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Tirzepatide Protects the Kidney by Modulating Oxidative Stress, Inflammation, and Apoptosis in an Induced Ischemia-Reperfusion Rat Model

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ABSTRACT

Renal ischemia-reperfusion (I/R) injury is major cause of acute kidney injury (AKI), driven by oxidative stress, inflammation, and cell death, and no effective pharmacological therapy available. Tirzepatide, a dual GIP/GLP-1 receptor agonist, has shown metabolic and anti-inflammatory benefits that may offer renal protection. This study investigate its effect against acute renal I/R injury in rats. Twenty-four male Sprague-Dawley rats were assigned to four groups: sham, I/R control, I/R + DMSO, and I/R + tirzepatide (1.35 mg/kg). Drug or vehicle was administered 24 hours and 1 hour before 40 minutes of bilateral renal ischemia followed by 2 hours of reperfusion. I/R markedly increased serum urea, creatinine, NGAL, oxidative stress markers, TNF-α, caspase-3, and tubular injury. Tirzepatide significantly improved renal function, reduced NGAL, oxidative stress, inflammation, apoptosis, with histological damage. These findings indicate strong renoprotective effect of tirzepatide, that support its potential as a therapeutic option for AKI associated with ischemic insults.

Keywords: Tirzepatide, Ischemia Reperfusion, acute kidney injury, apoptosis, inflammation.

Article Information

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INTRODUCTION

Acute kidney injury is still a major complication in patients in hospitals, resulting in chronic kidney disease development and high mortality (1). I/R injury, which can occur following renal surgery, transplantation, or shock, is a major cause of AKI both in intensive care and surgical settings (1). In renal I/R, the short restriction of blood flow (ischemia) followed by its restoration (reperfusion) triggers a complex pathophysiological cascade that involves oxygen deprivation and the subsequent formation of reactive oxygen species (ROS) after re-oxygenation. Excess ROS causes oxidative stress and peroxidation of lipids, which damages cellular membranes and organelles (1). Mitochondrial failure ensues, increasing ROS generation and activating cell death pathways (1). I/R triggers an extensive inflammatory response: damaged renal cells release damage-associated molecular patterns, which activate resident immune cells and infiltrating neutrophils and macrophages. This results in production of pro-inflammatory cytokines (including interleukin-1β and tumor necrosis factor-α) and chemokines (2). The inflammatory cascade worsens tissue injury and can slow the healing process. Other feature of I/R injury is apoptotic cell death in renal tubular epithelial cells, which is mediated by factors that include caspase-3 activation result from severe adenosine triphosphate (ATP) depletion and oxidative DNA damage (1). In the post-ischemic kidney, oxidative stress, inflammation, and apoptosis combine to form a sustaining injury cycle.

Despite extensive research into these pathways, there are actually no approved pharmaceutical therapies that can avoid or alleviate renal I/R injury in clinical settings (1). Supportive action (hydration, hemodynamic

optimization, and dialysis if necessary) stay the main focus. This gap led to research in medicines that can stop the I/R injury cascade. Interventions that concurrently lower oxidative stress, inflammation, and cell death would be extremely helpful to offer real tissue protection (1). One new treatment method is to repurpose medications with proven multi-system advantages, such as incretin-based therapies, for organ preservation.

Glucagon-like peptide-1 (GLP-1) receptor agonists gained in popularity as treatments for type 2 diabetes and obesity because of powerful glucose-lowering and weight-loss effects. along with metabolic management, GLP-1 receptor agonists showed cardiovascular and renal protective effects in large outcome studies and preclinical researches (3) (4). GLP-1 receptors exist in the kidney, particularly in proximal tubular cells, renal blood vessels, and juxtaglomerular apparatus cells (3). Activation of renal GLP-1 receptors induces cyclic AMP/PKA signaling, which may directly reverse injury processes. For example, protein kinase A (PKA) activation inhibits nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, decreasing superoxide production and oxidative damage in the kidney (3).

GLP-1 agonists also increase afferent arteriole vasodilation and natriuresis, which improves renal microcirculation and reduces ischemia hypoxia. GLP-1 agonism was demonstrated to diminish nuclear factor-kappa B (NF-κB) activation and production of cytokines including TNF-α, IL-1β, and monocyte chemoattractant protein-1 for both diabetic and ischemic nephropathy (2,3). In a mouse model of acute renal I/R, the GLP-1 agonist liraglutide significantly enhanced survival, preserved renal function, and decreased the production of pro-inflammatory mediators (TNF-α, IL-1β, IL-6, etc.) (2). Similarly, increasing endogenous GLP-1 by dipeptidyl peptidase-4 (DPP-4) inhibition (e.g., sitagliptin) protected rats from I/R damage by reducing oxidative stress indicators and inflammatory cell infiltration in the kidney (1). These findings demonstrate that activating the GLP-1 pathway can provide wide renoprotective advantages by targeting the same mechanisms that cause I/R injury (oxidative stress, inflammation, and apoptosis).

Tirzepatide is a novel dual incretin mimetic which stimulates both GLP-1 and GIP receptors ("twincretin"). It was recently permitted for type 2 diabetes following the SURPASS clinical trials revealed better glucose control and decreased body weight than a GLP-1 agonist alone (4,5). Tirzepatide's double action is anticipated to yield additional or synergistic benefits: but GLP-1 agonism is known to have anti-diabetic and organ-protective properties, GIP receptor activation could improve metabolic profile and maybe affect inflammatory processes. When GIP receptors, which are found in adipose tissue, are activated, they can lower adipose inflammation and inflammatory cytokines (3).

In rats, prolonged GIP analog treatment decreased levels of IL-1β, IL-6, and TNF-α at the same time increasing anti-inflammatory adiponectin (3). This shows that tirzepatide could successfully decrease systemic inflammation. Tirzepatide has demonstrated potential in chronic injury models related to the kidney: in streptozotocin induced diabetic mice, tirzepatide improved diabetic renal disease by dropping malondialdehyde, raising SOD and catalase, as well as lowering oxidative stress. It additionally suppressed pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) by preventing the IL-17 signaling pathway (3,5). A kidney outcomes analysis from the phase 3 study found that tirzepatide delayed the progression of chronic kidney disease (CKD) in type 2 diabetic patients, significantly lowering a composite endpoint of eGFR drop, kidney failure, or renal mortality compared to insulin. These findings raise the possibility that tirzepatide’s benefits extend to acute kidney injury settings as well, via concerted antioxidant and anti-inflammatory actions.

