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Insights Into Protective Mechanisms of Dandelion Leaf Extract Against Cisplatin-Induced Nephrotoxicity in Rats: Role of Inhibitory Effect on Inflammatory and Apoptotic Pathways

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Abstract

Cisplatin (CP) nephrotoxicity is associated with the induction of oxidative stress, inflammation, and apoptosis. Several studies demonstrated the antioxidant, anti-inflammatory, and antiapoptotic effects of dandelion leaf extract (DLE); therefore, this research aimed to investigate the protective effects of DLE against CP-induced nephrotoxicity. Thirty-two male Wistar rats were divided into 4 groups: normal control, DLE control received 500 mg/kg daily for 11 days, intoxicated group received vehicle daily for 11 days and a single dose of CP (7 mg/kg, intraperitonealp) on day 5, and DLE + CP group received DLE (500 mg/kg) daily for 11 days plus a single dose of CP (7 mg/kg) on day 5. The dose of DLE is selected based on a dose–effect study using different doses. Dandelion leaf extract pretreatment ameliorated CP-induced nephrotoxicity as evident by histopathological examination, alleviating CP-induced elevation in serum creatinine, blood urea nitrogen, oxidative stress marker (thiobarbituric acid reactive substances), tumor necrosis factor-α (as inflammatory cytokine), and caspase-9 and caspase-3 (as apoptotic markers). In addition, DLE reduced nuclear factor-κB and cytochrome c expression, and DNA fragmentation. It also maintained levels of reduced glutathione, superoxide dismutase, and serum albumin. Thus, the present study shows that DLE is a promising nephroprotective agent for CP-induced nephrotoxicity through antioxidant, anti-inflammatory, and antiapoptotic activities.

Keywords

cisplatin, dandelion leaf extract, anti-inflammatory, antiapoptotic; nephrotoxicity

Introduction

Cisplatin (CP) is a platinum-derived chemotherapeutic agent that has been widely used for the treatment of various malignancies.[1] It has frequently been used to increase survival in patients with head, neck, colon, testis, breast, ovary, cervix, lung, or bladder cancer.[2] Cisplatin is an alkylating agent that induces inter- and intrastrand cross-links in DNA, which arrest DNA replication.[3]

The cytotoxic activity of CP targets not only tumor cells but also normal somatic cells, particularly in the liver, heart, and kidneys. Cisplatin is renally eliminated and accumulated in the renal tubules,[4] leading to CP concentrations in the proximal tubule cells that are approximately 5 times those in the serum. Thus, proximal tubule cells are a major site of damage.[5] Once introduced into the cell, CP conjugates with glutathione and is metabolized to the corresponding reactive thiol, which shows

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potent nephrotoxic activity.[6] Its accumulation in the renal tubules enhances the production of reactive oxygen species (ROS) and decreases the antioxidant enzymes.[7] Reactive oxygen species initiate inflammation and mediate the activation of nuclear factor (NF)-κB, which upregulates the expression of several genes involved in the inflammatory response.[8] Inflammation worsens renal injury by enhancing renal expression of tumor necrosis factor-α (TNF-α) and other cytokines. Binding of TNF-α to the TNF-α death receptors (TNFR)1 and 2 leads to the activation of caspase-8, which in turn activates downstream caspases to induce apoptosis. Moreover, CP therapy can lead to both transient and permanent renal damage (apoptosis and necrosis) as indicated by both molecular and histological studies.[9]

It is expected that both antioxidants and anti-inflammatory compounds could help prevent CP-induced nephrotoxicity. Some of these antioxidants are naturally present in the body, while others are provided as supplements to augment the function of endogenous free radical scavengers.[7] Several phytochemicals act as antioxidants and protect cells against CP-induced nephrotoxicity in experimental animals.[10] Phytochemicals have been used in traditional medicine for centuries for treating various diseases. There is considerable evidence that phytochemicals may retard tumor growth and elicit antioxidant and anti-inflammatory effects.[11]

Plants belonging to genus *Taraxacum* have been used for long time as medicinal herbs. One of those is Taraxacum *officinale*, known as dandelion. It is widespread in the world and used traditionally in many cultures. It was used by Arabs in the 10th and 11th centuries to treat liver- and spleen-related conditions. It is considered to be a blood purifier, laxative, and is used to treat arthritic and rheumatic pains and for eczema and other skin conditions.[12] Decoction of the whole plant is traditionally used in the treatment of diabetes mellitus.

Dandelion is rich in wide variety of biologically active compounds, which explains its therapeutic potential. The most valuable active components are biotin, sesquiterpenic lactones, inositol, and vitamins B, D, and E in addition to phosphorous. The leaves are rich in β-carotene than carrot and pose more calcium and iron than spinach. The leaves of dandelion are characterized by high polyphenolic contents, especially hydroxycinnamic acid derivatives such as caffeic acid esters (chlorogenic, dicaffeoyl tartaric, and mono caffeoyl tartaric acids).[13,14] Moreover, various flavonoid glycosides have also been identified in dandelion leaves (such as luteolin 7-*O*-glucoside, luteolin 7-*O*-rutinoside, isorhamnetin 3-*O*-glucoside, quercetin 7-*O*-glucoside, and apigenin 7-*O*-glucoside).[15,16] This high phenolic content may explain many observed effects of dandelion leaf extract (DLE), such as antioxidant, anti-inflammatory, antiapoptotic, anticarcinogenic, and analgesic activity.[16] Based on this information, we aimed in this study to evaluate the role of DLE as a promising protective remedy against CP-induced nephrotoxicity and exploring its effect on inflammatory and apoptotic markers.

Materials and Methods

Preparation of DLE

Dandelion tree leaves (500 g) were obtained from Egypt and were evaluated by specialized botanist such as Dr Naglaa Saad El Din, Pharmacognosy Department, Ain Shams University, Cairo, Egypt, who also supervised extraction process. Leaves were washed, air-dried, and grounded with a mortar to a fine powder. The resultant powder was then macerated for 3 days in 2 L of methanol with shaking. The mixture was filtered daily and 2 L fresh methanol was added to the remaining pulp, while the collected fractions were put together. The methanolic extract was evaporated by rotary evaporator to obtain a semisolid mass, which was stored at −20°C until needed.[17]

Chemicals

Cisplatin was obtained as commercially used vials from Hospira (Lake Forest, Illinois). Methanol, potassium dihydrogen phosphate, n-butanol, 1,1′,3,3′-tetramethoxypropane, reduced glutathione (GSH), Ellman's reagent (5,5′-dithio-bis-2-nitrobenzoic acid), thiobarbituric acid (TBA), and trichloroacetic acid (TCA) were purchased from Sigma-Aldrich (St. Louis, Missourie). Kits for the determination of urea, creatinine, and albumin were obtained from Sigma-Aldrich. Superoxide dismutase (SOD) kit was obtained from assay kits (Biodiagnostic, Cairo, Egypt). Rat TNF-α, caspase-9, and caspase-3 enzyme-linked immunosorbent assay (ELISA) kits were purchased from Raybioech (Norcross, Georgia) and Cloud-Clone Corp (Houston, TX), respectively. Immunohistochemistry (IHC) antibodies for NF-κB and cytochrome c were purchased from Thermo Scientific (Waltham, Massachusetts). DNA extraction kit and RNAlater solution were obtained from Qiagen (Hilden, Germany). Other chemicals were of high analytical grade.

