

Effects of Grape Seed Proanthocyanidin Extract on Cisplatin-Induced Renal Oxidative Damage and Apoptosis in Rats

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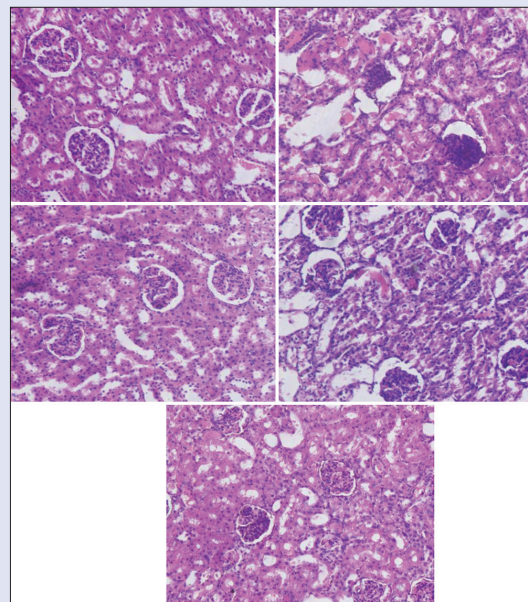
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ABSTRACT

Background: Grape seed proanthocyanidin extract (GSPE) shows a protective effect against cisplatin (cis-dichlorodiammine-platinum (II) [CDDP])-induced nephrotoxicity, but the protection mechanism is still not clear. **Objectives:** Herein, protective effects of GSPE on cisplatin (CDDP) relevant to oxidative Damage and apoptosis were investigated in rats. **Materials and Methods:** Randomly divide the experimental animals into five groups: Respectively are normal control group, CDDP model group, GSPE (400 mg/kg) group, CDDP + GSPE (200 mg/kg) group, and CDDP + GSPE (400 mg/kg) group. Each group was administered with distilled water or the corresponding doses of GSPE via gavage for 15 consecutive days. Subsequently, a single intraperitoneal injection of CDDP (8 mg/kg) was administered to the CDDP and CDDP + GSPE groups, and the remaining groups were administered with normal saline via intraperitoneal injection. **Results:** Based on the histopathological analysis, cisplatin caused structural and functional renal impairment and elevated the levels of serum creatinine and blood urea nitrogen, respectively. Renal injury was caused due to increased lipid peroxidation (LPO)/oxidative stress as evidenced by the increased levels of malondialdehyde and decreased levels of antioxidants including reduced superoxide dismutase, glutathione peroxidase and glutathione. Cisplatin administration also observably increased the rate of apoptosis of renal cortical cells, decreased the expression of Bcl-2, and increased the expression of Bax and caspase-3. The pretreatment of GSPE significantly improved renal function and attenuated the cisplatin-induced LPO/oxidative stress and apoptosis. **Conclusion:** Our results suggest that that GSPE had a good protective effect against CDDP-induced renal oxidative stress and apoptosis in rats. In addition, the co-administration of GSPE might prove to be a novel and promising preventive strategy against cisplatin-induced nephrotoxicity.

Key words: Antioxidant activity, apoptosis, cisplatin, grape seed proanthocyanidin extracts, nephrotoxicity



Abbreviations used: CDDP: Cis-dichlorodiammine-platinum (II); GSPE: Grape seed proanthocyanidin extracts; GSH: Reduced glutathione; GSH-Px: Glutathione peroxidase; SOD: Superoxide dismutase; MDA: Malondialdehyde; BUN: Blood urea nitrogen; Cr: Creatinine; RI: Renal index; PI: Propidium iodide.

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INTRODUCTION

Cisplatin (cis-dichlorodiammine-platinum (II), [CDDP]) plays a highly effective on diverse spectrum of malignancies and yet is one of the most potent agents in resisting tumor.^[1] It is widely used to treat malignancies of various solid tumors such as head and neck, esophageal, bladder, testicular, ovarian, and small cell lung cancer.^[2,3] However, it has been reported that the use of CDDP is often limited because of a number of adverse effects,^[4] including nausea, vomiting, sensitivity reactions,

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ototoxicity, neurotoxicity, and bone marrow suppression. Among these adverse effects, nephrotoxicity is the most important limiting factor in cancer treatment using CDDP.^[5] The primary manifestations are impairment in the functioning of renal tubular epithelial cells, including inhibition of protein synthesis, oxidative stress, mitochondrial dysfunction, cytoskeleton remodeling, changes of intercellular adhesion, and apoptosis of tubular epithelial cells. It has been suggested that oxidative damage related to oxidative stress may also be one of the causes of CDDP-induced nephrotoxicity. Therefore, it is important to prevent nephrotoxicity of cisplatin.

