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Protection by Indigenous Drugs against Hepatotoxic Effects of Carbon Tetrachloride – A Long term Study

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Drug therapy of liver cirrhosis has been mainly symptomatic and disappointing, since much is still obscure about its aetiology. Many agents (Rees 1962; Fiume *et al.* 1961) being selected show beneficial effects against hepatic damage induced by carbon tetrachloride.

Joglekar *et al.* (1963) have observed encouraging protective effects of Liv.52, a proprietary medicine, against carbon tetrachloride toxicity in mice. It is claimed to contain reputed indigenous hepatic stimulants and has been reported effective (Sheth *et al.* 1960; Patrao 1957; Sule *et al.* 1956).

We have now studied the long-term effects of Liv.52 therapy and promethazine (Phenergan) against carbon tetrachloride toxicity.

MATERIALS AND METHODS

Part A:

We used 40 albino female rats of weight range 150-180 g divided in four equal groups, one group as a control. Animals in the other three groups were given carbon tetrachloride 0.2 ml mixed with liquid paraffin 0.2 ml subcutaneously twice a week. One group of the three was given no protective agent.

The remaining groups received either promethazine elixir 0.7 ml (2.5 mg/rat) or Liv.52 Pediatric Drops 0.5 ml (30 mg/rat) daily by intragastric tube.

The animals were observed for twelve weeks. When death occurred during this period, the liver was examined both macroscopically and microscopically. The livers were weighed, and their volumes were measured by displacement method.

All animals surviving 12 weeks were killed and examined as outlined.

Part B:

We used eight male rabbits of weight range 1.5-1.8 kg to study the effects of Liv.52. All rabbits were given carbon tetrachloride 1 ml mixed with 1 ml of liquid paraffin subcutaneously twice a week as long as they survived. Six of these received 2 g of Liv.52 powder suspended in 30 ml of water daily by intragastric tube.

Histologic examinations were made of the livers.

RESULTS

1. Table 1 gives the figures for survival among rats.
2. Figure 1 gives the mortality of rats and rabbits.
3. Table 2 gives the weights and volumes of livers from rats.

Table 1: Liv.52 reduced the percentage mortality in rats due to carbon tetrachloride considerably more than promethazine did

	No. of animals living after 12 weeks	% mortality
Blank control	10	0%
Carbon tetrachloride control	2	80%
Carbon tetrachloride and Promethazine	2	80%
Carbon tetrachloride and Liv.52	7	30%

Table 2: Liv.52 did not significantly alter the weight and volume of liver. Promethazine showed significant increase in volume without a corresponding increase in weight

	Weight in g.	Volume in ml.
Blank control	6.39 ± 1.08	5 ±0.67
Carbon tetrachloride control	7 ± 1.4 (p>0.05)	5.9 ± 0.9 (p>0.05)
Carbon tetrachloride and Promethazine	8.45 ± 1.8 (p>0.05)	7.75 ± 1.2 (p<0.01)
Carbon tetrachloride and Liv.52	6.74 ± 0.9 (p>0.05)	5.45 ± 0.55 (p>0.05)

Macroscopic appearance (Fig. 2) showed that in the carbon tetrachloride group (No. 2) gross cirrhotic changes were noted in five animals and lobulation was obvious. In the promethazine groups cirrhotic changes were noticed in four animals, but there was only a suggestion of lobulation. The livers of the Liv.52 appeared almost like control livers.

Fig. 1: Liv.52 prolongs survival time in both rats and rabbits

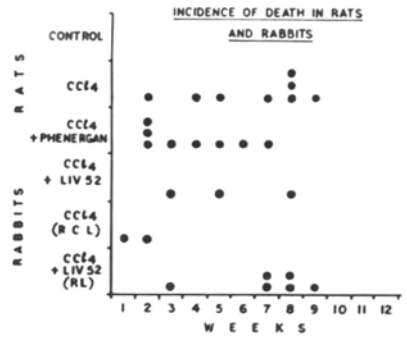

Fig. 2: (1-4). Liv.52-treated liver (4) appears like normal control liver (1). Carbon tetrachloride and promethazine treated livers (2 and 3) show gross cirrhotic changes

