Research Article

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Hesperidin and eugenol attenuate cadmium-induced nephrotoxicity via regulation of oxidative stress, Bax/Bcl2 and cleaved caspase 3 expression

[Hesperidin ve öjenol, oksidatif stres, Bax/Bcl2 ve bölünmüş kaspaz 3 ekspresyonunun düzenlenmesi yoluyla kadmiyum kaynaklı nefrotoksisiteyi zayıflatır]

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Abstract

Objectives: Cadmium (Cd) is a heavy metal that induces nephrotoxicity through stimulating the oxidative stress in kidney cells. In this study, we investigated the protective effects of hesperidin and eugenol on Cd-induced nephrotoxicity and their impact on modulating oxidative stress, Bax/Bcl2 ratio and cleaved caspase 3 level.

Methods: Male Wistar rats were divided into: Normal group; Cd-treated group; hesperidin & Cd-treated group and eugenol & Cd-treated group. Rats received CdCl₂ (5 mg/kg body weight/daily) via oral gavage for four weeks to induce kidney toxicity.

Results: The results showed that, both hesperidin & Cd and eugenol & Cd- treated groups exhibited lower serum creatinine and urea levels as well as better kidney tissue

integrity compared to the Cd-treated group. Moreover, they could preserve the cellular antioxidants to normal levels as was seen in a significantly lower malondialdehyde content, but significantly higher catalase and superoxide dismutase activities compared to the Cd-treated group. Furthermore, both groups significantly decreased the Cd-induced elevation in Bax/Bcl2 ratio and cleaved caspase 3 level.

Conclusion: Administration of hesperidin or eugenol effectively protected from the structural and functional kidney damage induced by Cd exposure via antioxidant and anti-apoptotic effects.

Keywords: apoptosis; Bcl2; cleaved caspase 3; heavy metal; oxidative stress.

Introduction

Environmental pollutants such as heavy metals represent a major health problem worldwide. Cadmium (Cd) is one of these heavy metals that is characterized by a long biological half-life (about 30 years in humans) and a low rate of excretion from the body [1]. Accumulation of Cd in the human body is very toxic to many organs such as liver, lung, kidney, brain and testis. However, Cd toxicity affects mainly the kidneys since 50% of the Cd that enters into the human body is deposited in the kidneys inducing nephrotoxicity that progresses into end-stage renal failure [2]. The toxic effects of Cd can be mediated via various mechanisms. Remarkably, Cd toxicity involves the production of reactive

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oxygen species (ROS) such as hydrogen peroxide, hydroxyl radicals and superoxide ions both *in vitro* and *in vivo*. This increased ROS production overwhelms the cellular antioxidant defence mechanisms leading to DNA damage and lipid peroxidation [3]. Moreover, it was found that, Cd disrupts the mitochondrial membrane permeability, inhibits the electron transport chain and impairs DNA repair mechanisms [4].

Apoptosis plays an important role in the control of kidney diseases. On the molecular level, Bcl2 family comprises many proteins that regulate the apoptosis in addition to other cellular processes such as mitochondrial metabolic rate, *de novo* biogenesis and organelle dynamics [5]. Bcl2 proteins are classified into two categories: anti-apoptotic proteins and pro-apoptotic proteins. Bcl2 (B cell lymphoma/leukemia 2) forms a homodimer and it functions as an anti-apoptotic protein to promote cell survival. On the other hand, Bax (Bcl2-associated protein X), that has sequence homology to Bcl2, is a pro-apoptotic protein and it promotes cell death. Hence, Bax/Bcl2 ratio serves as a determinant factor in the development of apoptosis and subsequent renal fibrosis [6].

Interestingly, oxidative stress plays a vital role in apoptosis induction. It was found that, Cd affects all apoptotic pathways since it stimulates the extrinsic apoptotic pathway leading to caspase 8 activation and it also stimulates the intrinsic apoptotic pathway through mitochondrial damage leading to caspase 9 activation. Moreover, Cd induces caspase independent apoptosis via stimulating the endonuclease activity of apoptosis inducing factor (AIF) and protease activity of calpain [7]. Remarkably, the oxidative stress induced by Cd activates the expression of activator protein-1 (AP-1), nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B) and mitogen-activated protein kinase (MAPK) which regulate the expression of apoptotic and proinflammatory genes both in cultured cells and in animals [8, 9].

Hence, natural products, particularly the antioxidants, are viewed as therapeutic and/or protective agents that may diminish the Cd toxicity.

