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RESEARCH ARTICLE OPEN ACCESS

The potential reno-protective effect of pirfenidone against renal ischemia/reperfusion injury in a mouse model

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Abstract
Objective: This study aimed to assess whether pirfenidone offers protection to the kidneys in a mouse model subjected to renal ischemia-reperfusion injury, focusing particularly on its anti-inflammatory and anti-apoptotic effects.
Methods: Twenty-eight adult male mice were randomly divided into four groups of seven: Sham, Ischemia, Vehicle (DMSO), and Pirfenidone. We induced bilateral renal ischemia for 30 minutes, followed by 2 hours of reperfusion. Pirfenidone at a dose of 300 mg/kg or the vehicle was administered orally 30 minutes before ischemia. After reperfusion, serum and kidney tissues were collected to analyse markers of renal function, urea and creatinine, along with indicators of kidney damage (KIM-1), inflammation (IL-6), and apoptosis (caspase-3). Histological examination was also performed to evaluate tubular injury.
Results: The ischemia and vehicle groups showed considerably higher levels of serum creatinine, urea, and KIM-1, and increased levels of IL-6 and caspase-3 in kidney tissues, compared to the sham group ($P<0.001$). Treatment with pirfenidone resulted in decreased biochemical parameters when compared to the ischemia and vehicle groups ($P<0.001$). Histologically, kidneys from the pirfenidone group exhibited a markedly lower injury score (score 2) relative to the ischemia and vehicle groups (score 4), indicating less tubular damage. The sham group showed no important histological abnormalities, with an injury score of 0.
Conclusion: Pirfenidone demonstrates substantial protective effects against renal ischemia-reperfusion injury in mice, evidenced by improvements in biochemical markers and decreased tissue damage. This suggests that pirfenidone holds potential as a therapeutic agent for preventing or ameliorating acute kidney injury.

Keywords: Caspase-3, Creatinine, IL-6, KIM-1, Pirfenidone, Renal ischemia/reperfusion injury, Urea

Plain English Summary

This study looked at whether the drug pirfenidone has kidney-damage-protecting effects. The kidney damage is being caused by a temporary lack of blood flow and reflow (called renal ischemia-reperfusion injury, or RIRI) in adult male mice. The researchers divided the mice into four groups: One group had surgery but no reduced blood flow, another group had reduced blood flow induced in both kidneys. The third group had induced reduced blood flow and received an agent (DMSO). The last group had an induced reduced blood flow and was treated with pirfenidone. Two hours after blood flow was restored, the researchers measured markers of kidney damage and inflammation in the blood and kidney tissue. They found that Mice with reduced blood flow or vehicle treatment had higher levels of kidney damage markers.

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Pirfenidone treatment reduced these damage markers significantly. Under the microscope, kidney tissue from the pirfenidone group looked healthier than tissue from the untreated group, with reduced blood flow. In conclusion, pirfenidone helped protect the kidneys from damage caused by temporary blood flow loss by reducing inflammation and cell death.

