Naunyn-Schmiedeberg's Archives of Pharmacology (2025) 398:11839–11853
https://doi.org/10.1007/s00210-025-03910-9

RESEARCH

Trans-sodium crocetinate attenuates acute kidney injury induced by rhabdomyolysis in rats: focusing on PI3K/AKT, apoptosis, and autophagy pathways

Tahereh Aminifard[1] · Soghra Mehri[1,2] · Abolfazl Khajavirad[3,5] · Zahra Moosavi[4] · Sara Hosseinian[3] · Hossein Hosseinzadeh[1,2]

Received: 7 January 2025 / Accepted: 10 February 2025 / Published online: 13 March 2025

Abstract

Rhabdomyolysis (RM) is a clinical disorder characterized by the release of potentially toxic muscle cell components into the bloodstream, with acute kidney injury (AKI). Trans-sodium crocetinate (TSC) is derived from the carotenoid crocetin known for its renoprotective, anti-inflammatory, and antioxidant properties. This study aimed to assess the protective effects of TSC on RM-induced AKI in rats. Six groups of rats ($n=6$) were used: control, AKI (50% glycerol 10 mL/kg, intramuscularly), AKI treated with TSC (10, 20, and 40 mg/kg, intraperitoneally), and TSC (40 mg/kg) alone groups. Two days after the initial injection, urine and blood samples were collected over 24 h to investigate creatine phosphokinase (CPK), kidney function markers, and electrolyte levels. Additionally, kidney tissue was collected to assess renal oxidative markers, histological alterations, and the expression of protein markers related to autophagy, apoptosis, renal injury, inflammation, and the PI3K/AKT signaling pathway. After glycerol administration, there was an increase in oxidative stress, autophagy, apoptosis, renal injury, and inflammatory marker levels, accompanied by a decrease in the proteins of the PI3K/AKT signaling pathway in the kidney. The co-administration of TSC with glycerol resulted in the improvement of renal dysfunction and structural abnormalities, achieved through a reduction in oxidative stress. TSC also down-regulated autophagy, apoptotic, renal injury, and inflammatory markers. Furthermore, TSC treatment led to a decrease in the renal expression of PI3K/AKT signaling pathway proteins. In conclusion, TSC exhibited a protective effect against RM-induced AKI by modulating oxidative stress, autophagy, apoptosis, and the PI3K/AKT pathway.

Keywords Rhabdomyolysis · Acute kidney injury · Trans-sodium crocetinate · Apoptosis · Autophagy

✉ Soghra Mehri
Mehris@mums.ac.ir

✉ Hossein Hosseinzadeh
hosseinzadehh@mums.ac.ir

[1] Department of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

[2] Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran

[3] Department of Physiology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[4] Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

[5] Applied Biomedical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Abbreviations

RM	Rhabdomyolysis
TSC	Trans-sodium crocetinate
MB	Myoglobin
AKI	Acute kidney injury
DTNB	5,5-Dithio-bis-2-nitrobenzoic acid
KCl	Potassium chloride
PVDF	Polyvinylidene difluoride
PMSF	Phenylmethanesulfonyl fluoride
TBST	Tris-buffered saline tween
ROS	Reactive oxygen species
SDS	Sodium dodecyl sulfate
TCA	Trichloroacetic acid
TBA	2-Thiobarbituric acid
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
PI3K	Phosphoinositide 3-kinases
SD	Standard deviation

BUN	Blood urea nitrogen
sCr	Serum creatinine
CPK	Creatine phosphokinase
Na	Sodium
K	Potassium
Ca	Calcium
uCr	Urine creatinine
sOsm	Serum osmolality
uOsm	Urine osmolality
GFR	Glomerular filtration rate
MDA	Malondialdehyde
GSH	Glutathione
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel
SOD	Superoxide dismutase
TBST	Tris-buffered saline Tween 20
MTT	3-(4,5-Dimethyl-thiazol-2-yl) 2,5-diphenyl tetrazolium bromide
P-AKT	Phospho-AKT
TNF-α	Tumor necrosis factor-α
PI3K	Phosphoinositide 3-kinases
P-PI3K	Phospho-PI3K
H&E	Hematoxylin and eosin
NGAL	Neutrophil-gelatinase-associated lipocalin
IL-6	Interleukin 6
KIM-1	Kidney injury molecule 1
IL-1β	Interleukin 1β

Introduction

Rhabdomyolysis (RM) is a clinical condition that occurs when striated skeletal muscle is extravagantly destructed (Zutt et al. 2014). This process results in the liberation of breakdown muscle cell content including, sarcoplasmic proteins, myoglobin (MB), and electrolytes into the plasma (Stahl et al. 2020). In more serious cases, potentially toxic compounds such as MB can adversely affect kidney function, leading to life-threatening emergencies (Scharman and Troutman 2013).

After RM, released muscle MB is metabolized in the kidney tubule cells and promotes lipid peroxidation and free radical generation. MB produced a synergistic effect on tubular obstruction, cast formation, and renal vasoconstriction leading to acute renal injury (AKI) (Panizo et al. 2015). Moreover, some studies showed that direct MB-induced cytotoxicity has a fundamental role in the progression of RM-induced AKI via evoking apoptotic responses, oxidative stress, and inflammation (Ahmad et al. 2021). Although the underlying mechanism of RM-induced AKI is complicated, well-timed therapeutic interventions can elevate recovery (Scharman and Troutman 2013).

The most frequently used experimental model for studying rhabdomyolysis-induced acute kidney injury (AKI) involves the intramuscular injection (IM) of glycerol in rats (Chen et al. 2018). In glycerol-induced AKI, the reabsorption of myoglobin (MB) by proximal tubule cells, coupled with the precipitation of MB in the distal tubules, leads to renal functional and structural damage (Guerrero-Hue et al. 2019). This slight revision maintains the original meaning while enhancing the flow of information.

Attention to the pathogenesis of RM-induced AKI suggests that therapeutic agents for RM-induced AKI treatment should have anti-inflammatory, antiapoptotic, and antioxidant properties in kidneys, especially tubular cells (Hebert et al. 2023).

A large number of investigations have suggested that ingredients of plants downregulate diverse health disorders related to the kidney (Alok et al. 2013). Saffron is derived from the stigmas of the *Crocus sativus* L. plant and is a type of perennial plant with several medicinal functions (Guo et al. 2022a). Saffron and its ingredients are commonly used to promote health in modern and traditional medicine (Mzabri et al. 2019).

Among the components of saffron, crocetin is primarily responsible for these therapeutic and pharmacological effects (Wani et al. 2021). Crocetin as a main constituent of saffron has a long history of useful biological activities such as antiapoptotic, anti-inflammatory, and antioxidation effects (Guo et al. 2022b). Furthermore, multiple reports showed that crocetin has the therapeutic potential for renal cell protection (Hashemi and Hosseinzadeh 2019).

In the last years, there has been remarkable interest in using trans sodium crocetinate (TSC) in the treatment of different disorders such as cancer (Colapietro et al. 2019), atherosclerosis (Mohler et al. 2011), and hyperlipidemia (Deng et al. 2015). Modern pharmacological investigations have reported that TSC is a bipolar salt that increases the bioavailability and the solubility of crocetin and promotes its remedial efficacy (Rajabian et al. 2023). Moreover, TSC as a potent antioxidant has a protective effect against renal injury in multiple reports (Shah et al. 2021).

Currently, epidemiological studies show that there is no any specific and effective therapy for RM-induced AKI (Chavez et al. 2016). It is necessary to find a renoprotective agent with minimal adverse effects for the treatment of RM-induced AKI (Panizo et al. 2015).

In this regard, the present research was undertaken to survey the potential nephroprotective effect of TSC against RM-induced AKI. We determined the effect of TSC renal function, structure, and oxidative stress, in an experimental AKI model of RM in rats. Additionally, the underlying molecular mechanisms for the antiapoptotic, autophagy regulation, and anti-inflammatory effects of TSC on glycerol-induced AKI in rats were evaluated.

Materials

The chemicals used in the current study, including thiobarbituric acid (TBA) and potassium chloride (KCl), were purchased from Merck Company, Germany. Trichloroacetic acid (TCA), and 5,5′-dithiobis 2-nitrobenzoic acid (DTNB) were obtained from the Sigma Company, Germany. TSC was bought from Tinab Shimi, Iran. Additionally, glycerol was prepared by Dr. Mojallali Company in Iran. All antibodies were acquired from Cell Signaling Company, while the polyvinylidene fluoride (PVDF) membranes were purchased from Bio-Rad, USA.

Experimental animals

Thirty-six male Wistar rats with background (6–8 weeks old and 250–270 g body weight) bred in the experimental animal center of the School of Pharmacy, Mashhad University of Medical Sciences (Mashhad, Iran), were used. The rats were maintained under standard and normal conditions with gratis access to clean water and regular rodent chow. The experimental protocols were conducted according to the guidelines of the laboratory animals' Ethics Committee at the Mashhad University of Medical Sciences (Ethical Number: IR.MUMS.PHARMACY.REC.1399.033).

Experimental design

The rats were randomly distributed into six groups of six individuals. All groups had access to food but were water deprived 12 before the glycerol or saline injection as follows:

Group 1 (control group) served as a normal control reference: the rats received an intraperitoneal (IP.) administration of normal saline (0.9% NaCl) and IM. administration of normal saline (equivalent volume for glycerol in each hind leg) under anesthesia.
Group 2 (AKI group) served as an RM-induced AKI control reference: the rats received a single administration of 50% glycerol (10 mL/kg, IM.) with no more treatment (Sharawy et al. 2018).
Groups 3–5 served as treatment groups (Amin et al. 2015):
Group 3: injected with 50% glycerol (10 mL/kg, IM.) and TSC (10 mg/kg, IP.)
Group 4: injected with 50% glycerol (10 mL/kg, IM.) and TSC (20 mg/kg, IP.)
Group 5: injected with 50% glycerol (10 mL/kg, IM.) and TSC (40 mg/kg, IP.)
Group 6 (TSC group) served as the TSC control reference: the rats received a single injection of TSC (40 mg/kg, IP.).

One day after the first injection, all the rats were separately placed in metabolic cages for 24 h to determine urine output. Two days after the IM. injection, the rats were sacrificed. Afterward, urine and blood samples were collected and both of the kidneys were isolated. The left kidney was stored in a 10% formalin solution for the histological analysis. While the right kidney was deep frozen at − 80 °C for western blot analysis and oxidative parameter measurement.

