Hepatoprotective activity of *Calotropis procera* flowers against paracetamol-induced hepatic injury in rats

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**Abstract**

Hydro-ethanolic extract (70%) of *Calotropis procera* flowers was prepared and tested for its hepatoprotective effect against paracetamol-induced hepatitis in rats. Alteration in the levels of biochemical markers of hepatic damage like SGPT, SGOT, ALP, bilirubin, cholesterol, HDL and tissue GSH were tested in both treated and untreated groups. Paracetamol (2 g/kg) has enhanced the SGPT, SGOT, ALP, bilirubin and cholesterol levels and reduced the serum levels of HDL and tissue level of GSH. Treatment with hydro-ethanolic extract of *C. procera* flowers (200 mg/kg and 400 mg/kg) has brought back the altered levels of biochemical markers to the near normal levels in the dose dependent manner.

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*Keywords*: Calotropis procera; Paracetamol; Hepatoprotective

1. Introduction

The liver is a vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification from the exogenous and endogenous challenges, like xenobiotics, drugs, viral infections and chronic alcoholism. If during all such exposures to the above mentioned challenges the natural protective mechanisms of the liver are overpowered, the result is hepatic injury.

Liver damage is always associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT, SGPT, triglycerides, cholesterol, bilirubin, alkaline phosphatase, are elevated [1,2].

In spite of phenomenal growth of modern medicine, there are no synthetic drugs available for the treatment of hepatic disorders. However there are several herbs/herbal formulations claimed have possess beneficial activity in treating hepatic disorders. In one of our field surveys we found that a wildly grown plant *C. procera* which was claimed...
to possess hepatoprotective property. It was found that this plant contains flavonoids, alkaloids, cardiac glycosides, tannins, sterols, triterpenes [1]. There are reports showed that flowers possess antiinflammatory, antipyretic, analgesic, antimicrobial properties and larvicidal activity [2,3]. The latex of the plant was reported to possess analgesic and wound healing activity [4,5]. The roots are reported to have anti-fertility and anti-ulcer activities [6,7]. However there are no scientific basis or reports in the modern literature regarding its usefulness as hepatoprotective agent. Thus the present study was conducted to evaluate the hepatoprotective activity of the hydro-ethanolic extract of the *C. procera* flowers by using paracetamol-induced hepatic injury in rats.

2. Experimental

2.1. Plant

The flowers were collected in September from the suburban out fields of Harapanahalli. K. Prabhu, Department of Pharmacognosy, SCS College of Pharmacy identified and a Herbarium specimen was deposited in the Department of Pharmacognosy with No. SCSCOP/P.COL/2/04-05.

2.2. Preparation of extract

The shade-dried powder of flowers extracted in a Soxhlet extracter with 70% EtOH gave 20% of extract.

2.3. Animals

Wistar rats (125–175 g) of either sex obtained from National Institute of Mental Health and Neuro Sciences were kept in standard environmental conditions, fed with standard rodent diet and with water ad libitum. Approval from the institutional animal ethical committee for the usage of animals in the experiments was obtained.

2.4. Hepatoprotective activity

The method of Chattopadhyay [8] was used in the study. Animals were divided into five groups of 6 animals each. The first group received saline 1 ml/kg for one week (control). The group II received saline 1 ml/kg for one week (positive control). The groups III, IV and V received silymarin (100 mg/kg p.o.) and 200 mg/kg and 400 mg/kg of *C. procera* flowers ethanolic extract respectively once a day for seven days. On the fifth day, after the administration of the respective treatments, all the animals of groups II, III, IV and V were administered with paracetamol 2 g/kg orally. On the seventh day after 2 h of respective treatments the blood samples were collected for the estimation of biochemical marker enzymes. Then animals under ether anesthesia were sacrificed. The livers from all the animals were collected, washed and used for the estimation of tissue GSH levels.

2.5. Biochemical analysis

The collected blood samples were used for the analysis of biochemical markers SGPT [9], SGOT [10], ALP [11], bilirubin, [12] cholesterol [13], and HDL [14] levels. The tissue GSH was evaluated according to Ayake et al. [15].

2.6. Statistical analysis

The results are expressed as mean±SEM, (N=6). Statistical significance was determined by one-way analysis of variance with *P*<0.05 considered significant. The analysis was performed by Prism software.

3. Results and discussion

Paracetamol has enhanced the levels of SGPT, SGOT, bilirubin (both total and direct bilirubin levels), Alkaline phosphatase level (ALP), total cholesterol, whereas HDL and tissue GSH levels are decreased significantly. Treatment
with silymarin and 200 mg/kg and 400 mg/kg of *C. procera* flowers (70% ethanolic extract) has significantly brought down the elevated levels of SGPT, SGOT, ALP, bilirubin, cholesterol and also significantly enhanced the decreased levels of tissue GSH and HDL. Results are reported in Tables 1 and 2.

