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RESEARCH ARTICLE

Modulation of inflammatory, oxidative, and apoptotic stresses mediates the renoprotective effect of daidzein against glycerol-induced acute kidney injury in rats

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Abstract

Acute kidney injury (AKI) is a life-threatening complication that accompanies rhabdomyolysis. Daidzein is a dietary isoflavone that has various biological activities. This study examined the therapeutic potential of daidzein and the underlying mechanisms against AKI induced by glycerol in male rats. Animals were injected once with glycerol (50%, 10 ml/kg, intramuscular) for induction of AKI and pre-treated orally with daidzein (25, 50, and 100 mg/kg) for 2 weeks. Biochemical, histopathological, immunohistopathological, and molecular parameters were assessed to evaluate the effect of daidzein. The results revealed that the model group displayed remarkable functional, molecular, and structural changes in the kidney. However, pre-administration of daidzein markedly decreased the kidney relative weight as well as the levels of urea, creatinine, K, P, kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), and cystatin C. Further, daidzein lessened the rhabdomyolysis-related markers [lactate dehydrogenase (LDH) and creatine kinase (CK)]. Notably, the enhancement of the antioxidant biomarkers [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and reduced glutathione (GSH) is accompanied by a decrease in malondialdehyde (MDA) and nitric oxide (NO) levels. Moreover, upregulated gene expression levels of nuclear factor erythroid 2-related factor 2 (*Nfe212*) and hemeoxygenase-1 (*Hmox1*) were exerted by daidzein administration. Rats who received daidzein displayed markedly lower interleukin-1β (IL-1β), tumor nuclear factor-α (TNF-α), myeloperoxidase (MPO), and nuclear factor kappa B (NF-κB) levels together with higher interleukin-10 (IL-10) related to the model group. Remarkably, significant declines were noticed in the pro-apoptotic (Bax and caspase-3) and rises in antiapoptotic (Bcl-2) levels in the group that received daidzein. The renal histological screening validated the aforementioned biochemical and molecular alterations. Our findings support daidzein as a potential therapeutic approach against AKI-induced renal injury via suppression of muscle degradation, oxidative damage, cytokine release, and apoptosis.

Keywords Acute kidney injury · Caspase-3 · Isoflavone supplements · Inflammation · Nrf2 · Rhabdomyolysis

Introduction

Acute kidney injury (AKI) is a serious syndrome that occurs because of the rapid incidence of renal dysfunction within a few hours or a few days and is associated with high morbidity and mortality (Al-Brakati et al. 2021, Yin et al. 2019). The major reasons for AKI include decreases in renal perfusion, altered renal histological structure, and obstruction of urinary outflow. The global incidence of AKI in patients has been evaluated at around 20% (Li et al. 2017). The mortality rate is about 20% in patients without kidney damage and reaches 59% when AKI is developed (Reis et al. 2019). The impairment in kidney function may evoke the accumulation of waste products in the blood and failure to maintain fluid and electrolytes homeostasis (Adedapo et al. 2020). AKI also negatively affects other organs such as the heart, brain, and lungs (Al-Brakati et al. 2021). However, the disturbance in kidney function is reversible

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in the majority of survived patients; the mortalities from AKI are still high (Wu et al. 2017). Until now, no effective therapy is developed to avoid or manage AKI. Rhabdomyolysis (RM) is the damage of striated skeletal muscle and liberation of its intracellular components including myoglobin, electrolytes, and sarcoplasmic proteins into the blood. It accounts for 10–40% of all cases of AKI and represents the major cause of their mortality (Yin et al. 2019). The myoglobinemia provokes arterial constriction and accumulation of myoglobin in renal tubules, with subsequent tubular necrosis and obstruction (Reis et al. 2019). Myoglobin induces renal toxicity by inducing oxidative injury, inflammation, vasoconstriction, and apoptosis (Al-Brakati et al. 2021, Wu et al. 2017). Furthermore, myoglobin exacerbates the overproduction of reactive radicals that activate nuclear factor kappa B (NF-κB) (Amirshahrokhi 2021). When activated, NF-κB increases the overproduction of inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), and interleukins during AKI (Sun et al. 2018). Antioxidant administration had been reported for effective prevention or treatment of myoglobinuria-induced AKI in renal tissue (Al-Brakati et al. 2021, Yin et al. 2019). Soybeans are enriched by unique isoflavones such as genistein and daidzein with various favorable health benefits (Liu et al. 2014b). It has been reported that consumption of a soy diet provoked notable renal protection in various animal models (Anderson 2008, Meng et al. 2017, Tomar et al. 2020). A former clinical trial revealed that short-time (less than 8 weeks) administration of soy proteins for patients with diabetes or chronic kidney disease was accompanied by less hyperfiltration and albuminuria, with subsequent improvement of renal functions (Liu et al. 2014a). A previous study (Liu et al. 2014b) found that consumption of whole soy for 6 months improved the renal functions in postmenopausal women with prehypertension. Daidzein, an isoflavone from soy, has outstanding antioxidant, anti-inflammatory, antiapoptotic, and anticancer activities (Zhang et al. 2021). A single dose of daidzein has been demonstrated to protect the kidney against cisplatin-induced nephrotoxicity in mice by alleviating oxidative stress, inflammation, and cell death (Meng et al. 2017). Guru et al. (2022) have found that daidzein protected against gentamicin-induced nephrotoxicity in Madin-Darby canine kidney cells via increasing cell viability, lowering ROS levels and counteracting cell apoptosis. In addition, they found marked increases in antioxidant biomarkers together with decreases in COX-2, TNF-α, and IL-1β in the kidney of gentamicin-injected zebrafish. Moreover, daidzein improved antioxidative mediators, and inflammatory cytokines in the serum and kidney tissue in rats subjected to ovariectomy and unilateral ureteral obstruction (Askaripour et al. 2022a). Daidzein also evoked marked hepatoprotection against lipopolysaccharide (Yu et al. 2020) or concanavalin A (Li et al. 2021)–induced liver injury via decreasing the formation of free radicals, enhancement of SOD activity, and upregulation of Nrf2 expression. Daidzein counteracted the myocardial damage induced by hypoxic-ischemic injury via the activation of NRF2/HO-1 signaling (Zeng et al. 2021). Earlier in vitro study revealed that daidzein alleviated the obesity-related neuroinflammation in human hypothalamic GnRH neurons through downregulating cell death, proinflammatory processes, oxidative stress, and apoptosis (Morelli et al. 2021). Despite that the nephroprotective potency of daidzein was formerly reported, its antagonistic action against glycerol induced renal injury was not investigated. In addition, the molecular mechanisms that underlie the daidzein-mediated renal protection involving Nrf2/HO-1, Nf-κB and apoptotic pathways were not formerly clarified. Hence, in our study, glycerol was used for the induction of the acute renal injury model to elucidate the therapeutic efficacy of daidzein. Along with the basic parameter evaluation, we also emphasized the mechanisms underlying daidzein-mediated nephroprotection in rats including oxidative damage, renal inflammatory, and apoptotic processes.

Materials and methods

Experimental animals

Glycerol and daidzein were purchased from Sigma-Aldrich Chemical Co. (St Louis, Mo, USA). All other used chemicals were of high analytical grade. Male Wistar albino rats weighing 180–200 g at 3 months old were used in this study. They were obtained from the VACSERA (Cairo, Egypt) and reared under controlled environmental circumstances of alternating 12-h dark-light cycle, 23±2 °C temperature, and 50±10% relative humidity. They were provided by standard diet and water ad libitum. Prior to the beginning of the experiment, they were acclimatized for one week. All experimental procedures were accepted by the department of zoology and entomology, faculty of science, Helwan University (Cairo, Egypt; approval no, HU2021/Z/RKA0921-01).

Induction of AKI and experimental design

The induction of AKI was performed via intramuscular injection of rats with 50% glycerol (10 ml/kg, single dose) into the hind limbs after being diluted in saline (0.9% NaCl). Rats were deprived of water 24 h before glycerol injection as described elsewhere (Kim et al. 2010). Thirty-five rats were assigned into five equal groups as follows (n=7):

Group 1 (CON): rats received intramuscular injection with physiological saline (0.9% NaCl).

Group 2 (AKI): rats were injected with glycerol (50%, 10 ml/kg, intramuscular).

Group 3 (AKI+ daidzein 25): rats were administered orally with daidzein at a dose of 25 mg/kg for 2 weeks, then injected intramuscularly with single dose of glycerol.

Group 4 (AKI + daidzein 50): rats were administered orally with daidzein at a dose of 50 mg/kg for 2 weeks, then injected intramuscularly with single dose of glycerol.

Group 5 (AKI+ daidzein 100): rats were administered orally with daidzein at a dose of 100 mg/kg for 2 weeks, then injected intramuscularly with single dose of glycerol.

The doses of daidzein were selected according to the study reported by Goel and Chaudhary (2020).

Animals were anesthetized 24 h after the glycerol treatment by intraperitoneal injection of pentobarbital at a dose of 100 mg/kg and then sacrificed. Blood samples were collected through retro-orbital plexus for conducting the biochemical tests and the kidneys were instantly collected and weighed. The right kidneys were used for carrying out the biochemical and molecular tests while the left kidneys were examined for histopathological screening.