Biochemical analysis

The blood levels of urea nitrogen (BUN), creatinine (sCr), creatine phosphokinase (CPK), sodium (Na), potassium (K), calcium (Ca), and urine creatinine (uCr) were measured spectrophotometrically using the commercially standard kits (Parsazmoon company, Iran).

Urine osmolality (uOsm) and serum osmolality (sOsm) were measured by the freezing method (The Advanced Osmometer, Norwood, USA).

Glomerular filtration rate (GFR) was measured by calculating creatinine clearance (Ccr) (mL/min) using the following equation (Shahbaz and Gupta 2019):

$$\varepsilon Ccr = [uCr(mg/dL) \times urineflow(mL/min)]/sCr(mg/dL)\varepsilon$$

Urine flow per minute was calculated by dividing 24-h urine volume by 1440 (the number of minutes in a day).

Lipid peroxidation assay in the kidney

Malondialdehyde (MDA) level was determined as an indicator of lipid peroxide in the kidney tissue. According to the procedure explained by Ohkawa et al. (1979), 10% homogenates of the kidney tissue in KCl (1.15%) were mixed with TBA (0.6%, 1 mL) and phosphoric acid (1%, 3 mL). After mixing, the homogenate was heated in a water bath (95 °C) for 45 min. Then, n-butanol (4 mL) was added to the mixture, strongly stirred for 2 min, and centrifuged for 30 min at 3000 rpm. Finally, the absorbance of samples was measured spectrophotometrically at 532 nm and the MDA content in the kidney was reported as nmol/g tissue (Mohammadi et al. 2018).

GSH assays in the kidney

The glutathione (GSH) content in the kidney tissue was determined based on the method described by Moron et al. (28). Kidney homogenate (10%) in phosphate buffer (pH = 7.4, 0.1 M) was combined with TCA (10%, 0.5 mL). After vortexing, the homogenate was centrifuged at 3000

g for 10 min. Then, DTNB (0.5 mL) and phosphate buffer (pH 8, 2.5 mL) were added to the supernatant fraction (0.5 mL). The absorbance of the yellow product was instantly measured using a spectrophotometer at 412 nm. The GSH levels were calculated based on a standard GSH curve and reported as nmol/g tissue (Tabeshpour et al. 2019).

SOD assay in the kidney

The colorimetric technique uses for the determination of superoxide dismutase (SOD) activity by using 96-well microplates. This method is based on the formation of SOD by auto-oxidation of pyrogallol and reliant on inhibition of the conversion of 3-(4,5-dimethyl-thiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT) to formazan and described as follows.

At first, samples were placed in the microplate wells, saturated with buffer and MTT, and finally incubated for 5 min at room temperature. Then, DMSO was added to the wells and the final solution was read at two wavelengths of 570 nm and 630 nm. One unit of SOD is determined as the amount of protein needed to inhibit a 50% reduction of MTT. The data were displayed as unit/gram tissues (Ghasemzadeh Rahbardar et al. 2023).

Western blotting analysis

Western blotting was done according to the procedure previously explained (Tabeshpour et al. 2019). Briefly, the total protein concentration was determined by the Bradford method (Kruger 2009). Then, equal amounts of protein extracts from kidney tissue were subjected to sodium dodecyl sulfate polyacrylamide gel (12% SDS-PAGE) electrophoresis and transferred to PVDF membranes. The membranes were blocked in Tris-buffered saline Tween 20 (TBST) containing 5% dry skim milk or 5% bovine serum albumin for 2 h at room temperature. After blocking, incubation of the membranes was performed with the primary antibodies at 1:1000 dilutions, against beclin-1 (Cell Signaling, #3495), LC3 II/I (Cell Signaling, #12,741), cleaved caspase-3 (Cell Signaling, #9664), phospho-AKT (P-AKT) (Cell Signaling, #9271), AKT (Cell Signaling, #9272), phospho-PI3K (P-PI3K) (Cell Signaling, #9271), phosphoinositide 3-kinases (PI3K) (Cell Signaling, #9271), tumor necrosis factor-α (TNF-α) (Cell Signaling, #3707), neutrophil-gelatinase-associated lipocalin (NGAL) (Abcam, #216,462), and beta-actin (Cell Signaling, #3700) overnight at 4 °C. Next, the membranes were incubated with anti-rabbit IgG conjugated with horseradish peroxidase or anti-mouse IgG conjugated with the enzyme at 1:3000 dilutions for 2 h at room temperature. Finally, visualization of protein bands was performed using an Alliance 4.7 Geldoc. Densitometric analysis for protein bands was done using UVtec software (UK). Protein levels in each membrane were normalized to the corresponding β-actin band (Tabeshpour et al. 2019).

Renal histopathological assessment

Histopathological analysis was conducted to assess tubular necrosis, cast formation, and glomerular atrophy. The left kidneys of rats from different groups were fixed in a 10% formalin solution and embedded in paraffin. Subsequently, 5-µm kidney sections were stained with hematoxylin and eosin (H&E) and examined blindly under a light microscope. The extent of tissue damage was evaluated based on the percentage area of sections. The progression in tubular lesion severity was determined using scores on a scale: none (0), grade 1 (0–25%), grade 2 (25–50%), grade 3 (50–75%), and grade 4 (more than 75%) (Yang et al. 2012).

Statistical analysis

The experimental results were analyzed using GraphPad Prism software version 8.0 (Software Inc., CA, USA) and presented as the mean ± standard deviation (SEM). To determine significant differences among different groups, a one-way analysis of variance (ANOVA) was conducted, followed by the Tukey–Kramer post hoc test. Pathological data

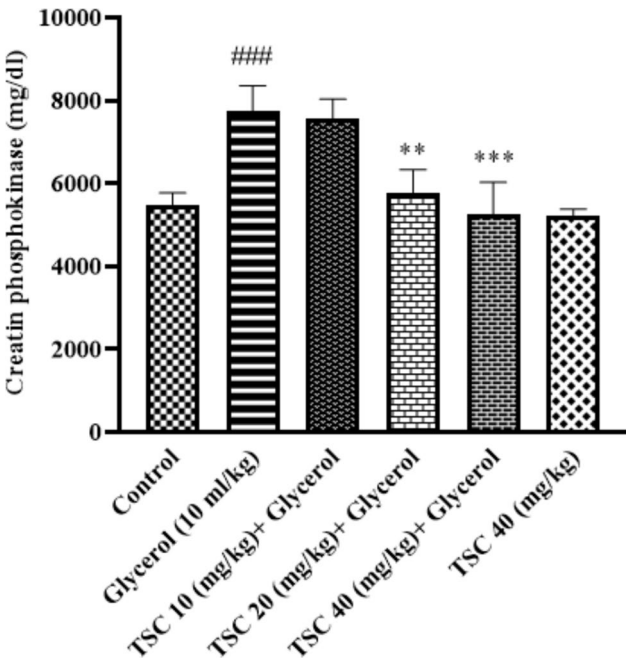

Fig. 1 Effect of TSC on CPK following glycerol-induced AKI. Data are presented as mean ± SD of 4–6 separate experiments. Data were analyzed by one-way ANOVA and the Tukey–Kramer post-test. $^{###}P<0.001$ vs control group and $^{***}P<0.001$ and $^{**}P<0.01$ vs AKI group. AKI: acute kidney injury, TSC: trans-sodium crocetinate, CPK: creatine phosphokinase

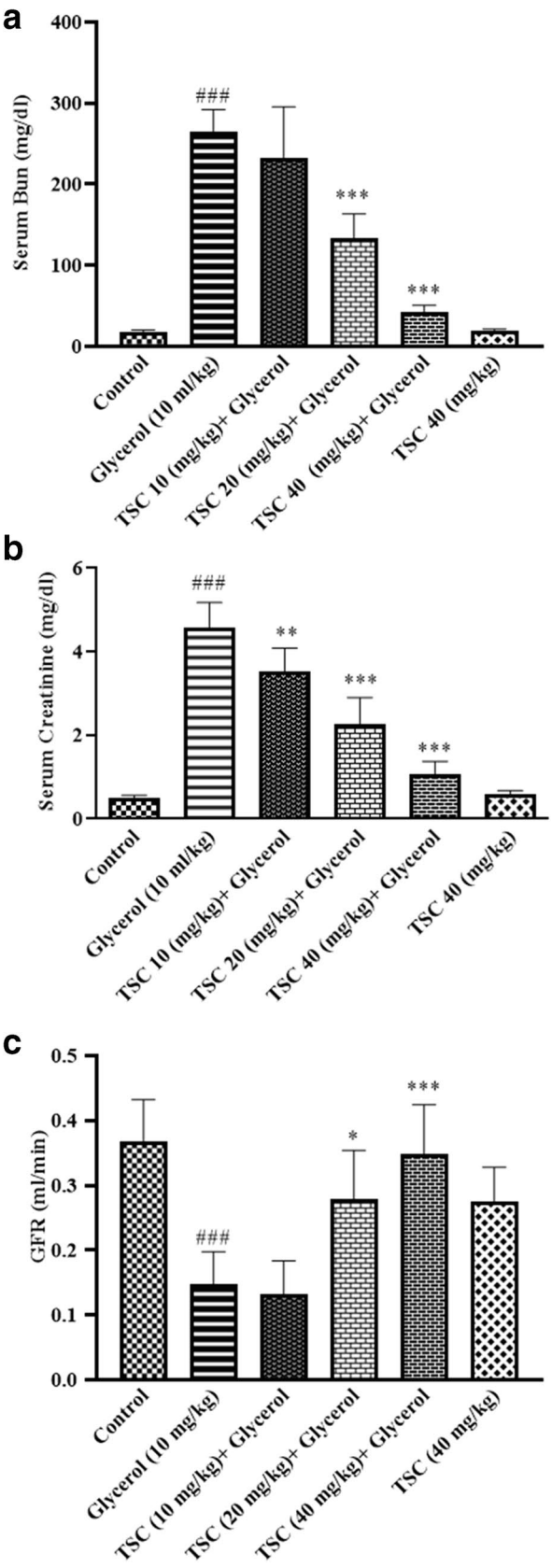

Fig. 2 Effect of TSC on BUN (**A**), sCr (**B**), and GFR (**C**), following glycerol-induced AKI. Data are presented as mean ± SD of 4–6 separate experiments. Data were analyzed by one-way ANOVA and the Tukey–Kramer post-test. $^{###}P < 0.001$ vs control group and $^{***}P < 0.001$, $^{**}P < 0.01$, and $^{*}P < 0.05$ vs AKI group. AKI: acute kidney injury, TSC: trans-sodium crocetinate, BUN: blood urea nitrogen, sCr: serum creatinine, GFR: glomerular filtration rate

were assessed using the median (interquartile range) with the Kruskal–Wallis nonparametric analysis, and Dunn's post hoc test was applied. *P* values less than 0.05 were considered statistically significant (Ghasemzadeh Rahbardar et al. 2023).

