HEPATOPROTECTIVE EFFECT OF *Leucas cephalotes* Spreng ON CCL₄ INDUCED LIVER DAMAGED IN RATS

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Summary

*Leucas cephalotes* (Labiatae) is an annual herb growing widely in India. It is traditionally used in liver disorder. The aim of present work is to study the hepatoprotective effect of methanolic extract of whole plant of *Leucas cephalotes* against carbontetrachloride induced liver damage in wistar rats. Preliminary phytochemical screening was done and extract was subjected to toxicity study. The methanolic extract at an oral dose of 100 mg/kg and 200 mg/kg produced significant (*P* < 0.05) protective effect as evidenced by lowering serum levels of SGOT, SGPT, ALKP, total bilirubin and total cholesterol in treated group as compared to positive control. These biochemical observations were supplemented by histopathological examination of liver sections. The activity may be due to the presence of flavonoid compound. Acute toxicity of extract show no sign of toxicity up to a dose level of 2000 mg/kg. Thus it could be concluded that *Leucas cephalotes* methanolic extract possesses significant hepatoprotective activity and prevent chemically (CCL₄) induced hepatic damaged in rats.

Key Words: *Leucas cephalotes*; Labiatae; Methanolic extract; Carbon tetrachloride

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Introduction

Liver is the key organ that regulates the homeostasis in the body. It is involved in almost all the biochemical pathway in body. Liver not only perform the physiological function but also it protect the body from various diseases and chemical toxins. Liver disease is a leading cause of death in many countries and the causative factors are alcohol consumption, malnutrition, anemia, hepatotoxic drugs and infections etc. More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market [1]. In spite of tremendous advances in modern medicine no effective drugs are available, which stimulate liver functions and offers protection to the liver from the damage or help to regenerate hepatic cells.

*Leucas Cephalotes* (Roth) Spreng. Syn. *Phlomis cephalotes* (Labiatae or Lamiaceae) rainy season weed mainly found in North India. It is commonly known as ‘Kubo or Kubi’ in traditional medicine of Gujarat. The genus *Leucas* includes about 100 Asiatic and African species. It is a valuable drug for snake bite. The plant is useful in bronchitis, inflammation, asthma, dyspepsia, paralysis and leucoma. The leaves are useful in fever and urinary discharge [2]. According to Ayurveda, the plant is mild stimulant, diaphoretic. Flowers mixed in honey are used as domestic remedy of cough and colds [3]. It is valuable homoeopathic drug and as such is used for the treatment of chronic malaria and asthma [4]. The plant was evaluated for *in vitro* antifilarial activity [5] and antidiabetic activity [6].

Bahadur and Sen, 1969 reported presence of Lauric acid, Tridecanoic acid, Adipic acid, Glutaric acid [7]. Labellenic acid (Octadeca-5,6-dienoic acid) has been reported in seed oil [8]. The plant was found to contain triterpenes, oleanolic acid, sterols and flavones [9]. The whole plant is used in the treatment of jaundice traditionally, but no systematic study has been carried out for this activity. The present work were carried out to investigate the effect of methanolic extract of *Leucas cephalotes* on liver function markers in serum and histopathology of rat liver showing CCl₄ induced liver damage.

Materials and Methods

Plant Material

The whole herbs of *Leucas cephalotes* were collected from Saurashtra University Campus, Rajkot, Gujarat, during August 2007 by uprooting. The plant was authenticated by Mr. N.J.Parmar, Botanical Survey of India, Jodhpur, Rajasthan, India. The voucher specimen (SU/DPS/HERB/18) was deposited at Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat, India.

Plant materials and extracts

The whole plant of *Leucas cephalotes* were washed thoroughly in tap water, shade dried and powdered and passed through 40 mesh sieve. The powder (200g) was defatted with petroleum ether (60-80°C) and then extracted with 1000 ml methanol using soxhlet apparatus till the extract is colorless for 12 hours. The *Leucas cephalotes* methanolic extract (LME) was filtered and concentrated under rotary vacuum evaporator. The percentage yield of the methanolic extract was found to be 15.89% (w/w). A preliminary phytochemical screening of LME was carried out by the methods described in Khandelwal [10]. Silymarin was used as a positive control at an oral dose of 200 mg/kg [11]. For animal study, extract and silymarin was suspended in vehicle i.e. 1% CMC, to required concentration.
Animals
Wistar albino rats of either sex, weighing 200–250 g maintained under standard husbandry conditions (temperature 23±2 °C and 12-h light:12-h dark cycle) were used for all experiments. Animals were allowed to take standard laboratory feed and tap water. The experiments were performed after the experimental protocol was approved by the institutional animal ethical committee as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Toxicity studies
The animals were kept fasted overnight providing only water, then after methanolic extract of plant was administered orally at the dose of 400 mg/kg body wt. and the rats were observed for signs of toxicity and mortality for 14 day. Such acute toxicity study was performed as per OECD guideline. If mortality was not observed then procedure was repeated for further higher dose. One-tenth of the maximum dose of the extract tested for acute toxicity was selected for evaluation of hepatoprotective activity [12].

