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Enavogliflozin attenuates early sepsis-associated acute kidney injury in a murine cecal ligation and puncture model

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Abstract. *Sepsis is often complicated by the onset of acute kidney injury (AKI) through processes involving inflammation, oxidative stress, tubular damage, and cell death. In this study, we investigated whether enavogliflozin might mitigate acute renal damage following Cecal Ligation and Puncture (CLP)-induced sepsis.*

Methods. Twenty-four male mice were allocated to the following four experimental groups: sham operation (Sham), CLP, CLP plus dimethyl sulfoxide (DMSO), and CLP plus enavogliflozin. Enavogliflozin was administered intraperitoneally at 1mg/kg, 1h before CLP surgery. At 24 hours after surgery, blood and kidney tissue samples were collected. Serum creatinine was measured to assess kidney function, while renal tissue levels of kidney injury molecule-1 (KIM-1), nuclear factor kappa B (NF-κB), malondialdehyde (MDA), caspase-3, and nuclear factor erythroid 2-related factor 2 (Nrf2) were measured using an enzyme-linked immunosorbent assay (ELISA). We also performed a kidney histopathological assessment using Hematoxylin & Eosin staining and a score assigned for renal damage.

Results. Serum creatinine was significantly elevated in mice undergoing CLP compared to sham controls (2.240 ± 0.2546 mg/dL vs 0.4307 ± 0.05516 mg/dL; $P < 0.0001$). Administration of enavogliflozin in CLP animals resulted in a decrease in serum creatinine levels (1.117 ± 0.1276 mg/dL vs 2.240 ± 0.2546 mg/dL; $P < 0.01$). Compared with the CLP group, enavogliflozin significantly reduced renal tissue levels of the tubular injury marker KIM-1 (359.3 ± 12.30 vs. 461.4 ± 18.32 pg/mL; $P < 0.001$), the inflammatory mediator NF-κB (844.7 ± 20.09 vs. 1429 ± 11.38 pg/mL; $P < 0.0001$), the oxidative stress marker MDA (204.5 ± 18.79 vs. 320.6 ± 23.51 ng/mL; $P < 0.01$), and the apoptosis-related protein caspase-3 (7.726 ± 0.4581 vs. 10.21 ± 0.5911 ng/mL; $P < 0.05$). In contrast, renal Nrf2 levels were significantly higher in enavogliflozin-treated mice than in the CLP group (4383 ± 123.6 vs. 3210 ± 117.6 pg/mL; $P < 0.0001$), indicating enhancement of the antioxidant response. Histologically, renal tissue of enavogliflozin-treated mice showed ameliorated injury scores compared to the CLP controls [0.0 (0.0–1.0) vs 3.5 (3.0–4.0); $P < 0.05$].

Conclusions. Treatment with enavogliflozin attenuates acute CLP-induced renal injury by ameliorating tubular damage, suppressing inflammation and oxidative stress, and promoting an antioxidative cellular response. Further studies are warranted to elucidate the mechanism of action and establish the clinical utility of enavogliflozin administration following sepsis.

Keywords: *sepsis, acute kidney injury, sodium-glucose transporter 2 inhibitors, enavogliflozin, cecal ligation and puncture, oxidative stress.*

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Енавогліфлозин зменшує тяжкість раннього сепсис-асоційованого гострого пошкодження нирок у мишачій моделі з перев'язуванням і пункцією сліпої кишки

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Резюме. *Сепсис часто ускладнюється гострим пошкодженням нирок (ГПН), у розвитку якого важливу роль відіграють запалення, оксидативний стрес, пошкодження ниркових канальців і загибель клітин. У цьому дослідженні оцінювали здатність енавогліфлозину зменшувати тяжкість ГПН у моделі сепсису, індукованого перев'язуванням і пункцією сліпої кишки.*

Методи. Двадцять чотири самці мишей були розподілені на чотири експериментальні групи: псевдооперовані тварини (Sham), перев'язування та пункція сліпої кишки (CLP), CLP із введенням диметилсульфоксиду та CLP із введенням енавогліфлозину. Енавогліфлозин вводили внутрішньоочеревинно в дозі 1 мг/кг за 1 годину до операції CLP. Через 24 години після операції відбирали зразки крові та тканини нирок. Для оцінки функції нирок визначали концентрацію креатиніну в сироватці крові, а в тканині нирок методом імуноферментного аналізу (ІФА) визначали рівні молекули пошкодження нирок-1 (KIM-1), ядерного фактора каппа B (NF-κB), малонового діальдегіду (MDA), каспази-3 та ядерного фактора, пов'язаного з еритроїдним фактором 2 (Nrf2). Також проводили гістологічне дослідження тканини нирок із забарвленням гематоксиліном та еозином і визначали ступінь пошкодження нирок за бальною шкалою.

