Inflammation Research (2019) 68:511–523
https://doi.org/10.1007/s00011-019-01241-z

Inflammation Research

ORIGINAL RESEARCH PAPER

Chicoric acid prevents methotrexate-induced kidney injury by suppressing NF-κB/NLRP3 inflammasome activation and up-regulating Nrf2/ARE/HO-1 signaling

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Received: 26 February 2019 / Revised: 22 April 2019 / Accepted: 24 April 2019 / Published online: 29 April 2019

Abstract

Objective Chicoric acid (CA) is a natural product with promising antioxidant and anti-inflammatory properties; however, its protective effect on methotrexate (MTX)-induced acute kidney injury (AKI) hasn't been reported. We investigated the effect of CA on MTX-induced AKI in rats, pointing to the role of NF-κB/NLRP3 inflammasome and Nrf2/ARE/HO-1 signaling.

Materials and methods Wistar rats received 25 mg/kg and 50 mg/kg CA for 15 days and a single injection of MTX at day 16. At day 19, the rats were killed, and samples were collected for analyses.

Results MTX induced a significant increase in serum creatinine and urea, and kidney Kim-1, reactive oxygen species (ROS), malondialdehyde and nitric oxide levels. In addition, MTX-induced rats exhibited multiple histopathological alterations, diminished antioxidant defenses, and decreased expression of Nrf2, NQO-1 and HO-1. CA prevented histological alterations, ameliorated kidney function markers, attenuated ROS production and lipid peroxidation, and boosted antioxidant defenses. CA suppressed the expression of NF-κB p65, NLRP3, caspase-1 and IL-1β in the kidney of MTX-induced rats. Furthermore, CA inhibited MTX-induced apoptosis as evidenced by the decreased expression of BAX and caspase-3, and increased Bcl-2 gene expression.

Conclusions CA prevented MTX-induced AKI through activation of Nrf2/ARE/HO-1 signaling, and attenuation of ROS-induced activation of NF-κB/NLRP3 inflammasome signaling.

Keywords Chicoric acid · Methotrexate · NLRP3 inflammasome · Nrf2 · ROS

Introduction

The kidney is a frequent site of drug toxicity due to its role in the concentration and excretion of drugs and toxic agents. The use of drugs is associated frequently with nephrotoxicity which assist the progress and development of acute and chronic kidney diseases (CKD) [1]. Tubular toxicity, interstitial nephritis, glomerular effects, urinary crystallization, and other manifestations associated with nephrotoxicity can lead to acute kidney injury (AKI), a rapidly growing health problem with socioeconomic consequences [2]. Drug-induced AKI accounts for 19–26% in adults [3] and 16% in pediatric cases of AKI [4]. AKI is associated with the development and progression of CKD and its related increased mortality [5]. The nephrotoxic potential of drugs is affected by the pharmacological properties, molecular characteristics, metabolism, dose, duration, route of administration, and other factors [6, 7]. Numerous drugs have been reported to be inherently nephrotoxic [6]. The metabolism of these

Responsible Editor: Mauro Teixeira.

Sanaa M. Abd El-Twab and Omnia E. Hussein participated as first author.

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drugs in the kidney produces various metabolites which can cause kidney injury through different mechanisms, including excessive production of reactive oxygen species (ROS) and oxidative damage [6]. The dihydrofolate reductase inhibitor methotrexate (MTX) and its 7-hydroxy metabolite have the tendency to crystallize and precipitate in the renal tubules in the setting of low urinary pH and sluggish urine flow rates [6]. Although crystallization in the renal tubules is the most commonly described mechanism of MTX nephrotoxicity [8], other mechanisms have been reported. MTX induces mitochondrial dysfunction [9], activates neutrophils [10] and triggers NADPH oxidase activity [11], resulting in excessive production of ROS. In addition, it has direct effects on the mesangial cells, tubular epithelial cells and afferent capillary constriction [12]. MTX is widely used in the treatment of multiple diseases, including cancer, rheumatoid arthritis, lupus erythematosus, and psoriasis [13, 14]; however, the adverse effects and toxicity often limit its therapeutic applications. Several studies have demonstrated AKI in 2–12% patients who received high dose MTX [15, 16]. For instance, 9.1% of patients with lymphoma on MTX therapy have been reported to develop renal injury [16].

Oxidative stress and inflammation have been implicated in MTX nephrotoxicity. Excess ROS can result in multiple deleterious effects and activate nuclear factor-kappaB (NF-κB) leading to increased production of pro-inflammatory cytokines [17]. NF-κB can activate NLRP3 inflammasome, a multiprotein oligomer promotes the maturation of pro-inflammatory cytokines [18]. This protein complex includes nucleotide-binding domain and NOD-like receptors (NLRs) that contain leucine-rich repeats, apoptosis-associated speck-like protein (ASC) and pro-caspase-1. In response to an inflammatory signal, the inflammasome functions as a molecular scaffold for the activation of caspase-1 which promotes the maturation of interleukin (IL)-1β and IL-18 from their inactive precursors [19]. IL-1β and other cytokines released as a result of the activation of inflammasome can promote programmed pro-inflammatory cell death called pyroptosis [20]. Multiple studies have reported that NLRP3 inflammasome is implicated in kidney diseases [21, 22]. ROS have been suggested to integrate different signals and elicit inflammasome activation [23]. Therefore, attenuation of drug-induced oxidative stress and its subsequent NF-κB/NLRP3 inflammasome activation can protect the kidney against inflammation and cell death.

Chicoric acid (CA), a dicaffeyltartaric acid, is a natural compound with promising beneficial properties. It occurs in a variety of plant species used in folk medicine, such as *Cichorium intybus* L., *Ocimum basilicum* L. and *Echinacea purpurea* [24]. CA exhibits multiple biological activities, including antioxidant, anti-inflammatory, anti-atherosclerotic, and antidiabetic [25–28]. In a mouse model of diabetes, CA ameliorated hyperglycemia and activated antioxidant defenses in the muscle [29]. CA improved glucose tolerance, insulin sensitivity and mitochondrial function in diet-induced obese mice [30]. In addition, it protected against in vivo cerebral ischemia/reperfusion (I/R) injury via its antioxidant effect [31]. Despite these beneficial effects, the potential protective effect of CA against MTX nephrotoxicity hasn't been studied. Therefore, this study was designed to investigate the protective effect of CA on MTX nephrotoxicity, pointing to the involvement of NF-κB/NLRP3 inflammasome signaling. In addition, we evaluated whether treatment with CA activates the nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) signaling in the kidney of MTX-induced rats. Nrf2 is a transcription factor that regulates the expression of antioxidant enzymes and protect against oxidative damage [32]. Under normal conditions, Nrf2 is sequestered in the cytosol by Kelch-like ECH-associated protein 1 (Keap1) and cullin 3. Upon exposure to mild oxidative stress, ROS disrupt the Keap1-Cullin 3 ubiquitination system and Nrf2 translocate into the nucleus, complex with a small MAF protein, bind to antioxidant response element (ARE), and activate the expression of antioxidant genes [33]. Therefore, activation of Nrf2/ARE/antioxidant signaling by CA can attenuate MTX-induced oxidative stress, inflammation and kidney injury.

Materials and methods

Experimental animals

Adult male Wistar rats, weighing 150–160 g, obtained from the National Research Centre (NRC, Giza, Egypt), were included in this study. The animals were housed in standard cages, supplied a standard chow diet and water ad libitum, and maintained on a 12-h light/dark cycle at normal temperature (23 ± 2 °C) and humidity (50–60%). The animals were acclimatized for 1 week before the start of the experiment.

Experimental design and treatments

Twenty-four rats were divided into four groups ($n = 6$) as follows (Fig. 1):

Control: received 0.5% carboxymethyl cellulose (CMC) for 15 days via oral gavage and a single intraperitoneal (i.p.) injection of physiological saline at day 16.

MTX: received 0.5% CMC for 15 days and a single ip injection of MTX (20 mg/kg; Shanxi PUDE Pharmaceutical Company, China) at day 16.

MTX + 25 mg/kg CA: received 25 mg/kg CA in 0.5% CMC for 15 days and MTX at day 16.

MTX + 50 mg/kg CA: received 50 mg/kg CA in 0.5% CMC for 15 days and MTX at day 16.

