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Antioxidative and antiperoxidative effects of *Spirulina platensis* against cadmium induced hepatotoxicity in rats

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Abstract

The study highlights the protective effect of Spirulina treatment on cadmium induced oxidative stress and lipid peroxidation in Wistar rats. The study consisted of four groups in all with eight animals in each group. Control animals received physiological saline orally for 15 days. Second group animals received $CdCl_2$ (2mg/kg in 0.9% NaCl s.c.) whereas, third group animals were administered Spirulina platensis extract alone (1000mg/5ml/kg, orally). Fourth group animals were treated with Spirulina extract for a week and thereafter Spirulina and cadmium chloride was administered concomitantly for another 15 days. Cadmium intoxicated rats showed significant increase ($p<0.05$) in lipid peroxidation (TBARS), aspartate amino phosphatase (AST) and alanine aminotransferase (ALT) activities whereas, a marked decline was observed in superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity. However, animals treated with Spirulina platensis extract and concomitant cadmium chloride intoxication showed a significant ($p<0.05$) decrease in LPO level, AST and ALT activity and increase in SOD and GPx activity. Cadmium intoxication produced various pathological lesions in the liver, whereas, Spirulina treated rats exhibited minimal histological changes in hepatic tissue. Thus, the results obtained from the present study suggest that oral administration of Spirulina platensis extract provides protection against $CdCl_2$ induced toxicity in Wistar rats.

Keywords: Cadmium, Histology, Lipid peroxidation, Oxidative stress, *Spirulina*, Toxicity.

INTRODUCTION

Cadmium (Cd) is a toxic trace metal of worldwide concern because of its extremely long half-life [1]. Most human Cd exposure comes from food, water as well as cigarette smoke and air

contaminations [2]. Cadmium administration has been shown to deplete glutathione (GSH) and protein binding sulfhydryl groups, which resulted in an increase in reactive oxygen species like hydrogen peroxide, hydroxyl radicals and superoxide ions leading to such events as an increase in lipid peroxidation, a change in intracellular stability, DNA damage, membrane damage and apoptosis. Cadmium initially accumulates in the liver and therefore acute exposure to toxic of cadmium produces apoptosis and necrosis in the liver [3]. Malondialdehyde (MDA), SOD and GPx levels are accepted as indicators of the oxidative stress resulting from lipid peroxidation [4]. Several previous studies show that changes in ALT, AST, SOD, alkaline phosphatase (ALP) and GPx levels were observed upon intake of Cd into the body [4-7]. It has been reported that cadmium caused morphological and functional damage in hepatic tissue [8], renal tissue [9], testicular necrosis, morphological and biochemical changes in lung and gastrointestinal tract. Also, cadmium exposure was shown to alter carbohydrate metabolism in liver [10] and the hepatic microsomal drug metabolism. The various toxic effects induced by cadmium in biological system may be due to increased lipid peroxidation [4]. Thus, previous studies confirm that there is a relationship between oxidative stress and hepatotoxicity.

Spirulina, microscopic blue-green algae, has a property of reducing heavy metals and nephrotoxic substance from the body. It is not only a whole food, but it seems to be an ideal therapeutic supplement. So far, no other natural food is found with such a combination and amazing concentration of so many unusual nutrients like protein, amino acid, iron, β-carotene, phycocyanin, gama lenolenic acid, vitamin B_1, B_2, B_3, B_6, B_{12}, essential fatty acid etc. In fact it is the highest known source of protein, β-carotene which is a precursor of vitamin A and only vegetable source of vitamin B_{12}. Beta-carotene concentration of *Spirulina* is ten times higher than carrot. It was evident that food rich in β-carotene can reduce the risk of cancer [11]. It was found in the laboratory that the natural carotene of *Spirulina* could inhibit, shrink and destroy oral cancer cells. In *Spirulina* extract plus zinc-treated group, the clinical scores for keratosis before and after treatment was statistically significant ($p<0.05$). The β-carotene in algae and leafy green vegetables has greater anti-oxidant effects than synthetic β-carotene [12]. However, no attention has been paid so far to explore its hepatoprotective activity in animals and human beings.

Therefore, the present study was undertaken to evaluate the hepatoprotective activity of *Spirulina platensis* on biochemical parameters against cadmium induced liver damage in Wistar male albino rats.

MATARIALS AND METHODS

Chemicals

Cadmium chloride ($CdCl_2$) was obtained from Merck India Ltd. (India). *Spirulina platensis* was purchased from the Sigma Chemical Co. India.

Animal and Experimental design

A total of 32 male Wistar rats (14-16 weeks old, 200-220 g) were obtained from the Defense Research and Development Establishment (DRDE) animal facility. Ethical permission was obtained from the local ethic committee before the study (Reg No. 37/99/CPCSEA, dated 11th Mar 1999, renewed 2011). 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*. Wistar rats were divided into the following four groups with eight rats in each group. 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 $CdCl_2$ 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 liver was 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 4ºC to remove the cell debris and then the supernatant was collected and used for various assays.