Результати. У мишей групи CLP концентрація креатиніну сироватки крові була значно вищою, ніж у псевдооперованих тварин (2,240 ± 0,2546 проти 0,4307 ± 0,05516 мг/дл; p < 0,0001). Введення енавогліфлозину тваринам групи CLP супроводжувалось зниженням концентрації креатиніну (1,117 ± 0,1276 проти 2,240 ± 0,2546 мг/дл; p < 0,01). Порівняно з групою CLP, введення енавогліфлозину супроводжувалось значним зниженням у тканині нирок рівня маркера канальцевого пошкодження KIM-1 (359,3 ± 12,30 проти 461,4 ± 18,32 пг/мл; p < 0,001), медіатора запалення NF-κB (844,7 ± 20,09 проти 1429 ± 11,38 пг/мл; p < 0,0001), маркера оксидативного стресу MDA (204,5 ± 18,79 проти 320,6 ± 23,51 нг/мл; p < 0,01) та пов'язаної з апоптозом каспази-3 (7,726 ± 0,4581 проти 10,21 ± 0,5911 нг/мл; p < 0,05). Водночас рівень Nrf2 у тканині нирок був значно вищим у мишей, які отримували енавогліфлозин, порівняно з групою CLP (4383 ± 123,6 проти 3210 ± 117,6 пг/мл; p < 0,0001), що свідчить про посилення антиоксидантної відповіді. За результатами гістологічного дослідження миші, які отримували енавогліфлозин, мали нижчий бал пошкодження ниркової тканини порівняно з тваринами групи CLP [0,0 (0,0–1,0) проти 3,5 (3,0–4,0); p < 0,05].

Висновки. Енавогліфлозин зменшував тяжкість ГПН, що проявлялось зменшенням канальцевого пошкодження, запалення та оксидативного стресу, а також посиленням антиоксидантної відповіді. Подальші дослідження необхідні для уточнення механізмів дії енавогліфлозину та оцінки перспектив його застосування у пацієнтів із сепсисом.

Ключові слова: *сепсис. гостре пошкодження нирок. інгібітори натрій-глюкозного котранспортера 2 типу. Енавогліфлозин. перев'язування та пункція сліпої кишки, оксидативний стрес.*

Introduction. Sepsis is a life-threatening condition that develops in the dysregulated host's response to infection. It remains one of the leading causes of organ failure and death worldwide [1, 2]. Sepsis-associated acute kidney injury (SA-AKI) is a common and critical condition that has a poor prognosis and mortality, along with limited therapeutic options [3]. Although reduced renal blood flow would appear to be the main cause of SA-AKI, inflammation, oxidative stress, endothelial injury, microcirculatory dysfunction, mitochondrial injury, and tubular epithelial cell injury all play a role in the occurrence of SA-AKI [4, 5].

Inflammation induced by sepsis has leukocytes going to renal tissue as an organ damage process mediated by nuclear factor kappa B (NF-κB) [4]. Simultaneously, the emergence of reactive oxygen species (ROS) induces lipid peroxidation, apoptosis, renal architecture disturbance and higher concentrations of malondialdehyde (MDA), caspase-3, and kidney injury molecule-1 (KIM-1) [6, 7]. Nuclear factor erythroid 2-related factor 2 (Nrf2) mediates cellular responses to oxidative stress and inflammatory stimulation, promoting increased antioxidant capacity and limiting oxidative/inflammatory cell damage [8, 9]. Recent experimental work suggests the association between

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Nrf2/NF-κB axis activation and the pathogenesis of SA-AKI [10]. However, changes in Nrf2 and NF-κB levels measured by enzyme-linked immunosorbent assay (ELISA) should be interpreted with caution. Such measurements can suggest an association with these pathways, but they do not confirm direct pathway activation or inhibition.

