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GLP-1 receptor activation protects against renal ischemia-reperfusion injury by suppressing oxidative stress and ferroptosis

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ABSTRACT

Objective(s): Renal ischemia–reperfusion (I/R) injury contributes to acute kidney injury through oxidative stress and regulated cell-death pathways, including ferroptosis. This study investigated the effects of linagliptin on renal I/R injury, with particular focus on oxidative stress and ferroptosis-associated changes.

Materials and Methods: Thirty-five adult male Wistar rats were randomly assigned to five groups (n=7/group): Sham, I/R, I/R+linagliptin (10 mg/kg, IP), I/R + Exendin(9–39) (160 µg/kg, IP), and I/R + linagliptin plus Exendin(9–39). Treatments were administered for three consecutive days before bilateral renal ischemia (45 min), followed by 24 hr of reperfusion. Renal function and injury biomarkers, oxidative stress markers, ferroptosis-associated biomarkers, and renal histopathology were assessed.

Results: Compared with untreated I/R animals, linagliptin lowered serum creatinine and renal injury biomarker levels, increased renal tissue SOD and CAT levels, raised GPX4, SLC7A11, and FTH1 levels, and reduced 4-HNE levels. These changes were accompanied by less histopathological injury. Co-administration of Exendin(9–39) partially attenuated linagliptin's effects, whereas Exendin(9–39) alone did not significantly improve the evaluated outcomes.

Conclusion: Linagliptin attenuated renal injury and altered biomarkers of oxidative stress and ferroptosis in this acute I/R model. The findings are consistent with a potential role for GLP-1 receptor signaling but do not establish definitive receptor dependence or ferroptotic cell death. Further studies are needed to clarify these mechanisms and assess their translational relevance.

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Introduction

Renal ischemia-reperfusion injury (I/R) is an unavoidable event during kidney transplantation and plays a pivotal role in delayed graft function, graft dysfunction, and rejection (1). Although timely restoration of renal perfusion is indispensable for tissue survival, reperfusion paradoxically aggravates tissue injury by triggering a complex cascade of molecular and cellular events (2). Excessive reactive oxygen species (ROS) generation, mitochondrial dysfunction, endothelial dysfunction, inflammatory activation, and regulated cell death collectively contribute to widespread cellular injury and tissue dysfunction (3). Consequently, the cumulative effects of these pathological processes progressively compromise renal function, ultimately increasing the risk of persistent kidney injury and chronic functional decline (4). Current pharmacological strategies for preventing renal I/R remain limited despite advances in perioperative management and supportive care. Novel therapeutic targets are therefore urgently needed.

Oxidative stress plays a pivotal role in the pathogenesis of renal ischemia-reperfusion injury by promoting mitochondrial dysfunction, inflammation, and structural renal damage (5). Reintroducing oxygen after ischemia triggers excessive reactive oxygen species (ROS) generation, overwhelming endogenous anti-oxidant defenses and causing oxidative damage through lipid peroxidation, protein oxidation, and DNA damage (6). These events impair tubular epithelial integrity, disrupt mitochondrial function, amplify inflammation, and ultimately accelerate renal dysfunction (7). Endogenous anti-oxidant enzymes, particularly superoxide dismutase (SOD) and catalase (CAT), normally protect against oxidative injury by scavenging ROS; however, their activities are markedly

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reduced during renal ischemia–reperfusion, shifting the intracellular redox balance toward oxidative stress (8). Consequently, restoration of anti-oxidant capacity has emerged as a promising therapeutic strategy (9). Among the regulated forms of cell death, ferroptosis has gained increasing attention as a key mechanism underlying renal I/R (9). Ferroptosis is characterized by iron-dependent lipid peroxidation driven by impaired anti-oxidant defenses, particularly the disruption of the GPX4/GSH axis (10). The SLC7A11–GPX4–FTH1 axis constitutes a major endogenous defense against ferroptosis by maintaining glutathione synthesis, detoxifying lipid hydroperoxides, and regulating intracellular iron homeostasis, whereas 4-hydroxynonenal (4-HNE) serves as a marker of lipid peroxidation (11). Growing evidence suggests that preserving this anti-oxidant axis suppresses ferroptosis and attenuates renal ischemia–reperfusion injury (12). Given the central role of oxidative stress and ferroptosis in renal I/R, therapeutic strategies targeting these pathways have attracted increasing interest.

Glucagon-like peptide-1 receptor (GLP-1R) signaling has attracted increasing attention because of its biological effects beyond glucose homeostasis (13). Although GLP-1 receptor agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors were originally developed for type 2 diabetes mellitus, accumulating evidence indicates that GLP-1R activation exerts anti-oxidant, anti-inflammatory, and cytoprotective effects in multiple organs, including the kidney (14). Experimental studies have shown that GLP-1R activation attenuates oxidative stress, suppresses inflammatory signaling, and enhances endogenous anti-oxidant defenses, thereby improving tissue tolerance to ischemic injury (15).

Linagliptin is a highly selective DPP-4 inhibitor characterized by minimal renal excretion (16). Beyond its glucose-lowering effects, linagliptin has demonstrated renoprotective properties by attenuating oxidative stress, suppressing inflammatory signaling, and improving renal function (17). However, whether linagliptin protects against renal ischemia–reperfusion injury by suppressing ferroptosis through GLP-1 receptor activation remains unclear. Therefore, this study investigated whether linagliptin protects against renal ischemia–reperfusion injury by pharmacologically activating GLP-1 receptor signaling and modulating oxidative stress and ferroptosis. We hypothesized that GLP-1 receptor activation attenuates renal ischemia–reperfusion injury by restoring anti-oxidant defenses, suppressing ferroptosis, and preserving renal structure and function.

Materials and Methods

Experimental animals

Thirty-five healthy adult male Wistar rats weighing 220–250 g were obtained from the Animal Research Center of Lorestan University of Medical Sciences (Khorramabad, Iran). Animals were housed in pathogen-controlled facilities under standardized environmental conditions (22 ±2 °C, 50–60% relative humidity, and a 12-hr light/12-hr dark cycle) with unrestricted access to standard laboratory chow and filtered water. Before experimentation, rats were acclimatized to the animal facility for one week to ensure physiological stabilization. Animal health and welfare were monitored daily throughout the experimental period. All procedures involving animals were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and are reported in compliance with the ARRIVE 2.0 guidelines. Every effort was made to minimize animal suffering and to reduce the number of animals used without compromising the scientific validity of the study. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee of Lorestan University of Medical Sciences (Approval No. IR.LUMS.REC.1405.131).