Experimental Design

Adult healthy male Wistar rats weighing 150 to 200 g were supplied by the animal house of King Saud University, Riyadh, Saudi Arabia. Animals were housed 4 per cage in standard cages of polypropylene and acclimatized for 1 week before the experiments. Animals were maintained at water and standard basal diet ad libitum and were maintained under controlled conditions of temperature, humidity, and light. Handling of animals was in compliance with the guidelines for the use of animals for scientific purposes, with ethical approval number: KSU-SE-191-02 for animal protocol, according to King Saud University instructions.

Thirty-two Wistar adult rats were randomly divided into 4 groups, 8 rats per group (4 animals per cage)

Group I (normal control) received normal saline daily via an oral tube. Group II (DLE control) received 500 mg/kg DLE daily for 11 days via oral tube; DLE was adjusted to suitable dose by dissolving measure amount of extract in normal saline. Group III (CP-intoxicated) received normal saline daily via

oral tube for 11 days, and CP was injected intraperitoneally (IP; 7 mg/kg) on day 5.[18] Group IV (DLE + CP) received 500 mg/kg DLE daily for 11 days via oral tube and a single dose of CP by IP injection (7 mg/kg) on day 5 of the experiment 1 hour prior to the DLE dose. The dose of DLE was selected based on a preliminary dose–response experiment in which 3 different doses of DLE (250, 500, and 750 mg/kg) were examined. Serum creatinine and urea results were used to compare the effect of different doses.

Animals were killed by decapitation on the 11th day, 1 hour after last DLE dose. Rats' blood samples were collected and allowed to stand for half an hour, and the serum was collected by centrifugation at 3000 rpm for 15 minutes at 4°C and then stored at −80°C before use. The kidneys were immediately removed, dried, and weighed. Part of the kidney tissue was transferred immediately to 10% buffered formaldehyde for histological and IHC examination. A second part was weighed and homogenized immediately on ice-cold medium containing 50 mM Tris–HCl at pH 7.4, to give 20% (w/v) homogenate to be used for biochemical determinations. The third part was submerged immediately in RNAlater solution to avoid DNA and RNA degradation and stored until use at −20°C.

Estimation of Kidney Weight to Body Weight Ratio

Rats were weighed before decapitation. After decapitation, the kidney of each rat was dried and weighed to calculate the kidney weight to body weight ratio. Ratio = (kidney weight/body weight) × 100.

Kidney Function Tests

Serum levels of creatinine, blood urea nitrogen (BUN), and albumin were determined using biochemical kits (Sigma-Aldrich) according to manufacturer's instructions.

Determination of Oxidative Stress-related Parameters

Twenty percent of liver homogenate was used for determination of SOD, lipid peroxidation (LPO), and GSH as markers of oxidative stress. Superoxide dismutase was measured using kits from Biodiagnostic. Lipid peroxidation was determined spectrophotometrically as thiobarbituric acid-reactive substances (TBARS), according to the method of Mihara and Uchiyama.[19] The colorimetric determination of TBARS was based on the reaction of malondialdehyde with TBA at low pH and high temperature. The resulting pink product was extracted with n-butanol, and the absorbance was determined spectrophotometrically at 535 nm.

The estimation of GSH was determined using a spectrophotometer, according to Ellman's method.[20] An aliquot of 0.5 mL of tissue homogenate was used. Proteins were precipitated using TCA, and samples were centrifuged at 3000 rpm for 10 minutes. The resulting supernatant was used for determination of GSH using Ellman's reagent. The absorbance was measured at 412 nm.

Determination of Renal Levels of TNF-α, Casapse-3, and Caspase 9 by ELISA Kits

All TNF-α, caspase-3, and caspase-9 were detected in kidney tissue homogenates using a standard Sandwich ELISA kit. Tests were performed according to the manufacturer's instructions.

Total DNA Preparation, Extraction, and Fragmentation

Kidney tissue (30 mg) was homogenized in RLT lysis buffer (Qiagen, Ambion, Courtabeuf, France) containing 1% 2-mercaptoethanol. Extraction of total DNA using a Prep DNA/RNA Mini kit (Qiagen, Hilden, Germany, cat #80204) was carried out, following the manufacturer's instructions, and DNA was eluted with 50 µL elution buffer. Quantification of extracted DNA was performed using NanoDrop-8000 (Waltham, USA) and its integrity was determined with agarose gel (1.5%) electrophoresis. Gels were illuminated using 300 nm ultraviolet light and a photographic record was obtained.[21]

Histopathological Examination

Kidney samples were fixed in 10% buffered formaldehyde for 24 hours and then washed with tap water. Dehydration was performed using serial dilutions with alcohol. Specimens were cleared with xylene and embedded in paraffin with hot-air oven at 56°C for 24 hours. Paraffin beewax tissue blocks were cut into sections of 5-µm thickness using a sledge microtome. The tissue sections were stained with hematoxylin and eosin (H&E) for histopathological examinations using light microscope.[22]

IHC Testing of NF-κB and Cytochrome C

Formalin-fixed kidney tissues embedded in paraffin were cut into 5-µm sections and fixed overnight, then deparaffinized with xylene and rehydrated in a graded concentrations of ethanol, and finally boiled in antigen unmasking solution (Vector Laboratories, Burlingame, California) for 5 minutes. Slides were then immersed in 0.075% peroxidase-blocking reagent (Dako, Botany Bay, Australia) for 10 minutes. Next, they were incubated with blocking goat serum (Dako) for 30 minutes. Sections were then treated and incubated with anti-NF-κB antibody (rabbit polyclonal; 1:500) in blocking solution for 14 hours at temperature of 4°C or cytochrome c antibody (Clone 7H8.2C1; mouse monoclonal) for detection of NF-κB and cytochrome c, respectively. Then sections were re-equilibrated to room temperature, washed with phosphate buffer solution (PBS), and incubated with horseradish peroxidase antibody conjugates, concentration (1:2500) in blocking solution without Tween-20 for 2 hours at room temperature.

Finally, specimens were washed with again PBS, incubated with a 0.2% solution of 3,3′-diaminobenzidine until desired stain developed intensity at room temperature, and washed in distilled water. Sections were then counterstained with H&E, dehydrated in a graded series of ethanol, and finally mounted with di-n-butylphthalate-polystyrenexylene.[23]

Statistical Analysis

Results are represented as mean ± standard error of the mean. Statistical analysis was done using 1-way analysis of variance, comparisons among groups were made using post hoc Tukey test. Statistical analyses and graphical representation were performed by Graph Pad Prism 5 software (Graph Pad Software, Inc).

