Hepatoprotective Effect of C-Phycocyanin: Protection for Carbon Tetrachloride and R-(-)-Pulegone-Mediated Hepatotoxicity in Rats

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Effect of C-phycocyanin (from Spirulina platensis) pretreatment on carbon tetrachloride and R-(-)-pulegone-induced hepatotoxicity in rats was studied. Intraperitoneal (i.p.) administration (200 mg/kg) of a single dose of phycocyanin to rats, one or three hours prior to R-(-)-pulegone (250 mg/kg) or carbon tetrachloride (0.6 ml/kg) challenge, significantly reduced the hepatotoxicity caused by these chemicals. For instance, serum glutamate pyruvate transaminase (SGPT) activity was almost equal to control values. The losses of microsomal cytochrome P450, glucose-6-phosphatase and aminopyrine-N-demethylase were significantly reduced, suggesting that phycocyanin provides protection to liver enzymes. It was noticed that the level of menthofuran, the proximate toxin of R-(-)-pulegone was nearly 70% more in the urine samples collected from rats treated with R-(-)-pulegone alone than rats treated with the combination of phycocyanin and R-(-)-pulegone. The possible mechanism involved in the hepatoprotection is discussed.

Key Words: Spirulina platensis; C-phycocyanin; carbon tetrachloride; R-(-)-pulegone, a monoterpenic ketone; hepatotoxicity; hepatoprotective effect.

Spirulina platensis, a unicellular filamentous blue-green algae is gaining more attention these days because of its nutritional and various medicinal properties(1,2). Spirulina maxima has preventive effect on the fatty liver induced by a fructose-rich diet in the rat (2) suggesting that this algae contains a factor or factors which affect the fructose-induced alterations of triglyceride metabolism. It has also been shown that C.

**Materials and Methods**

C. phycocyanin (isolated from Spirulina platensis) was a generous gift from Cyanotech Bio-products (p) ltd. Bangalore, India.

Animals and treatment. Male albino rats (2-3 months old) weighing 160-180 g were used throughout the course of this investigation. Rats were housed in groups and were fed ad libitum. Six groups (A, B, C, D, E, and F) of rats, each group with 6 animals were used in the following way. Unless otherwise mentioned, all treatments were carried out intraperitoneally (i.p.). Group A and B (control rats) received coconut oil (0.3 ml); group C and E received R-(-)-pulegone at a dosage of 250 mg/kg as a suspension in 0.3 ml of coconut oil; group D and F received carbon tetrachloride at a dosage of 0.6 ml/kg as a suspension in coconut oil (0.3 ml). Rats from group B, E and F were pretreated with phycocyanin (200 mg/kg) dissolved in water (0.5 ml) one hour prior to the administration of coconut oil (control rats), R-(-)-pulegone and carbon tetrachloride, respectively. 24 h After the administration of R-(-)-pulegone and carbon tetrachloride, the animals were sacrificed by cervical dislocation and blood was collected by cardiac puncture for SGPT determinations. The above experiment was repeated by pretreating the animals with phycocyanin three hours prior to the administration of R-(-)-pulegone.
### Results and Discussion

The effect of C. phycocyanin pretreatment on CCl4 and R-(-)-pulegone-induced hepatotoxicity in rats is shown in Table 1. A significant increase in GPT, alanine transaminase, and glucose-6-phosphatase activities and a significant decrease in cytochrome P450, aminopyrine-N-demethylase activity, and hydroxylamine-induced hepatic necrosis in rats pretreated with CCl4 and R-(-)-pulegone were analyzed by GC and the levels of all the activities tested were similar to those of control values (Tables 1 and 2). However, administration of CCl4 alone did not alter liver function. The levels of SGPT were almost equal to control values in rats treated with phycocyanin, one or three hours prior to R-(-)-pulegone or CCl4. Rats treated with CCl4 administered alone did not alter liver function. The increase in GPT, alanine transaminase, and glucose-6-phosphatase activities and a significant decrease in cytochrome P450, aminopyrine-N-demethylase activity, and hydroxylamine-induced hepatic necrosis in rats pretreated with CCl4 and R-(-)-pulegone were almost equal to control values. The response to R-(-)- pulegone (250 mg/kg) and CCl4 (0.6 ml/kg) was held at 80 °C for 10 min, and then it was raised to 200 °C at the rate of 5 °C/min.