Experimental design

After a one-week acclimatization period, animals were randomly allocated to five experimental groups (n = 7 per group) using a computer-generated randomization sequence prepared by an investigator not involved in the subsequent experimental procedures or outcome assessments. Drug doses and treatment schedules were selected based on previous studies demonstrating effective pharmacological modulation of GLP-1 receptor signaling. Pharmacological pretreatment was initiated three consecutive days before surgery and continued once daily until the induction of renal ischemia. Before group allocation, each rat was assigned a unique alphanumeric identification code. The identification codes were entered into an online randomization tool, which randomly allocated the animals to five experimental groups, with seven rats in each group. The allocation followed the computer-generated random sequence rather than investigator-directed assignment.

The experimental groups were as follows:

1. Sham group: Rats underwent identical surgical procedures, including midline laparotomy and bilateral renal pedicle isolation without vascular occlusion. Animals received an equivalent volume of vehicle on the same schedule.
2. I/R group: Rats were subjected to bilateral renal ischemia followed by reperfusion and received an equivalent volume of vehicle.
3. I/R + Linagliptin group: Rats received linagliptin (10 mg/kg, intraperitoneally (IP)) once daily for three consecutive days before surgery. The final dose was administered 1 hr before induction of renal ischemia.
4. I/R + Exendin (9–39) group: Rats received Exendin (9–39) (160 µg/kg, IP) once daily for three consecutive days before surgery. The final dose was administered 90 min before renal ischemia induction to match the combination group's treatment schedule.
5. I/R + Linagliptin + Exendin (9–39) group: Rats received Exendin(9–39) (160 µg/kg, IP) 30 min before each linagliptin injection (10 mg/kg, IP). This sequence was selected to allow GLP-1 receptor antagonism to precede DPP-4 inhibition and was informed by a previously published animal protocol in which Exendin-9 was administered 30 min before the test compound to investigate the involvement of GLP-1 receptor signaling (18). The present protocol was designed to assess whether pharmacological antagonism of GLP-1 receptors modified the effects of linagliptin on renal ischemia–reperfusion injury. Both agents were administered once daily for three consecutive days, and renal ischemia was induced 1 hr after the final linagliptin injection.

The combination-treatment group was included to investigate whether pharmacological antagonism of GLP-1 receptors with Exendin(9–39) modified the effects of linagliptin on renal ischemia–reperfusion injury. The combination group received the same doses of linagliptin and Exendin(9–39) as the respective single-treatment groups. The study examined the potential involvement of GLP-1 receptor signaling in the effects of linagliptin,

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rather than establishing dose equivalence or quantifying pharmacological additivity or synergy. To minimize experimental bias, investigators responsible for biochemical analyses, histopathological evaluation, morphometric measurements, and statistical analyses remained blinded to group allocation until completion of all data analyses.

Induction of renal ischemia–reperfusion injury

Following an overnight fast, rats were anesthetized with sodium pentobarbital (50 mg/kg, IP). Adequate anesthesia was confirmed by the absence of the pedal withdrawal reflex before surgery. During surgery, animals were placed on a thermostatically controlled heating pad to maintain core body temperature at 37±0.5 °C. Respiratory pattern, mucosal color, pedal withdrawal reflex, and general physiological status were continuously monitored to ensure adequate anesthetic depth and physiological stability. Following aseptic preparation of the surgical field, a midline abdominal incision was made to expose both kidneys. The bilateral renal pedicles were carefully isolated from the surrounding connective tissue using blunt dissection and occluded with atraumatic microvascular clamps for 45 min to induce warm renal ischemia. Successful induction of ischemia was verified by the rapid color change of both kidneys from bright red to dark purple, indicating complete interruption of renal blood flow.

At the end of the ischemic period, the vascular clamps were gently removed to initiate reperfusion. Successful reperfusion was confirmed by the prompt restoration of the normal reddish coloration of the kidneys, indicating re-establishment of renal perfusion. The abdominal cavity was irrigated with sterile warm normal saline to minimize tissue dehydration before the abdominal musculature and skin were closed separately using absorbable sutures under sterile conditions. Animals recovered in warmed cages under close postoperative observation until full recovery from anesthesia. During reperfusion, animals were monitored regularly for general appearance, spontaneous activity, food and water intake, and signs of pain or postoperative complications. Standard husbandry conditions were maintained throughout the 24-hr reperfusion period, after which animals were deeply anesthetized for blood collection by cardiac puncture and subsequent tissue harvesting (19).

Sample collection

Twenty-four hours after reperfusion began, all animals were deeply anesthetized with sodium pentobarbital, and whole blood was collected via cardiac puncture. Blood samples were allowed to clot at room temperature and subsequently centrifuged at 3,000 rpm for 15 min to obtain serum. The separated serum was aliquoted into sterile microcentrifuge tubes and stored at −80 °C until biochemical analysis. Following blood collection, both kidneys were rapidly excised and rinsed with ice-cold sterile normal saline to remove residual blood. The right kidney was fixed in 10% neutral-buffered formalin for histopathological and morphometric evaluations. The left kidney was immediately snap-frozen in liquid nitrogen and stored at −80 °C until biochemical analyses. Frozen renal tissues were homogenized in ice-cold phosphate-buffered saline (PBS) supplemented with a protease inhibitor cocktail using a glass homogenizer. The homogenates were centrifuged at 12,000 × g for 20 min at 4 °C, and the resulting supernatants were collected for subsequent biochemical analyses. All tissue processing procedures were performed on ice to preserve protein integrity, minimize enzymatic degradation, and prevent postmortem biochemical alterations. Total protein concentration in each homogenate was determined using the Bradford protein assay, and tissue biomarker concentrations were normalized to total protein content where appropriate.

Assessment of renal function and kidney injury biomarkers

Renal function was evaluated by measuring serum creatinine with a commercially available rat ELISA kit (Cat. No. ELK9274; ELK Biotechnology Co., Ltd., Wuhan, China) according to the manufacturer's instructions. Renal tissue injury was further assessed by measuring the concentrations of cystatin C (Cys-C; Cat. No. ELK11021) and kidney injury molecule-1 (KIM-1; Cat. No. ELK11099) in kidney tissue homogenates using commercially available rat ELISA kits (ELK Biotechnology Co., Ltd., Wuhan, China) following the manufacturer's protocols (20).