Introduction

Ischemia/reperfusion injury (IRI) transpires when an organ's blood flow is temporarily interrupted and subsequently restored, together with the oxygen supply, this results in the activation of leukocytes, infarction, sepsis, creation of ROS (the reactive oxygen species), they all exacerbate tissue destruction following the transplantation of the organs, inflammation, as well as any other medical procedure (1). IRI is a distinct phenomenon that elicits divergent responses in organs and cells, cumulatively contributing to the overall damage associated with IRI (2). This can induce several symptoms in the body, including cardiomyopathy, diminished cerebral function, reperfusion arrhythmias, and gastrointestinal barrier failure (3). The simultaneous presence of IRI post-renal transplantation increases the risk of complications, mortality, and length of hospital stay, ultimately leading to KF (kidney failure) and AKI (acute kidney injury) (4). AKI occurs in the kidneys whenever blood flow becomes impeded due to ischemia, resulting in hypoxia, which is defined as an oxygen deficiency. The prevalence of morbidity and mortality escalates when the glomerular filtration rate (GFR) and kidney output decline concurrently (5). Consequently, the progression of the ischemia-reperfusion (I/R) pathogenic mechanism encompasses the activation of neutrophils, ROS production, the attachment of molecules, and a variety of chemokine markers (6). Multiple signs of RIRI include increased vascular permeability, interstitial oedema, dysfunction of endothelial cells and epithelial cell of kidney tubules, as well as activation of tissue-resident leukocytes; All occurring in a lack of adenosine tri-phosphate (ATP), a glycogen, or oxygen, destroying deoxyribonucleic acid (DNA), blood vessel permeability, and immune stimulation (7). Specific conditions, like hypotension and shock, might induce this RIRI; nevertheless, renal failure may arise from thrombus or dissection of the main kidney artery (8). Pirfenidone is approved for therapeutic application in idiopathic pulmonary fibrosis (IPF) due to its protective impact on the lung against pulmonary fibrosis (PF) by the suppression of fibrotic mediator known as transforming growth factor-beta-1 (TGF-β1) expression (9), that had been documented in numerous animal models of progressing fibrotic conditions that its impact also exhibits anti-inflammatory and antioxidant effects (10). The prophylactic use of pirfenidone protects the kidney from IRI by preventing renal dysfunction and damage to the structures through its antioxidant properties, which are sustained by constant synthesis of an essential element in combating free radicals, which is nitric oxide (NO) (11), eliminating ROS, and preventing lipid peroxidation, therefore mitigating cellular damage in renal tubules (12). PFD exhibits an antiapoptotic effect; the caspase-3 marker promoted the expression of pro-apoptotic genes. The inhibition of apoptosis resulted in an increased survival rate in an acute lung injury (ALI) animal model study, attributed to PFD's capacity to reduce caspase-3 activity in vitro and mitigate the apoptotic effect (13). PFD also demonstrates efficacy in treating various diseases, such as cancer and inflammation-related conditions, exhibiting anti-tumour properties and the ability to inhibit the multiplication of carcinoma cell lines (14). Interleukin-six (IL-6) is a mini polypeptide (15), its production is primarily stimulated by two parameters, interleukin 1-β (IL-1β) and tumour necrosis factor (TNF-α); in addition, other mechanisms that enhance its production include, activation of receptors called toll-like receptors (TLRs), prostaglandins (PG), adipokines, responses for stress, and numerous other mediators (16), and elevated the level of IL-6 in ischemia-reperfusion injury (17).

KIM-1 is the most sensitive marker, characterised as a (38.7-kDa) class I-transmembrane glycoprotein. It features an extracellular immunoglobulin-like domain topped by an extended mucin-like domain. Typically, it is present at low levels in the renal system and other tissues; however, it is significantly upregulated after kidney injury, particularly after RIRI (18).

Caspase-3 is a protein family that is highly homologous to the C. elegans cell death abnormal-3 gene (CED-3) and serves a central role in apoptosis, with extrinsic activation starting the caspase cascade, particularly in this pathway (19). It is elevated following ischemia-reperfusion injury in a renal fibrosis mouse model (20).

Materials and Methods

Animal Preparation

Twenty-eight adult male Mus-Musculo mice weighing 25-35 g, aged 12-15 weeks, were acquired from the Iraqi Centre for Cancer

Research. The mice were housed in the animal facility and were maintained at the College of Science / Kufa University. Mice were kept in cages under controlled conditions; a 12-hour light and 12-hour dark cycle with temperatures ranging from 22 to 24 °C, and humidity levels between 60 and 65 %. The mice received standard access to hydration and sustenance; this study was performed at the laboratory unit of the Clinical Branch. Research Department-College of Pharmacy/ Kufa University from October 15, 2024, till February 15, 2025.

Study Design
Mice underwent a one-week acclimatisation period before being randomly allocated into four groups: Sham, Ischemia, Vehicle, and Pirfenidone (n = 7 per group):

Sham group
Mice received identical anaesthesia, and the kidneys were subjected to the same flank laparotomy procedures without ischemia induction; this was used as the negative laparoscopic control group.

Ischemia group
In this group, the mice had been anaesthetised, and a flank laparotomy was performed on the kidneys, which experienced bilateral renal ischemia for thirty minutes, subsequently followed by reperfusion for two hours.

Vehicle (DMSO) group
Mice received oral DMSO (dimethyl sulfoxide) as transport medium for pirfenidone 30 minutes before ischemia/reperfusion.