Results

Dose–Response Experiment

Serum BUN levels. Serum BUN levels were significantly higher in the CP-treated animals than in the control group. Treatment with DLE + CP significantly alleviated the high levels of BUN at all DLE doses except 250 mg/kg. Although the doses 500 and 750 mg/kg lowered the BUN levels, the differences showed no significant difference between their effects (Figure 1A).

Serum creatinine levels. Serum creatinine levels were significantly higher in the CP-treated animals than in the control group. Treatment with DLE + CP maintained the levels of creatinine at all different DLE doses. The 500 mg/kg dose was the most effective with a significant difference from the effect of 750 mg/kg (Figure 1B). Accordingly, the DLE dose of 500 mg/kg was selected for further assessments.

Effect of DLE (500 mg/kg) on CP Nephrotoxicity

Kidney weight/body weight ratio. Treatment with DLE (500 mg/kg) alone had no effect on the kidney weight–body weight (KW/BW) ratio compared to normal control, while this ratio significantly increased ($P < .01$) in the CP-treated group and significantly decreased ($P < .001$) upon pretreatment with DLE (Figure 2A).

The levels of serum creatinine, BUN, and albumin. The levels of serum BUN and creatinine were significantly higher in the CP-treated animals compared with control group. Treatment with DLE + CP significantly ameliorated the elevated levels of creatinine and BUN. In contrast, the levels of serum albumin were significantly lowered in the CP-treated animals, while animals treated with DLE + CP maintained normal albumin levels. Treatment with DLE alone did not induce any change in the levels of the tested parameters from the baseline (Figure 2B-D).

Kidney histopathology. Control group rats (Figure 3A) and rats treated with DLE (Figure 3B) had normal glomeruli and tubules, and no significant differences were found between them. Cisplatin administration resulted in histopathologic changes in kidney tissues including degeneration in the epithelial cells lining the renal tubules with desquamation of degenerated cells within the lumen of the tubules, vacuolization, apoptosis, severe necrosis, loss of brush border, blood congestion, debris in the intratubular area, and flattening of the tubular epithelium. It also caused tubular dilatation in corticomedullary junction (Figure 3C). Moreover, an eosinophilic amorphous material, debris (pyknotic and karyorrhectic), was also observed in the necrotic tubules of the examined tissues.

Pretreatment with DLE reduced the pathological changes observed in the kidney (Figure 3D), with visible mitosis and regeneration of tubular epithelium. These results demonstrate that DLE administration protected the kidney from renal injury that could occur by CP injection. An observable improvement in morphology of the examined kidney sections and reversion of renal injury was noticed.

Oxidative stress markers

Effect of CP and DLE treatment on LPO levels. Cisplatin treatment significantly increased LPO levels in kidney tissues compared with that in the control group; as evidenced by increased TBARS. The DLE pretreated rats showed a significant decline in LPO levels compared to that observed with CP treatment alone (Figure 4A).

Effect of CP and DLE treatment on GSH levels. Cisplatin treatment significantly reduced GSH levels in kidney tissue homogenates compared with control group animals. Pretreatment with DLE caused maintained GSH level comparable to the control group (Figure 4B). Dandelion leaf extract alone did not cause any changes in the levels of LPO and GSH from the levels seen in the control group (Figure 4B).

Effect of CP and DLE treatment on inflammatory mediators (NF-κB, TNF-α) and apoptotic markers (cytochrome c, caspase 9, caspase-3, and DNA fragmentation)

Nuclear factor-κB. Immunohistochemical analysis of the expression of NF-κB p65 subunit in rat kidney of the control group (Figure 5A) and DLE control (Figure 5B) showed low NF-κB immunoreactivity. In contrast, strong NF-κB immunoreactivity was observed in renal tubular cells of CP-intoxicated rats, with numerous positive reactions in nuclei (Figure 5C). Pretreatment of CP-intoxicated rats with DLE significantly reduced the NF-κB immunopositivity (Figure 5D).

Tumor necrosis factor-α. As shown in Figure 6, the levels of the cytokine TNF-α, as quantified by ELISA, were markedly increased (223% of control) in the kidneys of rats treated with CP. In contrast, DLE alone decreased the levels of TNF-α by 15% of control levels. Rats treated with DLE prior to CP exhibited a significant ($P < .001$) suppression in the levels of TNF-α compared to CP-treated rats.

Expression of cytochrome c. Figure 7 showed the expression of cytochrome c at protein level using IHC as a marker of apoptosis. Kidneys of control group rats and rats treated with DLE showed low cytochrome c immunoreactivity. In contrast, strong cytochrome c immunopositivity was observed in tubular cells of CP-treated animals, with many positive nuclei. Pretreatment of CP-intoxicated rats with DLE decreased protein expression.

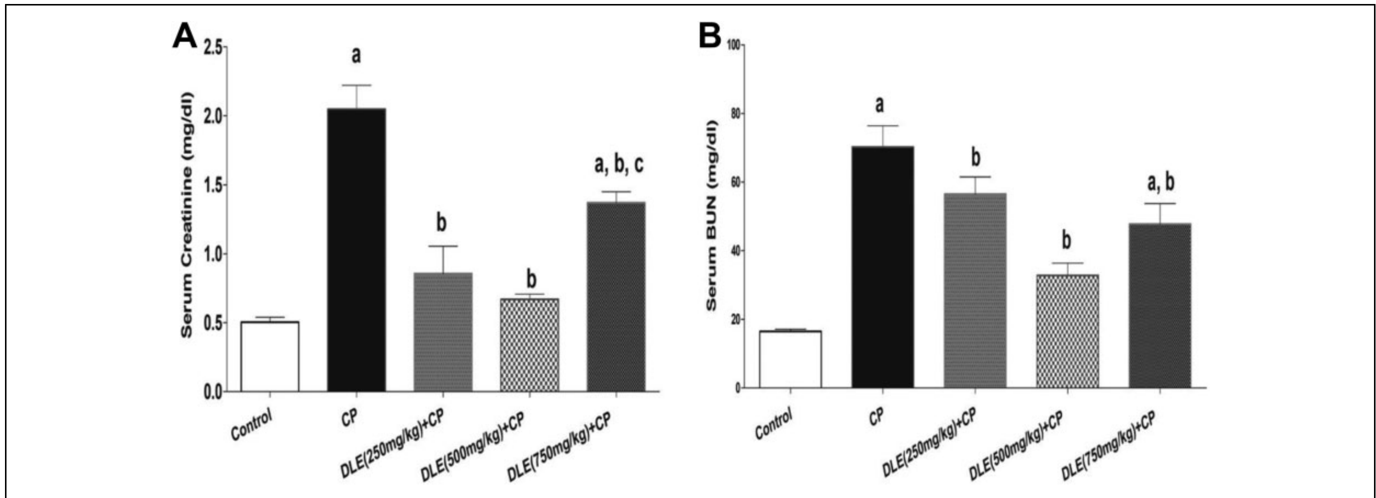

Figure 1. Effects of different doses of DLE on kidney function tests in CP-induced nephrotoxicity. (A) Creatinine (mg/dL) and (B) serum blood urea nitrogen (BUN; mg/dL) in different animal groups. Each value represents the mean of 6 records ± standard error of the mean. [a] $P < .05$, significant change with respect to control group; [b] $P < .05$, significant change with respect to CP group, [c] $P < .05$, significant change with respect to DLE (500 mg) + CP group. CP indicates cisplatin; DLE, dandelion leaf extract.

