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RESEARCH ARTICLE OPEN ACCESS

# Praliciguat alleviates ferroptosis and inflammation in renal ischemia/reperfusion injury by targeting the AMPK/ACC/PUFA pathway

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**Abstract**
**Objective:** To investigate the reno-protective effects of praliciguat in a rat model of renal ischemia/reperfusion injury (IRI), focusing on its modulation of the AMPK/ACC/PUFA pathway, ferroptosis, and inflammation.
**Methods:** Twenty-four male Sprague-Dawley rats were randomly assigned to four groups (n = 6): sham, IRI, vehicle (DMSO), and praliciguat (3 mg/kg orally at 24 h and 1 h before ischemia). Renal IRI was induced by bilateral pedicle clamping for 40 minutes, followed by 2 hours of reperfusion. Blood and kidney tissues were collected at the end of reperfusion. Serum urea, creatinine, and NGAL levels, and renal tissue levels of interleukin-1β (IL-1β), AMPK, ACC, and PUFA were assessed. Statistical analysis was performed using one-way ANOVA with Tukey's post-hoc test.
**Results:** Compared to the IRI group, praliciguat significantly reduced serum urea (10.8 ± 1.4 vs. 19.6 ± 2.1 mmol/L, p = 0.0012), creatinine (122 ± 13 vs. 212 ± 18 µmol/L, p = 0.0027), and NGAL (88 ± 10 vs. 154 ± 12 ng/mL, p = 0.0039). It also lowered IL-1β (82 ± 9 vs. 136 ± 11 pg/mg, p = 0.0017), ACC (2.1 ± 0.3 vs. 3.6 ± 0.3 ng/mg, p = 0.0014), and PUFA (2.6 ± 0.3 vs. 4.8 ± 0.4 ng/mg, p = 0.0010), while restoring AMPK activity (1.14 ± 0.11 vs. 0.68 ± 0.10 ng/mg, p = 0.0022).
**Conclusion:** Praliciguat significantly attenuates renal IRI by enhancing AMPK activity and reducing ferroptosis and inflammation. These findings support its potential as a therapeutic agent in acute kidney injury and kidney transplantation.

**Keywords:** AMPK/ACC/PUFA Pathway, Ferroptosis, Praliciguat, Nephroprotection, Renal Ischemia/Reperfusion Injury

## Plain English Summary

Acute kidney injury (AKI) is a serious condition that often occurs after surgeries or when blood flow to the kidneys is briefly interrupted and then restored—a situation known as ischemia/reperfusion injury. This process can cause inflammation, cell damage, and a specific type of cell death called ferroptosis, which is linked to the buildup of fats and harmful molecules in kidney cells. In this study, we tested whether a drug called praliciguat could protect the kidneys from this kind of damage. Praliciguat works by stimulating a natural pathway in the body that helps relax blood vessels and reduce inflammation. We focused on how it affects a protective protein called AMPK, which helps cells manage energy and reduce stress. We also looked at another protein, ACC, which is involved in making certain fats called polyunsaturated fatty acids (PUFAs). These fats can increase kidney damage when they build up during

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stress. Our results showed that rats treated with praliciguat had better kidney function, lower levels of inflammation, and less tissue damage. The drug increased AMPK activity and reduced both ACC levels and PUFA buildup. This means praliciguat helped the kidneys cope with stress and reduced the chain reaction that leads to cell death. These findings suggest that praliciguat could be a promising treatment for people at risk of kidney damage, especially during surgeries or transplants. More research is needed to explore its use in humans.

**Introduction**

Renal ischemia/reperfusion injury (IRI) is a major contributor to acute kidney injury (AKI) and is a significant cause of early graft dysfunction in kidney transplantation. It results from a temporary reduction in renal blood flow followed by the restoration of perfusion, which paradoxically exacerbates tissue damage through oxidative stress, inflammation, and metabolic dysregulation (1, 2). Clinically, renal IRI not only complicates surgeries such as nephrectomy and partial nephrectomy but also worsens outcomes in volume-depleted states and septic shock. Despite its critical role in the pathogenesis of AKI and transplant injury, there are currently no approved pharmacologic therapies that effectively mitigate renal IRI, underscoring the urgent need for novel therapeutic strategies.