Naively designed to address diabetes, sodium-glucose cotransporter-2 (SGLT2) inhibitors provide additional benefits in metabolic function beyond glycemic control, with beneficial effects in kidney and cardiovascular disease settings [11–13]. Clinical trials show anti-inflammatory response, antioxidant activity and protective effect of some SGLT2 inhibitors in acute organ injury models. Previous studies indicated that dapagliflozin could improve the prognosis of kidney injury in an experimental AKI model [14], and the use of ertugliflozin reduced sepsis-associated acute liver injury in mice [15], suggesting therapeutic potential.

The new SGLT2 inhibitor enavogliflozin (licensed recently in Korea for type 2 diabetes) was reported for its pharmacokinetic action, safety, biodistribution in the body, and even in the kidney [16–18]. It has also shown protective qualities in experimental inflammatory and metabolic disorders like diet-induced steatohepatitis [19]. However, the effect of enavogliflozin on SA-AKI has not yet been established.

The cecal ligation and puncture (CLP) model is the predominant polymicrobial model of sepsis in rodent species, and reproduces a large number of sepsis-related components, such as inflammatory/systemic/organ dysfunction responses [20]. While several SGLT2 inhibitors have been studied in AKI models and patients with sepsis-induced organ destruction [21]. The effectiveness of enavogliflozin in CLP-induced SA-AKI is not clear. Importantly, the mechanism by which enavogliflozin reduces early kidney dysfunction, tubular injury, oxidative stress, apoptosis, and inflammation after CLP-induced sepsis has not been definitively shown [21]. Accordingly, in this study, we attempted to determine whether enavogliflozin could attenuate early kidney injury in a mouse model of CLP-induced SA-AKI and to explore its possible association with inflammatory, oxidative stress, and apoptotic responses.

Materials and methods. *Animals and ethical approval*. This study was conducted on 24 adult male mice (aged 6-8 weeks; 25-35 g weight), which were obtained from the animal facility at the College of Science, University of Kufa. Before experimentation, animals were adapted for one week in laboratory conditions (maintained at stable temperature and humidity with a 12 hr light/dark cycle, and provided with unlimited access to food and water).

The whole experiment was performed at the laboratories of the Faculty of Pharmacy, University of Kufa, Najaf, Iraq. All the animal experiments were performed according to the Institutional Animal Care and Use Committee, University of Kufa, Najaf, Iraq (Approval number 2329 on February 3, 2026). The care of animals and experiments was performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Animals were observed for symptoms of marked distress, poor mobility, extreme weakness, and moribundity.

Sample size. Each experimental group included six mice. A formal sample size calculation was not performed before the study. The number of animals was chosen based on similar experimental studies using the CLP model, and with consideration of reducing animal use. For this reason, the study should be regarded as an exploratory preclinical experiment.

The model. Sepsis-associated acute kidney injury was induced by CLP, with minor modifications to the method described by Li et al [22]. Briefly, the mice were anesthetized using a mixture of ketamine (100mg/kg) and xylazine (10mg/kg). After the loss of deep surgical planes of anesthesia, the skin overlying the abdomen was shaved and surgically prepared with Betadine solution. A midline abdominal incision of approximately 1-2 cm length was performed, exposing the cecum. The cecum was exteriorized and ligated using a 6-0 silk suture at approximately 50-75% of its length from the ileocecal valve. It was then punctured two times with a 22-gauge needle, and a small amount of fecal material was gently extruded to ensure patency of the punctured sites. Following the procedure, the cecum was replaced into the abdominal cavity, and the abdominal incision was closed with surgical sutures.

After the surgery, the animals received 1 mL of normal sterile saline subcutaneously as supportive fluid. The animals were placed on a warming pad until they recovered from anesthesia. The sham-operated animals underwent an identical procedure except for the cecal ligation and puncture.

After adaptation, the mice were randomly divided into four groups of six animals per group. The sham group underwent only laparotomy without cecal ligation and puncture and without treatment. The CLP group underwent cecal ligation and puncture without treatment. The CLP + DMSO group received dimethyl sulfoxide (DMSO) vehicle intraperitoneally 1 h before CLP. The CLP + enavogliflozin group received enavogliflozin (1 mg/kg) intraperitoneally 1 h before CLP [15].

Drug preparation and administration. Enavogliflozin was purchased from TargetMol (Boston, MA, USA) with Cat No: T38843; purity 98%. Dimethyl sulfoxide was purchased from TargetMol (Cat. No.: T0341). Fresh stock solutions of enavogliflozin were prepared daily for the day of the experiment. The stock solution of enavogliflozin was prepared first with dimethyl sulfoxide, then diluted with normal sterile saline.