Hesperidin (Hesp; 3',5,7-trihydroxy-4'-methoxy-flavone-7-rhamnoglucoside) is a flavone glycoside derived from citrus fruits. It is composed of a disaccharide, rutinose, and aglycone part, hesperetin [10]. It was found that Hesp possesses anti-inflammatory, antioxidant, anti-allergic, anti-carcinogenic and neuroprotective activities. Interestingly, Hesp does not only exhibit free radical scavenging activity but it also affects the other antioxidant defence mechanisms [11].

Eugenol (Eug; 4-allyl-2-methoxy phenol) is a phenolic compound derived from the natural oils found in cinnamon, clove, nutmeg and basil [12]. Interestingly, Eug

was reported to have antioxidant, analgesic and antibacterial activities [13].

In the present study, we investigated the possible protective effects of Hesp and Eug on Cd-induced nephrotoxicity. In addition, we investigated the impact of these drugs on modulating oxidative stress, Bax/Bcl2 ratio and cleaved caspase 3 expression levels.

Materials and methods

Animals

Male albino Wistar rats weighing from 150–180 g (6 weeks old) were purchased from the animal breeding house of Nahda University, Beni-Suef, Egypt. Animals were kept under standard conditions of a 12/12 h light-dark cycle, 25 $\pm$ 2 °C temperature and 60 $\pm$ 10% humidity. Animals had access to water and food *ad libitum*.

Chemicals

Cadmium dichloride (CdCl₂, 98%) was purchased from LOBACHemie, India. Eugenol (E51791, 99%) and Hesperidin (H5254, $\geq$ 80%) were purchased from Sigma-Aldrich, St. Louis, USA.

Experimental design

Thirty-two animals were divided into four groups (eight rats each) as follows:

Normal group: Rats received the drug vehicles (equal volumes of 2% tween 80 dissolved in saline and corn oil) daily for four weeks via oral gavage.

Cd-treated group: Rats received CdCl₂ dissolved in saline (5 mg/kg body weight/daily) via oral gavage for four weeks. The dose of CdCl₂ was selected based on the previously published studies [14, 15].

Hesp+Cd-treated group: Rats received Hesp (200 mg/kg body weight/daily) suspended in 2% tween 80 in saline via oral gavage as the same dose used in [16–18]. Two hours after Hesp dose, rats received the same dose of CdCl₂ as Cd-treated group daily for four weeks.

Eug+Cd-treated group: Rats received Eug (80 mg/kg body weight/daily) dissolved in corn oil via oral gavage as the dose used by [19]. Two hours after Eug dose, rats received the same dose of CdCl₂ as Cd-treated group daily for four weeks.

Serum and tissue preparation

At the end of the experimental period, rats were anesthetized after an overnight fasting and blood samples were collected from retro-orbital plexus using non heparinized capillary tubes. Serum samples were prepared by centrifugation at 3000 $\times$ g for 20 min and collecting the supernatants for the assessment of kidney functions. After sacrificing rats by decapitation, kidneys were immediately dissected out and washed with saline. To prepare 25% w/v tissue homogenates, one part of the kidney was homogenized in phosphate buffered saline and centrifuged at 3000 $\times$ g for 20 min at 4 °C. The supernatants were then

collected and stored at -80°C until analysis. Another part of the kidney was washed with saline then immersed in 10% formal saline (10 mL formalin+90 mL neutral saline) for histopathology and immunohistochemistry analysis.

Determination of kidney functions

The serum creatinine and blood urea nitrogen (BUN) concentrations were measured colorimetrically by kits provided by Diamond Diagnostics, Germany according to the provided protocols.

Determination of the oxidative stress parameters

Determination of malondialdehyde (MDA) content: The kidney tissue homogenates were used to measure MDA content by spectrophotometric MDA assay kit provided by Biodiagnostics, Giza, Egypt. Briefly, it depends on the interaction of MDA with thiobarbituric acid in acidic pH and 95°C . The produced thiobarbituric acid reactive substances (TBARS) were measured colorimetrically at 534 nm according to [20]. MDA concentration was expressed as nmol/g wet tissue.

Determination of superoxide dismutase (SOD) activity: SOD activity was determined in the kidney tissue homogenates using spectrophotometric SOD assay kit provided by Biodiagnostics, Giza, Egypt. Briefly, the assay depends on SOD inhibition of nitroblue tetrazolium reduction mediated by phenazine methosulphate at 560 nm based on the method of [21]. SOD activity was expressed as U/g wet tissue.

