Taylor & Francis
Taylor & Francis Group

Renal Failure

ISSN: 0886-022X (Print) 1525-6049 (Online) Journal homepage: informahealthcare.com/journals/irnf20

Proanthocyanidin and fish oil potent activity against cisplatin-induced renal cell cycle arrest and apoptosis in rats

Hanaa A. Hassan, Gamal M. Edrees, Ezz M. El-Gamel & Elsamra A. El-Sayed

To cite this article: Hanaa A. Hassan, Gamal M. Edrees, Ezz M. El-Gamel & Elsamra A. El-Sayed (2015) Proanthocyanidin and fish oil potent activity against cisplatin-induced renal cell cycle arrest and apoptosis in rats, Renal Failure, 37:8, 1356-1362, DOI: 10.3109/0886022X.2015.1073528

To link to this article: https://doi.org/10.3109/0886022X.2015.1073528

RENAL FAILURE

http://informahealthcare.com/rnf
ISSN: 0886-022X (print), 1525-6049 (electronic)

Ren Fail, 2015; 37(8): 1356–1362
 DOI: 10.3109/0886022X.2015.1073528

Taylor & Francis
Taylor & Francis Group

LABORATORY STUDY

Proanthocyanidin and fish oil potent activity against cisplatin-induced renal cell cycle arrest and apoptosis in rats

Hanaa A. Hassan[1], Gamal M. Edrees[1], Ezz M. El-Gamel[2], and Elsamra A. El-Sayed[1]

[1]*Department of Zoology, Physiology Division, Faculty of Science, Mansoura University, Mansoura, Egypt and* [2]*Immunology Division, Urology and Nephrology Center, Mansoura University, Mansoura, Egypt*

Abstract

Cisplatin is an effective chemotherapeutic agent that displays dose-limiting nephrotoxicity. In the present study, the efficacy of grape seed proanthocyanidin extract (GSPE: 100 mg/kg/day) and fish oil (FO: 5 mL/kg/day) against cisplatin-induced nephrotoxicity was evaluated in terms of DNA damage, histopathological changes and expression levels of molecular markers of apoptosis. The administration of cisplatin (CP) (7 mg/kg) results in an increasing percentage of S-phase, G2/M and apoptosis. Furthermore, CP induces apoptosis as indicated by an elevation of renal caspase-3 and reduction in the expression of BCL-2. In addition to occurred renal histopathological changes as manifested by tubular degeneration, degenerative glomerulus, necrotic tubular cells, and cell debris. On the other hand, the administration of GSPE or FO pre-cisplatin treatment can be ameliorated the current DNA cell cycle alterations by the restoration of expression of proteins related to apoptosis and reduced the undesirable renal histopathological changes. So, it can be concluded that the consumption of GSPE or FO might be useful for minimizing nephrotoxicity caused by cisplatin chemotherapy through their anti-apoptotic and antioxidant properties.

Keywords

Cisplatin, DNA damage, FO, GSPE, nephrotoxicity apoptosis

History

Received 4 March 2015
Revised 2 June 2015
Accepted 30 June 2015
Published online 31 August 2015

Introduction

Cisplatin and other platinum derivatives are among the most effective chemotherapeutic agents widely used in the treatment of a variety of solid tumors including ovarian, testicular, head and neck carcinomas, and germ cell tumors,[1] However, the full clinical utility of cisplatin is limited by its nephrotoxicity.[2] The mechanism of cisplatin nephrotoxicity remains to be fully elucidated. In addition to direct tubular toxicity in the form of apoptosis and necrosis,[3] vascular factors,[4] and inflammation[5] that have been implicated in the pathogenesis of cisplatin-mediated nephrotoxicity. These complications are associated with cisplatin-induced oxidative stress.[1,6] Cisplatin chemotherapy induces a reduction in plasma antioxidant levels, leading to a failure of the antioxidant defense against free radical damage generated by antitumor drugs.[7] It has multiple intracellular effects, causing direct cytotoxicity with reactive oxygen species, activating mitogen-activated protein kinases, inducing apoptosis[8] and stimulating inflammation, but its major dose limiting side effect is nephrotoxicity. Cisplatin selectively damages proximal tubular epithelial cells.[9] Its concentration in proximal tubular cells is about five times the serum concentration,[10] this disproportionate accumulation of cisplatin in renal tissue contribute to cisplatin-induced nephrotoxicity.[3] Continued aggressive high-dose cisplatin chemotherapy necessitates the investigation of ways for prevention of the dose limiting side effects that inhibit the cisplatin administration at tumoricidal doses. There is a continuous search for agents that provides nephroprotection against CP and other platinum drugs.[2]

Recent studies suggested that diet supplemented with natural agents, such as grape seed extracts that have a high concentration of vitamin E, flavonoids, linoleic acid, polyphenols, including oligomeric proanthocyanidins recognized as antioxidants, which are naturally occurring polyphenolic compounds widely available in fruits, vegetables, nuts, seeds, flowers, and bark.[11] GSPE has been shown to serve as one of the most potent free radical scavengers and provides significant protection against damage of oxidative stress.[12] Also, a number of investigations have also demonstrated that fish oil (FO) enriched in ώ-3 fatty acids has profound beneficial health effects against various pathologies[13] including cardiovascular diseases, respiratory diseases, diabetes, depression, cancers, inflammatory, and immune renal disorders.[14] Thus, it was thought worthwhile to investigate the role of the supplementation of GSPE as a plant product or fish oil as animal product in alleviating the nephrotoxicity and other adverse effects of cisplatin in rats.

