Hepatoprotective activity of LIV-first against carbon tetra chloride-induced hepatotoxicity in albino rats

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Liver toxicity is a major health problem of worldwide proportions. Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases. In the present study, LIV-first (16.3 mg/kg, p.o.) was used to screen the hepatoprotective activity. Hepatotoxicity was induced in experimental animals by administration of carbon tetrachloride (CCl$_4$) (1 ml/kg, i.p.). Silymarin (25 mg/kg, p.o.) was used as the standard. Biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and serum bilirubin were measured. Cytotoxicity of CCl$_4$ was estimated by quantitating the release of malondialdehyde. The activity of tissue antioxidant enzymes namely superoxide dismutase, catalase and the level of total protein and glutathione were also measured. Histopathological evaluation of liver sections was also done. CCl$_4$ administration in rats elevated the levels of SGPT, SGOT, ALP and bilirubin. Administration of LIV-first significantly ($P<0.01$) prevented this increase. The activity of anti-oxidant enzymes in carbon tetrachloride CCl$_4$-treated group was decreased and these enzyme levels were significantly ($P<0.05$) increased in LIV-first-treated groups. Histopathological studies revealed that the concurrent administration of CCl$_4$ with the extract exhibited protection of the liver tissue, which further evidenced the above results. The study confirmed the hepatoprotective activity of LIV-first, which may be attributed to its antioxidant property.

Key words: Hepatoprotective activity, LIV-first Albino rats, carbon tetrachloride, LIV-first, silymarin

INTRODUCTION

The liver performs many functions vital to the health of the organism. The liver transforms and excretes many drugs and toxins. These substances are frequently converted to inactive forms by reactions that occur in the hepatocytes.$^{[1-3]}$ Historically, plants have been used in the folk medicine to treat various diseases. Experimental work on several plants has been carried out to evaluate their efficacy against chemically induced liver toxicity.

Carbon tetrachloride (CCl$_4$) was the first toxin for which it was shown that the injury it produces is largely or entirely mediated by a free-radical mechanism. Its main toxic effects are shown on the liver. Toxic levels administered to animals produce fatty accumulation in the liver due to a blockage in the synthesis of the lipoproteins that carry triglycerides away from this organ. It is believed that CCl$_4$ is metabolized by the P$_{450}$ system to give the trichloromethyl radical, a carbon-centred radical. Several P$_{450}$ are involved including CYP2E1, the ‘ethanol-inducible’ cytochrome P$_{450}$. Hence, CCl$_4$-induced hepatotoxicity serves as an excellent model to study the molecular, cellular and morphological changes in the liver.$^{[2,4]}$

LIV-first is a polyherbal Ayurvedic proprietary medicine which is used as a hepatoprotective agent. Each capsule contains Eclipta alba (100 g), Andrographis paniculata (50 mg), Tinospora cordifolia (100 mg), Picrorhiza kurroa (75 mg), Boerhavia diffusa (50 mg) and Berberis aristata (50 mg). The present study was to investigate the hepatoprotective activity of LIV-first against CCl$_4$-induced hepatotoxicity in rats.

MATERIALS AND METHODS

Animals

Adult Wistar male albino rats weighing between 150 and 200 g were used for the study. They were kept under standard laboratory conditions and were fed with commercial rat pellets and drinking water ad libitum. The animals were housed in polypropylene cages. Ethical committee in accordance with animal experimentation and care has approved all animal procedures.

Drugs and Chemicals

LIV-first capsule
Nectur Herbal Research Laboratories, Tamil Nadu
Carbon tetrachloride
SD Fine Chemicals, Mumbai
Silymarin
Microlabs, Bangalore
Reduced glutathione
HiMedia Laboratories Pvt. Ltd, Mumbai
All drugs and chemicals were purchased commercially and were of analytical grade.

**Experimental Design**  
**Induction of experimental hepatotoxicity**  
Hepatotoxicity was induced by injecting CCl₄ intraperitoneally at a dose of 1 ml/kg body weight for 7 consecutive days.