Determination of kidney weight

The relative kidney weight was calculated based on the method of Almeer et al. (2019) according to the following mathematical equation:

$$\text{Relative kidney weight} = \frac{\text{Left kidney}}{\text{Body weight}} \times 100$$

Preparation of kidney homogenate

The renal tissue 10% (w/v) was homogenized in 50 mM Tris–HCl (pH 7.4), and the homogenate was centrifuged at 4°C for 10 min at 3000 × *g*. The resultant supernatant was kept at −80 °C for further biochemical tests. The renal protein content was measured according to the method of Lowry et al. (1951) using bovine serum albumin as a reference protein.

Assessment of the intensity of rhabdomyolysis

Lactate dehydrogenase (LDH, Catalogue Number: LD3842, measuring ranges 8.8 - 635U/l) and creatine kinase (CK, Catalogue Number: CK3812, measuring ranges 9.16 - 2886U/l) were assessed by kits supplied by Randox/Laboratory, Crumlin, UK, according to the manufacturer's protocol. Reagents are stable to expiry at 2–8°C.

Determination of renal function biomarkers

Serum levels of urea (Catalogue Number: CR2336, measuring range: 11.4–2460µmol/l) and creatinine (Catalogue Number: UR446, measuring range: 0.866–56.7mmol/l) were measured by kits (Randox/Laboratory, Crumlin, UK) according to the manufacturer's instructions. Reagents are stable to expiry at 2–8°C. Furthermore, electrolyte (sodium/potassium/phosphorus) levels in serum were quantified using commercially available kits from Biodiagnostic, Giza, Egypt, and values were expressed as millimoles/liter. The reagents are stable when stored at +15 to +25 °C up to the expiry date. Moreover, ELISA kits were utilized for analysis of the plasma levels of kidney injury molecule (Kim-1; R&D Systems, Catalogue Number: AF3689, measuring range: 7.8–500 pg/mL, reagents are stable for 6 months at 20 to 70 °C under sterile conditions after reconstitution), neutrophil gelatinase-associated lipocalin (NGAL; MyBioSource, Catalogue Number: MBS260195, measuring range: 12–2000 pg/mL, reagents are stable for 12 months at −20 °C, intra and inter assay precision: ≤ 8% and 12%, respectively) and cystatin C (My BioSource, Catalogue Number: MBS733592) following the manufacturer's instructions.

Estimation of renal non-enzymatic oxidative stress markers

Lipid peroxidation was assessed in terms of malondialdehyde (MDA) according to the method of Ohkawa et al. (1979). Moreover, nitric oxide (NO) levels in renal tissues were measured by Griess reagent (Green et al. 1982). Additionally, reduced glutathione (GSH) in renal tissue was calculated according to Ellman (1959).

Measurement of kidney antioxidant enzymatic activities

The activities of superoxide dismutase (SOD) and catalase (CAT) enzymes were measured according to Nishikimi et al. (1972) and Aebi (1984), correspondingly. Furthermore, glutathione peroxidase (GPx) was assessed according to Paglia and Valentine (1967), and glutathione reductase (GR) was estimated following De Vega et al. (2002).

Assessment of inflammatory-related markers

The renal inflammatory response was evaluated by measurement of TNF-α (Catalogue Number: NBP1-92681), interleukin-1β (IL-1β; Catalogue Number: NBP1-92702), interleukin-10 (IL-10; Catalogue Number: NBP1-92701), and NF-κB (Catalogue Number: NB100-2176) following the manufacturers' instructions (Novus Biologicals, Centennial, CO, USA). Myeloperoxidase (MPO) activity was assayed based on the protocol reported by Bradley et al. (1982).

Assessment of the renal apoptotic markers

The levels of apoptotic proteins (Bax, caspase-3, and Bcl-2) in renal tissues of all tested groups were measured by ELISA kit (Cusabio (Wuhan, China)) following the manufacturer's information. The catalogue Number for Bax: CSB-EL002573RA, caspase-3: CSB-E08857r and Bcl-2: CSB-E08854r.

Quantitative real-time PCR

TRIzol reagent was used for extraction of total RNA from renal tissue followed by synthesis of cDNA using RevertAid™ H Minus Reverse Transcriptase (Fermentas, Thermo Fisher Scientific Inc., Canada) according to the manufacturer's protocol. qrt-PCR was employed using the Quanti-Fast SYBR Green RT-PCR kit (Qiagen, Hilden, Germany). All reactions were conducted in duplicate using the ViiA™ 7 System (Thermo Fisher Scientific, CA, USA). The PCR cycling conditions were initial denaturation at 95 °C for 12 min, followed by 40 cycles of denaturation at 94 °C for 60s and annealing at 58 °C for 60s, extension at 72 °C for 90s, then held for a final extension at 72 °C for 10 min. The relative gene expressions were determined between the different groups using the $^{\Delta\Delta}$Ct method (Livak and Schmittgen 2001). Glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) was used as a housekeeping gene. The primer sequences (Jena Bioscience (Jena, Germany)) for estimation of *Nfe212* and *Hmox-1* gene expressions are listed in Table 1 according to Abdel Moneim (2016).

Renal histopathological screening

Kidney samples were fixed in 10% neutral buffered formalin for 24 h. Next, tissues were dehydrated, embedded in paraffin wax, and sectioned at a thickness of 4–5 µm. Specimens were stained with hematoxylin and eosin (H&E) and examined under a Nikon Eclipse E200-LED (Tokyo, Japan) microscope at 400× magnification (Carleton and Montgomerie 1980). The prepared slides were separately examined by two blinded specialists.

Immunohistochemistry analysis

The immunohistochemical analysis following the procedures of Kim et al. (2016). Kidney sections were incubated in hydrogen peroxide (10%, 30 min) to deactivate endogenous peroxidase activity and blocked for one h with normal goat serum (10%) at room temperature. Sections were further incubated with primary anti-Bax antibodies (diluted 1:200) overnight at 4°C. Antibody detection was determined using the Histostain-Plus Bulk kit (Invitrogen) against rabbit IgG, and finally, 3,3'-diaminobenzidine was applied for visualization. A Nikon microscope was used to capture photomicrographs at 400× magnification (Eclipse E200-LED, Tokyo, Japan). Semiquantitative scoring of glomerular, tubulointerstitial lesions, and Bax immunohistochemical intensity in control and experimental rats has been presented in Table 2.

Molecular docking analysis

The docking experiment was carried out using MOE 2015 to correct active site errors caused by the structure preparation process in Molecular Operating Environment (MOE®) version 2014.09 (Montreal, QC, Canada). Hydrogens were added after the correction, and partial charges (Amber12: EHT) were estimated. The energy was minimized (AMBER12: EHT, root-mean-square gradient: 0.100). The receptor's binding site was discovered using the MOE. Site Finder program, which employs a geometric approach to calculate putative binding sites in a protein based on its tridimensional structure. This method does not use energy models and instead relies on alpha spheres, which are a generalization of convex hulls. The binding sites predicted by the MOE Site Finder module confirmed the binding sites defined by the co-crystallized ligands in the holo-forms of the proteins under study. The optimized 3D structure of the molecule was subjected to the triangular matcher placement method, which generates poses by aligning ligand triplets of atoms on triplets of alpha spheres represented in the receptor site points, with a random triplet of alpha sphere center being used to determine the pose during each iteration. The produced pose was scored again using the London dG scoring method.

Table 1 Primer sequences of genes analyzed in real time-PCR

Name	Accession number	Sense (5'---3')	Antisense (5'---3')
Gapdh	NM_017008.4	AGTGCCAGCCTCGTCTCATA	TCCCGTTGATGACCAGCTTC
Nfe2l2	NM_031789.2	CAGCATGATGGACTTGGAATTG	GCAAGCGACTCATGGTCATC
Hmox1	NM_012580.2	TTAAGCTGGTGATGGCCTCC	GTGGGGCATAGACTGGGTTC

Gapdh Glyceraldehyde 3-phosphate dehydrogenase; *Nfe2l2* nuclear factor-erythroid 2-related factor 2; *Hmox1* Heme oxygenase 1

Table 2 Effect of daidzein pretreatment on the kidney weight and the relative kidney weight in glycerol-induced AKI model in rats

Group	CON	AKI	AKI+ daidzein 25	AKI+ daidzein 50	AKI+ daidzein 100
Kidney weight (g)	0.65±0.02	1.19±0.03[a]	1.01±0.04[ab]	0.83±0.05[ab]	0.68±0.03[b]
Relative kidney weight (%)	0.37±0.01	0.59±0.03[a]	0.53±0.02[ab]	0.41±0.02[ab]	0.38±0.02[ab]

The statistical difference between groups was estimated at $P < 0.05$. a: represents the significant difference ($P < 0.05$) between all groups against the control group. b: indicates the significant difference ($P < 0.05$) between AKI-treated groups with daidzein (25, 50, and 100 mg/kg) and the AKI group. All results are presented as the mean ± standard deviation (SD), (n=7)

Statistical analysis

Data are expressed as the mean ± standard deviation (SD). The comparison between different groups was performed by one-way analysis of variance (ANOVA), followed by Duncan's post hoc test, using the statistical package SPSS version 23.0. P values less than 0.05 were considered significant.

