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Protective effects of bexagliflozin on renal function in a rat model of ischemia-reperfusion injury; an experimental animal study

Ghada A. Alkhafaji[1], **Ali M. Janabi**[2*]

[1]Pharmacy Department, Babylon Directorate of Health, Babel, Iraq
[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Kufa, Najaf, Iraq

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ABSTRACT

Introduction: Ischemia-reperfusion injury (IRI) is a significant clinical challenge that often leads to acute kidney injury (AKI), adversely affecting renal function and patient outcomes. Recent advancements in pharmacotherapy have highlighted the potential of sodium-glucose cotransporter-2 (SGLT2) inhibitors in providing renal protection.

Objectives: This study aimed to investigate the protective effects of bexagliflozin on renal function in a rat model subjected to IRI.

Materials and Methods: In an experimental study, 28 male rats, weighing between 200-300 g, were utilized in this experimental study and divided into four distinct groups. The sham group underwent identical anesthesia and surgical procedures without the induction of ischemia. The IRI group experienced 30 minutes of bilateral renal ischemia followed by 24 hours of reperfusion. The dimethyl sulfoxide (DMSO) group, serving as a vehicle for bexagliflozin, received an oral dose two hours before the ischemia induction and subsequently underwent the same reperfusion protocol. In the bexagliflozin pretreated group, rats were administered bexagliflozin at a dosage of 3 mg/kg orally two hours before ischemia induction, followed by 30 minutes of bilateral renal ischemia and 24 hours of reperfusion. After the reperfusion period, all rats were subjected to a laparotomy to collect blood and kidney samples, including urea, creatinine, interleukin-6 (IL-6), Akt, glutathione (GSH), caspase, light chain 3-B (LC3-B), kidney injury molecules-1 (KIM-1), and histopathological renal tubular injury.

Results: The study findings indicated that both the IRI and IRI+DMSO groups experienced significant renal impairment compared to the sham group, as evidenced by elevated levels of serum urea, creatinine, caspase, Akt, LC3-B, KIM-1, and IL-6, alongside decreased GSH levels. In contrast, the IRI + bexagliflozin treatment group demonstrated notable protective effects against renal injury, reflected in lower levels of these parameters and reduced renal tubular injury scores compared to the IRI and IRI+DMSO groups. Furthermore, bexagliflozin was associated with a smaller increase in GSH levels relative to the other groups, underscoring its potential therapeutic role in alleviating renal damage linked to IRI.

Conclusion: Bexagliflozin demonstrated promising protective effects against renal injury, as evidenced by lower levels of injury markers and reduced renal tubular damage. These findings suggest that bexagliflozin may serve as a viable therapeutic option for mitigating renal damage associated with IRI, warranting further investigation into its clinical applications.

Implication for health policy/practice/research/medical education:
In this experimental study, we found that bexagliflozin significantly decreased kidney damage by activating anti-inflammatory, anti-apoptotic, antioxidant, autophagy and Akt signaling pathways. The demonstrated protective effects of bexagliflozin against renal injury in ischemia-reperfusion models suggest that sodium-glucose cotransporter-2 (SGLT2) inhibitors should be considered as a therapeutic option in clinical settings, particularly for patients at risk of acute kidney injury (AKI). Policymakers should advocate for the integration of such pharmacotherapies into treatment guidelines to improve patient outcomes. Furthermore, the results highlight the need for ongoing research to explore the mechanisms by which bexagliflozin exerts its protective effects, potentially leading to advancements in renal protection strategies.

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***Corresponding author**: Ali M. Janabi, Email: alim.hashim@uokufa.edu.iq

Original

Introduction

Ischemia occurs when there is an inadequate supply of oxygen-rich blood, leading to tissue hypoxia, the accumulation of cellular waste, nutrient deficiencies, and a buildup of carbon dioxide (hypercapnia), which ultimately results in cellular death (1). The damage caused by ischemia-reperfusion injury (IRI) is exacerbated compared to ischemia alone due to a significant increase in reactive oxygen species (ROS). This surge contributes to endothelial dysfunction and organ damage across various clinical conditions, making IRI more severe than ischemia by itself (2). ROS are made in small amounts during ischemia compared to the whole IRI process. The reason behind this is a reduction in the activation of several enzymes, including cytochrome c, nitric oxide synthases, xanthine oxidase, and nicotinamide adenine dinucleotide phosphate (3). The normalization of pH and the rise in oxygen levels during reperfusion negatively impact previously ischemic cells. The ischemia-reperfusion process triggers multiple cell death pathways, including necrosis, apoptosis, and autophagy-related cell death. Necrosis is characterized by cellular swelling, which ultimately leads to the rupture of the cell membrane (4).