Pirfenidone group
Mice were pre-administered 300 mg per kg of PFD by oral gavage 30 minutes before ischemia/reperfusion.

Experimental Model of Ischemia
In this study, 0.01 mg per g of xylazine and 0.1 mg per g of ketamine were administered I.P. to create general anaesthesia in the mice (21). Ischemia was induced using a renal artery clamp. A vertical flank incision measuring 1.5 cm was made with surgical scissors, layer by layer, through the cutaneous, fascia, and muscle sections. A diameter of 0.3 cm was utilised to displace the kidney from the retroperitoneal fat. To get the renal hilum, a cotton pad was used to incise the peri-nephric fat on the midline aspect of the kidney, creating sufficient space for the pedicle clamp, after that cotton pads or tweezers were then inserted into the renal hilar fat both superior and inferior to the renal pedicle, followed by a (30-minute) period of bilateral renal ischemia. Subsequently, to achieve closure of the flank incision, a surgical suture with a diameter of 3-4 was implemented. At the end of reperfusion, the mice were verified after 2 hours.

Preparation of Pirfenidone
Pirfenidone powder (raw material) at a dose of 300 mg/kg (21) was dissolved with 100 mg/ml in DMSO, which is the standard solvent. It was kept until it was diluted in normal saline (the right medium) before being used, as directed by Target-Mol instruction, the company that produces the PFD medication (22).

Sample Collection:
Blood Sample
Blood samples were gathered via cardiac puncture before euthanising the mice. These samples were collected without anticoagulant, put within a gel tube, as well as allowed to remain standing at 25°C for 1 hr.; subsequently, the serum was separated via centrifugation at 6000 rpm for ten minutes, and this serum was utilized to evaluate (blood urea nitrogen and serum creatinine) by utilizing spectrophotometric techniques, as well as to assess KIM-1 marker levels via commercial operations ELISA kits.

Tissue Sample
The kidney tissues were stored at a temperature of −80 °C till homogenization, which was performed using the high-intensity ultrasonic liquid processor in a solution of phosphate-buffered saline (1:10 W/V) containing 1% Triton X-100 and a protease inhibitor cocktail added to it (23). The homogenate was then centrifuged at 5000 rpm for 10 minutes at 4 °C, and the supernatants were used to measure IL-6 and Caspase-3 levels by using the provided ELISA kits.

Histological Examination
The piece of kidney tissue sections underwent fixation in 10% formaldehyde, followed by dehydration through a series of alcohols, clearing in xylene, and embedding in paraffin. The paraffin-embedded kidney's tissues were subsequently sectioned into 5 µm-thick slices. The tissue section slices were stained with haematoxylin and eosin before examination under a light microscope (24). The histological examination was conducted at (X-100 and X-400) of original magnification (25). Histological alterations characterised by the

percentage of injured or damaged kidney tubules were assessed as scores:
• Score 0: 0% damage. No detectable tubular damage: histology is normal.
• Score 1: The damage is less than 25%. No interesting necrosis or interstitial oedema.
• Score 2: The damage ranges from 25 to 50%. Diffuse swelling of the nephrogenic tissue.
• Score 3: The damage is between 50% and 75%. Leukocyte infiltration and contraction bands.
• Score 4: The damage exceeds 75%. Leukocyte infiltration, haemorrhage, cellular swelling, cytoplasmic eosinophilia, and vascular congestion (26).

Statistical Analysis
The analysis of data in this study was conducted using GraphPad Prism 8.1 software (GraphPad Software, La Jolla, CA, USA). Results were expressed as mean ± Standard Error Mean (SEM), unless indicated otherwise. A One-Way Analysis of Variance (ANOVA) was conducted, followed by Bonferroni's multiple comparison test for post-hoc analysis of the data. Histopathological alterations have been assessed amongst groups using the non-parametric test, followed by Dunn's post hoc analysis. Statistical significance was determined at $P < 0.05$ for all analyses.

