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Protective effect of *Spirulina platensis* on cadmium induced renal toxicity in wistar rats

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Abstract

Spirulina, filamentous blue green mycobacterium belongs to the family Oscillatoriaceae and is generally known as a valuable additional food and medicinal, nutraceutical as well as a therapeutic agent. Besides, *Spirulina platensis* also possess potent antiviral, antimutagenic, anticancerous and cholesterol lowering activity. But, to date, there is no study demonstrating the protective effect of *Spirulina platensis* on cadmium induced nephrotoxicity. Protective effect of oral administration of *Spirulina platensis* extract (1000mg/5ml/kg, once daily) on cadmium (2mg/kg, *subcutaneously*, 15 days) induced renal toxicity was investigated in albino rats. Renal injury was assayed by measuring serum creatinine and serum urea. Renal oxidative stress was determined by renal thiobarbituric acid reactive substance levels, enzymatic activity of superoxidase dismutase and glutathione peroxidase. Statistically significant amelioration in all the serum and biochemical parameters supported by significantly improved renal cortical histology was observed in the *Spirulina platensis* treated nephrotoxic rats. It is suggested that some ingredients contained in the extract of *Spirulina platensis* effected in ameliorating the signs of nephrotoxicity and that the specific active principle of the *Spirulina platensis* responsible for this amelioration if obtained, would be more useful.

Key words: Cadmium, *Spirulina platensis*, Oxidative stress, Histology, Renal dysfunction

Introduction

Cadmium is a nonessential heavy metal, and serious environmental and industrial pollutant. Cadmium exposure such as working with cadmium-containing pigments, plastics, glass, metal-alloys, electrode material in nickel-cadmium batteries and non-occupational exposure such as food, water and cigarette smoke induces uptake of cadmium from the environment into the body through pulmonary and eternal pathways [1-4]. Cadmium accumulates in the kidneys. Human

kidney concentrations of cadmium have increased several folds during the last century [5]. Cadmium in pig kidney has been shown to have increased by about 2% per year [6]. Superoxide dismutase (SOD), Catalase and Glutathione peroxidase (GPx) are the enzymes that provide cellular protection against the damage caused by free radicals and reactive oxygen species (ROS). Measurement of these enzyme activities is an indirect and noninvasive method that could be used to assess oxidative stress [7]. Cadmium generates ROS which depletes endogenous ROS scavengers. ROS also damage a variety of transport proteins, including Na^+ / K^+ , ATPase, which are subsequently degraded by the endolysosomal proteases [8, 9]. Long term exposure to Cd increased lipid peroxidation and caused inhibition of SOD activity indicating oxidative damage in liver, kidney and testes. The increase in lipid peroxidation may be attributed to alterations in the antioxidant defense system. This defense system includes the enzymes *viz.*, glutathione peroxidase, glutathione-S-transferase, superoxide dismutase, catalase as well as glutathione, which normally protect against radical toxicity.

Vitamin C and Vitamin E are the primary components of the antioxidant system [10, 11] and Vitamin E is one of the major membrane protectants against ROS and lipid peroxidation. Until now, the studies regarding treatment of cadmium toxicity are restricted mainly to some sulfhydryl containing chelating agents, such as meso 2,3-dimercaptosuccinic acid (DMSA), and 2,3-dimercaptopropane-1-sulfonate (DMPS) or British Anti Lewisite (BAL; 2,3-dimercaprol) [12] administered either individually or in combination with few antioxidants such as Vitamin C, Vitamin E [13, 14] N-acetyl cysteine [15] and some micronutrients like zinc and selenium [16]. Most of the conventional metal chelating agents and antioxidants have been reported to possess toxic side effects [17]. Thus, there has been an increased interest in the therapeutic potential of plant products and medicinal plants having antioxidant properties in reducing free radical-induced tissue injury [18-20].

In recent years, *Spirulina* is gaining more attention from medical scientists as a nutraceutical and source of potential pharmaceutical. *Spirulina* is considered as a valuable additional food source of some macro and micronutrients including amino acids, chlorophyll, gamma linolenic acid, carotenoids, vitamins B₁ and B₂ and trace elements such as iron, iodine, selenium and zinc [21, 22]. It is rich in all the three types of nutrients *viz.*, proteins, lipids and carbohydrates and some vital elements such as zinc, magnesium, and selenium, and vitamins including β -carotene, riboflavin, cynocobalamin, and α -linolenic acid [23]. In addition *Spirulina* has also been reported to have biosorption capacity for cadmium and lead which enhance the *Spirulina*'s effectiveness to remove cadmium and lead from waste water. Recently, it was demonstrated to prevent lipid peroxidation and restored levels of endogenous antioxidants in liver, lungs and heart of cadmium exposed animals [24]. However, no attention has been paid so far to explore its renoprotective activity in animals and human beings, therefore the present paper reports protective effect of *Spirulina platensis* on cadmium induced renal toxicity in Wistar rats.

