Renoprotective Effects of Isoliquiritigenin against Glycerol-Induced Myoglobinuric Acute Kidney Injury in Rabbits

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ABSTRACT

Acute kidney injury (AKI) caused by myoglobinuria associated with rhabdomyolysis is a severe clinical condition with limited effective therapies. This study evaluates the nephroprotective effects of isoliquiritigenin (ISO) in a glycerol-induced model of AKI in male rabbits. Sixty healthy male albino rabbits were divided into five groups: control, induction (glycerol-induced AKI), vehicle, ISO 30, and ISO 50. Renal function was assessed by measuring serum urea, creatinine, and creatine phosphokinase (CK) levels. Electrolyte balance, oxidative stress markers malondialdehyde (MDA), antioxidant enzyme levels glutathione peroxidase (GPx), and pro-inflammatory cytokine levels (IL6, IL1β, and TNF-α) were also evaluated. Histopathological examination of kidney tissues was performed to assess structural damage. The induction group exhibited significantly elevated urea, creatinine, and CK levels, alongside electrolyte imbalances, increased malondialdehyde MDA and decreased GPx levels, indicating severe oxidative stress and inflammation. ISO treatment, particularly at a 50 mg dose, significantly reduced serum urea, creatinine, CK levels, and improved electrolyte homeostasis. ISO also demonstrated potent antioxidant effects, reducing MDA and increasing GPx levels, while lowering pro-inflammatory cytokines. Histopathological analysis showed marked protection against kidney damage in the ISO-treated groups, with near-normal renal architecture observed in the 50 mg dose group. ISO exhibits significant nephroprotective effects against glycerol-induced AKI by improving renal function, restoring electrolyte balance, reducing oxidative stress, and suppressing inflammation. These findings suggest that ISO could be a promising therapeutic agent for managing AKI, particularly in cases associated with rhabdomyolysis.

Keywords: Acute kidney injury, Myoglobinuria, glycerol, oxidative stress, inflammation.

INTRODUCTION

AKI, identified as a reversible, abrupt reduction in renal function during a short period from hours to days, manifested as an elevation in serum creatinine and/or decreased urine production (oliguria). This condition may persist for up to 7 days[1,2]. The etiology is complex and multifactorial. It may result from the traumatic or nontraumatic origin, such as hypovolemia, septic shock, drug toxicity, electrolyte disturbances, chronic diseases, acute injuries (trauma or ischemia), excessive muscle workout, and surgical procedures, which sometimes trigger it[3]. AKI is a debilitating condition in clinical practice with a rising mortality rate; although many medications were used to restore normal renal functions, death among patients with AKI is significantly high, about 25-75%[4]. Rhabdomyolysis (RML) is where the muscle has been destroyed, and its intracellular contents, including myoglobin (Mb), spill into the blood. Considered one of the common causes of AKI, about 10-50% of RML patients are associated with AKI. Pathogenesis of RML-AKI, mainly mediated by myoglobin nephrotoxicity, is complex with vasoconstriction, inflammation, oxidative stress, renal epithelial cell apoptosis, and free iron overload[5]. Overproduction of Myoglobin is excreted through the kidneys, which then accumulates and causes tubular obstruction, inflammation, and oxidative stress to produce AKI[6]. The model of glycerol-induced myoglobinuric AKI in animals is widely used to investigate the pathophysiology and treatment of RML-induced kidney injury[7].

Isoliquiritigenin (ISO), a flavonoid extract from licorice root (*Glycyrrhiza* spp.), is known to be anti-inflammatory, anti-oxidant, and anti-cancer[8] Several studies have investigated the effects of ISO, an active constituent of licorice roots, that can protect the renal tissues via its impact on different signaling pathways, mitigate oxidative stress, and pro-inflammatory cytokines[9,10]. In an updated study about diabetic kidney nephropathy, it has been reported that ISO can preserve the renal tissues and function via reduced oxidative stress-induced renal damage[11]. Moreover, ISO has been found to reduce oxidative damage by activating antioxidant enzymes, removing free radicals, and inhibiting critical signaling networks during inflammation[12]. In another investigation, ISO was also observed to reduce acute liver damage by reducing inflammation and oxidative stress-provoked lipid peroxidation[13]. The existing literature suggests that ISO might offer efficacious anti-nephrotoxicity, limiting oxidative stress and inflammation. However, its potential as a protective agent in glycerol-induced myoglobinuric AKI has yet to be fully explored, warranting further investigation into its therapeutic efficacy in such models.

The current study investigated the protective activity of ISO on glycerol-induced myoglobinuric AKI in male rabbits. More specifically, the effects of ISO on oxidative stress, inflammatory markers, and kidney function were assessed. The research seeks to determine whether ISO can mitigate kidney damage associated with myoglobinuria and offer a potential therapeutic approach for preventing or treating AKI.

MATERIALS AND METHODS

Sources of chemical reagents: Isoliquiritigenin, a purified flavonoid (≥98%). It was purchased from Chengdu Bio Purify Phytochemicals

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Ltd. (Bp0787). Dimethyl sulfoxide (DMSO), Tween 80, protease inhibitor, and glycerol (GLY) were obtained from Med Chem Express. Rabbit enzyme-linked immunosorbent assay (ELISA) kits were purchased from ELK Biotechnology Co., Ltd., Wuhan.

