The Protective Effects of Spirulina in Cyclophosphamide Induced Nephrotoxicity and Urotoxicity in Rats

Orhun Sinanoglu, Arzu Neşe Yener, Sinan Ekici, Ahmet Midi, and Fehime Benli Aksungar

OBJECTIVE To evaluate the role of *Spirulina*, a blue-green algae with antioxidant properties in the protection of cyclophosphamide-induced nephrotoxicity and hemorrhagic cystitis in rats.

METHODS The control group (C) was sacrificed 24 hours after being given a single dose of saline intraperitoneally (150 mg/kg) on the seventh day of the experiment. The rats in the second group (CP) were sacrificed 24 hours after being given a single dose of cyclophosphamide, intraperitoneally (150 mg/kg) on the seventh day of the experiment. *Spirulina* was administered to the third group (SP+CP) orally (1000 mg/kg bw/day) for 7 days and a single dose of cyclophosphamide was injected intraperitoneally (150 mg/kg) on the seventh day of the experiment. At the eighth day of the experiment, malondialdehyde, superoxide dismutase, and catalase levels in renal and urinary bladder tissues were measured. Histomorphology in urinary bladder, apoptosis by caspase 3 immunostaining, and TUNEL assay in kidney were also evaluated.

RESULTS Tissue levels of malondialdehyde in the SP+CP group were significantly lower versus CP group ($P < .05$). Tissue levels of superoxide dismutase and catalase in the SP+CP group were significantly higher vs the CP group ($P < .05$). The histomorphologic alteration in urinary bladder in the SP+CP group was significantly lower vs that in the CP group. In the kidney, apoptosis in the SP+CP group as shown with TUNEL assay and immunohistochemistry was significantly lower vs that in the CP group ($P < .05$).

CONCLUSION Pretreatment with *Spirulina* protects the rats from cyclophosphamide-induced nephro-urotoxicity via its antioxidant and anti-apoptotic properties. UROLOGY 80: 1392.e1–1392.e6, 2012. © 2012 Elsevier Inc.

Cyclophosphamide (Cyc) has been in clinical use since the late 1950s and is proved to be effective in the treatment of both neoplastic diseases, such as solid tumors and lymphomas, and nonneoplastic diseases, such as rheumatoid arthritis and systemic lupus erythematosus.[1] It is well known that this drug or its metabolites causes acute inflammation of the urinary bladder and may cause renal damage.[1] The damage is related to the Cyc itself and to its structural analogue ifosfamide, both of which are highly alkylating cytostatic compounds. Both are known to have severe urologic adverse effects, but the effects of Cyc on the kidney is controversial.[1-3] Cyc causes histopathologic changes in rat kidney, even if the plasma creatinine level, a reliable indicator of the glomerular function is not altered.[4] The renal damage in rats is recognized histopathologically as glomerular inflammation, epithelial cytoplasmic vacuolization in cortical tubules, interstitial edema, and mild hemorrhagic changes in the renal cortex and also biochemically, as reduced renal glutathione level and increased renal malondialdehyde (MDA) level 24 hours after Cyc treatment.[4] In children and elderly patients, Cyc treatment can also result in glomerular and tubular dysfunction because of its toxic effects on immature kidneys and because of the progressive decline in renal function with aging, respectively.[2] However, in most of the adult patients on Cyc treatment, the adverse effects of the drug or its metabolites in the kidney are usually neglected because of unchanged kidney function. As mentioned before, severe urinary bladder toxicity is also one of the most important adverse effects of Cyc, being the major limiting factor in its use. Clinically, this toxicity varies from transient irritative voiding symptoms and mild hematuria to life-threatening hemorrhagic cystitis.[5] The urotoxicity is related to the formation of toxic

Financial Disclosure: *The authors declare that they have no relevant financial interests.*

The study was approved by the Local Ethics Committee for Animals Study of Maltepe University (Project #2011/01).

