

# Protective Effect of *Spirulina* against Doxorubicin-induced Cardiotoxicity

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The generation of reactive oxygen species and mitochondrial dysfunction has been implicated in doxorubicin (DOX)-induced cardiotoxicity. The aim of the present study was to determine whether *Spirulina*, a blue-green algae, could serve as a cardioprotective agent during DOX treatment in a mouse model. Mice were treated with DOX (4 mg/kg bw, intraperitoneally), weekly, for 4 weeks. *Spirulina* was administered orally for 3 days twice daily, then for 7 weeks along with the four equal injections of DOX. Cardiotoxicity was assessed, at 3 weeks after the end of the DOX-treatment period, by mortality, volume of ascites, liver congestion, oxidative stress and ultrastructural changes of heart tissue. The DOX-treated animals showed higher mortality (53%) and more ascites. Myocardial damage, as assessed by ultrastructural changes, showed loss of myofibrils, cytoplasmic vacuolization and mitochondrial swelling. Myocardial superoxide dismutase and glutathione peroxidase activities were decreased and lipid peroxidation was increased. Pretreatment with *Spirulina* significantly protected the mice from DOX-induced cardiotoxic effects as evidenced from lower mortality (26%), less ascites, lower levels of lipid peroxidation, normalization of antioxidant enzymes and ultrastructural studies showing minimal damage to the heart. *In vitro* cytotoxic studies using ovarian cancer cells demonstrated that *Spirulina* did not compromise the anti-tumor activity of doxorubicin. These results suggest that *Spirulina* has a protective effect against cardiotoxicity induced by DOX and it may, therefore, improve the therapeutic index of DOX. Copyright © 2005 John Wiley & Sons, Ltd.

**Keywords:** doxorubicin, *Spirulina*, cardiotoxicity, free radicals, antioxidant.

## INTRODUCTION

Doxorubicin (DOX), an anthracycline antibiotic, is used primarily in the treatment of a variety of solid tumors including hemopoietic malignancies in children and adults (O'Bryan *et al.*, 1973; Tan *et al.*, 1973). However, its use has been limited primarily due to cardiotoxicity after an acute dose as well as cumulative doses (Lefrak *et al.*, 1973; Singal and Iliskovic, 1998). The chronic cardiotoxicity is dose-dependent and causes irreversible myocardial damage, resulting in dilated cardiomyopathy with fatal congestive heart failure (Von Hoff *et al.*, 1979). The DOX-induced cardiotoxicity has been shown to be mediated through different mechanisms including free radical generation, membrane lipid peroxidation, mitochondrial damage and iron-dependent oxidative damage to macromolecules (Kalyanaraman *et al.*, 1980; Jackson *et al.*, 1984; Doroshow, 1991; Xu *et al.*, 2001). Several studies have demonstrated that DOX induces the generation of a cascade of reactive oxygen species (ROS) such as O<sub>2</sub><sup>-</sup>, ·OH and H<sub>2</sub>O<sub>2</sub>, which are implicated in the DOX-

induced cardiotoxicity (Doroshow, 1983; Doroshow and Davies, 1986; Lee *et al.*, 1991; Kalivendi *et al.*, 2001; Wang *et al.*, 2004). The semiquinone form, produced by the reduction of DOX by several endogenous enzymes, generates O<sub>2</sub><sup>-</sup> radical by transferring electrons to molecular oxygen. The superoxide radicals are rapidly transformed, either spontaneously or enzymatically, into other forms of ROS such as ·OH and H<sub>2</sub>O<sub>2</sub> (Doroshow, 1983; Doroshow and Davies, 1986; Lee *et al.*, 1991). Studies have also demonstrated an increase in the myocardial lipid peroxidation and a decrease of antioxidant enzymes in DOX-treated mice (Myers *et al.*, 1977; Naidu *et al.*, 2002). The heart is more susceptible to free radical-induced damage, because it has relatively low antioxidant enzymes such as superoxide dismutase and catalase (Doroshow *et al.*, 1980; Simmons *et al.*, 1989; Naidu *et al.*, 2002; Abou-El-Hassan *et al.*, 2003).

