

Iyyapu Krishna Mohan · Mahmood Khan
Jagdish Chandra Shobha
Madireddy Umamaheswara Rao Naidu
Aruna Prayag · Periannan Kuppusamy
Vijay Kumar Kutala

Protection against cisplatin-induced nephrotoxicity by *Spirulina* in rats

Received: 4 January 2006 / Accepted: 1 March 2006 / Published online: 22 March 2006
© Springer-Verlag 2006

Abstract Purpose: isplatin (CP)-induced nephrotoxicity is associated with the increased generation of reactive oxygen metabolites and lipid peroxidation in kidney, caused by the decreased levels of antioxidants and antioxidant enzymes. The purpose of this study was to evaluate the role of *Spirulina*, blue-green alga with antioxidant properties, in the protection of cisplatin-induced nephrotoxicity in rat. **Methods:** Rats were treated with CP (6 mg/kg bw, single dose, intraperitoneally). *Spirulina* (1,000 mg/kg) was administered orally for 8 days and CP treatment was given on day 4. Nephrotoxicity was assessed, 6 days after the CP treatment, by measuring plasma urea, creatinine, urinary *N*-acetyl-(D-glucosaminidase) (β -NAG) and histopathology of kidney. **Results:** Rats treated with CP showed marked nephrotoxicity as evidenced from the significant elevation in plasma urea, creatinine and urinary β -NAG. Histological assessment revealed marked proximal tubular necrosis and extensive epithelial vacuolization in the kidney of CP-treated rats. Superoxide dismutase, catalase and glutathione peroxidase were decreased and lipid peroxidation was increased in kidney tissue. Pretreatment with *Spirulina* protected the rats from CP-induced nephrotoxicity. The rise in plasma urea, creatinine, urinary β -NAG, plasma and kidney tissue MDA and histomorphological changes were significantly attenuated by *Spirulina*.

In vitro studies using human ovarian cancer cells revealed that *Spirulina* did not interfere with the cytotoxic effects of CP on tumor cells. **Conclusions:** In summary, *Spirulina* significantly protected the CP-induced nephrotoxicity through its antioxidant properties.

Keywords Cisplatin · Nephrotoxicity · *Spirulina* · C-phycocyanin · Antioxidant

Introduction

Cisplatin (CP) is a potent antineoplastic drug, whose optimal clinical use is limited because of its ability to induce nephrotoxicity [1]. Acute renal failure has been observed after a single dose of cisplatin [2]. Primary targets of CP in kidney are proximal straight and distal convoluted tubules, where it accumulates and promotes cellular damage, and by involving multiple mechanisms including oxidative stress, DNA damage, apoptosis and inflammation [3–6]. Many in vivo and in vitro studies indicate an important role of reactive oxygen species (ROS) in the pathogenesis of nephrotoxicity [7]. CP induces free radical production causing oxidative renal damage, possibly due to depletion of non-enzymatic and enzymatic antioxidant systems [8]. Antioxidants have been shown to be protective in CP nephrotoxicity [9, 10]. Several radical scavengers and antioxidants such as vitamin E, vitamin C [11], selenium [12], edaravole [13], amifostine [14], superoxide dismutase (SOD) [10] and caffeic acid phenylethyl ester [15] are reported to attenuate CP-induced renal toxicity.

Spirulina (SP), a blue-green alga, is popularly used as a nutritional supplement as well as in therapeutic applications [16]. *Spirulina* contains proteins, lipids, carbohydrates, some vital minerals, vitamins including β -carotene and a pigmented protein, C-phycocyanin [17]. The antioxidant potential of *Spirulina* was demonstrated in both in vitro and in vivo studies [18]. *Spirulina* is known for its wide-ranging biological

I. K. Mohan · J. C. Shobha · M. U. R. Naidu · V. K. Kutala
Department of Clinical Pharmacology and Therapeutics,
Nizam's Institute of Medical Sciences,
500 082 Hyderabad, India

M. Khan · P. Kuppusamy · V. K. Kutala (✉)
Davis Heart and Lung Research Institute,
Comprehensive Cancer Center, The Ohio State University,
420 West 12th Ave, Room 126, Columbus, OH 43210, USA
E-mail: vijay.kutala@osumc.edu
Tel.: +1-614-2928383
Fax: +1-614-2928454

A. Prayag
Department of Pathology, Nizam's Institute of Medical Sciences,
500 082 Hyderabad, India

activities and antioxidative [18], anti-inflammatory [19], antimutagenic [20], antiviral [21], immune enhancing [18], cardioprotective [22] and anticancer properties [23].

Many studies have suggested that the plasma and tissue concentrations of various antioxidants including β -carotene were decreased during CP-based therapy in cancer patients and in experimental animal models [24], which, in part, may contribute to the development of nephrotoxicity by CP. Hence, the present study was carried out to determine the nephroprotective role of *Spirulina* against CP-induced nephrotoxicity in rats. Results revealed that *Spirulina* significantly attenuated the CP-induced nephrotoxicity in rats and further supported the crucial role of antioxidants in protecting nephrotoxicity induced by CP.

