Received: 03 July 2023, Accepted: 06 August 2023

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 8, 1497-1506

Nephroprotective Properties of Palm Dates and Olive Leaves Extracts on Cadmium-Induced Acute Renal Toxicity in Albino Rats

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Abstract

Cadmium (Cd) is a well-known industrial and environmental pollutant having hazardous and poisonous properties in various forms of kidney impairment. Consequently, this study aimed to evaluate the nephroprotective effect of palm dates extract (PDE) and olive leaves extract (OLE) against cadmium chloride ($CdCl_2$)-induced nephrotoxicity. A total of 36 adult male albino rats were divided into six equal groups. Group 1: control group injected with physiological saline, group 2: oral gavage with PDE, group 3: orally administrated with OLE, group 4: injected daily with $CdCl_2$ (3 mg/kg, i.p.). Groups 5 and 6: were orally treated with either PDE or OLE, respectively one hour prior to ip administration of $CdCl_2$. After one-week samples were collected from all groups for serum biochemical analysis of kidney function as well as investigation of malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx). Kidney tissue specimens were obtained for histopathological examination. $CdCl_2$ exposure caused a significant elevation ($P<0.05$) in serum creatinine, urea, uric acid and MDA levels with marked reduction in CAT, SOD and GPx indicating renal damage. $CdCl_2$ induced severe congestion and vasculitis with marked perivascular edema, inflammation, hemorrhage, glomerular shrinkage, massive degeneration, necrosis and apoptosis in the renal tubular epithelium. PDE and OLE treatment resulted in significant improvement in kidney function and oxidative markers with a marked reduction of MDA level. Histopathological changes were also ameliorated. In conclusion, PDE or OLE treatment significantly reduced the deleterious effects of acute $CdCl_2$ renal damage by reducing oxidative stress as a protective mechanism.

KEYWORDS
$CdCl_2$, Histopathology, OLE, Oxidative stress, PDE, Renal injury

INTRODUCTION

Cadmium (Cd) is a highly toxic heavy metal that poses a significant risk to renal tissue. It has been shown to affect not only the proximal tubules but also distal tubules and glomeruli (Thévenod and Lee, 2013; Eom *et al*., 2017). Studies have reported that exposure of renal cells to Cd results in cytotoxicity and morphological changes, as well as overexpression of markers associated with renal injury (Lemaire *et al*., 2020). The exact mechanisms behind Cd-induced nephrotoxicity remain unclear and multifactorial, potentially involving oxidative stress, inflammation, mitochondrial dysfunction, and apoptosis (Erboga *et al*., 2016). Despite numerous previous studies on the mechanisms of Cd-induced nephrotoxicity, further investigation is required to fully understand this complex issue (Luo *et al*., 2017; Guo *et al*., 2022).

According to the available literature, there are no effective and safe therapeutic strategies currently available for treating Cd toxicity, accordingly, the management of Cd nephrotoxicity must focus on its prevention. Supplementation of the diet with natural agents has been reported to alleviate the effects of Cd toxicity and overcome the adverse effects of chelation therapy (Zhai *et al*., 2015). In recent years, there has been increased interest in the use of natural agents with antioxidant properties as potential strategies for managing Cd toxicity (Brewer, 2011).

Date Palm (*Phoenix dactylifera*) is a member of the palm tree family and is known to be a rich source of natural antioxidants with high scavenging activity, as reported by Al-Farsi *et al*., (2018). Palm dates extract (PDE) contains a variety of compounds, including anthocyanins, phenolics, sterols, carotenoids, procyanidins, and flavonoids, which contribute to its nutraceutical potential (Baliga *et al*., 2011; El-Far *et al*., 2016; Al-Alawi *et al*., 2017). These compounds have been shown to have a range of health benefits, including anti-inflammatory, anti-cancer, gastroprotective, cardioprotective, hepatoprotective, and nephroprotective activities, as reported by Hussain *et al*. (2020) and Idowu *et al*. (2020). The nephroprotective effect of PDE has been studied in several previous studies, demonstrating its ability to protect against nephrotoxic agents such as dimethoate (Saafi-Ben Salah *et al*., 2012), trichloroacetic acid (El Arem *et al*., 2014), paracetamol (Oseni *et al*., 2017), doxorubicin (Wang *et al*., 2019) and gentamicin (Celik and Irak, 2018a; Al-Asmari *et al*., 2020). However, there have been limited studies on the protective effect of PDE against Cd-induced nephrotoxicity.

Olive leaves (*Olea europaea* L.) have been reported to be a rich source of antioxidant polyphenols and scavenging ability among the different parts of the olive tree (Sabry, 2014). Olive leaves extract (OLE) has garnered attention in the field of healthcare due to its wide range of polyphenols, with oleuropein and its derivatives such as hydroxytyrosol and tyrosol being the pri-

ISSN: 2090-6277/2090-6269/

mary phenolic constituents (Borjan *et al*., 2020). These phenolic compounds are believed to be responsible for the various therapeutic effects of OLE, including antiarrhythmic, antihypertensive, anti-inflammatory, immune-stimulating, spasmolytic, hypoglycemic, hypocholesterolemic, cardioprotective and nephroprotective activities (Vogel *et al*., 2015; Özcan and Matthäus, 2017). Consequently, this study aimed to evaluate the protective effects of PDE and OLE in alleviating acute renal injury in rats induced by Cd through serum biochemical analysis, detection of oxidative stress and histopathological examination.

MATERIALS AND METHODS

Drugs and reagents

Cadmium Chloride ($CdCl_2$) Monohydrate 98% was procured from LOBA CHEME PVT.LTD in India. All chemicals utilized in this study were of superior analytical grade and premium quality.

Preparation of palm dates ethanolic extract

The fresh date palm fruit (*Phoenix dactylifera* L.) used in this study was procured at the Tamr phase from Haye*et al* Madina Company in Al Madinah Al Munawara, Saudi Arabia. The Department of Horticulture of the Faculty of Agriculture, Benha University, approved the botanical identity and verification.

