Hepatoprotective Activity of *Cardiospermum helicacabum* Stem Extracts Against Carbontetrachloride-induced Hepatotoxicity in Wistar Rats

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**ABSTRACT:** Natural remedies from medicinal plants are considered to be effective and safe alternative treatments for liver toxicity. Our aim was to demonstrate the hepatoprotective effect of various extracts of *Cardiospermum helicacabum* (sapindaceae) stem. The various extracts of stem in arachis oil were administered orally for 7 days and the hepatoprotective activity was studied in carbon tetrachloride induced hepatic damage model in male wistar rats. The hepatoprotective activity was assessed using various biochemical parameters like serum bilirubin, protein, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and alkaline phosphatase (ALP) along with histopathological studies of liver tissue. There was a significant increase in serum levels of bilirubin, SGOT, SGPT and ALP with a decrease in total protein level, in the CCl4 treated animals, reflecting liver injury. In the stem extracts treated animals there was a decrease in serum levels of the markers and significant increase in total protein, indicating the recovery of hepatic cells. These biochemical observations were supplemented by histopathological examination of liver section. The effects of extracts were compared with standard drug silymarin. The ethyl acetate stem extract (400mg/kg) of *Cardiospermum helicacabum* afforded significant protection against CCl4 induced hepatocellular injury compared to all extracts.

**KEYWORDS:** *Cardiospermum helicacabum*, hepatoprotective activity, carbon tetrachloride, acute toxicity

**Introduction**

Drug-induced liver injury is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies. Different types of drugs such as acetaminophen, chloroquine and isoniazid induce hepatotoxicity. These drugs account for approximately one-half of the cases of acute liver failure and mimic all forms of acute and chronic liver disease (Kaplowitz, 2001). According to the United States Acute Liver Failure Study Group, drug-induced liver injury accounts for more than 50% of acute liver failure, including hepatotoxicity caused by overdose of acetaminophen (39%) and idiosyncratic liver injury triggered by other drugs (13%) (Michael and Cynthia, 2005). Therefore, studies with plant extracts are useful to know their efficacy and mechanism of action and safety. Natural remedies from medicinal plants are considered to be effective and safe alternative treatments for hepatotoxicity. The plant *Cardiospermum halicacabum* (Family: Sapindaceae), is an annual or sometimes perennial climber. The plant is distributed in America, extending to Africa and Asia. It also occurs throughout the plains and in lower elevations (up to 1200m) of India, Bangladesh and Pakistan (Kirtikar and Basu, 1999; Parrota, 2000).

The whole plant of *Cardiospermum halicacabum* is used as diuretic, stomach ache, rheumatism, lumbago, nervous disease, piles, fever, chronic bronchitis, hydrocele, amenorrhoea, sprains, edema, and jaundice (Guha et al.,1999; Joshi, 2002; Puliaiah, 2002). Herb juice is used in ear ache and in cancer therapy. Leaves find its use in emesis, stomach ache, healing wounds and diuretic. Whole plant contains saponins, traces of alkaloids, flavonoids, proanthocya-nidines, apigenin and phytosterols and in the leaves larger amount of saponins, alkaloids (+) pinitol, apigenin luteolin and chrysoeriol were reported (Srinivas et al., 1998). Here, we report detailed studies on the hepatoprotective activity of various extracts from stem of *Cardiospermum halicacabum*, with a view to provide scientific evidence on modern lines.

**Materials and Methods**

**Plant collection and authentication**
The stem of *Cardiospermum halicacabum* was collected from the Kommala village in Warangal (A.P), India, and was identified by Dr. Raja S.Vastavaya, Taxonomist, Department of Botany, Kakatiya University, Warangal (A.P) India and authenticated by comparing with the voucher specimen. The collected plant material was thoroughly checked for any foreign matter and were separated and shade dried. The completely shade dried stems were powdered separately with laboratory mixer and passed through sieve and used for further studies.