Materials and methods

Spirulina, a fine dark blue-green spray-dried powder, was prepared from *Spirulina platensis* (New Ambadi Estates, India). The biochemical analysis of *Spirulina* used in our experiments revealed a composition of proteins (65.38%), crude phycocyanin (15.37%), minerals (7.95%), total carotenoids (0.43%), β -carotene (0.17%) and total pheophorbide (0.02%). Required quantity of *Spirulina* powder was dissolved in sterile distilled water and used as the *Spirulina* preparation to treat the animals. Cisplatin was purchased from Sigma Chemical Co. (St. Louis, USA).

Animals and treatment

Adult male Wistar rats (weight 200–250 g) were used in the study. The rats were housed under conditions of controlled temperature and a 12-h lighting cycle and were fed with standard rat chow. The animals were divided into four groups of six animals each. The control group received only normal saline orally. The second group received *Spirulina* (1,000 mg/kg bw/day) orally for 8 days. The third group received a single dose of cisplatin (6 mg/kg bw) intraperitoneally and the fourth group received *Spirulina* (1,000 mg/kg bw/day) daily for 8 days and cisplatin (6 mg/kg bw, single dose, intraperitoneally) on day 4. On the fifth day after CP treatment, all the animals were kept in metabolic cages for 24 h urine collection. Blood was collected on the sixth day after CP treatment, by ocular puncture, for biochemical analysis and the kidneys were collected in buffered formalin for histopathological examination. The study was approved by the Institutional Ethics Committee for the use of laboratory animals at Nizam's Institute of Medical Sciences.

Biochemical assays

Plasma urea and creatinine levels were measured spectrophotometrically by using commercially available kits. The urinary β -NAG activity was measured as per the reported procedure [25].

Lipid peroxidation products

Kidney tissue was homogenized (10%, w/v) in PBS (pH 7.4) containing 20-mmol/l butylated hydroxytoluene. The homogenate was centrifuged at 3,000 rpm for 10 min

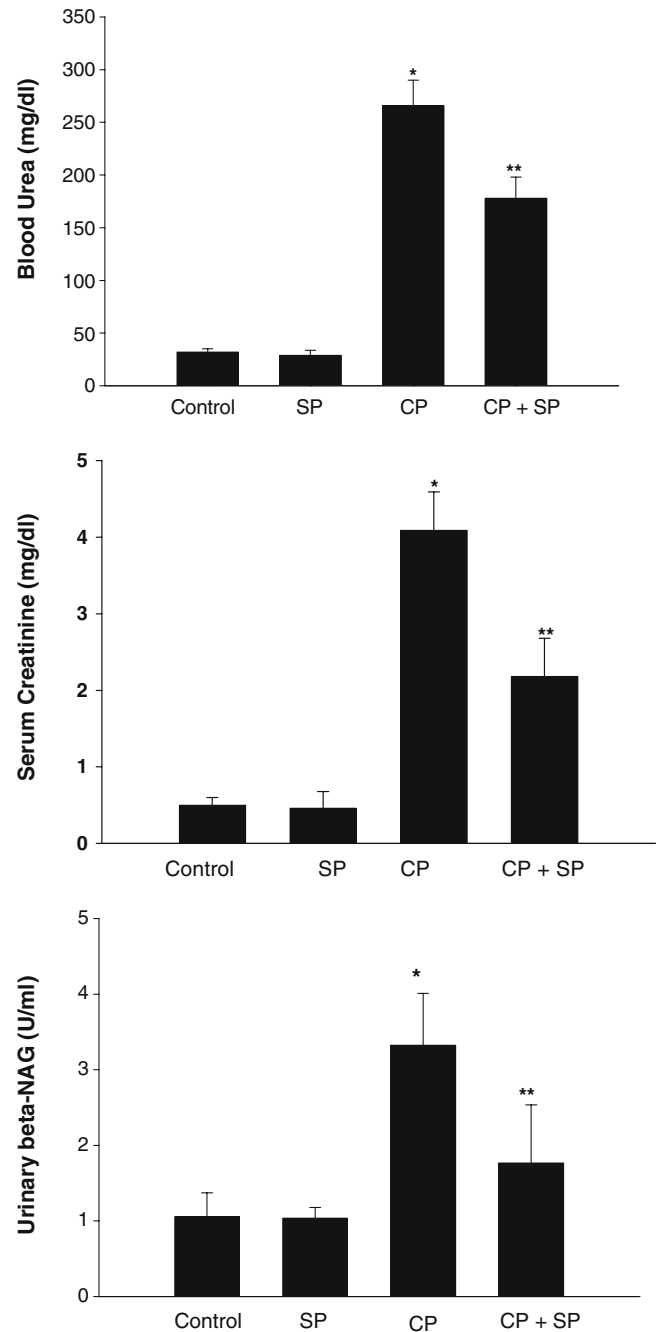


Fig. 1 Effect of *Spirulina* on cisplatin (CP)-induced nephrotoxicity as measured by: **a** plasma urea; **b** plasma creatinine; and **c** urinary β -NAG. Nephrotoxicity in rat was induced by CP (6 mg/kg bw single dose, intraperitoneally) and *Spirulina* (1,000 mg/kg) was administered orally, 3 days prior to CP treatment and continued till the end of the experiment. Values are expressed as mean \pm SD ($n = 6$), * $P < 0.05$ versus control; ** $P < 0.05$ versus CP. The results show that *Spirulina* treatment attenuated CP-induced nephrotoxicity

