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Protection by Indigenous Drugs against Hepatotoxic effects of Carbon tetrachloride in Mice

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In recent years much work has appeared in the literature about the induction of liver damage by carbon tetrachloride and protection against it by a wide variety of agents, such as antihistaminics, phenothiazines, quinine and procaine (Rees 1962; Fiume *et al.* 1961). Liv.52 (Product of The Himalaya Drug Co., Bombay) a proprietary medicine consisting of indigenous drugs, has been claimed to be effective in hepatic disorders (Sheth *et al.* 1960; Patrao 1957; Sule *et al.* 1956).

In our work we proposed to study the protection offered by promethazine (Phenergan), chlorpromazine (Largactil) and Liv.52 against the effects of carbon tetrachloride in mice.

MATERIALS AND METHODS

We used 100 albino mice, of average weight-range 25-40 g. For the short term study 50 male mice were divided into five equal groups. One group was kept as a control receiving no treatment. Animals in each of the other four groups were given carbon tetrachloride 0.1 ml orally by intragastric tube. One group out of these four was given no protective agent.

The remaining three groups received Phenergan elixir (1 mg/mouse) Largactil (0.4 mg/mouse) or Liv.52 pediatric drops (0.4 ml i.e. 25 mg/mouse) immediately after (i.e. 0 hrs) and again 4 hours after the administration of the carbon tetrachloride.

The animals were observed for 72 hours. Those surviving at the end of the observation period were killed and subjected to histological examination.

A similar study has been intended on 50 female mice. However, at the end of 72 hours they were all alive, and it was therefore decided to extend the study by repeated administration of carbon tetrachloride.

Distributions into groups and the administration of carbon tetrachloride and other drugs was done in the same way as in the short term experiment. In this study carbon tetrachloride was administered five times at the interval of three days for 21 days. To the animals in the three groups, 0 hour, and 4 hours after each carbon tetrachloride administration, Phenergan, Largactil or Liv.52 was given orally in the doses already mentioned. The livers of those dying during the study period and those surviving to the end were examined histologically.

RESULTS

In Table 1 and 2 are given the figures for survival in the two experiments. Tables 3 and 4 summarize the results of the histological study.

Table 1

Group (50 ô mice)	No. of animals living after 72 hours	% mortality
Blank control	10	0
Carbon tetrachloride control	2	80
Carbon tetrachloride and Phenergan	2	80
Carbon tetrachloride and Largactil	2	80
Carbon tetrachloride and Liv.52	7	30

Table 2

Group (50 O+ mice)	No. of animals living after 21 hours	% mortality
Blank control	10	0
Carbon tetrachloride control	1	90
Carbon tetrachloride and Phenergan	0	100
Carbon tetrachloride and Largactil	2	80
Carbon tetrachloride and Liv.52	7	30

Table 3: Histological changes in liver after 72 hours 50 ô mice, five groups of 10 numbers showing change

	Control	CCl_4	CCl_4 and Phenergan	CCl_4 and Largactil	CCl_4 and Liv.52
Diffuse necrosis	Nil	10	10	10	10
Hydropic degeneration	Nil	3	5	4	2
Fatty degeneration	2	7	7	6	9
Hemorrhages	Nil	5	2	4	6
Congestion	3	10	8	7	9

Table 4: Histological changes in liver after 21 days, 50 O+ mice, five groups of 10 numbers showing change

	Control	CCl_4	CCl_4 and Phenergan	CCl_4 and Largactil	CCl_4 and Liv.52
Areas of Active Necrosis	Nil	10	8	10	2
Chronic cell infiltration	Nil	10	9	8	Nil
Localised Regenerative activity	Nil	8	6	8	2
Generalised Regenerative activity	Nil	Nil	Nil	Nil	8
Lobulation	Nil	8	6	7	1
Fatty degeneration	3	7	4	4	5
Reticulum	-	Normal	Normal	Normal	Normal

72 hours study: In the carbon tetrachloride group diffuse necrosis of the liver parenchyma was noticed as acidophilic pink areas. However, it was especially marked in the layers of cells surrounding the central vein.

Other areas showed degenerative changes as hydropic and fatty degeneration. haemorrhages were also present in few areas.