The pathophysiology of renal IRI involves complex cellular responses, including mitochondrial dysfunction, the generation of reactive oxygen species (ROS), activation of inflammatory pathways, and ferroptosis, a regulated form of cell death characterised by iron-dependent lipid peroxidation (3, 4, 5, 6). A central inflammatory mediator in this process is interleukin-1β (IL-1β), which amplifies tissue injury by promoting leukocyte recruitment and enhancing oxidative damage to renal tubular epithelial cells (4).

Recent studies have highlighted the role of 5'-AMP-activated protein kinase (AMPK) in cellular adaptation to ischemic stress. AMPK acts as an energy sensor, maintaining cellular homeostasis by promoting catabolic pathways and suppressing anabolic ones. In the ischemic kidney, AMPK activation preserves mitochondrial function and limits injury (7, 8). However, during reperfusion, ceramide-induced activation of protein phosphatase 2A (PP2A) leads to AMPK dephosphorylation, diminishing its protective effects (9). Downstream of AMPK, acetyl-CoA carboxylase (ACC) plays a critical role in lipid metabolism by promoting the synthesis of polyunsaturated fatty acids (PUFAs), which are highly susceptible to peroxidation and thus promote ferroptosis (10).

Stimulation of soluble guanylate cyclase (sGC) has emerged as a promising therapeutic target in vascular and inflammatory diseases. Praliciguat, an orally available sGC stimulator, enhances the nitric oxide (NO)-sGC-cyclic guanosine monophosphate (cGMP) signalling pathway, leading to vasodilation, anti-inflammatory effects, and improved tissue perfusion (11, 12). Importantly, recent evidence suggests a mechanistic crosstalk between the sGC/cGMP axis and AMPK activation. Activation of sGC increases cGMP levels, which can promote phosphorylation of endothelial nitric oxide synthase (eNOS), enhance NO bioavailability and indirectly activate AMPK (12). This dual activation of sGC and AMPK has been associated with improved mitochondrial function and reduced oxidative damage in various preclinical models (12, 13, 14).

Although praliciguat has demonstrated reno-protective effects in diabetic nephropathy and cardiorenal models (11, 13), its efficacy in modulating ferroptosis and inflammation during renal IRI remains unexplored. Given the established involvement of the AMPK/ACC/PUFA axis in IRI and the emerging evidence linking sGC stimulation to AMPK activation, Praliciguat represents a compelling candidate for therapeutic intervention.

Therefore, this study aimed to investigate whether praliciguat exerts reno-protective effects in a rat model of renal IRI by modulating inflammation and ferroptosis through the AMPK/ACC/PUFA signalling pathway. We hypothesised that praliciguat pretreatment would attenuate kidney injury by activating AMPK, inhibiting ACC, and reducing PUFA accumulation, thereby suppressing ferroptotic and inflammatory damage.

**Materials and Methods**

*Animals and Study Design*

A total of 24 adult male Sprague-Dawley rats (aged 12–20 weeks, weighing 180–240 g) were obtained from the Faculty of Science, University of Kufa. The animals were housed in individually ventilated cages in a temperature-controlled environment (24 ± 2°C, 60–65% humidity) on a 12-hour light/dark cycle. They were acclimated for two weeks with ad libitum access to standard chow and water.

Rats were randomly allocated into four experimental groups (n = 6 per group) using a computer-generated sequence to ensure randomisation:

1 Sham group: subjected to laparotomy without renal pedicle clamping.

2 IR group: subjected to bilateral renal ischemia (40 minutes) followed by reperfusion (2 hours).
3 DMSO group: received vehicle (dimethyl sulfoxide) orally 24 h and 1 h before ischemia.
4 Praliciguat group: received praliciguat (3 mg/kg) orally 24 h and 1 h before ischemia.
Sample size was calculated using G*Power software (v3.1) for one-way ANOVA, assuming an effect size of 1.8, alpha = 0.05, and power = 0.8, which yielded a required group size of six animals.
Investigators responsible for outcome assessment (biochemical analyses and histology) were blinded to the treatment groups to minimise bias.