The date palm fruits were thoroughly cleaned with tap water and the seeds were removed. The fruit was then chopped into small pieces and dried. About 1856 g of dried powder was macerated twice for three days with 10 liters of 70% ethyl-alcohol (B.P 78.37°C) using a Heidolph stirrer for six hours daily. The alcoholic extract was filtered, decanted, and evaporated under a vacuum using a Heidolph rotavapor at 50-55°C until dryness to yield a hydroalcoholic extract. The dried hydroalcoholic extract was then dissolved in 2% Tween 80 in distilled water using a sonicator at 50-55°C and stored in the refrigerator at 4°C until use (Stephen *et al*., 2018).

Preparation of olive leaves ethanolic extract

Fresh olive leaves (*Olea europaea*) were collected from the Faculty of Agriculture, Benha University, Egypt, where botanical identification and authentication were performed by the Department of Horticulture. The plant was identified as belonging to the family Oleaceae, the genus Olea, and the species *Olea europaea* L. The process for preparing the OLE was based on the method described by Tavafi *et al*. (2012). The leaves were first washed to eliminate any foreign matter and then dried and ground into a fine powder. The resulting 600 g of dry powder was macerated with 10 L of 70% ethyl alcohol (B.P 78.37°C) twice for three days, using a Heidolph stirrer for six hours daily. The resulting alcoholic extract was decanted, filtered, and evaporated under a vacuum using a Heidolph rotavapor until dryness, resulting in the hydroalcoholic extract. The dried hydroalcoholic extract was then dissolved in 2% Tween 80 in distilled water using a sonicator at 50-55°C and stored at 4°C until use.

Estimation of total phenolic content

A gallic acid stock solution of 1 mg/ml in methanol was prepared followed by the following dilutions: 25, 50, 100, 200, 400, 600,800 and 1000 µg/ml for calibration curve. PDE and OLE samples were prepared at the concentration of 5 mg/mL in methanol. The total phenolic content was determined using the Folin–Ciocalteu method as described by Attard, (2013). Briefly, the procedure consisted of mixing 10 µL of sample/standard with 100 µL of Folin-Ciocalteu reagent (Diluted 1: 10) in a 96-well microplate. Then, 80 µL of 1M Na2CO3 was added and incubated at room temperature (25°C) for 20 min in the dark. At the end of incubation time, the resulting blue complex color was measured at 630 nm. Data are represented as means ± SD and the results were recorded using microplate reader FluoStar Omega.

Estimation of total flavonoids content

A stock solution of standard rutin was prepared at 2000µg/mL in methanol, from which the following dilutions were prepared: 1000, 500, 200, 125, 62.5,31.4, 15.625 and 7.81 µg/mL for calibration curve. PDE and OLE samples were prepared in Methanol at a concentration of 5 mg/mL. The total flavonoid content was assessed using the aluminium chloride method reported by Kiranmai *et al*. (2011), with minor modifications for microplate testing. Briefly, 15 µL of sample/standard was placed in a 96-well microplate, then, 175 µL of methanol was added followed by 30 µL of 1.25 % AlCl3. Finally, 30 µL of 0.125 M $C2H_3NaO_2$ was added and incubated for 5 min. At the end of incubation time, the resulting yellow color was measured at 420 nm. Data are represented as means ± SD and the results were recorded using microplate reader FluoStar Omega.

Qualitative HPLC identification

Different authentic flavonoids and phenolic acids were used for the HPLC analysis to detect their presence in olive leaves and palm dates extracts. The stock solution of 10 different standards in methanol was prepared and filtered using a 0.22 µm syringe filter then 10 µl was injected. Extracts of each plant (100 mg/ml) were accurately weighed and sonicated for 15 min., filtered using 0.22 µm Nylon syringe filter then 10µl of each were separately injected into HPLC Waters 2690 Alliance HPLC system equipped with auto sampling injector, Column C18 Inertsil ODS 4.6x250 mm, 5 µm, and Waters 996 photodiode array detector at 280 nm. The column temperature was maintained at 35˚C. Gradient separation was carried out with 0.1 % Phosphoric acid in water: Methanol as a mobile phase at a flow rate of 1 ml / min (Nogata *et al*., 1994; Mattila *et al*., 2000).

DPPH free radical scavenging assay

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical assay was carried out according to the method of Faso (2016). Briefly, 100 µL of freshly prepared DPPH reagent (0.1% in methanol) was added to 100 µL of the samples and blank (ethanol 70%) in 96 microplates, the reaction was incubated at room temp for 30 min in the dark. At the end of incubation time, the resulting reduction in DPPH color intensity was measured at 540 nm by a microplate reader. Data are represented as means ± SD (µg/ml) according to the following equation:

Percentage inhibition= (Average absorbance of blank-average absorbance of the test)/(Average absorbance of blank) X 100

Percentages of inhibition were plotted against concentrations of extracts to calculate the concentration providing 50% inhibition (IC50). The assay was done in triplicate and data were analyzed according to Chen *et al*. (2013).

Animals

Thirty-six adult male albino rats with a weight of 200 ± 10

g were purchased from the Nile Pharmaceutical Company, Al-Sawah Street, Al-Amiriya, Cairo, Egypt. The rats were housed in stainless-steel wire cages under controlled environmental conditions of 21±2°C and 50±5% relative humidity in a 12-hour light and dark cycle for one week of acclimatization prior to experimentation. The rats were provided ad libitum access to standard rat rations and water. All experimental procedures were carried out in accordance with international guidelines for laboratory animal care and were approved by the Ethical Committee of the Faculty of Veterinary Medicine at Benha University, with an ethical approval number (BUFVTM 17-10-22).

Experimental design

Rats were randomly divided into six equal groups, six rats per group (N=6): Group 1: the control non-intoxicated group and each rat was administered 1ml of physiological saline intraperitoneally (ip) as a vehicle. Group 2: received oral administration of PDE at a dose of 400 mg/kg body weight (bw) daily for a week, as described by Alhaider *et al.* (2017). Group 3: received daily oral gavage of OLE at a dose of 400 mg/kg bw for a week, as reported by Khattab *et al.* (2020). Group 4: intoxicated with $CdCl_2$ ip at a dose of 3 mg/kg bw daily for one week was chosen according to Ansari *et al.*, (2021). Group 5: was treated with oral administration of PDE (400 mg/kg bw) followed by ip administration of $CdCl_2$ at a dose of 3mg/kg bw daily, one hour later for one week. Group 6: Received oral administration of OLE (400 mg/kg bw) one-hour before ip administration of $CdCl_2$ at a dose of 3 mg/kg bw daily for one week. Throughout the experimental period, rats were monitored daily for any clinical signs and mortalities. A graphical scheme of the study design was illustrated in Fig. 1.