Across various conditions, including trauma, acute kidney injury (AKI), ischemic stroke, and myocardial infarction, IRI significantly contributes to increased morbidity and mortality (5). Through its involvement in various biological responses, such as inflammation, cell apoptosis, and free radical accumulation, renal IRI is a critical factor in developing AKI (6). The kidneys become significantly depleted of oxygen after meeting the demands of the counter-current exchange system, making them particularly vulnerable to ischemia and hypoxia. Additionally, the rapid decline in glomerular filtration rate (GFR) due to ischemia exacerbates renal injury and functional impairment. When blood flow is restored, this can trigger a "second hit," commonly referred to as IRI (7). Numerous complex mechanisms underlying renal IRI include ATP depletion, intracellular Ca^{2+} and ROS buildup, mitochondrial malfunction, activation of various enzyme systems, and the generation of pro-inflammatory cytokines (8). Dehydration, infections, and drug toxicity in communities are the main causes of AKI, which is a silent killer. Severe AKI usually results from specific hospital-related events, including major surgery, septic shock, or medication poisoning. Almost all body systems are affected by kidney malfunction, which causes several organ failures (9). Activating autophagy has been shown to help cells survive in some real-life scenarios. For instance, starting up autophagy can help protect ischemic cell damage (10). In AKI, the effectiveness of autophagic flux within the autophagy pathway is not well understood. The precise kinetics of autophagy activation during the progression of AKI in experimental models have yet to be clearly defined. Consequently, the status of autophagy and the potential impairment of flux during AKI remains ambiguous, as efficient autophagic flux is essential for cellular survival (11). Tissues include the kidneys, liver, brain, and heart often exhibit elevated autophagy levels when IRI is present. Modulating autophagy levels successfully managed Acute IRI and apoptosis (12,13).

Recently, sodium-glucose cotransporter 2 inhibitors (SGLT2is) have been discovered as an oral glucose-lowering medication that benefits the kidneys and heart (14). bexagliflozin, as a SGLT2i, plays a crucial role in preventing renal injury by enhancing renal protection through various mechanisms. It has been shown to improve renal outcomes by reducing inflammation and oxidative stress, which are significant contributors to kidney damage, particularly in conditions such as diabetic kidney disease and AKI (15). By promoting diuresis and natriuresis, bexagliflozin helps to alleviate the burden on the kidneys, thereby mitigating the risk of injury during ischemic events. Additionally, its ability to lower albuminuria and slow the progression of renal impairment further underscores its therapeutic potential in preserving kidney function and improving overall patient outcomes (16). In this study, we investigated the potential kidney-protective effects of bexagliflozin against IRI in male rats. We assessed its impact on various mechanisms, including anti-inflammatory, anti-apoptotic, and antioxidant activities, as well as its role in activating the autophagy and Akt signaling pathways.

Objectives

The objective of this study is to evaluate the renal protective effects of bexagliflozin in a controlled experimental setting. Specifically, the study aims to determine whether pre-treatment with bexagliflozin can mitigate renal damage and improve renal function following IRI in male rats. This objective was assessed by measuring various biomarkers associated with renal injury, inflammation, oxidative stress, and histopathological changes in kidney tissue. The findings are expected to provide insights into the potential therapeutic role of bexagliflozin in preventing AKI induced by ischemic events.

Materials and Methods

Study design and samples

This experimental animal study was approved by the institutional ethics committee of University of Kufa, Iraq, in 2024. A total of 28 male Sprague Dawley rats were randomly assigned to one of four groups: sham, IRI, dimethyl sulfoxide (DMSO) serving as a vehicle for bexagliflozin + IRI, or bexagliflozin + IRI pretreatment. Each group consisted of seven rats, allowing for a controlled comparison of the effects of bexagliflozin on renal function following IRI.

Animal preparation

Twenty-eight male Sprague-Dawley rats, both adults and juveniles, weighing between 200-300 g and aged 15-20

weeks, were included in this study. The rats were housed in the animal facility of the Faculty of Pharmacy at the University of Kufa. They were maintained under controlled conditions with a 12-hour light/dark cycle, a temperature set at 24 ± 2 °C, and humidity levels maintained between 60%-65%. The animals were kept in a separate chamber utilizing a group caging system, which facilitated social interaction. Their diet consisted of standard food and water provided ad libitum.

Drug preparation

Bexagliflozin powder was sourced from Shanghai Macklin Biochemical Technology Co., Ltd. Following the manufacturer's guidelines, bexagliflozin was dissolved in DMSO to create a stock solution at a concentration of 100 mg/mL. The appropriate dose was then administered orally to the rats based on their body weight (17).