Antioxidants have been reported to have beneficial effects against DOX-induced cardiotoxicity in mice and rats (Speyer *et al.*, 1988; Siveski-Iliskovic *et al.*, 1994; Liu *et al.*, 2002). Antioxidants such as vitamin E provide protection from cardiac cell damage with a simultaneous decrease in lipid peroxidation (Speyer *et al.*, 1985). N-acetyl cysteine also showed protection against DOX-induced cardiotoxicity (Speyer *et al.*, 1988). Free radical scavengers such as melatonin and alpha-lipoic acid have been shown to ameliorate myocardial toxicity induced by doxorubicin (Liu *et al.*, 2002). Although the iron-chelator ICRF-187 has been

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found to be effective in protecting animals (Herman *et al.*, 1985) and patients from DOX-induced cardiotoxicity, the use of IRCF-187 is limited because, in combination with DOX, it causes hematological toxicity (Speyer *et al.*, 1992). Probucol®, a lipid-lowering drug and an antioxidant, protects animals from DOX-induced cardiotoxicity (Siveski-Iliskovic *et al.*, 1994). In our previous study, *Gingko biloba* extract, containing potent antioxidant activity, significantly protected the mice from doxorubicin-induced cardiotoxicity (Naidu *et al.*, 2002). In contrast, lazaroid, with potent antioxidant activity, did not show any significant protection against DOX cardiotoxicity in rats (Falcone *et al.*, 1998).

*Spirulina* (SP), a blue-green algae used in the daily diet of natives of Africa and America, has been found to be a rich source of proteins, carotenoids and other micronutrients (Ciferri, 1983). It is a rich source of  $\beta$ -carotene and C-phycocyanin. This blue-green algae has been used both as a dietary supplement and as a source of potential pharmaceutical applications. The extracts of *Spirulina* possess antioxidant (Miranda *et al.*, 1998), anticancer (Schwartz *et al.*, 1988), antiviral (Ayeahunie *et al.*, 1998), hepatoprotective (Gonzalez de Rivera *et al.*, 1993), immune enhancing (Qureshi *et al.*, 1996) and lipid-lowering (Iwata *et al.*, 1990) effects. Experimental studies in animal models have demonstrated an inhibitory effect of *Spirulina* in oral carcinogenesis (Mathew *et al.*, 1995). *Spirulina* has also been found to reduce the hepatic cytochrome P<sub>450</sub> content and to increase the hepatic glutathione S-transferase activity involved in the activation/detoxification of chemical mutagens/carcinogens (Mittal *et al.*, 1999). C-phycocyanin, one of the major biliproteins present in *Spirulina*, is known to possess significant antioxidant and free-radical scavenging properties (Bhat and Madyastha, 2000). C-phycocyanin effectively inhibits carbon tetrachloride-induced lipid peroxidation in rat liver and prevents hepatotoxicity (Vadiraja *et al.*, 1998). It also inhibits oxalate-mediated lipid peroxidation and renal cell injury in rats (Farooq *et al.*, 2004). The marked protective effect of *Gingko biloba* extract and CardiPro®, a polyherbal extract containing antioxidants, against doxorubicin-induced cardiotoxicity in mice was reported previously (Naidu *et al.*, 2002; Mohan *et al.*, 2005). The aim of the present study was to evaluate the potential role of *Spirulina* as a cardioprotective agent during doxorubicin treatment in mice using biochemical and histomorphological parameters indicative of cardiotoxicity and oxidative stress. The effect of *Spirulina* on the antitumor activity of DOX was also examined *in vitro* in order to evaluate the interference of *Spirulina* with the antitumor activity of DOX.

## MATERIALS AND METHODS

*Spirulina*, a fine dark blue-green spray-dried powder, was prepared from *Spirulina platensis* (Batch No 0473/02-03, New Ambadi Estates, Tamil Nadu, India). The composition of the *Spirulina*, used in our experiments, was made up of proteins (65.38%), crude phycocyanin (15.37%), minerals (7.95%), total carotenoids (4.3 mg/g),  $\beta$ -carotene (1.67 mg/g) and total pheophorbide (0.02%). Pure C-phycocyanin was obtained from Sigma Chemicals, St Louis, MO.

**Experimental protocol.** Female Swiss albino mice (weight 30–40 g) were housed under conditions of controlled temperature and a 12 h lighting cycle and fed with standard diet *ad libitum*. The animals were divided into four groups of 20 animals each. The control animals received only normal saline once weekly for 4 weeks. The second group received four equal injections (each containing 4 mg/kg bw DOX) intraperitoneally, once weekly for 4 weeks (cumulative dose 16 mg/kg) and the third group received *Spirulina* (250 mg/kg bw) orally, twice a day for 7 weeks. The fourth group received *Spirulina* (250 mg/kg bw) orally, twice daily for 3 days, then for 7 weeks along with four injections of DOX similar to the second group. All animals were observed 3 weeks after the last injection of DOX for changes in body weight, general appearance and mortality. The surviving animals were killed and the heart tissues were evaluated for lipid-peroxidation products, antioxidant enzymes, total antioxidant activity and morphological appearance. The study was approved by Institutional Ethics Committee for the use of laboratory animals.

**Lipid-peroxidation products.** Myocardial tissue was homogenized (10% w/v) in PBS (pH 7.4) containing 20 mmol/L butylated hydroxytoluene. The homogenate was centrifuged at 3000 rpm for 10 min and the supernatant was mixed with equal volumes of 20% trichloroacetic acid, vortexed vigorously and centrifuged at 5000 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 (Bernheim *et al.*, 1948). Freshly diluted tetramethoxy propane (Sigma Chemical) was used as the standard and data was expressed in nmol MDA/g of heart tissue.