Carbontetrachloride- induce liver damage in rats
Rats were divided into five groups of six each, control, hepatotoxin, positive control and two test groups. The control group received oral vehicle treatment at 0, 24 and 48 h. The animals in hepatotoxin-treated group received vehicle at 0 h and at 24 h vehicle followed by carbon tetrachloride diluted in liquid paraffin (1:1, i.p.) at a dose of 1.25 ml/kg, while at 48 h these animals received only vehicle. The standard (positive control) group has received the first dose of silymarin (200 mg/kg) at 0 h, at 24 h the second dose of silymarin followed by a dose of carbon tetrachloride and at 48 h the third dose of silymarin. The test groups have received the first dose of extracts at 0 h, second dose of extracts at 24 h, which was followed by a dose of carbon tetrachloride and at 48 h the third dose of extracts [13,14]. After 72 h blood was collected from all the groups by puncturing retro-orbital plexus, and allowed to clot for the separation of serum. Serum was separated by centrifugation at 2500rpm at 37°C for 15 min. The serum was used for estimation of biochemical parameters.

Histopathological study
One animal from each of the treated groups showing maximum activity as indicated by improved biochemical parameters was used for this purpose. The animals were sacrificed and the abdomen was cut open to remove the liver. The liver was fixed in 10% neural buffer formalin. After 12 hours liver was embedded in paraffin using conventional methods [21] and cut into 5µm thick sections and stained using haematoxylin–eosin dye and finally mounted in di-phenyl xylene. Then the sections were observed under microscope for histopathological changes in liver architecture and their photomicrographs were taken.

Statistical Analysis
The mean values ± S.E.M. are calculated for each parameter. For determining the significant inter-group difference each parameter was analyzed separately and one-way analysis of variance (ANOVA) [22] was carried out and the individual comparisons of the group mean values were done using Bonferroni’s test.
Results

LME was found to be non-toxic up to 2000 mg/kg and were considered as safe (OECD, 1996). From Table 1 it is evident that CCl₄ intoxication in normal rats elevated the levels of SGOT, SGPT, ALKP, TB and TC whereas decrease in the levels of TP and ALB was observed significantly indicating acute hepato cellular damage and biliary obstruction. Pretreatment of rats with LME (100 and 200 mg/kg) and silymarin (200 mg/kg) prior to carbon tetrachloride administration cause significant reduction in all the elevated SGOT, SGPT, ALKP, TB and TC levels and significant increase in ALB levels. However there was no significant change occurs in TP level. The degree of protection is maximal with higher dose of extract.

Table 1 Effect of *Leucas cephalotes* methanolic extract (LME) on CCl₄ induced toxicity in Rats

<table>
<thead>
<tr>
<th>Biochemical Parameter</th>
<th>Control</th>
<th>Hepatotoxin</th>
<th>Standard</th>
<th>LME 100</th>
<th>LME 200</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGOT (IU/I)</td>
<td>289.33 ± 9.22</td>
<td>389 ± 19.1a</td>
<td>309.56 ± 3.69b</td>
<td>334.67 ±4.73b</td>
<td>322.5 ± 7.85b</td>
</tr>
<tr>
<td>SGPT (IU/I)</td>
<td>51.5 ± 2.95</td>
<td>314.17 ± 11.71a</td>
<td>60.33 ± 2.10b</td>
<td>181.0 ± 7.19b</td>
<td>69.33 ± 3.72b</td>
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<tr>
<td>ALKP (IU/I)</td>
<td>16.92 ± 0.36</td>
<td>31.24 ± 3.10b</td>
<td>14.86 ± 0.38b</td>
<td>16.72 ± 2.47b</td>
<td>15.91 ± 2.97b</td>
</tr>
<tr>
<td>TB (mg/dl)</td>
<td>1.01 ± 0.04</td>
<td>2.156 ± 0.04a</td>
<td>0.69 ± 0.01b</td>
<td>0.93 ± 0.04b</td>
<td>0.70 ± 0.02b</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>50.41 ± 0.64</td>
<td>72.18 ± 4.99a</td>
<td>54.06 ± 3.70b</td>
<td>57.270 ± 3.54b</td>
<td>55.51 ± 2.07b</td>
</tr>
<tr>
<td>TP (mg/dl)</td>
<td>6.53 ± 0.07</td>
<td>6.25 ± 0.06</td>
<td>6.57 ± 0.05</td>
<td>6.37 ± 0.10</td>
<td>6.59 ± 0.09</td>
</tr>
<tr>
<td>ALB (g/dl)</td>
<td>4.34 ± 0.18</td>
<td>3.88 ± 0.04a</td>
<td>4.56 ± 0.03b</td>
<td>4.55 ± 0.04b</td>
<td>4.55 ± 0.04b</td>
</tr>
</tbody>
</table>