and the supernatant was mixed with equal volumes of 20% trichloroacetic acid, vortexed vigorously and centrifuged at 5,000 rpm for 30 min. To the protein-free supernatant, 0.33% thiobarbituric acid (TBA) was added and boiled for 1 h at 95°C. The TBA-reactive products were extracted in butanol and the intensity of the pink color was read at 520 nm [26]. Freshly diluted tetramethoxy propane (Sigma) was used as the standard and data were expressed in nmol of MDA/g of heart tissue.

Estimation of antioxidant enzymes

Superoxide dismutase activity in kidney tissue homogenate was determined spectrophotometrically according to the method of McCord and Fridovich [27]. This method is based on the ability of SOD to inhibit the reduction of cytochrome *c* in the presence of xanthine and xanthine oxidase. One unit was defined as the amount of enzyme that inhibits the reduction of cytochrome *c* by 50% and activity was expressed in units/mg protein. Catalase activity was determined by the method of Aebi [28], with H₂O₂ (10 mM) and phosphate buffer (0.05 M, pH 7.0) at 210 nm. A unit is defined as the amount of enzyme that catalyzed the dismutation of 1 μmol of H₂O₂/min. The specific activity is expressed in units/mg protein [28]. Glutathione peroxidase activity was measured by the NADPH oxidation method [29], and expressed as nmol of NADPH oxidized to NADP/mg protein. Protein was determined by method of Lowry et al. [30].

Histopathological studies

Histological sections (5 μm) of kidney from all the treated groups were stained with hematoxylin and eosin and periodic acid Schiff's stain. The microscopic scoring of the kidney sections was carried out in a blinded fashion by a pathologist who is unaware of the

treatment groups and assigned a score as described [31], which represents the approximate extent of necrotic area in the cortical proximal tubules on a scale of 0–4 (0, no necrosis; 1, a few focal necrotic spots; 2, necrotic area was about one half; 3, necrotic spots was about two thirds; 4, nearly the entire area was necrotic).

Effect of *Spirulina* on antitumor activity of CP in vitro

Human ovarian cancer cells were seeded into 96-well plates at a starting density of 2.5×10⁴ cells/well and cultured overnight in phenol red-free RPMI-1640 medium, containing 5% fetal calf serum at 37°C, humidified with 5% CO₂. The following day, CP (6 μM) and/or *Spirulina* (50 μg/ml) was added to the medium. Twenty-four and forty-eight hours later, 0.5% of MTT was added, incubated for 3 h, and then the medium was removed. The water-insoluble blue formazan dye formed was solubilized in DMSO, and the absorbance was read using a 96-well plate ELISA reader (Beckmann Coulter, AD 340) at 550 nm. All experiments were run in at least four parallels and repeated three times.

Statistical analysis

The statistical significance of differences among values of individual parameters was evaluated by using the Student's *t* test. All the values are expressed as mean ± SD. The significance was set at *P* < 0.05.

Results

To assess CP-induced nephrotoxicity, the plasma urea and creatinine levels were determined. Figure 1 shows significant elevation of plasma urea and creatinine levels in rats treated with CP alone as compared with untreated control (*P* < 0.05). Urinary β-NAG, a marker of renal tubular damage, was also significantly increased

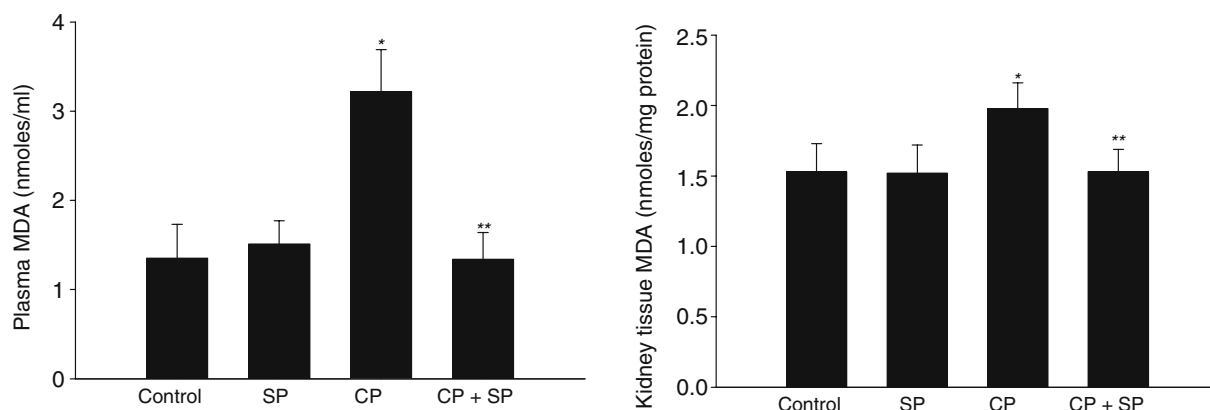


Fig. 2 Effect of *Spirulina* on cisplatin (CP)-induced lipid peroxidation (MDA). Rats were treated with CP and *Spirulina* as described in Fig. 1. Values are expressed as mean ± SD (*n* = 6),