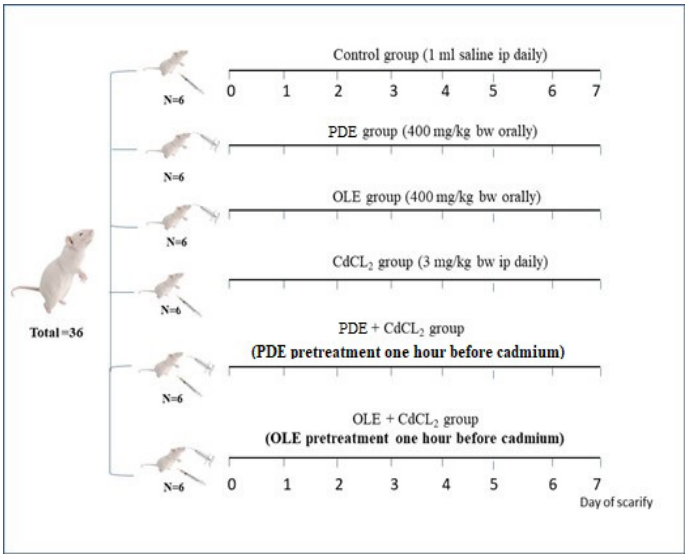

Fig. 1. Graphical scheme of the study design and animal grouping. Cadmium Chloride ($CdCl_2$), palm dates extract (PDE) and Olive leaves extract (OLE).

Body and kidney weights

All animals' body and kidney weights were recorded in this study. The body weight was reported prior and post-treatment by placing each animal in a closed plastic container and weighing it. At the end of the experiment, the kidney weight and kidney weight ratio (kidney weight per body weight × 100) of each animal was calculated (Bharathiraja *et al.*, 2013).

Serum biochemical analysis

Blood samples were collected from each animal at the end of the experiment using retro-orbital venous plexus puncture under diethyl ether anesthesia and without the use of anticoagulants. The serum was obtained by blood centrifugation for 10 minutes at 2000 g, transferred to clean and dry ependroph, and stored at -20°C for subsequent serum biochemical analysis. Creatinine concentration in serum was determined by using a special kit Spectrum–creatinine kit, Egypt-IFUFCC10, by using a device spectrophotometer sesil, England measured by Semi-auto chemistry analyzer (Dunn *et al.*, 2004). Serum urea was measured by Semi-auto chemistry analyzer using Spectrum-urea kit, Egypt-IFUFCC40 (Jing *et al.*, 2018). Uric acid levels in sera were estimated based on the method of Tiffany *et al.* (1972) by reagent kits purchased from Spectrum Company, Egypt-IFUFCC46.

Serum oxidative stress

The serum level of the oxidative marker malondialdehyde (MDA) was measured based on the methods described by Karatas *et al.*, (2002). Additionally, the activities of enzymatic antioxidant markers, including superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) were assayed in serum samples using Biodiagnostic kit (Cairo, Egypt), according to the methods described by Sies, (1999), Marklund and Marklund, (1974) and Aebi, (1974), respectively.

Histopathological studies

At the end of the experiment, specimens were obtained from the kidney of rats in all groups and immediately fixed in 10% neutral buffered formalin. The specimens underwent proper fixation before being dehydrated in ascending grades of ethyl alcohol, cleared in xylol, and embedded in paraffin wax. Subsequently, 5 µm sections of tissue paraffin were prepared and stained with hematoxylin and eosin (H&E) (Suvarna *et al.*, 2018) and periodic acid-Schiff (PAS) (Carleton, 1980) for microscopic examination.

Statistical analysis

Statistical analysis was conducted using Prism GraphPad

Table 1. Total phenolic contents of palm dates and olive leaves extracts.

Sample	Average reading at 630 nm	Substitution in cal. curve equation (µg GA/mL sample solution)	Total phenolics content (µg gallic acid per 1mg extract)	Standard deviation
Palm dates	0.33	136.48	27.29	1.74
Olive leaves	0.10	385.67	77.13	6.99

Table 2. The total flavonoid contents of palm dates and olive leaves extracts.

Sample	Average reading at 420 nm	Total flavonoids content (µg rutin eq/mg extract)	Standard deviation
Palm dates	0.01	2.23	0.11
Olive leaves	0.15	17.27	1

software version 9.0 (San Diego, California, USA). Statistical comparisons between the groups were performed using a one-way analysis of variance (ANOVA) followed by a Tukey-Kramer post hoc test. The results are expressed as the mean ± SEM. P-values < 0.05 were considered statistically significant.

RESULTS

Total phenolic contents

The phenolic compounds of PDE and OLE were 27.29 and 77.13 µg/ mg or mg/ g extract expressed in gallic acid equivalent, respectively (Table 1).

Total flavonoid contents

The palm dates and olive leaves extracts contents of flavonoids compounds were 2.23 and 17.27 µg/ mg or mg/ g extract expressed in rutin equivalent, respectively, as shown in Table 2.

HPLC analysis

The chromatographic results obtained by the HPLC analysis of palm dates extract showed the presence of gallic and caffeic acids while olive leaves extract showed the presence of chlorogenic and Ellagic acids, rutin, quercetin and apigenin (Table 3) and (Fig. 2).

DPPH radical scavenging assay

DPPH radical scavenging activity of the sample of both palm dates and olive leaves is presented in Table 4.

Changes in the body and kidney weights

The results in Table 5 revealed a significant difference in the final body and kidney weights among different treated groups were recorded, as a significant ($p < 0.05$) reduction in the final body weight of $CdCl_2$ intoxicated rats in comparison to control rats. Interestingly, an increase in the final body weight was observed in Cd-intoxicated rats treated with the PDE, or OLE.

