Prevention of cadmium bioaccumulation by herbal adaptogen:

*Spirulina platensis*

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ABSTRACT

To evaluate the effect of herbal adaptogen such as Spirulina platensis on cadmium intoxicated rats and its accumulation in blood, liver and kidney. The study consisted of four groups in all with eight animals in each group. Control animals received physiological saline orally for 30 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. Body weight gain, hepatic (aspartate amino phosphatase, AST and alanine aminotransferase, ALT) and renal (creatinine, CRE and urea, URE) function marker and concentration of cadmium in liver and kidney were investigated. Pre and post treatment of Spirulina platensis orally significantly improved the body weight gain and restored liver and kidney function marker to normal levels as compared to Cd exposed rats. Administration of herbal adaptogen at the rate of 1gm significantly prevented the bioaccumulation of cadmium and reversed the Cd induced toxicity in Wistar rats.

Keywords: Spirulina platensis, Accumulation, Toxicity, Renal marker, Hepatic marker, Kidney.

INTRODUCTION

In the recent past, extensive mining and indiscriminate industrialization have increased cadmium contamination of environment. Most of the metals present in environment enter the biological system on exposure mainly through air, drinking water, pesticides, batteries, piping, paints, petrochemicals, articles etc. Multifractional mechanisms are involved in cadmium-induced
reactive oxygen species (ROS) generation. Cadmium inhibits Ca\(^{2+}\)-ATPase in cell membrane/endoplasmic reticulum and prevents Ca\(^{2+}\) export from the cytoplasm. Cadmium also participates in Fenton reaction leading to generation of hydroxyl radical (HO\(^-\)) \[1\]. Cadmium once absorbed gets accumulated in major organs of the living system. Cadmium exerts its toxic effects on tissues like red blood cells, liver kidney and heart \[2 \& 3\]. Recently, it has been realized that certain essential metals, amino acids, vitamins and antioxidants can play a significant protective role in the treatment of metal induced oxidative stress or damage. Dietary nutrients can also behave as efficient chelators. Therefore, the oxidizing property together with the chelating capacity of antioxidants makes them a strong candidate for decontamination of toxic metals. The herbs which increase the ability to adapt and avoid damage by environmental factors are called adaptogens e.g. Withania somnifera, Ocimum sanctum, Asperagus recemosus, Andrographis paniculata are potent antioxidants which reduce lipid peroxidation (LPO) \[4-7\] by their potential radical scavenging activity. Spirulina is being considered as one of the nutritionally enriched naturally occurring food consisting of protein, minerals, vitamins, amino acids, essential, and essential fatty acids and rich source of antioxidants which has been proved to combat oxidant damage and protected against Cd induced nephro-, hepato- and haematoxtoicity \[8\]. Currently, the possible toxicity of synthetic antioxidants has been criticized. The interest in natural antioxidants, especially of plant origin, has greatly increased in the recent years \[9\]. Hence, the present study was conducted to evaluate the effect of herbal adaptogens on Cd bioaccumulation and on scavenging of Cd-induced free radicals.

**EXPERIMENTAL SECTION**

**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**

Male Wistar albino rats weighing 210±10g were obtained from the Defense Research and Development Establishment (DRDE) animal facility, Gwalior (Reg No. 37/99/CPCSEA, dated 11th Mar 1999, renewed 2011). The rats were fed with commercial pellet and water *ad libitum*. The room temperature was kept at 26 °C ± 2 °C. Rats were divided into eleven groups of 8 rats each.

**Group I** normal saline, orally (negative control); **Group II** 2mg/kg CdCl\(_2\), sc (positive control); **Group III** 1000mg/5ml/kg *Spirulina platensis* extract, orally; **Group IV** Animals were pretreated with *Spirulina* extract (1g kg\(^{-1}\)) for seven days and thereafter *Spirulina* and cadmium chloride was administered concomitantly for another 15 days.

After the completion of treatment, animals were sacrificed under light ether anesthesia. The wet tissue weight and volume of blood was recorded for cadmium determination in blood, liver and kidney.

**Bioaccumulation of metals**

The concentration of cadmium, copper and zinc in liver and kidney was measured by standard method of Parker \[10\].
Assessment of Hepatic Functions
The concentration of AST, ALT, CRE and URE in serum was measured using commercial kit (Ranbaxy, India Ltd.).