Results
Ischemia was sustained for 30 minutes, followed by a reperfusion period of 2 hours. Thirty minutes before ischemia, the animals received either DMSO (as a vehicle) or pirfenidone (as therapy) or were left unaddressed (sham and ischemia cohorts). Several kinds of biochemical and histological-based markers have been examined to assess the severity of this RIRI as follows:

The Effect of PFD Treatment on IL-6 Levels
Mice in the ischemia group showed significantly higher renal tissue IL-6 levels compared to the sham group (P< 0.001). There were no statistically significant differences in the IL-6 levels in renal tissues between the vehicle and ischemia groups (Figure 1). The group treated with PFD exhibited significantly lower levels of IL-6 in renal tissues compared to both ischemic and the vehicle groups (P<0.001).

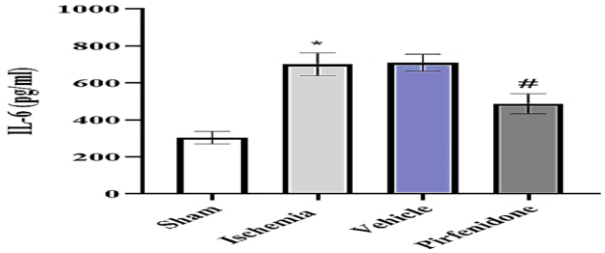

Figure 1: Renal tissue level of IL-6 in the study groups
** Significant versus sham group (P<0.001): # Significant versus ischemic or vehicle groups (P<0.001)*

The Effect of PFD on KIM-1 Level
Mice in the ischemia group had significantly higher serum levels of KIM-1, compared to the sham group (P< 0.001). There were no statistically significant differences in serum levels of KIM-1 among the vehicle and ischemia groups (Figure 2). PFD-treated group demonstrated significantly lower serum KIM-1 levels compared to both vehicle and ischemic groups (P<0.001).

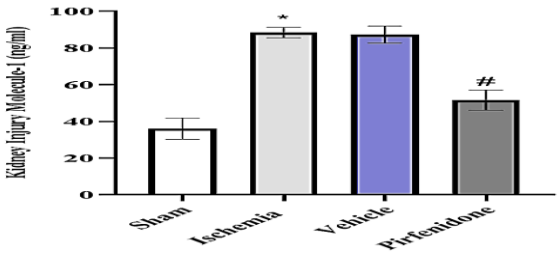

Figure 2: Serum level of KIM-1 in the study groups(n=7)
** Significant versus sham group (P<0.001): # Significant versus ischemic or vehicle groups (P<0.001)*

The Effect of PFD on Caspase-3 Level

Mice in the ischemia group showed significantly higher renal tissue caspase-3 levels in contrast to the sham group (P< 0.001). There were no statistically significant differences in the renal tissue caspase-3 concentration between the vehicle and ischemia groups (Figure 3). The PFD-treated group demonstrated significantly lower concentrations of renal tissue caspase-3 as compared to vehicle and ischemic groups (P<0.001).

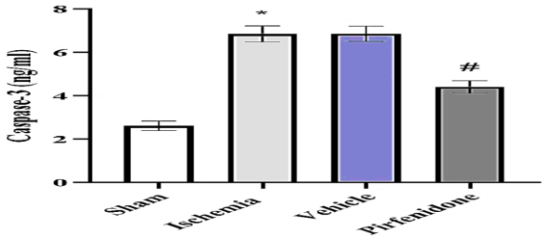

Figure 3: Renal tissue caspase-3 level in the study groups (n=7).

** Significant versus sham group (P<0.001): # Significant versus ischemic or vehicle groups (P<0.001)*

The Effect of PFD on Urea Level

Mice in the ischemia group exhibited significantly higher serum urea levels compared to the sham group (P < 0.001). There were no statistically significant differences in serum urea values among the vehicle and ischemia groups (Figure 4). The group treated with PFD showed significantly lower serum urea levels compared to vehicle and ischemic groups (P<0.001).

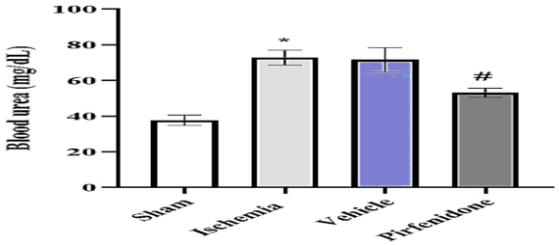

Figure 4: Serum urea level in the experimental groups (n=7)

** Significant versus sham group (P<0.001): # Significant versus ischemic or vehicle groups (P<0.001)*

The Effect of PFD on Creatinine Level

Mice in the ischemia group showed significantly higher serum creatinine levels compared to those of the sham group (P < 0.001). There were no statistically significant differences in S. Cr. values among the ischemia and vehicle groups (Figure 5). It was found that the group given PFD had significantly lower levels of serum creatinine than both the ischemic and vehicle groups (P<0.001).