Determination of catalase (CAT) activity: CAT activity was measured in the kidney tissue homogenates by spectrophotometric CAT assay kit provided by Biodiagnostics, Giza, Egypt. CAT helps in H_2O_2 hydrolysis into O_2 and H_2O . The remaining amount of H_2O_2 was measured colorimetrically at 510 nm based on the method mentioned in [22]. CAT activity was expressed as U/g wet tissue.

Histopathological examination of kidney tissues

Kidney tissue samples were dissected out, washed with normal saline and fixed in 10% formal saline (10 mL formalin+90 mL neutral saline) for 72 h. Samples were then trimmed and dehydrated in alcohol, cleared in xylene, in filtered in synthetic wax and finally blocked out into Paraplast tissue embedding media. $5\text{ }\mu\text{m}$ thick sections of each sample were cut by a rotatory microtome. The sections were stained with hematoxylin and eosin (H&E) stain according to the previously published protocol [23]. Slides were examined under a Nikon microscope at $400\times$ magnification by a skilled experimentally blinded observer. Scores were given to each sample to determine the degree of kidney injury.

Immunohistochemistry analysis of Bax and Bcl2

Deparaffinized $5\text{ }\mu\text{m}$ thick tissue sections were treated by 0.03% H_2O_2 for 20 min, followed by incubation with Bax monoclonal antibody (Cat #: MA5 -14003, Thermo Fisher Scientific, USA) and Bcl2 polyclonal antibody (Cat #: PA1-30411, Thermo Fisher Scientific, USA) separately

overnight at 4°C . Slides were then washed and incubated with secondary antibody (HRP Envision kit, DAKO, Agilent, USA) for 20 min. After that, slides were washed with PBS and incubated with 3,3'-diaminobenzidine (DAB, Sigma Aldrich, USA) for 10 min. Finally, slides were washed thoroughly by PBS, counterstained with hematoxylin, dehydrated and cleared in xylene then coverslipped for microscopic examination. For quantification, five non-overlapping fields ($400\times$ magnification) were randomly selected in each $5\text{ }\mu\text{m}$ section for determination of the immuno-expression levels of Bax and Bcl2. Morphological measurements were analyzed and photographs were taken using Leica Application Suite attached to Full HD microscopic imaging system (Leica Microsystems GmbH, Germany).

Western blot analysis of cleaved caspase 3

Thirty μg proteins, extracted from the kidney tissue, were used to run 15% SDS-PAGE. After gel electrophoresis, PVDF membranes were used to transfer the proteins from the gel at 25 v for 2 h. The membranes were then blocked with 3% BSA in TBST for 1 h. The membranes were separately incubated overnight at 4°C with the following primary antibodies:

Cleaved caspase 3 antibody (#9661, Cell Signaling Technology, USA) and beta actin antibody (ab8229, Abcam, USA). Next day, the blots were washed thoroughly and incubated with the secondary conjugated antibody poly HRP anti-rabbit IgG (31460, Thermo Fisher Scientific, USA) for 1 h. Finally, the blots were rinsed with TBST and incubated with chemiluminescent substrate (Clarity™ Western ECL substrate, BIO-RAD, USA cat#170-5060). The bands densitometry was quantified using Bio-Rad image analysis software.

Statistical analysis

Prism 5 (GraphPad Software, USA) was used for data analysis. One-way analysis of variance (ANOVA) followed by Tukey-Kramer post hoc test were used to calculate the significant differences among groups. Data were expressed as mean $\pm$ SE and p values <0.05 were considered as statistically significant.

Results

The effect of Hesp and Eug on Cd-induced functional and structural kidney damage

The results showed that Cd-treated rats exhibited significantly higher serum creatinine and BUN concentrations compared to normal rats (400 and 144.5% respectively at $p<0.05$). However, administration of either Hesp or Eug together with Cd for four weeks could effectively protect from the deterioration of kidney function as evidenced by the significantly lower creatinine concentrations (26.6 and 35.6% for Hesp+Cd and Eug+Cd treated groups respectively) as well as lower BUN concentrations (56.3% and 68.8% for Hesp+Cd and Eug+Cd treated groups respectively) compared to Cd-treated rats at $p<0.05$ (Table 1).

Table 1: Kidney functions among normal, Cd, Hesp+Cd and Eug+Cd treated groups.