P* < 0.05 versus control; *P* < 0.05 versus CP. The results show that *Spirulina* treatment attenuated the CP-induced increase in lipid peroxidation

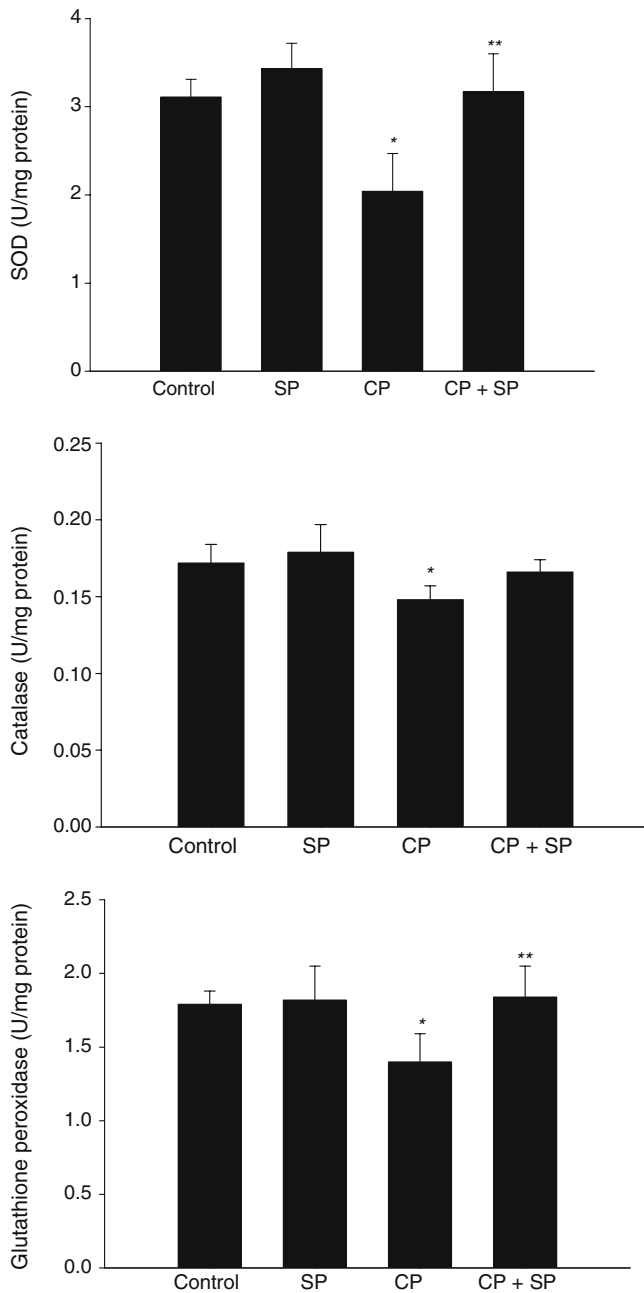


Fig. 3 Effect of *Spirulina* on Cisplatin (CP)-induced changes in SOD, catalase and glutathione peroxidase in kidney tissue. Rats were treated with CP and *Spirulina* as described in Fig. 1. Values are expressed as mean \pm SD ($n=6$), * $P < 0.05$ versus control; ** $P < 0.05$ versus CP. The results show that *Spirulina* treatment attenuated the CP-induced decrease in SOD, catalase and glutathione peroxidase

in CP-treated animals (Fig. 1). In a dose–response study, we found that *Spirulina* at 1,000 mg/kg was the most effective dose in ameliorating the CP-induced nephrotoxicity (data not shown). Hence all our experiments were performed using this dose. The CP-induced rise in the levels of plasma urea, creatinine and urinary β -NAG was reduced significantly by *Spirulina* treatment. As shown in Figs. 2 and 3, there was a significant increase

in lipid peroxidation in plasma and kidney tissues and a significant decrease in antioxidant enzymes, SOD, catalase and glutathione peroxidase in CP-treated animals. These findings are similar to those obtained by others, showing increased lipid peroxidation and decreased antioxidant enzymes in kidney tissues of CP-treated rats [15, 32]. However, pretreatment of *Spirulina* significantly attenuated the plasma and kidney tissue MDA levels. Furthermore, SOD, catalase and glutathione peroxidase levels were also restored to control levels.

The impaired renal function induced by CP was further confirmed by histological examination of kidney, from the regions of cortex and cortico-medullary junction (Fig. 4). As shown in Fig. 4a, kidney from control rats showed no abnormality whereas kidney in CP-treated animals revealed a marked proximal tubular necrosis, extensive epithelial vacuolization, swelling and tubular dilation and renal tubules with hyaline casts (Fig. 4b). The glomerulus and tubular epithelial changes were less severe in the group treated with CP + *Spirulina* when compared with CP-treated rats (Fig. 4c). The histological changes were graded as described in the Materials and methods section and are summarized in Table 1.