Assessment of oxidative stress biomarkers

Renal oxidative stress status was evaluated by measuring the tissue concentrations of superoxide dismutase (SOD; Cat. No. ELK8178) and catalase (CAT; Cat. No. ELK5986) in kidney homogenates using commercially available rat ELISA kits (ELK Biotechnology Co., Ltd., Wuhan, China), according to the manufacturer's instructions. Where appropriate, the measured values were normalized to the total protein content of each homogenate (21).

Determination of ferroptosis-associated biomarkers

To investigate the involvement of ferroptosis in renal ischemia–reperfusion injury, the renal tissue concentrations of glutathione peroxidase 4 (GPX4; Cat. No. ELK7935), ferritin heavy chain 1 (FTH1; Cat. No. ELK6788), solute carrier family 7 member 11 (SLC7A11/xCT; Cat. No. ELK7274), and 4-hydroxynonenal (4-HNE; Cat. No. ELK0115) were quantified in kidney tissue homogenates using commercially available rat ELISA kits (ELK Biotechnology Co., Ltd., Wuhan, China) according to the manufacturer's instructions. For all ELISA assays, absorbance was measured at 450 nm using a calibrated microplate reader. Biomarker concentrations were calculated from the corresponding standard curves generated for each assay according to the manufacturer's protocols. Investigators performed all biochemical measurements in duplicate and were blinded to the experimental groups. Investigators calibrated instruments and performed internal quality-control procedures before each analytical session to ensure analytical accuracy and reproducibility. The mean value of duplicate measurements was used for all statistical analyses (21).

Histopathological evaluation

The right kidneys were fixed in 10% neutral-buffered formalin for 48 hr, routinely processed, embedded in paraffin, and sectioned at 5 µm. Tissue sections were stained with hematoxylin and eosin (H&E) according to standard histological procedures. An experienced renal pathologist performed histopathological examination using a light microscope and was blinded to the experimental groups. Renal injury was assessed in the renal cortex and outer medulla by examining tubular epithelial degeneration, including cytoplasmic vacuolization, tubular epithelial necrosis, tubular dilatation, intraluminal cast formation, and

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inflammatory cell infiltration. Tubules showing one or more of these histopathological abnormalities were considered affected for semiquantitative scoring. The severity of tubular injury was graded according to the percentage of affected tubules using a predefined scale: 0, normal histology; 1, <10% affected tubules; 2, 10–25%; 3, 26–50%; 4, 51–75%; and 5, >75%. The average score across multiple randomly selected, non-overlapping microscopic fields was calculated for each animal and used for statistical analysis. In addition, morphometric analysis was performed using digital image analysis software to quantify the percentage of damaged tubular area in randomly selected microscopic fields examined at identical magnification (5).

Statistical analysis

All statistical analyses were performed using GraphPad Prism version 8 (GraphPad Software Inc., San Diego, CA, USA). Data are presented as mean ± standard error of the mean (SEM). Sample size was determined a priori using power analysis based on the expected differences in renal injury biomarkers observed in preliminary experiments and previous studies. A minimum sample size providing 80% statistical power at a two-sided significance level of α=0.05 was calculated, and seven animals were included in each experimental group to compensate for potential surgical losses while maintaining adequate statistical power. Comparisons among multiple groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple-comparison post hoc test. Pearson's correlation analysis was performed where appropriate to evaluate the association between ferroptosis-related biomarkers and indices of renal injury. A two-tailed P<0.05 was considered statistically significant.

Results

Effects of linagliptin on kidney injury biomarkers

As shown in Figure 1, renal ischemia–reperfusion (I/R) injury resulted in marked renal dysfunction, as evidenced by significant increases in serum creatinine (Figure 1A), cystatin C (Figure 1B), and KIM-1 (Figure 1C) compared with the Sham group (P<0.001 for all comparisons). Pretreatment with linagliptin significantly reduced serum creatinine (P<0.01), cystatin C (P<0.001), and KIM-1 (P<0.001) compared with the untreated I/R group, indicating a substantial improvement in renal function. Administration of Exendin(9–39) alone did not significantly alter any of the evaluated renal biomarkers compared with the I/R group. However, serum creatinine, cystatin C, and KIM-1 remained significantly higher than those observed in both the Sham group (P<0.001) and the linagliptin-treated group (P<0.001). Co-treatment with linagliptin and Exendin(9–39) significantly increased serum creatinine (P<0.05), cystatin C (P<0.01), and KIM-1 (P<0.001) compared with linagliptin treatment alone. Nevertheless, all three biomarkers remained significantly lower than those observed in the untreated I/R group (creatinine, P<0.05; cystatin C, P<0.001; KIM-1, P<0.05) and were also significantly reduced compared with the Exendin(9–39) group (creatinine, P<0.05; cystatin C, P<0.001; KIM-1, P<0.01). These findings indicate that Exendin(9–39) partially, but not completely, attenuated linagliptin's renoprotective effects following renal I/R injury.

Effects of linagliptin on oxidative stress biomarkers

As shown in Figure 2, renal ischemia–reperfusion (I/R) injury markedly impaired the renal anti-oxidant defense system, as evidenced by significant reductions in tissue catalase (CAT; Figure 2A) and superoxide dismutase (SOD; Figure 2B) levels compared with the Sham group (P<0.001 for both biomarkers). Pretreatment with linagliptin significantly restored renal anti-oxidant capacity, with marked increases in CAT (P<0.001) and SOD (P<0.001) compared with the untreated I/R group. Administration of Exendin(9–39) alone did not significantly affect CAT or SOD levels compared with the I/R group. However, both anti-oxidant enzymes remained significantly lower than those observed in the Sham and linagliptin-treated groups (P<0.001). Co-administration of linagliptin with Exendin(9–39) significantly attenuated the anti-oxidant effects of linagliptin. Compared with linagliptin monotherapy, CAT (P<0.001) and SOD (P<0.01) levels were significantly reduced in the combination treatment group. Nevertheless, CAT and SOD remained significantly higher than in the untreated I/R group (P<0.001). They were also significantly higher than in the Exendin(9–39) group (P<0.001), indicating that GLP-1 receptor blockade only partially diminished linagliptin's anti-oxidant effects

