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RESEARCH ARTICLE

The ameliorative effect of kaempferol against $CdCl_2$- mediated renal damage entails activation of Nrf2 and inhibition of NF-kB

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Abstract

This study evaluated the nephroprotective effect of kaempferol against cadmium chloride ($CdCl_2$) -induced nephropathy in rats. It also investigated if activation of Nrf2 is a common mechanism of action. Adult male rats ((150 ± 15 g) were divided into 4 groups ($n = 8$/each) as a control (1% DMSO, orally), control + kaempferol (200 mg/kg, orally), $CdCl_2$ (50 mg/l in drinking water), and $CdCl_2$ + kaempferol (200 mg/kg)-treated rats. All treatments were conducted for 8 weeks. Kaempferol significantly attenuated $CdCl_2$-induced weight loss, reduction in kidney weights, and the injury in the glomeruli, proximal tubules, and distal tubules in the treated rats. It also significantly lowered serum levels of urea and creatinine, increased urine output and urinary creatinine levels and clearance but reduced urinary levels of albumin urinary albumin exertion (UAER), and urinary albumin/creatinine ratio (UACR) in these rats. In parallel, kaempferol downregulated renal levels of cleaved caspase-3 and Bax and unregulated those of Bcl2. In the kidney tissues of the control animals and $CdCl_2$ rats, kaempferol significantly attenuated oxidative stress, inflammation and significantly boosted levels of manganese superoxide dismutase and glutathione. Also, and in both groups, kaempferol suppressed the nuclear levels of NF-κB p65, downregulated Keap1, and stimulated the nuclear activation and protein levels of Nrf2. In conclusion, kaempferol is a potential therapeutic drug to prevent $CdCl_2$-induced nephropathy due to its anti-inflammatory and anti-oxidant effects mediated by suppressing NF-NF-κB p65 and transactivating Nrf2.

Keywords Nephropathy · Kaempferol · $CdCl_2$ · Kidney · Oxidative stress · Inflammation · NF-κB · Nrf2

Introduction

Chronic kidney disease (CKD), is a medical condition that is characterized by impaired kidney function, reduced estimated glomerular filtration rate (eGFR), albuminuria, and glomerular hypertension (Afkarian et al. 2016). The recently available updates indicate a total global CKD prevalence of about 11–13% (Alsuwaida et al. 2010; Hill et al. 2016). However, the molecular mechanisms underlying CKD are complex, but include, at least, oxidative stress activation, inflammation, intestinal fibrosis, and apoptosis (Gajjala et al. 2015). Although diabetes mellitus (DM), smoking, hypertension, and cardiovascular disorders (CVDs) are major risk factors for the development of CKD, it is currently well-established that exposure to heavy metals is an independent risk factor (Gajjala et al. 2015).

Cadmium ($Cd^{2+)}$ ion is the most known heavy metal that is widely distributed in the soil, air, smoking, and industrial products (e.g., pigment and batteries) (El-Kott et al. 2020b, Prozialeck and Edwards 2012, Satarug 2018). As an environmental contaminant, experimental and human studies have shown that exposure to Cd^{2+} increases the risk for the development of nephropathies and CKD by promoting oxidative stress, inflammation, interstitial fibrosis, and apoptosis (Diamond et al. 2019; Jiao et al. 2019; Pavón et al. 2019). However, overproduction of reactive oxygen species (ROS), depletion of antioxidants, and suppression of nuclear factor erythroid 2 related factor-2 (Nrf2) antioxidant pathway are the best-described mechanisms underlying the pro-oxidant potential of Cd2 + , which is

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assumed to be the major mechanism behind its effect of all renal damaging pathways (Diamond et al. 2019, Hagar and Al Malki 2014, Jiao et al. 2019, Pavón et al. 2019, Satarug 2018, Wang et al. 2013, Yuan et al. 2016).

Nonetheless, oxidative stress is identified by the overproduction of reactive oxygen species (ROS) as compared to elimination (Newsholme et al. 2016). In the majority of the cells, the major source of ROS is the mitochondria (Zorov et al. 2014). However, the generation of ROS can be increased under several stressful conditions including exposure to radiation, toxic drugs, injury, ischemia, hypoxia, etc. (Zorov et al. 2014). On the other hand, animal cells are well-equipped with enzymatic and non-enzymatic antioxidant systems that are distributed in the cytoplasm and organelles to fight oxidative stress. These include glutathione (GSH), thioredoxin (TRx), heme-oxygenase-1 (HO-1), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GRx) (Birben et al. 2012). The effect of ROS in the cell is well-established and includes activation of inflammation and apoptosis. Indeed, ROS can promote cell inflammation by activating the NRLP3 inflammasome and the nuclear factor kappa-beta (NF-κB) which can translocate to the mitochondria to stimulate the transcription of numerous inflammatory cytokines and mediators. In addition, NF-κB is positively crossed talk with ROS that induced persistent oxidative stress and inflammatory status within the cells (Morgan and Liu 2011). In addition, ROS can induce intrinsic (mitochondria-mediated cell death) by damaging the DNA and upregulation of p53 and Bax signaling (Redza-Dutordoir and Averill-Bates 2016).

On the other hand, Nrf2 is a ubiquitous transcription factor that plays a significant role in the majority of the cells through stimulating cell survival and inhibiting cell inflammation and apoptosis (Bellezza et al. 2018). In the cytoplasm, Nrf2 is always bound to keap-1 protein (Li et al. 2018). Under normal conditions, keap-1 stimulates the ubiquitination and proteasome degradation Nrf2. However, under stress, ROS and electrophiles can phosphorylate keap-1, thus inhibiting its association with Nrf2 (Li et al. 2018). This promotes the nuclear translocation of Nrf2 to initiate the transcription process. In this regard, Nrf2 is the best-known antioxidant transcription factor that stimulates the synthesis of GSH and other phases II antioxidant enzymes (e.g., HO-1, SOD, CAT) (Li et al. 2018; Bellezza et al. 2018). Also, Nrf2 is a potent anti-inflammatory molecule that can suppress NF-κB (Alshehri et al. 2021; Li et al. 2008). Also, Nrf2 inhibits cell apoptosis by direct upregulation of the anti-apoptotic protein Bcl2 (Niture and Jaiswal 2012). Of note, keap-1/Nrf2 signaling is extremely inhibited in the majority of renal disorders including those induced by Cd^{2+} whereas the activation of this pathway was protective (Alshehri et al. 2022; Liu et al. 2019; Nezu et al. 2017).