Though the urea and creatinine levels were not normal in *Spirulina* + CP-treated rats, they were significantly less as compared with CP-treated rats. However, histopathological data and urinary β -NAG (indicative of tubular necrosis) clearly indicated that *Spirulina* protected against CP-induced nephrotoxicity.

To evaluate whether *Spirulina* could modify the chemotherapeutic efficacy of CP, we studied the effect of *Spirulina* on CP-induced cell-killing in human ovarian cancer cells in vitro. The results demonstrated that the cell survival was reduced significantly with CP treatment after 24 and 48 h of incubation, and co-administration of CP with *Spirulina* had no significant effect on CP-induced cell death (Fig. 5).

Discussion

The production of ROS and oxidative stress in kidney tissue have been implicated in the pathogenesis of CP-induced renal injury [33] including lipid peroxidation, enzyme inactivation, changes in the cellular non-enzymatic and enzymatic antioxidant system [10, 15, 31]. It has been reported that the administration of CP to rats resulted in the depletion of GSH in kidney tissue [34]. CP has been shown to inhibit the function of mitochondrial respiratory complex in normal tubular cells [35], resulting in ROS generation and mitochondrial dysfunction [36]. The involvement of oxidative stress was further supported by the fact that antioxidants such as melatonin [37], vitamin E, vitamin C [11], selenium [12], diethyldithiocarbamate [32] and MnSOD prevented CP-induced nephrotoxicity [10]. Overexpression of heme oxygenase-1 (HO-1) ameliorated [38] the renal damage induced by CP, while deficiency of HO-1

Fig. 4 Histological examination of rat kidney. *Left panel* shows cortical region (H&E 40×); *Right panel* shows cortico-medullary junction (H&E, 100×). **a** Control rat. *Left and right panels* show normal morphology; **b** cisplatin-treated rat. *Left and right panels* show renal tubules with hyaline casts, swelling and vacuolization and proximal tubular necrosis; and **c** cisplatin + *Spirulina*-treated rat. *Left and right panels* show minimal tubular necrosis

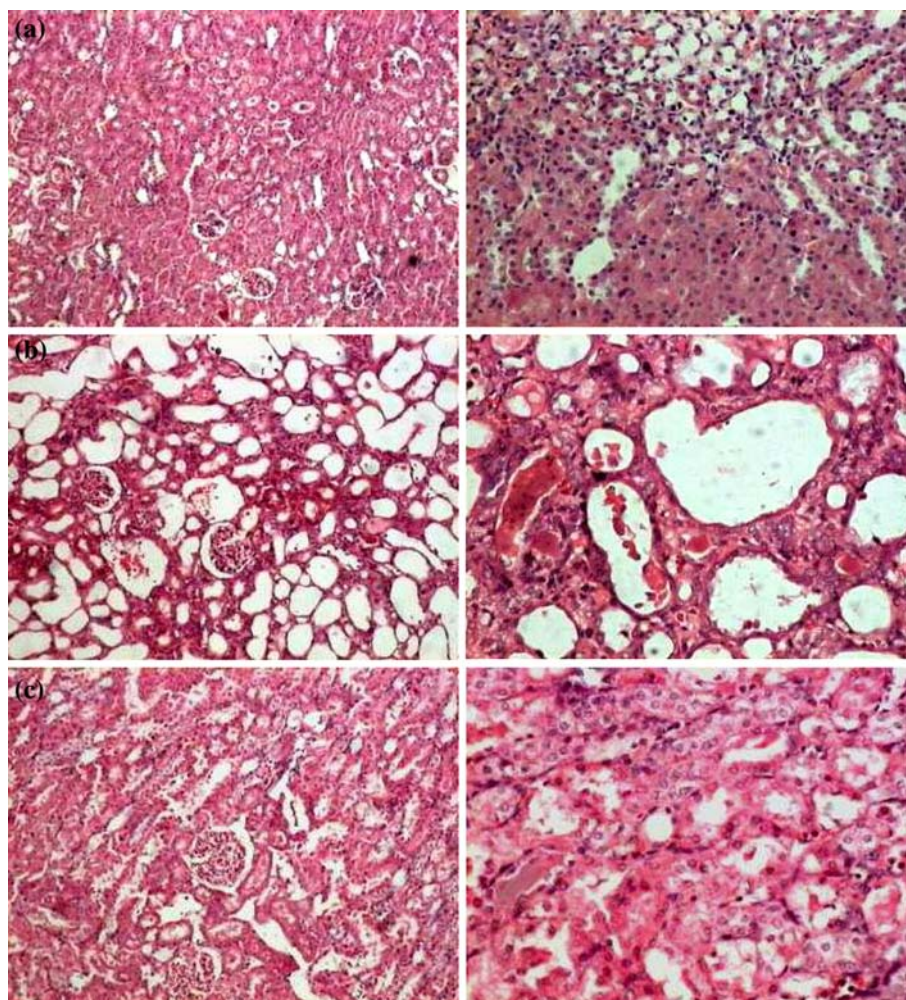


Table 1 Semiquantitative analysis of histology of rat kidney

Group	Treatment	Score
1	Control	0+
2	<i>Spirulina</i>	0+
3	Cisplatin	3+
4	Cisplatin + <i>Spirulina</i>	1+

Rats were treated with cisplatin and *Spirulina* and histological grading was performed as described in [Materials and methods](#) section

[39] worsened it, supporting the involvement of oxidative stress in the experimental model of CP toxicity. The protective effect of dimethylthiourea (OH radical scavenger) against CP-induced proximal tubular (LLC-PK1) cell toxicity in vitro, also demonstrated the crucial role of ROS in CP-induced toxicity [7].

