JOURNAL OF APPLIED TOXICOLOGY
J. Appl. Toxicol. **24**, 27–35 (2004)
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/jat.941

Role of Caffeic Acid Phenethyl Ester, an Active Component of Propolis, against Cisplatin-induced Nephrotoxicity in Rats

Süleyman Özen,[1] Ömer Akyol,[2]* Mustafa Iraz,[3] Sadık Söğüt,[4] Fikret Özuğurlu,[5] Hüseyin Özyurt,[5] Ersan Odacı[6] and Zeki Yıldırım[7]

[1] Department of Pathology, Yüzüncü Yıl University Medical School, Van, Turkey
[2] Department of Biochemistry, Inonu University Medical School, Malatya, Turkey
[3] Department of Pharmacology, Inonu University Medical School, Malatya, Turkey
[4] Department of Biochemistry, Mustafa Kemal University Medical School, Hatay, Turkey
[5] Department of Biochemistry, Gaziosmanpaşa University Medical School, Tokat, Turkey
[6] Department of Histology and Embryology, Ondokuz Mayıs University Medical School, Samsun, Turkey
[7] Department of Pulmonary, Inonu University Medical School, Malatya, Turkey

Key words: caffeic acid phenethyl ester; cisplatin; nephrotoxicity; nitric oxide; catalase; superoxide dismutase; glutathione peroxidase; malondialdehyde; myeloperoxidase.

We have investigated the effect of caffeic acid phenethyl ester (CAPE) on cisplatin-induced nephrotoxicity in rats. Administration of a single dose of cisplatin resulted in the elevation of blood urea nitrogen and creatinine in serum, as well as nitric oxide in kidney tissue of rats. Cisplatin also caused reduction of catalase ($P < 0.0001$), superoxide dismutase ($P = 0.149$) and glutathrone peroxidase ($P < 0.0001$) activities in kidney tissue. Although cisplatin caused elevation in malondialdehyde levels and myeloperoxidase activities in kidney tissue, they were not statistically significant. Caffeic acid phenethyl ester was found to be protective against cisplatin-induced antioxidant enzyme reductions. Treatment with free-radical scavenger CAPE attenuated the increase in plasma blood urea nitrogen and kidney nitric oxide levels, and showed histopathological protection against cisplatin-induced acute renal failure. Extensive epithelial cell vacuolization, swelling, desquamation and necrosis were observed in the kidney of the cisplatin-treated rat. There were also larger tubular lumens in cisplatin-treated rats than those of the control and the CAPE groups. Caffeic acid phenethyl ester caused a marked reduction in the extent of tubular damage. It is concluded that administration of cisplatin imposes an oxidative stress to renal tissue and CAPE confers protection against the oxidative damage associated with cisplatin. This mechanism may be attributed to its free-oxygen-radical scavenging activity. Copyright © 2004 John Wiley & Sons, Ltd.

INTRODUCTION

Cisplatin (cis-diamminedichloroplatinum II) is one of the most important antineoplastic agents used for several types of solid tumours (Baliga *et al.*, 1998). The most common adverse effect limiting the use of cisplatin is nephrotoxicity, which develops primarily in the proximal tubule. Although the mechanism underlying this nephrotoxicity is still not clear, reactive oxygen species (ROS) have been implicated extensively in the toxicity (Sugihara and Gemba, 1986). Administration of cisplatin causes an increase in lipid peroxidation products and a decrease in the activity of enzymes protecting lipid peroxidation in the kidney (Hara *et al.*, 2001). On the other hand, decreased levels of reduced glutathione indicate the formation of ROS in cisplatin nephrotoxicity (Huang *et al.*, 1997). Iron is one of the unique elements that have redox potential. Injuries mediated by ROS in the kidney have been suggested to be connected with iron (Herbert *et al.*, 1994), and iron chelators were found to protect against cisplatin nephrotoxicity (Baliga *et al.*, 1998). Therefore, antioxidant enzymes and compounds such as superoxide dismutase, glutathione peroxidase, glutathione, selenium, flavonoids and diethyldihtiocarbamates have been tested for protection against cisplatin-induced nephrotoxicity by using them prior to administration of cisplatin in experimental animals (Anand and Bashey, 1993).

Clinically, several agents such as amifostine have been approved as cytoprotectants to reduce the side-effects of cisplatin (Orditura *et al.*, 1999). Although amifostine was introduced into clinical usage, the prices of the treatment regimen with cisplatin plus amifostine are very expensive when compared with cisplatin alone. Thus, it is not judged to be a cost-effective agent for nephroprotection in chemotherapeutic regimens. Caffeic acid phenethyl ester (CAPE) is an active component of honeybee propolis extracts and has been used for many years as a folk medicine. It has antiinflammatory, immunomodulatory, antiproliferative and antioxidant properties and has been shown to inhibit lipooxygenase activities as well as suppress lipid peroxidation (Sud'ina *et al.*, 1993; Hepsen *et al.*, 1997; Hepsen *et al.*, 1999; Ilhan *et al.*, 1999; Koltuksuz

*Correspondence to: Ö. Akyol, Inonu University Medical School, Department of Biochemistry, Turgut Ozal Medical Center, 44069 Malatya, Turkey.
E-mail: oakyol@inonu.edu.tr

Received 18 February 2003
Revised 26 May 2003
Accepted 27 May 2003

et al., 1999, 2000). In addition, CAPE was shown to inhibit the growth of different types of transformed cells (Grunberger *et al.*, 1988; Khayyal *et al.*, 1993) and can also inhibit phorbol ester-induced hydrogen peroxide production and tumour promotion (Frenkel *et al.*, 1993). At a concentration of 10 μM, CAPE completely blocks the production of ROS in human neutrophils and the xanthine/xanthine oxidase system (Sud'ina *et al.*, 1993).

The aim of the present study was therefore to investigate whether treatment of rats with CAPE prior to cisplatin administration prevents cisplatin-induced nephrotoxicity. For this purpose, we have investigated the histopathological effects of cisplatin and the possible protective effect of CAPE on tissue damage of rat kidney. We have also examined various oxidative/antioxidative markers such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), nitric oxide (NO) and myeloperoxidase (MPO) in the kidney tissue of rats subjected to cisplatin-induced toxicity with or without pretreatment with CAPE.

MATERIALS AND METHODS

Animals

Female Wistar albino rats weighing 200–250 g were purchased from the Experimental Research Centre, University of Erciyes (Kayseri, Turkey) and housed in individual cages in the animal laboratory of Inonu University. They were acclimatized for 1 week prior to experimentation, during which time they had free access to tapwater and normal rat food. The animals were fed with a standard diet, kept on a physiological day/night rhythm and maintained in an ambient temperature of 24 °C during the experimental procedures. The experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (DHEW Publication No. 85–23, 1985). Rats were divided into three groups: rats given cisplatin as a cisplatin-induced acute renal failure model ($n = 8$); rats given cisplatin plus CAPE ($n = 8$); and rats given isotonic saline solution alone as a control group ($n = 6$).