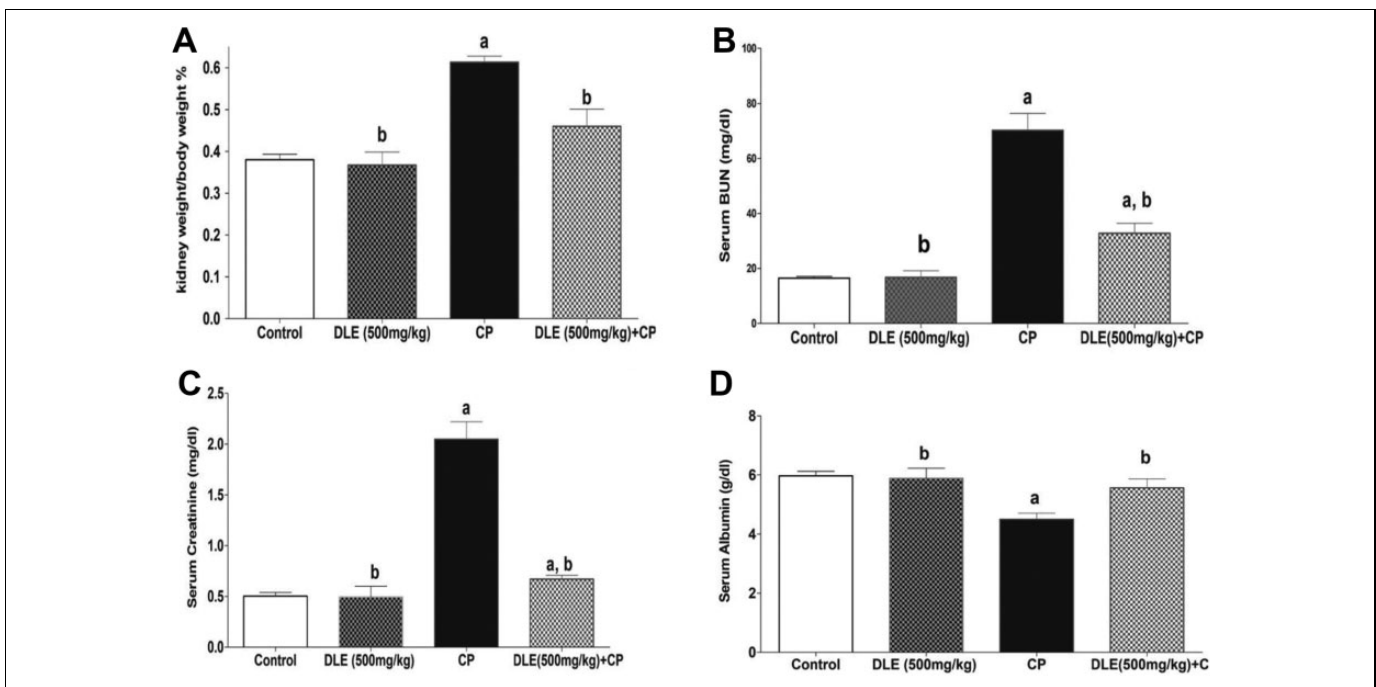

Figure 2. Effects of DLE (500 mg/kg) on kidney function tests in CP-induced nephrotoxicity. (A) The kidney weight/body weight ratio (%), (B) serum blood urea nitrogen (BUN; mg/dL), (C) serum creatinine, and (D) albumin (g/dL) in different animal groups. Each value represents the mean of 6 records ± standard error of the mean. [a] $P < .05$, significant change with respect to the control group; [b] $P < .05$, significant change with respect to the CP group. CP indicates cisplatin; DLE, dandelion leaf extract.

Casapase-3 and caspase-9 levels. The elevation in renal levels of caspase-3 and caspase-9 is known as the index of apoptosis. The group treated with CP showed high concentration of both caspases. The group of animals treated with DLE prior to CP administration showed significant decrease in caspase-9 and caspase-3 to levels comparable to the control and compared to rats treated with CP alone (Figure 8A and B).

DNA fragmentation. DNA fragmentation is known as a marker of apoptosis. The qualitative measurement of the integrity of the renal genomic DNA has been studied by agarose gel electrophoresis (Figure 8C). The results demonstrated that CP treatment causes DNA damage as indicated by smearing of DNA fragments (lanes 3 and 4), while the DNA isolated from control rats (lane 1) and DLE treated (lane 2) showed good-

