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Effect of Carbon tetrachloride and Liv.52 on Liver Microsomal Protein, Total Protein and Nucleic Acids

Subbarao, V.V. and **Gupta, M.L.**
Upgraded Department of Physiology, S.M.S. Medical College, Jaipur, India.

The liver is the key organ concerned with various metabolic processes including food and drug metabolism. The activity of the drug-metabolising or microsomal enzymes is markedly affected by factors such as starvation, adrenaline stress, hyperthyroidism, and hepatectomy (Kato, *et al.*, 1964 and Anthony, 1972) as also by the dietary status (Marshal and McLean, 1971). The drug metabolizing activity in the animal liver increases with the administration of drugs such as phenobarbital (Orrenious *et al.,* 1969).

Various chemicals are known to cause serious hepatotoxicity. Carbon tetrachloride is one such classical hepatotoxin. However, very little literature is available on substances or diets, which can offer protection against chemically-induced hepatotoxicity.

For the past several years an indigenous compound, Liv.52 (The Himalaya Drug Co.) has been used by various workers as an effective hepatic protective and stimulant (Sheth *et al.,* 1960; Joglekar *et al.,* 1963; Karandikar *et al.,* 1963) showing that Liv.52 affords quite considerable protection against hepatic damage caused by carbon tetrachloride; Patrao (1957) found it beneficial in patients suffering from severe hepatic damage. Sule and Sathe (1957) found that in patients on Liv.52 deranged liver function tests rapidly return to normal or near normal. Bearing this in mind, an attempt has been made in this study to re-examine the hepatoprotective action of the indigenous compound Liv.52 against carbon tetrachloride-induced hepatotoxicity by assessing and studying certain altered biochemical parameters.

Each 2.5 ml of Liv.52 syrup contains:

Exts.	*Capparis spinosa*	17 mg
	Cichorium intybus	17 mg
	Solanum nigrum	8 mg
	Cassia occidentalis	4 mg
	Terminalia arjuna	8 mg
	Achillea millefolium	4 mg
	Tamarix gallica	4 mg

Processed in: *Eclipta alba, Phyllanthus niruri, Boerhaavia diffusa, Tinospora cordifolia, Berberis aristata, Raphanus sativus, Phyllanthus emblica, Plumbago zeylanica, Embelia ribes, Terminalia chebula* and *Fumaria officinalis.*

MATERIALS AND METHODS

Experiments were performed on 32 adult male albino rats weighing between 150-200 g. These were divided into 4 groups of 8 animals each. Group I served as the control group. Group II was administered CCl_4 0.2 ml/100 g orally and its effects were studied 24 hours after administration.

Group III was pre-treated with Liv.52 syrup 5 ml daily for 5 days prior to the administration of CCl_4 0.2 ml/100 g and again a similar dose of Liv.52 syrup after the challenge. The pre- and post-treatment effects with Liv.52 and the challenge with CCl_4 were studied 24 hours after the administration of the last dose. The animals in Group IV were orally fed 5 ml of Liv.52 syrup for 6 days prior to studying its effects. At the end of the study period, all the animals were killed by decapitation and their livers were immediately chilled in ice.

The following investigations were carried out:

1. Liver proteins – Total and microsomal.
   Liver microsomal protein (9000 x g supernatant fraction) was estimated using the biuret method (Layne, 1957).

2. The nucleic acids were estimated following the method of Schmidthannahauser and Scheider (Glick, 1967). The acid soluble materials were removed with 5% trichloroacetic acid and the lipids by an ethanol-ether mixture. The deoxyribonucleic acid (DNA) was separated from the proteins by using 5% hot perchloric acid and then estimated by diphenylamine reaction of Burton (1956) at 600µ. The ribonucleic acid (RNA) was estimated by Ceriotti's orcinol reaction at 657µ (Ceriotti, 1955).

RESULTS

Table 1

Experiment		Total RNA mg/100 g net weight	Total DNA mg/100 g	Total protein g/100 g	Microsomal protein mg/g
Control	(8)	781.7 ± 7.48	302.0 ± 3.58	19.6 ± 0.54	155.9 ± 2.58
CCl_4	(8)	763.4 ± 3.38 $p<0.01$	301.4 ± 3.18 NS	15.9 ± 0.56 NS	98.4 ± 4.40 $p<0.01$
CCl_4 + Liv.52	(8)	770.2 ± 4.56 NS	305.1 ± 4.81 NS	18.2 ± 0.63 NS	144.6 ± 4.20 NS
Liv.52	(8)	780.7 ± 2.38 NS	307.9 ± 3.47 NS	20.1 ± 0.78 NS	158.1 ± 7.88 NS
Number in parentheses denotes the number of animals ± SE.					

From Table 1, it is seen that CCl_4 administration significantly reduces liver microsomal protein and RNA but total protein and DNA remain practically unaltered.

Treatment of animals with Liv.52, 5 days prior to the challenge with CCl_4 and once after the challenge prevents this reduction in ribosomal protein and RNA seen after the administration of CCl_4 alone.

Liv.52 by itself does not cause an alteration in these parameters but it significantly protects against the damaging effects of CCl_4.

DISCUSSION

Carbon tetrachloride administration is used as a standard laboratory procedure to cause hepatic damage and to evaluate the effect of protective agents. In the present study, CCl_4 decreased the hepatic RNA as well as microsomal protein. Thus, the effect of CCl_4 may primarily be on the RNA

and may secondarily be causing a decrease of RNA-dependant synthesis of microsomal proteins. Or, CCl_4 may be having an independent, specific effect on the synthesis of microsomal enzymes which effect may be prevented by Liv.52.

From the present study, it is clear that prior and subsequent administration of Liv.52 prevents the changes caused by CCl_4 but Liv.52 alone does not cause any significant change in the liver proteins and nucleic acids.

It, therefore, appears that Liv.52 either causes accelerated regeneration of damaged liver tissue or specifically antagonizes the effects of CCl_4. From the literature it is seen that Liv.52 affords similar protection against a variety of noxious agents (Joglekar and Leevy, 1970) and the same effect is seen even when Liv.52 is administered only subsequent to the noxious agent. Thus, it is probable that the action of Liv.52 seen here is the one responsible for protection against damage and for rapid regeneration but is also likely that the drug may have a specific action against CCl_4.

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