Hepatoprotective effect of *Pergularia daemia* (Forsk.) ethanol extract and its fraction

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Ethanol extract and its ethanol fraction from aerial parts of *P. daemia* exhibited significant hepatoprotective effect against CCl₄ induced hepatotoxicity in rats. Glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, total bilirubin, total cholesterol, total protein and albumin in serum indicated hepatoprotective effect of the ethanol extract and its ethanol fraction. Histopathological examination of liver sections confirmed that, pre-treatment with ethanol extract and its ethanol fraction prevented hepatic damage induced by CCl₄. The results were comparable with the standard hepatoprotective drug silymarin. The extract and its fraction showed no signs of toxicity up to a dose level of 2000 mg/kg. It is suggested that, the presence of flavonoids in ethanol extract and its ethanol fraction may be responsible for hepatoprotective properties. High Performance Thin Layer Chromatography profile of flavonoids of bio-active extracts was developed using quercetin-3-glucoside as a marker. Results indicate hepatoprotective properties of ethanol extract of *P. daemia*.

**Keywords**: CCl₄, Ethanol extract, Hepatoprotective, *P. daemia*, Silymarin

*Pergularia daemia* Forsk. (Asclepiadaceae) known as *Pergularia* in English, *Uttaravaruni* in Sanskrit and *Utranajutuka* in Hindi is a perennial twining herb, grows wild throughout Andhra Pradesh state, India. The plant is used as folk medicine for liver disorders in Chittoor district of Andhra Pradesh. Presence of cardenolides, alkaloids, triterpenes¹, saponins, steroidal compounds² and flavonoids³ has been reported in the plant. The plant is described as anthelmintic, laxative, anti-pyretic, expectorant, anti-diarrhoeal, anti-malarial⁴, anti-pyretic, analgesic, anti-inflammatory¹, and anti-diabetic³. Preliminary investigations on aerial parts of *P. daemia* showed significant hepatoprotective activity of ethanol extract at a fixed dose level of 200 mg/kg⁶. In the present study the bioactive ethanol extract has been further fractionated in an attempt to enrich hepatoprotective activity and to identify active constituents. The hepatoprotective activity of ethanol extract and its ethanol fraction at various dose levels was assessed using carbon tetrachloride (CCl₄) as hepatotoxin. Silymarin was used as a positive control.

**Materials and Methods**

**Plant material**—The aerial parts of *P. daemia* were collected from foot hills of Tirumala, Andhra Pradesh state in the month of December 2004 and their identity was confirmed at The Botanical Survey of India, Southern circle, Coimbatore, India. The voucher specimen (BSI/SC/5/21/05-06/Tech 1512) was deposited at The Madras Herbarium, The Botanical Survey of India, Coimbatore.

**Preparation of extracts**—Powdered (500 g) aerial parts of the plant was defatted first with petroleum ether (60°-80°C), and then completely extracted with 95% ethyl alcohol using soxhlet apparatus. The ethanol extract concentrated in vacuum yielded solid mass (13.9%; w/w). About 60 g of ethanol extract was adsorbed on to the 300 g of silica gel (60-120 mesh) and fractionated using chloroform and 95% ethyl alcohol. The recovered fractions were finally evaporated to give yields of 1.48 g and 43.1 g solid respectively. Preliminary thin layer chromatographic (TLC) studies⁷ of ethanol extract (EE) revealed the presence of flavonoids, cardenolides, and terpenoids; the chloroform fraction (CFEE) showed the presence of cardenolides and terpenoids; while the ethanol fraction (EFEE) showed flavonoids and cardenolides. The EE was tested for hepatoprotective activity at dose levels of 100, 200 and 300 mg/kg, po, whereas EFEE was tested at dose levels of 50, 150 and 250 mg/kg, po.

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mg/kg, po, Chloroform fraction being rich in cardenolides with very minor concentration of terpenoids was not selected for the hepatoprotective activity. The results were compared with a standard hepatoprotective drug silymarin (100 mg/kg). All the test substances were suspended in vehicle (5% acacia mucilage).

**Animals**—Wistar albino rats of either sex weighing 175-225 g, maintained under standard husbandry conditions (23°±2°C, 55±10% RH and 12:12 hr L:D cycle) were used. Animals were allowed to take standard laboratory feed and tap water. The experiments were performed after the experimental protocol was approved by the institutional animal ethics committee.

