-arginine (Arg) on mitochondrial indices in the kidney of unilateral ureter obstruction (UUO) model of acute kidney injury.
Data are represented as mean ± SD (n = 6).Data sets with different alphabetical superscripts are significantly different (P < 0.05)..

Inflammatory cell infiltration, tubular glomerular atrophy, and necrosis were the most prominent histopathological alterations in the kidneys (Table 1). A significant increase in the fibrotic tissue was also evident in the UUO group, as revealed by trichrome staining and elevation in the kidney hydroxyproline content (Table 1 and Figure 4). It was found that ARG (100, 250, and 500 mg/kg) significantly decreased renal histopathological alterations induced by UUO surgery (Table 1). The effect of ARG on renal histopathology was not dose-dependent in the current study (Table 1).

4. Discussion

Acute kidney injury (AKI) is a severe clinical complication that needs appropriate pharmacological interventions. Unilateral ureter obstruction (UUO) is a suitable experimental tool for investigating the mechanisms of AKI and the application of nephroprotective agents. The current study found that administration of the amino acid l-arginine (ARG) significantly protected renal tissue in an animal model of UUO. The effects of ARG on oxidative stress biomarkers and its role in modulating mitochondrial function seem to be fundamental mechanisms for the nephroprotective properties of this amino acid.

In a recent study, our research team found that oxidative stress and mitochondrial dysfunction play an essential role in the pathogenesis of AKI induced by UUO surgery (34). The role of oxidative stress and its associated complications in the pathogenesis of renal injury in various models of AKI, including UUO,

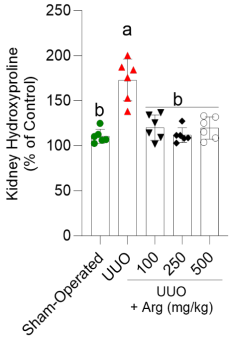

Figure 4. Renal hydroxyproline levels in unilateral ureter obstructed (UUO) mice.
Data are represented as mean ± SD (n = 6). Data sets with different alphabetical superscripts differ significantly (P < 0.05).

Table 1. Effects of l-arginine (Arg) on renal histopathological changes in unilateral ureter obstruction (UUO) model of acute kidney injury.

Treatment	Inflammation	Tubular Atrophy	Glomerular Atrophy	Fibrosis (Masson Stain)
Control	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
UUO	3 (3, 3) #	3 (3, 3) #	3 (3, 3) #	1 (1, 1) #
UUO + Arg 100 mg/kg	1 (1, 2)	1 (1, 2)	1 (0, 1)	0 (0, 0)
UUO + Arg 250 mg/kg	1 (1, 1)	1 (1, 1)	1 (0, 1)	0 (0, 0)
UUO + Arg 500 mg/kg	1 (0, 1)	1 (0, 1)	1 (0, 1)	0 (0, 0)

Renal histopathological changes in the unilateral ureter obstruction (UUO) model were scored based on a protocol described by Li et al. as described in the materials and methods (33). Arg: l-arginine. Data are represented as median and quartiles for five random pictures/group. #: indicates significant differences as compared with all investigated groups.

also has been mentioned in previous studies (35, 36). Oxidative stress is characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify these reactive intermediates or repair the damage. Elevated ROS levels can damage cellular components, including lipids, proteins, and DNA, leading to cellular dysfunction and death (35, 36). The current study found that UUO surgery caused significant oxidative stress in mice kidneys, characterized by ROS formation, lipid peroxidation, protein carbonylation, and depleted tissue antioxidant capacity. These data indicate the significance of oxidative stress and its related events in the pathogenesis of renal injury in the UUO model of AKI.

The effect of ARG on oxidative stress in biological systems has been widely investigated. In this regard, it has been found that ARG could protect the heart, nervous system, liver, and kidneys against oxidative stress and its consequences (12-17). In cardiovascular health, ARG has been shown to improve endothelial function, reduce blood pressure, and enhance vascular compliance (37, 38). Studies have demonstrated that ARG supplementation can decrease biomarkers of oxidative stress, such as malondialdehyde (MDA) and oxidized low-density lipoprotein, in patients with hypertension and atherosclerosis (39, 40). In other tissues, such as the liver and kidneys, ARG has also been shown to suppress ROS formation and enhance antioxidant defense (41). In the current model of renal injury, we found that ARG administration significantly blunted oxidative stress in the kidneys of UUO animals. This data indicates that the effect of ARG on oxidative stress plays an essential role in its protective properties observed in the current AKI model.

