Abrogation of cisplatin-induced nephrotoxicity in rats by Berne date extract through ameliorating oxidative stress, inflammation and apoptosis

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Abstract

Our study aimed to investigate the possible protective effect of Berne date extract on cisplatin-induced nephrotoxicity. A single dose of cisplatin (6 mg/kg) was given intraperitoneally (i.p) to male rats produced significant elevation in serum urea, creatinine and TNF-α levels with significant reduction in serum albumin. It also increased kidney contents of lipid peroxides measured as malondialdehyde (MDA) and caspase-3 contents accompanied by a significant decrease in kidney contents of reduced glutathione (GSH) as well as enzymatic activities of catalase (CAT) and superoxide dismutase (SOD) compared to that of the control group. On the other hand, administrations of Berne date extract and vitamin E (a standard reference antioxidant drug) given per os (p.o) in doses of 300 mg/kg and 1g/kg, respectively for 14 days before cisplatin and 7 consecutive days after cisplatin injection ameliorated the cisplatin-induced nephrotoxicity as indicated by the restoration of kidney function and oxidative stress parameters. Furthermore, they reduced the histopathological changes induced by cisplatin. In conclusion, Berne date extract showed protective effect against cisplatin-induced nephrotoxicity; effects that may be attributed to antioxidant, anti-inflammatory and antiapoptotic activities.

KEYWORDS: Antioxidant; Caspase-3; Cisplatin; Berne date extract.

Introduction

Cisplatin is still used as a fist-line chemotherapeutic agent for solid tumors such as nasopharyngeal cancer, lung cancer and ovarian cancer [1]. However, cisplatin has severe side effects such as gastrointestinal toxicity, bone marrow suppression, ototoxicity, neuropathy and nephrotoxicity. Nephrotoxicity is the major side effect that may restrict the therapeutic use of cisplatin [2-4]. The most severe nephrotoxicity of cisplatin is acute kidney injury, occurring in 20-30% of patients treated with cisplatin [5]. Nephrotoxicity is found in 28-36% patients who received a single dose (50 mg/m^2) of cisplatin [6].

In addition to direct tubular toxicity, cisplatin induces two models of cell death: apoptosis and necrosis. Necrosis has been mainly associated with high doses of cisplatin, whereas apoptosis is associated with therapeutic doses [7]. Also, inflammation has been implicated in the pathogenesis of cisplatin-induced nephrotoxicity [8]. Several studies have also reported that cisplatin-induced oxidative stress is involved in the development of renal tubule injury [3, 9]. The involvement of oxidative stress was further supported by the fact that free radical scavengers and antioxidants prevent cisplatin -induced nephrotoxicity [10, 11].

Because of the importance of cisplatin, many studies have focused on protective strategies targeting the cisplatin-induced nephrotoxicity. Hydration was developed to reduce the cisplatin-induced nephrotoxicity, however many hydration protocols for cisplatin are available; some components such as duration and volume of hydration remain controversial. Also, there is no standard regimen for cisplatin hydration [2, 12, 13].

Many antioxidant agents were investigated for their preventive abilities against cisplatin-induced nephrotoxicity. Some researches recommended the use of enriched diets with natural antioxidants like methionine, vitamin E and ascorbic acid [14, 15]. Others reported that the use of sulfhydryl-containing drugs, such as N-acetylcysteine, captopril, sodium thiosulfate and lipoic acid, could also exert antioxidant activity [16, 17].

The fruit of the date palm (Pheonix dactylifera L.) is an important commercial crop in many countries of Middle East. Dates are a good source of energy, vitamins, and elements [18]. Besides nutritional value, date fruits are rich in phenolic compounds possessing antioxidant activity. Several studies have reported such activity of date fruits from several countries [19-25]. These studies showed that fresh and dried dates varied quantitatively and qualitatively in their phenolic acids content. The antioxidant activity is attributed to the wide range of phenolic compounds in dates including ferulic, p-coumaric and sinapic acids, flavonoids and procyanidins [21-28].

Therefore, the current study was conducted to investigate the possible efficacy of Berne date extract in ameliorating the cisplatin-induced nephrotoxicity *via* inhibition of oxidative damage, inflammation, and apoptosis in rats.