Value are mean ± SEM.; n=6

a significant reduction compared to hepatotoxin (P< 0.05)

b significant increase compared to hepatotoxin (P < 0.05)

Effect of LME on SGOT level on CCl₄ induced toxicity in Rats

Effect of LME on SGPT level on CCl₄ induced toxicity in Rats
Effect of LME on ALKP level on CCl₄ induced toxicity in Rats

Effect of LME on TB level on CCl₄ induced toxicity in Rats

Effect of LME on TC level on CCl₄ induced toxicity in Rats

Effect of LME on TP level on CCl₄ induced toxicity in Rats
The hepatoprotective activity of LME was confirmed by histopathological examination of liver tissue of control and treated animals. The liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and a central vein. The cells have well preserved cytoplasm, prominent nucleus and nucleolus [Fig.1] which is completely lost in rat treated with CCl₄ [Fig.2]. Vacuolization, fatty change and centrilobular necrosis were severe in CCl₄ treated group compared control groups. The liver of the rat treated with LME at a dose of 200 mg/kg [Fig.3], 100 mg/kg [Fig.4] or silymarin 200 mg/kg [Fig.5] showed significant recovery from CCl₄ induced liver damage evidence by normal hepatocyte with nuclei. Vacuolization and fatty degeneration were prevented by the treatment with extract and silymarin. The section of rat treated with LME 200 mg/kg shows the regeneration of hepatocytes.
Traditionally many herbs used to treat liver disorder but all herbs are not evaluated in a scientific manner. In the present study one such drug *Leucas cephalotes* taken for the study. The present investigation on the extracts showed the presence of phytosterol and flavanoids in the methanolic extract.

Hepatic cells appear to participate in a variety of enzymatic metabolic activities. The hepatotoxicity of CCl₄ has been reported to be due to the formation of the highly reactive trichloro free radical, which attacks polyunsaturated fatty acids. It produces hepatotoxicity by altering liver microsomal membranes in experimental animals [23]. The disturbance in the transport function of the hepatocyte as a result of hepatic injury causes the leakage of enzymes from cells due to altered permeability of membrane [24]. The effect of CCl₄ is generally observed after 24 h of its administration. Hence the withdrawal of the blood for biochemical parameters should be carried out only after 24 h of CCl₄ intoxication. Protection of hepatic damage caused by carbon tetrachloride administration was observed by recording serum level of SGOT, SGPT, ALKP, TC, TP and TB in different groups because such enzyme have been reported to be sensitive indicators of liver injury [25].
The result represents in Table-1 shows that pretreatment with LME at 100 and 200 mg/kg, p.o., was able to reduce all elevated biochemical parameter due to CCl4 intoxication. The levels of total proteins and albumin were reduced due to the hepatotoxin intoxication. The reduction is attributed to the damage produced and localized in the endoplasmic reticulum which results in the loss of $P_{450}$ leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides. Intoxication with CCl4 also resulted in inhibition of synthesis of the bile acids from cholesterol which is synthesized in liver or derived from plasma lipids, leading to increase in cholesterol levels. Suppression of cholesterol levels suggests the inhibition of the synthesis of bile acids from cholesterol is reversed by the extract. Reduction in the levels of SGOT and SGPT towards the normal value is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by CCl4. The reduction of ALKP levels with concurrent depletion of raised bilirubin level suggests the stability of the biliary function during injury with CCl4. The raise in protein and albumin levels in treated rat suggests the stabilization of endoplasmic reticulum leading to protein synthesis. The protective effect exhibited by the methanolic extract is similar to silymarin treatment.

Histological examination of the liver sections reveals that the normal liver architecture was disturbed by hepatotoxin intoxication. Treatment of rat with LME before CCl4 intoxication exhibit regenerative changes like normal appearance of hepatic cells with nucleus, less vacuolization and fatty change supplements the protective effect of the extract. However the results strongly suggest an initiation of the process of liver regeneration, which is also evident from the various biochemical parameter results.

Conclusions

In conclusion, the result of this study demonstrated that LME (200mg/kg) shows significant ($P<0.05$) hepatoprotective activity against CCl4 induced liver damage rats. It may also be hypothesized that flavanoids, which are present in the methanolic extract, could be considered responsible for the hepatoprotective activity. The present study also justified the traditional use of *Leucas cephalotes* in the treatment of liver diseases.

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References