Source of animals: The research was conducted between December 1, 2023, and July 31, 2024, with 60 healthy male albino rabbits collected from the animal house at Basra College of Medicine, AL-Basra University. Two weeks before the experiments, the rabbits were acclimated. This experiment was carried out according to the Guide for the Care and Use of Laboratory Animals and in accordance with the ethical policies and procedures of the Animal Ethics Committee of the College of Medicine, Baghdad University. Sixty adult male albino rabbits weighing 1-2 kg, 6 months-1 year old, were kept at 25±2°C and relative humidity of 50 ± 5%, with 12 hours of light/dark cycle. The animals were kept in 5 cages (12 rabbits in a cage) with free water and suitable food.

Experimental grouping: All of the rabbits were randomly assigned to five groups of 12 animals. All the animals in the study were deprived of water 24 hours before GLY administration to mimic the RML situation in humans. All the groups except the control were injected once deep intramuscularly with 8 ml/kg of (50% v/v) hypertonic GLY solution on the third day of the experiment, evenly divided between both hind limbs[14,15]. The control group was given distilled water IM. The vehicle solution consisting of (DMSO, Tween 80, and distilled water) (10:15:75) ratios was used to dissolve ISO. The control group received no treatment other than 5 ml of distilled water administered intraperitoneally (IP). The GLY group was given a single IM injection (8 ml/kg) of 50% hypertonic GLY solution on the third day. The vehicle group received the solvent solution IP for five days, along with an 8 ml/kg GLY injection on the third day. The ISO30+GLY group was administered 30 mg/kg ISO IP for five days, along with an 8 ml/kg GLY injection on the third day. Similarly, the ISO50+GLY group received 50 mg/kg ISO IP for five days, along with an 8 ml/kg GLY injection on the third day.

Collection of serum samples: All of the animals were fasted for two hours before anesthesia and 24 hours after the last dose. The rabbits were anesthetized according to the euthanasia protocol by using 35 mg/kg ketamine, 5 mg/kg xylazine hydrochloride, and 0.1 mg/kg acepromazine maleate[16] .About 3 ml of blood was collected from each animal, transferred into a gel tube, allowed to clot for 15 minutes, and then centrifuged for 10 minutes at 4,000 g. The supernatant serum was removed, aliquoted in Eppendorf tubes labeled, and stored at -20°C until biochemical testing. The samples collected were used for biochemical analyses, including serum urea, creatinine, and electrolytes (Na^+, K^+, Ca^{2+}), as well as serum CK, which served as a marker for predicting RML, and AKI[14].

Determination of biochemical markers: Kidney function was assessed by measuring serum urea and creatinine levels using specific kits, following the manufacturer's instructions. Serum CK was measured using a specific kit to determine the onset and extent of RML, following the manufacturer's instructions. Serum electrolytes (Na^+, K^+, and Ca^{+2}) were measured using specific biochemical kits, following the manufacturer's instructions. All the biochemical parameters were measured using the Mindray BS-240 clinical biochemistry analyzer.

Collection and preparation of kidney tissue samples: Both kidneys were removed soon after euthanasia, washed in normal saline to flush out blood, decapsulated, and sectioned horizontally[17]. Left kidneys were fixed in 10% formalin for histopathological examination. Meanwhile, the right kidneys were sorted in a clean tub with ice phosphate-buffered saline (PBS) and stored at -80°C until tissue homogenates were prepared. These samples were later used to determine renal tissue levels of oxidative stress markers (MDA, GPX) and inflammatory markers (TNF-α, IL-1β, IL-6).

Preparation of tissue homogenates for ELISA marker testing: Sections of rabbit's right kidney tissue frozen at -80°C were used as tissue homogenates. The tissue was then rinsed again with ice-cold PBS after defrosting it to wash away clots or RBCs. The kidneys were chopped up, weighed, and homogenized in a new lysis buffer (PBS, pH 7.4) at 1:9 tissue-to-buffer (100 mg tissue/900 µL buffer) according to the manufacturer's instructions using a tissue homogenizer[18]. The suspensions were sonicated at 4°C for 5 minutes at 10,000 g, the supernatant discarded, aliquoted to avoid further freeze-thaw cycles, and stored at -20°C until ELISA measurement of inflammatory and oxidative markers.

Histopathological examination of kidney tissues: Left kidney tissue was cut into 5 µm slices and fixed in 10% formalin. These sections were stained with hematoxylin and eosin according to the Bancroft Manual of Histological Techniques and their Diagnostic Applications[19]. A pathologist scored the histopathological changes in renal tissues semi-quantitatively using a scoring system. Tissue damage characteristics examined under a light microscope included interstitial vascular congestion, glomerular atrophy, glomerular congestion, interstitial inflammation, hyaline cast formation, acute tubular necrosis, and epithelial vacuolar degeneration. Scores were calculated by the average percent of each histopathological alteration in 10 fields per section for each animal in the group. The scoring system was based on five points (based on the severity of renal tissue damage): score 0 (no change), score 1 (1-25% damage), score 2 (26-50% damage), score 3 (51-75% damage), and score 4 (>75% damage)[20].

