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Hepatoprotective effect of Liv.52 Against CCl_4-induced Lipid Peroxidation in Liver of Rats

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ABSTRACT

Effect of oral feeding of Liv.52, on lipid peroxidation in normal liver and damaged liver induced by CCl_4 of albino rats was studied. While Liv.52 did not show any effect on normal healthy liver cells, it had a significant protective effect against damage by CCl_4 as shown by significant decrease in malonaldialdehyde content.

Liv.52 an herbal indigenous preparation (The Himalaya Drug Company, Bombay) is reputed to possess a hepatic stimulant effect in chronic liver disease[1], corrects liver dysfunction in acute hepatitis and protects liver against several hepatotoxins, alcohols[3], heavy metals[4], paracetamol etc.

Carbon tetrachloride exerts its damaging effect on the liver cells by its conversion to radicals i.e., CCl_3 (trichloromethyl radical) and $^{\bullet}O_2CCl_3$ (trichloromethylperoxy radical). The $^{\bullet}O_2CCl_3$ is the most reactive species and causes damage to biological macromolecules by combining with them thereby causing covalent modification and setting the chain reactions of lipid peroxidation[6].

Since the above-mentioned hepatotoxins are frequently used and protective effect of Liv.52 against them is well documented. Liv.52 protects cellular membranes by lowering lipid peroxidation[8,9]. The present study was carried out with the objective to assess the protective effect of Liv.52 after CCl_4 damage in terms of lipid peroxidation (malonaldialdehyde) an index of damage by free radicals.

Adult healthy albino rats of either sex weighing 70-80 were used for the study. The animals were fed pellet diet and water was allowed *ad libitum*[10]. Prior to drug administration animals were fasted for 24 hours.

The study was carried out in two sets of experiments. In the first set (considered as pilot experiment to see the effect of Liv.52 on healthy liver cells) animals were divided into 4 groups of five animals each. One group served as control and was given 2% gum acacia solution orally. the remaining three groups (II, III, IV) were fed Liv.52 suspension in 2% gum acacia solution orally at a dose of 100, 200, 400 mg/kg for 6 days.

In the second set of experiment animals were divided into 5 groups of five animals each. The control group received only 2% gum acacia solution and groups I, II, III, IV received CCl_4 orally in dose of 2 ml/kg body weight (i.e. 20 mmole CCl_4/kg body weight) along with an equal quantity of liquid paraffin following the method of Singh *et al.*[11]. Groups (II, III, IV) received Liv.52 suspension in 2% gum acacia orally in the doses of 100, 200 and 400 mg/kg after 2 hours of CCl^4 administration. The dose treatment was done for 6 x days once daily.

After 6th day all rats were sacrificed by decapitation, the livers were removed, washed with chilled normal saline and were weighed. 30% w/v liver homogenates were prepared in ice-cold 0.15 M KCl.

Lipid peroxidation (MDA) – Malonaldialdehyde (MDA) level was estimated in whole homogenate by the TBA method of Warvedhkar and Sashw[11] on DU model spectrophotometer. Suitable aliquots of the plasma were mixed with equal volume of 50% of TCA, vortexed thoroughly and the tubes were kept in cold. After ½ hour tubes were centrifuged at 800 g for 10 minutes. To the supernatant, 0.67% TBA was added, vortexed and then kept in boiling water bath for 15 minutes followed rapid cooling. Absorbance was recorded at 535 nm. The results are expressed in nmoles of MDA/mg protein.

Protein estimation – Protein content in each liver was determined by the method of Lowery *et al*.[12] using BSA as standard.

All the reagents used in biochemical estimations were of AR grade.

Statistical analysis – The data were statistically analysed by using Student's test.

A small decrease in the level of lipid peroxides (MDA) content in normal livers was observed in Liv.52 fed group which was not dose dependent no matter whatsoever the dose was used, but MDA levels were significantly decreased when Liv.52 was given to rats having liver injury induced by CCl_4. This response was dose dependent (Table 1) and statistically significant.

Table 1: Effect of Liv.52 on lipid peroxidation in normal livers and CCl_4 induced injury in the livers of albino rats

Group	Treated with Liv.52 (Set 1)	Treated with CCl_4 + Liv.52 (Set 2)
Control	8.48 ± 1.01	13.52 ± 0.88
Group I (CCl_4)	–	19.52 ± 0.52[c]
Group II (Liv.52; 100 mg/kg)	7.95 ± 0.61	12.88 ± 0.54[a]
Group III (Liv.52; 200 mg/kg)	8.22 ± 0.71	12.07 ± 0.79[a]
Group IV (Liv.52; 400 mg/kg)	8.09 ± 0.62	9.78 ± 1.22[b]

1. In set 2, all the animals were given CCl_4 (20 mmole) and Liv.52 at different doses after 2 hours for 6 days.
2. Statistically significant [a]$p<0.001$, [b]$p<0.005$ on comparison with group 1 and [c]$p<0.001$ for normal control in set 2.
3. Groups of rats for both sets of experiments were different.

Liv.52 is known to prevent hepatic cell necrosis following carbon tetrachloride administration by providing protection against decreased levels of mitochondrial and microsomal enzymes in CCl_4 hepatotoxicity[15,16]. The effect produced by CCl_4 may be due to the fact that it is substrate for Cyt.P_{450}[14], which is dominant in liver. CCl_4 causes rapid peroxidation of microsomal lipids accompanied by the inactivation of enzymes and destruction of Cyt.P_{450} itself. Else than Liv.52 could maintain normal proteins. RNA and DNA, which are significantly lowered following CCl_4, induced hepatic insult[17].

However, its role in free radical damage of liver with respect to CCl_4 induced injury has not been assessed so far. The above results indicate that Liv.52 does not effect normal cells as MDA levels of control and Liv.52 treated were almost similar whereas it showed marked protective effect on the damaged liver cells (induced by CCl_4) by depressing the lipid peroxidation process. The study further adduces evidence that Liv.52 is a potent hepatic stimulant.

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