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ORIGINAL RESEARCH

Amelioration of cisplatin-induced nephrotoxicity by grape seed extract and fish oil is mediated by lowering oxidative stress and DNA damage

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Abstract Cisplatin (CP) is a chemotherapeutic drug used in treatment of malignancies. However, its clinical utility is limited by nephrotoxicity. The purpose of the present study was to investigate the protective role of grape seed proanthocyanidin extract (GSPE) (100 mg/kg/day) or fish oil (FO) (5 ml/kg/day) against cisplatin induced nephrotoxicity in terms of biochemical parameters, oxidative stress and DNA damage. CP nephrotoxiciy is manifested by increased levels of serum creatinine, urea and uric acid, accompanied by their decrease in urine. Na, K and Ca levels were altered in both serum and urine. In addition, cisplatin caused a decrease in renal GSH, SH-group, SOD, GST, and Na–K–ATPase levels. However the levels of MDA, H_2O_2 and NO were increased. Also, we assessed the renal genotoxic potential of cisplatin as manifested by an increase in the tail length of DNA, tail intensity (DNA %) and tail moment. On the other hand, administration of GSPE or FO pre-cisplatin treatment ameliorated the current changes in most of the above tested parameters, particularly oxidative stress, endogenous antioxidant defense system and DNA damage indicating their curative effect. Thus, it can be concluded that the consumption of GSPE or FO might be useful for preventing nephrotoxicity caused by cisplatin treatment.

Keywords Cisplatin · GSPE · FO · Nephrotoxiciy · Antioxidants · DNA damage

List of abbreviations

CP	Cisplatin
GSPE	Grape seed proanthocyanidin extract
FO	Fish oil
DNA	Deoxyribonucleic acid
SH	Sulfhydryl group
SOD	Superoxide dismutase
GST	Glutathione-S-transferase
ATPase	Adenosine Triphosphatase
MDA	Malondialdehyde
H_2O_2	Hydrogen peroxide
NO	Nitric oxide

Introduction

The use of chemotherapeutic agents for the treatment of cancer has opened new prospective for improvement of the quality of life of cancer patients. However, besides its success, many anticancer drugs have been shown to be teratogenic and carcinogenic in experimental systems (Cherry et al. 2004). Cisplatin (cis diamminedichloroplatinum II, CP) is a major

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antineoplastic chemotherapy drug for the treatment of various forms of cancers such as ovarian, testicular, bladder, head and neck, and uterine cervix carcinomas (Taguchi et al. 2005; Pabla and Dong, 2008). However the clinical use of cisplatin is limited because of its unwanted side effects such as nephrotoxicity (Arany and Safirstein 2003; Pabla and Dong 2008), neurotoxicity (Barabas et al. 2008), ototoxicity (Rybak et al. 2009) and hepatotoxicity (Liao et al. 2008). Thus, there is a continuous search for agents that provide nephroprotection against CP and other platinum drugs.

Grape seed extracts are industrial derivatives from whole grape seeds that have a high concentration of vitamin E, flavonoids, linoleic acid, and oligomeric proanthocyanidins (OPCs). Typically, the commercial opportunity of extracting grape seed constituents has been done for chemicals known as polyphenols, including oligomeric proanthocyanidins recognized as antioxidants, which are naturally occurring polyphenolic compounds widely available in fruits, vegetables, nuts, seeds, flowers and bark (Zhang et al. 2005). GSPE has been shown to serve as one of the most potent free radical scavengers and provide significantly greater protection against damage of oxidative stress than vitamins C, E and beta-carotene (Hassan et al, 2013). Furthermore proanthocyanidins have been reported to exert antibacterial, antiviral, anticarcinogenic, antimutagenic, anti-inflammatory, anti-allergic, and vasodilatory actions (Fine 2000; Hassan and Al-Rawi 2013).

Furthermore, a number of investigations have also demonstrated that diet supplemented with fish oil (FO) enriched in ω-3 fatty acids has profound beneficial health effects against various pathologies (Simopoulos 1991) including cardiovascular diseases, respiratory diseases, diabetes, depression, cancers, inflammatory and immune renal disorders (Thakkar et al. 2000). Thus, it was thought worthwhile to investigate in detail the role of the supplementation of GSPE or fish oil in amelioration of cisplatin induced nephrotoxicity in an experimental rat model.