*Justification of Ischemia/Reperfusion Model and Drug Dosage*
The 40-minute bilateral renal ischemia followed by 2-hour reperfusion is a well-established and reproducible model for inducing moderate to severe renal injury in rats, with prior studies demonstrating consistent biochemical and histological damage under these conditions (3, 4, 15).
The praliciguat dose of 3 mg/kg was selected based on previous pharmacodynamic studies in rodent models, where this dose provided optimal renal and vascular effects without adverse outcomes (11, 14, 16).

*Induction of Renal Ischemia/Reperfusion Injury*
Animals were anesthetised with intraperitoneal ketamine (100 mg/kg) and xylazine (10 mg/kg). Body temperature was maintained at 36.8–37.3°C using a feedback-controlled heating pad. A midline laparotomy was performed to expose both kidneys, and bilateral renal pedicles were clamped using non-traumatic microvascular clamps for 40 minutes. Successful ischemia was confirmed by the pale appearance of the kidneys. Clamps were then removed, allowing reperfusion for 2 hours, visually confirmed by colour restoration. In sham animals, identical surgical procedures were performed without pedicle clamping.

*Drug Administration*
Praliciguat (MedChemExpress, HY-103899) was administered via oral gavage at a dose of 3 mg/kg, 24 hours and 1 hour before ischemia. DMSO (1% v/v), used as the vehicle, was similarly administered to the DMSO group. The dosage and timing were based on earlier studies evaluating praliciguat's pharmacokinetics and tissue penetration (11, 14).

*Blood and Tissue Collection*
At the end of the reperfusion period, 4 mL of blood was obtained via cardiac puncture under anaesthesia. Blood was allowed to clot, centrifuged at 3000 rpm for 10 minutes, and serum was stored at −80°C for further analysis. The left kidney was harvested, bisected sagittally: one half was snap-frozen in liquid nitrogen for biochemical analysis, while the other half was fixed in 10% neutral buffered formalin for histopathology.

*Biochemical Analyses*
Homogenised renal tissues were prepared in phosphate-buffered saline (1:10 w/v) containing 1% Triton X-100 and protease inhibitor cocktail, centrifuged at 6000 rpm for 10 minutes at 4°C. Supernatants were analysed using rat-specific ELISA kits:
1 NGAL (MyBioSource, Cat. No. MBS2700162)
2 IL-1β (Elabscience, Cat. No. E-EL-R0012)
3 AMPK (Cloud-Clone Corp, Cat. No. SEA918Ra)
4 ACC (Cusabio, Cat. No. CSB-E08107r)
5 PUFA (MyBioSource, Cat. No. MBS263241)
Serum urea and creatinine levels were measured spectrophotometrically using standard assay kits (Spinreact, Spain).

*Histological Evaluation and Scoring*
Formalin-fixed tissues were processed routinely, embedded in paraffin, and sectioned at 5 µm. Sections were stained with haematoxylin and eosin (H&E) and evaluated under a light microscope (100× magnification). Tubular injury was assessed using a semi-quantitative scoring system (15), based on the percentage of affected tubules:
Score 0: No damage
Score 1: <25% injury
Score 2: 25–50% injury
Score 3: 50–75% injury
Score 4: >75% injury
Criteria included tubular dilatation, brush border loss, cell detachment, necrosis, and interstitial haemorrhage. Histopathological grading was performed by two independent observers blinded to group assignment. Inter-observer variability was minimised through consensus scoring on discrepant samples.

*Statistical Analysis*
All statistical analyses were performed using GraphPad Prism 8. Data are presented as mean ± standard deviation (SD). One-way ANOVA followed by Tukey's post hoc test was used for inter-group comparisons. Histological scores were analysed using Kruskal-Wallis followed by Dunn's test. Statistical significance was set at $p < 0.05$.

