ORIGINAL ARTICLE

Hepatoprotective potential of *Saraca ashoka* (Roxb.) De Wilde bark by carbon tetrachloride induced liver damage in rats

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**KEYWORDS**
- Hepatotoxicity;
- *Saraca ashoka*;
- Carbon tetrachloride;
- Hepatocytes

**Abstract**  
*Objective:* *Saraca ashoka* [SA] (Family: Caesalpiniaceae) is widely used in skin infection, CNS function, uterus pain during painful periods, in bacterial infection and for hepatoprotective activity. The present study was carried out to investigate possible hepatoprotective activity of methanolic and hydroalcoholic extract of *Saraca ashoka* stem bark.

**Methods:** Hepatoprotective activity of hydroalcoholic and methanolic extract of stem bark of *S. ashoka* (HAESA and MESA) was demonstrated by using *CCL* 4 induced hepatotoxicity model. Normal group was given only vehicle and *CCL* 4 was given at 0.5 mL/kg, s.c. as toxic dose to induce hepatotoxicity. Liv-52 was given as standard drug (1 mL/kg/day, p.o.). Two doses of MESA and HAESA (200 and 400 mg/kg/day, p.o.) were tested for hepatoprotective activity for nine days.

**Results:** Administration of hepatoxin *CCL* 4 showed significant biochemical and histological deterioration in the liver of experimental animals. Pretreatment with methanolic extract more significantly and to a lesser extent hydroalcoholic extract reduced the elevated levels of serum enzymes like serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT) and alkaline phosphatase (ALP) and reversed the hepatic damage which evidenced the hepatoprotective activity of stem bark of *S. ashoka*.

**Conclusion:** The results suggest that MESA and HAESA extracts at doses of 200 and 400 mg/kg, p.o. have a significant effect on the liver of *CCL* 4 induced hepatotoxicity animal model.

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1. Introduction

The liver plays a pivotal role in the regulation of vital physiological functions such as metabolism, secretion of bile, storage of vitamins and detoxification of drugs.  

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among more serious ailments affecting a large population of world and can be classified as acute or chronic hepatitis and cirrhosis. Hepatotoxicity implies chemical-driven liver damage and can occur due to overdose and sometimes even in therapeutic ranges, may injure the organ. Other chemical agents, such as those used in laboratories and industries can also induce hepatotoxicity. Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures.

In spite of tremendous strides in modern medicine, there are very less drugs that stimulate liver function, offer protection to the liver from damage or help regeneration of hepatocytes. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. Traditional medical systems such as Ayurveda, Siddha and Unani are predominantly based on the use of plant materials. Many formulations containing herbal extracts are sold in the Indian market for liver disorders. One such formulation is Liv-52 which is very commonly used as hepatoprotective.

Saraca ashoka is an evergreen plant belonging to family caesalpinaceae and found throughout India, especially in Himalayas, Kerala, Bengal and whole South region. The plant exhibit hepatoprotective, antibacterial and anti-cancer activities as well as is used to treat skin infection, CNS function, uterus pain during painful periods, clots and amenorrhea. Phytochemical study of the bark extract showed the presence of phytosterols, carbohydrates, tannins and glycosides. Glycosides (polyethanoid) are known to possess anti-inflammatory, hepatoprotective and neuroprotective activities. In some of the plants of family caesalpiniaceae biological activities such as anti-leukemic, anti-inflammatory and hepatoprotective have been reported. Some of the important plants under this family are Cae salpinia bonducella, Delonix regia, and Bauhinia racemosa. Literature reviews indicated that the hepatoprotective activity of the bark of S. ashoka has not been scientifically evaluated so far. In view of this, the present study was aimed at evaluating the hepatoprotective activity of the bark of S. ashoka plant against CCl4 induced hepatotoxicity in albino rats.

2. Materials and methods

2.1. Chemical reagent

* Methanol and petroleum ether were purchased from SD Fine Chemical Ltd. Carbon tetra chloride was obtained from Himedia Laboratories Pvt. Ltd. and all biochemical estimation kits were purchased from Erba diagnostic kits, Transasia biomedicals Ltd.

2.2. Plant material

The bark of S. ashoka was collected from Haryana Agriculture University, Hisar district, Haryana and authenticated by Dr. H.B Singh, Head, Raw Material Herbarium & Museum, (RHMD), National Institute of Science Communication and Information Resources, New Delhi, (India). Voucher specimen Ref. No. NISCAIR/RHMD/CONSULT-2010-11/1639/237 has been preserved there for future references.

2.3. Preparation of plant extracts

The bark was washed under running tap water so as to remove any type of contamination. Then washed plant material was air dried under shade, powdered in grinder and passed through the sieve of mesh size No. 40. The bark powder was first defatted by petroleum ether and then successive extraction was done with methanol and hydroalcohol by the hot Soxhlet extraction method. The extracts were filtered and concentrated by rotary evaporator under reduced pressure and preserved in airtight container at 4–8 °C for further investigation.