Given this context, we anticipated that tirzepatide might reduce acute renal ischemia-reperfusion damage by targeting the injury's primary pathological mediators: oxidative stress, inflammation (including inflammasome activation), and apoptosis. To investigate this, we used a rat bilateral renal I/R model and measured the effect of tirzepatide pretreatment on functional, biochemical, and histological indicators of kidney damage. We assessed renal failure indicators (serum urea, creatinine, NGAL) as well as tissue markers for oxidative injury (F2-isoprostane), inflammation (TNF-α), and apoptosis (caspase-3). By examining these many end goals We intended to find out more about how tirzepatide affects the complicated injury pathway in renal I/R. Our findings offer information on the therapeutic potential of GLP-1/GIP dual agonism as an approach for kidney protection against ischemic damage.

This study aimed to discover whether tirzepatide administration enhances kidney function and histology after I/R and decreases molecular indicators of damage in comparison to untreated I/R controls.

To the best of our knowledge, this work is the first to examine how tirzepatide affects acute tubular injury as well as oxidative stress markers, F2-isoprostane and NGAL particularly, in a rat model of early-phase renal ischemia-reperfusion injury. Although tirzepatide's nephroprotective effects documented in models of diabetes or chronic kidney injury in previous studies, this study is the first to assess its early protective effects in acute I/R conditions with a dual-time-point pretreatment strategy.

METHODS

Ethics approval

The experiments conducted in this study were reviewed and approved by the Ethics Committee of the Faculty of Pharmacy at The Kufa University, Najaf, Iraq (Approval No. 3177/2025-2-4).

Animals' preparation

A total of 24 adult male Sprague-Dawley rats, weighing between 200 and 250 grams, were sourced from the University of Kufa's Faculty of Science. They were housed at the animal house of the College of Science, University of Kufa, under standard conditions. The daily temperature was maintained at 24 ± 2 °C, and 60 - 65% humidity with the rats having free access to food and tap water. The animals were kept in cages, with a 12-hour light-dark cycle.

Experimental design

Twenty-four adults male SD rats were assigned randomly to four groups using a simple randomization method (n = 6 each group) The sample size of n=6 per group was chosen based on previous studies of I/R injury that detected significant differences with similar group sizes (6,7): Sham, I/R control (+ve control), I/R + DMSO, and I/R + Tirzepatide. The Sham group went through surgical operation without renal ischemia, while the I/R control group underwent renal ischemia-reperfusion injury without any drugs or vehicle interference. The I/R + DMSO group experienced I/R injury and got injection of dimethyl sulfoxide (DMSO) as medication solvent, whereas I/R + Tirzepatide group suffered I/R injury and received 1.35 mg/kg Tirzepatide (TargetMol Chemicals, China). Tirzepatide (or an equal DMSO vehicle) was injected intraperitoneally twice 24 hours and 60 minutes before the onset of ischemia to guarantee systemic drug exposure throughout the I/R insult. The dose of 1.35 mg/kg chosen based on prior studies indicating efficacy of tirzepatide at this dose in rats (8). At the conclusion of the experimental period the blood samples and kidneys were collected for a further assessment, and then all animals were sacrificed.

Renal ischemia/reperfusion model

Animals received ketamine (100 mg/kg) (Interchem, Holland) and xylazine hydrochloride (10 mg/kg) (Alfasan, Holland) intraperitoneally to induce anesthesia (9). After

anesthesia induction, the limbs were secured, the area around the incision was shaved, and it was then disinfected with iodine spray. Subsequently, performing a retroperitoneal flank incision. The renal pedicles were cautiously recognized using blunt dissection to gently separate the connective tissues and adipose tissue surrounding the right and left renal arteries and veins, both anteriorly and posteriorly. The abdominal cavity was accessed via a retroperitoneal flank incision exposing both kidneys. Bilateral renal occlusions were performed for 40 minutes using non-traumatic microvascular clamps (Minechina/Germany). The kidney's color shifted from red to dark maroon within 10 minutes, and the whole surface blanching was an obvious indication of occlusion. A sterilized gauze was then placed on the rat. Upon completing the ischemia phase and withdrawing the clamp, the kidney color would rapidly change from a dark maroon to a dark pink, signifying successful reperfusion. Upon verification of reperfusion, the incision was sutured and bandaged with sterilized gauze saturated with 0.9% saline to prevent dehydration.

Blood sample collection

Upon 2 hours of reperfusion, blood was collected directly from the heart for parameter assessments, and the kidneys were harvested for experimental parameter assessment; afterwards, the rats were euthanized by cardiac puncture. At the end of the procedure, when the rats were still under anesthesia approximately 2-4 milliliters of blood were collected directly from the heart. The blood sample was put in a gel tube without anticoagulant agents and centrifuged at 3000 rpm for about 10 min to extract serum. The serum was next used to evaluate urea and creatinine spectrophotometrically, and NGAL levels using commercial ELISA kit (Shanghai Ideal Medical Technology Co., Ltd. /China., Cat No: ADLEL-RT00241).

Tissue sample preparation

Samples of kidney tissue were taken after blood samples were collected. The kidney was sagittal sectioned into two parts; one was preserved in 10% formaldehyde for histological examination, while the other was frozen at -80°C in phosphate-buffered saline (PBS) for enzyme-linked immunosorbent assay (ELISA) analysis (10). For histopathological evaluation, one portion of the tissue was fixed in 10% formaldehyde, and the other part was frozen at -80°C before being homogenized at high intensity ultrasonic liquid processor in 1:10 W/V phosphate buffered saline which included 1% Triton X-100 and a mixture of protease inhibitors (Roche Germany) (11).The homogenate went through a centrifuge at 5000 rpm at 4˚C for 10 minutes. The resulting supernatants were used to assess F2-Isoprostane, TNF-α, and Caspase-3 using available ELISA Kits (Shanghai Ideal Medical Technology Co., Ltd. /China., Cat No: ADL-EL-RT01064, Cat No: ADL-EL-RT01063, Cat No : ADL-EL-RT00752).