Materials and methods

Chemicals and drugs

Cisplatin (CP) was purchased from Mayne Pharmaceuticals (Warwickshire, UK), Fish oil (FO) [Menhaden, Sigma Chemical Company, St. Louis, MO, USA] and a dried powdered grape seed proanthocyanidin extract (GSPE) commercially known as Noxy life was obtained from Pharco Pharmaceuticals (Mansoura, Egypt).

Experimental animals

Adult male Wistar rats, weighing 150–180 g, were kept under a photoperiod of 12 h light: 12 h darkness schedule with lights-on from 06.00 to 18.00 h. They were housed in stainless cages in a windowless room with automatically regulated temperature (22–25 °C). The animals received standard chow and water ad libitum.

Experimental design

After 2 weeks of acclimatization, the animals were divided at random into six groups (eight animals each group): group I served as control, group II was orally treated with GSPE (100 mg/kg BW/day) dissolved in water for 6 weeks (Yamakoshi et al. 2002). Group III was orally treated with fish oil (5 ml/Kg BW/day) for 6 weeks (El-Daly 1996). Group IV received a single intraperitoneal dose of cisplatin (7.5 mg/kg BW) (Yilmaz et al. (2004); Atessahin et al. 2005). Group V received GSPE (100 mg/kg BW/day) dissolved in water orally for 6 weeks in addition to cisplatin (7.5 mg/kg BW) as a single intraperitoneal dose after the 5th week of the experiment. Group VI was orally treated with fish oil (5 ml/Kg BW/day) for 6 weeks in addition to cisplatin (7.5 mg/kg BW) as a single intraperitoneal dose after the 5th week of the experiment. All the animals were sacrificed after 1 week of cisplatin administration.

Sample collection and tissue preparations:

Urine samples were collected for 24 h in standard metabolic cages a day before the sacrifice of rats. After that, animals were sacrificed and blood was withdrawn from left jugular vein and put into chilled non-heparinized tubes, which were centrifuged at 860 g for 10 min for separation of serum. The sera were frozen at −20 °C for future measurements. Then the animals were dissected and the kidneys were removed and decapsulated. One of the two kidneys was stored at −80 °C for the Comet assay. 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 at 10,000*g* for 20 min for biochemical parameters analysis.

Biochemical parameters

Serum creatinine, urea, uric acid, sodium, potassium, calcium levels were measured using kits purchased from Diamond Diagnostics Company (Gizza, Egypt). Na–K–ATPase activity was measured by the method of Bonting et al. (1961). Superoxide dismutase (SOD) activity was assayed according to Misra and Fridovich (1972), Glutathione-S-transferase activity was assayed spectrophotometrically using 1-chloro-2-4-dinitrobenzene (CDNB) and glutathione as described by Habig et al. (1974). Reduced glutathione (GSH) content was assayed according to the method of Beutler et al. (1963), SH- group was measured by the method of Ellman (1959). The amount of malondialdehyde (MDA) was measured by the thiobarbituric acid assay (Ohkawa et al. 1982). Hydrogen peroxide was determined using the colorimetric kit purchased from Biodiagnostic (Gizza, Eygpt) according to the technique used by Aebi (1984). The nitric oxide level was estimated according to the method of Montgomery and Dymock (1961), using the nitric acid kit obtained from Biodiagnostic company for laboratory services, Egypt.

Single-cell gel electrophoresis (comet essay):

The kidneys were removed, decapsulated and immediately stored at −80 °C for Comet assay. The specimens were homogenized in chilled homogenization buffer, pH 7.5 containing 75 mM NaCl and 24 mM Na2EDTA, pH 13, to obtain a 10 % tissue solution. A potter-type homogenizer was used and samples were kept on ice during and after homogenization. Six microliters of kidney homogenate were suspended in 0.5 % low melting agarose and sandwiched between a layer of 0.6 % normal-melting agarose and a top layer of 0.5 % low melting agarose on fully frosted slides. The slides were kept on ice during the polymerization of each gel layer. After the solidification of the 0.6 % agarose layer, the slides were immersed in a lysis solution (1 % sodium surcosinate, 2.5 m NaCl, 100 mM Na2EDTA, 10 mm Tris-HCl, 1 % TritonX-100 and 10 % DMSO) at 4 °C. After 1 h, the slides were placed in electrophoresis buffer (0.3 M NaOH, 1 mM Na2EDTA, pH 13) for 10 minutes at 0 °C to allow DNA to unwind. Electrophoresis was performed for 10 min at 300 mA and 1 V/cm. The slides were neutralized with Tris–HCl buffer, pH 7.5, and stained with 20 μg/ml ethidium-bromide. Each slide was analyzed using a Leitz Orthoplan epifluorescence microscope (Wetzlar, Germany). One hundred cells were analyzed on each slide using the comet assay II automatic digital analysis system. Perspective tail length (μm) is the distance of DNA migration from the center of the body of the nuclear core and is used to evaluate the DNA damage. The tail moment is defined as the product of the tail length and the fraction of total DNA in the tail (tail moment = tail length × % of DNA in the tail). Both tail length and tail intensity are measured automatically by image analysis software (Sasaki et al. 1997; Robbiano et al. 2004).