**Estimation of antioxidant enzymes.** Superoxide dismutase (SOD) activity was determined spectrophotometrically according to the reported method (McCord and Fridovich, 1969). The 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 as units/mg protein. Glutathione peroxidase activity was measured by the NADPH oxidation method (Paglia and Valentine, 1967) and expressed as nmol NADPH oxidized to NADP/mg protein. Protein was determined by method of Lowry *et al.* (1955).

**Total antioxidant activity.** The total antioxidant activity in plasma and heart tissue homogenate was measured by ABTS decolorization assay (Re *et al.*, 1999). The pre-formed radical monocation of 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS<sup>•+</sup>) is generated by oxidation of ABTS (Sigma Chemical Co, USA) with potassium persulfate and is reduced in the presence of hydrogen-donating antioxidants. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Aldrich Chemical Co., Gillingham, Dorset, UK), a water soluble vitamin E analog was used as a standard.

**Histopathology of heart.** Hearts from mice of all the groups were fixed in 10% buffered formalin and

embedded in paraffin. Sections (5  $\mu\text{m}$ ) were stained with hematoxylin and eosin and were examined under a light microscope by a histopathologist who did not know the details of the treatment groups.

**Ultrastructural heart examination.** Hearts from mice treated with DOX in the presence or absence of *Spirulina* were fixed with 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, at room temperature for 3 h and were processed as described previously (Naidu *et al.*, 2002). Samples were observed under a JEOL 1000 CX electron microscope. At least 2 to 3 tissue blocks from each treatment group were taken for ultrastructural study.

**Determination of superoxide-scavenging property of *Spirulina* and C-phycoyanin by EPR spectroscopy.** Xanthine (X)-xanthine oxidase (XO) system was used to generate superoxide radical and the scavenging activity of *Spirulina* was determined by a spin-trapping EPR technique using DMPO as a spin trap. The assay medium contained X (0.5 mM), XO (0.01 U/mL) DTPA (100  $\mu\text{M}$ ), DMPO (40 mM) in PBS at pH 7.4, in the absence and presence of SOD (200 U/mL), *Spirulina* (10–100  $\mu\text{g}/\text{mL}$ ) and C-phycoyanin (10–75  $\mu\text{M}$ ). The DMPO-OH adduct formation was measured at 10 min after the addition of XO using a Bruker ER 300 EPR spectrometer operating at X-band with a TM110 cavity and a quartz flat cell. The quantification of the DMPO-OH adduct was done as described previously using TEMPO (1  $\mu\text{M}$ ) as a standard measured under identical conditions (Roubaud *et al.*, 1997).

**Effect of *Spirulina* on the antitumor activity of doxorubicin, *in vitro*.** The *in vitro* study used human ovarian cancer (HOCCs) cells, maintained in RPMI-1640 medium with 5% fetal-calf serum. The cells were seeded into 96-well plates at a starting density of  $2.5 \times 10^4$  cells/well and cultured overnight in a phenol red-free RPMI-1640 medium with 5% fetal-calf serum at 37 °C humidified with 5% CO<sub>2</sub>. The following day, doxorubicin (10 and 50  $\mu\text{M}$ ) and/or *Spirulina* (50  $\mu\text{g}/\text{mL}$ ) was added to the medium. Then 24 h 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 thrice.

**Statistical analysis.** Results are presented as mean  $\pm$  SD. The significance of the difference among the four groups was assessed using one-way ANOVA and Tukey's HSD test to identify differences between the groups. Statistical significance was set at  $p < 0.05$ .

## RESULTS

The general appearance of mice from all four groups was observed daily after the treatment with DOX. At the end of the treatment period, the surviving mice in the DOX-treated groups appeared sick, weak, lethargic and had weight loss and ascites. However, these symptoms were less severe in the group of mice treated with DOX + *Spirulina*. In addition, the liver was enlarged and congested in all the mice treated with DOX alone. During the 3-week post-treatment period, the mortality was significantly higher in the DOX group (53%) compared with the *Spirulina* + DOX group (26%) as shown in Table 1. No deaths were observed in the group treated with *Spirulina* only or in the untreated control group. Treatment with DOX resulted in a significant decrease in heart weight (38%) and the heart-to-body weight ratio (27%) compared with the control. In the *Spirulina* + DOX group, the heart-to-body weight ratio was similar to that of control and *Spirulina*-treated groups. Animals surviving in the DOX group developed marked ascites with a mean ascites fluid volume of  $2.05 \pm 0.58$  mL (Table 1), whereas less ascites fluid ( $0.91 \pm 0.25$  mL) was observed in the *Spirulina* + DOX group.

