Antihepatotoxic effect of \( \beta \)-carotene on paracetamol induced hepatic damage in rats

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Received 24 March 2004; revised 10 December 2004

Beta carotene (BC), a carotenoid pigment functions mainly as provitamin A in animals. It also acts as a powerful free radical scavenger and chain breaking antioxidant. It protects against heart diseases, may also provide prevention from heart attacks and strokes, slows down the progression of cataracts and prevents macular degeneration. Dietary BC may also slow down the progression of osteoarthritis and most effective naturally occurring quencher of singlet oxygen and is highly reactive energized molecule.

Reports suggest that BC protects against cancer and enhances the immune response and reduces photo induced neoplasm and tumor growth. The function of carotenoid as radical scavenging antioxidants can protect the cells from oxidative damage. This communication deals with antihepatoxic effect of BC on paracetamol induced hepatic damage in rats.

Materials and Methods

Animals—Albino rats of Wistar strain weighing about 150-200g were obtained from Perundurai Medical College, Perundurai, Erode, Tamilnadu and kept under standard laboratory conditions at 12:12 hr L:D cycles at 25°-28°C and 60-80% RH. Animals were reared with robust health by providing pellet diet (Lipton, India) and water ad libitum.

Experimental protocol—The rats were divided into 3 groups of 6 rats each. The animals in group 1 served as control and given distilled water, po, for 10 days in succession. The group 2 rats served as test and were administered distilled water similarly followed by oral administration of paracetamol @ 3g/kg body weight, 1hr after distilled water administration. The animals in group 3 served as experimental and treated orally with BC (10mg/kg body weight) once in a day for 10 days in succession followed by a single oral administration of paracetamol (3g/kg body weight), 1hr after BC administration.

Preparation of BC—BC was separated by chromatography of a hexane solution.

Assessment of liver function—After 24hr of paracetamol administration rats of all groups were sacrificed by cervical dislocation, blood was collected from the carotid arteries in the neck blood vessels, and centrifuged at 2000 rpm for 10 min to separate the serum, which was kept at 4°C to assay the activities of serum enzymes. Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and serum bilirubin were estimated.
After the collection of blood, the liver was immediately excised, washed with cold saline, blotted and weighed. A piece of liver from each rat was taken and homogenized to make liver homogenate; this was then subjected to biochemical analysis. Hepatic glycogen, reduced glutathione (GSH), glutathione-S-transferase (GST), glutathione peroxidase (GPX), and GSH-R were determined.

**Histological investigations**—The remaining portions of livers were quickly removed and preserved in 10% formosaline and processed for paraffin embedding following the standard microtechnique. Sections (5µm thick) of livers stained with Haematoxylin and Eosin were evaluated for histopathological under a light microscope.

**Statistical analysis**—Results of the biochemical estimations are reported as mean ± SD. Total variations, present in a set of data were estimated by one-way analysis of variance (ANOVA), Student’s t-test was used for determining significance. The percentage of the protection is calculated as $100 \times (\text{values of paracetamol control} - \text{values of sample})/(\text{values of paracetamol control} - \text{values of normal control})$.

**Results**

The results are presented in Table 1.

**Biochemical**—Rats treated with a single dose of paracetamol alone developed significant hepatocellular damage as evidenced from a significant ($P<0.05$) increase in the serum SGOT, SGPT, ALP and bilirubin when compared with control (Table 1). Pretreatment of rats with BC reduced the elevated serum levels of these hepatospecific enzymes, in a dose responsive manner. Treatment with paracetamol caused a reduction in hepatic glycogen and GSH levels (Table 1). Pretreatment of rats with BC (10 mg/kg body weight) exhibited a high degree of protection by reversing the altered levels of glycogen and GSH. The activities of GST, GPX, and GSH-R showed significant reduction in liver of paracetamol treated rats as compared to the control group. Pretreatment of rats with BC significantly increased the enzyme activities (Table 1).

**Histopathology**—Histology of the liver sections of control animals (Group 1) showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus and nucleolus and conspicuous central vein (Fig. 1A). The liver sections of paracetamol treated animals (Group 2) showed hepatic cells with severe toxicity characterized by perilobular necrosis, dilated sinusoidal spaces, and diffuse hyaline necrosis with blood pooling in sinusoidal spaces and central venule (Fig. 1B). The histopathological pattern of the livers of the rats treated with BC showed a normal lobular pattern with minimal pooling of blood in the sinusoidal spaces (Fig. 1C).

