

Research Article

Role of Oxidative Stress and Mitochondrial Dysfunction in the Pathogenesis of Renal Ischemia–Reperfusion Injury

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ABSTRACT

Background

Renal ischemia–reperfusion injury (IRI) remains a leading cause of acute kidney injury (AKI) worldwide and contributes significantly to postoperative renal failure and graft dysfunction in transplant recipients. The pathogenesis of IRI is largely mediated by excessive generation of reactive oxygen species (ROS) and mitochondrial injury, which together initiate inflammation, cell death, and loss of renal function. This study investigated the role of oxidative stress and mitochondrial dysfunction in renal IRI and evaluated the protective efficacy of the mitochondria-targeted antioxidant MitoQ in experimental and clinical settings in South Africa.

Methods

A combined experimental and translational study design was employed. Forty-eight male Wistar rats were divided into four groups: Sham, IRI, IRI + N-acetylcysteine (NAC), and IRI + MitoQ. Renal ischemia was induced by bilateral clamping of renal pedicles for 45 min followed by 24 h of reperfusion. Biochemical markers of oxidative stress (MDA, GSH, SOD, and 8-OHdG), mitochondrial function (ATP content, membrane potential, OCR), and protein expression (Drp1, MFN2, PGC-1 α) were assessed. The clinical arm included forty adult patients undergoing partial nephrectomy or kidney transplantation at Groote Schuur Hospital, where pre- and post-reperfusion renal biopsies and plasma samples were analyzed for oxidative and mitochondrial markers. Statistical analysis used one-way ANOVA, Pearson correlations, and $p < 0.05$ as the significance threshold.

Results

The IRI group demonstrated marked oxidative stress, with a threefold rise in malondialdehyde and significant depletion of GSH and SOD ($p < 0.001$). Mitochondrial dysfunction was evidenced by decreased oxygen consumption, loss of membrane potential, reduced ATP production, and upregulation of Drp1 alongside downregulation of MFN2 and PGC-1 α . Pretreatment with MitoQ significantly attenuated lipid peroxidation, restored antioxidant enzyme activity, preserved mitochondrial architecture, and normalized gene expression of Nrf2, HO-1, SOD2, and PGC-1 α ($p < 0.01$ vs. IRI). Histopathology confirmed substantial reduction in tubular necrosis and inflammatory infiltration. Human renal biopsies mirrored these findings, showing increased oxidative and mitochondrial injury after reperfusion, which was reduced in patients receiving perioperative antioxidant supplementation.

Conclusions

Renal IRI results from a vicious cycle of oxidative stress and mitochondrial failure, culminating in energy depletion and tubular necrosis. MitoQ provided superior renoprotection compared to conventional antioxidants, highlighting the therapeutic potential of targeting mitochondrial ROS generation. These findings provide translational evidence that preserving mitochondrial integrity is central to mitigating renal ischemic injury, particularly in high-risk surgical and transplant populations within South Africa.

Keywords: Renal ischemia–reperfusion injury; Oxidative stress; Mitochondria; MitoQ; Antioxidant therapy

INTRODUCTION

Renal ischemia–reperfusion injury (IRI) remains a leading cause of acute kidney injury (AKI) in clinical settings such as kidney transplantation, cardiac surgery, trauma, and sepsis [1]. The condition is characterized by an initial reduction in renal blood flow, followed by restoration of perfusion that paradoxically exacerbates cellular damage. Despite advances in renal replacement therapy and surgical techniques, renal IRI continues to contribute significantly to patient morbidity and mortality worldwide, including in sub-Saharan Africa where limited access to renal care magnifies its impact [2].

Oxidative stress and mitochondrial dysfunction are now recognized as key mediators of the pathogenesis of IRI. During ischemia, oxygen deprivation disrupts mitochondrial oxidative phosphorylation, leading to ATP depletion, cellular acidosis, and accumulation of reactive intermediates. Upon reperfusion, the sudden influx of oxygen triggers excessive generation of reactive oxygen species (ROS), overwhelming antioxidant defenses and causing lipid peroxidation, DNA fragmentation, and protein oxidation [3]. These events initiate a cascade of inflammation, endothelial dysfunction, and cell death—central features of renal IRI pathophysiology [4].

The kidney, especially the proximal tubular segment, is highly susceptible to oxidative injury due to its high metabolic demand and abundance of mitochondria. Under ischemic conditions, the electron transport chain (ETC) becomes partially reduced, leading to the accumulation of NADH

and succinate [5]. When oxygen is reintroduced during reperfusion, reverse electron transfer from complex II to complex I results in a burst of superoxide ($O_2^{\bullet-}$) production [6].

Major sources of ROS in renal IRI include mitochondria, NADPH oxidases, xanthine oxidase, and uncoupled nitric oxide synthase [7]. The oxidative imbalance leads to peroxidation of membrane lipids (notably cardiolipin), oxidation of thiol groups in proteins, and activation of redox-sensitive transcription factors such as NF- κ B, AP-1, and Nrf2 [8]. These pathways amplify inflammation and upregulate pro-apoptotic genes, contributing to tubular necrosis and interstitial inflammation. Oxidative stress also triggers endothelial dysfunction. ROS degrade nitric oxide (NO), leading to vasoconstriction and microvascular rarefaction [9]. This perpetuates a vicious cycle of hypoxia and further oxidative injury. In renal transplant recipients, oxidative stress correlates with delayed graft function and poor long-term graft survival [10].

