Comparative hepatoprotective activity of Liv-52 and livomyn against carbon tetrachloride-induced hepatic injury in rats

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INTRODUCTION

Liver plays a major role in the detoxification and excretion of many endogenous and exogenous compounds; any type of injury (due to systemic drugs, food preservatives, agrochemicals and addiction to alcohol) or impairments of its functions may lead to many complications in one's health. There is a no rational therapy available for liver disorder, and it is a still challenge to modern medicine.[1] Hepatic injury can be life threatening when the entirely or most of the liver is exposed to any hepatotoxin, including CCl₄. Carbon tetrachloride is used to study hepatotoxic potential because it is life threatening when an entire liver or most of the liver is exposed to CCl₄; this requires metabolic activation, particularly by liver cytochrome P-450 enzyme, to form reactive toxic metabolites that in turn cause liver injury in experimental animals and humans.[2] Hepatotoxicity by CCl₄ is connected with severe impairment of the cell protection mechanism.

Liver disease is one of the serious health problems. Herbs play a major role in the management of various liver disorders. The present study was conducted to compare the hepatoprotective activity of two marketed formulations, Liv-52 and Livomyn. The formulations showed significant hepatoprotective effect by reducing elevated serum enzyme levels such as glutamate pyruvate transaminase (SGPT), glutamate oxaloacetate transaminase (SGOT), bilirubin content (direct and total) and total protein. These biochemical observations were supplemented by weight and histopathological examination of liver sections. Various pathological changes such as steatosis, centrilobular necrosis and vacuolization observed in rats treated only with CCl₄, but the groups treated with the CCl₄ and hepatoprotective formulations were produced to a moderate extent from such pathological changes. Silymarin was used for positive control. It was concluded from study that Liv-52 has shown more significant hepatoprotective activity against CCl₄-induced hepatotoxicity in rats in comparison to Livomyn.

Key words: Carbon tetrachloride, hepatoprotective activity, Liv-52, livomyn, silymarin

MATERIALS AND METHODS

Animals

Wistar strain male albino rats having a weight range of 150-180 g were used for the experiment. The animals were well housed in polypropylene cages under hygiene conditions and maintained at 28 ± 2°C temperature. The animals were allowed to have food and water ad libitum. The Institutional Animals Ethics Committee approved all the experimental protocols.

Chemicals and Drugs

Liv-52 syrup (The Himalaya Drug Company)) and Livomyn suspension (Charak Pharma Ltd) were used to evaluate the
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hepatoprotective activity, and carbon tetrachloride were used to induce hepatotoxicity. Silymarin was used as a standard (Micro Labs, Tamil Nadu, India). All the biochemicals and chemicals used were of analytical grade.

Experimental Design
Animals are divided into five groups (n = 6). Considering human dose (HD) of Liv-52 (HD-15 ml daily) and Livomyn (HD-12 ml daily), the rat dose was calculated on the basis of the surface area ratio (Ghosh MN, 2005).

Group I: Normal control vehicle i.e. distilled water, Group II: CCl4 (1 ml/kg, i.p.)
Group III: Silymarin (25 mg/kg, p.o.)
Group IV: Liv-52 (0.216 ml/kg, p.o)
Group V: Livomyn (0.216 ml/kg, p.o.)

Treatment duration was 10 days, and the dose of CCl4 was administered after every 72 h.[12] Animals were sacrificed after 24 hrs of the last injection of CCl4. Blood was collected, allowed to clot and the serum is separated. The liver was dissected out and used for biochemical and histopathological studies.

Biochemical Estimation
The blood was obtained from all the animals by puncturing the retro-orbital plexus. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm for 15 min at 30°C and utilized for the estimation of various biochemical parameters, namely, SGOT, SGPT, Total Protein (Beacon diagnostic kit, Navsari) and Bilirubin (Biolab diagnostic kit, Maharashatra).

Histopathological Examination
All animals were sacrificed on the last day of the study; blood was collected and liver was removed and washed with saline. Liver pieces were preserved in 10% formalin for histopathological study. The sections were approximately 4-6 micron in thickness. They were stained with hematoxylin and eosin and photographed.

Statistical Analysis
The data were expressed as mean ± S.D.; for obtaining this data, biochemical and physiological parameters were statistically analyzed using one-way ANOVA followed by Dunnet's test.[13] For comparison with the control group and CCl4-treated group, P < 0.001 was considered as significant.

RESULTS

The results of CCl4-induced hepatotoxicity are shown in Table 1. CCl4 intoxication in normal rats significantly elevated the serum levels of SGOT, SGPT, bilirubin (total and direct) and total proteins, whereas there was a significant decrease in level of total proteins that indicated acute hepatocellular damage and biliary obstruction. The rats treated with Liv-52 and Livomyn showed a significant reduction in all biochemical parameters, whereas increase in the level of total proteins was observed in comparison to that of the standard group (silymarin-treated).