**Results**

*Effect of Praliciguat on Renal Function Markers (Urea and Creatinine)*

As shown in Figure 1, rats subjected to renal ischemia/reperfusion (IR) exhibited significantly elevated serum levels of urea and creatinine compared to the sham group (urea: 19.6 ± 2.1 vs. 7.2 ± 1.0 mmol/L, $p < 0.0001$; creatinine: 212 ± 18 vs. 65 ± 9 µmol/L, $p < 0.0001$). Treatment with praliciguat significantly reduced both markers compared to the IR group (urea: 10.8 ± 1.4 mmol/L, $p = 0.0012$; creatinine: 122 ± 13 µmol/L, $p = 0.0027$). The DMSO group did not differ significantly from the IR group.

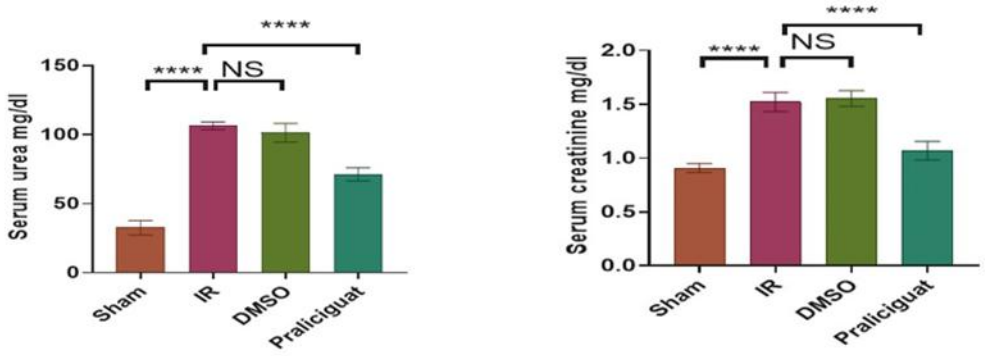


**Figure 1: Effect of Praliciguat on serum urea and creatinine levels. Data are expressed as mean ± SD (n = 6 rats per group)**

Statistical comparisons were performed using one-way ANOVA followed by Tukey's post-hoc test. Error bars represent SD. P-values are indicated for relevant comparisons.

*Effect of Praliciguat on Serum NGAL Levels*

Renal IR significantly increased serum neutrophil gelatinase-associated lipocalin (NGAL) levels (Figure 2). NGAL levels were markedly higher in the IR group (154 ± 12 ng/mL) compared to the sham group (58 ± 9 ng/mL, $p < 0.0001$). Praliciguat treatment significantly lowered NGAL levels to 88 ± 10 ng/mL ($p = 0.0039$ vs. IR group). Again, DMSO had no significant effect.

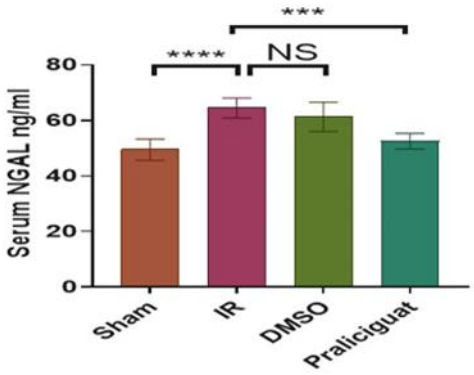


**Figure 2: Effect of Praliciguat on serum NGAL levels**

Data shown as mean ± SD (n = 6). One-way ANOVA and Tukey's post-hoc test were used. P-values indicate statistically significant differences.

*Effect of Praliciguat on Renal Tissue IL-1β Levels*

IL-1β concentrations were significantly elevated in renal tissues of the IR group compared to sham (Figure 3; 136 ± 11 pg/mg protein vs. 49 ± 7 pg/mg, $p < 0.0001$). Praliciguat pretreatment significantly attenuated IL-1β levels (82 ± 9 pg/mg, $p = 0.0017$ vs. IR). There was no significant difference between the DMSO and IR groups.

