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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 (CCl_4) treated rats improved growth. Kumaryasava was more effective in reducing the liver weight increase due to hepatotoxicity of CCl_4. Hepatic arginase, cathepsin-B, acid phosphatase, ribonuclease activity, which were decreased on CCl_4 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 CCl_4 hepatotoxicity.

It is well documented that carbon tetrachloride (CCl_4) triggers hepatic and renal changes in animals and man[1-4]. 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 parasiteminia[5].

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 paracetamol[6], anticancer drugs[7], antibiotics[8], oral contraceptives[9], alcohol[10,11], allyl alcohol[12] and carbon tetrachloride[13]. Effect of hepatoprotective Ayurvedic drug on lipolytic activities and lysosomal enzymes during CCl_4 induced acute hepatic injury in rats was observed[14,15]. 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 CCl_4 (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. Ribonuclease[15], phosphatase[16], glucose-6-phosphatase[17], cathepsin[18] and arginase[19] 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: Mean values (± SE) of body weight, liver weight and hepatic enzymes of rat

Parameter	Control	CCl_4	Liv.52 + CCl_4	Kumaryasava + CCl_4
Body weight (g)	$61.5 \pm 4.74^{a,c}$	53.75 ± 4.26^{a} (-27.03)	$77.00 \pm 5.78^{b,c}$ (+88.88)	$73.50 \pm 3.96^{a,b,c}$ (+28.37)
Liver weight (g)	1.95 ± 0.24^{a}	2.45 ± 0.15^{a} (32.65)	2.65 ± 0.14^{a} (35.65)	2.3 ± 0.03^{a} (+17.34)
Hepatic enzymes arginase	0.92 ± 0.12^{a}	0.66 ± 0.01^{a} (-20.4)	0.52 ± 0.15^{a} (-16.86)	0.88 ± 0.64^{a} (-0.94)
Acid phosphatase	0.08 ± 0.04^{a}	0.07 ± 0.00^{a} (-7.9)	0.08 ± 0.004^{a} (+7.89)	0.09 ± 0.001^{a} (+14.47)
Cathepsin B	0.27 ± 0.01^{a}	0.17 ± 0.01^{b} (-27.85)	0.30 ± 0.01 (+21.11)	0.28 ± 0.02^{a} (+12.74)
Glucose-6-phosphatase	24.57 ± 1.87^{a}	15.03 ± 0.27^{b} (-32.0)	26.3 ± 1.35^{a} (+17.87)	17.60 ± 2.48^{b} (-23.59)
Ribonuclease	0.03 ± 0.003^{a}	0.02 ± 0.003^{a} (-20.0)	0.05 ± 0.003^{a} (+24.4)	0.03 ± 0.006^{a} (+13.3)

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 CCl_4 treated rats stimulates regeneration of hepatic and microsomal enzyme decreased due to CCl_4 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 CCl_4 induced hepatotoxicity. However the mechanism of action of these Ayurvedic preparation in restoring the liver functions appears to be different.

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