Address correspondence to Dr Hanaa A. Hassan, Department of Zoology, Physiology Division, Faculty of Science, Mansoura University, Mansoura, Egypt. Tel: 00201001059907; E-mail: drhanaa hassan@yahoo.com; drhanaahassan@mans.edu.eg

Materials and methods

Chemicals and drugs

Cisplatin (CP) was purchased from Mayne Pharmaceuticals (Warwickshire, UK), Fish oil (FO) [Menhaden, Sigma Chemical Co., Los Angeles, CA] and a dried powdered grape seed proanthocyanidin extract (GSPE) commercially known as Noxylife was obtained from Pharco Pharmaceuticals (Mansoura, Egypt).

Animals

Healthy male albino rats *(Rattus rattus*), 8 weeks old (150–170 g) were purchased from Institute of Ophthalmic Disease Research, Cairo, Egypt. Rats were housed in a stainless steel cages in a windowless room with automatically regulated temperature (22–25 °C). They were kept under good ventilation under a photoperiod of 12 h light:12 h darkness schedule with lights-on from 06.00 to 18.00. They received a standard laboratory diet composed of 60% ground corn meal, 15% ground beans, 10% bran, 10% corn oil, 3% casein, 1% mineral mixture, and 1% vitamins mixture, purchased from Meladco Feed Company (Aubor City, Cairo, Egypt) and supplied with water *ad libitum* throughout the experimental period. Animals were provided with humane care and the present study complies with the instruction's guidelines. The local committee approved the design of the experiments, and the protocol conforms to the guidelines of the National Institutes of Health (NIH).

Experimental design

After two weeks of acclimatization, the animals were divided at random into six groups (six animals each group): group I served as control, group II was orally received GSPE (100 mg/kg BW/day) dissolved in water for six weeks.[15] Group III was orally received fish oil (5 mL/kg BW/day) for six weeks.[16] Group IV treated with a single intraperitoneal dose of cisplatin (7.5 mg/kg BW).[17] Group V received GSPE (100 mg/kg BW/day) dissolved in water orally for six weeks in addition to cisplatin treatment (7.5 mg/kg BW) as a single intraperitoneal dose after five weeks of the experiment. Group VI was orally received fish oil (5 mL/kg BW/day) for six weeks in addition to cisplatin treatment (7.5 mg/kg BW) as a single intraperitonealdose after the 5th week of the experiment. All the animals were sacrificed after one week of cisplatin administration.

Sample collection and tissue preparations

Animals were sacrificed and blood was withdrawn from left jugular vein and put into chilled non-heparinized tubes, which were centrifuged for separation of serum. The sera were frozen at −20 °C for future measurements. Then animals were dissected and the kidneys were removed and decapsulated, then they buffered to remove blood and other materials. One of the two kidneys was fixed with 10% formalin solution. The other was minced and homogenized (10% w/v), separately, in ice-cold sodium, potassium phosphate buffer (0.01 M, pH 7.4) containing 1.15% KCl in a Potter–Elvehjem type homogenizer. The homogenates were centrifuged and used for further analysis.

Flow cytometric DNA cell cycle analysis

The flow cytometry analysis was performed in the Mansoura University Hospital using flow activated cell sorter (FACS) Calibur Flow Cytometer (Becton Dickinson, Sunnyvale, CA) equipped with a compact air-cooled low power 15 mWArgon ion laser beam (488 nm). The average number of evaluated nuclei per specimen was 20.000 and the number of nuclei scanned was 120/s. DNA histogram derived from flow cytometry was obtained with a computer program by Dean and Jett mathematical analysis.[18] Data analysis was conducted using DNA analysis program MODFIT (Verity Software House, Inc., Topsham, ME, version: 2.0 powers Mac with 131,072 kb; Registration No: 42000960827-16193213; Date made: 16 September 1996). This software calculated the coefficient of variation (CV) around the G0/G1 peak and the percentage of cells in each phase (G0/G1, S and G2/M) of the DNA cell cycle for each sample. The analysis of apoptotic cell death was performed by measuring the DNA contents using FACS Calibur Flow Cytometer.[19] Flow cytometry P53 and Bcl2 protein expression was performed using fluorescence threshold using FACS Calibur Flow Cytometer (Becton Dickinson, Sunnyvale, CA) as described by Brotherick et al.[20]

Histological analysis

The kidneys were excised after sacrifice and fixed in 4% paraformaldehyde, prior to being embedded in paraffin. Paraffin sections (5 μm thick) were prepared according to routine methods and were stained with hematoxylin and eosin according to the standard procedures.[21] All sections were examined under a light microscope.

Immunohistochemical staining to determine caspase-3 and Bcl-2 expression

Kidney sections (5 μm thick) were prepared according to routine methods. Immunostaining (Maryland Chemical Company, Inc., Los Angeles, CA) was performed according to previously described methods.[22] Briefly, sections were deparaffinized in xylene and rehydrated by immersion in descending concentrations of ethanol. After routine antigen retrieval procedures, sections were treated with 3% hydrogen peroxide to block endogenous peroxidase and were then incubated with a primary antibody raised against caspase 3 or Bcl-2 overnight at 4 °C. After washing with phosphate buffer saline (PBS) (PH 7.4), the sections were incubated with a secondary antibody-linked to horseradish peroxidase (HRP) at room temperature for 30 min. Then, the immunoreactivity of the cells was visualized using diaminobenzidine (DAB). The cells that displayed brown precipitation were considered to be positive for caspase 3 or Bcl-2 expression. Scoring was determined by the percentage of the positivity and negativity to the stain.

Statistical analysis

All data were statistically analyzed by one-way ANOVA (analysis of variance) test and post comparison was carried out with Waller–Duncan k ratio using SPSS program (Statistical Package for Social Science) version 11 (Chicago, IL). The results are presented as mean ± SE. The values of

$p \leq 0.05$ were considered statistically significant based on least significant difference (LSD) probability.