From the Departments Urology, Pathology, and Biochemistry, Maltepe University School of Medicine, Maltepe/Istanbul, Turkey

Reprint requests: Orhun Sinanoglu, M.D., Maltepe University School of Medicine, Department of Urology, Feyzullah Caddesi, No: 39, PK:34843 Maltepe, Istanbul, Turkey. E-mail: orhundr@hotmail.com

Submitted: February 14, 2012, accepted (with revisions): June 28, 2012

© 2012 Elsevier Inc.
All Rights Reserved

http://dx.doi.org/10.1016/j.urology.2012.06.053

metabolites of Cyc in the liver and partly to the direct alkylating activity of the drug metabolites on the urinary tract.[5] In the liver, the cytochrome p-450 monooxygenase system converts Cyc to 4-hydroxy-cyclophosphamide and its tautomer aldophosphamide. Aldophosphamide undergoes β elimination to release acrolein and the alkylating compound phosphoramide mustard.[5] The mechanism of acrolein toxicity is that it binds to and causes the depletion of the cellular antioxidant nucleophiles, such as glutathione and it initiates the lipid peroxidation that results in hemorrhagic cystitis.[6] Many cytokines, such as tumor necrosis factor, interleukins, and transcription factors, also play a role in the pathogenesis of this inflammatory process.[5,7] By contrast, superoxide dismutase (SOD) and catalase are 2 powerful antioxidant enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide and the hydrogen peroxide to water and oxygen, respectively. Because of the plasma and the tissue, concentrations of various antioxidants were experimentally shown to be decreased during Cyc therapy.[2] The application of plant extracts that contain antioxidants to scavenge the harmful effects of Cyc attracted worldwide interest.[8,9] Among these, a blue-green algae, *Spirulina*, has been demonstrated to have antioxidant effects in many in vitro and in vivo studies.[10] It has a complex structure that contains mainly proteins, lipids, carbohydrates, vitamins, and a pigmented protein, C-phycocyanin. It has been used as a nutritional supplement and also for many therapeutic purposes.[10,11] Meanwhile, its nephroprotective benefits were also reported.[12,13] Its possible uroprotective effects have not yet been studied.

The present study was designed to show the adverse effects of Cyc on kidney and also to show the possible protective role of *Spirulina* against Cyc-induced nephrotoxicity and hemorrhagic cystitis in rats. To the best of our knowledge, the effects of *Spirulina* on Cyc-induced nephrotoxicity and urotoxicity in rats have not been reported in the English literature.

MATERIAL AND METHODS

A fine, dark blue–green powder of Hawaiian *Spirulina-Arthrospira platensis pacifica* (Algbiotek, Istanbul, Turkey) was dissolved in sterile distilled water and given orally. Cyc was purchased from Eczacibaşı/Baxter Chemical, Co. (Istanbul, Turkey). The study was approved by the Local Ethics Committee for Animal Experiments of Maltepe University Medical School, Istanbul, Turkey (Project# 2011/01). The dosage and the route of administration of Cyc were determined from that described in the literature.[14]

Animals and Treatment

Eighteen adult female Wistar albino rats (180-210 g) purchased from the Experimental Animal Laboratory of Maltepe University School of Medicine were used. The rats were housed under conditions of controlled temperature in individual cages in a room with a daily 12-hour light-dark cycle. Food and water were available ad libitum. The rats were divided randomly into 3 groups of 6 rats each. After acclimatization for 2 weeks, the experiment was started. The control group (C) were sacrificed 24 hours after being given a single dose of saline, intraperitoneally (ip) (150 mg/kg) on the seventh day of the experiment. The rats in the second group (CP) were sacrificed 24 hours after being given a single dose of Cyc, ip (150 mg/kg) on the seventh day of the experiment. The rats in third group (SP+CP) received *Spirulina* (1000 mg/kg bw/day) orally for 7 days and were sacrificed 24 hours after being given a single dose of Cyc (150 mg/kg ip) on the seventh day of the experiment. They were anesthetized with 50 mg/kg ketamine and 10 mg/kg xylasize before sacrifice by exsanguination. The kidneys and the urinary bladders were collected for histologic examination and immunohistochemical, biochemical, and terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL) assays.

Tissue Homogenization

Fresh tissues were washed with ice-cold phosphate-buffered saline (PBS) solution (10 mM Na_2HPO_4, 10 mM KH_2PO_4, 0.9 g NaCl/100 mL, pH 7.4) and weighed. After weights were recorded, homogenization was done with a tissue homogenizator (Heidolph DIA $\times$ 900, Schwabach, Germany) in ice-cold PBS immediately (1 mL/mg-volume/weight tissue) and they were kept at -70°C until assayed.