Cisplatin-induced acute renal failure

A cisplatin model of acute renal failure was induced in rats as described by Zhang *et al.* (1999) and Yildirim *et al.* (2003). The animals received an intraperitoneal injection of cisplatin (Cisplatinum Ebewe, 0.5 mg ml^{-1}) at a dose of 7 mg kg^{-1} body wt and were sacrificed 5 days after cisplatin injection. Blood and kidneys were obtained for the various measurements after sacrificing the rats by decapitation. Renal impairment was assessed by blood urea nitrogen (BUN) and plasma creatinine levels, as well as kidney histology. The BUN and creatinine were determined by the use of Sigma diagnostic kits (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany). Kidney histology was performed as described in the following section.

Application of CAPE in cisplatin model of acute renal failure

The CAPE was synthesized by the standard method of Grunberger (Grunberger *et al.*, 1988) and administered intraperitoneally once a day at a dose of 10 μmol kg^{-1} body wt. The first dose of CAPE was given 24 h prior to cisplatin injection and continued until sacrifice.

Control rats

Isotonic saline solution (an equal volume of cisplatin) was administered by intraperitoneal injection.

Kidney histology

The kidneys were sectioned, fixed in 10% formalin, dehydrated and embedded in paraffin. Tissues were then sectioned at 3 μm, stained with haematoxylin and eosin (H&E) and examined for tubular necrosis and dilatation. The slides were coded and semiquantitative analysis of the kidney sections was performed without knowledge of the treatment protocol, as described previously (Baliga *et al.*, 1998). The changes seen were limited to the tubulointerstitial areas and graded as follows: 0, normal; I, areas of tubular epithelial cell swelling, vacuolar degeneration, necrosis, and desquamation involving <25% of cortical tubules; II, similar changes involving >25% but <50% of cortical tubules; III, similar changes involving >50% but <75% of cortical tubules; IV, similar changes involving >75% of cortical tubules.

Enzymes and chemicals

Xanthine oxidase, xanthine, nitroblue tetrazolium (NBT), thiobarbituric acid, 1,1,3,3-tetramethoxy propane, NADPH, glutathione (reduced form), hexadecyltrimethyl ammonium bromide, *N*-naphthyl ethylene diamine dihydrochloride, sodium azide, sulphanilamide, 4-aminoantipyrine, glycine and glutathione reductase were purchased from Sigma Chemical Co. (St Louis, MO, USA) and $CuCl_2$, bovine serum albumin, H_2O_2, EDTA, Na_2CO_3, $(NH_4)_2SO_4$, chlorofom, ethanol, NaCl, zinc sulphate, cadmium granules, cupric sulphate pentahydrate, phenol, HCl, NaOH, potassium nitrite, KH_2PO_4 and $Na_2HPO_{4\cdot2}H_2O$ were from Merck (Germany).

Preparation of kidney tissue for biochemical measurements

All tissues were washed twice with cold saline solution, placed into glass bottles, labelled, and stored in deep freezer (–30 °C) until processed (maximum of 10 h). Tissues (after cutting into small pieces with scissors) were homogenized in four volumes of ice-cold TRIS·HCl buffer (50 mM, pH 7.4) using a glass Teflon homogenizer (Tempest Virtishear, Model 278069; The Virtis Company, Gardiner, NY) for 2 min at 5000 rpm after weighed. Nitric oxide and MDA measurements were made at this stage. The homogenate was then centrifuged at 5000 *g* for 60 min to remove debris. Clear upper supernatant fluid was taken and CAT, GSH-Px activities and protein concentration were carried out at this stage. The supernatant solution was then extracted with an equal volume of ethanol–chloroform mixture (5 : 3, v/v). After centrifugation at 5000 *g* for 30 min, the clear upper layer (the ethanol phase) was taken and used in the SOD activity and protein assays. Some of the kidney tissue was homogenized in 5 volumes of 0.5% hexadecyltrimethyl ammonium bromide at 8000 *g* for 2 min for MPO activity

analysis. All preparation procedures were performed at +4 °C.

Determination of MDA

Kidney MDA levels were determined by Wasowicz's method (Wasowicz *et al.*, 1993) based on the reaction of MDA with thiobarbituric acid at 95–100 °C. Fluorescence intensity was measured in the upper *n*-butanol phase by a fluorescence spectrophotometer (Hitachi, Model F-4010) with adjusted excitation at 525 nm and emission at 547 nm. Arbitrary values obtained were compared with a series of standard solutions (1,1,3,3-tetramethoxypropane). Results were expressed as nmol g^{-1} wet tissue.

Determination of NO

Because NO measurement is very difficult in biological specimens, tissue nitrite (NO_2^-) and nitrate (NO_3^-) were estimated as an index of NO production. The method for kidney nitrite and nitrate levels was based on the Griess reaction (Cortas and Wakid, 1990). Samples initially were deproteinized with Somogyi reagent. Total nitrite (nitrite + nitrate) was measured after conversion (reduction) of nitrate to nitrite by copporized cadmium granules using a spectrophotometer at 545 nm (Ultraspec Plus, Pharmacia LKB Biochrom Ltd, UK). A standard curve was established with a set of serial dilutions (10^{-8}–10^{-3} mol l^{-1}) of sodium nitrite. Linear regression was done by using the peak area from nitrite standard. The resulting equation then was used to calculate the unknown sample concentrations. Results were expressed as μmol g^{-1} wet tissue.

Determination of SOD activity

Total (Cu–Zn and Mn) SOD (EC 1.15.1.1) activity was determined according to the method of Sun *et al.* (1988), with a slight modification by Durak *et al.* (1993). The principle of the method is based on the inhibition of NBT reduction by the xanthine–xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the supernatant after 1.0 ml of ethanol–chloroform mixture (5 : 3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. The SOD activity was also expressed as U g^{-1} protein.

Determination of CAT activity

Catalase (EC 1.11.1.6) activity was determined according to Aebi's method (1974). The principle of the method was based on determination of the rate constant k (s^{-1}) of the H_2O_2 decomposition rate at 240 nm. Results were expressed as k g^{-1} protein.

Determination of GSH-Px activity

The GSH-Px (EC 1.6.4.2) activity was measured by the method of Paglia and Valentine (1967). The enzymatic reaction in the tube that contained NADPH, reduced glutathione (GSH), sodium azide and glutathione reductase was initiated by the addition of H_2O_2 and the change in absorbance at 340 nm was monitored by a spectrophotometer. Activity was expressed as U g^{-1} protein.