2.4. Preliminary phytochemical screening

Qualitative phytochemical investigation of MESA and HAESA was carried out using standard procedure given by Kokate and Harbone.

2.5. Experimental animals

Wistar albino rats (100–150 g) were purchased from the animal house of Chaudhary Charan Singh Haryana Agriculture University, Hisar, Haryana (India). Animals were housed in animal house, Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra, Haryana (India) and were fed with available rat feed. The standard condition of temperature (25 °C ± 5 °C) humidity (55 ± 10%) and light/dark cycle (12:12) was maintained properly. The study protocol was approved by Institutional Animal Ethics Committee (IAEC) (Register Number: 536/02/a/CPCSEA) and was conducted according to the CPCSEA (Committee for the Purpose of Control and Supervision of Experimental on Animals) guidelines.

2.6. Selection of doses and administration

Doses of 200 and 400 mg/kg were selected based on the literature survey of previous research.

2.7. Hepatoprotective activity

Hepatoprotective effect of MESA and HAESA extracts of bark of S. ashoka was demonstrated by using the CCl4 induced hepatotoxicity model and phenobarbitone induced sleeping time. CCl4 activates by Cytochrome P-450 into a free radical (CCl3) which causes peroxidative degradation in the adipose tissue resulting in fatty infiltration of the hepatocytes. Liver toxicity also affects the metabolism of barbiturates thus enhancing the sleeping time due to delay in excretion of phenobarbitone. So, the present study was aimed to investigate the hepatoprotective activity of the bark of S. ashoka against CCl4 induced hepatotoxicity in rats.

2.7.1. Carbon tetrachloride induced hepatotoxicity model

The effects of MESA and HAESA extracts were studied in CCl4 induced hepatotoxicity. Wistar albino rats of either sex weighing from 100 to 150 g were used in this method. Animals were divided into seven groups containing six animals each. All the groups were treated orally for 9 days. Rats in group I (normal control) received only distilled water (1 mL/kg, p.o.);
group II (toxic group) received distilled water and CCl₄ (0.5 mL/kg, s.c.) for 9 days. Group III received standard drug Liv-52 (1 mL/kg/day, p.o.) with CCl₄ (0.5 mL/kg, s.c.) for 9 days. Group IV and V received MESA extract (200 and 400 mg/kg/day, p.o.) and CCl₄ (0.5 mL/kg, s.c.) for 9 days; group VI and VII received HAESA extract (200 and 400 mg/kg/day, p.o.) and CCl₄ (0.5 mL/kg, s.c.) for 9 days. On the 10th day, all the animals were sacrificed under anesthesia and blood as well as liver samples were collected for biochemical and histopathological investigation.

### 2.7.2. Phenobarbitone induced sleeping time

Liver toxicity affects the metabolism of barbiturates thus enhancing the sleeping time due to delay in excretion of phenobarbitone. In this model, phenobarbitone sodium (40 mg/kg) was administered after 48 h of last treatment of both the extracts. The time interval between onset of sleep and regain of righting reflex was measured.

### 2.7.3. Biochemical investigation

Blood samples were taken by retro orbital plexus and allowed to clot at room temperature for 45 min. Centrifugation was done at 1200–1500 rpm for 20 min for separation of serum. The serum was used for the estimation of biochemical parameters namely serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT) and alkaline phosphatase (ALP) by using autoanalyzer.

### 2.7.4. Histopathology

After draining the blood, liver samples were excised and washed with normal saline and processed separately for histological observations. Initially the samples were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h. Liver tissues were dehydrated with alcohol and their paraffin section were cut in 5 mm thickness, and were stained with alum hematoxylin and eosin. The sections were examined microscopically for histopathological parameters at Anupam Memorial Garg Diagnostic Center, Sirsa.

### 2.8. Statistical analysis

Statistical analysis was carried out by a one-way analysis of variance (ANOVA) followed by Dunnett’s test. Results were expressed as mean ± S.E.M for six rats in each group. *p* value < 0.05 was considered significant.

# 3. Results

### 3.1. Preliminary phytochemical screening

Preliminary screening revealed the presence of carbohydrates, steroids, tannin, saponin, flavonoids, protein and amino acids in methanolic and hydroalcoholic extracts (Table 1).

### 3.2. Pharmacological evaluation

#### 3.2.1. CCl₄ induced hepatotoxicity

In CCl₄ treated rats, the levels of SGOT, SGPT and ALP increased as compared to the vehicle control group. Pretreatment with Liv-52 reduced SGOT significantly (*p* < 0.01) as compared with diseased control. Both MESA and HAESA extracts also decreased the SGOT significantly (*p* < 0.01) at both the doses but effect of MESA at dose 400 mg/kg was superior to dose 200 mg/kg and comparable to the standard group. MESA and HAESA extracts at the dose of 400 mg/kg showed significant changes (*p* < 0.01) and reduced the level of SGPT and ALP (Table 2).

