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ORIGINAL ARTICLE: NEPHROLOGY

Mitochondrial Impairment and Oxidative Stress Are Essential Mechanisms Involved in the Pathogenesis of Acute Kidney Injury

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Abstract

Acute kidney injury (AKI) is an emergency condition that requires restrictive and appropriate clinical interventions. Identifying mechanisms of organ injury is a critical step in developing clinical interventions. Unilateral ureter obstruction (UUO) is widely used as an animal model for investigating AKI. The current study was designed to evaluate the role of mitochondrial impairment and oxidative stress in the pathogenesis of renal injury in UUO model. Mice underwent UUO surgery. Then, kidney tissue histopathological changes, plasma biomarkers of renal injury, oxidative stress, and different renal mitochondrial indices were evaluated at scheduled time intervals (3, 7, 14, and 21 days after UUO surgical procedure). Significant increase in plasma creatinine and blood urea nitrogen levels was evident in UUO mice. The UUO surgery induced severe kidney tissue histopathological alterations, including necrosis, severe tubular atrophy, and interstitial inflammation. Moreover, kidney biomarkers of oxidative stress included reactive oxygen species formation, lipid peroxidation, protein carbonylation, decreased glutathione reservoirs (GSH), and increased oxidized glutathione (GSSG) observed in UUO mice. On the other hand, significant mitochondrial depolarization, decreased mitochondrial dehydrogenases activity, mitochondrial permeabilization, and decreased adenosine triphosphate and GSH/GSSG levels were discovered in mitochondria isolated from the kidneys of UUO mice. The data obtained from the current study demonstrated a pivotal and interconnected role for oxidative stress and mitochondrial dysfunction in the pathogenesis of renal injury in UUO model. Therefore, these directions could serve as therapeutic targets in animal models or patients of acute renal failure.

Keywords: antioxidants; bioenergetics; mitochondria; nephrotoxicity; renal failure

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Introduction

Renal disease refers to a wide range of disorders ranging from mild kidney function abnormalities to failure of this vital organ. In this context, acute kidney injury (AKI) is an emergency clinical condition that needs appropriate and restricted clinical interventions to prevent renal failure (1, 2). A wide range of diseases and xenobiotics could cause AKI (1–6). The ultimate goal in patients with severe renal disease could be organ transplantation. However, detection of early tissue damage and understanding of the disease's mechanisms could prevent/delay organ transplantation by developing appropriate therapeutic interventions.

The term oxidative stress refers to the situation where the balance between production and removal of reactive oxygen species (ROS) in biological environments is impaired (7). There is a plethora of evidence indicating the central role of oxidative stress and its associated complications in the pathogenesis of AKI (8–14). Increased levels of biomarkers of oxidative stress, including ROS formation, lipid peroxidation, oxidative damage of proteins, and other intracellular components (e.g., DNA), as well as a significant impairment of renal antioxidant defense mechanisms, have been documented in various models of AKI (15–19). Therefore, the role of oxidative stress and its association to mitochondrial impairment is highlighted in the current AKI model of unilateral ureter obstruction (UUO).

The association between oxidative stress and damage to vital organelles, such as mitochondria, is well documented (20–24). Mitochondria act as a powerhouse for producing cell energy. Meanwhile, mitochondria are also cells' most critical ROS production center (20, 25–27). Therefore, there is a robust connection between oxidative stress and mitochondrial impairment.

Kidney tissue contains many mitochondria that, by producing sufficient energy, enable vital processes, such as reabsorbing substances in the kidney (27–30). Loss of mitochondrial function in renal tissue and consequent insufficient production of adenosine triphosphate (ATP) can lead to the urinary loss of vital electrolytes, vitamins, and minerals (29). Eventually, mitochondrial damage can lead to the release of cell death mediators from this organelle, leading to cytotoxicity, organ injury, and renal failure (29, 31, 32). The role of renal mitochondrial impairment and its association with oxidative stress are evaluated in the current model of UUO.

Obstructive renal injury and nephropathy are frequent clinical complications that could lead to AKI (33, 34). Despite their etiology, these complications could lead to irreversible consequences, such as renal failure. Therefore, identifying the mechanisms involved in the pathogenesis of obstructive kidney damage might help develop novel therapeutic strategies to control renal diseases. The current study aimed to evaluate the role of oxidative stress and mitochondrial impairment in the pathogenesis of renal injury in the UUO model of AKI. The data obtained from this study could help develop novel therapeutic strategies to combat AKI with different etiologies.

Materials and Methods

Reagents

2′,7′-Dichlorofluorescein diacetate (DCFH-DA), reduced glutathione (GSH), trichloroacetic acid (TCA), malondialdehyde (MDA), 3-[4,5dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), sucrose,3-(N-morpholino) propanesulfonic acid (MOPS), D-mannitol, ferric chloride hexahydrate ($FeCl_3.6H_2O$), rhodamine123 (Rh 123), thiobarbituric acid (TBA), 2,4,6-tripyridyl-s-triazine (TPTZ), dithiothreitol (DTT), coomassie brilliant blue, 2,4-dinitrophenyl hydrazine (DNPH), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma (Sigma-Aldrich, USA). Kits for measuring serum biomarkers of renal injury were obtained from Pars-Azmun® (Tehran, Iran). Meta-phosphoric acid, n-propanol, ethylenediaminetetraacetic acid (EDTA), perchloric acid, and 2-amino-2-hydroxymethyl-propane-1, 3-diol-hydrochloride (Tris-HCl) were purchased from Merck (Darmstadt, Germany).

Animals

Male BALB/c mice (n = 60, 25±3 g) were obtained from Shiraz University of Medical Sciences, Shiraz, Iran. Animals were kept at an environmental temperature of 23±1°C with ≈40% relative humidity and adequate ventilation. Animals had free access to tap water and a standard pellet chow during experiments. The Institutional Laboratory Animal Care and Use Committee at Shiraz University of Medical Sciences Shiraz, Iran, approved all animal experiments (Code: IR.SUMS.REC.1398.1220). The Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines for the care and use of experimental animals were also followed.