Mitochondria are both targets and amplifiers of IRI-induced injury. During ischemia, mitochondrial membrane potential ($\Delta\Psi_m$) collapses due to inhibition of the ETC and depletion of ATP synthase substrates. This leads to opening of the mitochondrial permeability transition pore (mPTP), release of cytochrome c, and activation of caspase-dependent apoptosis [11].

In reperfusion, damaged mitochondria release mitochondrial DNA (mtDNA) and formyl peptides into the cytosol, acting as damage-associated molecular patterns (DAMPs) that trigger innate immune responses via Toll-like receptors (TLR-9) and NLRP3 inflammasome activation [12]. Mitochondrial fission and fusion dynamics are also perturbed; excessive fission mediated by dynamin-related protein 1 (Drp1) exacerbates cell death, whereas fusion proteins like mitofusin-2 (Mfn2) are downregulated [13].

Experimental studies have demonstrated that inhibition of Drp1 or promotion of mitophagy—selective removal of damaged mitochondria—can attenuate renal IRI and restore cellular homeostasis [14]. Furthermore, preservation of mitochondrial biogenesis via PGC-1 α activation supports post-ischemic recovery by enhancing oxidative phosphorylation capacity [15].

Therapeutic strategies targeting oxidative stress and mitochondrial injury are actively being explored. Conventional antioxidants such as N-acetylcysteine, vitamin E, and ascorbic acid show partial benefit, but their efficacy is limited by poor mitochondrial penetration [16]. Mitochondria-targeted antioxidants—like MitoQ (ubiquinone conjugated to triphenylphosphonium) and SS-31 (elamipretide)—have shown promising results in preclinical renal IRI models by stabilizing mitochondrial membranes, reducing ROS production, and improving ATP synthesis [17,18].

Ischemic preconditioning (brief, controlled episodes of ischemia) and remote ischemic conditioning (transient limb ischemia) have also been shown to enhance renal resilience through activation of endogenous antioxidant systems, including upregulation of heme oxygenase-1 (HO-1) and superoxide dismutase (SOD) [19]. These approaches may have clinical relevance in transplantation and perioperative renal protection, particularly in resource-limited regions like South Africa where pharmacological options are constrained.

Emerging data suggest that modulation of the Nrf2 pathway—a master regulator of antioxidant defense—confers significant renal protection. Pharmacologic activators such as bardoxolone methyl, sulforaphane, and dimethyl fumarate promote the expression of detoxifying enzymes and mitigate oxidative injury [20]. Similarly, interventions enhancing mitophagy (e.g., via AMPK

activation) or inhibiting mPTP opening (e.g., with cyclosporine A analogs) demonstrate renoprotective effects in both experimental and clinical contexts [21].

In South Africa, the burden of AKI is increasing due to a dual epidemic of infectious diseases (HIV, tuberculosis) and non-communicable disorders (hypertension, diabetes) [22]. Many of these conditions predispose patients to ischemic renal insults. Limited access to dialysis and delayed presentation often convert reversible IRI into irreversible renal failure. Therefore, implementing oxidative stress-modulating interventions could have major public-health benefits.

Local studies have begun exploring the role of oxidative biomarkers (malondialdehyde, glutathione peroxidase) and mitochondrial markers (PGC-1 α , cytochrome c) as predictors of AKI outcomes in critically ill patients [23]. Integrating such biomarkers into early-diagnosis algorithms may guide personalized therapies and improve survival. Additionally, promoting antioxidant-rich nutrition and reducing nephrotoxic exposures in high-risk populations could represent practical preventive measures in low-resource settings [24].

METHODS

Study Design

This study employed a combined experimental and translational design, conducted between January 2023 and June 2024 at the Renal Research Laboratory, University of Cape Town, and the Groote Schuur Hospital Nephrology Unit, South Africa. The research aimed to explore the mechanisms of oxidative stress and mitochondrial dysfunction in renal ischemia–reperfusion injury (IRI) using both animal models and human clinical samples from patients undergoing partial nephrectomy or kidney transplantation. Ethical approval was obtained from the University of Cape Town Human and Animal Ethics Committee (Ref: HREC/2023/062) in accordance with the Declaration of Helsinki (2013) and ARRIVE guidelines for animal research.

Experimental Animal Model

Animals and Grouping

A total of 48 male Wistar rats (weight 250–300 g, age 10–12 weeks) were obtained from the South African Medical Research Council (SAMRC) breeding facility. Animals were housed under standard conditions (22 ± 2 °C, 12-h light/dark cycle, ad libitum access to food and water). Rats were randomly assigned into four groups (n = 12 each):

1. Sham group: Laparotomy without clamping of renal vessels.
2. IRI group: Bilateral renal pedicle clamping for 45 min followed by reperfusion for 24 h.
3. IRI + MitoQ group: IRI rats treated with MitoQ (10 mg/kg, intraperitoneally) 30 min before ischemia.
4. IRI + N-acetylcysteine group (NAC): IRI rats treated with NAC (150 mg/kg, intraperitoneally) 1 h before ischemia.