Statistical analysis: The data were analyzed using the Statistical Package for Social Sciences (SPSS; version 21), and the values are expressed as means ± standard errors of the mean (SEM). Differences between groups were evaluated by analysis of variance (ANOVA). Statistical significance was set at $p < 0.05$.

Ethical Approval: All animal procedures were conducted in accordance with the ethical guidelines for the care and use of laboratory animals and were approved by the Animal Ethics Committee of the College of Medicine, Baghdad University Approval No. (03-30). The study adhered to internationally accepted standards for laboratory animal care and welfare

RESULTS

Isoliquiritigenin improved renal function and muscle injury

The assessment of renal function through urea and creatinine levels revealed significant elevations in the induction group, displayed an elevated mean urea and creatinine levels, both significantly ($p < 0.001$) higher than those in the control group. These findings underscore the renal damage induced by glycerol. The vehicle group did not exhibit significant change in comparison with the induction group. Treatment with ISO demonstrated protective effect. In the 30 ISO 30 group, urea and creatinine levels significantly ($p < 0.001$) decreased compared with the induction group. This effect was even more pronounced in the ISO 50 group, where urea and creatinine levels were significantly ($p < 0.01$) reduced compared with the induction group (Table1). Furthermore, serum CK levels, a marker of muscle injury and RML, were significantly ($p < 0.01$) elevated in the induction group compared to the control. In the ISO30 group, CK levels showed no significant ($p < 0.05$) change from the induction group. However, the 50 mg dose (ISO 50 group) significantly ($p < 0.01$) reduced CK levels (Table 1).

Table 1. Effects of isoliquiritigenin on renal function biochemical parameters and serum CK levels.

Groups	Urea (mg/dL)	P-value	Creatinine (mg/dL)	P-value	CK U/L	P-value
Control	24.08 ± 7.66	-	0.61 ± 0.20	-	767.250±247.6321	-
Induction	89.16 ± 26.00	<0.001[a]	2.05 ± 0.69	<0.001[a]	30442.333±7651.8310	<0.001[a]
Vehicle	85.37 ± 13.53	0.87	1.93 ± 0.46	0.37	30442.333±7651.8310	1.00
ISO30	55.16 ± 28.24	<0.001[b]	0.90 ± 0.39	<0.001[b]	32883.33±8223.23	<0.41
ISO50	45.21 ± 10.77	<0.001[b]	0.80 ± 0.40	<0.001[b]	22811.25±9558.04	<0.01[b]

*Values are mean ± SD. (a) significantly different compared with the control group; (b) significantly different compared with the induction group

Table 2. Effects of isoliquiritigenin on electrolyte homeostasis.

Groups	Sodium (mmol/L)	P-value	Potassium (mmol/L)	P-value	Calcium (mg/dL)	P-value
Control	139.66±2.77	-	4.63 ±0.89	-	13.11 ±0.51	-
Induction	68.64±20.66[a]	<0.001[a]	8.89±1.63[a]	<0.001[a]	8.23±0.99	<0.001[a]
Vehicle	67.80±20.04	0.87	9.06±1.46	0.72	8.04±0.96	0.72
ISO 30	123.83±9.53[b]	<0.001[b]	6.39 ± 1.0[b]	<0.001[b]	12.41±1.5	<0.001[b]
ISO 50	120.33±9.0[1]b	<0.001[b]	4.99 ± 0.86[b]	<0.001[b] <0.01[c]	12.4±1.24	<0.001[b]

*Values are mean ± SD. (a) significantly different compared with the control group; (b) significantly different compared with the induction group; (c) significantly different compared with ISO30 group.