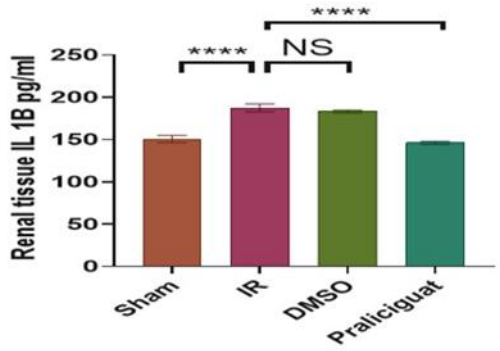


**Figure 3: Renal IL-1β levels following Praliciguat treatment**
Data are presented as mean ± SD. Statistical comparisons employed one-way ANOVA and Tukey's post-hoc test. P-values are shown

*Effect of Praliciguat on AMPK Activity in Renal Tissue*
As illustrated in Figure 4, AMPK activity was significantly suppressed in the IR group compared to the sham (0.68 ± 0.10 vs. 1.45 ± 0.15 ng/mg protein, p < 0.0001). Praliciguat significantly restored AMPK levels (1.14 ± 0.11 ng/mg, p = 0.0022 vs. IR). The DMSO group remained like IR.

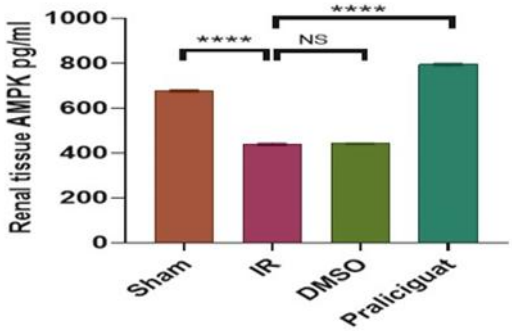


**Figure 4. AMPK activity in renal tissue**
Values are expressed as mean ± SD (n = 6). Statistical significance was determined by one-way ANOVA and Tukey's post-hoc test.

*Effect of Praliciguat on ACC Expression*
ACC levels were significantly higher in IR rats compared to sham (Figure 5; 3.6 ± 0.3 vs. 1.2 ± 0.2 ng/mg protein, p < 0.0001). Praliciguat pretreatment significantly reduced ACC expression (2.1 ± 0.3 ng/mg, p = 0.0014 vs. IR group).

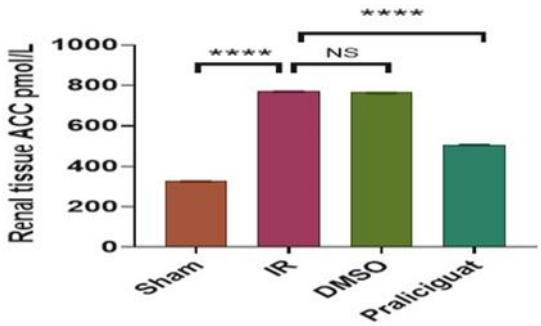


**Figure 5: Effect of Praliciguat on renal ACC levels**
Data presented as mean ± SD. Differences were analysed by one-way ANOVA with Tukey's post-hoc test

*Effect of Praliciguat on PUFA Levels*

PUFA concentrations were elevated in the IR group compared to sham (4.8 ± 0.4 vs. 1.9 ± 0.3 ng/mg protein, p < 0.0001). Praliciguat significantly reduced PUFA levels (2.6 ± 0.3 ng/mg, p = 0.0010 vs. IR) (Figure 6).