Currently, no definite treatment is available to treat CKD. Therefore, international efforts in experimental science and clinical medicine are carried out during the last decades to develop suitable and safe therapies to treat CKD. In this view, much interest in drug discovery to treat CKD from plant flavonoids is rapidly increasing and well-reported (Vargas et al. 2018). Kaempferol is a plant flavonoid that is abundantly found in vegetables and plants such as tea, tomato, grapes, broccoli, and beans (Devi et al. 2015). Kaempferol attenuated liver, lung, kidney, brain, and heart damage in a variety of animal models by silencing oxidative stress and inflammation. It also attenuated renal oxidative, inflammatory, and fibrotic damage in diabetic, aged, and cisplatin and tacrolimus-treated rodents (Al-Numair et al. 2015; Ali et al. 2020; Park et al. 2009; Sharma et al. 2020; Wang et al. 2020; Zhang et al. 2019). Similar protective effects of kaempferol were also reported in D-ribose-induced mesangial cell apoptosis (Zhang et al. 2019). In all these studies, the nephroprotective effects of kaempferol were mediated by several mechanisms, including scavenging of ROS, activation of Nrf2/antioxidants axis, inhibiting the nuclear factor kappa-beta (NF-κB), and modulating of the mitogen-activated protein kinase (MAPK) apoptotic members (i.e., such as p38, ERK, and JNK).

Despite these studies, the renoprotective effect of kaempferol against Cd^{2+} ions-kidney damage was poorly investigated. Therefore, in this study, we tested whether the treatment with kaempferol could attenuate Cd-chloride ($CdCl_2$)-induced nephropathy in rats. Besides, we tested the hypothesis that this protection involves activation of Nrf2/ and or inhibiting NF-κB.

Materials and methods

Animals

Mature male Wistar rats (150 ± 15 g) were included in this investigation. All rats were provided from the animal facility complex at King Khalid University (KKU), Abha, Kingdome of Saudi Arabia. The rodents were housed in groups of 4 rats/cage under stable, controlled conditions (21 ± 1 ℃, 60% humidity, and 12 h dark/light cycle and always had free access to the chow and drinking water. Rats were adapted for 1 week. All procedures were approved by the ethics committee at the College of Science at KKU.

Experimental design

All animals were randomly selected and divided into 4 groups of rats ($n = 10$ each) as (1) control rats: orally (gavage) administered 0.25 ml of 1% DMSO (dimethyl sulfoxide) (cat 472,301, Sigma Aldrich, St. Louis, MO, USA) prepared

in phosphate-buffered saline (PBS); (2) Kaempferol-treated rats: orally administered an 0.25 ml of kaempferol (200 mg/kg) (cat K0133, Sigma) dissolved in 1% DMSO solution; (3) $CdCl_2$-treated rats: administered $CdCl_2$ (cat 202,908, Sigma) in the drinking water (50 mg/l) and co-treated orally with 0.25 ml of 1% DMSO 4) $CdCl_2$ + kaempferol-treated rats: animals treated with $CdCl_2$ as in group 3 but also co-received the kaempferol solution (200 mg/kg). All drugs were given daily for eight weeks.

Selection of the treatment regimen

The chosen regimen of $CdCl_2$ was adopted for the study of Wang et al. (Wang et al. 2009) who confirmed nephropathy and renal fibrosis by the end of week 8 after daily administration of this dose of Cd ions. The regimen of kaempferol was approved from our previous studies (Alshehri 2021), confirming its therapeutic effect against diabetes mellitus-induced nephropathy.

24-h urine collection

On the last day of week 8, all rats were placed individually in metabolic cages (Lab Products, USA). The 24-h urine samples were collected in 50-ml tubes containing sodium azide. The volume of urine was calculated and all tubes were centrifuged (10 min/1400 × *g*/room temperature). The collected clear supernatants were aliquot and maintained at − 20 until use.

Collection of the blood and tissues processing

The next day, all rats were fasted overnight and then anesthetized with a mixture of xylazine hydrochloride and ketamine hydrochloride (10 mg/kg and 90 mg/kg) (Cat. No. 61763–23-3. Sigma Aldrich, MO, USA). Blood samples (1 ml) were directly collected by cardiac puncture into empty glass tubes and centrifuged (10 min/1300 × *g*/room temperature) to collect clear serum which then was stored at − 80 ℃. All animals were then ethically authenticated and both kidneys were isolated on ice, weighed, and cut in transverse sections. Parts of the kidneys were directly placed in 10% buffered formalin and forwarded to the pathology laboratory. All other sections were snap-frozen and kept at − 80 ℃ until use. Later, some parts of these frozen kidney parts were homogenized either in phosphate buffered saline (PBS) or in radioimmunoprecipitation (RIPA) buffer containing protease inhibitor cocktail, centrifuged (12,000 × *g*/20 min/4℃) to isolate total cell homogenates or protein extracts for the biochemical and western blotting analyses, respectively. All extracts were frozen at – 80 ℃ until use. In addition, the cytoplasmic and nuclear fractions were prepared to form frozen kidney parts using the NE-PER commercially available kit (cat 78,833, ThermoFisher Scientific, USA) as per the manufacturer's instructions.

Biochemical analysis

Serum and urinary levels of creatinine (Cr) were assessed using a colorimetric-based kit (cat Ab65240, Abcam, UK). The concentrations of albumin in the serum and urine were analyzed using a rat-specific ELISA kit (cat Ab108789, Abcam, Cambridge, UK). The 24-h urinary Cr excretion was calculated using the following formula, UCrE (mg/24 h) = urinary Cr (mg/dl) × urinary volume in 24 h (dl). The 24-h creatinine clearance (Ccr) was calculated according to this formula; Ccr (ml/min) = (urinary Cr (mg/dL) × urine Vol (ml/min))/(serum Cr (mg/dl)). Urinary albumin excretion rate (UAER) and urinary albumin/creatinine ratio (UACR) were calculated using the following equations: [UACR = Urinary albumin (mg/dl) /urinary creatinine (g/dl)] and [UACR = Urinary albumin (mg/dl)/urinary creatinine (g/dl)] (Kim et al. 2016).

