



ORIGINAL PAPER

# Sotagliflozin prevents acute kidney injury by suppressing oxidative stress, inflammation, and apoptosis in renal ischemia/reperfusion rat model

Nisreen Abd Alhassan Hamza Alshimary , Thu-Alfeqar Razzaq Tweij 

Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Kufa, Najaf, Iraq

## ABSTRACT

**Introduction and aim.** Acute kidney injury (AKI) is a life-threatening condition with limited effective pharmacological options. Although sodium-glucose cotransporter 2 (SGLT2) inhibitors have shown renal protective effects, the potential role of the dual SGLT1/2 inhibitor sotagliflozin in ischemia/reperfusion injury (IRI) has not been previously investigated. We aimed to evaluate its nephroprotective properties in a rat model of renal IRI.

**Material and methods.** Twenty-four male Sprague-Dawley rats were randomized into four groups (sham, control, dimethyl sulfoxide [DMSO], sotagliflozin). Renal IRI was induced by 40 min ischemia followed by 2 h reperfusion. Rats received either DMSO or sotagliflozin (10 mg/kg, intraperitoneally) 24 h and 1 h before surgery. Kidney function (urea, creatinine, neutrophil gelatinase-associated lipocalin [NGAL]), oxidative stress (8-iso-prostaglandin F<sub>2α</sub> [8-iso-PGF<sub>2α</sub>]), inflammation (tumor necrosis factor-α [TNF-α]), apoptosis (caspase-3), and histopathology were assessed.

**Results.** In the control group, serum urea ( $106.5 \pm 2.9$  mg/dL), creatinine ( $1.52 \pm 0.09$  mg/dL), and NGAL ( $64.5 \pm 3.6$  ng/mL) were significantly higher than in the sham group ( $32.6 \pm 5.3$ ,  $0.89 \pm 0.06$ ,  $49.5 \pm 3.8$ , respectively;  $p < 0.0001$ ). Tissue 8-iso-PGF<sub>2α</sub> ( $63.8 \pm 5.9$  pg/mL), TNF-α ( $186 \pm 7$  pg/mL), and caspase 3 ( $120.3 \pm 6.5$  pmol/L) were also elevated vs. sham ( $35.6 \pm 3.6$ ,  $137 \pm 7$ ,  $92.3 \pm 4.9$ ;  $p < 0.0001$ ). Sotagliflozin pretreatment reduced urea ( $53.8 \pm 2.8$  mg/dL), creatinine ( $1.04 \pm 0.07$  mg/dL), NGAL ( $49.6 \pm 6.4$  ng/mL), 8-iso-PGF<sub>2α</sub> ( $41.3 \pm 3.9$  pg/mL), TNF-α ( $140 \pm 6.6$  pg/mL), and caspase 3 ( $89.7 \pm 2.4$  pmol/L; all  $p < 0.0001$  vs. control). Histological injury scores improved from 4.0 in control to 1.0 in the sotagliflozin group ( $p < 0.05$ ).

**Conclusion.** Sotagliflozin significantly improved renal function and histopathological damage in rats with renal IRI by attenuating oxidative stress, inflammation, and apoptosis. These findings support its potential as a candidate for further investigation in the prevention of AKI.

**Keywords.** acute kidney injury, apoptosis, oxidative stress, sotagliflozin

## Introduction

Acute kidney injury (AKI) is common in emergency department patients, raising the risk of chronic kidney disease, and is responsible for an estimated 2 million deaths annually worldwide.<sup>1</sup> It can be defined as a rapid decline in kidney function typically occurring over a short period, leading to acute damage to the renal pa-

renchyma, disturbances in electrolyte balance, fluid retention, electrolyte imbalances, and nitrogenous metabolic waste product accumulation.<sup>2-4</sup> Several factors can impair blood flow, including organ transplantation, systemic infections, trauma or crush injuries, and atherosclerotic plaque buildup in coronary arteries that restricts oxygen delivery to the heart.<sup>5-6</sup>

**Corresponding author:** Nisreen Abd Alhassan Hamza Alshimary, e-mail: [nisreena.alshimary@student.uokufa.edu.iq](mailto:nisreena.alshimary@student.uokufa.edu.iq)

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Renal ischemia is especially harmful as the kidneys are highly metabolic organs that depend mostly on a continuous blood flow to operate. Particularly in the proximal tubule, the thick ascending limb, and tubular epithelial cells, whose high energy needs make them most vulnerable when blood flow is decreased.<sup>7</sup> By starting the ischemia phase, ATP levels decreased, and the energy-dependent processes like ion transport, protein synthesis, and maintenance of membrane integrity began to fail. The loss of cell polarity and the inability of Na<sup>+</sup>/K<sup>+</sup>-ATPase pump to maintain ionic gradients result from cytoskeletal disruption. This, in turn, causes intracellular sodium and calcium excess, which leads to cell swelling and activates harmful protease enzymes.<sup>8</sup>