However, the administration of PDE or OLE in addition to $CdCl_2$ resulted in a significant ($p < 0.05$) rise in kidney weights compared to the $CdCl_2$-treated group, whereas Cd-intoxicated rats had a significant (p 0.05) decrease in kidney weight.

Effect of PDE and OLE on biomarkers of kidney function

The impact of PDE and OLE on biomarkers of intoxicated kidney function was further evaluated. The levels of serum urea, uric

Table 3. HPLC analysis of palm dates and olive leaves extracts.

No.	Compounds	Retention time (R_t)		
		Standards	Date palms extract	Olive leaves extract
1	Gallic acid	10.92	10.95	--
2	Catechin	25.44	--	--
3	Chlorogenic acid	27.74	--	27.01
4	Caffeic acid	30.83	31.63	--
5	Rutin	47.26		47.15
6	Hespiridin	47.51	--	--
7	Ellagic acid	49.08	--	50.13
8	Quercetin	56.10	--	57
9	Kampeferol	58.37	--	--
10	Apigenin	58.74	--	58.72

Table 4. DPPH radical scavenging activity of palm dates and olive leaves.

Sample	IC50 (µg/ml)	Standard deviation
Palm dates ex.	1629	49.34
Olive leaves ex.	38.92	2.12
St Trolox (µg/ml)	7.22	0.2

Table 5. The effect of $CdCl_2$, PDE, and OLE on body and kidney weights.

Groups	Body weight (g)		Kidney weight	
	Initial	Final	Absolute (g)	Relative (%)
Control	192.25±19.25	243.3±13.74*	2.000±0.169 [a]	0.8034±0.1907[a]
PDE	195.25±13.07	253.5±25.11*	2.079±0.202 [a]	0.8134±0.1542 [a]
OLE	188.5±8.88	246.0±22.55*	1.915±0.094	0.8134±0.0281[a]
Cd	184.5±17.40	149.3±10.56*	1.003±0.037[c]	0.6730± 0.0700 [c]
PDE+ Cd	181.0±4.69	210.0±12.25*	1.509±0.10 [b]	0.7772±0.0368 [b]
OLE+ Cd	174.5±7.23	192.5±9.574*	1.354±0.041 [b]	0.7593±0.0202[b]

Values (means ± SD) within the same column carrying different superscripts are significantly different ($p \leq 0.05$). * Significant increase ($p \leq 0.05$).

acid, and creatinine were assessed in rats treated with $CdCl_2$ only for 7 days, as the obtained results indicated a significant ($p < 0.05$) increase in these biomarkers. However, the administration of PDE or OLE in $CdCl_2$-intoxicated rats resulted in a significant improvement in kidney function. Notably, the serum levels of creatinine, urea and uric acid were significantly reduced, as shown in Fig. 3.

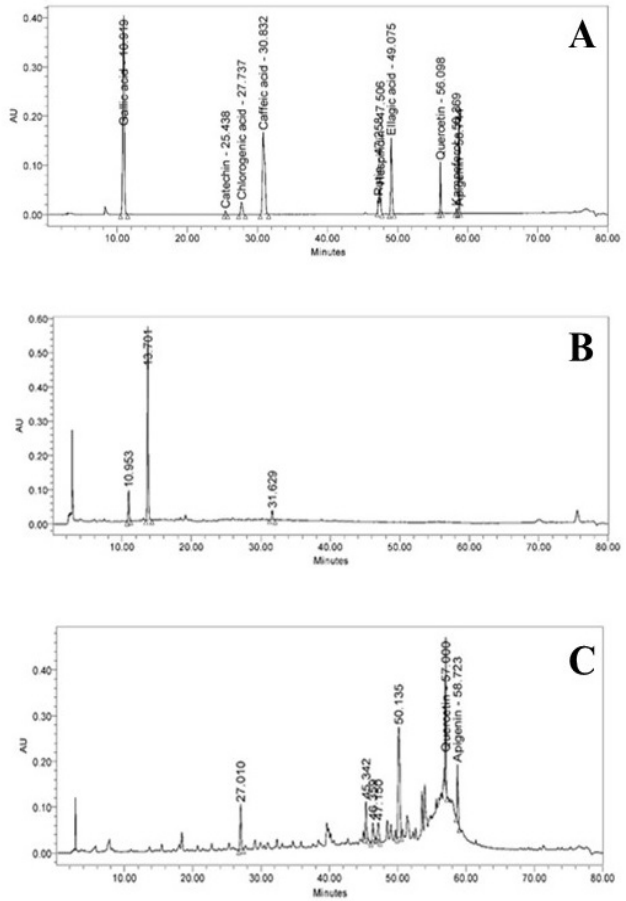

Fig. 2. HPLC analysis chromatogram of (A) standards, (B) palm dates extracts and (C) olive leaves extract.

Effect of PDE and OLE on oxidative stress markers

As shown in Fig. 4. The serum contents of MDA significantly enhanced ($P<0.05$) in Cd-intoxicated rats in comparison to the control group, meanwhile, those of antioxidant enzymes such as CAT, SOD and GPx were drastically decreased in the same Cd-intoxicated group. Interestingly, MDA level was significantly reduced in the treated Cd-intoxicated group with PDE or OLE. Meanwhile, the levels of CAT, SOD and GPx were markedly ($P<0.05$) elevated in these groups in comparison to the Cd- intoxicated group.

Histopathological findings

The microscopical examination of the kidneys from the control, PDE and OLE groups revealed normal histological appearance of glomeruli, proximal and distal convoluted tubules as well as the renal blood vessels (Fig. 5). The investigated rat's kidney displayed various histological abnormalities after a week of $CdCl_2$ treatment, as congestion and dilation of the renal blood vessels and intertubular blood capillaries in association with vacuolation of their tunica media and proliferation of their endothelial cell lining with perivascular edema admixed with mononuclear leukocytic cellular infiltrations (Fig. 6A), along with marked perivascular hemorrhage was also seen in other cases (Fig. 6B). Multifocally, in the cortex, glomeruli exhibited vacuolation of the lining endothelial cell of the glomerular tuft (Fig. 6C), as well as shrinkage and necrosis of the glomerular tuft of some glomeruli (Fig. 6D). Furthermore, widening of the Bowman's space and hypertrophy of the Bowman's capsule lining epithelium, as well as hypersegmentation of the glomerular tuft with periglomerular lymphocytic infiltration (Fig. 6E) were determined. Vacuolar and hydropic degeneration of proximal and distal convoluted tubules together with desquamation of their lining epithelial cell, with the presence of eosinophilic debris in their lumina (Fig. 6E). Interestingly, other renal tubules displayed extensive coagulative necrosis with pyknotic nuclei (Fig. 6F) and lymphocytic infiltration. Multifocally, numerous renal tubules exhibited apoptotic bodies with cytoplasmic and nuclear condensation in association with nuclear fragmentation. Apoptotic cells detached from basal membranes and admixed with necrotic cells (Fig. 6G and H).

