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Evaluation of the nephroprotective effect of dapagliflozin in renal ischaemia-reperfusion injury in male rats: *Foxo1* modulation and pyroptosis

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ABSTRACT

Renal ischaemia-reperfusion injury (RIRI) significantly contributes to acute kidney injury, leading to high morbidity and mortality rates. This study has investigated the nephroprotective effects of dapagliflozin, a sodium-glucose co-transporter 2 inhibitor, on RIRI in male Wistar rats. Twenty four rats were divided into four groups (n=6 each): a sham group (without an induction of RIRI), a control group (with an induction of RIRI), a vehicle group (receiving an intraperitoneal injection of 10% v/v dimethyl sulfoxide in isotonic saline solution, 1 h before ischaemia), and a dapagliflozin-treated group (receiving an intraperitoneal injection of dapagliflozin at 1 mg/kg, 1 h before ischaemia). Ischaemia was induced by clamping the renal pedicles for 30 min, followed by 2 h of reperfusion. Histopathological analysis revealed marked renal tissue injury in the control and vehicle groups, while dapagliflozin administration reduced these injuries, suggesting that less than 50% of the renal tubules were affected as a result. Kidney injury molecule-1, caspase-1, and caspase-11 levels were found to be significantly elevated in the control and vehicle groups, but decreased in the dapagliflozin group. The forkhead box O1 gene (*Foxo1*) expression was also found significantly reduced as a result of the treatment. These findings suggest that dapagliflozin mitigates renal injury through the suppression of *Foxo1* expression and pyroptosis, thereby improving renal cell integrity. This study highlights dapagliflozin's potential as a nephroprotective agent against acute renal failure, as it can enhance renal cell survival and mitigate injury pathways.

KEY WORDS:
renal ischaemia-reperfusion; dapagliflozin; nephroprotection; pyroptosis; FOXO1

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1. Introduction

Renal ischaemia-reperfusion injury (RIRI) can cause acute kidney injury and is associated with high morbidity and mortality[1,2]. During RIRI, oxidative stress (recognized by the production of reactive oxygen species) triggers inflammatory responses, which interfere with tissue repair and regeneration and disrupt renal function. Kidney injury molecule-1 (KIM-1), a proximal tubular protein, peaks following RIRI, acting as a vital biomarker for the early identification of the latter[3]. Pyroptosis, a programmed cell death, takes up a significant role in RIRI; it is principally mediated by caspase-1 and caspase-11, contributing to tubular epithelial cell death[4].

Dapagliflozin, a sodium-glucose co-transporter 2 inhibitor, is a novel therapeutic option exhibiting efficacy in glycaemic management and probable renal protection[5]. The forkhead box O1 (FOXO1) gene (humans: *FOXO1*; rats: *Foxo1*) expression is induced in RIRI, leading to mitochondrial dysfunction. Inhibition of the *Foxo1* expression is known to retard apoptosis, enhance mitochondrial function, and hasten renal function[6]. The aim of this study was to assess the ability of dapagliflozin in potentially mitigating RIRI in a rat model, by focusing on *Foxo1* expression and pyroptosis.

2. Methodology

2.1. Study design and experiments

Twenty four adult male albino Wistar rats (weighting 200–250 g) were utilized in this study. The rats were randomly allocated into four groups (n=6 each): (i) a sham group (without an induction of RIRI), (ii) a control group (with an induction of RIRI), (iii) a vehicle group (receiving an intraperitoneal injection of 10% v/v dimethyl sulfoxide in isotonic saline solution, 1 h before ischaemia), and (iv) a dapagliflozin-treated group (receiving an intraperitoneal injection of dapagliflozin at 1 mg/kg, 1 h before ischaemia). Anaesthesia was administered *via* an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Ischaemia was induced through the clamping of both renal pedicles for 30 min, followed by 2 h of reperfusion.

Following reperfusion, the rats were euthanized under anaesthesia and kidney samples were collected for analysis. The right kidneys were imbedded in formalin for histological examination, while the left kidneys were kept at -80°C for the undertaking of ELISA and genetic analysis.