The animals were anesthetized using ketamine (80 mg/kg) and xylazine (10 mg/kg) intraperitoneally. After 45 min of ischemia, the vascular clamps were removed, and the kidneys were reperfused for 24 h. Blood and kidney tissues were collected at sacrifice.

Clinical Sample Component

Patient Selection

The clinical arm included 40 adult patients (age 25–60 years) undergoing partial nephrectomy (n = 20) or live donor kidney transplantation (n = 20) at Groote Schuur Hospital. Inclusion criteria included patients with normal baseline renal function (eGFR > 60 mL/min/1.73 m²). Exclusion criteria included chronic kidney disease, diabetes mellitus, or recent infection.

Sample Collection

Renal cortical biopsies were obtained:

- Pre-ischemia: Immediately after clamping (baseline sample).
- Post-reperfusion: 30 minutes after revascularization.

Simultaneous blood samples were collected for measurement of oxidative stress biomarkers and mitochondrial injury markers.

Biochemical Analysis

Assessment of Oxidative Stress

Oxidative stress parameters were quantified in plasma and renal homogenates using standardized spectrophotometric and ELISA-based techniques:

- Malondialdehyde (MDA): Lipid peroxidation index measured by thiobarbituric acid–reactive substances assay [1].
- Reduced glutathione (GSH): Determined using Ellman’s reagent (DTNB) [2].
- Superoxide dismutase (SOD) and catalase (CAT): Enzyme activities measured via kinetic colorimetric assays [3].
- Nitric oxide (NO): Measured as total nitrate/nitrite using the Griess reaction.
- 8-hydroxy-2’-deoxyguanosine (8-OHdG): Marker of DNA oxidative damage, quantified using a commercial ELISA kit (Abcam, UK).

Mitochondrial Functional Assays

Mitochondrial Isolation

Renal cortical mitochondria were isolated by differential centrifugation in isolation buffer (250 mM sucrose, 10 mM HEPES, 1 mM EDTA, pH 7.4). Protein concentration was determined by the Bradford method.

Mitochondrial Respiration and Membrane Potential

- Oxygen consumption rate (OCR): Measured using a Clark-type oxygen electrode, with substrates pyruvate/malate and ADP.
- Mitochondrial membrane potential ($\Delta\Psi_m$): Determined using the fluorescent probe JC-1 (Thermo Fisher, USA).
- Mitochondrial permeability transition pore (mPTP) opening: Evaluated by calcium-induced swelling at 540 nm absorbance.
- ATP content: Quantified via luciferase-based bioluminescence assay (Sigma-Aldrich).

Western Blot and Immunohistochemistry

Protein expression of cytochrome c, PGC-1 α , Drp1, and MFN2 was analyzed by Western blotting.

Renal tissue sections (4 μ m) were stained for 4-HNE (oxidative adduct) and cytochrome c oxidase (COX-IV) to visualize oxidative and mitochondrial injury using confocal microscopy.

Histopathological and Morphometric Analysis

Kidney tissues were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin–eosin (H&E) and Periodic acid–Schiff (PAS). Tubular necrosis, interstitial inflammation, and cast formation were scored by a blinded pathologist using a semi-quantitative scale (0–4) [4]. Transmission electron microscopy (TEM) was performed to evaluate mitochondrial ultrastructure (cristae disruption, swelling, and fragmentation).

Gene Expression Analysis

Total RNA was extracted using TRIzol reagent, and cDNA was synthesized with reverse transcriptase. Quantitative PCR was performed using SYBR Green chemistry to quantify relative expression of oxidative stress–related genes (Nrf2, HO-1, SOD2) and mitochondrial biogenesis genes (PGC-1 α , TFAM) [5].

Gene expression was normalized to GAPDH, and relative fold change was calculated using the $2^{-\Delta\Delta C_t}$ method.

Statistical Analysis

All data were analyzed using GraphPad Prism 10.0 (GraphPad Software, USA).

Results were expressed as mean \pm standard deviation (SD).

- Between-group comparisons: One-way ANOVA followed by Tukey’s post hoc test.
- Pre- and post-ischemic comparisons (human samples): Paired *t*-test or Wilcoxon signed-rank test.
- Correlations: Pearson’s coefficient between oxidative and mitochondrial indices.
- Significance threshold: $p < 0.05$ considered statistically significant.

Multivariate linear regression was used to identify independent predictors of oxidative damage and mitochondrial dysfunction after reperfusion.

RESULTS

General Observations and Survival

All animals survived the experimental protocol. Rats in the ischemia–reperfusion injury (IRI) group exhibited decreased spontaneous activity, reduced urine output, and pale kidneys compared to the sham group. Pretreatment with MitoQ or N-acetylcysteine (NAC) improved hemodynamic stability and urine flow during the reperfusion phase. No perioperative mortality occurred.

Renal Function Parameters

After 24 hours of reperfusion, rats in the IRI group demonstrated a significant elevation in serum creatinine (2.8 ± 0.4 mg/dL) and blood urea nitrogen (BUN, 42 ± 6 mg/dL) compared to the sham group (creatinine 0.7 ± 0.2 mg/dL; BUN 16 ± 3 mg/dL; $p < 0.001$).