Single-cell gel electrophoresis assay, also known as the "comet" assay, is a fairly recent, rapid, simple, and reliable biochemical technique for evaluating DNA

Table 1 Serum biochemical parameters in control and different treated rat groups

Animal groups		Control	GSPE	Fish oil	Cisplatin	GSPE + Cisplatin	Fish oil + Cisplatin
Creatinine (mg/dl)	Mean ± SE	1.08 ± 0.043^{a}	1.08 ± 0.051^{a}	1.21 ± 0.078^{a}	6.26 ± 0.44^{b}	3.96 ± 0.27^{d}	4.54 ± 0.40^{d}
Urea (mg/dl)	Mean ± SE	63.45 ± 2.28^{a}	60.36 ± 3.04^{a}	62.25 ± 2.81^{a}	123.35 ± 2.73^{b}	109.13 ± 3.30^{d}	103.83 ± 3.06^{d}
Uric acid (mg/dl)	Mean ± SE	1.50 ± 0.15^{a}	1.71 ± 0.19^{a}	1.24 ± 0.08^{a}	3.02 ± 0.27^{b}	1.88 ± 0.12^{a}	1.99 ± 0.29^{a}
Na (mmol/L)	Mean ± SE	147.13 ± 2.73^{a}	168.61 ± 6.77^{b}	162.44 ± 7.85^{a,b}	84.72 ± 3.36^{c}	120.80 ± 6.30^{d}	120.64 ± 8.32^{d}
K (mmol/L)	Mean ± SE	6.44 ± 0.31^{a}	6.59 ± 0.35^{a}	5.95 ± 0.28^{a}	4.33 ± 0.41^{b}	6.05 ± 0.12^{a}	4.65 ± 0.25^{b}
Ca (mg/dl)	Mean ± SE	5.01 ± 0.14^{a}	5.25 ± 0.36^{a}	4.72 ± 0.17^{a}	3.35 ± 0.29^{b}	4.16 ± 0.21^{c}	4.01 ± 0.35^{d}

GSPE Grape seed proanthcyanidin extract

Data are expressed as mean ± SE of 6 rats. Within each row, means with different superscript (a, b, c, d, e) were significantly different at $p < 0.05$, whereas means superscripts with the same letters mean that there is no significant difference at ($p > 0.05$)

Table 2 Urine biochemical parameters in control and different treated rat groups

Animal groups		Control	GSPE	Fish oil	Cisplatin	GSPE + Cisplatin	Fish oil + Cisplatin
Creatinine (mg/dl)	Mean ± SE	38.24 ± 1.89a	39.49 ± 1.92^{a,b}	44.95 ± 3.08^{b}	16.72 ± 1.65^{c}	27.13 ± 1.74^{d}	33.66 ± 1.08^{d}
Urea (g/dl)	Mean ± SE	1.87 ± 0.09a	2.72 ± 0.30^{b}	2.80 ± 0.23^{b}	0.31 ± 0.04c	1.63 ± 0.08^{a}	0.97 ± 0.07^{d}
Uric acid (mg/dl)	Mean ± SE	124.61 ± 3.33a	126.06 ± 5.36^{a}	114.73 ± 5.36^{a}	34.24 ± 2.93^{b}	56.81 ± 2.59^{c}	58.13 ± 2.67^{c}
Na (mmol/L)	Mean ± SE	65.29 ± 2.82a	58.20 ± 2.92^{a}	44.57 ± 2.00^{b}	151.41 ± 3.75^{c}	105.41 ± 3.75^{d}	92.32 ± 8.32^{e}
K (mmol/L)	Mean ± SE	85.65 ± 4.86a	97.90 ± 2.83^{a}	98.48 ± 6.40^{a}	139.55 ± 4.15^{b}	115.46 ± 3.36^{d}	115.91 ± 5.51^{d}
Ca (mg/dl)	Mean ± SE	0.79 ± 0.11a	0.65 ± 0.19^{a}	0.84 ± 0.13^{a}	2.77 ± 0.14^{b}	2.10 ± 0.13^{d}	1.94 ± 0.17^{d}
crcl (ml/min)	Mean ± SE	0.24 ± 0.01a	0.29 ± 0.026^{b}	0.27 ± 0.022^{a}	0.01 ± 0.003^{c}	0.07 ± 0.013^{d}	0.06 ± 0.016^{c}