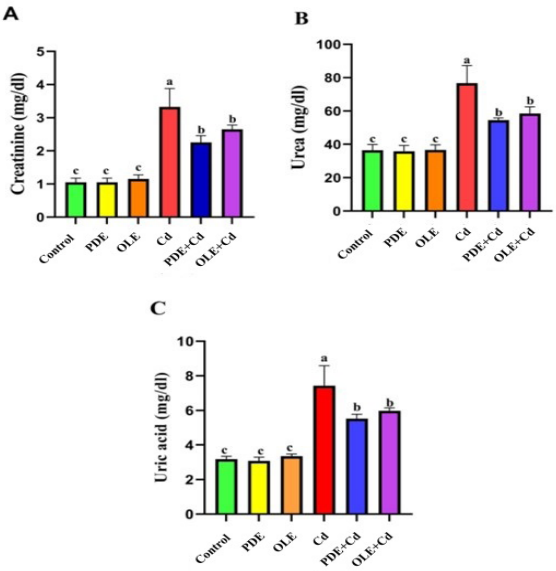

Fig. 3. The levels of creatinine (A), urea (B) and uric acid (C) in serum from Control, PDE, OLE, PDE plus Cd and OLE plus Cd groups. Data are expressed as the mean ± SD. Differences were considered statistically significant at P values < 0.05. a significant change at $p < 0.05$ from the control group. b Significant change at $P < 0.05$ from the $CdCl_2$ group.

However, kidney sections obtained from the cadmium-treated group with PDE displayed a marked reduction in the severity of pathological alterations induced by cadmium. Whereas there was mild congestion of renal blood vessels and perivascular edema. Additionally, vacuolation and mild segmentation of the glomerular tuft (Fig. 7A). Cloudy swelling of epithelial cells lining renal tubules was also observed (Fig. 7B). Interestingly, there were focal areas of normal renal tubules with regenerated tubular epithelium. In contrast, when cadmium-intoxicated rats were treated with OLE, the histological structure of the renal tissue improved in comparison to the cadmium group, with only slight degeneration in glomeruli, mild vacuolation of endothelial cells lining the glomerular tuft, and degeneration in the renal epithelium were also noticed, along with mild cystic dilatation of some renal tubules (Fig. 7C and D).

PAS staining of renal tissue obtained from the control, PDE and OLE groups revealed normal glomerular and tubulointerstitial structure without deposition of any proteinaceous materials (Fig. 8A). While the kidney sections from the Cd-intoxicated group stained with PAS revealed abnormal morphological changes in the glomeruli and renal tubules. The glomeruli exhibited deposition of proteinaceous material in Bowman's space with complete degeneration of the glomerular tuft (Fig. 8B) in

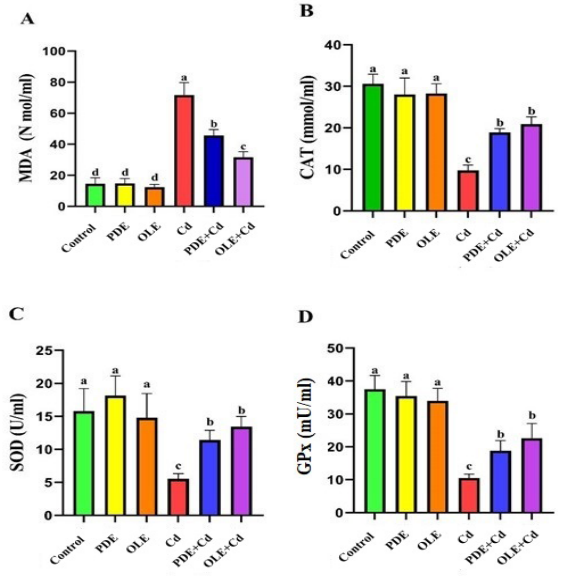

Fig. 4. The levels of MDA (A), CAT (B), SOD (C) and GPx (D) in serum from Control, PDE, OLE, PDE plus Cd and OLE plus Cd groups. Data are expressed as the mean ± SD. Differences were considered statistically significant at P values < 0.05. a significant change at p < 0.05 from the control group. b Significant change at P < 0.05 from the $CdCl_2$ group.

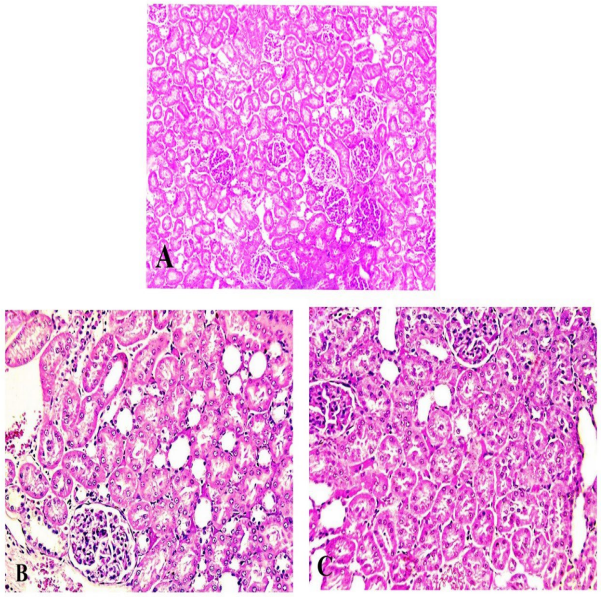

Fig. 5. Photomicrographs of rat's renal tissue stained by H&E obtained from (A) control group ×100. (B): PDE-treated group ×200. (C): OLE-treated group×200. Note: normal histological structure of glomeruli (normal glomerular tuft, capsule and space) with normal proximal and distal renal tubules.