**Evaluation of hepatoprotective activity**  
Animals were divided into five groups, consisting of six animals each. The rat dose was calculated on the basis of the surface area ratio.⁵

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>ALP (U/L)</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Control (Normal saline 10 ml/kg, p.o)</td>
<td>36.46</td>
<td>26.02</td>
<td>16.77</td>
<td>0.46</td>
</tr>
<tr>
<td>Group II</td>
<td>CCl₄ (1 ml/kg, i.p)</td>
<td>95.26</td>
<td>75.46</td>
<td>74.65</td>
<td>1.07</td>
</tr>
<tr>
<td>Group III</td>
<td>Silymarin (25 mg/kg, p.o)+CCl₄ (1 ml/kg, i.p)</td>
<td>58.26</td>
<td>48.46</td>
<td>27.39</td>
<td>0.83</td>
</tr>
<tr>
<td>Group IV</td>
<td>LIV-first (0.5ml/kg, p.o)+CCl₄ (1 ml/kg, i.p)</td>
<td>35.13</td>
<td>36.46</td>
<td>17.43</td>
<td>0.67</td>
</tr>
<tr>
<td>Group V</td>
<td>LIV-first (1ml/kg, p.o)+CCl₄ (1 ml/kg, i.p)</td>
<td>25.14</td>
<td>18.45</td>
<td>10.94</td>
<td>0.41</td>
</tr>
</tbody>
</table>

All the groups were treated for 7 consecutive days.⁶ At the end of this period, animals were kept overnight fasting and were sacrificed. Blood samples were withdrawn, serum separated and estimated for biochemical parameters. Liver tissues were removed for the determination of antioxidant enzyme levels and histopathological examinations.

**Measurement of Biochemical Parameters**  
Blood samples were collected from retro-orbital plexus under ether anaesthesia and the serum was used for the assay of marker enzymes namely SGPT, SGOT, ALP and bilirubin. The enzyme levels were assayed using standard kits obtained from Agappe Diagnostics Pvt. Ltd, Kerala.⁷⁻¹⁰

The liver homogenate was prepared and the clear supernatant was used for the estimation of lipid peroxidation (MDA),¹¹⁻¹³ total protein,¹⁴ reduced glutathione (GSH)¹⁴⁻¹⁵ and antioxidant enzymes viz. catalase (CAT)¹⁴⁻¹⁶ and superoxide dismutase (SOD)¹⁷⁻¹⁸ levels.

**Histopathological Examination**  
A portion of liver tissue from each group was preserved in a 10% formaldehyde solution for histopathological studies. Haematoxylin and eosin were used for staining and later the microscopic slides of the liver cells were photographed at a magnification of ×100.

**Statistical Analysis**  
Values were represented as mean±SEM. Data were analysed by one-way analysis of variance (ANOVA) followed by Dunnnett’s test using statistical package for social sciences (SPSS) version 10.0. P<0.05 was considered significant.¹⁹

**RESULTS**  
**Biochemical Parameters**  
The animals treated with CCl₄ exhibited a significant (P<0.01) rise in SGOT, SGPT, ALP and bilirubin levels when compared to the control group. This was significantly (P<0.01) reduced after treatment with LIV-first, which was almost similar to that of silymarin [Table 1].

**Lipid Peroxidation**  
The liver MDA, which is an index of tissue lipid peroxidation, was found to be significantly (P<0.01) higher in the CCl₄-treated group than measured in the control group. Treatment with LIV-first decreased the elevated MDA levels. The MDA level for silymarin was also found to be significantly decreased.

**Total Protein**  
Total protein level was significantly (P<0.01) reduced in the CCl₄-treated group when compared to the control and was significantly elevated in the LIV-first-treated groups. This was comparable to that of silymarin-treated group.

**Antioxidant Enzymes and Glutathione Levels**  
The levels of antioxidant enzymes such as CAT and SOD and GSH were decreased significantly (P<0.05) after CCl₄ treatment and was significantly (P<0.01) elevated in LIV-first-treated group. This was comparable with that of silymarin-treated group [Table 2].

**Histopathology**  
The histopathological examination showed that treatment with CCl₄ caused typical centrallobular hepatic steatosis (both macrovesicular and microvesicular) and necrosis, limiting plate necrosis, apoptosis, especially in the perportal hepatocytes and portal triaditis as compared with control liver. Liver tissues exposed to LIV-first and silymarin were almost similar to the control in histology, size and
staining properties and showed only mild congestion. In the formulation-treated group, there was reduction in inflammation and it significantly prevented the degeneration of hepatocytes. Thus, histological examination clearly demonstrated the protection of liver against CCl$_4$ cytotoxicity.