**Discussion**

Paracetamol (N-acetyl p-amino phenol, acetaminophen) a widely used analgesic and antipyretic drug is known to cause hepatotoxicity in experimental animals and humans at high doses. It is mainly metabolized in the liver to excretory glucuronide and sulphate conjugates. However, hepatotoxicity of paracetamol has been attributed to formation of toxic metabolites when a part of paracetamol is activated by

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Control (Gr.1)</th>
<th>Test Paracetamol (Gr.2)</th>
<th>Experimental (10mg/kg bw) + Paracetamol (Gr.3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
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<tr>
<td>SGOT (IU/l)</td>
<td>94.2 ± 5.1</td>
<td>252.8 ± 4.03c</td>
<td>193.4±6.2a (37.4)</td>
</tr>
<tr>
<td>SGPT (IU/l)</td>
<td>58.40 ± 2.4</td>
<td>172.5 ± 3.6c</td>
<td>128.4±4.8b (38.6)</td>
</tr>
<tr>
<td>ALP (IU/l)</td>
<td>126 ± 8.2</td>
<td>275.2 ± 1.4c</td>
<td>176.4±4.2b (66.2)</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>0.89 ± 0.02</td>
<td>3.48 ± 0.12b</td>
<td>1.3±0.04b (84.1)</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
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<tr>
<td>Glycogen (mg/100g tissue)</td>
<td>6.76 ± 0.97</td>
<td>2.18 ± 0.29e</td>
<td>3.62±0.7a (31.4)</td>
</tr>
<tr>
<td>GSH (nmol/mg protein)</td>
<td>30.5±1.8</td>
<td>14.4 ± 2.4a</td>
<td>23.2±0.8a (54.6)</td>
</tr>
<tr>
<td>GST (u/g tissue)</td>
<td>107.8 ± 2.1</td>
<td>91.6 ± 1.8b</td>
<td>95.2±1.2a (22.2)</td>
</tr>
<tr>
<td>GPX (u/mg protein)</td>
<td>8.8 ± 0.8</td>
<td>5.6 ± 0.6b</td>
<td>7.24±0.8b (51.2)</td>
</tr>
<tr>
<td>GSH-R (µmol NADPH min⁻¹/g tissue)</td>
<td>172 ± 3.2</td>
<td>110 ± 4.8b</td>
<td>142.6±2.8a (52.5)</td>
</tr>
</tbody>
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$P$ values: *<0.05; **<0.01; ***<0.001 as compared with Group 2 animals.
hepatic Cyt-p 450 (ref. 29) to a highly reactive metabolite N-acetyl-p-benzoquinoneimine, which is normally conjugated with GSH and excreted in the urine as conjugates. Overdose of paracetamol leads to mitochondrial dysfunction followed by acute hepatic necrosis. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver. Damage to liver cells cause leakage of cellular enzyme into serum. A significant rise in SGOT, SGPT could be taken as an index of liver damage. The reversal of increased serum transaminases returns to normal by BC supplementation with healing of hepatic parenchyma and regeneration of hepatocytes.

ALP and bilirubin concentration have been used to evaluate chemically induced hepatic injury. More than 90% of ALP activity has been found to be elevated in serum of common laboratory animals used in toxicity studies. BC prevented the paracetamol effect on ALP activity in serum. It is reasonable to suggest that BC limited the severity of liver injury. Stabilization of serum bilirubin levels through the administration of BC is further a clear indication of the improvement of the functions of the liver cells.

In the present study, the decrease in the hepatic glycogen content due to induced action of hepatotoxin findings agree with previous reports. The present results support that the recovery of hepatic glycogen content was observed in the pretreatment of BC, while signs of histological and biochemical recuperation were present in the liver of rats treated with BC.

GSH in the cytosolic pool consists of 85% hepatocellular GSH and 15% mitochondrial GSH. Hepatic GSH depletion or even extra hepatic GSH depletion can provide useful information on the protective role of GSH against toxic foreign compounds. Thus, GSH, be regarded as an endogenous protective agent against drugs. In the present study decreased level of reduced GSH in liver was decrease in paracetamol-induced animals, while pretreatment of BC clearly enhanced the GSH levels. GST is a soluble protein located in cytosol, which plays an important role in the detoxification of excretion of xenobiotics. It increases the solubility of hydrophobic substances and metabolises toxic compounds to non-toxic ones, which mean they have an increasing protective activity of the liver. The increased hepatic GST activity, induced by BC supplementation can, therefore, reduce the paracetamol hepatotoxicity. There was a decrease...
in GPX activity in animals administered with paracetamol, which could be due to the higher production of toxicity. In presence of BC, GPX levels were restored back to control levels. The increase in hepatic GSH-R activities were shown in BC supplemented rats as compared with the liver of paracetamol-induced rats.

These results suggest the hepatoprotective action of BC, which protect hepatic cells from paracetamol-induced damage and the degree of hepatoprotection improved with increasing dosage. Further these data provide information regarding the possible use of BC as a nutritional supplement and as hepatoprotectant in Indian systems of medicine.

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