### Biochemical measurements

MDA was measured as a marker of lipid peroxidation and an indicator of oxidative injury. The MDA levels in heart tissue were increased significantly in the DOX-treated group compared with the untreated control group. The increase in MDA by DOX was significantly attenuated by *Spirulina* (Table 2). The total antioxidant activity, as a measure of antioxidant status, was significantly decreased in the heart tissue of the DOX-treated group. In the *Spirulina* + DOX group, the total antioxidant activity was significantly increased compared with the control and the DOX groups (Table 2). Different antioxidant enzymes were examined in the heart tissue from all the groups and the data are shown in Table 2. The DOX-treated mice showed a significant decrease in SOD and glutathione peroxidase levels compared with the controls. The DOX-induced decrease in SOD and glutathione peroxidase levels was attenuated by *Spirulina*.

### Morphological study

Electron and light microscope analyses of the left ventricles of all four groups were carried out. Light microscopic examination of hearts stained with hematoxylin and eosin of the control and *Spirulina*-treated animals displayed a normal morphological appearance, whereas

**Table 1.** Effect of *Spirulina* on doxorubicin-induced changes in mortality, heart weight and ascites

Parameter	Control	DOX	<i>Spirulina</i>	DOX + <i>Spirulina</i>
Mortality (%)	0%	53%	0%	26%
Ascitic fluid (mL)	0	$2.05 \pm 0.58^a$	0	$0.91 \pm 0.25^b$
Heart weight (mg)	$166 \pm 25$	$101 \pm 15^a$	$164 \pm 23$	$146 \pm 30^b$
Heart/body wt ratio ( $\times 10^2$ )	$5.21 \pm 0.10$	$4.20 \pm 0.28^a$	$5.19 \pm 0.47$	$4.98 \pm 0.48^b$

All values are expressed as mean  $\pm$  SD. <sup>a</sup>  $p < 0.05$  vs control; <sup>b</sup>  $p < 0.05$  vs DOX.

**Table 2. Effect of doxorubicin (DOX) and *Spirulina* on myocardial lipid peroxidation, antioxidant activity and antioxidant enzymes**

Parameter	Control	DOX	<i>Spirulina</i>	DOX + <i>Spirulina</i>
MDA (nmol/g of heart tissue)	18.7 ± 2.6	31.3 ± 4.1 <sup>a</sup>	19.0 ± 2.3	25.8 ± 1.7 <sup>b</sup>
Total antioxidant activity				
Plasma (nmol/L)	1.39 ± 0.29	0.90 ± 0.11 <sup>a</sup>	1.51 ± 0.19 <sup>b</sup>	1.12 ± 0.15
Heart tissue (nmol/mg protein)	1.64 ± 0.32	1.20 ± 0.22 <sup>a</sup>	1.86 ± 0.24	1.68 ± 0.32 <sup>b</sup>
SOD (U/mg protein)	37.8 ± 1.6	29.0 ± 3.1 <sup>a</sup>	36.2 ± 1.4	38.0 ± 2.4 <sup>b</sup>
Glutathione peroxidase (nmol/mg protein)	56.6 ± 4.0	41.1 ± 2.8 <sup>a</sup>	58.1 ± 5.9	52.5 ± 6.3 <sup>b</sup>

All values are expressed as mean ± SD. <sup>a</sup>  $p < 0.05$  vs control; <sup>b</sup>  $p < 0.05$  vs DOX.

the hearts of the DOX-treated animals showed myocardial degeneration including the loss of myofibrils and focal cytoplasmic vacuolization (Fig. 1b). In mice treated with *Spirulina* + DOX, a significant reduction in the severity of myocardial degeneration was noted (Fig. 1c). Electron microscopic analysis of the ventricles showed that administration of DOX induced significant morphological changes in the heart structure. Typical DOX-induced cardiomyopathy included swelling of mitochondria, loss of myofibrils and damage to the sarcoplasmic reticulum and sarcomere (Fig. 2b). The ultrastructure of the hearts of the *Spirulina* + DOX treated mice showed only minimal changes compared with the untreated control mice (Fig. 2c).