Pretreatment of *Spirulina* attenuated CP-induced nephrotoxicity and this effect is attributed to its antioxidant property. Several studies have demonstrated that *Spirulina* possess significant antioxidant activity both in vitro and in vivo [22, 40–42]. Earlier studies have shown that *Spirulina* exhibits antioxidant property in various oxidative conditions that can cause tissue injury

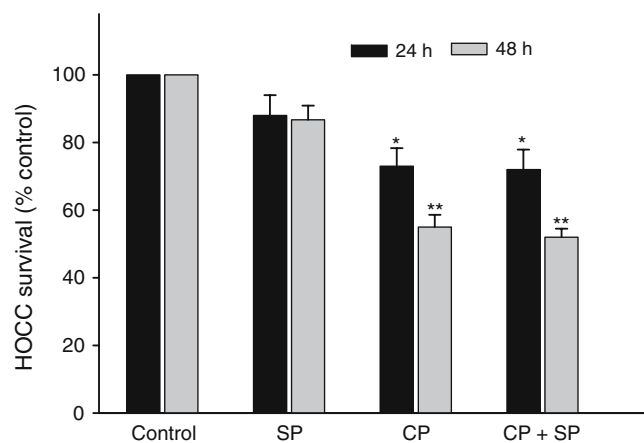


Fig. 5 Effect of *Spirulina* (SP) on the antitumor potency of CP. Human ovarian cancer cells were plated in 96-well plates and allowed to attach overnight. The cells were treated with CP and/or *Spirulina* for a period of 24 or 48 h and cell proliferation was determined by MTT assay. Control cell growth was set at 100%. Values are expressed as mean \pm SD ($n=3$), * $P<0.01$ versus control; ** $P<0.001$ versus control. The results show that *Spirulina* does not interfere with the cytotoxic effect of CP on tumor cells

[18, 43–45]. *Spirulina* prevented the cyclophosphamide and mitomycin-C toxicity [45] and CP- and urethane-induced genotoxicity in mice [46]. Rats fed with a *Spirulina*-enriched diet showed reduced ischemia–reperfusion-induced cerebral infarction [47]. In our recent studies, we have demonstrated that mice pretreated with *Spirulina* significantly protected doxorubicin-induced cardiotoxicity [22], and also attenuated doxorubicin-induced ROS generation and apoptosis in isolated rat cardiomyocytes in vitro [42]. Recent study has demonstrated that C-phycoerythrin, one of the main constituents of *Spirulina*, inhibited oxalate-mediated lipid peroxidation and protected renal cell injury [48]. In addition, the results of the present study also indicated that *Spirulina* did not interfere with the inhibition of cell proliferation induced by CP in human ovarian cancer cells in vitro.

Though the results of the present study suggest *Spirulina* offers protection against CP-induced nephrotoxicity through the inhibition of oxidative stress, other possible mechanism(s) cannot be ruled out. It is evident from many studies that apoptosis is involved in CP-induced renal injury [6, 49]. A recent study has indicated that treatment with a *Spirulina*-enriched diet reduced the ischemia–reperfusion-induced apoptosis and cerebral infarction by inhibiting caspase-3 activity [47]. The chemoprotective agent, *N*-acetylcysteine blocks the CP-induced apoptosis through the caspase signaling pathway [50]. In our recent study, we have demonstrated that *Spirulina* and C-phycoerythrin significantly inhibited the doxorubicin-induced free radical generation and apoptosis by attenuating caspase-3 activity in isolated rat cardiomyocytes [42]. It is likely that *Spirulina* might be altering the oxidative stress-mediated apoptotic pathway, thereby inhibiting the CP-induced apoptosis. However, this requires further investigation.

In summary, our study demonstrated that *Spirulina* protected CP-induced nephrotoxicity in rats by its antioxidant properties. Furthermore, *Spirulina* does not compromise the antitumor effect of CP. Further studies are required to examine the clinical use and exact mechanisms behind a possible protective effect of *Spirulina* on CP-induced nephrotoxicity.

Acknowledgment We thank M/s Parry Nutraceuticals, Chennai, India for providing the pure powder of *Spirulina* for our study and Jing Fang for her help in the study with ovarian cancer cells. This work was partially supported by the National Institutes of Health Grant CA-102264.