Unilateral ureter obstruction model of acute kidney injury

Animals were randomly divided into the UUO and sham-operated groups. The UUO model was induced based on the previously reported protocol (35). Briefly, mice were anesthetized with a mixture of 8 mg/kg xylazine and 60 mg/kg ketamine. The left ureter was isolated and doubly ligated. The sham-operated mice underwent an identical surgical intervention for ureter identification and manipulation without ligation (35). Animals were recovered under Infrared (IR) light and received normal saline (2.5 mL/kg subcutaneous [s.c.]). Mice had access to easy food on the first day after surgery (36). No mortality rate was discovered in the current study.

Experimental setup and sample collection

Animals were intensely anesthetized with thiopental, 100 mg/kg, at different time intervals (3, 7, 14, and 21 days after UUO procedure). Blood samples (1 mL from the inferior vena cava) were transferred to sodium citrate-coated tubes, and the plasma was prepared (4000 g, 20 min, 8°C). Afterward, five mice in each group were randomly selected for kidney mitochondria isolation. Kidneys of five other mice were used to assess histopathological alterations and oxidative stress biomarkers. All mentioned markers were also evaluated in the right intact kidney (without ureter obstruction; as a control in each animal) on day 21 post-UUO surgery of right kidney (UUO-RK).

Reactive oxygen species formation

Reactive oxygen species were estimated in the kidneys of UUO mice using the 2′,7′-dichlorofluorescein diacetate (DCF-DA) method (9, 37–41). 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 900 µL of Tris-HCl buffer (40 mM, pH = 7.4) and 10 µL of DCF-DA (final concentration of 10 µM) (3, 42–47). Samples were incubated in dark for 10 min (37ºC shaker incubator). Finally, the fluorescence intensity was assessed using a FLUOstar Omega® fluorimeter (λ_{excit} = 485 nm and λ_{emiss} = 525 nm) (9, 37, 40, 48, 49).

Protein carbonylation

Renal tissue protein carbonylation in UUO mice was assessed based on the dinitrophenyl hydrazine (DNPH) test (50–52). Briefly, kidney tissue (200 mg) was homogenized in 5-mL phosphate buffer (pH = 7.5, containing 0.1% v:v of triton X-100). Samples were centrifuged (700 ×*g*, 10 min, 4ºC) and the resulting supernatant was treated with 1500 µL of 10-mM DNPH solution (dissolved in 6-M HCl). Samples were incubated in a shaker incubator (for 1 h, 25ºC, protected from light) (50, 51, 53–56). Afterward, 500-µL trichloroacetic acid (20% w:v) was added and centrifuged (17,000 ×*g*, 5 min, 4ºC). The pellet was washed for five times with ethanol:ethyl acetate (1 mL of 1:1 v:v) and redissolved in guanidine chloride (6 M, pH = 2.3). Finally, samples were centrifuged (17,000 ×*g*, 1 min, 4ºC), and the absorbance of the supernatant was assessed (λ = 370 nm) (51, 57).

Lipid peroxidation

The thiobarbituric acid reactive substances (TBARS) test was used to assess lipid peroxidation in the kidneys of UUO mice (9, 47, 58–62). For this purpose, 0.5 mL of tissue homogenate (10% w:v in Tris-HCl buffer, 40 mM, pH = 7.4) was treated with 1 mL of TBARS assay reagent (a mixture of 0.4% w:v of thiobarbituric acid, 50% w:v of trichloroacetic acid, and 1% w:v of meta-phosphoric acid, pH = 2) (9, 42, 63–70). Samples were vortexed (1 min) and heated (100 C water bath) for 45 min. Then, 1 mL of n-butanol was added. Samples were mixed considerably and centrifuged (10,000 ×*g*, 20 min, 25°C). Finally, absorbance of the upper phase was measured (λ = 532 nm) (9, 71–74).

Renal hydroxyproline levels

Renal hydroxyproline content was assessed as an index of tissue fibrosis. Briefly, 500 µL of tissue homogenate (10% w:v in Tris-HCl buffer) was digested in 1 mL of hydrochloric acid (6 N) at 120°C (12 h). Afterward, an aliquot of digested homogenate (250 µL) was treated with 250 µL of citrate-acetate buffer (pH = 6) and 500 µL of chloramines-t (56 mM) and incubated at room temperature for 20 min. Then, 500 µL of 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 λ = 550 nm (75).

Total antioxidant capacity of the kidney tissue

The ferric-reducing antioxidant power (FRAP) assay measured the total antioxidant capacity of renal tissue (9, 76). Tissue was homogenized in an ice-cooled (4ºC) 40-mM Tris-HCl buffer. Afterward, 100 µL of tissue homogenate (10% w:v in Tris-HCl buffer) was added to 900 µL of freshly prepared FRAP reagent (77–82). Samples were incubated at 37ºC (5 min, protected from light). Finally, the absorbance was measured at λ = 595 nm (9, 83).

Myeloperoxidase enzyme activity in the kidney

Myeloperoxidase (MPO) activity of the renal tissues of UUO mice was assessed based on the previously reported procedure (84). Briefly, tissue specimens (100 mg) were homogenized in 1 mL of hexadecyl-trimethyl-ammonium bromide (HTAB) solution (0.5% w:v of HTAB; dissolved in 50-mM potassium phosphate buffer; pH = 6, at 4°C) and centrifuged (3000 ×*g*, 20 min, at 4°C). Then, 100 µL of the supernatant was added to 2.9 mL of 50-mM potassium phosphate buffer (pH = 6; containing 16.7-mg/100 mL of O-dianisidine hydrochloride and 0.0005% v:v of H_2O_2). Samples were incubated in dark for 5 min (25°C). Then the reaction was stopped by HCl (100 µL of 1.2 M). Finally, the absorbance was measured at λ = 400 nm (84).