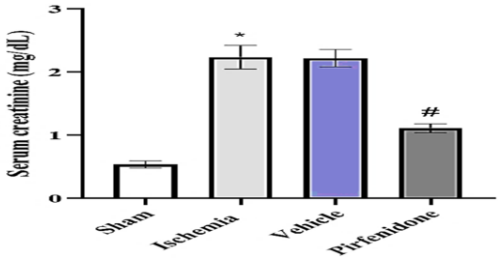

Figure 5: Serum creatinine level in the study groups

** Significant versus sham group (P<0.001); # Significant versus ischemic or vehicle groups (P<0.001)*

Histopathological Alterations of Renal Tissue in Study Group

The analysis of the kidney tissues was conducted to support the reno-protective effects of PFD. Seven mice were included in each group, and a minimum of four sections from each mouse were examined, obeying the Zingarelli protocol.

Sham Group

Renal tissue demonstrated significantly normal architectural histology of the renal tubules (Figures 6A and B), with no areas of damage found (0% renal tubular damage), resulting in a score of 0 (Figure 10) ($P < 0.001$).

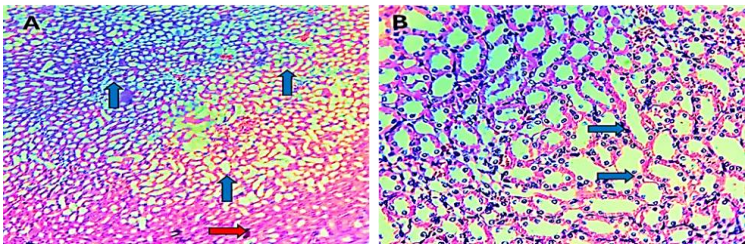

Figure 6: Histopathological examination of the renal section for the sham group

Mice kidney with score-0 (0% renal tubular damage). Damaged area (red arrow), normal histology of renal tubules (blue arrows). The section stained with Haematoxylin& Eosin. A. 100x. B. 400x.

Ischemia Group

The ischemia group showed significantly severe renal tissue injury, with 90% renal tubular damage and a score of 4 (Figure 10) ($P<0.001$). This injury was characterised by cellular swelling, cytoplasmic eosinophilia, vascular congestion, leukocyte infiltration, haemorrhage, and inflammation (Figures 7A, B, and C).

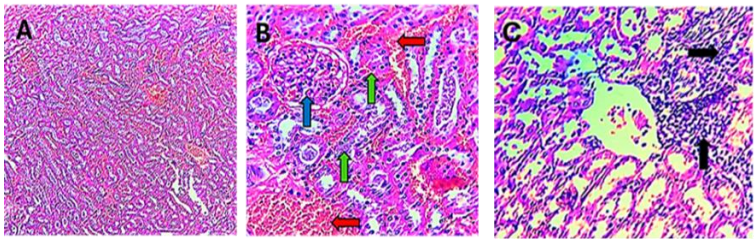

Figure 7: Histopathological examination of the renal section for the Ischemia group

Mice kidney with score-4 (90% renal tubular damage). Cellular oedema and a cytoplasmic eosinophilia (green arrows), inflammation (black arrow), leukocyte infiltration (blue arrow), and congestion of the vascular and haemorrhage (red arrows). The section stained with Haematoxylin& Eosin. A. (100x.), B., and C. (400x.)

Vehicle Group

The vehicle group demonstrated a significantly severe renal injury, with a score of 4 indicating 90% renal tubular damage (as shown in Figure 10) ($P < 0.001$). This was demonstrated by cellular swelling, cytoplasmic eosinophilia, leukocyte infiltration, vascular congestion, haemorrhage, and inflammation (as shown in Figures 8: A, B, C, and D).

