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BIOLOGICAL SCIENCES

Involvement of redox status and the nuclear-related factor 2 in protecting against cadmium-induced renal injury with Sana Makki (*Cassia senna* L.) pre-treatment in male rats

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Abstract: This study was designed to investigate the potential defensive strategy of Sana Makki extract (SME) against Cd-induced *in vivo* nephrotoxicity and its underlying mechanisms. Male albino rats were used in a thirty days study comparing control, SME-treated, $CdCl_2$-treated, and combined SME and Cd treatment. Pre-treatment with SME significantly reduced serum kidney biomarkers (urea and creatinine), the concentration of renal KIM-1, and kidney index values. Additionally, SME also attenuated $CdCl_2$-induced oxidative and nitrosative stress in renal tissue; significantly reducing malondialdehyde (MDA) and nitric oxide (NO) concentrations and significantly increasing antioxidant enzymes in kidney tissue. Molecularly, SME significantly upregulated antioxidant gene expression (SOD2, GR, GPx1, and CAT) caused by Cd. Notably, the augmented mRNA expression of nuclear-related factor 2 (Nrf2) by Cd was enhanced by SME administration. SME markedly suppressed the Cd-induced rise in pro-inflammatory cytokines. The combination of Cd and SME relieved the Cd-induced apoptotic damage by enhancing Bcl2 and suppressing Bax and Cas-3 levels in renal tissue. The renal tissue histoarchitecture confirmed the biochemical and molecular findings. Collectively, our data indicate that SME can counteract Cd-induced renal intoxication through anti-oxidative, anti-inflammatory, and anti-apoptotic mechanisms.

Key words: Sana Makki, cadmium, apoptosis, oxidative stress, nephrotoxicity.

INTRODUCTION

Nephrotoxicity is a major reason for kidney injury and subsequently leads to renal disease, and finally renal failure. Exposure to environmental pollutants, particularly industrial ones, represents a significant public health concern (Almeer et al. 2019a). Cadmium (Cd) is an extremely toxic industrial metal and is considered the seventh most toxic agent among all known hazardous pollutants (Al Olayan et al. 2020). The main sources for Cd exposure are contaminated food, water, tobacco products, as well as the occupational risks from mining activities, electroplating, and working in the battery industry (Dkhil et al. 2014). Chronic Cd exposure causes the accumulation of this metal in proximal renal tubules of the kidney, its primary target organ (Elkhadragy et al. 2018). The Cd bound to metallothionein, a cysteine-rich metal-binding protein, is filtered through the glomeruli then presented for tubular reabsorption (Prozialeck et al. 2016). The majority of Cd is accumulated in the epithelial cells within the proximal tubules at a level of 450–600 µg/g dry weight and has a slow excretion rate of approximately 1-2 µg per day (Roels et al. 1983, Zhu et al. 2019).

The generation of free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS), leads to oxidative stress when their presence exceeds the antioxidant capacity of the cells to counterbalance their effects (Azab et al. 2013, Mumtaz et al. 2020). Almeer et al. (2019a) stated that Cd enhances the production of free radicals as well as suppresses the scavenging system in cells, resulting in oxidative stress, lipid peroxidation, and apoptosis by disturbing mitochondrial function and redox metabolism. Additionally, Cd can induce the inflammatory cascade, with a subsequent rise in interleukins and TNF-α levels. High TNF-α contributes to further renal damage through activation of the pro-inflammatory signaling pathways, vasoconstriction, and cytotoxicity (Mehaffey & Majid 2017).

A recent focus involves understanding the role of plant-based medicines with antioxidant properties to ameliorate the undesirable effects of drugs and xenobiotics. Sana Makki (*Cassia senna* L.), a widely used herbal drug with many pharmacological activities, is found in Africa, India, and Asia. All parts of *Cassia,* including leaves, pods, and fruits are being used pharmacologically (Wang et al. 2020). The leaves and pods are the main constituents of commercial herbal teas which are used for the treatment of constipation. Moreover, it can be used as a purgative, an antipyretic, or a diuretic (Farag et al. 2015, Balasankar et al. 2013). Farag et al. (2015) investigated the metabolic profiling of Sana Makki extract (SME) and many phytochemical molecules present in the extract have been shown to combat oxidative damage to DNA in vitro by stabilizing the $^{-}$OH moiety (Lin et al. 2014). Thus, the present study aimed to evaluate the toxic effects of cadmium chloride on male renal function and the possible nephroprotective role of SME in albino rats.

MATERIALS AND METHODS

Chemicals and reagents

Cadmium chloride was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

All other reagents used were of high analytical grade.

Plant material

Leaves and pods of Sana Makki were purchased from a local herbal seller in Riyadh, SA. The leaves and pods were identified and authenticated by a taxonomy specialist in the Botany Department at Saud University, Riyadh. After carefully washing and drying the plant material, they were pulverized into fine pieces using an electrical blender. Subsequently, the fine powder was extracted three consecutive times using 70% ethanol at 4°C at a 1:10 (w/v) ratio of plant powder to ethanol. The resulting alcoholic extract was filtered, concentrated in a rotary evaporator, and lyophilized to get rid of any remaining methanol. The final Sana Makki extract was a semi-solid that was stored at 4°C until use.