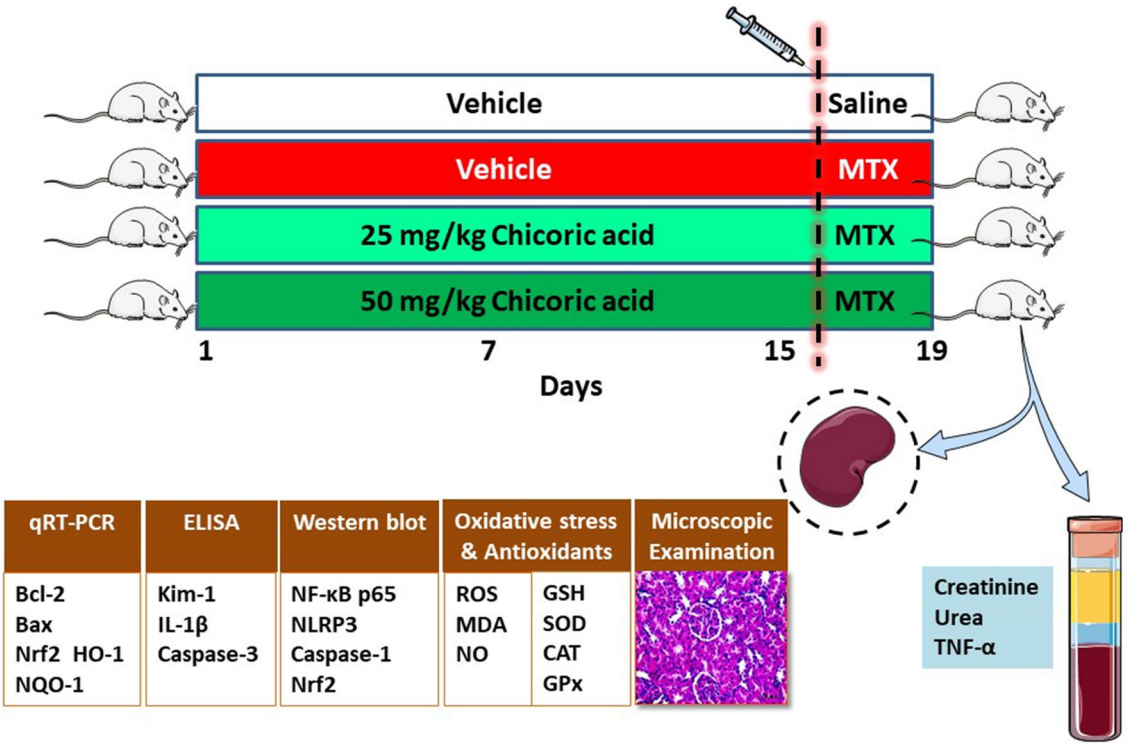

Fig. 1 Schematic diagram showing the experimental design

The MTX-induced AKI model was established as reported previously [34], and the doses of CA were selected based on previous studies showed the in vivo antioxidant effect of CA at doses between 10 and 100 mg/kg/day [29, 31]. CA (≥98% purity) was obtained from Chengdu Purify Phytochemicals Ltd (Chengdu, China) and dissolved in 0.5% CMC. Previous studies have dissolved CA in drinking water and saline [26, 28, 29]. At the end of the experiment (day 19), the animals were killed under anesthesia and blood samples were collected. Serum was isolated from blood and kept at − 20 °C until used for the measurement of creatinine, urea, and tumor necrosis factor alpha (TNF-α) levels. The animals were immediately dissected, and kidneys were excised and washed in cold phosphate buffered saline (PBS). Samples from the kidney were fixed in 10% neutral buffered formalin for histological examination, whereas other samples were kept at − 80 °C. For Western blotting and biochemical assays, samples from the kidney were homogenized in RIPA buffer supplemented with proteinase inhibitors or PBS (10% w/v), respectively.

Biochemical assays

Measurement of kidney function markers, caspase-3 and cytokines

Creatinine [35] and urea [36] were assayed using reagent kits purchased from Spinreact (Girona, Spain). TNF-α and IL-1β were measured using ELISA kit (R&D systems, USA). Kidney injury molecule-1 (Kim-1) and caspase-3 levels were determined using ELISA kits supplied by Cusabio (Wuhan, China). All assays were carried out according to the manufacturers' instructions.

Measurement of oxidative stress and antioxidant markers

ROS were measured using the fluorescent probe 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA) [37, 38]. To 1 ml PBS, 100 µl of the kidney homogenate and 5 µl H_2DCF-DA (final concentration 10 µM) were added, and the mixture was incubated for 30 min protected from light. The fluorescence intensity was measured at excitation 490 nm and emission 540 nm using a plate reader. Malondialdehyde (MDA) [39] and NO, assayed using Griess reagent [40], reduced glutathione (GSH) [41], superoxide dismutase (SOD) [42], catalase (CAT) [43] and glutathione peroxidase (GPx) [44] were measured in the kidney homogenate samples.

Histological examination

The kidney samples were fixed in 10% neutral buffered formalin for 48 h and then processed via routine histological procedures (dehydration, clearing and paraffin embedding).

5 µm sections were cut, stained with hematoxylin and eosin (H&E), and examined using a light microscope.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

The gene expression levels of Nrf2, HO-1, NAD(P)H quinone dehydrogenase 1 (NQO-1), B cell lymphoma 2 (Bcl-2) and Bcl-2-associated X protein (Bax) were assessed by qRT-PCR as previously described [45]. Total RNA, isolated from the frozen kidney samples using TRIzol reagent (Invitrogen, USA), was purified by RNeasy purification kit (Qiagen, Germany), and quantitated by measuring absorbance at 260 and 280 nm. RNA samples with A260/280 ≥ 1.8 were selected for cDNA synthesis. Two µg RNA was reverse transcribed into cDNA using RevertAidTM First Strand cDNA Synthesis Kit (Fermentas, USA). The amplification of cDNA was performed by SYBR Green master mix (Fermentas, USA) and the primers set listed in Table 1, using β-actin as a house-keeping gene. The obtained amplification data were analyzed by the $2^{-\Delta\Delta C_t}$ method [46].

Western blotting

The expression levels of NF-κB p65, NLRP3, caspase-1 and Nrf2 were estimated in the kidney samples of control and experimental rats. Protein content was determined in the kidney homogenate using Bradford reagent and 50 µg protein was separated using SDS-PAGE. The isolated proteins were transferred to PVDF membranes followed by blocking for 1 h in 5% milk/Tris-buffered saline-tween 20 (TBST) at room temperature. The membranes were probed with primary antibodies (Novus Biologicals, USA) overnight at 4 °C, washed in TBST and incubated with the secondary antibodies (Novus Biologicals, USA). After washing with TBST, the membranes were developed using enhanced chemiluminescence kit (BIO-RAD, USA). The band intensity was determined using ImageJ (version 1.32j; NIH, USA) and presented as percent of control.

Table 1 Primers used for qRT-PCR

Gene	Sequence (5′–3′)
NRF2	F: TTGTAGATGACCATGAGTCGC R: TGTCCTGCTGTATGCTGCTT
NQO-1	F: GGCCATCATTTGGGCAAGTC R: TCCTTGTGGAACAAAGGCGA
HO-1	F: GTAAATGCAGTGTTGGCCCC R: ATGTGCCAGGCATCTCCTTC
BAX	F: AGGACGCATCCACCAAGAAG R: CAGTTGAAGTTGCCGTCTGC
BCL-2	F: ACTCTTCAGGGATGGGGTGA R: TGACATCTCCCTGTTGACGC
β-actin	F: AGGAGTACGATGAGTCCGGC R: CGCAGCTCAGTAACAGTCCG

Statistical analysis

GraphPad Prism 5 software (San Diego, CA, USA) was used for the statistical analysis. The results were presented as mean ± the standard error of the mean (SEM). All statistical comparisons were performed using one-way ANOVA test followed by Tukey's post hoc analysis. A P value less than 0.05 was considered significant.

Results

CA prevents MTX-induced kidney injury in rats

To evaluate the protective effect of CA on MTX-induced AKI, we determined the levels of creatinine, urea, and Kim-1, and performed a histological study (Fig. 2). MTX-induce rats showed a significant increase in serum creatinine ($P < 0.001$; Fig. 2A) and urea ($P < 0.001$; Fig. 2B) when compared with the control group. Similarly, the administration of MTX induced a significant increase in Kim-1 levels in the kidney of rats ($P < 0.001$; Fig. 2C). Pre-treatment of the MTX-induced rats with either 25 mg/kg or 50 mg/kg CA ameliorated the levels of creatinine, urea and Kim-1 significantly ($P < 0.001$).

The ameliorative effect of CA on MTX-induced kidney injury was confirmed by the histological examination. While the H&E-stained section from the control group revealed normal structure of the glomeruli and renal tubules (Fig. 2D, a), MTX-induced rats showed interstitial hemorrhage, inflammatory cell infiltration, glomerular atrophy, dilation of renal tubules and Bowman's capsule, congested blood vessels and others (Fig. 2D, b–d). Rats received 25 mg/kg (Fig. 2D, e) and 50 mg/kg CA (Fig. 2D, f) showed a remarkable improvement in the histological structure of the kidney.

CA attenuates MTX-induced oxidative stress and inflammation in rats

The administration of MTX promoted a significant increase in the production of ROS in the kidney of rats when compared with the control group ($P < 0.001$; Fig. 3A). Consequently, MDA levels showed a significant increase in the kidney of MTX-induced rats ($P < 0.001$; Fig. 3B). Pre-treatment of the rats with 25 mg/kg and 50 mg/kg CA remarkably ($P < 0.001$) prevented MTX-induced excess production of ROS (Fig. 3A) and lipid peroxidation (Fig. 3B). In addition, both doses of CA significantly ($P < 0.001$) reduced NO levels in the kidney of MTX-induced rats (Fig. 3C).

