ABSTRACT
Hepatoprotective activity of the chloroform extract of the shade dried aerial parts of Solanum trilobatum Linn. was studied in wister rats using the CCl₄ induced hepatotoxicity. The activity was evaluated by using biochemical parameters such as serum aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin and gamma glutamate transpeptidase (GGTP). The extract exhibited significant hepatoprotective activity at 300 mg/kg, p.o. body weight, which was comparable to the control and activity exhibited by the reference standard Silymarin in carbon tetrachloride induced hepatotoxicity model.

Keywords: Solanum trilobatum, chloroform extract, hepatoprotective, CCl₄ induced.

INTRODUCTION
Solanum trilobatum Linn. (Solanaceae) is a small plant widely distributed throughout India. Herbal drugs are being proved as effective as synthetic drugs with lesser side effects and they are in line with nature, with no hazardous reactions. The roots and leaves are bitter and prescribed in consumptive cases in the form of electuary, decoction and powder. A decoction of the entire plant 1 in 10 was administered to cases of acute and chronic bronchitis and found to be useful in those diseases. This is considered to be a home remedy for all kinds of cough (1). It’s anti-asthmatic (2) and anti-cancer (3) activities are already proved. A review of literature afforded no information on the hepatoprotective aspects of this plant. So the present study is therefore an attempt to assess the efficacy of this indigenous herb for its hepatoprotective activity against CCl₄ induced toxicity model (4) in rats.

MATERIALS AND METHODS
Plant material
The aerial parts of the plant were collected from the foothill of Yercaud, Salem, in the month of December 2010 and cleaned to remove the debris. The collected plant was identified and authenticated by a botanist Dr. A. Marimuthu, Department of Botany, Government Arts College, Attur. A voucher specimen (STM-1) has been kept in our museum for future reference. The plant parts were dried at room temperature for 10 d and coarsely powdered with the help of a hand-grinding mill and the powder was passed through sieve No. 60.

Preparation of the extract
The powder of aerial parts of S. trilobatum was extracted separately by continuous hot extraction process using soxhlet apparatus with different solvents in increasing order of polarity from petroleum ether, chloroform, acetone, alcohol, to finally chloroform:water (5). After extraction, the extracts were concentrated under reduced pressure in
tared vessel. The marc of crude drug powder was then once again subjected to successive extraction with other solvents and the extractive values were calculated with reference to the air-dried drug. The dry extracts were subjected to various chemical tests to detect the presence of different phytoconstituents.

Test animals
Wister rats of either sex and of approximately the same age, weighing about 150-175 g were used for the study. They were housed in polypropylene cages and fed with standard chow diet and water ad libitum. The animals were exposed to alternate cycle of 12 h of darkness and light each. Before each test, the animals were fasted for atleast 12 h. Male mice weighing about 20-25 g each were used for acute toxicity studies. The experimental protocols were subjected to the scrutinization of the Institutional Animal Ethics Committee and were cleared by the same.

Acute toxicity studies
The animals were divided into control and test groups containing six animals each. The control group received the vehicle (1 % acacia) while the test groups got graded doses of different extracts orally and were observed for mortality till 48 h and the LD₅₀ was calculated.

Hepatoprotective study
For determining the hepatoprotective activity animals were divided into four groups containing 6 animals each. Group I served as normal control and received 1 ml/kg of propylene glycol daily for 7 consecutive days. Group II was served as positive control and received CCl₄ followed by 1 ml/kg of propylene glycol. Group III and IV were treated with chloroform extract of S. trilobatum (300 mg/kg, p.o.) and reference compound Silymarin (200 mg/kg, p.o.), respectively for 7 consecutive days.

On the seventh day 2 ml/kg, p.o. of CCl₄ (7) was administered 30 min of the last dose to all the rats except in group I. After 36 h, blood samples were withdrawn from all groups by cardiac puncture of nonanaesthetized rats. The biochemical parameters such as ALT (8), AST (9), ALP (10), total bilirubin (11), total protein (12, 13) and GGTP (14) were estimated as reported earlier. A small portion of liver was cut from the animals from each group and preserved in neutral buffered formalin and was processed for paraffin embedding, following the standard microtechnique (15). 5 μ section of the livers stained with alun haemotoxylin and eisin and studied for degenerative and necrotic changes. Statistical analysis (16) was performed using student’s t-test. The values are represented as mean±SEM. Level of significance was set at P<0.001.