Results

Flow cytometric DNA cell cycle analysis

The data showed no significant change in the percentage of G0/1, S-phase G2/M, and apoptosis in GSPE or FO administered rat groups compared with control rats group. However, there was a significant decrease in the percentage of G0/1 and a significant increase in S-phase, G2/M and apoptosis percentage in cisplatin-treated rats group compared with the control group. Meanwhile, there was a significant increase in the percentage of G0/1 but significant decrease in G2/M, S-phase and apoptosis percentage in case of rats administered with GSPE or FO pre-cisplatin comparing with cisplatin only (Table 1; Figures 1 and 2).

Histopathological findings

The cisplatin-nephrotoxicity was characterized by degenerative glomerulus (DG), necrotic tubular cells (N), and cell debris (CD). The intake of GSPE or FO to cisplatin-treated group ameliorated the undesirable renal histopathological changes produced after cisplatin-intoxication (Figure 3).

Immunohistochemical findings

The immunohistochemical investigation showed more intense expression of caspase-3 and poor expression of Bcl2 in rats subjected to cisplatin comparing with control rats. However, these changes are reversed in GSPE or FO treated rats' pre-cisplatin compared with cisplatin only treated rats (Figures 4 and 5).

Discussion

Cisplatin is a potent antitumor drug. The therapeutic effects of cisplatin are significantly improved by dose calculation. However, high-dose therapy with cisplatin is limited by its cumulative nephrotoxicity and neurotoxicity.[23] Cisplatin is toxic to the renal proximal tubules.[24] Although several studies have been carried out to elucidate the molecular mechanism that causes cisplatin nephrotoxicity; however, the factors responsible for this are not fully understood. Recently, it has been postulated that oxidative stress and reactive oxygen species (ROS) are involved in the pathogenesis of cisplatin-induced nephrotoxicity.[25] The nephrotoxicity induced by cisplatin is characterized by morphological destruction of intracellular organelles, cellular necrosis, loss of microvilli, alterations in the number and size of the lysosomes, and mitochondrial vacuolization, followed by functional alterations including inhibition of protein synthesis, GSH depletion, lipid peroxidation, and mitochondrial damage.[10]

The observed DNA damage and arresting of the cell cycle of kidney cells due to CDDP treatment is in agreement with the work by De Martinis and Bianchi,[26] Chang et al.,[27] Satoh et al.,[28] and Hassan et al.[29]. This damage may be due to that cisplatin binds to DNA to form covalent platinum DNA adducts and also acts as a DNA alkylator. In addition, cisplatin generates reactive oxygen species (ROS), which are known as one of the pathogenic intermediates triggering DNA damage following chemotherapy. Through these mechanisms, cisplatin triggers cellular responses involving multiple pathways, including DNA repair, transcription inhibition, cell cycle arrest, cellular transport system impairment, ATPase activity reduction, and mitochondrial damage.[30] Furthermore, cisplatin is a DNA damaging agent which forms cisplatin DNA adducts that destroys cells via numerous mechanisms resulting in apoptosis.[29,31]

The degradation of cellular DNA by endonucleases is an important component of renal tubular epithelial cell death induced by ischemia or nephrotoxins.[32] Renal tubule cell apoptosis has recently been observed in an increasing array of renal disorders and is emerging as a final common pathway in response to a wide variety of cellular stresses applied at intensity below the threshold for necrosis.[33] This observation also holds true for cisplatin nephrotoxicity, in which necrotic cell death is encountered with higher doses, whereas lower concentrations induce apoptosis.[34]

The improvement in DNA by administration of GSPE or FO is in accordance with the finding of Bagchib et al.,[35]which reported that GSPE is a better as free radical scavenger and inhibitor of oxidative tissue damage, DNA fragmentation, and subsequent apoptosis than all the antioxidant vitamins. Thus, the results suggest that GSPE is bioavailable, and its significant potential to prevent CDDP-induced acute renal failure may be attributed to the attenuation of renal tubular damage and enhancement of the regenerative response of the damaged tubular cells.[36] This beneficial effect may also be due to scavenging activities especially for hydrolytic radical, such as peroxyl radical[36] and also scavenge superoxide radicals, quenches singlet oxygen.[37]

In this study, the histopathological observations of cisplatin-treated rats displayed a model of acute renal failure represented in necrosis of the renal tubular cells, pyknotic nuclei, degeneration of the glomeruli, intertubular hemorrhage, desquamation of the tubular epithelium forming cell

Table 1. Renal DNA cell cycle and apoptosis % in control and different treated rat groups.

Animal Groups	C	GSPE	FO	CP	GSPE + CP	FO + CP
Mean ±SE						
G0/1%	78.41 ± 2.28^{a}	80.96 ± 1.41^{a}	79.22 ± 2.44^{a}	42.62 ± 0.92^{b}	62.96 ± 1.61^{c}	63.66 ± 1.61^{c}
S-phase%	15.64 ± 0.64^{a}	13.71 ± 1.11^{a}	14.30 ± 0.79^{a}	34.38 ± 0.72^{b}	25.78 ± 1.63^{c}	22.18 ± 0.57^{c}
G2/M%	6.72 ± 0.55^{a}	6.59 ± 0.45^{a}	6.17 ± 0.37^{a}	23.59 ± 0.99^{b}	14.55 ± 1.04^{c}	12.79 ± 0.47^{c}
Apoptosis%	5.57 ± 0.13^{a}	4.50 ± 0.13^{a}	5.51 ± 0.27^{a}	32.07 ± 5.50^{b}	10.42 ± 0.66^{c}	8.57 ± 0.64^{c}

Notes: Data are expressed as mean ± SE of six rats. Within each row, means with different superscript (a, b, c) were significantly different at $p < 0.05$. Where means superscripts with the same letters mean that there is no significant difference at ($p > 0.05$). C: control GSPE: grape seed proanthocyanidin extract.
FO: fish oil; CP: cisplatin.