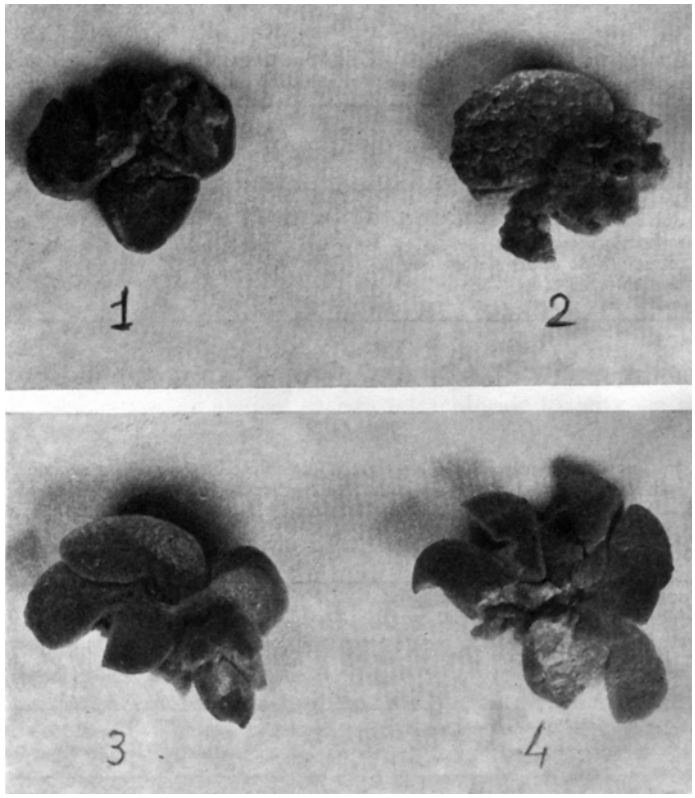

Table 3 – Summarises the results of the histological examinations.

Table 3: Histological changes in rat and rabbit livers. Liv.52 shows protective effects and marked regenerative activity, the histological changes due to carbon tetrachloride being less severe

	Blank Control (10)	CCl_4 (7)	CCl_4 & prome-thazine (9)	CCl_4 & Liv.52 (10)	CCl_4 (Rabbits) (2)	CCl_4 & Liv.52 (Rabbits) (6)
Congestion	Nil	Moderate	Moderate	Nil	Moderate	Nil
Fatty change	Nil	Marked	Severe	Moderate	Severe	Moderate
Necrosis	Nil	Marked	Marked	Moderate	Marked	Moderate
Infiltration by chronic inflammatory cells	Nil	Moderate	Moderate	Moderate	Moderate	Moderate
Connective tissue (Reticulum stain)	Nil	*Thick bands	*Thick bands	*Thin bands	Nil	Nil
Lobulation	Nil	Marked	Marked	Moderate	Nil	Nil
Regeneration	Nil	Moderate	Moderate	Marked	Nil	Moderate

Numbers in parenthesis indicate the number of livers of which histological examination was possible.
*See photograph micro, figures 8, 9 and 10.

COMMENTS

In a previous short-term study on mice (Joglekar *et al.* 1963) it was observed that Liv.52 conferred a significant reduction in mortality and also appreciable protection against liver damage induced by carbon tetrachloride.

This work was extended on a long-term basis, and the period of study being increased to twelve weeks to discover whether or not Liv.52 has any effect on the cirrhosis induced by carbon tetrachloride. For the manifestation of cirrhotic changes a period of at least 8 weeks is necessary (Wahi 1956; Fiume 1961). Female rats were thus intentionally selected, since it was observed by us (Joglekar *et al.* 1963) that male mice succumbed quickly to the toxic effects of carbon tetrachloride. Gyorgi *et al.* (1946) have noted a similar effect of sex on carbon tetrachloride toxicity.

Though Rees (1962) has reported the beneficial effects of promethazine against carbon tetrachloride, we were unable to confirm it in our earlier study. Promethazine was included to study its effect in a long-term trial.