Grape seed proanthocyanidin extract (GSPE) condensed by different numbers of flavan-3-ol units (i.e., catechin, epicatechin, epicatechin gallic acid, and gallic acid ester), they are all polyphenol bioflavonoids. These compounds are diverse in chemical structure and properties and pharmacological effects. Proanthocyanidins can be extracted from various plant parts, especially from fruits and flowers.^[6] However, GSPE shows better scavenging activity against free radicals, including hydroxyl radicals, peroxy radicals, and superoxide anion than that of Vitamins A, C, and E.^[7,8] Furthermore, GSPE shows various therapeutic potential such as radical scavenging and antitumor activities and protects renal tissue against oxidative injury and inflammation.^[9,10] GSPE is safe and is highly effective and bioavailable has been reported in GSPE.^[38,39] GSPE acts by promoting apoptosis of cancer cells, blocking the cell cycle, and regulating signal molecules. Previous studies have shown that GSPE can significantly inhibit the oxidative damage of human embryonic kidney cells induced by CDDP. It has been proposed to develop GSPE as a functional food which can reduce the CDDP-induced nephrotoxicity, enhance its chemotherapeutic effect, and enhance its antitumor activity. Previous research has showed that GSPE shows protective effect against CDDP-induced nephrotoxicity, but the action mechanism is still obscure. In this study, the GSPE mechanism was designed to explore the function to inhibit nephrotoxicity induced by CDDP.

MATERIALS AND METHODS

Reagents and chemicals

Shandong Qilu Pharmaceutical Factory in China provided us with CDDP for experiments. Its dosage freeze-dried form is powder, grade is for injection.

Tianjin Shanding Natural Products Research and Development Co., Ltd., provided us with GSPE for experiments. The purity of GSPE detected by ultraviolet exceeds 95%, the content of dimer, trimer, and tetramer of GSPE is 56%, 12%, and 6.6% respectively. Further, high-molecular-weight monomers and other oligomers based on HPLC analysis are 20.4%.

Following chemicals were obtained from Nanjing Jiancheng Bioengineering Institute: Coomassie brilliant blue, glutathione (GSH, # A006-2-1), malondialdehyde (MDA, # A003-1-2), superoxide dismutase (SOD, # A001-1-2), and glutathione peroxidase (GSH-Px, # A005-1-2) kits.

The following primary antibodies (they were all purchased from Santa Cruz-Chinese Sequoia Jinqiao Biotech Corp) were listed as follows: Bax monoclonal antibody (NO.SC-7480) and Bcl-2 polyclonal antibody (NO.SC-492). In addition, caspase-3 polyclonal antibody (NO.SC-7148) was used to detect full-length procaspase-3, and p11, p17, and p20 subunits of caspase-3.

Animals and experimental design

This research was conducted in accordance with our institutional guidelines associated with research using live animals. The experimental protocol got approval from the Experimental Animal

Ethical Committee which belonged to Function Test Center for Functional Food of College of Arts and Science of Beijing Union University. The serial number of the approval of the ethical committee is 2014-01. In this study, 50 male Sprague Dawley rats (weighing around 140–160 g) were obtained from Animal Center of Academy Laboratory, Military Medical Sciences of China. Feed and water were provided *ad libitum*. These rats were housed under the conditions (24°C ± 3°C and half-light and half dark in 24 h a day), and fed a standard laboratory bag pellet commercial feed.

The experimental animals were divided into five groups randomly, and each group included 10 animals. After dissolving CDDP with normal saline, the experimental animals were injected intraperitoneally (i. p.) according to the dose known to cause nephrotoxicity.^[11] The dose of CDDP solution was 8 mg/kg bw. The distilled water was used to formulate GSPE administered to animals with the condition (a dose of 200 or 400 mg/kg body weight by gavage). In this study, the concentration of GSPE was selected based on previous studies.^[12,13] Group 1 was used as control, On the 10th day of the experiment, groups 2, 4, and 5 were treated with a single intraperitoneal dose of CDDP (7 mg/kg bw). Groups 3, 4 and 5 received oral GSPE (400, 200, and 400 mg/kg bw, respectively) for 15 consecutive days.

Sample collection

Our previous study on the course of CDDP nephrotoxicity showed that: nephrotoxicity began to appear on the 3rd day after the injection of CDDP in rats, reached the peak on the 5th day, and the kidney was damaged the hardest, consistent with the results of other studies.^[14] Therefore, the sacrifice time for all animals in all groups was chosen 5 days after CDDP injection. The kidneys were quickly harvested and weighed to calculate the ratio of organ weight. After this, part of the kidney tissue was immobilized in 70% ethanol to detect apoptosis, another part was immobilized in 10% formalin for histopathological examination, and the remaining kidney tissue was stored in a sealed bottle at –80°C and then used in the assessment of oxidative stress markers. Collect blood samples to assess the levels of creatinine (Cr) and blood urea nitrogen (BUN).

Measurement of nephrotoxicity markers

The blood sample was allowed to stand for 30 min at room temperature, and then the serum was obtained by centrifuging blood samples at 3000 rpm for 10 min. The serum levels of BUN and Cr were measured to assess kidney function. All biochemical assays were performed spectrophotometrically by using available commercial kits (Nanjing Built Biological Engineering Research Institute, Jiangsu, China). According to the formula: (kidney weight/total bodyweight) × 100%, relative kidney weight renal index (RI) was calculated.

Measurement of oxidative stress markers

The kidney tissue was taken out of the deep-freezer and weighed. Furthermore, they were transferred to cold glass tubes and then diluted with a nine-fold volume of phosphate buffer (pH 7.4). The kidney tissue was chopped up for enzymatic hydrolysis analysis, and then homogenized by using a Teflon-glass homogenizer at 16000 × g for 3 min with cold physiological saline when it on ice. The homogenized tissue solution was centrifuged in a 4°C centrifuge at 3000 rpm for 15 min, then the supernatant was collected and cryopreserved at –20°C for subsequent testing and analysis.