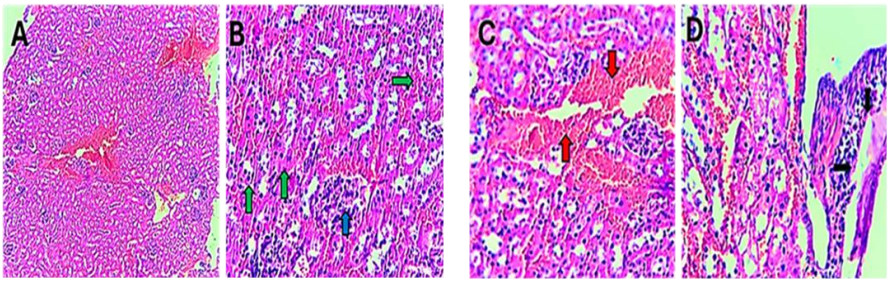

Figure 8: The histopathological examination of the renal section for the vehicle (DMSO) group.

Mice kidney with score-4 (90% renal tubular damage). Cellular oedema and a cytoplasmic eosinophilia (green arrows), inflammation (black arrows), leukocyte infiltration (blue arrow), and congestion of the vascular and haemorrhage (red arrows). The section stained with Haematoxylin & Eosin. A. (100x.); B., C, and D. (400x.)

Pirfenidone-Treated Group

The PFD-treated group exhibited mild kidney injury in mice, characterised by a score of 2 (indicating less than 30% renal tubular damage) (Figure 10) ($P < 0.001$). Additionally, this is demonstrated by a significantly lower average in histopathological alterations compared to the ischemic and vehicle groups (as shown in Figures 9: A, B, and C).

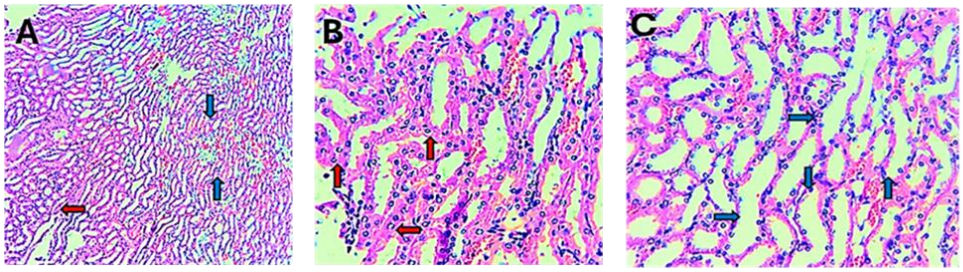

Figure 9: The histopathological examination of the renal section for the **Pirfenidone group**
Mice kidney with score-2 (less than 30% renal tubular damage). Damaged area (red arrows), normal renal tubules (blue arrows). The section stained with Haematoxylin & Eosin. A. (100x.); C. and B (400x.)

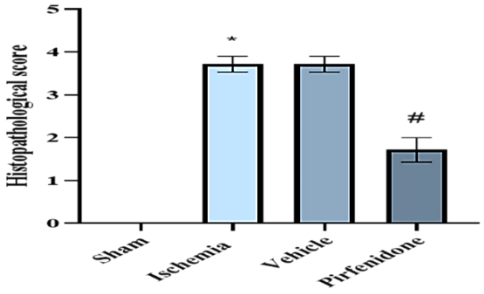

Figure 10: Histopathological scores in the study groups
** Significant versus sham group (P<0.001); # Significant versus ischemia or vehicle groups (P<0.001)*

Discussion

Renal ischemia-reperfusion injury is a condition characterised by increased intratubular pressure in the proximal tubule, during which the ischemia leads to damage of the renal microvasculature, particularly affecting the peritubular capillaries (27). This illness is prevalent, especially in hospitalised patients, accounting for approximately 30% of Intensive Care Unit patients and 7% of hospitalised cases (8).