When blood flow is restored during the reperfusion phase, a high level of reactive oxygen species (ROS) is created. Along with the reduction in the body's antioxidant enzyme activity, these harmful compounds damage DNA, peroxidize lipid membranes, and cause protein oxidation.<sup>9</sup> 8-iso-prostaglandin F2 $\alpha$  (8-iso-PGF2 $\alpha$ ) is a part of a group of chemical compounds called f2-isoprostanes, formed in kidney tissue by non-enzymatic oxidation of arachidonic acid in membranes and released into the circulation and urine.<sup>10</sup> Aside from acting as indicators of oxidative stress, 8-iso-PGF2 $\alpha$  actively worsens renal ischemia and reperfusion injury (IRI) by causing vasoconstriction and inflammation. It also impairs glomerular filtration, disturbs tubular function, and increases endothelial dysfunction and fibrosis, all of which contribute directly to the progression of renal impairment.<sup>11</sup> Inflammation is an early response to IRI. A pro-inflammatory cytokine such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is rapidly elevated in renal tissue following ischemia/reperfusion. It primarily communicates through TNF receptor 1 (TNFR1), which features a death domain that triggers apoptotic pathways. TNFR1 activation attracts adaptor proteins that convert procaspase 8 into its active form, caspase 8. The active caspase 8 then cleaves and enhances caspase 3 activation, leading to apoptotic cell death.<sup>12</sup> Now, treatments for renal ischemia are limited to supportive measures, including hydration and renal replacement therapy; no effective therapeutic alternatives are available. Many drugs have been studied for their possible kidney-protecting effects, but the dual inhibition of sodium-glucose cotransporter (SGLT1/2) has not been studied enough in this context. Sotagliflozin is a new oral dual SGLT1/2 inhibitor that uniquely acts on both glycemic and non-glycemic pathways, including modulation of inflammation, oxidative stress, and sodium handling, which are central in IRI pathogenesis.<sup>13</sup> It lowers blood glucose levels in people with type 2 diabetes mellitus by inhibiting SGLT2 in the proximal tubule and blocking SGLT1 in the small intestine and kidney, leading to decreased postprandial glucose absorption and enhanced

incretin release, which supports glycemic control, blood pressure reduction, and weight loss alongside favorable renal outcomes.<sup>14</sup> Furthermore, emerging evidence indicates that sotagliflozin shows unique cardiovascular advantages that are not uniformly seen with selective SGLT2 inhibitors.<sup>15,16</sup> Apart from its strong blood glucose-lowering action, new data indicate that sotagliflozin also lowers inflammation, slows down cell death, and provides antioxidative effects.<sup>17</sup> While those outcomes are encouraging, more research is needed to better understand their long-term safety and establish the appropriate therapeutic application across a wide range of patient conditions. While renal protective effects of selective SGLT2 inhibitors have been reported, the potential of the dual SGLT1/2 inhibitor sotagliflozin in ischemia/reperfusion injury has not yet been systematically evaluated. This study explores this novel application in a rat model.

## Aim

This study aims to evaluate the nephroprotective potential of sotagliflozin in an experimental model of renal ischemia/reperfusion by assessing histological changes and key biochemical markers, including serum urea, creatinine, NGAL, as well as renal tissue 8-iso-PGF2 $\alpha$ , TNF- $\alpha$ , and caspase 3, employing spectrophotometric and enzyme linked immunosorbent assay (ELISA) methodologies.

## Material and methods

### Animal preparation

The University of Kufa, Faculty of Science, provided 24 male Sprague Dawley adult rats, weighing approximately 180–250 g and aged 12 to 18 weeks. The rats were housed in the animal facility. Equipped with a 12-hour light-dark cycle, they were maintained at room temperature (approximately 24 $\pm$ 2°C) and humidity (approximately 60 $\pm$ 5%). Housed separately using a group-caging approach, the animals had free access to water and food. Female rats and those with other abnormalities were excluded from consideration. All experimental procedures and animal handling were carried out after receiving approval from the Institutional Animal Care and Use Committee (IACUC) at Kufa University, following the submission of the required documents (NO.2126) on January 23, 2025.

### Study design

After 7 days of adaptation, a computer-generated sequence was used to assign twenty-four Sprague-Dawley rats into four experimental groups (n = 6 each group) to ensure that the groupings were random. The sham group underwent identical procedures to the control group, excluding the clamping of the renal pedicles. However, the control group experienced bilateral re-

nal ischemia for 40 min, followed by 2 h of reperfusion, based on previous preclinical studies.<sup>10,18</sup> While in the dimethyl sulfoxide (DMSO) group, the rats underwent the same procedures as the control group but were given two doses of DMSO intraperitoneally (IP), the first 24 h before surgery and the second one hour before, which was considered pharmacologically inert and often used in preclinical studies.<sup>19</sup> The sotagliflozin group was injected with two doses of sotagliflozin (10 mg/kg), the first 24 h and the second, one hour before surgery. A dose of 10 mg/kg was selected based on previous *in vivo* studies.<sup>19-20</sup>