Animals and experimental protocol

Adult male albino rats (n=40), weighing 200 to 220 g (9-11 weeks old), were obtained from the Biological Department of Faculty of Science, at Princes Nourah bint Abdulrahman University. They were raised in plastic cages under optimal environmental conditions as follows: the temperature was 22-25°C, the humidity was 50-60%, and they were maintained in a 12-hour alternating light/dark cycle. Rats were fed a standard balanced diet with *ad libitum* access to food and water. All experimental procedures were performed at Princes Nourah bint Abdulrahman University (Riyadh, KSA; approval no, H-01-R-059/19-0168). The experimental rats were acclimatized for one week prior to the

experiment and then randomly allocated into four groups, with ten rats per group (n=10) as follows:

Group 1 (control; CTR): the rats were first administered orally 3 ml isotonic NaCl solution kg^{-1} day^{-1} and after 2 h they intraperitoneally (i.p.) injected with 1 ml isotonic NaCl solution kg^{-1} day^{-1} over 30 successive days.

Group 2 (Sana Makki extract; SME): the rats received 100 mg SME, kg^{-1} bw day^{-1} orally by gastric tube for 30 successive days. The selected dose was based on a pilot study conducted to mitigate $CdCl_2$ toxicity. The rats of this group were also received i.p. injection of 1 ml isotonic NaCl solution kg^{-1} day^{-1} after 2 h of SME administration.

Group 3 (Cadmium chloride; $CdCl_2$): the rats received $CdCl_2$ (dissolved in an isotonic NaCl solution) at a dose of 3.5 mg kg^{-1} bw day^{-1} via i.p. injections for 30 successive days (Kara et al. 2005), at the same time 2 h before the $CdCl_2$ injection, the rats were received orally 3 ml isotonic NaCl solution kg^{-1} day^{-1}.

Group 4 (a mixture of SME and $CdCl_2$): the rats were orally administered 100 mg SME kg^{-1} bw day^{-1} two hour before the i.p. injection of $CdCl_2$ at 3.5 mg kg^{-1} bw day^{-1} for 30 successive days.

One-day after receiving the last treatment, rats were euthanized with pentobarbital (400 mg/kg bodyweight). Blood samples were collected from the inferior vena cava of each rat, clots were allowed to form, and then samples were centrifuged. Serum was harvested and used in renal functional tests. The kidney was immediately dissected and weighted. Renal tissue was placed in ice-cold 50 mM Tris-HCl buffer (pH 7.4) and homogenates were generated using a tissue homogenizer, producing a 10% (w/v) tissue homogenate. The homogenate was centrifuged at 3,000 × g for 10 min at 4°C and the supernatant was stored at -70°C for biochemical tests.

Kidney functional and injury assays

Kidney functional biomarkers were measured in the serum samples using commercial kits from RANDOX Reagents (USA) for urea (Cat. No. UR3825) and creatinine (Cat. No. CR510) and were performed following the manufacturer's protocols.

Kidney injury molecule-1 (Kim-1) assay

The concentration of kidney injury molecule-1 (Kim-1) in renal tissue homogenates was assessed using an enzyme-linked immunosorbent assay (ELISA) kit purchased from Abcam (Catalog No. ab119597; Cambridge, UK). The manufacturer's instructions were followed when performing the test.

Kidney index

The kidney index was estimated using the following formula:

$$\text{Kidney index} = \frac{\text{Left kidney weight}}{\text{Bodyweight}} \times 100$$

Renal oxidative stress and antioxidant biomarkers

The activities of enzymatic antioxidant markers, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) were assessed using a spectrophotometer according to the methods described by Sun et al. (1988), Luck (1965), Paglia & Valentine (1967), and Factor et al. (1998), respectively. Additionally, the levels of non-enzymatic markers such as glutathione (GSH), in addition to the oxidative markers namely, malondialdehyde (MDA), and nitric oxide (NO) were measured based on the methods described by Ellman (1959), Ohkawa et. al. (1979), and Green et al. (1982), respectively.

Biomarkers for renal inflammation and apoptosis

The levels of the pro-inflammatory cytokines TNF-α (tumor necrosis factor-α) and IL-1β (interleukin-1β) were estimated using ELISA kits (Peprotech, Rocky Hill, NJ, USA) and following the manufacturer's instructions.

Apoptotic protein markers

The concentrations of Cas-3, Bax, and Bcl-2 were measured. Commercial ELISA kits (Elabscience, Houston, TX, USA) were used for measuring Bax and Bcl-2 according to the manufacturer's instructions. The caspase-3 concentration was estimated using a colorimetric assay (Sigma-Aldrich, St. Louis, MO, USA).

Gene expression studies

Total RNA was isolated from the renal tissue using TRIzol reagent (Sigma-Aldrich, St. Louis, MO, USA). Reverse transcription of the total RNA was performed immediately using the EasyScript Plus cDNA Synthesis Kit (ABM, Richmond, BC, Canada) to obtain cDNA. Real-time PCR was performed using Power SYBR Green (Life Technologies, CA, USA) on an Applied Biosystems 7500 instrument. The PCR program was as follows: 95°C for 4 minutes, 40 cycles of 95°C for 10 seconds, 60°C for 30 seconds, and 72°C for 10 seconds. The forward and reverse primers used in the PCR reactions were purchased from Sigma-Aldrich (Louis, MO, USA) and the NCBI Primer-BLAST program was used to design the primers. The relative gene expression of SOD2, CAT, GPx1, GR, Bcl2, Bax, Cas3, iNOS, and Nrf2 was determined in comparison with the control. Primer sequences and accession numbers used in the PCR reaction were previously published (Almeer & Abdel Moneim 2018). The housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was used as it had fixed concentrations in all treatments.