Materials and methods

Animals

A total of 32 male Wistar rats (14-16 weeks old, 210±10 g) were obtained from the Defense Research and Development Establishment (DRDE) animal facility, Gwalior (India). Rats were housed in a temperature control room (22±28°C) with a 12:12 light: dark cycle; water and food

were given *ad libitum*. All experiments were performed according to the norms of the local ethical committee.

Drugs

Cadmium chloride was obtained from Merck India Ltd. and suspended in 0.9% NaCl. Powdered *Spirulina platensis* was obtained commercially from the Sigma Chemical Co. India, and was suspended in distilled water.

Experimental protocol

Animals were divided into the following four groups with eight rats in each group. Group I consisted of control animals that were given 0.9% NaCl orally. Group II animals received single dose of CdCl₂ 2mg/kg in 0.9% NaCl subcutaneously. Group III animals were given *Spirulina platensis* extract (1000mg/5ml/kg, orally) in distilled water and Group IV were treated with *Spirulina* extract (1000mg/5ml/kg) and cadmium chloride, concomitantly for 15 consecutive days.

After the completion of treatment, animals were sacrificed under light ether anaesthesia. Blood samples were collected by cardiac puncture and the organs of interest were taken out for biochemical assays. The kidney and liver were removed, washed in 0.25 M sucrose solution and weighed. A 10% tissue homogenate was prepared in 0.25M sucrose by a motor driven Teflon pestle glass homogenizer. The tissue homogenate was centrifuged at 10,000xg for 15 min at 4°C to remove the cell debris and then the supernatant was collected and used for various assays.

Assessment of Renal Functions

The concentration of creatinine and urea in serum was measured using commercial kit (Ranbaxy, India Ltd.).

Assessment of Oxidative Stress

Estimation of Lipid peroxidation

Tissue lipid peroxidation was measured by the method of Onkawa [25]. Tissue homogenate was incubated with 8.1% SDS (w/v) for 10 min followed by addition of 20 % acetic acid (pH 3.5). Reaction mixture was incubated with 0.6% TBA (w/v) for 1hr in boiling water bath. Pink color chromogen so formed was extracted in butanol/ pyridine (15:1) solution and read at 532 nm. The amount was calculated using a molar extinction coefficient of 1.56×10^5 M/cm.

Estimation of Superoxide dismutase

Tissue superoxide dismutase was assayed by the method of Kakkar [26]. Reaction mixture contained 1.2ml of (0.052 mM) sodium pyrophosphate buffer, 0.1ml of (186 μM) phenazine methosulfate and 0.3ml of nitro blue tetrazolium (300 μM). Reaction was initiated by adding 0.2ml of NADH (780 μM) and stopped by the addition of 1ml glacial acetic acid. Color intensity of the chromogen was measured at 560 nm and activity was expressed as units/min mg protein.

Estimation of Glutathione peroxidase

Glutathione peroxidase activity was measured by the procedure of Flohe and Gunzler [27]. Reaction mixture consisted of 0.3 ml of phosphate buffer (0.1 M, pH 7.4) 0.2ml of GSH (2 mM), 0.1ml of sodium azide (10 mM), 0.1ml of H₂O₂ (1 mM) and 0.3 ml of tissue homogenate was

incubated for 15 min at 37°C. Reaction was stopped by addition of 0.5ml of TCA (5%). The mixture was centrifuged at 1500 x g for 5 min and to the supernatant 0.7 ml of DTNB (0.4 mg/ml) and 0.2 ml of phosphate buffer (0.1 M, pH 7.4) was added. After vortexing absorbance was recorded at 420nm.

Histopathological examination

For microscopic evaluation, kidneys were fixed in 10% formalin for 24 h, and standard dehydration in ascending series of ethanol (70, 80, 95, and 100%). Tissue samples were then cleared in xylene and embedding in paraffin-wax. Sections (5 µm) were cut in a microtome and stained with hematoxylin and eosin (H-E).