### *Model of induction ischemia/reperfusion injury*

Male rats were anesthetized using IP injections of ketamine hydrochloride (100 mg/kg) from (Sigma-Aldrich, USA, Cat. No K2753) combined with xylazine hydrochloride (10 mg/kg) from (Sigma-Aldrich, USA, Cat. No X1251).<sup>21</sup> The level of anesthesia was monitored throughout the surgery by looking at the pedal withdrawal response, corneal reaction, and breathing rate. Body temperature was maintained between 36.8°C and 37.3°C using a feedback-controlled heating pad, with the animal positioned prone. Renal ischemia was induced surgically by making flank incisions to expose both kidneys. Bilateral renal pedicles were occluded for 40 min using non-traumatic microvascular clamps to minimize tissue damage. The successful start of ischemia was proven by the appearance of uniform kidney pallor, consistent with previously documented models.<sup>22-23</sup> Once the ischemic period ended, the clamps were removed to initiate reperfusion, which was maintained for 2 h. Reperfusion was visually confirmed by the return of a reddish coloration to the kidneys. Surgical incisions were then closed using standard procedures.

### *Drug preparation*

Sotagliflozin (TargetMol, Cat. No. T3547) was administered IP as two doses. It exhibits a solubility of 50 mg/mL in DMSO, the standard solvent, according to the manufacturer's instructions, and we prepared it immediately before use. The dosage was dissolved in DMSO (10% v/v) and administered based on body weight. The time and dose were based on previous investigations that looked at how sotagliflozin works in the body and how well it gets into tissues.<sup>20,24</sup>

### *Blood sample collection*

After surgery, while the rat was still anesthetized, we obtained around 4 milliliters of blood from the heart of the rat by direct cardiac puncture.<sup>25</sup> To separate the serum from the blood, it was placed in a gel tube from (Medic-Home, China) that does not include any anticoagulant. Then the rat was immediately euthanized un-

der ethical guidelines, and kidney tissue was harvested promptly to assess additional experimental parameters. The blood samples were kept for half an hour at ambient temperature, then centrifuged at 5000 rpm for about 10 min using a centrifuge (Hettich, Germany). We then used the resulting serum to identify urea, creatinine by using a spectrophotometer (Emclab/ Germany), as well as to identify NGAL by using an accessible rat-specific enzyme-linked immunosorbent assay (ELISA) kit (Ideal Medical Technology, Shanghai, Cat No. ADL-EL-RT00241).

### *Tissue sample preparation*

After euthanizing the rats and taking the kidneys, the excised left kidneys were cut sagittally: one portion was frozen at -80°C for homogenization and utilized for further biochemical investigations, while the other portion was fixed by immersion in 10% formaldehyde for further histological examination. The frozen portion was homogenized in a 1:10 weight/volume phosphate-buffered saline containing 1% Triton X-100 and a protease inhibitor cocktail.<sup>26</sup> The homogenate was centrifuged for 10 minutes at 4°C and 6000 rpm. The supernatants were utilized for the assessment of the following parameters using rat-specific ELISA kits from (Ideal Medical Technology, Shanghai): 8-iso-PGF2 $\alpha$  (Cat No: ADL-EL-RT00985), TNF- $\alpha$  (Cat No: ADL-EL-RT00160), and caspase 3 (Cat No: ADL-EL-RT00268). The results were obtained by following the instructions provided by the kit's manufacturer. To reduce bias, the assessors responsible for assessment of outcomes of biochemical tests were blinded to the groups of therapy.

### *Preparation of tissue for histopathological examination*

After fixation in formaldehyde, the kidney tissues were rinsed in phosphate-buffered saline (PBS) and dehydrated through a graded ethanol series followed by xylene. The tissues were then embedded in paraffin. Sections of 5  $\mu$ m thickness were cut using a microtome and stained with hematoxylin and eosin (H&E) for examination in a blinded method by a pathologist using a light microscope to assess kidney histological injury. The level of renal tubular injury was determined according to the severity of alterations in tubular cells, such as necrosis, hypertrophy, glomerular atrophy, and inflammatory cell infiltrate. Scores were given to these alterations as follows: score 0 (no pathological alterations), score 1 (<25%), score 2 (25–50%), score 3 (50–75%), score 4 (>75%).<sup>27</sup>

### *Statistical analysis*

GraphPad Prism, version 10 (GraphPad Software, San Diego, CA, USA) was used for the statistical analysis. Mean with standard deviation (SD) was the method of presentation of the findings. A one-way analysis of vari-

ance (ANOVA) was utilized to verify statistical significance across various treatment groups, with Tukey’s multiple comparisons serving as a post-analytical test. A p-value of less than 0.05 was statistically significant. The histopathological variations were also compared among groups using Kruskal-Wallis and Dunn’s test.

Results

Effect of sotagliflozin on renal function test

The control and DMSO groups exhibited a substantial increase in urea and creatinine levels in their serum, when compared with the sham group ( $p<0.0001$ ), contributing to impaired renal function, as illustrated in Figure 1. Sotagliflozin substantially decreased serum levels of creatinine and urea in contrast to each of the control and DMSO groups ( $p<0.0001$ ). Compared to the control group, the DMSO group did not demonstrate any evident differences in levels of urea ( $p=0.276$ ) and creatinine ( $p=0.828$ ).