GSPE Grape seed proanthcyanidin extract

Data are expressed as mean ± SE of 6 rats. Within each row, means with different superscript (a, b, c, d, e) were significantly different at $p < 0.05$, whereas means superscripts with the same letters mean that there is no significant difference ($p > 0.05$)

damage in mammalian cells. Perspective tail length (μm) is the distance of DNA migration from the center of the body of the nuclear core and is used to evaluate the of DNA damage. The tail moment is defined as the product of the tail length and the fraction of total DNA in the tail (Tail moment = tail length × % of DNA in the tail). Both tail length and tail intensity are measured automatically by image analysis software (Sasaki et al. 1997; Robbiano et al. 2004).

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 (Waller and Duncan 1969) using SPSS program (Statistical Package for Social Science) version 11. 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

Cisplatin treatment to rats resulted in a significant increase in serum creatinine, urea and uric acid compared with control rat group, but excretion of them in urine were decreased (Tables 1, 2). Also, cisplatin affects reabsorption of some electrolytes which manifested itself by decreasing serum sodium and calcium and increasing serum potasium, while their levels increased in urine. Pretreatment with GSPE or FO significantly reduced CP-induced high levels in creatinine, urea and uric acid in serum and increased their excretion in urine. The levels of sodium and calcium were significantly increased in serum and potassium was decreased in the groups pretreated with GSPE and FO as compared to the groups treated with cisplatin, but their levels significantly decreased in urine.

As shown in Table 3 the levels of kidney GSH and SH group were significantly decreased in rats treated with cisplatin when compared with the control rats. Also, the activities of SOD, GST and Na–K ATPase were significantly decreased in the rats treated with cisplatin. In contrast, administration of GSPE or FO significantly increased renal GSH and SH group levels and SOD, GST and Na–K ATPase activities as compared to the cisplatin treated rats. Also, cisplatin caused oxidative stress through a significant increase in kidney MDA, H_2O_2 and NO as compared to control rats. However, pretreatment with GSPE or FO significantly decreased their levels in kidney compared to the cisplatin group.

Concerning the renal genotoxic potential of cisplatin using the comet essay there was a significant increase in the tail length of DNA, tail intensity (DNA %) and tail moment in the cisplatin treated rats compared to the control. On the other hand pretreatment with GSPE or FO significantly decreased DNA tail length, intensity and moment as compared to the cisplatin treated rats (Table 4 and Fig. 1).

Discussion

In the present study, the observed nephrotoxicity due to cisplatin treatment was manifested by marked increases in serum creatinine, urea and uric acid

Table 3 Renal antioxidant biomarkers and oxidative stress in control and different treated rat groups

Animal groups		Control	GSPE	Fish oil	Cisplatin	GSPE + Cisplatin	Fish oil + Cisplatin
GSH (mg/g wet tissue)	Mean ± SE	1.09 ± 0.06^{a}	1.02 ± 0.03^{a}	0.99 ± 0.04^{a}	0.78 ± 0.01^{b}	0.87 ± 0.03^{b}	0.87 ± 0.02^{b}
SH (mM/g wet tissue)	Mean ± SE	11.83 ± 0.52^{a}	12.99 ± 0.49^{a}	11.45 ± 0.67^{a}	8.59 ± 0.20^{b}	10.60 ± 0.55^{c}	9.93 ± 0.51^{b}
SOD (U/g wet tissue)	Mean ± SE	164.81 ± 2.37^{a}	160.45 ± 1.79^{a}	168.03 ± 8.50^{a}	105.59 ± 2.91^{b}	128.03 ± 3.06^{d}	110.91^{b} ± 5.56^{b}
GST (μmol/min/g tissue)	Mean ± SE	5.55 ± 0.27^{a}	5.79 ± 0.23^{a}	5.54 ± 0.21^{a}	3.98 ± 0.16^{b}	4.48 ± 0.13^{b,d}	4.78 ± 0.10^{d}
Na–K ATPase (μmol/Pi/min/gm wet tissue)	Mean ± SE	59.94 ± 2.31^{a}	59.84 ± 2.60^{a}	60.02 ± 2.49^{a}	27.09 ± 1.82^{b}	41.07 ± 2.65^{c}	33.37 ± 1.87^{b}
MDA (nmol/g wet tissue)	Mean ± SE	59.8 ± 3.17^{a}	53.74 ± 4.14^{a}	59.30 ± 3.02^{a}	198.49 ± 7.08^{b}	121.70 ± 6.10^{c}	139.08 ± 5.40^{d}
H_2O_2 (mM/g wet tissue)	Mean ± SE	3.26 ± 0.25^{a}	3.21 ± 0.21^{a}	4.04 ± 0.27^{a}	14.83 ± 0.44^{b}	9.21 ± 0.42^{d}	7.74 ± 0.30^{c}
NO (mmol/g wet tissue)	Mean ± SE	0.84 ± 0.01^{a}	0.77 ± 0.02^{a}	0.81 ± 0.04^{a}	3.48 ± 0.15^{b}	2.07 ± 0.21^{d}	2.12 ± 0.37^{c}