Enavogliflozin was prepared at a final dose of 1mg/kg, according to a recent research study that illustrated its antioxidant and anti-inflammatory characteristics in experimental models [23]. For the control vehicle

group, the same volume of DMSO was injected intraperitoneally. This administration took place 1 hr before sepsis induction.

Sample preparation. Twenty-four hours after surgery, mice were deeply anesthetized, and blood was collected by cardiac puncture as a terminal procedure. Immediately after blood collection, mice were euthanized under deep anesthesia with a high dose of ketamine and xylazine, and death was confirmed before kidney collection.

Blood samples were collected and centrifuged at 1500 rpm for 15 min at 4°C. Serum was separated and stored at -80°C until the renal function assessment was performed. The kidneys were quickly removed and flushed with normal cold sterile saline solution. Kidney tissue was divided into two parts, one used for biochemical measurements and the second one fixed in 10% buffered formalin solution. The parts of kidney samples designated for biochemical analysis were weighed and homogenized in cold phosphate-buffered saline solution at a ratio of 1:9 weight/volume, and then centrifuged for 15 minutes at 12,000 rpm at 4°C [24]. The supernatanti were collected and kept at -80°C until use.

Blood and kidney samples were coded prior to lab testing to minimize assessment bias; this was performed on serum and kidney tissue samples. Biochemical measurements were taken blindly. The group identities of the samples were unknown. Histological sections were also examined by a pathologist blinded to the groups under study to ensure minimized evaluation bias.

Measurement of renal injury, inflammatory, oxidative stress, antioxidant, and apoptotic markers. The renal injury was assessed by measuring serum creatinine. Serum creatinine was assayed using a UV-1900i spectrophotometer (Shimadzu, Japan) according to the manufacturer's instructions. The results were expressed as mg/dL.

Concentration of KIM-1, NF-κB, MDA, caspase-3, and Nrf2 in the renal tissue homogenate supernatant was determined using commercial ELISA kits (SunLong Biotech Co., Ltd., Hangzhou, China), and catalog numbers are SL0339Mo (KIM-1), SL0723Mo (NF-κB), SL0370Mo (MDA), SL0679Mo (caspase-3), SL1027Mo (Nrf2). The procedures followed are as per the kit instructions. The absorbance at a wavelength of 450nm was measured with a microplate Humareader HS, HUMAN Diagnostics, Wiesbaden, Germany.

Standard curves were used for each marker in order to extrapolate the concentration in the unknown sample. Values are expressed as pg/mL for KIM-1, NF-κB, Nrf2, and ng/mL for MDA, caspase-3.

Histopathological examination. Kidney samples fixed in 10% buffered formalin solution were sectioned at 5 m thickness using a microtome after paraffin embedding and stained with Hematoxylin & Eosin [25]. The sections were observed under a light microscope. The renal damage was graded on a semi-quantitative scale from 0 to 4, based on observed pathologist findings, which were: tubular epithelial degeneration, tubular swelling, vascular congestion, and inflammatory cell infiltration [26]. The scale was as follows: 0 for normal renal tissue, 1 for mild injury which occurred in less than 25% of the field, 2 for moderate injury, which occurred in 25% to 50% of the field, 3 for marked injury which occurred in 51% to 75% of the field, and 4 for severe injury which occurred in >75% of the field.

Statistical analysis. Data analysis was performed using GraphPad Prism version 10.6.1 (GraphPad Software, Boston, MA, USA). Data were analyzed for normal distribution using the Shapiro-Wilk test. Normally distributed biochemical data were presented as mean ± standard error of the mean. Comparisons among groups were performed using one-way analysis of variance followed by Bonferroni's post hoc test.

Histopathological scores were presented as median with interquartile range. These data were analyzed using the Kruskal–Wallis test followed by Dunn's multiple comparison test. A p-value less than 0.05 was considered statistically significant.

Results. *Enavogliflozin improved kidney function after CLP*. To evaluate kidney function after CLP, serum creatinine was assessed 24 h post-CLP. There was a significant increase in serum creatinine levels in the CLP group compared to the sham group, indicating renal dysfunction induced by sepsis. CLP + DMSO groups had similar elevation in serum creatinine levels compared to the CLP group, revealing that the vehicle itself did not attenuate kidney function. In contrast, in the enavogliflozin group, the serum creatinine levels were lower compared to the CLP group (Fig. 1). The numerical values corresponding to the graphical data are provided in **Supplementary Tables S1 and S2**.