Histopathological Examination

Kidney tissues have been extracted and preserved in 10% neutral-buffered formalin. The samples were fixed in paraffin and sliced into 5 µm thick slices. The tissue slices were stained using hematoxylin and eosin (H&E) for histological examination. The kidney tissues were examined for histological changes including tubular necrosis, inflammation, and damage severity. Injury scores were given depending on the level of damage, and tubular necrosis and inflammation were documented in every section. Histological changes were evaluated by the percentage of damaged renal tubules.

Tissue damage was scored semi-quantitatively as follows: 0 = no damage (intact tubules); 1 = <25% damage; 2 = 25 - 50% damage; 3 = 50 - 75% damage; and 4 = >75% damage (12). All outcome assessments, including histopathological scoring and biomarker measurements, were performed by investigators blinded to group allocations.

Statistical analysis

Version 8.1 of GraphPad Prism was employed to conduct statistical analysis. The data were expressed as mean ± standard deviation (SD) for normally distributed variables. The Shapiro–Wilk test was used to assess normality of data distribution. A one-way ANOVA was applied to assess differences across groups, then followed by Tukey's multiple comparison test. Non-parametric Kruskal Wallis and Dunn's post hoc tests were employed to compare the histopathological changes in different groups. A p-value < 0.05 was considered the threshold for statistical significance.

RESULTS

Effect of Tirzepatide on renal function (urea, creatinine, NGAL)

Effect of Tirzepatide on Urea and Creatinine

In comparison with the sham group, the I/R control group's serum urea and creatinine level was considerably higher (p<0.0001). The I/R control and vehicle (DMSO) groups did not differ significantly (p>0.05). In comparison of the rats treated with Tirzepatide with the I/R control group, the rats' blood urea levels were considerably lower (p<0.0001) **(Figure 1).**

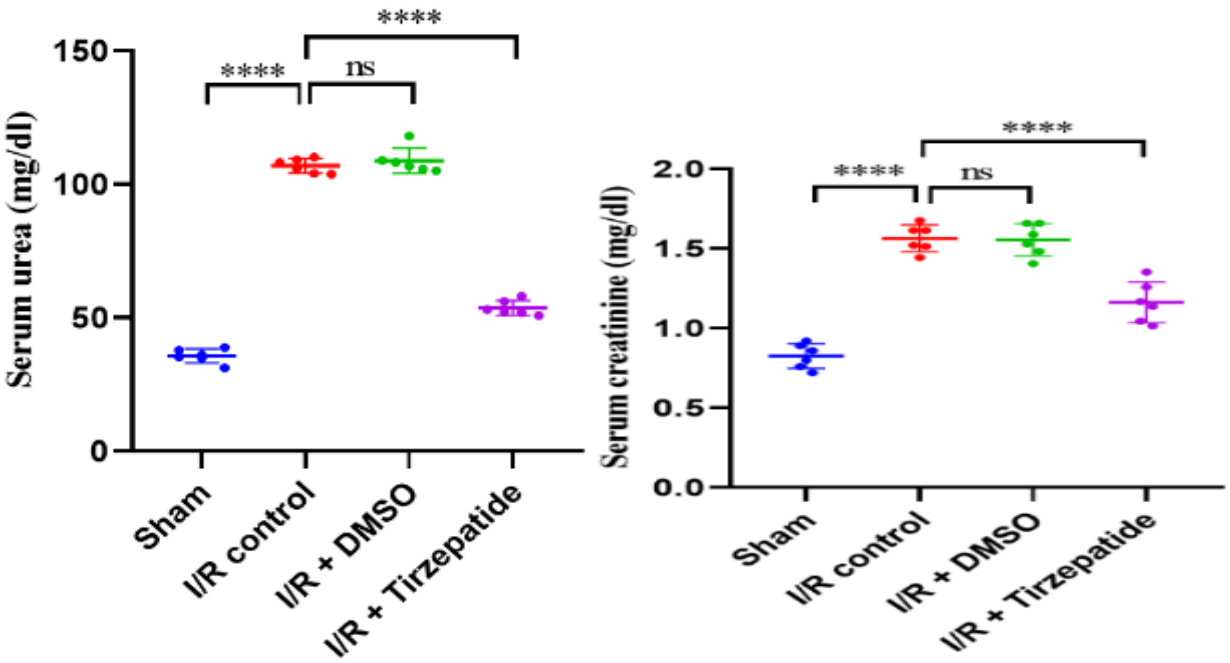

Figure 1. Means of serum urea and creatinine concentration in different groups.
Data represent mean ± SD; n=6 biological replicates. ****p<0.0001, and ns = p>0.05 (nonsignificant) vs. I/R control group (one-way ANOVA, Tukey's test).

Effect of Tirzepatide on NGAL level

In contrast to the sham group, the I/R control group's NGAL level was considerably higher (p<0.0001). There was no apparent difference between the vehicle and I/R control groups (p>0.05). NGAL levels were significantly less in the rats treated with Tirzepatide than in the I/R control group (p<0.0001) **(Figure 2)**.