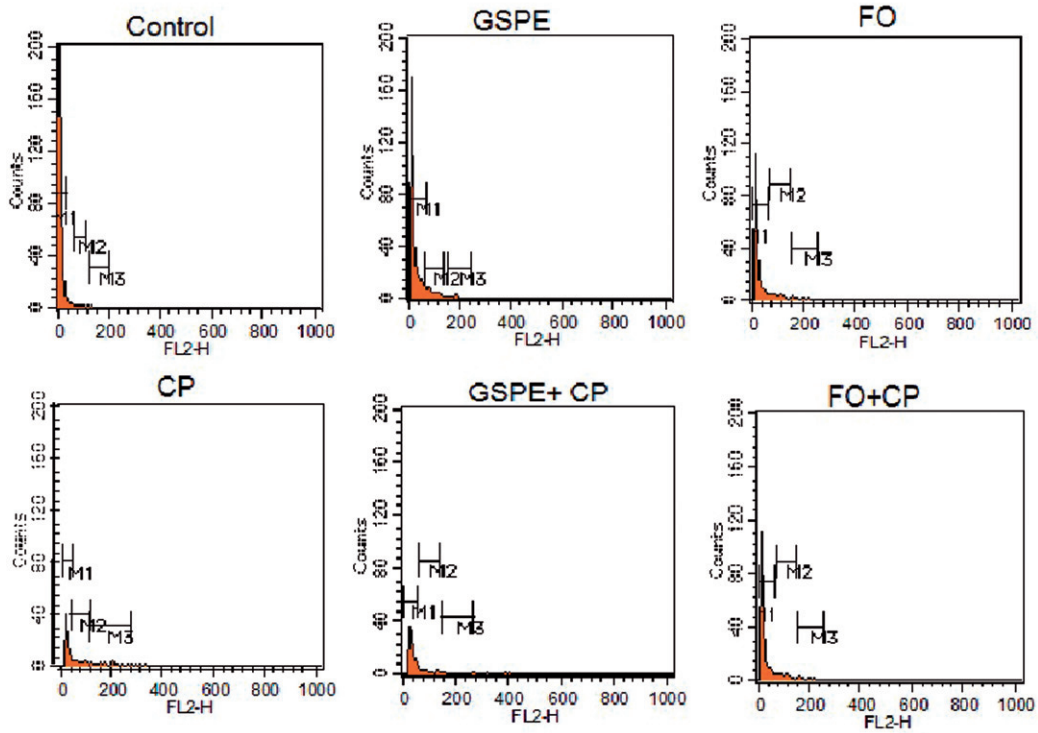

Figure 1. Renal DNA cell cycle analysis in control and different treatment groups of rats. Note: M1: G0/1%, M2 S phase %, M3 G2/M%.

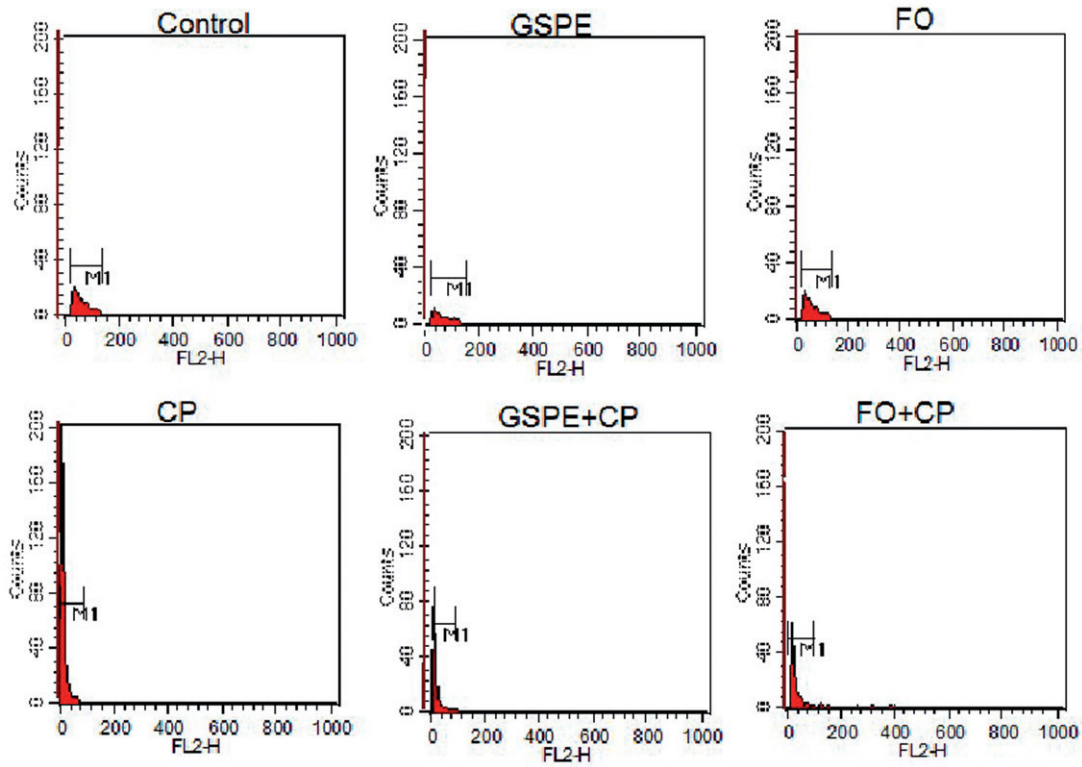

Figure 2. Renal apoptosis % in control and different treatment groups of rats. Note: M1: apoptosis %.

debris in the lumen, and leukocytic infiltration. These histopathological changes are parallel to the findings of many investigators.[38] These changes may be due to that cisplatin provokes a loss of tubular epithelial cells by necrosis and apoptosis, followed by inflammatory cell infiltration and fibroproliferative changes.[39,40] Also, Fouad et al.[41] reported that cisplatin administration caused severe and wide spread necrosis with dilatation of proximal tubules, vacuolization, tubular cell desquamation, and intraluminal cast formation.