In the histopathological examination of liver sections of control group [Fig. 1], the central vein was prominent with normal hepatocytes. In the CCl4-intoxicated group [Fig. 2], centriolobular necrosis was observed. In the histopathological profile of silymarin-treated groups [Fig. 3], there was no centriolobular necrosis and hepatocytes showing regenerative activity. In the groups treated with the formulations (Liv-52 [Fig. 4] and Livomyn) [Fig. 5], the section showed liver tissue hepatocytes with regenerative activity. These formulations were able to control this necrotic change that was comparable to that of Livomyn. Thus, the biochemical observations correlate well with the histopathology results of the liver samples. Thus, these observations confirmed the potent hepatoprotective activity of Liv-52 than that of Livomyn.

DISCUSSION

In this study, rats treated with a single dose of CCl4 developed significant hepatic damage and oxidative stress, which was observed from a substantial increase in the activities of serum, SGOT, SGPT, total protein and bilirubin. This is indicative of cellular leakage and loss of the functional integrity of the cell membrane in liver.[14-16]

CCl4 is one of the most commonly used hepatotoxins

### Table 1: Effect of Liv-52 and Livomyn on CCl4-induced Hepatotoxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>Biochemical parameters mean ± SEM</th>
<th>Total</th>
<th>Direct</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SGOT (IU/L)</td>
<td>SGPT (IU/L)</td>
<td>Total protein (g/dl)</td>
</tr>
<tr>
<td>Vehicle Control</td>
<td>56.34 ± 3.83</td>
<td>189.50 ± 22.47</td>
<td>7.10 ± 0.25</td>
</tr>
<tr>
<td>CCl4</td>
<td>260.50 ± 26.12</td>
<td>412.23 ± 7.41</td>
<td>2.26 ± 0.39</td>
</tr>
<tr>
<td>Silymarin</td>
<td>75.25 ± 6.00*</td>
<td>238.25 ± 13.95*</td>
<td>6.65 ± 0.20*</td>
</tr>
<tr>
<td>Liv-52</td>
<td>84.21 ± 4.23*</td>
<td>332.25 ± 25.2*</td>
<td>4.52 ± 0.13*</td>
</tr>
<tr>
<td>Livomyn</td>
<td>90.12 ± 2.35*</td>
<td>362.20 ± 21.6*</td>
<td>4.36 ± 0.42*</td>
</tr>
</tbody>
</table>

b.w. = Body weight, CCl4 = Carbon tetrachloride, “Significant (P < 0.001) reduction compared to CCl4

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in experimental study of liver disease.\textsuperscript{[17]} The lipid peroxidative degradation of biomembrane is one of the principle causes of hepatotoxicity of CCl\textsubscript{4}.\textsuperscript{[18,19]} This is evident from an elevation in the serum marker analysis, namely AST, ALT, total proteins and bilirubin. The formulations significantly reduced this serum enzyme in groups. The simultaneous administration of formulations and CCl\textsubscript{4} produced significant recovery of the liver damage induced by CCl\textsubscript{4}. The hepatotoxic effect of CCl\textsubscript{4} is largely due to its active metabolite trichloromethyl radical\textsuperscript{[20]} that binds to the macromolecule and induces peroxidative degradation of the membrane lipids of endoplasmic reticulum that is rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxide, which in turn produces a toxic aldehyde that causes damage to liver.\textsuperscript{[21]} This was evident by an increase in the level of lipid peroxidation in the CCl\textsubscript{4} group and there was a significant decrease in lipid peroxidation in the groups treated with CCl\textsubscript{4} and formulations.\textsuperscript{[22]}

The comparative histopathological study of the liver from different groups of rats corroborated the hepatoprotective efficacy of formulations. Various pathological changes
such as steatosis, centrilobular necrosis and vacuolization observed in group II rats were prevented to a moderate extent in groups III, IV and V. All the effects of formulations were comparable with Silymarin as a positive control.

The biochemical studies in albino rats revealed that CCl₄-induced hepatic injury was inhibited significantly ($P < 0.001$) by Liv-52 and Livomyn. All the results can be compared with the standard drug silymarin. The above results also state that Liv-52 has shown significant hepatoprotective activity in comparison to Livomyn. In support, the histopathological reports also revealed that there is a regenerative activity in the liver cells.

**CONCLUSION**

Overall, the results of the present study indicate that Liv-52 and Livomyn demonstrated a significant hepatoprotective activity against CCl₄-induced hepatotoxicity in rats. Moreover, Liv-52 has shown significant hepatoprotective activity in comparison to Livomyn.

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**REFERENCES**


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