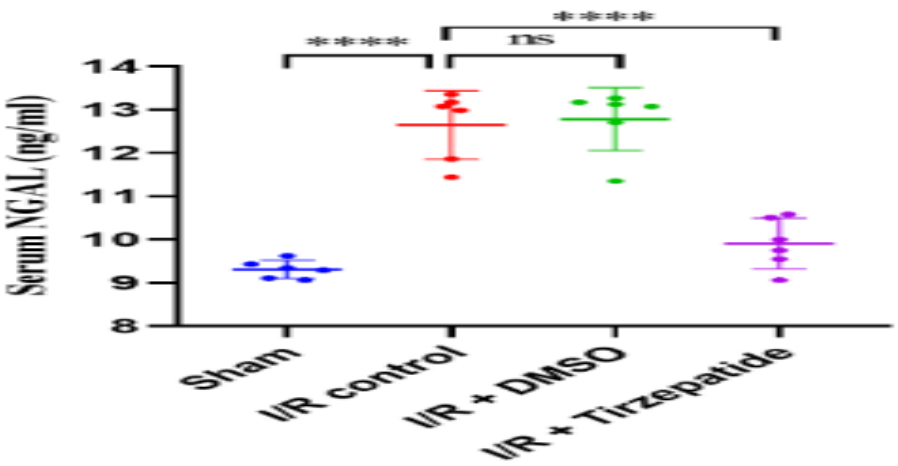

Figure 2. Means of serum NGAL concentration in different groups

Data represent mean ± SD; n=6 biological replicates. ****p<0.0001, and ns = p>0.05 (nonsignificant) vs. I/R control group (one-way ANOVA, Tukey's test).

Effect of Tirzepatide on Inflammatory biomarker F2-Isoprostane level

The I/R control group's tissue homogenate F2-Isoprostane concentration was significantly greater than that of the sham group (p<0.0001). When comparing the DMSO group to the I/R control group, there was no noticeable difference in the F2-Isoprostanelevel (p>0.05). Rats treated with Tirzepatide group showed a significant decrease in F2-Isoprostane levels in comparison to the I/R control group (p<0.01) **(Figure 3).**

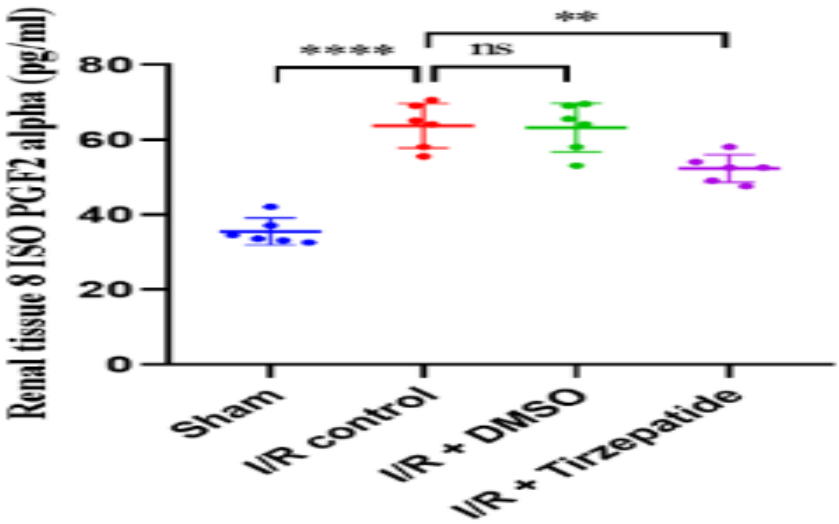

Figure 3. Means of tissue F2-Isoprostane concentration in different groups

Data represent mean ± SD; n=6 biological replicates. ****p<0.0001, **p<0.01, and ns = p>0.05 (nonsignificant) vs. I/R control group (one-way ANOVA, Tukey's test).

Effect of Tirzepatide on TNF-α level

The I/R control group's tissue homogenate TNF-α concentration was significantly greater (***p<0.001) than that of the sham group. When comparing the DMSO group to the I/R control group, there was no noticeable difference in the TNF-α level (p>0.05). Rats treated with Tirzepatide group showed a significant decrease in TNF -α levels in comparison to the I/R control group (***p<0.001) (**Figure 4**).

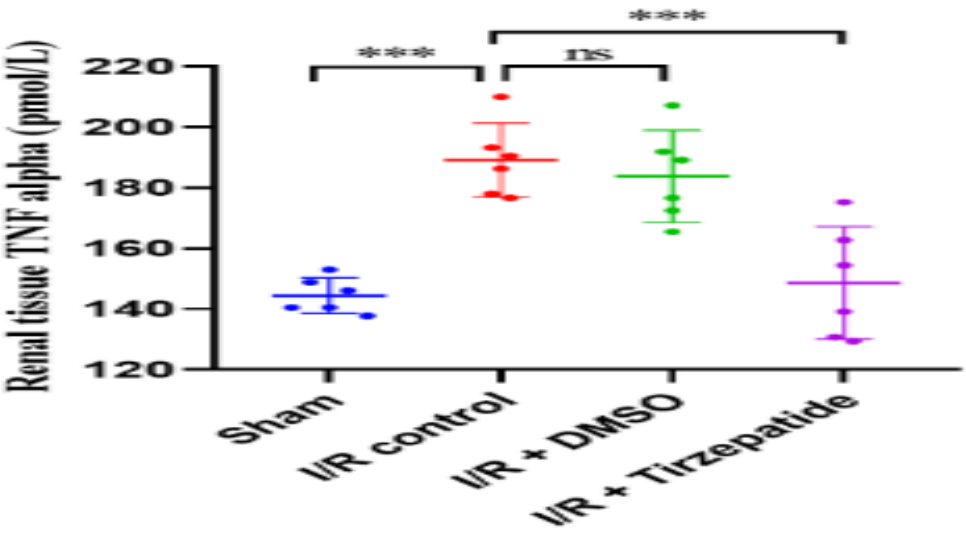

Figure 4. Means of tissue TNF-α concentration in different groups
Data represent mean ± SD; n=6 biological replicates. ***p<0.001, and ns = p>0.05 (nonsignificant) vs. I/R control group (one-way ANOVA, Tukey's test).

Effect of Tirzepatide on Pyroptosis Biomarker Caspase-3 level

The I/R control group's tissue homogenate Caspase-3 concentration was significantly greater than that of the sham group (****p<0.0001). When comparing the DMSO group to the I/R control group, there was no noticeable difference in the Caspase-3 level (p>0.05). Rats treated with Tirzepatide group showed a significant decrease in Caspase-3 levels in comparison to the I/R control group (****p<0.0001) **(Figure 5)**.