Measurement of MDA

Samples were thawed and centrifuged. Supernatants were used for the measurements. MDA assay was performed with a spectrophotometric assay (Catalog #NWK-MDA01, Northwest Life Science Canada, Vancouver, WA). The assay was based on the reaction of MDA with thiobarbituric acid (TBA), forming an MDA-TBA2 complex, which absorbs light strongly at 532 nm. The absorbance was directly proportional to the concentration MDA present. Intra-assay coefficient of variability (CV) was 3.2% and interassay CV was 2.5%. Data were expressed in nmol of MDA per 1 g of tissue.

Measurement of SOD ACTIVITY

Homogenates were thawed and centrifuged. Superoxide dismutase enzyme activity was measured immediately. Enzyme activity was measured by a colorimetric assay of superoxide dismutase (Catalog #NWK-SOD2, Northwest Life Science-Canada). The assay was based on monitoring the autoxidation rate of hematoxylin. In the presence of SOD enzyme, the rate of autoxidation is inhibited and the percentage of inhibition is linearly proportional to the amount of SOD present within a specific range. Sample SOD activity is determined by measuring the ratios of autoxidation rates in the presence and absence of the sample. The assay had an intra-assay CV of 8% and interassay CV of 12%. Data were expressed in U SOD per 1 g of tissue.

Measurement of Catalase Activity

The measurement of the catalase activity in the tissue homogenates were performed with a colorimetric assay. In this assay, the decomposition of peroxide is monitored at 240 nm (Catalog #NWK-SOD2, Northwest Life Science-Canada). The absorbance of hydrogen peroxide at 240 nm is measured directly to calculate the reaction rate because water and oxygen do not absorb at this wavelength. In the presence of catalase, the reaction rate is proportionally enhanced. The assay has an

intra-assay CV of 6.12% and interassay CV of 8%. Data were expressed in U catalase per 1 g of tissue.

Histomorphologic Evaluation

Kidneys and urinary bladders from all groups were fixed in 10% formaldehyde and after tissue processing, they were embedded in paraffin. Five-μm sections were prepared and stained with routine hematoxylin and eosin. Light microscopic evaluation was done by two researchers in a blinded fashion. Toxic effects of Cyc on the glomeruli, the tubules, and the interstitium were evaluated semiquantitatively on a scale of 0–3. For the kidney, glomerular inflammation, tubular necrosis, and interstitial inflammation were assessed. For the urinary bladder, the ratio of the intact to involved mucosa was given for each rat. The mucosal involvement was assessed by evaluating the epithelial desquamation, congestion, edema, and neutrophile leukocyte infiltration in lamina propria.[15]

Determination of Apoptosis

TUNEL assay was performed to detect apoptosis in situ. DNA fragmentation was detected by the labeling of DNA breaks in apoptotic nuclei in paraffin-embedded tissue sections using apoptosis detection kit (Unmatched termTACS 2 TdT-DAB in situ Apoptosis Detection Kit, Trevigen, Gaithersburg, MD). Apoptotic cells were identified as the cells with brown-black–stained nuclei. To quantitate the extent of the apoptosis, we recorded the number of apoptotic cells (TUNEL-positive cells) under high power magnification (x400). We totalled all TUNEL-positive and intact cells in those areas and then calculated the apoptotic index (AI) by means of an average count per slide. AI was calculated according to the formula stated below:

$$AI = AC \times 100$$

$$AC = IC$$

(AI = apoptotic index; AC = apoptotic cell count; IC = Intact cell count.)[16]

Immunohistochemistry. The renal and urinary bladder tissue sections were placed onto poly-L-lysine–coated slides. Caspase 3 activity was determined immunohistochemically according to the manufacturer's instructions (GeneTex, GTX22302, Lometa, TX). The immunoreactivity was evaluated semiquantitatively on a scale of 0-3 in all sections.

