Phytochemical Studies and Hepatoprotective activity of *Melia azedarach* Linn, against CCl₄ induced Hepatotoxicity in rats

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ABSTRACT

The aim of the study is to investigate the phytochemicals and hepatoprotective activity of *Melia azedarach* L leaves extracts against carbon tetrachloride (CCl₄) induced hepatotoxicity. The phytochemical screening was carried on the leaves extracts of *Melia azedarach* revealed the presence of some active ingredients such as Alkaloids, Tannins, Saponins, Phenols, glycosides, steroids, terpenoids and flavonoids. Leaves of *Melia azedarach* was successively extracted with ethanol against carbon tetrachloride (CCl₄) induced hepatotoxicity using Standard drug Silymarin (25 mg/kg). There was a significant changes in biochemical parameters (increases in serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT), alanine phosphatase (ALP),serum bilirubin and decrease the total proteins content.) in CCl₄ treated rats, which were restored towards normalization in *Melia azedarach* (500 mg/kg) treated animals. Thus the present study ascertains that the leaf extract of *Melia azedarach* possesses significant hepatoprotective activity.

Key words: *Melia azedarach*, phytochemical screening, hepatoprotective activity, CCl₄, ethanol and Silymarin.

INTRODUCTION

Medicines that are used today are not definitely the same as those that were used in ancient times or even in the recent past. India has a wealth of medicinal plants most of which have been traditionally used in Ayurveda, Unani systems of medicine and by tribal healers for generation. In ancient Indian literature, it is mentioned that every plant on this earth is useful for human beings, animals and other plants. The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against diseases, nutrient supply, energy provision and reproduction.⁶ The liver is expected not only to perform physiological functions but also to protect the hazards of harmful drugs and chemicals. In spite of tremendous scientific advancement in the field of hematology in recent years, liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate.⁷ Presently only a few hepatoprotective drugs and those from natural sources are available for the treatment of liver disorders.⁸ The disorders associated with the liver are also numerous and varied.⁹ CCl₄ is toxic to the liver and its toxicity is dose dependent and based on time of exposure.⁴,⁶ In the liver, CCl₄ is metabolized in to the highly reactive trichloromethyl radical. This free radical causes autooxidation of the fatty acids present in the cytoplasmic membrane phospholipids resulting in functional and morphological changes in the cell membrane.⁷ The metabolism of CCl₃ free radical released from CCl₄ initiates peroxidation and cleavage of fatty acids in membranes. Thus, trichloromethylperoxyl free radical elicits lipid peroxidation, the destruction of Ca²⁺ homeostasis, and finally, results in cell death.⁹

*Melia azedarach* linn (meliaceae; Neem) is an indigenous plant possessing several medicinal properties. Melia azedarach linn (synonym: Melia dubia Cav, Indian lilac, Persian lilac) belonging to the family Meliaceae is a tree found in India. It is popular as Indian lilac. Different phytochemicals present in leaf, root and stem, are meliacarpins, limonoids, sendanins, trichilins and azedarachins.⁴ The plant is traditionally used for the treatment of leprosy, inflammations, Analgesics and cardiac disorders. Its fruits extracts possess ovicidal and larvicidal activity.⁵ The leaves extracts also possess antiviral and antifertility activity.⁶ Hence the present study was aimed at investigating the phytochemicals and hepatoprotective activity of *Melia azedarach* L leaves extracts against carbon tetrachloride (CCl₄) induced hepatotoxicity.

MATERIALS and METHOD

Plant materials

The basic plant material of *Melia azedarach* Linn used for the investigation was obtained from Mount Opera Garden, Near Ramoji Film City, and Nalgonda Dist. The plant can be identified authenticated by Department of Botany, research office (Botanist), Anwar-ul- loom College of Pharmacy, Hyderabad.