The remaining three groups in which the protective agents were given after the carbon tetrachloride showed almost similar picture.

21 days' study: In the carbon tetrachloride group (Fig. 1) the areas of necrosis were being gradually replaced by bands of cells consisting of histiocytes and other chronic inflammatory cells, including plasma cells. Certain areas still showed evidence of active necrosis, whereas the intervening cells showed regenerative activity. The whole picture suggested early cirrhotic changes, since an attempt at lobulation was clear.

The Phenergan- and Largactil-treated groups (Figs. 2 and 3) also showed invasion of chronic inflammatory cells in the necrosed area and regenerative activity in the surrounding cells. However, the changes were little different from those noticed with carbon tetrachloride alone.

On the other hand, in the Liv.52 group (Fig. 4) regenerative activity was marked even in the previously necrosed areas, and replacement by chronic inflammatory cells was almost negligible. The liver pictures showed striking resemblances to those of the control group.

Reticulum staining in all the groups did not reveal any abnormality.

On the basis of the experience gained during this study, a long-term investigations on rats is in progress.

Fig. 1: Carbon tetrachloride effect "Protection Studies against hepatotoxic effects of carbon tetrachloride in mice by indigenous drugs"

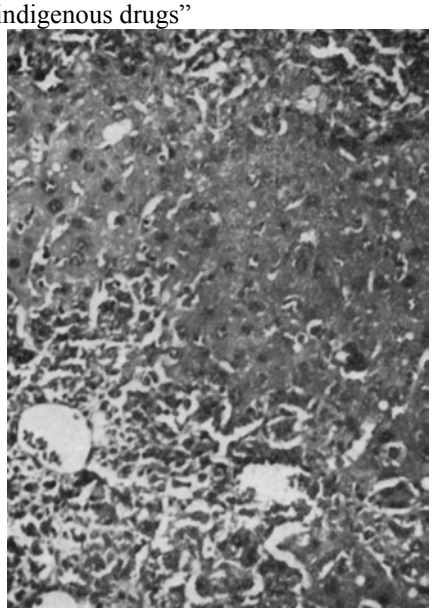


Fig. 2: Carbon tetrachloride and Phenergan "Protection studies against hepatotoxic effects of carbon tetrachloride in mice by indigenous drugs"

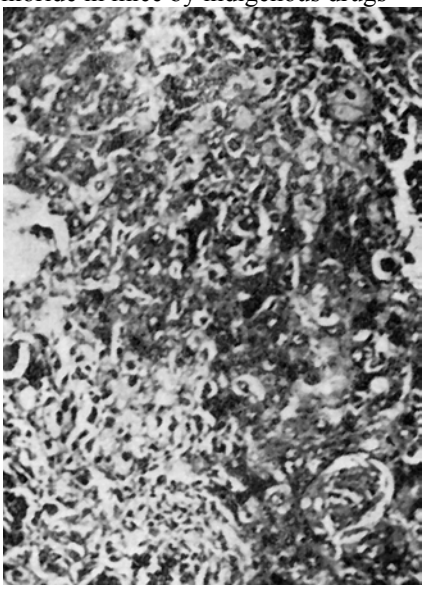


Fig. 3: Carbon tetrachloride and chlorpromazine "Protection studies against hepatotoxic effects of carbon tetrachloride in mice by indigenous drugs"

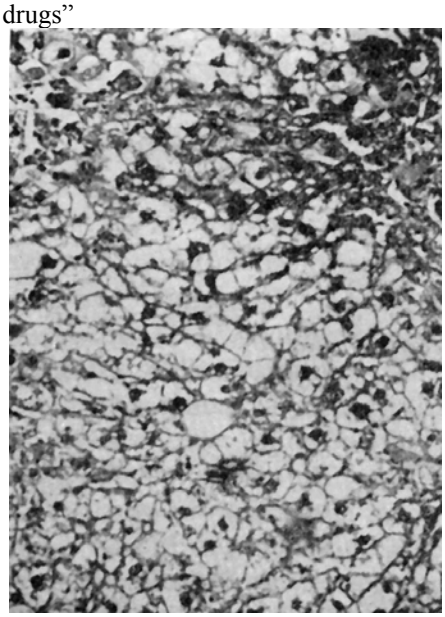
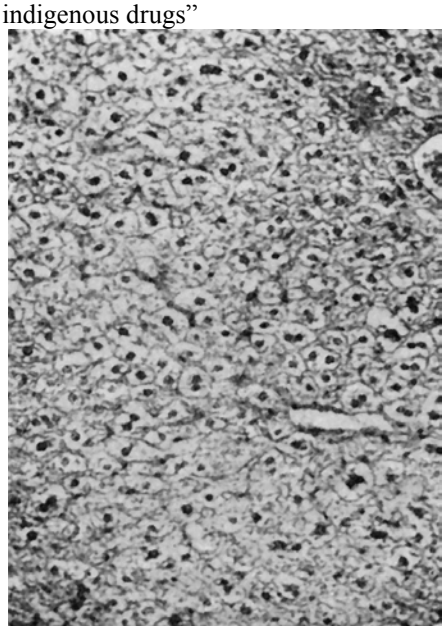


