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Reno-protective effect of Roflumilast against kidney injury induced by ischemia/reperfusion in rats: Evidence from biochemical and histological investigations

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ABSTRACT

Oxidative stress, inflammation, and apoptosis are major contributors to renal ischemia/reperfusion (I/R) injury. This study aimed to investigate the effects of pretreatment with the PDE4 inhibitor roflumilast (Rof) on renal I/R and its underlying mechanisms. Sprague-Dawley rats were subjected to 30 minutes of unilateral renal ischemia followed by 45 minutes of reperfusion. Rof (1.5 and 3 mg/kg) was administered for seven days prior to I/R induction. The findings showed that Rof significantly and dose-dependently attenuated kidney damage by reducing blood urea nitrogen and creatinine levels. Rof also exhibited antioxidant and anti-inflammatory effects, as evidenced by improved glutathione and malondialdehyde levels and decreased proinflammatory cytokines (IL-6 and TNF-α). Furthermore, Rof prevented the downregulation of HO-1 and Nrf2 expression. These results suggest that Rof therapy could protect the kidneys from I/R-induced injury through its antioxidant and anti-inflammatory properties, providing a potential therapeutic approach for the management of renal I/R damage.

1. Introduction

Acute kidney damage (AKI) is primarily caused by renal ischemia-reperfusion injury (I/R injury), which has been associated with significant morbidity and mortality in both adults and children. In addition to sepsis, trauma, hypovolemic shock, and other conditions, it frequently happens following renal transplantation [1]. Renal I/R causes significant changes in cell function, metabolism, and proximal tubule structural regularity [2]. The complex process of renal ischemia/reperfusion injury involves oxidative stress [3], inflammation [4], and apoptosis [5]. Following renal I/R, critical events that may trigger further damage contain decreased activity of antioxidant enzymes and/or increased levels of reactive oxygen species (ROS) [6]. Lipid peroxidation is the result of oxidative damage caused by excess ROS during the renal I/R process [7] and cell death brought on by ferroptosis, pyroptosis, autophagy, and apoptosis conveyed by damage of proteins and DNA in hypoxia tissue [8]. Leukocyte infiltration, tissue damage, and excessive production of proinflammatory cytokines are further factors that contribute to renal I/R injury, as well as inflammation [9].

The occurrence of acute kidney damage is associated with an increase in blood urea nitrogen (BUN) and creatinine levels. Elevated BUN and creatinine are commonly observed markers in clinical assessments, signaling impaired kidney function. These indicators are indicative of the kidneys' reduced ability to filter and excrete waste products from the bloodstream effectively. Monitoring BUN and creatinine levels is crucial in diagnosing and assessing the severity of acute kidney damage, guiding healthcare professionals in implementing appropriate interventions and treatments [10,11].

According to evidence, activating endogenous antioxidant systems could be a means of achieving reno-protection from I/R damage [12]. An inducible transcription factor, NF-E2-related factor (Nrf2), controls several cellular antioxidant mechanisms to prevent oxidative stress during I/R-induced kidney injury [13]. Normally, the Kelch-like ECH-associated protein-1 (Keap1)-dependent pathway detachment from

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Nrf2 [14]. As oxidants are present, Nrf2 is released from Keap1. It moves the nucleus and attaches itself to ARE sequences to start the transcription of genes that respond to AREs, comprising glutathione peroxidase (GSH-PX), HO-1, glutathione-*S*-transferase (GST), and superoxide dismutase (SOD) [15–17]. The primary defense mechanism in countering oxidative stress during the pathophysiological events of I/R is suggested to be the Nrf2. Nrf2 plays a pivotal role in orchestrating the cellular response to oxidative stress by regulating the expression of genes involved in antioxidant defense and detoxification pathways. Its activation represents a crucial adaptive response aimed at mitigating the damaging effects of reactive oxygen species generated during I/R [13,18].

Roflumilast (Rof), a second-generation selective PDE4 inhibitor, has anti-antioxidant properties. It has a distinct method of action on the inflammation caused by eosinophils, neutrophils, and remodeling [19]. By triggering the cAMP/PKA pathways and inhibiting LPO, Rof has been shown to reduce inflammatory reactions [20]. In a dose-related manner, the release of TNF-α and the chemokines CCL2, CCL3, CCL4, and CXCL10 from human lung macrophages was inhibited by Rof [21]. The objective of this work was to evaluate Rof's ability to prevent I/R damage and look into any possible underlying molecular mechanisms.

2. Materials and methods

2.1. Chemicals

Roflumilast was acquired from Sigma-Aldrich (St. Louis, MO, USA). Every chemical and solvent used in this investigation is of the highest quality and commercially available.