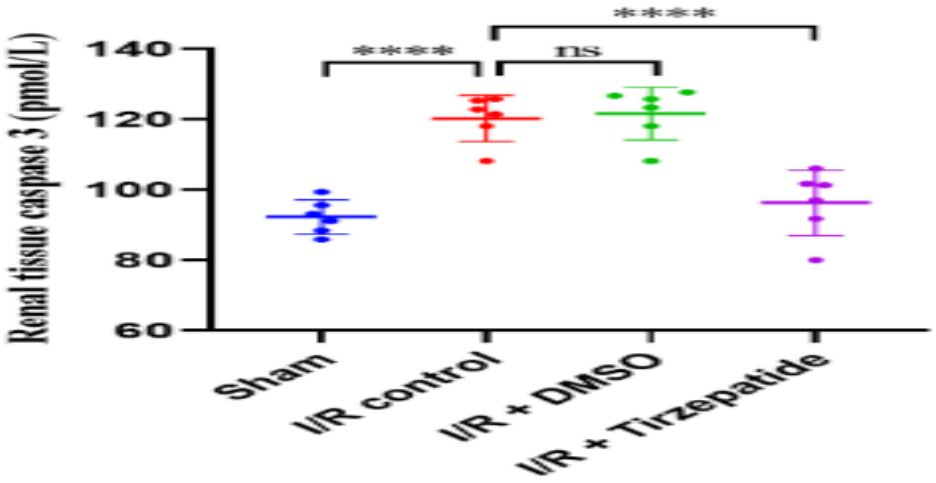

Figure 5. Means of tissue Caspase-3 concentration in different groups
Data represent mean ± SD; n=6 biological replicates. ****p<0.0001, and ns = p>0.05 (nonsignificant) vs. I/R control group (one-way ANOVA, Tukey's test).

Histopathological findings

The I/R control and DMSO groups showed a significant histological change (Scores of renal tubular damage) compared with normal tissue of sham group (***p<0.001). Further comparison that according to the histopathology and scoring system, it was found that the score of renal tubular damage, was significantly lower in Tirzepatide group compared to I/R control and vehicle groups (*p<0.05) **(Figure 6,7,8,9,10).**

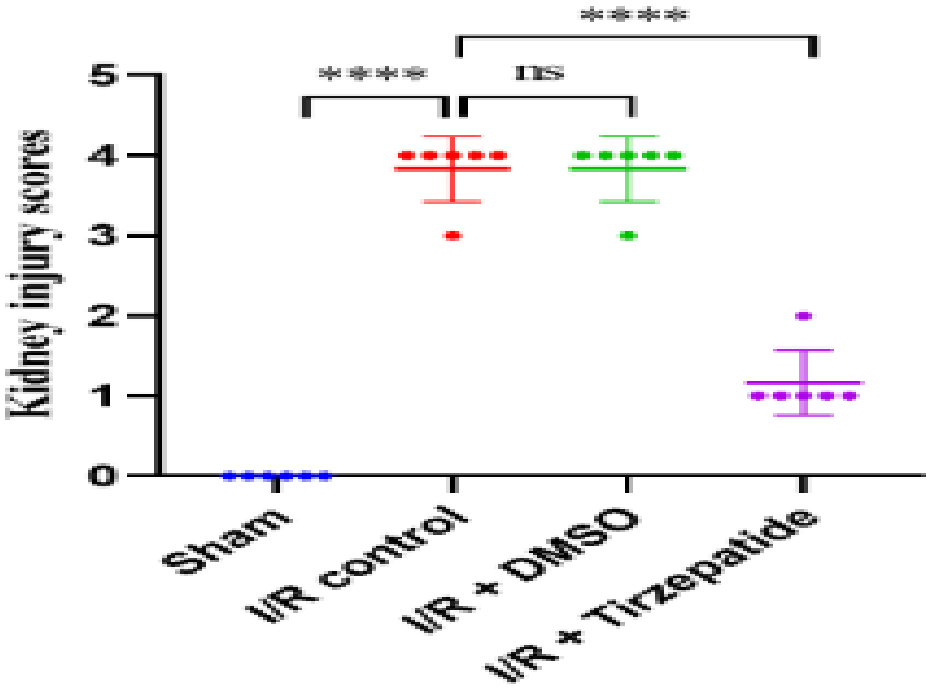

Figure 6. Scores of kidney injury in different groups
Data represent mean ± SD; n=6 biological replicates. ****p<0.0001, and ns = p>0.05 (nonsignificant) vs. I/R group (Non-parametric Kruskal Wallis and Dunn's post hoc tests).