Results

Daidzein alleviates glycerol-induced alterations in kidney weight and functions

Table 2 clarifies the modulating effect of daidzein on kidney weight in AKI-challenged rats. Significant increases ($P < 0.05$) were recorded in the absolute and relative kidney weights in the AKI group (70.76%) in comparison with the untreated control group. Further, glycerol injection provoked significantly higher plasma creatinine (98.63%) and urea (166.82%) levels ($P < 0.05$) than their corresponding values in the control group. Meanwhile, it was noted that daidzein treatment significantly improved renal function biomarkers in treated AKI groups related to the untreated group. The increases in serum creatinine and urea levels in the model group were attenuated by daidzein pretreatment at doses of 25 and 50 mg/kg, nevertheless, it is still notably higher than those of the model group. In addition, daidzein at the highest dose (100 mg/kg) remarkably decreased the levels of creatinine and urea (−43.47% and −56.64%, respectively) compared to the AKI values (Fig. 1).

Daidzein treatment corrected the serum electrolytes, NGAL, KIM-1, and cystatin-c levels in glycerol-challenged rats

As compiled in Fig. 2, the AKI group demonstrated noteworthy increases ($P < 0.05$) in serum potassium (23.28%) and phosphorus (15.07%) levels despite no detectable changes in sodium levels compared to the control one. Concerning the sodium levels, noteworthy increases ($P < 0.05$) were detected in the AKI group treated with daidzein at doses of 50 and 100 mg/kg only without any observable changes at the dose of 25 mg/kg. In addition, daidzein administration, especially at the higher dose, notably decreased ($P < 0.05$) the serum levels of both potassium and phosphorus (−15.14% and −12.62%, respectively) related to the AKI

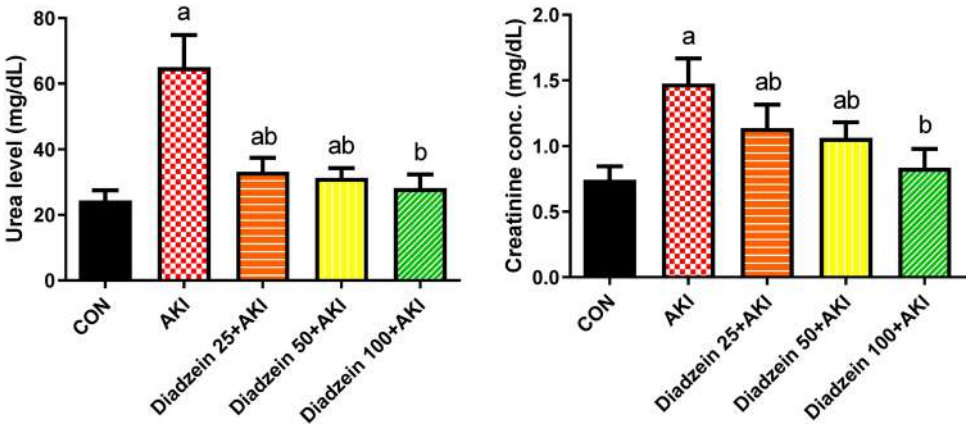

Fig. 1 Effect of daidzein pretreatment on the kidney weight, urea, and creatinine in glycerol-induced AKI model in rats. The statistical difference between groups was estimated at $P < 0.05$. **a** The significant difference ($P < 0.05$) between all groups against the control group. **b** The significant difference ($P < 0.05$) between AKI-treated groups with daidzein (25, 50, and 100 mg/kg) and the AKI group. All results are presented as the mean ± standard deviation (SD), (n=7)

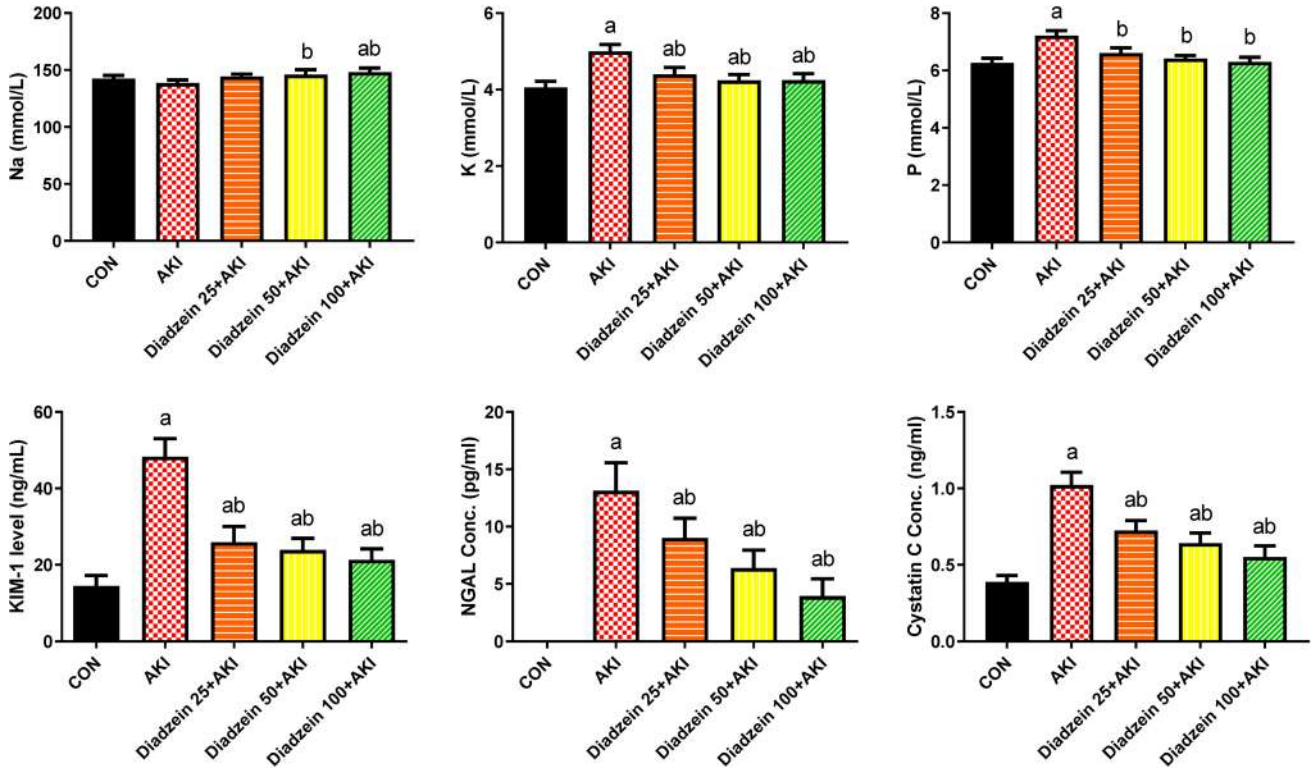

Fig. 2 Effect of daidzein pretreatment on the serum electrolytes, NGAL, Kim-1, and cystatin-c in glycerol-induced AKI model in rats. **a** The significant difference ($P < 0.05$) between all groups against the control group. **b** The significant difference ($P < 0.05$) between AKI-treated groups with daidzein (25, 50, and 100 mg/kg) and the AKI group. All results are presented as the mean ± standard deviation (SD), (n=7)

model group. Noteworthy increments ($P < 0.05$) were recorded in the levels of NGAL, KIM-1 (233.5%), and cystatin C (163.4%) in the serum of the glycerol-treated group in relation to the sham group. Adversely, daidzein pre-administration to the AKI group noticeably counteracted ($P < 0.05$) the glycerol mediated decreases (−70.03%, −55.88%, and −46.18%) in their levels compared to the AKI group, especially at 100 mg/kg, but they did not reach normal levels. In sum, these results revealed that daidzein supplementation could help to alleviate AKI-mediated renal damage and dysfunction.

Daidzein administration decreased the levels of LDH and CK in glycerol-treated rats

The effect of daidzein supplementation on rhabdomyolysis-related biomarkers CK and LDH in the glycerol-challenged group is displayed in Fig. 3. Glycerol injection-induced notable augmentations ($P < 0.05$) in serum CK (237.07%) and LDH (154.32%) levels with respect to the control group. Meanwhile, co-treatment with daidzein, especially at 100 mg/kg, significantly decreased CK (−56.13%) and LDH (−46.37%) levels when compared to the AKI group. These findings supported the potential of daidzein administration to protect against muscle fiber damage resulting from glycerol injection.

