Hepatoprotective effect of *Epaltes divaricata* extract on carbon tetrachloride induced hepatotoxicity in mice

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**Background & objectives**: *Epaltes divaricata* is widely used in Sri Lanka as an Ayurvedic medicine. In the present study the hepatoprotective and antioxidative effects of an aqueous extract of *E. divaricata* plant (Family-Compositae) were investigated against carbon tetrachloride induced hepatocellular injury in mice.

**Methods**: Healthy male mice (30-35 g body weight, 6-8 wk old) were used. A single dose of carbon tetrachloride (CCl₄ 0.5 ml/kg in olive oil) was administered ip to induce hepatotoxicity and the plant extract at a dose of 0.9 g/kg was administered orally by gavage. Animals were sacrificed 24 h and 4 days after the administration of CCl₄. Blood and liver tissue were collected for the assessment of serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and liver reduced glutathione level. The liver tissue was used for histopathological assessment of liver damage.

**Results**: Pre-treatment of mice with the plant extract of *Epaltes* (0.9 g/kg) orally for 7 days significantly reduced serum levels of ALT \((P<0.01)\), AST \((P<0.01)\) and ALP \((P<0.001)\) enzymes by 21.40, 47.36 and 71.12 per cent respectively and significantly increased \((P<0.001)\) the liver reduced glutathione level by 42.32 per cent, 24 h after the administration of carbon tetrachloride. A marked improvement in the enzyme activities and the liver reduced glutathione level was observed in the *Epaltes* pre-treated mice 4 days after the administration of carbon tetrachloride. Histopathological studies provided supportive evidence for the biochemical analysis.

**Interpretation & conclusion**: The results of the present study indicated that under the present experimental conditions, aqueous extract of *Epaltes divaricata* showed hepatoprotective abilities against carbon tetrachloride induced liver damage in mice.

**Key words** Antihepatotoxic effect - carbon tetrachloride - *Epaltes divaricata* - liver injury

*Epaltes divaricata* (Family Compositae) is found in Sri Lanka, India, Myanmar, Java and China. *Epaltes* is used in traditional Ayurvedic medicine to alleviate jaundice, urethral discharges and acute dyspepsia. It is also regarded as a diaphoretic, diuretic and a stimulating expectorant\. *E. divaricata* is widely used in Sri Lanka not only as an Ayurvedic medicine but also as a delicacy in villages. Though five closely related eudesmane derivatives have been isolated from the acetone extract of the plant\(^2\), no information is available on their therapeutic action.

Carbon tetrachloride is a widely used chemical to induce liver damage in experimental studies, and its toxicity has been studied extensively. The resulting hepatic injury was characterized by leakage of cellular enzymes into the blood stream and by centrilobular necrosis\(^3,4\).

The present study was undertaken to evaluate the use of *E. divaricata* as a hepatoprotective and antioxidative agent in carbon tetrachloride induced hepatotoxicity in mice.
Material & Methods

Plant extract: Whole plants of *E. divaricata* were collected from Elpitiya in the Galle district, Sri Lanka, and identity of plants was confirmed by comparing with the authentic samples from the Royal Botanical Gardens, Peradeniya, Sri Lanka. The whole plants were cut into pieces and refluxed in distilled water for one hour to prepare the aqueous extract.

Experimental animals: Healthy, 6-8 wk old male mice (30-35g), were obtained from the animal house of the Faculty of Medicine, University of Ruhuna, Sri Lanka. Animals were maintained on a standard laboratory diet. Food and water were given *ad libitum*. Animals were fasted for 16 h prior to administration of CCl₄. All protocols used in this study were approved by the ethics committee of the University of Ruhuna, Sri Lanka.

Chemicals: Assay kits for the estimation of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were purchased from Randox, UK. 5,5’ dithio bis-(2-nitrobenzoic acid) was purchased from Sigma Chemical Co., USA. All other chemicals were of analytical grade.

