HEPATOPROTECTIVE EFFECT OF THE METHANOLIC EXTRACT OF CURCULIGO ORCHIOIDES IN CCl₄-TREATED MALE RATS

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ABSTRACT

Objective: To evaluate the hepatoprotective effect of the methanolic extract of Curculigo orchioides rhizomes (MEC) in rats treated with carbon tetrachloride. 

Methods: In hepatotoxic rats, liver damage was studied by assessing parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transpeptidase (GGT) in serum, and concentrations of total proteins, total lipids, phospholipids, triglycerides and cholesterol in both serum and liver. The effect of co-administration of MEC on the above parameters was further investigated. Histopathological study of the liver in experimental animals was also undertaken.

Results: Hepatic damage as evidenced by a rise in the levels of AST, ALT, ALP and GGT in serum, and also changes observed in other biochemical parameters in serum and liver showed a tendency to attain near normalcy in animals co-administered with MEC. The normal values for AST (IU/L), ALP (IU/L), protein (g/100 ml) and total lipids (mg/100 ml) in serum (i.e., 21.24, 71.04, 6.72 and 136.54 respectively) were found to alter towards values 33.61, 128.11, 4.83 and 266.91 in hepatotoxic rats. These parameters attained near-normal values (i.e., 23.82, 80.3, 6.22 and 152.24 for AST, ALP, protein and total lipids respectively) in MEC co-administered rats. Profound steatosis, ballooning degeneration and nodule formation observed in the hepatic architecture of CCl₄ treated rats were found to acquire near-normalcy in drug co-administered rats, thus corroborating the biochemical observations.

Conclusion: The study substantiates the hepatoprotective potential of MEC.

KEYWORDS Carbon tetrachloride marker enzymes total lipids total protein

INTRODUCTION

Liver, the largest organ in vertebrate body, is the major site of intense metabolic activities. Liver injury caused by toxic chemicals and certain drugs has been recognised as a toxicological problem. Herbal drugs are playing an important role in health care programmes world wide, and there is a resurgence of interest in herbal medicines for treatment of various ailments including hepatopathy. India, the abode of Ayurvedic system of medicine, assigns much importance to the pharmacological aspects of many plants. Hepatoprotective effect of some plants like Spirulina maxima, Eclipta alba, Boehmeria nivea, Cichorium intybus and Picrorhiza kurroa etc. has been well established. Yet there is paucity of information regarding the activity of C. orchioides in hepatic protection. This study was undertaken to fill the lacuna in this regard.

Curculigo orchioides Gaertn. of family Amaryllidaceae, is a herbaceous, tuberous, geophilous, perennial widely distributed all over India. Its rootstock bears several fleshy lateral roots (rhizomes). The rhizomes are sweet, cooling, diuretic, aphrodisiac, viriligenic, antipyretic and tonic and can be used against haemorrhoids, leucorrhoea, pruritis, skin diseases, bronchitis, jaundice and diarrhoea. An efficient method has been developed for large-scale multiplication of this medicinal plant through direct bulbil
formation from leaf explant in shake flask culture. *Curculigo saponin* G, isolated from rhizomes of this plant has been reported to increase weight of thymus gland *in vivo* in mice. Ethanolic extract of *C. orchioides* has been reported to have adaptive effects, such as enhancing tolerance towards hypoxia and hyperthermia and also sedative, anticonvulsant and androgen-like effect.

A number of pharmacological and chemical agents act as hepatotoxin and produce variety of liver ailments. Carbon tetrachloride (*CCl₄*) intoxication in rats is an experimental model widely used to study necrotic and steatotic changes in hepatic tissue. Accordingly, our experiment was designed to use *CCl₄*-intoxicated rat liver as model.

### MATERIALS AND METHODS

**Plant materials:** Rhizomes of *C. orchioides* were collected from Thattekkad, Ernakulam district of Kerala, India. The plant was previously identified and authenticated by experts in the Post Graduate and Research Department of Botany, St. Thomas College Pala, Kottayam. The collected materials were washed thoroughly in water, chopped, air dried for a week at 35-40°C and pulverized in electric grinder. The powder obtained was successively extracted in petroleum ether (60-80°C), benzene, chloroform, methanol and distilled water. The extracts were then made to powder by using rotary evaporator under reduced pressure. Rhizomes of *C. orchioides* yielded 0.6, 0.9, 2.2, 2.6 and 2.4% w/w powdered extract with petroleum ether, benzene, chloroform, methanol and distilled water respectively. A pilot study using the various extracts at the dose of 70 mg/kg body weight revealed the methanolic extract offering maximum hepatoprotection. Accordingly, powdered methanolic extract of *C. orchioides* (MEC) was prepared in sufficient quantity and stored in refrigerator for further use.

