**Hepatoprotective Effect of Centella asiatica (L) in Carbon Tetrachloride-Induced Liver Injury in Rats**

B. ANTONY*, G. SANTHAKUMARI, B. MERINA, V. SHEEBA AND J. MUKKADAN

R & D Laboratory, Arjuna Natural Extracts Ltd., P.B. No. 126, Bank Road, Alwaye-683 101, ‘Little Flower Hospital & Research Centre, Angamaly-683 572, Kerala, India.

The present study was conducted to evaluate the hepatoprotective effects of the Centella asiatica extract in carbon tetrachloride-induced liver injury in rats. Sprague Dawley rats were treated with alcoholic extract of Centella asiatica orally in two doses (20 and 40 mg/kg/day) for 3 mo along with intraperitoneal injection of carbon tetrachloride (1 ml/kg). Biochemical parameters such as serum total protein, albumin and marker enzymes (aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase) were estimated both before and after the experiment. Histopathological studies of liver were also carried out to confirm the biochemical changes. Carbon tetrachloride-induced hepatotoxic effects were evident by a significant (p < 0.05) increase in the serum marker enzymes and a decrease in the total serum protein and albumin. Administration of extract of Centella asiatica effectively inhibited these changes in a dose-dependent manner; maximum effect was with 40 mg/kg. Histopathological examination of liver tissue corroborated well with the biochemical changes. Hepatic steatosis, hydropic degeneration and necrosis were observed in carbon tetrachloride-treated group, while these were completely absent in the treatment group. Centella asiatica extract exhibited hepatoprotective action against carbon tetrachloride-induced liver injury. This effect is attributed to the presence of asiaticoside (14.5%) in the extract.

*For correspondence
E-mail: benny@arjunanatural.com

**MATERIALS AND METHODS**

All chemicals used for the experiment were of analytical grade. Methanol, hexane and acetonitrile were purchased from Merck India Ltd.

**Preparation of the extract of Centella asiatica:**
Centella asiatica plants were collected from local suppliers during the months of August/September and pharmacognostically identified with the help of herbarium sample of the species kept at R&D Laboratory of Arjuna Natural Extracts, Aluva, Kerala. Dried leaves of Centella asiatica (300 g) were refluxed with 2 litres of 80% methanol for 3-4 h. After refluxing, the contents were cooled and filtered. The filtrate was collected and the residue was refluxed again with 1 litre of 80% methanol for 2 h. The process was repeated; the filtrates were pooled and concentrated up to 70% total dissolved solid (TDS) level. The crude extract thus obtained was refluxed with hexane for about 1 h at 80°. The residue obtained after hexane extraction was then kept for crystallization in a refrigerator for 4-5 d. The crystals formed were separated and dried under vacuum. The dried material was then powdered and kept as Centella.
**asiatica** extract. The yield was 28.5 g (9.5%). The asiaticoside was estimated using HPLC (Shimadzu-class VP system) and found to be 14.5%.

Male Sprague Dawley rats weighing 250-280 g were used for the experiment. Animals were purchased from Small Animal Breeding Station, Veterinary College, Mannuthy, Thrissur, Kerala. They were housed in a temperature-controlled room (28 ± 1°C) in clean polypropylene cages with 12 h light and 12 h dark cycles and fed with normal rat chow (Amrut Animal Feed, Maharashtra) and water ad libitum.

The study was conducted at Little Flower Medical Research Centre, Angamaly, after obtaining clearance from Institutional Animal Ethics Committee. After an acclimatization period, animals were divided into five groups of six each, comprising normal control, vehicle control, toxic control and treatment groups. Normal control group was given distilled water, while vehicle control group was administered coconut oil. CCl₄ was given to toxic control group, while treatment groups were administered **Centella asiatica** extract along with CCl₄. Liver injury was induced by an intraperitoneal injection (1 ml/kg) of CCl₄ in coconut oil (1:1 ratio). Injection was given twice a week for 3 mo. Oral feeding of **Centella asiatica** extract was started 3 d prior to intraperitoneal injection of CCl₄ and continued for 3 mo.

**Estimation of biochemical parameters:**

Blood samples were collected from the caudal vein before starting the experiment under light ether anaesthesia and by direct cardiac puncture after the completion of the study. Serum was separated for estimation of biochemical parameters. Total protein and albumin were estimated by Biuret and bromo cresol green methods respectively. The marker enzymes (AST, ALT and ALP) were studied by Bergmeyer method and King and Amstrong method respectively.

### Histopathological studies:

Anatomy of the liver was studied immediately after sacrificing all the animals. A small portion was fixed in 10% neutral buffered formalin as described by Luna. Thin sections of 4-5 µm were taken, stained with Haematoxylin and Eosin and histology was studied.