#### Table 1

<table>
<thead>
<tr>
<th>Enzyme Activity</th>
<th>Control</th>
<th>Phycocyanin</th>
<th>R-(-)-Pulegone</th>
<th>Phycocyanin + R-(-)-Pulegone</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPT (units/ml)</td>
<td>32.2 ± 3.1</td>
<td>32.2 ± 3.1</td>
<td>25.5 ± 2.3</td>
<td>13.2 ± 2.3</td>
</tr>
<tr>
<td>ALP (units/ml)</td>
<td>490.0 ± 30.2</td>
<td>490.0 ± 30.2</td>
<td>341.2 ± 15.2</td>
<td>213.2 ± 15.2</td>
</tr>
<tr>
<td>Glucose-6-PH (units/ml)</td>
<td>65.6 ± 5.5</td>
<td>65.6 ± 5.5</td>
<td>34.6 ± 4.5</td>
<td>19.5 ± 4.5</td>
</tr>
<tr>
<td>Cytochrome P450 (nmol/mg)</td>
<td>0.78 ± 0.01</td>
<td>0.78 ± 0.01</td>
<td>0.34 ± 0.05</td>
<td>0.12 ± 0.06</td>
</tr>
<tr>
<td>Aminopyrine-N-Demethylase (nmol/mg)</td>
<td>0.45 ± 0.19</td>
<td>0.45 ± 0.19</td>
<td>0.07 ± 0.04</td>
<td>0.03 ± 0.02</td>
</tr>
</tbody>
</table>

Note: Rats were pretreated with C. phycocyanin (200 mg/kg) one or three hours prior to the administration of R-(-)-pulegone (250 mg/kg) and CCl4 (0.6 ml/kg). Experiments were carried out on a Shimadzu GC instrument equipped with a hydrogen flame ionization detector. The instrument was fitted with a Shimadzu HR-1 wide-bore column (20 m x 0.5 mm diameter). N2 at a flow rate of 0.5 ml/min was used as the carrier gas. The temperature of the pretreatment was made three hours prior to the administration of CCl4 and R-(-)- pulegone. Microsomes were prepared from liver by differential centrifugation method (11). The microsomes were suspended in Tris-HCl buffer (0.05 M, pH 7.8) containing 0.25M sucrose and EDTA (0.1 M, pH 7.8). The enzyme activities were measured using the methods as mentioned under "methods". All statistical analyses were performed using Student’s t-test and levels of significance determined at p < 0.05.
TABLE 2

Effect of Phycocyanin Pretreatment† on CCl4 (0.6 ml/kg) and R-(-)-Pulegone (250 mg/kg)-Induced Hepatotoxicity in Rats

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>Control†</th>
<th>Phycocyanin</th>
<th>R(+)-Pulegone</th>
<th>CCl4</th>
<th>R(+)-Pulegone</th>
<th>(+) Phycocyanin</th>
<th>(+) Phycocyanin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome P450 (nmol/mg)</td>
<td>0.71 ± 0.02</td>
<td>0.61 ± 0.01</td>
<td>0.27 ± 0.01</td>
<td>0.26 ± 0.01</td>
<td>0.58 ± 0.01</td>
<td>0.51 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>% change</td>
<td></td>
<td>-15.0</td>
<td>-61</td>
<td>-64</td>
<td>-17</td>
<td>-28.0</td>
<td></td>
</tr>
<tr>
<td>SGPT (units/ml)</td>
<td>33.25 ± 1.1</td>
<td>28.0 ± 2.0</td>
<td>504.0 ± 36.0</td>
<td>485.3 ± 18.7</td>
<td>31.27 ± 1.0</td>
<td>30.34 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>Fold change</td>
<td></td>
<td>+15.0</td>
<td>+14.6</td>
<td>+14.6</td>
<td>-0.06</td>
<td>-0.09</td>
<td></td>
</tr>
<tr>
<td>G-6-Phosphatase (nmol/min/mg)</td>
<td>225.1 ± 7.6</td>
<td>228.5 ± 7.5</td>
<td>152.2 ± 7.9</td>
<td>126.6 ± 4.6</td>
<td>193.3 ± 7.3</td>
<td>147.5 ± 12.2</td>
<td></td>
</tr>
<tr>
<td>% change</td>
<td></td>
<td>+1.5</td>
<td>-32.3</td>
<td>-43.7</td>
<td>-14.0</td>
<td>-34.5</td>
<td></td>
</tr>
<tr>
<td>Aminopyrine-N-demethylase (nmol/min/mg)</td>
<td>7.33 ± 0.33</td>
<td>6.0 ± 0.33</td>
<td>3.24 ± 0.45</td>
<td>3.58 ± 0.24</td>
<td>6.16 ± 0.32</td>
<td>5.83 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>% change</td>
<td></td>
<td>-18.0</td>
<td>-55.7</td>
<td>-51.0</td>
<td>-16.0</td>
<td>-20.3</td>
<td></td>
</tr>
</tbody>
</table>

