Prophylactic and curative effects of *Moringa oleifera* Lam. pods in CCl₄ damaged rat liver

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The present research work was designed to establish the possible preventive and curative hepatoprotective efficacy of 70% hydro-alcoholic extract of pods of *Moringa oleifera* Lam. at different dose levels (100, 250 and 500 mg/kg) on CCl₄ induced liver injury in rats. Antihepatotoxic potential was assessed by the estimation of biochemical markers, viz. SGPT, SGOT, ALP, ACP and bilirubin (Direct & Total). In addition liver GSH and MDA levels and histopathological examination were also studied. The dose dependent significant reduction of biochemical markers and bilirubin levels were seen in rats subjected to both pre and post treatments of test extract. Though, the extract at a dose of 100 mg/kg exhibited considerable reduction in concentration of ACP, TB and DB in pre and post treatment models, the results were found to be statistically not significant. CCl₄ poisoned rats showed significant decrease in hepatic GSH level and elevation of MDA (Malondialdehyde) level compared to normal control group. Pre and post treatment of extract dose dependently reversed the CCl₄ mediated altered hepatic GSH and MDA levels. The histopathological study supported the hepatoprotective activity of the test extract. In conclusion, the findings of the present study suggest that *M. oleifera* pods extract possesses potent prophylactic and curative hepatoprotective efficacy against CCl₄ rendered liver injury in rats.

**Keywords:** Drumstick, Hepatoprotective, Liver injury, *Moringa oleifera* pods, Prophylactic.

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**Introduction**

Liver diseases are large public health problem in the world¹. Towards these pathologies modern medicine does not find any curative treatments². Hence, searching the safe and potent remedies from the herbal origin for the treatment of hepatic disorders has become most fascinating and desired area of research for the pharmacologists. Literature review showed that some of the Indian medicinal plants used traditionally in the management of liver disorders have been scientifically investigated and reported for their measurable hepatoprotective effects against various experimental animal models. However, still more numbers of medicinal plants are needed to be screened for their hepatoprotective efficacy.

*Moringa oleifera* Lam. (Family — Moringaceae) commonly known as Drumstick is one such plant cultivated for different purposes such as medicine, vegetable, spice, for cooking and cosmetic oil³. The leaves, fruits, flowers and immature pods of this tree are used as a highly nutritive vegetable in many countries, particularly in India, Pakistan, Philippines, Hawaii and many parts of Africa⁴-⁶. All the parts of the tree are used in folk medicine practices for the treatment of various diseases such as UTI, HIV-AIDS, external sores and ulcers, diabetes, cancer, gastritis, diarrhoea, liver diseases⁷, etc. The plant is reported to possess anti-inflammatory, antioxidant, antiulcer, anticancer, antihyperlipidaemic and cardiotonic properties⁸-¹³. A study on ethanol and aqueous extracts of whole pods and its parts, i.e. coat, pulp and seed revealed that the blood pressure lowering effect of seed was more pronounced with comparable results in both ethanol and water extracts indicating that the activity is widely distributed¹⁴.

In vivo hepatoprotective activity of the *M. oleifera* leaves, flowers, roots and seeds have been already documented in the literature¹⁵-¹⁸. *In vitro* antihepatotoxic property has also been reported from its fruits¹⁹. However, comparative studies on prophylactic (preventive) and curative potential of
pods on CCl₄ damaged rat liver has not been investigated so far. Hence, the present investigation was undertaken.

Materials and Methods

Plant materials

The pods of *M. oleifera* (Plate 1) were collected from the surrounding gardens of the Harapanahalli, Karnataka, India and they were authenticated by Professor K. Prabhu, Department of Pharmacognosy, S.C.S. College of Pharmacy, Harapanahalli. A voucher specimen has been deposited in the museum of the college.

Preparation of extract

Fresh mature pods were shade dried at room temperature, coarse powdered and extracted with 70% hydro-alcohol by Soxhlet’s extraction method. Thereafter, the extract was concentrated using rotary flash evaporator to obtain semisolid crude extract. The percentage yield of the extract was found to be 7.8. The extract was stored in airtight container in refrigerator below 10°C. Appropriate concentrations of stock solutions was prepared using distilled water and used for the following studies:

01. Preliminary phytochemical investigations.
02. Acute toxicity studies in mice.
03. Evaluation of hepatoprotective activity against CCl₄ induced liver injury in rats.