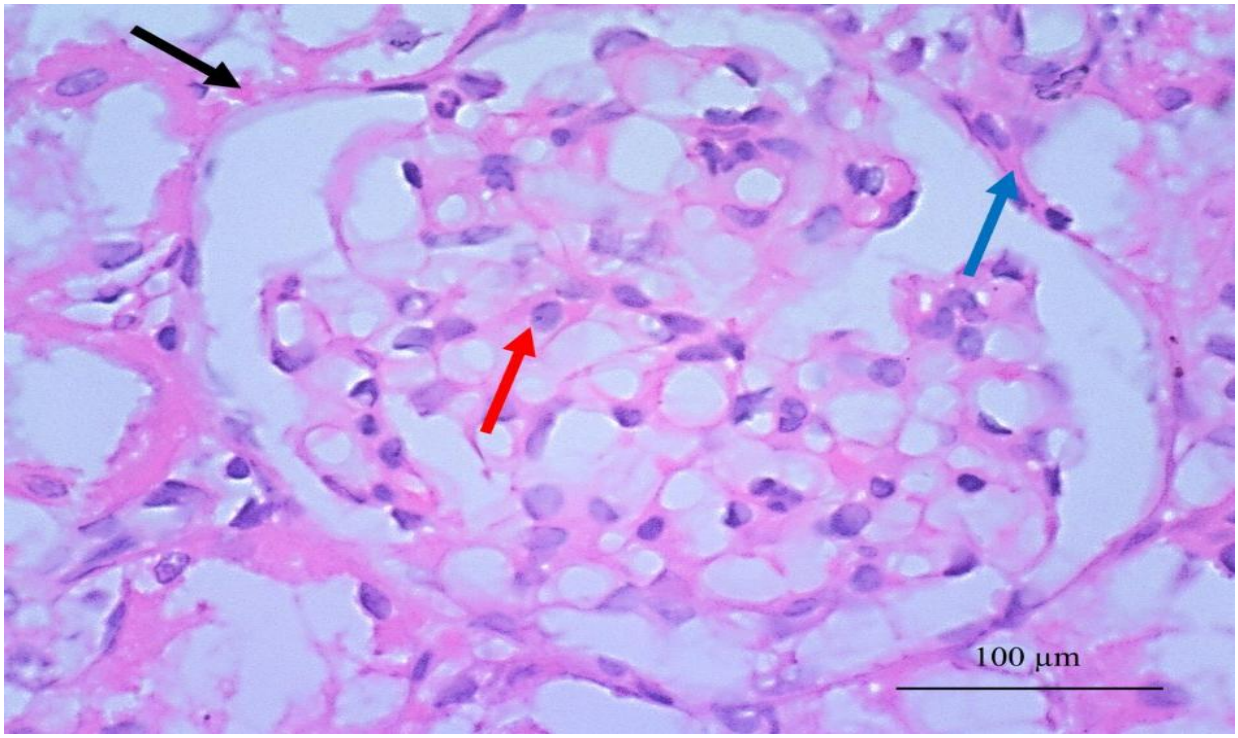

Figure 7. The histological section in kidney of rat in sham group.

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 lining the tubules with normal lumen (green arrows). The tissue is stained by H&E stain and the section was captured using digital camera attached to a light microscope at 40X magnifier scale.

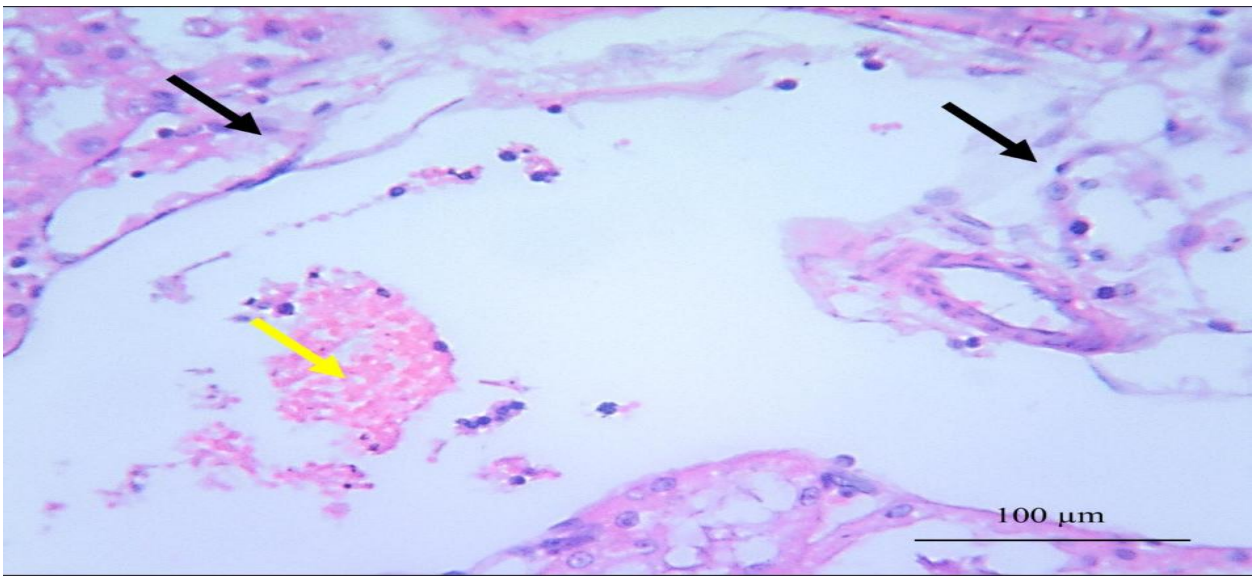

Figure 8. The histopathological section of kidney in rats of I/R control group

(Rats underwent renal artery clamping using the dorsal approach to cause ischemia for 40 min and reperfusion for 2 hours). The section shows clear necrotic lesion in the renal tissue (coagulative necrosis, Black arrows) with glomerular atrophy can be seen in the section of renal tissue (Red arrows). Glomerular tuft shows severe necrotic change for epithelial cells of tuft (Blue arrow). clumping of erythrocytes as severe pathological changes can be seen in the section (Yellow arrows). The tissue is stained by H&E stain and the section was captured using digital camera attached to a light microscope at 40X magnifier scale.

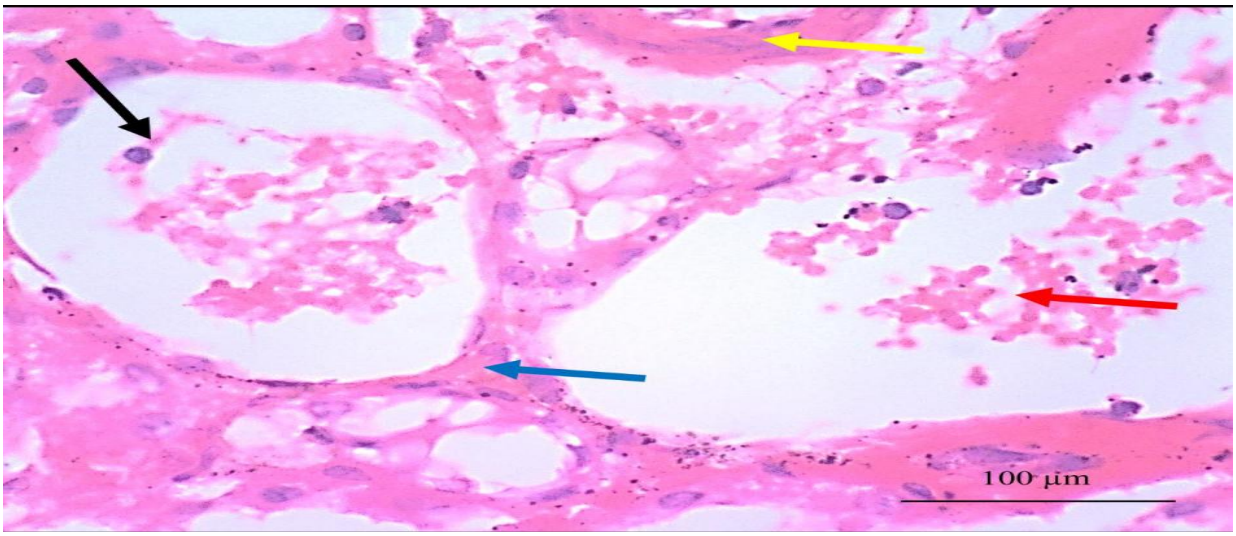

Figure 9. The histopathological section of kidney in rats of DMSO group.