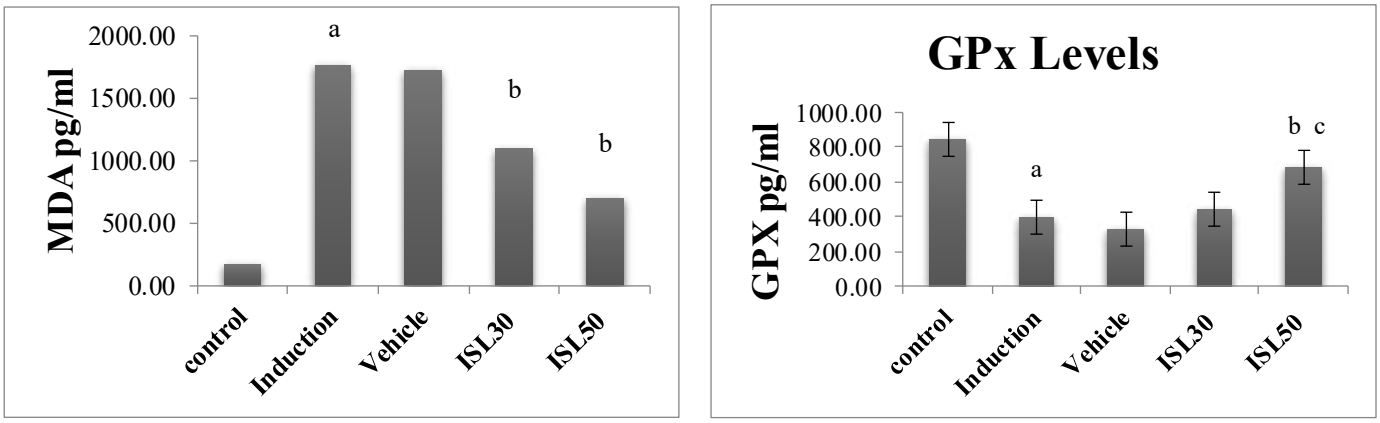

Figure 1. Oxidative stress marker levels in renal tissue. Values are expressed as mean ± SD. (a) significantly different compared with the control group. (b) significantly different compared with induction group. (C) significantly different compared with ISO30

Impact of isoliquiritigenin on serum electrolyte homeostasis

Electrolyte levels were assessed to understand further the impact of glycerol-induced AKI and ISO treatment on renal function. Sodium levels were markedly decreased ($p < 0.001$) in the induction group compared to the control group reflecting electrolyte imbalance due to renal dysfunction (Table 2). The administration of 30mg or 50mg of ISO helped restore sodium levels, which significantly ($p < 0.001$) increased, approaching control group levels.

Potassium levels, which are typically affected by renal impairment, were significantly ($p < 0.001$) elevated in the induction group. Both ISO-treated groups significantly ($p < 0.01$) reduced potassium levels, compared with induction group, and the ISO 50 group reaching near-normal levels. Calcium levels, which were significantly ($p < 0.001$) reduced in the induction group compared with the control, improved in both ISO-treated groups, with the highest restoration when the ISO given in 50 mg dose ($p < 0.001$) (Table 2).

Modulation of oxidative stress and antioxidant enzyme by isoliquiritigenin

To investigate the oxidative damage induced by GLY and the protective effect of ISO, the oxidative stress marker MDA and the antioxidant enzyme GPx were measured.

MDA shows a significant increase in the induction group, ($p < 0.001$) compared with the control group. Treatment with ISO leads to a reduction in MDA levels. The ISO 30 group showed a significant ($p < 0.05$) decrease in MDA levels. In comparison, ISO 50 group exhibited a marked reduction ($p < 0.001$) compared with the induction group.
Conversely, GPx levels were significantly ($p < 0.01$) lower in the induction group, compared with control group, and improved in both ISO-treated groups. The 50 mg group demonstrated the most substantial improvement, with GPx levels significantly ($p < 0.001$) restored compared to the induction group. Significant lower ($p \leq 0.001$ compared with ISO 30), as shown in (Figure 1).

Effects of isoliquiritigenin on pro-inflammatory markers

The pro-inflammatory cytokine levels (IL6, IL1β, TNF-α) were elevated in the induction group, which were significantly higher than those in the control group ($p < 0.001$). Treatment with ISO reduced cytokine levels, particularly at the 50 mg dose, significantly lowering cytokine concentrations ($p < 0.001$ compared to the induction group, $p < 0.001$ compared with ISO 30), demonstrating ISO's anti-inflammatory effect. (Figure 2).