Preliminary phytochemical screening

Preliminary phytochemical tests were conducted on test extract to detect the presence of phytochemicals by following the standard methods described by Trease and Evans²⁰.

Experimental animals

Male albino Wistar rats (150-200 g) and female albino Swiss mice (20-25 g) were used in the experiments. They were procured from Sri Venkateshwar Enterprises, 4304, 13th main 2nd cross, Subramanya nagar, Bangalore-21 (237/CPCSEA), India. After randomization into various groups and before initiation of experiment, the animals were acclimatized for a period of 10 days. Animals were housed in polypropylene cages and maintained under standard environmental conditions such as temperature (26 ± 2°C), relative humidity (45-55%) and 12 h dark/light cycle. The animals were fed with rodent pellet diet (Golden Mohur Lipton India Ltd.) and water *ad libitum*. The study protocol was approved from the Institutional Animal Ethics Committee (IAEC) before commencement of experiment.

Determination of acute toxicity (LD₅₀)³¹

The acute toxicity of test extract was determined in female albino mice following fixed dose method of CPCSEA, (OECD, guideline No. 420, Annexure-2d). The mice weighing 20-25g were fasted overnight prior to experiment and based on the LD₅₀ cutoff value 1/25th, 1/10th and 1/5th screening doses of the extract were selected for the hepatoprotective study.

Evaluation of hepatoprotective activity on CCl₄ rendered hepatic damage in rats²²

Albino rats of Wistar strain weighing 150-200 g were allocated to 08 groups of six each as shown below:

*Group 1:* Control - Untreated.
*Group 2:* CCl₄ control - Injected with fresh mixture of equal volume of CCl₄ and liquid paraffin by 3 i.p. injections at a doses of 2 ml/kg body wt.

*Pre treatment of M. oleifera pods extract (Prophylactic study)*

*Group:* 3, 4 and 5 were given orally 100, 250 and 500 mg/kg, respectively for 14 consecutive days and injected intraperitonially CCl₄ (2 ml/kg in equal volume of liquid paraffin) on days 12, 13 and 14.

Plate 1—*Moringa oleifera*: a-Twig bearing pods, b-Dried pods
Post treatment of *M. oleifera* pods extract (Curative study)

Group: 6, 7 and 8 were given orally 100, 250 and 500 mg/kg, respectively for 14 consecutive days and injected intraperitoneally CCl<sub>4</sub> on days 1, 2 and 3.

During the period of treatment the rats were maintained under normal diet and water. Twenty four hours after the last treatment i.e. on 15<sup>th</sup> day, the blood samples were collected by puncturing the retro-orbital plexus under the influence of light ether anaesthesia and allowed to coagulate for 30 min at 37°C. Plasma was separated by centrifugation at 3000 rpm for 15 min and used for estimation of biochemical parameters such as SGPT, SGOT, ALP, ACP, bilirubin (Total and Direct) using ready Erba Diagnostics Mannheim GmbH – Germany kits by STAR 21 PLUS semi autoanalyzer.

The screening doses selected for the hepatoprotective activity of pod extracts of the plant were:

- 100 mg/kg-1/25<sup>th</sup> dose of 2500 mg/kg b.w.;
- 250 mg/kg-1/10<sup>th</sup> dose of 2500 mg/kg b.w.;
- 500 mg/kg-1/5<sup>th</sup> dose of 2500 mg/kg b.w.

Effect of *M. oleifera* pods extract on biochemical parameters in CCl<sub>4</sub> damaged rat liver

The CCl<sub>4</sub> challenged animals exhibited significant elevation of serum marker enzymes SGPT, SGOT, ALP, ACP and increased concentration of bilirubin (Total & Direct) indicating hepatocellular damage when compared with normal control rats. The dose dependent significant reduction of elevated SGPT, SGOT, ALP, ACP, total and direct bilirubin levels monitored in rats subjected to both pre- and post-treatment with 70% hydro-alcoholic extract of *M. oleifera* pods. Though, the extract at doses of 100 mg/kg exhibited considerable reduction in concentration of ACP, total and direct bilirubin in pre- and post-treatment modes, but the results were found to be statistically not significant. The results are represented in Table 1.

Effect of test extract on liver GSH content

In CCl<sub>4</sub> poisoned rats a significant decrease in GSH level was observed compared to normal control group. Pre- and post-treatment with the test extract significantly attenuated CCl<sub>4</sub> induced hepatocellular damage when compared with normal control rats. The dose dependent significant reduction of elevated SGPT, SGOT, ALP, ACP, total and direct bilirubin levels monitored in rats subjected to both pre- and post-treatment with 70% hydro-alcoholic extract of *M. oleifera* pods. Though, the extract at doses of 100 mg/kg exhibited considerable reduction in concentration of ACP, total and direct bilirubin in pre- and post-treatment modes, but the results were found to be statistically not significant. The results are represented in Table 1.