**Experimental animals:** Twenty-four male albino rats of Sprague-Dawley strain weighing 100-120 g were purchased from Small Animals' Breeding Centre of Kerala Agricultural University, Mannuthy, Trichur. The animals were housed in polypropylene cages and maintained in controlled temperature (27±2°C) and light cycle (12 h light and 12 h dark). They were fed with Amrut Laboratory Animal Feed [Nav Maharashtra Chakan Oil Mills Ltd, Pune]. Water was supplied *ad libitum*. They were given a week's time to get acclimatized with the laboratory conditions. Initial body weight of each animal was recorded. Ethical clearance for the use of animals was obtained from the committee constituted for the purpose.

**Experimental induction of hepatic damage:** Liver damage was induced in rats by administering *CCl₄* subcutaneously (sc) in the lower abdomen in a suspension of liquid paraffin (LP) in the ratio 1:2 v/v at the dose of 1 ml *CCl₄*/kg body weight of each animal. *CCl₄* was administered twice a week, on every first and fourth day of all the 13 weeks.

**Experimental design:** Rats were divided into 3 groups of 8 animals each as follows: Group I animals served as control and received sc administration of LP only at the dose of 3 ml/kg body weight, twice a week for a duration of 13 weeks. Group II animals constituted hepatotoxic rats and received sc administration of LP+*CCl₄* twice a week for a total of 13 weeks. Group III animals were the herb-treated animals and received sc administration of LP+*CCl₄* as in group II rats. Besides they received orally by intubation MEC in a suspension of 1 ml water at the dose of 70 mg/kg body weight daily for 89 days.

Replenishing a known quantity of fresh food daily at 10.30 a.m. and thereby measuring the food intake of the previous day carried out measurement of daily food consumption. LP, LP+*CCl₄* and LP+*CCl₄* + MEC were administered at the same time between 10-11 a.m. Body weight of rats was recorded weekly to assess percentage of weight gain in each group. General well being and behaviour of the animals were observed daily throughout the period of study. The litter in the cage was renewed twice a week to ensure maximum comfort for the animals.

Animals were kept starved overnight on the 89th day. On the next day, after recording the weight in each case, they were sacrificed by decapitation by making an incision on jugular vein to collect blood. The liver tissue was dissected out, blotted off blood, washed in saline and weighed instantaneously. This was kept in frozen containers and proceeded for biochemical estimations.

**Biochemical estimations:** Serum was prepared from the collected blood and subjected to biochemical estimations of different parameters like aspartate aminotransferase (AST), alanine aminotransferase...
HEPATOPROTECTIVE EFFECT OF CURCULIGO ORCHIOIDES

(ALT)¹¹, alkaline phosphatase (ALP)¹², gamma glutamyl transpeptidase (GGT)¹³ total proteins¹⁴, total lipids¹⁵, triglycerides¹⁶, phospholipids¹⁷ and cholesterol¹⁸. Liver homogenates were also subjected to various biochemical estimations.

Histopathology: A portion of liver tissue in each group was fixed in 10% formaal (formalin diluted to 10% with normal saline) and proceeded for histopathology. After paraffin embedding and block making, serial sections of 5μ thicknesses were made, stained with Haematoxylin and Eosin and examined under microscope. A few photomicrographs of representative types were also taken.

Statistical analysis: One-way analysis of variance (ANOVA) followed by Scheffe’s test was applied for determining the statistical significance of difference in enzymes, protein and lipid levels between different groups. The level of significance was set at 0.05.

RESULTS

Food consumption and weight gain: We observed that food consumption and weight gain significantly increased in group III animals as compared to other groups. In-group II rats there was a lesser weight gain as compared to group I animals.

Serum marker enzymes: All the marker enzymes, viz., AST, ALT, ALP and GGT registered enhanced activity in CCl₄-treated rats as compared to control group (Table 1). In MEC co-administered group, the levels of these enzymes were found retrieving towards normalcy.

Other biochemical parameters: The total protein concentration of the serum and liver was lesser in group II animals, when compared with normal control. (Tables 1 and 2) and it attained an almost normal value in group III rats. The level of total lipids, triglycerides and cholesterol in serum as well as liver recorded significant increment in CCl₄-administered rats as compared to those of group I. All these biochemical changes showed signs of returning towards the normalcy in group III animals. There was a significant decline in the concentration of phospholipids in liver tissues of CCl₄-treated rats as compared to normal control. In group 3 animals phospholipid concentration attained normalcy.