RESULTS
The plant S. trilobatum was collected from the foothill of Yercaud, Salem, air-dried and extracted by continuous hot extraction process using soxhlet apparatus. The average percentage yield of chloroform extract of S. trilobatum was found to be 3.2 % w/w. The LD₅₀ was found to be 2893 mg/kg for chloroform extract of S. trilobatum. The chloroform extract did not exhibit and toxic effects up to 1000 mg/kg when administered to mice as a single i.p. dose. The results of biochemical parameters revealed to the elevation of enzyme level in CCl₄ treated group indicating that CCl₄ induces damage to the liver. Liver tissue rich in both transaminase increased in patients with acute and hepatic diseases. AST, which is slightly elevated by cardiac necrosis is a more specific indicator of liver disease (17). A significant reduction was observed in AST, ALT, ALP, GGTP, total bilirubin and total protein levels in the animals treated with chloroform extract of S. trilobatum. The enzyme levels were almost restored to the normal. So the animals treated with chloroform extract of S. trilobatum exhibited statistically significant (P<0.001) protection against CCl₄ induced hepatotoxicity in rats, which is comparable to the reference compound Silymarin. The histopathological studies support the biochemical findings. Hepatotoxicity induced by CCl₄ manifested itself by the 8th d with the liver showing massive degeneration enveloping the not so visible necrotic areas as compared to the normal. The liver sections of rats treated with the ethanolic extract were similar to liver sections of group IV and showed micro vesicular changes with mild congestion and widening of the sinusoids. There was no evidence of necrosis.

DISCUSSION
Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver disease. The hepatotoxic effects of CCl₄ are largely due to its active metabolite, trichloromethyl radical (17, 18). These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides. This lipid peroxidative degradation of biomembranes is one of the principal causes of hepatotoxicity of CCl₄ (19). This is evidenced by an elevation in the serum marker enzymes namely AST, ALT, ALP, GGTP, total bilirubin and total protein. Estimation of serum
transaminase levels gives a fairly good idea about the functional study of liver. The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or maintaining the normal hepatic physiology, which has been disturbed by a hepatotoxin. The extracts decreased CCl₄ induced elevated levels of the enzymes in groups III and IV, indicates the production of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells by the extracts. Histopathological examination of the liver section of the rats treated with toxican showed intense centrilobular necrosis and vasculisation. The rats treated with extracts alone with toxicant showed sign of protection against these toxicants to considerable extent as evident from formation of normal hepatic cards and absence of necrosis and vasculoses. Decrease in serum bilirubin after treatment with extract in liver damage indicated the effectiveness of the extracts in normal functional status of the liver. So, the result of present investigation indicates that the chloroform extract of *S. trilobatum* possess good hepatoprotective activity. Further investigation are required to characterize the active hepatoprotective principle and its mechanism of action.

### Table 1: Effect of chloroform extract of *S. trilobatum* on CCl₄ induced hepatotoxicity in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg, p.o.</th>
<th>AST U/L</th>
<th>ALT U/L</th>
<th>ALP U/L</th>
<th>GGTP U/L</th>
<th>Total Protein mg/dl</th>
<th>Total bilirubin mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1 ml</td>
<td>112.17±4.49</td>
<td>40.17±1.88</td>
<td>174.33±6.53</td>
<td>112.67±4.41</td>
<td>5.93±0.06</td>
<td>0.47±0.003</td>
</tr>
<tr>
<td>Control (CCl₄)</td>
<td>1.25 ml/kg</td>
<td>185.50±4.98</td>
<td>110.67±3.27</td>
<td>268.67±5.22</td>
<td>240.50±3.51</td>
<td>2.77±0.10</td>
<td>0.98±0.02</td>
</tr>
<tr>
<td>Chloroform extract of <em>S. trilobatum</em></td>
<td>300</td>
<td>130.33±5.72*</td>
<td>51.50±2.37*</td>
<td>196.83±8.49*</td>
<td>132.50±7.11*</td>
<td>3.83±0.12*</td>
<td>0.78±0.002*</td>
</tr>
<tr>
<td>Silymarin</td>
<td>200</td>
<td>121.67±4.90*</td>
<td>45.33±1.79*</td>
<td>182.50±8.41*</td>
<td>124.33±5.23*</td>
<td>4.62±0.03*</td>
<td>0.67±0.003*</td>
</tr>
</tbody>
</table>

*P<0.001 when compared with control. Number of individuals used=6 in each group. Days of drug treatment=7. Values are expressed as mean±S.E.

### REFERENCES