Paracetamol is normally eliminated mainly as sulfate and glucoronide. Only 5% of the paracetamol is converted into \(N\)-acetyl-\(p\)-benzoquineimine. However, upon administration of toxic doses of paracetamol the sulfation and glucoronidation routes become saturated and hence, higher percentage of paracetamol molecules are oxidized to highly reactive \(N\)-acetyl-\(p\)-benzoquineimine (NAPQI) by cytochrome-450 enzymes. Semiquinone radicals, obtained by one electron reduction of NAPQI, can covalently binds to macromolecules of cellular membrane and increases the lipid peroxidation resulting in the tissue damage. Higher dose of paracetamol and NAPQI can alkylate and oxidize intracellular GSH and protein thiol group, which results in the depletion of liver GSH pool subsequently leads to increased lipid peroxidation and liver damage [16]. In our experiments it is observed that tissue GSH levels in the paracetamol group is decreased to the extent of around 65%. This clearly indicates that there is a significant hepatic damage due to paracetamol. This is further evident from the fact that there is elevation in the levels of various biochemical markers of hepatic damage like SGPT, SGOT, bilirubin, and cholesterol. Treatment with silymarin and *C. procera* flowers (70% ethanolic extract) has increased tissue GSH level and the elevated levels of above mentioned biochemical markers to the near healthy levels. The treatment has also demonstrated the reduced hepatic damage or improvement in the hepatic architecture (data not shown).

It may be concluded that the hepatoprotective effect of *C. procera* flowers (70% ethanolic extract) is due to the prevention of the depletion in the tissue GSH levels. Upon literature review it is found that the flowers of *C. procera* contains quercetin-3-rutinoside and other flavonoids which are still present in the ethanolic extract. Therefore there is a possibility that the flowers extract may possess antioxidant property, which may be involved in the hepatoprotective property [17,18]. In addition it is necessary to carry-out further studies to rule out if treatment with ethanolic extract is able to inhibit oxidation of paracetamol to highly reactive NAPQI.

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SGPT U/l</th>
<th>SGOT U/l</th>
<th>ALP U/l</th>
<th>Total bilirubin mg/dl</th>
<th>Direct bilirubin mg/dl</th>
<th>Cholesterol mg/dl</th>
<th>HDL mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline 0.5 ml p.o., seven days)</td>
<td>68.57±0.42</td>
<td>131.76±0.43</td>
<td>135.57±1.05</td>
<td>0.92±0.01</td>
<td>0.25±0.01</td>
<td>103.42±2.71</td>
<td>47.14±1.60</td>
</tr>
<tr>
<td>Paracetamol (PCM, 2 g/kg s.c)</td>
<td>281.18±0.65</td>
<td>403.16±1.15</td>
<td>436.33±1.33</td>
<td>3.42±0.11</td>
<td>0.69±0.01</td>
<td>157.85±1.84</td>
<td>28.47±0.91</td>
</tr>
<tr>
<td>Silymarin (100 mg/kg p.o., seven days)+PCM</td>
<td>74.57±0.61*</td>
<td>135.26±0.96*</td>
<td>166.35±0.70*</td>
<td>1.04±0.03*</td>
<td>0.26±0.01*</td>
<td>116.19±2.43*</td>
<td>45.12±1.59*</td>
</tr>
<tr>
<td>CPA (200 mg/kg p.o. seven days)+PCM</td>
<td>161.28±0.94*</td>
<td>285.16±0.56*</td>
<td>286.43±1.27*</td>
<td>1.45±0.06*</td>
<td>0.38±0.01*</td>
<td>136.34±1.95*</td>
<td>33.41±1.62 (NS)</td>
</tr>
<tr>
<td>CPA (400 mg/kg p.o. seven days)+PCM</td>
<td>86.86±0.63*</td>
<td>152.35±0.60*</td>
<td>181.65±1.00*</td>
<td>1.07±0.04*</td>
<td>0.31±0.01*</td>
<td>118.69±1.73*</td>
<td>41.25±0.97*</td>
</tr>
</tbody>
</table>

\(N=6, *P<0.01\) vs PCM group, NS non significant.

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/kg (p.o.)</th>
<th>Tissue levels of GSH Absorbance 412 nm</th>
<th>% Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0.5 ml</td>
<td>0.932±0.06</td>
<td>–</td>
</tr>
<tr>
<td>Paracetamol (PCM)</td>
<td>2 g/kg</td>
<td>0.316±0.03</td>
<td>–</td>
</tr>
<tr>
<td>Silymarin + PCM</td>
<td>100 mg/kg</td>
<td>0.564±0.09*</td>
<td>78.74</td>
</tr>
<tr>
<td>CPA + PCM</td>
<td>200 mg/kg</td>
<td>0.452±0.03*</td>
<td>43.17</td>
</tr>
<tr>
<td>CPA + PCM</td>
<td>400 mg/kg</td>
<td>0.544±0.04*</td>
<td>72.2</td>
</tr>
</tbody>
</table>

\(N=6, *P<0.01\) vs PCM group, NS non significant.
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References