Histopathological observation: Histopathological study of liver from group I animals showed a normal hepatic architecture. (Figure 1a). In CCl₄-treated group, severe hepatotoxicity was evidenced by profound steatosis, centrilobular necrosis, ballooning degeneration, nodule formation and fibrosis. (Figure 1b). In-group III animals, the liver exhibited an almost normal architecture, barring a little deformity of hepatocytes with pyknosis and clearing of cytoplasm (Figure 1c).

DISCUSSION

The changes associated with CCl₄-induced liver damage are similar to that of acute viral hepatitis. CCl₄, a widely used experimental hepatotoxicant, is biotransformed by the cytochrome P-450 system to produce the trichloromethyl free radical, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation, disturb Ca²⁺ homeostasis, and finally result in cell death¹⁹.

Animals of group II (received CCl₄ alone) significantly lost their body weight and showed reduced food consumption as compared to control. Animals of group III which received both (CCl₄ + MEC) showed a significant increase in body weight and food consumption when compared to group II, and the values were even significantly higher than that of group I rats. These findings suggested that MEC administration has significantly neutralised the toxic effect of CCl₄ and helped in regeneration of hepatocytes.

Estimating the activities of serum marker enzymes, like AST, ALT, ALP and GGT, can make assessment of liver function. When liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage²¹. The enhanced activities of these serum marker enzymes observed in CCl₄-treated rats in our study correspond to the extensive liver damage induced by the toxin. The tendency of these enzymes to return towards a near normal level in group III rats is a clear manifestation of anti-hepatotoxic effect of MEC.
Table 1. Effect of Curculigo orchioides on different biochemical parameters in the serum of rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I Control with LP only</th>
<th>Group II LP+CC\textsubscript{4} only</th>
<th>Group III LP+CC\textsubscript{4}+MEC</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (IU/L serum)</td>
<td>21.24 ± 0.58</td>
<td>33.61 ± 1.34*</td>
<td>23.82 ± 0.76@</td>
<td>5.32</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ALT (IU/L serum)</td>
<td>24.62 ± 1.34</td>
<td>61.91 ± 3.68*</td>
<td>32.11 ± 2.61@</td>
<td>4.79</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ALP (IU/L serum)</td>
<td>71.04 ± 4.61</td>
<td>128.11 ± 5.24*</td>
<td>80.30 ± 4.04@</td>
<td>5.20</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>GGT (IU/L serum)</td>
<td>2.92 ± 0.41</td>
<td>24.63 ± 0.16*</td>
<td>3.67 ± 0.72@</td>
<td>5.79</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Total protein (g/100 ml)</td>
<td>6.72 ± 0.31</td>
<td>4.83 ± 0.16*</td>
<td>6.22 ± 0.33@</td>
<td>4.98</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Total lipids (mg/100 ml)</td>
<td>136.54 ± 6.16</td>
<td>266.91 ± 9.20*</td>
<td>152.24 ± 6.41@</td>
<td>4.66</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Triglycerides (mg/100 ml)</td>
<td>8.43 ± 0.72</td>
<td>13.14 ± 1.14*</td>
<td>9.19 ± 0.66@</td>
<td>5.06</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cholesterol (mg/100 ml)</td>
<td>66.93 ± 3.61</td>
<td>99.04 ± 5.02*</td>
<td>89.72 ± 4.01@</td>
<td>5.68</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Phospholipids (mg/100 ml)</td>
<td>120.21 ± 6.91</td>
<td>270.93 ± 14.21*</td>
<td>132.62 ± 9.43@</td>
<td>5.09</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*P<0.05 as compared to group I.
@P<0.05 as compared to group II.
Values are mean±SEM of 8 animals in each group.
LP = liquid paraffin; CCl\textsubscript{4} = carbon tetrachloride; MEC = methanol extract of rhizomes of C. orchioides.