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
Effect of simultaneous administration and pretreatment of *Spirulina platensis* in Cd intoxicated rats on body weight and organ-body weight ratio. The results demonstrate that Cd intoxication caused significant inhibition in the percentage of body weight gain (85 %, p<0.05) and enhanced organ-body weight ratio (0.6 and 0.7 fold in liver and kidney respectively, p<0.05) as compared to controls indicating retardation in growth. No significant differences have been observed for body weight and weight of organs studied (Fig. 1 & Table 1) in *Spirulina platensis* alone treated rats.

Table 1 Organs weight of albino rats treated with cadmium chloride and *Spirulina platensis*, during four weeks of exposure

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>Liver weight (gms)</th>
<th>Weight percentage (%)</th>
<th>Kidney weight (gms)</th>
<th>Weight percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>1.26±0.32</td>
<td>0.96</td>
<td>0.51±0.03</td>
<td>0.39</td>
</tr>
<tr>
<td>II</td>
<td>Cd alone</td>
<td>2.39±0.83</td>
<td>2.15</td>
<td>0.78±0.03</td>
<td>0.70</td>
</tr>
<tr>
<td>III</td>
<td>SP alone</td>
<td>1.24±0.81</td>
<td>0.94</td>
<td>0.56±0.07</td>
<td>0.43</td>
</tr>
<tr>
<td>IV</td>
<td>Cd+SP</td>
<td>1.79±0.81</td>
<td>1.33</td>
<td>0.68±0.01</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M. (n=8). *p<0.05, compared to control animal, **p<0.01 compared to cadmium treated rats.

However, pretreatment with *Spirulina platensis* significantly increased the body weight (120 %) and organs weight (180 % liver and 130 % kidney) as compared to Cd exposed rats. Serum variables indicative of renal and heptotoxicity are depicted in table 2.

Table 2 Cd induced changes in hepatic and renal markers and their response to treatment with dietary nutrients alone or in combination in serum of albino rats, during four weeks of exposure

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>Urea (mg/dl)</th>
<th>CRE (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>8.17±0.26</td>
<td>8.59±0.19</td>
<td>29.69±0.59</td>
<td>0.76±0.01</td>
</tr>
<tr>
<td>II</td>
<td>Cd alone</td>
<td>11.11±0.39</td>
<td>13.8±0.11</td>
<td>38.00±2.03</td>
<td>0.93±0.017</td>
</tr>
<tr>
<td>III</td>
<td>SP alone</td>
<td>7.86±0.32</td>
<td>8.56±0.15</td>
<td>28.86±4.45</td>
<td>0.75±0.01</td>
</tr>
<tr>
<td>IV</td>
<td>Cd+SP</td>
<td>8.13±0.32</td>
<td>9.17±0.27</td>
<td>29.69±1.27</td>
<td>0.77±0.02</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.M. (n=8). *p<0.05, compared to control rats.
Fig. 1 Body weight gain of albino rats treated with cadmium chloride and herbal adaptogen \textit{(Spirulina platensis)}, during four weeks of exposure.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure1.png}
\caption{Body weight gain of albino rats treated with cadmium chloride and herbal adaptogen \textit{(Spirulina platensis)}, during four weeks of exposure.}
\end{figure}

Results are expressed as mean $\pm$ S.E.M. \((n=8)\). $^a$p<0.05, compared to control animal, $^b$p<0.01 compared to cadmium treated rats.

Treatment with cadmium significantly increased \((p<0.05)\) the activities of serum AST, ALT, URE and CRE \((136, 161, 128 \text{ and } 122 \% \text{ respectively})\) as compared to control. Administration of \textit{Spirulina platensis} alone did not show any significant change in the serum levels of liver and kidney enzymes. Whereas, pretreatment with \textit{Spirulina platensis} attenuated the cadmium induced decrease of serum AST, ALT, URE and CRE \((73, 66, 78 \text{ and } 82 \% \text{ respectively})\) as compared to their levels in cadmium treated rats.

