Effect of Spirulina and Liv.52 on Cadmium induced Toxicity in Albino Rats

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Oral administration of cadmium (6 mg/kg body weight/day) as cadmium chloride (CdCl₂) for 30 days resulted in a significant increase in thiobarbituric acid reactive substances (TBARS) level and a decrease in the levels of copper, zinc, iron, selenium, glutathione, superoxide dismutase, catalase, glutathione peroxidase when compared to normal control. Administration of either Liv.52 alone or in combination with spirulina produced a well pronounced protective effect in respect to these parameters in cadmium intoxicated rats. The protective effect of spirulina and Liv.52 in respect to biochemical changes were also confirmed by histopathological study in the liver and kidney sections.

Keywords: Cadmium chloride (CdCl₂), Liv.52, Spirulina, Thiobarbituric acid reactive substances (TBARS)

Cadmium, a heavy metal well known to be highly toxic to both human and animals, is distributed widely in the environment due to its use in various industries. Some of the toxic effects of cadmium exposure are testicular atrophy, renal dysfunction, hepatic damage, hypertension, central nervous system injury and anemia¹.

Cadmium may induced oxidative damage in different tissues by enhancing peroxidation of membrane lipids in tissues and altering the antioxidant systems of the cells. The peroxidative damage to the cell membrane may cause injury to cellular components due to the interaction of metal ions with the cell organelles². Cadmium depletes glutathione and protein bound sulfhydryl groups resulting in enhanced production of reactive oxygen species such as superoxide ions, hydroxyl radicals and hydrogen peroxides. These reactive oxygen species result in increased lipid peroxidation³.

Biological compounds with antioxidant properties contribute to the protection of cells and tissues against deleterious effects of reactive oxygen species and other free radicals. Protective agents from plant origin with antiperoxidative and antioxidant properties play an important role in protecting the liver against toxicity⁴.

Although, high incidence of low level exposure to cadmium takes place, proper therapeutic intervention remains obscure. Most of the synthetic antidotes in the management of cadmium toxicity have met with limited success due to their inherent toxicity and nonspecificity⁵.

Traditional medicines are effective in certain disorders and are based on experience in the use of plant products in amelioration of common diseases. Liv.52, an Ayurvedic multiherbal formulation is widely used in various hepatic disorders⁶,⁷.

Spirulina is a microscopic, multicellular filamentous blue green algae (cyanobacterium). It is unique among blue green algae because of it has a long history of safe use⁸. The antioxidant properties of spirulina have attracted the attention of researchers recently. The antioxidant effect of spirulina against carbon tetrachloride⁹ and mercury induced toxicity¹⁰ in rats have been studied.

However, the antioxidant and protective effect of spirulina against cadmium induced toxicity in respect to lipid peroxidation, antioxidant status, trace elements in tissues remains unexplored.
Therefore, the present study has been undertaken to delineate its antioxidant and protective role against cadmium induced toxicity in rats.

MATERIALS AND METHODS

Chemicals: Cadmium chloride (CdCl$_2$), thiobarbituric acid (TBA), nitroblue tetrazolium (NBT), reduced glutathione (GSH), 5,5'-dithio-2-nitrobenzoic acid (DTNB) were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). Other chemicals used were of analytical grade and purchased locally. Liv.52 tablets (500 mg each) were obtained commercially from The Himalaya Drug Company, Bangalore, India. Each Liv.52 tablet is composed of *Capparis spinosa* (65 mg), *Cichorium intybus* (65 mg), *Solanum nigrum* (32 mg), *Cassia occidentalis* (16 mg), *Terminalia arjuna* (32 mg), *Achillea millefolium* (16 mg), and *Tamarix gallica* (16 mg). Spirulina tablets (100 mg each) were obtained commercially from Parrys Neutraceuticals Ltd., Chennai, India.