### Table 1 — Preventive and Curative effects of *Moringa oleifera* pods extract on biochemical markers

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGPT</th>
<th>SGOT</th>
<th>ALP</th>
<th>ACP</th>
<th>TB</th>
<th>DB</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>117.98 ± 4.13</td>
<td>145.1 ± 4.67</td>
<td>291.25 ± 1.33</td>
<td>30.45 ± 1.27</td>
<td>0.370 ± 0.007</td>
<td>0.283 ± 0.012</td>
</tr>
<tr>
<td>CCl&lt;sub&gt;4&lt;/sub&gt; control</td>
<td>250.00 ± 2.33</td>
<td>350.0 ± 6.16</td>
<td>560.80 ± 6.60</td>
<td>55.60 ± 1.22</td>
<td>0.639 ± 0.004</td>
<td>0.551 ± 0.007</td>
</tr>
<tr>
<td>Preventive (mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>220.30±1.87***</td>
<td>302.87±2.87***</td>
<td>486.10±3.87***</td>
<td>43.01 ± 3.13&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.520 ± 0.09&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.490 ± 0.017&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>250</td>
<td>180.13±3.14***</td>
<td>270.13±4.90***</td>
<td>349.80±4.90***</td>
<td>35.01 ± 4.70&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.498 ± 0.07&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.349 ± 0.03&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>500</td>
<td>120.70±3.80***</td>
<td>157.19±4.83***</td>
<td>301.10±3.83***</td>
<td>33.07 ± 2.87&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.376±0.03***</td>
<td>0.285±0.03***</td>
</tr>
<tr>
<td>Curative (mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>223.19±2.10***</td>
<td>300.30±5.13***</td>
<td>470.83±4.10***</td>
<td>40.08 ± 4.10&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.517 ± 0.001&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.483 ± 0.02&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>250</td>
<td>189.27±4.17***</td>
<td>269.83±5.31***</td>
<td>335.90±3.30***</td>
<td>33.70 ± 3.10&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.510 ± 0.003&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.360 ± 0.07&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>500</td>
<td>125.60±5.17***</td>
<td>153.93±3.87***</td>
<td>310.90±2.87***</td>
<td>32.90 ± 3.10&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.365±0.01***</td>
<td>0.275±0.01***</td>
</tr>
</tbody>
</table>

Results are Mean ± SE, n = 6, * P < 0.05, ** P < 0.01 and *** P < 0.001 compared to CCl<sub>4</sub> control. ns: Statistically not significant.
decrease of GSH content in dose dependent manner compared to \( \text{CCl}_4 \) intoxicated group. The results are presented in Table 2.

**Effect of test extract on liver LPO content**

The effect of test extract on \( \text{CCl}_4 \) mediated LPO was examined through monitoring the levels of MDA (Malondialdehyde). The extract of title plant on pre- and post-treatments dose dependently reversed the \( \text{CCl}_4 \) intoxicated elevation of hepatic MDA level. Though, there was considerable decrease in LPO content monitored in rats with pre- and post-treatments of test extract at a dose of 100 mg/kg, but the results were found to be non-significant statistically. The results are tabulated in Table 2.

**Effect of \textit{M. oleifera} pods extract on histopathological profile**

Histopathological profile of liver from \( \text{CCl}_4 \) (Hepatic control group) intoxicated rats reveal hepatic globular architecture disrupted, hepatic cells has shown various degree of fatty degeneration like ballooning of hepatocytes, fatty cyst, infiltration of lymphocytes, proliferation of Kupffer cells and congestion of liver sinusoids. Pre and post treatment of test extract was confirmed by histopathological examination of liver sections. Administration of test extract at the dose of 500 mg/kg exhibited a significant improvement of the hepatic architecture and areas of Kupffer cell proliferation and sinusoid appeared normal compared to 100 and 250 mg/kg. Histological studies of liver sections are shown in Plate 2 (a-d).