Histopathological and immunohistochemical examinations

Samples from the kidney tissue were immersed in 10% neutral-buffered formalin for 24 h at 25°C for fixation. The samples were embedded in paraffin and sliced into sections. The tissue sections (5 μm thickness) were deparaffinized and stained with hematoxylin and eosin (H&E) and caspase-3 antibody as described previously (Yuan et al. 2014). Kidney sections were examined under a light microscope (Kontron Electronic Imaging System KS300, Zeiss, Germany) for studying the pathological histoarchitecture at a magnification of 400×.

Statistical analysis

All the obtained data were analyzed using a one-way ANOVA followed by Duncan's post hoc test. *P* values less than 0.05 were considered to be statistically significant. The analyzed values were expressed as the mean ± standard deviation (SD).

RESULTS

Bodyweight and clinical signs

Neither mortalities nor abnormal signs were observed in all treated rats. However, Cd-treated rats consumed less food when compared to the controls or SME-treated rats. A non-significant drop in the bodyweight of Cd-treated rats was noticed compared to control rats.

Kidney function, kidney index, and Kim-1 assays

Intraperitoneal injections of rats with Cd for 30 days resulted in a significant elevation ($p<0.05$) in serum urea and creatinine levels compared to the controls. In addition, the Kim-1 concentration and kidney index were significantly higher ($p<0.05$) in Cd-treated rats compared to the

controls. Pre-treatment with SME before Cd treatment resulted in a significant decline ($p<0.05$) in all of the above mentioned renal parameters (Figure 1).

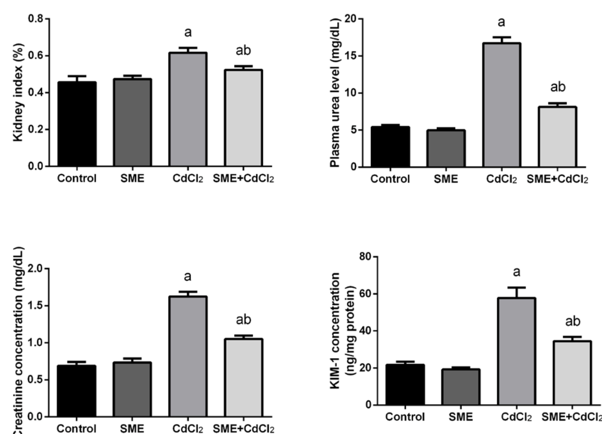

Figure 1. The kidney index, and serum level of kidney function markers (urea and creatinine) and kidney injury molecule-1 in adult male rats treated with $CdCl_2$ (3.5 mg/kg) and/or SME (100 mg/kg). Values are expressed as the mean ± SD (n = 10). [a]$p<0.05$ vs. the control group; [b]$p<0.05$ vs. the $CdCl_2$-treated rats.

Renal oxidative stress parameters

When compared to the control rats, the Cd-injected rats exhibited a significant reduction ($p<0.05$) in the renal activities of SOD, CAT, GPx and GR, and content of GSH, in addition to a significant elevation ($p<0.05$) in the MDA and NO content. In contrast, administration of SME shortly before $CdCl_2$ markedly improved the renal oxidative state. It significantly increased the antioxidant parameters and lowered the NO and MDA in renal tissue. Further, oral SME administration alone significantly increased the activity of CAT and the content of GSH only when compared to the control group (Figures 2, 3).

As shown in Figure 4, quantitative real-time polymerase chain reaction (qRT-PCR) analysis revealed that the fold change in gene expression of renal antioxidant genes showed marked downregulation ($p<0.05$) in Nrf2, SOD2, CAT, GPx1, and GR mRNA following Cd treatment compared to control rats. Meanwhile, the rats exposed to both Cd and SME exhibited upregulated gene expression of Nrf2, SOD2, CAT, GPx1, and GR compared to the rats injected with Cd only. The treatment with SME alone enhanced gene expression of Nrf2 and SOD2 in renal tissue compared to the control group (Figure 4).

Kidney inflammatory assays

Cd-intoxicated rats demonstrated markedly high levels of renal IL-1β and TNF-α compared with control rats. Similarly, iNOS mRNA expression in renal tissue was significantly upregulated after Cd treatment. Contrastingly, pre-treatment with SME significantly reduced levels of both renal inflammatory cytokines and iNOS mRNA expression (Figure 5).

Apoptotic protein markers

Cd intoxication was associated with a significant disruption to apoptotic signaling pathways. A significant increase ($p<0.05$) in renal tissue Bax concentrations was noticed, while Bcl-2 levels declined ($p<0.05$) in Cd-treated rats compared to the control group. Further, significant increases ($p<0.05$) in mRNA expression of Bax and Cas3 were detected with a significant downregulation of Bcl2 after Cd injection in respect to the control group. These alterations were notably reversed following SME administration shortly before $CdCl_2$ (Figure 6). Constant with the biochemical and molecular findings, caspase-3 detected with immunohistochical method was upregulated with Cd injection (Figure 7c) and downregulated with SME pretreatment (Figure 7d).