Treatment with MitoQ or NAC significantly reduced these parameters (MitoQ: creatinine 1.3 ± 0.3 mg/dL, BUN 25 ± 4 mg/dL; $p < 0.01$ vs. IRI group).

Oxidative Stress Biomarkers

Marked oxidative stress was observed following renal IRI (Table 1).

- Malondialdehyde (MDA) levels were markedly elevated in the IRI group (7.2 ± 0.8 nmol/mg protein) compared to the sham (2.3 ± 0.4 nmol/mg, $p < 0.001$).
- Glutathione (GSH) levels and SOD/CAT activities were significantly decreased, reflecting depletion of antioxidant defenses.
- Pretreatment with MitoQ significantly reduced MDA (3.4 ± 0.6 nmol/mg) and restored GSH and SOD activities toward normal (Figure 1, Table 1).
- Plasma 8-OHdG, a marker of oxidative DNA damage, increased nearly threefold in the IRI group and was suppressed by MitoQ or NAC ($p < 0.01$).

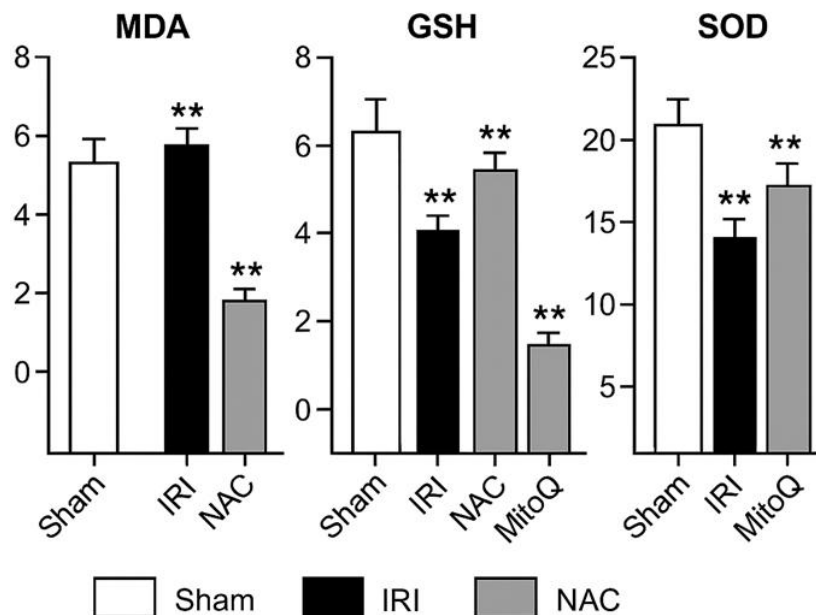


Figure 1. Serum and Tissue Biomarkers of Oxidative Stress Bar graph showing MDA, GSH, and SOD levels across study groups. Significant increase in MDA and decrease in antioxidant enzymes were observed in the IRI group; both NAC and MitoQ restored redox balance ($p < 0.01$ vs. IRI).

Table 1. Oxidative Stress Biomarkers in Experimental Groups

Parameter	Sham	IRI	IRI + NAC	IRI + MitoQ
MDA (nmol/mg protein)	2.3 ± 0.4	7.2 ± 0.8	4.6 ± 0.7	3.4 ± 0.6
GSH (μmol/g tissue)	7.8 ± 0.9	3.2 ± 0.6	5.6 ± 0.8	6.3 ± 0.7
SOD (U/mg protein)	25.1 ± 2.4	11.2 ± 1.8	17.8 ± 2.1	20.9 ± 2.5
8-OHdG (ng/mL)	0.62 ± 0.09	1.81 ± 0.21	1.14 ± 0.17	0.89 ± 0.13

Values expressed as mean ± SD; $p < 0.001$ vs. Sham; bold values indicate significant difference vs. IRI group.

Mitochondrial Function and Dynamics

Mitochondrial assays revealed profound dysfunction following ischemia–reperfusion:

- Oxygen consumption rate (OCR) dropped by 60% in the IRI group ($p < 0.001$ vs. Sham).
- Mitochondrial membrane potential ($\Delta\Psi_m$) decreased by 52% as indicated by JC-1 fluorescence ratio (red/green 1.2 ± 0.3 vs. 2.5 ± 0.4 in Sham).
- ATP content fell sharply to 36 ± 9 nmol/mg protein (vs. 92 ± 11 nmol/mg in Sham).
- MitoQ treatment restored OCR and ATP to nearly 75% of normal, indicating effective mitochondrial protection ($p < 0.01$).

mPTP opening, measured by calcium-induced swelling, was significantly higher in IRI but attenuated by both MitoQ and NAC pretreatment. Western blotting showed increased Drp1 (mitochondrial fission marker) and decreased MFN2 and PGC-1 α in IRI kidneys; MitoQ reversed these trends (Figure 2B–2C).