Assessment of Hepatic Functions

The concentration of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum was measured using commercial kit (Ranbaxy,India Ltd.).

Lipid peroxidation

Tissue lipid peroxidation was measured by the method of Onkawa [13]. 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.

Superoxide dismutase

Tissue superoxide dismutase was assayed by the method of Kakkar [14]. Reaction mixture contained 1.2ml of (0.052 mM) sodium pyrophosphate buffer, 0.1ml of (186 µM) phenazine methosulpfate 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.

Glutathione peroxidase

Glutathione peroxidase activity was measured by the procedure of Flohe and Gunzler [15]. 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 H_2O_2 (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.

Histopathology

The tissues were fixed in 10% formalin for 24 h, and standard dehydration and paraffin-wax embedding procedures were used. Sections (5 µm) were cut in a microtome. Hematoxylin and eosin-stained slides were prepared by using standard methods and evaluated by light microscopy.

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

Treatment with cadmium significantly (p<0.05) increased the activities of serum ALT and AST (161% and 136% respectively) compared to the control. Administration of *Spirulina* alone did not show any significant change in the serum levels of these enzymes whereas, treatment with *Spirulina* attenuated the cadmium induced increase of serum AST and ALT (79% and 91% respectively) compared to their levels in cadmium treated groups (Table. 1).

Table 1. Cadmium induced changes in serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) and their response to administration of *Spirulina platensis* (SP) in rats.

Groups	Treatments	AST U/L	ALT U/L
I	Control	8.17±0.26	8.59±0.19
II	Cd treated	11.97±0.39$^{\#}$	13.8±0.11$^{\#}$
III	SP	7.86±0.32^{ns}	8.56±0.15 ^{ns}
IV	Cd+SP	8.13±0.32 ^{ns}	9.17±0.27 ^{ns}

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.

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

Groups	Treatments	TTBARS nmol MDA/mg of protein	SOD units/min mg of protein	GPx µg/min/mg protein
I	Control	13.91±0.42	23.69±1.7	13.07±1.44
II	Cd treated	27.37±0.8$^{\#}$	18.67±0.37$^{\#}$	5.64±0.19$^{\#}$
III	SP	13.36±0.43^{ns}	26.04±2.23 ^{ns}	12.21±0.53 ^{ns}
IV	Cd+SP	19.18±0.42*	26.46±0.89*	10.8±0.32*

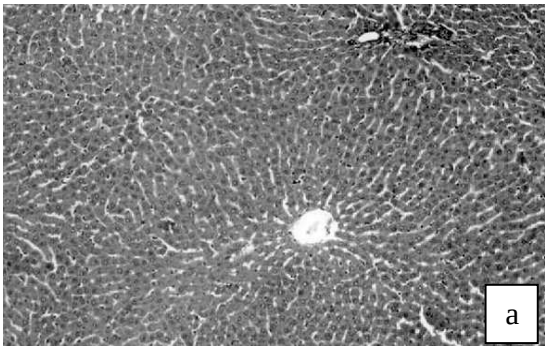
Values are expressed means ± SE; $^{\#}$p<0.01, $^{\#\#}$p<0.0001, ns (non significant) compared with control; * p<0.0001 compared with cadmium (Cd) treated animals.

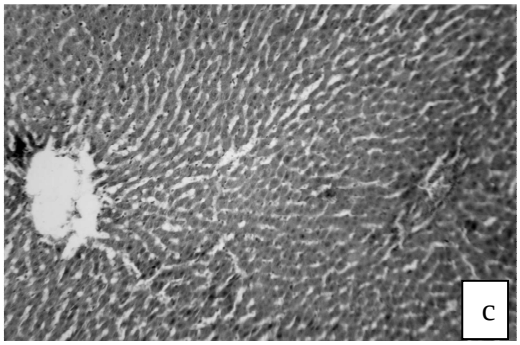
Biochemical studies

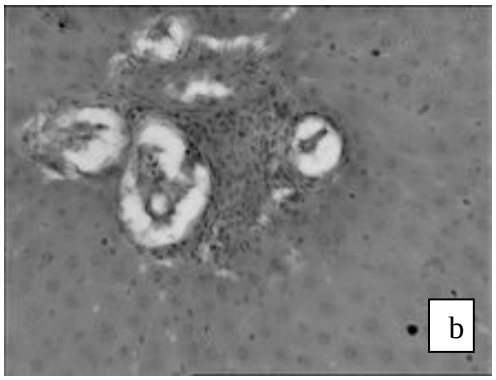
Results indicated that lipid peroxidation level (LPO) was significantly increased in the liver ($p<0.05$ and $p<0.001$) of rats treated with cadmium (Table. 2). Treatment with *Spirulina* was very effective in the prevention of oxidative damage induced by cadmium which resulted in significantly lower LPO level.