In this study, there was a significantly higher renal tissue level of IL-6 in the vehicle and ischemia and vehicle groups in contrast with sham group, (P<0.001); Conversely, the PFD-treated group before ischemia induction showed a significantly lower tissue level of pro-inflammatory mediator IL-6 when compared to the vehicle and ischemia groups, (P<0.001). thus, indicating an anti-inflammatory effect against IRI. In their recent study, the authors, Li et al. (2019), revealed that the amount of IL-6 was significantly higher in a rat model with induced ischemic damage (28). Jallawee & Janabi found that the renal tissue levels of IL-6 were elevated in the induced-ischemia group in contrast to the sham group in the RIRI rat model (29). Hong et al., showed that pirfenidone significantly inhibits the synthesis of proinflammatory mediators, which involves the cytokine IL-6 (25). Another study by Zhao et al., indicated that PFD modulates multiple pathways, involving IL-6 (30). This result aligns with the study by Liu & Shi, which showed PFD decreases IL-6 levels in the IRI model (31).

This study shows that the serum levels of KIM-1 is significantly higher in the ischemia group and vehicle groups as compared to the sham group (P<0.001); in contrast, a significantly lower serum concentration level of KIM-1 of PFD group when compared with vehicle and ischemia groups, (P<0.001); thus, showing the reno-protective effect of PFD against RIRI. In 2020, Dase J *et al.* demonstrated that KIM-1 serves as a sensitive marker for detecting inflammatory responses and tubular damage in renal ischemia-reperfusion injury (RIRI). Their study showed an elevation of the KIM-1 molecule in a rat model's kidneys during the early stages of RIRI (32). In 2024, Wahyuni *et al.* showed that rats with induced renal ischemia

had significantly higher levels of KIM-1 compared to the normal and control groups (33). This investigation concurred with the findings of Jallawee & Janabi, indicating that the level of KIM-1 is raised in RIRI (29). In addition, Kadhim *et al.*'s study showed that the ischemia group in rats RIRI model had significantly higher serum KIM-1 levels than in a control group (34), since KIM-1 serves as a specific, sensitive biomarker for hypoxic injury in proximal tubular cells.

The study by Lima-Posada et al., demonstrated that prophylactic administration of PFD prevented acute kidney injury (AKI) in rats subjected to bilateral renal ischemia-reperfusion injury (RIRI) by significantly inhibiting damage to tubular cells (11). Endre, proved that concentrations of certain AKI biomarkers decrease in association with restoration (35). This study backs up what Melo et al., found by showing that PFD has a protective effect against the RIRI in an animal model via significantly lower KIM-1 levels when measured after surgery. This shows that PFD has an inhibitory effect on this marker (36).

The study found that after RIRI, caspase-3 concentrations in kidney tissue were significantly higher in vehicle and ischemia groups compared with the sham group (P<0.001). In contrast, the PFD-pretreated group exhibited significantly lower levels of caspase-3 in renal tissues compared to both the vehicle and ischemia groups (P<0.001). This indicates that PFD has anti-apoptotic properties concerning RIRI.

Alsaaty & Janabi, showed that renal tissue caspase-3 level is significantly increased in the induced I/R group compared to the sham group in the RIRI rat model (37). Jallawee & Janabi, discovered that the caspase-3 value in the kidney tissue was higher in an induced-renal IRI group in the rat model (29). The findings of this study agreed with those of Shan et al., which utilised a rat RIRI model, underwent 45 minutes of renal ischemia after 12 hours of reperfusion. Their results stated a significant increase in caspase-3 levels within the ischemia group compared with the sham group (38). Antar et al. demonstrated that PFD exerts an antiapoptotic effect by significantly downregulating caspase-3 expression, primarily observed in renal tubular epithelial cells (39). This study agreed with Komiya *et al.*, which found that the PFD pretreated group had a significant inhibition of caspase-3 activity when compared to the ischemia group. This result showed that PFD inhibits the inflammatory mediator-induced apoptosis in primary hepatocytes as well as reduces caspase-3 activities (40).

This study's findings showed the blood urea nitrogen and serum creatinine values are significantly higher in the vehicle and ischemia groups when contrasted to the sham group, (*P<0.001*); Conversely, the PFD group had significantly lower blood urea nitrogen (BUN) and serum creatinine (S. Cr.) values when compared to both, vehicle and ischemia groups, (*P<0.001*).