Table 2. Effect of Curculigo orchioides on different biochemical parameters in the liver of rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I Control with LP only</th>
<th>Group II LP+CC\textsubscript{4} only</th>
<th>Group III LP+CC\textsubscript{4}+MEC</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/100 g)</td>
<td>7.48 ± 0.38</td>
<td>4.91 ± 0.23*</td>
<td>7.68 ± 0.31@</td>
<td>5.53</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Total lipids (mg/100 g)</td>
<td>6290 ± 641</td>
<td>8432 ± 199*</td>
<td>6766 ± 119@</td>
<td>4.99</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Triglycerides (mg/100 g)</td>
<td>613.2 ± 84.1</td>
<td>902.6 ± 56.8*</td>
<td>718.1 ± 43.6@</td>
<td>4.61</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cholesterol (mg/100 g)</td>
<td>602.9 ± 29.1</td>
<td>886.2 ± 36.1*</td>
<td>662.8 ± 31.5@</td>
<td>5.16</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Phospholipids (mg/100 g)</td>
<td>2410 ± 102.8</td>
<td>1478 ± 62.6*</td>
<td>2216 ± 103.2@</td>
<td>5.40</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*P<0.05 as compared to group I.
@P<0.05 as compared to group II.
Values are mean±SEM of 8 animals in each group.
LP = liquid paraffin; CCl\textsubscript{4} = carbon tetrachloride; MEC = methanol extract of rhizomes of C. orchioides.

The site specific oxidative damage of some of the susceptible aminoacids of proteins is now regarded as the major cause of metabolic dysfunction during pathogenesis\textsuperscript{22}. Hypoalbuminaemia is most frequent in the presence of advanced chronic liver diseases. Hence decline in total protein content can be deemed as a useful index of the severity of cellular dysfunction in chronic liver diseases. The lowered level of total proteins recorded in the serum as well as liver of CCl\textsubscript{4}-treated rats reveals the severity of hepatopathy. The attainment of near normalcy in total protein content of serum and liver of herb-treated rats further elucidates the hepatoprotective effect of C. orchioides.

Treatment with CCl\textsubscript{4} increases the levels of total lipids, total triacyl glycerols and total cholesterol in liver\textsuperscript{24}. Presence of significantly high concentration
Figure 1. Photomicrographs of liver sections of rat stained with haematoxylin and eosin (x 100).

a. Liver section from normal rat showing normal liver architecture with portal triad.

b. Liver section from CCl$_4$-treated rat showing severe steatosis and focal necrosis. Central vein with minimal perivenular fibrosis.

c. Liver section from CCl$_4$ + C. orchioides-treated rats showing almost normal liver architecture.
of total lipids and cholesterol in the serum and liver tissue of group II animals and its recovery towards near normal values in MEC - administered rats coincides with the above observations, thus unearthing the hepatoprotective effect of C. orchioides once again. Hepatotoxins like CCl₄ can interfere with the hepatic phospholipid synthesis. The phospholipid content in serum registered a significant hike and that of liver showed a diminution in CCl₄-administered group, which was retrieved to near normalcy in MEC - treated rats. This observation also indicates the hepatoprotective potential of C. orchioides.

A comparative histopathological study of liver from different groups further corroborated the hepatoprotective efficacy of C. orchioides. Works are in progress here to unravel the antioxidant activity of C. orchioides, and also to isolate and purify the active principle involved in hepatoprotection of this promising plant.

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**INDIAN MEDLARS CENTRE**

The Indian MEDLARS Centre (IMC) was set up, in 1986, as a collaborative project between Indian Council of Medical Research (ICMR), New Delhi and National Informatics Centre (NIC), to provide biomedical information services from the MEDLARS databases of National Library of Medicine (NLM), USA.

IMC’s webpage was launched in August 2000 (http://indmed.nic.in) catering specifically to the information needs of the medical researchers and medical professionals in India. Since its launch the page has crossed 23,000 hits and links to the webpage feature in international sites like the Public Health Library, University of Berkeley, University of Portsmouth’s Online Information Service and University Hospital Benjamin Franklin Medical Library (University of Berlin). The IndMED database has been accepted as a competing IT-project in the 2002 Stockholm Challenge Award (a very prestigious IT award). The Google Directory has ranked the site amongst the top 5 Indian health websites. The Indian MEDLARS Centre’s website has also received a lot of recognition from international associations and has received awards for its content as well as for its design. The salient features of the webpage are:

- **Access to IndMED database** (indigenously developed bibliographic database of 76 peer reviewed Indian biomedical journals).
- **Chat room** for medical professionals ([Live@IndMED](http://live.indmed.nic.in)).
- **Links to Internet biomedical/health resources** (both for the professional and the consumer, nursing professionals, health librarians). This includes links to NLM’s resources. **Links to medical journals** available on the Internet.
- **Lectures** by medical specialists.
- **Web-enabled training tutorials** with downloadable Training Manual.
- **Search request forms** (which can be submitted directly from the webpage).