2.2. Animals and ethical statement

Twenty-four male albino Wistar rats weighing 220 ± 20 g were obtained from the "Animal House Colony at the National Research Centre (NRC, Egypt)". After being placed in stainless steel cages, the rats were given a week to acclimate to room temperature (25 ± 2 °C) and a 12-hour cycle of light and dark. Rats were fed rodent chow and had unlimited access to drinking water. The way the rats were treated adhered to ethical standards. All investigational procedures were carried out in compliance with the ethical guidelines endorsed by the "NRC's Committee on Animal Care and Use's ethics committees" (No.: 1415062022).

2.3. Surgical procedure

Inducement of bilateral renal IR was performed using the protocol outlined by Chok et al.[22]. Animals were anesthetized by intraperitoneal injection of chloral hydrate (400 mg/kg) via using a warming pad. After a midline abdominal incision, the left renal pedicle was partially dissected and surgical clamped for 30 min to keep it closed. Following that, the surgical clamps were removed to allow for 45 min of blood flow (reperfusion). The abdomen was briefly closed to prevent further fluid loss during the process of causing renal ischemia. After the arterial clip was removed, the kidney's color changed from dark purple to reddish-brown in just five minutes, indicating that blood perfusion had been effectively restored.

2.4. Experimental design

This study was conducted in accordance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines for reporting animal research. The experimental design involved four groups: a sham control group (I), an ischemia/reperfusion (I/R) group (II), and two treatment groups receiving Roflumilast at doses of 1.5 group (III) and 3 mg/kg group (IV) body weight prior to I/R induction. A total of 24 male Wistar rats were used, with 6 rats allocated to each group. After that, rats were brought to left kidney ischemia for half an hour accompanied by ¾ hour of reperfusion, group III and group IV (I/R group + Rof): rats underwent renal I/R as in group II after receiving Rof (1.5 and 3 mg/kg body weight) orally for seven days. Samples of blood were withdrawn, and the left kidneys were removed from all rats after 45 min of reperfusion.

Inclusion and exclusion criteria were established a priori according to the specified weights, and no animals or data points were excluded from the analysis. Randomization was employed to allocate the rats to the respective groups, and potential confounders, such as the order of treatments and measurements, were minimized. Blinding procedures were implemented during the experiment, outcome assessment, and data analysis. Clearly defined outcome measures included renal function tests, oxidative stress markers, inflammatory cytokine levels, gene expression analysis, and histopathological evaluation. Appropriate statistical methods were used for data analysis, with details provided in the manuscript. Comprehensive information about the experimental animals, including species, strain, sex, and age, is reported. The experimental procedures, including surgical interventions, drug administration, tissue collection, and analytical techniques, are described in sufficient detail to allow replication by other researchers. Results are presented with measures of variability and effect sizes where applicable.

2.5. Homogenization of tissues

Following the reperfusion phase, blood samples from every animal underwent a 15-minute centrifugation at 4000 rpm and 4 °C. Following collection, the serum was utilized to determine renal function tests. All animals were euthanized after the blood was collected, and each rat's kidney was removed. Renal tissues were split in two: one portion was fixed in 10 % phosphate-buffered formalin for histopathological analysis, and the other portion was taken and homogenized in ice-cold saline to identify oxidative stress markers and inflammation-associated cytokines. For Western blot analysis, at −80°C, the third part was kept frozen.

2.6. Determination of renal function

A commercial kit was used to measure the serum levels of BUN colorimetrically, Catalog# ab83362, and creatinine, Catalog # E4370–100 (BioVision, Milpitas Boulevard, Milpitas, USA) in order to assess renal function.

2.7. Determination of renal function

Total protein was extracted from kidney tissue samples using RIPA buffer with protease inhibitors. Protein concentrations were determined using the Bradford assay Catalog #23200 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) with bovine serum albumin as the standard.

2.8. Determination of oxidative stress markers

Colorimetric techniques were applied in compliance with the guidelines provided by the manufacturer to measure the MDA level, Catalog # K739–100, and GSH content, Catalog # K464–100 (BioVision, Milpitas Boulevard, Milpitas, USA).

2.9. Determination of inflammatory markers

Following the guidelines provided by the manufacturer, commercial colorimetric sandwich ELISA kits were used for the measurement of inflammation-associated cytokines, TNF-α (BioLegend, San Diego, USA, Cat. No. 438205) and IL-6 (Cloud-Clone Corp. (CCC), Wuhan, China, Cat. No. SEA079Ra).

Table 1
List of oligonucleotide primers used in qPCR.