Renal mitochondria play a critical role in maintaining kidney function by providing the energy necessary for various cellular processes, including filtration, reabsorption, and secretion (9). The importance of renal mitochondria as a target in the pathogenesis of renal disease has become increasingly evident. Mitochondrial dysfunction is implicated in various renal pathologies, such as AKI, CKD, and diabetic nephropathy (42, 43). Meanwhile, targeting mitochondrial pathways offers potential therapeutic strategies to preserve mitochondrial function, reduce oxidative stress, and improve energy metabolism, thereby mitigating the progression of renal diseases (44). Understanding and modulating mitochondrial health in the kidneys can provide novel insights and approaches to treating and preventing renal disease, highlighting the mitochondria as a crucial target in nephrology research and therapy (44). Mitochondria are also the primary source of ROS within cells. It is well-known that mitochondrial dysfunction significantly contributes to oxidative stress, creating a vicious cycle of damage and impaired energy production (7). Hence, there is an interconnection between mitochondrial impairment and the occurrence of oxidative stress in the kidneys of mice in the UUO model.

The effect of the amino acid ARG on mitochondrial function has been investigated in various studies. In this regard, it has been found that ARG improves mitochondrial function by enhancing mitochondrial biogenesis and reducing mitochondrial ROS production, thereby protecting the heart from ischemia-reperfusion injury (7).In previous studies, ARG has shown promise in regulating mitochondrial function. For instance, in neurodegenerative diseases, such as Alzheimer's and Parkinson's, associated with high levels of oxidative stress and mitochondrial dysfunction, ARG has been found to improve mitochondrial activity, thereby slowing disease progression and improving cognitive function (45, 46). ARG supplementation has also been shown to protect against liver damage induced by various toxins and diseases. The hepatoprotective properties of ARG seem to be mediated through enhancing antioxidant defenses, decreasing lipid peroxidation, and improving mitochondrial function in hepatocytes (22, 47, 48). The current study found that ARG significantly improves renal mitochondrial function in UUO animals. Therefore, the positive effects of this amino acid on mitochondria play an essential role in its nephroprotective properties.

5. Conclusion

This study demonstrated that ARG significantly blunted renal dysfunction in an animal model of AKI. ARG administration improved renal function by reducing plasma creatinine and blood urea nitrogen levels, enhanced mitochondrial health, and mitigated oxidative stress markers. These findings suggest that ARG has substantial therapeutic potential in managing AKI by preserving mitochondrial function and reducing oxidative damage. This study focused on the nephroprotective effects of the amino acid l-arginine in the UUO model of AKI as one of the most critical/acute types of renal injury. Thus, the data obtained from this model could be more readily extrapolated to clinical situations as the UUO model is similar to conditions associated with urinary tract obstruction-induced renal injury (e.g., kidney stones obstructed in the urinary tract). Moreover, the involvement of mitochondrial impairment in the pathogenesis of AKI in the UUO model, its interconnection to oxidative stress, and finally, the effects of l-arginine on these parameters as basic mechanisms involved in the nephroprotective properties of this amino acid are novel data provided in the current study. Identifying these mechanisms could pave the way for the clinical application of safe molecules such as the amino acid l-ARG in the clinic. Future investigations should focus on elucidating the precise molecular mechanisms underlying ARG's protective effects, optimizing dosing strategies, and exploring its efficacy in other kidney injury models and clinical settings.

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Authors contributions

Seyed Mohammad Amin Kashani, Narges Abdoli, Forouzan Khodaei, Zahra Honarpishefard, Tahereh Golzar, Nazi Dastkhosh, Azad Salimi, and Negar Azarpira contributed to the experimental work, data acquisition, analysis, and/or interpretation. They also participated in drafting and critically revising the manuscript. Hossein Niknahad and Reza Heidari (supervisors) conceived and designed the study, supervised the research, provided intellectual input, and finalized the manuscript. All authors reviewed and approved the final version of the manuscript, agreeing to be accountable for all aspects of the work.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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