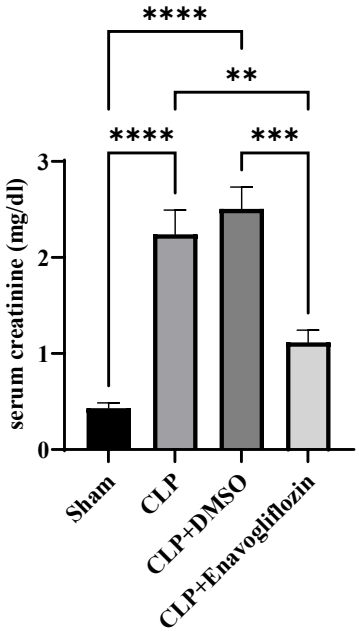

Fig. 1. Effect of enavogliflozin on serum creatinine levels.

*Data are presented as M ± SEM, n = 6 per group. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's post hoc test. **P < 0.01, ***P < 0.001, and ****P < 0.0001.*

Enavogliflozin decreased renal KIM-1 levels. Renal KIM-1 was measured as a marker of tubular injury. Compared to the sham group, the KIM-1 expression increased significantly in the CLP group. The CLP + DMSO group had a similar significant up-regulation compared to the sham group. Enavogliflozin treatment decreased KIM-1 expression significantly compared to the CLP group, showing that the damage of renal tubules is attenuated (Fig. 2).

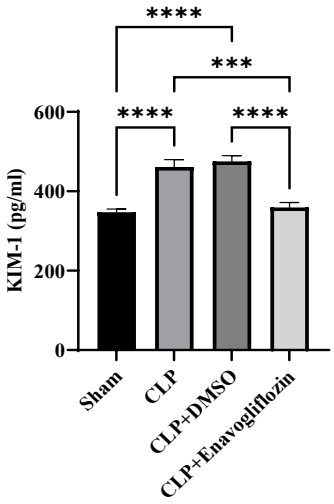

Fig. 2. Effect of enavogliflozin on renal KIM-1 levels after CLP.
*Data are presented as M ± SEM, n = 6 per group. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's post hoc test. ***P < 0.001 and ****P < 0.0001.*

Enavogliflozin reduced renal NF-κB expression and upregulated renal Nrf2 expression. Renal NF-κB and Nrf2 expressions were measured to analyze the renal inflammatory and antioxidant status. Compared to the sham group, NF-κB levels increased in the CLP group and CLP + DMSO group significantly. The treatment of enavogliflozin attenuated NF-κB expression compared to the CLP group (Fig. 3).

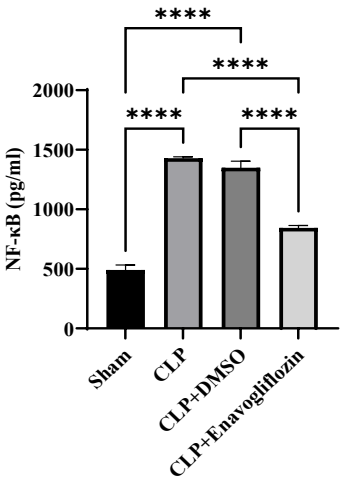

Fig. 3. Effect of enavogliflozin on renal NF-κB levels after CLP.
*Data are presented as M ± SEM, n = 6 per group. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's post hoc test. ****P < 0.0001.*

Conversely, compared to the sham group, Nrf2 levels decreased in the CLP group, indicating attenuated antioxidant response following sepsis. In the enavogliflozin treatment group, Nrf2 expression increased significantly compared to the CLP group (Fig. 4).

Enavogliflozin attenuated oxidative stress and apoptosis-related markers in the kidneys. MDA was evaluated to analyze oxidative stress levels. Similar to other parameters, MDA levels significantly increased after CLP compared to the sham group. The elevated MDA levels in CLP + DMSO groups compared to the sham group showed that the vehicle did not ameliorate the oxidative injury. Enavogliflozin treatment ameliorated the increase in MDA levels compared to the CLP group, indicating that it significantly decreased the oxidative stress in the renal tissue (Fig. 5).