The undertaken histopathological assessment involved kidney samples processing, followed by staining with haematoxylin and eosin for microscopic examination. Quantitative and qualitative renal tissue damage assessment were achieved through the scoring of tissue degeneration.

Tissue homogenates from the frozen kidneys were washed, homogenized in phosphate buffered saline with protease inhibitors and Triton X-100, and they were subsequently centrifuged. Supernatants were aliquoted and frozen for the measurement of the levels of KIM-1 and of pyroptosis biomarkers (i.e., caspase-1 and caspase-11) through ELISA. In addition, total RNA extraction was undertaken for a real-time PCR analysis of the expression of *Foxo1*.

2.2. Statistical analysis

The SPSS v.26 software was utilized for the analysis of the current study's data. All values are expressed as mean ± standard deviation. One-way ANOVA (followed by a Tukey test) was used in order to assess the differences in the variables' means among the study groups, and a significance level was considered for p-values that were <0.05.

2.3. Ethical approval

Ethical approval for the present study was granted by the Central Committee for Bioethics of the University of Kufa (approval number: 20552; date: August 29, 2024).

3. Results and Discussion

The histopathological assessment of the renal tissue injury in both the control and vehicle groups has rec-

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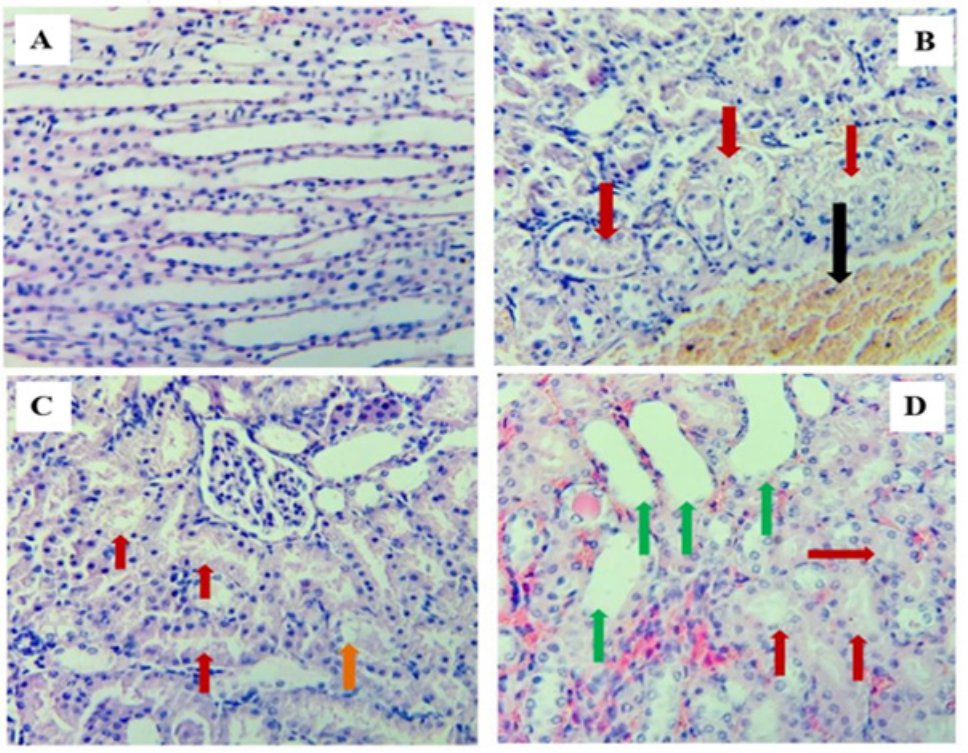

Figure 1. *Representative microphotographs from the histopathological evaluation (using haematoxylin and eosin) of kidney tissues from the four herein studied rat groups. (A): The sham group tissue samples exhibited normal glomeruli and tubules. (B,C): The control (B) and the vehicle (C) group tissue samples exhibited significant alterations involving the development of cytoplasmic swelling and eosinophilia (red arrows), vascular congestion (black arrow), and cytoplasmic vacuoles (orange arrow). (D): The dapagliflozin-treated group tissue samples exhibited a significant improvement of the renal tissue damage when compared to that observed in the control group; damaged tubules (red arrows) and normal tubules (green arrows) are highlighted. Caption magnification: x400 (A–D).*

ognized cytoplasmic swelling and eosinophilia, vascular congestion, and marked alterations in glomerular and tubular structures. In contrast, the dapagliflozin-treated group samples exhibited a reduction in the number of these pathological changes, with injury scores indicating that less than 50% of the renal tubules were affected by comparison (Figure 1).