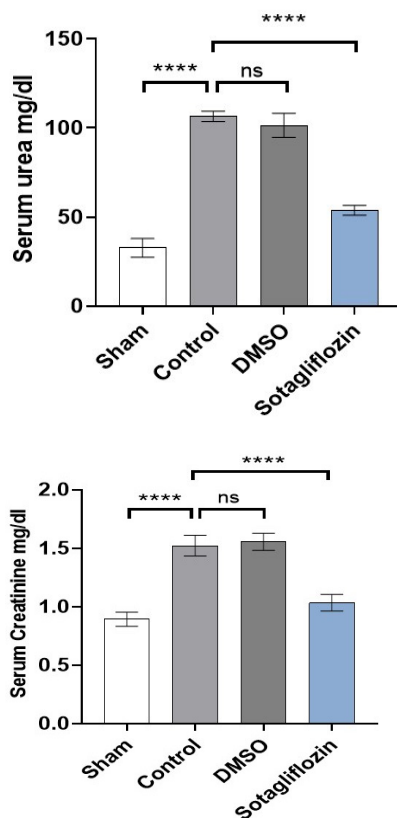


Fig. 1. Mean serum levels of urea and creatinine among experimental groups, ns – no significant difference, \*\*\*\* –  $p<0.0001$ , results are presented as the mean±standard deviation (n=6 per group)

Effect of sotagliflozin on neutrophil gelatinase-associated lipocalin (NGAL)

The study demonstrated that the control and DMSO groups exhibited substantially elevated serum NGAL levels compared to the sham ( $p<0.001$ ), which is an ear-

lier biomarker of tubular injury as highlighted in Figure 2. Pretreatment with sotagliflozin significantly reduced NGAL relative to the control group ( $p<0.001$ ). The DMSO group did not exhibit any significant alterations concerning the control group ( $p=0.318$ ).

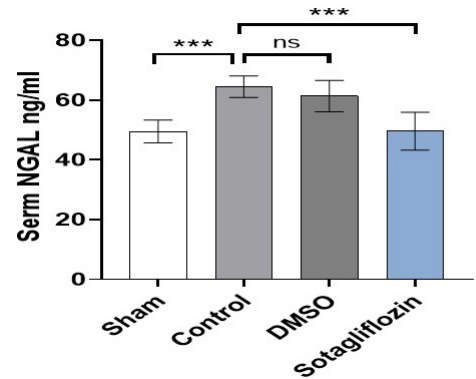


Fig. 2. Mean serum levels of NGAL among experimental groups ns. – no significant difference, \*\*\* –  $p<0.001$ , results are presented as the mean±standard deviation (n=6 per group)

Effect of sotagliflozin on 8-iso-PGF2α

Our results showed that there was an elevation in the level of 8-iso-PGF2α in the renal tissue homogenate of the control and DMSO groups compared to the sham group ( $p<0.0001$ ), which is considered an indicative biomarker of lipid peroxidation and oxidative damage to renal tissues due to ischemia/reperfusion, as seen in Figure 3. Sotagliflozin significantly reduced these levels ( $p<0.0001$ ) as opposed to the control. However, no observable variations in renal tissue 8-iso-PGF2α in the DMSO group compared to the control group ( $p=0.999$ ).

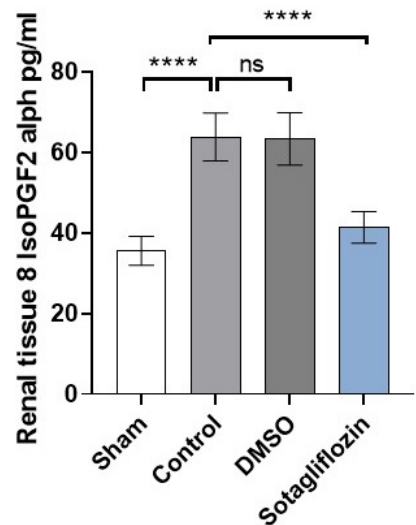
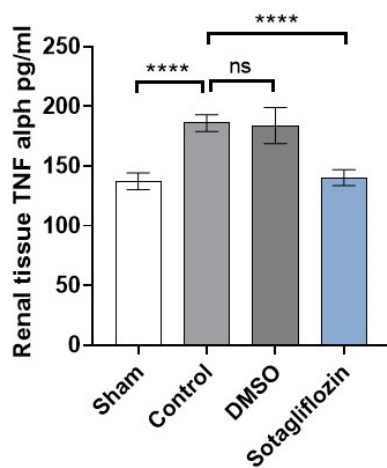