GSPE Grape seed proanthcyanidin extract

Data are expressed as mean ± SE of 6 rats. Within each row, means with different superscript (a, b, c, d, e) were significantly different at $p < 0.05$, whereas means superscripts with the same letters mean that there is no significant difference ($p > 0.05$)

Table 4 Renal DNA tail length, tail intensity (% of total genomic DNA found in the tail of the comets), and tail moment (tail length × tail intensity/100) measured by the comet essay in control and different treated rat groups

Animal groups		Control	GSPE	Fish oil	Cisplatin	GSPE + Cisplatin	Fish oil + Cisplatin
Tail length (μm)	Mean ± SE	2.43 ± 0.02^{a}	2.47 ± 0.02^{a}	2.46 ± 0.01^{a}	5.00 ± 0.07^{b}	3.66 ± 0.04^{c}	3.94 ± 0.01^{d}
Tail intensity (%)	Mean ± SE	2.37 ± 0.03^{a}	2.40 ± 0.02^{a}	2.49 ± 0.01^{b}	4.89 ± 0.01^{c}	3.72 ± 0.05^{d}	3.58 ± 0.02^{e}
Tail moment (UNIT)	Mean ± SE	0.058 ± 0.0008^{a}	0.059 ± 0.0007^{a}	0.062 ± 0.001^{a}	0.244 ± 0.004^{b}	0.135 ± 0.001^{c}	0.141 ± 0.001^{d}

Data are expressed as mean ± SE of 6 rats. Within each row, means with different superscript (a, b, c or d) were significantly different at $p < 0.05$, whereas means superscripts with the same letters mean that there is no significant difference ($p > 0.05$). Grape seed proanthcyanidin extract

accompanied by a decrease in urine creatinine, urea, uric acid and creatinine clearance. This may be due to the decrease in the glomerular filtration rate or may be secondary due to the increase of the reactive oxygen species (Noori and Mahboob 2010) which induce mesangial cells contraction, altering the filteration surface area and modifying the ultrafiltration coefficient factors that thereby decrease the glomerular filtration rate (Aydogan et al. 2008). These results are in accordance with previous studies (Fouad et al. 2008).

Also, the destruction of proximal and distal tubules observed in the cisplatin treated mice preceded the renal homodynamics, suppressed the reabsorption, increased vascular resistance and caused the elevation in BUN and creatinine levels (Daugaard and Abildgaard 1989). The current results also indicated that administration of cisplatin decreased the activity Na–K ATPase and induced disturbances in the electrolytes which are manifested by a decrease in the Na and Ca and K levels in the serum and an increase in their levels in urine. Daugaard and Abildgaard (1989) recorded that cisplatin causes decreased mitochondrial functions, decreased ATPase activity, altered cell cation content, and altered solute transport. Also, Arany and Safirstein (2003) reported that cispaltin treatment decreased sodium reabsorption in the proximal tubule and decreased sodium and water reabsorption in the distal tubule. These disturbances may be due to impairment in proximal and distal reabsorption and increased vascular resistance after cisplatin administration (Daugaard and Abildgaard 1989).