#### Scavenging of superoxide anion by *Spirulina* and C-phycoyanin

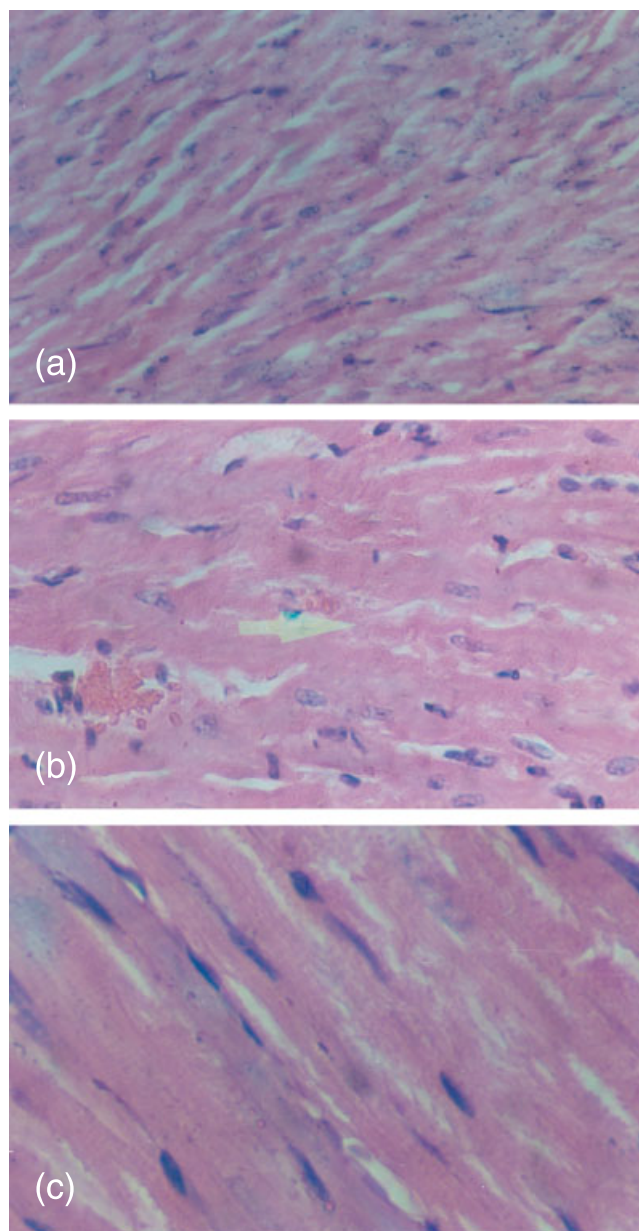
The effect of *Spirulina* (10–100 µg/mL) and C-phycoyanin (10–75 µM) was studied for their ability to scavenge the superoxide generated by the X+XO system. The superoxide was measured by EPR spectroscopy using the DMPO spin-trapping technique. As shown in Fig. 3, the DMPO-adduct formed from the X/XO-generated superoxide anion was decreased significantly by the addition of *Spirulina* in a concentration dependent manner. Similarly, C-phycoyanin, one of the main constituents of *Spirulina*, was also able to decrease the DMPO adduct significantly. The DMPO adduct formed was completely inhibited by SOD confirming that the adduct formed was from superoxide.

#### Anticancer effect of doxorubicin in the presence of *Spirulina*

To evaluate whether *Spirulina* could modify the chemotherapeutic efficacy of DOX, the effect of *Spirulina* on the DOX-induced cell killing in human ovarian cancer cells was investigated *in vitro*. The results, shown in Fig. 4, demonstrated that the cell survival was reduced with an increased dose of DOX and the co-administration of DOX with *Spirulina* had no significant effect on DOX-induced cell death. These results suggest that the antitumor activity of DOX was not compromised by *Spirulina*.