Biochemical measurements in total kidney homogenates

Levels of manganese superoxide dismutase (Mn SOD); tumor necrosis factor-α (TNF-α); interlukine-6 (IL-6); GSH; and MDA were analyzed using rats special ELISA kits (cat MBS729914, cat MBS175908, cat MBS175904, cat MBS046356; and cat. MBS268427, MyBioSource, CA, USA, respectively). The assessment of the nuclear concentration of NF-κB p65 and Nrf2 was conducted using commercial ELISA kits (cat. 40,096 and cat. 31,102, respectively, Active Motif, Tokyo, Japan). A commercial fluorometric kit was used to measure levels of free radicals (ROS/RNS) (cat. No. E-BC-K138-F, Elabscience, USA).

Western blots

Proteins from the nuclear and total kidney homogenates were boiled and then diluted in the loading (2 µg/µL). Equal protein concentrations were loaded and separated by SDS-PAGE (8–12%) and then transferred to nitrocellulose membranes (cat Ab133413, Abcam, Cambridge, UK), blocked by skimmed milk (5%), washed with 1X Tris-buffered saline with 0.1% Tween (TBST). The individual membranes were incubated with 1st and 2nd antibodies (prepared in TBST buffer) for 2 h at room temperature. The primary antibodies were Bcl2 (cat. 8276, 28 kDa), Bax (Cat. 20 kDa), cleaved Caspase-3 (Asp175) (cat. 9664, 17/19 kDa), cytochrome-c (cat. 11,940, 14 kDa, 1:1000), NF-κB p65 Antibody (cat. 3034, 65 kDa, 1:1000), keap1 (sc-365626, 69 kDa), Nrf2 (cat. 17,212, 100 kDa, 1:1000), and β-actin (Cat. No. 4970,

45 kDa) (Cell signaling technology), and Lamin A (Cat. No. sc-293162, 69 kDa, 1:1000) (Santa Cruz Biotechnology). After washing, the bands were developed and measured using enhanced Chemiluminescence ECL detection reagent (cat 32,109, ThermoFisher Scientific, MA, USA) and C-Di Git blot scanner (LI-COR, NE, USA). Expressions of nuclear and total proteins were presented as relative to Lamin A and β-actin, respectively.

Histological evaluation

Parts of the kidney section were fixed 10% buffered formalin for 24 h and then were dehydrated in ethanol (70–100%). All samples were then cleared with xylene, embedded in paraffin, cut into fine Sects. (3–5 µm), and then routinely stained with hematoxylin and eosin (H&E) for routine pathological morphology detection. Slides were examined and photographed under a Nikon Eclipse E200, light microscope, Tokyo, Japan.

Statistical analysis

GraphPad Prism statistical software (V8, Sydney, Australia) was used. The normality of the data was tested using the Kolmogorov–Smirnov test. Analysis was accomplished by a 2-way ANOVA analysis followed by the Tukey *t*-test. Data were presented as means ± SD. Significance was considered at values $P < 0.05$.

Results

Changes in weights and other urinary and serum-renal parameters

No rat death was viewed in all experimental groups during the whole period of the treatment. Final body weight (FBW), kidney weights, and urine volume, as well as all measured renal-related markers, were not significant when kaempferol-treated rats when compared to the control rats (Table 1). However, FBW and the weights of both kidneys were significantly lower in the $CdCl_2$-treated rats in comparison to the control rats (Table 1). Besides, $CdCl_2$-treated rats showed a significantly less urine volume (oliguria), urinary creatinine levels, and creatinine clearance (Ccr) that was parallel with a significantly higher concentration of serum urea and creatinine (Table 1). Besides, a significant increment in the urinary concentrations of albumin, NAG, and β2-MG, and values of UAER and UACR were depicted in $CdCl_2$-treated rats in contrast to the control rats (Table 1). The levels of all these parameters were significantly reversed in the $CdCl_2$ + kaempferol-treated rats compared to $CdCl_2$-treated rats. Except for FBW, kidney weights, and urine volume, which returned to their basal levels, the values of all other markers remained significantly different (higher/lower) in $CdCl_2$ + kaempferol as compared to control rats (Table 1).

Table 1 Alterations in rat's final body weights, kidney weights, serum, and urinary parameters in all experimental groups

Parameter	Control	Control + kaempferol	$CdCl_2$	$CdCl_2$ + kaempferol
Final body weights (g)	314.8 ± 26.4^a	324.4 ± 21.9^a	257.5 ± 13.3^b	311.5 ± 19.1^a
Average water intake (ml/4 rats/day)	153 ± 18.7^a	161 ± 16.9^a	158 ± 15.1^a	157 ± 17.3^a
Average weekly food intake (g/4 rats/day)	98.8 ± 7.9^a	104 ± 9.1^a	78.2 ± 6.9^b	82.5 ± 7.1^b
Kidneys' weights (g)	2.91 ± 0.29^a	2.76 ± 0.31^a	2.34 ± 0.27^b	2.81 ± 0.32^a
Serum markers				
Urea (mg/dl)	10.84 ± 1.5^b	9.34 ± 1.8^b	34.6 ± 5.1^a	14.4 ± 2.9^c
Creatinine (mg/dl)	0.56 ± 0.08^b	0.43 ± 0.05^b	2.65 ± 0.47^a	0.74 ± 0.12^c
Urine markers				
Volume (ml)	12.4 ± 2.8^a	10.8 ± 2.1^a	4.9 ± 0.93^b	11.4 ± 1.9^a
Albumin (µg/dl)	11.2 ± 0.25^c	12.3 ± 0.31^c	108 ± 8.7^a	21.2 ± 3.6^b
Creatinine (mg/dl)	41.3 ± 5.6^a	39.8 ± 3.9^a	18.7 ± 3.9^c	31.3 ± 3.7^b
NAG (mIU/ml)	8.9 ± 1.1^c	7.9 ± 1.5^c	33.2 ± 6.8^a	13.2 ± 3.6^b
β2-MG (ng/ml)	227.1 ± 17.5^c	251.3 ± 22.8^c	1093 ± 115^a	382 ± 47.7^b
UCrER (mg/24 h)	4.9 ± 0.72^b	4.2 ± 0.92^b	0.91 ± 0.12^a	3.1 ± 0.73^c
Ccr (ml/min)	0.61 ± 0.09^a	0.68 ± 0.07^a	0.005 ± 0.001^c	0.33 ± 0.04^b
Ccr (ml/min/kg)	1.9 ± 0.39^a	2.1 ± 0.58^a	0.02 ± 0.002^c	1.1 ± 0.14^b
UAER (µg/24 h)	97.3 ± 15.4^c	109.4 ± 17.3^c	2204 ± 154.4^a	182 ± 28.4^b
UACR (mg/g)	2.8 ± 0.51^c	3.1 ± 0.67^c	57.4 ± 8.1^a	6.7 ± 1.4^b

