LIST OF PUBLICATIONS


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Paper presentation and Participation in Seminars/Conferences/Workshops.

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- International Conference on Molecular Mechanism of Disease, Defence Research & Development Establishment, Gwalior, India, 15th-16th Dec 2008.
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- Sophisticated analytical instrumental facility, Central Drug Research Institute, Lucknow, training programme 21st -25th June. 2010.
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, neutraceutical 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, cyanocobalamin, 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 10,000 x g 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 1 hr 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 x 10⁵ M/cm.

**Estimation of Superoxide dismutase**

Tissue superoxide dismutase was assayed by the method of Kakkar [26]. Reaction mixture contained 1.2 ml of (0.052 mM) sodium pyrophosphate buffer, 0.1 ml of (186 μM) phenazine methosulfate and 0.3 ml of nitro blue tetrazolium (300 μM). Reaction was initiated by adding 0.2 ml of NADH (780 μM) and stopped by the addition of 1 ml 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) 0.2 ml of GSH (2 mM), 0.1 ml of sodium azide (10 mM), 0.1 ml 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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Serum Urea (mg/dl)</th>
<th>Serum Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>29.69±0.59</td>
<td>0.75±0.009</td>
</tr>
<tr>
<td>II</td>
<td>Cadmium</td>
<td>42.19±2.03**</td>
<td>0.93±0.001***</td>
</tr>
<tr>
<td>III</td>
<td>SP</td>
<td>28.86±0.45**</td>
<td>0.75±0.014**</td>
</tr>
<tr>
<td>IV</td>
<td>Cd + SP</td>
<td>29.83±1.27</td>
<td>0.77±0.023***</td>
</tr>
</tbody>
</table>

Values are expressed means ± SE; **p<0.01, ***p<0.0001, ns (non significant) compared with control; $^a$ 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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>TBARS (nmol MDA/g)</th>
<th>SOD (units/min mg protein)</th>
<th>GPx (μg/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>13.82±0.61</td>
<td>24.16±1.59</td>
<td>13.55±0.92</td>
</tr>
<tr>
<td>II</td>
<td>Cadmium</td>
<td>26.67±0.48</td>
<td>11.20±1.19</td>
<td>4.88±0.30</td>
</tr>
<tr>
<td>III</td>
<td>SP</td>
<td>13.32±0.34ns</td>
<td>30.02±2.64ns</td>
<td>10.23±1.43ns</td>
</tr>
<tr>
<td>IV</td>
<td>Cd+SP</td>
<td>19.73±0.69</td>
<td>32.69±1.82</td>
<td>8.71±0.08</td>
</tr>
</tbody>
</table>

Values are expressed means ± mean. *p<0.001, **p<0.0001 (non-significant) compared with control. †p<0.0001 compared with cadmium (Cd) treated animals.

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 selenoglутathione, selenocystein, 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.

Acknowledgements

The authors are thankful to Dr. S.J.S. Flora, Scientist ‘F’, DRDE, Gwalior (India), for his support and encouragement and Prof. V.G. Das, Director, Dayalbagh Educational Institute (Deemed University), Dayalbagh, Agra (India) for providing necessary facilities for this work. We are also thankful to UGC, New Delhi for providing Senior Research Fellowship to the first author.
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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 CdCl₂ (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 CdCl₂ 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 lenolic acid, vitamin B₁, B₂, B₃, B₆, B₁₂, 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 B₁₂. 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.

MATERRIALS AND METHODS

Chemicals
Cadmium chloride (CdCl₂) 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. 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 4°C 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 x 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 methosulphate 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$_2$O$_2$ (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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>AST U/L</th>
<th>ALT U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>8.17±0.26</td>
<td>8.59±0.19</td>
</tr>
<tr>
<td>II</td>
<td>Cd treated</td>
<td>11.97±0.39(^*)</td>
<td>13.8±0.11(^*)</td>
</tr>
<tr>
<td>III</td>
<td>SP</td>
<td>7.86±0.32(^{ns})</td>
<td>8.56±0.15(^{ns})</td>
</tr>
<tr>
<td>IV</td>
<td>Cd+SP</td>
<td>8.13±0.32(^{ns})</td>
<td>9.17±0.27(^{ns})</td>
</tr>
</tbody>
</table>

Values are expressed means ± SE; \(^* p<0.01\), \(^{ns} p<0.0001\), 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.

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

Values are expressed means ± SE; \(^* p<0.01\), \(^{ns} p<0.0001\), 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 entrilobular 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 B1 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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