Hence, cisplatin treatment results in impaired tubular reabsorption and decreased urinary concentration. Moreover, the disruptive action of ROS induced by cisplatin may cause alterations in the membrane

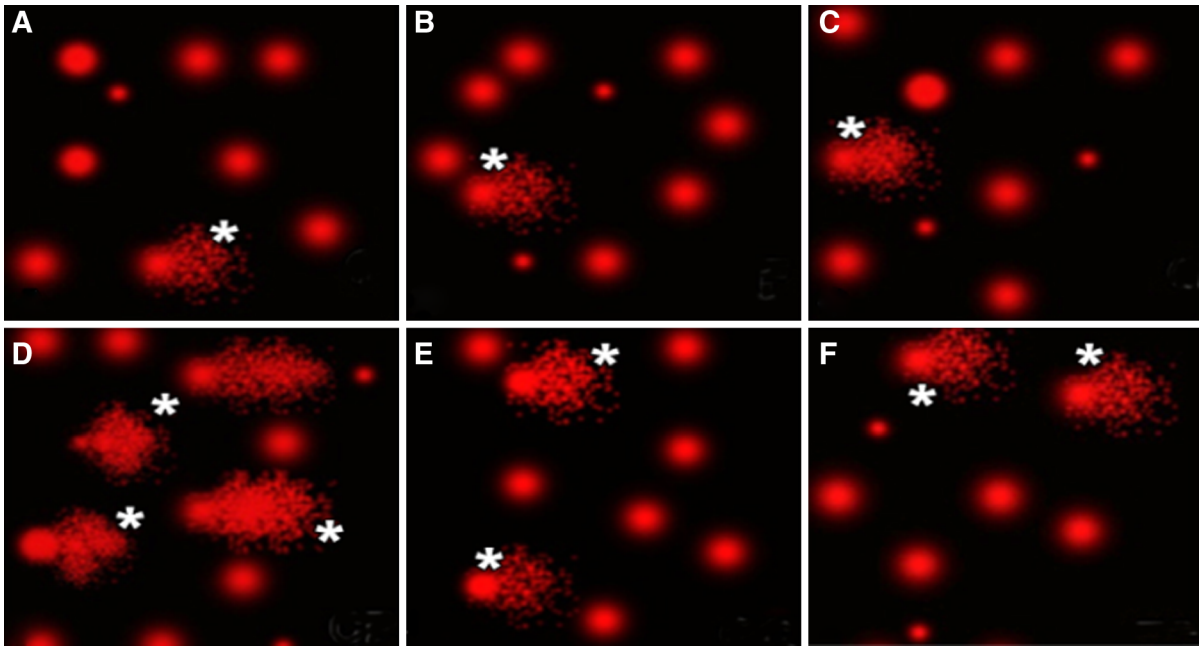

Fig. 1 Image from the comet essay on kidney cells: **a** Control group, **b** GSPE group and **c** FO group [No DNA damage], **d** Cisplatin group [Significant damage and movement of DNA (DNA tail significantly increased)], **e** GSPE + CP group and **f** FO + CP group [There is a significant decrease in DNA tail and movement]. * indicates DNA tail

structure and function, including fluidity, permeability, and activity of enzymes, channels, and receptors. It has also been shown that ROS could inhibit Na^+, K^+-ATPase and Ca^{2+}-ATPase of in vitro membrane ion transport systems (Lehotsky et al. 1999). However, pre-administration of GSPE or FO improved the kidney function through decreasing the concentration of serum creatinine, urea, uric acid and creatinine clearance, and improved the reabsorption of electrolytes such as Na, K and Ca and increasing the concentration of Na–K–ATPase (Priyamvada et al. 2008). This improvement in kidney function may be due to the antioxidant properties of proanthocyanidins and FO enriched in ω-3 fatty acids which enhanced the resistance to free radical attack generated by cisplatin (Guendez et al. 2005).

It was shown that ROS generated during normal cellular processes are immediately detoxified by endogenous antioxidants like GSH, catalase, GR, GRx, GST etc., but excessive ROS accumulation by cisplatin causes an antioxidant status imbalance leading to lipid peroxidation and GSH depletion (Kim et al. 2006). Also, the increased reactive oxygen species that attack the cell membrane lipids leads to increased tissue lipid peroxides as manifested by increased MDA level and overaccumulation of lipid peroxides in tissue causes overconsumption and depletion of GSH and inhibition of antioxidant enzymes (Noori and Mahboob 2010). The present study showed that administration of cisplatin decreased the activity of antioxidant enzymes, such as SOD and GST, depletion of, both, GSH and SH group and enhancement of MDA production in renal tissue, and increase of kidney H_2O_2 and NO. This may be due to increased activity of NADPH oxidase, xanthine oxidase and adenosine deaminase that leads to decline in the activity of the antioxidant enzymes (catalase, SOD and GPx), depletion of, both, the GSH and protein thiols and enhancement of MDA production in renal tissue (Ali et al. 2007; Chirino et al. 2008). A significant decline in antioxidant enzymes activity and increase in free radicals in experimental models as well as in subjects is typical during the regimens of commonly used chemotherapy, and this is particularly related to cisplatin treatment (Partibha et al. (2006). Ajith et al. (2007) also, reported a significant increase in kidney malondialdehyde (MDA) and a decrease in the activity of antioxidant enzymes due to cisplatin treatment. Similarly, Yuce et al. (2007) have also reported an increase in MDA and decrease in the