Data were analyzed by two-way ANOVA followed by Tukey's *t*-test. All data are presented as mean ± SD ($n = 8$)

Changes in renal markers of oxidative stress and inflammatory

A significant increment in the renal levels of ROS, MDA, IL-6, and TNF-α, and nuclear protein levels of NF-κB p65 with a parallel reduction in the levels of MnSOD and GSH were observed in $CdCl_2$-treated rats in contrast to the control group (Fig. 1A–D and Fig. 2A–D). A significant reduction in the ROS levels, IL-6, MDA, and TNF-α, and the nuclear protein levels of NF-κB p65 with increased levels of MnSOD and GSH were shown in the renal homogenates of both kaempferol- and $CdCl_2$ + kaempferol-treated rodents in contrast to the control rats or $CdCl_2$-treated rats, respectively (Fig. 1A–D and Fig. 2A–D). Interestingly, no significant differences in the values of all these markers were observed when kaempferol-treated rats $CdCl_2$ were compared to controls.

Changes in Nrf2 signaling

Significantly lower cytoplasmic and nuclear protein levels of Nrf2 with stimulated levels of keap1 and keap1/Nrf2 ratio were depicted in the renal homogenates of $CdCl_2$-treated rats when a comparison was done versus the control group (Fig. 3A–D). On the contrary, higher cytoplasmic and nuclear protein levels of Nrf2 with significant suppression of kepa1 levels and keap1/Nfr2 ratio were observed in the kidneys of the kaempferol- and $CdCl_2$ + kaempferol-treated animals when compared with their vehicle-treated controls (Fig. 3A–D). However, control and $CdCl_2$ + kaempferol-treated rats showed non-significant cytoplasmic and nuclear levels of this transcription factor, as well as in the levels of keap-1 when compared against each other (Fig. 3A–D).

Changes in apoptotic markers

Kaempferol-treated rats seen normal protein levels of all measured apoptotic/anti-apoptotic markers when compared to control animals (Fig. 4A–D). While Bcl2 protein levels were significantly repressed, significantly higher protein levels of cleaved caspase-3, Bax, and Bax/Bcl2 ratio were seen in the renal tissues of the $CdCl_2$-treated rats (Fig. 4A–D). This was reversed in the kidneys of $CdCl_2$ + kaempferol rats when compared to CdCl2-intoxicated animals, values which were within their normal expression depicted in the control rats (Fig. 4A–D).

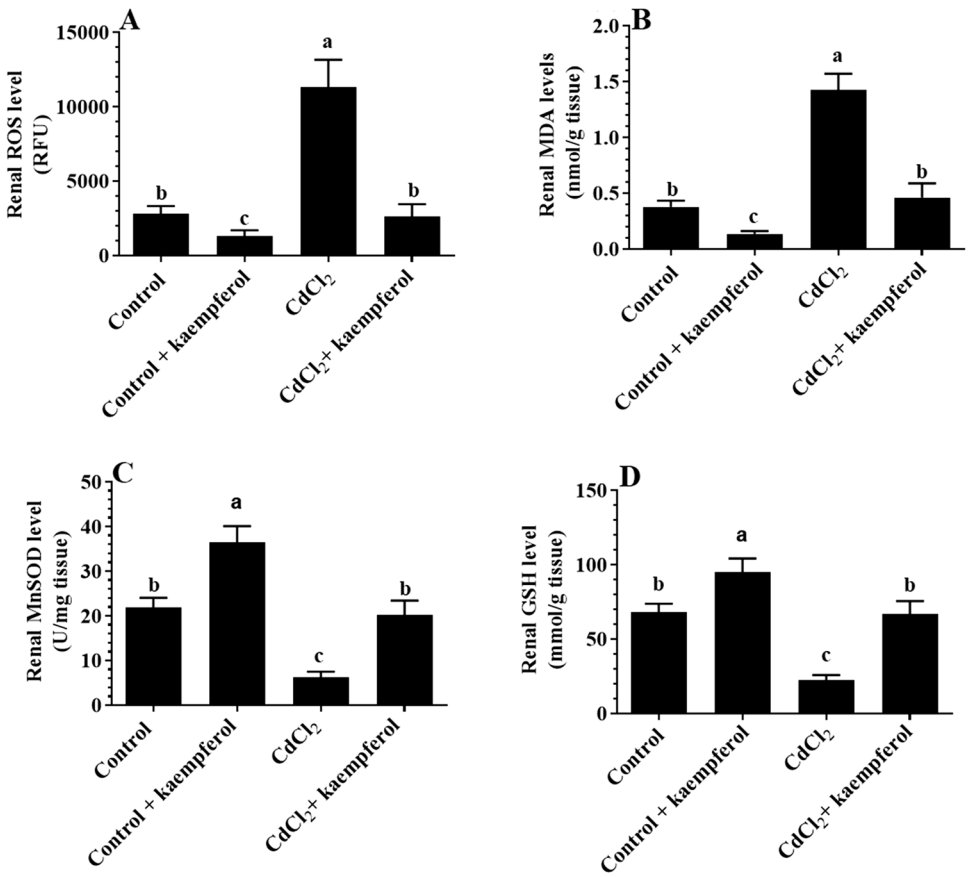

Fig. 1 Levels of reactive oxygen species (ROS), malondialdehyde (MDA) (**B**), manganese superoxide dismutase (MnSOD) (**C**), and total glutathione (GSH) in the kidneys of all groups of rats. Data were analyzed by two-way ANOVA followed by Tukey's *t*-test. All data are presented as mean ± SD ($n = 8$)