Preparation of ethanolic extract

The leaves were collected and shadow dried. The shade leaves were subjected to pulverization to get coarse powder. The coarsely powder leaves of *Melia azedarach* were used for extraction. The shade dry coarsely leaves of *Melia azedarach* were used for extraction with ethanol. *Melia azedarach* leaf powder (250 g) was loosely packed in the thimble of soxhlet apparatus and extracted with ethanol at 55°C for 18 h. The extract was air dried at 25-30°C and weighed. For oral administration, extract was dissolved in 10 mL Phosphate Buffer Saline (PBS) at different concentrations. To make the extract soluble in PBS, 1% tween 80 was used.
**Phytochemical investigation**

The preliminary qualitative phytochemical studies were performed for testing the different chemical groups such as alkaloids, tannins, glycosides and saponins etc present in ethanol extracts. (14, 15 & 16)

**Experimental Animals**

Wistar albino rats (150-200 g) of both sexes were obtained from the animal house of NIZAM INSTITUTE OF PHARMACY, Deshmukhi, Ramoji film city, Hyderabad. Before and during the experiment, rats were fed with standard diet (Gold Moher, Lipton India Ltd). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 h ad libitum. All animals experiment was carried out in accordance with the guidelines of CPCSEA and study was approved by the IAEC (Institutional animal ethical committee) with registration number. (1330/AC/10/CPCSEA)

**Acute toxicity study**

Melia azedarach in the dose range of 110 mg-630 mg/kg were administered orally to different group of mice comprising of ten mice in each group. Mortality was observed after 72 h. Acute toxicity was determined according to the method of Litchfield and Wilcoxon. (17)

**Experimental design for hepatoprotective activity**

Animals are divided into 4 groups, each comprising 6 rats.

- **Group I**: Control group
- **Group II**: CCl<sub>4</sub>, treated group
- **Group III**: CCl<sub>4</sub> + Melia azedarach leaf extract (500mg/kg, p.o)
- **Group IV**: CCl<sub>4</sub> + Silymarin (25mg/kg, p.o)

Hepatoprotective study was carried out as described by Brijesh et al. (2008) (18). Albino rats of either sex (150-200 gm) were selected and divided into four groups of six animals each. The first group was fed with 1 ml/kg p.o of saline solution (S.S.) for 4 days. The second group was fed with 1 ml/kg p.o. of S.S. for 4 days along with 2 ml/kg of CCl<sub>4</sub> by S.C. on the second and third days. The third group was fed with azedarach leaf extract (500mg/kg, p.o) for 4 days along with 2 ml/kg of CCl<sub>4</sub> by S.C. on the second and third days. Fourth and fifth groups were fed with Silymarin (25mg/kg, p.o) for 4 days along with 2 ml/kg of CCl<sub>4</sub> by S.C. on the second and third days. On the fifth day, all the animals were sacrificed by mild ether anesthesia.

**Blood biochemistry**

Blood samples were collected in glass tube from retro-orbital puncture to obtain haemolysis free clear serum for the analysis of SGOT and SGPT (19) with 2 ml/kg of CCl<sub>4</sub> and fifth groups were fed with Silymarin (25mg/kg, p.o) for 4 days along with 2 ml/kg of CCl<sub>4</sub> by S.C. on the second and third days. On the fifth day, all the animals were sacrificed by mild ether anesthesia.

**Histopathology**

Histopathology of liver was carried out by a modified Luna (Luna LG, 1999) (21). In brief, the autopsied livers were washed in normal saline and fixed in 10% formalin for 2 days followed by bovine solution for 6 h. Then the livers were paraffin embedded and 5 μ thickness microtome sections were made. The sections were processed in alcohol-xylene series and stained with haematoxylin and eosin. The slides were studied under a light microscope for any histological damage/protection.

**Statistical analysis**

The data obtained were analyzed by One way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test using computerized program. P-value <0.05 or was taken as the criterion of significance.