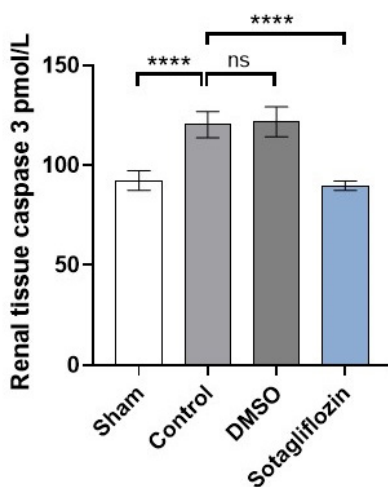
Fig. 3. Mean tissue levels of 8-Iso-PGF2α among experimental groups, ns – no significant difference, \*\*\*\* –  $p<0.0001$ , results are presented as the mean±standard deviation (n=6 per group)

**Effect of sotagliflozin on TNF- $\alpha$**

Following ischemia/reperfusion, tissue proinflammatory cytokine (TNF- $\alpha$ ) was significantly raised in the control and vehicle groups versus the sham group ( $p<0.0001$ ), as shown in Figure 4. Sotagliflozin significantly reduced tissue (TNF- $\alpha$ ) compared with a control group ( $p<0.0001$ ). The DMSO and control groups did not vary significantly ( $p=0.705$ ).



**Fig. 4.** Mean tissue levels of TNF- $\alpha$  among experimental groups, ns – no significant difference, \*\*\*\* –  $p<0.0001$ , results are presented as the mean $\pm$ standard deviation ( $n=6$  per group)



**Fig. 5.** Mean tissue levels of caspase 3 among experimental groups, ns – no significant difference, \*\*\*\* –  $p<0.0001$ , results are presented as the mean $\pm$ standard deviation ( $n=6$  per group)

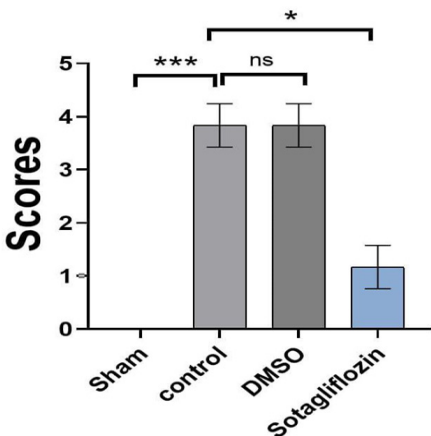
**Effect of sotagliflozin on caspase-3**

Rats that underwent ischemia showed a significant up-regulation of apoptotic marker caspase 3 in their kidney homogenate compared to sham rats ( $p<0.0001$ ). This upregulation suggests that tubular cell death and kidney damage progression were accelerated in the DMSO and control groups, as shown in Figure 5. Sotagliflozin

considerably decreased caspase 3 expression compared to the control ( $p<0.0001$ ). There was no noticeable variance between the control and vehicle groups ( $p=0.902$ ).

**Histopathological examination**

As seen in Figures 6 and 7A, 7B, 7C, and 7D, histological studies showed that the sham rats had normal glomeruli, tufts, and capsules, indicating a normal renal tissue microstructure a score of 0 ( $p<0.001$ ). However, the control group's renal tubules developed inflammation, loss of brush border of proximal tubules, epithelial hypertrophy of lining cells, and the lumen of the proximal renal tubules became narrowed, with coagulative necrosis, and a vascular congestion with a score of 4. Similar histopathological abnormalities were seen in the DMSO group versus the control group. In contrast, the sotagliflozin group showed improvement in renal tubular architecture with preservation of the typical glomerular capsule and tuft. Moreover, mild cytoplasmic vacuolation and slight hypertrophy were observed in the tubular epithelial cells. These changes corresponded with a statistically significant reduction in injury scores compared to the control group, a score of 1, and  $p<0.05$ .

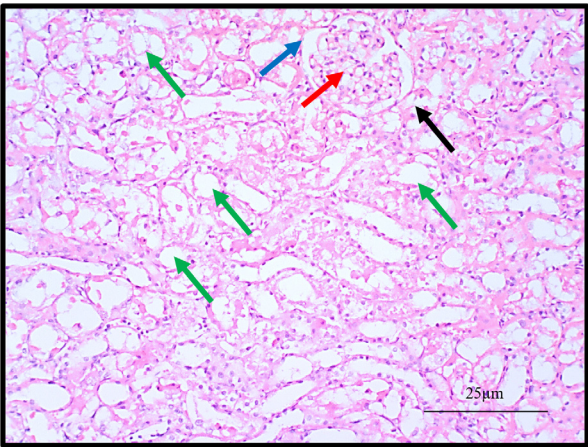


**Fig. 6.** Scores of renal tubular injuries in the studied groups, ns – no significant difference, \*\*\* –  $p<0.001$  vs. sham group, \* –  $p<0.05$  vs. control group, the Kruskal-Wallis and Dunn's tests were used for analyzing the data, the data are presented as the mean $\pm$ standard deviation ( $n=6$  per group)