Materials and methods

Plant material

The fruits of Berne dates (Phoenix dactylifera) were purchased from Almadina market and they were identified and authenticated by Dr. Albaraa Elsaied (Lecturer of Plant Ecology, Department of Botany, Faculty of Science, Al-Azhar University, Cairo, Egypt), and voucher specimens were deposited in the regional center for mycology and biotechnology, AL-Azhar University. One hundred grams of Berne dates were homogenized using a blender and mixed with 500 ml of mixture of ethanol and distilled water (50:50). These extraction media were chosen because ethanol and water are the two extraction media used for phenolic compounds. The mixture was macerated for 48h at room temperature after being vortexed for 10 min and filtered using a funnel. The funnel was rinsed with 5 ml aliquot of extraction solvents prior to filtration. The filtrate was evaporated to dryness in vacuo at 40 °C using Soxlet evaporator and concentrated solution was measured and calculated for rat dose.

Identification and characterization of plant constituents

Analysis of phenolic compounds (antioxidants) was performed by HPLC (Agilent 1000) on a reverse phase Zorbax Eclipse XDB-C18 column (4.6 x 150 mm, 5 µm), using a gradient program with two solvents system (A: 0.5 % acetic acid in acetonitrile: water (1:1), B: 2 % acetic acid in water) at a constant solvent flow rate of 1.2 ml/min. Injection volume was 20 µl. The signals were detected at 280 nm by UV-VIS detection through comparison with standard authentic phenolic samples (purity > 99.0%) purchased from Sigma-Alderich for HPLC analysis as follows: gallic acid, itaconic acid, protocathechuic acid, catechin, esculetin, catechol, tannic acid, ferulic acid, pyrogallol and cinnmaic acid. The analysis of the extract indicates the presence of phenolic acids such as gallic acid, ferulic acid, coumaric acid, caffeic acid, hydroxybenzic acid, hydroxycinnamic acid, cinnamic acid, Itaconic acid and protochatechuic acid as well as caratonoids (lutein, β-carotene and neoxanthin) in addition to vitamins (A, B_1, B_2, B_3, B_5, B_6 C, E, K and folate). The extract also contains many minerals including Ca^{3+}, Fe^{3+}, Mg^{2+}, P^{3+}, K^{+}, Na^{+} and Zn^{2+} as well as proteins, fatty acids sterols, triterpene and carbohydrates. Finally, it contains many essential, non-essential amino acids and many essential oils (Table 1).

Drugs and Chemicals

Cisplatin was obtained from EIMC United Pharmaceuticals, Egypt, and given i.p. in a single dose of 6 mg/kg [29]. Berne dates were purchased from Almedina market for dates (Kingdom of Saudi Arabia). The total Berne dates extract was obtained using equal volumes of ethanol and water, identified for its contents at the regional center for mycology and biotechnology, AL-Azhar University and given orally in a dose of 300 mg/kg daily for 14 days before cisplatin injection and 7 days after cisplatin injection [30]. Vitamin E was purchased from El-Gomhoria Co. for chemicals (Cairo, Egypt) and was administered orally in a dose of 1g/kg, daily for 14 days before cisplatin injection and 7 days after cisplatin injection [31]. Ellman's reagent, thiobarbituric acid (TBA), GSH, 1,1,3,3-tetraethoxypropane, bovine serum albumin (BSA), pyrogallol and trichloroacetic acid (TCA) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). All other chemicals are of the standard grades.

Animals

Male adult Swiss albino rats weighing 200–225 g were obtained from the breeding colony and maintained at the animal house of the Nile, Egypt. Animals were caged in six groups, given a standard diet and water *ad libitum* and maintained at 21–24°C and 40–60% relative humidity with 12h light–dark cycles. Animals were subjected to an adaptation period of 2 weeks in the animal house of Faculty Pharmacy, Al-Azhar University, Cairo, Egypt, before experiments. The experiments were conducted in accordance with the ethical guidelines for investigations in laboratory animals and comply with the Guide for the Care and Use of Laboratory Animals [32].

Experimental Design

Forty-eight male adult Swiss albino rats were allocated into six groups (eight rats each); two rats from each group were used for histopathological examination as follows:

Group 1: received saline and served as control.