Kidney mitochondria isolation

The differential centrifugation method was used to isolate kidney mitochondria (85–89). For this purpose, 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% bovine serum albumin (BSA), pH = 7.4). Then, the minced tissue was transported into a fresh buffer (5-mL buffer/1 g of the kidney) and homogenized. The homogenized tissue was centrifuged (1000 ×*g*, 20 min, 4°C), and the supernatant was collected (90–93). The supernatant was centrifuged again (10,000 ×*g*, 20 min, 4°C) to pellet mitochondrial fraction. The mitochondrial pellet was washed for at least three times using fresh isolation buffer to increase mitochondrial purity and yield. Finally, isolated mitochondria were suspended in buffer and used for further evaluation.

Mitochondrial dehydrogenases activity

The 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay was applied to determine renal mitochondrial dehydrogenases activity (8, 44, 94–96). Briefly, a mitochondrial suspension (0.5-mg protein/mL) was incubated with 40 µL of 0.4% w:v of MTT solution (37°C, 30 min, protected from light) (85, 97). Then the samples were centrifuged (10,000 ×*g*, 20 min), and the pellet was dissolved in dimethyl sulfoxide (DMSO, 1000 µL). Finally, the absorbance was measured at λ = 570 nm (98–100).

Mitochondrial depolarization

Briefly, mitochondrial fractions (0.5-mg protein/mL) were incubated with 10 µM of Rh 123 at 37°C in a shaker incubator (10 min, protected from light) (101–104). Afterward, samples were centrifuged (10,000 ×*g*, 10 min, 4°C), and the fluorescence intensity of the supernatant was assessed (FLUOstar Omega® Fluorimeter; $\lambda_{excitation}$ = 485 nm and $\lambda_{emission}$ = 525 nm) (9, 105–107).

Mitochondrial swelling

Analysis of mitochondrial swelling was estimated through changes in light scattering (105, 108–114). Briefly, isolated mitochondria samples (0.5-mg protein/mL) were added to pre-warmed buffer. The absorbance was monitored at λ = 540 nm for 30 min at 30°C with orbital shaking (EPOCH® microplate reader; Agilent Technologies, Santa Clara, CA, USA). Primary and final absorbance difference (ΔOD) was calculated (28, 105, 115).

Renal tissue and isolated mitochondrial glutathione content

The reduced (GSH) and oxidized (GSSG) glutathione content in the kidneys of UUO mice was measured using the high-performance liquid chromatography (HPLC) method based on derivatization with dinitrofluorobenzene (DNFB) (3, 116, 117). An amine column (NH_2, 25-cm Bischoff chromatography; Leonberg, Germany) was used as a stationary phase (118). Buffer A (acetate buffer:water; 1:4 v/v) and buffer B (methanol:water; 4:1 v/v) were mobile phases. Gradient method with a regular increase of buffer B to 95% in 30 min was used, and the flow rate was 1 mL/min (119). The ultraviolet (UV) detector was set at λ = 254 nm.

Mitochondrial ATP content

The previously described HPLC protocol assessed mitochondrial ATP level (120, 121). Briefly, isolated mitochondria (0.5-mg protein/mL) were treated with ice-cooled 0.2-M perchloric acid, incubated in ice (5 min) and centrifuged (10 min, 17,000 ×*g*, 4°C). The supernatant was treated with an equivalent volume of ice-cooled 1-M KOH solution. Samples were filtered and injected (30 µL) into an HPLC system consisting of an LC-18 column (µ-Bondapak, 15 cm). The mobile phase comprised KH_2PO_4 (215 mM), tertiary butyl ammonium sulfate (2.3 mM), KOH (1 M, 0.4%), and acetonitrile (4% v:v). The constant flow rate was 1 mL/min, and the UV detector was set at λ = 254 nm (5).

Tissue histopathology and organ weight index

Kidney samples were fixed in buffered formalin (10% w:v formaldehyde in 0.1-mM phosphate buffer, pH = 7.4). Then, paraffin-embedded kidney sections (5 µm) were prepared and stained with hematoxylin and eosin (H&E) (122). Renal histopathological changes in UUO model were scored based on the previously reported following protocol: score 0, none; score 0.5, <10%; score 1, 10–25%; score 2, 25–50%; score 3, 50–75%, and score 4, >75% (123). Masson's trichrome staining determined fibrotic kidney changes in UUO (21). Periodic acid–Schiff (PAS) staining was applied to assess kidney cast formation. A pathologist analyzed samples blindly. Kidney weight index was measured as follows:

$$\text{Organ weight index} = \frac{\text{Wet organ weight (g)}}{\text{Whole body weight (g)}} \times 100 \quad (95)$$

Statistical analysis

Data were presented as mean ± SD. Data comparison was accomplished by one-way analysis of variance (ANOVA) with *post hoc* Tukey's multiple comparison test. The normality of data sets was assessed by Kolmogorov–Smirnov test. Scores for histopathological changes in renal tissue were presented as median and quartiles for five random scores. The analysis of tissue histopathological alterations was performed by the Kruskal–Wallis test, followed by the Mann–Whitney U test. $P < 0.05$ was considered significant.

Results

Kidney weight index was significantly decreased in UUO mice after 7, 14, and 21 days of surgery (Figure 1). No significant changes were recorded in the right kidney of UUO mice (UUO-RK), compared to the sham-operated group (Figure 1).

Plasma blood urea nitrogen (BUN) and creatinine levels as biomarkers of renal injury were significantly elevated at different time points of post-ureteral obstruction (Figure 1). On the other hand, oxidative stress markers were altered in both sham-operated and UUO model (Figure 2). A time-dependent increase in renal tissue ROS, lipid peroxidation, protein carbonylation, and increased GSSG levels was evident in UUO mice (Figure 2). Moreover, renal antioxidant capacity reduced GSH content and GSH–GSSG ratio was significantly decreased at different time intervals after UUO surgical procedure in a time-dependent manner (Figure 2). No significant changes in renal biomarkers of oxidative stress were recorded in the right kidney of UUO mice (UUO-RK), compared to the sham-operated group (Figure 2).