References

- Loehrer PJ, Einhorn LH (1984) Drugs five years later Cisplatin. *Ann Intern Med* 100:704–713
- Heidemann HT, Muller S, Mertins L, Stepan G, Hoffmann K, Ohnhaus EE (1989) Effect of aminophylline on cisplatin nephrotoxicity in the rat. *Br J Pharmacol* 97:313–318
- Safirstein R, Winston J, Moel D, Dikman S, Guttenplan J (1987) Cisplatin nephrotoxicity: insights into mechanism. *Int J Androl* 10:325–346
- Schaaf GJ, Maas RF, de Groene EM, Fink-Gremmels J (2002) Management of oxidative stress by heme oxygenase-1 in cisplatin-induced toxicity in renal tubular cells. *Free Radic Res* 36:835–843
- Xiao T, Choudhary S, Zhang W, Ansari NH, Salahudeen A (2003) Possible involvement of oxidative stress in cisplatin-induced apoptosis in LLC-PK1 cells. *J Toxicol Environ Health A* 66:469–479
- Cummings BS, Schnellmann RG (2002) Cisplatin-induced renal cell apoptosis: caspase 3-dependent and -independent pathways. *J Pharmacol Exp Ther* 302:8–17
- Baliga R, Zhang Z, Baliga M, Ueda N, Shah SV (1998) In vitro and in vivo evidence suggesting a role for iron in cisplatin-induced nephrotoxicity. *Kidney Int* 53:394–401
- Rybak LP, Ravi R, Somani SM (1995) Mechanism of protection by diethyldithiocarbamate against cisplatin ototoxicity: antioxidant system. *Fundam Appl Toxicol* 26:293–300
- Antunes LM, Araujo MC, Darin JD, Bianchi ML (2000) Effects of the antioxidants curcumin and vitamin C on cisplatin-induced clastogenesis in Wistar rat bone marrow cells. *Mutat Res* 465:131–137
- Davis CA, Nick HS, Agarwal A (2001) Manganese superoxide dismutase attenuates Cisplatin-induced renal injury: importance of superoxide. *J Am Soc Nephrol* 12:2683–2690
- Appenroth D, Frob S, Kersten L, Splinter FK, Winnefeld K (1997) Protective effects of vitamin E and C on cisplatin nephrotoxicity in developing rats. *Arch Toxicol* 71:677–683
- Antunes LM, Francescato HD, Darin JD, de Lourdes PBM (2000) Effects of selenium pretreatment on cisplatin-induced chromosome aberrations in wistar rats. *Teratog Carcinog Mutagen* 20:341–348
- Sueishi K, Mishima K, Makino K, Itoh Y, Tsuruya K, Hirakata H, Oishi R (2002) Protection by a radical scavenger edaravone against cisplatin-induced nephrotoxicity in rats. *Eur J Pharmacol* 451:203–208
- 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
- Ozen S, Akyol O, Iraz M, Sogut S, Ozugurlu F, Ozyurt H, Odaci E, Yildirim Z (2004) Role of caffeic acid phenethyl ester, an active component of propolis, against cisplatin-induced nephrotoxicity in rats. *J Appl Toxicol* 24:27–35
- Khan Z, Bhadouria P, Bisen PS (2005) Nutritional and therapeutic potential of *Spirulina*. *Curr Pharm Biotechnol* 6:373–379
- Ciferri O (1983) *Spirulina*, the edible microorganism. *Microbiol Rev* 47:551–578
- Miranda MS, Cintra RG, Barros SB, Mancini Filho J (1998) Antioxidant activity of the microalga *Spirulina maxima*. *Braz J Med Biol Res* 31:1075–1079
- Remirez D, Gonzalez R, Merino N, Rodriguez S, Ancheta O (2002) Inhibitory effects of *Spirulina* in zymosan-induced arthritis in mice. *Mediators Inflamm* 11:75–79
- Chamorro G, Salazar M, Favila L, Bourges H (1996) Pharmacology and toxicology of *Spirulina* alga. *Rev Invest Clin* 48:389–399
- Ayehunie S, Belay A, Baba TW, Ruprecht RM (1998) Inhibition of HIV-1 replication by an aqueous extract of *Spirulina platensis* (*Arthrospira platensis*). *J Acquir Immune Defic Syndr Hum Retrovirol* 18:7–12
- Khan M, Shobha JC, Mohan IK, Naidu MU, Sundaram C, Singh S, Kuppasamy P, Kutala VK (2005) Protective effect of *Spirulina* against doxorubicin-induced cardiotoxicity. *Phytother Res* 19:1030–1037
- Schwartz J, Shklar G, Reid S, Tricker D (1988) Prevention of experimental oral cancer by extracts of *Spirulina*–*Dunaliella* algae. *Nutr Cancer* 11:127–134