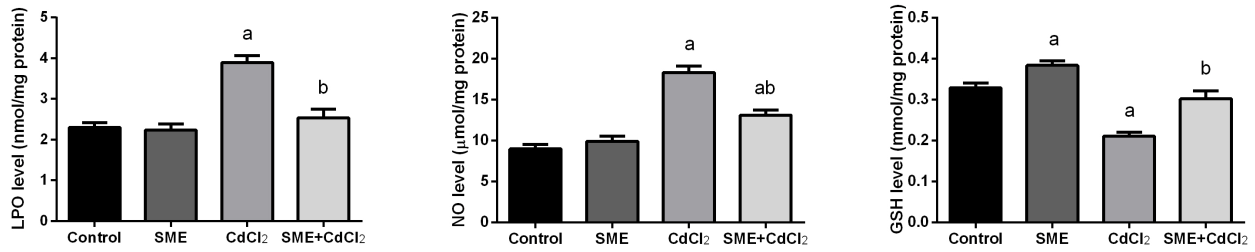

Figure 2. The oxidative indices (malondialdehyde (MDA), nitric oxide, and glutathione) in the renal tissue of adult male rats treated with $CdCl_2$ (3.5 mg/kg) and/or SME (100 mg/kg). Values are presented as mean ± SD (n = 10). $^a p < 0.05$ compared to the control group; $^b p < 0.05$ compared to the $CdCl_2$-alone treatment group.

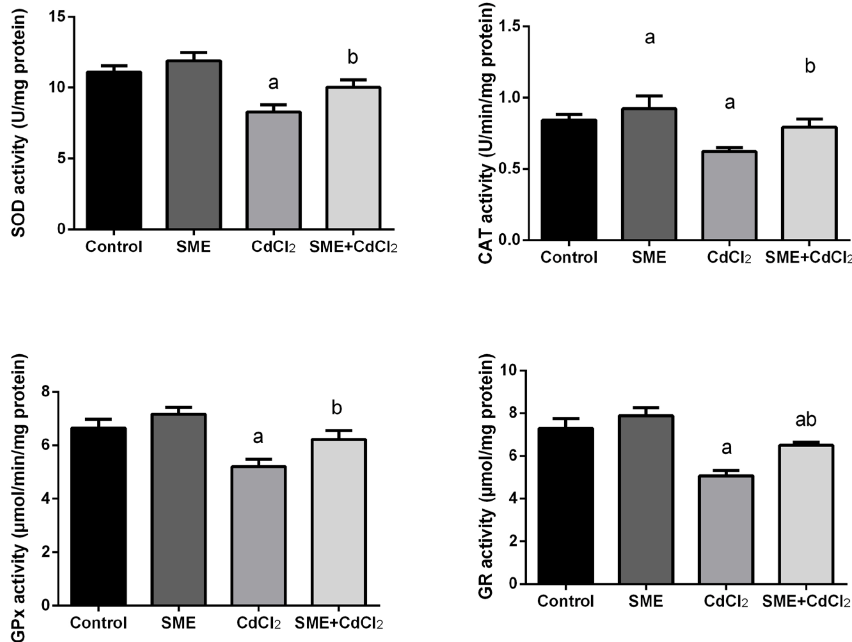

Figure 3. The activity of superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase in the kidney of adult male rats treated with $CdCl_2$ (3.5 mg/kg) and/or SME (100 mg/kg). Values are presented as mean ± SD (n = 10). $^a p < 0.05$ compared to the control group; $^b p < 0.05$ compared to $CdCl_2$-alone treatment group.

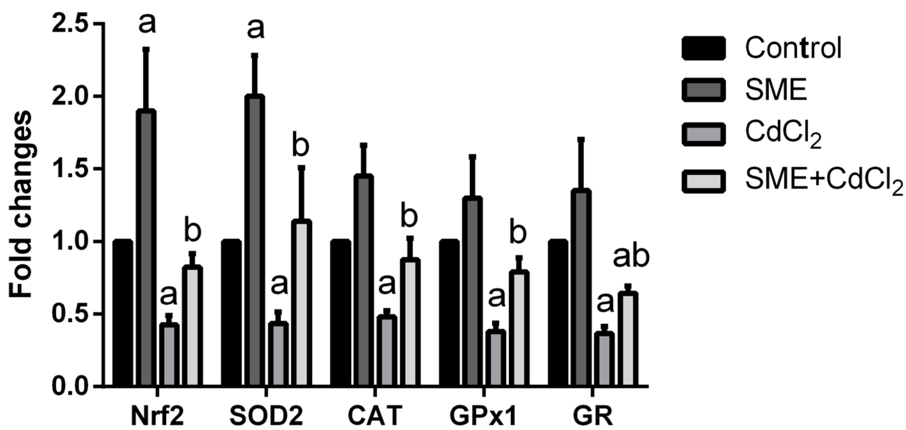

Figure 4. The Nrf2/ARE antioxidant signaling pathway gene expression in the renal tissue of adult male rats treated with $CdCl_2$ (3.5 mg/kg) and/or SME (100 mg/kg). Values are presented as mean ± SD of two assays and normalized to GAPDH and expressed as fold change (log2 scale) relative to mRNA levels in controls; $^a p < 0.05$ compared to the control group; $^b p < 0.05$ compared to the $CdCl_2$-alone treatment group.