Results

Effect of TSC on biochemical markers following glycerol-induced AKI

The data show that glycerol administration (10 mL/kg) significantly elevated CPK levels ($P < 0.001$) compared to the control group (Fig. 1). Furthermore, kidney function markers, including BUN levels ($P < 0.001$) and sCr levels ($P < 0.001$), were significantly increased, and GFR was significantly reduced ($P < 0.001$) in the AKI group compared to the control rats (Fig. 2).

In Fig. 3, it is evident that serum Na levels ($P < 0.001$) significantly decreased, while K levels ($P < 0.001$) significantly increased in the AKI group compared to the control. Additionally, AKI groups' rats showed decreased uOsm ($P < 0.001$ vs control) and increased sOsm ($P < 0.001$ vs control) (Fig. 4). The administration of TSC (40 mg/kg) with glycerol (10 mL/kg) induced a remarkable decrease ($P < 0.001$) in the serum levels of BUN, Cr, K, CPK, and Osm, and a marked increase ($P < 0.001$) in GFR, Na, and uOsm compared to the AKI group. Furthermore, in the AKI + TSC (20 mg/kg) group, the values of sCr ($P < 0.001$), BUN ($P < 0.001$), CPK ($P < 0.01$), and K ($P < 0.01$) significantly decreased, while GFR ($P < 0.05$) and Na ($P < 0.01$) significantly increased compared to the AKI group. Notably, TSC administration alone (40 mg/kg) did not alter biochemical markers compared to the control group.

Effect of TSC on the oxidative status following glycerol-induced AKI in the kidney

The results, as shown in Fig. 5, demonstrate an increase in MDA levels in the kidneys of rats that received glycerol compared to the control group ($P < 0.001$). Simultaneous administration of 10, 20, and 40 mg/kg TSC resulted in a reduction in MDA levels compared to the

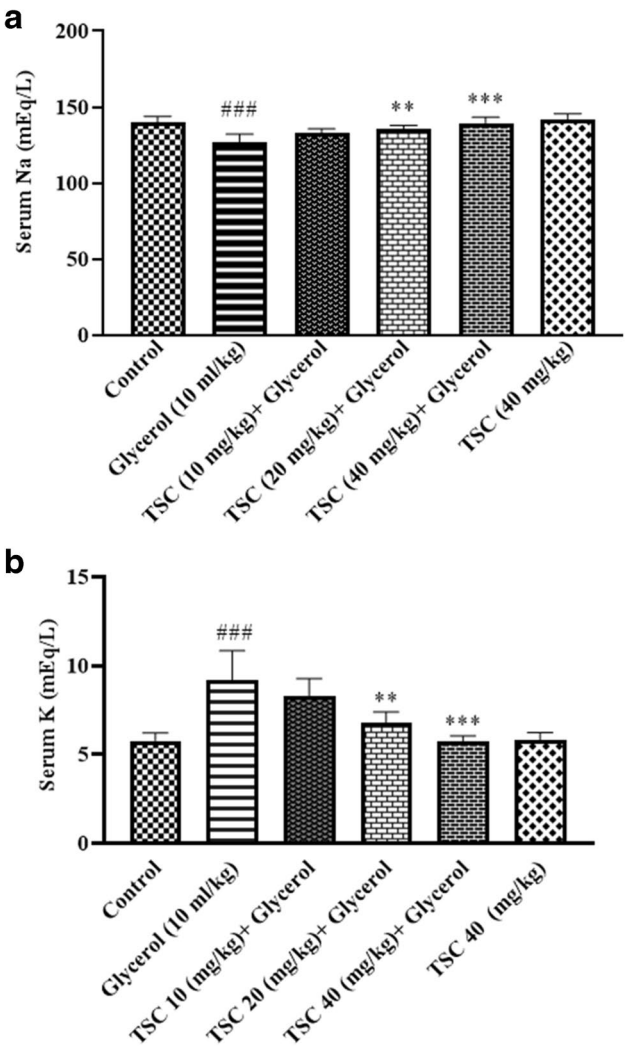

Fig. 3 Effect of TSC on Na (**A**) and K (**B**), following glycerol-induced AKI. Data are presented as mean ± SD of 4–6 separate experiments. Data were analyzed by one-way ANOVA and the Tukey–Kramer post-test. $^{###}P < 0.001$ vs control group and $^{***}P < 0.001$ and $^{**}P < 0.01$ vs AKI group. AKI: acute kidney injury, TSC: trans-sodium crocetinate, Na: sodium, K: potassium

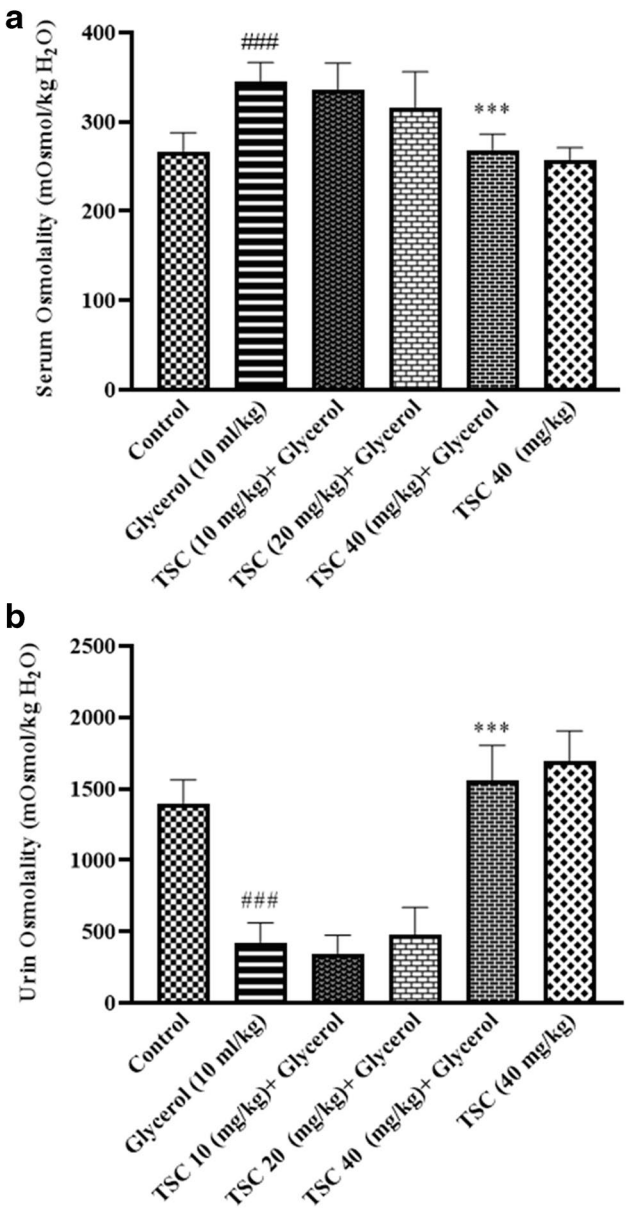

Fig. 4 Effect of TSC on sOsm (**A**) and uOsm (**B**) following glycerol-induced AKI. Data are presented as mean ± SD of 4–6 separate experiments. Data were analyzed by one-way ANOVA and the Tukey–Kramer post-test. $^{###}P < 0.001$ vs control group and $^{***}P < 0.001$ vs AKI group. AKI: acute kidney injury, TSC: trans-sodium crocetinate, uOsm: urine osmolality, sOsm: serum osmolality

AKI group ($P < 0.01$, $P < 0.001$, and $P < 0.001$, respectively). Glycerol injection notably attenuated GSH levels and SOD activity in renal tissue compared to the control group ($P < 0.05$ and $P < 0.01$, respectively). The administration of TSC at 20 and 40 mg/kg with glycerol resulted in a remarkable enhancement in GSH content ($P < 0.001$) and SOD activity ($P < 0.01$ and $P < 0.001$, respectively) compared to the AKI group (Fig. 5). Interestingly, a notable elevation in renal GSH levels was detected in the TSC (40 mg/kg) alone group compared to control animals.

Effect of TSC on glycerol-induced renal histopathological changes

The data obtained from the histopathological experiment in the different groups are summarized in Table 1 and shown in Fig. 6. The kidney tissue sections from control rats and TSC (40 mg/kg) alone group did not display any morphological changes. In contrast, kidney tissue of the AKI group showed widespread damage, evidenced by tubular necrosis