24. Weijl NI, Hopman GD, Wipkink-Bakker A, Lentjes EG, Berger HM, Cleton FJ, Osanto S (1998) Cisplatin combination chemotherapy induces a fall in plasma antioxidants of cancer patients. *Ann Oncol* 9:1331–1337
25. Horak E, Hopfer SM, Sunderman FW Jr (1981) Spectrophotometric assay for urinary *N*-acetyl-beta-D-glucosaminidase activity. *Clin Chem* 27:1180–1185
26. Bernheim FM, Bernheim BL, Wilbur KM (1948) The reaction between TBA and the oxidation products of certain lipids. *J Biol Chem* 174:257–264
27. McCord JM, Fridovich I (1969) Superoxide dismutase. An enzymic function for erythrocyte (hemocuprein). *J Biol Chem* 244:6049–6055
28. Aebi H (1984) Catalase in vitro. *Methods Enzymol* 105:121–126
29. Paglia DE, Valentine WN (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 70:158–169
30. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–275
31. Baliga R, Ueda N, Walker PD, Shah SV (1999) Oxidant mechanisms in toxic acute renal failure. *Drug Metab Rev* 31:971–997
32. Somani SM, Ravi R, Rybak LP (1995) Diethyldithiocarbamate protection against cisplatin nephrotoxicity: antioxidant system. *Drug Chem Toxicol* 18:151–170
33. Yilmaz HR, Uz E, Yucel N, Altuntas I, Ozcelik N (2004) Protective effect of caffeic acid phenethyl ester (CAPE) on lipid peroxidation and antioxidant enzymes in diabetic rat liver. *J Biochem Mol Toxicol* 18:234–238
34. Nakano S, Gemba M (1989) Potentiation of cisplatin-induced lipid peroxidation in kidney cortical slices by glutathione depletion. *Jpn J Pharmacol* 50:87–92
35. Kruidering M, Van de Water B, de Heer E, Mulder GJ, Nagelkerke JF (1997) Cisplatin-induced nephrotoxicity in porcine proximal tubular cells: mitochondrial dysfunction by inhibition of complexes I to IV of the respiratory chain. *J Pharmacol Exp Ther* 280:638–649
36. Richter C, Gogvadze V, Laffranchi R, Schlapbach R, Schweizer M, Suter M, Walter P, Yaffee M (1995) Oxidants in mitochondria: from physiology to Diseases. *Biochim Biophys Acta* 1271:67–74
37. Sener G, Satiroglu H, Kabasakal L, Arbak S, Oner S, Ercan F, Keyer-Uysa M (2000) The protective effect of melatonin on cisplatin nephrotoxicity. *Fundam Clin Pharmacol* 14:553–560
38. Shiraishi F, Curtis LM, Truong L, Poss K, Visner GA, Madsen K, Nick HS, Agarwal A (2000) Heme oxygenase-1 gene ablation or expression modulates cisplatin-induced renal tubular apoptosis. *Am J Physiol Renal Physiol* 278:F726–F736
39. Agarwal A, Nick HS (2000) Renal response to tissue injury: lessons from heme oxygenase-1 GeneAblation and expression. *J Am Soc Nephrol* 11:965–973
40. Bhat VB, Madyastha KM (2000) C-phycoerythrin: a potent peroxyl radical scavenger in vivo and in vitro. *Biochem Biophys Res Commun* 275:20–25
41. Romay C, Armesto J, Ramirez D, Gonzalez R, Ledon N, Garcia I (1998) Antioxidant and anti-inflammatory properties of C-phycoerythrin from blue-green Algae. *Inflamm Res* 47:36–41
42. Khan M, Varadharaj S, Ganesan LP, Shobha JC, Naidu MU, Parinandi NL, Tridandapani S, Kutala VK, Kuppusamy P (2006) C-phycoerythrin protects against ischemia-reperfusion injury of heart through involvement of p38 MAPK and ERK signaling. *Am J Physiol Heart Circ Physiol* (in press)
43. Mathew B, Sankaranarayanan R, Nair PP, Varghese C, Somanathan T, Amma BP, Amma NS, Nair MK (1995) Evaluation of chemoprevention of oral cancer with *Spirulina fusiformis*. *Nutr Cancer* 24:197–202
44. Premkumar K, Pachiappan A, Abraham SK, Santhiya ST, Gopinath PM, Ramesh A (2001) Effect of *Spirulina fusiformis* on cyclophosphamide and mitomycin-C induced genotoxicity and oxidative stress in mice. *Fitoterapia* 72:906–911
45. Upasani CD, Khera A, Balaraman R (2001) Effect of lead with vitamin E, C, or *Spirulina* on malondialdehyde, conjugated dienes and hydroperoxides in rats. *Indian J Exp Biol* 39:70–74
46. Premkumar K, Abraham SK, Santhiya ST, Ramesh A (2004) Protective effect of *Spirulina fusiformis* on chemical-induced genotoxicity in mice. *Fitoterapia* 75:24–31
47. Wang Y, Chang CF, Chou J, Chen HL, Deng X, Harvey BK, Cadet JL, Bickford PC (2005) Dietary supplementation of with blueberries, spinach, or spirulina reduces ischemic brain damage. *Exp Neurol* 193:75–84
48. Farooq SM, Asokan D, Sakthivel R, Kalaiselvi P, Varalakshmi P (2004) Salubrious effect of C-phycoerythrin against oxalate-mediated renal cell injury. *Clin Chim Acta* 348:199–205
49. Lau AH (1999) Apoptosis induced by cisplatin nephrotoxic injury. *Kidney Int* 56:1295–1298
50. Wu YJ, Muldoon LL, Neuwelt EA (2005) The chemoprotective agent *N*-acetylcysteine blocks cisplatin-induced apoptosis through caspase signaling pathway. *J Pharmacol Exp Ther* 312:424–431