association with thickening of the glomerular tuft which takes a strong positive reaction by PAS stain (Fig. 8C) as well as thickening of the parietal layer of Bowman's capsule (Fig. 8D) with either complete loss or fragmentation of the brush border of proximal convoluted tubules (Fig. 8C and 8D). PAS staining also identified apoptotic bodies in proximal tubular cells, which take deep magenta staining of the cytoplasm surrounded by a clear halo (Fig. 8D). However, the renal tissues from rats challenged with $CdCl_2$ and treated with PDE or OLE revealed a nearly normal glomerular structure with a slight thickening of glomerular tuft but the Bowman's space and capsule free from any depositions. Furthermore, the majority of the proximal convoluted tubules have intact brush borders. Only mild fragmentation of the brush border of some renal tubules was detected in a few examined cases (Fig. 8E-F).

DISCUSSION

Environmental contamination by toxic heavy metals such as cadmium (Cd) is a pervasive global issue that leads to toxicity through ingestion and inhalation (Satarug *et al*., 2010). Cd metabolism and accumulation in the kidneys increase their susceptibility to Cd toxicity. That is why it is important to counteract the harmful effects of Cd, consequently, the current study aimed to evaluate the protective effects of PDE and OLE on acute renal injury induced by Cd.

PDE and OLE contain varying quantities of phenolic and flavonoids, which may account for their notable antioxidant effect. Moreover, the antioxidant activities of ethanol 70% extracts of palm dates and olive leaves were evaluated by the DPPH free radical assay as an indicator of their protection ability against numerous diseases. Various hypotheses are suggested for the beneficial effects of these compounds in improving renal injury (Vogel *et al*., 2015; Al-Alawi *et al*., 2017; Özcan and Matthäus, 2017).

The findings of the current study revealed a significant reduction in the body weight of the Cd-intoxicated group. While treatment with PDE or OLE in the Cd group exhibited an increase in the final body weight. These results align with previous research conducted by Bharathiraja *et al*. (2013). The reduction in the body weight of Cd-intoxicated rats could be attributed to loss of appetite and malabsorption of nutrients (Sajjad *et al*. 2014). Additionally, a significant decrease in kidney weights was observed in the $CdCl_2$-treated group. Contrary, the co-administration of PDE and OLE with $CdCl_2$ has a protective effect on the kidneys, as evidenced by the marked elevation in kidney weights compared to the nephrotoxic group. This finding could account for the ability of $CdCl_2$ to induce oxidative injury in the kidney tissue resulting in apoptosis (Sakr *et al*., 2015; Almeer *et al*., 2019) or if the organ mass, including the kidney, is maintained by a balance of differentiation, proliferation, and death processes, a shift toward apoptosis can result in a decrease in organ mass (Almeer *et al*., 2019).

Serum urea and creatinine are well-established serum biochemical markers used to assess kidney function (Sahu *et al*., 2020). Creatinine is a nitrogenous compound formed during muscular metabolism and primarily eliminated through glomerular filtration (Dasgupta and Sepulveda, 2019). That is why, in the present research, the levels of serum creatinine, urea, and uric acid were assessed in all groups. A significant elevation in the levels of serum creatinine, urea, and uric acid were recorded in Cd-intoxicated rats. These findings could be attributed to renal damage induced by cadmium leading to leakage of this product into the bloodstream resulting in their marked elevation in the serum (Bashandy *et al*., 2021) as well as acute kidney injury is associated with a sudden reduction in kidney function resulting in the accumulation of creatinine and urea (Tiong *et al*., 2014). The observed results were corroborated with previous studies (Ansari *et al*., 2019; Ansari *et al*., 2021) revealing acute renal dysfunction.

Treatment with either PDE or OLE in Cd-intoxicated groups resulted in a significant reduction in uric acid, urea and creatine in serum. These findings suggest that PDE and OLE have the potential to attenuate $CdCl_2$-induced nephrotoxicity, possibly by enhancing renal function and reducing the toxic metabolites accumulation in the kidneys. These results are in line with (Celik and Irak, 2018) who found that date extract attenuates acute nephrotoxicity in rats. Besides, OLE ameliorated acute renal injury with a significant decrease in serum creatinine, urea and uric acid concentration (Abdel-Gayoum *et al*., 2015; Geyikoglu *et al*., 2017).

Increase oxidative stress is also related to cadmium nephrotoxicity (Ozbek, 2012). Herein, MDA, a lipid peroxidation marker, was found to be considerably greater in the Cd-intoxicated group. Furthermore, rats exposed to $CdCl_2$ had reduced antioxidant enzyme activity (CAT, SOD, and GPx). Meanwhile, $CdCl_2$ treatment with PDE or OLE improved antioxidant defense by normalizing the activity of these antioxidant enzymes. These findings are in the same vein as those who indicated that OLE therapy modulated oxidative stress to restore normal kidney function (Geyikoglu *et al*., 2017). The OLE promising nephroprotective agent via

