HEPATOPROTECTIVE ACTIVITY OF METHANOLIC EXTRACT OF *MADHUCA INDICA* ON CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY IN RATS

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Summary

The methanol extracts of *M. indica* studied for hepatoprotective activity against albino rats with liver damage induced by carbon tetrachloride (CCl₄). It was found that the methanol extract of *M.indica* at a dose of 300 mg/kg body weight exhibited moderate protective effect by lowering the serum levels of Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum bilirubin and Serum alkaline phosphate (SALP) to a significant extent. Present finding demonstrated the methanolic bark extract of *Madhuca indica* could afford significant dose-dependent protection against CCl₄ induced hepatocellular injury.

Keywords: *Madhuca indica*, Hepatoprotective activity, carbon tetrachloride.
Introduction

In 1948, World Health Organization (WHO) defined health as a state of complete physical, mental and social wellbeing and not merely the absence of disease or infirmity. The WHO defines health as “A state of complete physical, mental and social wellbeing” now is conventional wisdom. Health is no longer defined simply in physical terms, as the absence of disease or disability, but now includes mental and social dimensions (1). According to WHO, about 18,000 people die every year due to liver diseases. The common ailments of liver are cirrhosis, cholestasis, hepatitis, portal hypertension, hepatic encephalopathy, fulminant hepatic failure and certain tumors like hepatoma. It is estimated that two billion people around the world are infected with hepatitis B. About 350 million of these have the chronic form of the disease. In modern medicine, corticosteroids and immunosuppressant are commonly used to treat liver disease in allopathic form of medicine. But, these drugs are associated with adverse effects such as immunosuppressant and bone marrow depression. Further, the success rate of treating liver diseases is disappointing (2). In recent times, focus on plant research has increased all over world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. More than 13,000 plants have been studied during the last 5 year period (3).

**Madhuca indica** Syn. **Madhuca latifolia** (Sapotaceae), commonly known as ‘mahua’ in the India, is an important economic plant growing throughout the subtropical region of the Indo-Pak subcontinent (4). Different parts of this plant are used as stimulants, demulcents, emollients, heating and astringents (5). The bark is a good remedy for itching, swellings, fractures and snake bites, as well as for diabetes mellitus (6). Mahua oil is used for the treatment of skin diseases, rheumatism, headache and as a laxative. Fruits are astringent and largely employed as a lotion for chronic ulcers, in acute and chronic tonsillitis, and in pharyngitis. The constituents reported from *M. indica* include fatty acids (7, 8), sapogenins (9), sugars (6), triterpenoids steroids (10, 11, and 12), saponins (13, 14), flavonoids and glycosides (11, 12, and 15).
Material and Methods

Plant material:
*Madhuca indica* (Sapotaceae) was purchased from local market and identified by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. A voucher specimen (BIT/Anu/2009/MI-520) was deposited in the herbarium of School of Pharmacy, Bharat Institute of Technology, Meerut, UP, India.

Preparation of plant extract:
Dried *Madhuca indica* bark samples was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. Equal quantity of powder was passed through 40 mesh sieve and extracted with methanol in soxhlet apparatus at 60°C. The solvent was completely removed by rotary vacuum evaporator. The extract obtained with methanol was 0.39 % w/w. The extract was freeze dried and stored in vacuum desiccators.

Animals:
Wistar rats of both sexes (150-200 g) were maintained under uniform laboratory conditions in standard polypropylene cages and provided food and water *ad libitum*. The animals were acclimatized for a period of 14 days prior to performing the experiments. The experimental protocol was approved by Institutional Animal Ethics Committee (Regn No: 1147/ab/07/CPCSEA).

Acute toxicity:
The acute toxicity studies were carried out in adult female albino rats weighing 150-200 g, by up and down method as per OECD 425 guidelines (16). Overnight fasted animals received test drug at a dose of 50, 300, 1000 and up to 3000 mg/kg body weight orally. Then the animals were observed continuously once in half an hour for the next 4 hours and then after 24 hours for general behavioral, neurologic and autonomic profiles and to find out mortality. The extract was found safe to up to a dose of 3000 mg/kg body weight.

Hepatoprotective activity:
The animals were divided in four groups. Group I and II, served as Control and Carbon tetrachloride control, and received the vehicle (water: propylene glycol, 4:1) by gastric intubation once daily for 7 days. Group III and IV, were given 1 ml suspension of methanolic extract of *Madhuca indica* at a dose level of 150 and 300 mg/kg b.w. and V served as Silymarin at a dose level of 100 mg/kg b.w. (17) once daily for 7 days. On the 8th day one hr after administration of the last dose of drug, the animals of groups II, III, IV were
given an intraperitoneal injection of CCl₄ (0.5 ml/kg b.w.). All the animals were then fasted for 24 hrs. After that they were anaesthetized and the blood was collected by cardiac puncture. The blood samples were allowed to coagulate at room temperature for one hour. Serum was separated by centrifugation at 4°C, 12000 rpm for 5 minutes (18).