Statistical analysis

The data are presented as mean ± S.E.M. value. Number of animals per group stated in the table or figure legends. One way analysis of variance (ANOVA) followed by Student-Newman-Keuls test was used to analyze mean differences between experimental groups for each parameter separately after ascertaining the homogeneity of variance between treatment groups by Bartlett's test.

Results

Biochemical analysis

Treatment with cadmium significantly ($p < 0.0001$) increased the activities of serum urea and creatinine (128 and 109% respectively) compared to the control. Administration of *Spirulina* alone did not show any significant change in the serum levels of these enzymes whereas, concomitant treatment with *Spirulina* attenuated the cadmium induced increase in serum urea (100%; $p < 0.0001$) and creatinine (105%; $p < 0.0001$) compared to their levels in cadmium treated groups (Table. 1).

Table 1. Cadmium induced changes in serum urea and serum creatinine and their response to administration of *Spirulina platensis* (SP) in rats

Groups	Treatment	Serum Urea(mg/dl)	Serum Creatinine (mg/dl)
I	Control	29.69±0.59	0.75±0.009
II	Cadmium	42.19±2.03**	0.93±0.001***
III	SP	28.86±0.45 ^{ns}	0.75±0.014 ^{ns}
IV	Cd + SP	29.83±1.27 [#]	0.77±0.023 [#]

Values are expressed means ± SE ; * $p < 0.01$, ** $p < 0.0001$, ns (non significant) compared with control; # $p < 0.0001$ compared with cadmium (Cd) treated animals.

TBARS level was increased significantly ($p < 0.0001$) by CdCl₂ administration compared with control group. Concomitant administration of *Spirulina platensis* was very effective in the prevention of oxidative damage induced by cadmium which resulted in significantly lower LPO. Treatment with cadmium significantly decreased the superoxide dismutase ($p < 0.01$) and glutathione peroxidase ($p < 0.0001$) levels while this reduction was significantly ($p < 0.0001$) and alleviated by concomitant treatment with *Spirulina platensis* (Table. 2).

Table. 2. Cadmium induced changes in renal oxidative stress parameters and their response to administration of *Spirulina platensis* (SP) in rats

Groups	Treatment	TBARS (nmol MDA/g)	SOD (units/ min mg protein)	GPx ($\mu\text{g}/\text{min}/\text{mg}$ protein)
I	Control	13.82 \pm 0.61	24.16 \pm 1.59	13.55 \pm 0.92
II	Cadmium	26.67 \pm 0.48 ^{**}	11.20 \pm 1.19 [*]	4.88 \pm 0.30 ^{**}
III	SP	13.32 \pm 0.34 ^{ns}	30.02 \pm 2.64 ^{ns}	10.23 \pm 1.43 ^{ns}
IV	Cd+SP	19.73 \pm 0.69 [#]	32.69 \pm 1.82 [#]	8.71 \pm 0.08 [#]

Values are expressed means \pm mean.; p<0.001, ^{**}p<0.0001, ns (non-significant) compared with control.; [#]p<0.0001 compared with cadmium (Cd) treated animals.

Histopathological examination

Transverse sections of kidney in control and *Spirulina* alone treated rats showed normal glomeruli and renal tubules. Whereas, cadmium intoxicated rats showed cellular glomeruli, congestion cortex and outer medulla such as tubular brush border loss, interstitial edema and necrosis of epithelium. On the other hand, concomitant treatment with *Spirulina*, exhibited minimal histological changes in kidney limited only to tubular cells (Fig.1).