Statistical Analysis

SPSS statistical software (SPSS for Windows, version 16.0, IBM, Armonk, NY) was used to analyze the data. Kruskal-Wallis test was used to analyze the differences among the 3 groups, and multigroup comparisons were further analyzed by the Mann-Whitney *U* test. Values were expressed as mean ± standard error of the mean. A value of $P < .05$ was considered significant.

RESULTS

Tissue MDA levels were significantly higher in the CP group among all groups ($P < .05$). In rats pretreated with *Spirulina* (SP+CP group), mean MDA value in SP+CP was significantly lower compared with the CP group but was higher compared with that of the kidney and urinary bladder homogenates in the C group ($P < .05$). SOD and catalase activities were significantly lower in the CP group among all groups ($P < .05$). In the SP+CP group, SOD and catalase were significantly higher compared with the CP group but were lower compared with those of kidney and urinary bladder homogenates in the C group) ($P < .05$) (Tables 1 and 2).

Histopathologically, the kidney and the urinary bladder damages were significantly higher in the CP group among all groups ($P < .05$). They were significantly at lower levels in the SP+CP group compared with the CP group but at higher levels compared with the C group ($P < .05$) (Fig. 1, Tables 1 and 2). AI was significantly higher as shown with TUNEL assay in the CP group when compared with the other groups ($P < .05$). It was significantly lower in the SP+CP group compared with the CP group but higher compared with the C group ($P < .05$) (Figure 1a and Table 1). Similarly, the caspase 3 was most intensely stained in the CP group when compared with the other 2 groups ($P < .05$). The caspase 3 immunostaining and AI in the cortical renal tubule epithelium with TUNEL was significantly lesser in the SP+CP compared with the CP group ($P < .005$) (Table 1). Caspase 3 immunostaining and TUNEL methods showed no statistically significant difference in the urinary bladder among the C, CP, and SP+CP groups.

COMMENT

In the present study, we showed both biochemically, histopathologically and/or immunohistochemically, and/or with the TUNEL method that Cyc causes kidney and urinary bladder damage. *Spirulina* reversed the adverse effects of Cyc in the kidney and urinary bladder in rats.

Histopathologically, Cyc causes the urinary bladder inflammation in the mucosal and the suburothelial connective tissues and leads to vascular damage and subsequent bleeding.[1]

In the kidneys, acrolein and chloroacetaldehyde derived from Cyc causes cell death in the proximal tubule epithelium.[17] The cellular mechanisms for Cyc toxicity are first the direct adverse effect of acrolein to tubular epithelium and urinary bladder epithelium, and second the increased production of free oxygen radicals through intracellular phosphoramide mustard, the principal alkylating metabolite of Cyc.[15,18,19] Among the pathogenetic pathways of this toxicity, release of cytokines, growth factors, inflammatory mediators, and nitric oxide, as well as polyadenosine diphosphate-ribose polymerase, which plays a key role in cellular stress response, are significant.[20] MDA, an indicator of oxidative stress, increases in body systems after administration of Cyc and leads to the eventual destruction of membrane lipids with the formation and propagation of lipid radicals, and increased uptake of oxygen, causing rearrangement of the double bonds in unsaturated lipid.

Antioxidant enzymes protect the tissues against oxidative injury and the tissue damage occurs when these

Table 1. Effect of *Spirulina* on MDA, catalase, SOD in kidney homogenates, and the AI in the renal tubuli

Parameters/Groups	MDA (nmol/mL)	Catalase (U/g tissue)	SOD (U/g tissue)	AI (TUNEL)
C (#6)	0.82 ± 0.05	53.41 ± 3.22	42.66 ± 3.12	1.93 ± 0.31
CP (#6)	3.39 ± 0.44*	22.76 ± 4.34*	19.98 ± 1.71*	4.06 ± 0.55*
SP+CP (#6)	1.57 ± 0.30†	39.58 ± 2.51†‡	40.86 ± 0.30†	2.35 ± 0.20†

Values are expressed as mean ± SEM.
* $P < .05$, control vs CP group.
† $P < .05$, CP vs SP+CP group.
‡ $P < .05$, control vs SP+CP group.