Animals and treatment: Male albino rats (Wistar strain) weighing 150-175 g were obtained from animal breeding centre, P.S.G. Institute of Medical Sciences & Research, Coimbatore, Tamilnadu, India. They were housed in KMCH College of Pharmacy, Coimbatore, Tamilnadu, India, in controlled temperature (27°C ± 2°C), humidity (55 ± 10%) and light with 12:12 hr L:D cycle. Animals were fed with standard pellet (Hindustan Lever Ltd., India). They were given a week time to get acclimatized with laboratory condition. Ethical clearance for the handling of experimental animals was obtained from the committee constituted for the purpose (685/02/a/CPCSEA/2002).

After acclimatization the animals were divided into the following 8 groups of 6 rats each: Gr A: normal control; Gr B: cadmium (6 mg/kg body weight/day) as CdCl$_2$ orally for 30 days; Gr C: Spirulina (500 mg/kg body weight/day) orally for 30 days; Gr D: Cadmium as in group B + spirulina as in group C orally for 30 days; Gr E: Liv.52 (500 mg/kg body weight/day) orally for 30 days; Gr F: Cadmium as in group B + Liv.52 as in group E orally for 30 days; Gr G: Spirulina as in group C + Liv.52 as in group E orally for 30 days; and Gr H: Cadmium as in group B + Spirulina as in group C + Liv.52 as in group E orally for 30 days.

At the end of the experimental period, the rats were deprived of food overnight and sacrificed by light ether anaesthesia. Serum samples were collected to estimate the amount of copper$^{11}$, zinc$^{12}$, iron$^{13}$ and selenium$^{14}$. Liver and kidney were removed and cleaned in normal saline. A known weight of these tissues were quickly weighed and then homogenized (10% w/v) in ice cold phosphate buffer (0.1 M, pH 7.4) using potter Elvehjem teflon homogenizer. The homogenate was centrifuged at 5000 rpm at 4°C for 30 minutes and supernatant obtained was used for the assay of various enzymes. Lipid peroxidation was estimated by the method of Das *et al.*$^{15}$ Superoxide dismutase (SOD; EC.1.15.1.1)$^{16}$ Catalase (CAT; EC.1.11.1.6)$^{17}$, reduced glutathione (GSH)$^{18}$ and glutathione peroxidase (GPx; EC.1.11.1.9)$^{19}$ were assayed. Protein was quantified as per Lowry *et al.*,$^{20}$ using bovine serum albumin as standard.

Histopathology: Small pieces of liver and kidney tissues were fixed in 10% formalin solution, dehydrated with 90% ethanol, embedded in paraffin, cut into thin sliced section (5 µm thickness), and stained with haematoxylin-eosin dye$^{21}$.

Phytochemical analysis: Preliminary quantitative measurement of total phenols$^{22}$, flavonoids$^{23}$, vitamin C$^{24}$, vitamin E$^{25}$ and glutathione (GSH)$^{18}$ was done in spirulina and Liv.52 samples.

Mineral analysis in spirulina and Liv.52: Spirulina and Liv.52 samples (0.5 g each) were digested with 5 ml perchloric acid and nitric acid mixture (1:3) in a microjeldhal digestion flask. The digestion was continued till no more brown fumes evolved and the solution in the flask become colourless. The digested mixture was transferred to a 100 ml standard flask and made upto 100 ml
with double distilled water. The aliquots of the sample was used for the estimated of copper, zinc, iron and selenium.

**Statistical analysis:** Statistical analysis was performed by one way analysis of variance (ANOVA). Critical difference (CD) was calculated at 1% level according to the method of Gomez et al. and results were expressed as mean ± SD of six rats in each group.

**RESULTS AND DISCUSSION**

Spirulina and Liv.52 are good sources of flavonoids, total phenols, vitamin C, vitamin E, reduced GSH, copper, zinc, iron and selenium (Table 1).