Experimental model of renal ischemia/reperfusion injury

Experimental surgery was conducted on the body's dorsal (retroperitoneal) areas. Rats were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) to alleviate pain (18) by intraperitoneal injection into the abdominal cavity; sterile instruments were performed for all procedures. A 1.5 cm vertical flank incision was made by surgical instruments, layer by layer, through the skin, fascia, and muscle layer. Atraumatic microvascular clamps can be employed to induce renal IRI during surgical procedures (19). After 24 hours of reperfusion, the dorsal lesion was closed, and the animals were rehydrated with 1 mL of pre-warmed 0.9% saline at 37 °C in the retroperitoneal space before wound closure. The procedure was repeated on the contralateral side in the animal model designed for bilateral renal IRI. AKI occurs within the first 24 hours after renal reperfusion (20). The uniformity of the kidney's coloration to a dusky appearance within a few minutes proved the efficacy of the ischemia. The procedure was repeated on the contralateral side of the animal model intended for bilateral renal IRI (21). After 24 hours of reperfusion, the dorsal lesion was closed, and the animals were rehydrated with 1mL of 37 °C pre-warmed 0.9% saline administered into the retroperitoneal region before wound closure (22). Evaluating creatinine and blood urea nitrogen (BUN) levels, blood was taken from the hearts of the animals after they were euthanized under deep anesthesia. The histological examination of the right kidney was conducted in 10% formaldehyde, while the biomarkers in the renal tissues were measured in the left kidney, which was stored at -80 °C.

Procedure

The sham group of rats underwent the same surgical procedures and anesthesia as the other groups; however, they were not subjected to the 30 minutes of bilateral renal ischemia or the subsequent 24 hours of reperfusion. In the IRI groups, the rats underwent 30 minutes of bilateral renal ischemia (23-25), followed by a 24-hour reperfusion period (26). For the IRI+DMSO group, DMSO was administered to the rats two hours before the ischemic event, after which they experienced the same 30 minutes of ischemia and subsequent 24 hours of reperfusion. Similarly, the bexagliflozin pretreated group received an oral dose of bexagliflozin at 3 mg/kg two hours before ischemia induction and followed the same ischemia-reperfusion protocol as the IRI+DMSO group. After the 24-hour reperfusion period (27), a laparotomy incision was made to collect blood and kidney samples for further analysis. Throughout the procedure, all rats were maintained under complete anesthesia using an intraperitoneal injection of 100 mg/kg of ketamine and 10 mg/kg of xylazine.

Blood samples collection for measurement of renal function

Approximately 2-4 mL of blood was collected directly from the hearts of the rats while they were still under anesthesia following the surgical procedure. To isolate serum, the blood samples were placed in gel tubes devoid of anticoagulants and subsequently centrifuged. The resulting serum was then analyzed for urea and creatinine levels using a spectrophotometric technique at an absorbance wavelength of 550 nm. This quantitative method is effective for accurately determining the concentrations of these compounds in the samples.

Tissue preparation for measurement of inflammatory, apoptotic, autophagy, and oxidative parameters

Kidney tissue samples came next after the blood sample collection. Every animal had its kidney removed and then cut in half. One-half was preserved in 10% formalin for a histological examination. In contrast, the remaining half was frozen at −80 °C then homogenized under high intensity in 1:10 W/V phosphate-buffered saline, including 1% Triton X-100 and a protease inhibitor cocktail. Using available enzyme-linked immunosorbent assay (ELISA) technology from Sunlong Biotech Co., Ltd., China, the homogenate was centrifuged at 2000–3000 rpm at 4 °C for 10–20 minutes, and supernatants were conducted to determine kidney injury molecule 1 (KIM-1), interleukin 6 (IL-6), Caspase-3, PKB/Akt, light chain 3-B (LC3-B) and glutathione (GSH) kits according to manufacturer specifications.

Preparing tissues for histopathology

A portion of kidney tissue fixed in 10% formalin, dehydrated via an alcohol series, cleared with xylene, and embedded in paraffin. Following paraffin embedding, kidney tissues were sliced into 5-µm thick pieces. The sections were subsequently stained with hematoxylin, eosin, and trichrome stain.

Parameter measurement

A spectrophotometric technique was conducted to estimate serum urea and creatinine, which depends on measuring light absorption or transmission of chemical reactions within a specific wavelength. This method is a quantitative measurement and very helpful in determining the compounds' concentrations; urea and creatinine levels were measured at 550 nm absorbance minutes. The levels of KIM-1, IL-6, caspase-3, Akt, LC3-B, and GSH were measured by following the manufacturer's instructions with ELISA kits for these biomarkers obtained from Sunlong Biotech Co., Ltd. (China).

Statistical analysis

The data were analyzed utilizing GraphPad Prism 9.5 software (GraphPad Software, La Jolla, CA, USA) and the Statistical Package for the Social Sciences (SPSS) software version 27 (IBM, Corp, USA). The significance of mean differences between groups was evaluated using analysis of variance (ANOVA), followed by a post hoc least significant difference (LSD) test. Statistical significance was established with a P value < 0.05 for all analysis.