**RESULTS**

The acute oral toxicity study of Melia azedarach showed no mortality up to 610 mg/kg. The phytochemical screening of Melia azedarach shows the presence of Alkaloids, Carbohydrates, Steroids, Tannins, Flavonoids and Glycosides (Table 1). The effect of ethanol extract of Melia azedarach an serum transaminases, alkaline phosphates, bilirubin and total protein level in CCl<sub>4</sub> intoxicated rats are summarized in Table 2. There was a significant increase in bilirubin level, SGOT, SGPT and ALP, in CCl<sub>4</sub>-intoxicated group compared to the normal control group (Graph-1, 2, 3, and 4). The total protein levels were significantly decreased to 5.4 g/dl in CCl<sub>4</sub> intoxicated rats from the level of 7.1 g/dl in normal group (Graph 5). On the other hand the groups with received both Melia azedarach leaf extract (500mg/kg, p.o) and CCl<sub>4</sub> (Group III) and CCl<sub>4</sub> + Silymarin (25mg/kg, p.o) (GROUP IV) showed significantly decreased the elevated serum marker enzymes when given orally and reversed the altered total protein to almost normal level (Table 2). Group III and Group IV treated also reduced the level of bilirubin in 1.08 and 1.05 mg/dl respectively from the level of 3.29 mg/dl in the untreated group.

**Table 1. Preliminary Phytochemical Screening.**

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Constituents</th>
<th>Ethanol Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Terpenes</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Reducing Sugar</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Antraquinone</td>
<td>+</td>
</tr>
</tbody>
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**Table 2. Effect of Melia azedarach on some serum chemical parameters of paracetamol intoxicated rats.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
<th>Bilirubin (mg/dl)</th>
<th>Total protein (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>38.2±1.50</td>
<td>59.5±1.21</td>
<td>155±1.50</td>
<td>0.17±0.42</td>
<td>7.1±0.30</td>
</tr>
<tr>
<td>CCl&lt;sub&gt;4&lt;/sub&gt;</td>
<td>198±16.77</td>
<td>102±10.5</td>
<td>253±11.5</td>
<td>3.29±0.33</td>
<td>5.4±0.37</td>
</tr>
<tr>
<td>CCl&lt;sub&gt;4&lt;/sub&gt;+Vinca</td>
<td>109±2.66</td>
<td>67±2.21</td>
<td>186±1.21</td>
<td>1.08±1.2</td>
<td>6.98±0.21</td>
</tr>
<tr>
<td>CCl&lt;sub&gt;4&lt;/sub&gt; +Silymarin</td>
<td>95±2.25</td>
<td>57±1.11</td>
<td>163±2.21</td>
<td>1.05±0.27</td>
<td>7.0±0.37</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. number of rats = 6.

**GRAPH I: BLOOD SERUM SGPT (U/L)**

**GRAPH II: BLOOD SERUM ALP (U/L)**

**GRAPH III: BLOOD SERUM BILIRUBIN (mg/dl)**

**GRAPH IV: BLOOD SERUM TOTAL PROTEIN (mg/dl)**

**Fig 1: Section of liver of control group**

**Fig 2: Section of the liver of CCl₄ treated group**

**Fig 3: Section of liver of CCl₄ and *Melia azedarach* treated group**

**Fig 4: Section of liver of CCl₄ and silymarin group**
DISCUSSION
The liver can be injured by many chemicals and drugs. In the present study, CCl₄ was selected as a hepatotoxicant to induce liver damage, since it is clinically relevant. CCl₄ produces a constellation of dose related deleterious effects in the liver. During hepatic damage, cellular enzyme like SGOT, SGPT, ALP and serum bilirubin present in the liver cell, leak into the serum resulting to increase in concentration. Histological changes such as steatosis (fatty changes in hepatocytes) and perivenular fibrosis were observed in CCl₄ control group. Ethanolic extract of Melia azedarach leaf extract (500mg/kg, p.o) prevented these histological changes, further indicating their hepatoprotective activity. All the histological changes observed were in correlation with the biochemical and functional parameters of the liver.

CONCLUSION
In conclusion, the results of present study demonstrate that Melia azedarach leaf extract (500mg/kg, p.o) has potent hepatoprotective activity against CCl₄ induced liver damage in rats. The results also imply that the hepatoprotective effects of Melia azedarach may be due to its antioxidant property. Further investigation is in progress to determine the exact phytoconstituents responsible for hepatoprotective effect.

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