Histopathological improvements caused by isoliquiritigenin

As presented in Table 3, ISO treatment prevented kidney pathology

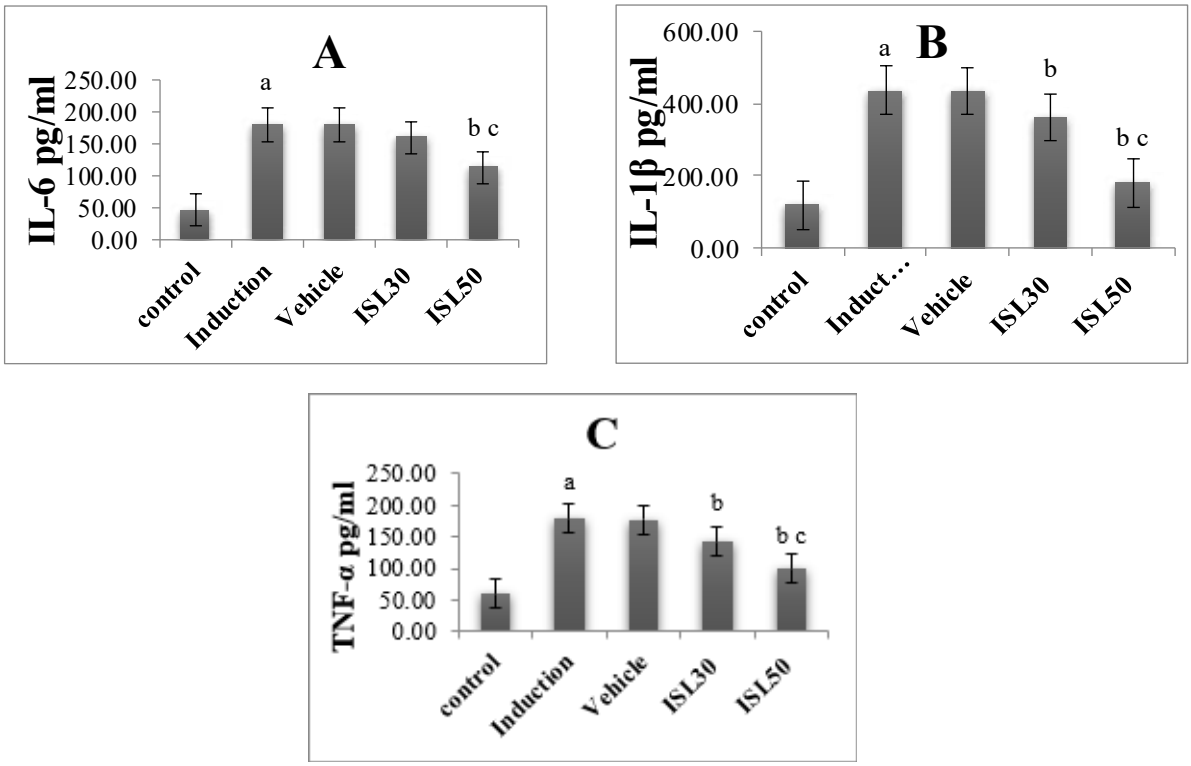

Figure 2. Pro-inflammatory cytokine levels in the renal tissue Values are expressed as mean ± SD: (a) significantly different compared with the control group; (b) significantly different compared with the induction group; (c) significantly different compared with LQ30 group.

Table 3. Histopathological changes of kidney scores in study groups.

Pathological change	Control (%)	Vehicle (%)	induction (%)	ISO30 (%)	ISO50 (%)
Inflammation	0 (0)	69±3.41a (3)	62±1.45[a] (3)	59±1.25 (3)	42±2.7[b] (2)
Glomerular atrophy	0 (0)	81±0.15[a] (4)	77±0.93[a] (4)	63±2.37 (3)	34±0.2[b] (2)
Glomerular congestion	0 (0)	93±0.09[a] (4)	81±2.4[a] (4)	47±0.12[b] (2)	31±1.18[b] (2)
Epithelial hyaline degeneration	0 (0)	89±4.63[a] (4)	93±3.1[a] (4)	70±0.78[b] (3)	55±0.96[b] (3)
Epithelial vacuolar degeneration	0 (0)	82±2.77[a] (4)	82±1.11[a] (4)	70±0.19[b] (3)	60±2.09[b] (3)
Hyaline cast formation	0 (0)	91±0.4[a] (4)	95±0.83[a] (4)	68±2.3[b] (3)	72±1[b] (3)
Interstitial vascular congestion	0 (0)	65±5.54[a] (4)	69 ±4.12[a] (3)	22±0.11[b] (1)	20±0.15[b] (1)

in groups with induced damage. The control kidneys showed no abnormality, while the induction and vehicle groups developed inflammatory, glomerular atrophy, congestion, hyaline degeneration, vacuolar degeneration, and cast formation (scores ranged from 62 to 95%). Isoliquiritigenin treatment improved these parameters, with 50 mg/kg giving the most significant ($p < 0.01$) reductions. For example, inflammation declined to 42%, glomerular atrophy to 34%, and congestion to 31% in the ISO50 group, indicating that ISO protected renal structure and function, especially at higher doses.

The values are expressed as a mean ± SD; ISO30: isoliquiritigenin (30 mg/kg dose); ISO50: isoliquiritigenin group (dose 50 mg/kg); (a) significantly different ($p \leq 0.05$) compared with the control group. (b) significantly different ($p \leq 0.05$) compared with the induction group.

The micrographs (Figure 3: A-O) results supported the findings of the histopathological scores. As shown in Figure A1, the kidney sections of the control group had normal glomeruli (black arrow) and intact proximal (blue arrow) and distal (green arrow) renal tubules in the cortex. Similarly, Figure A2 highlights the cortex's normal structure of glomerular capillaries (black arrow) and proximal renal tubular epithelium (blue arrow). In addition, Figure A3 reveals normal medullary renal tubules (black arrow) in the control group. In contrast, Figure B1 depicts the kidney sections of the induction group, showing significant vacuolation of the renal tubular epithelium (black arrow), hyaline degeneration of cortical renal tubules (red arrow), and protein casts within the proximal (green arrow) and distal (yellow arrow) renal tubules. Figure B2 further confirms these results, with marked vacuolation of the renal tubular epithelium (black arrow), hyaline degeneration of cortical renal tubules (red arrow), and protein casts in both proximal (green arrow) and distal (yellow arrow) tubules. Additionally, Figure B3 demonstrates hyaline degeneration of renal tubules (black arrow) and interstitial inflammation (yellow arrow) in the induction group.