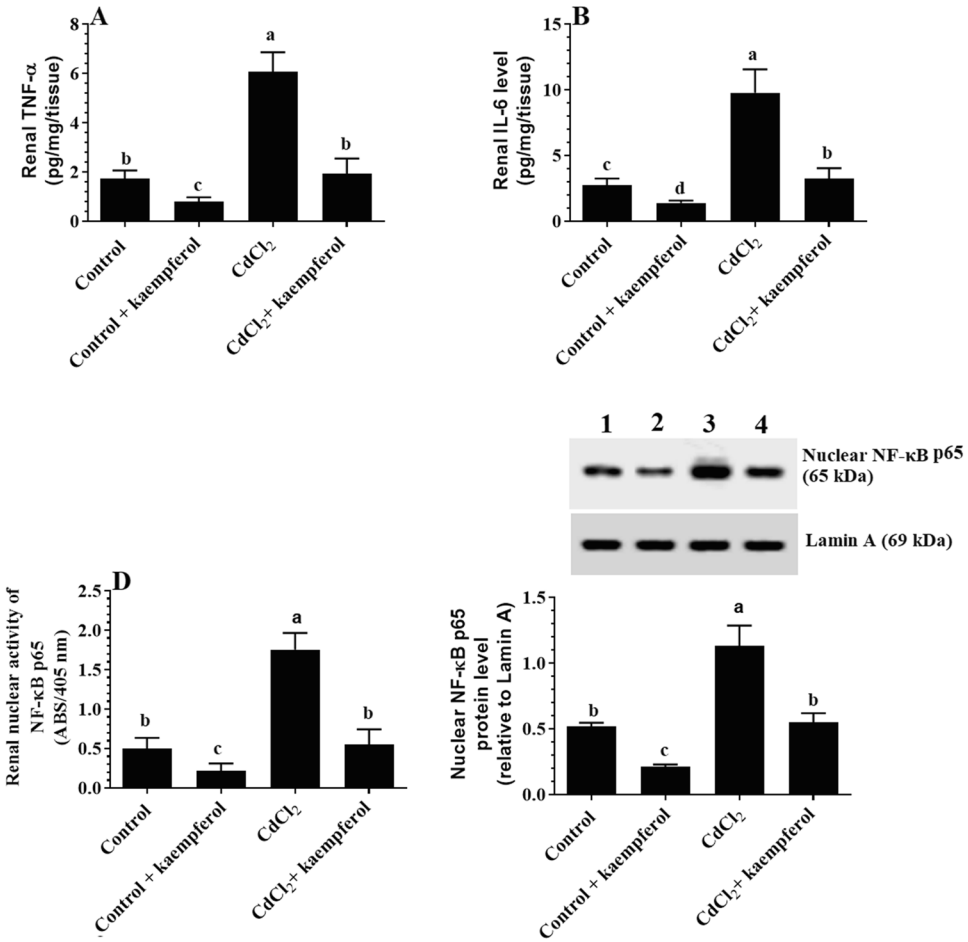

Fig. 2 Levels of tumor necrosis factor-α (TNF-α) (**A**) and interleukin-6 (IL-6) (**B**), as well as the nuclear activity of Nrf2 NF-Kb P65 (d) in the kidneys of all groups of rats. Data were analyzed by two-way ANOVA followed by Tukey's *t*-test. All data are presented as mean ± SD ($n = 8$)

Histological changes

Control and kaempferol-treated animals showed normal histological features including normally appeared glomeruli, glomerular membranes, proximal convoluted tubules (PCTs), and distal convoluted tubules (DCTs) (Fig. 5A, B). Severe decrease in mesangial mass with damaged glomerular membrane and degenerated proximal and distal convoluted tubules (PCTs and DCTs, respectively) were seen in $CdCl_2$-treated rats (Fig. 5C). Almost normal histological findings like those seen in the control rats were seen in the $CdCl_2$ + kaempferol-treated rats (Fig. 5D).

Discussion

Data derived from this investigation confirm the protection of kaempferol against $CdCl_2$-induced nephropathy in rats. In addition, it illustrates that the mechanism of protection involves suppressing oxidative stress and inflammation. In addition, the results showed that these effects are associated with activation suppression of Keap1 and NF-κB p65 and concomitant upregulation and activation of Nrf2.

Generally, the kidneys are the major target of Cd^{2+} ions intoxication which can lead to AKI, CKD, and renal failure (Lentini et al. 2017, Prozialeck and Edwards 2012, Satarug 2018, Wang et al. 2009). Once it reaches the kidneys, Cd^{2+} ions not only induce proximal tubule injury but the damage also the glamorous and distal convoluted tubules (Diamond et al. 2019, Jiao et al. 2019, Pavón et al. 2019, Prozialeck and Edwards 2012, Satarug 2018). A reduction in food intake due to decreased appetite, loss of weight gain, a decrease in kidney weights, oliguria, micro/macroalbuminuria, and reduced creatinine clearance (Ccr) are the major clinical manifestation for Cd^{2+} ions-induced nephropathy (Diamond et al. 2019; Pollack et al. 2015; Satarug 2018). Besides, several authors have shown Cd^{2+}ions-induced renal injury leads to a significant release of special damaging markers named, NAG and β2-MG (Bernard et al. 1983, Hagar and Al Malki 2014, Milnerowicz et al. 2008, Prozialeck et al. 2007, Satarug 2018, Yuan et al. 2016). All these alterations were also shown in our animal model, thus validating it. Interestingly, all these changes were independent of water intake, thus dissipating the role of water consumption from the observed effect of $CdCl_2$.