Fig. 4: Carbon tetrachloride and Liv.52 "Protection studies against hepato-toxic effect of carbon tetrachloride in mice by indigenous drugs"



COMMENTS AND METHODS

The present day drug treatment of liver cirrhosis is still in the experimental stage. No drug can prevent the cirrhotic change in the hepatic tissue once it begins.

Experimental liver cirrhosis is commonly produced by administering carbon tetrachloride by various routes. Rees (1962) has reported the protective effects of certain phenothiazine compounds and other agents. On the other hand, chlorpromazine, a phenothiazine derivative, has produced liver toxicity in certain clinical cases (Kelsey *et al.* 1955; Cohen *et al.* 1955; Isaacs *et al.* 1955).

An indigenous proprietary medicine, Liv.52, containing reputed hepatic stimulants (Kirtikar and Basu 1933) was clinically tested and showed some value. The Liv.52 drops contain *Capparis spinosa* 1.67%, *Tamarix gallica* 0.42%, *Cassia occidentalis* 0.42%, *Terminalia arjuna* 0.84%, *Achilea millefolium* 0.42%, *Cinchorium intybus* 1.67% and *Solanum nigrum* 0.84%.

In the investigation we have studied the effects of promethazine (Phenergan), chlorpromazine (Largactil) and Liv.52 on carbon tetrachloride induced liver damage in mice. The short study of male mice for 72 hours (Table 1) clearly shows that the percentage mortality with Liv.52 is markedly less than that in the other groups. Phenergan and Largactil failed to protect the mice against carbon tetrachloride, since the same number of deaths occurred in all the three groups. Histologically, however, no clear protective effects of Liv.52 were noted.

When a similar study was carried out on female mice, no death occurred in 72 hours. György *et al.* (1946) have observed a similar longer survival and less hepatic damage in female rats than in male ones after carbon tetrachloride treatment. When this study was continued for 21 days, even after the repeated administration of carbon tetrachloride, the percentage mortality with Liv.52 was the same as after 72 hours (Table 2) and considerably less than in the other groups. Phenergan and Largactil again failed to protect the animals.

Histological study of these groups, showing early cirrhotic change as evidenced by infiltration of chronic inflammatory cells, suggestion of lobulation and localised regenerative activity, clearly indicates that promethazine and chlorpromazine were both unable to stop the changes occurring after carbon tetrachloride administration. In the Liv.52 group the liver picture had a remarkable similarity to that of control livers there being no areas of chronic cell infiltration and the necrosis that might have resulted was completely replaced by active regeneration spread throughout. This suggests that Liv.52 can possibly prevent liver damage and may even be useful in preventing further fibrotic changes.

Since our study was only for of limited duration, reticulum staining showed no change, which requires more than 8 weeks to become obvious (Fiume *et al.* 1961; Wahi *et al.* 1956).

The mechanism of this protective effect of Liv.52 remains unexplained, but the results have encouraged us to pursue a long-term study.

SUMMARY

Protective effects of Liv.52 – an Indian indigenous proprietary medicine – were noticed against carbon tetrachloride in mice both in a 72 hours and in a 21 days study. Histological changes due to carbon tetrachloride were also prevented.

Promethazine and chlorpromazine showed no protection against carbon tetrachloride as evidenced by similar percentage mortality and the nature of hepatic damage.

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