Biochemical studies:

The activity of Serum transaminases (SGOT, SGPT) were estimated by Reitman and Frankel method (19). Serum bilirubin (total and direct) was determined by Melloy and Evelyn method (20). Serum alkaline phosphate (SALP) level was also determined (21).

Histopathology

A portion of liver tissue in each group of rats was selected and fixed paraffin embedding. Sections were stained with hematoxylin and eosin and observed under microscope (22).

Statistical analysis

The values were expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. P values < 0.05 were considered as significant.

Results

Administration of *M. indica* Bark extract in two different dosages remarkably prevented CCl₄ induced elevation of serum enzyme in a dose dependent manner. As given in Table 1, compared with the control group and it attained an almost near the normal value in groups which were treated with *M. indica* Bark extract. Histopatological study of liver from the control group animals showed a normal hepatic architecture with distinct hepatic cells, sinusoidal spaces and a central vein (Figure 1a). However, CCl₄–intoxicated treatment exhibited severs histopathological change, such as centrilobular hepatic necrosis, fatty change, apoptotic bodies and ballooning degeneration (Figure 1b). Pretreatment with 150 mg/kg and 300 mg/kg of methanolic extract of *M. indica* bark showed significant recovery. Ballooning degeneration, fatty change, centrilobular hepatic necrosis and apoptotic bodies in hepatocytes were scarce (Figure 1c & d).
Table No-1 Effect of various Extracts on SGOT, SGPT, and SALP in CCl_4 induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>SGOT (mg/dl)</th>
<th>SGPT (mg/dl)</th>
<th>SALP (mg/dl)</th>
<th>Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
<td>Total</td>
</tr>
<tr>
<td>Control</td>
<td>33.98±1.12</td>
<td>45.06±2.71</td>
<td>115.62±2.91</td>
<td>0.39±0.06</td>
</tr>
<tr>
<td>CCl_4</td>
<td>83.10±2.40</td>
<td>87.60±1.87</td>
<td>212.41±4.31</td>
<td>8.96±0.41*</td>
</tr>
<tr>
<td>Standard</td>
<td>36.5±1.7</td>
<td>39.6±0.9</td>
<td>6.74±0.3</td>
<td>0.42±0.02</td>
</tr>
<tr>
<td>MEMI (150 mg/kg)</td>
<td>68.28±0.8**</td>
<td>82.74±1.6ns</td>
<td>155.62±2.8***</td>
<td>0.43±0.02</td>
</tr>
<tr>
<td>MEMI (300 mg/kg)</td>
<td>62.24±0.3***</td>
<td>70.52±1.1**</td>
<td>162.84±1.2***</td>
<td>0.43±0.005**</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=6) one way ANOVA followed by Tukey’s multiple coparision column test. Where, * represents significant at p<0.05, ** represents highly significant at p<0.01, *** represents very significant at p<0.001 and ns represents non significant.

Fig-1. Histopathological studies of the rat liver in CCl_4 induced hepatotoxicity

Normal (a)  
CCl_4 (b)  
M. indica 150 mg/kg (c)  
M. indica 300 mg/kg (d)
Discussion

In the present study it was noted that the administration of CCl$_4$ increased the levels of SGOT, SGPT, SALP and bilirubin (total and direct). A significant reduction was observed in SGPT, SGOT, SALP, total and direct bilirubin levels in the groups treated with silymarin and methanolic extract of *M. indica*. The enzyme levels were almost restored to the normal.

The changes associated with CCl$_4$-induced liver damage are similar to those of acute viral hepatitis. In the liver, microsomal oxidizing systems produce reactive metabolites of CCl$_4$ such as trichloromethyl radical (CCl$_3$) or trichloroperoxy radical (CCl$_3$O$_2$). These free radicals cause lipid peroxidation which produces hepatocellular damage and enhances production of fibrotic tissue (23, 24). When liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into the bloodstream. A comparative histopathological study of liver from different groups (Figure 1-4) demonstrated the protective action of the crude extract against liver damage.

In conclusion, the results of the present study suggest that the *M. indica* extract possess hepatoprotective activity against CCl$_4$ intoxication rats. According to these results it can be suggested that hepatoprotective action of *M. indica* may be possess significant protective effect against hepatotoxicity induced by CCl$_4$ which may be attributed to the individual or combined action of Phytoconstituents present in it. Further elucidation of structure of active component(s) is under progress in the laboratory.

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