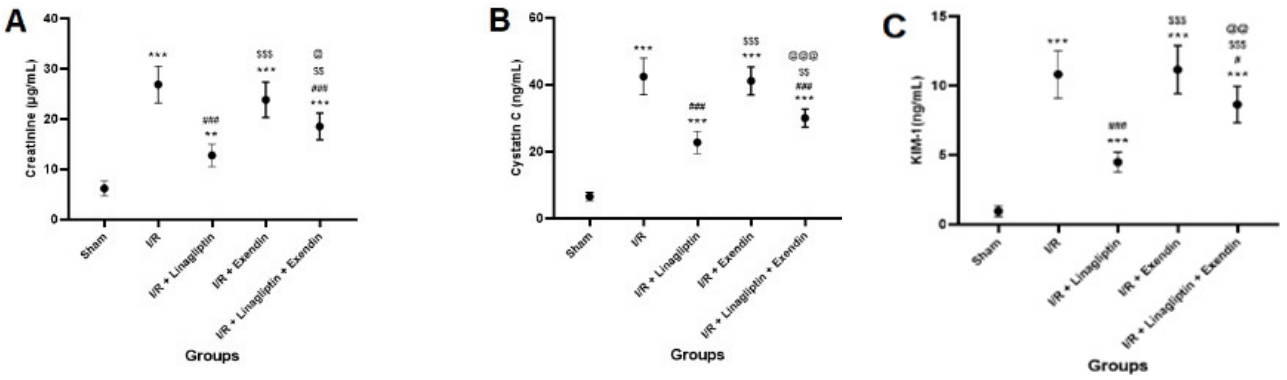


Figure 1. Effects of linagliptin on rat renal function and kidney injury biomarkers following renal ischemia–reperfusion injury
(A) Serum creatinine concentration and renal tissue concentrations of (B) cystatin C (Cys-C) and (C) kidney injury molecule-1 (KIM-1) were determined 24 hr after reperfusion. Renal ischemia–reperfusion markedly increased serum creatinine and renal tissue Cys-C and KIM-1 levels compared with the sham group. Linagliptin significantly attenuated these alterations, whereas Exendin(9–39) alone failed to confer protection. Co-administration of Exendin(9–39) partially reversed the protective effects of linagliptin. Data are presented as mean±SEM (n=7 per group). *P<0.05, **P<0.01, ***P<0.001 vs. Sham; #P<0.05, ##P<0.01, ###P<0.001 vs. I/R; $$P$<0.05, $$$P$<0.01, $$$$P$<0.001 vs. I/R+Linagliptin; @P<0.05, @@P<0.01, @@@P<0.001 vs. I/R+Exendin (9–39).

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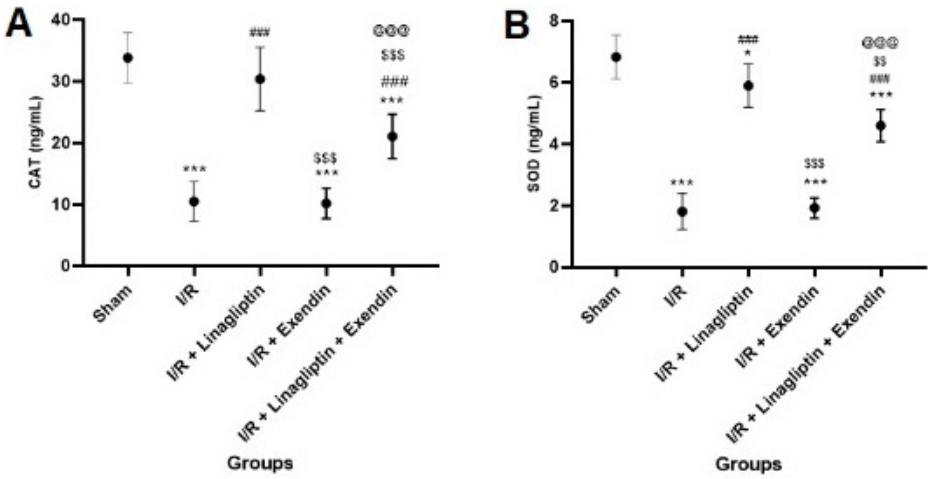


Figure 2. Effects of linagliptin on rat renal oxidative stress biomarkers following renal ischemia–reperfusion injury
Renal tissue concentrations of (A) catalase (CAT) and (B) superoxide dismutase (SOD) were quantified 24 hr after reperfusion. Renal ischemia–reperfusion significantly reduced antioxidant enzyme levels compared with the sham group. Linagliptin markedly restored CAT and SOD levels, whereas Exendin(9–39) alone did not improve oxidative stress. Co-treatment with Exendin(9–39) partially attenuated the antioxidant effects of linagliptin. Data are presented as mean±SEM (n=7 per group). *P<0.05, **P<0.01, ***P<0.001 vs. Sham; #P<0.05, ##P<0.01, ###P<0.001 vs. I/R; $$P$<0.05, $$$P$<0.01, $$$$P$<0.001 vs. I/R + Linagliptin; @P<0.05, @@P<0.01, @@@P<0.001 vs. I/R + Exendin(9–39).

following renal I/R injury.

Effects of linagliptin on ferroptosis-associated biomarkers

As illustrated in Figure 3, renal ischemia–reperfusion injury markedly promoted ferroptotic responses in kidney tissue. Compared with the Sham group, renal I/R significantly increased tissue 4-hydroxynonenal (4-HNE) levels (Figure 3A; P<0.001), while significantly decreasing ferritin heavy chain 1 (FTH1; Figure 3B), glutathione peroxidase 4 (GPX4; Figure 3C), and solute carrier family 7 member 11 (SLC7A11/xCT; Figure 3D) (P<0.001 for all comparisons). Pretreatment with linagliptin significantly attenuated these ferroptosis-associated alterations. Specifically, linagliptin markedly reduced renal 4-HNE levels (P<0.01 vs. I/R) while significantly restoring the expression of FTH1, GPX4, and SLC7A11 compared with the untreated I/R group (P<0.001 for all comparisons). Administration of Exendin(9–39) alone did not significantly modify any ferroptosis-associated biomarker compared with the I/R group.