Even in this study we have observed the significant protection offered by Liv.52 against carbon tetrachloride if the percentage mortality is taken as a criterion (Table 1). Promethazine does not produce any difference in percentage mortality from that due to carbon tetrachloride alone. Indeed, if the incidence of death is examined (Fig. 1) it seems clear that promethazine has apparently potentiated carbon tetrachloride, since the deaths occurred quite early. Both in rats and rabbits Liv.52 prolonged survival time considerably.

There was no significant difference between the weights of the livers in any of the groups. However the promethazine group showed a significant increase in the volume of liver (Table 2). This may have been due to an increase in fat content of the liver, which could increase the volume without similarly altering the weight. Severe fatty degeneration observed histologically in the Phenergan group (Table 3) confirms this possibility.

On histological examination (Figs. 3, 4, 5 and 6) it was clear that the Liv.52 treated group showed considerably reduced fatty change and necrosis. Hepatic structure was maintained in Liv.52 group. Regenerative changes were marked in this group compared with the others. On reticulum staining (Figs. 7, 8, 9 and 10), the thick bands of reticulum observed in the carbon tetrachloride and promethazine group were clearly absent in the Liv.52 group, where we found thin compressed streaks of reticulum due to extensive regenerative changes in the surroundings. Similarly, the lobulation so clearly seen both macroscopically and microscopically in the carbon tetrachloride and promethazine group was not observed in the Liv.52 group.

The mechanism of this protective effect of Liv.52 remains to be elucidated, but we are impressed with its beneficial effect against the hepatotoxic action of carbon tetrachloride in prolonging the survival and changing the gross and the histological appearances of liver tissue. This salutary effect is observed even in rabbits, the other species studied (Figs. 11 and 12).

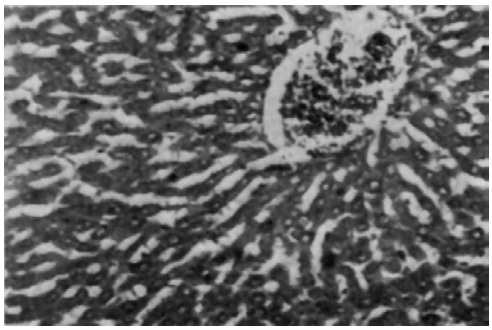

Fig. 3: Blank control

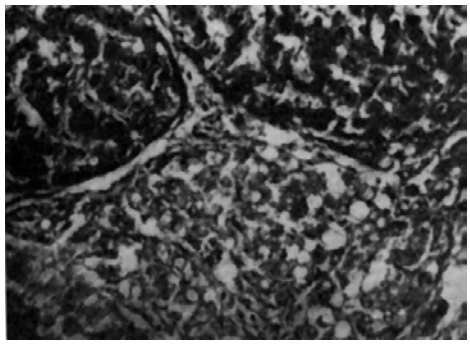

Fig. 4: Carbon tetrachloride control

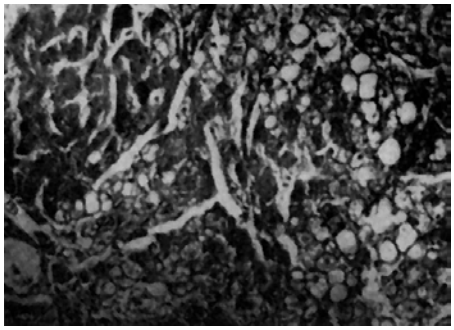

Fig. 5: Carbon tetrachloride plus promethazine

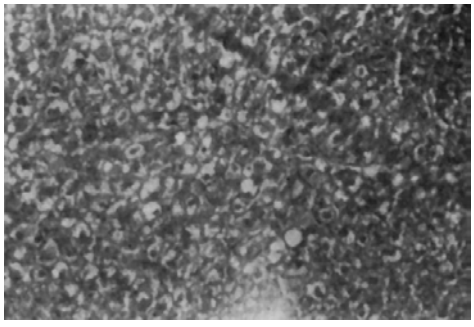

Fig. 6: Carbon tetrachloride plus Liv.52

Figs. 3 to 6: The Liv.52 group shows remarkable protective effects histologically (Fig. 6) compared with carbon tetrachloride group (Fig. 4) and promethazine group (Fig. 5). Promethazine group shows excessive fatty infiltration