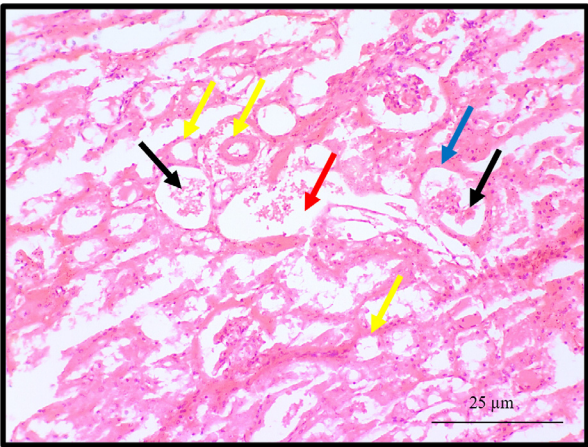
**Discussion**

To our knowledge, this is the first experimental evidence that sotagliflozin attenuates biochemical and histological features of renal IRI, expanding current understanding of SGLT inhibition beyond selective SGLT2 blockers. A major challenge in the proficiency of organ transplantation is the ischemia/reperfusion storm. So, it is essential to improve transplant techniques while developing an innovative treatment to avoid IRI.<sup>28</sup> There is a surge in local production of proinflammatory cytokines after injury from ischemia/reperfusion. These cytokines

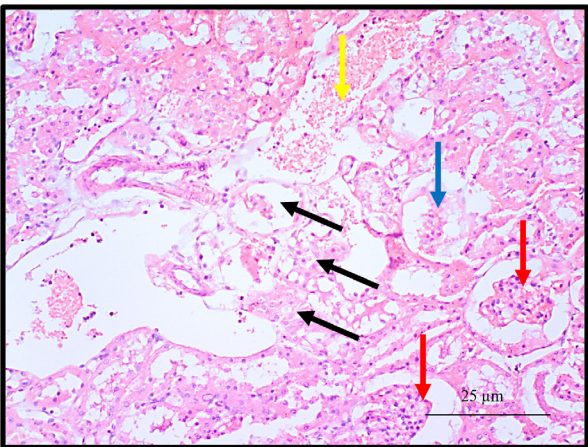




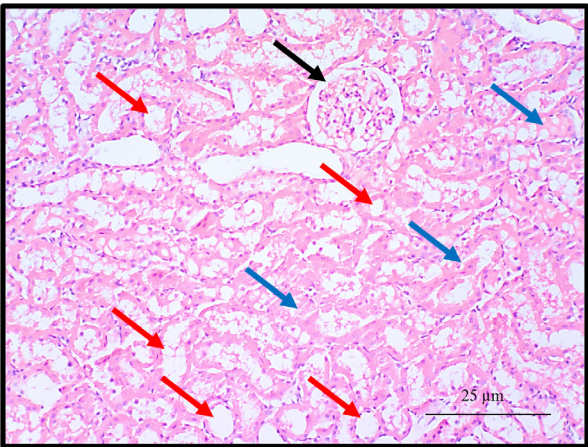
**Fig. 7A.** The histological section of kidney of a rat in sham group, the section shows normal renal tissue texture including normal glomeruli (black arrow), normal glomerular tuft (red arrow), and normal glomerular capsule (blue arrow), the proximal renal tubules show normal epithelial cells that line the tubules with a normal lumen (green arrows), the tissue was stained with H&E, and a digital camera and light microscope were used to observe the slice at a magnification of 100×



**Fig. 7C.** The histopathological section of kidney of a rat in DMSO group, this section of renal tissue shows severe atrophic lesion of renal glomeruli and glomerular tufts (black arrows) with severe hypertrophic changes in the glomerular capsule (blue arrow), the blood vessels show clear wall damage and congestion (red arrow), the proximal renal tubules show narrowing in the lumen with epithelial hypertrophy of lining cells (yellow arrows), the tissue was stained with H&E, and a digital camera and light microscope were used to observe the slice at a magnification of 100×



**Fig. 7B.** The histopathological section of kidney of a rat in control group, the section shows clear necrotic lesions in the renal tissue as coagulative necrosis (black arrows) with glomerular atrophy can be seen in the section of renal tissue (red arrows), the glomerular tuft shows severe necrotic change in epithelial cells of tuft (blue arrow), blood vessel congestion, as severe pathological changes can be seen in this section (yellow arrow), the tissue was stained with H&E, and a digital camera and light microscope were used to observe the slice at a magnification of 100×