(Intraperitoneal injection with DMSO solvent of tirzepatide 24 hours prior to ischemia and then one hour prior to ischemia, then underwent the surgical procedure, followed by 40 minutes of bilateral renal ischemia then 2 hours of reperfusion). The section of renal tissue shows severe atrophied lesion of renal glomeruli and glomerular tuft (Black arrows) with severe hypertrophic changes in the glomerular capsule (Blue arrow). The blood vessels show clear wall damage congestion (Red arrow). The proximal renal tubules show narrowing in the lumen with epithelia hypertrophy of lining cells (yellow arrows). The tissue is stained by H&E stain and the section was captured using digital camera attached to a light microscope at 40X magnifier scale.

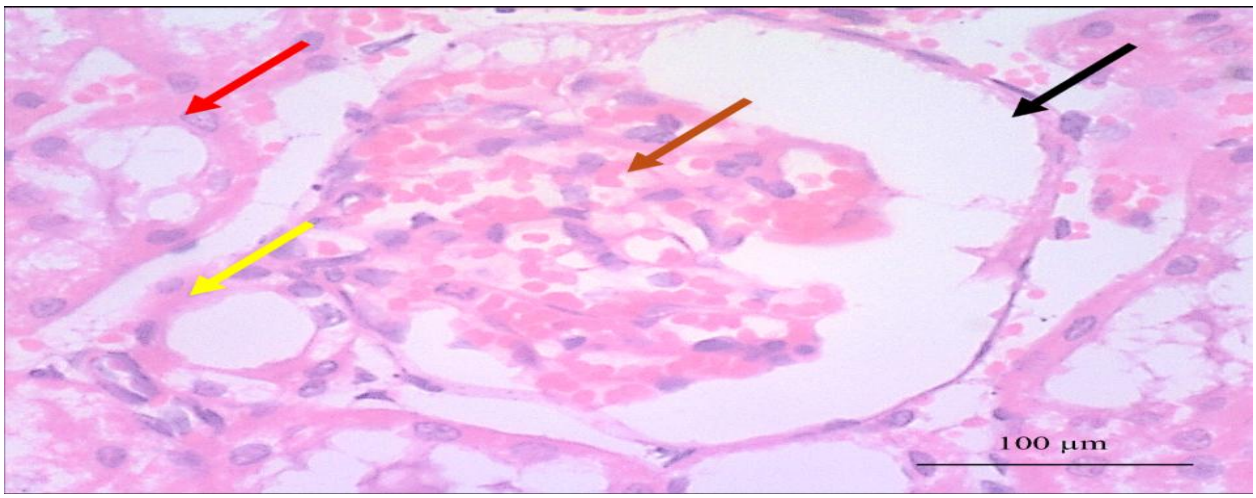

Figure 10. The histopathological section of kidney in rat of the treated group with Tirzepatide

(Intraperitoneal Injection with Tirzepatide (1.35 mg/kg) both 24 and 1 hour previous to induction of ischemia). The section shows abnormal glomerular capsule and tuft (Black arrow) with RBCs accumulation in the glomerular tuft (Brown arrow) and normal proximal renal tubule lumen (Red arrows) with mild hypertrophic lesion of epithelial cells that lining the renal tubules (Yellow arrows). The tissue is stained by H&E stain and the section is captured using digital camera and light microscope at 40X magnifier scale.

DISCUSSION

In this study, the results showed serum urea, serum creatinine and serum NGAL concentrations were significantly raised in the control and DMSO groups compared to the sham group, reflecting impaired kidney function and tubular damage. These results are comparable with Shiva et al in (2020) that showed significant increases in serum levels of urea and creatinine and NGAL (13). In addition to Jeon et al., in (2024) which noted similar results (14). The noted increase in NGAL levels in the renal IRI group corresponds with past research showing its significant relevance for ischemia-related kidney injury (15). NGAL released as consequence of tubular damage, whereas urea and creatinine levels rised due to diminished renal function. These indicators indicate renal dysfunction during ischemia-reperfusion injury.In the results of this research it was founded that the administration of tirzepatide 24 hours and 1 hour before the induction of renal ischemia reperfusion injury lead to significant decrease in serum levels of urea and creatinine in comparison with the control and DMSO group, indicating a protective effect on renal function. This finding in line with the results of Alkhafaji and Janabi (2025) that found in their study where they examined the possible renoprotective benefits of tirzepatide on rats with renal ischemia-reperfusion injury (Alkhafaji and Janabi, 2025). Additionally, Yang et al (2024) was noted that tirzepatide exhibited kidney function-preserving effects in diabetic nephropathy models treated with tirzepatide, with reductions in creatinine levels (17). This noted decrease in these markers indicate that tirzepatide could have a protective effect on renal function after ischaemic injury, by avoided or decreasing the cellular disorders that linked to IRI. The current study offers a novel perspective on role of tirzepatide in modulating NGAL during renal ischemia-reperfusion injury, filling a gap in the literature. In which the result showed that the pretreatment of rats with tirzepatide significantly reduced the serum level of NGAL. This result is corresponding with those that described by Tian et al. (2025), tirzepatide found to reduce levels

of NGAL, which is marker of kidney injury, in diabetic nephropathy models (18). Also, Hassan et al. (2024) was studied the impact of tirzepatide in lessening kidney damage brought by colistin, which is antibiotic recognised for its nephrotoxic adverse effect, and there findings proved that tirzepatide treatment lowered kidney injury significantly, as reflected by decreased levels of NGAL (19). Depending on the improvement of the level of renal functions and decreasing of kidney damage indicator, these results primarily indicate that tirzepatide can prevent kidney damage. These characteristics could make it possible to protect kidney from ischemic injury with tirzepatide.