#### 3.2.2. Phenobarbitone induced sleeping time

Due to hepatic injury, barbiturate metabolism is delayed and excretion is slow. The CCl₄ control group has prolonged sleeping time when compared to the vehicle control group. MESA extracts reduced the sleeping time (*p* < 0.05) at the dose of 400 mg/kg significantly as compared to CCl₄ induced hepatotoxic group (Table 3).

### 3.3. Histopathological studies

Vehicle control group showed normal central vein and hepatocytes. CCl₄ toxic group showed degeneration of hepatocytes with fatty changes (Fig. 1). Liv-52 treated group showed normal central vein and mild changes in hepatocytes. MESA and HAESA (200 mg/kg) treated animals showed normal hepatocytes and less or mild fatty changes. MESA and HAESA (400 mg/kg) showed dilated vein and mild degenerate hepatocytes.

### Table 1  Chemical constituents present in MESA and HAESA extracts of *S. ashoka*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phytoconstituents</th>
<th>Method</th>
<th>Methanolic extract</th>
<th>Hydroalcoholic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Dragendorff’s test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mayer’s test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hager’s test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrates</td>
<td>Molish’s test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Feinberg’s test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Steroids</td>
<td>Lieberman-Burchard reaction</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Tannins</td>
<td>Ferric chloride test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>Foam test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Proteins and amino acids</td>
<td>Millon’s test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ninhydrin test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Flavonoids</td>
<td>Shinoda test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lead acetate test</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ indicates positive; – indicates negative.
is a potent hepatotoxin.

Table 2  Effect of MESA and HAESA extracts of *S. ashoka* on serum SGOT, SGPT and ALP in CCl₄ induced liver toxicity.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>SGOT(IU/L)</th>
<th>SGPT(IU/L)</th>
<th>ALP(IU/L)</th>
<th>Liver weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Vehicle control</td>
<td>–</td>
<td>95.04 ± 3.3</td>
<td>37.41 ± 2.1</td>
<td>17.05 ± 0.8</td>
<td>2.98 ± 0.1</td>
</tr>
<tr>
<td>Group 2</td>
<td>CCl₄ control</td>
<td>–</td>
<td>189.43 ± 12.8</td>
<td>134.43 ± 8.2</td>
<td>100.53 ± 3.8</td>
<td>4.08 ± 0.1</td>
</tr>
<tr>
<td>Group 3</td>
<td>CCl₄ + Liv 52</td>
<td>–</td>
<td>109.43 ± 5.8**</td>
<td>47.13 ± 2.8**</td>
<td>35.04 ± 1.3**</td>
<td>3.09 ± 0.2**</td>
</tr>
<tr>
<td>Group 4</td>
<td>CCl₄ + MESA</td>
<td>200</td>
<td>129.43 ± 9.1</td>
<td>75.03 ± 3.3**</td>
<td>62.76 ± 5.3</td>
<td>3.02 ± 0.2**</td>
</tr>
<tr>
<td>Group 5</td>
<td>CCl₄ + MESA</td>
<td>400</td>
<td>103.53 ± 7.8**</td>
<td>43.46 ± 2.3**</td>
<td>39.66 ± 2.9</td>
<td>3.60 ± 0.0</td>
</tr>
<tr>
<td>Group 6</td>
<td>CCl₄ + HAESA</td>
<td>200</td>
<td>149.83 ± 5.9**</td>
<td>73.63 ± 6.8**</td>
<td>70.03 ± 5.8**</td>
<td>3.54 ± 0.1</td>
</tr>
<tr>
<td>Group 7</td>
<td>CCl₄ + HAESA</td>
<td>400</td>
<td>128.03 ± 8.3**</td>
<td>48.74 ± 4.8**</td>
<td>44.87 ± 3.4**</td>
<td>4.15 ± 0.0</td>
</tr>
</tbody>
</table>

Values was mean ± S.E.M, n = 6 in each group, all groups were compared with disease control, *(p < 0.05), **(p < 0.01)* (One way ANOVA followed by Dunnett’s Test).