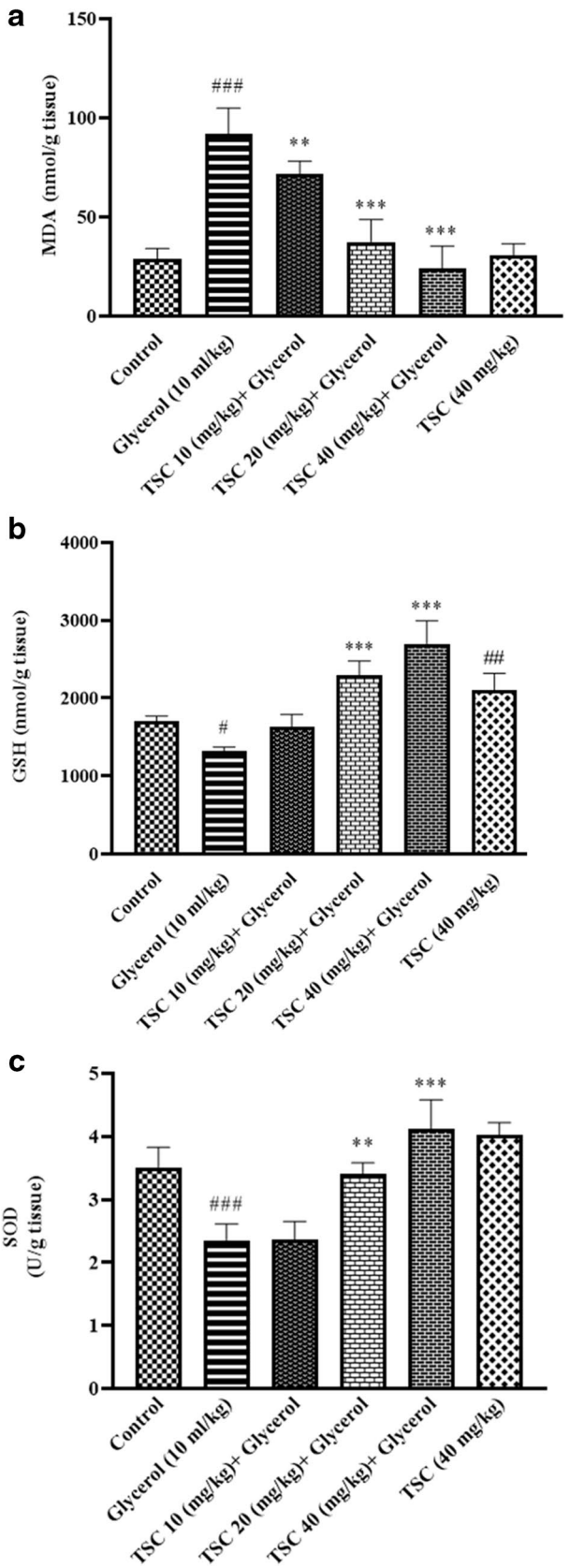

Fig. 5 Effect of TSC and glycerol on MDA (**A**), GSH (**B**), and SOD (**C**) in the kidney. Data are presented as mean ± SD of 4–6 separate experiments. Data were analyzed by one-way ANOVA and the Tukey–Kramer post-test. $^{###}P < 0.001$ and $^{\#}P < 0.05$ vs control group and $^{***}P < 0.001$ and $^{**}P < 0.01$ vs AKI group. AKI: acute kidney injury, TSC: trans-sodium crocetinate, MDA: malondialdehyde, GSH: glutathione, SOD: superoxide dismutase

($P < 0.001$ vs control) and glomerular atrophy ($P < 0.01$ vs control), and MB casts ($P < 0.001$ vs control). TSC (20 and 40 mg/kg) administration with glycerol (10 mL/kg) significantly relieved the severity of tubular damage and renal lesions in the rat. A remarkable decrease in tubular necrosis ($P < 0.001$ vs AKI group) and glomerular atrophy ($P < 0.05$ vs AKI group) in renal tissue was observed in AKI + TSC (40 mg/kg) group. Also, administration of TSC (20 mg/kg) with glycerol (10 mL/kg) reduced tubular necrosis ($P < 0.05$ vs AKI group) and MB casts ($P < 0.05$ vs AKI group).

Effect of TSC and glycerol on PI3K/AKT signaling pathway proteins in kidney

We measured the level of PI3K/AKT signaling pathway proteins, including P-AKT, AKT, P-PI3K, and PI3K in kidney samples. Western blot analysis revealed that the P-AKT/AKT ratio ($P < 0.001$ vs control) decreased meaningfully in rats that suffered AKI following glycerol administration. A significant increase in the P-AKT/AKT ratio ($P < 0.01$ vs AKI) was observed in the AKI + TSC (40 mg/kg) group (Fig. 7 A and B).

The injection of glycerol (10 mL/kg) resulted in a reduction of the P-PI3K/PI3K ratio ($P < 0.05$) compared with the rats in the control group. Simultaneous administration of TSC (40 mg/kg) with glycerol (10 mL/kg) increased the P-PI3K/PI3K ratio ($P < 0.05$) in comparison with the AKI group (Fig. 7 C and D). As shown in Fig. 7, the levels of PI3K/AKT signaling pathway proteins were not statistically changed in the TSC (40 mg/kg) alone group compared with the control.

Effect of TSC and glycerol on apoptotic marker in kidney

The cleaved caspase-3 as apoptotic marker was measured in kidney tissue by western blot analysis. As shown in Fig. 8, the cleaved caspase-3 ($P < 0.01$ vs control) was enhanced in kidney tissues of the AKI group. Contrary, co-administration of TSC (40 mg/kg) with glycerol (10 mL/kg) diminished the cleaved caspase-3 level ($P < 0.05$) in the kidney compared to the AKI group. There was no change in cleaved caspase-3 levels in the TSC (40 mg/kg) alone group in comparison to the control rats.

Table 1 Effect of TSC on glycerol-induced renal histopathological changes

Groups						Injury
TSC 40 (mg/kg)	TSC 10 (mg/kg) + AKI	TSC 20 (mg/kg) + AKI	TSC 40 (mg/kg) + AKI	AKI	Control	
0 (1)	2.5 (0)	1* (1)	1** (0)	$3^{\#\#\#}$ (1)	0 (1)	Tubular necrosis
0 (0.75)	2 (0)	1 (0.25)	0.5* (1)	$2^{\#\#}$ (0.25)	0 (0)	Glomerular atrophy
0 (0.75)	3.5 (1)	1.5* (0.25)	2.5 (0.5)	$4^{\#\#\#}$ (0.5)	0 (0)	MB cast

The histopathologic data were described with median (interquartile range) using the Kruskal–Wallis nonparametric analysis and Dunn's post hoc test. Statistical significance was considered at $^{\#\#\#}P < 0.001$ and $^{\#\#}P < 0.01$ vs the control group and $^{**}P < 0.01$ and $^{*}P < 0.05$ vs the AKI group

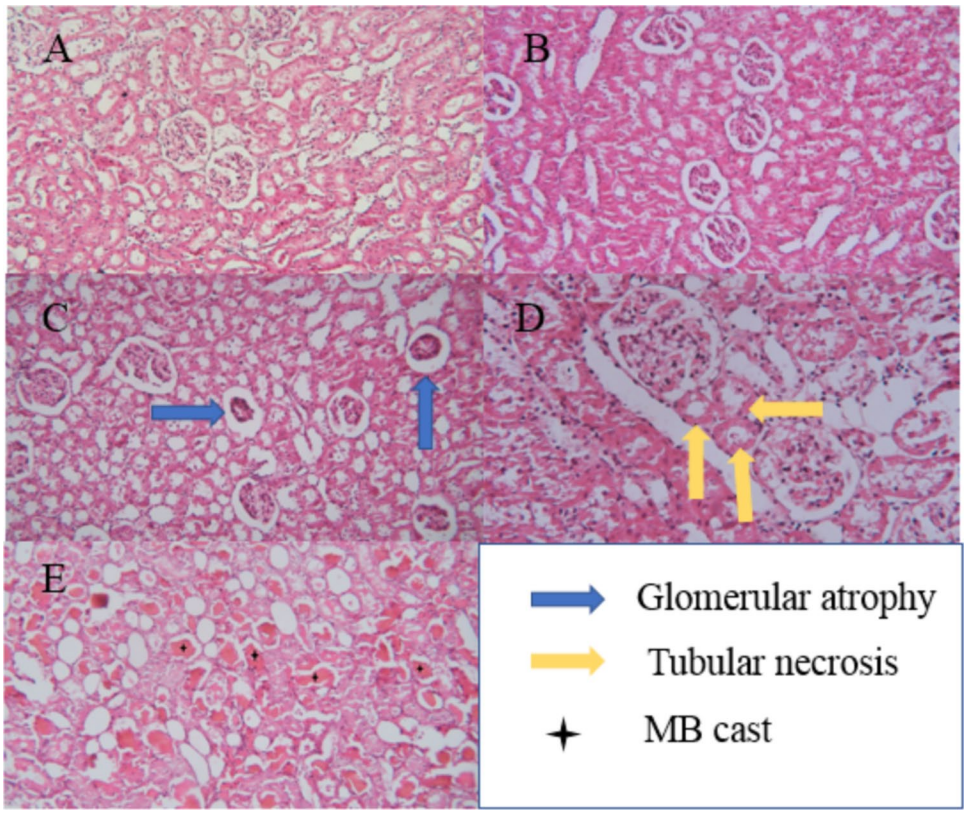

Fig. 6 Effect of TSC on glycerol-induced renal histopathological changes stained with hematoxylin and eosin. (**A**) Control group. (**B**) AKI + TSC (40 mg/kg) group. (**C**, **D** and **E**) AKI group

Effect of TSC and glycerol on NGAL protein level in kidney

Glycerol injection significantly increased renal NGAL values compared to the control group ($P < 0.05$), while IP. administration of TSC (40 mg/kg) with glycerol (10 mL/kg) led to a significant reduction of NGAL level ($P < 0.001$ vs AKI group) in the kidney. Notably, no significant change was observed in the TSC (40 mg/kg) alone group, in comparison to the control (Fig. 9).

Effect of TSC and glycerol on TNF-α protein level in kidney

According to the results of Fig. 10, elevated TNF-α content ($P < 0.001$ vs control) when the animals received glycerol (10 mL/kg). However, co-administration of TSC (40 mg/kg) with glycerol (10 mL/kg) reduced TNF-α level ($P < 0.01$) in comparison to the AKI group. Administration of TSC (40 mg/kg) alone did not affect TNF-α level compared to the control group.