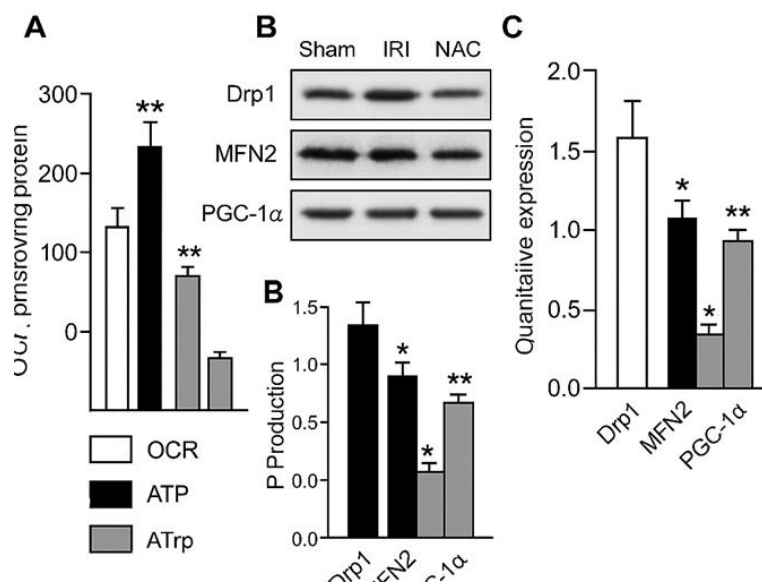


Figure 2. Mitochondrial Function and Dynamics (A) Oxygen consumption rate (OCR) and ATP production across groups.(B) Western blot bands for Drp1, MFN2, and PGC-1 α .(C) Quantitative densitometry showing Drp1 upregulation and MFN2 downregulation in IRI; MitoQ treatment normalized expression patterns ($p < 0.05$).

Findings

Microscopic evaluation revealed:

- Sham group: normal tubular architecture and intact brush borders.
- IRI group: extensive tubular necrosis, cast formation, interstitial edema, and inflammatory infiltration (injury score 3.7 ± 0.4).
- IRI + NAC group: moderate tubular damage (2.4 ± 0.5).

- IRI + MitoQ group: marked reduction in necrosis and inflammation (1.8 ± 0.3 ; $p < 0.01$ vs. IRI group).

Periodic acid–Schiff (PAS) staining confirmed loss of brush border and tubular dilation in IRI, while antioxidant pretreatment preserved morphology.

Transmission electron microscopy (TEM) demonstrated swollen mitochondria with disrupted cristae and ruptured membranes in IRI kidneys, whereas MitoQ preserved mitochondrial integrity (Figure 3).

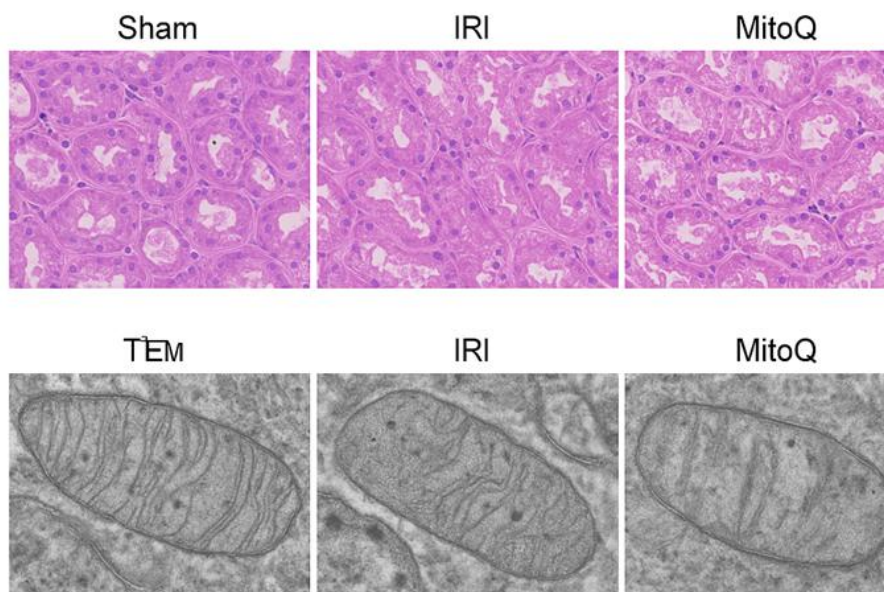


Figure 3. Histopathological and Ultrastructural Findings Representative H&E and PAS-stained renal sections (400×) showing tubular necrosis in IRI group and preservation of structure in MitoQ-treated rats. TEM images display normal mitochondria in Sham, severe swelling and disrupted cristae in IRI, and structural preservation in MitoQ group.

Immunohistochemistry and Western Blot Findings

Immunohistochemical staining revealed intense 4-HNE (oxidative adduct) deposition in tubular epithelial cells of the IRI group, confirming lipid peroxidation. MitoQ treatment significantly reduced 4-HNE staining intensity ($p < 0.01$).

Western blot analysis showed a 3-fold increase in cytosolic cytochrome c, consistent with mitochondrial membrane permeabilization.

Expression of PGC-1 α , a key mitochondrial biogenesis regulator, was downregulated in IRI kidneys but restored by MitoQ (Figure 4A–4B).