Results

The study compares various biochemical and histopathological parameters across different treatment groups, including sham, IRI, IRI with DMSO, and IRI with bexagliflozin. The serum levels of urea and creatinine were notably elevated in the IRI group compared to the sham group, indicating significant renal impairment. Additionally, the serum concentrations of KIM-1 and IL-6 were markedly higher in the IRI group, suggesting an inflammatory response associated with renal injury. The caspase levels, indicative of apoptosis, also showed substantial increases in the IRI groups. Furthermore, the serum analysis of Akt and LC3-B levels revealed alterations consistent with cellular stress and autophagy processes. Histopathological evaluations indicated varying degrees of renal tubular injury across the treatment groups, highlighting the potential protective effects of bexagliflozin against IRI (Table 1).

The findings indicated that the IRI group experienced significant renal impairment, as evidenced by elevated levels of urea and creatinine compared to the sham group. Additionally, KIM-1 levels were significantly increased in the IRI group, reflecting a heightened inflammatory response associated with kidney damage. The analysis also revealed that the IRI with DMSO and IRI with bexagliflozin groups exhibited varying effects on these parameters, with bexagliflozin showing potential protective properties against renal injury. Furthermore, histopathological assessments demonstrated varying degrees of renal tubular injury across the groups, reinforcing the impact of ischemia-reperfusion on kidney health and highlighting the therapeutic potential of bexagliflozin in mitigating such injuries (Table 2 and Figure 1).

The findings revealed significant differences in IL-6 levels among the various treatment groups, particularly between the sham and IRI groups, as well as between the IRI and other treatment combinations, such as IRI with DMSO and bexagliflozin. Notably, while the differences in IL-6 levels between the sham group and IRI combined with DMSO and also the sham and IRI combined with bexagliflozin groups were not statistically significant, the IRI and IRI+bexagliflozin group exhibited a marked increase in IL-6 levels compared to the sham group. In terms of caspase levels, all groups subjected to IRI, including those treated with DMSO and bexagliflozin, showed substantial increases relative to the sham group; however, no significant difference was observed between the IRI and IRI+DMSO groups. The IRI+bexagliflozin group demonstrated lesser increases in caspase levels compared to both the IRI and IRI+DMSO groups. Regarding Akt levels, both the IRI and IRI + DMSO groups presented significant increases compared to the sham group, although no significant differences were found

Table 1. Laboratory data of included rats

Parameters	Group							
	Sham		IRI		IRI+DMSO		IRI+bexagliflozin	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Serum urea (mg/dL)	34.08	6.71	78.43	8.55	75.85	9.69	58.74	17.82
Serum Cr (mg/dL)	0.51	0.14	2.35	0.50	2.43	0.53	1.34	0.25
KIM-1(pg/mL)	36.60	16.10	94.66	17.90	99.23	26.29	47.40	13.45
IL-6 (ng/L)	32.08	13.52	76.38	18.63	75.59	19.54	45.08	16.58
Caspase (ng/mL)	2.53	0.70	7.05	1.10	6.95	1.35	4.33	0.52
Akt (pg/mL)	634.15	337.59	1115.13	108.12	1077.31	79.38	699.53	89.29
LC3-B (pg/mL)	350.55	80.90	643.56	140.31	679.48	68.12	418.59	86.15
GSH (ng/l)	108.64	19.19	33.60	5.62	29.60	6.07	83.07	20.51
Histopathological renal tubular injury score	0	0	3.86	0.37	3.71	0.48	2.36	1.66

IRI, Ischemia-reperfusion injury; DMSO, Dimethyl sulfoxide; Sham, control; Cr, Creatinine; IL-6, Interleukin-6; KIM-1, Kidney injury molecules-1; GSH, Glutathione; LC3-B, Light chain 3-B.

Table 2. Comparison of the serum urea, creatinine, and kidney injury molecules-1 of included rats between four groups

First group		Second group	Mean difference	*P* value*
Urea (mg/dL)	Sham	IRI	44.35	<0.001
		IRI+DMSO	41.76	<0.001
		IRI+bexagliflozin	24.66	<0.001
	IRI	IRI+DMSO	2.58	0.679
		IRI+bexagliflozin	19.68	0.004
	IRI + DMSO	IRI+bexagliflozin	17.10	0.010
Cr (mg/dL)	Sham	IRI	1.84	<0.001
		IRI+DMSO	1.92	<0.001
		IRI+bexagliflozin	0.83	<0.001
	IRI	IRI+DMSO	0.07	0.721
		IRI+bexagliflozin	1.01	<0.001
	IRI + DMSO	IRI+bexagliflozin	1.08	<0.001
KIM-1(pg/mL)	Sham	IRI	58.05	<0.001
		IRI+DMSO	62.62	<0.001
		IRI+bexagliflozin	10.80	0.300
	IRI	IRI + DMSO	4.56	0.658
		IRI+bexagliflozin	47.25	<0.001
	IRI + DMSO	IRI+bexagliflozin	51.82	<0.001

IRI, Ischemia-reperfusion injury; DMSO, Dimethyl sulfoxide; Sham, control; Cr, Creatinine; KIM-1, Kidney injury molecules-1; *ANOVA and post hoc LSD.

when comparing the sham group with IRI+bexagliflozin or between the IRI and IRI+DMSO groups. Nevertheless, a significant elevation in Akt levels was recorded in both the IRI and IRI+DMSO groups when compared to the IRI+bexagliflozin group (Table 3 and Figure 2).