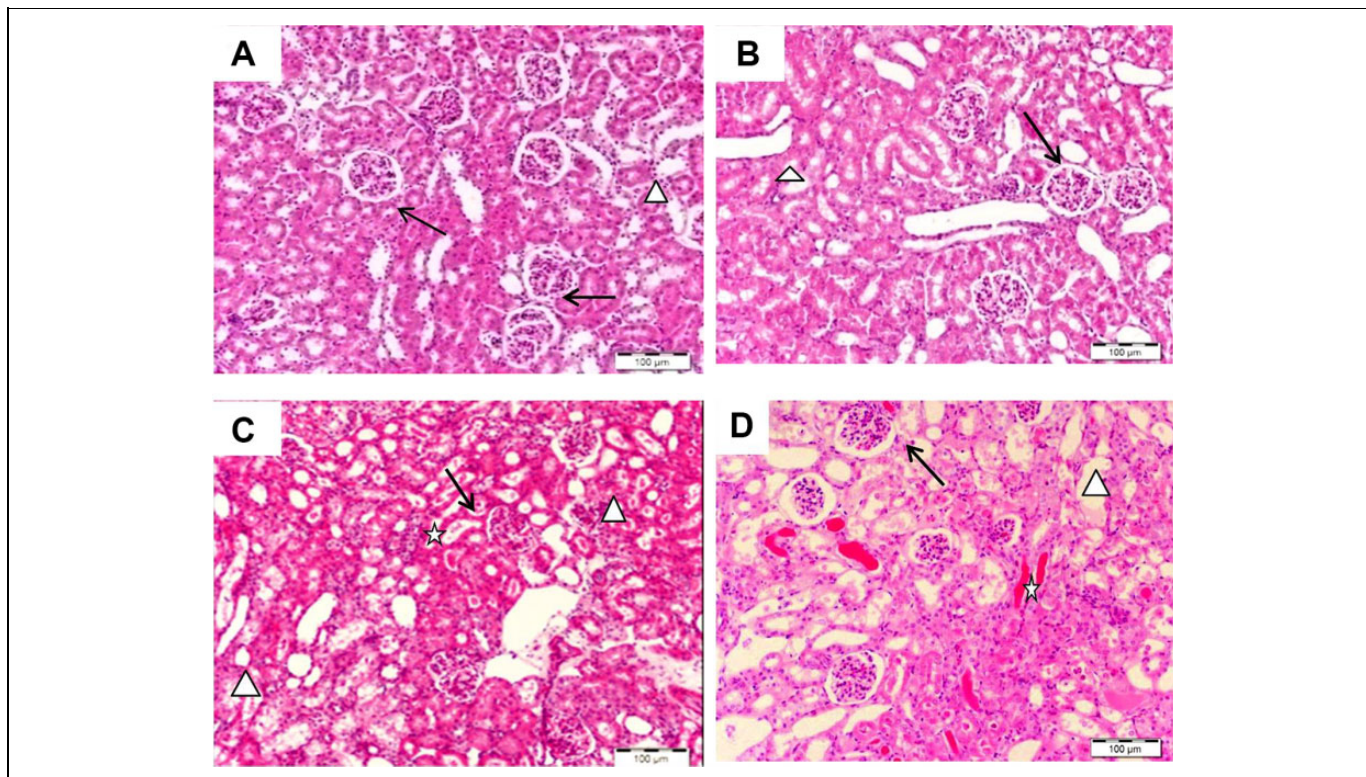

Figure 3. Histological examinations using hematoxylin and eosin staining to demonstrate the effect of dandelion leaf extract (DLE) on cisplatin (CP)-induced renal damage in rats. Light micrographs showing rats treated with vehicle (A); DLE 500 mg/kg (B) showing normal glomeruli (↑) and renal tubules lined by tubular cells with vesicular nuclei (Δ); CP (C) showing necrotic glomeruli (↑), and renal tubules with marked vacuolation of their lining epithelium, dense nuclei, and congested blood vessels (star); DLE (500 mg/kg) +CP (D) showing decreased renal damage as evidenced by normal glomeruli (↑) and renal tubules (Δ), that in rats receiving the highest dose DLE was resembled tissues from the control group. Original magnification is ×40.

quality DNA separation without smearing. DNA of rats treated with DLE prior to CP (lane 5) showed less DNA damage with only mild smearing.

Discussion

Cisplatin is one of the highly efficient and widely used cytotoxics for the treatment of solid tumors. Although it also causes dose-dependent nephrotoxicity, its survival benefits outweigh the risks involved. Nephrotoxicity mechanisms include increased ROS generation, DNA damage, tubular inflammation, and apoptosis. It affects drug tolerability and lowers maximum dose that can be used.[24] Therefore, it is an essential requirement to develop modalities that can ameliorate CP-induced nephrotoxicity. The protective effect of DLE has been investigated in this study. Alcoholic DLE's active constituents were well characterized by Jassim et al.[25] Dandelion leaf extract contains a wide variety of active constituents, including glycosides, phenolic compounds, alkaloids, tannins, flavonoids, trace elements, and vitamins, many of which are reported to have many biological activities including antioxidant, antidiabetic, hepatoprotective, and anti-inflammatory activity. Dandelion is widely distributed in many countries, making it a good candidate for further elucidation of potential uses and effects. In this study, we aimed to investigate the effect of DLE as possible natural remedy to protect against CP-induced nephrotoxicity.

The administration of a single dose of CP (7 mg/kg) resulted in hypertrophy as indicated by the significant increase in the KW–BW ratio. Cisplatin also resulted in renal dysfunction as revealed by a significant alteration of renal function parameters (increased serum creatinine, elevated BUN, and decreased serum albumin; Figures 1 and 2). These results agree with previous works.[26] Increased serum BUN and creatinine levels reflect the defective excretory function of the kidney. Reduced albumin levels indicate altered glomerular permeability and impairment in the filtration. Pretreatment with different doses of DLE maintained KW–BW ratio and corrected impaired renal function tests. However, a dose of 500 mg/kg showed no significant difference from 750 mg/kg. Therefore, the lower dose was selected for further investigation.

Nephrotoxicity was also evidenced by histopathological examination (Figure 3C) that showed excessive renal tissue damage, including vacuolization, necrosis, and degenerative changes in the epithelium lining of renal tubules with desquamation of degenerated cells. Cisplatin nephrotoxicity is mainly attributed to the metabolism of CP in the kidney to more potent toxins that lead to cell death and degeneration.[27] Treatment