**Table 1:** None enzymic antioxidants & antioxidant minerals in spirulina and Liv.52

<table>
<thead>
<tr>
<th>Samples</th>
<th>Flavonoids (mg/g)</th>
<th>Total phenols (mg/g)</th>
<th>Vit C (mg/g)</th>
<th>Vit E (mg/g)</th>
<th>GSH (nm/g)</th>
<th>Copper (µg/g)</th>
<th>Zinc (mg/g)</th>
<th>Iron (mg/g)</th>
<th>Selenium (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liv.52</td>
<td>6.92</td>
<td>12.96</td>
<td>1.30</td>
<td>0.838</td>
<td>67.30</td>
<td>302</td>
<td>6.80</td>
<td>1.26</td>
<td>0.02</td>
</tr>
<tr>
<td>Spirulina</td>
<td>8.98</td>
<td>9.65</td>
<td>0.21</td>
<td>0.150</td>
<td>123.80</td>
<td>12</td>
<td>1.50</td>
<td>30</td>
<td>1.50</td>
</tr>
</tbody>
</table>

In rats treated with cadmium (Gr. B), there was a significant decrease (p<0.01) in the level of these trace elements (copper, iron, zinc and selenium) as compared to normal control (Gr. B) (Table 2). This may be due to interference of cadmium on absorption and transport of these trace elements, which would have resulted in the depletion of these metals in this group of rats. Cadmium may inhibit zinc activities at many stages, interfering with absorption, distribution and transport of zinc into cells or into several intracellular structure. Bannis et al. have suggested that cadmium impairs iron absorption and utilization or both. Cadmium induced decrease in the level of copper and selenium have been reported. Co-administration of either spirulina (Gr. D) or Liv.52 (Gr. F), or both Liv.52 and spirulina (Gr. H), along with cadmium, caused a significant increase (p<0.01) in the level of these trace elements in serum as compared to cadmium intoxicated rats (Gr. B). This may be due to interaction and antagonistic action of these trace elements, which are present in spirulina and Liv.52 with cadmium and resulted in elevated level of these trace elements in these groups of rats. Studies have shown that supplementation of zinc, copper, selenium, iron offered a protective effect against toxic effects of cadmium.

**Table 2:** Levels of trace elements in serum of different experimental groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Copper (µ/dl)</th>
<th>Zinc (µg/dl)</th>
<th>Iron (µg/dl)</th>
<th>Selenium (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Normal control</td>
<td>102.18 ± 0.32a</td>
<td>94.21 ± 0.41a</td>
<td>156.35 ± 1.19a</td>
<td>74.31 ± 0.15a</td>
</tr>
<tr>
<td>B</td>
<td>Cadmium treated</td>
<td>42.55 ± 0.50b</td>
<td>31.62 ± 0.22b</td>
<td>80.20 ± 0.82b</td>
<td>36.24 ± 0.66b</td>
</tr>
<tr>
<td>C</td>
<td>Spirulina alone</td>
<td>102.35 ± 0.62c</td>
<td>94.62 ± 0.76c</td>
<td>157.15 ± 0.98c</td>
<td>74.81 ± 0.32c</td>
</tr>
<tr>
<td>D</td>
<td>Cadmium + Spirulina</td>
<td>81.40 ± 0.45c</td>
<td>76.78 ± 1.05c</td>
<td>122.12 ± 1.12c</td>
<td>62.42 ± 0.46c</td>
</tr>
<tr>
<td>E</td>
<td>Liv.52 alone</td>
<td>103.10 ± 1.16d</td>
<td>95.20 ± 0.80d</td>
<td>158.17 ± 0.59d</td>
<td>74.63 ± 1.10d</td>
</tr>
<tr>
<td>F</td>
<td>Cadmium + Liv.52</td>
<td>94.31 ± 0.93d</td>
<td>83.24 ± 0.52d</td>
<td>143.53 ± 1.23d</td>
<td>70.12 ± 0.52d</td>
</tr>
<tr>
<td>G</td>
<td>Spirulina + Liv.52</td>
<td>103.66 ± 0.65d</td>
<td>95.81 ± 0.91d</td>
<td>159.75 ± 0.72d</td>
<td>75.14 ± 0.12d</td>
</tr>
<tr>
<td>H</td>
<td>Cadmium + Spirulina + Liv.52</td>
<td>96.72 ± 0.49d</td>
<td>96.06 ± 0.21a</td>
<td>146.79 ± 1.10d</td>
<td>71.37 ± 0.71d</td>
</tr>
</tbody>
</table>

Values with same superscript did not differ significantly (p<0.01).