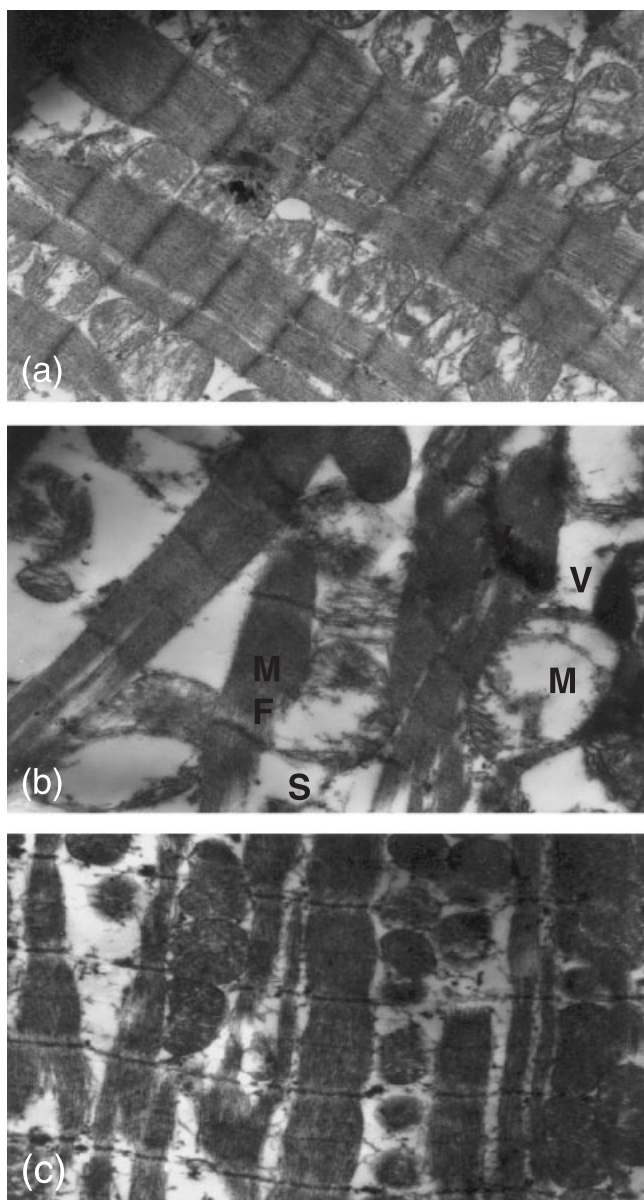
## DISCUSSION

Doxorubicin-induced cardiac toxicity has been demonstrated in cultured cells (Chan *et al.*, 1996), isolated



**Figure 1.** Histopathological examination of mouse hearts (H&E × 60). (a) Control mice showing normal morphological appearance, (b) DOX-treated mice showing focal fibrillar loss and cytoplasmic vacuolization, (c) DOX + *Spirulina* treated mice showing minimal focal fibrillar loss.

heart preparations (Repine, 1991), whole animal models (Singal *et al.*, 1987; Pouna *et al.*, 1996), and in humans (Lefrak *et al.*, 1973; Ferrans, 1978; Mazzarello and Morra, 1998; Bu'Lock *et al.*, 1999). Previous reports showed that the free radicals and peroxy-nitrite



**Figure 2.** Electron photomicrograph of mouse hearts. Ultrastructure of myocardial tissue from (a) control mice showing normal morphology; (b) doxorubicin-treated mice showing myofibril loss (MF), swelling of mitochondria (M), vacuolization of cytoplasm (V) and dilation of sarcotubular system (S); (c) *Spirulina* plus doxorubicin-treated mice showing occasional intracytoplasmic vacuoles. The results show that *Spirulina* protected the DOX-induced myocardial ultrastructural alterations.

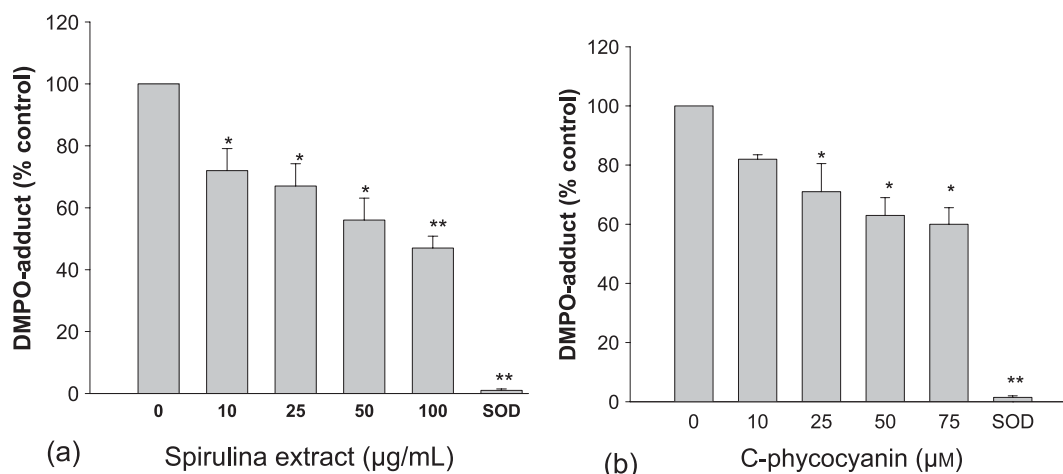
formed by DOX interact with the phospholipids of the cell membrane resulting in membrane damage (Kalyanaraman *et al.*, 1980; Kalyanaraman *et al.*, 1984; Sterrenberg *et al.*, 1984). Tissues with less-developed antioxidants, such as in the heart, are particularly susceptible to injury by DOX-induced free radical generation (Olson and Mushlin, 1990). Several reports also suggested that DOX-induced apoptosis plays an important role in the cardiotoxicity and that the apoptosis is linked to the formation of ROS (Kalyanaraman *et al.*, 1980). Many attempts have been made to ameliorate DOX-induced cardiotoxicity using antioxidants (Herman *et al.*, 1985; Speyer *et al.*, 1985; Speyer *et al.*, 1988; Siveski-Iliskovic *et al.*, 1994; Liu *et al.*, 2002). Although several antioxidants showed

promising effects in reducing the DOX-induced cardiotoxicity, to date none has been shown to act selectively at the site of toxicity, the heart. The present study was designed to increase the levels of antioxidants thereby aiming to overcome free radical-induced cardiac tissue damage.