Daidzein boosted the antioxidant status of renal tissue in glycerol-challenged rats

Antioxidant enzymatic activities were evaluated in the renal tissue of the model and treated groups (Fig. 4). Noteworthy decreases ($P < 0.05$) were noticed in the activities of SOD (−27.59%), CAT (−34.05%), GPx (−38.07%), and GR (−32.48%) enzymes in glycerol-treated rats related to the sham group. On contrary, pre-treated rats with daidzein enhanced significantly ($P < 0.05$) their enzymatic activities compared to their corresponding activities in the model group. Interestingly, the activities of SOD and GR in the group co-treated with daidzein at doses of 50 and 100 mg/kg were close to the control group. Regarding CAT activity, daidzein at 100 mg/kg restored ($P < 0.05$) its renal levels in the glycerol group near the control levels. Further, all tested doses of daidzein were successful to reinstate ($P < 0.05$) the

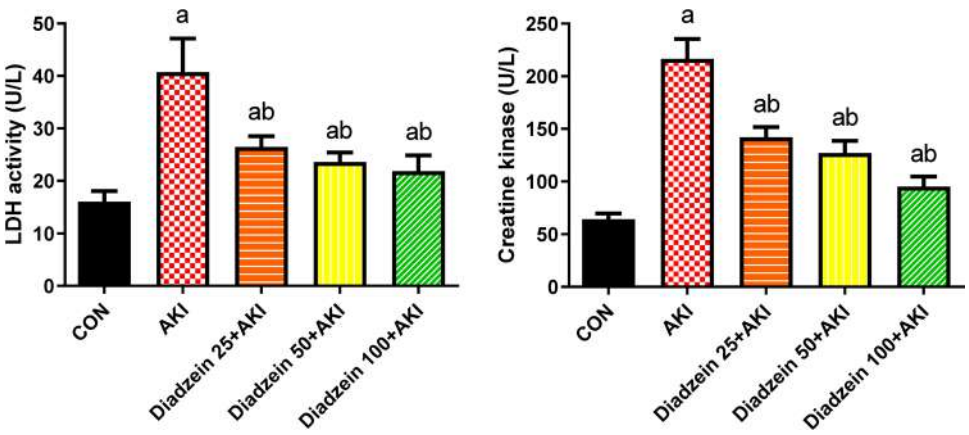

Fig. 3 Effect of daidzein pretreatment on the rhabdomyolysis-related biomarkers in glycerol-induced AKI model in rats. **a** The significant difference ($P < 0.05$) between all groups against the control group. **b** The significant difference ($P < 0.05$) between AKI-treated groups with daidzein (25, 50, and 100 mg/kg) and the AKI group. All results are presented as the mean ± standard deviation (SD), (n=7)

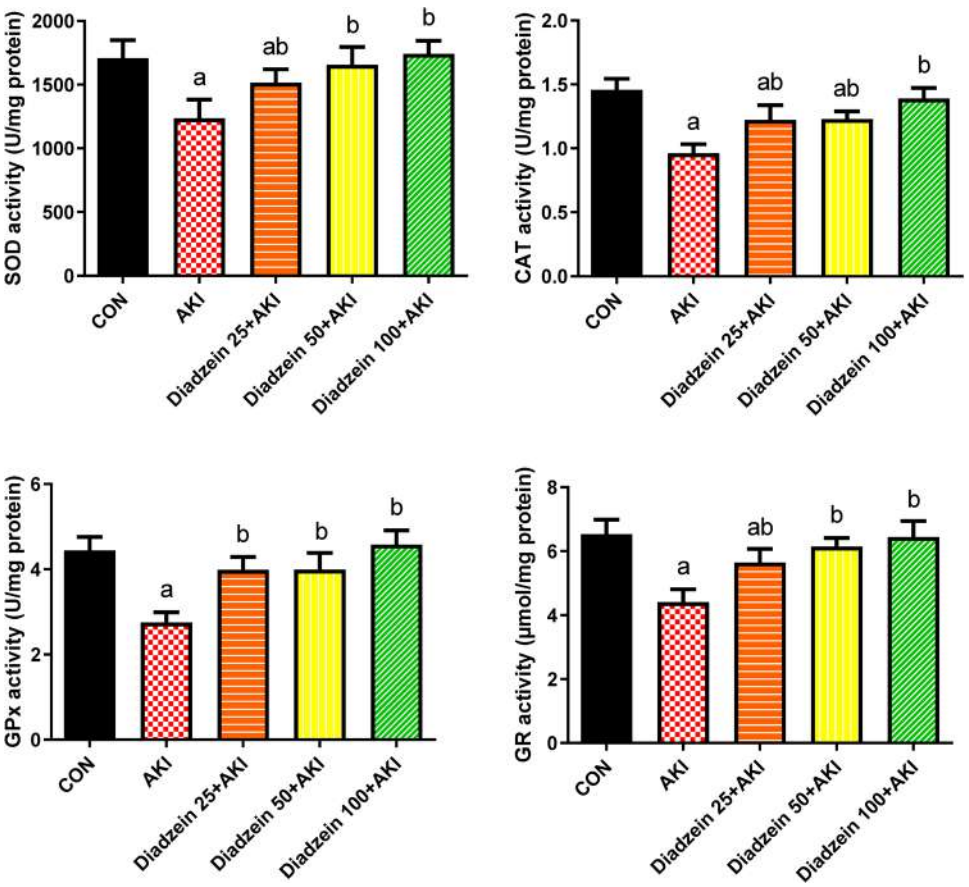

Fig. 4 Effect of daidzein pretreatment on the renal enzymatic antioxidant biomarkers (SOD, CAT, GPx, and GR) in glycerol-induced AKI model in rats. **a** The significant difference ($P < 0.05$) between all groups against the control group. **b** The significant difference ($P < 0.05$) between AKI-treated groups with daidzein (25, 50, and 100 mg/kg) and the AKI group. All results are presented as the mean ± standard deviation (SD), (n=7)

GPx enzymatic activities in the kidneys of rats with AKI. The levels of non-enzymatic oxidant biomarkers were examined in the kidneys of AKI and treated groups (Fig. 5). Remarkably, glycerol induced substantial decreases ($P < 0.05$) in the contents of GSH (−49%) together with raised levels ($P < 0.05$) of MDA (50.84%) and NO (63.16%) in comparison with the sham group. Nevertheless, daidzein particularly at the highest dose (100 mg/kg) reversed the changes in the aforementioned parameters compared to the AKI group. These results indicated that daidzein abolished the oxidative stress related to rhabdomyolysis by boosting the antioxidant enzymes and decreasing the oxidant markers in renal tissue.

For further clarification of the antioxidant potential of daidzein against AKI-associated oxidative injury, Nrf2/

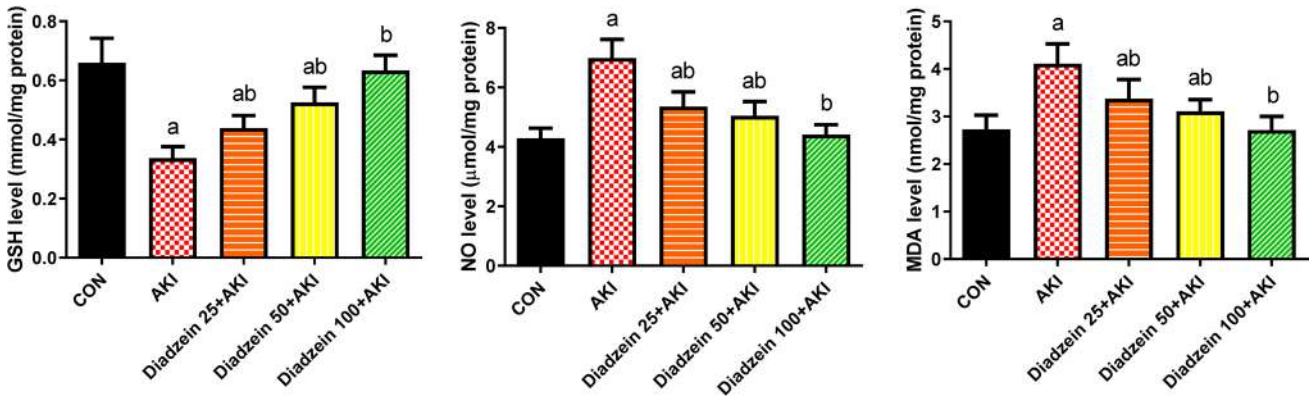

Fig. 5 Effect of daidzein pretreatment on the non-enzymatic antioxidant biomarkers (GSH, NO, and MDA) in glycerol-induced AKI model in rats. **a** The significant difference ($P < 0.05$) between all groups against the control group. **b** The significant difference ($P < 0.05$) between AKI-treated groups with daidzein (25, 50, and 100 mg/kg) and the AKI group. All results are presented as the mean ± standard deviation (SD), (n=7)

HO-1 signaling pathway was assessed (Fig. 6). Our molecular results revealed noticeable downregulations ($P < 0.05$) in the gene expressions of Nrf-2 (−65.75%) and HO-1 (−50.96%) in the renal tissue of glycerol-injected rats in relation to the control rats. On the contrary, daidzein pre-treatment, at all tested doses, augmented markedly ($P < 0.05$) the expression levels of these genes that encode antioxidant molecules compared to the model group. Notably, daidzein administration at 100 mg/kg upregulated markedly ($P < 0.05$) the expression of HO-1 that exceed the expression level of the control untreated rats.

Daidzein pre-administration counteracted the glycerol-dependent inflammation in the rat kidney

Glycerol-mediated renal inflammation and the counteracting effect of daidzein were evaluated in the kidneys of the AKI and treated groups (Fig. 7). Noteworthy increases ($P < 0.05$) in the pro-inflammatory cytokines [IL-1β (129.48%), TNF-α (112.06%), and MPO (114.55%)] with declines ($P < 0.05$) in the anti-inflammatory cytokine (IL-10, −73.2%) in kidneys of the AKI group. In addition, substantial increases ($P < 0.05$) were observed in the levels of NF-κB (147.14%) in glycerol-injected rats related to the sham rats. Remarkably, the treatment of nephrotoxic rats with different doses of daidzein reversed the levels for all tested inflammatory markers in the renal tissue compared with the AKI group. Daidzein at doses of 50 and 100 mg/kg decreased (−26.3% and −42.82%, respectively) notably the renal NF-κB levels with respect to the model group. These outcomes indicate the potent anti-inflammatory action of daidzein administration in AKI treatment.