Gene		Sequence (5′-3′)	
Nrf2	F	AAAATCATTAACCTCCCTGTTGAT	NM_010902.5
	R	CGGCGACTTTATTCTTACCTCTC	
HO − 1	F	CATCCGTGCAGAGAATTCTG	NM_012580.2
	R	CTGGTATGGGCCCCTGGC	
GAPDH	F	CCTCGTCTCATAGACAAGATGGT	NM_001394060.2
	R	GGGTAGAGTCATACTGGAACATG	

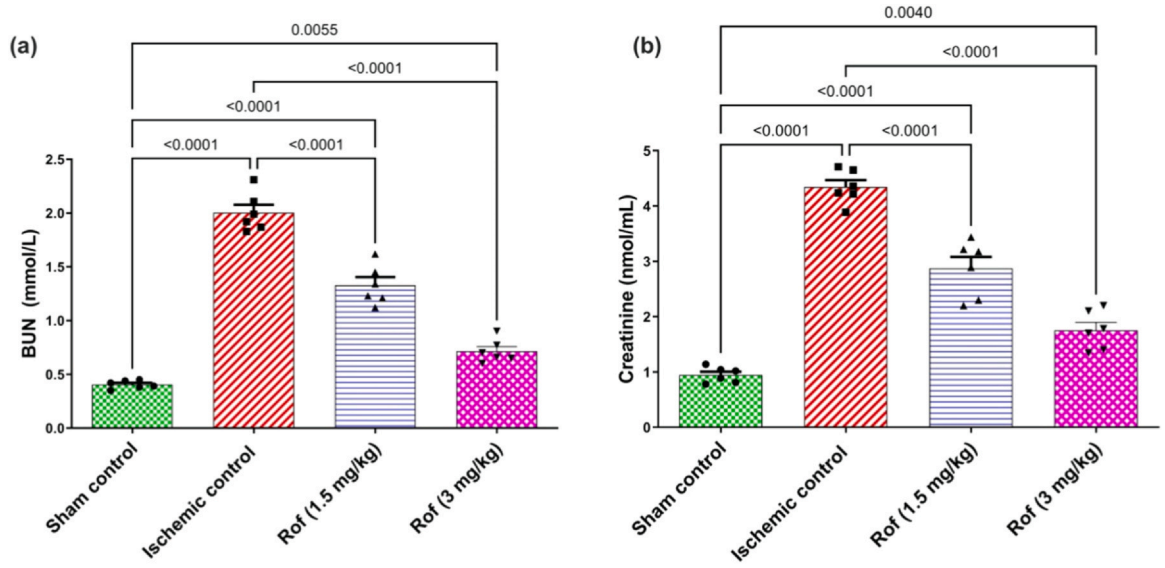

Fig. 1. Impacts of Rof on serum levels of (a) Blood urea nitrogen and (b) creatinine. Panels represent mean ± SE of six rats analyzed by One-way ANOVA followed by Tukey's multiple comparisons tests (a) BUN [F = 153.1 (DFn = 3, DFd = 20), p < 0.0001] and (b) creatinine [F = 104.3 (DFn = 3, DFd = 20), p < 0.0001]. The exact *p*-value between each compared pairwise group is stated above the pairwise bar. Rof: Roflumilast.

2.10. Real-time PCR determination of Nrf-2 and HO-1

For PCR of the isolated RNA, Thermo Fisher Scientific, Waltham, Massachusetts, USA, supplied the SuperScript IV One-Step RT-PCR kit. With StepOne (Applied Biosystems, USA) 96-well plate equipment, the following thermal profile was performed: Ten minutes are needed for reverse transcription at 45 ˚C, 2 min are required for RT inactivation (98 ˚C), and 40 repeated cycles of 10 s (98 ˚C and 55 ˚C) and 30 s (72 ˚C) were performed for the initial denaturation phase of the amplifying step. Following the RT-PCR run, the cycle threshold (Ct) was utilized to convey the information regarding the target and housekeeping genes. The forward and reverse primer oligonucleotide sequences are shown in Table 1. Target genes Nrf2 and HO-1 were normalized for variation in expression using the mean critical threshold (CT) expression. GAPDH was used as the housekeeping gene for normalization. The expression level of each target gene was calculated relative to GAPDH and then expressed as fold change compared to the control group

2.11. Histological assessment

Twenty-four hours were spent fixing the renal tissues in 10 % buffered formalin. After that, it was cleared in xylene, dehydrated in various alcohol grades, and encased in paraffin wax. H&E stain (hematoxylin and eosin) was applied to the Section (4 μm). A blinded pathologist examined the cells under a light microscope in order to prevent bias using Olympus BX-53 Xand 400.

2.12. Statistical analysis

The means ± SEM are used to express all the variables. Prism Software, version 9.5.1 (California, USA), was used to analyze the data. ANOVA was used to compare three or more groups, and the Tukey test was used as a post hoc analysis after assuring the normality and homogeneity of data. Differences were considered statistically significant at $p \leq 0.05$.

3. Results

3.1. Impacts of Rof on serum levels of BUN and creatinine

Renal I/R caused obvious renal impairment in comparison to the control group, as demonstrated by noticeably higher levels of creatinine and BUN in the serum by 4.9 and 4.6-fold, matching the normal group. Rof (1.5 or 3 mg/kg) pretreated groups demonstrated a dose-related remarkable restoration of certain renal impairment as compared with I/R rats by 34 or 64 % for the BUN and by 34 and 60 % for the creatinine (Fig. 1).