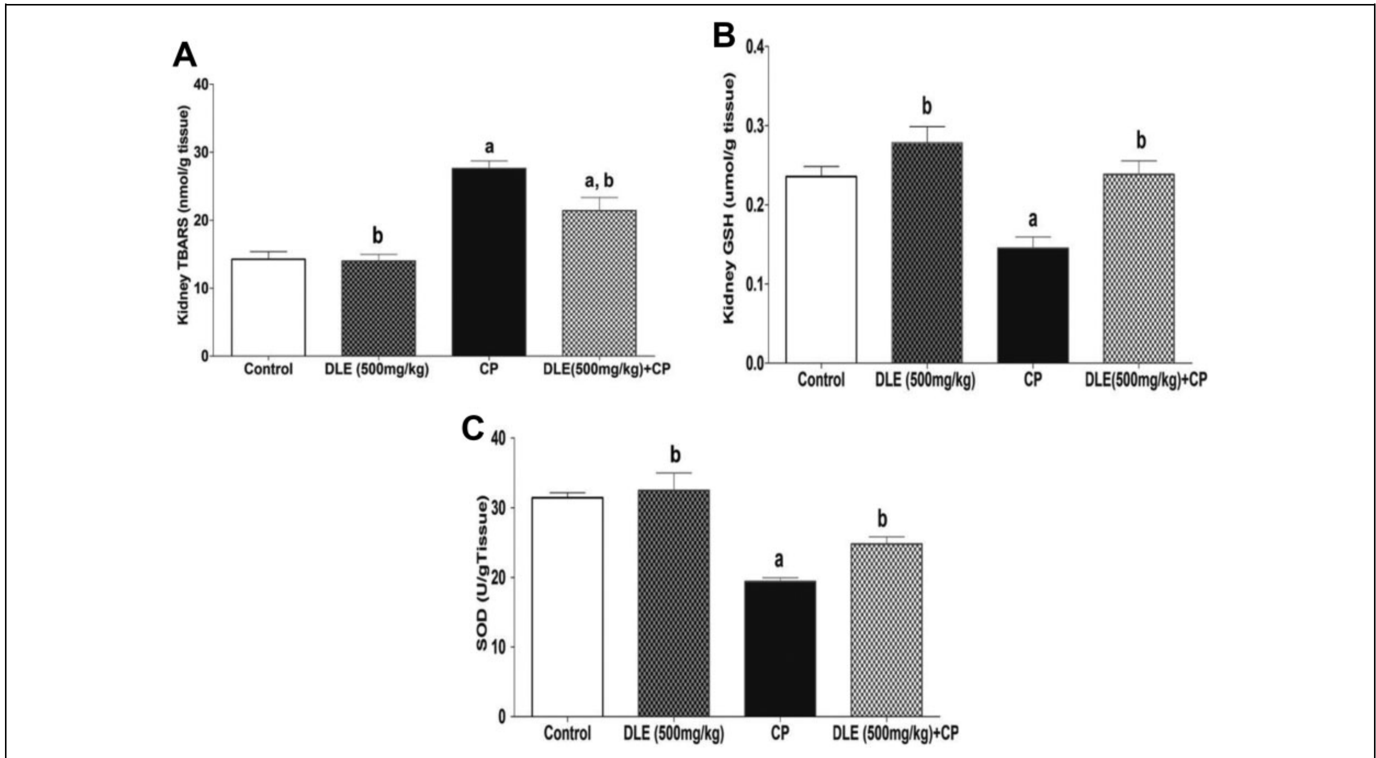

Figure 4. Effects of DLE (500 mg/kg) on oxidative stress-related parameters in CP-induced nephrotoxicity. (A) Thiobarbituric acid reactive substance (TBARS; nmol/g tissue), (B) kidney GSH (μmol/g tissue), and (C) kidney SOD in different animal groups. Each value represents the mean of 6 records $\pm$ standard error of the mean. [a] $P < .05$, significant change with respect to the control group; [b] $P < .05$, significant change with respect to CP group. CP indicates cisplatin; DLE, dandelion leaf extract; SOD, superoxide dismutase.

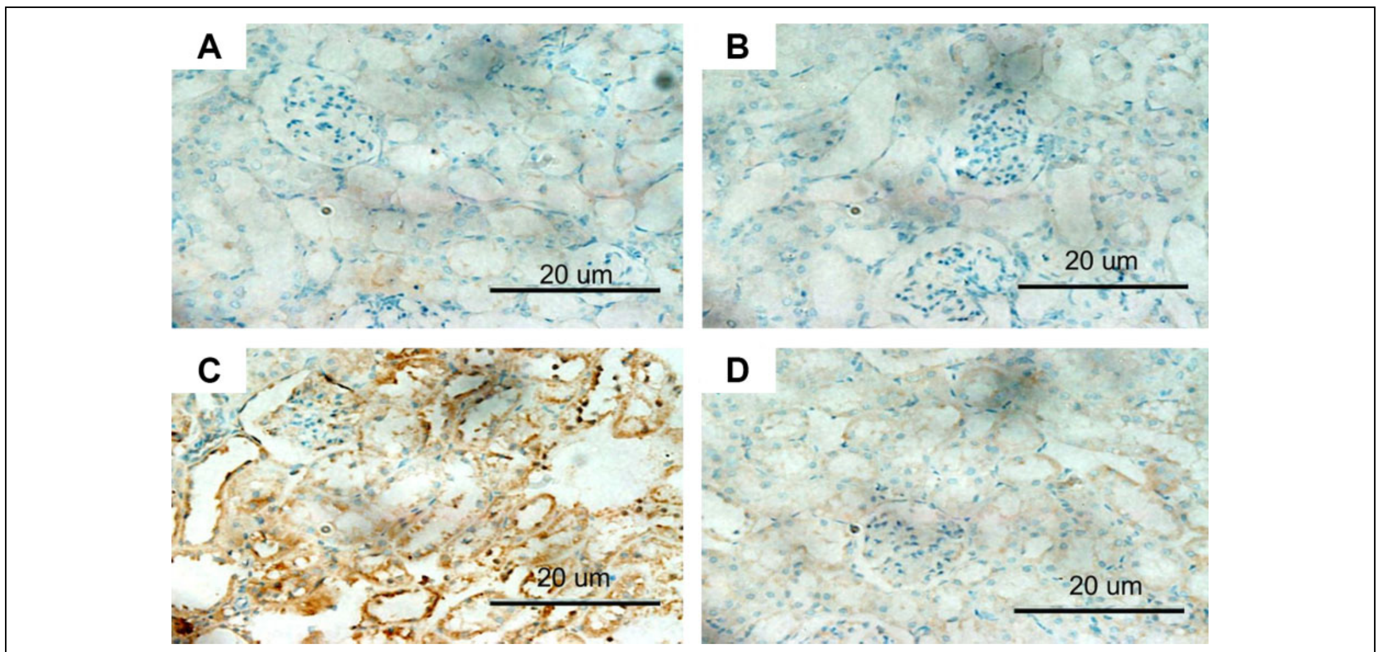

Figure 5. Effects of DLE (500 mg/kg) on NF-κB expression in rat kidneys in CP-induced nephrotoxicity using immunohistochemistry in different groups. Rats treated with vehicle (A), DLE 500 mg/kg (B), CP 7 mg/kg (C), DLE (500 mg/kg) + CP (7 mg/kg) (D). Immunohistochemistry staining, original magnification ×40 (A). CP indicates cisplatin; DLE, dandelion leaf extract.

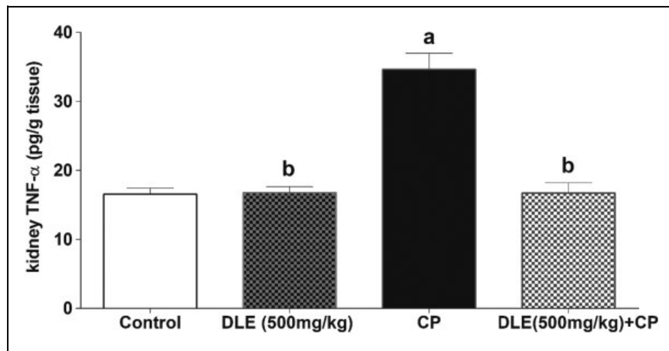

Figure 6. Effects of DLE (500 mg/kg) on TNF-α levels (pg/g tissue) in rat kidneys in CP-induced nephrotoxicity using enzyme-linked immunosorbent assay (ELISA) in different groups. Each value represents the mean of 6 records ± standard error of the mean. [a] $P < .05$, significant change with respect to control group; [b] $P < .05$, significant change with respect to CP group. CP indicates cisplatin; DLE, dandelion leaf extract; TNF-α, tumor necrosis factor-α.

with DLE preserved renal tissue integrity and counteracted the effect of CP (Figure 3D), and this explains the improvement of renal function in intoxicated rats treated with DLE. Dandelion leaf extract alone did not induce any changes in renal tissue architecture, compared with that in the control group (Figure 3A and B).