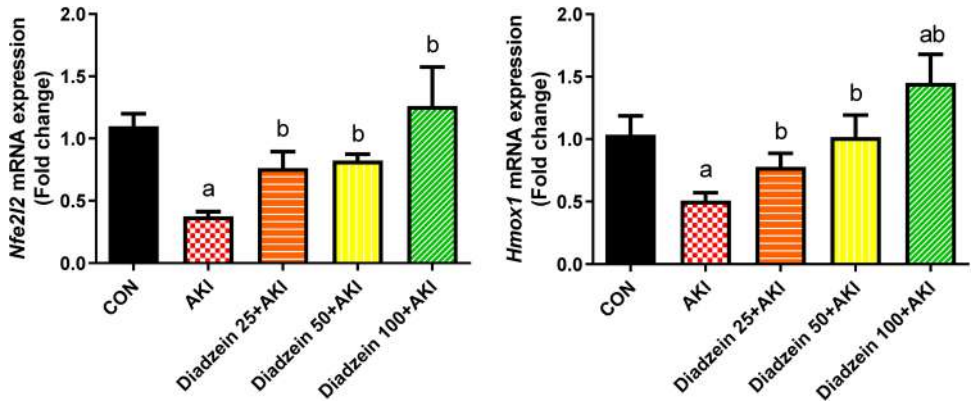

Fig. 6 Effect of daidzein pretreatment on the gene expression levels of *Nfe212* and *Hmox-1* in glycerol-induced AKI model in rats. **a** The significant difference ($P < 0.05$) between all groups against the control group. **b** The significant difference ($P < 0.05$) between AKI-treated groups with daidzein (25, 50, and 100 mg/kg) and the AKI group. All results are presented as the mean ± standard deviation (SD) of triplicate experiments and were referenced to Gapdh and represented as a fold change (log2 scale) with respect to mRNA levels in the control group, (n=7)

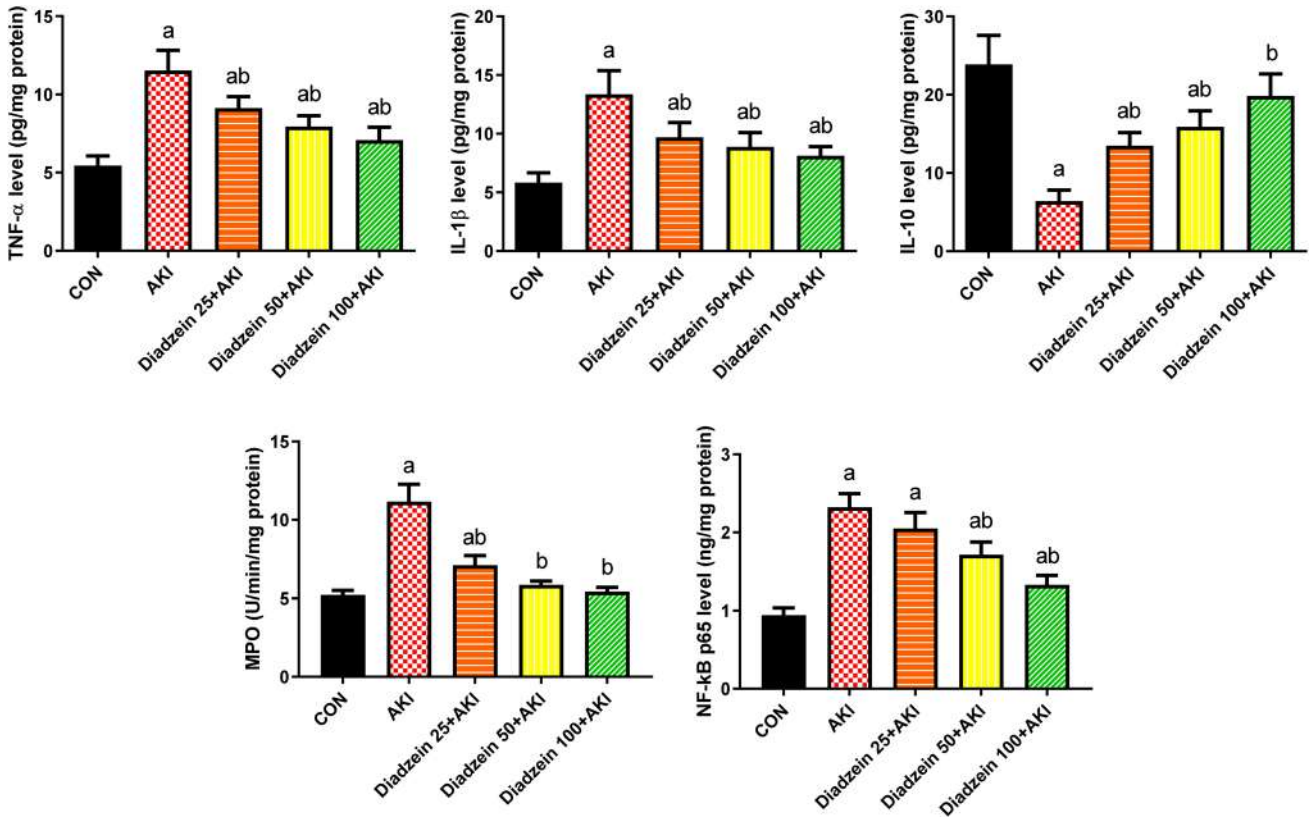

Fig. 7 Effect of daidzein pretreatment on the levels of inflammatory mediators (IL-1β, TNF-α, IL-10, MPO, and NF-κB) following glycerol-induced AKI model in rats. **a** The significant difference ($P < 0.05$) between all groups against the control group. **b** The significant difference ($P < 0.05$) between AKI-treated groups with daidzein (25, 50, and 100 mg/kg) and the AKI group. All results are presented as the mean ± standard deviation (SD), (n=7)

Daidzein reversed the apoptotic changes in the kidney induced by glycerol injection in rats

Our results revealed remarkable apoptotic evidence in the kidney of rats injected with glycerol as indicated by augmented levels ($P < 0.05$) of Bax (103.6%) and caspase-3 (132.12%) with decreased levels ($P < 0.05$) of Bcl-2 (−62.54%) compared to the sham group. On the contrary, co-administration of daidzein effectively suppressed apoptosis in the renal tissue of the AKI group. Remarkably, daidzein at a dose of 100 mg/kg lessened the levels of Bax (−42.42%) and increased the levels of Bcl-2 (150%) in the nephrotoxic group close to their values in the control group (Fig. 8). Additionally, immunohistochemical analysis exhibited that glycerol injection enhanced the expression of Bax compared to the control group. Notably, the administration of daidzein lessened its expression in the kidney tissue as compared to the model group (Fig. 9; Table 3).

Daidzein improved the renal histopathological changes in glycerol-injected rats

The histological appearances in renal tissues of AKI and daidzein co-administered rats are illustrated in Fig. 10. The kidney of the control group displayed normal tissue structure and healthy renal tubular epithelium without any pathological lesions in the glomerular or renal interstitial tissues (Fig. 10A). Adversely, the kidney from the glycerol group presented notable enlargement in the kidney tubular cavity and infiltration of inflammatory cells in the interstitial space together with glomerular hypertrophy and tubular epithelial cell edema (Fig. 10B). Pre-administration of daidzein at a dose of 25 mg/kg evoked some improvement in the histological changes of the renal cortex and medulla (Fig. 10C). The group received 50 mg/kg daidzein has significantly improved renal histology while little vacuolation and luminal cellular debris were still detected in some tubules (Fig. 10D). Interestingly, sections from rats pretreated with 100 mg/kg of daidzein displayed normal components of both renal cortex and medulla like the control group (Fig. 10E).

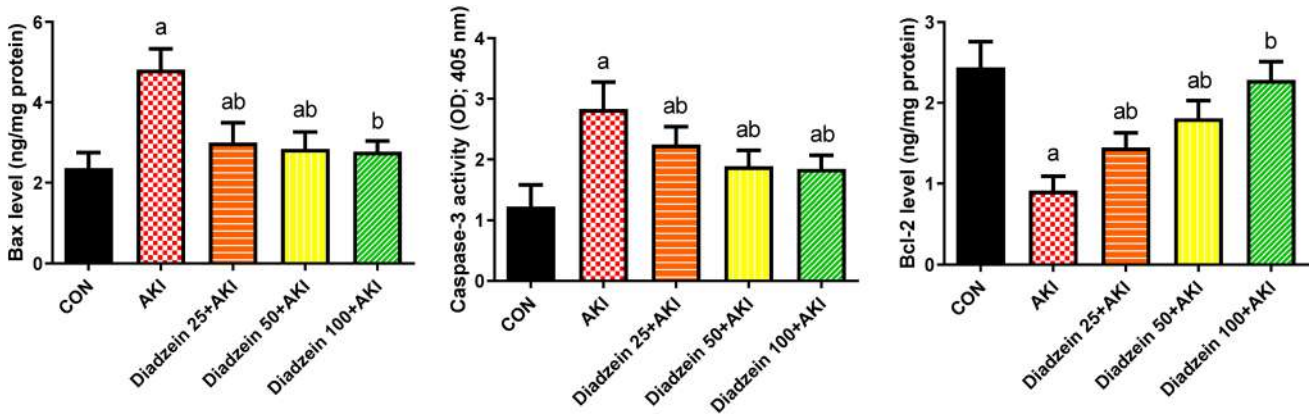

Fig. 8 Effect of daidzein pretreatment on the levels of apoptotic markers (Bax, caspase-3, and Bcl-2) in glycerol-induced AKI model in rats. **a** The significant difference ($P < 0.05$) between all groups against the control group. **b** The significant difference ($P < 0.05$) between AKI-treated groups with daidzein (25, 50, and 100 mg/kg) and the AKI group. All results are presented as the mean ± standard deviation (SD), (n=7)