### Statistical analysis:

Statistical analyses were carried out using ANOVA. Results were expressed as means ± SE. One-way ANOVA with repeated measures was used to analyse the variance over a period of time and for intergroup comparisons. Paired ‘t’ test was used to compare the biochemical parameters both before and after experiment. Level of significance was set at p < 0.05.

### RESULTS AND DISCUSSION

Results of the present study show that serum total protein and albumin levels remained the same in groups A and B (controls) both before and after the treatment period. The serum protein level significantly (p < 0.05) decreased in group C (toxic control). In rats treated with **Centella asiatica** extract, the protein level reversed partially (Table 1). The serum albumin level was significantly decreased in toxic control group, which was reversed partially in group E (40 mg/kg), but in group D (20 mg/kg) there was no significant effect (Table 1).

The serum marker enzyme such as ALP, AST and ALT levels remained normal in groups A and B both before and after experiment, but they were increased in all the other groups during the experimental period. In the CCl₄-treated rats, there was a significant (p < 0.05) increase in ALT levels, which was decreased when treated with a dose of 20 mg/kg extract and back to almost normal level with 40 mg/kg dose of extract.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Total protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>AST (U/L)**</th>
<th>ALT (U/L)**</th>
<th>ALP (KA)**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 month</td>
<td>3 month</td>
<td>0 month</td>
<td>3 month</td>
<td>0 month</td>
</tr>
<tr>
<td>Control (A)</td>
<td>6.8±0.5</td>
<td>6.7±0.5</td>
<td>4.3±0.3</td>
<td>3.9±0.3</td>
<td>32.7±2.1</td>
</tr>
<tr>
<td>V. control (B)</td>
<td>6.4±0.4</td>
<td>6.4±0.4</td>
<td>3.8±0.1</td>
<td>3.7±0.1</td>
<td>32.7±1.5</td>
</tr>
<tr>
<td>CCl₄ alone (C)</td>
<td>7.0±0.1</td>
<td>*3.1±0.1 (66%)</td>
<td>3.5±0.1</td>
<td>*1.1±0.1 (68%)</td>
<td>32.5±2.0</td>
</tr>
<tr>
<td>CCl₄+CA (D)</td>
<td>7.7±0.3</td>
<td>*5.1±0.2 (34%)</td>
<td>3.4±0.2</td>
<td>*1.4±0.1 (60%)</td>
<td>35.7±1.8</td>
</tr>
<tr>
<td>CCl₄+CA (E)</td>
<td>7.3±0.2</td>
<td>*6.2±0.4 (15%)</td>
<td>2.5±0.4</td>
<td>*1.9±0.4 (24%)</td>
<td>35.2±0.2</td>
</tr>
</tbody>
</table>

* Asterisk indicates significant difference from control group at p < 0.05.

**TABLE 1: EFFECT OF CENTELLA ASIatica ON BIOCHEMICAL PARAMETERS IN CCl₄-INDUCED LIVER INJURY IN RATS**
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The ALP level increased \((p < 0.05)\) in all three experimental groups. It was observed that in the group treated with 40 mg/kg of *Centella asiatica* extract, ALP level was partially inhibited to a significant \((p < 0.05)\) level. The enzyme AST increased \((p < 0.05)\) in all experimental groups after 3 mo. The percentage of increase observed was lower \((p < 0.05)\) in the extract-treated groups when compared to toxic control group (C) (Table 1).

Macroscopic examination of liver in groups A and B showed normal architecture. In toxic control group (group C), liver was pale in colour with micro- and macro-nodules on the liver surface. In group D (20 mg/kg of extract), pale colour was not so prominent, but the
The surface of the liver was rough and showed nodules in the CCl₄-treated group, while the intensity of damage was not so high in the treatment group. A dose-dependent hepatoprotection was observed in the present study. Rats treated with 40 mg of the extract had a higher degree of protection than those treated with 20 mg extract.

Centella asiatica extract provides hepatoprotective action against CCl₄-induced liver injury in rats. This was evidenced from the present study by the inhibition of decrease in serum albumin and protein level and elevation in the serum marker enzymes - AST, ALT and ALP. Administration of the extract of Centella asiatica effectively inhibited fatty changes and round cell infiltration in hepatocytes in a dose-dependent manner. The results of the present study are comparable with studies conducted with silymarin¹² and curcumin¹³.

Previous studies showed that administration of asiaticoside, an isolated constituent of Centella asiatica, significantly increased the levels of antioxidant enzymes like super oxide dismutase, catalase, glutathione peroxidase in excision-type cutaneous wounds in rats¹⁴. Antioxidants such as ellagic acid¹⁵ and curcumin had been reported to protect liver injury and fibrosis induced by hepatotoxins¹⁶. Hence the hepatoprotective effects of Centella asiatica in the present study might be due to the potent antioxidant action of asiaticoside present (14.5%) in the extract.

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REFERENCES