Next, we determined the levels of reduced GSH, MDA, and lipid peroxidation (LPO) in the cytosolic supernatant which were stored at –20°C. In addition, the supernatant was used to measure GSH-Px activities and SOD. The levels of MDA and GSH, as well as the activities of SOD and GSH-Px were detected by kits, and the concentration

of total protein was assayed by using a BCA protein assay kit (purchased from Nanjing built biological engineering research institute, Jiangsu, China).

Histopathological examination of the kidney

Kidney samples were fixed in 10% neutral-buffered formalin, followed by routine treatment and stained with hematoxylin and eosin stain. The evaluation was performed using light microscopy.

Measurement of apoptotic cells by flow cytometry

Single cell suspension preparation

The kidney tissue samples were first removed from 70% ethanol, cut it into very small pieces, and then gently ground them on the cellular grid. The cell suspension formed was mixed with saline and the cell count was recorded to $1 \times 10^6/\text{mL}$.

Detection of apoptosis

The cell suspension was subjected to propidium iodide staining in binding buffer and placed in the dark at 4°C for 30 min. The stained cells were analyzed by flow cytometry (Epics-XLII Beckman Coulter, USA). The percentage of cells denoted the apoptosis rate in sub-G1 phase.

The emission wavelength for flow cytometry was 488 nm, and the 15 mW ion laser was as the emission resource. Then we used Expo 32 ADC software to analyze immunofluorescence data, and employed Muticycle AV software to detect the cell cycle of DNA. Flow-check Fluorpheres (10 μm) Fluorescent Microspheres (REF 6605359, Beckan Coulter Inc. Fullerton, CA, USA) was used to adjust coefficient of variation of the machine to <2%.

Bcl-2, Bax, and caspase-3 expression detection

In this experiment, 100 μL of Bax monoclonal antibody (1:100) or Bcl-2 polyclonal antibody (1:100) or caspase-3 polyclonal antibody (1:100) was added to 100 μL of cell suspension and mixed the solution thoroughly. After incubation for 30 min at room temperature, 10 mL of phosphate-buffered saline (PBS) was added to wash the cells. Then we added 100 μL goat anti-mouse FITC-IgG antibody (1:50) into cells, and

incubate them at room temperature for 30 min in a dark environment. As mentioned above, the cells are washed again. The supernatant was decanted after centrifugation at $132 \times g$ for 3–5 min. Finally, the precipitate was resuspended using 100 μL PBS. Flow cytometry performed to detect the expression of caspase-3, Bcl-2, Bax. The flow cytometer data analysis software and setting parameters are identical to the flow cytometer for detecting apoptotic cells (b).

Statistical analysis

Data were analyzed using the SPSS statistical program (Version 12.0 software) (IBM). All data were presented as mean $\pm$ standard error. The data were analyzed with a one-way analysis of variance test with multiple samples. Statistical significance was indicated by $P < 0.05$.

RESULTS

Parameters of renal function changes

The protective effects of GSPE prevent from nephrotoxicity caused by cisplatin were evaluated in kidney tissues of animals. After oral administration, GSPE alone did not cause any kidney dysfunction. In contrast, the RI, serum BUN and Cr were increased significantly when we treatment with cisplatin. Compared to cisplatin group [Table 1], the RI and serum BUN and Cr were significantly decreased after using CDDP + GSPE (400 mg/kg). The RI, Cr, and BUN of the group of CDDP + GSPE (200 mg/kg) also decreased when we compared it to the group of CDDP, but there was no statistical difference between the Cr and BUN group, and the effect was not as obvious as that of the 400 mg/kg group.

Levels of GSH, activity of MDA, SOD and GSH-Px, in renal tissue

To determine the antioxidant effects of GSPE on cisplatin-induced oxidative stress, we measured the levels of GSH and MDA in kidney tissues and biological activities of SOD and GSH-Px. As shown in Table 2, the cisplatin group showed a significant increase of the level of MDA in the kidney tissue in comparison with the control. CDDP + GSPE (400 mg/kg) reduced the levels of MDA significantly in kidney tissues as compared with cisplatin-induced kidney. CDDP significantly decreased the activity of GSH, GSH-Px, and SOD in kidney tissue samples as compared with the control group. However, the activity of these antioxidants did not reduce in rats treated by CDDP after the administration of GSPE (200 and 400 mg/kg).

Histopathological observations

In histopathological analysis 5 days after CDDP administration, kidney tissue was showed tubular epithelial desquamation, remarkable vacuolation, hyaline casts in some tubules, and lymphocytic infiltration in the renal interstitium as comprised with the control group [Figure 1a and b]. The kidney tissue of the GSPE (400 mg/kg)

Table 1: Effects of grape seed proanthocyanidin extracts with or without cis-dichlorodiammine-platinum treatment on renal index, blood urea nitrogen and creatinine levels in rats (mean $\pm$ standard deviation, $n=10$)

Groups (mg/kg)	RI (%)	Cr ($\mu\text{mol/L}$)	BUN (mmol/L)
Control	0.77 $\pm$ 0.03	69.60 $\pm$ 8.52	5.19 $\pm$ 1.11
CDDP	1.28 $\pm$ 0.16 ^a	729.38 $\pm$ 140.64 ^a	49.67 $\pm$ 3.85 ^a
GSPE (400)	0.76 $\pm$ 0.03	65.13 $\pm$ 3.96	5.02 $\pm$ 0.93
CDDP + GSPE (200)	1.06 $\pm$ 0.78 ^b	540.33 $\pm$ 152.87	38.55 $\pm$ 7.81
CDDP + GSPE (400)	1.04 $\pm$ 0.95 ^b	452.00 $\pm$ 82.64 ^b	34.19 $\pm$ 6.33 ^b