The vehicle-treated group exhibited glomerular atrophy (black arrow; C1), marked hyaline degeneration of the renal tubular epithelium (black arrow), and protein casts in the medullary tubules (blue arrow; C2). Renal vascular congestion was also observed (black arrow; Figure 3: C3). In the ISO (30 mg/kg) treated group, kidney sections revealed sloughing of the renal tubular epithelium (black arrow) and glomerular destruction (blue arrow), as shown in Figure D1. Figure D2 further highlights protein casts in the renal tubular lumen (black arrow), consistent with Figure D3, which also showed protein casts in the renal tubular lumen (black arrow). In contrast, the ISO (50 mg/kg) treated group displayed normal renal tubular epithelium in all

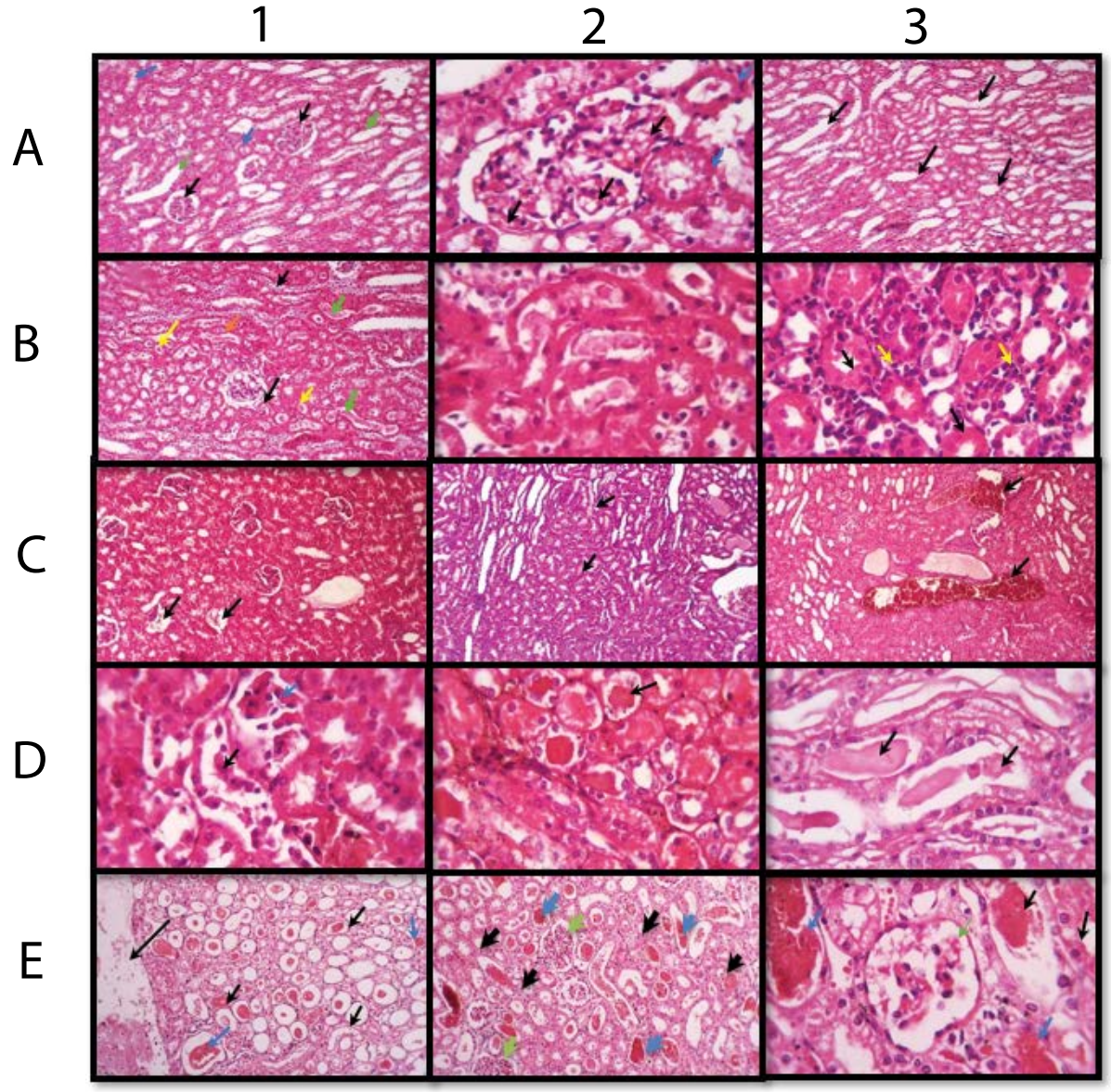

Figure 3. Histopathological features of kidney sections. A1-3: control group; B1-3: induction group; C1-3: vehicle treated group; D1-3: ISO (30 mg/kg) treated group; E1-3: ISO (50 mg/kg) treated group. The kidney sections were stained with hematoxylin and eosin.

medullary tubules (black arrow) alongside protein casts in the renal tubules (blue arrow), as depicted in Figure E1. Similarly, Figure E2 illustrates normal renal tubular epithelium in all cortical tubules, with protein casts in the renal tubules (blue arrow) and normal glomeruli (green arrow). Finally, Figure E3 shows that the 50 mg/kg ISO-treated group maintained normal renal tubular epithelium in all cortical tubules (black arrow), protein casts in the renal tubules (red arrow), and an atrophied glomerulus (green arrow).