**Preparation of the extracts**

The powder of *Cardiospermum halicacabum* was successively extracted using different solvents (petroleum ether, chloroform, ethyl acetate, methanol and water) by soxhlation method. Extraction process was performed for 8 hours. The extracts were filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in desiccators.

**Preliminary Phytochemical evaluation**

All the extracts were screened for the presence of various secondary metabolites like alkaloids, steroids, carbohydrates, flavonoids, amino acids and proteins using standard methods (Brunston, 1999; Harborne, 2005).

**Animals**

The Wistar rats and albino mice (for acute toxicity study) were obtained from Mahaveera enterprises, Hyderabad (A.P) India. All the animals were stored in standard cages and maintained at 27°C ± 2°C under 12hrs dark/light cycle. The animals were fed with standard rat feed and water was given *ad libitum*. Ethical clearance for handling of animals and the procedures used in the study was obtained from the institutional animal ethical committee prior to the study.

**Acute Toxicity Study**

Acute toxicity studies were conducted using albino mice of either sex weighing between 20 and 25g. These rats were divided into different groups comprising six animals each. The control group received suspension of acacia. The other groups received 200, 400, 800, 2000, 3000, 4000 mg/kg of the test extracts, respectively. The animals were fasted over night prior to the experimental procedure. The test extracts were given orally in the form of suspension in the arachis oil. Immediately after dosing, the animals were observed continuously for the first 4 hrs for any behavioural changes and there after, they were then kept under observation up to 48 hrs.

**Hepatoprotective Effect Against CCl4-induced Hepatotoxicity in Rats**

Animals were divided into thirteen groups of six rats each. Group I and II served as normal and intoxicated control, respectively and received only the vehicle ((1 ml/kg/ day of Arachis oil; p.o.). Group III animals were treated with standard silymarin at an oral dose of 100 mg/kg and group IV to X111 were treated with petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of stem of *Cardiospermum halicacabum* at doses of 200 mg/kg and 400 mg/kg as a fine suspension in arachis oil. The treatment was continued for 7 days, once daily. On the day 7 for groups II- X111, 30 min post-dose of extract administration animals received CCl4 at a dose of 1.5 ml/kg (1:1 of CCl4 in olive oil) orally (Tirupati et al., 2006). The animals were sacrificed after 36 h after administration of acute dose of CCl4. The blood was collected from carotid artery. The serum was separated by centrifugation at 3000 rpm for 30 minutes and then analyzed for total bilirubin (Jendrassik and Grof, 1938), Lactate dehydrogenase (LDH), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase, and total protein and albumin levels by span diagnostic kits (Comb and Bowers, 1972; Peters, 1968). The animals were then dissected and the livers were carefully removed and washed with 0.9% saline solution and preserved in 10% formalin solution for histopathological studies.

**Histopathological Studies**

The tissues of liver were fixed in 10% formalin and embedded in paraffin wax. Sections of 4-5 microns thickness were made using rotary microtome and stained with haematoxylin-eosin and histological observations were made under light microscope.

**Results**

The results of the preliminary phytochemical analyses of stem extracts of petroleum ether showed the presence of carbohydrates, steroids, terpenoids, trace amount of alkaloid and glycosides. Chloroform extract gave positive tests for carbohydrates, steroids, terpenoids, alkaloids, glycosides, phenols and tannins. The ethyl acetate extract responded positively to all the tests for carbohydrates, steroids, terpenoids, phenolics, tannins, proteins, amino acids, glycosides, flavonoids and alkaloids. Methanolic extract of the stem powder produced positive tests for carbohydrates, amino acids, proteins, steroids - terpenoids, phenolics, tannins, alkaloids and glycosides. Aqueous extract of the stem showed the presence of carbohydrates, flavonoids, aminosacids, proteins, steroids - terpenoids, phenolics, tannins, glycosides and alkaloids.
In acute toxicity studies, none of the extracts studied showed any toxic symptoms or caused death of mice even after 48 hrs at all the doses of the extracts studied.