	Normal group	Cd- treated group	Hesp+Cd- treated group	Eug+Cd- treated group
Serum creatinine conc. (mg/dL)	1.27±0.2	5.08±0.4*	1.35±0.2 ^a	1.813±0.2 ^a
Blood urea nitrogen (BUN) (mg/dL)	27.32±1.9	39.48±2.2*	22.23±1.9 ^a	27.16±2.9 ^a

n=8 rats per group, One Way Analysis of Variance (ANOVA) followed by Tukey-Kramer post hoc test were used for analysis of differences among groups. *Significant values at $p<0.05$ when compared to the normal group. ^aSignificant values at $p<0.05$ when compared to the Cd-treated group.

Moreover, the Cd-treated group showed severe kidney damage including atrophy of the glomerular tufts, increased Bowman's space and necrosis of tubular cells of proximal as well as distal convoluted tubules when compared to the normal group. Remarkably, both Hesp+Cd and Eug+Cd treated groups showed variable degrees of protection against Cd-induced kidney damage in which Hesp seems to be more protective to the kidney tissues than Eug. Altogether, these results indicate that both Hesp and Eug exhibit protective effects against Cd-induced functional and structural kidney damage (Figure 1 & Table 2).

The effect of Hesp and Eug on Cd-induced oxidative stress

We found that Cd-treated rats exhibited significantly higher MDA content (145.4%) as well as lower CAT (40.5%) and lower SOD (43.1%) activities compared to normal rats, $p<0.05$. Interestingly, both Hesp+Cd and Eug+Cd treated groups showed significantly lower contents of MDA (84.2 and 87.5% respectively) in addition to higher activities of CAT (161.3 and 197.9% respectively) and SOD (191 and 191% respectively) compared to the Cd-treated group, $p<0.05$ (Figure 2A–C). There was no statistically significant difference between the protective effect of Hesp and that of Eug against Cd-induced oxidative stress. These results confirm the antioxidant activities of both Hesp and Eug.

The effect of Hesp and Eug on Bax/Bcl2 ratio

The immunohistochemical staining of Bax (first row) and Bcl2 (second row) in normal, Cd, Hesp+Cd and Eug+Cd-

Figure 1: Histopathological examination of kidney tissues from the studied groups.

Sections of kidney tissues were examined by H&E stain as follows: (A) Normal group showed renal cortex with normal histological architecture including renal corpuscle with intact glomerular tuft (star) and intact tubular lining epithelium of proximal/distal convoluted tubules. (B) Cd-treated group showed a renal cortex with severe atrophy of glomerular tufts with increased Bowman's space (stars) associated with necrosis of tubular epithelial cells (arrow) and vacuolation of other cells (arrowheads); (C) Hesp+Cd- treated group showed renal cortex with apparent intact glomerular tuft and Bowman's space with fewer necrotic tubular epithelial cells (arrow) and mild vacuolation of other cells (arrow head); (D) Eug+Cd- treated group showed a renal cortex with mild atrophy of glomerular tufts, congested tuft capillaries with increased Bowman's space (stars) associated with necrosis of tubular epithelial cells (arrow) and vacuolation of other cells (arrow head). 400×.

treated groups are depicted in Figure 3A. in which the expression level of Bax was decreased while Bcl2 expression was increased in Hesp+Cd and Eug+Cd-treated groups compared to the Cd-treated group. The quantification showed that Cd-treated group exhibited higher Bax (155.5%) and lower Bcl2 (33.3%) expression when compared to the normal group at $p<0.05$. Remarkably, both Hesp+Cd and Eug+Cd treated groups showed significantly lower Bax (17.9 and 50% respectively) and higher Bcl2 expression levels

Table 2: The degree of kidney tissue damage in normal, Cd, Hesp+Cd and Eug+Cd treated groups.

	Normal group	Cd-treated group	Hesp+Cd treated group	Eug+Cd treated group
Glomerular tufts atrophy	–	+++	–	+
Bowman's space	–	+++	+	++
Degenerative and necrotic changes	–	+++	+	+

–: no lesions; +: mild lesions; ++: moderate lesions; +++: severe lesions.

Figure 2: Determination of the oxidative stress biomarkers among studied groups. Column figures show the concentration of (A) MDA; (B) CAT; (C) SOD in kidney tissue homogenates from normal, Cd, Hesp+ Cd and Eug+ Cd-treated groups.

*: Significant values when compared to the normal group at $p < 0.05$, a: Significant value when compared to the Cd-treated group at $p < 0.05$.