This study exhibited significant increase in tissue homogenate TNF-α level in control group in comparison with sham and DMSO group, which contribute in inflammatory damage. This inflammatory response exacerbate renal injury. This finding are in line with a study by Rahmani Moghadam et al., (2025) which examined the role of TNF-α in a rat model of IR induced renal failure. A noticeable reduction in renal function was reported after 24 hours of reperfusion in ischemically damage kidneys, following an increase in TNF-α levels compared to the sham group (20). A research by Ozgen et al., in (2025) on rats model that exposed to renal I/R. TNF-α level significantly increased in tissue of control group compared to sham group (21). Also, Ha et. al (2024) in other study showed the level of TNF-α in ischemic-reperfusion injury with partial nephrectomy rat models after 24 hours of reperfusion was elevated significantly in the control group (22). This research demonstrated that the pretreatment of the rats with tirzepatide has led to a significantly lower level of TNF-α when compared to the control and DMSO groups. This results are comparable to what Alkhafaji and Janabi, in (2025) found in their study, which show that tirzepatide significantly reduces TNF-α levels in rats model of renal ischemia-reperfusion injury (RIRI), helping in reduce inflammation and improve renal recovery (Alkhafaji and Janabi, 2025).

A research by Yang et al., in (2025) who was found that "tirzepatide reduced the expression of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) in both mouse serum and kidney homogenates" (17).

This findings of the study point that tirzepatide may prevent renal injury, particularly through the modulation of inflammatory mediators such as TNF-α , that may decrease the production or release of ROS and renal damage. The renal IR induction in this study lead to significant elevation in F2-isoprostane levels in tissue homogenate of control group in contrast to sham group, which reflect an elevation in oxidative stress that contributes to renal tissue injury. Consistent with the work of Alawadi et al. in (2020) and Lamaan M. Abbas et al. in (2021) which are both reported significant elevation in F2- isoprostane level after renal ischemia reperfusion induction (Lamaan Et Al., 2021; Alawadi et al., 2020). Also Hasan et al. in (2020) noticed significant increase in F2-isoprostane level after induction of renal IR when he investigated the nephroprotective potential effect of quercttin in renal ischemia reperfusion injury in rat model (Rusul et al., 2020).

In this study, the administration of tirzepatide 24 hours and 1 hour prior to ischemia induction has resulted in a significant decrease in tissue F2-isoprostane levels when compared to the control group and DMSO group. This study represent an original investigation to the effects of tirzepatide on F2-isoprostane levels in the context of renal ischemia-reperfusion injury, there is no directly related studies found in prior research. Additionally, Abd Uljaleel et al. (2023) reported that activation of GLP-1 receptors reduced F2-isoprostane levels and inflammatory markers in a mouse model of endotoxemia-induced lung injury. Further substantiating the potential to attenuate

oxidative stress in renal ischemia using GLP-1 receptors agonists (Abd Uljaleel et al., 2023). Furthermore, the research conducted by Rojas-Solé et al. in (2024) on rodent models of heart failure, found that treatment with GLP-1 receptor agonists reduced levels of F2-isoprostane significantly (27). The research results indicate that tirzepatide can prevent renal damage, primarily by reducing oxidative stress marker levels including F2-Isoprostane. The anti-oxidative features of tirzepatide may mitigate the effect of renal IR.

This research exhibited that RIRI results in significant increase in renal marker of apoptosis caspase-3 levels in control and DMSO groups when compared to the sham group, indicating apoptosis of renal tubular cells. This apoptotic cell death contributes in tissue damage after ischemia. Alaasam et al., in (2024) found comparable effect in their study, confirmed that renal IR injury increases caspase-3 levels (28). In addition to Kim et al., in (2024) their results pointed that IR injury caused marked increase in caspase-3 level, an indication to heightened tubular apoptosis (29). In current study, it was observed that pretreatment with Tirzepatide had lower levels of caspase-3 significantly when compared to control and DMSO groups. This findings indicated that tirzepatide has cytoprotective effect against ischemia and reperfusion. These outcomes reflect similar patterns as reported in the study conducted by Alkhafaiji and Janabi (2025) which displayed the nephroprotective effects of tirzepatide in renal ischemia-reperfusion injury rats models. The authors observed that caspase3 level significantly decreased, indicating protective effect from apoptosis (Alkhafaji and Janabi, 2025). Urkon et al., (2025) The study revealed significant reduction in caspase 3 level due to treatment with tirzepatide in their experiment animal models of Alzheimer's disease (30). Hegab et al. in (2024) they investigated the effect of adropin and tirzepatide combination in models of polycystic ovarian syndrome (PCOS), it observed that tirzepatide reduced the expression of caspase-3, suggesting its potential to decrease apoptosis in cells of heart tissue (31). The results of this research indicate that tirzepatide can prevent renal injury. Illustrated by reduction of the level of caspase-3 which can decrease cells death and renal damage.

There are several limitations in this study. First, future research with longer reperfusion times is advised because the brief reperfusion period (2 hours) might not accurately represent the progression and recovery phases of renal injury. Second, statistical power may be restricted for identification of smaller effects, even though our sample size (n = 6 per group) aligns with similar studies. Third, generalizability across male and female may be limited due to the restricted use of male rats. Lastly, only two dose and pretreatment strategy employed; additional dose-response and therapeutic timing research is required to confirm clinical applicability.

CONCLUSION

This research showed that tirzepatide has significant nephroprotective properties in rat models with induced renal ischemia-reperfusion injury. Tirzepatide efficiently reduces kidney damage by simultaneously attenuating oxidative injury, inflammation, and apoptosis, thereby interrupts the vicious cycle of I/R-induced AKI.

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Statement of Permission and Conflict of Interests

The authors declare that they have no conflict of interest.

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