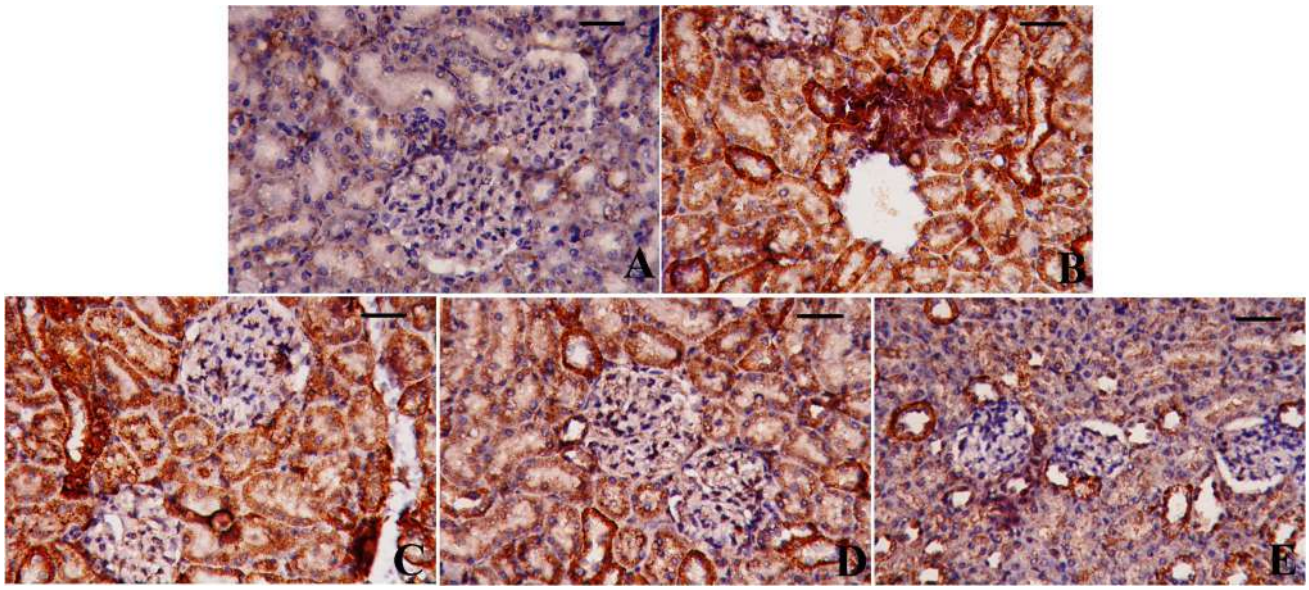

Fig. 9 Effects of daidzein pretreatment on the immunoreactivity of Bax of kidneys of AKI rats. **A** Control, **B** AKI, **C** daidzein 25+AKI, **D** daidzein 50+AKI, and **E** daidzein 100+AKI. Scale bar = 80 μm

Table 3 Semiquantitative scoring of glomerular, tubulointerstitial lesions, and Bax immunohistochemical intensity in control and experimental rats

Group	CON	AKI	AKI+ daidzein 25	AKI+ daidzein 50	AKI+ daidzein 100
Glomerular congestion	-	+++	++	+	-
Glomerular atrophy	-	++	+	-	-
Tubular damage	-	+++	++	++	+
Inflammatory cells infiltration	-	+++	++	+	+
Bax immunohistochemical intensity	+	++++	++++	+++	++

Scoring scale: none (-), mild (+), moderate (++), severe (+++), and highly sever (++++)

The analysis was determined on 10 filed per kidney, 2 kidneys per slide and 3 slides per group

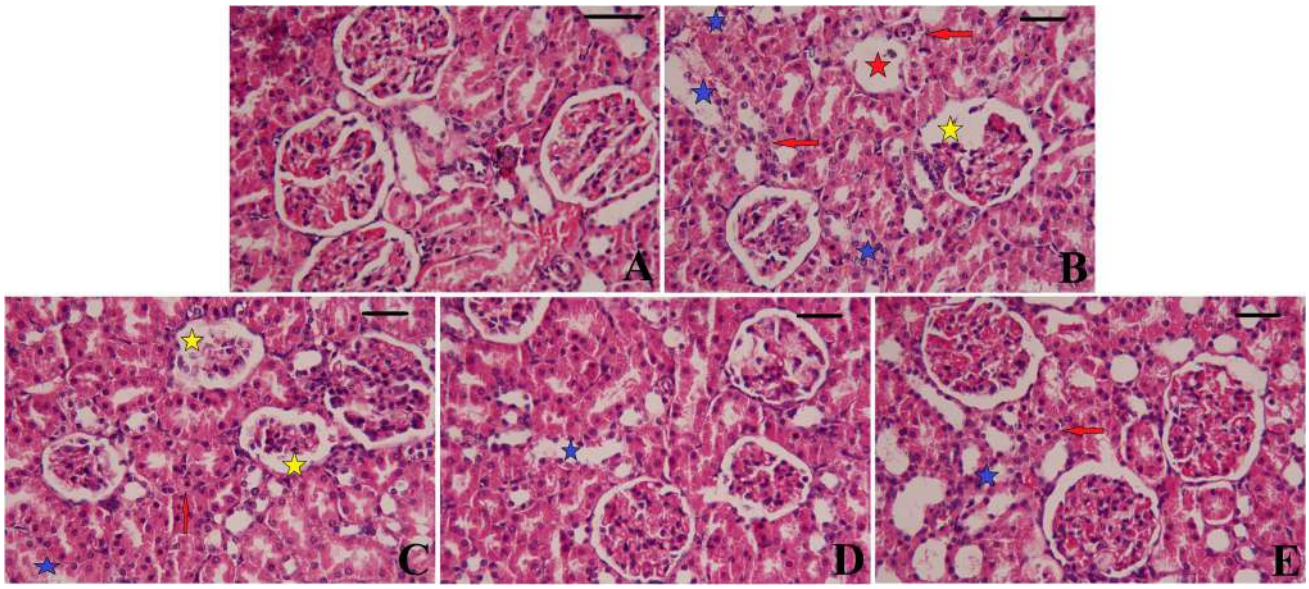

Fig. 10 Effects of daidzein pretreatment on the histopathological features of kidneys of AKI rats. **A** Control, **B** AKI, **C** daidzein 25+AKI, **D** daidzein 50+AKI, and **E** daidzein 100+AKI. Hematoxylin and eosin (H&E), Scale bar = 80 μm. Red stars indicate degenerated glomerulus. Yellow stars indicate damaged and shrunk glomerulus. Blue stars indicate desquamated and damaged tubules. Red arrows indicate information of inflammatory cells

Molecular docking analysis

The docking study was performed to investigate the biological action of daidzein against Keap1–Nrf2, NF-κB, and caspase-3. The different docking energies were listed in Table 4. The crystal structure of Keap1–Nrf2 (PDB: 3VNG), NF-κB (PDB: 1LE5), and caspase-3 (PDB: 3GJQ) proteins were obtained as a good template for docking experiment. Daidzein was successfully redocked and compared with reference inhibitors. To validate docking experiment, we select pose with a root mean square deviation (RMSD) less than 2 Å (Table 4). The ligand-protein-interaction-fingerprint was assessed depending on the binding score "ΔG" Van Dar wall interaction "E.vdw", interaction between pose and receptor "E.Int" and energy of H-bond and receptor "E.H.B". Daidzein was fruitfully localized into active sites of the enzymes. The poses that have the lowest binding free energy "ΔG" with the lowest root means quart deviation (RMSD) between the pose initial and final refinement. Finally, the highest MOE scoring function for the tested compounds applied to evaluate the binding affinities of the tested compound.

Table 4 The Docking energy scores (kcal/mol) for isolated component

	ΔG	rmsd	E.vdw	E.Int	$E._{H.B}$
Keap1–Nrf2 (PDB: 3VNG)					
Daidzein	−5.11	1.55	−6.35	−19.07	−8.03
NF-κB (PDB: 1LE5)					
Daidzein	−5.27	0.72	−6.0	−23.58	9.28
Caspase-3 (PDB: 3GJQ)					
Daidzein	−5.94	1.69	−6.26	−42.55	−5.94

For Keap1–Nrf2

Daidzein was docked successfully with the same manner for the reference inhibitor "FUU". Daidzein showed higher binding energy (ΔG =-5.1Kcal/mol.) with lower (RMSD 0.57 Å) compared to FUU (ΔG=4.93 Kcal/mol and RMSD= 1.23 Å). Daidzein was stabilized in binding pocket by formation important three H-bond interactions with vital amino acids Arg 94, Arg.59 and Asn.91 (Fig. 11). Compounds stabilized in active binding sites for tested enzymes with the same manner for the reference inhibitor.