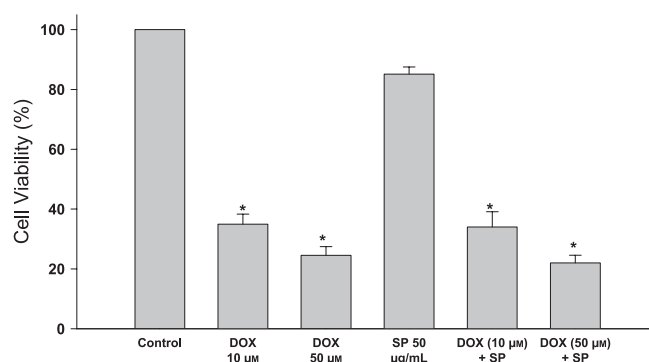
In this study, it was observed that a cumulative dose of DOX (16 mg/kg) induced cardiotoxicity in mice as evidenced from an increase in mortality, accumulation of ascitic fluid, decrease in heart weight and loss of cardiac myocytes. Treatment of *Spirulina* ameliorated the cardiotoxic effects of DOX and protected the heart from oxidative stress-induced damage. The protective effect of *Spirulina* could be due to the presence of antioxidants such as  $\beta$ -carotene and C-phycoyanin.

A large body of evidence points towards the formation of oxygen free radicals which can damage the cells by lipid peroxidation (Horenstein *et al.*, 2000). Heart homogenates from rats treated with DOX showed a significant degree of lipid peroxidation as observed by an increase in MDA levels. *Spirulina* inhibited this effect and significantly lowered the levels of lipid peroxidation induced by DOX. The tissue levels of antioxidant enzymes SOD and glutathione peroxidase were decreased in DOX-treated animals. However, in *Spirulina*-treated animals, SOD and glutathione peroxidase levels were restored to normal. The total antioxidant activity in heart tissue was significantly decreased in DOX-treated animals, whereas in *Spirulina*-treated animals, the total antioxidant activity in heart tissue was significantly high compared with untreated control and DOX treated animals. This could be due to the presence of antioxidants such as  $\beta$ -carotene and C-phycoyanin in *Spirulina*. The result of this study indicates that *Spirulina* can protect the cardiac myocytes against oxidative stress induced by DOX and confirms the earlier findings showing that induction of oxidative stress and lipid peroxidation are among the basic mechanisms responsible for the cardiotoxicity (Myers *et al.*, 1977; Singal *et al.*, 1987; Nowak *et al.*, 1995).

Previous studies have demonstrated that *Spirulina* exhibits antioxidant property in various oxidative conditions that cause tissue injury (Mathew *et al.*, 1995; Miranda *et al.*, 1998; Premkumar *et al.*, 2001; Upasani *et al.*, 2001). The extract of *Spirulina* has been shown to be effective in free radical-induced lipid peroxidation by lead and to have protective activity in the major organs including the heart (Upasani *et al.*, 2001). It has been well established that C-phycoyanin, one of the major biliproteins of *Spirulina*, possesses significant antioxidant and radical scavenging properties (Bhat and Madyastha, 2000). It was also found that both *Spirulina* and C-phycoyanin possess antioxidant activity as evidenced from the scavenging of superoxide measured by EPR spin-trapping technique. *Spirulina* has been shown to not only scavenge peroxy, hydroxyl (Bhat and Madyastha, 2000; Romay and Gonzalez, 2000), superoxide (Romay *et al.*, 1998) and peroxy nitrite (Bhat and Madyastha, 2001), but also acts as a potent antioxidant and inhibits lipid peroxidation mediated by ROS (Bhat and Madyastha, 2000). Previous studies have shown that C-phycoyanin has the ability to chelate metals including free iron (Bhat and Madyastha, 2000). These results indicate that the cardioprotective effect of *Spirulina* could also be due to the scavenging of hydroxyl and peroxy nitrite and chelating of free iron



**Figure 3.** Effect of *Spirulina* and C-phycoerythrin on the scavenging of superoxide radicals generated by xanthine (X)-xanthine oxidase (XO) system measured by EPR spectroscopy. The assay medium contained X (0.5 mM), XO (0.01 U/ml), DTPA (100 µM), DMPO (40 mM) in PBS (pH 7.4), in absence and presence of SOD (200 U/ml), (a) spirulina (10–100 µg/ml), (b) phycoerythrin (10–75 µM). Values are expressed as Mean ± SD ( $n = 4$ ). \*  $p < 0.05$  vs control; \*\*  $p < 0.01$  vs control. The results show that *Spirulina* and C-phycoerythrin significantly decreased the DMPO-adduct in a concentration dependent manner.