The results indicated statistically significant differences in the levels of LC3-B and GSH among the treatment groups, emphasizing the effectiveness of bexagliflozin in mitigating injury markers when compared to both the IRI and DMSO groups. Specifically, while LC3-B levels were significantly elevated in the treatment groups relative to the sham group, GSH levels exhibited a decrease. Notably, the differences between the IRI+bexagliflozin group and the other two groups (IRI and IRI+DMSO) were statistically significant, whereas no significant difference was found between the IRI and IRI+DMSO groups. Furthermore, bexagliflozin resulted in a smaller increase in LC3-B levels and a lesser reduction in GSH compared to both the IRI and IRI+DMSO groups. The histopathological evaluation of renal tubular injury scores also revealed significant differences between the IRI+bexagliflozin group and the other two groups; however, there was no significant difference between the IRI and IRI+DMSO groups. The histopathological assessment indicated a notable reduction in renal tubular injury scores in the bexagliflozin group relative to the other groups, suggesting its potential protective effects against renal damage (Table 4 and Figure 3).

Discussion

Acute kidney injury is a significant concern in the intensive care unit, where its prevalence has been reported to affect approximately 20% to 50% of hospitalized patients. This growing incidence underscores the critical nature of AKI

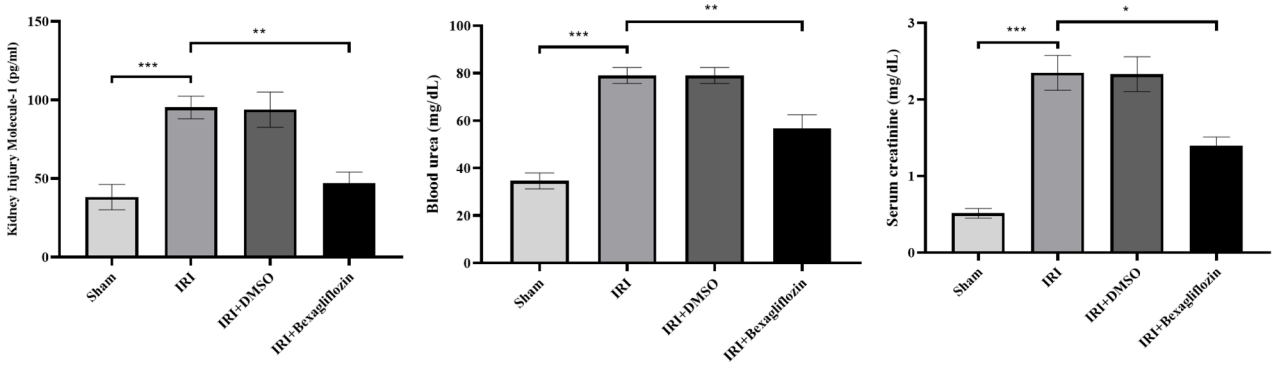

Figure 1. Frequency distribution of urea, creatinine, and kidney injury molecule-1 of studied rats. *P<0.05, **P<0.01 and ***P<0.001.

Table 3. Comparison of the interleukin-6, caspase, and Akt of included rats between four groups

First group		Second group	Mean difference	*P* value*
IL-6 (ng/L)	Sham	IRI	44.30	<0.001
		IRI+DMSO	43.51	<0.001
		IRI+bexagliflozin	13.00	0.171
	IRI	IRI+DMSO	0.78	0.933
		IRI+bexagliflozin	30.51	0.002
	IRI + DMSO	IRI+bexagliflozin	1.97	0.003
Caspase (ng/mL)	Sham	IRI	4.51	<0.001
		IRI+DMSO	4.41	<0.001
		IRI+bexagliflozin	1.79	0.002
	IRI	IRI+DMSO	0.09	0.852
		IRI+bexagliflozin	2.71	<0.001
	IRI + DMSO	IRI+bexagliflozin	2.62	<0.001
Akt (pg/mL)	Sham	IRI	480.97	<0.001
		IRI+DMSO	443.15	<0.001
		IRI+bexagliflozin	65.37	0.519
	IRI	IRI + DMSO	37.82	0.709
		IRI+bexagliflozin	415.60	<0.001
	IRI + DMSO	IRI+bexagliflozin	377.78	<0.001

IRI, Ischemia-reperfusion injury; DMSO, Dimethyl sulfoxide; Sham, control; Cr, Creatinine; IL-6, Interleukin-6; *ANOVA and post hoc LSD.