Treatment of animals: Mice were randomly divided into eight groups with 10 animals in each. Group 1 served as normal control and was given distilled water orally. Group 2, the drug control group was given the plant extract orally for 7 days and sacrificed on the following day. Animals were administered 0.9 g/kg of the plant extract once daily, which is equivalent to 0.2 ml/30g mouse. Groups 3 and 4 were given carbon tetrachloride (0.5 ml/kg in olive oil) intraperitoneally (ip) and the mice in group 3 were sacrificed 24 h later. Group 4 mice were sacrificed four days after the administration of carbon tetrachloride. Mice in groups 5 and 6 were given the plant extract orally for 7 days after the administration of carbon tetrachloride. Mice in groups 5 and 6 were given the plant extract orally for 7 days. On the seventh day, carbon tetrachloride was administered ip half an hour after the administration of the last dose of plant extract. Mice in group 5 were sacrificed 24 h later and those of group 6 were sacrificed 4 days after the administration of carbon tetrachloride. Groups 7 and 8 were administered the same dose of carbon tetrachloride ip and half an hour later the plant extract was administered orally. Mice in group 7 were sacrificed 24 h later. Mice in group 8 were administered the plant extract for two more days at 24 h intervals and sacrificed four days later.

Blood samples were drawn from ether anaesthetized mice by cardiac puncture and liver tissue was excised for the determination of reduced glutathione and a part was fixed in buffered formalin for histopathological assessment of liver damage.

Assessment of liver damage: Liver damage was assessed by the estimation of serum activities of ALT, AST and ALP using commercially available test kits (Randox, UK). Liver reduced glutathione level (GSH) was assessed by the method of Sedlak and Lindsay. Histopathological assessment of liver damage was done by studying haematoxylin and eosin stained slides of liver tissue.

Statistical analysis: Statistical comparisons of data were made by means of Student’s t-test; *P*<0.05 was regarded as significant.

Results & Discussion

There was no significant change in the activities of serum ALT, AST, ALP and liver reduced glutathione levels in group 2 mice compared to group 1 (Table). A significant increase (*P*<0.001) in the activities of serum enzymes and a significant decrease (*P*<0.001) in the liver reduced glutathione level occurred within 24 h of exposure to carbon tetrachloride (group 3).

Post-treatment with *Epaltes* (group 7) decreased the CCl₄ induced alterations in ALT, AST and ALP by 8.7, 20.96 and 68.82 per cent respectively while pre-treatment (group 5) decreased the respective enzyme levels by 21.40, 47.36 and 71.12 per cent, 24 h after the administration of CCl₄ compared to group 3. The percentage increase in liver reduced glutathione level in the post-treated group was 27.53 per cent whereas it was increased by 42.32 per cent in the pre-treated group. The same pattern was observed in the serum enzyme activities and liver reduced glutathione levels 4 days after the administration of CCl₄. The percentage decrease in ALT, AST, ALP and increase in GSH levels were 8.28, 10.39, 26.65 and 77.27 per cent respectively in the post-treated group (group 8) and 33.59, 63.94, 57.28 and 93.76 per cent respectively in the pre-treated group (group 6) compared to group 4 (Table).

Histopathological studies also provided supportive evidence for the biochemical analysis. The drug control group (group 2) showed the normal parenchymal
architecture with cords of hepatocytes, portal tracts and central veins without noticeable alterations compared to the normal control group (Fig. 1a). Centrilobular necrosis accompanied by fatty changes and ballooning degeneration were observed in the remaining hepatocytes in the livers of mice treated with carbon tetrachloride (group 3, Fig. 1b). The toxin mediated changes in livers of mice pre-treated with Epaltes extract (group 5, Fig. 1c), 24 h after the administration of CCl4 were of much less intensity than those observed in the livers of carbon tetrachloride treated mice (group 3). Though the extent of cellular necrosis was less compared to the carbon tetrachloride group, post-treatment was not as good as the pre-treatment. A similar pattern was observed in the liver histopathology of mice 4 days after the administration of CCl4. Compared to the post-treated groups, pre-treated groups showed a faster recovery. The areas of necrosis were much less in the pre-treated group, 4 days after the administration of CCl4 (group 6). Though in the post-treated group, the extent of damage was less compared to the CCl4 treated mice, the areas of necrosis were visible.

