Prevention of carbon tetrachloride-induced hepatic injury in mice by *Picrorhiza kurrooa*

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**Objective:** *Picrorhiza kurrooa* (Pk) has been used in liver diseases in the Indian indigenous system of medicine. We undertook this study to determine whether Pk extract possesses hepatoprotective function and if so to determine its nature and mechanism. **Methods:** Liver injury was induced in 18 mice by thrice-a-week injection of carbon tetrachloride (CCL₄) for nine weeks. Eight of them were given daily feeding of Pk extract (12 mg/Kg) 10 days prior to CCL₄ injection. Control mice (n=6) were injected with olive oil for the same period. Serum markers of liver injury and histology of liver tissues were studied. Hepatic glutathione (GSH), total thiol (-SH), glucose 6-phosphate dehydrogenase (G6PD), catalese, lipid peroxidation and plasma membrane-bound Na⁺/K⁺ ATPase were also determined. **Results:** CCL₄ treatment resulted in significant elevation of serum ALT and AST. Liver GSH [6.3 (0.7) vs control 10.5 (1.1) μg/mg protein], -SH, G6PD, catalese and membrane-bound Na⁺/K⁺ ATPase [164.3 (23.2) vs control 358.4 (12.9) n mole pi released/min/mg protein] were significantly reduced. Significant increase of lipid peroxidation [3.0 (0.6) vs control 1.0 (0.3) n mole MDA/mg protein] and histologic changes characteristic of liver injury were also seen. Feeding of Pk extract in CCL₄-treated mice caused significantly less alteration of serum ALT, AST, liver GSH [8.9 (0.7) μg/mg protein], -SH, G6PD, catalese and membrane-bound Na⁺/K⁺ ATPase [270.8 (21.3) n mole pi released/min/mg protein]. Histologic lesions of liver and lipid peroxidation [1.7 (0.4) n mole MDA/mg protein] were also significantly less in these animals. **Conclusion:** The extract of Pk appears to offer significant protection against liver damage by CCL₄, it probably acts as free-radical scavenger and inhibitor of lipid peroxidation of liver plasma membrane. **Animal experiments** Twenty two BALB/C mice (6-7 weeks old, weighing 20-22 g) were studied in three groups. All the mice were housed in a constant temperature and humidity environment with a 12-h photo period. The mice were maintained on basal diet and water ad libitum.

In the Pk group, eight animals were treated with intraperitoneal (IP) injection of CCL₄ (diluted in olive oil in a ratio of 1:10) three times a week for 9 weeks. CCL₄ was administered in increasing doses of 0.3 mL/Kg during the first week, 0.4 mL/Kg during the second week, and 0.5 mL/Kg from the third to the ninth week. These mice were also treated orally with Pk extract (12 mg/Kg/day) starting...
10 days preceding the injection of CCl₄, and continued to the end of the experiment. One mouse in this group died in the second week of this study due to fault in feeding Pk extract. In the CCl₄ group, eight animals were treated with CCl₄ as described above. The mice were not given any Pk extracts but were fed with equivalent volumes of water each day. In the control group, six animals were injected IP with equal volume of olive oil thrice a week for 9 weeks.

All the mice were sacrificed following ether anesthesia 72 hours after the last injection. Blood was collected from each animal by cardiac puncture before sacrificing them; total protein, albumin, ALT and AST were estimated by standard laboratory procedures. A small piece of liver tissue from each animal was fixed in 10% buffered formalin for histologic examination. The remaining portion of the liver was rapidly perfused through the vena portae with 20 mL of ice-cold physiological saline, removed, blotted with gauze, weighed and divided into two portions.

A portion of liver tissue from each animal was minced, suspended in 0.15M KCl solution (pH 7.4) and homogenized in Teflon homogenizer for 2 min at below 4°C. The homogenate was divided into three parts; one part was used for the assay of glutathione (GSH) and total thiol (-SH). The second part was centrifuged at 2000 g for 15 min in a cold centrifuge (Remi C24) and the supernatant was used for study of lipid peroxidation. The third part was centrifuged at 3000 g for 15 min in a cold centrifuge (Remi C24). The pellet was reconstituted and the supernatant was reconstituted at 105,000 g for 1 h (Spinco Model L ultracentrifuge) and the pellet discarded. The soluble supernatant was used for assay of the activities of glucose-6-phosphate dehydrogenase (G6PD) and catalase.