DISCUSSION

AKI is one of the most significant complications associated with RML. Diverse mechanisms are involved in the pathogenesis of RML-induced AKI, primarily the Mb nephrotoxic effect, iron overload, oxidative stress, inflammation, Vasoconstriction, intratubular cast formation, tubular necrosis, and apoptosis[21,22]. Although renal function remission is possible after an RML episode, studies reported structural deformability, such as fibrosis, due to the precipitation of extracellular materials and the stimulation of pro-inflammatory cytokines and pro-fibrotic materials. Accordingly, urgent management to accelerate kidney tissue recovery and prevent life-threatening complications is mandatory[23]. Unfortunately, no effective treatment is currently available to cure or mitigate AKI, although several studies have been conducted in previous years for this reason[24]. "Developing Mechanism-Based Treatments" such as Antioxidant and anti-inflammatory products are of high value in managing RML-associated AKI due to their central role in the pathophysiology of AKI[25]. Our results exhibited a significant elevation in renal function parameters urea and creatinine in addition to Structural alterations, including tubular necrosis, hyalin degeneration, tubular cast formation, and glomerular atrophy.

Our results agree with earlier studies that reported an elevation in renal functional parameters and kidney histopathological alterations after GLY Administration[5,26,27]. GLY-induced AKI is the most widely used laboratory experimental model to understand AKI's diverse pathological mechanisms and investigate nephroprotective agents[26]. GLY administration associated with the release of numerous intracellular toxic materials, specifically Mb, leads to renal functional impairment that negatively affects kidney excretory function through various mechanisms, specifically oxidative stress-mediated

renal tubular damage and renal vasoconstriction due to the release of vasoactive materials like (MDA, Ang Π, TNF-α, vasopressin, isoprostane-F2), as well as vasodilators scavenging effects of Mb, consequently leads to hypoperfusion and renal ischemic-injury. This hypoperfusion decreases the tubular fluids, which are considered a proper media for Mb cast formation, causing tubular occlusion and reducing the glomerular filtration rate[21,28]. Moreover, structural damage of kidney tissues, such as diminishing brush borders, tubular necrosis, and tubular epithelial cell damage, leads to kidney excretory function failure and accumulation of waste products[29]. The ISO-treated groups (30, 50mg) significantly reduced renal function parameters, suggesting possible nephroprotective effects against GLY-mediated AKI. The findings of our study are compatible with many earlier studies that reported ISO's ability to attenuate GLY-induced elevation in serum urea and creatinine[30,31]. The ability of ISO to restore renal function could be explained by its vasorelaxant activity that attenuates renal vasoconstriction through stimulation of cyclic Guanosine monophosphate (cGMP), as well as nitric oxide (NO), thereby mediating renal vascular smooth muscle relaxation, improving renal blood flow and GFR[32]. Moreover, flavonoids, including ISO, have astonishing reactive oxygen species (ROS) scavenging ability, preventing ROS-induced lipid peroxidation and subsequent tubular epithelial cell damage, which maintains its role in regulating water and waste product elimination[33].

Serum CK levels were significantly increased, confirming muscle damage and release of intracellular contents into blood circulation, since CK elevation ≥1000 u/l indicates RML. Which was consistent with those of previous studies[34,35]. The result indicates that treatment with 50 mg dose still show high level of CK (22811.25±9558.04), which is above 1000 u/l. Polyphenolic compounds in previous studies exhibited CK reduction properties that could support our findings in the present study[36].