For NF-κB

Induced fit docking analysis into the NF-κB (PDB: 1SVC) has displayed that the significant level of docking score and binding energies (Table 4). The binding score against NF-κB (PDB: 1SVC) were found to be (ΔG =-5.27 Kcal/mol) and RMSD =0.7 Å for daidzein (Table 4 and Fig. 11). Its compound showed promising energy scores and H-bond interaction with catalytic active site (Gln277 and Lys147), as represented in Fig. 11.

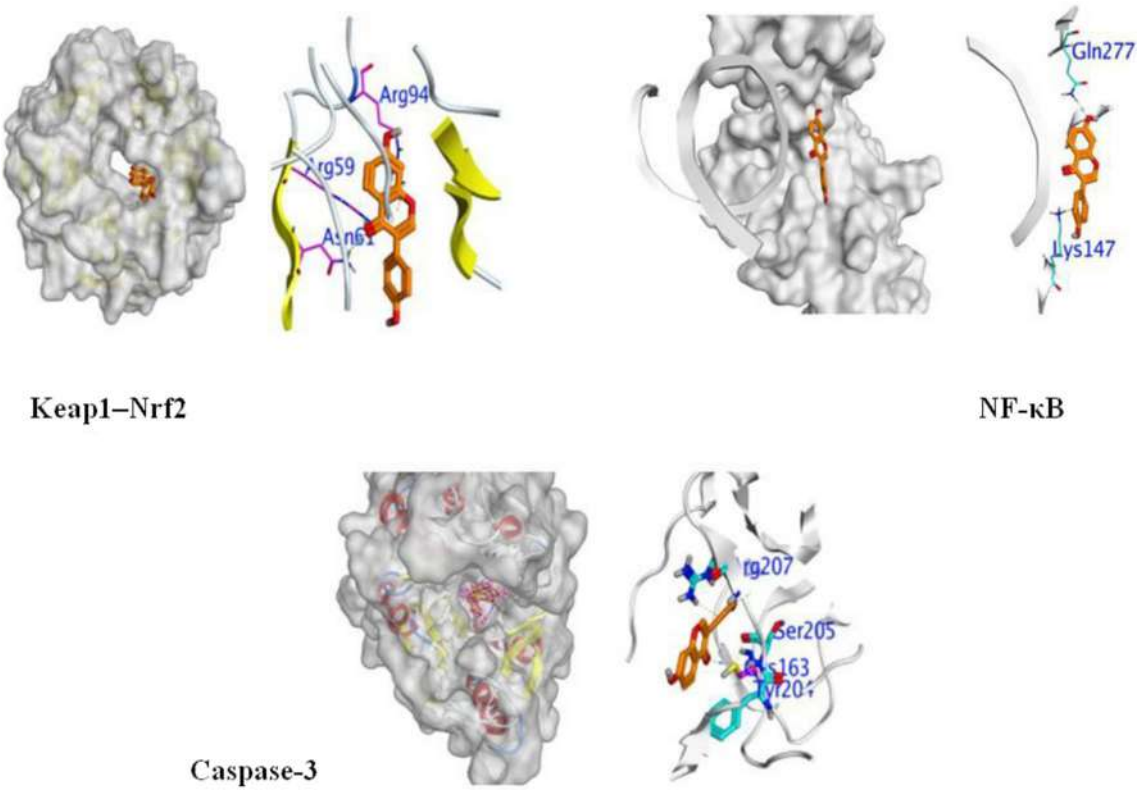

Fig. 11 Binding mode for daidzein with Keap1–Nrf2 (PDB:3VNG), NF-κB (PDB: 1LE5), and caspase-3 (PDB: 3GJQ)

For caspase-3

The mechanistic antiapoptotic pathway was proved by a docking study. Our study based on these aspects; (i) interaction with active site of caspase-3 (ARG207, SER205, TRP206, PHE250, GLN161, GLY122, HIS121, SER65, ARG64) (ii) RMSD value lower than 2 Å, which reflect the high stability fitting of daidzein into binding pocket. The investigated compound has nearly the same binding affinity (ΔG= −5.94 Kcal/mol) with the reference inhibitor DEVD (ΔG= −5.90 Kcal/mol). Daidzein form important H-bond with Ser205, that amino acid interacted with vital amino acids (Cys163 and Tyr 204) in catalytic pocket, other π-π stacky interaction between Arg207 and hydroxy group of daidzein (Fig. 11).

Discussion

AKI is associated with rapid decreases in the rate of glomerular filtration and retention of nitrogenous waste products inside the body (Abugomaa and Elbadawy 2020). In order to study AKI, animal models using nephrotoxic drugs such as gentamycin and cisplatin are mostly conducted, but none of these models mimic the exact pathology of AKI as that occurs in RM. RM is one of the leading causes of AKI that results from the breakdown of skeletal muscles and muscle enzymes leakage. In our study, the model of RM-induced AKI was achieved by glycerol injection that evoked substantial precipitation of myoglobin in renal tubules with subsequent tubular obstruction that mimics the clinically observed alterations. On the contrary, the administration of daidzein counteracted functionally, biochemically, and histopathologically RM-induced AKI. The muscle damage was prominent in the AKI group as indicated by higher CK and LDH levels than in the control group and this is following former reports (Sharawy et al. 2018; Wu et al. 2017). The massive injury of skeletal muscles exceeds the capacity of plasma protein binding for myoglobin. This increases in the tubular reabsorption and precipitation of myoglobin in the distal tubules, finally provoking tubular cell death (Khan 2009). Our results revealed that the AKI group showed marked increases in the absolute and relative kidney weight as well as elevated serum urea and creatinine levels. These results indicated the existence of kidney edema, and this came in line with previously reported studies (Abd-Ellatif et al. 2019; Al-Brakati et al. 2021; Elsayed et al. 2014). In addition, rhabdomyolysis was reported to induce other biochemical abnormalities such as hyperpotassemia, hyperkalemia, and hypocalcemia (Ayvaz et al. 2012). As such, we observed

significant elevations in P and K levels in the myoglobinuric AKI group, but not in Na levels. The histological examination of glycerol-treated animals revealed enlargement in the kidney tubular cavity and infiltration of inflammatory cells in the interstitial space together with glomerular hypertrophy and tubular epithelial cell edema, these results supported the biochemical changes and were in accordance with other studies (Mard et al. 2022; Ohtani et al. 2022). On the other side, the daidzein administration alleviated the changes in kidney weight and electrolytes caused by glycerol injection and maintained the renal muscle functions. Liu et al. (2014b) found that administration of whole soy and its constituent daidzein improved markedly the renal functions in a clinical trial on prehypertensive postmenopausal women. Further, pretreatment with daidzein normalized serum creatinine and blood urea nitrogen in cisplatin-induced nephrotoxicity (Meng et al. 2017; Tomar et al. 2020). The results reported elsewhere (Askaripour et al. 2022b) showed that daidzein substantially reduced fractional urine excretion of sodium, potassium, and urine calcium levels in postmenopausal rats. Substituting soy protein for animal protein was associated with declines in hyperfiltration and decreased urine albumin excretion in patients with diabetes mellitus and nephropathy (Teixeira et al. 2004). In addition, cystatin C, a proteinase inhibitor, is considered an appropriate kidney disease marker because it is undergoing complete glomerular filtration and tubular reabsorption (Alhusaini et al. 2020). Its level is not affected by muscle mass, age, sex, and food type. Cystatin-c is a more accurate renal function biomarker because it senses the renal injury two days before the increases in urea and creatinine levels (Al-Kuraishy et al. 2020). Higher cystatin-C levels were previously reported in gentamycin and lead acetate-induced AKI (Al-Kuraishy et al. 2020; Alhusaini et al. 2020). Also, Kim-1 and NGAL are early diagnostic markers that reflect tubular kidney injury (Al-Brakati et al. 2021). In the current investigation, notable increases were observed in the levels of cystatin-c, KIM-1, and NGAL in the kidney tissue of the glycerol-injected group. These outcomes are in accordance with the results of previous reports (Al-Brakati et al. 2021; Sharawy et al. 2018; Yin et al. 2019). However, these markers displayed notable decreases in the daidzein administered group, and this is in harmony with former reports (Khan and Sultana 2004; Meng et al. 2017). These findings illustrate the nephroprotective effect of daidzein that may be linked to its anti-oxidative action and free radical scavenging ability.