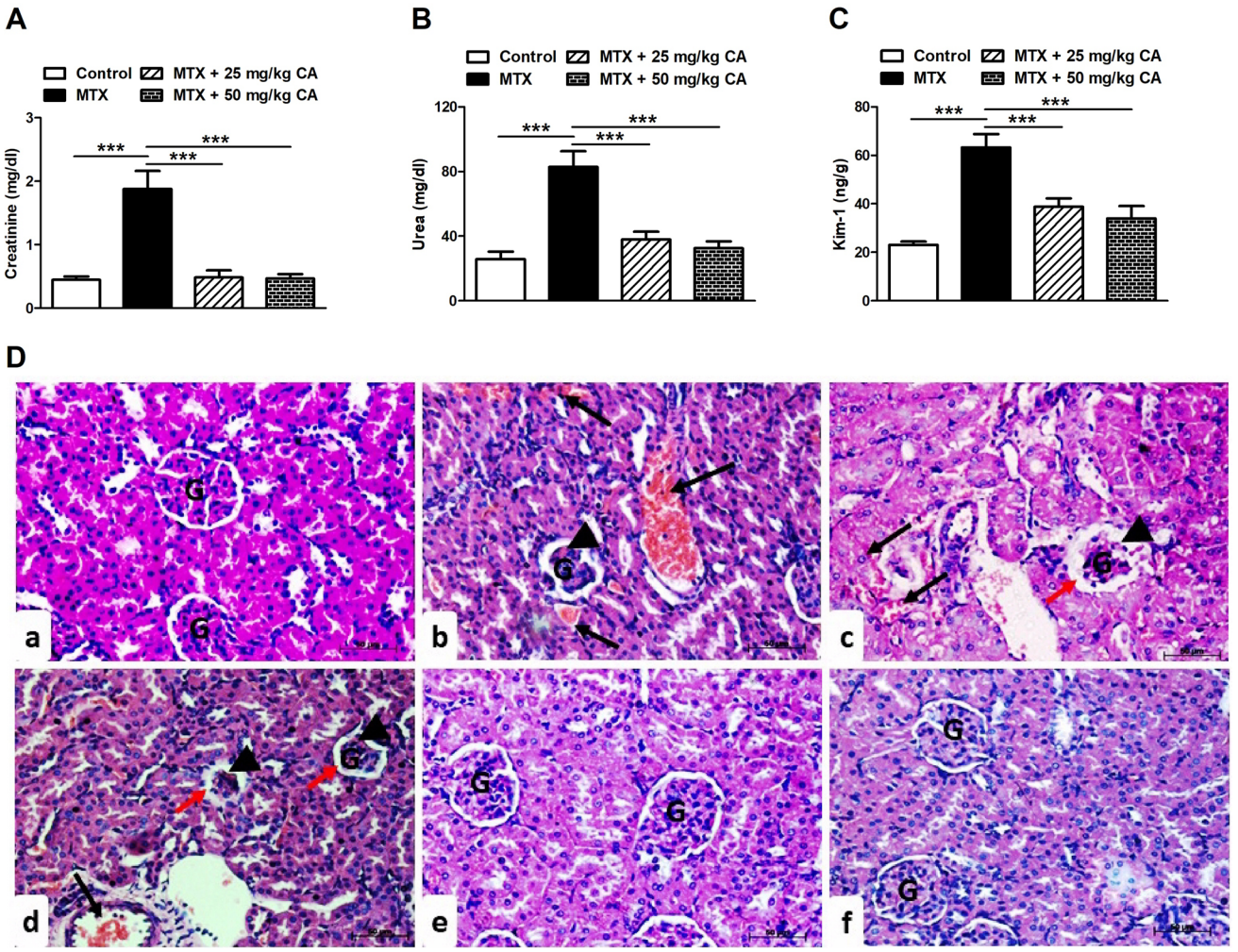

Fig. 2 CA prevents MTX-induced kidney injury in rats. **A**–**c** Pre-treatment with CA ameliorated serum creatinine (**A**) and urea (**B**), and kidney Kim-1 levels (**C**) in MTX-induced rats. Data are expressed as Mean ± SEM, $n = 6$. ***$P < 0.001$. **D** CA inhibits MTX-induced histopathological changes in the kidney of rats. Photomicrographs of sections in the kidney of control rats (**D**/*a*) showing normal glomeruli (G) and renal tubules, (**D**/*b*–*d*) MTX-induced rats showing interstitial hemorrhage (black arrow), glomerular atrophy (arrow head), dilation of renal tubules and Bowman's capsule (red arrow), congested blood vessels and inflammatory cell infiltration, and (**D**/*e*, *f*) MTX-induced rats pre-treated with 25 mg/kg (**D**/*e*) and 50 mg/kg CA (**D**/*f*) showed improved histological structure of the kidney. Scale bar = 50 µm

The pro-inflammatory cytokine TNF-α was significantly ($P < 0.001$) increased in the serum of MTX-induced rats when compared with the control group as represented in Fig. 3D. Rats received CA before the administration of MTX showed a significant ($P < 0.001$) amelioration of serum TNF-α levels.

CA enhances antioxidants in the kidney of MTX-induced rats

Given the significant amelioration of ROS, MDA and NO in the kidney of MTX-induced rats treated with CA, we determined the changes in GSH and antioxidant enzymes (Fig. 4). The kidney of rats received MTX showed remarkable reduction in GSH ($P < 0.001$; Fig. 4A), SOD ($P < 0.001$; Fig. 4B), CAT ($P < 0.001$; Fig. 4C) and GPx ($P < 0.001$; Fig. 4D) when compared with the control rats. Pre-treatment of the MTX-administered rats with 25 mg/kg and 50 mg/kg CA ameliorated GSH ($P < 0.01$; $P < 0.01$), SOD ($P < 0.001$; $P < 0.001$), CAT ($P < 0.001$; $P < 0.001$) and GPx ($P < 0.001$; $P < 0.001$). The 50 mg/kg CA exerted a significant effect on SOD activity ($P < 0.05$) when compared with the lower dose.

CA suppresses NF-κB/NLRP3 inflammasome signaling in the kidney of MTX-induced rats

The expression levels of NF-κB p65 was significantly ($P < 0.001$; Fig. 5A, B) increased in the kidney of

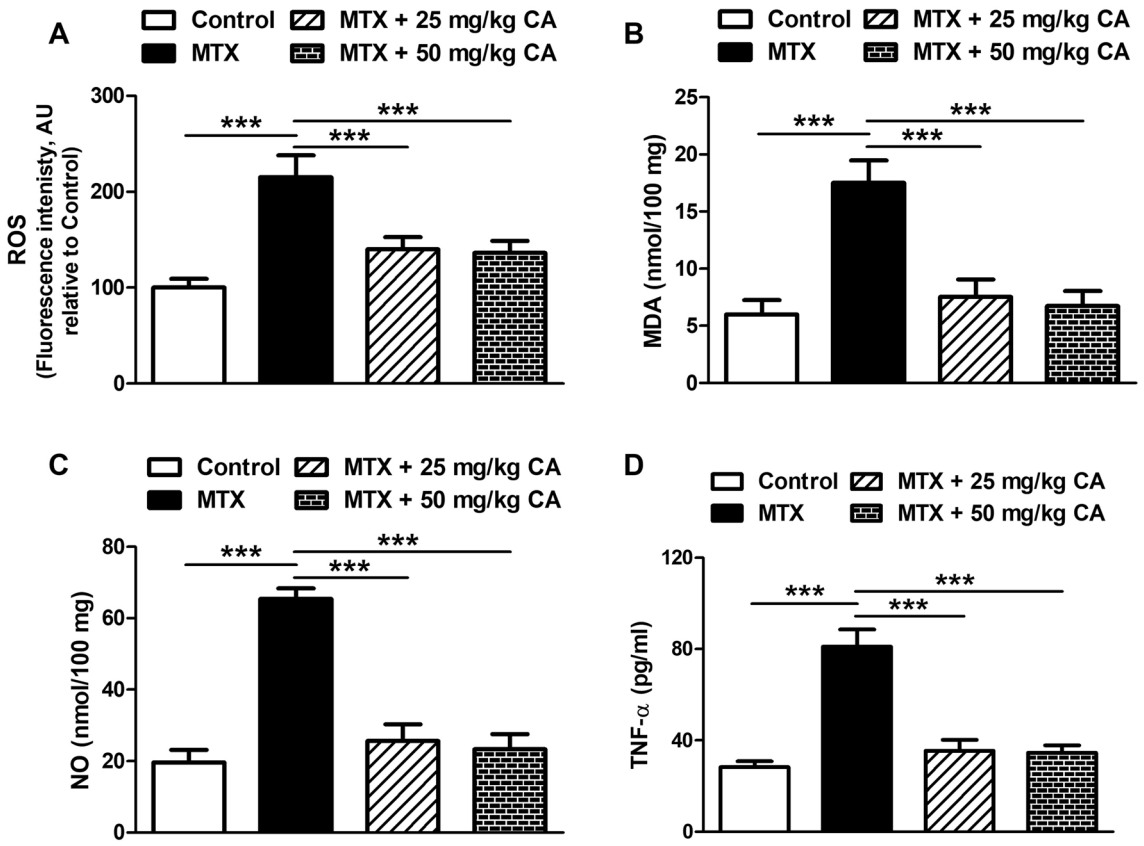

Fig. 3 CA attenuates MTX-induced oxidative stress and inflammation in rats. CA significantly reduced kidney **A** ROS, **B** MDA and **C** NO levels, and **D** serum TNF-α in MTX-induced rats. Data are expressed as Mean ± SEM, $n = 6$. ***$P < 0.001$

MTX-induced rats when compared with the control group. Similarly, MTX-induced rats exhibited significant up-regulation in the expression levels of NLRP3 ($P < 0.001$; Fig. 5A, C), caspase-1 p20 ($P < 0.001$; Fig. 5A, D) and IL-1β ($P < 0.001$; Fig. 5E). Rats received 25 mg/kg and 50 mg/kg CA showed remarkable decrease in the expression levels of NF-κB p65 ($P < 0.001$; $P < 0.001$), NLRP3 ($P < 0.001$; $P < 0.001$), caspase-1 p20 ($P < 0.001$; $P < 0.001$) and IL-1β ($P < 0.001$; $P < 0.001$). CA exerted a dose-dependent decrease in the expression of caspase-1 p20 in the kidney of MTX-induced rats ($P < 0.01$; Fig. 5D).