Table 2. Effect of *Spirulina* on MDA, catalase, SOD in urinary bladder homogenates, and the intact/involved mucosa

Parameters/Groups	MDA (nmol/mL)	Catalase (U/g tissue)	SOD (U/g tissue)	Viable/Necrotic Mucosa (%)
C (#6)	0.54 ± 0.10	6.31 ± 0.83	3.68 ± 0.22	93 ± 1.37
CP (#6)	1.36 ± 0.17*	2.78 ± 0.60*	1.85 ± 0.26*	12.00 ± 3.15*
SP+CP (#6)	0.79 ± 0.16†	6.22 ± 0.73†	3.50 ± 0.15†	77.40 ± 5.55†‡

Values are expressed as mean ± SEM.
* $P < .05$, control vs CP group.
† $P < .05$, CP vs SP+CP group.
‡ $P < .05$, control vs SP+CP group.

enzymes are depleted. SOD provides the first line of defense against superoxide generated in mitochondria. It competes with nitric oxide, which is a free radical and converts superoxide anion to hydrogen peroxide. The final effect is the prevention of generation of peroxynitrite, which interacts with lipids, DNA, and proteins and subsequently commits cells to necrosis or apoptosis.[21] Thus, sufficient amounts of catalytically competent SOD are required to prevent tissue damage. In addition to SOD, catalase, another endogenous antioxidant enzyme, detoxifies the hydrogen peroxide and protects the cells from being damaged through the reactive oxygen species (ROS). A Cyc derivative, acrolein, activates intracellular ROS and nitric oxide production, which then leads to peroxynitrite production. This further causes the vital cellular pathways to collapse and result in cell death in the kidney and urinary bladder.[5] By contrast, oxidative stress initiates the apoptotic process via migration of a cellular p53 fraction, a tumor suppressor protein into mitochondria. In other words, the death signals make the pro-apoptotic bcl-2 proteins (especially Bax) allow cytochrome C to leak out of the mitochondria.[19,22] The released cytochrome *c* and apoptotic protease-activating factor-1 (Apaf-1) bind to caspase 9, an initiator caspase. Then, caspase 9 activates the further caspase cascade, including caspase 3. Caspase 3, which is an effector caspase, then cleaves its target substrates and finally leads to apoptotic cell death.[22]

The protective and improving effects of *Spirulina* on apoptosis and on oxidative stress have long been studied widely.[23] This blue-green algae has a complex structure composed primarily of two species of cyanobacteria: *Arthrospira platensis* and *Arthrospira maxima*. These eubacterial species have various components, such as B-complex vitamins, chlorophyl, β-carotene, vitamin E, superoxide dismutase, and numerous minerals. They are involved in tissue protection when ingested. C-phycocyanin, a protein-bound pigment found in *Spirulina*, accounts for >20% of its dry weight and is a 20 times more powerful antioxidant molecule than vitamin C.[24,25] C-phycocyanin inhibits oxalate-mediated lipid peroxidation and prevents renal injury.[17] It also reduces the apoptotic cell death of pancreatic β cells by preventing the overproduction of ROS and enhancing the SOD and glutathione peroxidase enzymes.[26]

In the present study, the parameters of oxidative stress, ie, MDA, was found as markedly increased and the activities of SOD and catalase enzymes were markedly decreased in the kidney and urinary bladders of Cyc-treated rats, suggesting that Cyc treatment caused oxidative damage to the lipids and proteins in these organs. In the SP+CP group, MDA levels were significantly lower than in the CP group but were higher than in the C group ($P < .05$). We believe that the pretreatment with *Spirulina* prevented the Cyc-induced elevation of MDA and restored SOD and catalase. This can be explained by the free radical scavenging properties of the *Spirulina*. Histopathologic changes in the kidney and urinary bladder were significantly reversed when the rats were pretreated with *Spirulina*. *Spirulina* reduced MDA and restored SOD and catalase values in SP+CP bladders, which were not significantly different from the values in the C group, whereas viable/necrotic cell ratio in the SP+CP group was found to be superior to the CP group and inferior to the C group, with statistically significant differences among the 3 groups. The apoptotic activity in the SP+CP group was significantly reduced compared with the CP group. On the basis of the studies showing that the synergistic action of combined antioxidants is more efficient than the activity of a single antioxidant, the antioxidants in *Spirulina*—mainly C-phycocyanin, SOD, B-complex vitamins, chlorophyl, β-carotene, and vitamin E—may act synergically to restore the antioxidant status of the tissues.[27] These compounds probably reduced the formation of the