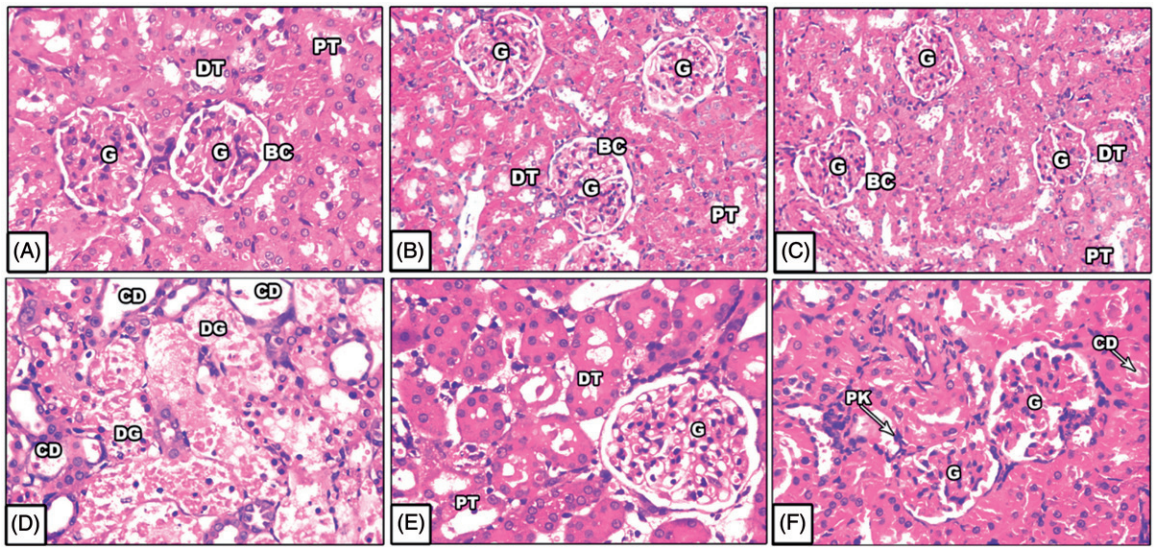

Figure 3. Representative photomicrographs of kidney by staining with hematoxylin and eosin of control and different treated rat groups. Notes: [A] Normal control untreated rats (normal glomerular architecture and normal proximal and distal convoluted tubules with cuboidal epithelial cells); [B] GSPE-treated rats; [C] FO-treated rats (normal glomerular architecture and normal proximal and distal convoluted tubules with cuboidal epithelial cells); [D] Cisplatin-intoxicated rats (tubular degeneration degenerative glomerulus, necrotic tubular cells, and cell debris); [E] Cisplatin-GSPE-treated rats and [F] Cisplatin-FO treated rats (amelioration the undesirable changes produced after cisplatin-intoxication).

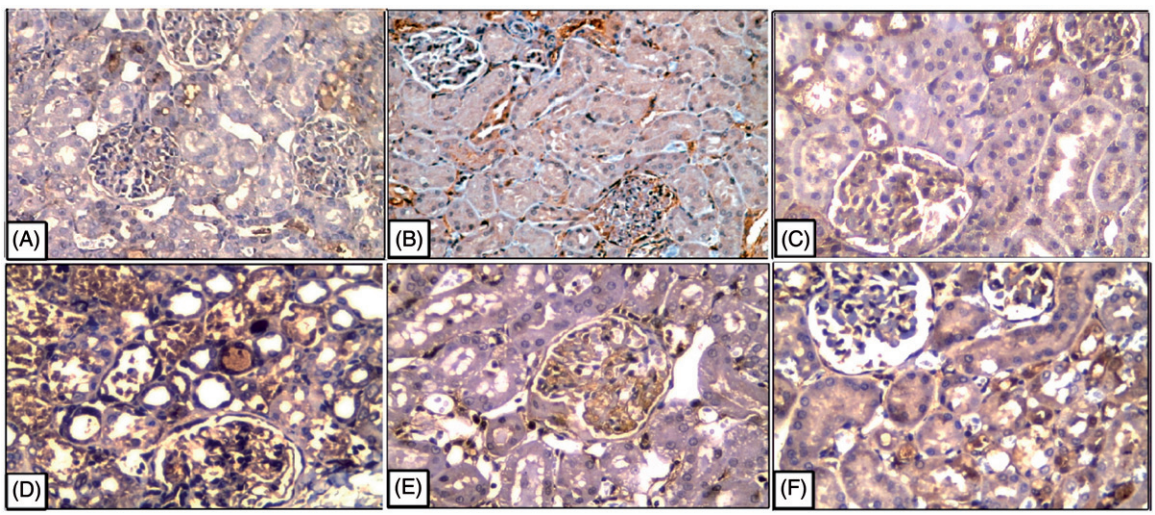

Figure 4. Representative photomicrographs of caspase-3 expression determined by immunohistochemistry. Notes: [A, B and C] There are 26, 23, and 21% of caspase-3 expressions in the cortical regions of kidney of control rats and in GSPE- or FO-treated rats, respectively. [D] Cisplatin administration increased strongly caspase-3 expression about 70% in inner cortical and outer medullary areas especially in the proximal convoluted tubules, whereas in the glomerular structure there was less caspase-3 expression. [E, F] There was inhibition of caspase-3 expression as evidenced by weak immunostaining about 45 and 49% in the distal tubules in the cortical regions of rat kidneys treated GSPE or FO, respectively.