The results of carbon tetrachloride-induced hepatotoxicity are represented in Table 1. Carbon tetrachloride (CCl4) intoxication in normal rats elevated the levels of SGOT, SGPT, ALP, TBL, and, where as significant decrease in the levels of TPTN and ALB levels were observed indicating acute hepato cellular damage and biliary obstruction. All the extracts showed a significant decrease in all the elevated SGOT, SGPT, ALP, and TBL levels and significant increase in reduced TPTN and ALB levels as compared to silymarin. The rats which received ethyl acetate extract (400mg/kg/bw) showed significant changes in the levels of biochemical parameters compared to all other extracts. Histopathological examination of liver sections of control group showed normal cellular architecture with distinct hepatic cells, well preserved cytoplasm, sinusoidal spaces and central vein (Fig.1). Disarrangement of normal hepatic cells with necrosis and vacuolization are observed in CCl4 intoxicated liver (Fig.2). The liver sections of the rat treated with 400mg/kg of ethyl acetate extract followed by CCl4 intoxication (Fig.4), showed almost normal liver architecture, less vacuole formation and absence of necrosis and overall less visible changes observed as compared to that treated with silymarin (Fig. 3), supplementing the protective effect of the extract.

### Table 1  Effect of different stem extracts of *Cardiospermum halicacabum* on different serum biochemical parameters in CCl4 induced liver toxicity

<table>
<thead>
<tr>
<th>Name of the drug</th>
<th>Dose (mg/kg)</th>
<th>Total bilirubin (mg/dl)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
<th>SGOT (U/L)</th>
<th>LDH (U/L)</th>
<th>Total protein (gm/dl)</th>
<th>Albumin (gm/dl)</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>0.65±0.02</td>
<td>58.0±6.8</td>
<td>174±5.0</td>
<td>32.0±2.1</td>
<td>3140±5.2</td>
<td>7.8±0.2</td>
<td>3.8±0.1</td>
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<tr>
<td>CCl4</td>
<td>-</td>
<td>0.90±0.07</td>
<td>402±52</td>
<td>368±2.8</td>
<td>214±1.5</td>
<td>7480±16</td>
<td>5.2±0.1</td>
<td>2.4±0.4</td>
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<tr>
<td>Standard</td>
<td>100</td>
<td>0.70±0.02</td>
<td>94.0±2.5</td>
<td>286±4.0</td>
<td>156±3.5</td>
<td>5140±59</td>
<td>6.5±0.2</td>
<td>3.2±0.1</td>
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<tr>
<td>PEE</td>
<td>200</td>
<td>0.74±0.08</td>
<td>198±5.1</td>
<td>350±2.0</td>
<td>203±3.6</td>
<td>7120±8.1</td>
<td>5.8±0.2</td>
<td>2.6±0.3</td>
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<tr>
<td>PEE</td>
<td>400</td>
<td>0.72±0.08</td>
<td>158±3.7</td>
<td>284±2.6</td>
<td>182±4.0</td>
<td>6290±3.6</td>
<td>5.9±0.2</td>
<td>2.8±0.3</td>
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<tr>
<td>CE</td>
<td>200</td>
<td>0.75±0.03</td>
<td>361±4.5</td>
<td>362±5.6</td>
<td>150±3.5</td>
<td>7140±2.6</td>
<td>5.8±0.6</td>
<td>2.6±0.3</td>
</tr>
<tr>
<td>CE</td>
<td>400</td>
<td>0.69±0.02</td>
<td>292±10.2</td>
<td>332±5.6</td>
<td>92±4.1</td>
<td>6240±1.5</td>
<td>6.0±0.8</td>
<td>2.9±0.3</td>
</tr>
<tr>
<td>EAE</td>
<td>200</td>
<td>0.68±0.03</td>
<td>86.0±1.5</td>
<td>305±5.5</td>
<td>138±2.0</td>
<td>7260±7.6</td>
<td>6.0±0.8</td>
<td>2.5±0.6</td>
</tr>
<tr>
<td>EAE</td>
<td>400</td>
<td>0.65±0.07</td>
<td>68.0±3.0</td>
<td>1462±4.1</td>
<td>64±3.5</td>
<td>31802.2</td>
<td>7.12±0.2</td>
<td>3.22±0.15</td>
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<td>ME</td>
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<td>0.67±0.01</td>
<td>318±3.5</td>
<td>359±7.2</td>
<td>205±1.5</td>
<td>7210±2.6</td>
<td>5.5±0.2</td>
<td>2.9±0.26</td>
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<tr>
<td>ME</td>
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<td>0.66±0.01</td>
<td>124±5.0</td>
<td>356±3.6</td>
<td>182±1.5</td>
<td>5320±2.6</td>
<td>5.9±0.26</td>
<td>3.0±0.2</td>
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<tr>
<td>AE</td>
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<td>0.69±0.02</td>
<td>118±3.0</td>
<td>348±4.7</td>
<td>215±1.5</td>
<td>7380±5.5</td>
<td>5.8±0.2</td>
<td>2.6±0.3</td>
</tr>
<tr>
<td>AE</td>
<td>400</td>
<td>0.68±0.05</td>
<td>86.0±3.0</td>
<td>214±3.0</td>
<td>211±1.5</td>
<td>7340±4.0</td>
<td>6.1±0.2</td>
<td>2.8±0.26</td>
</tr>
</tbody>
</table>