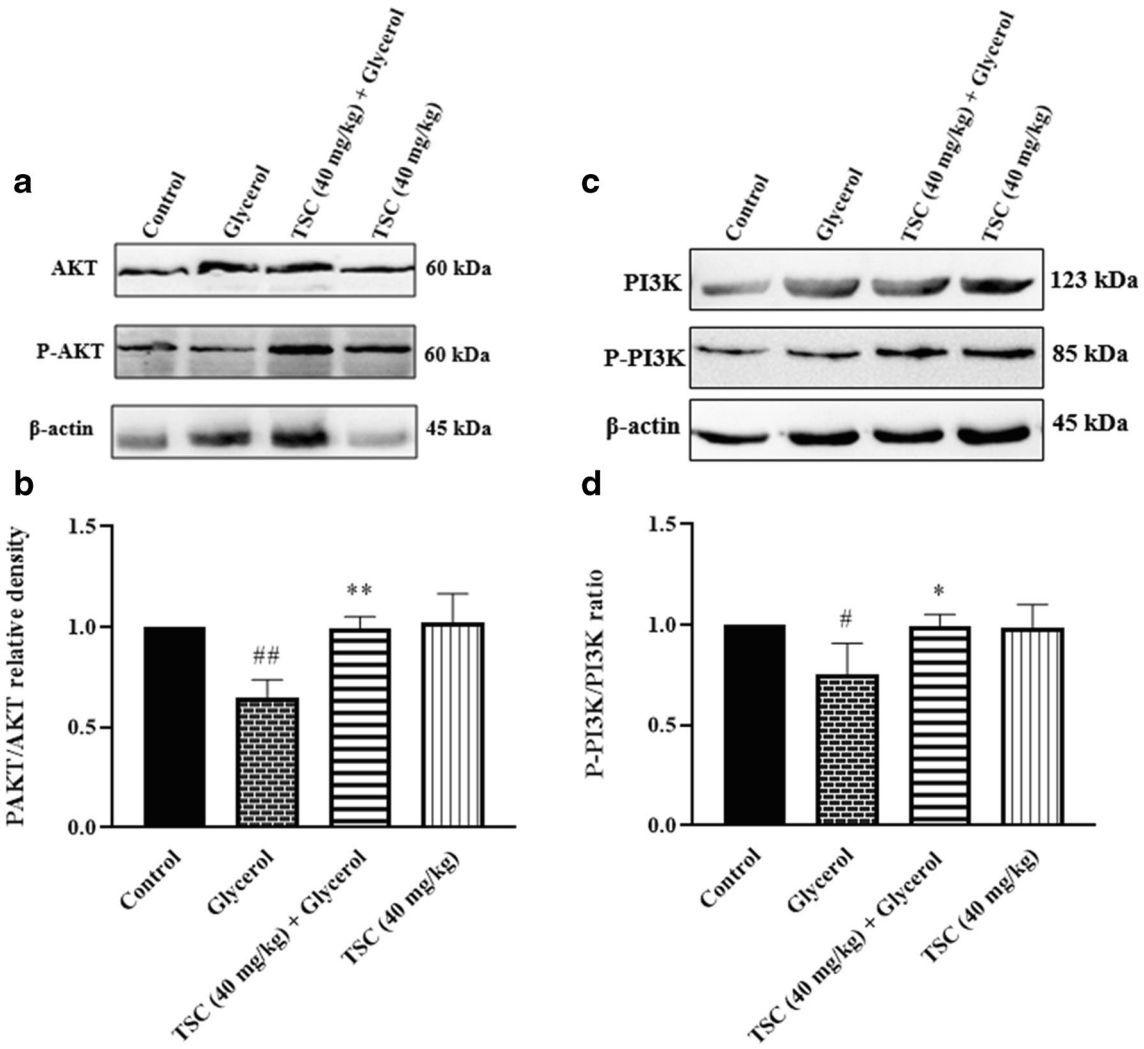

Fig. 7 Effect of TSC and glycerol on PI3K/AKT signaling pathway proteins in the kidney. (**A**, **B**) Western blot bands of AKT, P-AKT, PI3K, P-PI3K, and beta-actin in the kidney. (**C**, **D**) Western blot analysis of P-AKT/AKT and P-PI3K/PI3K ratio in the kidney. Data are presented as means ± SD ($n = 4$). Data were analyzed by one-way ANOVA and the Tukey–Kramer post-test. $^{\#}P < 0.05$ vs control group and $^{*}P < 0.05$ vs AKI group. AKI: acute kidney injury, TSC: trans-sodium crocetinate

Effect of TSC and glycerol on autophagy markers in kidney

To appraise the role of autophagy in glycerol-induced AKI, the level of beclin-1 and the LC3-II/I ratio in kidney tissue were analyzed. A statistically remarkable increase in beclin-1 level ($P < 0.05$ vs control) in the kidney was observed after glycerol injection. TSC (40 mg/kg) treatment ($P < 0.05$) diminished beclin-1 protein level in comparison to the AKI group (Fig. 11 A and B). Also, the renal LC3 II/I ratio was increased in the AKI group ($P < 0.001$ vs control) but in animals treated with TSC (40 mg/kg), this ratio was significantly reduced ($P < 0.001$) (Fig. 11 C and D). On the other hand, the levels of autophagy markers were not statistically changed in the TSC (40 mg/kg) alone group compared with the control (Fig. 11).

Discussion

This research assessed the renoprotective effect of TSC on RM-induced AKI following glycerol administration in rats. The glycerol-induced RM model in this study was evidenced by increased CPK levels after glycerol injection. Moreover, glycerol administration can induce AKI, characterized by renal functional disorder and electrolyte perturbations. Our results also revealed that glycerol administration led to alterations in renal structure. The increase in MDA levels was accompanied

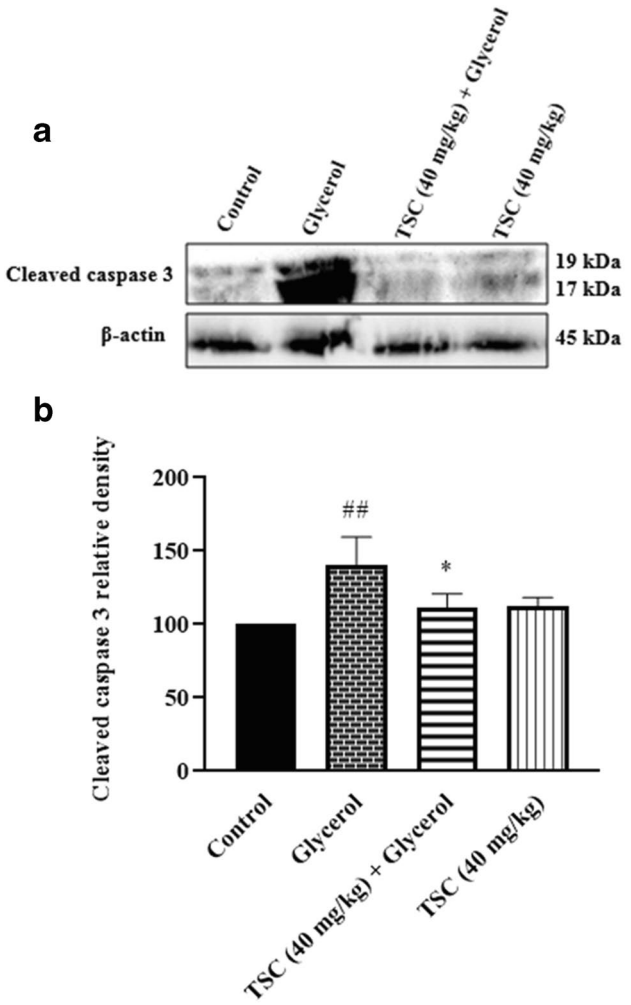

Fig. 8 Effect of TSC and glycerol on the apoptotic marker in the kidney. (**A**) Western blot bands of cleaved caspase-3 and beta-actin in the kidney. (**B**) Western blot analysis of cleaved caspase-3 in the kidney. Data are presented as means ± SD ($n = 4$). Data were analyzed by one-way ANOVA and the Tukey–Kramer post-test. $^{\#\#}P < 0.01$ vs control group and $^{*}P < 0.05$ vs AKI group. AKI: acute kidney injury, TSC: trans-sodium crocetinate

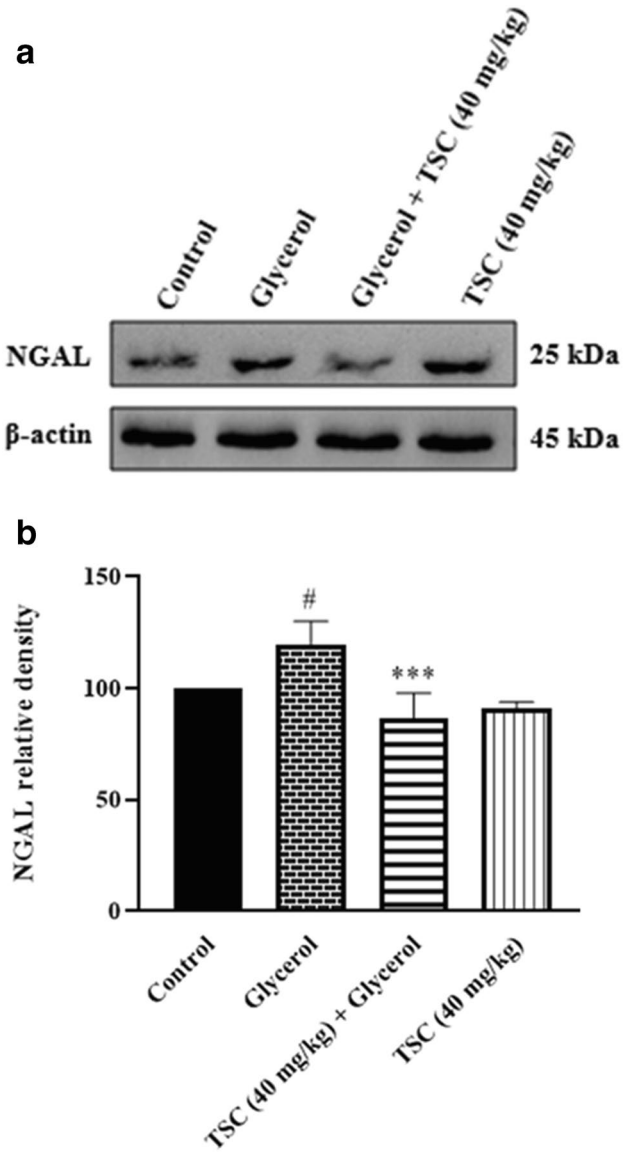

Fig. 9 Effect of TSC and glycerol on NGAL level in kidney. (**A**) Western blot bands of NGAL and beta-actin in the kidney. (**B**) Western blot analysis of NGAL in the kidney. Data are presented as means ± SD ($n = 4$). Data were analyzed by one-way ANOVA and the Tukey–Kramer post-test. $^{\#}P < 0.05$ vs control group and $^{***}P < 0.001$ vs AKI group. AKI: acute kidney injury, TSC: trans-sodium crocetinate

by decreased SOD activity and GSH content in the kidney tissue of the AKI group, suggesting oxidative stress involvement. Additionally, elevated apoptotic markers, inflammatory markers, renal injury markers, and autophagy markers were observed in the kidney of the AKI group. In contrast, there was a significant decrease in PI3K/AKT signaling pathway proteins following glycerol injection in the kidney. On the other hand, TSC administration biochemically, structurally, and functionally mitigated glycerol-induced AKI and improved the extent of renal injury by modulating oxidative stress, autophagy, apoptosis, and the PI3K/AKT pathway (Figs. 12 and 13).