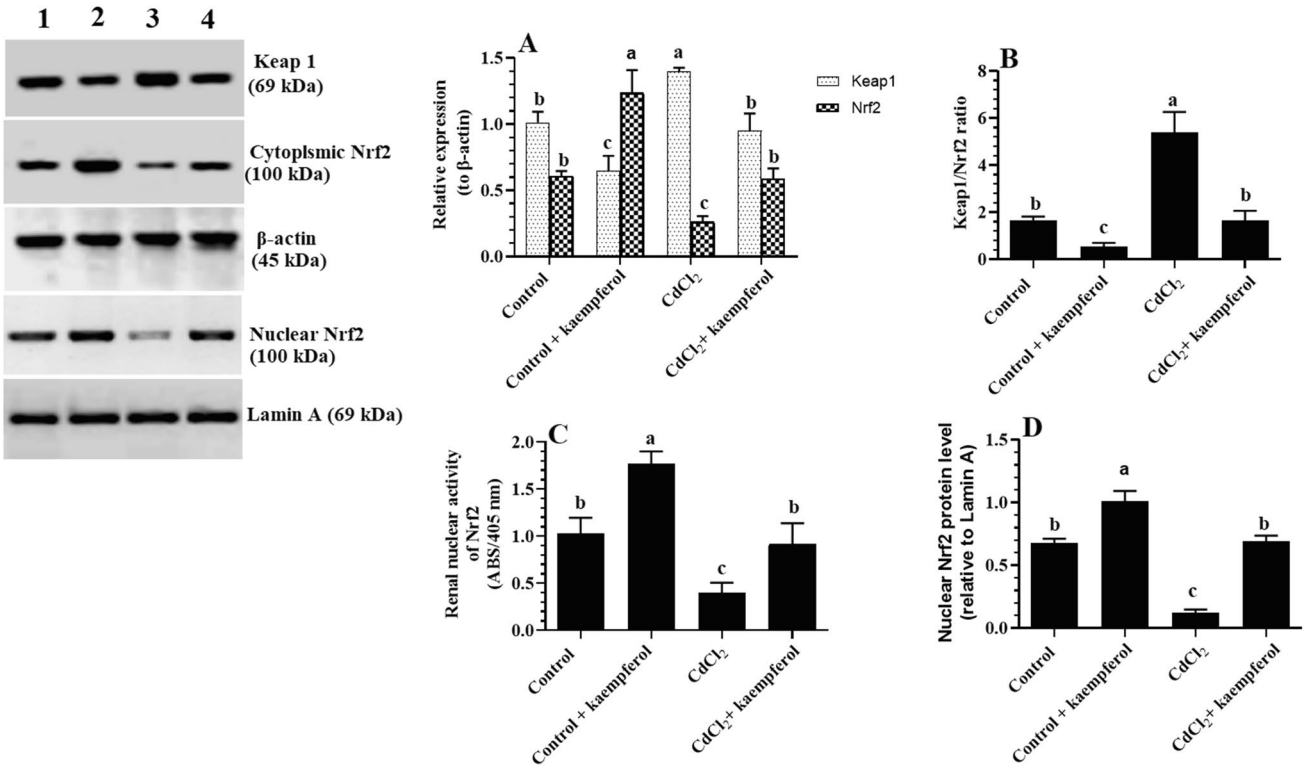

Fig. 3 Total protein levels of Nrf2 and keap1 (**A**), the ratio of keap1/Nrf2 (**B**), and nuclear protein levels of Nrf2 and NF-κB p65 (**D**) in the kidneys of all groups of rats. Data were analyzed by two-way ANOVA followed by Tukey's *t*-test. All data are presented as mean ± SD ($n = 8$)

Independent of water intake or improving satiety, kaempferol was not only able to alleviate the oliguria and the alteration in all measured urinary and serum markers, but also prevented the loss in the final body and kidney weights and suppressed the renal damage of the glomeruli and tubules. These data were our first evidence that supports the direct protective impacts of kaempferol against weight loss and $CdCl_2$-induced nephrotoxicity. Although this nephroprotective effect of kaempferol is novel to be shown in this animal model, several studies performed in other tissues of this animal model or kidneys of other animal models can support our findings. Indeed, kaempferol prevented the reduction in rats' body weights and prevented cortical and hippocampal injury in $CdCl_2$-treated rats without improving food intake (Al-Brakati et al. 2021). Also, kaempferol prevented renal damage and attenuated the alterations in all serum and renal kidney-related markers in diabetic and aged rats, as well as in rats exposed to cisplatin, and tacrolimus (Al-Numair et al. 2015; Ali et al. 2020; Park et al. 2009; Sharma et al. 2020; Wang et al. 2020; Zhang et al. 2019).

Nonetheless, oxidative stress and inflammation are the two leading mechanisms underlying the genotoxic effect of $CdCl_2$ and are associated with suppression of Nrf2/antioxidant and activation of NF-κB signaling (Brzóska et al. 2016; Liu et al. 2019, 2015; Luo et al. 2017; Rani et al. 2014). This has been also confirmed in the $CdCl_2$-treated animals in this study. Herein, the data derived from this study shows that the observed nephroprotective properties of kaempferol involve antagonizing these damaging pathways. Besides, kaempferol has a potent stimulatory potential to enhance the expression and activity of the Nrf2/GSH/SOD axis while it causes repression of NF-κB in the kidneys of control rats too. This suggests that kaempferol is a potent activator of Nrf2 and an inhibitor of NF-κB under the basal and intoxicated conditions. In addition, our data strongly inspire that the stimulatory effect of kaempferol on Nrf2 is mediated via a downregulation of kepa1, which normally stimulates the proteasome degradation of Nrf2 in the cytoplasm (Deshmukh et al. 2017). Therefore, it seems reasonable that $CdCl_2$ suppresses the cellular antioxidants by either direct savaging through overproducing ROS or via modulating the keap1/Nrf2 axis. So far, the antioxidant potential of kaempferol seems to be mediated by downregulating kepa1 and subsequent transactivation of Nrf2. In support, Liu et al. (Liu et al. 2019) have also shown that $CdCl_2$ induces liver injury by activating NF-κB and keap1 and subsequent inhibition of Nrf2. Also, kaempferol is a potent stimulator of Nrf2 while being a common inhibitor of NF-κB (Saw et al. 2014). However, NF-κB and Nrf2 are negatively crossed-talked with each other (Wardyn et al. 2015). Therefore, it could also be possible that kaempferol stimulates Nrf2 by suppressing