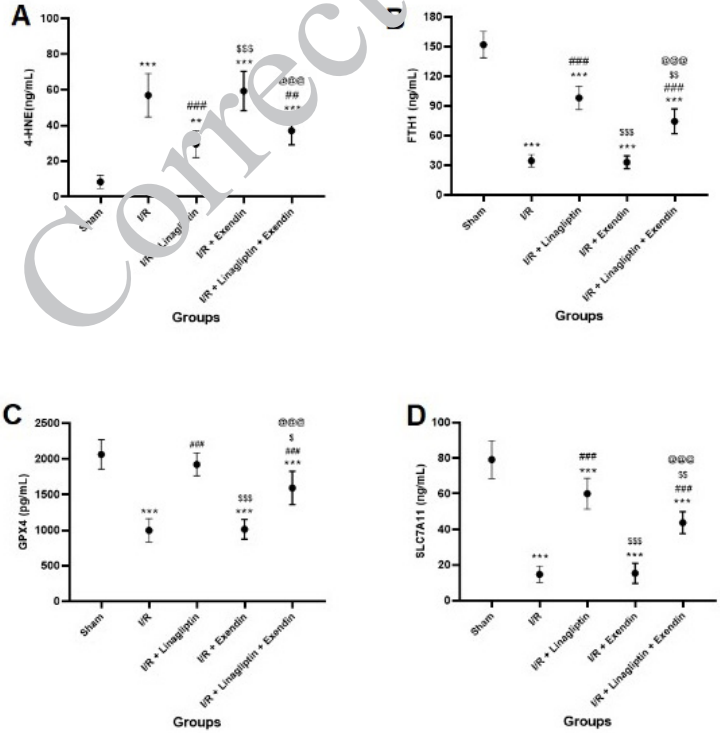


Figure 3. Effects of linagliptin on rat renal ferroptosis-associated biomarkers following renal ischemia–reperfusion injury
Renal tissue concentrations of (A) 4-hydroxynonenal (4-HNE), (B) ferritin heavy chain 1 (FTH1), (C) glutathione peroxidase 4 (GPX4), and (D) solute carrier family 7 member 11 (SLC7A11/xCT) were measured 24 hr after reperfusion. Renal ischemia–reperfusion significantly increased lipid peroxidation, as shown by elevated 4-HNE levels, while markedly decreasing GPX4, FTH1, and SLC7A11 expression compared with the sham group. Linagliptin substantially ameliorated these ferroptosis-associated alterations. Exendin(9–39) alone failed to exert protective effects, whereas co-administration of Exendin(9–39) partially reversed the beneficial effects of linagliptin. Data are presented as mean±SEM (n=7 per group). *P<0.05, **P<0.01, ***P<0.001 vs. Sham; #P<0.05, ##P<0.01, ###P<0.001 vs. I/R; $P<0.05, $$$P$<0.01, $$$$P$<0.001 vs. I/R+Linagliptin; @P<0.05, @@P<0.01, @@@P<0.001 vs. I/R+Exendin(9–39).

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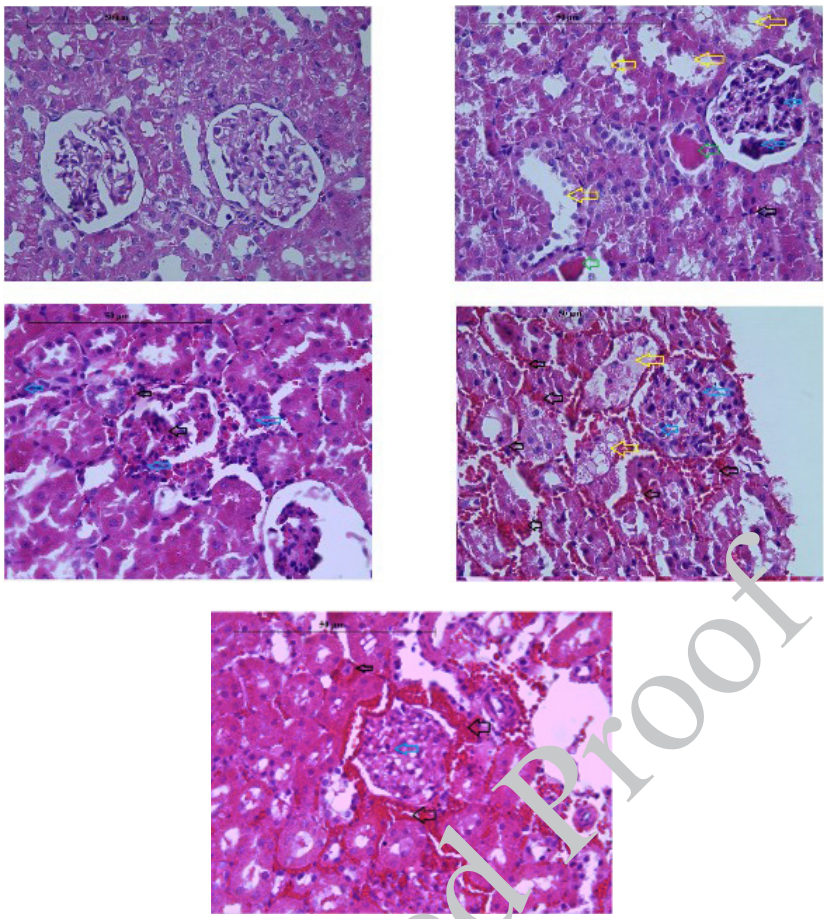


Figure 4. Representative H&E-stained rat renal sections (scale bar=50 µm)
The sham group exhibits normal renal architecture. The I/R group demonstrates severe tubular epithelial degeneration, cytoplasmic vacuolization (yellow arrows), tubular cast formation (green arrows), interstitial inflammatory cell infiltration (black arrows), and glomerular congestion (blue arrows). Linagliptin markedly ameliorated these histopathological alterations, whereas Exendin(9–39) alone failed to provide protection. Co-treatment with linagliptin and Exendin(9–39) partially attenuated the protective effects of linagliptin, resulting in moderate renal injury

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Nevertheless, renal 4-HNE remained significantly higher, whereas FTH1, GPX4, and SLC7A11 remained significantly lower than those observed in both the Sham and linagliptin-treated groups ($P<0.001$). Co-administration of linagliptin with Exendin(9–39) significantly attenuated the beneficial effects of linagliptin. Compared with linagliptin alone, renal 4-HNE levels increased significantly ($P<0.001$), whereas FTH1 ($P<0.01$), GPX4 ($P<0.05$), and SLC7A11 ($P<0.01$) decreased significantly. Despite this attenuation, all four biomarkers remained significantly improved compared with the untreated I/R group, with lower 4-HNE and higher FTH1, GPX4, and SLC7A11 levels ($P<0.001$ or $P<0.01$, as indicated in Figure 2). Moreover, the combination treatment also showed significant improvement compared with Exendin(9–39) alone, indicating that GLP-1 receptor blockade only partially reversed linagliptin's anti-ferroptotic effects.