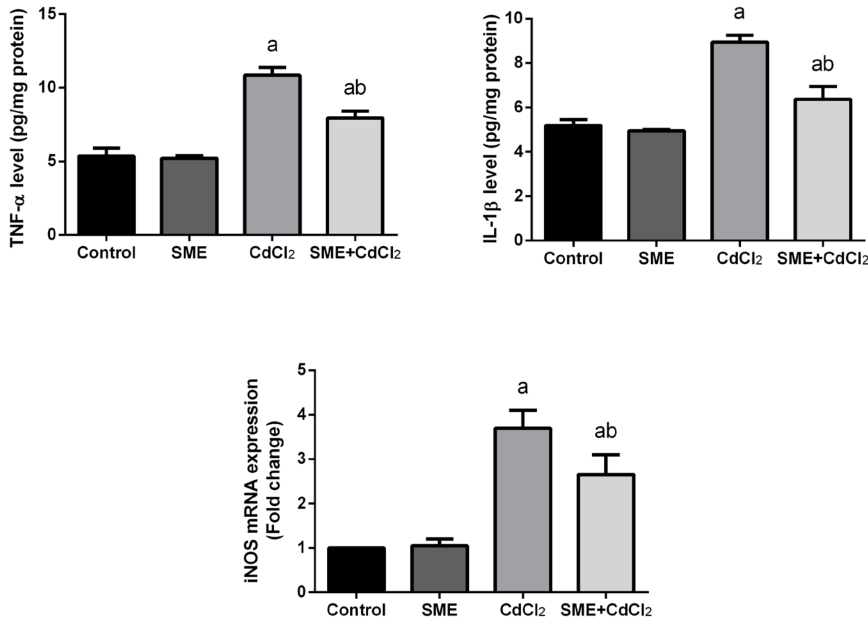

Figure 5. Pro-inflammatory cytokine production (TNF-α and IL-1β) and iNOS mRNA expression in the renal homogenate of adult male rats treated with $CdCl_2$ (3.5 mg/kg) and/or SME (100 mg/kg). Values are presented as mean ± SD (n = 10). $^{a}p < 0.05$ compared to the control group; $^{b}p < 0.05$ compared to the $CdCl_2$-alone treatment group. iNOS mRNA expression is presented as mean ± SD of two assays relative to GAPDH in the control group.

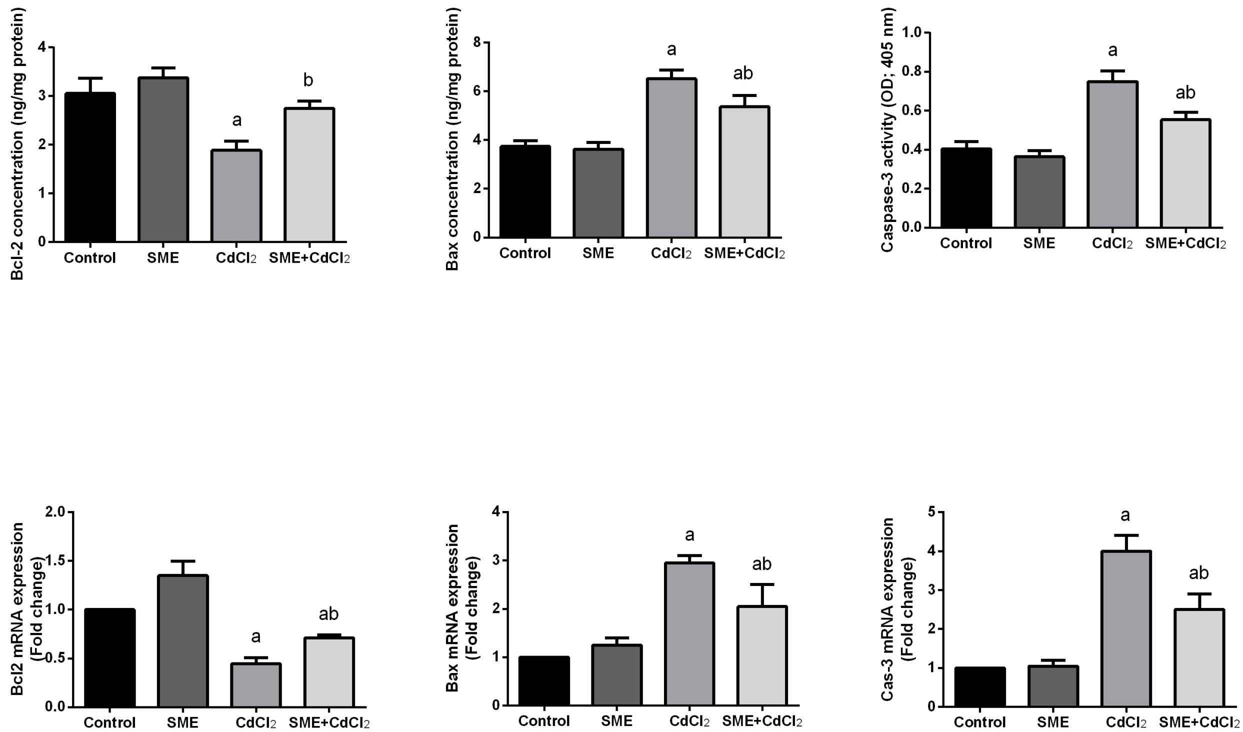

Figure 6. The apoptotic markers (Bcl-2, Bax, and caspase-3) and Bcl2, Bax, and Cas-3 mRNA expression in the renal homogenates. Values are presented as mean ± SD (n = 10). $^{a}p < 0.05$ compared to the control group; $^{b}p < 0.05$ compared to the $CdCl_2$-alone treatment group. Cas-3, Bax and Bcl2 mRNA levels are presented as mean ± SD of two assays relative to GAPDH in the control group.