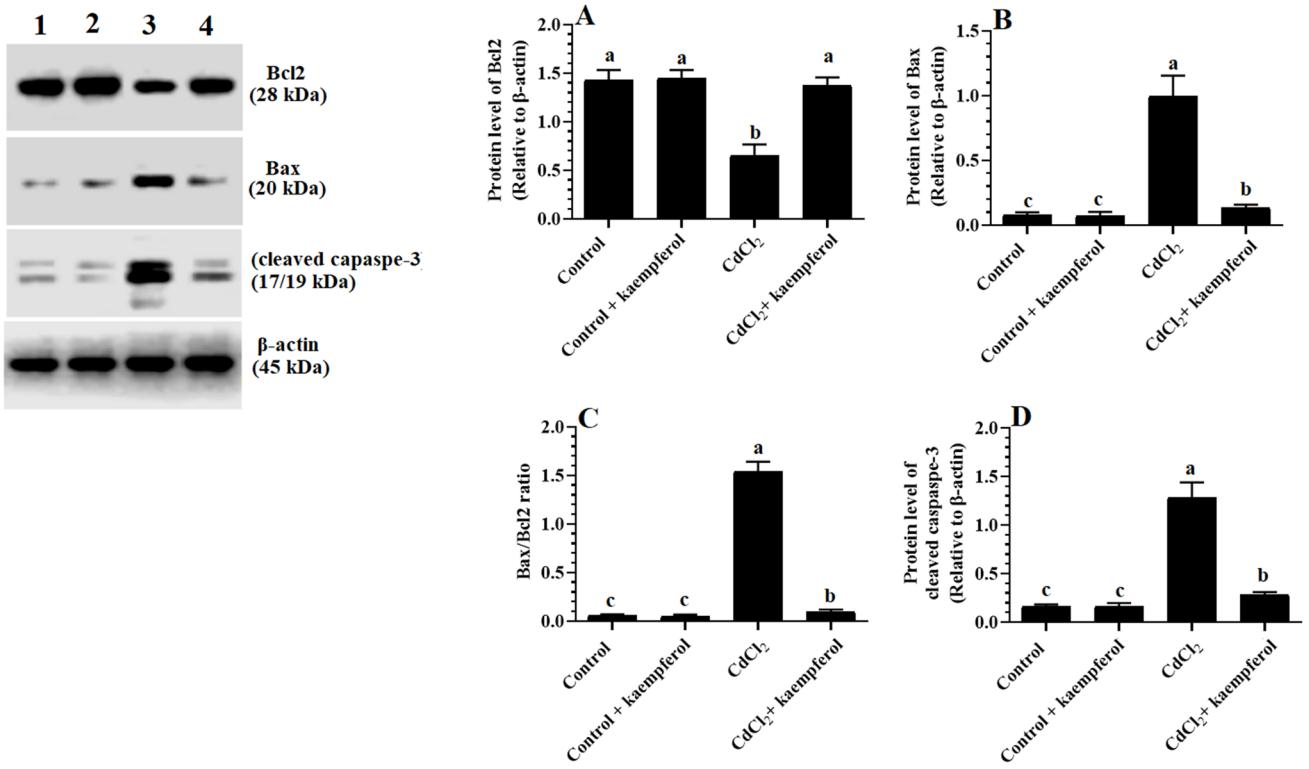

Fig. 4 Total protein levels of Bcl2 (**A**) and Bax (**B**), the ratio of Bax/Bcl2 (**C**), and cytoplasmic protein levels of cleaved caspase-3 in the kidneys of all groups of rats. Data were analyzed by two-way ANOVA followed by Tukey's *t*-test. All data are presented as mean ± SD ($n = 8$)

NF-κB p65 as shown here. However, the opposite is correct, and maybe kaempferol suppresses NF-κB p65 by activating Nrf2. This cannot be confirmed from these data and requires further investigation.

Supporting our report, pharmacological activation of Nrf2/antioxidant axis is a confirmed strategy to inhibit $CdCl_2$-induced reno-hepatic damage in rats (Diamond et al. 2019; Jiao et al. 2019; Pavón et al. 2019; Wu et al. 2012). Also, accumulating data demonstrates an exceptional ability of kaempferol to attenuate renal, cardiac, hepatic, and neural injury by its antioxidant and anti-inflammatory effects [22]. Similar stimulatory effects of Nrf2 that is coincided with increased antioxidant synthesis and reduced activity of NF-κB were seen in the brains of the control and $CdCl_2$-intoxicated rats after treatment with kaempferol (El-Kott et al. 2020a, 2020c). Also, kaempferol prevented chlorpyrifos-induced brain damage by upregulating/activating Nrf2 and subsequently increasing the antioxidant enzymes. In other animal models, kaempferol prevented doxorubicin (DOX)-induced mitochondria damage, carbon-tetrachloride CCL_4-induced hepatic damage, and streptozotocin (STZ)-induced hepatic apoptosis by upregulation of antioxidant and GSH enzymes (Wang et al. 2020). Also, kaempferol prevented cisplatin-induced nephropathy, isoproterenol-induced cardiomyocytes apoptosis in diabetic rats, and oxidative stress-induced umbilical vein endothelial cells (HUVECs) injury by inhibiting NF-κB and concomitant activation of Nrf-2 (Al-Numair et al. 2015; Zhang et al. 2019). Furthermore, kaempferol prevented aged kidney disease by suppressing NF-κB inflammatory cascade and apoptotic MAPKs pathways (P38 and JNK) (Park et al. 2009).

The mitochondria-mediated (intrinsic) cell death is the dominant cell death modality in the kidneys of rodents after intoxication with $CdCl_2$ (Almeer et al. 2019; Fujiwara et al. 2012; Joardar et al. 2019; Lee et al. 2006). Bcl2 is located on the mitochondrial outer membrane where it forms heterodimers with both Bad and Bax to inhibit their apoptotic effects and subsequent mitochondria leakiness and damage (Galluzzi et al. 2018). A higher cytoplasmic ratio of Bax/Bcl2 stimulates the opening of the mitochondria anion channel proteins which end up with the release of cytochrome-c and activating the caspase cascade (Galluzzi et al. 2018). In this investigation, we have confirmed the activation of intrinsic cell death in the kidney of $CdCl_2$-treated rats by the more expression of Bax and cleaved caspase-3 and the concomitant reduction in Bcl2 and the increase in Bax/Bcl2 ratio. Typical data have been previously shown in the kidneys of rats and chickens after $CdCl_2$ administration (Almeer et al. 2019; Bao et al. 2017; Joardar et al. 2019).

However, the potential of kaempferol to attenuate the increment of the expression of Bax and cleaved caspase-3 and to