^a $P < 0.01$ compared with the control group; ^b $P < 0.05$ compared with the CDDP group. RI: Renal index; Cr: Creatinine; BUN: Blood urea nitrogen; CDDP: Cis-dichlorodiammine-platinum; GSPE: Grape seed proanthocyanidin extract

Table 2: Reduced glutathione and malondialdehyde levels and superoxide dismutase and glutathione peroxidase activities in the renal cortex of rats (mean $\pm$ standard deviation, $n=10$)

Groups (mg/kg)	GSH (mg/g protein)	MDA (nmol/mg protein)	SOD (units/mg protein)	GSH-PX (units/mg protein)
Control	563.35 $\pm$ 51.90	4.64 $\pm$ 0.44	115.42 $\pm$ 5.76	564.18 $\pm$ 73.53
CDDP	364.70 $\pm$ 59.53 ^a	5.81 $\pm$ 0.92 ^a	76.83 $\pm$ 9.25 ^a	434.00 $\pm$ 59.33 ^a
GSPE (400)	545.40 $\pm$ 40.89	4.49 $\pm$ 0.58	111.15 $\pm$ 5.56	569.91 $\pm$ 83.30
CDDP + GSPE (200)	407.86 $\pm$ 59.05	5.00 $\pm$ 0.77 ^b	87.64 $\pm$ 7.80 ^b	479.97 $\pm$ 44.43
CDDP + GSPE (400)	436.85 $\pm$ 94.12 ^b	4.90 $\pm$ 0.53 ^c	87.52 $\pm$ 9.11 ^b	519.08 $\pm$ 52.00 ^b

^a $P < 0.01$ compared with the control group; ^b $P < 0.05$; ^c $P < 0.01$ compared with the CDDP group. GSH: glutathione; MDA: Malondialdehyde; SOD: Superoxide dismutase; GSH-PX: GSH-peroxidase; CDDP: Cis-dichlorodiammine-platinum; GSPE: Grape seed proanthocyanidin extract

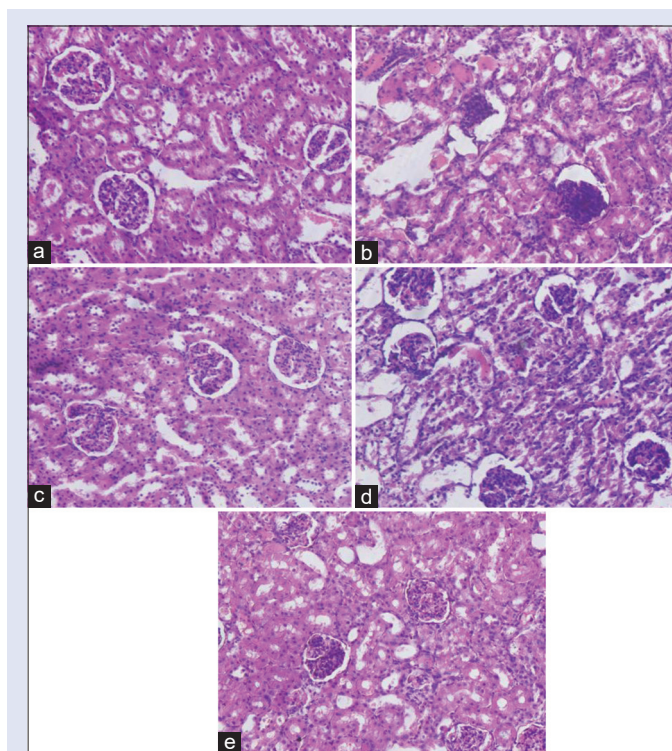


Figure 1: Pathological changes in the kidney tissue samples (H and E, $\times 200$). (a) Normal control group. (b) Cis-dichlorodiammine-platinum (II) model group. (c) Grape seed proanthocyanidin extract (400 mg/kg) group. (d) Cis-dichlorodiammine-platinum (II) + grape seed proanthocyanidin extract (200 mg/kg) group. (e) Cis-dichlorodiammine-platinum (II) + grape seed proanthocyanidin extract (400 mg/kg) group

group has no significant difference as compared with the control group [Figure 1c]; the structure of the renal tubules and glomeruli were found to be normal. CDDP + GSPE (400 mg/kg) reduced the nephrotoxic effect of CDDP and there were no distinct pathological changes except mild glomerular congestion and turbidity of the epithelial cells of the proximal convoluted tubules [Figure 1d]. However, CDDP + GSPE (200 mg/kg) showed pathological changes induced by CDDP in comparison with the CDDP-alone group [Figure 1e], although some glomerular atrophy in rat kidneys can still be seen, transparent casts can be seen in the lumen of renal tubules.

Rate of apoptosis

Apoptosis was measured by the analysis of flow cytometry. As shown in Table 3, whereas treatment with GSPE alone had no effect on apoptotic rate, exposure to cisplatin alone resulted in a significant increase in apoptosis rate. However, the group, pretreated with GSPE (400 mg/kg) before injection of CDDP, can observably reduce the apoptosis rate of CDDP induced renal cells ($P < 0.05$) in comparison with the CDDP group. When CDDP + GSPE (200 mg/kg) was used, the rate of apoptosis also decreased, but there was no statistical difference.