Determination of MPO activity

The MPO (EC 1.11.1.7) activity was determined using a 4-aminoantipyrine–phenol solution as the substrate for MPO-mediated oxidation by H_2O_2, and changes in absorbance at 510 nm (A_{510}) were recorded (Wei and Frenkel, 1993). One unit of MPO activity is defined as that which degrades 1 μmol of H_2O_2 per minute at 25 °C. Data were presented as mU g^{-1} protein.

Protein determinations

Protein assays were made by the method of Lowry *et al.* (1951).

Statistical analysis

Data are expressed as means ± standard deviation. The one-way analysis of variance (ANOVA) and *post hoc* multiple comparison tests (least-squares difference) were performed on the data of biochemical variables to examine the differences among groups. The semiquantitative analyses of histological grades of the histological samples were also made using one-way ANOVA analysis of variance and *post hoc* multiple comparison tests (least-squares difference). All analyses were made using the SPSS statistical software package; $P < 0.05$ was considered statistically significant.

RESULTS

All the rats survived the experimental period until sacrifice. Cisplatin administration at a dose of 7 mg kg^{-1} body wt resulted in acute renal failure similar to a previous study (Zhang *et al.*, 1999). Table 1 shows significant elevations in the mean plasma creatinine and BUN levels after treatment with cisplatin alone compared with the control ($P < 0.001$ for BUN). Caffeic acid phenethyl ester attenuated increases in serum BUN levels but did not return to the control level in a statistically significant manner ($P = 0.051$ compared with the cisplatin group). Similarly, NO and MDA levels in the kidney tissue were increased by the cisplatin management ($P < 0.0001$ and $P = 0.094$, respectively). Caffeic acid phenethyl ester prevented these increments at a statistically significant level for NO ($P < 0.0001$) and a non-statistically significant level for MDA ($P = 0.224$) (Figs 1 and 2). In the control group, the mean kidney tissue activities of CAT, SOD and GSH-Px were found to be

Table 1—Serum BUN and creatinine levels in rats subjected to cisplatin-induced nephrotoxicity

	BUN (mg dl^{-1})	Creatinine (mg dl^{-1})
1. Control (n = 6)	19.5 ± 1.18	0.408 ± 0.020
2. Cisplatin (n = 8)	82.5 ± 15.56	1.312 ± 0.512
3. Cisplatin + CAPE (n = 8)	53.37 ± 13.39	1.187 ± 0.171
P values		
1–2	0.001	NS
1–3	0.037	NS
2–3	0.051	NS

Values are expressed as mean ± standard error of mean.

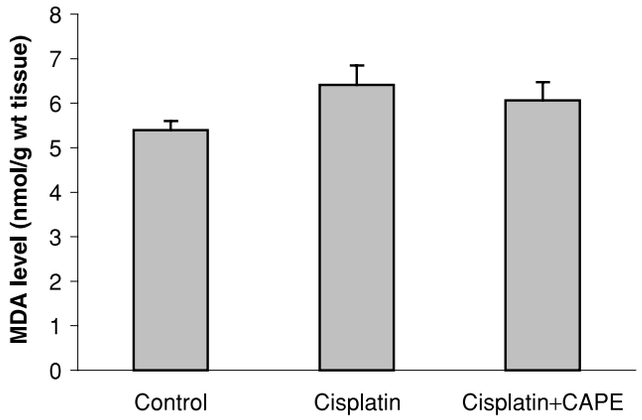

Figure 1. Malondialdehyde (MDA) levels in kidney tissues of rats divided into control, cisplatin and cisplatin + CAPE subgroups (CAPE, caffeic acid phenethyl ester; there are no significant differences between the groups).

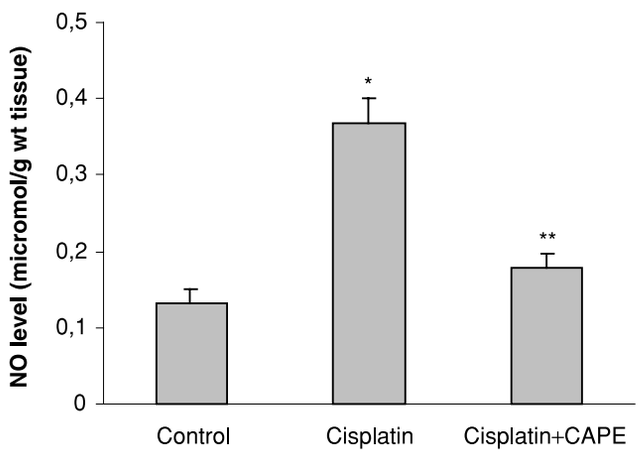

Figure 2. Nitric oxide (NO) levels in kidney tissues of rats divided into control, cisplatin and cisplatin + CAPE subgroups (CAPE, caffeic acid phenethyl ester; * $P < 0.0001$ compared with control group; ** $P < 0.0001$ compared with cisplatin group).

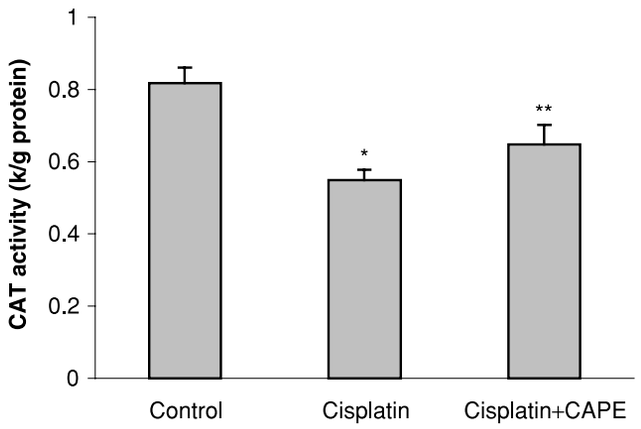

Figure 3. Catalase (CAT) activities in kidney tissues of rats divided into control, cisplatin and cisplatin + CAPE subgroups (CAPE, caffeic acid phenethyl ester; * $P < 0.0001$ and ** $P < 0.014$ compared with control group).

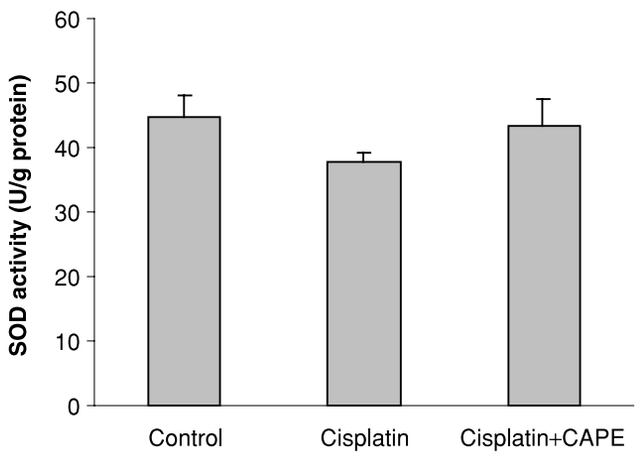

Figure 4. Superoxide dismutase (SOD) activities in kidney tissues of rats divided into control, cisplatin and cisplatin + CAPE subgroups (CAPE, caffeic acid phenethyl ester; there are no significant differences between the groups).