Previous reports have illustrated that the oxidative stress induced by ROS is strongly implicated in the initiation of glycerol-mediated kidney injury (Al-Brakati et al. 2021; Yin et al. 2019). The released products of muscle degradation (myoglobin and heme derivatives) into the bloodstream initiate ROS production and oxidative injury in renal tubules (Yin et al. 2019). In our study, glycerol injection–induced oxidative stress was witnessed by a marked increase in MDA and NO levels as well as profound decreases in GSH levels. In addition, the AKI group showed marked suppression in the activities of SOD, CAT, GPx, and GR, and this agrees with previous studies (Adedapo et al. 2020; Wu et al. 2017). In contrast, our results indicate that daidzein showed notable antioxidant effects against glycerol-induced kidney injury. The remarkable enhancement in the activities of SOD, CAT, GPx, and GR enzymes and the levels of GSH in the daidzein co-administered group. A previous work Meng et al. (2017) reported that daidzein improved cisplatin-mediated depletion of GSH and the reduction in SOD and GPx activities in mouse kidneys. In addition, daidzein evoked marked increases in the activities of SOD and GPx, and CAT as well as total antioxidant capacity in the serum and kidney tissue of ovariectomized rats (Askaripour et al. 2022a). Another study Guru et al. (2022) found that daidzein counteracted the oxidative stress and ROS generation elicited by gentamicin in Madin-Darby canine kidney (MDCK) cells in vitro and zebrafish model in vivo. Also, daidzein prevented the suppression of the antioxidants (GSH, GST, and CAT) in the intestinal mucosa of 5-FU-administered mice (Atiq et al. 2019). These effects may be directly endorsed by the phenolic ring in daidzein's chemical structure, which protects against lipid peroxidation, or by its ability to upregulate antioxidant genes (Khan et al. 2012). Further, the antioxidant potential of daidzein may refer to equol which is a specific metabolite of daidzein produced by intestinal bacteria. Equol has the highest antioxidant potential of all identified and tested isoflavones (Tanaka et al. 2022).

Our results also illustrated that daidzein markedly increased the gene expressions of HO-1 and Nrf2 which suggest that daidzein protected the kidney from oxidative stress via activation of these two molecules. Nrf2 is a master regulator for antioxidant and detoxification genes to act in synergy to scavenge free radicals and other electrophilic molecules (El-Khadragy et al. 2021; Kassab et al. 2021). HO-1 is a crucial enzyme that is responsible for the breakdown of prooxidant heme with subsequent relive of oxidative, inflammatory, and apoptotic stresses (Kassab et al. 2020). The downregulation of Nrf2 and HO-1 gene expressions in animal models of AKI has been reported in former studies (Al-Brakati et al. 2021, Yin et al. 2019). The activation of the NRF-2/HO-1 pathway was involved in the cardioprotective effect of daidzein against myocardial infarction induced by hypoxic-ischemic injury in a rat model (Zeng et al. 2021). Further, daidzein was reported to inhibit lipopolysaccharide-induced hepatic injury via activation of Nrf2/Keap-1 signaling with subsequent reduction of oxidative stress (Yu et al. 2020). Similar effects of daidzein were detected in mice models of autoimmune hepatitis induced by concanavalin A (Li et al. 2021). Equol induced noteworthy activation of Nrf2, HO-1, and NQO1 in endothelial cells

transfected with Nrf2-siRNA transfection plasmid that indicated its potent cytoprotective effects (Zhang et al. 2013). Thus, it could be concluded that NRF-2/HO-1 is a key pathway by which daidzein overwhelmed the glycerol-mediated oxidative insult in the damaged kidney. Renal inflammation and leukocyte infiltration are typical characteristics of the pathogenesis of RM-induced AKI. Excess ROS production by the released products of muscle breakdown aggravates the progress of inflammatory conditions (Amirshahrokhi 2021). ROS can exacerbate the inflammatory reaction with subsequent tissue damage through activation of the NF-κB pathway. NF-κB, a transcriptional factor, controls the expressions of many pro-inflammatory genes that provoke tubular cell destruction and death (Othman et al. 2021a). In harmony with former studies (Al-Brakati et al. 2021; Amirshahrokhi 2021), we found profound increments in the levels of IL-1β, TNF-α, IL-10, NF-κB, and MPO in glycerol-injected group. MPO is a pro-inflammatory enzyme released from neutrophils and is involved in the progress of AKI (Adedapo et al. 2020). It enhances the reaction of hydrogen peroxide and chloride anion to form hypochlorous acid. These reactive molecules act as a strong oxidant that induces lipid peroxidation and cellular redox imbalance. Interestingly, daidzein treatment markedly decreased these inflammatory markers in renal tissue. The anti-inflammatory effect of daidzein was previously observed in former studies (Atiq et al. 2019; Li et al. 2021; Yu et al. 2020). Daidzein treatment significantly downregulated the expression levels of TNFα, IL-10, IL-18, and MCP-1 as well as decreased the protein expression of TNFα in renal tissue of cisplatin-exposed mice (Meng et al. 2017). Daidzein was also observed to alleviate gentamicin-induced kidney inflammation in zebrafish via downregulation of pro-inflammatory cytokines such as COX-2, TNF-α, IL-1β, and iNOS (Guru et al. 2022). An in vitro study stated that daidzein suppressed the pro-inflammatory cytokines in human hypothalamic GnRH neuronal cells (Morelli et al. 2021). The present findings show that daidzein pretreatment strongly suppressed NF-κB levels in injured renal tissues, and this is in accordance with former reports (Khan et al. 2012; Liu et al. 2016). Daidzein also inhibits the activation of NF-κB, the gene expression of iNOS, and NO production in macrophages exposed to lipopolysaccharide (Hämäläinen et al. 2007). Another study Guo et al. (2020) also reported that daidzein-rich isoflavones aglycone inhibited the proliferation of lung cancer via suppression of the NF-κB signaling pathway. Our study evidently supports that daidzein effectively suppressed the production of cytokines and NF-κB in the AKI model which implicated its anti-inflammatory potential. In this study, apoptotic biomarkers were also explored to determine the nephroprotective effects of daidzein in RM-induced AKI in a rat model. The cross-link between the oxidative and inflammatory response–mediated cell loss has been suggested to play a crucial role in the progression of AKI (Al-Brakati et al. 2021). Significant increases were observed in renal caspase-3 and Bax levels in the glycerol group together with a decline in Bcl-2 that indicate the shift towards apoptotic cell death. This is in harmony with former reports (Abd-Ellatif et al. 2019; Al-Brakati et al. 2021). Bcl-2 and Bax are two apoptosis-regulating proteins with opposite apoptotic actions (Lokman et al. 2022; Othman et al. 2021b). The balance between proapoptotic and antiapoptotic molecules has a critical role in cell survival and apoptosis (Al-Megrin et al. 2020; Albarakati et al. 2020). Adversely, these apoptotic markers were reversed by daidzein pretreatment as documented in earlier studies (Meng et al. 2017; Tomar et al. 2020; Zeng et al. 2021). Another study by Pang et al. (2020) found that soy isoflavones (daidzein and genistein) exerted in vitro and in vivo antiapoptotic activities against high fat diet–evoked hypothalamic apoptosis in obese male mice. Daidzein improved notably the expression levels of both genes and proteins of Bcl-2, Bax, and caspase-3 in brain infarct tissue induced by cerebral ischemia-reperfusion (Zeng et al. 2021). Further, a former in vitro study revealed that daidzein protected MDCK cells from gentamicin-induced cellular apoptosis (Guru et al. 2022). These findings suggest that the regulation of the expressions of Bax, Bcl-2, and caspase-3 by daidzein may contribute to the protection against glycerol-mediated renal apoptotic cell loss. Docking study against (PDB:3VNG (Satoh et al. 2015), NF-κB (PDB: 1SVG (Breitenlechner et al. 2004)), and caspase-3 (PDB: 3GJQ (Fang et al. 2009) showed that daidzein interacted with vital amino acid residues of 3VNG, 1SVG, and 3GJQ. The variation in the interaction mode between daidzein and hydrophobic protein backbone assumed that the efficiency of the tested daidzein is due to an increase in its hydrophilicity. Overall docking analysis showed that the tested compound is able to stabilize in binding sites in the same manner as reference drugs. These results are in line with the experimental findings. The present study focused on pretreatment with daidzein for 2 weeks prior to administration of glycerol which has a certain limitation on the translational relevance. This limitation suggest further research on modulation of inflammatory, oxidative, and apoptotic stresses mediates the renoprotective effect of daidzein administrated after or at the same time of induction of acute kidney injury in rats by glycerol.

Conclusion

The current study showed that daidzein supplementation offered significant renoprotection against glycerol-induced renal damage in rats. The protective impact of daidzein may come from the interplay of its anti-inflammatory, antioxidant, and antiapoptotic effects. Administration of daidzein

improved the biomarkers related to rhabdomyolysis and renal functions. Furthermore, daidzein lessened the renal oxidative damage biomarkers and upregulated the expressions of Nrf2 and HO-1. Daidzein evoked substantial declines in inflammatory cytokines and NF-κB levels and decreases in apoptotic changes in rat kidneys. Collectively, this study assumes that daidzein could be considered an effective and promising modality for managing AKI-related renal tissue damage. Docking analysis showed that the daidzein efficiency is due to increasing its hydrophilicity which interacts with the hydrophobic part. Overall docking analysis showed that the tested compound is efficiently stabilized in binding sites in the same manner as reference drugs. Further future studies should be conducted to address the protection by daidzein when administered after and/or at the same time of the single injection of glycerol.

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Author contribution All authors were involved in the conception of the idea, performed data analysis and interpretation, provided resources, were responsible for data curation, wrote and edited the manuscript, and reviewed drafts of the paper. All authors read and approved the final version of the manuscript.

Data availability All the relevant data are presented within the article.

Declarations

Institutional review board statement and ethics approval All experimental procedures were accepted by the department of zoology and entomology, faculty of science, Helwan University (Cairo, Egypt; approval no, HU2021/Z/RKA0921-01).

Consent to participate Not applicable.

Consent for publication Consented.

Informed consent Not applicable.

Conflict of interest The author declares no competing interest.

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