Expression levels of apoptosis-related protein

Table 4 shows the expression levels of caspase-3, Bax, Bcl-2 and in the kidney tissue samples. The apoptosis-related data and the expression levels of apoptosis-related proteins caspase-3, Bax and Bcl-2 are shown in Figure 2-5. Compared with the control group, the levels of Bax ($P < 0.05$) and Caspase-3 ($P < 0.01$) in CDDP group were significantly increased, and the level of Bcl-2 ($P < 0.01$) showed a significant decrease. GSPE (400

Table 3: Anti-apoptosis of grape seed proanthocyanidin extract on renal apoptosis induced by cis-dichlorodiammine-platinum (mean $\pm$ standard deviation, $n=10$)

Groups (mg/kg)	Apoptosis rate (%)
Control	0.99 $\pm$ 0.27
CDDP	3.10 $\pm$ 0.69 ^a
GSPE	1.06 $\pm$ 0.35
CDDP + GSPE (200)	2.68 $\pm$ 0.71
CDDP + GSPE (400)	2.39 $\pm$ 0.53 ^b

^a $P < 0.01$ compared with the control group; ^b $P < 0.05$ compared with the CDDP group. CDDP: Cis-dichlorodiammine-platinum; GSPE: Grape seed proanthocyanidin extract

Table 4: Expression levels of apoptosis-related proteins in rat renal cells in response to treatment of cis-dichlorodiammine-platinum and grape seed proanthocyanidin extracts (mean $\pm$ standard deviation, $n=10$)

Groups (mg/kg)	Fluorescence intensity		
	Caspase-3	Bcl-2	Bax
Control	44.61 $\pm$ 6.11	67.68 $\pm$ 6.19	76.09 $\pm$ 7.19
CDDP	62.6 $\pm$ 12.09 ^b	55.34 $\pm$ 10.27 ^b	87.31 $\pm$ 9.16 ^a
GSPE	45.32 $\pm$ 9.05	66.88 $\pm$ 7.15	74.5 $\pm$ 6.66
CDDP + GSPE (200)	55.23 $\pm$ 11.07	59.67 $\pm$ 8.59	84.09 $\pm$ 7.13
CDDP + GSPE (400)	51.49 $\pm$ 10.08 ^c	64.37 $\pm$ 6.7 ^c	79.43 $\pm$ 6.19 ^c

^a $P < 0.05$; ^b $P < 0.01$ compared with control group; ^c $P < 0.05$ compared with CDDP group. CDDP: Cis-dichlorodiammine-platinum; GSPE: Grape seed proanthocyanidin extract; Bcl-2: B-cell lymphoma-2; Bax: Bcl-2-Associated X

mg/kg) observably inhibited the increase of Bax and Caspase-3 levels ($P < 0.05$), and decreased Bcl-2 levels ($P < 0.05$).

DISCUSSION

CDDP is primarily excreted through the kidneys, which explains its nephrotoxicity. It can cause renal injury and reactive oxygen species-induced acute renal failure, interstitial inflammation of the renal tubules, and induction of apoptosis.^[15] The generation of free radicals and oxidative stress might cause CDDP-induced nephrotoxicity. It reduces the ability of the renal cortex to scavenge free radicals and enhances LPO, thus damaging membrane protein and mitochondrial function,^[16] resulting in the decrease of renal function and changes in pathologic structure. This study showed that the levels of BUN and Cr in the serum of healthy male rats were significantly increased after intraperitoneal injection of CDDP (7 mg/kg). BUN and Cr are the important indexes of renal function, and their increased levels indicate that CDDP causes renal injury in rats. In addition, GSH-Px and SOD are important indexes to judge the antioxidant level of the organism. SOD primarily scavenges free radicals, whereas GSH-Px primarily scavenges low levels of H_2O_2 , and both are in the dynamic equilibrium state.^[17] When the metabolism of free radicals is disturbed, excess oxygen radicals damage renal tubular epithelial cells. Reactive oxygen species increase the amount of LPO leading to the formation of MDA, which is the characteristic index to determine the level of LPO under *in vivo* conditions. In addition, as the second line of defense, non-enzymatic antioxidants such as glutathione, can protect cells from oxidative damage induced by oxidative stress by scavenging free radicals or converting toxic free radicals into non-toxic end products.^[18] The results indicated that the renal cortex of rats injected intraperitoneally with CDDP (7 mg/kg) increased of MDA content, significantly decreased in GSH content, and reduced in SOD and GSH-PX activity significantly, which verified that CDDP caused oxidative damage in the kidney and overutilization of the antioxidant GSH in the scavenging of free radicals. It was demonstrated that CDDP-induced nephrotoxicity was related to oxidative stress.