n=six animals in each group; values are Mean ± SEM, when compare to control.

PEE – Petroleum ether extract, CE – Chloroform extract, EAE – Ethyl acetate extract, ME – Methanol extract, AE – Aqueous extract.
Fig. 1 Section of the liver tissue of normal control group showed a normal cellular architecture and central vein of the liver.

Fig. 2 Section of the liver tissue of animals treated with carbon tetrachloride showing necrosis and fatty changes.
Fig. 3 Section of the liver tissue of Silymarin treated animals showing normal hepatocytes and architecture.

Fig. 4 Section of the liver tissue of ethyl acetate (400mg/kg) stem extract treated animals showing almost normal liver architecture, less vacuole formation and absence of necrosis.
Discussion

The present studies were performed to assess the hepatoprotective activity of stem extracts of 
*Cardiospermum halicacabum* in Wistar rats against carbon tetrachloride as hepatotoxin to prove its claims in folklore practice for liver disorders. The extent of hepatic damage was assessed by histological examination and the level of various biochemical parameters in circulation. Highly reactive trichlor methyl free radical formation, which attacks polyunsaturated fatty acids of the endoplasmic reticulum, is responsible for the hepatotoxicity of CCl4. It produces hepatotoxicity by altering liver microsomal membranes in experimental animals (Pandit et al., 2004; Rajesh and Latha, 2001). From the study it was evident that ethyl acetate extract was able to reduce all the elevated biochemical parameters caused by the hepatotoxin intoxication. The levels of total proteins and albumin were reduced due to the CCl4 induced hepatotoxicity. The reduction is attributed to the initial damage produced and localized in the endoplasmic reticulum which results in the loss of P450 leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides in protein synthesis and accumulation of triglycerides leading to fatty liver (Rackengel and Glender, 1973). Reduction in the levels of SGOT and SGPT towards the endoplasmic reticulum leading to protein synthesis. The protein and albumin levels were also raised suggesting the stabilization of endoplasmic reticulum leading to protein synthesis. The protective effect exhibited by ethyl acetate extract at a dose of 400mg/kg was comparable with the standard drug silymarin. The histological examination of the liver sections reveals that the normal cellular architecture was retained as compared to silymarin, there by confirming the protective effect of the extract. Based on the results, among the tested extracts ethyl acetate extract of *Cardiospermum halicacabum* at a dose of 400 mg/kg body weight possess significant protective effect against hepatotoxicity induced by carbon tetrachloride which may be attributed to the individual or combined action of phytoconstituents present in it. Further investigations are needed for the identification of the active compounds responsible for hepatoprotective action.

References