A significant decrease in mitochondrial dehydrogenase activity and ATP levels was evident in the kidney of UUO model (Figure 3). On the other hand, severe mitochondrial permeabilization and depolarization were also detected in mitochondria isolated from UUO mice in a time-dependent manner (Figure 3). The GSH reservoirs were also depleted, and the GSH–GSSG ratio decreased considerably in the mitochondria isolated from UUO mice (Figure 3). No significant changes in mitochondrial indices were discovered when the right kidney of UUO mice (UUO-RK) was compared with that of the sham-operated group (Figure 3).

Renal tissue histopathological assessments revealed significant interstitial inflammation, tubular atrophy, and tissue necrosis on different days post-UUO surgery (Figure 4 and Table 1). A mild inflammatory response was also apparent only in the right kidney of UUO mice (UUO-RK) on day 21 after UUO surgery (Table 1). Markers of kidney fibrosis were also evaluated in both sham-operated and UUO mice (Figure 5). Collagen deposition was significantly elevated in the kidneys of UUO mice than in the kidneys of sham-operated mice (Figure 5). Renal tissue hydroxyproline content was also considerably increased in UUO mice (Figure 5). MPO enzyme activity as an index of tissue inflammation was also significantly higher than in the control animals at various time intervals post-UUO surgery (Figure 5).

Periodic acid–Schiff staining of renal tissue revealed significant cast formation in UUO mice (Figure 6). It was discovered that the number of kidney casts was time-dependently increased in the kidney after UUO surgery (Figure 6).

Discussion

Acute kidney injury is a severe clinical complication requiring restricted and emergent interventions (1, 2). Determination of the mechanisms involved in the pathogenesis of the diseases is a critical step in identifying and developing therapeutic targets. UUO is a model investigated widely for evaluating the modality of renal injury. Inflammatory

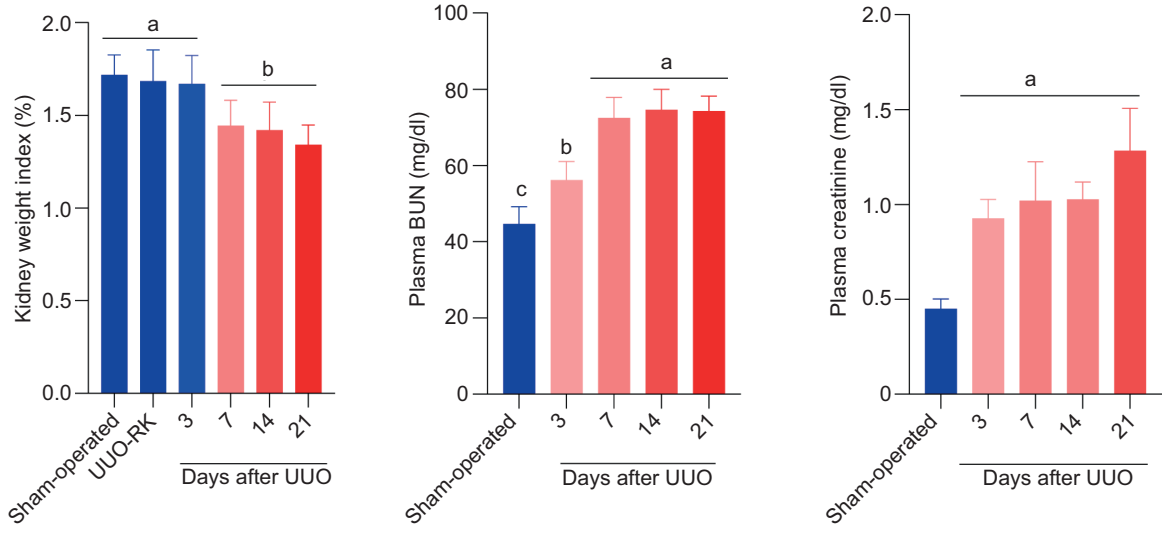

Figure 1: Serum biochemical measurements and renal weight index in unilateral ureter obstruction (UUO) model of acute kidney injury. UUO-RK: Right kidney of UUO model. Data are presented as mean ± SD (n = 5). Groups with different alphabetical superscripts are significantly different ($P < 0.05$).