CA prevents MTX-induced apoptosis in the kidney of rats

The kidney of MTX-induced rats showed a significant down-regulation of the mRNA abundance of the anti-apoptosis marker Bcl-2 when compared with the control rats ($P < 0.001$; Fig. 6A). In contrast, MTX induced a significant increase in Bax mRNA expression ($P < 0.001$; Fig. 6B), Bax/Bcl-2 ratio ($P < 0.001$; Fig. 6C) and protein levels of caspase-3 ($P < 0.001$; Fig. 6D). Pre-treatment of the MTX-induced rats with 25 mg/kg and 50 mg/kg CA increased the expression levels of Bcl-2 ($P < 0.05$; $P < 0.01$), and decreased Bax ($P < 0.001$; $P < 0.001$), Bax/Bcl-2 ratio ($P < 0.001$; $P < 0.001$) and caspase-3 ($P < 0.001$; $P < 0.001$).

CA activates Nrf2/ARE/antioxidant signaling in the kidney of MTX-induced rats

Rats received MTX showed decreased mRNA ($P < 0.01$; Fig. 7A) and protein expression ($P < 0.001$; Fig. 7B) levels of Nrf2 in the kidney when compared with the control group. Pre-treatment of the MTX-induced rats with 25 mg/kg and 50 mg/kg CA resulted in a significant increase in the gene ($P < 0.01$; $P < 0.001$) and protein ($P < 0.01$; $P < 0.001$) expression levels of Nrf2.

mRNA abundance of NQO-1 (Fig. 7C) and HO-1 (Fig. 7D) showed a significant ($P < 0.01$; $P < 0.01$) decrease in the kidney of MTX-induced rats when compared with the control group. In contrast, MTX-induced rats pre-treated with 25 mg/kg and 50 mg/kg CA showed remarkable improvement in the expression levels of NQO-1 ($P < 0.05$; $P < 0.05$) and HO-1 ($P < 0.05$; $P < 0.01$).

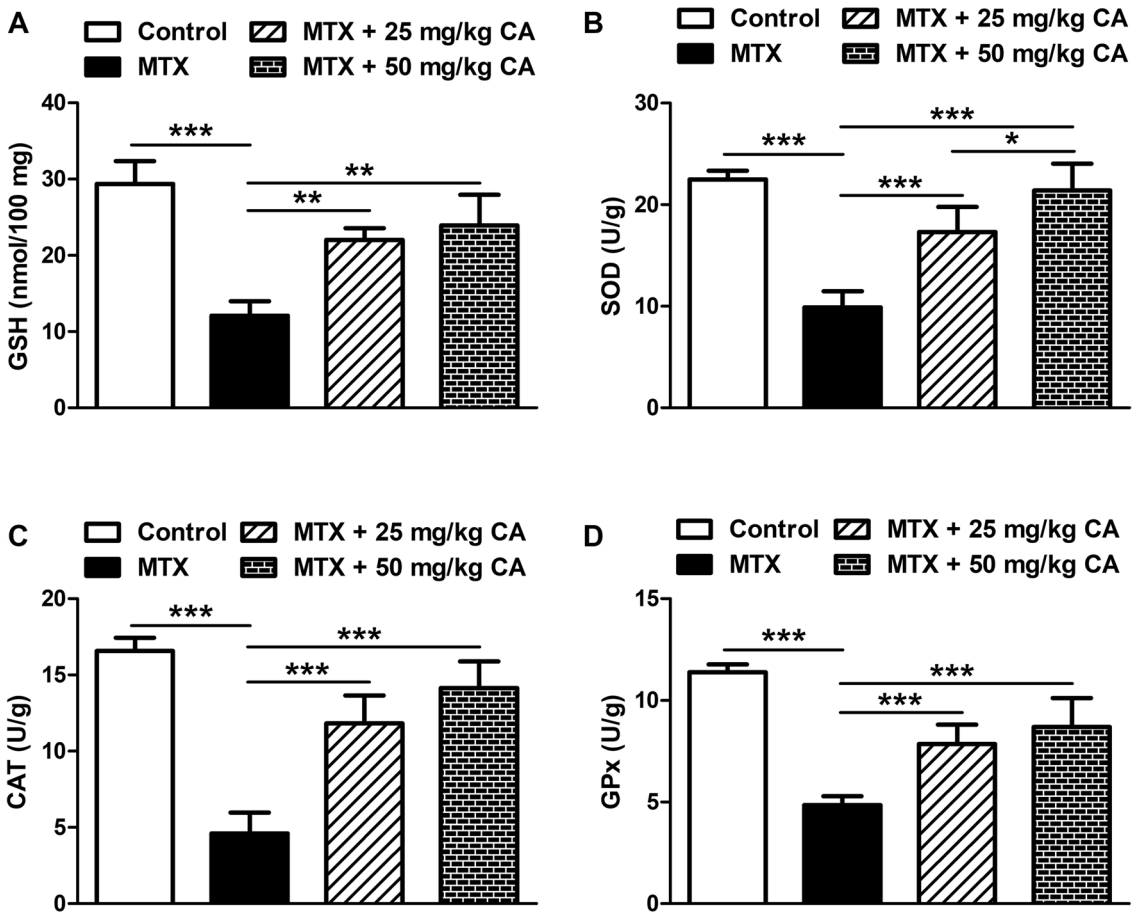

Fig. 4 CA enhances antioxidant defenses in the kidney of MTX-induced rats. Pre-treatment with CA significantly alleviated **A** GSH, **B** SOD, **C** CAT and **D** GPx in the kidney of MTX-induced rats. Data are expressed as Mean ± SEM, $n = 6$. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$

Discussion

CA is a hydroxycinnamic acid with antioxidant and anti-inflammatory efficacies. Recent studies have demonstrated the ability of CA to ameliorate nonalcoholic steatohepatitis [47], lipopolysaccharide (LPS)-induced acute lung injury (ALI) [26] and oxidized LDL (oxLDL)-induced endothelial dysfunction [25] through suppression of oxidative stress and inflammation. However, its protective effect on MTX-induced AKI hasn't been studied. We investigated the role of CA in attenuating MTX nephrotoxicity in rats, pointing to the involvement of oxidative stress, NF-κB/NLRP3 inflammasome axis and Nrf2/ARE/HO-1 signaling.

Rats received a single injection of MTX showed renal dysfunction evidenced by the significant increase in serum creatinine and urea as previously reported [45, 48]. MTX nephrotoxicity was confirmed by the increased levels of the transmembrane protein Kim-1 and the histological alterations in the kidney of rats. The microscopic examination revealed several histological alterations, including inflammatory cells infiltration, interstitial hemorrhage, glomerular atrophy, dilation of renal tubules and Bowman's capsule, congested blood vessels and others. The proximal convoluted tubules (PCT) are vulnerable to drug toxicity due to their role in the uptake and metabolism of drugs. Kim-1 is a sensitive marker of renal injury and has been reported to increase in response to injury of the PCT [49]. In support of our findings, MTX injection caused glomerular atrophy, focal tubular necrosis, interstitial hemorrhage, proteinaceous material in renal tubules and hypertrophy of glomerular tuft as demonstrated by previous studies [11, 45, 48]. The nephrotoxicity of MTX and its 7-hydroxy metabolite is directly attributed to their precipitation and crystallization in renal tubules [12]. The tendency of MTX to crystallize in the renal tubules is promoted by the low urine volume, acidic pH and high concentration of MTX [6].