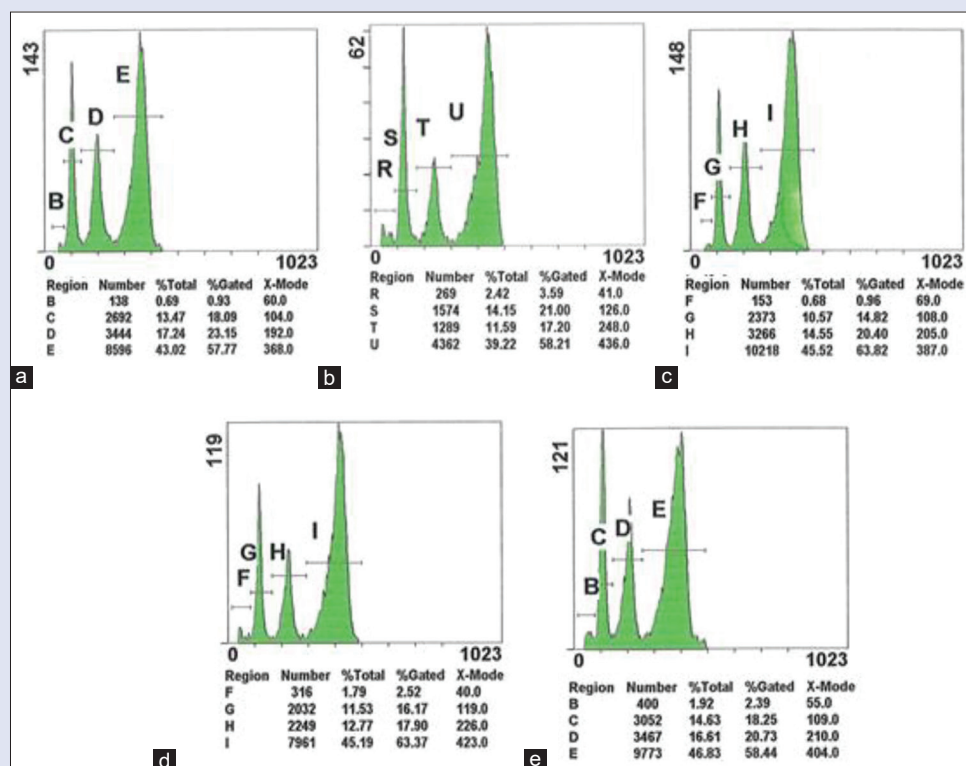


Figure 2: Apoptosis data map. (a) Normal control group. (b) Cis-dichlorodiammine-platinum (II) model group. (c) grape seed proanthocyanidin extract (400 mg/kg). (d) Cis-dichlorodiammine-platinum (II) + grape seed proanthocyanidin extract (200 mg/kg) group. (e) Cis-dichlorodiammine-platinum (II) + grape seed proanthocyanidin extract (400 mg/kg) group

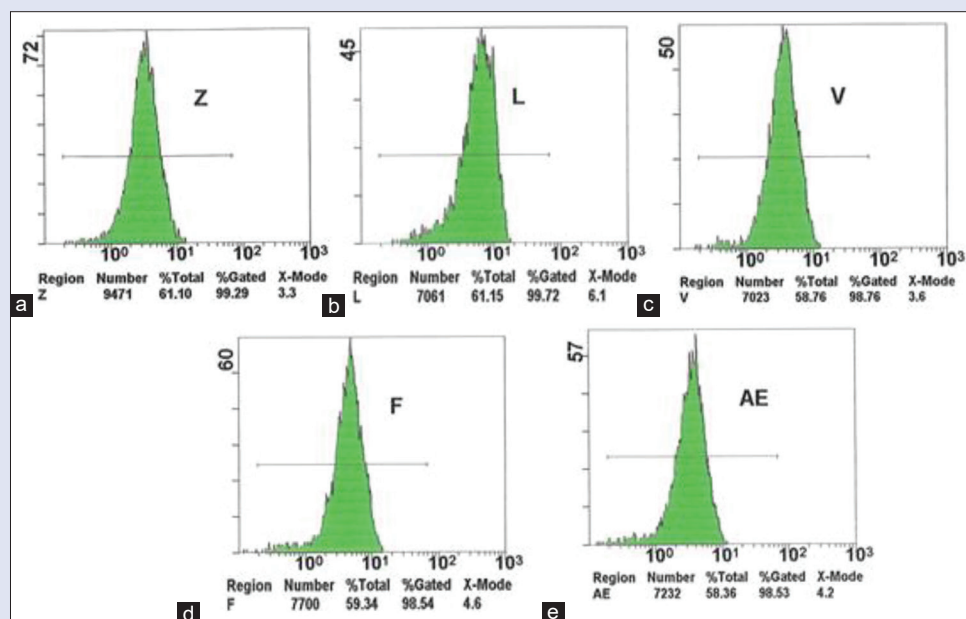


Figure 3: Expression levels of apoptosis-related protein caspase-3. (a) Normal control group (b) cis-dichlorodiammine-platinum (II) model group (c) grape seed proanthocyanidin extract (400 mg/kg) group (d) cis-dichlorodiammine-platinum (II)+ grape seed proanthocyanidin extract (200 mg/kg) group (e) cis-dichlorodiammine-platinum (II)+ grape seed proanthocyanidin extract (400 mg/kg) group

Combination treatment is one of the measures to improve the quality of tumor chemotherapy. In recent years, some natural active substances have shown synergistic effects on tumor chemotherapy because of their unique mechanism of action.^[19,20] For example, anthocyanins of grape

seed belong to biological flavonoids; their molecular structure contains phenolic hydroxyl groups. It is the phenolic hydroxyl group which show antioxidant activity. GSPE can donate electrons to free radicals, thereby blocking LPO. At the same time, GSPE provides hydrogen for lipid free