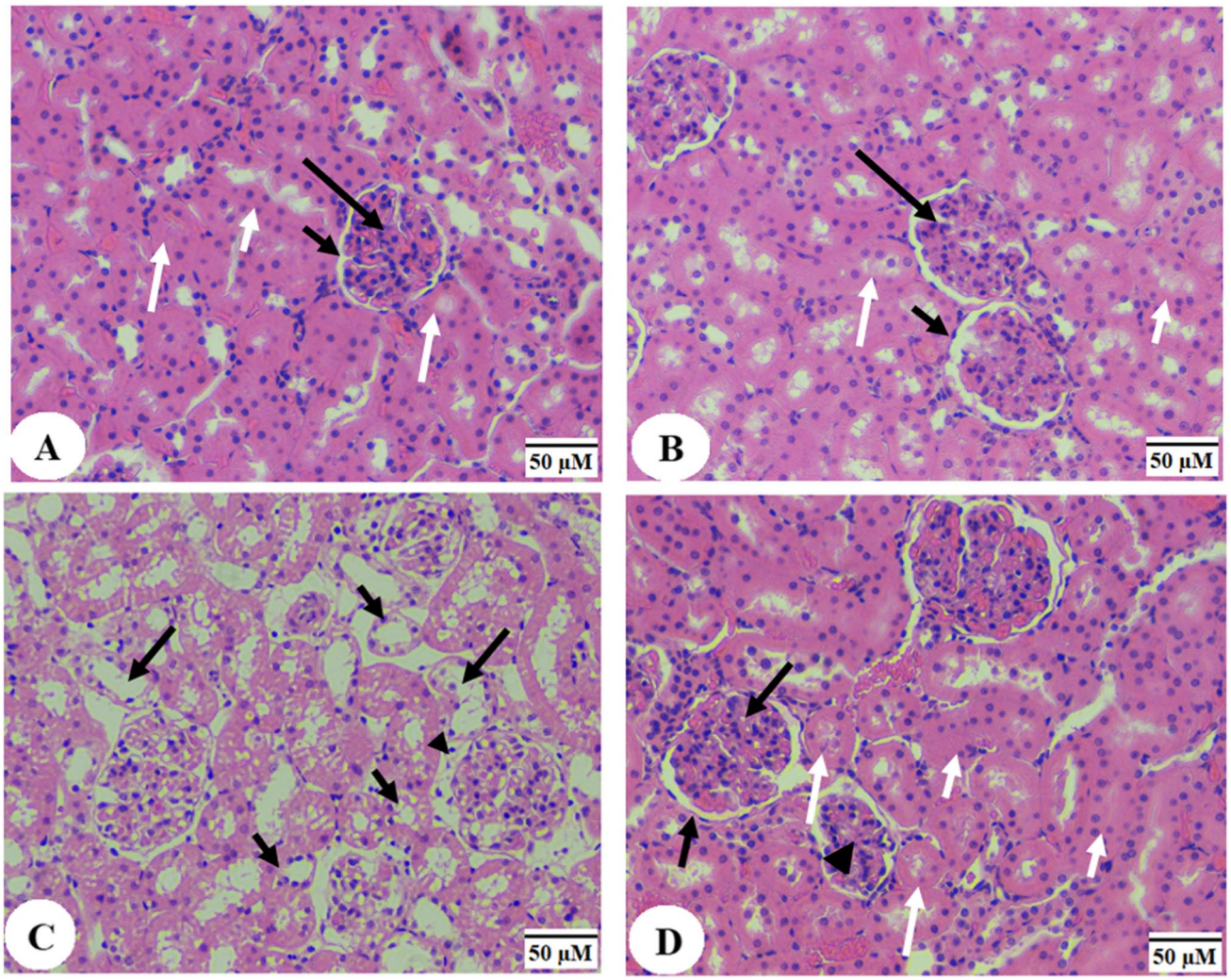

Fig. 5 Histological images of all experimental groups of rats. *A* and *B* were taken from control and control + kaempferol-treated rats and showed normal histological features with intact glomeruli (large black arrow), and glomerular membranes (short black arrow), proximal convoluted tubules (PCTs) having brush boarders (long white arrow), and distal convoluted tubules (DCTs) (short white arrow). *C* was taken from a $CdCl_2$-treated rat and showing decrease mesangial mass and damaged glomerular membrane (arrowhead) and severe damage in both the PCTs and DCTs. *D* was taken from a $CdCl_2$ + kaempferol-treated rats and showed almost normal architectures like those seen in the control group. However, some abnormalities in the shape of some glomeruli are still seen (star)

stimulate Bcl2 is the strongest evidence for its anti-apoptotic effect. However, kaempferol did not modulate the expression of any of these markers in the kidneys of control rats. These data suggest that the anti-apoptotic effect afforded by kaempferol is secondary to antioxidant and anti-inflammatory effects. Indeed, ROS can directly induce intrinsic cell death by upregulating p53/Bax axis and Bad (Redza-Dutordoir and Averill-Bates 2016). Also, ROS can activate mitochondria apoptosis by stimulating various MAPKs including p38 and JNK, which may also reduce the Bcl2 levels (Redza-Dutordoir and Averill-Bates 2016). In the same manner, the nuclear translocation of NF-κB triggers both intrinsic and extrinsic cell apoptosis by stimulating Bad and prompting its mitochondria translocation, as well as by raiseing the expression of FAS (Brzóska et al. 2016). Besides, TNF-α is an apoptotic cytokine that stimulates intrinsic renal cell death suppressing Bcl2 and Bcl-xL and activating of MAPKs (Campbell et al. 2008; Pastore et al. 2015).

Study limitations

Despite these data, this study still has some limitations. Importantly, our data are still observational. Besides, based on these data, it is still impossible to determine the upstream mechanism by which kaempferol affords its nephroprotection. Therefore, more focusing on these mechanisms using Nrf2 knockdown animals or cells will be more valuable. The used dose of kaempferol was based on a previously tested nephroprotective dose in diabetic rats. However, further

experiments using a dose–response curve are highly recommended. In addition, identifying other pathways regulating inflammation and oxidative stress such as AMPK and SIRT1 may present an excellent scope for future studies. This could be supported by the findings of others who have demonstrated evidence that kaempferol attenuates neural oxidative damage by activating SIRT1 and AMPK (El-Kott et al. 2020a). Finally, this study examined only the preventive effect of kaempferol against CdCl2-induced renal damage. However, it could be more valuable to examine this effect on the kidney structure and function, as well as on all measured markers at different time intervals. In addition, further studies examining the therapeutic effect in rats with pre-established nephropathy of the extract will expand our knowledge about such effect and the mechanisms of action of this drug.

Conclusion

Data obtained from these findings suggest the ability of kaempferol to mitigate $CdCl_2$-induced nephrotoxicity in animals mainly by attenuating oxidative stress and inflammation. However, these findings also demonstrate that the mechanism of action is due to activation of the keap1/Nrf2/antioxidant axis and the concomitant suppressing NF-κB p65. Given the high safety of kaempferol, these data encourage further preclinical and clinical studies to validate this effect in patients with CKD in a hope to discover a new safe, cheap, and effective drug. This could be correct knowing that the keap-1/Nrf2 axis is inhibited in the majority of kidney disorders.

Author contribution ASA, AFE, and AEE: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Supervision, Project administration, Funding acquisition, Writing—original draft. MSAZ, KM, RAG, MAD, MSM, and ETS: Conceptualization, Validation, Formal analysis, Investigation, Writing—original draft. ERE, EHI, HSK and AEA, MSM, HIMA: Conceptualization, Investigation, Methodology, Writing—review & editing. ASA, AFE, AEE, and EMA: Investigation, Methodology, Writing—review & editing.

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Data availability The data used to support the outcomes of this study are included within the article.

Declarations

Ethics approval All applicable international, national, and/or institutional guidelines for the care and use of animals and cell lines were followed and approved by the ethics committee at King Khalid University.

Consent to participate All authors equally participate in the study.

Consent for publication All authors allow the publication of the paper.

Conflict of interest The authors declare no competing interests.

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