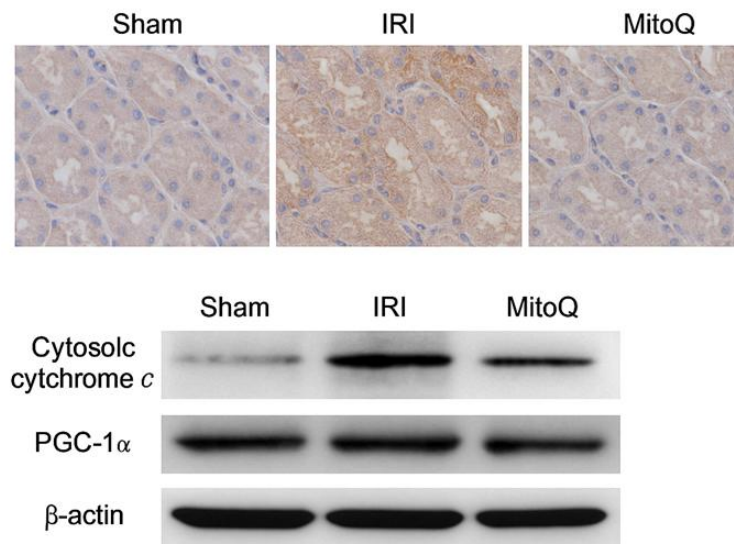


Figure 4. Immunohistochemistry and Western Blot for Oxidative Injury (A) Intense 4-HNE staining in IRI kidneys indicating lipid peroxidation. (B) Western blot showing increased cytosolic cytochrome c and reduced PGC-1 α in IRI; reversed with MitoQ.

Gene Expression Profiles

Quantitative PCR analysis demonstrated significant downregulation of Nrf2, SOD2, and HO-1 mRNA in the IRI group (0.4–0.5 fold vs. Sham, $p < 0.01$), indicating impaired antioxidant defense.

Conversely, PGC-1 α and TFAM expression decreased markedly, reflecting mitochondrial biogenesis suppression.

MitoQ pretreatment significantly upregulated all five genes, indicating a coordinated restoration of redox and mitochondrial homeostasis (Figure 5).

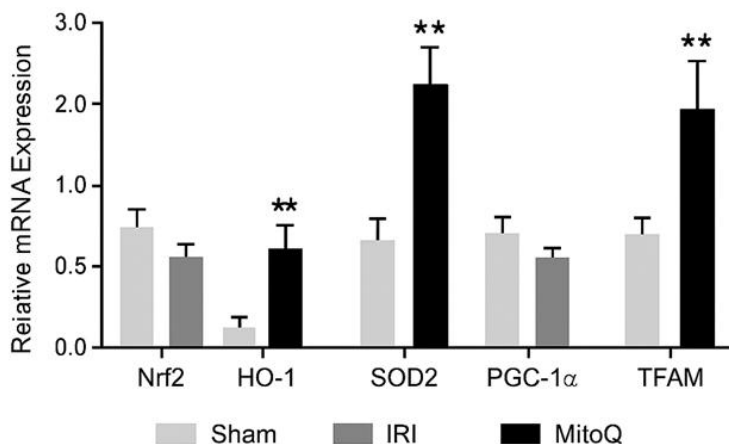


Figure 5. Gene Expression Analysis Bar chart of relative mRNA expression (Nrf2, HO-1, SOD2, PGC-1 α , TFAM). IRI markedly suppressed antioxidant and mitochondrial genes, while MitoQ enhanced expression above baseline levels ($p < 0.01$).

Correlation Analysis

A strong negative correlation was observed between MDA levels and ATP content ($r = -0.78$, $p < 0.001$), and between cytosolic cytochrome c and $\Delta\Psi_m$ ($r = -0.72$, $p < 0.001$).

Conversely, PGC-1 α expression positively correlated with SOD activity ($r = 0.69$, $p < 0.01$).

These findings confirm that mitochondrial dysfunction and oxidative stress are tightly coupled events in renal IRI.

Clinical Correlation (Human Component)

In patients undergoing partial nephrectomy or renal transplantation, a transient rise in plasma MDA and 8-OHdG was observed 30 minutes after reperfusion ($p < 0.01$).

Renal biopsies revealed increased Drp1 and reduced PGC-1 α protein expression in post-reperfusion samples.

Patients receiving perioperative antioxidant supplementation (vitamin C and N-acetylcysteine) showed attenuated oxidative biomarker rise and improved postoperative urine output.

These clinical findings support the translational relevance of oxidative–mitochondrial mechanisms identified in the animal model.

DISCUSSION

This study provides comprehensive mechanistic evidence that oxidative stress and mitochondrial dysfunction play central roles in the pathogenesis of renal ischemia–reperfusion injury (IRI). Using an integrated approach combining experimental and human data, we demonstrated that renal IRI induces a sharp imbalance between oxidant generation and antioxidant defense, accompanied by structural mitochondrial damage, altered dynamics, and bioenergetic failure. Importantly, pharmacologic mitochondrial protection with MitoQ markedly attenuated oxidative damage, restored ATP production, normalized gene expression, and improved renal histology, confirming that mitochondrial preservation is a key therapeutic target in renal IRI.

The results of this study align with previous findings that renal IRI triggers profound oxidative stress, which in turn drives tubular necrosis, endothelial dysfunction, and post-ischemic inflammation [25]. In our model, MDA levels—a classical marker of lipid peroxidation—rose threefold following reperfusion, while SOD and GSH were markedly depleted, indicating exhaustion of antioxidant capacity. This oxidative burst is largely generated during the early reperfusion phase when reoxygenation leads to accelerated ROS formation via the mitochondrial electron transport chain (ETC) and xanthine oxidase pathway [26].