Effects of linagliptin on renal histopathological alterations

Histopathological examination of H&E-stained renal sections revealed normal renal architecture in the sham group, with intact glomeruli and well-preserved renal tubules and no evidence of structural damage or inflammatory cell infiltration. In contrast, the I/R group exhibited severe renal injury, including extensive tubular epithelial degeneration, cytoplasmic vacuolization, tubular cast formation, interstitial inflammatory cell infiltration, and glomerular congestion. Similar histopathological alterations were observed in the Exendin(9–39)-treated group, indicating that GLP-1 receptor blockade alone failed to protect against ischemia–reperfusion-induced renal damage. In contrast, pretreatment with linagliptin markedly attenuated these pathological changes, preserving tubular architecture and substantially reducing tubular degeneration, inflammatory infiltration, and cast formation. Co-administration of Exendin(9–39) with linagliptin partially reversed these renoprotective effects, resulting in moderate histopathological injury that remained significantly less severe than that observed in the untreated I/R group but more pronounced than in the linagliptin-treated group. Overall, the histopathological findings closely aligned with the biochemical analyses, further supporting the pivotal role of GLP-1 receptor activation in mediating linagliptin's renoprotective effects following renal ischemia–reperfusion injury (Figure 4).

Discussion

The present study investigated the effects of linagliptin on renal ischemia–reperfusion (I/R) injury, with particular emphasis on renal injury markers, oxidative stress, histopathological changes, and ferroptosis-associated biomarkers. Linagliptin treatment was associated with improvements in selected biochemical and histopathological outcomes, together with changes in oxidative stress and ferroptosis-associated biomarkers.

However, the magnitude of the response varied across the measured parameters, and the improvement in creatinine was modest. The changes in cystatin C and KIM-1 should be interpreted according to the statistical significance of the respective between-group comparisons. Linagliptin treatment was also associated with changes in SOD and CAT measurements and in the ferroptosis-associated markers GPX4, SLC7A11, FTH1, and 4-HNE. However, the magnitude and statistical significance of these responses should be considered individually. Histopathological findings provided complementary evidence of attenuation of renal tissue injury in the linagliptin-treated group. The effects observed in the presence of Exendin(9–39) suggest that GLP-1 receptor signaling may contribute to some of the responses to linagliptin, whereas Exendin(9–39) alone did not significantly improve the outcomes compared with the untreated I/R group. Overall, these findings support a potential protective effect of linagliptin against selected aspects of renal I/R injury. At the same time, the extent of functional protection and the contribution of GLP-1 receptor signaling and ferroptosis-associated pathways require further investigation. Integrating functional, histopathological, and molecular biomarkers in the present study provides a mechanistic framework that strengthens the translational interpretation of linagliptin-mediated renoprotection (22).

Oxidative stress is widely recognized as a central driver of renal I/R injury and represents a major upstream trigger of ferroptotic cell death (23). Excessive reactive oxygen species production during reperfusion disrupts intracellular redox homeostasis, promotes lipid peroxidation, impairs mitochondrial function, and accelerates tubular epithelial injury (2). Because ferroptosis is associated with oxidative membrane damage and dysregulated iron metabolism, preservation of endogenous anti-oxidant defenses is considered a key mechanism limiting ferroptotic injury in ischemic tissues (24). Consistent with this concept, the present study showed profound suppression of the endogenous anti-oxidant system following renal I/R, as reflected by reduced SOD and CAT activities and marked disruption of ferroptosis-regulating proteins (25, 26). Linagliptin shifted these parameters toward sham-group values, although the magnitude and statistical significance of the responses should be considered separately for each outcome. These findings are consistent with a possible contribution of altered redox homeostasis to linagliptin's effects. Still, the present data do not establish whether these changes directly mediated the observed histopathological and functional responses. This effect is biologically plausible because activation of GLP-1 receptor signaling has been shown to enhance intracellular anti-oxidant responses, preserve mitochondrial function, and suppress oxidative stress, thereby creating a cellular environment that is less permissive for oxidative stress-mediated cellular injury (27). Consistent with this concept, context-dependent regulation of oxidative stress is increasingly recognized as a critical determinant of tissue protection during ischemia–reperfusion injury (28). Immunometabolic reprogramming highlights the interconnected roles of mitochondrial dysfunction, oxidative stress, and inflammatory signaling in promoting tissue injury (29).

Among the endogenous defense mechanisms against ferroptosis, the SLC7A11–GSH–GPX4 axis plays a pivotal role in maintaining phospholipid redox homeostasis (30). In parallel, FTH1 limits the availability of catalytically active iron, thereby reducing lipid radical propagation (31). Disruption of these protective pathways has been increasingly implicated in ferroptosis-associated kidney injury (32). Reduced SLC7A11 and GPX4 activity weakens cellular anti-oxidant defense, whereas disruption of iron homeostasis involving FTH1 compromises intracellular iron sequestration and increases susceptibility to iron-dependent oxidative injury (32). Consequently, excessive accumulation of lipid peroxidation products such as 4-HNE can further aggravate tubular epithelial injury, promote ferroptotic cell death, and accelerate renal dysfunction (33). Accordingly, preserving these endogenous anti-oxidant and iron-regulatory systems has emerged as an important therapeutic strategy to limit ferroptosis during renal ischemia–reperfusion injury (34). Interventions that restore this protective network may reduce ferroptotic susceptibility and mitigate ischemia-induced renal damage (35).

The present study demonstrated that renal I/R was associated with reduced levels of GPX4, SLC7A11, and FTH1, together with increased accumulation of the lipid peroxidation marker 4-HNE. These changes are consistent with disruption of anti-oxidant and iron-regulatory mechanisms associated with ferroptosis in ischemic renal tissue. Linagliptin treatment attenuated these alterations, with higher levels of ferroptosis-protective proteins and lower 4-HNE levels than those observed in the untreated I/R group. Taken together, these findings suggest that modulating oxidative stress and ferroptosis-associated pathways may contribute to linagliptin's effects in this model. However, because the assessment was based on molecular markers rather than direct measurements of ferroptotic cell death, the involvement of ferroptosis should be interpreted cautiously. Furthermore, although the findings in the Exendin(9–39) co-treatment group are consistent with a possible contribution of GLP-1 receptor signaling, they do not establish definitive receptor-mediated effects. Further studies using direct assessments of ferroptosis and complementary approaches to investigate GLP-1 receptor involvement are needed to clarify these mechanisms. Such multidimensional assessment of therapeutic effects is also relevant to clinical interventions, where treatment efficacy and safety may vary according to the characteristics and setting of the intervention (36).

Although linagliptin and GLP-1 receptor agonists act through different mechanisms within the incretin system, their expanding clinical use highlights the importance of individualized prescribing, careful patient assessment, and ongoing safety monitoring (37).