activity of antioxidant enzymes upon similar cisplatin treatments of rats. This impairment may be due to accumulation of cisplatin in the human kidney cells (Stewart et al. 1982), resulting in the enhanced production of ROS and the decrease in the antioxidant enzymes (Weijl et al. 1997).

Furthermore, GSH may be decreased due to its consumption in the detoxification of toxicants including chemotherapeutic drugs, metabolism of nutrients and regulation of various pathways to maintain cellular homeostasis (Wu et al. 2005). Thiols such as the sulfur of GSH will bind to the platinum molecule, replacing one of the chloride ions and preventing binding to other cellular nucleophiles (Berners-Price and Kuchel 1990). Increased intracellular GSH concentrations correlate with decreased platinum–DNA binding in freshly isolated peripheral blood mononuclear cells (Sadowitz et al. 2002). Different enzymatic and non-enzymatic reactions could be involved in GSH mediated scavenging of free radicals and other oxygen species. Therefore, lipid peroxidation due to cisplatin administration is a consequence of GSH depletion and impaired antioxidant enzyme activity. These observations support the evidence that part of the mechanism of nephrotoxicity in cisplatin-treated rats is related to depletion of the antioxidant system. The decrease in SOD activity could cause the initiation and propagation of lipid peroxidation in the cisplatin treated rats (Ajith et al. 2007). Cisplatin has been demonstrated to cause loss of copper and zinc in the kidneys (Badary et al. 2004). The reduction in the activity of the antiperoxidative enzymes (SOD and catalase) may be due to the increased generation of ROS such as superoxide and hydrogen peroxide, which in turn leads to the inhibition of the activity of these enzymes (Karthikeyan et al. 2007a, b).

Moreover, cisplatin is known to accumulate in mitochondria of renal epithelial cell; it is the primary target for cisplatin-induced oxidative stress resulting in loss of mitochondrial protein-SH, inhibition of calcium uptake and a reduction in the mitochondrial membrane potential (Saad et al. 2004). The reduction observed in the activitiy of GST accompanied with CP-induced injury might be due to decreased availability of its substrate, reduced glutathione (Karthikeyan et al. 2007a, b). GST catalyses the conjugation of reduced glutathione via the sulfhydryl group, to electrophilic centers on a wide variety of substrates. This activity is useful in the detoxification of endogenous compounds such as peroxidised lipids, as well as the metabolism of xenobiotics (Valavanidisa et al. 2006). In addition to that the increased content of MDA may result from an increase of hydroxyl radicals ($^{\cdot}OH$) which intiate lipid peroxidation in tissues (Celik and Tuluce 2006), and MDA is a major oxidation product of peroxidized polyunsaturated fatty acids and increased MDA content is an important product of lipid peroxidation. Glutathione S-transferase (GST) catalyses the conjugation of reduced glutathione via the sulfhydryl group, to electrophilic centers on a wide variety of substrates. This activity is useful in the detoxification of endogenous compounds such as peroxidised lipids, as well as the metabolism of xenobiotics (Valavanidisa et al. 2006). Excess NO reacts with superoxide anion to generate peroxynitrite radical that causes further cell damage by oxidizing and nitrating cellular macromolecules. Also, excess NO depletes intracellular GSH increasing the susceptibility to oxidative stress (Jung et al. 2009). NO reacts with free oxygen radicals and this lead to the formation of the most harmful peroxynitrite anion and this anion leads to lipid peroxidation, cellular damage and apoptosis (Groeneveld et al. 1996).

On the other hand, the administration of proanthocynidin (PAC) exhibited a clear protective action against the deleterious effects resulting from the administration of cisplatin on the antioxidant status. This observation is in harmony with Karthikeyan et al. (2007a, b); Yousef et al. (2009). The last author investigated that combined treatment of rats with proanthocyanidin and cisplatin increased the level of GSH and SOD activity in the liver compared to rats treated with cisplatin only.