0.818 ± 0.043 k g^{-1} protein, 44.8 ± 3.28 U g^{-1} protein, and 3.334 ± 0.172 U g^{-1} protein, respectively (Figs 3–5). In the cisplatin group, these enzyme activities were decreased in the kidney tissues ($P < 0.0001$ for CAT; $P = 0.149$ for SOD; $P < 0.0001$ for GSH-Px). There were increases in CAT, SOD and GSH-Px activities in the cisplatin + CAPE group when compared with that of the cisplatin group but the differences between the two groups were not statistically significant ($P = 0.113$ for CAT; $P = 0.224$ for SOD; $P < 0.009$ for GSH-Px). Finally, although a moderate elevation in the cisplatin group compared with the control group ($P = 0.258$) was seen in Fig. 6, there were no statistically significant changes in tissue MPO activity in cisplatin and cisplatin + CAPE groups compared with each other.

As shown in Fig. 7, control rats showed no abnormality for the kidney histology. Cisplatin administration to the rats revealed a remarkable proximal tubular necrosis with extensive epithelial vacuolization, swelling and tubular dilatation compared with the controls and CAPE-treated rats. The wall height of proximal tubules in control kidneys is higher than both the cisplatin and CAPE groups. The glomeruli appeared normal in all the groups studied. The

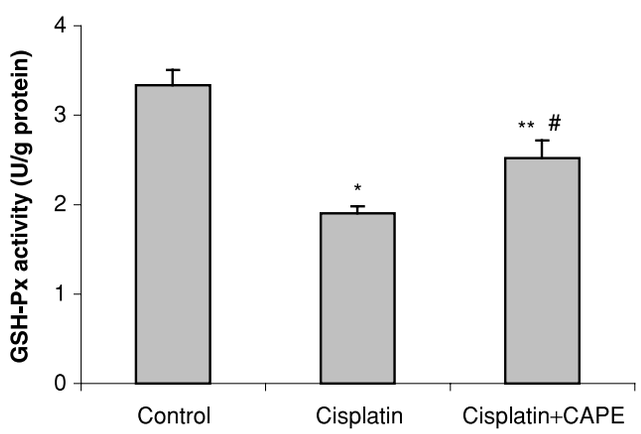

Figure 5. Glutathione peroxidase (GSH-Px) activities in kidney tissues of rats divided into control, cisplatin and cisplatin + CAPE subgroups (CAPE, caffeic acid phenethyl ester; * $P < 0.0001$ and ** $P < 0.002$ compared with control group; # $P < 0.009$ compared with cisplatin group).

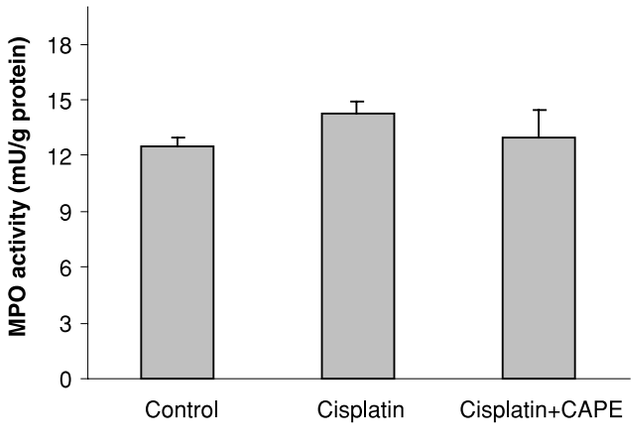

Figure 6. Myeloperoxidase (MPO) activities in kidney tissues of rats divided into control, cisplatin and cisplatin + CAPE subgroups (CAPE, caffeic acid phenethyl ester; there are no significant differences between the groups).

Table 2—The distribution of individual histological grades of kidney damage in controls and study groups

		Histological grades				
	n	0	I	II	III	IV
Control	6	5	1			
Cisplatin	8				5	3
Cisplatin + CAPE	8	2	4	2		

Table 3—The statistical means of histological grade of kidney damage in controls and study groups

Group	Grade of damage
Control ($n = 6$)	0.167 ± 0.408
Cisplatin ($n = 8$)	3.375 ± 0.517*
Cisplatin + CAPE ($n = 8$)	1.000 ± 0.756†,‡

Values are expressed as mean ± standard deviation; * $P < 0.0001$ compared with the control group; † $P < 0.0001$ compared with the cisplatin group; ‡ $P < 0.018$ compared with the control group.

histological changes, graded as described in the methods, are summarized in Tables 2 and 3. Compared with the control rats, there were statistically significant changes in semiquantitative histological analysis of the kidney of the cisplatin and cisplatin + CAPE groups.

DISCUSSION

This study showed that cisplatin causes cytotoxicity in rats, as measured by BUN, creatinine and NO elevations and by CAT, SOD and GSH-Px activity depletions. The possible protective properties of CAPE (which is known as an antioxidant agent) against cisplatin-induced nephrotoxicity were studied in this experimental study. The involvement of hydrogen peroxide in cisplatin-induced nephrotoxicity in outer medullary cortical tubule cells was demonstrated in a previous study (Tsutsumishita *et al.*, 1998). Thus, the enzymes that detoxify hydrogen peroxide are partiality