KIM-1 levels were found to be significantly raised in the control (1,546.87 ± 71.71 pg/mL) and vehicle (1,488.52 ± 54.93 pg/mL) group kidney samples as compared to those of the sham group (1,308.73 ± 95.58 pg/mL; p=0.005), while dapagliflozin significantly decreased the kidney KIM-1 levels (1,185.98 ± 140.49 pg/mL) as compared to those of the control group (p<0.001).

The rat kidney caspase-1 levels were found to be significantly raised in the control (8,402.76 ± 681.21 pg/mL) and the vehicle (8,152.56 ± 239.96 pg/mL) groups as compared to those of the sham group (6,998.98 ± 229.23 pg/mL; p=0.005), while dapagliflozin significantly decreased the kideny caspase-1 levels (6,271.62 ± 281.37 pg/mL) as compared to those of the control group (p<0.001). The caspase-11 levels were found to be significantly raised in the control (2,941.23 ± 203.70 pg/mL) and the vehicle (2,929.38 ± 235.67 pg/mL) rat groups as compared

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to those of the sham group (2,542.00 ± 229.70 pg/mL; p=0.020), while dapagliflozin significantly decreased the kideny caspase-11 levels (2,305.92 ± 201.21 pg/mL) as compared to those of the control group (p<0.001).

Finally, the rat kidney *Foxo1* expression was found to be significantly diminished in the control (0.30 ± 0.03 mg/dL) and the vehicle (0.18 ± 0.02 mg/dL) groups as compared to that of the sham group (1.01 ± 0.15 mg/dL; p<0.001). Dapagliflozin administration further reduced *Foxo1* expression (0.14 ± 0.02 mg/dL) as compared to that of the control group (p=0.012).

Our study has aimed at evaluating the effect of dapagliflozin on acute renal failure. Our results suggest that the pre-RIRI administration of dapagliflozin can alleviate renal tissue damage and minimize the mean tissue score damage to the moderate level of renal impairment. Furthermore, dapagliflozin has herein been found to suppress the expression of the *Foxo1* and prevent pyroptosis in the renal tubular epithelium. No studies exist that have assessed the effects of dapagliflozin on the same parameters in rats with RIRI. Nevertheless, Jallawee and Janabi (2024)[3] have recently reported that a pretreatment with dapagliflozin can significantly mitigate the renal tubular injuries, as evidenced by histological evidence in rats with RIRI.

Our study has shown that the *Foxo1* expression was reduced following RIRI, which is consistent with previous studies reporting that the FOXO1 gene expression is regulated by FOXO proteins: following ischaemic conditions, the PI3K/Akt pathway is activated, triggering the nuclear export of FOXO and the transcriptional suppression of FOXO1 subsequently to the PI3K-dependent FOXO phosphorylation in the cytoplasm[7,8]. Cheng[6] has demonstrated that, FOXO1 regulates target genes implicated in oxidative stress and energy metabolism, thereby affecting mitochondrial function and integrity: it downregulates the antioxidant enzyme expression (which protects against oxidative damage) and plays a critical role in the deterioration of the mitochondrial function. Studies have also concluded that the FOXO1 overexpression can worsen ischaemic injury through the upregulation of gasdermin D; a pyroptosis-related protein[9]. Therefore, the further suppression of the FOXO1 gene expression may diminish pyroptosis and oxidative stress, and could mitigate RIRI by maintaining mitochondrial homeostasis in the renal tubular epithelial cells.

4. Conclusion

Dapagliflozin exhibits nephroprotective effects against RIRI through the suppression of *Foxo1* expression and pyroptosis in rats, thereby improving renal cell integrity and mitigating tissue damage.

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Conflicts of interest

None exist.

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