(875 and 300% respectively) when compared to Cd-treated rats (Figure 3B). Calculation of Bax/Bcl2 ratio revealed that Cd-treated group exhibited 4.7 fold increase in Bax/Bcl2 ratio compared to the normal group and that Hesp+ Cd and Eug+ Cd treated groups exhibited lower Bax/Bcl2 ratio estimated as 0.1 and 0.8 folds respectively compared to the normal group (Figure 3C). It is worthy to mention that Bax/Bcl2 ratio in Hesp+ Cd treated group was significantly lower compared to that of Eug+ Cd treated group.

The effect of Hesp and Eug on cleaved caspase 3 expression

To confirm the effect of Hesp and Eug on the apoptotic pathway, we measured the expression level of cleaved caspase 3 by Western blotting. The results showed that Cd-treated group exhibited significantly higher levels of cleaved caspase 3 (3.9 fold, $p < 0.05$) compared to the normal group. However, both Hesp+ Cd and Eug+ Cd treated groups showed significantly lower cleaved caspase 3 levels (1.8 and 2 folds respectively, $p < 0.05$) compared to the Cd-treated group. There was no significant difference between Hesp+ Cd and Eug+ Cd treated groups although the cleaved caspase 3 level was a little higher in Eug+ Cd treated group (Figure 4A, B). These results indicate that both Hesp and Eug are effective in inhibiting the apoptosis pathway.

Discussion

The kidney is the major organ affected by Cd toxicity in environmentally or occupationally exposed humans as well as in experimental animals [24]. This can be attributed to the continuous susceptibility of the kidneys to toxic compounds passing through the renal tubular cells for excretion [25]. In particular, Cd has a very long half-life therefore, it has the chance to accumulate in the kidneys causing severe damage [14]. However, it seems that the degree of structural and/or functional kidney damage is dependent on the dose and duration of Cd exposure. Several studies that used low Cd doses, ranging from 500 μg to 2 mg/kg body weight, in rats for periods not exceeding 10 days showed only histological damage that was not accompanied by altering the kidney function [26]. However, administration of Cd for longer periods that reached 24 weeks or at higher doses was sufficient to impair both the kidney function and tissue integrity [25]. Our results are compatible with the studies that showed both structural and functional kidney damage after Cd administration.

Although Cd is a redox inactive metal, ROS are generated in the kidney tubular cells after Cd exposure. It was found that, Cd indirectly increases the oxidative stress through depleting the cellular antioxidant enzymes leading to ROS overproduction as hydroxyl radicals, nitric oxide and

Figure 3: Immuno-histochemical analysis of Bax and Bcl2 in kidney tissues.

(A) Photomicrographs show the immunohistochemical staining of Bax and Bcl2 in normal, Cd, Hesp+Cd and Eug+Cd-treated groups. Scale=50 μ m. (B) Column figure shows the quantification of the expression level of Bax and Bcl2 in normal, Cd, Hesp+Cd and Eug+Cd-treated groups relative to the normal group. (C) Quantification of Bax/Bcl2 ratio in the studied groups relative to the normal group. *: Significant values when compared to the normal group at $p < 0.05$, Φ : Significant value when compared to the Cd-treated group at $p < 0.05$ and #: Significant values when Hesp+Cd treated group was compared to Eug+Cd treated group at $p < 0.05$.

superoxide radicals in addition to the non-radical hydrogen peroxide that becomes a source of free radicals after Fenton reaction. Moreover, Cd can replace the iron and copper from their cellular proteins causing their release and production of more oxidative stress [1]. Several other mechanisms are involved in Cd-induced nephrotoxicity such as disruption of Na^+ - K^+ -ATPase function, inhibition of DNA repair mechanisms and altered DNA methylation pattern [27, 28]. Remarkably, the generated ROS can target various enzymes, proteins and calcium channels resident in the endoplasmic reticulum (ER) leading to the release of Ca^{+2} into the cytosol.

The increased cytosolic Ca^{+2} concentration allows Ca^{+2} to enter the mitochondria to stimulate the mitochondrial metabolism, the production of more ROS and importantly the release of cytochrome c that eventually stimulates the induction of apoptosis [29]. Furthermore, ER stress induces the activation of C Jun N terminal Kinase (JNK) and its target inflammatory genes via phosphorylation of AP-1 which promotes the expression of multiple proinflammatory genes, cytokines and chemokines [29].