RM is a syndrome characterized by the destruction of skeletal muscle fiber and the release of cellular contents into the blood eventually leading to AKI (Nance and Mammen 2015). AKI, as a serious complication of RM, is associated with morbidity and high mortality (Kellum et al. 2021). Hence, the management of AKI with available and safe drugs is very necessary (Panizo et al. 2015). Recently, plants and their effective ingredients have been considered therapeutic options for AKI (Alok et al. 2013). Also, numerous researches indicated the potential role of plant ingredients in decreasing renal dysfunction and pathological damage after AKI (Scharman and Troutman 2013). The beneficial effects of saffron as a famous herb on various kidney diseases have

been proven in animal models and clinical investigations (Zarei and Elyasi 2022). So, crocetin as a most effective saffron constituent can be useful in the treatment of RM-induced AKI (Hashemi and Hosseinzadeh 2019). The rodent model of glycerol-induced AKI is the most usually used for studying AKI which produces clinical symptoms such as AKI similar to RM disorder (Sharawy et al. 2018). Therefore, the rat RM model was used in this investigation to research the renoprotective effects of TSC against AKI-induced glycerol injection. In the current study, CPK as a sensitive index of RM and skeletal muscle damage was meaningfully increased after glycerol administration in rats. Likewise, our study revealed renal function disorder indicated by remarkably elevated levels of BUN, sCr, and sOsm and decreased uOsm and GFR. Additionally, increased potassium (K) and decreased sodium (Na) levels were observed in rats, confirming substantial toxic injury to the kidneys, consistent with earlier investigations (Rojas-Valverde et al. 2021). Chen et al. reported a notable disorder in renal function accompanied by an increase in CPK following glycerol injection in rats (Chen et al. 2018). According to the data, rats receiving TSC with glycerol exhibited lower sCr, BUN, uOsm, Na, and CK levels, along with higher GFR, sOsm, and K compared to the AKI group. Thus, our results indicate that TSC improved renal function in glycerol-induced AKI. Renoprotective effects of crocetin have been demonstrated previously, aligning with the current findings (Guo et al. 2022b). Crocetin treatment reduced BUN and sCr, and it restored kidney function in rats with hemorrhagic shock (Wang et al. 2012).

The renal histological examination of the AKI group revealed structural damage such as glomerular atrophy, tubular necrosis, and MB cast which these results were in accordance with previous studies (Ahmad et al. 2021). It has been reported that glycerol administration caused different forms of renal lesions such as lumina dilatation, tubular ischemia, and glomerular injury, leading to their pursuant tubular necrosis (Mousleh et al. 2018). Notably, the morphological injuries in the rats' kidneys were improved when TSC was administered. This protection has been indicated in previous reports and other models of renal injury induced by metabolic syndrome (Razavi and Hosseinzadeh 2017), cisplatin (Mohajeri and Doustar 2012), and cyclosporine (Pradhan et al. 2019). In support, Liu et al. showed that crocetin was able to diminish renal histological alterations in arsenic trioxide-induced nephrotoxicity (Liu et al. 2020).

Several mechanisms, such as the induction of oxidative stress, have been implicated in the pathogenesis of RM-induced AKI (Ahmad et al. 2021). To appraise the antioxidant capacity of the kidney, this research quantitated the activity of SOD and the contents of GSH and MDA. Enhanced lipid peroxidation and decreased levels of SOD and GSH occurred in the kidney after glycerol injection. In previous studies, renal oxidative stress induced by glycerol

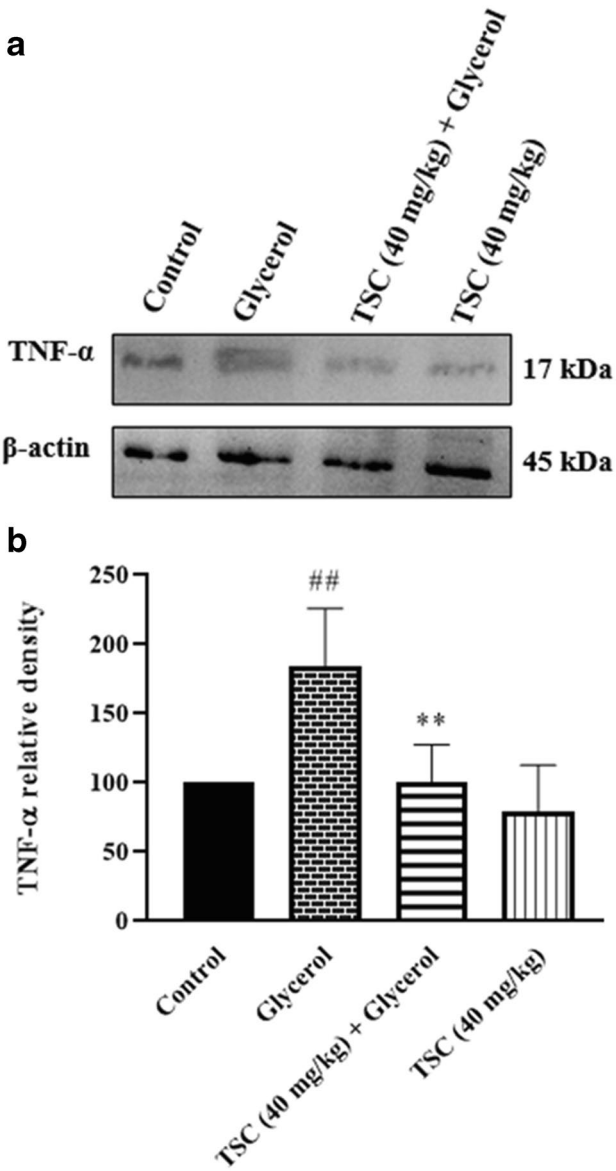

Fig. 10 Effect of TSC and glycerol on TNF-α level in kidney. (**A**) Western blot bands of TNF-α and beta-actin in the kidney. (**B**) Western blot analysis of TNF-α in the kidney. Data are presented as means ± SD ($n = 4$). Data were analyzed by one-way ANOVA and the Tukey–Kramer post-test. $^{\#\#}P < 0.01$ vs control group and $^{**}P < 0.01$ vs AKI group. AKI: acute kidney injury, TSC: trans-sodium crocetinate

injection led to an imbalance between oxidants and antioxidants, subsequently aggravating tubular damage (Hebert et al. 2023). Gu et al. confirmed the participation of oxidative stress and kidney injury following glycerol injection in rats (Gu et al. 2014). Interestingly, in our study, these oxidative/antioxidative markers were reversed by TSC administration, which refers to its powerful antioxidative capacity and its suppression effect on lipid peroxidation (Guo et al.

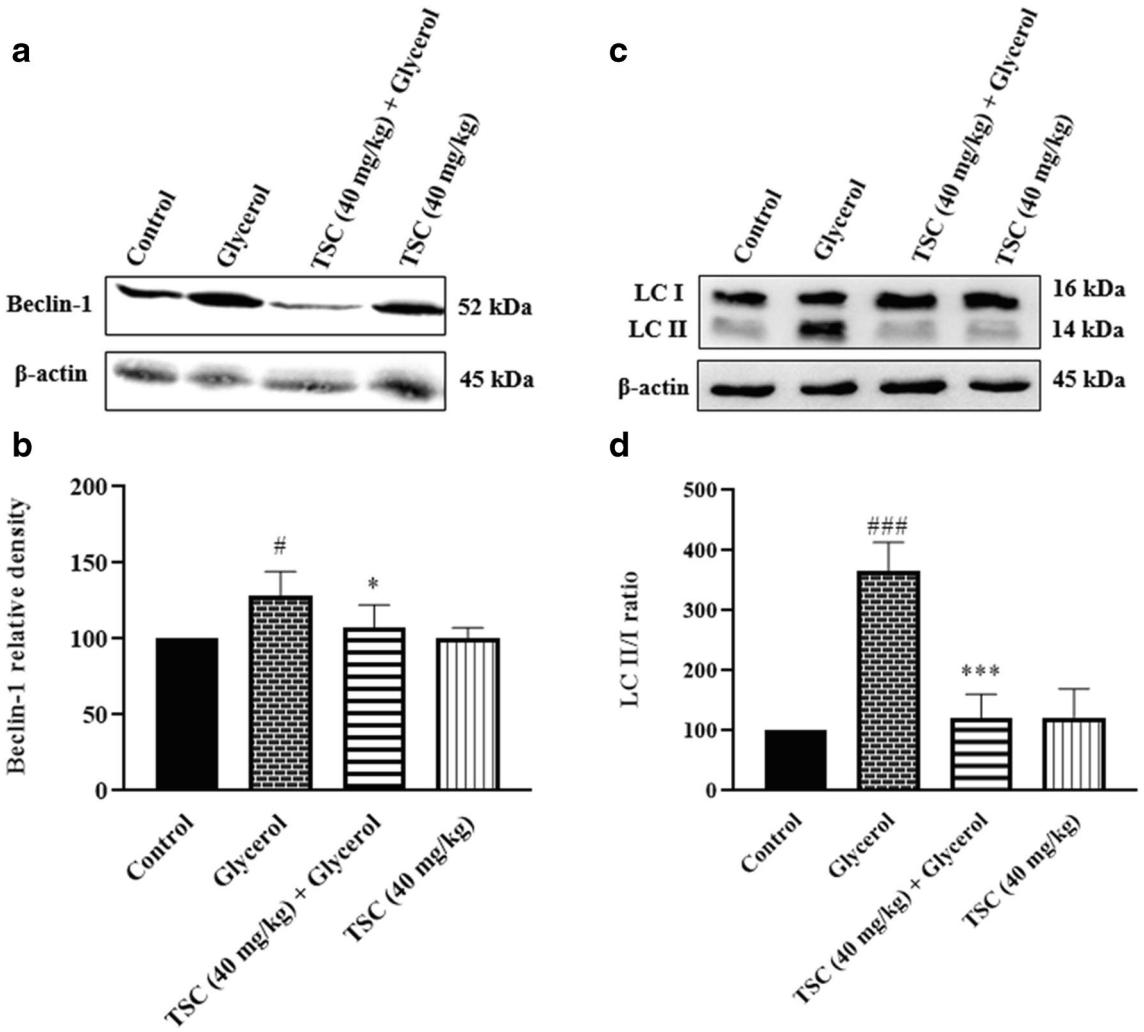

Fig. 11 Effect of TSC and glycerol on autophagy marker in the kidney. (**A**, **B**) Western blot bands of beclin-1, LC3 I, LC3 II, and beta-actin in the kidney. (**C**, **D**) Western blot analysis of beclin-1 and LC3 II/I ratio in the kidney. Data are presented as means ± SD ($n = 4$). Data were analyzed by one-way ANOVA and the Tukey–Kramer post-test. $^{###}P < 0.001$ vs control group and $^{***}P < 0.001$ vs AKI group. AKI: acute kidney injury, TSC: trans-sodium crocetinate