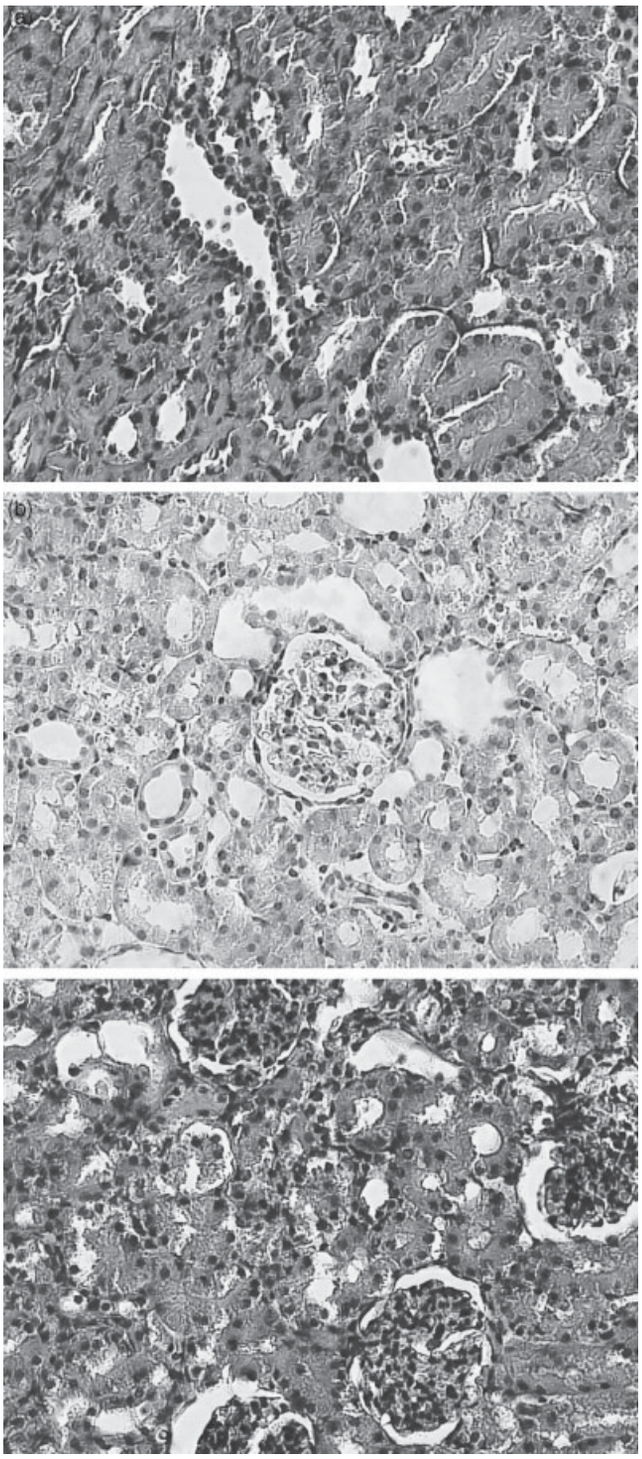

Figure 7. Three micrographs taken from the cortex of kidney in control (A), cisplatin (B) and cisplatin + CAPE (C) groups. Control rats show no abnormality (A). Extensive epithelial cell vacuolization, swelling, desquamation and necrosis are clearly observed in the kidney of the cisplatin-treated rat (B). The larger tubular lumens in cisplatin-treated rats than those of the control and the CAPE groups show extensive necrosis (B). The wall height (between two opposite-printing arrow heads) of proximal tubules in the control kidney is higher than both the cisplatin and CAPE groups (A). The free-radical scavenger CAPE shows a marked reduction in the extent of tubular damage (C).

important in cisplatin toxicity (Fig. 8). The balance between the production and catabolism of oxidants by cells and tissues is critical for maintenance of the biological integrity of the tissues (Fadillioglu *et al.*, 2003). Cisplatin