Substantial articles demonstrate the critical role of oxidative stress in CP-induced nephrotoxicity.[26,28] This was consistent with our results as CP increased the levels of kidney TBARS; the product of LPO (Figure 4A) and decreased the levels of GSH (Figure 4B), one of the main cellular antioxidants and SOD, one of the most important free radical scavengers. Decrease in GSH is consistent with earlier studies[29] and is probably due to the formation of CP-GSH conjugation products.[30] Indeed, GSH plays a significant role in CP-induced nephrotoxicity.[29] The metabolism of CP starts by binding with GSH forming GSH conjugates, which are further metabolized into cysteinyl glycine conjugates followed by its cleavage into cysteine conjugates, which finally metabolized into reactive thiols in the proximal tubules.[31] These reactive metabolites bind to macromolecules, triggering an increase in cytosolic-free calcium and ultimately cell death.[27]

According to previous data, CP-induced nephrotoxicity can be ameliorated by powerful antioxidants.[26,30] In the present study, pretreatment with DLE protected kidney cells against CP-induced oxidative stress, as revealed by the maintenance of GSH levels, and SOD compared to that of control, and the significant decrease in TBARS levels compared to that in the CP group. This protection could be attributed to the antioxidant activity of DLE, which has been previously reported by several researchers.[32,33] The ability of DLE to scavenge free radicals is mainly due to the presence of several phenolic and flavonoid antioxidant compounds, such as chicoric acid, chlorogenic acid, caffeic acid, quercetin glycoside, and sesquiterpene lactones.[14,15] Chlorogenic acid is the most abundant and potent antioxidant phenolic component in DLE. Sesquiterpenes mediate their antioxidant properties via their α-methyl-γ-lactone structure that reacts with cysteine sulfhydryl groups; the primary target group of sesquiterpene lactones.[34]

The cell death associated with oxidative stress leads to inflammatory response, which plays a key role in the

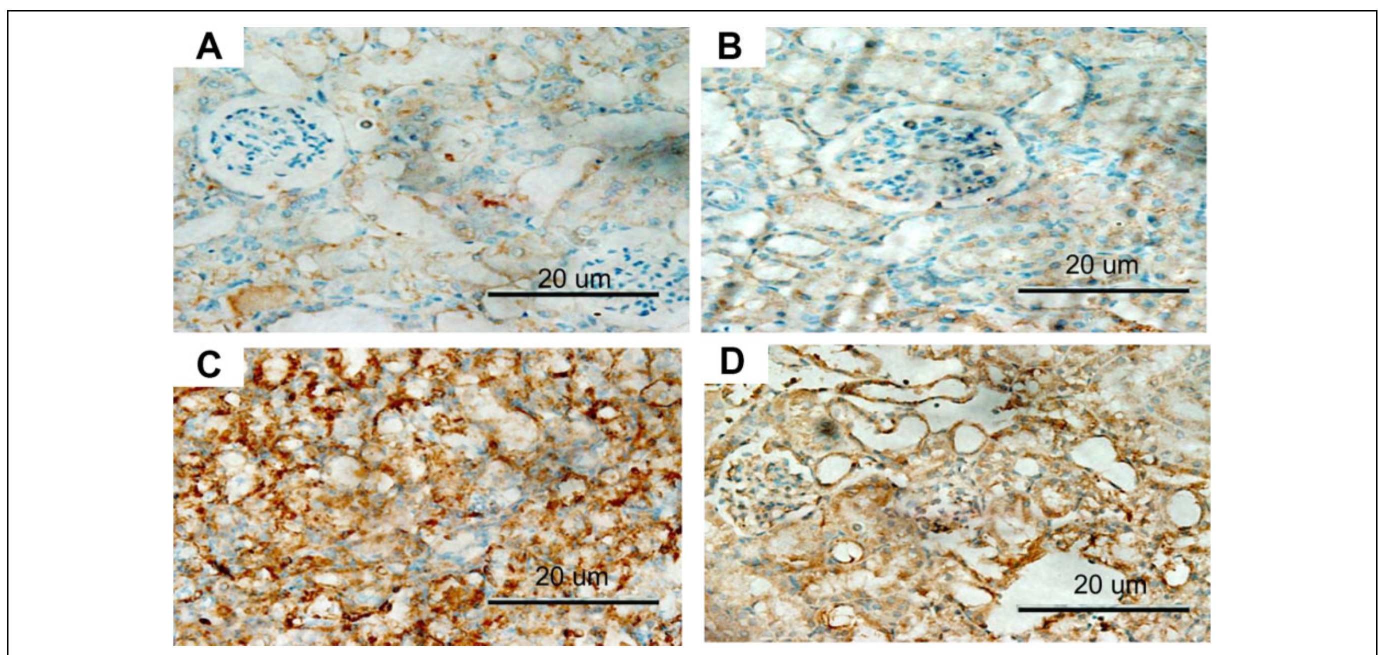

Figure 7. Effects of DLE (500 mg/kg) on the expression of cytochrome c in rat kidney tissue using immunohistochemistry in different groups. Rats treated with vehicle (A), DLE 500 mg/kg (B), CP 7 mg/kg (C), or DLE (500 mg/kg) + CP (D). Immunohistochemistry staining, original magnification ×40. CP indicates cisplatin; DLE, dandelion leaf extract.