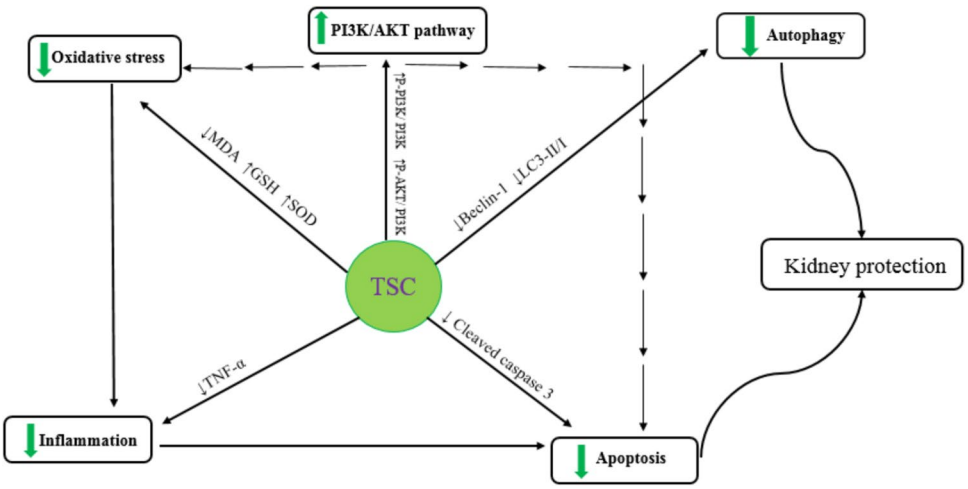

Fig. 12 Protective effect of TSC on glycerol-induced AKI via oxidative stress, PI3K/AKT, autophagy, and apoptosis signaling pathways

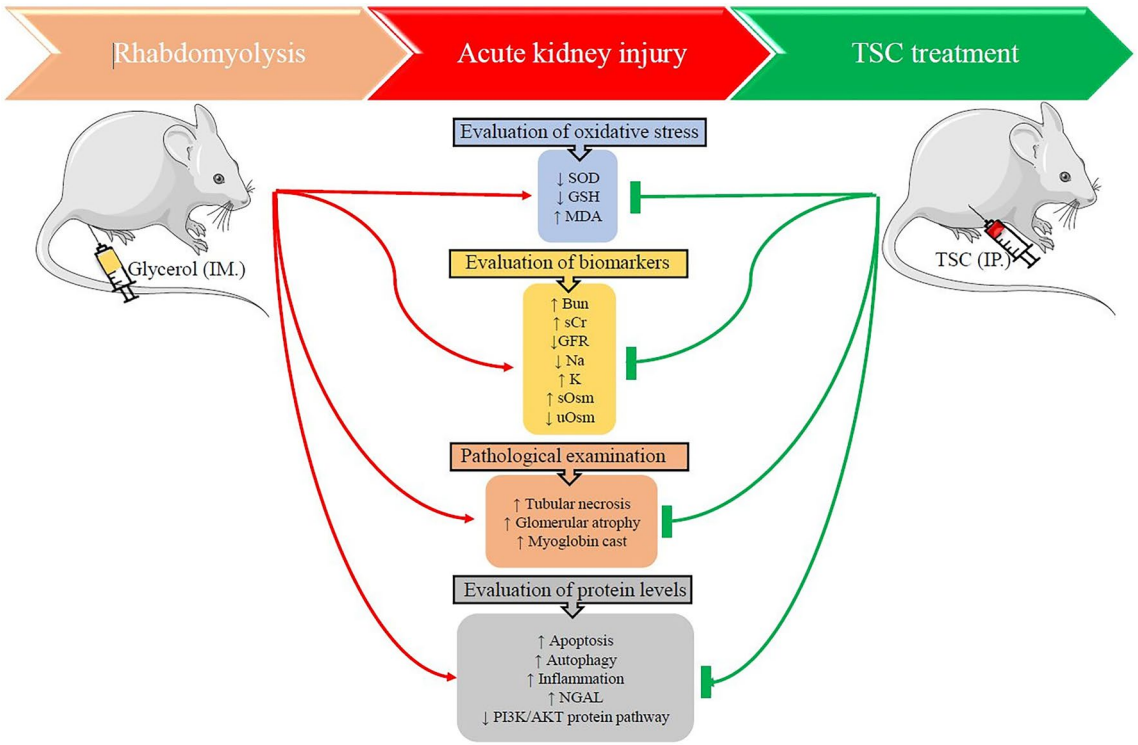

Fig. 13 Effects of trans sodium crocetinate on acute kidney injury induced by rhabdomyolysis in rats

2022b). This data is concordant with former investigations, suggesting that crocetin can diminish renal oxidative stress by improving several antioxidant enzymes in arsenic trioxide-induced nephrotoxicity (Liu et al. 2020).

Different markers such as NGAL, cystatin C, and kidney injury molecule 1 (KIM-1) relate to the AKI pathophysiological process that under normal conditions, have lower expression levels in the kidney (Kellum et al. 2021). These indicators are important and necessary in developing targeted treatment and designing in vivo studies for AKI (Panizo et al. 2015). In our research, NGAL, a sensitive and localized marker in the kidney, was used to assess renal injury and repair after AKI. Glycerol administration led to an enhancement in renal NGAL level confirming AKI. This result is in agreement with other publications in which glycerol-induced renal damage by increasing NGAL content (Sharawy et al. 2018). On the other hand, we also observed that TSC decreases the level of NGAL supporting its renoprotective property. Furthermore, other ingredients of saffron that possess antioxidant effects can reduce the renal NGAL concentration after insult (Esfandiary et al. 2022). For example, renal expression of NGAL was diminished during cadmium exposure by crocin treatment in the Omidali et al. study (Omidali and Korani 2021).

Numerous investigations have demonstrated that oxidative injury can stimulate the production of inflammatory cytokines such as TNF-α, which in conjunction with reactive oxygen species (ROS) generation promote AKI progression (Kellum et al. 2021). Interestingly, in this study, we observed a rise in TNF-α expression following glycerol injection. It is well known that inflammation plays a fundamental role in RM-induced AKI (Panizo et al. 2015). It was reported that glycerol induced inflammation by upregulating the expressions of TNF-α, interleukin 1β (IL-1β), and interleukin 6 (IL-6) in rat kidney tissues (Huang et al. 2017). In the current research, TSC was able to attenuate the TNF-α level in the kidney under conditions of glycerol-induced AKI.

Glycerol injection disrupted the permeability of the mitochondrial membrane in the renal cell and triggered the tubular apoptotic process (Chang et al. 2022). Caspase-3 is a significant marker of the start of the apoptotic signaling pathway (Dolka et al. 2016). The markable enhancement in cleaved caspase-3 level, as an important marker of apoptosis, was observed in rat kidneys that supported the induction of renal cell apoptosis. Another study has also shown that glycerol administration led to renal injury mainly through the apoptosis pathway (Chang et al. 2022). In our study, TSC administration decreased glycerol-induced apoptosis. Likewise, the antiapoptotic effect of crocetin was demonstrated in a rat model of ischemic myocardial damage (Wang et al. 2014). Therefore, using antioxidant agents has been suggested as a means of treatment or minimizing renal damage (Zarei and Elyasi 2022).

According to the previous investigation, the PI3K/AKT signal cascade mediates the expressions of different apoptosis-related factors, that lead to cell apoptosis and subsequently renal injury (Margaria et al. 2020). So, we evaluated the role of glycerol and TSC in the activation of the PI3K/AKT signal pathway. Our results indicated that this signaling cascade is inhibited in glycerol-induced AKI in renal, which suggested that released MB can manipulate the PI3K/AKT pathway to induce renal cell apoptosis. However, western blotting results indicate that TSC meaningfully increased the P-AKT/AKT and P-PI3K/PI3K ratio in this study. Similarly, the crocetin-mediated elevation of PI3K/AKT pathway protein levels was verified in Panpan et al., investigation (Liu et al. 2020).

Autophagy is an important degradation phenomenon in which damaged organelles and dysfunctional proteins are delivered to lysosomes to maintain normal cell function (Noda and Inagaki 2015). According to a previous publication, in acute kidney injury (AKI), autophagy plays a protective role in renal tissue (Ichimiya et al. 2020). Our results show that glycerol-induced renal autophagy is mediated through the upregulation of the LC3-II/I ratio and beclin-1 expression. Consistent with the current data, it has been reported that cisplatin increases the autophagy marker in rat renal cells (Liu et al. 2016). On the other hand, the administration of TSC (add what TSC stands for) decreased the autophagy response in renal tissue after glycerol injection. In line with this, several studies have reported that under autophagy-inducing conditions, crocetin regulates autophagy marker levels (49–50).

Conclusion

In this investigation, we assessed RM-induced acute kidney injury (AKI) and the potential renoprotective effects of TSC against this disorder through various signaling pathways in the AKI rat model induced by glycerol. Our results reveal alterations in renal morphology, RM-related markers, kidney function indices, and electrolyte levels following glycerol injection. Additionally, an imbalance between antioxidant and oxidant contents is observed. Furthermore, glycerol injection induces renal damage, characterized by increased inflammation, renal injury markers, autophagy-related proteins, and apoptotic events. In contrast, TSC administration along with glycerol reverses these changes through its anti-inflammatory, antiapoptotic, and autophagy-regulating activities, as well as its potent antioxidant capacity. In summary, our data confirm that TSC deserves significant attention as a promising therapeutic agent for the treatment of AKI.

Acknowledgements The data reported in this article are part of the Ph.D. thesis.