**Figure 4.** Effect of *Spirulina* on the antitumor potency of DOX. Human ovarian cancer cells were plated in 96-well plates and allowed to attach overnight. The cells were exposed to DOX and/or *Spirulina* for a period of 24 h and cell proliferation was determined by MTT assay as per the procedure described in methods. Control cell growth was set at 100%. Values are expressed as mean ± SD ( $n = 4$ ). \*  $p < 0.05$  vs control. The results show that *Spirulina* does not compromise the antitumor effect of DOX.

that are key mediators of DOX-induced cardiotoxicity (Speyer *et al.*, 1988; Pacher *et al.*, 2003).

Many compounds possessing antioxidant properties are able to protect the myocardium against DOX-induced cardiotoxicity. Amifostine by virtue of its potential to scavenge oxygen radicals has been shown to reduce DOX-associated cardiotoxicity in cultured neonatal myocytes (Dorr *et al.*, 1996) and in mice (Bhanumathi *et al.*, 1992). In our recent study, it was shown that *Ginkgo biloba* extract with its potent antioxidant activity protected mice from DOX-induced cardiotoxicity (Naidu *et al.*, 2002). Administration of CardiPro®, a polyherbal preparation containing antioxidants, reduced DOX-induced mortality in mice (Mohan *et al.*, 2005). Similarly, Probuco®, a lipid-lowering drug and antioxidant and melatonin, a potent antioxidant, have also been shown to reduce DOX-induced mortality in rats (Siveski-Iliskovic *et al.*, 1994; Morishima *et al.*, 1998). Both *in vitro* and *in vivo* studies have shown that vitamin A is a potent membrane lipid antioxidant and provides protection against cardiac

tissue damage by DOX (Tsuchiya *et al.*, 1992; Ciaccio *et al.*, 1993). In support of a primary role of free radicals in DOX toxicity, the over-expression of MnSOD (Yen *et al.*, 1996), metallothionein (Kang *et al.*, 1997), catalase (Abou-El-Hassan *et al.*, 2003) and lecithinized Cu-Zn-SOD (den Hartog *et al.*, 2004) have been shown to be cardioprotective in DOX-treated mice.

The present study demonstrates that *Spirulina* inhibits ultrastructural alterations induced by DOX in many organelles, including the disruption of the mitochondrial fine structure. Myocardial damage is specific to all anthracycline antibiotics, including myofibrillar degeneration, mitochondrial dilatation, cellular vacuolization and, finally, myocyte dropout (Billingham *et al.*, 1978). As seen in the present study, DOX treatment caused significant histological changes including marked myofibrillar loss and cytoplasmic vacuolization. In mice treated with *Spirulina*, these DOX-induced histological changes were minimal, suggesting protection from cellular damage by DOX. The changes demonstrated microscopically in the DOX-treated group were similar to those observed by us and others (Zbinden and Brandle, 1975; Danesi *et al.*, 1992; Naidu *et al.*, 2002). The reversal of DOX-induced ultrastructural changes and the restoration of antioxidant status by *Spirulina* demonstrated that *Spirulina* could ameliorate the DOX-induced myocardial damage. A reduction in scores of myocardial lesions-induced by DOX was also reported with Venoruton and ICRF-187 (van Acker *et al.*, 1993). It was shown that *Spirulina* preserved the normal myocardial structure and the histomorphological results supported our hypothesis that *Spirulina* protects DOX-induced cardiotoxicity.

A main prerequisite for any compound to be used as a cardioprotective agent during the treatment of cancer is that it should not interfere with the antitumor activity of the chemotherapy. Our *in vitro* studies with human ovarian cancer cells showed that *Spirulina* did not interfere with the inhibition of cell proliferation induced by DOX. Furthermore, tumor cells treated with *Spirulina* alone showed some level of cytotoxicity suggesting that *Spirulina* may also have some antitumor properties. This conforms with several studies in

animals and in humans that demonstrated that *Spirulina* has anticancer properties (Schwartz and Shklar, 1987; Schwartz *et al.*, 1988). *Spirulina* extracts have been shown to inhibit buccal cancers in animal models (Schwartz and Shklar, 1987; Schwartz *et al.*, 1988; Shklar and Schwartz, 1988) and DMBA-induced buccal squamous cell carcinoma (Schwartz *et al.*, 1988). Oral supplementations of *Spirulina* in humans prevented oral cancer (Mathew *et al.*, 1995). *Spirulina* was also reported to act as an antimutagen against cyclophosphamide and mitomycin-C (Mathew *et al.*, 1995).

In summary, our study, for the first time, demonstrates that *Spirulina* ameliorates DOX-induced

cardiotoxicity in mice. This effect could be due to the presence of the antioxidant components, C-phycoerythrin and  $\beta$ -carotene. Furthermore, *Spirulina* does not compromise the antitumor effect of DOX. The combined treatment of DOX and *Spirulina* holds promise as a safe and effective chemotherapeutic strategy.

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