In cadmium treated rats (Gr. B), there was a significant increase (p<0.01) in the level of TBARS in both liver and kidney, as compared to normal control (Gr. A) (Tables 3 and 4). This could be possibly due to excessive formation of free radicals, which leads to deterioration of biological macromolecules. Manca et al. have reported that lipid peroxidation is considered as a sensitive index of cadmium exposure. Simultaneous administration of either spirulina (Gr. D) or Liv.52 (Gr. F), or both Liv.52 and spirulina (Gr. H), along with cadmium, caused a significant decrease (p<0.01) in the level of TBARS when compared to rats treated with cadmium alone (Gr. B). This
may be attributed to the presence of phenolic compounds, flavonoids and antioxidant vitamins (Vitamins C and E) in Liv.52 and spirulina.

Vitamins E and C are potent free radical scavengers and prevent oxidative damage by utilizing the free radicals. Flavonoids and phenolic compounds have long been recognized as excellent scavengers of superoxide, hydroxyl ion and peroxy radicals and as potent inhibitors of lipid peroxidation.

Suja et al. reported that administration of Liv.52 reduced the peroxidative effects of hydrogen peroxide and inhibited the deleterious effects of lipid peroxidation by enhanced supply of reduced GSH.

In cadmium treated animals (Gr. B), there was a significant decrease (p<0.01) in the activities of SOD and CAT when compared to normal control (Gr. A) (Tables 3 and 4). This may be due to either the antagonistic effect of cadmium with copper and zinc, which are important metals for the activity of SOD molecule or inactivation of SOD by cadmium induced lipid peroxidation.

Whanger et al. reported that the decrease in catalase activity may reflect decreased absorption of iron, an essential trace element required for the activity of catalase. These findings support the results of the present study. Co-administration of either spirulina (Gr. D) or Liv.52 (Gr. F) or both spirulina and Liv.52 (Gr. H) along with cadmium significantly increased the activities of SOD and CAT when compared to rats treated with cadmium alone (Gr. B). This could be attributed to the presence of nonenzymic antioxidants (vitamin C, vitamin E, phenols, and flavonoids) and antioxidant minerals (copper, zinc, selenium and iron) in spirulina and Liv.52.