Author Contribution The study was conceptualized and designed by H. H. Animal study and laboratory tests conducted by T.A and acquired data were analyzed and interpreted by the supervision of S.M. and A.K. S.H. helped for laboratory tests. Z.A supervised the pathological study. The final version of the manuscript was reviewed and approved by all authors before submission for publication. The authors declare that all data were generated in-house and that no paper mill was used. During the preparation of this work, the author(s) used AI-assisted technologies to rephrase to reduce plagiarism and improve language and grammar. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Funding We thank Mashhad University of Medical Sciences Vice-Chancellor for Research (Grant No. 991907) for financial support.

Data Availability All source data for this work (or generated in this study) are available upon reasonable request.

Declarations

Conflict of Interest The authors declare no competing interests and also H.H. is the Editor of Naunyn-Schmiedebergs Arch Pharmacol and followed the submission guidelines of the journal (https://link.springer.com/journal/210/submission-guidelines#Instructions%20for%20Authors_Competing%20Interests).

References

Ahmad S, Anees M, Elahi I, Fazal EM (2021) Rhabdomyolysis leading to acute kidney injury. J Coll Physicians Surg Pak 31:235–237

Alok S, Jain SK, Verma A et al (2013) Pathophysiology of kidney, gallbladder and urinary stones treatment with herbal and allopathic medicine: a review. Asian Pacific J Trop Dis 3:496–504

Amin B, Nakhsaz A, Hosseinzadeh H (2015) Evaluation of the antidepressant-like effects of acute and sub-acute administration of crocin and crocetin in mice. Avicenna J Phytomedicine 5:458

Chang SN, Haroon M, Dey DK, Kang SC (2022) Rhabdomyolysis-induced acute kidney injury and concomitant apoptosis induction via ROS-mediated ER stress is efficaciously counteracted by epigallocatechin gallate. J Nutr Biochem 110:109134

Chavez LO, Leon M, Einav S, Varon J (2016) Beyond muscle destruction: a systematic review of rhabdomyolysis for clinical practice. Crit Care 20:1–11

Chen X, Sun J, Li H et al (2018) Curcumin-loaded nanoparticles protect against rhabdomyolysis-induced acute kidney injury. Cell Physiol Biochem 43:2143–2154

Colapietro A, Mancini A, D'Alessandro AM, Festuccia C (2019) Crocetin and crocin from saffron in cancer chemotherapy and chemoprevention. Anti-Cancer Agents Med Chem (Formerly Curr Med Chem Agents) 19:38–47

Deng J, Xiong L, Zuo Z (2015) Trans-sodium crocetinate provides neuroprotection against cerebral ischemia and reperfusion in obese mice. J Neurosci Res 93:615–622

Dolka I, Król M, Sapierzyński R (2016) Evaluation of apoptosis-associated protein (Bcl-2, Bax, cleaved caspase-3 and p53) expression in canine mammary tumors: An immunohistochemical and prognostic study. Res Vet Sci 105:124–133

Esfandiary S, Shojaei L, Rouzbahani M et al (2022) Evaluation of crocin effect on contrast-induced nephropathy following coronary

angiography or angioplasty: a randomized controlled trial. Iran J Pharm Res 10(2):123–130
Ghasemzadeh Rahbardar M, Razavi BM, Naraki K, Hosseinzadeh H (2023) Therapeutic effects of minocycline on oleic acid-induced acute respiratory distress syndrome (ARDS) in rats. Naunyn Schmiedebergs Arch Pharmacol 15(3):1–10
Gu H, Yang M, Zhao X et al (2014) Pretreatment with hydrogen-rich saline reduces the damage caused by glycerol-induced rhabdomyolysis and acute kidney injury in rats. J Surg Res 188:243–249
Guerrero-Hue M, García-Caballero C, Palomino-Antolín A et al (2019) Curcumin reduces renal damage associated with rhabdomyolysis by decreasing ferroptosis-mediated cell death. FASEB J 33:8961–8975
Guo H, Ruan C, Zhan X et al (2022a) Crocetin protected human hepatocyte LO2 cell from TGF-β-induced oxygen stress and apoptosis but promoted proliferation and autophagy via AMPK/m-TOR pathway. Front Public Heal 10:909125
Guo Z-L, Li M-X, Li X-L et al (2022b) Crocetin: a systematic review. Front Pharmacol 12:745683
Hashemi M, Hosseinzadeh H (2019) A comprehensive review on biological activities and toxicology of crocetin. Food Chem Toxicol 130:44–60
Hebert JF, Burfeind KG, Malinoski D, Hutchens MP (2023) Molecular mechanisms of rhabdomyolysis-induced kidney injury: from bench to bedside. Kidney Int Reports 8:17–29
Huang R-S, Zhou J-J, Feng Y-Y et al (2017) Pharmacological inhibition of macrophage toll-like receptor 4/nuclear factor-kappa B alleviates rhabdomyolysis-induced acute kidney injury. Chin Med J (Engl) 130:2163–2169
Ichimiya T, Yamakawa T, Hirano T et al (2020) Autophagy and autophagy-related diseases: a review. Int J Mol Sci 21:8974
Kellum JA, Romagnani P, Ashuntantang G et al (2021) Acute kidney injury. Nat Rev Dis Prim 7:52
Kruger NJ (2009) The Bradford method for protein quantitation. The protein protocols handbook, pp 17–24
Liu H, Gu L, Tu Y et al (2016) Emodin ameliorates cisplatin-induced apoptosis of rat renal tubular cells in vitro by activating autophagy. Acta Pharmacol Sin 37:235–245
Liu P, Xue Y, Zheng B et al (2020) Crocetin attenuates the oxidative stress, inflammation and apoptosis in arsenic trioxide-induced nephrotoxic rats: implication of PI3K/AKT pathway. Int Immunopharmacol 88:106959
Margaria JP, Campa CC, De Santis MC et al (2020) The PI3K/Akt/mTOR pathway in polycystic kidney disease: a complex interaction with polycystins and primary cilium. Cell Signal 66:109468
Mohajeri D, Doustar Y (2012) Protective effect of ethanolic extract of Crocus sativus L. (Saffron) stigma against Cisplatin induced hepatotoxicity in rats. Med Sci J Islam Azad Univesity-Tehran Med Branch 21:251–261
Mohammadi E, Mehri S, Bostan HB, Hosseinzadeh H (2018) Protective effect of crocin against d-galactose-induced aging in mice. Avicenna J Phytomedicine 8:14
Mohler ER III, Gainer JL, Whitten K et al (2011) Evaluation of trans sodium crocetinate on safety and exercise performance in patients with peripheral artery disease and intermittent claudication. Vasc Med 16:346–353
Mousleh R, Al Laham S, Al-Manadili A (2018) The preventive role of pioglitazone in glycerol-induced acute kidney injury in rats during two different treatment periods. Iran J Med Sci 43:184
Mzabri I, Addi M, Berrichi A (2019) Traditional and modern uses of saffron (Crocus sativus). Cosmetics 6:63
Nance JR, Mammen AL (2015) Diagnostic evaluation of rhabdomyolysis. Muscle Nerve 51:793–810
Noda NN, Inagaki F (2015) Mechanisms of autophagy. Annu Rev Biophys 44:101–122
Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358
Omidali M, Korani M (2021) Study the effect of crocin on the expression of PAX2 and WT1 genes in renal tissues and serum levels of NGAL and cystatin C in cadmium-treated rats. Mol Cell Toxicol 10(2):1–9
Panizo N, Rubio-Navarro A, Amaro-Villalobos JM et al (2015) Molecular mechanisms and novel therapeutic approaches to rhabdomyolysis-induced acute kidney injury. Kidney Blood Press Res 40:520–532
Pradhan J, Mohanty C, Sahoo SK (2019) Protective efficacy of crocetin and its nanoformulation against cyclosporine A-mediated toxicity in human embryonic kidney cells. Life Sci 216:39–48
Rajabian F, Mehri S, Razavi BM et al (2023) Effect of trans-sodium crocetinate on contrast-induced cytotoxicity in HEK-293 cells. Iran J Basic Med Sci 26:148
Razavi BM, Hosseinzadeh H (2017) Saffron: a promising natural medicine in the treatment of metabolic syndrome. J Sci Food Agric 97:1679–1685
Rojas-Valverde D, Sánchez-Ureña B, Crowe J et al (2021) Exertional rhabdomyolysis and acute kidney injury in endurance sports: a systematic review. Eur J Sport Sci 21:261–274
Scharman EJ, Troutman WG (2013) Prevention of kidney injury following rhabdomyolysis: a systematic review. Ann Pharmacother 47:90–105
Shah HM, Jain AS, Joshi SV, Kharkar PS (2021) Crocetin and related oxygen diffusion-enhancing compounds: review of chemical synthesis, pharmacology, clinical development, and novel therapeutic applications. Drug Dev Res 82:883–895
Shahbaz H, Gupta M (2019) Creatinine clearance. J Nephrol 15(3):200–205
Sharawy MH, Abdelrahman RS, El-Kashef DH (2018) Agmatine attenuates rhabdomyolysis-induced acute kidney injury in rats in a dose dependent manner. Life Sci 208:79–86
Stahl K, Rastelli E, Schoser B (2020) A systematic review on the definition of rhabdomyolysis. J Neurol 267:877–882
Tabeshpour J, Mehri S, Abnous K, Hosseinzadeh H (2019) Neuroprotective effects of thymoquinone in acrylamide-induced peripheral nervous system toxicity through MAPKinase and apoptosis pathways in rat. Neurochem Res 44:1101–1112
Wang Y, Yan J, Xi L et al (2012) Protective effect of crocetin on hemorrhagic shock–induced acute renal failure in rats. Shock 38:63–67
Wang Y, Sun J, Liu C, Fang C (2014) Protective effects of crocetin pretreatment on myocardial injury in an ischemia/reperfusion rat model. Eur J Pharmacol 741:290–296
Wani A, Al Rihani SB, Sharma A et al (2021) Crocetin promotes clearance of amyloid-β by inducing autophagy via the STK11/LKB1-mediated AMPK pathway. Autophagy 17:3813–3832
Yang F-L, Subeq Y-M, Chiu Y-H et al (2012) Recombinant human erythropoietin reduces rhabdomyolysis-induced acute renal failure in rats. Injury 43:367–373
Zarei B, Elyasi S (2022) Saffron nephroprotective effects against medications and toxins: a review of preclinical data. Iran J Basic Med Sci 25:419
Zutt R, Van Der Kooi AJ, Linthorst GE et al (2014) Rhabdomyolysis: review of the literature. Neuromuscul Disord 24:651–659