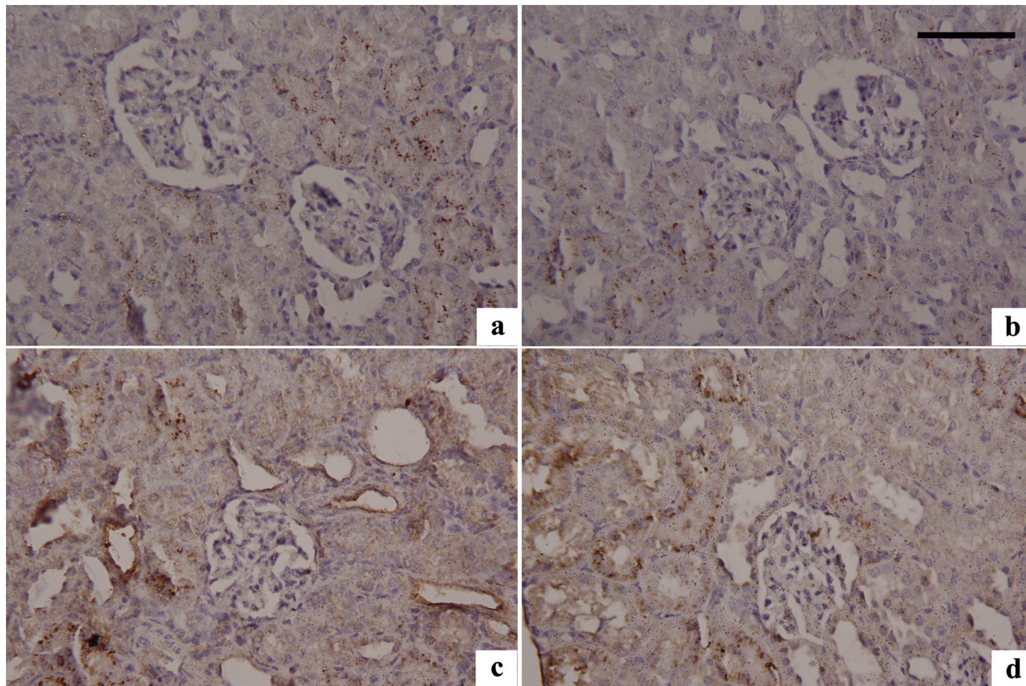

Figure 7. The expression of caspase-3 in the renal tissue of adult male rats treated with $CdCl_2$ (3.5 mg/kg) and/or SME (100 mg/kg). (a) Control group; (b) SME administered group; (c) $CdCl_2$ treatment group; (d) SME+$CdCl_2$ treatment group; scale bar = 100.

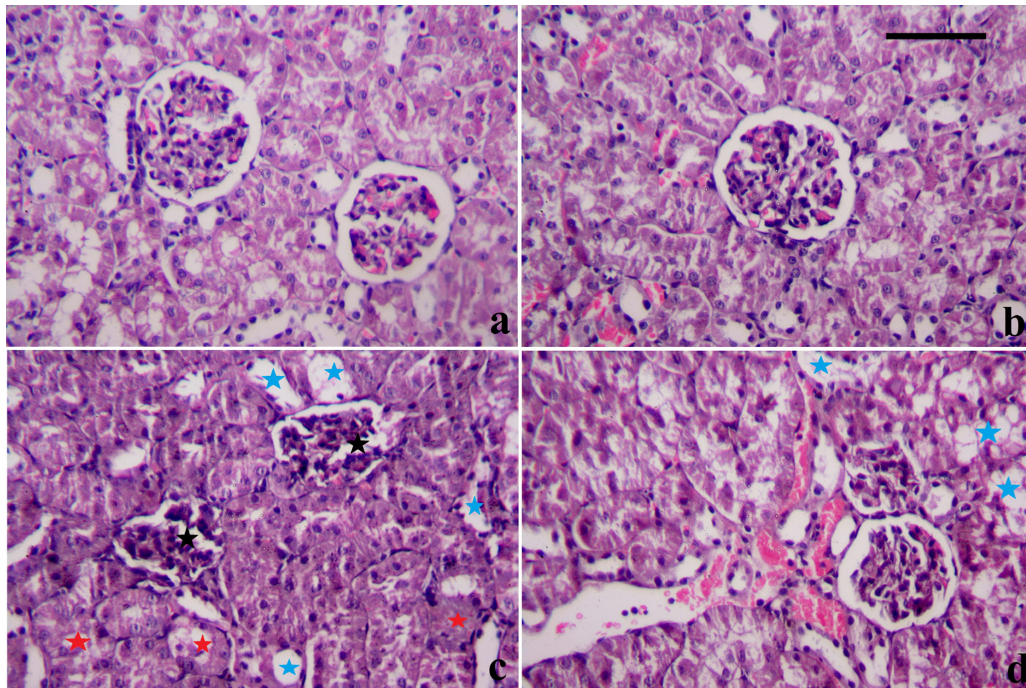

Figure 8. The histopathological alterations in the renal tissue of adult male rats treated with $CdCl_2$ (3.5 mg/kg) and/or SME (100 mg/kg). (a) Control group; (b) SME administered group; (c) $CdCl_2$ treatment group; (d) SME+$CdCl_2$ treatment group; scale bar = 100.