CA significantly prevented MTX-induced kidney injury and alleviated serum levels of creatinine and urea, demonstrating its renoprotective efficacy. The protective role of CA against drug-induced kidney injury hasn't been adequately studied. Our findings introduced an evidence that CA can prevent MTX nephrotoxicity. The beneficial

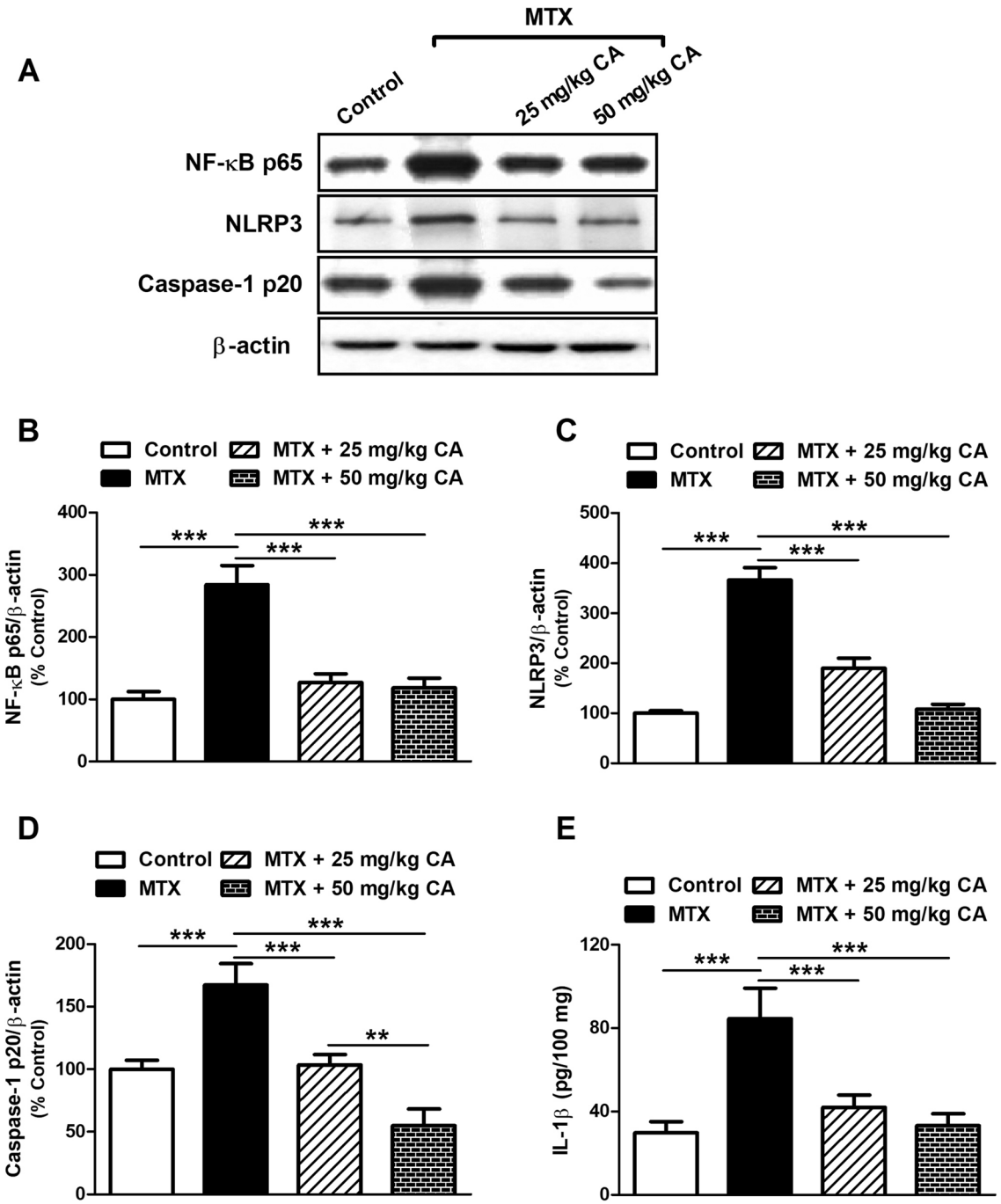

Fig. 5 CA suppresses NF-κB/NLRP3 inflammasome signaling in the kidney of MTX-induced rats. **A** representative gel images of NF-κB p65, NLRP3, caspase-1 p20 and β-actin. CA suppressed the expression of **B** NF-κB p65, **C** NLRP3, **D** caspase-1 p20 and **E** IL-1β in the kidney of MTX-induced rats. Data are expressed as mean ± SEM, $n = 6$. $^{**}P < 0.01$ and $^{***}P < 0.001$

effects of CA could be explained through its potent antioxidant and anti-inflammatory effects. MTX promotes ROS generation and oxidative stress via activation of NADPH oxidase [11] and neutrophils [10], and impairment of the mitochondrial function [9]. Excess ROS can damage cellular lipids, proteins and DNA, leading to cell death. In this context, our findings showed increased MDA, a lipid peroxidation marker, in the kidney of MTX-induced rats. In addition, the antioxidants GSH, SOD, CAT and GPx were significantly declined in response to MTX administration. GSH is kept in its reduced form via the action of glutathione reductase using NADPH [50]. Therefore,

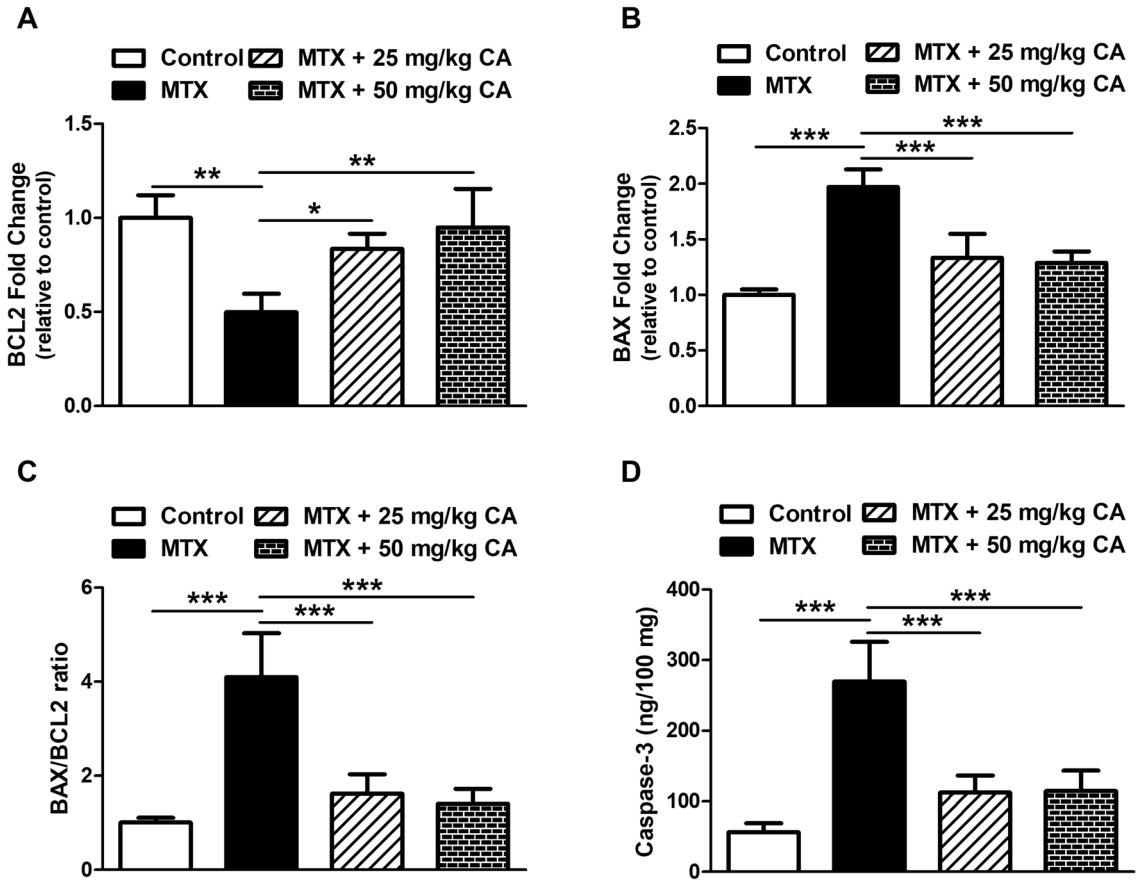

Fig. 6 CA prevents MTX-induced apoptosis in the kidney of rats. Pre-treatment with CA significantly increased the expression of Bcl-2 (**A**), and decreased Bax mRNA expression (**B**), Bax/Bcl-2 ratio (**C**) and caspase-3 protein levels (**D**) in the kidney of MTX-induced rats. Data are expressed as mean ± SEM, $n = 6$. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$

increased NADPH oxidase activity and ROS production explains the decreased levels of GSH. ROS can also induce oxidative damage of the cellular proteins, including antioxidant enzymes [51] which were diminished in the current study in conjunction with excessive ROS production. GSH and antioxidant enzymes prevent ROS-induced mitochondrial dysfunction and renal impairment [52]. Interestingly, pre-treatment with CA attenuated MTX-induced ROS and NO production, and lipid peroxidation, and boosted antioxidant defenses in the kidney of rats. Accordingly, CA attenuated ROS generation and enhanced cellular antioxidants in oxLDL-induced endothelial cells [25], doxorubicin (DOX)-treated human skin fibroblast cell line [53], and platelet-derived growth factor type BB (PDGF-BB)-induced vascular smooth muscle cells (VSMCs) [54]. In addition, CA improved mitochondrial function in palmitate-induced myotubes [30], activated antioxidants in the muscle of diabetic mice [29], and suppressed ROS levels in cerebral I/R injury in rats [31]. Therefore, attenuation of oxidative damage mediated to a great extent the protective effect of CA against MTX nephrotoxicity.