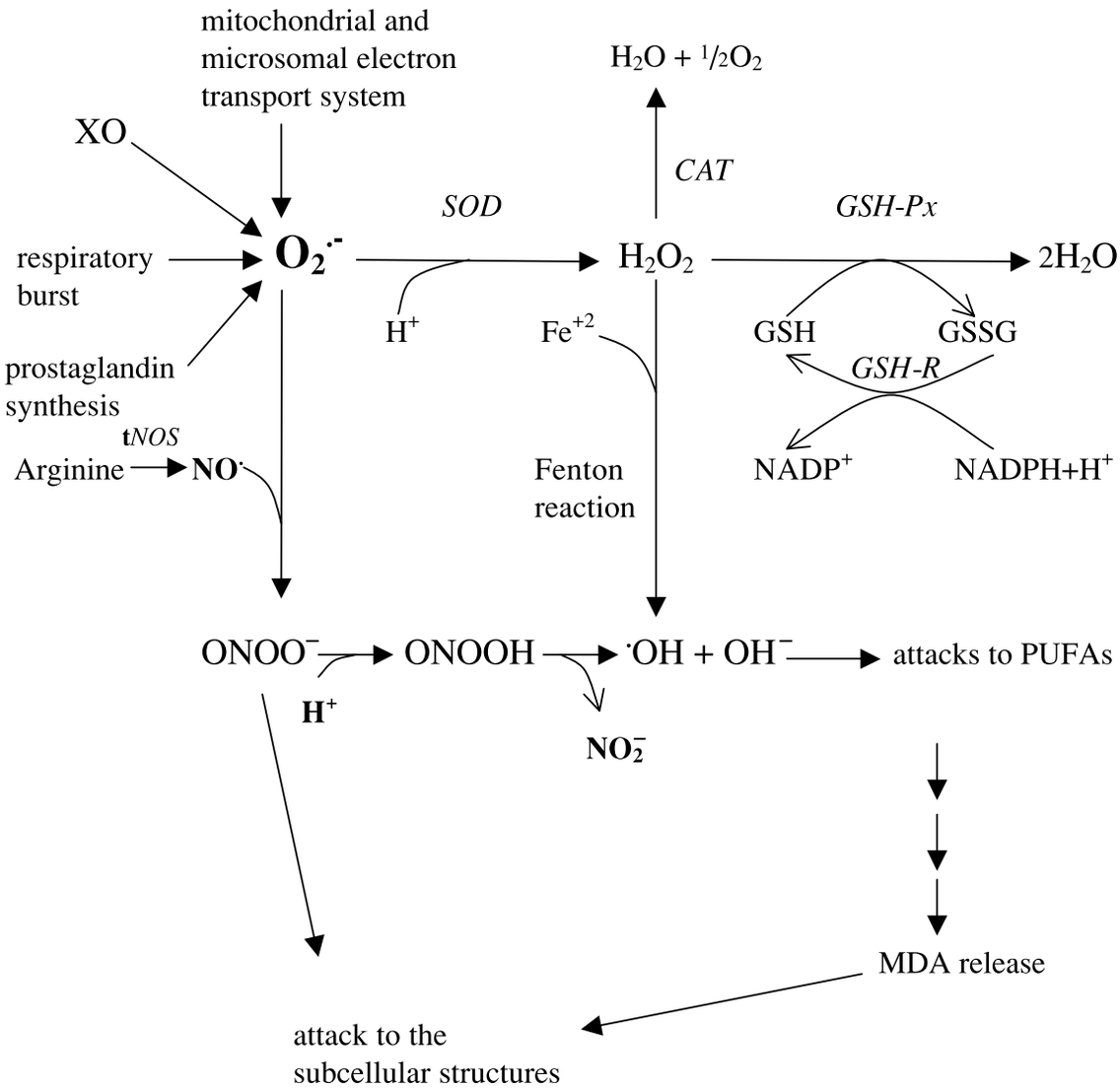

Figure 8. Schematic representation of oxidant and enzymatic antioxidant mechanisms: $O_2^{\cdot-}$, superoxide anion radical; O_2, molecular oxygen; H^+, hydrogen ion, proton; H_2O, water; SOD, superoxide dismutase; CAT, catalase; H_2O_2, hydrogen peroxide; GSH-Px, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; GSH-R, glutathione reductase; NADPH + H^+, reduced nicotinamide adenine dinucleotide phosphate; $NADP^+$, oxidized nicotinamide adenine dinucleotide phosphate; Fe^{2+}, ferrous iron; OH^-, hydroxyl ion; $^{\cdot}OH$, hydroxyl radical (the most potent free oxygen radical); tNOS, total nitric oxide synthases; NO, nitric oxide radical; $ONOO^-$, peroxynitrite; MDA, malondialdehyde (the last product of lipid peroxidation of membrane phospholipids); NO_2^-, nitrite; PUFA, polyunsaturated fatty acid; XO, xanthine oxidase.

treatment may cause both depletion of antioxidants and elevation of ROS in the kidney tissue. Kidney tissue contains antioxidants that prevent damage from excessive oxygen metabolites. They act either by decomposing peroxide or trapping the free radicals. Both CAT and GSH-Px together convert hydrogen peroxide to water. The activities of CAT and GSH-Px enzymes in kidney tissues from rats were found to be decreased significantly after cisplatin administration. The reduction of GSH-Px, CAT and SOD activities in renal tissue of animals treated with cisplatin alone was prevented by co-treatment with CAPE. We have reported previously in ischaemia–reperfusion models of several organs that CAPE caused an elevation in CAT (Ilhan *et al.*, 1999; Koltuksuz *et al.*, 1999) and SOD activities (Koltuksuz *et al.*, 1999). The exact mechanism of CAPE on the enzyme activities is not known yet. However, it can be speculated that CAPE may affect the transcriptional and/or translational pathways of these antioxidant enzymes.

Acute renal injury from ischaemia–reperfusion and cisplatin are known to share many common features, including similar histological changes, gene induction and gene repression (Safirstein, 1999). Moreover, some researchers suggested that the toxic effect of cisplatin resembles renal ischaemia (Kelly *et al.*, 1999). Our previous studies on renal ischaemia–reperfusion injuries and the present study supported this hypothesis (Irmak *et al.*, 2001; Ozyurt *et al.*, 2001). Therefore, the agents that have a protective effect for ischaemia–reperfusion of kidney can be used safely for cisplatin-induced nephrotoxicity. Like some other antioxidant compounds (i.e. vitamins C and E), CAPE shows antioxidant activity with its two hydroxyl groups located in one of the ring structures (Fig. 9). We showed previously the protective effect of CAPE both histologically and biochemically in ischaemia–reperfusion injury of rat kidney (Irmak *et al.*, 2001).

Non-enzymatic lipid peroxidation is an example of a ROS-associated process through which oxidative stress

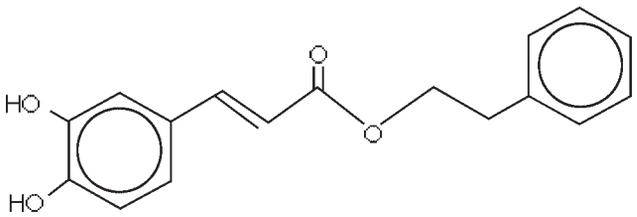

Figure 9. The structure of caffeic acid phenethyl ester. Note that the two hydroxyl groups located in the first ring show antioxidant activity. The antioxidant activity of the compound depends not only on the hydroxyl groups or catechol rings but also on the partition coefficient or hydrophobicity of CAPE.

promotes cellular damage. Tissue MDA content is a reliable marker for the breakdown of the major chain reactions, leading to significant oxidation of polyunsaturated fatty acids such as linoleic and linolenic acid. Contrary to the other studies on cisplatin nephrotoxicity, we did not find statistically significant increases in MDA content although there was a slight increase in tissue MDA. We have no idea on this contrary result but we may speculate that MDA released by the lipid peroxidation in kidney tissue might be metabolized immediately by a mitochondrial MDA-metabolizing enzyme (low specific aldehyde dehydrogenase, ALDH) and thus MDA increase was not seen. Oxidation of aldehydes to carbonylic acids by ALDH might be an important route of metabolism of aldehydes. Townsend *et al.* demonstrated that expression of class 3 ALDH, but not class 1 ALDH, can be an important determinant of cellular resistance to toxicity mediated by aldehydes of intermediate chain length that are produced during lipid peroxidation (Townsend *et al.*, 2001).

In the present study, the NO level was found to be increased in the kidney after cisplatin application alone. Nitric oxide is a free radical produced enzymatically by NOS in biological systems from the guanidine group of L-arginine. Its large spectrum of biological effects is achieved through chemical interactions with different targets, including oxygen, superoxide and other ROS, transition metals and thiols. It may react with thiol (-SH) groups of amino acids and proteins and form relatively stable nitroso-thiols (-S-NO). Superoxide anions (O_2^-) have been reported to react with NO to produce peroxynitrite anions ($ONOO^-$) that can decompose to form nitrogen dioxide (NO_2) and hydroxyl radical ($^{\cdot}OH$). Peroxynitrite and its further products have been linked to several interactions that may contribute to cellular injury, including lipid peroxidation, nitrosylation of some molecules and interactions with different metals that have redox potential, such as iron and copper (Kirkebo'en and Strand, 1999). Peroxynitrite has been hypothesized to play an important role in renal ischaemia–reperfusion injury (Walker *et al.*, 2000).

Peroxynitrite generated in the tubular epithelium during ischaemia–reperfusion or cisplatin administration has the potential to impair the adhesion properties of tubular cells, which then may contribute to the tubular obstruction in acute renal failure (Wangsiripaisan *et al.*, 1999). On the other hand, because the breakdown of superoxide anions by NO could participate in the decrease of ROS levels, NO can be accepted as a potential protecting agent in kidney injuries. Various tools have been developed that enable improved characterization of the action of NO. These tools include inhibitors of NOS (such as L-NAME), NO scavengers (such as methylene blue) or NO donors (such as nitroglycerine or arginine). Some inhibition studies confirm that NO may be detrimental. For example, L-arginine supplementation was deleterious in both *in vivo* and *in vitro* renal ischaemia, suggesting that injurious effects had overcome the beneficial effects during excess NO exposure (Tome *et al.*, 1999). Recently, it has been found that inducible NOS inhibitors L-N6-(1-iminoethyl)lysine and aminoethyl-isothiourea reduced the renal dysfunction and injury associated with ischaemia–reperfusion of the kidney via inhibition of inducible NOS activity and subsequent reduction of NO and also peroxynitrite generation (Chatterjee *et al.*, 2002). Selective inhibition, depletion or deletion of inducible NOS, not affecting constitutive isoforms of NOS, clearly demonstrates its renoprotective effect against ischaemia. This effect is due, at least in part, to the rescue of tubular epithelial cells from injury induced by a product of inducible NOS (Goligorsky *et al.*, 2002). Caffeic acid phenethyl ester was shown to inhibit NO production and protect renal tissues against cisplatin-induced toxicity in this experimental study.