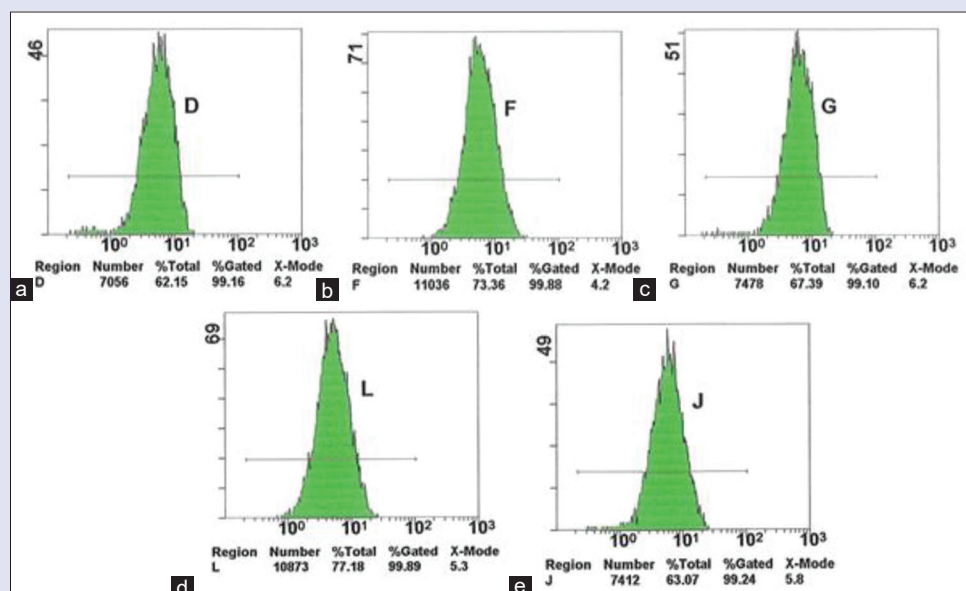


Figure 4: Expression level graph of apoptosis-related protein Bcl-2. (a) Normal control group (b) cis-dichlorodiammine-platinum (II) model group (c) grape seed proanthocyanidin extract (400 mg/kg) group (d) cis-dichlorodiammine-platinum (II) + grape seed proanthocyanidin extract (200 mg/kg) group (e) cis-dichlorodiammine-platinum (II) + grape seed proanthocyanidin extract (400 mg/kg) group

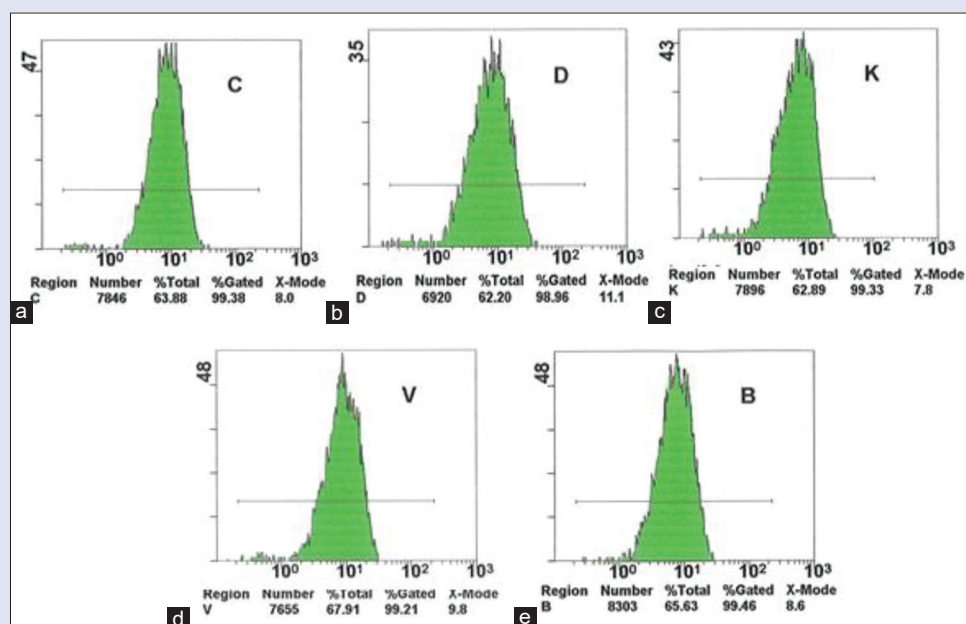


Figure 5: Expression levels of apoptosis-related protein Bax. (a) Normal control group (b) cis-dichlorodiammine-platinum (II) model group (c) grape seed proanthocyanidin extract (400 mg/kg) group (d) cis-dichlorodiammine-platinum (II) + grape seed proanthocyanidin extract (200 mg/kg) group (e) cis-dichlorodiammine-platinum (II) + grape seed proanthocyanidin extract (400 mg/kg) group

radicals, which prevents the prolongation of LPO chain, slows down the lipid oxidation process, and reduces the harm caused by free radicals.^[21] A previous study has shown that GSPE is a potent antioxidant agent which blocks LPO and regulates antioxidant enzyme activity.^[22] Therefore, the effects of GSPE on CDDP-induced nephrotoxicity were studied in rats (200 and 400 mg/kg for 10 days). Our results indicated that the pretreatment of GSPE could decrease Cr and BUN levels significantly in rats administered with CDDP. GSPE inhibits CDDP-induced GSH depletion, increases MDA content and decreases the activities of GSH-Px