The antioxidant activities of both Hesp and Eug were previously demonstrated, however, our results

Figure 4: Western blot analysis of cleaved caspase 3. (A): Immunoblot showing the expression level of cleaved caspase 3 among Normal, Cd, Hesp+Cd and Eug+Cd treated groups. (B): Column figure shows the quantification of the cleaved caspase 3 bands relative to the normal group. *: Significant value when compared to the normal group at $p < 0.05$, a: Significant value when compared to the Cd-treated group at $p < 0.05$.

demonstrate for the first time that Eug possesses protective antioxidant activity against Cd-induced nephrotoxicity. It was previously found that, Eug was effective in reducing the kidney tubular necrosis and inflammation induced by gentamicin [30]. Additionally, the diabetic nephropathy induced by streptozotocin in rats was ameliorated by Eug via decreasing TGF- β 1 expression in the kidney cells [31]. On the same way, the nephroprotective effect of hesp was previously demonstrated against the kidney damage induced by different agents [32–34].

It was found that ROS regulate the main apoptotic pathways mediated by death receptors, mitochondria and endoplasmic reticulum. Under stress conditions, the ratio of pro- to anti-apoptotic Bcl2 proteins is modified where most of the pro-apoptotic proteins are activated favoring apoptosis [35]. Specifically, ROS control the release of cytochrome c from the mitochondrial membrane which subsequently causes the activation of several caspases and induction of cell death via a mechanism involving Bax oxidation. More relevant to the current study, the redox imbalance resulting from the increased production of ROS in kidney tissues induced apoptosis, cellular damage and ultimately

nephropathy [36]. We found that Cd administration for 4 weeks exhibited higher Bax/Bcl2 ratio and cleaved caspase 3 expression which confirmed the induction of apoptosis in an agreement with the previously published study [37].

Interestingly, the administration of Hesp or Eug concomitantly with Cd was successful in inhibiting the apoptosis to significant levels. Eug was previously found to modulate the cellular apoptosis and proliferation-associated genes such as caspase 3, c-Myc, p53 and H-ras, in agreement with our results [38]. Similarly, Hesp induced apoptosis through increasing the activities of caspases 3, 8 and 9 while decreasing the activity of Bcl-xL in a dose dependent manner in a panel of cell lines [39]. Moreover, Hesp protected from the ischemic heart diseases via Bax downregulation and Bcl2 upregulation [40].

Collectively, the protective effects of Hesp or Eug could be attributed to their antioxidant potential as evidenced from normalizing MDA content as well as SOD and CAT activities. In addition, the two drugs targeted the apoptosis pathway as seen from normalizing the Bax/Bcl2 ratio and active caspase 3 expression. Notably, we found that Hesp exhibited a better protective effect than Eug. This noticed effect could be attributed to the different doses of both antioxidants that were used throughout the study.

One of the limitations of this study is that, only a single dose was tested and the ability of our drugs to treat rather than protect from Cd nephrotoxicity was not investigated.

Conclusion

The current study demonstrates that Cd administration for four weeks to Wistar rats induces both structural and functional kidney damage. However, co-administration of either Hesp or Eug effectively alleviates the decline of kidney function and renal damage. The antioxidant and anti-apoptotic properties may constitute an important part of their protective efficacy. They enhanced the antioxidant status of the kidney and reduced the apoptosis signaling through the regulation of apoptotic and anti-apoptotic proteins (Bax/Bcl2 ratio) and lowering the cleaved caspase 3 expression. In addition, the histological changes induced by Cd were significantly improved. Therefore, Hesp and Eug could be explored as protective agents against the nephrotoxicity induced by Cd (Figure 5).

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Figure 5: Graphical representation about the protective effects of Hesp and Eug against Cd-induced nephrotoxicity. Cd induces oxidative stress in kidney tissues (evidenced by high MDA but low SOD and CAT levels). This oxidative stress and ROS overproduction trigger the stimulation of apoptosis pathway (evidenced by high Bax and low Bcl2 levels that lead to high levels of the active form of caspase 3). Therefore, Cd administration for four weeks causes a damage on kidney tissues (evidenced by histopathological lesions) and kidney dysfunction (evidenced by high serum creatinine and urea levels). Administration of Eug or Hesp with Cd could successfully protect from all the kidney damage induced by Cd. ↑ Increased; ↓ Decreased.

draft; MK: performed the experiments and revised the manuscript; WM: performed the experiments, analyzed the data and revised the manuscript.

Competing interests: Authors have no conflict of interest regarding this study.

Ethical Considerations: All the experimental procedures performed in this study were in agreement with the guidelines of National Institutes of Health guide for use and care of Laboratory animals revised in 1978 and they were approved by the Animal Ethics Committee at the Faculty of Science, Beni-Suef University (Approval no: BSU/FP/2016/1).

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