3.2. Impacts of Rof on oxidative stress indicators

When compared to sham-operated animals, renal I/R caused a remarkable decrease in renal GSH levels by 78 % and a considerable increase in renal MDA by 6.5-fold compared with sham-operated rats. However, compared to the renal I/R group, pretreatment with Rof (1.5 or 3 mg/kg) considerably reduced the elevated renal MDA level by 57 or 78 %. It replenished the reduced renal GSH content by 2.8 and 4-fold in relation to the I/R control group (Fig. 2).

3.3. Impacts of Rof on the inflammation markers TNF-α and IL-6

The kidney damage that was caused by I/R procedures was linked to a notable surge in the levels of the renal inflammatory cytokines TNF-α

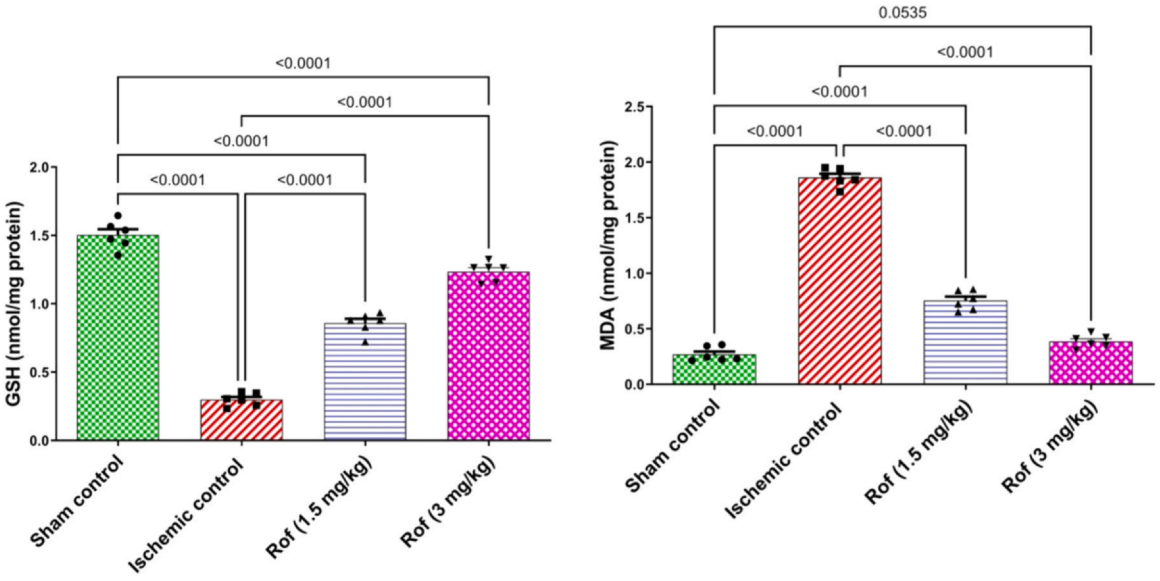

Fig. 2. Impacts of Rof on renal (a) glutathione and (b) malondialdehyde. Panels represent mean ± SE of six rats analyzed by One-way ANOVA followed by Tukey's multiple comparisons tests (a) GSH [F = 277.8 (DFn = 3, DFd = 20), p < 0.0001] and (b) MDA [F = 592.7 (DFn = 3, DFd = 20), p < 0.0001]. The exact *p*-value between each compared pairwise group is stated above the pairwise bar. Rof: Roflumilast.