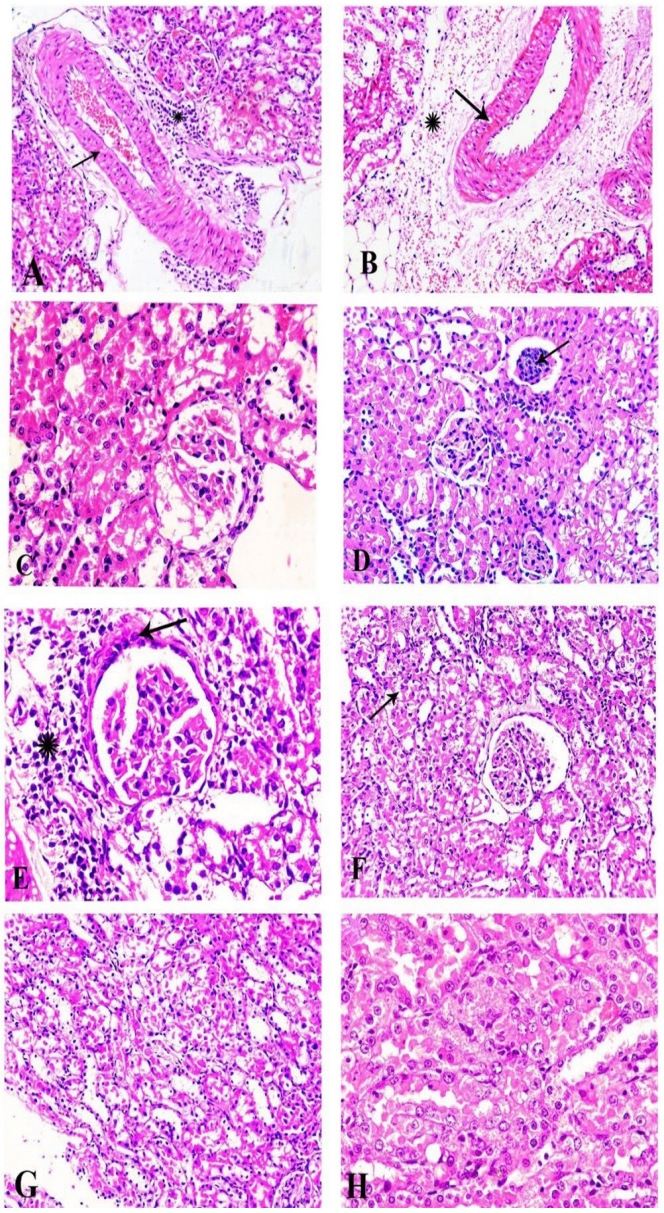

Fig. 6. Photomicrograph of renal tissue stained by H&E obtained from cadmium chloride intoxicated rats for 7 consecutive days. (A): Dilation and congestion of the renal blood vessel, vesiculation in the muscular layer (arrow) with mild perivascular leucocytic infiltration (asterisk) ×200. (B): Perivascular hemorrhage and edema (asterisk) ×200. (C) Degeneration of glomerular tuft with hydropic degeneration and desquamation of the epithelial cell lining proximal convoluted tubules ×200. (D): Necrosis of the glomerular tuft (arrow) with lymphocytic infiltration ×200. (E): Hypertrophy of the epithelial cell lining the Bowman's capsule (arrow), hypersegmentation of glomerular tuft with periglomerular leucocytic infiltration (asterisk) ×200. (F): severe degeneration and necrosis of epithelial cells lining of renal tubules (arrow) ×200. (G): coagulative necrosis of the epithelial cells lining renal tubules represented by pyknotic nuclei with desquamation of its epithelial lining×200. (H): Renal epithelium showing apoptotic bodies ×400.

several pathways including inhibition of oxidative damage of DNA caused by hydrogen peroxide (H_2O_2) and free-radical scavenging properties (Anter *et al*., 2011), reduction of reactive oxygen species (ROS) generation (Ranieri *et al*., 2019; Abugomaa and Elbadawy, 2020), increased activity of renal antioxidant enzymes as CAT and GPx (Tavafi *et al*., 2012; Karanovic *et al*., 2021) and enhancing the antioxidant defenses (Badr and Fouad, 2016; Alhaithloul *et al*., 2019). The prevention of oxidative stress-induced nephrotoxicity of PDE may explain its therapeutic activity (Saafi-Ben Salah *et al*., 2012; Abdeen *et al*., 2021).

Various histopathological alterations were demonstrated in the renal tissues of rats injected with $CdCl_2$ for seven days. This study also provides clear evidence of the damaging effects of $CdCl_2$ exposure on the renal blood vessels and intertubular capillaries. May be the result of increased microvascular permeability,

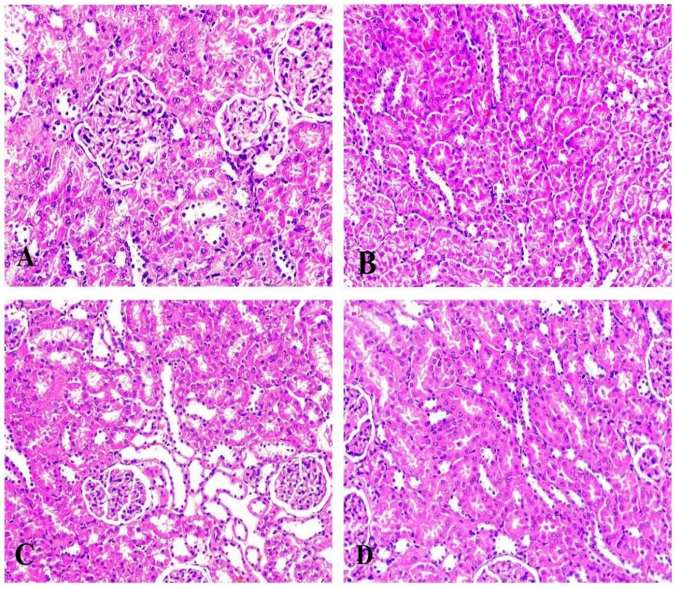

Fig. 7. Photomicrograph of renal tissue stained by H&E obtained from rats. (A-B): treated with PDE and $CdCl_2$ for a week. (A): a mild proliferation of the glomerular tuft. (B): mild degenerative changes in form of cloudy swelling in the epithelium lining proximal convoluted tubules. (C - D): OLE administration in $CdCl_2$-intoxicated rats. (C): mild cystic dilation of renal tubules. (D): mild degenerative changes in the renal tubular epithelium ×200.