On the other hand, the administration of proanthocyanidin caused significant histopathological improvement in kidney tissue. This result is in agreement with Dulundu et al.'s[42] work. This is may be due to reduction of extensive hemorrhage, necrosis and disruption of tissue architecture by administration of proanthocyanidins.[43,44] This also may be related to the reduction of lipid peroxidation by its antioxidant activity.[43] Moreover, it can exert its effect by scavenging ROS.[45] Additionally, fish oil pretreatment improved the histopathological changes of kidney. This improvement may be due to ω-3 fatty acid-rich fish oil that enhanced resistance to free radical attack generated by cisplatin administration.[46]

Furthermore, the occurred data demonstrated that treatment with cisplatin decreased the expression of Bcl-2 anti-apoptotic protein in renal glomeruli of rats. Apoptosis is a physiologic form of cell death, occurs in the individual cell in an endogenously programmed pattern.[47] It can be triggered by a variety of physiologic stimuli; however, the pathologic factors also affect the apoptotic pathways and then contribute to the induction or progression of many diseases.[47–49] Among the factors affecting apoptotic pathways,

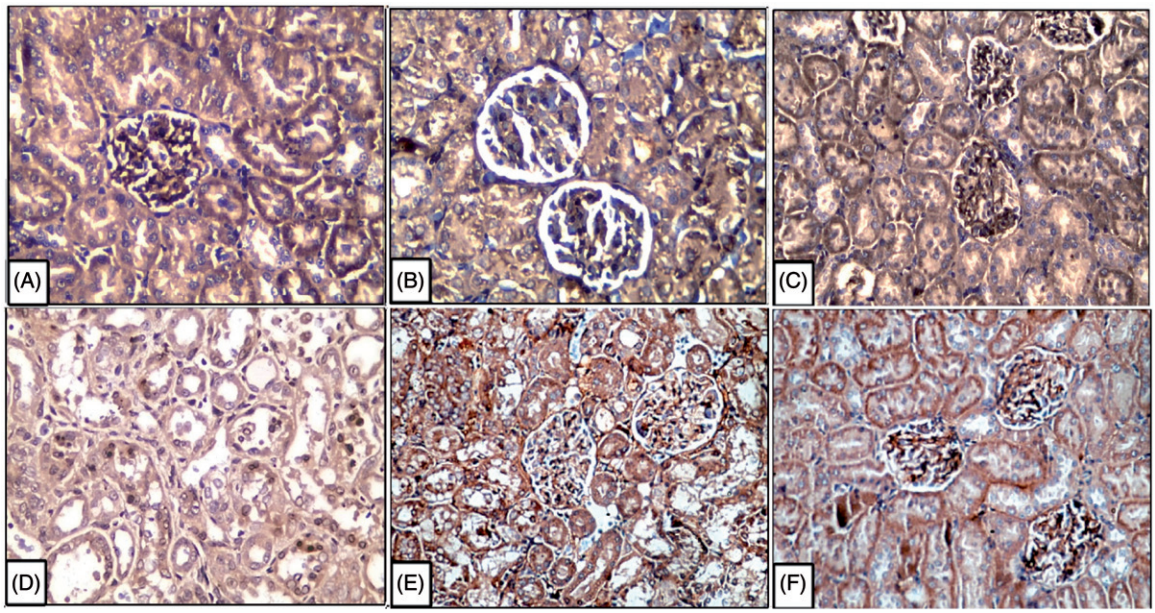

Figure 5. Representative photomicrographs of Bcl-2 expression determined by immunohistochemistry. Notes: [A, B, and C] There is normal expression of Bcl-2 in the cortical regions of kidney in control, GSPE, and FO treated rats about 80, 82, and 79%, respectively. However, cisplatin administration decreased strongly Bcl-2 expression which is about 30% in inner cortical and outer medullary areas especially in the proximal convoluted tubules [D]. On the other hand, there was an increase of Bcl-2 expression as evidenced by strong immunostaining in the distal tubules in the cortical regions of rat kidneys treated with GSPE or FO before cisplatin which are about 50 and 53% [E, F]. Brown color indicates immunopositivity.

Bcl-2 is a protein encoded by Bcl-2 proto-oncogene and one of the molecular members of the cell survival factors in the Bcl-2 family.[50] Bcl-2 is an important regulator of apoptosis and can block programmed cell death.

Moreover, caspases are crucial mediators of programmed cell death (apoptosis). Among them, caspase-3 is a frequently activated death protease, catalyzing the specific cleavage of many key cellular proteins. Pathways to caspase-3 activation have been identified that are either dependent on or independent of mitochondrial cytochrome c release and caspase-9 function. Caspase-3 is also required for some typical hallmarks of apoptosis, and is indispensable for apoptotic chromatin condensation and DNA fragmentation in all cell types examined. Previous studies have indicated that cisplatin-induced apoptosis in cultured renal proximal tubular cells proceeds via both caspase-dependent and caspase-independent pathways.[51] In our study, cisplatin increased the caspase-3 expression in renal tubular cells in the same manner as in earlier studies. This induction in caspase-3 expression in renal tubular cells by cisplatin may be due to its direct cytotoxicity with reactive oxygen species activating mitochondria-activated protein kinases and then inducing apoptosis.[8] Caspase-3 mediate most of the events in the execution phase of apoptosis, including cytoskeleton disruption, membrane blebbing, externalization of eat-me signals (such as phosphatidylserine), chromatin condensation, internucleosomal DNA fragmentation, nuclear rupture and cell dismantling into membrane-sealed apoptotic bodies.[52] Furthermore, cisplatin is a DNA damaging agent which forms cisplatin DNA adducts that destroys cells via numerous mechanisms resulting in apoptosis.[31]

However, pretreatment with GSPE or fish oil decreased the apoptosis, which manifested by increasing the expression of Bcl-2 and decreasing the expression of caspase-3. This may be attributed to scavenging activities especially for hydrophilic radical, such as peroxyl radical[36] and also scavenge superoxide radical.[37] Moreover, grape seed extract can block cell death signaling mediated through the proapoptotic transcription factors and genes, such as c-jun.[53]