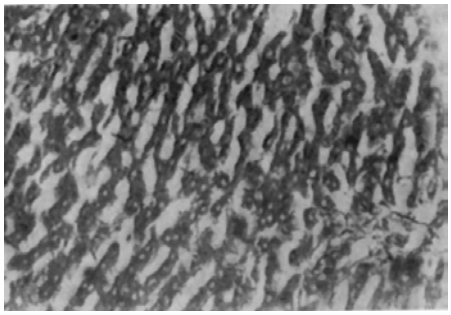

Fig. 7: Blank control

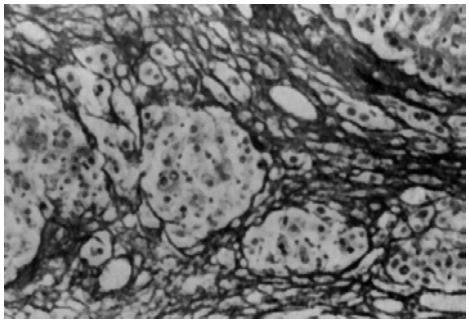

Fig. 8: Carbon tetrachloride control

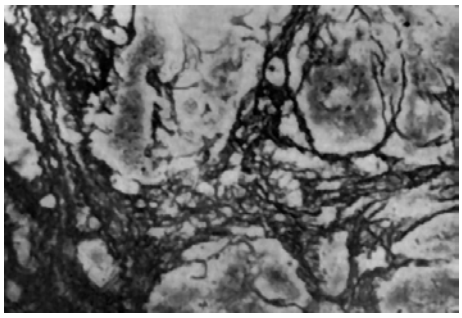

Fig. 9: Carbon tetrachloride plus promethazine

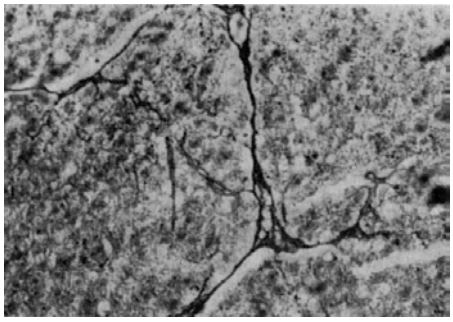

Fig. 10: Carbon tetrachloride plus Liv.52

Figs. 7 to 10: (Reticulum stain). The Liv.52 group shows thin streaks of reticulum indicative of marked regenerative activity in the surrounding areas (Fig. 10). Carbon tetrachloride and promethazine groups (Figs. 8 and 9) show thick bands of reticulum and lobulation

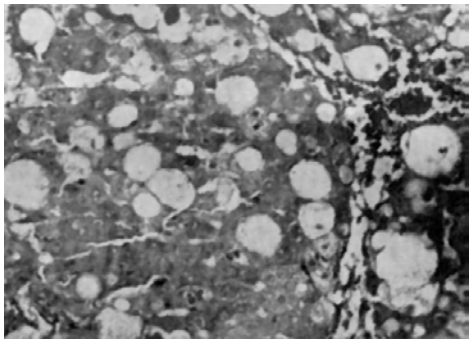

Fig. 11: Carbon tetrachloride control (rabbit)

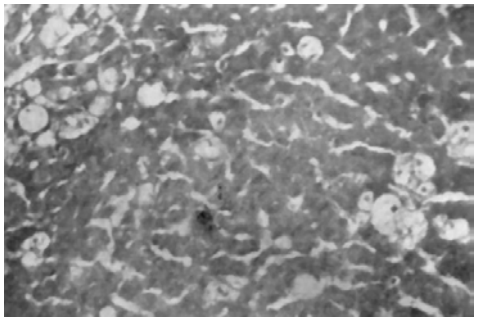

Fig. 12: Carbon tetrachloride plus Liv.52

Figs. 11 and 12: Liv.52 – treated rabbit livers show less necrosis and fatty change (Fig. 12) compared with those four rabbits receiving carbon tetrachloride alone (Fig. 11)

SUMMARY

A protective effect of Liv.52 – an Indian indigenous proprietary medicine – is noticed against carbon tetrachloride poisoning in rats and rabbits, even in a long-term study.

ACKNOWLEDGEMENTS

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