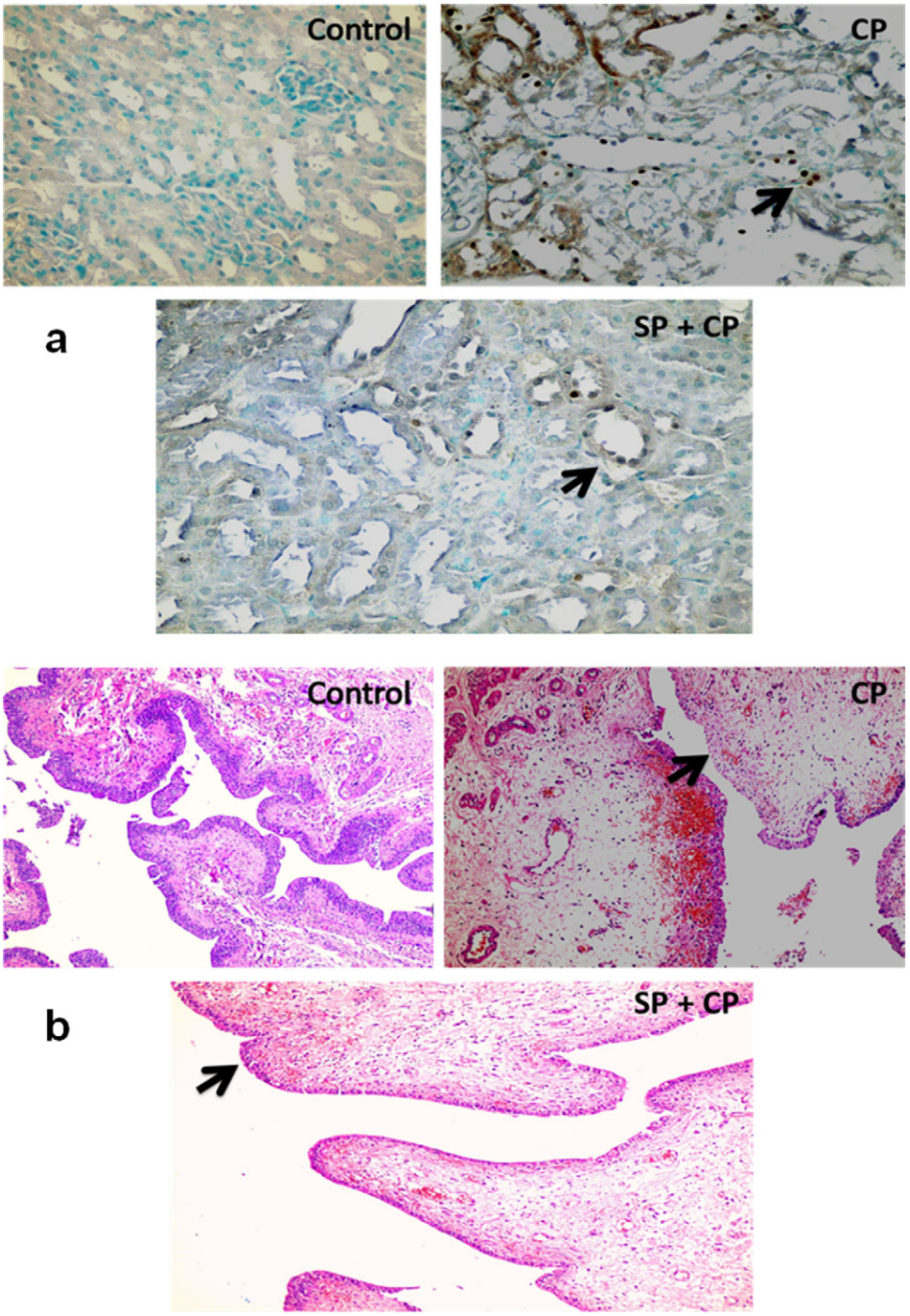

Figure 1. Effect of *Spirulina* on Cyc-induced lipid peroxidation (MDA) and changes in SOD and catalase in kidney tissue. Values are expressed as mean ± SEM (n = 6). *$P < .05$ vs C group; **$P < .05$ vs CP group.

potent oxidant peroxynitrite, which is produced by the reaction of nitric oxide with superoxide anion by scavenging the superoxide anion with SOD activity.