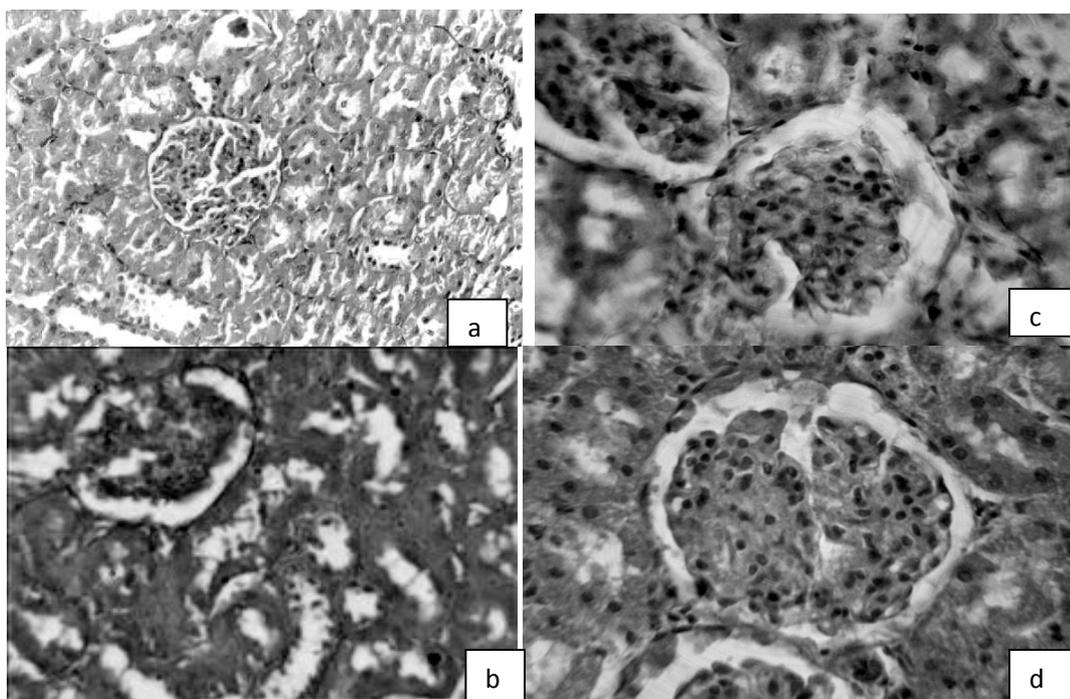


Fig.1. a- control kidney (normal histology), b- Cadmium treated kidney (Shows cellular glomeruli congestion), c- *Spirulina* alone treated kidney (shows normal glomeruli and renal tubules), d- Cadmium with *Spirulina* treated kidney (Shows prominent recovery) (H&E X 400)

Discussion

Our study strongly suggests that aqueous extract of *Spirulina platensis* exhibit a protective action on cadmium induced renal dysfunction. It is evident from the results of present investigation that

concomitant treatment with *Spirulina platensis* significantly protected cadmium induced nephrotoxicity in rats. To our knowledge this is first study that demonstrated beneficial effect of *Spirulina platensis* against renal toxicity.

The role of *Spirulina* in reversing the oxidative stress may be due to presence of several active components. The active components found in *Spirulina* may provoke the activity of free radical scavenging enzyme systems and provides protection against cadmium induced tissues damages. The metallo- protective role of *Spirulina* may be attributed to the presence of β -carotene, vitamin E [28] and vitamin C and selenium [29, 30]. β - Carotene is known to act as powerful quencher of singlet oxygen and a scavenger of free radicals [31]. Similarly, vitamin E of *Spirulina* prevents cadmium induced lipid peroxidation and maintains intracellular thiols and ascorbic acid levels in damaged tissues by inhibiting free radical formation and oxidative damage. Selenium component in *Spirulina* induces selenium containing enzymes glutathione peroxidase, protein or compound such as selenogluthathione, selenocystien, and selenodimethylselenide which are known to modulate the toxic effects of heavy metals [32, 33].

Cadmium injection at dosage 2mg/kg showed severe renal damage associations with marked increase in the serum activity of urea and creatinine, is mainly due to the leakage of these enzymes into the blood stream, which gives an indication of the renal toxicity. This phenomenon was also evidenced in the histological sections of cadmium treated kidney in this study. These characteristic features of cadmium induced renal toxicity were similar to those previously reported by other toxicologists [6] and [34]. Results of the present study showed that *Spirulina* significantly decreased the elevated levels of creatinine and urea. It may be possible, that *Spirulina*, due to its potential antioxidant properties, improved renal function via attenuating oxidative stress- mediated decline in kidney.

The effect of *Spirulina* is attributable both to its being a SOD and GPx stimulator and its radical scavenging activity. Further, the enzyme SOD constitutes the first line of defense against free radical induced damage and the restoration of this enzyme activity by *Spirulina* may account for its protective effect. Lipid peroxidation is one of the main manifestation of oxidative damage, which plays an important role in the toxicity of many xenobiotics [35, 36]. It has been reported that *Spirulina* possess strong antioxidant and free radical scavenging properties [37].

In conclusion, the present study provides convincing evidence for the oxidative stress- related renal dysfunction and morphological alteration in rats. Moreover, our results clearly indicated renoprotective potential of *Spirulina platensis* against Cd-induced oxidative stress and renal dysfunction in rats.

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