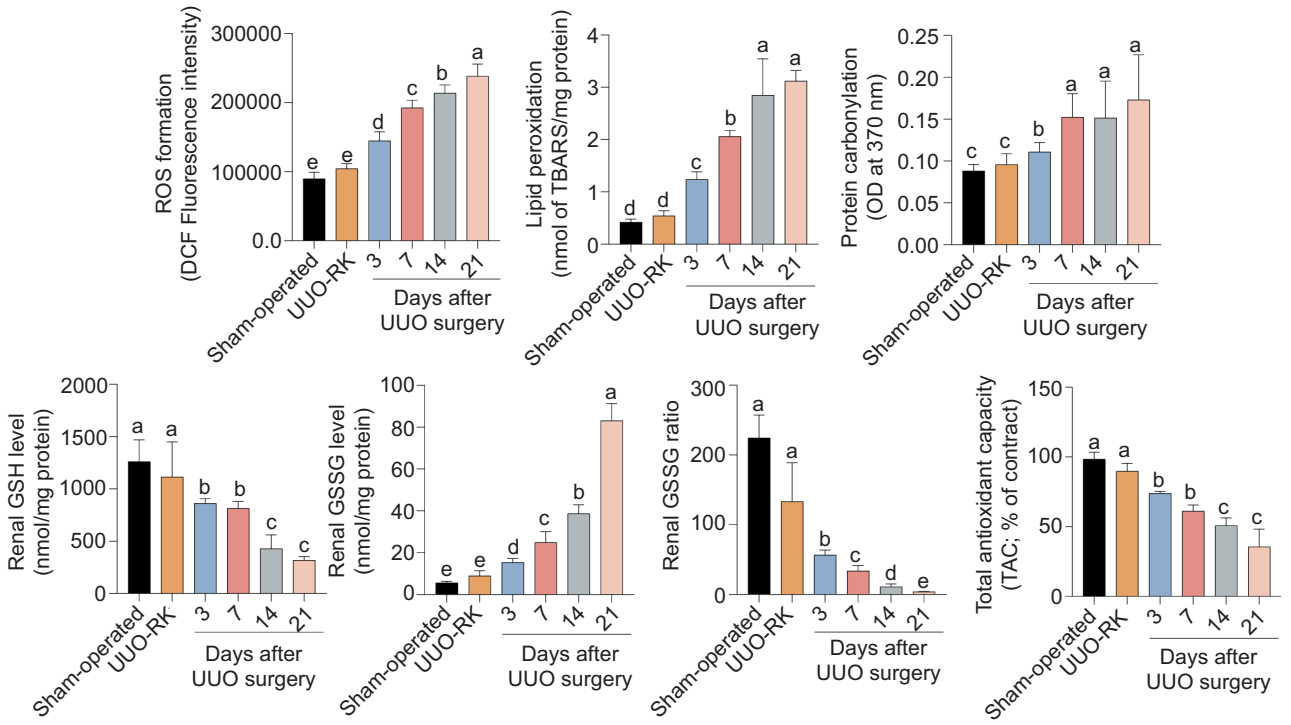

Figure 2: Biomarkers of oxidative stress in the renal tissue of unilateral ureter obstruction (UUO) mice model of acute kidney injury. UUO-RK: Right kidney of UUO model. Data are presented as mean ± SD (n = 5). Groups with different alphabetical superscripts are significantly different (P < 0.05).

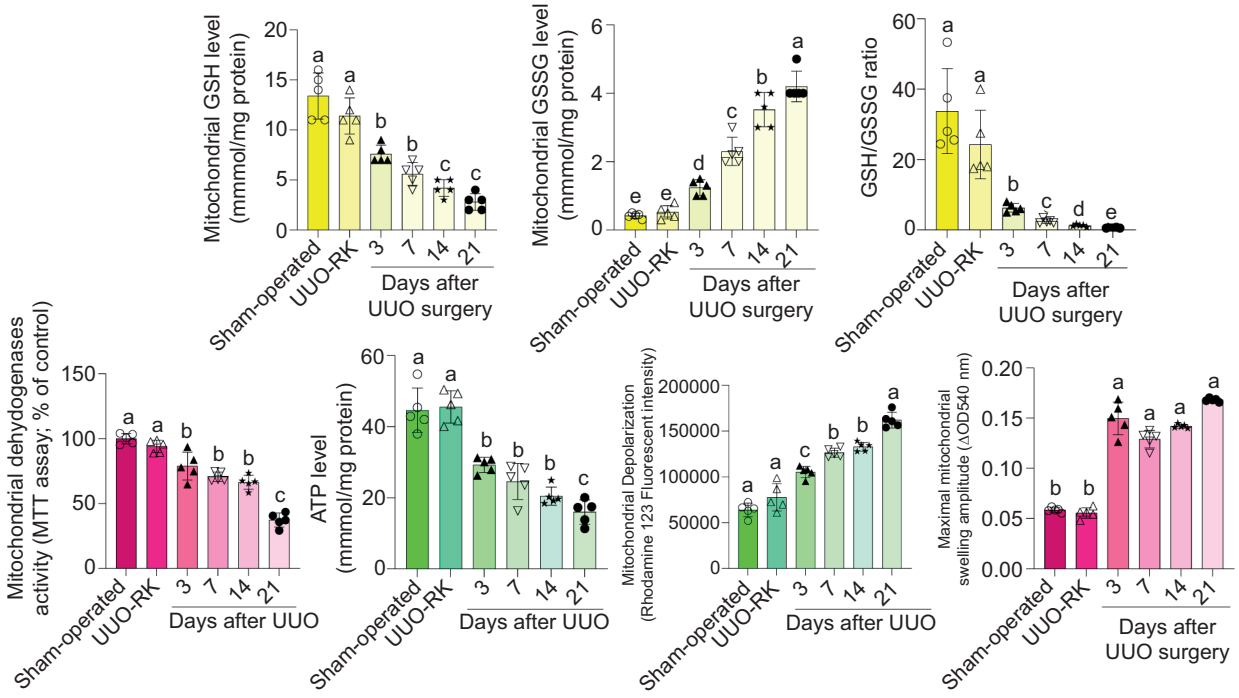

Figure 3: Mitochondrial indices in the kidney of unilateral ureter obstruction (UUO) mice. UUO-RK: Right kidney of UUO model. Data are presented as mean ± SD (n = 5). Groups with different alphabetical superscripts are significantly different (P < 0.05).

Table 1: Renal histopathological alterations in unilateral ureter obstruction (UUO) mice.

Treatments	Glomerular Damage	Tubular Damage	Inflammation
Control	0 (0, 0)[a]	0 (0, 0)[a]	0 (0, 0)[a]
UU-RK	0 (0, 0)[a]	0 (0, 0)[a]	0.5 (0, 1)[a]
3 days after UUO	2 (1, 2)[b]	2 (2, 2)[b]	3 (3, 3)[d]
7 days after UUO	3 (2, 3)[d]	3 (3, 3)[d]	4 (4, 4)[e]
14 days after UUO	3 (3, 3)[d]	4 (4, 4)[e]	4 (4, 4)[e]
21 days after UUO	4 (4, 4)[e]	4 (4, 4)[e]	4 (4, 4)[e]

Data are presented as median and quartiles for five pictures/groups.
Data sets with different alphabetical superscripts are significantly different ($P < 0.05$).
Renal histopathological changes in UUO model were scored based on a protocol described by Li et al. (2019) in the materials and methods section (123). UU-RK: Right kidney of UUO mice.