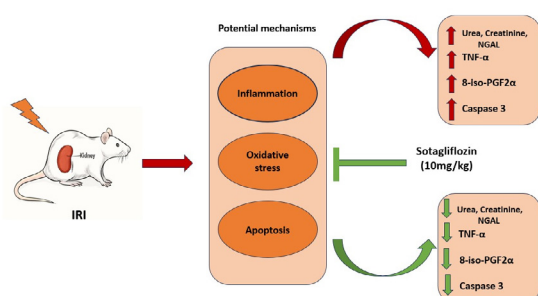


**Fig. 7D.** The histopathological section of kidney of a rat in sotagliflozin-treated group, this section shows a normal glomerular capsule and tuft (black arrow) with normal proximal renal tubule lumen (red arrows), the epithelial lining cells of renal tubules show moderate hypertrophy and vacuolation of cytoplasm (blue arrows), the tissue was stained with H&E, and a digital camera and light microscope were used to observe the slice at a magnification of 100×

can either intensify organ damage and dysfunction via the promotion of ROS production and the recruitment of inflammatory cells, or they may initiate protective physiological responses that limit and mitigate tissue damage.<sup>21</sup> The current study demonstrated that functional and structural kidney biomarkers like urea, creatinine, and NGAL levels were considerably higher in the groups undergoing renal ischemia/reperfusion surgery, indicating deterioration in renal function and tubular damage. A previous experimental study, harmonious with our findings, found that rats in control and vehicle groups demonstrated significant elevation in urea, Cr, and NGAL levels, as a consequence of renal ischemia/reperfusion.<sup>29</sup> However, sotagliflozin greatly lowered these renal biomarkers and improved filtration function. To the best of our knowledge, no previous research has focused on investigating the impact of sotagliflozin on urea, creatinine, and NGAL, in the context of renal IRI. Nevertheless, an earlier study demonstrated that mice administered SGLT inhibitors, such as empagliflozin, following renal ischemia and reperfusion exhibited a significant decrease in serum urea, creatinine, and NGAL.<sup>30</sup> When blood flow is restored in reperfusion period, the quick oxygen supply inversely causes a surge in the generation of ROS, mostly from mitochondrial complexes I and III, as well as from xanthine oxidase and NADPH oxidase pathways. These ROS start a chain reaction of cell damage, the most important of which is lipid peroxidation.<sup>31</sup> 8-iso-PGF2 $\alpha$  is produced when the excessive ROS generation attacks arachidonic acid in cell membranes, leading to oxidative damage to renal tissues that weakens these membranes, making them more permeable, causing ionic equilibrium to be lost, and eventually causing necrotic and apoptotic cell death.<sup>9,32</sup> The current study revealed a significant elevation in 8-iso-PGF2 $\alpha$  levels in the homogenate of renal tissue for the control and DMSO groups. This result is in line with findings of Basu and colleagues, who showed 8-iso-PGF2 $\alpha$  levels were substantially raised upon reperfusion of the transplanted graft after human renal transplantation.<sup>33</sup> Interestingly, sotagliflozin reduced 8-iso-PGF2 $\alpha$ , therefore displaying antioxidative properties. As far as we are aware, no previous projects have detailed how sotagliflozin affects the 8-iso-PGF2 $\alpha$  in a rat model of renal IRI. However, several studies provided evidence that SGLT inhibitors such as dapagliflozin and canagliflozin have antioxidative impacts by lowering the production of ROS and these isoprostane compounds in various experimental models and settings.<sup>34-35</sup> At the same time, ROS is considered a powerful signaling molecules that turn on redox-sensitive transcription factors that include nuclear factor-kappa B (NF- $\kappa$ B), thereby stimulating pro-inflammatory cytokines, such as TNF- $\alpha$ , which makes the inflammatory response stronger, contributing to leukocyte recruitment, endo-

thelial dysfunction, and tubular cell damage.<sup>12,36</sup> Our study demonstrated a significant increase in TNF- $\alpha$  in the rats after undergoing ischemia. This finding aligns with Woods and coworkers, who observed that TNF- $\alpha$  was significantly elevated in mice following renal IRI.<sup>35</sup> By dramatically reducing TNF- $\alpha$ , our findings exhibited that sotagliflozin has potential nephroprotective effects by mitigating inflammation and improving kidney function after renal IRI. To our knowledge, no prior research has examined the impact of sotagliflozin on TNF- $\alpha$  levels in renal IRI. Nonetheless, previous studies indicated that SGLT inhibitors like empagliflozin and dapagliflozin decrease the mRNA expression of TNF- $\alpha$  and IL-1 $\beta$  and demonstrate anti-inflammatory effects in diabetic renal proximal tubular cells.<sup>30,34</sup> Activation of caspases is a crucial component in cell death following renal IRI. In this study, levels of caspase 3 in the control and DMSO groups were significantly elevated compared with the sham group, indicating enhanced apoptosis in tubular cells. This finding aligns with the research conducted by Shan and colleagues in a rat model of renal IRI.<sup>37</sup> The rats that received sotagliflozin showed a notable reduction in caspase 3 levels. This indicates that sotagliflozin suppressed apoptosis in renal tubular cells. Currently, there are no published studies directly investigating the effect of sotagliflozin on caspase 3 expression in the context of renal IRI. However, earlier studies have shown that SGLT inhibitors significantly lower caspase 3 levels and reduce renal damage, suggesting that they have a protective effect on the kidneys.<sup>38-39</sup> Several studies revealed the protective effects of SGLT2 inhibitors like dapagliflozin and canagliflozin in diabetic and chronic kidney impairment models, and their main impact was only on the proximal tubule, where SGLT2 is found.<sup>34-35</sup> However, sotagliflozin works by blocking both SGLT1 in the small intestine as well as SGLT2 in the kidney. These mechanisms slow down the absorption of glucose in the intestines and change the levels of hormones like incretins that come from the gut. This dual beneficial effect may provide additional anti-inflammatory, anti-oxidative, and metabolic impacts than SGLT2 inhibitors, especially in renal IRI, where inflammation and oxidative stress make the damage worse. The histological examination displayed no aberrant pathological alterations in the sham group; the glomeruli and tubules were healthy. However, in the control group, there was evidence of loss of brush border in proximal tubules, renal interstitial hemorrhage, inflammatory cell infiltration, blood vessel congestion, cellular edema, and different intensities of coagulative necrosis. Our findings were in agreement with the findings of Zeid and coworkers.<sup>18</sup> Interestingly, the rats pre-treated with sotagliflozin exhibited intact tissue integrity compared to the control and DMSO groups. Our findings are in agreement with several studies that demonstrated the