as a major contributor to morbidity and mortality in critically ill individuals (28). The causes of AKI can be separated into three categories, including ischemic (like renal IRI), inflammatory (like sepsis), and nephrotoxic (29). An important factor in IR-induced tissue damage is the inflammatory response (30), which plays a key mediator of various problems including pancreatitis (31). According to previous studies, the main cause of AKI is renal injury, which led to renal tubule dilatation, inflammatory insult, apoptotic cell death, and, subsequently, renal failure (32). In this study, we observed that the groups subjected to IRI or treated with a vehicle exhibited significantly elevated levels of serum BUN and creatinine compared to the sham group. These findings indicate that renal IRI leads to substantial renal impairment, as reflected by the increased concentrations of these biomarkers, which are commonly associated with kidney dysfunction. This result is in line with the study by Yang et al that showed significantly elevated serum creatinine and BUN in IRI and vehicle groups when compared with the sham group in a mouse model exposed to 45 min intraperitoneal renal ischemia and 24 hour reperfusion (33). Furthermore, in this study, bexagliflozin significantly reduced levels of urea and creatinine compared to the other IRI groups, indicating its preservation of renal function. This finding aligns with the study by Munteanu et al, which demonstrated that SGLT2 inhibitors can effectively lower blood glucose, blood pressure, uric acid, and creatinine levels, while also reducing the development of albuminuria (34). The ability of bexagliflozin to mitigate renal injury suggests its potential as a therapeutic agent in preventing the progression of kidney dysfunction associated with IRI, thereby highlighting its importance in clinical applications for patients at risk of AKI.

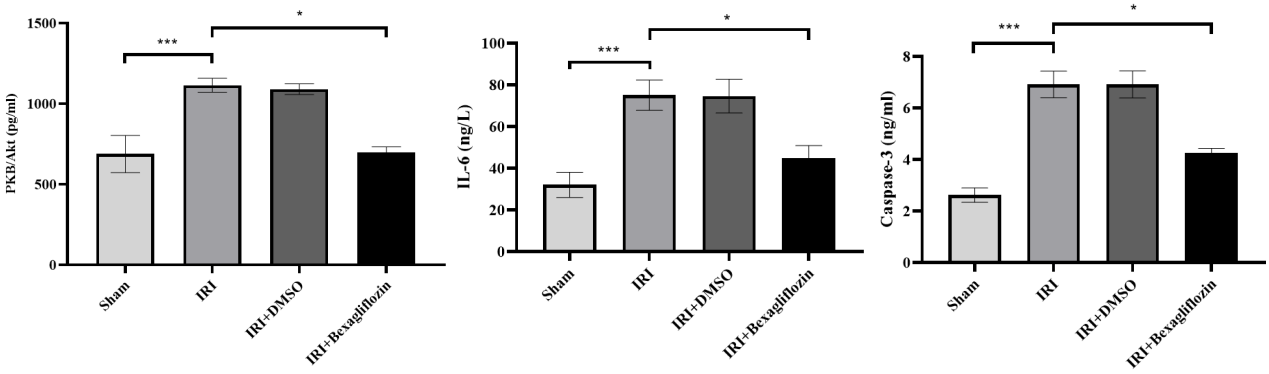

Figure 2. Frequency distribution of interleukin-6, caspase, and Akt of studied rats.*P<0.05, **P<0.01 and ***P<0.001.

Table 4. Comparison of the light chain 3-B, glutathione, and histopathological renal tubular injury score of included rats between groups

First group		Second group	Mean difference	*P* value*
LC3-B (pg/mL)	Sham	IRI	292.99	<0.001
		IRI+DMSO	328.93	<0.001
		IRI+bexagliflozin	68.04	0.206
	IRI	IRI+DMSO	35.93	0.499
		IRI+bexagliflozin	224.95	<0.001
	IRI + DMSO	IRI+bexagliflozin	260.88	<0.001
GSH (ng/»l)	Sham	IRI	75.04	<0.001
		IRI+DMSO	79.03	<0.001
		IRI+bexagliflozin	25.57	0.003
	IRI	IRI+DMSO	3.99	0.615
		IRI+bexagliflozin	49.46	<0.001
	IRI + DMSO	IRI+bexagliflozin	53.46	<0.001
Histopathological renal tubular injury score	Sham	IRI	3.85	<0.001
		IRI+DMSO	3.71	<0.001
		IRI+bexagliflozin	1.85	<0.001
	IRI	IRI + DMSO	0.14	0.569
		IRI+bexagliflozin	2.00	<0.001
	IRI + DMSO	IRI+bexagliflozin	1.85	<0.001

IRI, Ischemia-reperfusion injury; DMSO, Dimethyl sulfoxide; Sham, control; *ANOVA and post hoc LSD.

The results of this study revealed a significant reduction in KIM-1 levels in the bexagliflozin group compared to both the IRI and vehicle groups, indicating its potential protective effects on renal function. However, it is noteworthy that no prior studies have specifically examined the impact of bexagliflozin on urea, creatinine, or KIM-1 levels, highlighting a gap in the existing literature. This lack of previous research underscores the importance of further investigations to elucidate the mechanisms through which bexagliflozin may confer renal protection, as well as to establish its efficacy in managing biomarkers associated with AKI. The findings contribute valuable insights into the therapeutic potential of SGLT2 inhibitors in preventing renal damage and warrant additional studies to explore their clinical relevance. A study by Jha et al about diabetes and renal complications showed that SGLT2 inhibitors had significant renal protective effects (35).