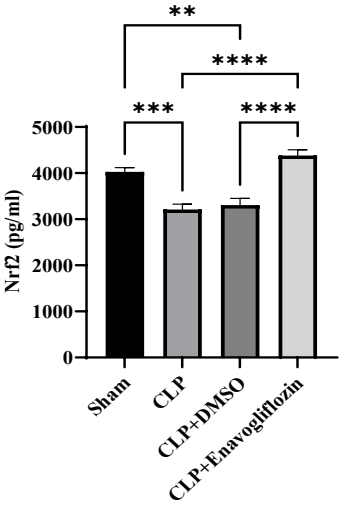

Fig. 4. Effect of enavogliflozin on renal Nrf2 levels after CLP.
*Data are presented as M ± SEM, n = 6 per group. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's post hoc test. **P < 0.01, ***P < 0.001, and ****P < 0.0001.*

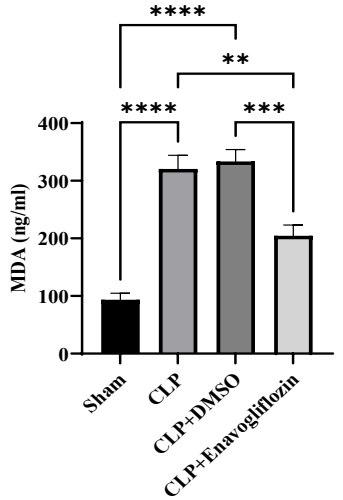

Fig. 5. Effect of enavogliflozin on renal MDA levels after CLP.
*Data are presented as M ± SEM, n = 6 per group. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's post hoc test. **P < 0.01, ***P < 0.001, and ****P < 0.0001.*

In addition, cleaved caspase-3 expression levels were detected as an indicator of apoptosis-related events. Compared to the sham group, caspase-3 levels increased in the CLP and CLP+DMSO groups, demonstrating increased apoptosis-related activity in kidney tissues following CLP. Enavogliflozin treatment attenuated caspase-3 levels compared to the CLP group (Fig. 6).

Enavogliflozin improved renal histology. In the sham group, the renal tissues exhibited preserved structure, and the glomeruli were morphologically normal, whereas the tubules were intact. The renal tissues were obviously damaged in the CLP group and CLP + DMSO group, with degeneration of renal tubular epithelium, tubular swelling, vascular congestion, and infiltration of inflammatory cells.

The renal injury score in the CLP and CLP + DMSO groups was significantly higher compared with that of the sham group. Compared with the CLP and CLP + DMSO groups, enavogliflozin administration decreased the degree of these damages in the renal tissue. The renal tissues of the enavogliflozin treatment group were observed with milder tubular injury, vascular congestion, infiltration of inflammatory cells, and well-preserved structure compared with the CLP group (Fig. 7 and 8).

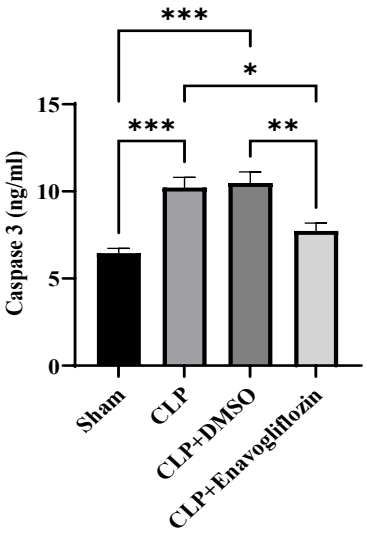

Fig. 6. Effect of enavogliflozin on renal caspase-3 levels after CLP.

*Data are presented as M ± SEM, n = 6 per group. Statistical analysis was performed using one-way ANOVA followed by Bonferroni's post hoc test. *P < 0.05, **P < 0.01, and ***P < 0.001.*

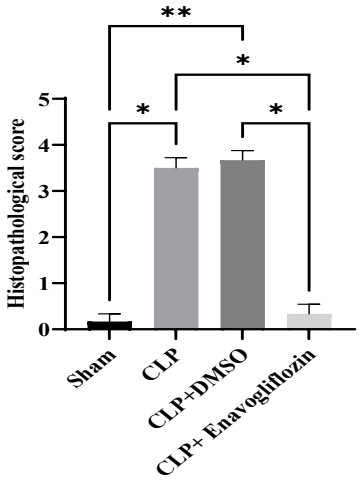

Fig. 7. Effect of enavogliflozin on renal histopathological injury score.