Therefore, it was concluded that GSPE or fish oil offered sufficient protection against nephrotoxicity caused by cisplatin chemotherapy through their free radical scavenging, antioxidant activity and membrane stabilizing by mechanisms that include up-regulation of the key apoptotic regulators and modulate the cytotoxic effects of cisplatin.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Pabla N, Dong Z. Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. *Kidney Int.* 2008;73:994–1007.
2. Ali BH, Al Moundhri SM. Agents ameliorating or augmenting the nephrotoxicity of cisplatin and other platinum compounds: A review of some recent research. *Food Chem Toxicol.* 2006;44:1173–1183.
3. Arany I, Safirstein RL. Cisplatin nephrotoxicity. *Semin Nephrol.* 2003;23:460–464.
4. Luke DR, Vadiel K, Lopez-Berestein G. Role of vascular congestion in cisplatin induced acute renal failure in the rat. *Nephrol Dial Transplant.* 1992;7:1–7.
5. Ramesh G, Reeves WB. TNFR2-mediated apoptosis and necrosis in cisplatin induced acute renal failure. *Am J Physiol Renal Physiol.* 2003;285:F610–F618.
6. Chirino Y, Pedraza-Chaverri J. Role of oxidative and nitrosative stress in cisplatin-induced nephrotoxicity. *Exp Toxicol Pathol.* 2009;61:223–242.
7. Masuda H, Tanaka T, Takahama U. Cisplatin generates superoxide anion by interaction with DNA in a cell-free system. *Biochem Biophys Res Commun.* 1994;203:1175–1180.