Researchers Tiba et al., & Alaasam et al. found that urea and S. Cr. Levels had been much elevated in the vehicle and the induced-ischemia groups than within the sham group. This happened in rats that had bilateral renal ischemia for 30 minutes and then reperfusion for 2 hours (23, 41), and another study performed by Fu et al. reported that the ischemia significantly increased urea and creatinine levels, suggesting an impaired glomerular function (42). Sun et al.'s study showed that urea and creatinine levels were significantly higher in the I/R group compared to the sham group in the RIRI rat model (43). A study by Matsumoto et al. demonstrated that PFD suppresses the decline in renal function in the treatment group through inhibiting urea and creatinine levels (44). Kane-Gill et al. found that PFD significantly lowers the concentrations of urea and creatinine in the group receiving treatment with it (45). The study, as reported by Hazem et al., indicates that PFD eliminates nephrotoxicity in rats by reducing serum urea and creatinine levels in the group that received PFD treatment, in contrast with an induced nephrotoxic group (46).

This study indicated that the cumulative intensity score of the kidney damage and the injury in tubular tissue in the ischemia and vehicle groups was significantly higher in comparison to the sham group, with a score of 4 for tubular damage in the vehicle and ischemic groups. A much higher score of 0 for tubular damage in the sham group (P<0.001). Histological scanning after renal ischemia demonstrated a loss of brush borders, cellular swelling, tubular dilation, cytoplasmic eosinophilia, development of eosinophilic casts, haemorrhage, inflammation, vascular congestion, and cytoplasmic vacuolization. In contrast, the PFD pre-treatment group had a significantly lower score of tissue injuries/tubular damages to score-2 (P<0.001). This study confirmed that PFD administered 30 min before renal IRI prevents renal injury via significantly lowered histopathological parameters.

A study by Alsaaty and Janabi showed that RIRI caused more tissue damage in the induced group (score-4) than in the sham group (score-0) (37). The study was conducted on a rat model by Jallawee & Janabi, revealed that the histological

analysis of the induced and vehicle groups exhibited significant alterations when compared to the sham group. The alterations encompass coagulative necrosis impacting the cortex and medulla, disruption of normal architecture, infiltration of inflammatory cells, and significant haemorrhaging impacting both the renal cortex and medulla (29). Sharawy and Serrya demonstrated that pirfenidone successfully mitigates induced renal injury via its improved kidney function and histological architecture, which could arise from the inhibition of ROS and NF-kB (47).

The study by Lima-Posada et al., demonstrated that prophylactic administration of PFD prevents renal injury induced by bilateral RIRI in rats by inhibiting extensive tubular damage (11). This result coincides with the findings of Wu et al.,) & Qi et al., which demonstrated that the kidneys of I/R-induced mice exhibit tubular cell swelling, cellular vacuolization, and medullary congestion. Beginning treatment with PFD maintained the normal morphology of the kidney, showing slight oedema of the tubular cells and very mild necrosis (48, 49). An investigation that is currently underway by Mohamed et al., showed the pirfenidone-treated group against induced I/R, which has kidney dysfunction, the PFD group showed moderate improvement in renal activity (50). Manawy et al., reported that the renal cortex from the PFD-group demonstrated that pirfenidone mitigated induced renal damage and histological alterations, regulated relevant pathways, and reduced oxidative stress, inflammatory, and apoptotic markers (51).

Conclusion:

Pirfenidone has a significant reno-protective effect against RIRI, This is evidenced by improved kidney function, demonstrated through the significant inhibition in urea and creatinine concentration, attenuation of kidney injury via a marked reduction in KIM-1, exerting an anti-inflammatory effect indicated by a significant decline in the concentration of proinflammatory marker IL-6, and exerting a potential anti-apoptotic effect via a significant inhibition of pro-apoptotic marker caspase-3.

List of Abbreviations

IRI: Ischemia/reperfusion injury
RIRI: renal ischemia-reperfusion injury
AKI: Acute Kidney Injury)
PFD: Pirfenidone
GFR: Glomerular Filtration Rate

Declarations

Ethics approval and consent to participate

All experimental protocols received approval from the Institutional Animal Care and Use Committee (IACUC) at Kufa University following the filing of the requisite applications (3178 on 4/2/2025).

Consent for Publication

All the authors gave consent for the publication of the work under the Creative Commons Attribution Non-Commercial 4.0 license.

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

ALA: Conceptualisation, methodology, investigation, data curation, writing, original draft preparation.
MBB: Formal analysis, validation, writing, review and editing, visualisation, supervision.

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