**Toxicity studies**—Acute toxicity study was performed for EE and EFEE according to the acute toxic classic method described by OECD. Female albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water. The rats were divided into two groups of 3 animals each. The group of rats were administered orally with appropriate extracts of *P. daemia* at a dose of 300 mg/kg. The animals were observed continuously after dosing during first 30 min, periodically for first 24 hr with special attention given during first 4 hr and daily thereafter, for a total of 14 days. As there was no mortality seen at this dose level, the procedure was repeated with further dose (2000 mg/kg) using fresh animals.

**Hepatotoxins and test substances**—All the test substances including silymarin were suspended in vehicle i.e. 5% acacia mucilage for administration. CCl4 at a dose level of 1.25 ml/kg diluted in liquid paraffin (1:1) was administered intraperitoneally.

**Carbon tetrachloride-induced hepatotoxicity**—Rats were divided into 9 groups of 6 each, control, CCl4, silymarin and test groups.

The rats of control and CCl4 groups received three doses of 5% acacia mucilage (1 ml/kg, po) at 12 hr intervals (0 hr, 12 hr and 24 hr). The rats of CCl4 group received a single dose of CCl4 (1.25 ml/kg, ip) diluted in liquid paraffin (1:1) 30 min after the administration of first dose of vehicle. The animals in silymarin and test groups received three doses of respective test substances (silymarin 100 mg/kg; EE 100, 200 and 300 mg/kg; EFEE 50,150 and 250 mg/kg, po) at 0 hr, 12 hr and 24 hr. CCl4 (1.25 ml/kg ip) was administered 30 min after the first dose of the respective test substances. After 36 hr of administration of CCl4, blood was collected and serum was separated for estimation of biochemical parameters.

**Assessment of liver function**—Glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) were estimated by a UV-Kinetic method based on the reference method of International Federation of Clinical Chemistry. Alkaline phosphatase (ALKP), total bilirubin (TBL), total cholesterol (CHL), total protein (TPTN), albumin (ALB) were estimated. All the estimations were carried out using standard kits on auto analyser of Merck make (300 TX, E.Merck-Micro Labs, Mumbai).

**Histopathological studies**—Animals were sacrificed to remove the liver. The liver was fixed in Bouin’s solution for 12 hr, and then embedded in paraffin using conventional methods cut into 5 µm thick sections and stained using haematoxylin-eosin dye. The sections were then observed for histopathological changes.

**Statistical analysis**—The mean values ± SEM were calculated for each parameter. Percentage restoration against the hepatotoxin by the test samples was calculated by considering the difference between hepatotoxin treated group and the control group as 100% restoration. For determining the significant inter group difference one-way analysis of variance was carried out and the individual comparisons of the group mean values were done using Dunnett’s test.

**HPTLC analysis**—The bioactive guided EE and EFEE containing flavonoids were subjected for High Performance Thin Layer Chromatography (HPTLC) finger printing by using quercetin-3-glucoside as a marker as its presence in the plant was detected and also reported by Samia et al. EE (16 µl) and EFEE (12 µl) at a concentration of 2 µg/µl and 8 µl of quercetin-3-glucoside (4 ng/µl conc.) were spotted as bands of 8 mm on silica gel 60F 254 pre-coated TLC plate (Merck) using Linomat V. The plate was developed in twin trough chamber over a path of 69.8 mm using ethyl acetate: formic acid: acetic acid: water (100: 11: 11: 27) as mobile phase. Natural product-polyethylene glycol reagent (NP-PEG) was used as visualisation agent. Densitometric analysis was carried out using Camag scanner III at 366 nm. The data was processed with WinCATS software.

**Results**

EE and EFEE did not cause any mortality at the dose levels tested i.e. 2000 mg/kg and were considered safe.
The rats treated with EE at dose levels of 200 and 300 mg/kg; EFEE at dose levels of 150 and 250 mg/kg showed a significant ($P<0.01$) restoration of altered biochemical levels due to CCl$_4$ intoxication, as observed with silymarin 100 mg/kg. Further, the activities exhibited by EE and EFEE were statistically ($P<0.01$; Table 1) similar.

Histopathological examination of liver sections of the rats treated with EE (200 and 300 mg/kg) and EFEE (150 and 250 mg/kg) followed by CCl$_4$ intoxication resulted in the absence of necrosis, vacuole formation and less disarrangement and degeneration of hepatocytes which were observed with CCl$_4$ intoxication indicating marked protective activity as observed with silymarin treated group (Fig. 1a–g).

The percentage restorations of various biochemical parameters showed by EE and EFEE at various dose levels against CCl$_4$-induced hepatotoxicity are represented in Table 2. The activity was increased in dose dependant manner, EE (300 mg/kg) and EFEE (250 mg/kg) exhibited maximum protection.