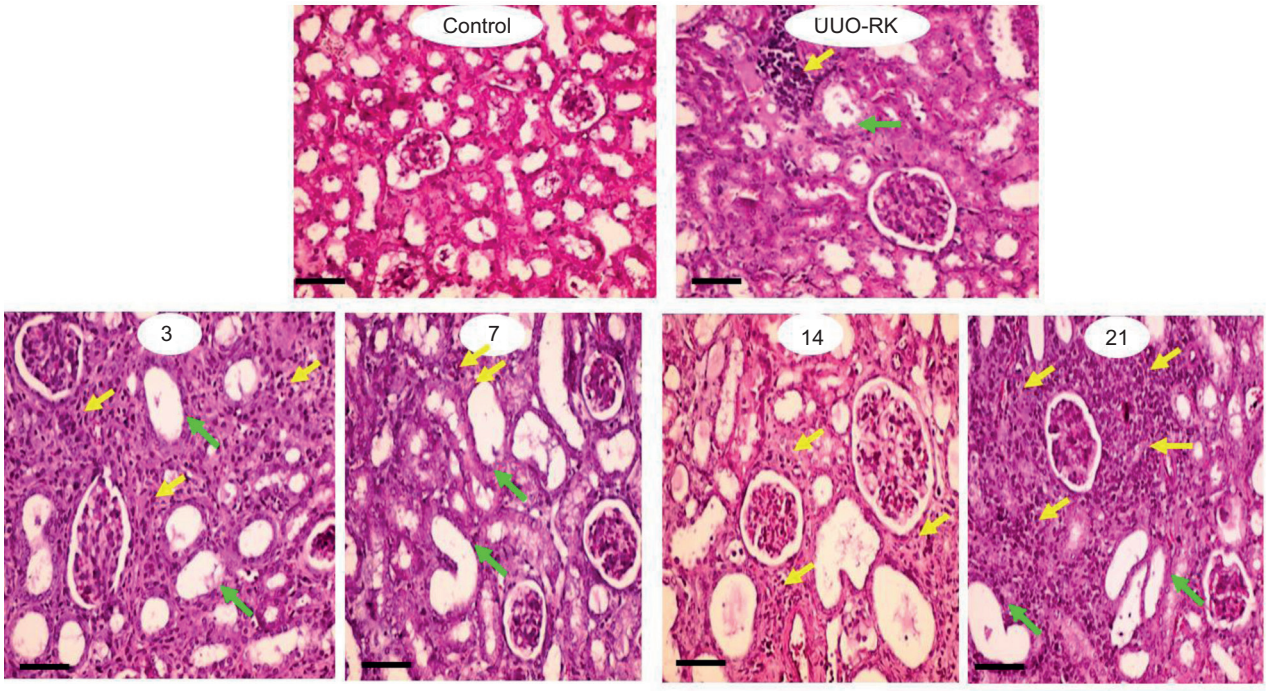

Figure 4: Renal histopathological changes in unilateral ureter obstructed (UUO) mice. Numbers (3, 7, 14, 21) indicate days after the UUO surgery. Inflammatory cell infiltration (yellow arrows) and tubular atrophy (green arrows) were prominent histopathological changes in UUO mice. UUO-RK: Right kidney of UUO model. Scale bar = 100 µm.

response, mechanical stress, and oxidative stress are commonly assessed in the UUO model of renal injury. On the other hand, there is no report on the role of mitochondrial impairment in cell death and organ injury in UUO model. In the current study, we discovered that severe oxidative stress, mitochondrial impairment, inflammation, and renal tissue histopathological alterations occurred in the kidney of UUO model. These findings demonstrated essential role of mitochondrial function disturbances and oxidative stress in the pathogenesis of renal injury in UUO model.