Nuclear transcription factor kappa B (NF-κB) plays an important role in various responses, leading to host defence through rapid induction of gene expression. It controls the expression of various inflammatory cytokines, the major histocompatibility complex genes and adhesion molecules involved in tumour metastasis. Dysregulation of NF-κB and its dependent genes has been associated with various pathological conditions, including toxic shock, graft versus host reaction, acute inflammatory conditions, acute-phase response and cancer. The activation of NF-κB promotes inflammation in rats (Muller *et al.*, 2000). The activation of NF-κB proteins (which exist in cytoplasm as inactive proteins) is induced by many factors, such as inflammatory cytokines (e.g. interleukin 1, tumour necrosis factor), bacterial products, protein synthesis inhibitors, oxidative stress (H_2O_2) and phorbol esters (Grilli *et al.*, 1993). Thus, the agents that can downregulate the activation of NF-κB have potential for therapeutic interventions. Caffeic acid phenethyl ester has been shown to be a pharmacologically safe compound with known antiinflammatory, immunomodulatory, anticarcinogenic and antioxidant properties and NF-κB inhibition is involved in most of these activities. Caffeic acid phenethyl ester has been shown to block specifically and completely the activation of NF-κB induced by a wide variety of inflammatory agents, including tumour necrosis factor and hydrogen peroxide (Natarajan *et al.*, 1996; Orban *et al.*, 2000). The inhibition of NF-κB activation by CAPE may be ascribed to its known ability to alter the redox state of the cell (Chiao *et al.*, 1995). According to our study results, we may speculate that CAPE may inhibit the activation of NF-κB in cisplatin-induced renal damage model.

Taken together, although the preventive effect of CAPE on the plasma BUN and creatinine levels is not evident, we think that CAPE may be a promising drug against cisplatin-induced renal failure and oxidative renal damage. Further studies are warranted to define the exact mechanism of the protecting effect of CAPE on cisplatin-induced nephrotoxicity and the optimum dosage of this compound. In addition, these data indicate that NO may play a role in the pathogenesis of cisplatin nephrotoxicity. The effect of CAPE on NO inhibition of cisplatin-induced nephrotoxicity should be investigated further.

REFERENCES

Aebi H. 1974. Catalase. In: Methods of Enzymatic Analysis, Bergmeyer HU (ed.). Academic Press: New York; 673–677.

Anand AJ, Bashey B. 1993. Newer insights into cisplatin nephrotoxicity. *Ann. Pharmacother.* **27**: 1519–1525.

Baliga R, Zhang Z, Baliga M, Shah SV. 1998. *In vitro* and *in vivo* evidence suggesting a role for iron in cisplatin-induced nephrotoxicity. *Kidney Int.* **53**: 394–401.

Chatterjee PK, Patel NSA, Kvale EO, Cuzzocrea S, Brown PA, Stewart KN, Mota-Filipe H, Thiemermann C. 2002. Inhibition of inducible nitric oxide synthase reduces renal ischemia/reperfusion injury. *Kidney Int.* **61**: 862–871.

Chiao C, Carothers AM, Grunberger D, Solomon G, Preston GA, Barrett JC. 1995. Apoptosis and altered redox state induced by caffeic acid phenethyl ester (CAPE) in transformed rat fibroblast cells. *Cancer Res.* **55**: 3576–3583.

Cortas NK, Wakid NW. 1990. Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. *Clin. Chem.* **36**: 1440–1443.

Durak I, Yurtarslani Z, Canbolat O, Akyol O. 1993. A methodological approach to superoxide dismutase (SOD) activity assay based on inhibition of nitroblue tetrazolium (NBT) reduction. *Clin. Chim. Acta.* **214**: 103–104.

Fadillioglu E, Erdogan H, Sogut S, Kuku I. 2003. Protective effects of erdosteine against doxorubicin-induced cardiomyopathy in rats. *J. Appl. Toxicol.* **23**: 71–74.

Frenkel K, Wei H, Bhimani R, Ye J, Zadunaisky JA, Huang M, Ferraro T, Conney AH, Grunberger D. 1993. Inhibition of tumor promoter-mediated processes in mouse skin and bovine lens by caffeic acid phenethyl ester. *Cancer Res.* **53**: 1255–1261.

Goligorsky MS, Brodsky SV, Noiri E. 2002. Nitric oxide in acute renal failure: NOS versus NOS. *Kidney Int.* **61**: 855–861.

Grilli M, Chiu JJS, Lenardo MJ. 1993. NF-kappa B and Rel: participants in a multiform transcriptional regulatory system. *Int. Rev. Cytol.* **143**: 1–62.

Grunberger D, Banerjee R, Eisinger K, Oltz EM, Efros L, Caldwell M, Estevez V, Nakanishi K. 1988. Preferential cytotoxicity on tumor cells by caffeic acid phenethyl ester isolated from propolis. *Experientia.* **44**: 230–232.

Hara M, Yoshida M, Nishijima H, Yokosuka M, Ligo M, Ohtani-Kaneko R, Shimada A, Hasegawa T, Akama Y, Hirata K. 2001. Melatonin, a pineal secretory product with antioxidant properties, protects against cisplatin-induced nephrotoxicity in rats. *J. Pineal Res.* **30**: 129–138.

Hepsen IF, Bayramlar H, Gultek A, Ozen S, Tilgen F, Evereklioglu C. 1997. Caffeic acid phenethyl ester to inhibit posterior capsule opacification in rabbits. *J. Cataract Refract. Surg.* **23**: 1572–1576.

Hepsen IF, Er H, Cekic O. 1999. Topically applied water extract of propolis to suppress corneal neovascularization in rabbits. *Ophthal. Res.* **31**: 426–431.

Herbert V, shaw S, Jayatilleke E, Stopler-Kasdan T. 1994. Most free-radical injury is iron-related: It is promoted by iron, hemin, holoferritin and vitamin C, and inhibited by desferoxamine and apoferritin. *Stem Cells* **12**: 289–303.