*Tubular degeneration, tubular swelling, vascular congestion, and inflammatory cell infiltration were evaluated using a semi-quantitative score from 0 to 4. Data are presented as median with interquartile range, n = 6 per group. Statistical analysis was performed using the Kruskal–Wallis test followed by Dunn's multiple comparison test. *P < 0.05 and **P < 0.01.*

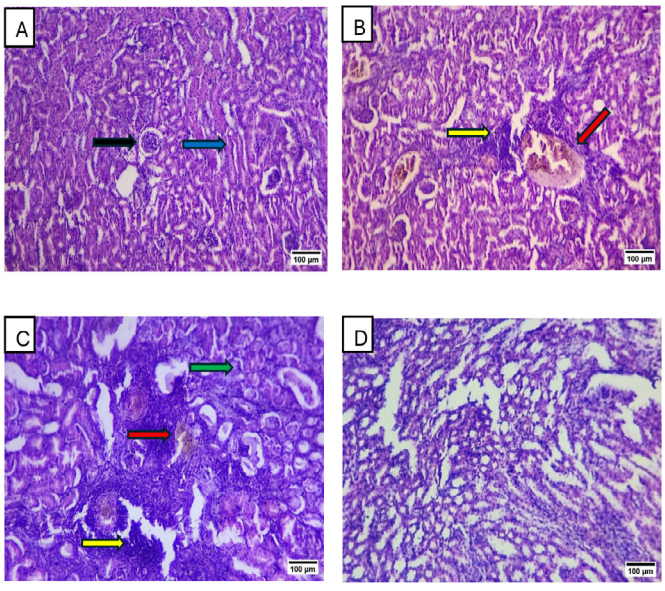

Fig. 8. Representative kidney histology after CLP-induced sepsis.

Representative hematoxylin and eosin-stained kidney sections from the experimental groups are shown. The sham group showed normal renal architecture with preserved glomeruli and tubules. The CLP group showed marked renal injury, including tubular epithelial degeneration, vascular congestion, and inflammatory cell infiltration. The CLP + DMSO group showed similar tissue damage. The CLP + enavogliflozin group showed milder renal injury, with reduced tubular damage, less congestion, and better preservation of renal structure. Magnification: 100×.

Discussion. The present study showed that enavogliflozin attenuates early renal injury in the mouse model of CLP-induced SA-AKI. The presence of CLP resulted in renal impairment with high serum creatinine, as well as elevated renal KIM-1, which suggested tubular damage. Histological injury, including tubular epithelial degeneration, vascular congestion, and inflammatory cell infiltration, supported these biochemical processes. A pretreatment of enavogliflozin lowered serum creatinine and KIM-1 levels and improved renal histology. It decreased renal NF-κB, MDA, and caspase-3 and raised Nrf2. Taken together, the study showed that enavogliflozin has anti-early septic AKI activity by inflammation, oxidative stress, antioxidant, and apoptosis effects. As far as we know, our study is the first to assess the effect of enavogliflozin against SA-AKI in a CLP model.

Previous work demonstrated improved renal injury due to dapagliflozin in experimental models of sepsis and lipopolysaccharide-induced AKI [14, 27]. Empagliflozin additionally lowered oxidative stress and enhanced antioxidant pathways in kidney ischemia/reperfusion injury [28], and ertugliflozin lowered liver injury markers in a mouse model of sepsis-induced acute liver injury [15]. Our results are consistent with these studies and extend a possible protective effect of SGLT2 inhibitors to enavogliflozin in septic kidney injury.

Improvement in serum creatinine and KIM-1 levels indicates that enavogliflozin decreased both functional and tubular components of renal injury. KIM-1 is an early marker of tubular epithelial injury and typically rises after proximal tubular damage [29]. Tubular injury is not a consequence of a single mechanism in septic AKI, but rather involves the multijunctive effects of an inflammatory mechanism, microcirculatory dysfunction, oxidative stress and damage to cells [3, 5]. Thus, the KIM-1 deficiency and improved histological structure align with the hypothesis that enavogliflozin attenuated early renal tissue insult after CLP.

Inflammation plays a major role in the pathophysiology of SA-AKI. NF-κB is a cornerstone in inflammatory signaling and has been associated with kidney injury in studies of sepsis [10]. In the present study, CLP increased the level of renal NF-κB and enavogliflozin suppression on that level was observed. This suggests that the protective renal effects of enavogliflozin may, at least in part, be associated with inhibition of the inflammatory response. Comparable anti-inflammatory effects have been described with the other SGLT2 inhibitors in experimental organ injury. For instance, empagliflozin lowered inflammatory NF-κB-dependent cytokine induction in sepsis-induced myocardial injury [30], and enavogliflozin is also noted to attenuate neuroinflammation in an experimental model of Alzheimer's disease [31].