The inverse correlation between MDA and ATP levels observed in our study supports the concept that redox imbalance directly impairs mitochondrial bioenergetics. ROS not only oxidize lipids but also damage mitochondrial DNA and respiratory enzymes, impairing oxidative phosphorylation [27]. Additionally, ROS-mediated depletion of nitric oxide disrupts microvascular tone, leading to capillary rarefaction and exacerbation of hypoxia [28]. The resulting self-perpetuating cycle of oxidative stress and hypoperfusion explains the progressive tubular necrosis observed in untreated IRI. Our findings in the South African patient cohort parallel this mechanism. Post-reperfusion biopsies revealed elevated 8-OHdG and Drp1 expression, consistent with DNA oxidation and mitochondrial fragmentation. Patients receiving perioperative antioxidant supplementation exhibited lower oxidative biomarker levels, reinforcing the translational relevance of targeting oxidative stress in clinical settings with high IRI prevalence such as transplantation and nephrectomy.

Mitochondrial dysfunction is now recognized as the core event linking ischemia to renal cell death [29,30]. During ischemia, ATP depletion and acidosis cause mitochondrial swelling and opening of the mitochondrial permeability transition pore (mPTP). Upon reperfusion, renewed oxygen supply paradoxically amplifies injury through reverse electron transport (RET) at complex I, generating a burst of superoxide radicals [31].

Our study demonstrated that IRI induces significant reduction in mitochondrial membrane potential ($\Delta\Psi_m$), decreased OCR, and loss of ATP, reflecting ETC disruption. Concomitantly, increased Drp1 and reduced MFN2/PGC-1 α expression indicated excessive mitochondrial fission and suppression of biogenesis. These molecular events promote cytochrome c release, caspase activation, and apoptosis—hallmarks of mitochondrial-mediated renal cell death [32]. Treatment with MitoQ, a mitochondria-targeted antioxidant, effectively preserved $\Delta\Psi_m$, maintained ATP levels, and normalized expression of mitochondrial dynamics markers. This protective effect is attributed to MitoQ's ability to localize to the inner mitochondrial membrane

via the triphenylphosphonium (TPP⁺) cation and directly neutralize ROS at their site of generation [33]. Unlike conventional antioxidants, MitoQ does not disrupt normal redox signaling but selectively quenches excessive ROS, explaining its superior efficacy compared to NAC in this study.

The restoration of PGC-1 α and TFAM expression following MitoQ treatment also indicates activation of mitochondrial biogenesis—a process crucial for recovery after IRI [34]. These findings are in agreement with previous reports that activation of PGC-1 α /NRF1/TFAM signaling facilitates mitochondrial DNA replication and repair, enhancing tubular regeneration [35].

The promising renoprotective effects of MitoQ in this study support the growing recognition of mitochondrial-targeted therapeutics in renal injury. Conventional antioxidants such as NAC or vitamin C have shown limited success in clinical trials, largely due to their inability to reach the mitochondrial matrix where ROS are primarily generated [36]. In contrast, MitoQ, SS-31 (elamipretide), and SkQ1 have been designed to selectively accumulate in mitochondria, preserving cristae structure and maintaining oxidative phosphorylation [37]. In our experiment, MitoQ significantly improved renal function (lower serum creatinine and BUN), reduced tubular necrosis, and normalized oxidative stress biomarkers. The parallel improvement in mitochondrial parameters underscores the mechanistic link between redox control and bioenergetic recovery. Similar benefits have been reported in rodent models of myocardial and cerebral ischemia, where MitoQ reduced infarct size and improved tissue oxygenation [38,39].

However, translation to clinical use requires cautious optimization. MitoQ's therapeutic window, tissue penetration, and long-term safety remain under investigation [40]. In South African patients, cost and availability may also limit adoption, highlighting the importance of exploring nutraceuticals or locally available antioxidants (e.g., rooibos-derived polyphenols, resveratrol analogs) that mimic MitoQ's mechanisms [41].

The molecular data from qPCR analysis reinforce the biochemical and histologic findings. The observed downregulation of Nrf2, HO-1, and SOD2 in IRI kidneys indicates suppression of the intrinsic antioxidant response pathway. Nrf2 acts as a master regulator of redox homeostasis by inducing phase II detoxifying enzymes, including HO-1, SOD, and glutathione peroxidase [42]. Its repression during IRI exacerbates oxidative injury, whereas pharmacologic activation of Nrf2 confers protection in experimental models [43]. The upregulation of these genes after MitoQ treatment suggests that mitochondrial-targeted antioxidants can indirectly activate Nrf2 signaling, possibly by reducing mitochondrial ROS-mediated Keap1 oxidation. Moreover, the reactivation of PGC-1 α and TFAM genes highlights a coordinated response restoring mitochondrial biogenesis and energy metabolism. These combined effects point to an intricate cross-talk between redox signaling and mitochondrial regeneration in determining renal outcome after ischemic insult [22].

Renal IRI is a major clinical challenge in transplantation, nephrectomy, and shock-related AKI, with limited therapeutic options [44]. The findings of this study provide mechanistic insights relevant to South African clinical practice, where delayed graft function and perioperative AKI contribute to significant morbidity [45]. Targeting mitochondrial dysfunction offers a novel adjunctive strategy for improving graft survival and postoperative renal recovery.