The current study demonstrated a significant reduction in IL-6 levels in the dapagliflozin-treated group compared to both the IRI and vehicle groups. These findings suggest that pretreatment with bexagliflozin effectively mitigates the release of pro-inflammatory cytokines following renal IRI, highlighting its potential as an anti-inflammatory therapeutic strategy. Despite the compelling results observed in this study, it is noteworthy that there is a lack of existing literature specifically addressing the effects of bexagliflozin on IL-6 and other inflammatory markers in the context of renal injury. This gap underscores the necessity for further research to elucidate the mechanisms by which bexagliflozin exerts its protective effects and to establish its clinical relevance in managing inflammation

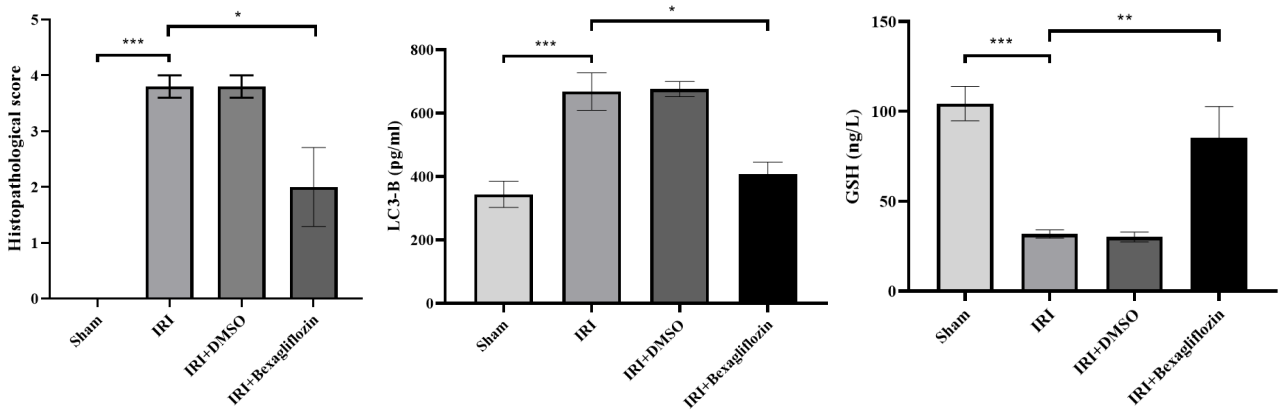

Figure 3. Frequency distribution of the light chain 3-B, glutathione, and histopathological renal tubular injury score of studied rats. *P<0.05, **P<0.01 and ***P<0.001.

associated with AKI. Increased inflammatory mediators are associated with increased oxidative stress (36,37). No study was found to discuss the effect of bexagliflozin on the inflammatory marker IL-6, specifically in IRI. This protective effect is comparable to that study by Wojciechowska et al which showed that SGLT-2 inhibitors are now seen as pleiotropic drugs showing multidirectional nephron-cardioprotective and anti-inflammatory effects, SGLT2is had a significant reduction in the inflammatory mediator IL-6 (38).

This study demonstrated that the group pretreated with bexagliflozin exhibited a significant reduction in caspase-3 levels compared to both the IRI and DMSO groups, indicating a potential anti-apoptotic effect of bexagliflozin following renal IRI. This finding is particularly noteworthy as it suggests that bexagliflozin may confer protective effects against apoptosis in renal tissues subjected to ischemic conditions. However, it is essential to note that, despite these promising results, there remains a lack of corroborative studies in the literature to confirm the anti-apoptotic properties of bexagliflozin in this context.

Although there was no statistically significant difference in Akt levels between the bexagliflozin and sham groups, the bexagliflozin pretreatment resulted in a notable decrease in Akt levels compared to both the IRI and DMSO groups, effectively reaching the levels observed in the sham group. This observation may indicate a potential protective mechanism afforded by bexagliflozin, suggesting its role in modulating the Akt signaling pathway in the context of renal IRI. However, it is important to note that no studies have specifically investigated the effects of bexagliflozin on Akt within renal IRI models. This finding aligns with the study of Weintraub et al (39), which demonstrated that SGLT2 inhibitors significantly inhibit the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway in hyperglycemic patients, thereby supporting the hypothesis that bexagliflozin may exert similar effects in renal contexts.