### Table 1 — Effect of EE and EFEE from *P. daemia* against CCl$_4$-induced hepatotoxicity in rats

<table>
<thead>
<tr>
<th>Group/Dose (mg/kg)</th>
<th>GOT (I/U/L)</th>
<th>GPT (I/U/L)</th>
<th>ALKP (I/U/L)</th>
<th>TBL (mg/dl)</th>
<th>CHL (mg/dl)</th>
<th>TPTN (g/dl)</th>
<th>ALB (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1)</td>
<td>103.50 ± 5.07</td>
<td>90.16 ± 7.86</td>
<td>217.83 ± 10.56</td>
<td>1.21 ± 0.14</td>
<td>108.12 ± 6.54</td>
<td>6.93 ± 0.71</td>
<td>4.55 ± 0.22</td>
</tr>
<tr>
<td>CCl$_4$ (1.25)</td>
<td>314.17 ± 20.64</td>
<td>247.00 ± 20.01</td>
<td>442.33 ± 24.56</td>
<td>3.01 ± 0.32</td>
<td>290.47 ± 34.61</td>
<td>2.95 ± 0.28</td>
<td>1.95 ± 0.13</td>
</tr>
<tr>
<td>Silymarin (100)</td>
<td>107.00 ± 5.48*</td>
<td>104.17 ± 10.05*</td>
<td>212.82 ± 10.77*</td>
<td>1.50 ± 0.18*</td>
<td>113.33 ± 7.62*</td>
<td>7.10 ± 0.67**</td>
<td>4.12 ± 0.27**</td>
</tr>
<tr>
<td>EE (100)</td>
<td>300.33 ± 17.07</td>
<td>211.33 ± 32.11</td>
<td>386.17 ± 29.22</td>
<td>2.43 ± 0.35</td>
<td>276.50 ± 34.03</td>
<td>3.81 ± 0.34</td>
<td>2.23 ± 0.23</td>
</tr>
<tr>
<td>EE (200)</td>
<td>132.67 ± 7.66*</td>
<td>135.50 ± 7.07*</td>
<td>252.33 ± 16.71*</td>
<td>1.80 ± 0.24*</td>
<td>250.50 ± 34.03</td>
<td>3.46 ± 0.37</td>
<td>2.51 ± 0.24</td>
</tr>
<tr>
<td>EE (300)</td>
<td>294.83 ± 23.01</td>
<td>199.67 ± 31.18</td>
<td>362.67 ± 28.63</td>
<td>2.35 ± 0.34</td>
<td>250.50 ± 24.00</td>
<td>3.46 ± 0.37</td>
<td>2.51 ± 0.24</td>
</tr>
<tr>
<td>EFEE (50)</td>
<td>122.33 ± 7.69*</td>
<td>129.33 ± 6.50*</td>
<td>240.50 ± 15.01*</td>
<td>1.67 ± 0.23*</td>
<td>133.17 ± 6.62*</td>
<td>6.05 ± 0.46**</td>
<td>3.98 ± 0.29**</td>
</tr>
<tr>
<td>EFEE (150)</td>
<td>109.31 ± 5.34*</td>
<td>111.33 ± 8.24*</td>
<td>223.17 ± 12.49*</td>
<td>1.57 ± 0.20*</td>
<td>116.83 ± 3.67*</td>
<td>6.78 ± 0.45**</td>
<td>3.72 ± 0.26**</td>
</tr>
</tbody>
</table>

EE = Ethanol extract; EFEE = Ethanol fraction of ethanol extract
GOT = glutamic oxaloacetic transaminase; GPT = glutamic pyruvic transaminase; ALKP = alkaline phosphatase; TBL = total bilirubin; CHL = cholesterol; TPTN = total protein; ALB = albumin

* Significant reduction compared to CCl$_4$ ($P<0.01$). ** Significant increase compared to CCl$_4$ ($P<0.01$).