### Table 3: Effect of spirulina and Liv.52 against cadmium induced toxicity in liver

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>TBARS (µM/mg protein)</th>
<th>SOD (Units/mg protein)</th>
<th>CAT (Units/mg protein)</th>
<th>GSH (µg/mg protein)</th>
<th>Gx (Units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Normal control</td>
<td>0.66 ± 0.01</td>
<td>6.99 ± 0.40</td>
<td>70.68 ± 1.99</td>
<td>5.29 ± 0.14</td>
<td>6.83 ± 0.64</td>
</tr>
<tr>
<td>B</td>
<td>Cadmium treated</td>
<td>2.12 ± 0.02</td>
<td>3.75 ± 0.15</td>
<td>34.61 ± 0.81</td>
<td>3.18 ± 0.16</td>
<td>3.41 ± 0.40</td>
</tr>
<tr>
<td>C</td>
<td>Spirulina alone</td>
<td>0.65 ± 0.04</td>
<td>7.08 ± 0.03</td>
<td>71.11 ± 0.66</td>
<td>5.42 ± 0.13</td>
<td>6.99 ± 0.53</td>
</tr>
<tr>
<td>D</td>
<td>Cadmium + Spirulina</td>
<td>1.09 ± 0.11</td>
<td>5.70 ± 0.13</td>
<td>63.18 ± 0.98</td>
<td>4.46 ± 0.10</td>
<td>4.78 ± 0.33</td>
</tr>
<tr>
<td>E</td>
<td>Liv.52 alone</td>
<td>0.64 ± 0.03</td>
<td>7.17 ± 0.10</td>
<td>71.50 ± 0.82</td>
<td>5.37 ± 0.11</td>
<td>7.08 ± 0.48</td>
</tr>
<tr>
<td>F</td>
<td>Cadmium + Liv.52</td>
<td>0.75 ± 0.06</td>
<td>6.17 ± 0.37</td>
<td>66.26 ± 1.64</td>
<td>4.79 ± 0.25</td>
<td>5.84 ± 0.45</td>
</tr>
<tr>
<td>G</td>
<td>Spirulina + Liv.52</td>
<td>0.63 ± 0.03</td>
<td>7.23 ± 0.09</td>
<td>71.62 ± 1.45</td>
<td>5.46 ± 0.12</td>
<td>7.19 ± 0.38</td>
</tr>
<tr>
<td>H</td>
<td>Cadmium + spirulina + Liv.52</td>
<td>0.73 ± 0.05</td>
<td>6.43 ± 0.20</td>
<td>67.14 ± 0.28</td>
<td>4.92 ± 0.31</td>
<td>6.08 ± 0.56</td>
</tr>
</tbody>
</table>

*a=Units/mg protein (amount of enzyme required to inhibit 50% reduction of NBT)
*b=Units/mg protein (µm of H2O2 decomposed/mg protein/min)
$=Units/mg protein (µg of GSH consumed/min/mg protein)
Values with same superscript did not differ significantly (p<0.01).

Hasan et al. indicated that adverse effect of heavy metals (cadmium, lead, arsenic, mercury) would be antagonized by the administration of zinc, copper and selenium. Spirulina contains superoxide dismutase (SOD) that can prevent the cell damage by free radicals. The enzymes SOD and CAT constitute the first line of defense against free radical induced damage and the restoration of these enzyme activity by Liv.52 and spirulina may account for their protective effect.
Table 4: Effect of spirulina and Liv.52 against cadmium induced toxicity in kidney
(Values are mean ± SD of 6 rats)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>TBARS (nm/mg protein)</th>
<th>SOD(^a) (Units/mg protein)</th>
<th>CAT(^b) (Units/mg protein)</th>
<th>GSH (µg/mg protein)</th>
<th>GSx(^c) (Units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Normal control</td>
<td>1.14 ± 0.03(^a)</td>
<td>5.43 ± 0.21(^a)</td>
<td>61.38 ± 1.62(^a)</td>
<td>4.62 ± 0.11(^a)</td>
<td>5.23 ± 0.65(^a)</td>
</tr>
<tr>
<td>B</td>
<td>Cadmium treated</td>
<td>2.79 ± 0.04(^b)</td>
<td>2.41 ± 0.45(^b)</td>
<td>29.58 ± 2.42(^b)</td>
<td>2.45 ± 0.08(^b)</td>
<td>1.52 ± 0.19(^b)</td>
</tr>
<tr>
<td>C</td>
<td>Spirulina alone</td>
<td>1.12 ± 0.03(^a)</td>
<td>5.56 ± 0.18(^a)</td>
<td>61.96 ± 0.94(^a)</td>
<td>4.70 ± 0.07(^a)</td>
<td>5.39 ± 0.52(^a)</td>
</tr>
<tr>
<td>D</td>
<td>Cadmium + Spirulina</td>
<td>1.68 ± 0.02(^a)</td>
<td>4.23 ± 0.13(^a)</td>
<td>52.47 ± 1.36(^a)</td>
<td>3.86 ± 0.08(^a)</td>
<td>3.62 ± 0.41(^a)</td>
</tr>
<tr>
<td>E</td>
<td>Liv.52 alone</td>
<td>1.11 ± 0.07(^c)</td>
<td>5.60 ± 0.10(^c)</td>
<td>62.27 ± 0.57(^c)</td>
<td>4.68 ± 0.13(^c)</td>
<td>5.48 ± 0.79(^c)</td>
</tr>
<tr>
<td>F</td>
<td>Cadmium + Liv.52</td>
<td>1.29 ± 0.08(^d)</td>
<td>4.58 ± 0.10(^d)</td>
<td>59.99 ± 1.38(^d)</td>
<td>4.24 ±0.09(^d)</td>
<td>4.33 ± 0.48(^d)</td>
</tr>
<tr>
<td>G</td>
<td>Spirulina + Liv.52</td>
<td>1.10 ± 0.04(^a)</td>
<td>5.63 ± 0.06(^a)</td>
<td>62.86 ± 0.68(^a)</td>
<td>4.72 ± 0.10(^a)</td>
<td>5.77 ± 0.57(^a)</td>
</tr>
<tr>
<td>H</td>
<td>Cadmium + spirulina + Liv.52</td>
<td>1.25 ± 0.17(^a,d)</td>
<td>4.77 ± 0.77(^d)</td>
<td>58.53 ± 2.82(^d)</td>
<td>4.34 ± 0.10(^d)</td>
<td>4.52 ± 0.37(^a,c)</td>
</tr>
</tbody>
</table>