which causes fluid, protein and blood cells to leak from capillaries into the interstitial space (Prozialeck *et al*., 2006) with endothelial injury which may be consequences of toxicity in parenchymal cells of various organs, such as kidney (Shinkai *et al*., 2016). Cd accumulation in kidneys causes nephritis and nephrosis, as supported by the current study findings. Degenerative alterations in the glomerular tufts, an increase in the Bowman's space, and thickening of the Bowman's capsule are signs of damage to the kidney's filtration system, decreased filtration capacity and altered kidney function. This is in concur with Li *et al*. (2016) who reported that Cd after entering the body circulates in the blood as a free ion or bound with plasma proteins such as metallothionein (MT), directly affecting the glomerular endothelium. Consistent with prior study, $CdCl_2$ for seven successive days induced severe renal injury (Bekheet *et al*., 2011). The findings of Bharathiraja *et al*. (2013) and Bekheet *et al*. (2011) revealed shrinkage of the glomerulus and increased glomerular space in rats exposed to $CdCl_2$. These results are also in line with previous studies (Kim *et al*., 2018; Yildirim *et al*., 2018; Aqeel *et al*., 2020). Severe tubular degeneration and necrosis, particularly the proximal one indicates that it is the primary target for Cd renal toxicity as reported by Ansari *et al*. (2021). The administration of $CdCl_2$ for 5 days induced inflammation, degeneration and apoptosis (Elkhadragy *et al*., 2018). These findings imply that Cd exposure disrupts all kidney function and alters cellular processes, contributing to the development of kidney diseases.

Apoptosis has been widely recognized as a critical factor in the pathogenesis of renal diseases (Havasi and Borkan, 2011). Cd exposure leads to apoptosis in proximal tubular cells via multiple pathways and may be associated with mitochondrial injury (Yuan *et al*., 2014; Lee *et al*., 2019). The results of Elkhadragy *et al*. (2018) and Ke *et al*. (2019) are in line with our findings suggesting that the mechanisms underlying $CdCl_2$-induced renal toxicity involve the activation of apoptotic pathways in the kidney. In addition, PAS staining revealed abnormal deposition of PAS-positive materials in uriniferous space, thickening of the glomerular basement membrane and Bowman's capsule, with weakening or loss of brush borders of proximal convulsed epithelium. PAS staining was superior for assessing tubule morphology, brush borders and basement membranes (Bland *et al*., 2017). Accordingly, the obtained results confirmed the marked deleterious effect of cadmium on the renal tubules.

The renal tissue from Cd-intoxicated rats treated with palm

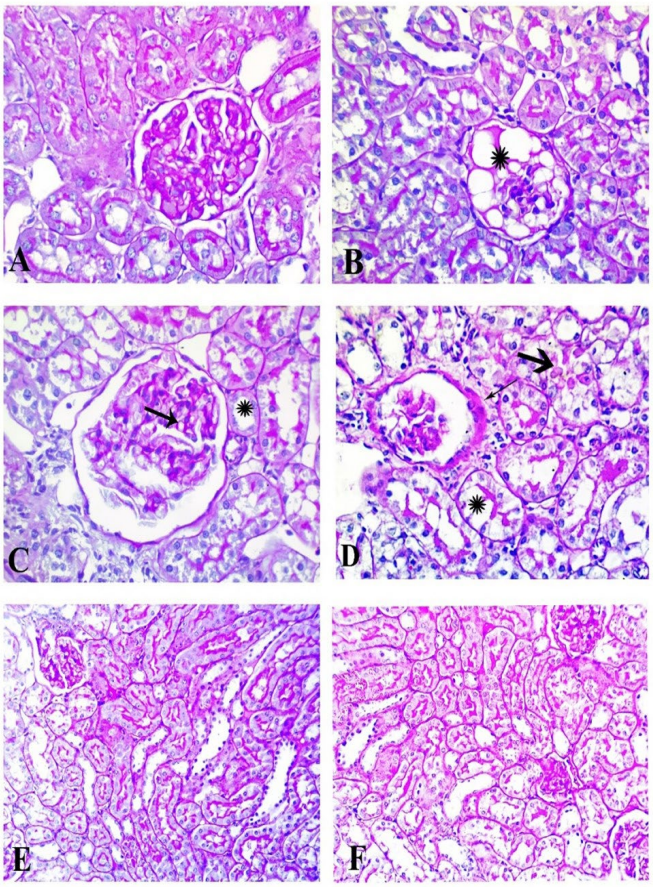

Fig. 8. Photomicrographs of renal tissues stained with PAS. (A): negative control group. Note: normal glomeruli and intact brush border of proximal convoluted tubules which take positive reaction by PAS. (B-D): cadmium-intoxicated rats. Note: (B): vesiculation of glomerular tuft with PAS-positive material in bowman's space (asterisk). (C): thickening of the segmented glomerular tuft (arrow) with loss of brush border of affected proximal convoluted tubules (asterisk). (D): severe thickening of Bowman's capsule (thin arrow), apoptotic bodies take positive reactions in degenerated renal tubules (thick arrow) with degeneration and loss of brush border of affected proximal convoluted tubules (asterisk). (E): PDE and $CdCl_2$. Note: normal glomeruli with mild thickening of tuft and intact brush border of proximal convoluted tubules. (F): OLE and $CdCl_2$. Note: some mild thickening of the glomerular tuft and the brush border of proximal convoluted tubules is partially intact. PAS ×200.

dates extract appeared nearly normal with only slight degenerative alterations, confirming PDE's ability to minimize the extent of cadmium damage. PDE therapy has renoprotective effects by modulating the abnormal histological structure (Celik and Irak, 2018). OLE, on the other hand, was able to promote renal tubule regeneration and significantly minimize abnormal findings in renal tissue (Geyikoglu *et al*., 2017). Furthermore, OLE considerably reduced inflammation and apoptosis (Badr and Fouad, 2016; Alhaithloul *et al*., 2019).

CONCLUSION

This study revealed that acute $CdCl_2$ exposure results in severe renal injury via marked induction of oxidative stress with severe histopathological alteration in renal tissues. Meanwhile, treatment with PDE has a greater protective effect on the kidney and significantly reduces the alternations in the renal histopathology while the OLE antioxidant effect is predominant. These results suggest that PDE and OLE's nephroprotective effects may be mediated by antioxidant and inflammatory activities with anti-apoptotic properties. Accordingly, these findings have important implications for the use of natural plant extracts such as PDE and OLE in the treatment of renal toxicity and as good sources of natural antioxidants with a wealth of pharmacological benefits.

ACKNOWLEDGMENTS

The authors would like to thank all staff members of the Pathology Department, Faculty of Veterinary Medicine, Benha University, Egypt.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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