Group 2: received cisplatin in a single dose of 6 mg/kg,i.p.

Group 3: received Berne date extract in a dose of 300 mg/kg, p.o. for 21 consecutive days.

Group 4: received vitamin E in a dose of 1g/kg, p.o. for 21 consecutive days.

Group 5: pretreated with Berne date extract in a dose of 300 mg/kg p.o., for 14 days, followed by a single dose of cisplatin (6 mg/kg, i.p.) and Berne date extract in a daily dose 300mg/kg p.o for 7 consecutive days.

Group 6: pretreated with vitamin E in a dose of 1g/kg, p.o., for 14 days, followed by a single dose of cisplatin (6 mg/kg, i.p.) and vitamin E in a daily dose 1g/kg p.o for 7 consecutive days.

Serum and Tissue Preparation

On the seventh day after the cisplatin injection, blood samples were collected from retro-orbital venous plexus under light ether anesthesia in non-heparinized tubes. Serum was separated by centrifugation for 20 min at 4000g and stored at –20°C. The kidneys were rapidly isolated and washed with ice-cold isotonic saline (0.9%). Then, they were stored at –80°C until they were homogenized in ice-cold 0.15M KCl (w/v) using a Sonicator homogenizer (4710 Ultrasonic homogenizer; Cole-Parmer instrument Co., USA) to prepare 10% (w/v) homogenate. The homogenate was then divided into aliquots and used for the determination of kidney contents of caspase-3, MDA and GSH and enzymatic activities of CAT and SOD.

Biochemical Analysis

Serum urea nitrogen, creatinine, and albumin were estimated colorimetrically according to methods of Fawcett and Scott [33], Bartles et al. [34], and Doumas and Peters [35], respectively, using aqueous primary standard urea solution (50 mg/dL), standard creatinine (2 mg/dL), and standard albumin (4 g/dL). TNF-α was estimated in serum using rat TNF-α ELISA kit provided by Alpico® (India); this is a solid- phase sandwich ELISA that utilizes a monoclonal antibody specific for rat TNF-α coated on a 96-well plate, and the concentrations were measured from the standard curve. The total protein content of kidney tissue was determined according to the method of Lowry et al. [36]. The kidney homogenate was used for the determination of TBA reactive substances levels measured as MDA according to the method of Mihara and Uchiyama [37], and the concentrations were measured from the standard curve, which was constructed using serial dilutions of 1,1,3,3-tetraethoxypropane. The GSH contents were assessed by the method of Ellman [38] and the concentrations were measured from the standard curve constructed using serial dilutions of GSH. The CAT activity was determined colorimetrically using hydrogen peroxide as a substrate according to the method of Claiborne [39], and the SOD activity was determined using the method of Marklund [40], which relies on the ability of the enzyme to inhibit the pyrogallol autooxidation. The caspase-3 content was measured using an ELISA kit from Cusabio® Biotech (Wuhan, People's Republic of China). The concentration of caspase-3 was determined from a standard curve constructed from a set of serial dilutions of the standard.

Histopathological Examination of the Kidney

Autopsy samples were taken from the kidney of rats in different groups and fixed in 10% neutral buffered formalin for 24h, and decalcification was carried out on formic acid. Washing was done with tap water, and then serial dilutions of alcohol (methyl, ethyl, and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56°C in a hot air oven for 24h. Paraffin bees wax tissue blocks were prepared for sectioning at 4 μm thicknesses by a sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin and eosin, and then examination was done through the light electric microscope [41].

Statistical Analysis of Data

All values were presented as means ± standard error of the means (SEM). Statistical analysis was performed using GraphPad Prism version 5 (Graph- Pad, San Diego, CA, USA). The comparison between different groups was carried out using one-way analysis of variance (ANOVA), followed by Tukey's test. The difference is considered significant when p was ≤ 0.05.

Results

Table 2 shows that injection of cisplatin (i.p.) in a single dose of 6 mg/kg caused significant increases in serum urea (241%), creatinine (334%) & TNF-α (93%) and kidney–body weight ratio (105%) as well as significant decrease in final body weight (45%) and serum albumin level (38%) after 7 days of treatment as compared with the control group. Moreover, cisplatin (6 mg/kg) produced a significant increase in the caspase-3 (296%) and MDA contents (129%). Additionally, cisplatin injection induced significant decreases in the GSH renal content (61%) and the enzymatic antioxidant activities, SOD and CAT in the kidney (52% and 62% respectively) in comparison with the control group (Table 3).