Glycerol injection resulted in electrolyte disturbances attributed to reduced glomerular filtration rate (GFR) and impairment of tubular fluid dynamics, specifically reabsorption[7,37]. The present study revealed electrolyte disturbances in the induction and vehicle groups, reflected as hyponatremia, hypocalcemia, and hyperkalemia. Tubular epithelial cells undergo structural damage due to Mb nephrotoxicity, which affects their function in controlling electrolytes and fluid homeostasis through tubular reabsorption. Hyponatremia is attributed to tubular damage leading to reabsorption impairment[38]. Our results were compatible with the findings of earlier studies that exhibited a significant reduction in serum Na^+ levels in rats model of GLY-induced AKI[39,40]. In contrast, some previous studies exhibited hypernatremia following GLY administration, which could be a result of dehydration and GLY hypertonicity, triggering renal fluid restriction to compensate for the hypovolemia. In addition, reduced renal functional units due to myoglobinuria-induced renal tubular damage lead to hyperfunction of remaining intact tubules, augmenting Na+ reabsorption and hypernatremia[7,41]. At the same time, ISO-treated groups showed a significant increase in Na^+ levels, supporting the ISO nephroprotective effects. Hyperkalemia, the usually manifested electrolyte disturbance associated with GLY-induced AKI, is evoked by the leakage of intracellular K^+ into interstitial fluid after muscle breakdown. Moreover, decreased urine output is another mechanism for elevating serum K^+ levels associated with RML. Hyperkalemia, a significant complication that may lead to arrhythmia, should be managed urgently[42,43]. Isoliquiritigenin treatment groups significantly reversed the hyperkalemia shown in the induction group. Additionally, hypocalcemia, another electrolyte disturbance shown in our study, is attributed to the trapping of calcium intracellularly in the early stage of AKI (oliguric phase); studies have shown that hypocalcemia is temporary at the beginning stage of RML, as the Ca^{+2} diffuses after muscle breakdown, which may result in hypercalcemia[44]. Isoliquiritigenin treatment groups reversed the hypocalcemia seen in the induction group significantly. Isoliquiritigenin's ability to reverse serum Na^+ and K^+ could be explained by mineralocorticoid-like effects exhibited by licorice active constituents, which stimulate potassium excretion and preserve sodium and water reabsorption[45,46]. Moreover, Studies have demonstrated that flavonoids could effectively ameliorate electrolyte disturbances associated with GLY-induced AKI. In addition, the ability of ISO to retrieve normal kidney function can reverse the electrolyte disturbances as it enhances the filtration and reabsorption mechanisms[27]. Oxidative stress is related to redox homeostasis disruption, which leads to lipid peroxidation, DNA, and protein damage. Lipid membrane peroxidation increases membrane permeability, which affects cellular ion channels and enzyme secretion, contributing to cell dysfunction and apoptosis[47]. Results of the current study revealed significant elevation in oxidative stress markers MDA, the lipid peroxidation by-product induced by an attack of ROS on lipid membrane, and downregulation of antioxidant enzyme level GPx due to overproduction of ROS, overwhelming the antioxidant defense capacity of renal tissue[48] Indicating heightened oxidative stress due to GLY-induced renal injury. These results demonstrate that ISO counteracts oxidative damage by enhancing antioxidant defenses. Our results were consistent with those of previous studies, which mentioned alteration in MDA and GPx levels in renal tissue due to GLY administration[7,49]. ISO-treated groups reversed these changes of oxidative stress markers in a dose-dependent manner, specifically for GPx[50]. Previous studies exhibited a compatible result; al-qahtani et al. demonstrate the protective effect of ISO against doxorubicin mediated nephrotoxicity through enhancing the gene transcription of antioxidant enzymes, replenishing the antioxidant enzyme[27]. Another study mentioned the role of ISO treatment in increasing levels of the antioxidant GPx enzyme and reducing MDA levels[51]. Flavonoids have astonishing ROS scavenging properties that explain the ability of ISO treatment in mitigating oxidative stress markers[10].

In response to harmful exciters like trauma, toxic damage, oxidative stress, inflammation develops by releasing several pro-inflammatory mediators. It may lead to severe complications, particularly in acute response[52]. IL1β, IL6, and TNF-α, the major pro-Inflammatory cytokines involving in the pathophysiology of GLY- induced AKI. They stimulate inflammation and exacerbate AKI by promoting leukocyte infiltration, causing cellular damage. In the current study, these cytokines were markedly elevated in the induction group, highlighting an inflammatory response associated with AKI; these findings were in line with the earlier research, which showed an elevation in renal tissue levels of these cytokines[44]. Isoliquiritigenin-treated groups successfully reversed an elevation in these cytokines in dose dependent manner, which is consistent with previous studies[31]. TNF-α, produced by macrophages, monocytes, T cells, as well as endothelial, and glomerular cells, which enhances ROS generation, increases albumin permeability, and stimulation of cellular necrosis and apoptosis[53]. Studies reported the central role of TNF-α in AKI associated with GLY injection; it is triggered fast, stimulates immune cell infiltration, traps neutrophils, and aggravates the secretion of other cytokines. The released TNF-α can amplify inflammatory response through its activation impact on nuclear factor kappa b (NF-κB), this factor regulates the expression of several pro-inflammatory cytokines; consequently, its activation leads to transcriptional stimulation of its downstream cytokines (IL6, IL1β, TNF-α). Evidence has shown that mitigating TNF-α can protect against GLY-AKI[44]. Similarly, studies mentioned the pivotal impact of (IL1β and IL6) in the development of AKI due to GLY administration, which is released in response to inflammation of renal tissues and infiltrated immune cells. Mentioned

the inhibition of these markers could alleviate the severity of AKI[54,55]. Previous reports proved that ISO can inhibit the production of IL-6 and TNF-α stimulated by the lipid A portion of lipopolysaccharides[56]. Wu Yangping reported that ISO can reduce IL-6 and IL-8 via attenuating the activity of NF-kB in the psoriasis model, proposing that ISO could be effective for managing psoriasis[57].

Even though the current study's results were very important, it is suggested that a wider range of doses be used. This will help in figuring out the lowest dose that works and finding any toxicity at higher levels. This will help in creating a safer dosing profile for future use. It is also recommended to expand the intervention period for more than five days to reach about 10, starting 7 days prior to the induction of AKI.

CONCLUSION

The current study supports ISO's nephroprotective activity in a GLY-induced kidney injury model. Isoliquiritigenin improves renal function, electrolyte disturbances, and histopathological impairment and attenuates oxidative stress and inflammation. The findings support other research showing ISO's antioxidant, anti-inflammatory, and tissue-protective activities. The dose-dependent nature of ISO's effects suggests that higher doses may offer more potent protective benefits. These findings suggest ISO as a potential treatment for kidney damage while offering the opportunity for future clinical use.

Authorship Contribution: All authors share equal effort contribution towards (1) substantial contributions to conception and design, acquisition, analysis and interpretation of data; (2) drafting the article and revising it critically for important intellectual content; and (3) final approval of the manuscript version to be published. Yes.

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Competing Interest: None

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