Although the protective effects of *Spirulina* against cisplatin- and gentamycin-induced nephrotoxicity were studied previously, it was not demonstrated that it had protective effects on Cyc-induced urinary bladder and kidney toxicities concomitantly.[12,28] The mechanism of this protection seems to be based on the complex molecular structure of the blue-green algae because it contains many antioxidants acting on the oxidative stress–stimulated apoptotic pathways.

One limitation of our study was that we did not study the serum creatinine and blood urea nitrogen levels to assess the renal dysfunction. Instead, we used histomorphologic and immunohistochemical parameters. One puzzling point for our study is based on a longstanding debate about whether the antioxidants should be used together with anticancer drugs because the former might

protect cancer cells from treatment modalities. However, there is no valid scientific evidence showing that antioxidants interfere with standard treatment methods.[29] Indeed, evidence collected from scientific studies showed that the antioxidants enhance the killing power of the chemotherapeutic agents, including that of Cyc.[30] Nevertheless, this is beyond our aim and should be the subject of another study.

CONCLUSIONS

The present study is the first to show that *Spirulina* protected Cyc-induced nephrotoxicity and hemorrhagic cystitis via its antioxidant and anti-apoptotic properties. Biochemical and ultrastructural basis and the exact mechanisms of the synergistic action of the antioxidant components of this algae may be the subject of further studies.

Acknowledgments. We thank Algbiotek Company, Istanbul, Turkey, for providing us with *Spirulina*. We also thank Baset Sigirci, the histology technician, for his kind help in histomorphologic processes.