Histopathological findings

The control and SME-treated alone groups showed normal morphology with no evidence of histopathological changes in the renal tissue (Figure 8a and 8b). However, the rats intoxicated with $CdCl_2$ showed severe alterations in the renal tubules and glomeruli, including hydropic degeneration (blue star) and hypertrophy of the proximal tubular epithelium (red star), tubular swelling, glomerular shrinkage (black

star), and cellular vacuolization. In addition, desquamation and subsequent degeneration of cells and focal necrosis were detected in some of the tubules (Figure 8c). In contrast, SME pre-treatment minimized the changes of $CdCl_2$ in renal tubules and glomeruli (Figure 8d).

DISCUSSION

The kidney is a vital organ responsible for the eradication of metabolic waste products from the body, management of body fluids, and maintenance of electrolyte balance (Sindhu et al. 2015). Cd is a heavy metal and represents one of the most hazardous pollutants, particularly in industrialized countries. Chronic Cd exposure results in injuries to multiple organs, especially the kidney. Cd can persist in the body for up to 30 years due to its prolonged half-life (Wongmekiat et al. 2018). Previous studies demonstrated the negative impact of Cd exposure, including oxidative status, inflammation, mitochondrial damage, and apoptosis in renal tissue (Almeer et al. 2019b, Rani et al. 2014, Ansari et al. 2017, Joardar et al. 2019).

In the current study, the pre-treatment with SME was tested to determine whether it can protect the renal system from the toxicological insult of Cd. The results showed that Cd injections for 30 days resulted in marked alterations in renal structure and function, with an increase in oxidative stress, inflammation, and apoptosis. Further, the nephroprotective efficacy of SME reversed the renal damage created by Cd injections.

After absorption, Cd exists in the blood in two forms, free Cd and Cd-MT complexes. This complex undergoes glomerular filtration and reabsorption by the proximal tubule via endocytosis. In the epithelial cells of the proximal tubules, the acid protease dissociates the Cd-MT complexes liberating cadmium, which is transported back to the cytosol of the proximal tubule by the aid of Zn^{2+} and/or Cu^{2+} transporters (Thevenod 2003). Previous studies concluded that the majority of the transported Cd was detected in the epithelial lining of the proximal tubules. According to the findings of Almeer et al. (2019b) and Jafarpour et al. (2017), Cd concentrations increased significantly in Cd-injected rats compared to the control. Farag et al. (2015) detected the existence of flavonoids, such as kaempferol, quercetin, and isorhamentin, in the ^{1}H-NMR spectrum of *Cassia senna*. Furthermore, Cherrak et al. (2016) stated that such flavonoids can bind with metals in human red blood cells in vitro.

The present study supports the results of previous studies (Poontawee et al. 2016, Wongmekiat et al. 2018, Sadek et al. 2017), where the Cd injection elevated serum biomarkers of renal malfunction (urea and creatinine) as well as the renal Kim-1 concentrations and kidney index in respect to control. Kim-1 is a transmembrane protein that is not detectable in the healthy kidney. However, with renal injury, it is upregulated and is detected in the urine (Yin et al. 2020). It was reported that Cd exposure enhances the expression of Kim-1, even at a mild stage of apoptosis in the proximal renal tubule and before the onset of cell death (Prozialeck et al. 2009). Moreover, the present renal histopathological findings showed severe damage in renal tissue treated with Cd owing to its disturbing effect on glomerular filtration and tubular reabsorption. Meanwhile, pre-administration with SME markedly augmented the adverse impacts of Cd on renal function and morphology.

Cadmium induced its nephrotoxicity via indirect enhancement of ROS and RNS formation and exhaustion of anti-oxidant defenses (Sanjeev et al. 2019). The marked rise

in tissue MDA along with NO concentration were indications of oxidative and nitrosative stress created by Cd. Due to the competition on the binding site, Cd can displace iron resulting in a high content of free redox-active ions. These free redox metals directly boost the generation of hydroxyl radicals via the Fenton reaction (Valko et al. 2016). Further, Cd enhances the NADPH oxidase activity resulting in the production of superoxide anion and upregulation of inducible nitric oxide synthase, which synthesizes excess NO and active nitrogen for increased lipid peroxidation (Li et al. 2017). In parallel with the high MDA and NO, significant suppression of SOD, CAT, GPx, and GR activities and GSH levels were detected after Cd exposure. Glutathione is the major thiol antioxidant and is considered to be the primary line of defense against Cd toxicity. Cd has a high affinity for thiol-containing compounds. Therefore, GSH is primarily attacked by free Cd-ions (Cuypers et al., 2010). Cd can also substitute the Zn^{2+} bound to SOD, increasing the free radical output and reducing its antioxidant activity. Catalase is an iron-containing enzyme responsible for hydrogen peroxide (H_2O_2) detoxification by converting H_2O_2 into water and divalent oxygen (Cuypers et al. 2010). The present findings revealed obvious exhaustion of the antioxidant capacity, leading to an overwhelming overproduction of free radicals induced by Cd. These results are in concordance with previous studies (Wongmekiat et al. 2018, Sadek et al. 2017, Li et al. 2017).