In contrast, administration of Berne date extract 14 days before cisplatin and 7 days after cisplatin significantly reduced the levels of urea, creatinine and TNF-α in serum by 73%, 72% and 57%, respectively, as well as kidney–body weight ratio (45%), and significantly increased the final body weight (30%) and the serum albumin level (54%) in comparison with the cisplatin-treated group. Furthermore, Berne date extract decreased the caspase-3 (76%) and MDA (40%) contents and increased GSH content (154%) as well as activities of SOD and CAT (220% and 196%, respectively) in kidney tissue in comparison with cisplatin-treated group (Table 2 & 3).

Moreover, administration of vitamin E (a standard reference drug) for 14 days before cisplatin and 7 days after cisplatin reduced the levels of urea, creatinine and TNF- α in serum by 67%, 68% and 53%, respectively as well as kidney-body weight ratio (46%) and increased the final body weight (22%) and the serum albumin level (53%) in comparison with cisplatin-treated group. Furthermore, vitamin E decreased the caspase-3 (67%) and MDA (40%) contents and increased GSH content (121%) as well as activities of SOD and CAT (151% and 117%, respectively) in the kidney tissue in comparison with cisplatin treated group (Table 2 & 3).

Histopathological findings of kidney tissues are illustrated in Table 4 & Figure 1. The histopathological examination of kidney sections of the control group (saline) showed a normal histological structure (Figure A). On the other hand, administration of cisplatin to rats revealed necrosis in tubules with cystic dilatation at the cortex (Figure B). Pretreatment of the rats with vitamin E (Figure C) or Berne date extract (Figure D) obviously mitigated the histopathological changes induced by cisplatin.

Discussion

Cisplatin is a major antineoplastic weapon used for the treatment of solid tumors. Its chief dose-limiting side effect is nephrotoxicity, which requires a reduction of dose or discontinuation of the treatment [42].

Our results revealed that cisplatin produced significant elevation in serum creatinine, urea levels, and kidney–body weight ratio and a significant decrease in the serum albumin level. The increased urea and creatinine levels suggest the reduction of glomerular filtration rate [43]. Also, the increase in kidney–body weight ratio could be attributed to the reduction of body weight from gastrointestinal toxicity [44]. Furthermore, our results revealed also that cisplatin caused a significant decline in the activity of the antioxidant enzymes (CAT and SOD), significant depletion of GSH, and enhancement of MDA production in the renal tissue. These findings are consistent with those of Ali et al. [45], Fouad et al. [46] and Yadav et al. [47]. It was evident that cisplatin nephrotoxicity occurs as a result of oxidative stress and increased generation of superoxide anion, hydrogen peroxide, and hydroxyl radicals due to the increased activity of NADPH oxidase, xanthine oxidase, and adenosine deaminase [11]. These free radicals damage the lipid components of the cell membrane *via* peroxidation and denaturing its proteins, which subsequently lead to enzymatic inactivation [48].

The results of this study revealed significant elevation of caspase-3 activity and TNF-α in the cisplatin-treated rats. It was reported that, cisplatin induces cascade of inflammatory reactions with increased production of TNF-α which is responsible for further renal tissue injury [49].

Moreover, apoptosis plays an important role in the pathogenesis of a variety of renal diseases [50]. These findings were consistent with a study showing that cisplatin-induced nephrotoxicity is mediated through caspase-3 dependent and independent apoptotic pathways [51].

The present study demonstrated that treatment with Berne date extract not only ameliorated cisplatin-induced alterations in serum creatinine and urea, but also had a positive effect on albumin, final body weight and kidney–body weight ratio. Similar studies were reported with Al-Qarawi et al. [52] who found that extracts of the flesh and pits of Phoenix dactyliferaon had ameliorating effect against gentamicin-induced nephrotoxicity in rats where antioxidant components in the date were suggested to be the basis of the nephroprotection.