Another portion of the liver tissue was homogenized in sodium bicarbonate/calcium chloride solution and centrifuged at 1500 g for 30 min and plasma membrane was prepared. Na⁺/K⁺ ATPase activities were determined at 37°C according to the method of Ismail-Beigi and Edelman as modified by Gonzalez-Calvin et al. NADPH-cytochrome C reductase and succinate dehydrogenase were determined according to the methods of Feo et al. The marker enzymes NADPH-cytochrome C reductase and succinate dehydrogenase for mitochondria and plasma membranes respectively were assayed to evaluate the degree of contamination of liver plasma membrane with these subcellular fractions. Protein content of liver homogenate and its cytosolic and plasma membrane fractions were estimated following the method of Lowry et al.

Histologic examination of the liver tissue was done after staining with hematoxylin-cosin and reticulin.

All procedures concerning animal experiment were reviewed and approved by the ethics committee of the institute as there is no separate animal welfare committee in this institute.

### Table 1: Serum ALT, AST, albumin and hepatic GSH, SH, G6PD, catalase levels and lipid peroxidation in different groups of mice

<table>
<thead>
<tr>
<th></th>
<th>Control n=6</th>
<th>CCl₄ n=6</th>
<th>Pk with CCl₄ n=7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ALT (U/L)</td>
<td>61.5</td>
<td>162***</td>
<td>97.4**</td>
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<tr>
<td>(6.5)</td>
<td>(15.5)**</td>
<td>(17.6)</td>
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<tr>
<td>AST (U/L)</td>
<td>56.7</td>
<td>185.4***</td>
<td>106.21***</td>
</tr>
<tr>
<td>(4.6)</td>
<td>(26.6)**</td>
<td>(19.4)</td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.7</td>
<td>2.8*</td>
<td>3.2</td>
</tr>
<tr>
<td>(0.6)</td>
<td>(0.8)</td>
<td>(0.5)</td>
<td></td>
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<tr>
<td>Liver</td>
<td></td>
<td></td>
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<tr>
<td>GSH (µg/mg protein)</td>
<td>10.54</td>
<td>6.27***</td>
<td>8.89*</td>
</tr>
<tr>
<td>(1.14)</td>
<td>(0.72)**</td>
<td>(0.68)</td>
<td></td>
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<tr>
<td>-SH (µg/mg protein)</td>
<td>12.56</td>
<td>8.32***</td>
<td>10.51**</td>
</tr>
<tr>
<td>(1.10)</td>
<td>(0.85)**</td>
<td>(0.80)</td>
<td></td>
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<tr>
<td>G6PD (nmol NADP)</td>
<td>10.08</td>
<td>7.29***</td>
<td>8.79*</td>
</tr>
<tr>
<td>(6.62)</td>
<td>(0.67)**</td>
<td>(0.55)</td>
<td></td>
</tr>
<tr>
<td>Catalase (nmol H₂O₂)</td>
<td>7.61</td>
<td>4.92***</td>
<td>6.20***</td>
</tr>
<tr>
<td>(0.47)</td>
<td>(0.70)**</td>
<td>(0.62)</td>
<td></td>
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<tr>
<td>Lipid peroxidation</td>
<td>1.00</td>
<td>3.04***</td>
<td>1.68***</td>
</tr>
<tr>
<td>(0.61)**</td>
<td>(0.61)**</td>
<td>(0.64)</td>
<td></td>
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</table>

*control vs CCl₄; **CCl₄ with Pk vs CCl₄; ***control vs CCl₄ with Pk; * p < 0.02; ** p < 0.01; *** p < 0.001; **** p < 0.0001; p < 0.05

### Statistical analysis

Data were expressed as mean (SD). Statistical analysis of the difference between sample means was done by the Student’s t-test.

### Results

Intraperitoneal treatment with CCl₄ caused significant elevation of ALT and AST and reduction of albumin levels. Feeding of Pk extracts to CCl₄-treated mice caused less elevation of enzymes and reduction of albumin (Table 1). Significant reduction of hepatic GSH, SH, cytosolic G6PD, catalase (Table 1) and membrane-bound NADP⁺/K⁺ ATPase (Table 2) associated with increase of lipid peroxidation was observed among mice treated with CCl₄. Feeding of...
Hepatoprotective function of Picrorhiza kurrooa

Fig 1: Liver histology of CCl₄-treated mice showing marked degeneration and necrosis with collection of mononuclear cells (H & E, 400X)

Plk extracts in CCl₄-treated mice caused less alteration of hepatic GSH, -SH and other enzymes, lipid peroxidation and membrane-bound Na⁺/K⁺ ATPase in these animals (Tables 1 and 2).