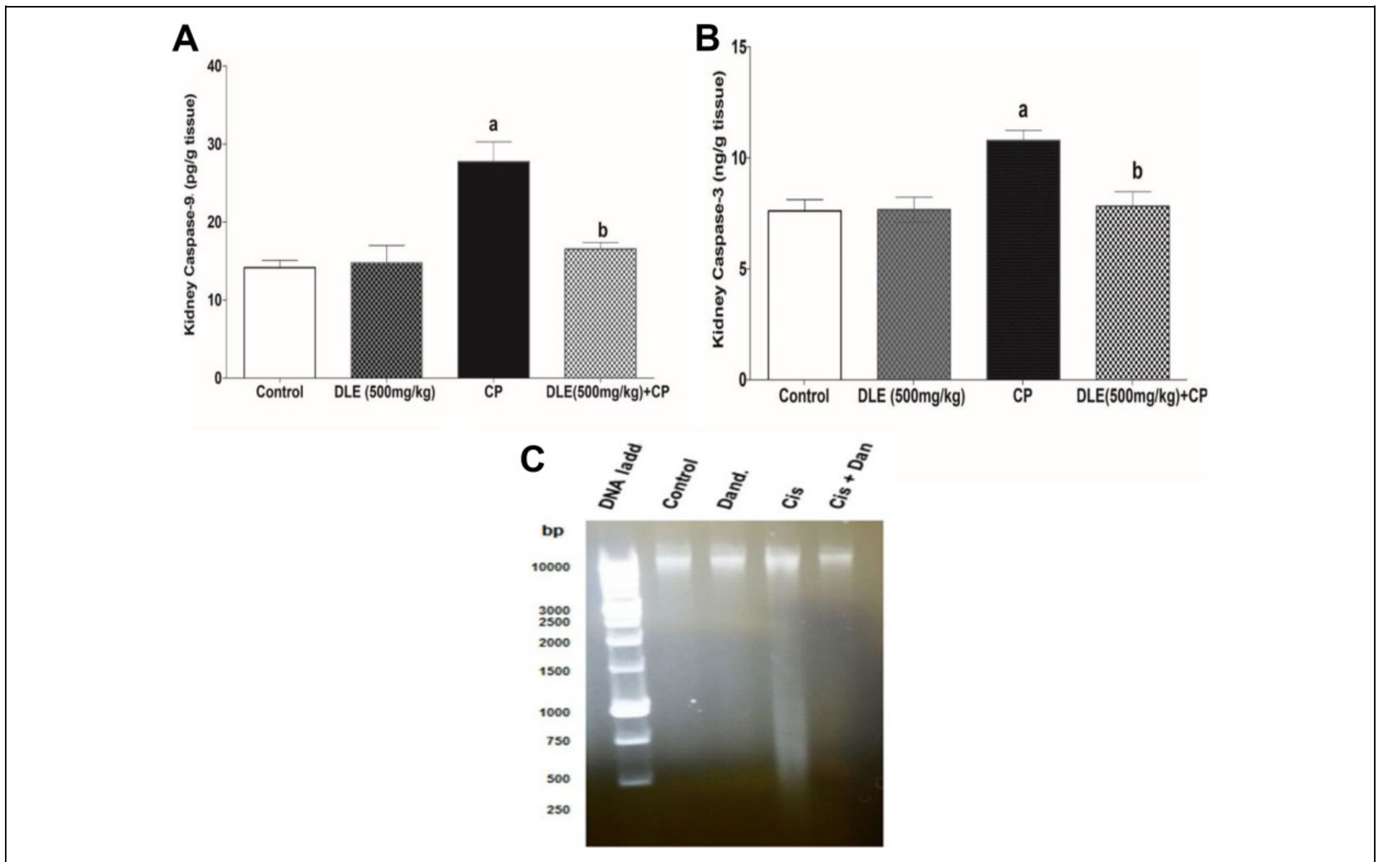

Figure 8. Effects of DLE (500 mg/kg) on apoptosis-related markers in rat kidneys in CP-induced nephrotoxicity. Results of enzyme-linked immunosorbent assay (ELISA) on kidney caspase-9 activity (pg/g tissue protein) (A) and caspase-3 (B) in different groups. Each value represents the mean of 6 records $\pm$ standard error of the mean. [a] $P < .05$, significant change with respect to control group; [b] $P < .05$, significant change with respect to CP group. (C) DNA fragmentation in control and experimental rats. Lane 1: control group; lane 2: group treated with DLE (500 mg/kg); lane 3: group treated with CP 7 mg/kg; lane 4: group treated with DLE (500 mg/kg) + CP (7 mg/kg). CP indicates cisplatin; DLE, dandelion leaf extract.

pathogenesis of CP-induced nephrotoxicity.[4,35] To study the anti-inflammatory effect of DLE in our experimental model, and its contribution to its protective effect, we determined the expressions of NF-κB and TNF-α in renal tissues (Figures 5 and 6). Both markers were significantly increased in CP treated rats, proving the importance of inflammation in mediating nephrotoxicity. Our findings are consistent with previous results.[36] Tumor necrosis factor-α appears to be a key regulator in the CP-induced inflammation, since the deletion of TNF-α receptors protects against CP nephrotoxicity. The increase in TNF-α expression is considered to be the direct result of ROS formation.[37] Tumor necrosis factor-α can also activate IκB kinase, leading to the activation of NF-κB, [38] which in turn exaggerates the inflammatory response via upregulating the genes involved in inflammatory response, including TNF, interleukins, and cyclooxygenases.

The inflammation caused by CP in rat tissues was significantly ameliorated by DLE through the inhibition of NF-κB activation and TNF-α production. The ability of DLE to suppress TNF-α and NF-κB demonstrates its anti-inflammatory activity, which is in agreement with previous studies in different experimental models[16,39] and can be attributed to the sesquiterpene lactones, taraxasterol, taraxerol, chlorogenic acid, and chicoric acid. These components are nontoxic and well known for their potential anti-inflammatory properties.[34]

Apoptosis is important for the maintenance of homeostasis in most multicellular organisms. This type of cell death can be triggered by an accumulation of ROS, and the activation of proinflammatory cytokines that ultimately leads to the opening of the mitochondrial permeability transition pore and the release of proapoptotic proteins such as cytochrome c. Cytochrome c activates procaspase-9, which in turn activates caspase-3, which is one of the major death proteases, thereby triggering the apoptotic pathway.[40,41] Tumor necrosis factor -α also activates apoptosis through extrinsic pathway, which culminates in caspase-3 activation. In addition, oxidative DNA damage can also lead to apoptosis, via a shift in the homeostasis of various cells leading to impaired signal transductions and apoptosis.[42]

Apoptosis plays an important role in the pathophysiology of CP-induced kidney renal injury especially after high doses,[43] and cellular DNA degradation represents an important marker

of the renal cell death induced by CP.[44] Consistent with these data, CP-intoxicated rats revealed significant increase in the expression of cytochrome c in the kidney (Figure 7), elevated renal levels of casapse-9 and caspase-3 (Figure 8A and B), and augmented DNA fragmentation (Figure 8C), reflecting the CP-induced apoptosis. It is noted that administration of DLE extracts prior to CP treatment markedly protected the renal tissues against apoptosis as indicated by the decreased amount of fragmented DNA (Figure 8C) and reduced apoptotic factors, which may be a consequence of its antioxidant and anti-inflammatory potential. These results agree with the most recent findings on the antiapoptotic effect of *T officinale* leaves extract[45] and with the growing evidence on free radical scavenging properties of many natural phenolic compounds.[46,47]

Our results suggest that antioxidant activity and the decrease in inflammatory mediators and apoptotic factors could be the key mechanisms for the nephroprotective activity of DLE against CP-induced nephrotoxicity. Dandelion leaf extract used alone didn't show any renal adverse effects at the used dose and was comparable to control regarding measured parameters.

Conclusion

In conclusion, to the best of our knowledge, this is the first study to demonstrate the nephroprotective effect of DLE against CP-induced nephrotoxicity. Dandelion leaf extract showed a protective effect against CP-induced kidney injury through the inhibition of the proinflammatory NF-κB pathway and the reduction in TNF-α level. Moreover, treatment with DLE reduced apoptosis via a decrease in cytochrome c expression, and subsequent inhibition of caspase-9 and caspase-3 activation, in addition to inhibition of DNA fragmentation. The oxidative stress changes in kidney function and histological changes were also ameliorated. Dandelion leaf extract itself did not cause any significant changes. Thus, DLE should be considered as a promising nephroprotective remedy in CP chemotherapy.

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Declaration of Conflicting Interests

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