Note. † Rats were pretreated with C. phycocyanin (200 mg/kg) 3h prior to the administration of CCl4 and R-(-)-pulegone. Other details are as in Table 1.

A typical gas chromatogram shows (Fig 1A and B) that the level of menthofuran was significantly higher (nearly 70% more) in the urine of rats treated with R-(-)-pulegone alone than in the urine of rats treated with the combination of phycocyanin and R-(-)-pulegone. However, there was only marginal changes in the levels of other major metabolites (Fig. 1). This is a significant observation since menthofuran is considered as the proximate toxin of R-(-)-pulegone and is responsible for at least half of the hepatocellular necrosis caused by R-(-)-pulegone (18). It is known that microsomal cytochrome P450 system carries out the regiospecific oxidation of R-(-)-pulegone to its allylic alcohol (9-hydroxymenthofuran) which upon cyclization followed by dehydration yields menthofuran (9,10). The cytochrome P-450 system further converts menthofuran to its epoxide which could easily give rise to an α, β-unsaturated-γ-ketoaldehyde, a highly reactive metabolite known to covalently interact with tissue macromolecules generating toxicity (19,20). So it is quite possible that phycocyanin could interact preferentially with individual species of cytochrome P-450 and thus could affect the formation of 9-hydroxymenthofuran which is the precursor of menthofuran. It is also possible that the cytochrome P450 mediated reaction involved in the conversion of menthofuran to its epoxide may be inhibited so that the reactive metabolite viz. α, β-unsaturated-γ-ketoaldehyde may not be formed in sufficient quantities to elicit toxicity. So it appears that prior administration of phycocyanin protects against CCl4 and R-(-)-pulegone mediated toxicity by lowering the biotransformation of these hepatotoxins into toxic intermediates. This assumption is supported by the fact that higher levels of menthofuran was shown to be present in the urine of rats treated with R-(-)-pulegone alone than in the urine of rats treated with the combination of phycocyanin and R-(-)-pulegone (Fig 1). It is also possible that the haloalkane free radicals produced from CCl4 and reactive metabolites formed from R-(-)-pulegone by the liver microsomal cytochrome P450 systems are being scavenged by phycocyanin. In fact recently it has been reported that phycocyanin has the ability to scavenge alkoxy and hydroxyl radicals (3).

FIG. 1. GC separation of major urinary metabolites from (A) R-(-)-pulegone treated rats, (B) R-(-)-pulegone was administered to C. phycocyanin pretreated rats. (1) p-Cresol, (2) R-(-)-menthofuran, (3) R-(-)-pulegone, (4) piperitone, and (5) piperitenone. Experimental conditions are as reported under “Methods.”
More experiments have to be carried out to establish the mechanism involved in the hepatoprotection by phycocyanin.

CONCLUSIONS

The results presented here demonstrate that C. phycocyanin, one of the major biliproteins of Spirulina platensis can significantly reduce R-(+)-pulegone and CCl₄ induced liver injury in rats. The responses to both of these hepatotoxins are significantly reduced in the presence of phycocyanin possibly due to lower levels of reactive metabolites formed. Phycocyanin may inhibit some of the cytochrome P450 mediated reactions involved in the formation of reactive metabolites. It is also possible that phycocyanin may act as an efficient radical scavenger.

ACKNOWLEDGMENTS

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REFERENCES