Previous studies have demonstrated that inflammation is implicated in MTX-induced liver and kidney injury [34, 45, 48, 55]. Our findings showed increased serum levels of the pro-inflammatory cytokine TNF-α and activated NF-κB/NLRP3 inflammasome signaling in the kidney of MTX-induced rats, demonstrating an inflammatory response. Increased levels of pro-inflammatory cytokines is a result of ROS-mediated activation of NF-κB, a redox sensitive transcription factor promoting the expression of inflammatory mediators and inducible NO synthase (iNOS) [17]. In accordance with our findings, NF-κB activation has been demonstrated in AKI in human [17] and animals [56]. Inflammatory cytokines can activate neutrophils and macrophages, increase ROS, and subsequently provoke neutrophil infiltration in the kidney [10]. In addition to NF-κB activation, ROS can integrate different signals resulting in inflammasome activation [23]. Here, MTX-induced rats showed increased expression of NLRP3, caspase-1 and IL-1β. These findings indicated that the activation of NF-κB/NLRP3 inflammasome signaling plays a role in MTX nephrotoxicity. NLRP3 inflammasome is a multiprotein

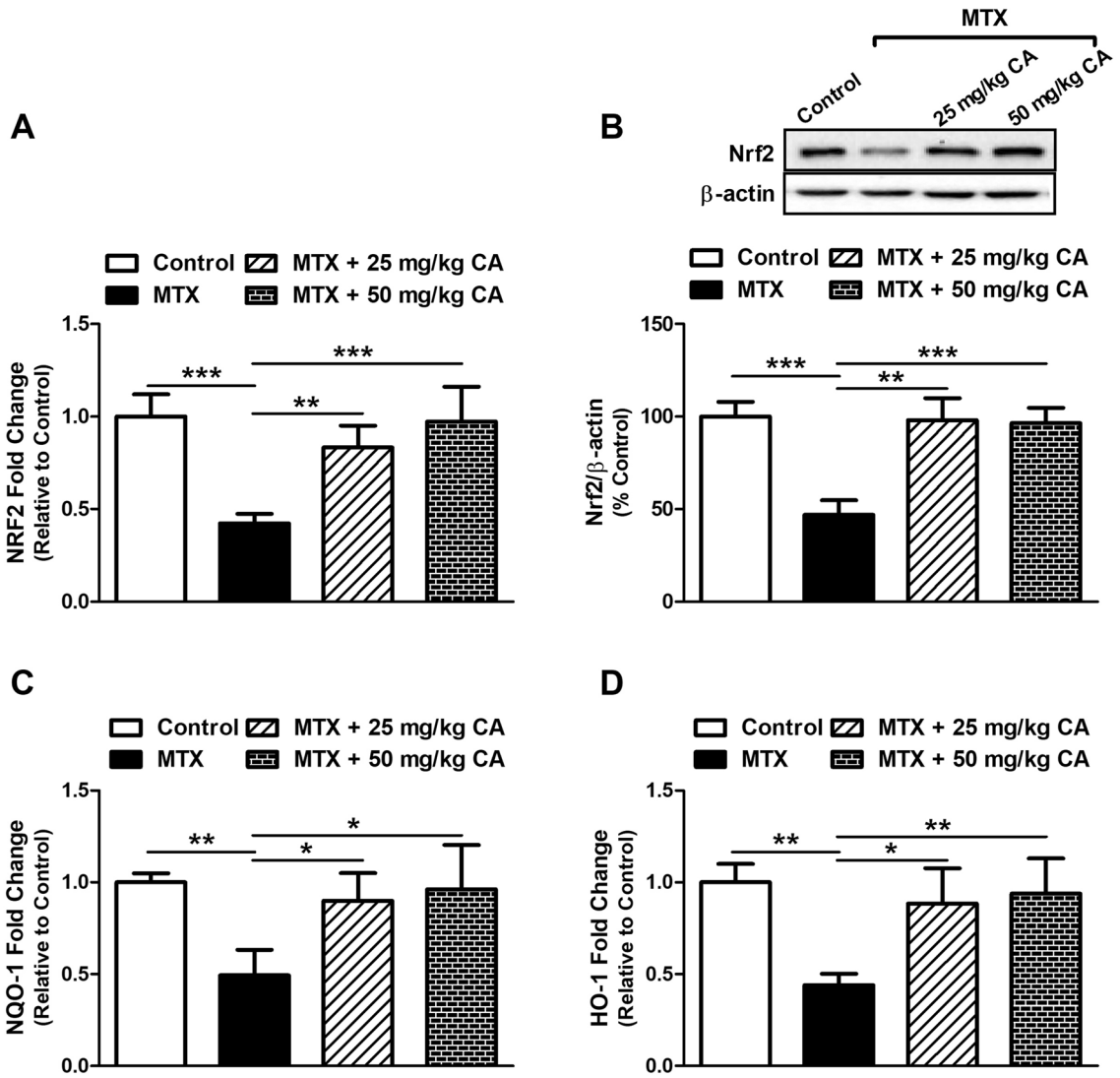

Fig. 7 CA activates Nrf2/ARE/antioxidant signaling in the kidney of MTX-induced rats. CA increased the mRNA abundance of **A** Nrf2, **C** NQO-1 and **D** HO-1 in the kidney of MTX-administered rats. **B** The protein expression of Nrf2 was significantly increased in the kidney of MTX-administered rats pre-treated with CA. Data are expressed as mean ± SEM, $n = 6$. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$

oligomer activated by NF-κB and promotes the secretion of IL-1β and IL-18 [18]. These cytokines induce pro-inflammatory cell death [20]; therefore, NLRP3 inflammasome has been implicated in the progression of chronic nephropathies [18]. Both ROS and IL-1β can activate cell death pathways [20, 57]. Our study demonstrated increased expression of the pro-apoptotic markers Bax and caspase-3, along with down-regulation of the anti-apoptotic protein Bcl-2 in the kidney of MTX-induced rats. Bax is activated by ROS and pro-inflammatory cytokines, resulting in increased release of cytochrome *c* from the mitochondria and activation of caspase-3. In contrast, Bcl-2 inhibits the activation of caspases by suppressing the release of cytochrome *c* from the mitochondria [58]. The balance between members of the Bcl-2 protein family controls the susceptibility of cells to apoptosis [59, 60]. The kidney of MTX-induced rats in this study showed increased Bax/Bcl-2 ratio, demonstrating activated apoptosis. Therefore, activation of NF-κB and its subsequent stimulation of NLRP3 inflammasome and release of inflammatory cytokines provoked apoptosis in the kidney of MTX-induced rats. Increased ROS production could be considered as the main culprit behind activation of the NF-κB/NLRP3 inflammasome signaling in the kidney of MTX-induced rats.

CA prevented MTX-induced activation of NF-κB/NLRP3 inflammasome signaling and production of pro-inflammatory cytokines. In addition, CA mitigated apoptosis in the kidney of rats through suppression of Bax and caspase-3, and up-regulation of Bcl-2. In agreement with these findings, treatment of endothelial cells with CA suppressed

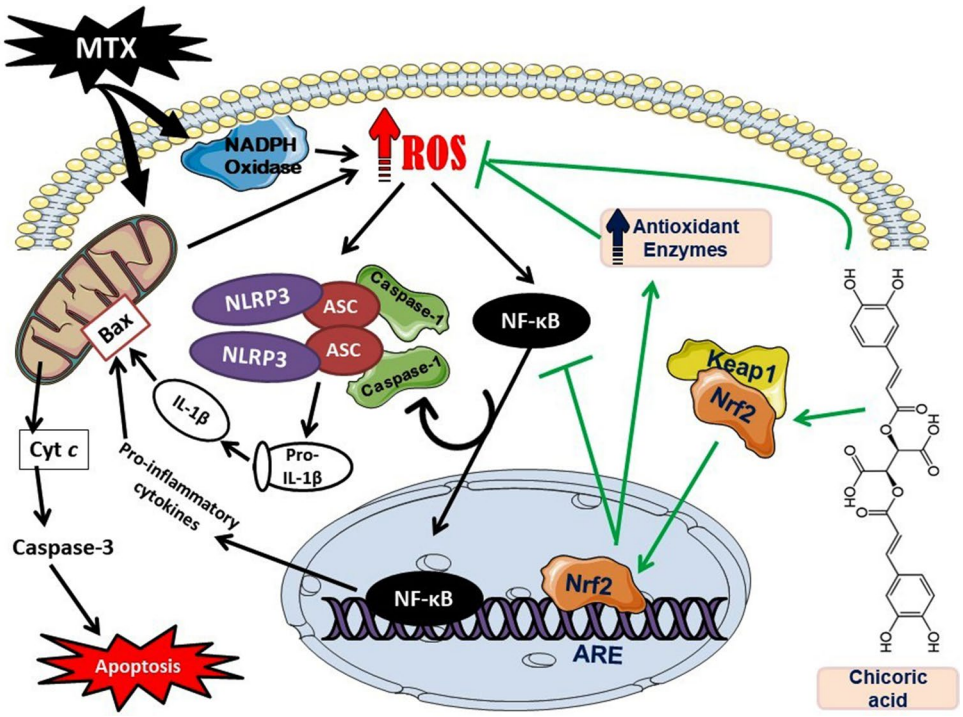

Fig. 8 A proposed schematic diagram illustrating the protective mechanism of CA against MTX-induced AKI. *MTX* methotrexate, *ROS* reactive oxygen species, *NF-κB* nuclear factor-kappaB, *Bax* Bcl-2-associated X protein, *NLRP3* nucleotide-binding domain, leucine-rich-containing family, pyrin domain (PYD)-containing-3, *IL-1β* interleukin-1beta, *Nrf2* nuclear factor (erythroid-derived 2)-like 2, *Keap-1* Kelch-like-ECH-associated protein 1, *ARE* antioxidant response element, *Cyt c* cytochrome *c*

oxLDL-induced activation of NF-κB, Bax and caspase-3 [25]. In PDGF-BB-induced VSMCs, CA inhibited ROS/NF-κB/mTOR/P70S6K signaling cascade [54], and suppressed NF-κB and inflammatory mediators and cytokines in the liver of methionine/choline-deficient (MCD) diet-fed mice, MCD-treated HepG2 and AML-12 cells [47], and the brain of LPS-induced mice [28]. Our findings conferred new information that CA can inhibit NLRP3 inflammasome activation in the kidney of MTX-induced rats secondary to suppression of oxidative stress.