Figure 2 show cadmium concentrations in blood liver and kidney of Cd exposed animals and the ability of \textit{Spirulina platensis} pretreatment to reduce its concentration. Rats exposed to cadmium alone showed significant elevation of cadmium in blood and soft tissues leading to 1.4, 1.5 and 1.4 fold increases in blood, liver and kidney respectively. \textit{Spirulina platensis} was found to have a protective effect by decreasing cadmium content in blood and tissues (blood; 69 \%, liver; 32 \%, and kidney; 62 \%) as compared to cadmium intoxicated rats.

There was a notable decrease in the level of copper which was 0.61 fold in blood, 0.58 fold in liver, and 0.8 fold in kidney as compared to cadmium intoxicated rats (Fig. 2). Whereas the increasing trend was observed in copper \((1.5 \text{ fold in blood}, 1.4 \text{ fold in liver, and } 1.1 \text{ fold in kidney})\) after \textit{Spirulina} supplementation prior to cadmium intoxication.

The presence of zinc was decreased by 0.5 fold in blood, 0.5 fold in liver, and 0.4 fold in kidney as compared to control rats when exposed to cadmium. The absorption of zinc was raised by 1.7 fold in blood, 1.8 fold in liver and 1.4 fold in kidney as compared to cadmium intoxicated rats (Fig. 2).
Fig. 2 Effect *Spirulina platensis* on cadmium, copper and zinc concentration in blood (µg/ml), liver and kidney (µg/gm) of control and Cd intoxicated rats. Data are expressed as mean ± S.E.M. (n=8).

Results are expressed as mean ± S.E.M. (n=8). *p*<0.05, *b* *p*<0.01 compared to cadmium treated rats.

**DISCUSSION AND CONCLUSION**

Both environmental pollution and scarcity of feed and fodder are increasing day by day. In view of the economics involved, discarding the contaminated feed is not possible always. Hence, means to reduce the toxicity likely to result from consumption of such feeds have to be developed. Antioxidant flavonoids in herbal adaptogens can chelate the catalytic metals and also can act as oxygen free radical scavengers [11,14] Changes in the body weight and organ/body weight ratio have been used as indices of chemical toxicity. The significant alterations observed in these parameters in Cd exposed rats are indicator of toxicity which exhibits a decreasing trend in the body weight and increase in organ/body weight ratio throughout the course of experiment. This reversal of the effect of Cd on organ weight expressed as organ/body weight ratio by the nutrients supplementation provided a strong indication that supplementation with dietary nutrients was helpful in ameliorating the effect of cadmium toxicity. Our results indicated a significant increase in the toxic metal level in the liver, kidney and blood with higher amount in the kidney which was evident from the data showing maximum accumulation of cadmium at the end of the experiment (Fig. 2). Our observations are in agreement with the findings of previous workers [15]. The activity of AST and ALT enzymes in blood serum may also be used as stress indicator. The significant changes in the activities of these enzymes in blood serum indicate tissue impairment caused by stress [16]. In the present study there were significant changes in AST and ALT activities in serum of rats exposed to Cd compared to the control group. In the present study, activity of urea and creatinine were increased significantly in the kidney of Cd exposed rats. This may be due to the damage of large number of nephrons. Only renal dysfunction changes the results, however, the serum creatinine level will not rise until at least half of the kidney’s nephron are destroyed or damaged [17]. The results of current study
demonstrated that *Spirulina platensis* itself significantly improved the damages of hepatocytes and renal tissues specifically glomerulus filtration for they normalized the activities of these hepatorenal markers [18].

Herbal adaptogens such as *W. somnifera, O. sanctum, Aspe. recemosus, An. paniculata, Asph. panjabinum* (Shilajith), *G. sylvestre, S. platensis*, and *P. ginseng* are known for their antioxidant properties. Results of the present study indicate that dietetic supplementation of the above herbs reduced the Cd accumulation in liver and kidney and protected them from subsequent Cd-induced peroxidative damage by free radicals. Herbal antioxidants might have scavenged the oxygen free radicals and averted GSH exhaustion during the process of Cd detoxification. It is further possible that the herbal antioxidants chelated Cd [11] and facilitated its elimination, thereby sparing –SH group of enzymes, GSH and proteins. It appears from this study that dietetic supplementation of certain antioxidant herbs to Cd intoxicated rats will prevent the Cd accumulation and oxidative stress.

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