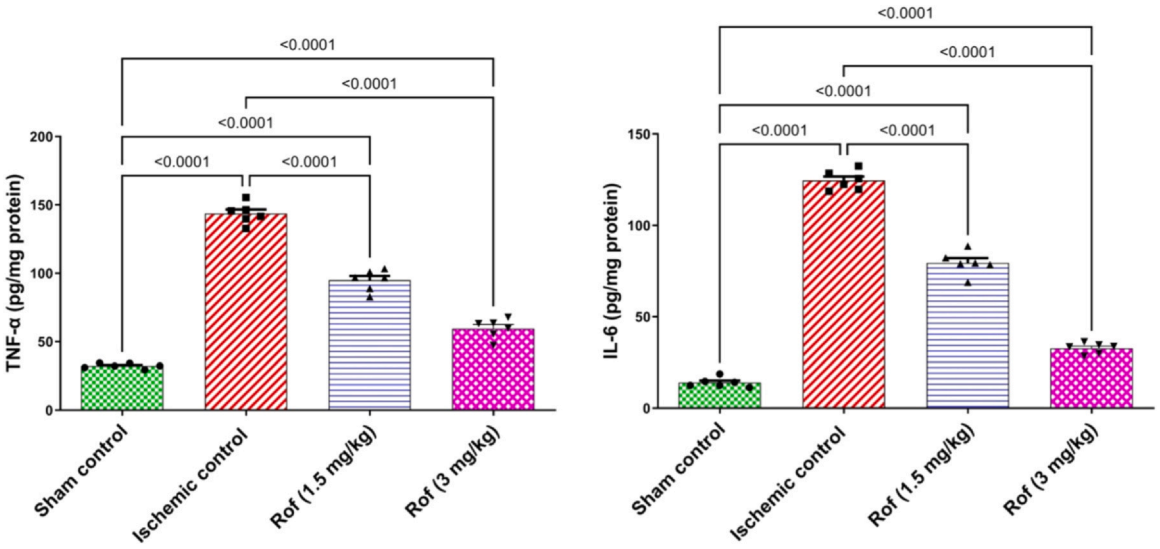

Fig. 3. The impacts of Rof on renal (a) TNF-α and (b) IL-6. Panels represent mean ± SE of six rats analyzed by One-way ANOVA followed by Tukey's multiple comparisons tests (a) TNF-α [F = 320.0 (DFn = 3, DFd = 20), p < 0.0001] and (b) IL-6 [F = 689.7 (DFn = 3, DFd = 20), p < 0.0001]. The exact *p*-value between each compared pairwise group is stated above the pairwise bar. Rof: Roflumilast.

and IL-6 by 4.4- and 8.9-fold when compared to rats that were not subjected to any surgical procedures (Fig. 3). When matched to the renal I/R group, the Rof (1.5 or 3 mg/kg) treatment had a notable anti-inflammatory effect, as seen by the noteworthy reduction in renal levels of TNFα by 34 or 59 % and IL-6 by 36 or 73 % after its administration, respectively.

3.4. Impact of Rof on the antioxidant markers Nrf2 and HO-1

Severe kidney damage caused by renal I/R was demonstrated by the marked downregulation of renal expression of the HO-1 by 84 % and Nrf2 by 88 %, matched to the normal control. However, compared to the renal I/R group, Rof (1.5 or 3 mg/kg) pretreatment demonstrated a substantially increased expression of the Nrf2 gene by 3 or 5.6-fold and consequent elevation in the expression of HO-1 by 2.8- and 5-fold compared to the normal group, indicating a protective and antioxidant effect on the kidneys (Fig. 4).

3.5. Impact of Rof on histopathological findings

As shown in Fig. 5, the renal tissue of the control group showed the normal histological structure of the kidney, including glomeruli and renal tubules. Histological examination of the ischemia kidney group showed peri-glomerular blood vessel congestion, increased glomerular urinary space, and some renal tubules showed necrobiotic changes. On the other hand, the ischemia kidney group treated with Rof (1.5 mg/kg body weight) showed mild peritubular congestion, increased urinary space, and some renal tubules showing degeneration. Moreover, histopathological examination of the ischemia kidney rat group, treated with Rof (3 mg/kg body weight), showed only an increase in urinary space. Table 2:

4. Discussion

In the current investigations, we aimed to explore the role of Rof against renal I/R injury in rats and to inspect its potential mechanisms.