and SOD in the renal cortex. It is suggested that GSPE can reduce CDDP-induced nephrotoxicity, and the mechanism may be to reduce oxidative stress by reducing free radicals and increasing antioxidant enzyme activity and antioxidant content, thus inhibiting lipid peroxide formation and slowing down the oxidative damage of the organism. GSPE can effectively improve the symptoms of ulcerative colitis induced by sodium dextran sulfate in mice by regulating the expression of oxidative stress-related proteins Nrf2, HO-1, and inflammatory pathway

protein NF- κ B, thereby affecting the changes of oxidative stress indexes SOD, MDA and inflammatory factors.^[45] In addition, grape seed proanthocyanidins can effectively inhibit the expression of p-p38 and p-JNK proteins, block the signal transduction pathway of MAPK cells, reduce the occurrence of hydrogen oxide-induced oxidative damage, and thus have a protective effect on human lens epithelial cells.^[46] GSPE also inhibits TGF- β -induced fibroblast activation by blocking Smad and JNK signaling pathways.^[47] It is clear that the signaling pathway is a very complex network, and researchers need to study it more carefully.

CDDP-induced cytotoxicity can ultimately cause cell necrosis, including apoptosis and swelling. Apoptosis is a programmed-cell death. It is a complex and orderly process involving many molecules. According to our results, the CDDP induces nephrotoxicity primarily via induction of apoptosis^[22-25] induced by CDDP. Lieberthal *et al.*^[26] demonstrated that CDDP might induce the death of primary cultured renal tubular epithelial cells (including apoptosis and necrosis). Park *et al.*^[27] found that low concentration of CDDP (8–100 μ mol/L)-induced apoptosis in a dose-dependent manner. Okuda *et al.*^[28] reported that CDDP (30 μ mol/L) induced apoptosis of LLC-PK1 cells and decreased the activities of alkaline phosphatase and γ -glutamine transferase. Thus, it is evident that CDDP induces renal toxicity via induction of apoptosis. Reducing apoptosis of renal tubular epithelium is an important measure to reduce renal toxicity of CDDP. Apoptosis is a programmed-cell death, usually accompanied by mitochondrial membrane rupture. Cytochrome C is released from mitochondria into the cytoplasm.^[29,30] Bcl-2, an anti-apoptosis-associated protein, can regulate apoptosis by altering mitochondrial membrane permeability and caspase family proteins in the feedback loop system.^[31,32] Bax protein is an important component of the apoptotic pathway. It has been suggested that the downregulation in the expression of Bcl-2 and the upregulation in the level of Bax increased the mitochondrial membrane permeability, which promotes the activation of caspase-3 which in turn induces apoptosis and the release of cytochrome C.^[33] These results show that CDDP increased the rate of apoptosis significantly, downregulated the level of Bcl-2, and upregulated the active caspase-3 gene and the level of Bax. It has been hypothesized that CDDP might induce apoptosis via the promotion of mitochondrial releasing factor. These results suggest that Bcl-2, Bax, and caspase-3 genes play an important role in CDDP-induced apoptosis.

Previous research demonstrates the protective effects of different natural polyphenols on the kidneys. Sahyon and Al-Harbi^[34] showed that the extract of *Phoenix dactylifera* shows ROS scavenging capacity. It can increase the endogenous antioxidant enzyme levels and the percentage of PD-1 protein in the kidney. Oral administration of the date extract can reduce the oxidative damage and renal apoptosis caused by Adriamycin. In addition, Xie *et al.*^[35] and Kanlaya and Thongboonkerd^[36] have shown the protective effect of tea polyphenols on mouse kidneys. Zhao *et al.*^[37] also indicated that resveratrol can significantly reduce renal interstitial fibrosis in mice, thus protecting kidneys from injury.

GSPE is one of the most widely studied natural polyphenols. It can scavenge free radicals under *in vivo* conditions. Previously, the micronucleus test [2000 mg/(kg • d)] and Ames test (500 μ g/plate) have shown that GSPE was not teratogenic or mutagenic.^[38,39] In addition, GSPE can not only inhibit the growth of tumor cells such as lung cancer, breast cancer, prostate cancer, oral epithelial cancer, and chronic bone marrow leukemia but also induce the apoptosis of tumor cells.^[40-42] Furthermore, GSPE does not induce apoptosis in normal cells, which is mainly used in the reduction of cell disease caused by excessive cell apoptosis, such as myocardial ischemia-reperfusion injury and Alzheimer's disease.^[43,44] Previous studies have shown that GSPE might upregulate the expression of Bcl-2, downregulate the levels of and caspase-3 Bax proteins, and inhibit the process of apoptosis induced by

CDDP, suggesting that GSPE might reduce nephrotoxicity induced by CDDP.

CONCLUSION

GSPE shows protective effects against CDDP-induced renal toxicity. According to this study, it was found that the protective mechanism of GSPE on cisplatin nephrotoxicity may be associated with its scavenging activity and antioxidant potential, thereby enhancing the activity of antioxidant enzymes (such as glutathione-S-transferase), upregulating the level of Bcl-2, and inhibiting cytochrome C release from mitochondria into the cytoplasm, and preventing apoptosis in normal cell. Therefore, we recommend pretreatment of patients with cancer with GSPE before administering chemotherapy.

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Conflicts of interest

There are no conflicts of interest.

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