Hepatoprotective Effect of Liv.52 and Kumaryasava on Carbon Tetrachloride induced Hepatic Damage in Rats

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ABSTRACT

Oral administration of Liv.52 and Kumaryasava to carbon tetrachloride (CCl₄) treated rats improved growth. Kumaryasava was more effective in reducing the liver weight increase due to hepatotoxicity of CCl₄. Hepatic arginase, cathepsin-B, acid phosphatase, ribonuclease activity, which were decreased on CCl₄ treatment was stimulated by both Liv.52 and Kumaryasava. Results indicate that Liv.52 and Kumaryasava have protective effect on hepatic enzyme induced due to CCl₄ hepatotoxicity.

It is well documented that carbon tetrachloride (CCl₄) triggers hepatic and renal changes in animals and man¹-⁴. Carbon tetrachloride in addition to its use as an industrial solvent is also used in disinfestation of grains and as a medical agent in cases of parasiteminia⁵.

Liv.52, an Ayurvedic preparation (The Himalayan Drug Co, Bombay) is a mixture of several herbal extracts and is prescribed as a liver tonic. It has been reported that Liv.52 protects liver from the hepatotoxicity of paracetamol⁶, anticancer drugs⁷, antibiotics⁸, oral contraceptives⁹, alcohol¹⁰,¹¹, allyl alcohol¹² and carbon tetrachloride¹³. Effect of hepatoprotective Ayurvedic drug on lipolytic activities and lysosomal enzymes during CCl₄ induced acute hepatic injury in rats was observed¹⁴,¹⁵. Kumaryasava, another Ayurvedic medicine (Dabur Ltd.) is used in case of colic, indigestion, constipation, besides several liver diseases.

In the present study the effect of Liv.52 and Kumaryasava on growth and hepatic enzymes of CCl₄ treated rats have been reported.

In this study, male rats (body weight, 26-30 g) were obtained from the Division of Laboratory Animal Research, IVRI, Izatnagar. Rats were divided into 4 groups. Group I (control) was given normal saline injection once a week for 5 weeks. Sublethal dose of CCl₄ (0.7 ml/kg body wt) was given ip once in a week for 5 weeks to rats of group II, III and IV. In addition to this, rats of group III were given Liv.52 (0.125 mg/kg body wt/day) and rats of group IV were given Kumaryasva (3.5 ml/kg body wt/day). All the rats had free access to pellets of control diet and water. Body weight of rats was recorded every week.