Table 3  Effect of pretreatment of Liv-52, MESA and HAESA extracts of *S. ashoka* on phenobarbton induced sleeping time in CCl₄ induced liver injury.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Duration of phenobarbion induce sleep in min (% reduction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Vehicle Control</td>
<td>–</td>
<td>278.78 ± 8.0°</td>
</tr>
<tr>
<td>Group 2</td>
<td>CCl₄ Control</td>
<td>–</td>
<td>359.02 ± 7.0</td>
</tr>
<tr>
<td>Group 3</td>
<td>CCl₄ + Liv 52</td>
<td>200</td>
<td>291.28 ± 15.4</td>
</tr>
<tr>
<td>Group 4</td>
<td>CCl₄ + MESA</td>
<td>200</td>
<td>277.95 ± 14.8*</td>
</tr>
<tr>
<td>Group 5</td>
<td>CCl₄ + MESA</td>
<td>400</td>
<td>304.61 ± 18.6</td>
</tr>
<tr>
<td>Group 6</td>
<td>CCl₄ + HAESA</td>
<td>200</td>
<td>289.61 ± 17.6</td>
</tr>
<tr>
<td>Group 7</td>
<td>CCl₄ + HAESA</td>
<td>400</td>
<td>289.61 ± 17.6</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SEM (n = 6 rats/group). Percentage reduction in parameters compared to Liv-52 and difference between CCl₄ & Liv-52 treatment group *(p < 0.05) (One way ANOVA followed by Dunnett’s Test).

4. Discussion and conclusion

Liver diseases like hepatotoxicity remains as one of the serious health problems and very year approximately 18,000 deaths occur due to liver cirrhosis. It is considered to be one of the most prevalent causes of human sufferings and death. Unfortunately synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects.

Herbs are one of the most promising sources of new drugs as these are free of or having very less side effects and adverse reactions. Various plant extracts and polyherbal formulations play a specific therapeutically active role in the management of liver disorder. Liver disease is a polyherbal used traditionally in the treatment of various liver disorders. It is a polyherbal formulation consisting of powders of *Capparis spinosa*, *Cichorium intybus*, *Solanum nigrum*, *Cassia occidentalis*, *Terminalia arjuna*, *Achillea millefolium*, *Tamarix gallica*, and Mandura bhasma. The hepatoprotective activity of esculentin and p-methoxybenzoic acid, the main constituents of *Cichorium intybus* and *Capparis spinosa*, respectively, has been reported by previous researchers, in chemically-induced hepatotoxicity in experimental animals. The hepatoprotective effects of Mandura bhasma and *Cassia occidentalis*, and the other two components of Liv-52 were also investigated against chemically-induced liver damage in experimental animals. Keeping in view the above facts, Liv-52 was selected as standard. The hepatoprotective effects of both MESA and HAESA extracts of *S. ashoka* were studied in rats by using the CCl₄ induced hepatotoxicity model at the doses of 200 and 400 mg/kg.

CCl₄ is a potent hepatotoxin activated by Cytochrome P-450 into a free radical (CCl₃) which causes peroxidoive degradation in the adipose tissue resulting in fatty infiltration of the hepatocytes. CCl₃ radical further interacts with molecular oxygen to form trichloromethyl peroxo radical. Both CCl₃ and peroxo radical are capable of binding to protein and lipid which elicit lipid peroxidation leading to functional and structural disruption of hepatocytes. During hepatic damage, cellular enzymes like SGOT, SGPT and ALP will leak into the serum resulting in elevation of their serum concentrations. Hepatopathy of the damaged liver showed histological changes, such as fatty changes in hepatocytes and perivenular fibrosis. We observed that pretreatment with Liv-52 and bark extracts of *S. ashoka* showed a significant protective effect against CCl₄ induced liver damage and significantly reduced the elevated liver enzymes indicating the hepatoprotective action. Both the extracts of the bark of *A. ashoka* prevented the histological changes caused by CCl₄ which further confirmed its hepatoprotective activity against CCl₄-induced hepatic damage. The possible mechanism of action may be associated with scavenging of free radicals responsible for CCl₄ toxicity.

In this investigation, we observed that there is marked liver damage due to CCl₄ intoxication with elevation in various markers like SGOT, SGPT and ALP. Pretreatment with bark extracts for 9 days and Liv-52 has significantly reduced lipid peroxidation and elevated biochemical markers.

These results showed that the treatment with MESA and HAESA extracts of *S. ashoka* possessed significant protection against liver damage caused by CCl₄. Hepatoprotective activity was confirmed by the results of biochemical, and histological studies. The hepatoprotective activity of *S. ashoka* may be due to flavonoids which were present in both the methanolic and hydroalcoholic extracts. Flavonoids are known for their powerful free radical scavenging activity.
and hydroxyl ion present in flavonoids show antioxidant property which effectively helps detoxify the toxic compounds. Thus these results showed that the extracts possess hepatoprotective activity.

Thus the result showed that the MESA and HAESA extracts of S. ashoka have hepatoprotective activity against CCl₄ induced hepatotoxicity at both the doses of 200 and 400 mg/kg. The antioxidant property of phytoconstituents of the bark of S. ashoka may be the possible mechanism for its hepatoprotective activity.

5. Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgement

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References