GSH is a critical determinant of tissue susceptibility to oxidative damage and the depletion of hepatic GSH has been shown to be associated with an enhanced toxicity to chemicals including CCl4. The significant impairment of hepatic GSH status associated with a substantial hepatocellular damage induced by CCl4 suggested the determinant role of hepatic GSH in the development of carbon tetrachloride toxicity. Cell injury induced by xenobiotics occurs only if mitochondrial GSH is depleted. In an earlier study, mice were fasted for 16 h to minimize the individual variation among mice of the same group where reduced glutathione level was concerned. In the present study, a significant decrease (P<0.001) in the liver GSH was observed 24 h and 4 days after the administration of CCl4 compared to normal controls and drug control groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (U/I)</th>
<th>AST (U/I)</th>
<th>ALP (U/I)</th>
<th>GSH (µg/g liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.06±0.65</td>
<td>12.35±0.83</td>
<td>14.23±2.06</td>
<td>2916.04±222.40</td>
</tr>
<tr>
<td>2</td>
<td>3.92±0.71</td>
<td>9.60±1.22</td>
<td>10.25±0.86</td>
<td>2626.64±109.47</td>
</tr>
<tr>
<td>3</td>
<td>974.82±28.8</td>
<td>1347.40±90.34</td>
<td>68.54±3.58</td>
<td>1015.16±137.47</td>
</tr>
<tr>
<td>4</td>
<td>31.62±2.38</td>
<td>45.20±5.24</td>
<td>27.32±5.27</td>
<td>1447.09±68.67</td>
</tr>
<tr>
<td>5</td>
<td>766.15±44.40</td>
<td>709.23±43.12</td>
<td>19.72±3.18</td>
<td>1444.82±127.63</td>
</tr>
<tr>
<td>6</td>
<td>21.00±1.76</td>
<td>16.30±1.62</td>
<td>11.67±1.85</td>
<td>2803.85±78.05</td>
</tr>
<tr>
<td>7</td>
<td>890.00±48.88</td>
<td>1064.90±94.48</td>
<td>21.37±3.27</td>
<td>1294.70±3.32</td>
</tr>
<tr>
<td>8</td>
<td>29.00±2.13</td>
<td>40.50±7.34</td>
<td>20.08±1.96</td>
<td>2565.28±141.63</td>
</tr>
</tbody>
</table>

n=10 mice in each group. Group 1: Normal control group, treated with distilled water; Group 2: Epaltes (0.9 g/kg, po) for 7 days. Group 3: a single dose of carbon tetrachloride (0.5 ml/kg in olive oil, ip) and sacrificed 24 h later; Group 4: a single dose of carbon tetrachloride (0.5 ml/kg in olive oil, ip) and sacrificed 4 days later; Group 5: Pre-treatment, sacrificed 24 h later; Group 6: Pre-treatment, sacrificed 4 days later; Group 7: Post-treatment, sacrificed 24 h later; Group 8: Post-treatment, sacrificed 4 days later. *No significant difference compared to group 1. † P<0.001 compared with group 1. ‡ P<0.05, § P<0.01, ¶ P<0.001 compared with group 3. ‡ P<0.01, ¶ P<0.001 compared with group 4. Results given as mean ±SEM. ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GSH, liver reduced glutathione.

Increase in hepatic GSH level in Epaltes treated mice could either be due to an effect on the de novo synthesis of GSH, its regeneration or both. As a consequence, hepatic GSH level could be sufficiently maintained to counteract the increased formation of free radicals as in the case of carbon tetrachloride toxicity. The dosage of the plant extract was calculated according to an internationally accepted calculation where the normal therapeutic dosage of humans was extrapolated to mice. The observed protective effect of the plant extract against carbon tetrachloride may be attributed to the presence of flavonoids, ascorbic acid, carotenoids, tannis and lignins among the plant constituents. Flavonoids are known to be antioxidants, free radical scavengers and antilipoperoxidants leading to hepatoprotection.
Carotenoids are also known to be antioxidants with antihepatotoxic activity\textsuperscript{11}. However, the active compounds of the \textit{Epaltes} responsible for the observed effects have not been identified in the present study.

Many compounds known to be beneficial against carbon tetrachloride-mediated liver injury exert their protective action by toxin-mediated lipid peroxidation either via a decreased production of carbon tetrachloride derived free radicals or through the antioxidant activity of the protective agents themselves\textsuperscript{12,13}. The mechanism by which \textit{Epaltes} exerts its protective action against carbon tetrachloride induced alterations in the liver may be due to the antioxidative effect of the plant extract. The fact that the plant extract was also capable of bringing about an accelerated recovery in the post-treatment regimen indicated that the protective effect might not be due to an antioxidant property alone.

The histopathological observations suggested the possibility of the plant extract being able to condition the hepatic cells to a state of accelerated regeneration thus decreasing the leakage of ALT, AST and ALP into the circulation.

In conclusion, the results of the present study indicated that under the present experimental conditions, aqueous extract of \textit{E. divaricata} showed hepatoprotective effects against carbon tetrachloride induced liver damage in mice.

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\textbf{References}


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