Conclusion

The present study suggests that linagliptin may attenuate selected aspects of renal ischemia–reperfusion injury in rats. Treatment was associated with improvements in some histopathological and biochemical outcomes, together with changes in oxidative stress and ferroptosis-associated biomarkers. However, the magnitude of improvement varied across the measured parameters, and the functional response, particularly the change in creatinine, was limited. The findings therefore do not establish uniform restoration of renal function or definitive inhibition of ferroptotic cell death. The effects observed in the presence of Exendin(9–39) suggest that GLP-1 receptor signaling may contribute to

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some of the responses to linagliptin, although receptor-specific mediation has not been conclusively demonstrated. Further studies incorporating comprehensive renal functional assessment and direct evaluation of ferroptosis are warranted to determine the extent and mechanisms of linagliptin's potential renoprotective effects.

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Limitations and Future Perspectives

Several limitations should be considered when interpreting these findings. First, although the effects of Exendin(9–39) support the potential involvement of GLP-1 receptor signaling, genetic approaches, such as receptor knockout or gene-silencing models, are needed to establish receptor dependence more definitively. Second, the study used an acute renal ischemia–reperfusion model involving 45 min of ischemia followed by 24 hr of reperfusion. Accordingly, the findings reflect early renal injury and do not establish linagliptin's longer-term effects on renal repair, fibrosis, or functional recovery. Furthermore, serum creatinine and cystatin C were assessed at a single time point, limiting the evaluation of changes in renal function over time. Studies incorporating serial measurements at multiple reperfusion time points would help characterize the progression of renal injury and subsequent recovery. Third, although changes in GPX4, SLC7A11, FTH1, and 4-HNE were consistent with modulation of ferroptosis-associated pathways, the study did not provide direct evidence of ferroptotic cell death. Future studies incorporating complementary assessments including iron accumulation, lipid ROS generation, mitochondrial ultrastructure, and ferroptosis-specific rescue experiments, would strengthen the mechanistic interpretation. Further investigation of upstream pathways, including Nrf2/HO-1, AMPK, and mitochondrial quality-control mechanisms, may also clarify how GLP-1 receptor signaling influences ferroptosis-associated processes. Finally, the combination group received the same individual doses of linagliptin and Exendin(9–39) as the respective monotherapy groups. Although this design allowed assessment of outcomes during combined treatment, the absence of dose-matched combination groups and a factorial design limits the ability to distinguish specific pharmacological interactions from the effects of adding one agent to the other. Therefore, the findings do not establish additivity or synergy. Future dose-ranging studies using factorial designs, followed by validation in clinically relevant preclinical models and clinical studies, are needed to clarify the underlying mechanisms and assess the translational potential of GLP-1 receptor signaling in ischemia-associated acute kidney injury.

Data Access and Responsibility

The corresponding author had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Authors' Contributions

M S conceived the study, developed the methodology, conducted the investigation, curated the data, and drafted the original manuscript. S D conducted the investigation, curated the data, and drafted the original manuscript. F B developed the methodology and contributed to reviewing and editing the manuscript. A RR conducted the investigation, curated the data, and contributed to reviewing and editing. Z H conducted the investigation, performed the histopathological assessment, and contributed to reviewing and editing. E G performed the formal and statistical analyses and contributed to reviewing and editing. A M provided supervision and project administration and contributed to reviewing and editing. All authors contributed to interpreting the results, critically reviewed the manuscript, approved its final version, and agreed to be accountable for all aspects of the work.

Conflicts of Interest

The authors declare that they have no conflicts of interest related to this study.

Declaration

The authors acknowledge the use of ChatGPT (OpenAI) solely for language editing and improving the clarity and readability of the manuscript. No AI tools were used for study design, data collection, data analysis, interpretation of the findings, or generation of scientific content. The authors take full responsibility for the accuracy and integrity of the manuscript.

References

1. Lasorsa F, Rutigliano M, Milella M, d'Amati A, Crocetto F, Pandolfo SD, *et al.* Ischemia-reperfusion injury in kidney transplantation: mechanisms and potential therapeutic targets. Int J Mol Sci 2024; 25: 4332.
2. Malek M, Nematbakhsh M. Renal ischemia/reperfusion injury; from pathophysiology to treatment. J Renal Inj Prev 2015; 4: 20-27.
3. Chen YS, Tian HX, Rong DC, Wang L, Chen S, Zeng J, *et al.* ROS homeostasis in cell fate, pathophysiology, and therapeutic interventions. Mol Biomed 2025; 6: 89.
4. An P, An Y, Chen M, Wu L, Wang R. Ischemia-reperfusion injury: molecular mechanisms and therapeutic interventions. MedComm (2020) 2026; 7: e70822.
5. Hasanvand A, Tajdar A, Mohtashami AZ, Haghighatian Z, Goodarzi E, Baharvand PA, *et al.* Investigating the role of empagliflozin in the pathological progress of ischemia-reperfusion injury in rat kidneys: the involvement of nitric oxide. Adv Pharm Bull 2025; 15: 939-947.
6. Maseko TL, Dlamini NP, Molefe SK. Role of oxidative stress and mitochondrial dysfunction in the pathogenesis of renal ischemia-reperfusion injury. J Adv Biomed Med 2025; 13: 292-310.
7. Chen Y, Li Z, Zhang H, Chen H, Hao J, Liu H, *et al.* Mitochondrial metabolism and targeted treatment strategies in ischemic-induced acute kidney injury. Cell Death Discov 2024; 10: 69.
8. Rashid H, Jali A, Akhter MS, Abdi SAH. Molecular mechanisms of oxidative stress in acute kidney injury: Targeting the loci by resveratrol. Int J Mol Sci 2024; 25: 3.
9. Pan Y, Zhu J. Mitochondria in renal ischemia-reperfusion injury: From mechanisms to therapeutics. Biomedicines 2026; 14: 310.
10. Artusi I, Rubin M, Cravin G, Cozza G. Ferroptosis in human diseases: Fundamental roles and emerging therapeutic perspectives. Antioxidants (Basel) 2025; 14: 1411.