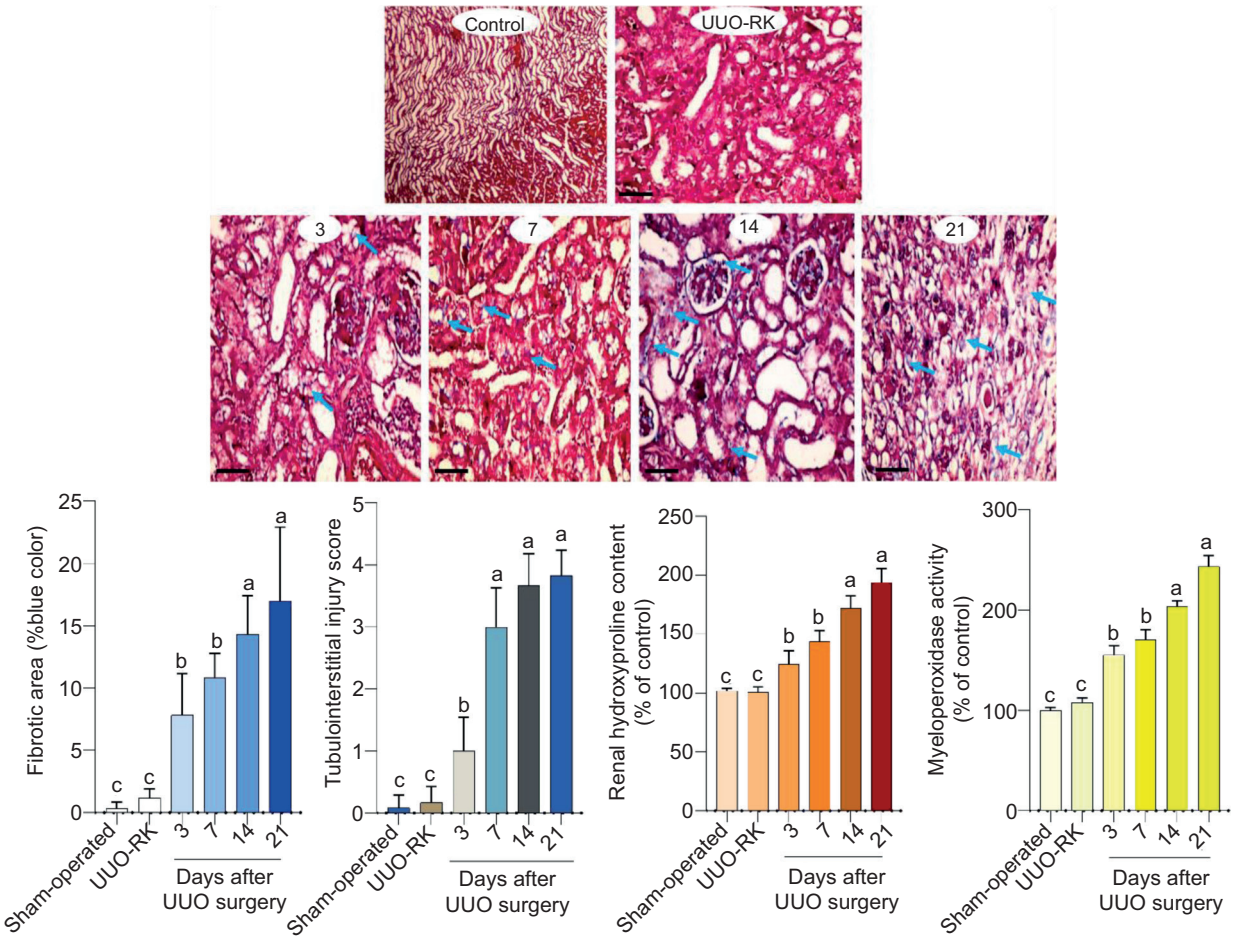

Figure 5: Renal fibrotic changes, collagen deposition (blue arrow), and hydroxyproline content in the unilateral ureter obstruction (UUO) model (Trichrome stain ×200). Numbers on histopathological pictures (3, 7, 14, 21) indicate days after UUO surgery. UUO-RK: Right kidney of UUO model. Data for fibrotic area, hydroxyproline content, myeloperoxidase activity, and tubulointerstitial injury score are given as mean ± SD (n = 5). Columns with various alphabetical superscripts are significantly different ($P < 0.05$). Scale bar = 100 μm.

As mentioned, identifying the cellular and molecular mechanisms involved in the pathogenesis of diseases is a critical step in developing therapeutic strategies. In this context, complications, such as prostatic hyperplasia and renal stones, could lead to urinary obstruction (124, 125). Although surgical intervention is an ultimate and promising therapeutic option for this condition, finding organ injury mechanisms could help develop pharmacological or ancillary treatments.

In the current study, we investigated the role of oxidative stress and mitochondrial impairment and their interconnection in the pathogenesis of AKI in an animal model. Previous evidence indicated the role of oxidative stress and its associated complications in UUO model (126–128). It was discovered that oxidative stress markers, such as ROS formation and damage to cellular components (e.g., lipids), occurred in UUO model (126–128). Moreover, it was discovered that antioxidant mechanisms in renal tissues were significantly impaired in UUO model (126, 127). However, no specific source(s) for ROS was identified in UUO model yet. In the current study, we determined that oxidative stress biomarkers were significantly elevated in the kidneys of UUO mice (Figure 3). Moreover, we tried to delineate a connection between oxidative stress and potential sources of ROS formation in UUO model. In this regard, we investigated the relation between inflammatory response, mitochondrial impairment, and oxidative stress in the UUO model of AKI.

An interesting point was the interconnection between mitochondrial impairment and oxidative stress (Figure 7). It is well known that mitochondria are the primary source of intracellular ROS formation. An average level

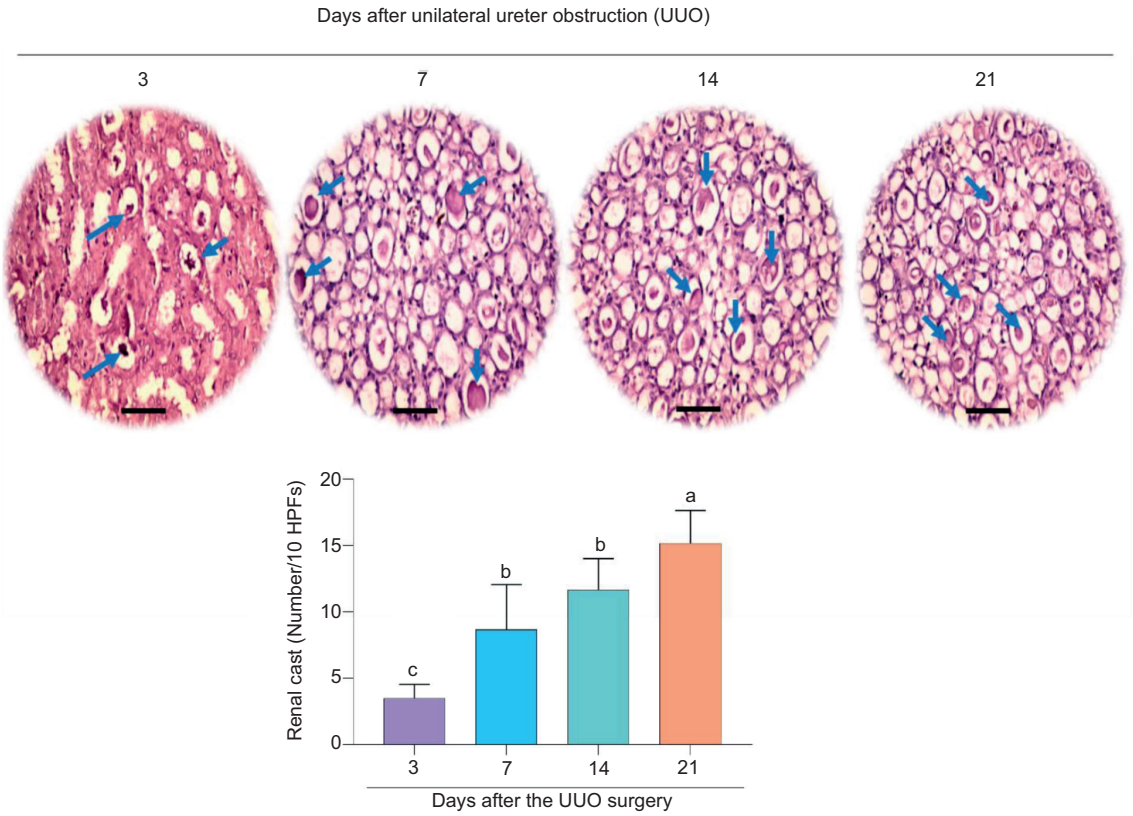

Figure 6: Cast formation in the kidney of unilateral ureter obstruction (UUO) model (periodic acid–Schiff [PAS] stain ×400). No significant cast formation was detected in the sham-operated group or right kidney of UUO model. HPFs: high power fields. Data for renal cast formation are presented as mean ± SD (n = 5). Columns with various alphabetical superscripts are significantly different ($P < 0.05$). Scale bar = 100 μm.