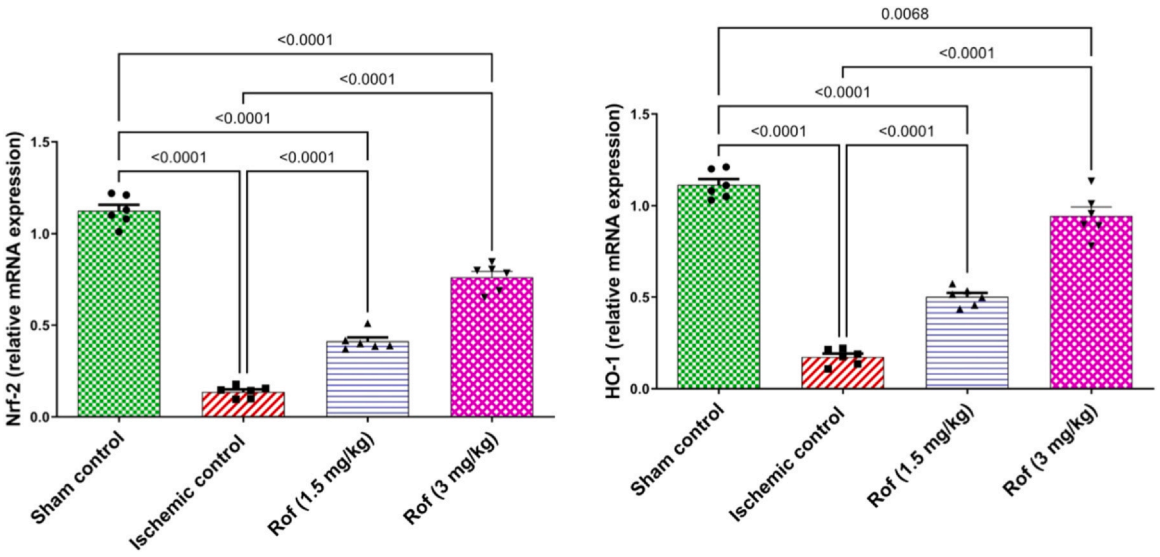

Fig. 4. Impacts of Rof on renal (a) Nrf-2 and (b) HO-1. Panels represent mean ± SE of six rats analyzed by One-way ANOVA followed by Tukey's multiple comparisons tests (a) Nrf2 [F = 279.2 (DFn = 3, DFd = 20), p < 0.0001] and (b) IL-6 [F = 176.2 (DFn = 3, DFd = 20), p < 0.0001]. The exact *p*-value between each compared pairwise group is stated above the pairwise bar. Rof: Roflumilast.

Our results revealed that I/R caused a sudden decrease in renal function, oxidative actions, and a notable rise in markers of inflammation that coincided with tissue pathological abnormalities. By lowering oxidative stress and inflammation, oral treatment of Rof prior to I/R significantly protects against I/R renal impairment and histopathological derangements in rats. Moreover, the antioxidant pathway Nrf2/HO-1 may be triggered by Rof following I/R damage. Thus, our findings suggest that Rof mitigates renal injury induced via I/R by activating the Nrf2/HO-1 signaling pathway; Rof has the potential to be a novel treatment for renal ischemia injury. The clinical syndrome known as acute kidney injury (AKI) has an elevated rate of disease and death rate that is illustrated by a rapid drop in the glomerular filtration rate [23].

These AKI animal models are developed in lab environments to model and investigate the features, processes, and possible therapies for acute kidney damage. Before beginning clinical trials, animal models are used to assess the effectiveness of treatment interventions and get a better understanding of the pathophysiology of illnesses. The use of rat models in renal research is common due to the physiological and genetic similarities between rats and humans, making the findings more translatable to human physiology and pathology [24,25]. AKI is frequently caused by renal I/R injury, which may be caused by a variety of clinical factors [26]. Numerous pathogenic mechanisms, such as oxidative stress, inflammation, apoptosis, vascular damage, and cell necrosis, are responsible for the renal I/R injury that results in AKI [27]. Consequently, reducing oxidative stress is a potential treatment for renal I/R injury.

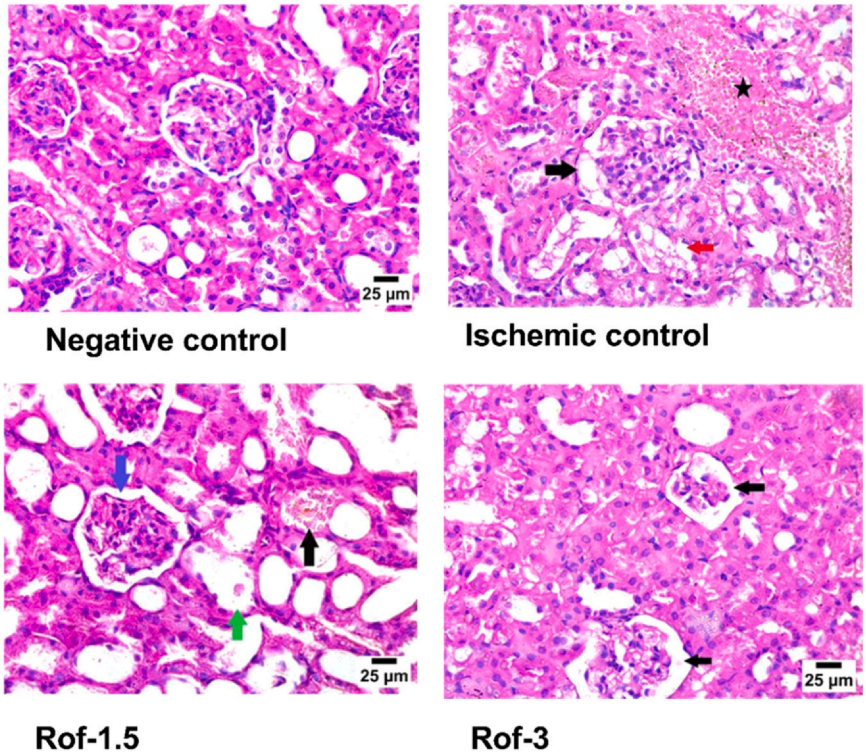

Fig. 5. Photomicrograph of normal control group, displaying normal histological configuration of kidney including glomeruli and renal tubules (Hematoxylin and Eosin stain), photomicrograph of ischemic control group showing peri-glomerular blood vessel congestion (star), increase glomerular urinary space (black arrow) and some renal tubules showed necrobiotic changes (red arrow) (Hematoxylin and Eosin stain), photomicrograph of Rof-1.5 group, showing mild peritubular congestion (black arrow), increase urinary space (blue arrow) and some renal tubules showing degeneration (green arrow) (Hematoxylin and Eosin stain), photomicrograph of Rof-3 group, showing increase urinary space (black arrows) (Hematoxylin and Eosin stain).