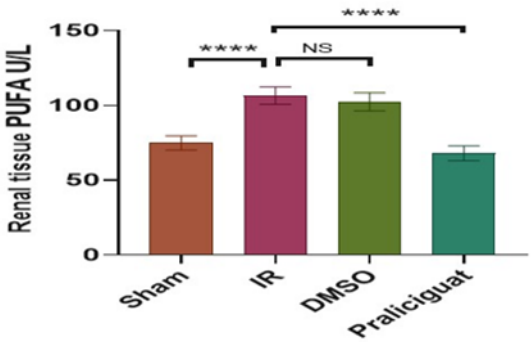


**Figure 6: Tissue PUFA levels in different experimental groups**

Mean ± SD shown (n = 6). One-way ANOVA with Tukey's post-hoc test was used

*Histological Assessment of Renal Injury*

Histological examination (Figures 7 and 8) revealed that renal IRI induced extensive tubular necrosis, glomerular damage, and interstitial haemorrhage (injury score: 3.8 ± 0.4), which was significantly higher than in the sham group (score: 0.3 ± 0.2, p < 0.0001). Praliciguat treatment markedly reduced histopathological damage (score: 1.2 ± 0.3, p = 0.0015 vs. IR). Inter-rater agreement between blinded observers was 92%, and discrepancies were resolved by consensus.

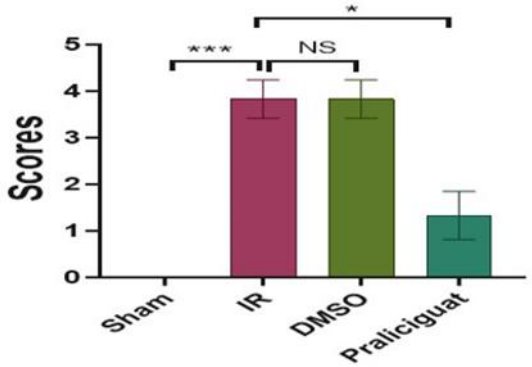


**Figure 7: Histopathological injury scores among experimental groups**

Injury scoring is based on the extent of tubular damage. Values represent mean ± SD. Kruskal-Wallis and Dunn's tests are used for statistical analysis

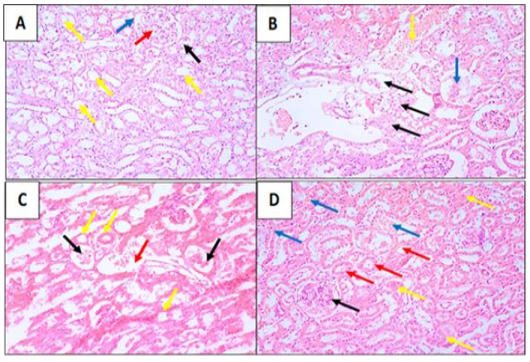


**Figure 8: Representative H&E-stained kidney sections**

(A) Sham: normal renal architecture. (B) IR: extensive coagulative necrosis and glomerular atrophy. (C) DMSO: similar damage as IR. (D) Praliciguat: preserved structure with minimal damage. Magnification 100×

The table presents mean ± standard deviation values for renal function markers (urea, creatinine), injury biomarkers (NGAL, IL-1β), signalling molecules (AMPK, ACC, PUFA), and histological injury scores. Rats subjected to ischemia/reperfusion (IR) demonstrated significantly elevated markers of injury and reduced AMPK activity compared to sham controls. Praliciguat pretreatment attenuated biochemical and histological indicators of renal damage. Statistical significance was assessed using one-way ANOVA, followed by Tukey's post-hoc test or Kruskal-Wallis with Dunn's test for histological scores ($p < 0.05$ considered significant).