Moreover, Berne date extract significantly mitigated the lipid peroxidation in the rat kidney induced by cisplatin as manifested by decreased MDA content accompanied by increased GSH content and enhanced enzymatic activities of CAT and SOD. Our data are in agreement with El Arem et al. [53] who found that date fruit aqueous extract has a nephroprotective role against trichloroacetic acid-induced oxidative stress in rat as manifested by reduction in MDA content and enhancement of antioxidant enzyme activity. Similar results were obtained with Saafi-Been Salah et al. [54] who revealed the antioxidant effect of date palm fruit extract on oxidative stress and nephrotoxicity induced by dimethoate in rat.

Furthermore, our study revealed for the first time that treatment with Berne date extract had marked reducing effect on serum TNF-α level. This observation was parallel with a similar study reported with Elberry et al. [55] showing the anti-inflammatory activity of date palm pollen (Phoneix dactylifera) on experimentally-induced atypical prostatic in rats. In addition, our results showed for the first time that treatment with Berne date extract decreased caspase-3 content in kidney tissue suggesting the antiapoptotic effect of the extract.

The histopathological findings demonstrated that administration of cisplatin induced various degenerative changes in kidney cells, which confirmed the biochemical evidence of the oxidative stress. In contrast, pretreatment with Berne date extract obviously mitigated the histopathological changes induced by cisplatin. Similar studies were obtained with El Arem et al. [53] and Saafi-Been Salah et al. [54].

Conclusion

These results indicate that Berne date extract has a protective effect on the kidney tissue against cisplatin-induced nephrotoxicity in rats through antioxidant, anti-inflammatory and antiapoptotic effect.

Therefore, Berne date extract represents a potential candidate to prevent renal injury, which is a major and dose-limiting problem during cisplatin course.

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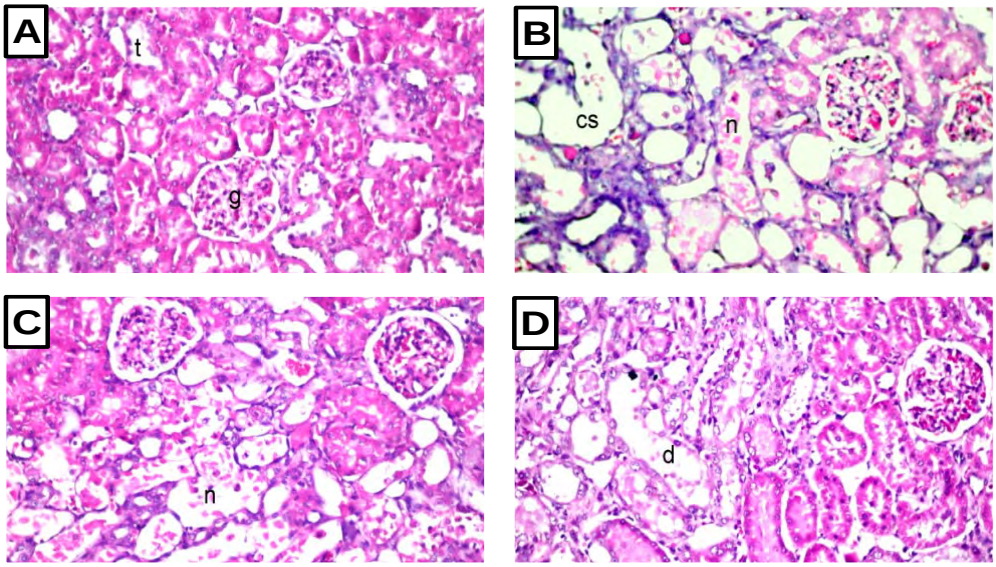

Fig. 1: Histology of kidney samples of the control (saline), cisplatin -treated group, Date extract + cisplatin -treated group, Vitamin E+ cisplatin -treated group. (A) Control group: normal histological structure of the glomeruli (g) and tubules (t); (B) cisplatin -treated group: necrosis in tubules (n) with cystic dilatation (cs) at the cortex; (C) Vitamin E + cisplatin -treated group: necrosis in the tubules lining epithelium (n) in the cortex; (D) Berne date extract + cisplatin -treated group: degeneration in lining epithelium of tubules (d) of the cortex. Hematoxylin–eosin staining, magnification: 40×.