Additionally, the fold-change in mRNA expression of antioxidant genes were assessed by qRT- PCR in kidney tissue to provide a better understanding regarding the molecular mechanism that regulates various enzymatic activities. Our results illustrated that there was a significant downregulation in mRNA for SOD2, CAT, GPx1, and GR following Cd injections. Nuclear factor erythroid 2-related factor 2 (Nrf2) regulates the gene expression of antioxidant response element (ARE)-dependent genes, which guard cells against oxidative damage (Almeer et al. 2019b). Nrf2 is a key factor in commencing Nrf2/ARE signaling pathway, one of the major antioxidant pathways. Normally, Nrf2 exists in an inactive form in the cytosol, as it is bound to Kelch-like ECH-associated protein 1 (keap1). During oxidative stress, the Nrf2 transcription factor is activated by its liberation from the Nrf2/keap1 complex. The free Nrf2 then moves to the nucleus and binds to the ARE and activates targets encoding antioxidant enzymes (Rao et al. 2010). In accordance with other authors (Rao et al. 2010, Yang et al. 2019), Cd exposure induced activation of the Nrf2/ ARE signaling pathway to protect the cellular components against oxidative insults.

Remarkably, the pre-treatment with SME before the Cd injection significantly reduced oxidative and nitrosative stress by enhancing the enzymatic and non-enzymatic antioxidant parameters as well as antioxidant gene expression levels. Flavonoids, the active compounds in SME, act as essential scavengers of free radicals through their ability to stabilize (Cherrak et al. 2016). The redox properties of flavonoid phenolic hydroxyl groups can be easily oxidized and lead to activation of detoxifying enzymes such as NAD(P)H-quinone oxidoreductase, GST, or UDP-glucuronosyl transferase, which all fight against cellular oxidative stress (Burda & Oleszek 2001, Nijveldt et al. 2001, Heim et al. 2002).

Supporting the results of former investigations (Sanjeev et al. 2019, Wongmekiat et al. 2018, Turley et al. 2019, Olszowski et al. 2012, Phuagkhaopong et al. 2017), this study revealed significant increases in the serum pro-inflammatory cytokines and renal iNOS gene expression following Cd treatment. TNF-α is produced by macrophages and considered to self-feed the inflammatory cycle because it

can start and control the expression of other pro-inflammatory genes involved its expression (Vazquez Prieto et al. 2015). IL-1β is mainly produced by bone marrow mononuclear cells and is rapidly synthesized and released by inactive tissue as a proactive cytoplasmic precursor (pro-IL-1β) when a pathogen triggers a Toll-like receptor (TLR) or other pattern recognition receptor (Dinarello 2018). Oxidative events can initiate an inflammatory cascade within the cell via the nuclear factor kappa-activated B cell (NF-κB) pathway, thus, activating pro-inflammatory cytokines. Previous reports showed that the high TNF-α levels elicit renal malfunction by direct cytotoxicity, limiting the blood supply, which triggers the inflammatory cycle and subsequently affects tubular function (Mehaffey & Majid 2017). Interestingly, SME could significantly reverse the elevation of IL-1β, TNF-α levels, and iNOS gene expression caused by Cd exposure. This may be attributed to the presence of anthraquinone substances that can be isolated from *Cassia* seeds and possess anti-inflammatory activities through modulation of the NF-κB pathway (Hou et al. 2018).

Apoptosis is a form of programmed cell death which can be induced by various stimuli, including physiologic and pathologic, causing a disruption in tissue homeostasis and affecting organ function. Caspases play a major role in mediating apoptotic death as they regulate the associated cellular alterations. Caspases are activated through two distinct pathways, which include the intrinsic (mitochondrial-dependent) pathway and the extrinsic (death receptor-dependent) pathway (Khafaga & El-Sayed 2018). The equilibrium between B-cell lymphoma-2 (Bcl-2) as an anti-apoptotic protein and Bcl-2-associated X (Bax) as a pro-apoptotic protein is essential for tissue homeostasis. Bcl-2 suppresses the deleterious impacts of ROS by preventing cytochrome-c liberation in the cytoplasm. Additionally, Bcl2 gene expression is increased was concomitantly high glutathione levels (Sadek et al. 2017). Cd can induce apoptosis in tissues and in vitro cell culture (Wongmekiat et al. 2018, Sadek et al. 2017, Teng et al. 2019, Liu et al. 2016). In accordance with other Cd-intoxication studies, this study revealed that Cd markedly upregulated Bax and Cas-3 and downregulated Bcl2, but after pre-treatment with SME a significant improvement was observed. This is consistent with a study which reported that *Cassia* species have anti-apoptotic properties by limiting the ROS formation and causing anti-inflammatory effects (Sobeh et al. 2017).

CONCLUSIONS

Collectively, the findings of the present study explained the nephroprotective efficacy of SME against renal injury elicited by 30-days Cd injections in male rats. This ameliorative effect was mediated by inhibition of lipid peroxidation, enhancement of antioxidant enzymes, and upregulation of genes related to antioxidant and apoptosis mechanisms, minimizing the damage to DNA and other cellular molecules. Therefore, SME can be used as a remedy to improve Cd-induced renal malfunction and toxicity in rats.

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