To further explore the underlying mechanism, we determined the effect of CA on Nrf2/ARE/HO-1 signaling in the kidney of MTX-induced rats. Our findings showed decreased expression of Nrf2, NQO-1 and HO-1, an effect that was reversed in CA-treated rats. Previous work from our lab showed the negative impact of MTX on Nrf2 signaling in the kidney of rats [45, 48]. Although activated by mild ROS levels, sustained oxidative stress has been reported to suppress Nrf2 activation [34, 37, 38, 48, 55, 61–67]. Therefore, it is noteworthy to assume that activation of Nrf2 by CA inhibited ROS-induced activation of NF-κB/NLRP3 inflammasome signaling, production of inflammatory mediators and apoptosis in the kidney of MTX-induced rats. This notion is supported by previous studies showed activation of NLRP3 by Nrf2 knockdown in cerebral I/R [68] and brain injury [69]. Very recently, Ding et al. [2] demonstrated that CA inhibited LPS-induced NLRP3 inflammasome activation and activated Nrf2 pathway in a mouse model of LPS-induced ALI. Given that the activation of NLRP3 inflammasome is promoted by NF-κB [18], the inhibition of NF-κB activation by Nrf2 can result in suppression of NLRP3. Nrf2 activates the expression of antioxidant enzymes leading to inhibition of ROS-induced activation of NF-κB and liberation of thioredoxin-interacting protein (TXNIP) which can interact with NLRP3 and elicits the assembly of inflammasome [70].

In conclusion, our findings show the involvement of NF-κB/NLRP3 inflammasome signaling activation, oxidative stress and inflammatory cytokines in MTX nephrotoxicity. CA prevented MTX-induced AKI through attenuation of ROS production, inflammation, and apoptosis. CA activated Nrf2/ARE/HO-1 signaling, enhanced antioxidant defenses and inhibited ROS-induced activation of NF-κB/NLRP3 inflammasome signaling in the kidney of MTX-induced rats (summarized mechanistic pathways are represented in Fig. 8). Therefore, our study conferred new information on the protective mechanism of CA against MTX nephrotoxicity. CA could be used as an adjuvant therapy or a supplement to protect against AKI in patients who receive MTX, pending further studies to assess its exact mechanisms of action.

Authors' contributions AMM conceived the study, designed experiments and wrote the manuscript. OEH and AMM carried out the animal model and treatments. SMA, OEH, WGH, MB-J, and AMM performed the assays. AMM and OEH analyzed the data and prepared figures. All authors commented on and revised the manuscript, and approved the submission.

Compliance with ethical standards

The experimental protocol and all animal procedures were approved by the Institutional Animal Ethics Committee of Beni-Suef University (Egypt).

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Paueksakon P, Fogo AB. Drug-induced nephropathies. Histopathology. 2017;70:94–108.
2. Koyner JL, Cerda J, Goldstein SL, Jaber BL, Liu KD, Shea JA, et al. The daily burden of acute kidney injury: a survey of U.S. nephrologists on World Kidney Day. Am J Kidney Dis. 2014;64:394–401.
3. Mehta RL, Awdishu L, Davenport A, Murray PT, Macedo E, Cerda J, et al. Phenotype standardization for drug-induced kidney disease. Kidney Int. 2015;88:226–34.
4. Moffett BS, Goldstein SL. Acute kidney injury and increasing nephrotoxic-medication exposure in noncritically-ill children. Clin J Am Soc Nephrol: CJASN. 2011;6:856–63.
5. Chawla LS, Eggers PW, Star RA, Kimmel PL. Acute kidney injury and chronic kidney disease as interconnected syndromes. N Engl J Med. 2014;371:58–66.
6. Perazella MA. Renal vulnerability to drug toxicity. Clin J Am Soc Nephrol: CJASN. 2009;4:1275–83.
7. Izzedine H, Perazella MA. Anticancer drug-induced acute kidney injury. Kidney Int Rep. 2017;2:504–14.
8. Pazhayattil GS, Shirali AC. Drug-induced impairment of renal function. Int J Nephrol Renovasc Dis. 2014;7:457–68.
9. Heidari R, Ahmadi A, Mohammadi H, Ommati MM, Azarpira N, Niknahad H. Mitochondrial dysfunction and oxidative stress are involved in the mechanism of methotrexate-induced renal injury and electrolytes imbalance. Biomed Pharmacother. 2018;107:834–40.
10. Kolli VK, Abraham P, Isaac B, Selvakumar D. Neutrophil infiltration and oxidative stress may play a critical role in methotrexate-induced renal damage. Chemotherapy. 2009;55(2):83–90.
11. Arab HH, Salama SA, Maghrabi IA. Camel milk attenuates methotrexate-induced kidney injury via activation of PI3 K/Akt/eNOS signaling and intervention with oxidative aberrations. Food Funct. 2018;9:2661–72.
12. Widemann BC, Adamson PC. Understanding and managing methotrexate nephrotoxicity. Oncologist. 2006;11:694–703.
13. Sakthiswary R, Suresh E. Methotrexate in systemic lupus erythematosus: a systematic review of its efficacy. Lupus. 2014;23(3):225–35.
14. Zhu H, Deng FY, Mo XB, Qiu YH, Lei SF. Pharmacogenetics and pharmacogenomics for rheumatoid arthritis responsiveness to methotrexate treatment: the 2013 update. Pharmacogenomics. 2014;15(4):551–66.
15. Howard SC, McCormick J, Pui CH, Buddington RK, Harvey RD. Preventing and managing toxicities of high-dose methotrexate. Oncologist. 2016;21:1471–82.
16. May J, Carson KR, Butler S, Liu W, Bartlett NL, Wagner-Johnston ND. High incidence of methotrexate associated renal toxicity in patients with lymphoma: a retrospective analysis. Leukemia Lymphoma. 2014;55:1345–9.
17. Loverre A, Ditonno P, Crovace A, Gesualdo L, Ranieri E, Pontrelli P, et al. Ischemia-reperfusion induces glomerular and tubular activation of proinflammatory and antiapoptotic pathways: differential modulation by rapamycin. J Am Soc Nephrol: JASN. 2004;15:2675–86.
18. Leemans JC, Kors L, Anders HJ, Florquin S. Pattern recognition receptors and the inflammasome in kidney disease. Nat Rev Nephrol. 2014;10:398–414.
19. Thornberry NA, Bull HG, Calaycay JR, Chapman KT, Howard AD, Kostura MJ, et al. A novel heterodimeric cysteine protease is required for interleukin-1βprocessing in monocytes. Nature. 1992;356:768–74.
20. Fink SL, Cookson BT. Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. Infect Immun. 2005;73:1907–16.
21. Sogawa Y, Nagasu H, Itano S, Kidokoro K, Taniguchi SI, Takahashi M, et al. The eNOS-NO pathway attenuates kidney dysfunction via suppression of inflammasome activation in aldosterone-induced renal injury model mice. PloS One. 2018;13:e0203823.
22. Anders HJ, Muruve DA. The inflammasomes in kidney disease. J Am Soc Nephrol: JASN. 2011;22:1007–18.
23. Tschopp J, Schroder K. NLRP3 inflammasome activation: the convergence of multiple signalling pathways on ROS production? Nat Rev Immunol. 2010;10:210–5.
24. Lee J, Scagel C. Chicoric acid: chemistry, distribution, and production. Frontiers in Chemistry 2013;1.
25. Tsai KL, Kao CL, Hung CH, Cheng YH, Lin HC, Chu PM. Chicoric acid is a potent anti-atherosclerotic ingredient by anti-oxidant action and anti-inflammation capacity. Oncotarget. 2017;8:29600–12.
26. Ding H, Ci X, Cheng H, Yu Q, Li D. Chicoric acid alleviates lipopolysaccharide-induced acute lung injury in mice through anti-inflammatory and anti-oxidant activities. Int Immunopharmacol. 2019;66:169–76.
27. Ferrare K, Bidel LPR, Awwad A, Poucheret P, Cazals G, Lazennec F, et al. Increase in insulin sensitivity by the association of chicoric acid and chlorogenic acid contained in a natural chicoric acid extract (NCRAE) of chicory (*Cichorium intybus* L.) for an antidiabetic effect. J Ethnopharmacol. 2018;215:241–8.
28. Liu Q, Chen Y, Shen C, Xiao Y, Wang Y, Liu Z, et al. Chicoric acid supplementation prevents systemic inflammation-induced memory impairment and amyloidogenesis via inhibition of NF-kappaB. FASEB J. 2017;31:1494–507.
29. Zhu D, Zhang X, Niu Y, Diao Z, Ren B, Li X, et al. Cichoric acid improved hyperglycaemia and restored muscle injury via activating antioxidant response in MLD-STZ-induced diabetic mice. Food Chem Toxicol. 2017;107:138–49.
30. Kim JS, Lee H, Jung CH, Lee SJ, Ha TY, Ahn J. Chicoric acid mitigates impaired insulin sensitivity by improving mitochondrial function. Biosci Biotechnol Biochem. 2018;82:1197–206.
31. Jia L, Chen Y, Tian YH, Zhang G. MAPK pathway mediates the anti-oxidative effect of chicoric acid against cerebral ischemia-reperfusion injury in vivo. Exp Ther Med. 2018;15:1640–6.
32. Satta S, Mahmoud AM, Wilkinson FL, Yvonne Alexander M, White SJ. The role of Nrf2 in cardiovascular function and disease. Oxidative Med Cell Longevity. 2017;2017:18.
33. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, et al. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun. 1997;236:313–22.
34. Mahmoud AM, Hussein OE, Hozayen WG, Abd El-Twab SM. Methotrexate hepatotoxicity is associated with oxidative stress, and down-regulation of PPARgamma and Nrf2: protective effect of 18beta-Glycyrrhetinic acid. Chem Biol Interact. 2017;270:59–72.
35. Larsen K. Creatinine assay in the presence of protein with LKB 8600 reaction rate analyser. Clin Chim Acta. 1972;38:475–6.
36. Coulombe JJ, Favreau L. A new simple semimicro method for colorimetric determination of urea. Clin Chem. 1963;9:102–8.