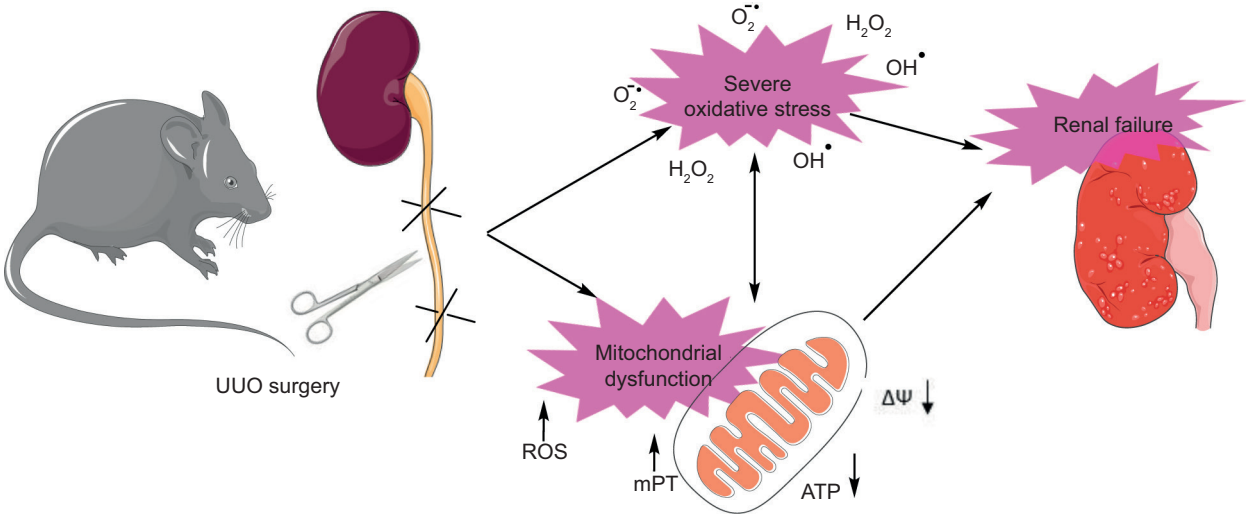

Figure 7: Schematic presentation of the central role of oxidative stress and mitochondrial impairment in the pathogenesis of unilateral ureter obstruction (UUO) model of acute renal injury. Oxidative stress and mitochondrial injury are two mechanistically interrelated events. ROS: reactive oxygen species; mPT: mitochondrial permeability transition; ATP: adenosine triphosphate; ΔΨ: mitochondrial membrane potential.

of mitochondrial-mediated ROS formation could act as signaling molecules essential for normal physiological processes (20). However, the mitochondrial-mediated ROS level increased dramatically if the mitochondrial function was impaired (20, 101). In the current UUO model, accumulating potently cytotoxic molecules routinely excreted from urine could affect mitochondrial function and lead to cytotoxicity and organ injury.

Previous studies revealed that inflammatory response is essential for renal injury in UUO model (128, 129). In the current study, we determined that the accumulation of inflammatory cells increased significantly at different time intervals after UUO surgery (Figure 4 and Table 1). The cytokines released by these cells could substantially cause oxidative stress in this model. On the other hand, it was discovered that cytokines, such as tumor necrosis factor-α (TNF-α), directly affected mitochondrial function (130, 131). These cytokines were also able to induce tissue fibrosis, finally leading to organ failure. It should also be mentioned that oxidative stress and inflammation are two interrelated processes that could lead to tissue damage (132, 133). The molecular connection between oxidative stress and inflammation is complicated and includes several signaling pathways (132, 133). The transcription factors nuclear factor erythroid 2-related factor 2 (Nrf-2) and nuclear factor kappa B (NF-κB) are critical oxidative stress and inflammation regulators, respectively (3, 6). Hence, more studies on these molecular pathways could provide viable therapeutic options to combat renal injury (e.g., in UUO model). Therefore, further research is needed to uncover this hypothesis.

Novel therapeutic options, such as mitochondrial replacement therapy, could be crucial in the future therapeutic strategies to control renal failure with different etiologies. These organelles provide enough energy for cell survival, prevent electrolyte imbalance, and provide enough time for basic organ transplantation strategies.

It has been well-documented that oxidative stress and the subsequent activation of extracellular matrix-producing cells (collagen deposition) are interconnected. Hence, a possible mechanism for fibrotic change in the current UUO model could be associated with oxidative stress (e.g., mitochondrial-mediated ROS formation). Kidney tissue also contains numerous mitochondria, guaranteeing the reabsorption of essential chemicals from urine (29). This process is an energy-dependent reaction. Hence, changes in renal mitochondrial function could lead to crucial electrolyte waste and serum electrolyte imbalance (8, 134, 135). Therefore, prior to surgical interventions, it is vital to preserve mitochondrial function and prevent kidney injury. Cellular mitochondria play a critical role in cell death (136).

Various studies revealed the significance of oxidative stress in UUO model (126, 127). Hence, our data on the role of oxidative stress markers are in line with these studies (Figure 7). More importantly, we found that renal mitochondrial indices of functionality were significantly hampered in UUO model. This represents mitochondrial function as a key mechanism in the pathogenesis of this complication (Figure 7). Recently, we tested different safe and clinically applicable agents with antioxidant and mitochondrial protecting agents in various experimental models or patients with renal disorders (5, 42, 91, 137–139). Some of these compounds even underwent clinical trials for different types of renal injury and nephropathy. Further studies are warranted to reveal the clinical significance of these data in complications leading to ureter obstruction and renal injury (e.g., prostate hyperplasia or kidney stones).

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Conflict of interest

The authors declared that they had no conflict of interest to disclose.

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