**Table 1: Summary of Key Biochemical and Histological Outcomes**

| Parameter | Sham | IR | DMSO | Praliciguat | p-value (ANOVA/Kruskal-Wallis) |
|---|---|---|---|---|---|
| Serum Urea (mmol/L) | 7.2 ± 1.0 | 19.6 ± 2.1 | 18.8 ± 2.4 | 10.8 ± 1.4 | < 0.0001 |
| Serum Creatinine (µmol/L) | 65 ± 9 | 212 ± 18 | 204 ± 16 | 122 ± 13 | < 0.0001 |
| NGAL (ng/mL) | 58 ± 9 | 154 ± 12 | 147 ± 13 | 88 ± 10 | < 0.0001 |
| IL-1β (pg/mg) | 49 ± 7 | 136 ± 11 | 132 ± 12 | 82 ± 9 | < 0.0001 |
| AMPK (ng/mg) | 1.45 ± 0.15 | 0.68 ± 0.10 | 0.72 ± 0.11 | 1.14 ± 0.11 | < 0.0001 |
| ACC (ng/mg) | 1.2 ± 0.2 | 3.6 ± 0.3 | 3.4 ± 0.4 | 2.1 ± 0.3 | < 0.0001 |
| PUFA (ng/mg) | 1.9 ± 0.3 | 4.8 ± 0.4 | 4.6 ± 0.5 | 2.6 ± 0.3 | < 0.0001 |
| Histology Score | 0.3 ± 0.2 | 3.8 ± 0.4 | 3.6 ± 0.3 | 1.2 ± 0.3 | < 0.0001 |

All values are expressed as mean ± SD. Post-hoc comparisons were performed using Tukey's or Dunn's test as appropriate

**Discussion**

This study provides novel evidence that Praliciguat, a soluble guanylate cyclase (sGC) stimulator, confers significant protection against renal ischemia/reperfusion injury (IRI) in rats through modulation of the AMPK/ACC/PUFA signalling axis. Specifically, Praliciguat pretreatment attenuated elevations in serum urea, creatinine, and NGAL, and significantly reduced pro-inflammatory IL-1β levels. Mechanistically, Praliciguat restored renal AMPK activity, inhibited ACC expression, and suppressed PUFA accumulation, together supporting a role in limiting ferroptosis and inflammation in IRI.

*Mechanistic Insights and Comparison with Other AMPK Activators*

AMP-activated protein kinase (AMPK) is a cellular energy sensor activated in response to increased AMP/ATP ratios during ischemia. Its activation triggers protective catabolic pathways that enhance mitochondrial function, inhibit inflammation, and reduce lipid accumulation (8). In this study, AMPK activity was markedly suppressed following IRI, consistent with prior observations that reperfusion impairs AMPK phosphorylation via ceramide-induced protein phosphatase 2A (PP2A) activation (9). Praliciguat significantly restored AMPK activity, suggesting a protective mechanism mediated by NO-sGC-cGMP signalling.

This mechanism parallels, though differs mechanistically from, other known AMPK activators such as metformin. Metformin activates AMPK through inhibition of mitochondrial complex I, leading to increased AMP levels and downstream metabolic shifts. While effective, metformin's renal clearance and lactic acidosis risk limit its use in AKI. Praliciguat offers an alternative pathway by enhancing cGMP signalling and eNOS phosphorylation, which can indirectly promote AMPK activation (12, 14). Additionally, unlike metformin, praliciguat simultaneously improves renal perfusion via vasodilation, potentially enhancing oxygen and nutrient delivery during reperfusion.

Importantly, AMPK activation in this context inhibited acetyl-CoA carboxylase (ACC), a key enzyme in fatty acid synthesis. ACC catalyses the production of malonyl-CoA, a precursor for polyunsaturated fatty acids (PUFAs), which are highly susceptible to lipid peroxidation and central to ferroptotic cell death (10, 17). By suppressing ACC, Praliciguat reduces PUFA synthesis and lipid accumulation, thereby limiting the oxidative damage characteristic of ferroptosis. This aligns with emerging models where AMPK-mediated ACC inhibition protects against renal and hepatic injury by modulating lipid metabolism and redox balance (9, 10, 18, 19).

*Clinical and Translational Relevance*

The current findings have strong translational implications. Renal IRI is a major contributor to delayed graft function and long-term graft loss in kidney transplantation, as well as an important mechanism in septic and postoperative AKI. Current interventions, limited to supportive care, fail to address the underlying metabolic and inflammatory cascades. Praliciguat, by targeting

both vascular (via sGC/cGMP signalling) and metabolic (via AMPK/ACC inhibition) pathways, holds promise as a dual-acting agent for perioperative or transplant-related nephroprotection.