37. Mahmoud AM, Wilkinson FL, Jones AM, Wilkinson JA, Romero M, Duarte J, et al. A novel role for small molecule glycomimetics in the protection against lipid-induced endothelial dysfunction: involvement of Akt/eNOS and Nrf2/ARE signaling. Biochim Biophys Acta. 2017;1861:3311–22.
38. Mahmoud AM, Wilkinson FL, McCarthy EM, Moreno-Martinez D, Langford-Smith A, Romero M, et al. Endothelial microparticles prevent lipid-induced endothelial damage via Akt/eNOS signaling and reduced oxidative stress. FASEB J. 2017;31:45.
39. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95:351–8.
40. Grisham MB, Johnson GG, Lancaster JR Jr. Quantitation of nitrate and nitrite in extracellular fluids. Methods Enzymol. 1996;268:237–46.
41. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963;61:882–8.
42. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. FEBS Eur J Biochem. 1974;47:469–74.
43. Cohen G, Dembiec D, Marcus J. Measurement of catalase activity in tissue extracts. Anal Biochem Anal Biochem. 1970;34:30–8.
44. Matkovics B, Szabo L, Varga IS. Determination of enzyme activities in lipid peroxidation and glutathione pathways (in Hungarian). Laboratoriumi Diagnosztika. 1998;15:248–9.
45. Mahmoud AM, Germoush MO, Al-Anazi KM, Mahmoud AH, Farah MA, Allam AA. Commiphora molmol protects against methotrexate-induced nephrotoxicity by up-regulating Nrf2/ARE/HO-1 signaling. Biomed Pharmacother. 2018;106:499–509.
46. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 2001;25(4):402–8.
47. Kim M, Yoo G, Randy A, Kim HS, Nho CW. Chicoric acid attenuate a nonalcoholic steatohepatitis by inhibiting key regulators of lipid metabolism, fibrosis, oxidation, and inflammation in mice with methionine and choline deficiency. Mol Nutr Food Res. 2017;61:1600632.
48. Abd El-Twab SM, Hozayen WG, Hussein OE, Mahmoud AM. 18beta-Glycyrrhetinic acid protects against methotrexate-induced kidney injury by up-regulating the Nrf2/ARE/HO-1 pathway and endogenous antioxidants. Ren Fail. 2016;8(1–12):2016.
49. Bonventre JV. Kidney Injury Molecule-1 (KIM-1): a specific and sensitive biomarker of kidney injury. Scand J Clin Lab Investig Suppl. 2008;241:78–83.
50. Babiak RM, Campello AP, Carnieri EG, Oliveira MB. Methotrexate: pentose cycle and oxidative stress. Cell Biochem Funct. 1998;16:283–93.
51. Wilson AJ, Gill EK, Abudalo RA, Edgar KS, Watson CJ, Grieve DJ. Reactive oxygen species signalling in the diabetic heart: emerging prospect for therapeutic targeting. Heart. 2018;104:293–9.
52. Ozbek E. Induction of oxidative stress in kidney. Int J Nephrol. 2012;2012:465897.
53. Jablonska-Trypuc A, Kretowski R, Kalinowska M, Swiderski G, Cechowska-Pasko M, Lewandowski W. Possible mechanisms of the prevention of doxorubicin toxicity by cichoric acid-antioxidant nutrient. Nutrients. 2018;10:44.
54. Lu QB, Wan MY, Wang PY, Zhang CX, Xu DY, Liao X, et al. Chicoric acid prevents PDGF-BB-induced VSMC dedifferentiation, proliferation and migration by suppressing ROS/NFkappaB/mTOR/P70S6 K signaling cascade. Redox Biol. 2018;14:656–68.
55. Mahmoud AM, Hozayen WG, Ramadan SM. Berberine ameliorates methotrexate-induced liver injury by activating Nrf2/HO-1 pathway and PPARgamma, and suppressing oxidative stress and apoptosis in rats. Biomed Pharmacother. 2017;94:280–91.
56. Abdel-Raheem IT, Khedr NF. Renoprotective effects of montelukast, a cysteinyl leukotriene receptor antagonist, against methotrexate-induced kidney damage in rats. Naunyn Schmiedebergs Arch Pharmacol. 2014;387(4):341–53.
57. Herrera B, Fernandez M, Alvarez AM, Roncero C, Benito M, Gil J, et al. Activation of caspases occurs downstream from radical oxygen species production, Bcl-xL down-regulation, and early cytochrome C release in apoptosis induced by transforming growth factor beta in rat fetal hepatocytes. Hepatology. 2001;34:548–56.
58. Yang J, Liu X, Bhalla K, Kim CN, Ibrado AM, Cai J, et al. Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. Science. 1997;275:1129–32.
59. Almeida OF, Conde GL, Crochemore C, Demeneix BA, Fischer D, Hassan AH, et al. Subtle shifts in the ratio between pro- and antiapoptotic molecules after activation of corticosteroid receptors decide neuronal fate. Faseb j. 2000;14:779–90.
60. Mahmoud AM, Ahmed OM, Galaly SR. Thymoquinone and curcumin attenuate gentamicin-induced renal oxidative stress, inflammation and apoptosis in rats. EXCLI J. 2014;13:98–110.
61. Kamel EM, Mahmoud AM, Ahmed SA, Lamsabhi AM. A phytochemical and computational study on flavonoids isolated from *Trifolium resupinatum* L and their novel hepatoprotective activity. Food Funct. 2016;7(4):2094–106.
62. Mahmoud AM, Germoush MO, Alotaibi MF, Hussein OE. Possible involvement of Nrf2 and PPARgamma up-regulation in the protective effect of umbelliferone against cyclophosphamide-induced hepatotoxicity. Biomed Pharmacother. 2017;86:297–306.
63. Mahmoud AM, Al Dera HS. 18β-Glycyrrhetinic acid exerts protective effects against cyclophosphamide-induced hepatotoxicity: potential role of PPARγ and Nrf2 upregulation. Genes & nutrition. 2015;10:1–13.
64. Mahmoud AM, Alqahtani S, Othman SI, Germoush MO, Hussein OE, Al-Basher G, et al. Commiphora molmol modulates glutamate-nitric oxide-cGMP and Nrf2/ARE/HO-1 pathways and attenuates oxidative stress and hematological alterations in hyperammonemic rats. Oxidative Med Cell Longevity. 2017;2017:15.
65. Mahmoud AM, Mohammed HM, Khadrawy SM, Galaly SR. Hesperidin protects against chemically induced hepatocarcinogenesis via modulation of Nrf2/ARE/HO-1, PPARgamma and TGF-beta1/Smad3 signaling, and amelioration of oxidative stress and inflammation. Chem Biol Interact. 2017;277:146–58.
66. Mahmoud AM, Zaki AR, Hassan ME, Mostafa-Hedeab G. Commiphora molmol resin attenuates diethylnitrosamine/phenobarbital-induced hepatocarcinogenesis by modulating oxidative stress, inflammation, angiogenesis and Nrf2/ARE/HO-1 signaling. Chem Biol Interact. 2017;270:41–50.
67. Aly MS, Galaly SR, Moustafa N, Mohammed HM, Khadrawy SM, Mahmoud AM. Hesperidin protects against diethylnitrosamine/carbon tetrachloride-induced renal repercussions via up-regulation of Nrf2/HO-1 signaling and attenuation of oxidative stress. J Appl Pharm Sci. 2017;7:7–14.
68. Hou Y, Wang Y, He Q, Li L, Xie H, Zhao Y, et al. Nrf2 inhibits NLRP3 inflammasome activation through regulating Trx1/TXNIP complex in cerebral ischemia reperfusion injury. Behav Brain Res. 2018;336:32–9.
69. Zeng J, Chen Y, Ding R, Feng L, Fu Z, Yang S, et al. Isoliquiritigenin alleviates early brain injury after experimental intracerebral hemorrhage via suppressing ROS- and/or NF-kappaB-mediated NLRP3 inflammasome activation by promoting Nrf2 antioxidant pathway. J Neuroinflamm. 2017;14:119.
70. Zhou R, Tardivel A, Thorens B, Choi I, Tschopp J. Thioredoxin-interacting protein links oxidative stress to inflammasome activation. Nat Immunol. 2010;11:136–40.