Table 2
The histopathology findings for the four conditions studied may be put in a table form.

Negative control		**Ischemic control**	**Rof 1.5**	**Rof. 3**
Normal histological structure of glomeruli and renal tubules		Showing peri-glomerular blood vessel congestion, increased glomerular urinary space, and some renal tubules showed necrobiotic changes	Showing mild peritubular congestion, increased urinary space, and some renal tubules showing degeneration	Increase urinary space
	Negative control	**Ischemic control**	**Rof 1.5**	**Rof. 3**
Glomeruli	Normal	Increase urinary space	Increase urinary space	Increase urinary space
Renal tubules	Normal	Necrobiotic changes	Degenerative changes	Normal
Interstitial blood vessels	Normal	Severe congestion	Mild congestion	Normal

In the current study, blood values of creatinine and BUN were considerably higher following I/R-induced renal injury, indicating that the renal functions were impaired. These results align with earlier research [24,28,29]. The impact of ischemia-reperfusion (IR) damage on creatinine levels and urea was found to be within the same elevated range as prior research that used thioacetamide as a model for inducing toxic kidney injury[30]. Meanwhile, the I/R-caused renal damage was appreciably reversed by Rof pretreatment at both doses, as evidenced by the decreased levels of creatinine and BUN in the serum. These findings support the findings of the earlier investigation [31], which showed how Rof could lessen renal toxicity caused by cadmium.

Renal I/R pathogenesis involves the inflammatory cascade, which damages kidney tissue by releasing several mediators [32]. Numerous inflammatory responses can be inhibited in vitro and in vivo by blocking PDE4, according to previous studies. Animal models of a number of disorders, including rheumatoid arthritis, psoriasis, asthma, inflammatory bowel disease, and chronic obstructive pulmonary disease, have been used to develop and validate the anti-inflammatory properties of PDE4 inhibitors [33]. In rats undergoing I/R, PDE4 inhibitors can ameliorate renal injury by upregulating cAMP expression [34]. PDE4 inhibitors are able to lessen renal tubular epithelial cell damage generated by transforming growth factor-β1 (TGF-β1) and renal interstitial fibrosis in mouse unilateral ureteral obstruction models [35]. Numerous studies have demonstrated that proinflammatory cytokines like TNF-α and IL-6 are released during the I/R phase as a result of kidney damage. It is crucial to manage the inflammatory response early and effectively in order to prevent and treat renal damage [36,37]. Thus, we investigated Rof's anti-inflammatory properties. As revealed by ELISA, Rof pretreatment could reduce renal I/R-triggered formation of proinflammatory cytokines, like TNF-α and IL-6. Cytokines measured are related to macrophage polarization and show a suggested beneficial role of Rof in shifting the inflammatory tissue environment in I/R injury into a quiescent climate [38]. In accordance with our findings, Ansari et al. confirmed the anti-inflammatory potential of Rof via NF-kB inhibition in cadmium-induced renal damage [31].

Another key pathway implicated in I/R renal injury is oxidative stress. I/R damage is characterized by the production of ROS, such as hydroxyl radicals and superoxide radicals [39]. These reactive radicals may damage proteins and membranes directly and indirectly by triggering pro-apoptotic signaling [40]. In addition to causing cellular damage, oxidative stress also causes inflammation by activating NF-kB and p38 mitogen-activated protein kinase, two crucial signaling molecules that produce cytokines like TNF-α [41,42], demonstrating how two pathways interact. Antioxidant therapy has been shown in experiments to attenuate AKI [43].

Reactive free radicals are eliminated in large part by antioxidant enzymes. Importantly, Nrf2 controls the gene activity of SOD, NQO-1, and HO-1, three enzymes with potent antioxidant activity [44]. Over 200 genes are coordinated by the Nrf2 transcription factor in both humans and animals [45], primarily concerned with processes of endogenous cellular defense and survival [46]. The relationship between the Nrf2/HO-1 signaling pathway and ROS scavenging during oxidative stress has been established. Consequently, it has been thought that a therapeutic target for renal I/R injury is the Nrf2/HO-1 pathway [47]. Our results showed that renal I/R caused a significant downregulation of the Nrf2 gene and its dependent gene, HO-1 [48]. We measured the Nrf2/HO-1 mRNA l,evel, and previous reports showed that measurement of Nrf2/HO-1 mRNA level reflects the changes in protein levels [49]. Our results also showed that Rof increased the induction of Nrf2 and its dependent genes, HO-1, which strongly suggested that Rof pretreatment offered renoprotection by activating Nrf2 and its target gene, HO-1. Our findings are in agreement with Abdel-Wahab et al., who investigated the protective effect of Rof against cisplatin-induced testicular toxicity in rats, and the results revealed that Rof increased intracellular HO-1 activities and Nrf2 and HO-1 gene expression [50].