This improvement in the antioxidant status may be due to its phenolic components (Cetin et al. 2008) which act as antioxidants not only because they are hydrogen and electron donators but also because they stabilize radical intermediators, thus preventing oxidation (Sun et al. 1999). In addition, procyanidin B1 has been assumed to be one of the most important radical scavenging in grape seed extracts (Guendez et al. 2005). Moreover, Hassan and Abdel-Aziz (2010) found that black berry juice (1.6 g/kg bw) increased GSH level, TAC and SOD activity in rats treated with sodium fluoride. The reason was probably due to the protection of sulfhydryl groups in glutathione from oxidative damages via the free radicals quenching

action of the di-OH (catechol) structure in the B ring of proanthocyanidin (Ishige et al. 2001).

Furthermore, the improvement in SOD activity may be due to the potential quenching of free radicals by proanthocyanidin (PAC) through the formation of resonance stabilized phenoxyl radicals, which significantly decrease the superoxide radical level (Rice-Evans et al. 1996). Also, grape seed proanthcyanidin extract can clear off free radicals and protect the over oxidative stress caused by free radicals (Spranger et al. 2008), and showed a generalized anti-peroxidative effect, which is effective against H_2O_2 (Roychowdhury et al. 2001).

Moreover, proanthocyanidin has been demonstrated to inhibit oxidative stress through modulation of metabolic functions, enhancement of detoxification pathways, and/or prevention of the interaction of xenobiotics with biological molecules (Bagchi et al. 2000; Hassan et al. 2013). In conclusion, the antioxidant function of PAC may work by increasing the activity of antioxidant enzymes of the body (Shan et al. 2010).

Also, the oral administration of fish oil (FO) to rats prevented cisplatin induced oxidative stress and suppression of antioxidant enzyme activity. This in accordance with Priyamvada et al. (2008) who reported that the feeding of FO diet to GM treated rats prevented GM-induced augmentation of lipid peroxidation (LPO) and suppression of antioxidant enzyme activity.

The protection against cisplatin effect by FO can be attributed to its intrinsic biochemical and natural antioxidant properties. Thus, it appears that FO enriched in ω-3 fatty acids enhanced resistance to free radical attack generated by cisplatin administration similarly as demonstrated in lupus nephritis and other pathologies (Chandrasekar and Fernandes 1994). Dietary FO supplementation has also been shown to strengthen antioxidant defense mechanisms in the plasma of normal rats (Erdogan et al. 2004). Recently, dietary FO has been shown to protect against acetaminophen (paracetamol)-induced hepatotoxicity (Speck and Lauterburgh 1991), ethanol-induced gastric mucosal injury in rats (Leung 1992), and a number of inflammatory diseases such as lupus nephritis (Chandrasekar and Fernandes 1994). Preliminary reports also showed partial protection by dietary FO ω-3 fatty acids against cyclosporine/GM-induced nephrotoxicity (Thakkar et al. 2000; Ali and Bashir 1994); however, the mechanism involved was not studied in detail. Our results thus support the rationale that ω-3 fatty acids enriched FO may be an effective dietary supplementation in the management of cisplatin nephrotoxicity and other pathologies in which antioxidant defense mechanisms are impaired. Moreover, the data including those from the comet assay showed that administration of cisplatin induced DNA damage through the increase in the migration of DNA (comet tail) compared with the control rats. The reported DNA damage due to CDDP treatment is in agreement with De Martinis and Bianchi (2001) and Satoh et al. (2003).

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 (Yin et al. 2007). The degradation of cellular DNA by endonucleases is an important component of renal tubular epithelial cell death induced by ischemia or nephrotoxins (Basnakian et al. 2005). This observation also holds true for cisplatin nephrotoxicity, in which necrotic cell death is encountered with higher doses whereas lower concentrations induce apoptosis (Razzaque 2007). Grape seed proanthocynidin extract administration prior to cisplatin decreased DNA damage in kidney cells due to scavenging free radicals and inhibition of oxidative tissue damage, DNA fragmentation, and subsequent apoptosis than all the antioxidant vitamins (Bagchi et al. 1997). This beneficial effect of GSPE or FO may be also due to scavenging activity especially for peroxyl and superoxide radicals (Ricardo da Silva et al. 1991; Ariga 2004). Furthermore, 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 (Ray et al. 1999).

In conclusion, the present data indicated that grape seed proanthocyanidin extract or fish oil may be effective to maximize the clinical use of cisplatin in the treatment of various malignancies without nephrotoxicity and other side effects. This may occur through intracellular pathways, involving suppression of oxidative stress and modulation of endogenous antioxidant defense mechanism However, the treatment by grape seed proanthocyanidin extract seems more effective than fish oil.

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