Table 1. Phenolic acids and flavonoids in Berne dates extract

Phenolic acids	Content (mg %)	Flavonoids	Content (µg/100 gm)
Coumaric acid	14.12	Quercetin	28.08
Ferulic acid	11.08	Catechin	16.08
Gallic acid	6.01	Lutein	11.89
Hydroxybenzic acid	3.18	Epicatechin	9.08
Hydroxycinnamic acid	0.121	Apigenin	5.01

TABLE 2. Effect of treatment of Berne date extract on serum urea, creatinine, albumin, TNF-α levels, final body weight, and kidney–body weight ratio in cisplatin-treated rats

	Urea *(mg/dl)*	Creatinine *(mg/dl)*	Albumin *(g/dl)*	TNF Alpha *(pg/mL)*	*Final body weight (g)*	Kidney–Body Weight Ratio *(1000×)*
Saline	34.5±2.5	0.76±0.07	4.2±0.13	31.1±1.5	224.8±1.81	5.90±0.13
Cisplatin	117.7±3.9[a]	3.3±0.08[a]	2.5±0.12[a]	60.6±2.2[a]	154.8±3.46[a]	12.3±0.26[a]
Berne date extract	28.6±1.4	0.86±0.06	4.5±0.12	27.8±1.5	212.7±2.96	7.08±0.47
Vitamin E	26.2±2.1	0.89±0.06	4.4±0.14	28.8±1.5	240.2±5.11	6.18±0.40
Berne date extract + cisplatin	31.5±1.6[b]	0.92±0.05[b]	4.1±0.17[b]	26.1±1.6[b]	201.2±4.67[a,b]	6.73±0.41[b]
Viamin E+ cisplatin	39.3±2.2[b]	1.1±0.06[a,b]	4.0±0.18[b]	28.0±1.4[b]	189.3±3.67[a,b]	6.61±0.10[b]

Data are expressed as means ± SEM of six rats per group.

[a] significantly different from the control saline group; [b] significantly different from the cisplatin -treated group, using one-way ANOVA followed by the Tukey's test for multiple comparison test at $p \leq 0.05$.

TABLE 3. Effect of treatment of Berne date extract on kidney contents of caspase-3, MDA, and GSH as well as activity of antioxidant enzymes (CAT and SOD) in cisplatin -treated rats

	Caspase-3 *(ng/g tissue)*	MDA *(nmol/g tissue)*	GSH *(μmg/g tissue)*	SOD *(U/mg protein)*	CAT *(U/mg protein)*
Saline	0.53±0.04	20.93±0.93	6.0±0.26	21.2±1.9	6.0±0.6
Cisplatin	2.07±0.09 [a]	48.0±1.4 [a]	2.4±0.19 [a]	10.3±1.1 [a]	2.2±0.1 [a]
Berne date extract	0.54±0.06	23.57±0.9	6.03±0.39	24.6±1.2	5.7±0.5
Vitamin E	0.59±0.04	21.10±1.81	5.26±0.15	20.7±1.8	5.0±0.5
Berne date extract + cisplatin	0.47±0.06 [b]	28.88±1.2 [a,b]	6.05±0.29 [b]	32.7±1.7 [a. b]	6.8±0.5 [b]
Viamin E+ cisplatin	0.67±0.04 [b]	28.73±0.8 [a,b]	5.32±0.34 [b]	25.6±2.3 [b]	4.91±0.2 [b]

Data are expressed as mean ± SEM of six rats per group.

[a] significantly different from the control saline group; [b] significantly different from the cisplatin -treated group, using one-way ANOVA followed by the Tukey's test for multiple comparison test at $p \leq 0.05$.

TABLE 4. Effect of treatment of Berne date extract and vitamin E on histopathological findings of kidney tissues of cisplatin-treated rats

	Control	Berne date extract	Cisplatin	Vitamin E + Cisplatin	Berne date extract + Cisplatin
Coagulation necrosis	0	0	++++	++	0
Renal cast	0	0	+++	0	0
Cystic tubules	0	0	+++	0	0
Focal hemorrhage	0	0	++	0	++

0=none; +=mild; ++=moderate; +++=severe; ++++=very severe.