Steadily, evidence shows that I/R decreased GSH levels and increased MDA content [51,52]. MDA is a significant oxidative stress marker. As a consequence of I/R damage-induced lipid peroxidation, MDA levels in the blood and tissues rise. Several studies have demonstrated that renal I/R-induced lipid peroxidation raises MDA levels and that this increase can be lowered by administering antioxidant and anti-inflammatory drugs [53,54]. Changes in GSH levels underlie the pathogenesis of many human diseases; on the other hand, GSH plays a protective role from the onset of tissue injury; on the other, it is involved in some pathology progression and therapy resistance. However, the increase in GSH is favorable in reversing I/R injury [55]. The investigation's findings showed that Rof pretreatment was an effective way to prevent the rise in MDA and decline in GSH levels that I/R induces, supporting that Rof may have antioxidant action against oxidative damage caused by renal I/R. Our findings are in agreement with previous studies [56,57]. As a result, our findings showed that Rof lessened oxidative damage induced via renal I/R by enhancing Nrf2 nuclear translocation and upregulating antioxidant enzymes.

5. Limitation of current study

One potential limitation of the current study is that it only measured the mRNA levels of Nrf2 and HO-1 but did not examine their protein levels through techniques such as western blotting or immunohistology. Evaluating only the mRNA levels may not fully reflect the actual protein levels and activity within the tissue, as there can be post-transcriptional regulatory mechanisms that affect protein expression, localization, and activity. Future studies could incorporate techniques to evaluate the protein levels and localization of Nrf2 and HO-1 to gain further insights into their regulation and role at the protein level, in addition to the mRNA measurements provided in this study. The study also did not investigate the mechanisms underlying the regulation of Nrf2 and HO-1 at the protein level or their interactions with potential binding partners. Additional experiments, for example, using an Nrf2 activator/inhibitor, antibody binding studies, or mutagenesis, could provide more insights into Nrf2 regulation and its interactions with proteins like Keap1 that are involved in modulating the Nrf2 pathway. This could help elucidate regulatory mechanisms at the protein level. The investigation of only two Rof dose levels is one of the study's limitations; more testing of additional dose levels might be helpful in determining the optimal dosage.

6. Conclusions

In summary, we examined the outcome of Rof on renal I/R in rats, taking into account several factors such as renal function, oxidative stress, inflammatory indicators, gene expression, and histological alterations. The findings showed that renal I/R resulted in markedly impaired renal function, oxidative stress, and inflammation, along with aberrant histopathology. On the other hand, pretreatment with Rof showed a dose-related reduction in these negative effects.

More specifically, a decrease in blood creatinine and BUN levels demonstrated that Rof pre-treatment successfully restored renal function. Moreover, Rof exhibited anti-inflammatory properties by considerably lowering proinflammatory cytokine levels (TNF-α and IL-6) in contrast to the renal I/R group. Rof's protective effect was further demonstrated by its capacity to mitigate oxidative actions, as shown by the recovery of GSH and reduction of MDA renal content.

The antioxidant pathway's essential components, Nrf2 and HO-1, were elevated in gene expression at the molecular level by Rof pretreatment, indicating a possible mechanism for its protective benefits against oxidative damage brought on by renal I/R. These results were corroborated by histopathological analysis, which revealed that groups treated with Rof had milder pathological alterations than the renal I/R group that was not treated.

Our research offers substantial proof of the antioxidant and protective properties of Rof versus renal I/R injury in rats. These results open the door to more investigation into the clinical uses of Rof in the setting of renal I/R injury by indicating that it may have therapeutic potential for reducing kidney damage brought on by ischemia-reperfusion episodes.

Taken together, it can be concluded that Rof stimulates the Nrf2 signaling pathway, which in turn increases the expression of HO-1 mRNA, which in turn guards the kidney against the oxidative stress response and renal tubule pathology associated with I/R injury.

Conflicts of interest

Authors declare no conflict of interest.

Informed consent statement

Not applicable.

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CRediT authorship contribution statement

Marawan Elbaset: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Rehab Abdel-Rahman:** Writing – review & editing, Writing – original draft, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rehab Abdel Diab:** Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Data curation. **Sherif Afifi:** Writing – review & editing, Resources. **Tuba Esatbeyoglu:** Writing – review & editing, Funding. **Hany Fayed:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation.

Data Availability

Data will be made available on request. All relevant data are within the manuscript, and all others are available with the corresponding author Rehab F.Abdel-Rahman upon reasonable request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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