This study showed that treatment with bexagliflozin two hour before ischemia induction significantly decreased the level of autophagic marker to that level in the sham group on ischemic renal tissues in comparison with those in both IRI and vehicle groups as a protective mechanism to promote cell survival comparison with those in both IRI and vehicle groups. No study explained the effect of bexagliflozin on LC3-B in renal IRI, but some studies explained the autophagic effect of SGLT2 inhibitors in other conditions. Guan et al found that autophagy was increased time-dependent and began before the beginning of cell apoptosis as an early response that served a renoprotective effect during renal I/R and cell H/R. Up-regulation of autophagy could be a viable method for treating acute renal damage (40).

Our recent description of the reduction of inflammation and oxidative stress indicators by SGLT2 inhibitors is consistent with their similar cardiorenal beneficial effects. This study found that renal tissue levels of GSH were significantly increased in the bexagliflozin pretreated group compared to the IRI and vehicle groups. This effect of bexagliflozin on oxidative stress levels is significantly compatible with the study by Bray et al, which showed that SGLT2 inhibitors have a nephroprotective effect by inhibiting ROS production and increasing antioxidant activity (41).

Rats pretreated with bexagliflozin before ischemia induction showed much less renal damage than IRI and vehicle groups, and the severity scores mean of this group verified minor renal damage. According to histopathological measures, they gave bexagliflozin two hours before renal IRI decreased renal injury. No previous studies explained the effect of bexagliflozin on renal tissue. This finding is in line with the study conducted by Wang et al on empagliflozin as a SGLT2i that produces a nephroprotective effect. The study was conducted on a mouse model subjected to 45 minutes of ischemia and reperfusion. Enlarged tubules, tubular architectural degeneration, tubular cell swelling, severe tubular necrosis, inflammation of the luminal chambers, and severe renal histology abnormalities were observed in the IRI group. There was less of a difference in the morphology of renal tissues in the treated group of renal mice compared to the IRI group (42).

Overall, the findings of this study highlight the potential of bexagliflozin as a protective agent against renal injury, particularly in the context of IRI. The observed reductions in injury markers and renal tubular damage suggest that bexagliflozin may exert its protective effects through multiple mechanisms, including anti-inflammatory and antioxidant actions, as well as the modulation of cellular pathways such as autophagy and Akt signaling. These results align with the growing body of evidence supporting the role of SGLT2 inhibitors in renal protection, indicating that bexagliflozin could be a viable therapeutic option for patients at risk of AKI. Further research is warranted to explore its clinical applications and to better understand the underlying mechanisms contributing to its renal protective effects, potentially leading to improved management strategies for renal injuries associated with various clinical conditions.

Conclusion

In conclusion, the study provides compelling evidence that bexagliflozin offers significant protective effects against renal injury in a rat model of ischemia-reperfusion, as indicated by improved biochemical and histopathological outcomes compared to the IRI and IRI+DMSO groups. The marked reduction in key injury markers and lower renal tubular injury scores in the bexagliflozin-treated group highlight its potential as a therapeutic agent for mitigating renal damage associated with ischemia-reperfusion events. These findings not only reinforce the role of SGLT2 inhibitors in renal protection but also pave

the way for further research into their clinical applications, ultimately contributing to better management strategies for patients at risk of AKI.

Limitations of the study

This study has several limitations that should be considered when interpreting the results. First, the use of a male rat model may limit the generalizability of the findings to female populations or humans, as sex differences can influence renal responses to ischemia and pharmacological interventions. Additionally, the relatively short duration of the observation period following IRI (24 hours) may not adequately capture the long-term effects of bexagliflozin on renal function and recovery. Furthermore, while various biomarkers were assessed, the study did not explore other potentially relevant molecular pathways or long-term outcomes associated with renal injury, which could provide a more comprehensive understanding of bexagliflozin's protective mechanisms. Lastly, the sample size of 28 rats may limit the statistical power to detect subtle differences between groups, warranting caution in drawing definitive conclusions from the data. These limitations highlight the need for further research to validate these findings and explore the broader implications of bexagliflozin in diverse populations and extended timeframes.

Authors' contribution

Conceptualization: Ali M. Janabi.
Data curation: Ghada A. Alkhafaji.
Formal analysis: Ali M. Janabi.
Investigation: Ali M. Janabi and Ghada A. Alkhafaji.
Methodology: Ghada A. Alkhafaji.
Project administration: Ali M. Janabi.
Resources: Ali M. Janabi and Ghada A. Alkhafaji.
Software: Ali M. Janabi.
Supervision: Ali M. Janabi.
Validation: Ghada A. Alkhafaji.
Visualization: Ali M. Janabi.
Writing–original draft: Ghada A. Alkhafaji.
Writing–review & editing: Ali M. Janabi and Ghada A. Alkhafaji.

Conflicts of interest

The authors declare no conflicts of interest.

Ethical issues

The research and the protocol of this study followed the guidelines of animal studies and were approved by the Ethics Committee of the University of Kufa, Pharmacy College, Iraq (Ethical code #6685). We tried to conduct the guidelines related to animal experiments, approved by the United States National Institutes of Health (NIH, 1978). Besides, the authors have ultimately observed ethical issues (including plagiarism, data fabrication, and double publication).

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