Corrected Proof

11. Duo K, Feng X, Tian X, Wang F, Zhao Y, Yu J, *et al*. Ferroptosis inhibitors: Mechanisms of action and therapeutic potential. Cell Mol Life Sci 2025; 82: 441.
12. Granata S, Votrico V, Spadaccino F, Catalano V, Netti GS, Ranieri E, *et al*. Oxidative stress and ischemia/reperfusion injury in kidney transplantation: Focus on ferroptosis, mitophagy and new antioxidants. Antioxidants (Basel) 2022; 11: 769.
13. Gulyaeva NV. Glucagon-like peptide-1 receptor agonists are promising for the treatment of brain diseases: An outlook from the perspective of integrative physiology. J Evol Biochem Physiol 2025; 61: 1326-1348.
14. Gaggini M, Sabatino L, Suman AF, Chatzianagnostou K, Vassalle C. Insights into the roles of GLP-1, DPP-4, and SGLT2 at the crossroads of cardiovascular, renal, and metabolic pathophysiology. Cells 2025; 14: 387.
15. Salmanoglu M, Ercan G, Genç HS, Gül SS, Aygün H. GLP-1 receptor agonist exenatide protects against doxorubicin-induced cardiotoxicity through the SIRT1 pathway: An electrocardiographic, 99mTc-PYP scintigraphic, and biochemical study. Medicina (Kaunas) 2026; 62: 143.
16. Fronc K, Listos P, Kasprzak P, Berger M, Słowik T, Kotlińska J, *et al*. Linagliptin, a selective DPP-4 inhibitor, attenuates ketamine- and diazepam-induced deficits in passive avoidance performance in mice. Brain Sci 2026; 16: 710.
17. Saeedi T, Almikhlafi M, Elbadawy HM, Albadrani MS, Abdel-Rahman RF, Asaad GF, *et al*. Linagliptin attenuates kidney cancer in rats via AMPK activation and suppression of YAP/TAZ/HIF-1α signaling. Eur J Pharm Sci 2026; 217: 107393.
18. Lee J, Li Y, Cheng JT, Liu IM, Cheng KC. Development of syringaldehyde as an agonist of the GLP-1 receptor to alleviate diabetic disorders in animal models. Pharmaceuticals (Basel) 2024; 17: 538.
19. Haghighatian Z, Goodarzi E, Hadian B, Hasanvand A. Investigating the nitric oxide pathway stimulated by rosuvastatin on the pathological effects of renal ischemia-reperfusion in rats. J Pharm Pharmacol 2026; 78: rgaf097.
20. Goudarzi M, Kalantar H, Fereidoonnezhad M, Kalantar M. Protective effect of gallic acid on mercuric chloride nephrotoxicity in experimental rats. Nexus of Pathophysiology and Therapeutics (NPT) 2026; 1: 016, 1-13.
21. Cakir M, Polat A, Tekin S, Vardi N, Taslidere E, Duran ZR, *et al*. The effect of dexmedetomidine against oxidative and tubular damage induced by renal ischemia reperfusion in rats. Ren Fail 2015; 37: 704-708.
22. Mohammadi Jouabadi S, Mokhtari S. Mechanistic clinical pharmacology: Bridging PK-PD modeling, biomarkers, and translational therapeutics. Nexus of Pathophysiology and Therapeutics (NPT) 2026; 1: 006, 1-5.
23. Li X, Wang H, Ma H, Sun J, Luo Y, Qin M, *et al*. USAG-1 aggravates renal ischemia-reperfusion injury via promoting GPX4 degradation-induced ferroptosis. Cell Death Dis 2026; 17: 646.
24. Tan R, Ge C, Yan Y, Guo H, Han X, Zhu Q, *et al*. Deciphering ferroptosis in critical care: mechanisms, consequences, and therapeutic opportunities. Front Immunol 2024; 15: 1511015.
25. Qiu Q, Zhou Y, Xia K, Yan Z, Hui Y, Chen Z, *et al*. Luteoloside attenuates renal ischemia-reperfusion injury by suppressing ferroptosis through disruption of the KEAP1-NRF2 interaction. Free Radic Biol Med 2026; 248: 177-193.
26. Ozhan O, Ekici C, Ates B, Yildiz A, Balcioglu S, Vardi N, *et al*. N-acetyl cysteine amide mitigates oxidative stress and apoptosis in a rat model of renal ischemia-reperfusion injury. Sci Rep 2026; 16: 6323.
27. Oh YS, Jun HS. Effects of glucagon-like peptide-1 on oxidative stress and Nrf2 signaling. Int J Mol Sci 2017; 19: 26.
28. Yaghoubi A, Aghaei Z. Nitric oxide signaling in inflammation and oxidative stress: Mechanistic insights and therapeutic opportunities. Nexus of Pathophysiology and Therapeutics (NPT) 2026; 1: 009, 1-16.
29. Mousavi SE, Davoudi S, Soleimani E. Immunometabolic reprogramming in disease: From energy homeostasis and inflammatory signaling to therapeutic targeting across organ systems. Nexus of Pathophysiology and Therapeutics (NPT) 2026; 1: 013, 1-19.
30. Zhang M, Zhong H, Jin H, Zhu X, Liu S, Chen Y, *et al*. Regulating SLC7A11/GSH/GPX4 axis by glucose dyshomeostasis to simultaneously promote disulfidptosis, cuproptosis and ferroptosis. Bioact Mater 2025; 54: 744-758.
31. Sahin I. Regulatory effects of plant-derived phenolic compounds on ferroptosis: A novel paradigm in cancer therapy. Pharmedicine J 2026; 3: 1-26.
32. Li X, Yan J, Xu Y, Ling Z, Zhang T, Shao X, *et al*. Formononetin alleviated cisplatin-induced acute kidney injury by orchestrating renal tubular cell ferroptosis via PI3K/AKT/NRF2 pathway. Front Pharmacol 2026; 17: 1777367.
33. Lai K, Chen Z, Lin S, Ye K, Yuan Y, Li G, *et al*. The IDH1-R132H mutation aggravates cisplatin-induced acute kidney injury by promoting ferroptosis through disrupting NDUFA1 and FSP1 interaction. Cell Death Differ 2025; 32: 242-255.
34. Pan Y, Wang X, Liu X, Shen L, Chen Q, Shu Q. Targeting ferroptosis as a promising therapeutic strategy for ischemia-reperfusion injury. Antioxidants (Basel) 2022; 11: 2196.
35. Wang W, Chen J, Zhan L, Zou H, Wang L, Guo M, *et al*. Iron and ferroptosis in kidney disease: Molecular and metabolic mechanisms. Front Immunol 2025; 16: 1531577.
36. Seyed Siamdoust SA, Zaman B, Noorizad S, Alimian M, Barekati M. Comparison of the effect of intercostobrachial nerve block with and without ultrasound guidance on tourniquet pain after axillary block of brachial plexus: A randomized clinical trial. Anesth Pain Med 2023; 13: e134819.
37. Gonzalez ES, Soltani R. Beyond weight loss: Rethinking the clinical pharmacology and medication safety of GLP-1 receptor agonists. Nexus of Integrated Clinical Science (NICS) 2026; 1: 018, 1-6.

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