Oxidative stress is a major mechanism in septic AKI. High ROS can harm the lipids, proteins and cellular structures, with tubular failure and cell death [5, 7]. MDA is often used as a marker for lipid peroxidation, and Nrf2 controls antioxidant defenses. In this study, CLP increased renal MDA levels and decreased renal Nrf2 levels. This suggests that CLP-induced sepsis caused oxidative stress and weakened the antioxidant response in kidney tissue. The changes were reversed by enavogliflozin with inhibition of MDA and enhanced Nrf2. Indeed, this pattern goes in line with previous studies indicating activation of antioxidant responses could safeguard against SA-AKI [8, 9]. It is also consistent with the antioxidant effects of the SGLT2 inhibitors reported in experimental renal injury models [28].

Apoptosis also plays its part in renal tubular injury during sepsis. Caspase-3 is a critical apoptosis marker, usually upregulated during sepsis-associated organ death [32]. Herein, levels of caspase-3 were increased following CLP and decreased after enavogliflozin treatment, indicating that enavogliflozin could moderate apoptotic tubular loss. Caspase-3 decrease also supported histological signs, where lower tubular injury in the enavogliflozin-treated group indicated better preservation of renal architecture. When combined, the current results indicate that enavogliflozin may shield the kidney in early sepsis by inhibiting multiple mediating pathways rather than an isolated pathway. Lower NF-κB levels might indicate less inflammatory response, lower MDA levels may suggest less lipid peroxidation, increased Nrf2 may indicate enhanced antioxidant response if the organism has a greater antioxidant response or increased Nrf2 value in the early sepsis phase, and therefore, less necrosis associated with apoptosis can indicate reduced cellular damage due to lower mitogenesis.

There are several limitations in our study. The first limitation is the small sample size, with only 6 mice per group; the findings must be considered as preliminary. Second, only male mice were used, and therefore, no assessment of sex-specific differences in enavogliflozin response can be made. Third, a single dose of enavogliflozin was used and only single time points 24h after CLP. The dose-response and late progression of kidney injury are thus unknown.

Another limitation is that the enavogliflozin was given 1h before CLP, which would test protective actions but is likely to be less representative of a clinical setting where therapy is initiated after the development of sepsis. Moreover, survival rate, bacterial load, systemic inflammation, blood pressure, urine output and renal perfusion were not measured. These would offer a comprehensive overview of disease progression and response.

Also, the mechanistic findings need to be interpreted with caution. NF-κB and Nrf2 were measured by ELISA in kidney tissue homogenate, but do not necessarily confirm activation or inhibition of the Nrf2/ NF-κB signaling pathway. Western blotting, PCR, immunohistochemistry, or pathway-specific assays are required for confirmation of the molecular

mechanism. Finally, this was an experimental study and direct translation of these findings to clinical S-AKI will require further preclinical and clinical investigation.

Conclusionis. Enavogliflozin may attenuate early SA-AKI in a mouse model of CLP, by improving serum creatinine and reducing renal KIM-1, NF-κB, MDA and caspase-3 levels. In addition, it increases renal Nrf2 expression and improves renal histology. Our findings suggest that the renoprotective effect may be attributed to attenuated tubular injury, inflammation, oxidative stress and apoptosis with a superior antioxidant response. However, in light of the small size of the study and the unconfirmed molecular pathways, this study should be considered to be exploratory. Further studies are needed to confirm the molecular mechanisms, test different doses and time-points, and demonstrate the therapeutic relevance of enavogliflozin in a post-sepsis scenario.

Supplementary materials. Supplementary Tables are available on Figshare: **https://doi.org/10.6084/m9.figshare.33295938**.

Ethics approval and consent to participate. All experimental and animal handling procedures were conducted in the laboratories of the Faculty of Pharmacy, University of Kufa, Najaf, Iraq. The study was approved by the Institutional Animal Care and Use Committee of the University of Kufa, Najaf, Iraq (approval No. 2329, dated February 3, 2026). All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Consent for publication. Not applicable.

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Authors' contributions.

Alaa Q. Aldhaheri: Investigation, data curation, formal analysis, visualization, and writing—original draft.

Ali M. Janabi: Conceptualization, methodology, supervision, project administration, data interpretation, and writing—review and editing. Both authors read and approved the final manuscript.

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