SGLT2 inhibitor mitigated kidney injury, fibrosis, macrophage infiltration, and coagulative necrosis.<sup>35,40</sup> Taken together, as explained in Figure 8, our findings indicated that the big drops in urea, Cr, NGAL, 8-iso-PGF2 $\alpha$ , TNF- $\alpha$ , and caspase-3 all point to the fact that sotagliflozin protects the kidney by not only improving metabolic balance through SGLT1/2 inhibition, but also by lowering oxidative stress, inflammation, and apoptotic pathways. These findings provide a rationale for further studies to determine whether sotagliflozin's dual mechanism may confer additional benefits compared to SGLT2 inhibitors in renal injury.



**Fig. 8.** Schematic diagram representing the mechanisms involved in the renoprotective effects of sotagliflozin following renal ischemia/reperfusion injury

### Study limitation

This research has some notable limitations that must be highlighted. The reperfusion period was restricted to 2 h, permitting the study of acute damage signs but precluding the measurement of long-term outcomes such as fibrosis, delayed cell death, or kidney function repair. The collection of tissue and serum samples at just one time point limits understanding of the temporal evolution of sotagliflozin's impacts. Future research should include serial measurements. Another limitation was using one dosage of sotagliflozin (10 mg/kg), and this may not show all of the drug's dose-dependent effects. We selected this dosage because previous research showed that it worked in animal models of metabolic and cardiovascular disorders. Furthermore, all studies were performed only on a male Sprague Dawley rat. We didn't include female animals since hormonal differences might make things even more complicated, especially in the healing and repairing of kidney damage. Our results showed that sotagliflozin lowers oxidative stress, inflammation, and apoptotic indicators in renal I/R damage, but we still do not know precisely how it does this at the molecular level. We did not directly study the expression or activity of renal SGLT1/2 proteins and the signaling pathways that could be implicated, such as NF- $\kappa$ B, Nrf2/ARE, and Bcl-2/Bax that may be very important in causing the effects that were seen. The relevance of these results to human clinical scenarios

is theoretical and requires additional confirmation in larger animal models and via extended investigations.

### Future directions

The future studies should apply longer reperfusion intervals, such as 24 to 72 hours or even a few weeks, to see how chronic alterations happen. Moreover, further studies are needed to find out whether sotagliflozin has different impacts on male and female animals, especially when it uses to protect the kidneys. Further research applying different doses of sotagliflozin are needed to find the best dose and better understand the dose-response relationship in renal IRI. Another future research using Western blotting or PCR to study NF- $\kappa$ B, Nrf2, and Bcl-2/Bax pathways to better understand how sotagliflozin protects the kidneys. Additionally, future studies should investigate the combination of sotagliflozin with anti-inflammatory, or antioxidant medications as well as studies comparing sotagliflozin effects with other SGLT inhibitors that might help find out whether it gives better protection.

### Conclusion

This study provided evidence that sotagliflozin has renoprotective effects by improving renal function and histopathological abnormalities, as well as regulating oxidative stress, inflammation, and apoptotic cell death. These findings suggest its potential therapeutic application in preventing and treating acute kidney injury.

### Declarations

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No particular funds were allotted to this study.

#### Author contributions

Conceptualization, N.A.H. and T.R.T.; Methodology, N.A.H.; Software, N.A.H.; Validation, N.A.H. and T.R.T.; Formal Analysis, N.A.H.; Investigation, T.R.T.; Resources, N.A.H.; Data Curation, N.A.H.; Writing – Original Draft Preparation, N.A.H.; Writing – Review & Editing, N.A.H.; Visualization, N.A.H.; Supervision, T.R.T.; Project Administration, T.R.T.; Funding Acquisition, N.A.H.

#### Conflict of interest

No conflict of interest has been indicated by the researchers.

#### Ethical approval

The research adhered to ethical principles and received approval from the Institutional Animal Care and Use Committee (IACUC) at the University of Kufa, Iraq, after the submission of the required requests (NO. 2126 on January 23, 2025).

#### Data availability

Data is supplied in response to reasonable requests.



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