Fig.1.

a- Control liver (normal histology)
b- Cadmium treated liver (Shows various pathological lesions i.e. cytoplasmic vacuolization, karyolysis, pycnosis and entrilobolar necrosis.)
c-*Spirulina* alone treated liver (shows normal hepatocyte, central vein and portal triad.)
d- Cadmium with *Spirulina* treated kidney (Shows prominent recovery and normal architecture with mild residual degeration) (H&E X 400)







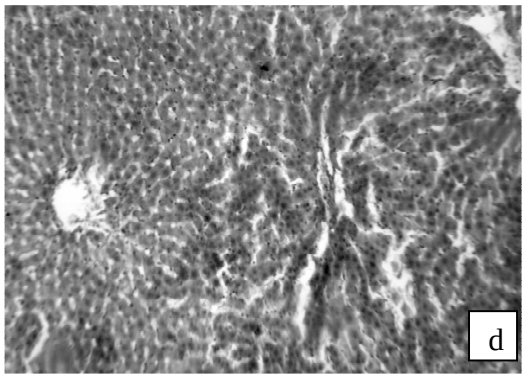


Table 2 shows significant changes in the activity of antioxidant defense system enzymes during the treatment of rats with cadmium, *Spirulina* and their combination. SOD and GPx activities were significantly decreased ($p<0.05$) in the liver. Treatment with *Spirulina* significantly increased hepatic SOD and GPx activities, reverted them very close to the normal level.

Histopathological studies

Histological sections of liver in control and *Spirulina* treated rats showed the normal hepatocytes, central vein and portal triad. Cadmium intoxication produced various pathological lesions in the liver such as cytoplasmic vacuolization, karyolysis, pycnosis and centrilobular necrosis. Concomitant treatment of *Spirulina* with cadmium showed prominent recovery and normal architecture with mild residual degeneration (Fig. 1).

DISCUSSION

The present study evaluates the protective effect of *Spirulina* against liver damage induced by cadmium in male Wistar rats. It has been shown to induce lipid peroxidation and cause excretion of lipid metabolites in urine as the superoxide dismutase and glutathione peroxidase are the most important enzymes against the toxic effect of oxygen metabolism. Therefore, a decrease in the activity of SOD can be attributed to elevated superoxide production during cadmium metabolism [15-16]. The present study has clearly demonstrated the ability of cadmium to induce oxidative stress in rat's liver as evidenced by increased lipid peroxidation. The production of reactive oxygen species may be associated with cadmium toxicity which increases the formation of TBARS in lungs, liver, kidney and brain. It has already been reported that urinary excretion of MDA, a product of lipid peroxidation by cadmium in rats is a consequence of decrease in antioxidant enzymes. Lipid peroxides that accumulate due to lipid peroxidation are known to be harmful to cells and tissues. The relation between the hepatic tissue damage and elevation of the liver enzymes is well documented [18].

Cadmium chloride has been widely used to induce experimental hepatic damage [19]. It induces liver cell necrosis and apoptosis and can be used to induce hepatic fibrosis or cirrhosis by repetitive administration. Liver is rich in transaminase, which increase in hepatic disease [20]. AST, which is slightly elevated by cardiac necrosis, is a more specific indicator of liver disease [21-22]. This phenomenon was also evidenced in the histological sections of cadmium treated liver in this study. These characteristic features of cadmium induced liver toxicity were similar to those previously reported by other toxicologists [3]. In the present investigation it was observed that cadmium intoxication significantly depletes GSH content in the blood and thus, reducing the antioxidant potential and accelerating the lipid peroxidation, resulting in hepatocyte damage.

The blue-green algae (Cyanobacterium) *Spirulina* has been used both as a dietary supplement and as a medicinal substance. In *Spirulina* supplemented (10-30%) diet, the rat did not show any abnormalities in organ weight of the liver, lung, kidney, heart and spleen. *Spirulina* fed rats showed 3 folds increase in lactobacillus content and a 43% increase in vitamin B_1 in the caecum of rats. Rats fed on *Spirulina* have reduced kidney toxicity from mercury poisoning [23]. *Spirulina* is rich in β-carotene and the bioavailability is as good as the pure β-carotene, vitamin E and vitamin C and selenium [24-25]. It has been suggested, that the *Spirulina* extracts could be effective against free radical induced lipid peroxidation which in turn may lead to cellular transformation.

In conclusion, the results of the present study indicated the antioxidant and antiperoxidative effects of multicomponent natural food supplement *Spirulina* in cadmium induced toxicity in rats. Although, administration of *Spirulina* alone or concomitantly with cadmium intoxicated rats

produced appreciable protective effects, further study may be needed to achieve optimal effects by increasing its dose.

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