References

1. Lawson M, Vasilaras A, De Vries A, et al. Urological implications of cyclophosphamide and ifosfamide. *Scand J Urol Nephrol*. 2008; 42(4):309-317.
2. Abraham P, Indirani K, Sugumar E. Effect of cyclophosphamide treatment on selected lysosomal enzymes in the kidney of rats. *Exp Toxicol Pathol*. 2007;59(2):143-149.
3. Mohrmann M, Ansorge S, Schmich U, et al. Toxicity of ifosfamide, cyclophosphamide and their metabolites in renal tubular cells in culture. *Pediatr Nephrol*. 1994;8:157-163.
4. Sugumar E, Kanakasabapathy I, Abraham P. Normal plasma creatinine level despite histological evidence of damage and increased oxidative stress in the kidneys of cyclophosphamide treated rats. *Clin Chim Acta*. 2007;376:244-245.
5. Korkmaz A, Topal T, Oter S. Pathophysiological aspects of cyclophosphamide and ifosfamide induced hemorrhagic cystitis; implication of reactive oxygen and nitrogen species as well as PARP activation. *Cell Biol Toxicol*. 2007;23:303-312.
6. Adams JD Jr., Klaidman LK. Acrolein-induced oxygen radical formation. *Free Radic Biol Med*. 1993;15:187-193.
7. Gomes TN, Santos CC, Souza-Filho MV, et al. Participation of TNF-alpha and IL-1 in the pathogenesis of cyclophosphamide-induced hemorrhagic cystitis. *Braz J Med Biol Res*. 1995;28(10): 1103-1108.
8. Oboh G, Akomolafe TL, Adefegha SA, et al. Attenuation of cyclophosphamide-induced neurotoxicity in rat by yellow dye extract from root of brimstone tree (Morinda lucida). *Exp Toxicol Pathol*. 2011 [Epub ahead of print].
9. Rehman MU, Tahir M, Ali F, et al. Cyclophosphamide-induced nephrotoxicity, genotoxicity, and damage in kidney genomic DNA of Swiss albino mice: the protective effect of ellagic acid. *Mol Cell Biochem*. 2012;365:119-27.
10. Belay A. The potential application of Spirulina (Arthrospira) as a nutritional and therapeutic supplement in health management. *JANA*. 2002;2:27-47.
11. Khan Z, Bhadouria P, Bisen PS. Nutritional and therapeutic potential of Spirulina. *Curr Pharm Biotechnol*. 2005;6(5):373-9.6:373-379.
12. Mohan IK, Khan M, Shobha JC, et al. Protection against cisplatin-induced nephrotoxicity by Spirulina in rats. *Cancer Chemother Pharmacol*. 2006;58:802-808.
13. Farooq SM, Asokan D, Sakthivel R, et al. Salubrious effect of C-phycocyanin against oxalate-mediated renal cell injury. *Clin Chim Acta*. 2004;348:199-205.
14. Abraham P, Isaac B, Ramamoorthy H, et al. Oral glutamine attenuates cyclophosphamide-induced oxidative stress in the bladder but does not prevent hemorrhagic cystitis in rats. *J Med Toxicol*. 2011; 7(2):118-124.
15. Abraham P, Rabi S, Selvakumar D. Protective effect of aminoguanidine against oxidative stress and bladder injury in cyclophosphamide-induced hemorrhagic cystitis in rat. *Cell Biochem Funct*. 2009;27:56-62.
16. Tunçdemir M, Demirkesen O, Oztürk M, et al. Antiapoptotic effect of angiotensin-II type-1 receptor blockade in renal tubular cells of hyperoxaluric rats. *Urol Res*. 2010;38(2):71-80.
17. Schwerdt G, Gordjani N, Benesic A, et al. Chloroacetaldehyde- and acrolein-induced death of human proximal tubule cells. *Pediatr Nephrol*. 2006;21(1):60-67.
18. Sadir S, Deveci S, Korkmaz A. Alpha tocopherol, beta carotene, and melatonin administration protects cyclophosphamide induced oxidative damage to bladder tissue in rats. *Cell Biochem Funct*. 2007;25:521-552.
19. Asiri YA. Probucol attenuates cyclophosphamide-induced oxidative apoptosis, p53 and Bax signal expression in rat cardiac tissues. *Oxid Med Cell Longev*. 2010;3:308-316.
20. Malley SE, Vizzard MA. Changes in urinary bladder cytokine mRNA and protein after cyclophosphamide-induced cystitis. *Physiol Genomics*. 2002;9:5-13.
21. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol Rev*. 2007;87(1):315-424.
22. Hussein MR. Apoptosis in the ovary: molecular mechanisms. *Hum Reprod Update*. 2005;11:162-178.
23. Chu WL, Lim YW, Radhakrishnan AK, et al. Protective effect of aqueous extract from Spirulina platensis against cell death induced by free radicals. *BMC Complement Altern Med*. 2010;10:53.
24. Marles RJ, Barrett ML, Barnes J, et al. United States Pharmacopeia safety evaluation of Spirulina. *Crit Rev Food Sci Nutr*. 2011;51:593-604.
25. Zhan PZ, Lu NL, Xiu LC, et al. Factors that affect antioxidant activity of c-phycocyanins from Spirulina platensis. *J Food Biochem*. 2005;29:313-322.
26. Muthuraman P, Senthilkumar R, Srikumar K. Alterations in beta-islets of langerhans in alloxan-induced diabetic rats by marine Spirulina platensis. *J Enzyme Inhib Med Chem*. 2009;24(6):1253-1256.
27. Gey KF. Vitamins E plus C and interacting conutrients required for optimal health. A critical and constructive review of epidemiology and supplementation data regarding cardiovascular disease and cancer. *Biofactors Biofactors*. 1998;7:113-74.
28. Karadeniz A, Yildirim A, Simsek N, et al. Spirulina platensis protects against gentamicin-induced nephrotoxicity in rats. *Phytother Res*. 2008;22(11):1506-1510.
29. Blaylock RL. A review of conventional cancer prevention and treatment and the adjunctive use of nutraceutical supplements and antioxidants: is there danger of a significant benefit? *JANA*. 2000; 3:17-35.
30. Prasad KN, Kumar A, Kochupillai V, et al. High doses of multiple antioxidant vitamins: essential ingredients in improving the efficacy of standard cancer therapy. *J Am Coll Nutr*. 1999;18:13-25.