Rats were sacrificed under chloroform anesthesia after 5 weeks. Livers were immediately excised, washed with normal saline and 10% homogenated (w/v) in 0.25 M sucrose was prepared. Homogenated was centrifuged at 2,000 rpm for 10 min. Ribonuclease¹⁵, phosphatase¹⁶, glucose-6-phosphatase¹⁷, cathepsin¹⁸ and arginase¹⁹ were analyzed in the supernatant. Protein was estimated by the method of Lowry et al.²⁰, using bovine serum as standard. The data were statistically analyzed using analysis of variance.
Growth of rats was reduced considerably on CCl$_4$ injection. Liv.52 and Kumaryasava improved the growth of CCl$_4$ treated rats. Liv.52 fed rats exhibited slightly better growth (Table 1). Increase in liver weight was observed in CCl$_4$ treated rats (Table 1) and the increase may be due to accumulation of lipid largely triglycerides i.e. formation of fatty liver. Liv.52 was found to be not much effective in reducing CCl$_4$ induced increase in liver weight whereas Kumaryasava reduced the weight of liver.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>CCl$_4$</th>
<th>Liv.52 + CCl$_4$</th>
<th>Kumaryasava + CCl$_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>61.5 ± 4.74$^{abc}$</td>
<td>53.75 ± 4.26$^{a}$ (-27.03)</td>
<td>77.00 ± 5.78$^{bc}$ (+88.88)</td>
<td>73.50 ± 3.96$^{abc}$ (+28.37)</td>
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<tr>
<td>Liver weight (g)</td>
<td>1.95 ± 0.24$^a$</td>
<td>2.45 ± 0.15$^a$ (32.65)</td>
<td>2.65 ± 0.14$^a$ (35.65)</td>
<td>2.3 ± 0.03$^a$ (17.34)</td>
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<tr>
<td>Hepatic enzymes</td>
<td>0.92 ± 0.12$^a$</td>
<td>0.66 ± 0.01$^a$ (20.4)</td>
<td>0.52 ± 0.15$^a$ (-16.86)</td>
<td>0.88 ± 0.64$^a$ (-9.4)</td>
</tr>
<tr>
<td>Arginase</td>
<td>0.08 ±0.04$^a$</td>
<td>0.07 ± 0.00$^a$ (-7.9)</td>
<td>0.08 ± 0.004$^a$ (+7.89)</td>
<td>0.09 ± 0.001$^a$ (+14.47)</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>0.27 ± 0.01$^a$</td>
<td>0.17 ± 0.01$^b$ (-27.85)</td>
<td>0.30 ± 0.01$^a$ (+21.11)</td>
<td>0.28 ± 0.02$^a$ (+12.74)</td>
</tr>
<tr>
<td>Glucose-6-phosphatase</td>
<td>24.57 ± 1.87$^a$</td>
<td>15.03 ± 0.27$^b$ (-32.0)</td>
<td>26.3 ± 1.35$^a$ (+17.87)</td>
<td>17.60 ± 2.48$^b$ (-23.59)</td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>0.03 ± 0.003$^a$</td>
<td>0.02 ±0.003$^a$ (-20.0)</td>
<td>0.05 ± 0.003$^a$ (+24.4)</td>
<td>0.03 ± 0.006$^a$ (+13.3)</td>
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</tbody>
</table>

The mean value with dissimilar superscripts differs significantly (p>0.05). Figures in parentheses are percentage of control. Acid phosphatase - µmole of p-nitro phenol released/min/mg protein. RNAase-O.D./min/mg protein. Cathepsin B - µmole of tyrosine released /min/mg protein. Arginase - µmole of urea released/min/mg protein. Glucose-6-phosphatase - µmole Pi liberated/min/mg protein.

Hepatic lysosomal enzyme, cathepsin B, acid phosphatase and ribonuclease activity decreased due to hepatotoxicity caused by CCl$_4$ (Table). Cathepsin B and acid phosphatase activity was significantly stimulated by Kumaryasava and Liv.52 (Table 1). Kumaryasava and Liv.52 showed slight stimulation in ribonuclease activity in liver decreased in response to CCl$_4$ toxicity (Table).

Liv.52 was more effective in regaining the cathepsin B activity than Kumaryasava. Whereas Kumaryasava was more effective in stimulating acid phosphatase activity in liver (Table). Chloroform extract of Liv.52 was found to be effective in increasing hepatic acid phosphatase, ribonuclease and cathepsin B activity in vitro system$^{24}$.

Hepatic microsomal enzyme, glucose-6-phosphatase, an important enzyme in the regulation of carbohydrate metabolism was decreased in CCl$_4$ treated rats (Table). Liv.52 significantly stimulated glucose-6-phosphatase activity in plasma in response to CCl$_4$ toxicity was reported by Reynolds and Lee$^{25}$. Formation of free radical CCl$_3$ may be associated with a decreased activity of glucose-6-phosphatase in the endoplasmic reticulum. A decrease in the activity of glucose-6-phosphatase can be expected to have severe consequences on the organized metabolism of normal liver cell which has a key role to play in maintaining the blood sugar by gluconeogenesis.
Results of the present study showed that oral feeding of Liv.52 and Kumaryasava to CCl₄ treated rats stimulates regeneration of hepatic and microsomal enzyme decreased due to CCl₄ toxicity. On the basis of changes in the activity of hepatic enzymes it seems that Liv.52 and Kumaryasva both provide certain amount of protection and correct liver dysfunction due to CCl₄ induced hepatotoxicity. However the mechanism of action of these Ayurvedic preparation in restoring the liver functions appears to be different.

REFERENCES