Huang Y, Zhou S, Qui L, Wu J, Xu C. 1997. Effects of zinc gluconate on nephrotoxicity and glutathione metabolism disorder induced by cisplatin in mice. *Drug Metab. Drug Interact.* **14**: 41–46.

Ilhan A, Koltuksuz U, Ozen S, Uz E, Ciralik H, Akyol O. 1999. The effects of caffeic acid phenethyl ester (CAPE) on spinal cord ischemia/reperfusion injury in rabbits. *Eur. J. Cardiothorac. Surg.* **16**: 458–463.

Irmak MK, Koltuksuz U, Kutlu NO, Yagmurca M, Ozyurt H, Karaman A, Akyol O. 2001. The effect of caffeic acid phenethyl ester on ischemia–reperfusion injury in comparison with alpha-tocopherol in rat kidneys. *Urol. Res.* **29**: 190–193.

Kelly KJ, Meehan SM, Colvin RB, Williams WW, Bonventre JV. 1999. Protection from toxicant-mediated renal injury in the rat with anti-CD54 antibody. *Kidney Int.* **56**: 922–931.

Khayyal MT, el-Ghazaly MA, el-Khatip AS. 1993. Mechanisms involved in the antiinflammatory effect of propolis extract. *Drugs Exp. Clin. Res.* **19**: 197–203.

Kirkebo'en KA, Strand QA. 1999. The role of nitric oxide in sepsis — an overview. *Acta Anaesthesiol. Scand.* **43**: 275–288.

Koltuksuz U, Ozen S, Uz E, Aydinc M, Karaman A, Gultek A, Akyol O, Gursoy MH, Aydın E. 1999. Caffeic acid phenethyl ester (CAPE) prevents intestinal reperfusion injury in rats. *J. Pediatr. Surg.* **34**: 1458–1462.

Koltuksuz U, Irmak MK, Karaman A, Uz E, Var A, Ozyurt H, Akyol O. 2000. Testicular nitric oxide levels after unilateral testicular torsion-detorsion in rats pretreated with caffeic acid phenethyl ester. *Urol. Res.* **28**: 360–363.

Lowry O, Rosenbraugh N, Farr L, Randall R. 1951. Protein measurement with the Folin-phenol reagent. *J. Biol. Chem.* **183**: 265–275.

Muller DN, Dechend R, Mervaala EM, Park JK, Schmidt F, Fiebeler A, Theuer J, Breu V, Ganten D, Haller H, Luft FC. 2000. NF-kappa B inhibition ameliorates angiotensin II-induced inflammatory damage in rats. *Hypertension.* **35**: 193–201.

Natarajan K, Singh S, Burke TR Jr, Grunberger D, Aggarwal BB. 1996. Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-κB. *Proc. Natl. Acad. Sci. USA.* **93**: 9090–9095.

Orban Z, Mitsiades N, Burke TR Jr, Tsokos M, Chrousos GP. 2000. Caffeic acid phenethyl ester induces leukocyte apoptosis, modulates nuclear factor-kappa B and suppresses acute inflammation. *Neuroimmunomodulation.* **7**: 99–105.

Orditura M, De Vita F, Roscigno A, Infusino S, Auriemma A, Iodice P, Ciaramella F, Abbate G, Catalano G. 1999. Amifostine: a selective cytoprotective agent of normal tissues from chemo-radiotherapy induced toxicity (Review). *Oncol. Rep.* **6**: 1357–1362.

Ozyurt H, Irmak MK, Akyol O, Sogut S. 2001. Caffeic acid phenethyl ester changes the indices of oxidative stress in serum of rats with renal ischemia-reperfusion injury. *Cell Biochem. Funct.* **19**: 259–263.

Paglia DE, Valentine WN. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* **70**: 158–170.

Safirstein RL. 1999. Lessons learned from ischemic and cisplatin-induced nephrotoxicityu in animals. *Renal Failure.* **21**: 359–361.

Sud'ina GF, Mirzoeva OK, Pushkareva MA, Korshunova GA, Sumbatyan NV, Varfolomeev SD. 1993. Caffeic acid phenethyl ester as a lipoxygenase inhibitor with antioxidant properties. *FEBS Lett.* **329**: 21–24.

Sugihara K, Gemba M. 1986. Modification of cisplatin toxicity by antioxidants. *Jpn. J. Pharmacol.* **40**: 353–355.

Sun Y, Oberley LW, Li Y. 1988. A simple method for clinical assay of superoxide dismutase. *Clin. Chem.* **34**: 497–500.

Tome LA, Yu L, de Castro I, Campos SB, Seguro AC. 1999. Beneficial and harmful effects of L-arginine on renal ischaemia. *Nephrol. Dial. Transpl.* **14**: 1139–1145.

Townsend AJ, Leone-Kabler S, Haynes RL, Wu Y, Szweda L, Bunting KD. 2001. Selective protection by stably transfected human ALDH3A1 (but not human ALDH1A1) against toxicity of aliphatic aldehydes in V79 cells. *Chem. Biol. Interact.* **130–132**: 261–273.

Tsutsumishita Y, Onda T, Okada K, Takeda M, endou H, Futaki S, Niwa M. 1998. Involvement of H2O2 production in cisplatin-inducednephrotoxicity. *Biochem. Biophys. Res. Commun.* **242**: 310–312.

Walker LM, Walker PD, Imam SZ, Ali SF, Mayeux PR. 2000. Evidence for peroxynitrite formation in renal ischemia-reperfusion injury: studies with the inducible nitric oxide synthase inhibitor L-N(6)-(1-iminoethyl)lysine. *J. Pharmacol. Exp. Ther.* **295**: 417–422.

Wangsiripaisan A, Gengaro PE, Nemenoff RA, Ling H, Edelstein CL, Schrier RW. 1999. Effect of nitric oxide donors on renal tubular epithelial cell–matrix adhesion. *Kidney Int.* **55**: 2281–2288.

Wasowicz W, Neve J, Peretz A. 1993. Optimized steps in fluorometric determination of thiobarbituric acid-reactive substances in serum: importance of extraction pH and influence of sample preservation and storage. *Clin. Chem.* **39**: 2522–2526.

Wei H, Frenkel K. 1993. Relationship of oxidative events and DNA

oxidation in SENCAR mice to *in vivo* promoting activity of phorbol ester-type tumor promoters. *Carcinogenesis*. **14**: 1195–1201.
Yildirim Z, Sogut S, Odaci E, Iraz M, Ozyurt H, Kotuk M, Akyol O. 2003. Oral erdosteine administration attenuates cisplatin-induced renal tubular damage in rats. *Pharmacol. Res.* **47**: 49–156.
Zhang JG, Viale M, Esposito M, Lindup WE. 1999. Tiopronin protects against the nephrotoxicity of cisplatin in the rat. *Hum. Exp. Toxicol.* **18**: 713–717.