\(^a\)=Units/mg protein (amount of enzyme required to inhibit 50% reduction of NBT)
\(^b\)=Units/mg protein (µm of H₂O₂ decomposed/mg protein/min)
\(^c\)=Units/mg protein (µg of GSH consumed/min/mg protein)

Values with same superscript did not differ significantly (p<0.01).

In rats treated with cadmium (Gr. B) there was a significant decrease (p<0.01) in the level GSH and GPx when compared to normal control (Gr.A) (Tables 3 and 4). This could be probably due to either increased utilisation of GSH by the cells to act as scavengers of free radicals caused by toxic chemical agents, or enhanced utilization of GSH by GPx, or decreased availability of selenium which leads to inefficient disposal of peroxides and results in elevated lipid peroxidation. Simultaneous administration of either spirulina (Gr.D) or Liv.52 (Gr.F) or both Liv.52 and spirulina (Gr.H), along with cadmium, significantly increased (p<0.01) the levels of GSH and GPx when compared to rats treated with cadmium alone (Gr.B). This may be due to either increased supply of GSH or selenium by these drugs for the activation of GPx. Selenium is an integral component of GPx which can capable of reducing peroxides and hydrogen peroxide.

Histopathology of liver section of control rats showed the normal hepatocytes, central vein and portal triad (Fig. 1a). Liver section of...
Fig. 2 – Kidney section of rats (a) control showing normal architecture (H&E x 100); (b) cadmium treated rats with cellular glomeruli (CG) (H&E x 450); (c) cadmium treated rats with tubular necrosis (TN). The interstitium appears normal (H&E x 100); (d) cadmium and spirulina treated rats showing normal architecture with mild residual tubular necrosis (MRTN) (H&E x 100); (e) cadmium and Liv-52 treated rats with normal architecture. (H&E x 100); (f) cadmium + Spirulina + Liv-52 treated rats showing normal architecture with reversal of cellular glomeruli and tubular necrosis. (H&E x 100).

In conclusion, the results of the present study indicated the antioxidant and antiperoxidative effects of both Liv.52 and spirulina in cadmium induced toxicity in rats. However, administration of either Liv.52 alone or in combination with spirulina in cadmium intoxicated rats caused a more pronounced protective effect. Though, rats treated with spirulina alone in cadmium intoxicated rats produced an appreciable effect, further study may be needed to achieve its optimal effect by increasing its dose.

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References

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