DISCUSSION

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine.

In the assessment of liver damage by CCl$_4$, the determination of enzyme levels was largely used. Serum SGPT, SGOT, ALP and bilirubin are the most sensitive markers employed in the diagnosis of hepatic damage because these are cytoplasmic in location and are released into the circulation after cellular damage. In this study, an increase in the activities of SGPT, SGOT, ALP and bilirubin in serum evidenced the CCl$_4$-induced hepatocellular damage. The reduction of CCl$_4$-induced elevated plasma activities of these enzyme levels in animals treated with the formulation showed their ability to restore the normal functional status of the damaged liver.

The determination of malondialdehyde (MDA) level is one of the most commonly used methods for monitoring lipid peroxidation. The result suggests that there was a dramatic increase in lipid peroxidation after CCl$_4$ treatment and it was inhibited by the treatment with the formulation revealing that it exhibits potent hepatoprotective activity.

Measurement of protein concentration was mainly used to calculate the level of purity of a specific protein. High doses of CCl$_4$ cause depletion of total proteins indicating tissue damage which was also evidenced in this study. Treatment with CCl$_4$ significantly depleted GSH, CAT and SOD stores indicating that they were used for the detoxification of toxic metabolites of the drug. The formulation restored the antioxidant enzyme levels significantly and reduced the CCl$_4$-induced oxidative injury, thus proving its antioxidant potential.

The histopathological examination of the liver of the control group showed normal hepatocytes with portal triad [Figure 1]. The liver section of CCl$_4$-treated rats showed typical centrilobular hepatocytic steatosis (both macrovesicular and microvesicular) and necrosis, limiting plate necrosis, apoptosis especially in the periportal hepatocytes and portal triaditis [Figure 2]. This could be due to the formation of highly reactive free radicals.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MDA (µmol/g tissue)</th>
<th>Protein (µg/ml)</th>
<th>GSH (µmoles of GSH/g wet tissue)</th>
<th>CAT (units/mg liver protein)</th>
<th>SOD (units/mg liver protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (10 ml/kg saline, p.o)</td>
<td>26.4±0.41</td>
<td>23.01±0.16</td>
<td>42.71±0.99</td>
<td>57.27±1.62</td>
<td>89.46±1.04</td>
</tr>
<tr>
<td>CCl$_4$ (1 ml/kg, p.o)</td>
<td>50.27±0.61</td>
<td>8.21±0.62</td>
<td>15.83±2.11</td>
<td>25.80±1.61</td>
<td>36.18±2.73</td>
</tr>
<tr>
<td>LIV-First (0.5 ml/kg, p.o)</td>
<td>35.29±0.50</td>
<td>14.09±0.57</td>
<td>20.83±0.71</td>
<td>36.91±1.07</td>
<td>71.72±1.24</td>
</tr>
<tr>
<td>LIV-First (1 ml/kg, p.o)</td>
<td>32.62±0.50</td>
<td>17.75±0.23</td>
<td>30.00±0.36</td>
<td>46.78±0.22</td>
<td>82.14±0.37</td>
</tr>
<tr>
<td>Silymarin (25 mg/kg, p.o)</td>
<td>30.41±0.22</td>
<td>19.7±0.18</td>
<td>33.85±1.52</td>
<td>46.28±0.51</td>
<td>78.92±4.70</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM; n=6 in each group; *P<0.01 and **P<0.05 vs control; *P<0.01 vs CCl$_4$
because of oxidative stress caused by \( \text{CCl}_4 \). Simultaneous administration of formulation along with \( \text{CCl}_4 \) prevented these effects [Figures 3 and 4]. Thus, histopathological studies revealed that concurrent administration of \( \text{CCl}_4 \) with the formulation exhibited protection of liver cells, which further confirmed the above results.

**CONCLUSION**

The results of this study clearly demonstrated that the formulation exhibited potent hepatoprotective activity against \( \text{CCl}_4 \)-induced hepatic damage in rats. This may be due to their antioxidant and free radical scavenging properties. Further studies are needed to isolate and purify the active principles involved in the individual plants of the formulation for confirming the hepatoprotective efficacy.

**REFERENCES**