**Discussion**

The aim of current investigation was to study the prophylactic (preventive) and curative effects of \textit{M. oleifera} pods extract on \( \text{CCl}_4 \) poisoned liver damage in rats. Liver injury induced by \( \text{CCl}_4 \) is perhaps widely used experimental model for the screening of hepatoprotective agent\(^{25}\). Several mechanisms underlying this toxicity have been suggested. \( \text{CCl}_4 \) the inactive metabolite is biotransformed by cytochrome P–450 enzymes to produce the trichloro methyl free radical (\( \text{CCl}_3 \)) that causes lipid peroxidation and there by produce liver damage\(^{26-28}\).

The dose dependent significant reduction of \( \text{CCl}_4 \) rendered elevated plasma activities of SGPT, SGOT, ALP, ACP, total and direct bilirubin levels in rats pre- and post-treated with 70% hydro-alcoholic extract of pods demonstrated their ability to restore the normal functional status of the poisoned liver, and also to protect against subsequent \( \text{CCl}_4 \) liver injury.

The development of hepatotoxicity induced by \( \text{CCl}_4 \) challenge was exacerbated following the depletion of glutathione. Therefore, in the current study glutathione level was measured to observe the preventive and curative effects of \textit{M. oleifera} pods in experimental animals. The results obtained from the present study clearly demonstrated that \( \text{CCl}_4 \) intoxication has significantly reduced the glutathione level compared to normal control animals. Rats on pre- and post-treatment with pods extract (100, 250 and 500 mg/kg, doses) have clearly restored the levels of glutathione significantly in a dose related manner.

The \( \text{CCl}_4 \) damaged liver toxicity was associated with marked increase in liver MDA level. The MDA elevation has been well accepted reliable marker of

<table>
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<tr>
<th>Groups</th>
<th>GSH</th>
<th>LPO</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Absorbance mean ± SEM</td>
<td>% increase</td>
</tr>
<tr>
<td>Control</td>
<td>0.783 ± 0.057</td>
<td>0.225 ± 0.008</td>
</tr>
<tr>
<td>( \text{CCl}_4 ) control</td>
<td>0.251 ± 0.032</td>
<td>0.527 ± 0.112</td>
</tr>
<tr>
<td>100 Preventive (mg/kg)</td>
<td>0.458 ± 0.039</td>
<td>78.48</td>
</tr>
<tr>
<td>250</td>
<td>0.603 ± 0.044***</td>
<td>140.23</td>
</tr>
<tr>
<td>500</td>
<td>0.658 ± 0.026***</td>
<td>162.15</td>
</tr>
<tr>
<td>100 Curative (mg/kg)</td>
<td>0.428 ± 0.06</td>
<td>87.25</td>
</tr>
<tr>
<td>250</td>
<td>0.623 ± 0.058***</td>
<td>148.20</td>
</tr>
<tr>
<td>500</td>
<td>0.663 ± 0.032***</td>
<td>164.14</td>
</tr>
</tbody>
</table>

Results are Mean ± SE, \( n = 6 \), \( * P < 0.05 \), \( ** P < 0.01 \) and \( *** P < 0.001 \) compared to \( \text{CCl}_4 \) control.

ns: Statistically not significant.
lipid peroxidation. MDA elevation is a result of oxidative stress demonstrated here through the decrease of GSH level in liver. Hence, in the present study MDA level was also estimated to evaluate prophylactic and curative properties of extract of the title plant. Results given in the Table 2 clearly indicated that CCl$_4$ intoxicated rats showed significant increase in the MDA level compared to normal control group. Rats on pre- and post-treatment with *M. oleifera* pods extract (100, 250 and 500 mg/kg, doses) has significantly decreased the MDA level in a dose dependent manner.

The findings of the present investigation were also consistent with previous reports, where different parts of *M. oleifera* treatment (but only pre-treatment mode) shown to protect liver from hepatotoxicity caused by ethanol, diclofenac and antitubercular drugs$^{18,29,30}$. The mechanism by which *M. oleifera* pods extract exhibited hepatoprotective activity is not clear from the present study. However, the important factor in the hepatoprotective property of any drug is the ability of its constituents to inhibit the aromatase activity of cytochrome P–450, thereby favouring liver regeneration$^{23}$. On this basis, it is suggested that flavonoids content in the test extract (evident by preliminary phytochemical screening) could be the reason for contributing hepatoprotective ability through inhibition of cytochrome P–450 aromatase$^{31}$. 

**Conclusion**

In conclusion, the findings of the present study suggest that pods extract of the *M. oleifera* possesses equally potent prophylactic and curative hepatoprotective efficacy against CCl$_4$ rendered liver injury in rats.

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