### Table 2 — Restoration of various parameters (%) showed by EE and EFEE against CCl$_4$-induced hepatotoxicity

<table>
<thead>
<tr>
<th>Group/Dose (mg/kg)</th>
<th>GOT</th>
<th>GPT</th>
<th>ALKP</th>
<th>TBL</th>
<th>CHL</th>
<th>TPTN</th>
<th>ALB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silymarin</td>
<td>98.19</td>
<td>90.98</td>
<td>102.13</td>
<td>83.80</td>
<td>97.07</td>
<td>104.24</td>
<td>83.45</td>
</tr>
<tr>
<td>EE (100)</td>
<td>06.56</td>
<td>22.71</td>
<td>24.99</td>
<td>32.21</td>
<td>07.65</td>
<td>21.60</td>
<td>10.76</td>
</tr>
<tr>
<td>EE (200)</td>
<td>86.03</td>
<td>71.02</td>
<td>84.55</td>
<td>67.21</td>
<td>88.48</td>
<td>71.59</td>
<td>55.76</td>
</tr>
<tr>
<td>EE (300)</td>
<td>99.38</td>
<td>82.06</td>
<td>95.41</td>
<td>75.54</td>
<td>95.14</td>
<td>97.46</td>
<td>72.30</td>
</tr>
<tr>
<td>EFEE (50)</td>
<td>09.17</td>
<td>30.14</td>
<td>35.44</td>
<td>36.66</td>
<td>21.90</td>
<td>12.81</td>
<td>21.53</td>
</tr>
<tr>
<td>EFEE (150)</td>
<td>90.93</td>
<td>74.95</td>
<td>89.81</td>
<td>74.43</td>
<td>86.20</td>
<td>77.87</td>
<td>78.07</td>
</tr>
<tr>
<td>EFEE (250)</td>
<td>97.09</td>
<td>86.42</td>
<td>97.52</td>
<td>79.99</td>
<td>98.16</td>
<td>96.21</td>
<td>68.07</td>
</tr>
</tbody>
</table>

Abbreviations are same as in Table 1.
was scanned for its presence in all the tracks between wave length 200 and 400 nm.

Fig 1—Photomicrographs of histopathological changes showing effect of test material on the rats liver intoxicated with CCl₄.
[normal liver (a); intoxicated with CCl₄ (b); silymarin and CCl₄ (c); EE (200 mg/kg) and CCl₄ (d); EE (300 mg/kg) and CCl₄ (e); EFEE (150 mg/kg) and CCl₄ (f); EFEE (250 mg/kg) and CCl₄ (g). cv = central vein, vc = vacuole, ss = sinusoidal spaces, hc = hepatocytes. E&H, × 400]
In order to ascertain the use of *P. daemia* as folk medicine in liver disorders the present studies were performed to assess the hepatoprotective activity of bioactive extract and its fraction at various dose levels in rats against CCl₄ induced hepatotoxicity. EE and EFEE restored all the biochemical parameters altered due to the CCl₄ intoxication (Table 1). Inhibition of bile acids synthesis from cholesterol leading to increase in its levels was resulted due to CCl₄ intoxication. Reduction of cholesterol levels by the EE and EFEE suggest the bile acids synthesis inhibition was reversed. Reduction in the levels of SGOT and SGPT towards the normal value is an indication of regeneration process. Reduction of ALKP levels with concurrent depletion of raised bilirubin level suggests the stability of the biliary function during injury with CCl₄. The protein and albumin levels reduced due to the CCl₄ induced hepatotoxicity were raised by the EE and EFEE suggesting the stabilization of endoplasmic reticulum leading to protein synthesis. The hepatoprotective effects exhibited by EE (200 and 300 mg/kg) and EFEE (150 and 250 mg/kg) were statistically (P<0.01) similar to that observed with standard drug silymarin at 100 mg/kg dose level. The histological examination of liver sections revealed that the normal liver architecture was disturbed by the hepatotoxin intoxication. In the liver sections of the rats treated with EE (200 and 300 mg/kg) and intoxicated with CCl₄; rats treated with EFEE (150 and 250 mg/kg) and intoxicated with CCl₄, the normal cellular architecture was retained as observed with silymarin, there by confirming the protective effect of the extract and its ethanol fraction. The ability of the extracts tested, to protect the liver against the CCl₄-induced hepatotoxicity may be due to stabilization of endoplasmic reticulum as well as repair of hepatic tissue damages caused by the CCl₄ leading to hepatic regeneration. The hepatoprotective effect of different flavonoids obtained from natural sources such as quercetin, rutin, Silymarin from *Silybum marianum* is documented. Samia *et al.* reported various flavonoid glycosides like quercetin-3-glucoside, quercetin-3-galactoside, kaempferol-3-glucoside, kaempferol-3-alactoside, kaempferol-3-malonylhexoside, isorhamnetin-3-glucoside and isorhamnetin-3-malonylhexoside etc. in *P. daemia*. The present phytochemical investigations also revealed the presence of flavonoids in the ethanol extract and its ethanol fraction of *P. daemia*. Therefore the hepatoprotective activity of aerial parts of *P. daemia* could be attributed to its flavonoid content. In order to standardise bio-active extract (EE) and fraction (EFEE), HPTLC profile of flavonoids was developed using quercetin-3-glucoside as marker.

**References**


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