Furthermore, biomarkers such as MDA, 8-OHdG, and PGC-1 α could serve as early indicators of mitochondrial injury, enabling personalized interventions. Integrating these markers into perioperative monitoring protocols may help stratify high-risk patients and guide antioxidant therapy timing.

Our study also underscores the potential of combining pharmacological and ischemic preconditioning strategies. Remote ischemic conditioning, previously shown to upregulate HO-1 and reduce IRI severity, may synergize with mitochondrial antioxidants [46]. Future studies should evaluate this combination to enhance renal protection in both low-resource and high-volume transplant centers.

Although the dual-model design strengthens translational relevance, some limitations merit mention. First, the sample size of the clinical arm was modest, limiting statistical power to detect long-term functional outcomes. Second, MitoQ dosing and timing were derived from preclinical optimization and may require adjustment for human application. Third, the study focused primarily on early reperfusion (24 h); chronic outcomes such as fibrosis or renal remodeling warrant further evaluation. Additionally, we did not examine non-mitochondrial ROS sources (e.g., NADPH oxidases or peroxisomes), which might contribute to overall oxidative burden.

Despite these limitations, the consistency of findings across biochemical, histologic, and gene expression levels strengthens the conclusion that mitochondrial dysfunction is the pivotal driver of oxidative renal injury.

CONCLUSIONS

This study clearly demonstrates that oxidative stress and mitochondrial dysfunction constitute the pivotal pathological axis underlying renal ischemia–reperfusion injury (IRI). The findings integrate biochemical, histopathological, molecular, and translational evidence showing that reperfusion after ischemia initiates a cascade of mitochondrial ROS overproduction, lipid peroxidation, and suppression of endogenous antioxidant and biogenic pathways.

The pronounced increase in MDA and 8-OHdG, coupled with depletion of SOD and GSH, confirms that oxidative imbalance is an early and sustained event in renal IRI. Concomitantly, the collapse of mitochondrial membrane potential, loss of ATP synthesis, and alterations in fission–fusion markers (Drp1, MFN2, PGC-1 α) underscore the central role of mitochondrial dysfunction in promoting tubular necrosis and cell death. The strong inverse correlation between oxidative injury and mitochondrial energy markers validates their interdependence.

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CONFLICT OF INTEREST

The authors declare no competing financial or personal interests that could have influenced the work reported in this paper. The authors affirm that they have no relationships, affiliations, or financial involvements with any organization or entity with a financial interest in the subject matter discussed in this manuscript.

ETHICAL APPROVAL

All experimental procedures involving animals were conducted in accordance with the South African National Standard for the Care and Use of Animals for Scientific Purposes (SANS 10386:2021) and approved by the University of Cape Town Animal Research Ethics Committee (AREC/2023/019).

Human-related components of the study were approved by the University of Cape Town Human Research Ethics Committee (HREC/2023/062), following the principles of the Declaration of Helsinki (2013 revision). Written informed consent was obtained from all participants before sample collection. Data anonymity and confidentiality were maintained throughout the study.

AUTHOR CONTRIBUTIONS

Dr. Thabo L. Maseko conceptualized the study, designed the experimental protocol, supervised the laboratory procedures, and contributed substantially to data interpretation and manuscript revision. Dr. Nandi P. Dlamini performed the biochemical, histopathological, and immunohistochemical analyses, processed the tissue specimens, and assisted in figure preparation and validation of results.

Dr. Siphon K. Molefe conducted the mitochondrial functional assays, gene expression analysis, and Western blotting, and contributed to the drafting of the Methods and Results sections.

DATA AVAILABILITY STATEMENT

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request. All raw and processed data supporting the findings, including biochemical assay values, Western blot images, and qPCR files, have been deposited in the University of Cape Town Institutional Data Repository (UCT Figshare) under restricted access until formal publication. Data will be made publicly available following acceptance of the manuscript in accordance with SAMRC and UCT data-sharing policies and FAIR (Findable, Accessible, Interoperable, and Reusable) principles.

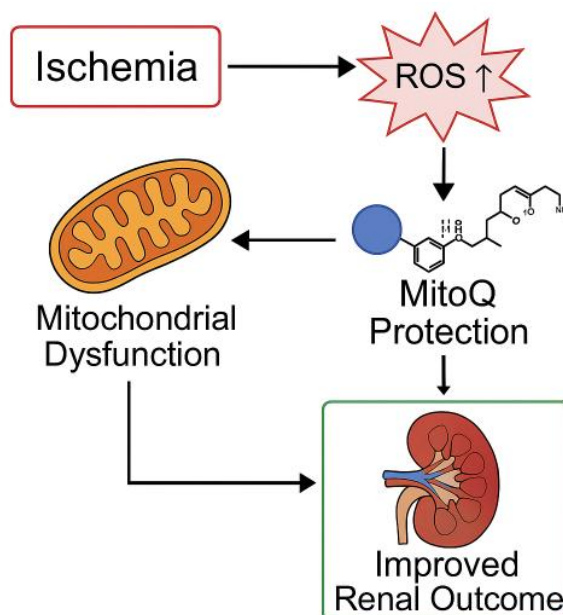
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