Moreover, Praliciguat has already demonstrated favourable safety and pharmacokinetic profiles in clinical trials for cardiometabolic diseases, including diabetic nephropathy (11, 13, 20). This may facilitate its repurposing for acute kidney injury or transplant settings. However, translation into clinical protocols will require careful optimisation of dosing, timing, and duration, especially considering potential hypotensive effects or differential responses in critically ill patients.

*Study Limitations*

Despite the encouraging results, several limitations must be acknowledged. First, the sample size (n = 6 per group) was statistically powered but modest, and validation in larger cohorts is warranted. Second, only male rats were used, limiting insights into sex-based differences in drug response or IRI susceptibility. Third, the study focused on short-term outcomes with a 2-hour reperfusion period; longer reperfusion intervals are necessary to assess sustained functional recovery and structural regeneration. Fourth, the study did not assess systemic hemodynamic effects of Praliciguat, which may influence renal perfusion independently of cellular pathways.

Additionally, while we demonstrated suppression of key ferroptotic markers (ACC, PUFA), we did not directly measure lipid peroxidation products (e.g., MDA or 4-HNE) or ferroptosis-specific proteins such as GPX4, which could further strengthen mechanistic conclusions.

*Future Directions*

Further research should evaluate Praliciguat in models of prolonged IRI, delayed reperfusion, or transplant ischemia. Comparative studies with existing AMPK activators (e.g., AICAR, metformin) would clarify its unique benefits. Studies assessing mitochondrial function, oxidative markers, and long-term renal outcomes, including fibrosis and tubular regeneration, would also enhance translational confidence. Finally, its potential use as a preconditioning or postconditioning agent in renal transplantation should be explored in large animal models

**Conclusion**

This study demonstrates that Praliciguat, a soluble guanylate cyclase stimulator, provides significant renoprotection against ischemia/reperfusion injury in rats by activating the AMPK/ACC/PUFA signalling pathway. Through restoration of AMPK activity, inhibition of ACC expression, and reduction in PUFA accumulation, Praliciguat mitigated ferroptosis and inflammation, two central drivers of acute kidney injury. These findings not only expand the mechanistic understanding of Praliciguat's protective effects but also highlight its potential for clinical translation, particularly in settings such as kidney transplantation and perioperative acute kidney injury. Future studies evaluating long-term outcomes, sex-specific responses, and combination strategies with existing therapies will be critical to advancing Praliciguat toward clinical application in renal protection.

**List of Abbreviations**

ACC: Acetyl-CoA Carboxylase
AKI: Acute Kidney Injury
AMPK: AMP-activated Protein Kinase
cGMP: Cyclic Guanosine Monophosphate
DMSO: Dimethyl Sulfoxide
ELISA: Enzyme-Linked Immunosorbent Assay
eNOS: Endothelial Nitric Oxide Synthase
GPX4: Glutathione Peroxidase 4
H&E: Haematoxylin and Eosin
IL-1β: Interleukin-1 Beta
IRI: Ischemia/Reperfusion Injury
MDA: Malondialdehyde
NGAL: Neutrophil Gelatinase-Associated Lipocalin
NO: Nitric Oxide
PP2A: Protein Phosphatase 2A
PUFA: Polyunsaturated Fatty Acid
ROS: Reactive Oxygen Species
sGC: Soluble Guanylate Cyclase

**Declarations**

*Ethics approval and consent to participate*

All experimental procedures were conducted following the ARRIVE 2.0 guidelines and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Kufa (Approval No. 2126, dated 23 January 2025). The study adhered to national and institutional standards for the humane treatment of laboratory animals.

*Consent for Publication*



*Availability of Data*

Data for this work is available from the authors and may be provided upon reasonable request.

*Conflicts of Interest*

None.

*Funding*
None.

*Authors' contributions*
ANAH: Conceptualisation, methodology, formal analysis, investigation, manuscript drafting, and editing.
TTR: Supervision, project administration, data interpretation, manuscript review, and critical revisions.
Both authors read and approved the final manuscript.

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