Abnormal histologic changes were observed in liver tissue of all the CCl₄-treated mice. Gross hepatic necrosis was evident, mostly involving the centrizonal areas of liver lobules. There was insignificant collection of inflammatory cells. Many hepatocytes showed hydropic degeneration marked by clear cytoplasm and pyknotic nuclei (Fig 1). Irregular reticulin condensation was seen in the liver lobules but there was no definite lobular disorganization of hepatic architecture. Pretreatment with Plk prevented these histologic changes (Fig. 2); the histology was essentially normal, with occasional spotty necrosis.

Discussion

Assessment of the hepatoprotective effect of any substance presents some difficulty. The importance of choosing appropriate criteria has been emphasized by Recknagel and Glende. Some authors have used survival time after large doses of CCl₄ as an easily defined end-point. But this does not appear to be appropriate since the effect of large doses of CCl₄ need not be limited to liver damage. We therefore used smaller doses of CCl₄ (0.3-0.5 mL/Kg) to simulate a situation of subacute liver damage than doses used by most workers (1 mL/Kg) for similar period for producing liver cirrhosis.

Data from the present study demonstrated that prior feeding of Plk extract to CCl₄-treated mice significantly reduced the degree of elevation of ALT and AST compared to CCl₄-treated animals. Similarly, reduction of serum albumin level was also prevented by Plk extract. Dwivedi et al. observed protection by Plk extract against CCl₄-induced increase in the levels of ALT and AST, though they did not observe much difference in serum albumin level. As they administered CCl₄ for a shorter period (4 weeks), the albumin-synthesizing capacity of the liver might not have been altered in their animals. Prevention of elevation of ALT in rats by Plk extract was also described by Ansari et al.

In the CCl₄-treated animals depletion of G6PD and catalase activities was observed simultaneously with membrane damage, as characterized by increased lipid peroxidation. Further, evidence of the latter was the decrease in Na⁺/K⁺ ATPase activity. Feeding of Plk extract before and during administration of CCl₄ decreased membrane damage; this has been described by Magra et al. earlier. Dwivedi et al. reported that feeding Plk significantly decreased the level of lipid peroxides in the liver of CCl₄-treated rats. They also observed that the activity of superoxide dismutase, a scavenger of free radicals, was stimulated by feeding Plk extract.

Oxidative damage and lipid peroxidation of membrane lipids are known to occur in a wide variety of liver disease induced by different etiological agents in humans as well as experimental animals. CCl₄-induced acute liver injury is known to be the prototype of free radical-mediated tissue damage. The hepatotoxicity of CCl₄ is directly related to its activation to trichloromethyl free radical (CCl₃) by the NADPH-dependent cytochrome 450-mediated monooxygenase system; CCl₃, in the presence of oxygen, is converted to trichloromethyl peroxo radical (CCl₃O₂⁻). These toxic and highly reactive free radicals initiate cell damage through the mechanism of covalent binding and lipid peroxidation.

Reactive metabolites of CCl₄ are detoxified by glutathione conjugate formation. Conjugation of reactive metabolites with glutathione was supported by the observation of reduction in its level after administration of CCl₄. Further, feeding of Plk extract to the CCl₄-treated mice caused significantly less reduction of hepatic GSH and
total thiol level. Chander et al.11 also reported restoration of liver cytosolic GSH and -SH level in Pk-treated Mastomys natalensis infected with Plasmodium berghei. Thus, partial cytoprotection by Pk extract, as demonstrated in this study and by other workers, may be caused either by increasing synthesis of GSH in the hepatocytes or by inhibiting the microsomal P450 system, causing less production of toxic metabolites of CCl4. Either of these mechanisms is possibly responsible for the decreased lipid peroxidation in Pk extract-fed animals.

Though less reduction of GSH, -SH, GDPD and diminished lipid peroxidation during administration of toxic substances along with Pk extracts have been reported earlier, evidence of protection against histologic damage in the liver by CCl4 has not been described previously. Most workers used experimental conditions for a shorter period. By prolonged administration of CCl4 in smaller doses we could produce histologic changes in the mice liver simulating subacute hepatic necrosis. Our study demonstrated that administration of Pk with CCl4 could prevent the occurrence of such changes.

References


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