8. Yao X, Panichpisal K, Kurtzman N, Nugent K. Cisplatin nephrotoxicity: A review. *Am J Med Sci.* 2007;334:115–124.
9. Camano S, Lazaro A, Moreno-Gordaliza E, et al. Cilastatin attenuates cisplatin-induced proximal tubular cell damage. *J Pharmacol Exp Ther.* 2010;334:419–429.
10. Kuhlmann MK, Horsch E, Burkhardt G, Wagner M, Kohler H. Reduction of cisplatin toxicity in cultured renal tubular cells by bioflavonoid quercetin. *Arch Toxicol.* 1998;72:536–540.
11. Zhang YY, Li WG, Wu YJ, Bai DC, Liu NF. Proanthocyanidin from grape seeds enhances doxorubicin-induced antitumor effect and reverses drug resistance in doxorubicin-resistant K562/DOX cells. *Cand J Physiol Phamacol.* 2005;83:309–318.
12. Hassan HA, Al Rawi MM. Grape seeds proanthocyanidin extract as a hepatic-reno-protective agent against gibberellic acid induced oxidative stress and cellular alterations. *Cytotechnology.* 2013;65: 567–576.
13. Simopoulos AP. Omega-3 fatty acids in health and disease and in growth and development. *Am J Clin Nutr.* 1991;54:438–463.
14. Thakkar RR, Wang OL, Zerouga M, Stillwell W, Haq A, Kissling R. Docosahexaenoic acid reverses cyclosporin-A induced changes in membrane structure and function. *Biochem Biophys Acta.* 2000;1474:183–195.
15. Yamakoshi J, Saito M, Kataoka S, Kikuchi M. Safety evaluation of proanthocyanidin-rich extract from grape seeds. *Food Chem Toxicol.* 2002;40:599–607.
16. El-Daly ES. Effect of methimazole and fish oil treatment on gentamicin nephrotoxicity. *J Islamic Acad Sci.* 1996;9:37–48.
17. Atessahin A, Yilmaz S, Karahan I, Ceribasi AO, Karaoglu A. Effects of lycopene against cisplatin-induced nephrotoxicity and oxidative stress in rats. *Toxicology.* 2005;212:116–123.
18. Dean PN, Jett JH. Mathematical analysis of DNA distributions derived from flow microfluorometry. *J Cell Biol.* 1974;60:523–527.
19. Nicoletti I, Mannucci R, Migloirati G, Riccardi C, Grignani F. Common method for measuring apoptotic cell death. *Purdue Cytom CD-ROM Ser.* 2001;3:1–9.
20. Brotherick I, Shenton BK, Cowan WK, et al. p53 expression measured by flow cytometry. A comparison of three monoclonal antibodies and the relationship with grade and DNA ploidy in breast cancer. *Cancer Immunol Immunother.* 1995;41:146–150.
21. Isitor GN, Rao S, Nayak SB, Sundaram V. Autofluorescent vesicular structures in hematoxylin and eosin stained duodenal mucosa of the domestic cat. *West Indian Veterinary J.* 2009;9:27–32.
22. Carter MR, Hornick JL, Lester S, Fletcher CD. Spindle cell (sarcomatoid) carcinoma of the breast: A clinico pathologic and immunohistochemicall analysis of 29 cases. *Am J Surg Pathol.* 2006;30:300–309.
23. O'Dwyer PJ, Stevenson JP, Johnson SW. Clinical status of cisplatin, carboplatin and other platinum-based antitumor drugs. In: Lippert B, ed. *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*. Zurich: Wiley-VCH; 1999:29–70.
24. Gonzales-Vitale JC, Hayes DM, Cvitkovic E, Sternberg SS. The renal pathology in clinical trials of cis-platinum (II) diammine-dichloride. *Cancer.* 1977;39:1362–1371.
25. Yilmaz HR, Iraz M, Sogut S, Ozyurt ZY, Akyol O, Gergerlioglu S. The effects of erdostiene on the activities of some metabolic enzymes during cisplatin-induced nephrotoxicity in rats. *Pharmacol Res.* 2004;50:287–290.
26. De Martinis BS, Bianchi M. Effect of vitamin C supplementation against cisplatin-induced toxicity and oxidative DNA damage in rats. *Pharmacol Res.* 2001;44:317–320.
27. Chang BJ, Nishikawa M, Sato E, Utsumi K, Inouea ML. Carnitine inhibits cisplatin-induced injury of the kidney and small intestine. *Arch Biochem Biophys.* 2002;405:55–64.
28. Satoh M, Kashihara N, Fujimoto S, et al. A novel free radical scavenger, edarabone, protects against cisplatin-induced acute renal damage in vitro and in vivo. *J Pharmacol Exp Ther.* 2003;305: 1183–1190.
29. Hassan H A, Edress GM, El-Gamal E, El-Sayed EA. Amelioration of cisplatin-induced nephrotoxicity by grape seed proanthocyanidin extract and fish oil is mediated by lowering oxidative stress and DNA damage. *Cytotechnology.* 2014;66:419–429.
30. Yin X, Apostolov EO, Shah SV, et al. Cisplatin-induced nephrotoxicity is mediated by DNase I and endonuclease G in mice. *J Am Soc Nephrol.* 2013;16:697–702.
31. Wang D, Lippard SJ. Cellular processing of platinum anticancer drugs. *Nat Rev Drug Discov.* 2005;4:307–320.
32. Basnakian AG, Apostolov EO, Yin X, Napirei M, Mannherz HG, Shah SV. Cisplatin nephrotoxicity is mediated by deoxyribonuclease. *J Am Soc Nephrol.* 2005;16:697–702.
33. Hanigen MH, Devarajan P. Cisplatin nephrotoxicity: Molecular mechanisms. *Cancer Ther.* 2003;1:47–61.
34. Park MS, De Leon M, Devarajan P. Cisplatin induces apoptosis in LLC-PK1 cells via activation of mitochondrial pathways. *J Am Soc Nephrol.* 2002;13:858–865.
35. Bagchib D, Gargb A, Krohn RL, Bagchi M, Tran MX, Stohs SJ. Oxygen free radical scavenging abilities of vitamins C and E, and a grape seed proanthocyanidin extract in vitro. *Res Commun Mol Pathol Pharmacol.* 1997;95:179–189.
36. Ricardo JM, Darmon N, Fenandez Y, Mitjavila S. Oxygen free radical scavenger capacity in aqueous models of different procyanidins from grape seeds. *J Agric Food Chem.* 1991;39: 1549–1552.
37. Ariga T. The antioxidant function, preventive action on disease and utilization of proanthocyanidins. *Biofactors.* 2004;21:197–201.
38. Chirini YI, Hernandez-Pando R, Pedraza-Chaverri J. Peroxynitrite decomposition catalyst ameliorates renal damage and protein nitration in cisplatin-induced nephrotoxicity in rats. *BMC Pharmacol.* 2004;4:20.
39. Lieberthal W, Triaca V, Levine J. Mechanisms of death induced by cisplatin in proximal tubular epithelial cells: Apoptosis vs. necrosis. *Am J Physiol Renal Physiol.* 1996;39:F700–F708.
40. Taguchi T, Nazneen A, Abid MR, Razzaque MS. Cisplatin-associated nephrotoxicity and pathological events. *Nephrology.* 2005;148:107–121.
41. Fouad AA, Morsy MA, Gomaa W. Protective effect of carnosine against cisplatin-induced nephrotoxicity in mice. *Environ Toxicol Pharmacol.* 2008;24:292–297.
42. Dulundu E, Ozel Y, Topalolu U. Grape seed extract reduces oxidative stress and fibrosis in experimentally biliary obstruction. *J Gastroenterol Hepatol.* 2007;22:885–892.
43. Shin MO, Yoon S, Moon JO. The proanthocyanidins inhibit dimethylnitrosamine induced liver damage in rats. *Arch Pharm Res.* 2010;33:167–173.
44. El-Kholy WM, Hassan HA, Nour SE. Impact of grape seed proanthocyanidin on adverse effects induced by the plant growth regulators: Gibberellic acid and indoleacetic acid in male rats. *J Egypt Ger Zool.* 2012;64A:29–52.
45. Hemmati AA, Nazari Z, Samei M. A comparative study of grape seed extract and vitamin E effects on silica induced pulmonary fibrosis in rats. *Pulmonary Pharm Ther.* 2008;21:668–674.
46. Xi S, Chen LH. Effects of dietary fish oil on tissue glutathione and antioxidant defense enzymes in mice with murine AIDS. *Nutr Res.* 2000;20:1287–1299.
47. White E. Life, death, and the pursuit of apoptosis. *Genes Dev.* 1996; 10:1–15.
48. Shinoura N, Yoshida Y, Asai A, Kirino T, Hamada H. Relative level of expression of Bax and Bcl-XL determines the cellular fate of apoptosis/necrosis induced by the overexpression of Bax. *Oncogene.* 1999;18:5703–5713.
49. Tsujimoto Y, Shimizu S. Bcl-2 family: Life or death switch. *FEBS Lett.* 2000;466:6–10.
50. Yang E, Korsmeyer SJ. Molecular than atopsis: A discourse on the BCL2 family and cell death. *Blood.* 2000;88:386–401.
51. Cummings BS, Schnellmann RG. Cisplatin-induced renal cell apoptosis: Caspase 3-dependent and-independent pathways. *J Pharmacol Exp Ther.* 2002;302:308.
52. Nunez R, Sancho-Martinez SM, Novoa JM, Lopez-Hernandez FJ. Apoptotic volume decrease as a geometric determinant for cell dismantling into apoptotic bodies. *Cell Death Differ.* 2010;17: 1665–1671.
53. Baines CP, Molkentin JD. stress signaling pathways that modulate cardiac myocyte apoptosis. *J Mol Cell Cardiol.* 2005;38:47–62.