[*Quarterly Journal of Crude Drug Research* (1978): (16), 1, 8-16]

An Experimental Evaluation of Protective Effects of Some Indigenous Drugs on Carbon Tetrachloride-induced Hepatotoxicity in Mice and Rats

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INTRODUCTION

The liver is the prime organ concerned with various states of metabolic and physiologic homeostasis of the organism. In modern medicine there is no specific cure for such fearful disease as infectious hepatitis and liver cirrhosis. Treatment of many liver diseases is symptomatic and often disappointing since much is still obscure about their etiology. There is, however, a plethora of drugs in the indigenous system of medicine said to be useful in these diseases. These are mixtures of several plant ingredients e.g. Liv.52, Livergen, Liver Cure etc. considering these facts, an experimental investigation on some indigenous drugs was carried out to explore the availability of a drug, which might be useful in protecting the liver from a hepatotoxic agent. This protection was judged by reduction in hexobarbital sleeping time in mice and carbon tetrachloride (CCl_4) induced liver toxicity in albino rats.

METHODS AND MATERIALS

1. *Effect of drugs on hexobarbital sleeping time in albino mice*

   The effect of drugs on hexobarbital sleeping time was investigated in albino mice by the method of Brodie (1956). Healthy albino mice of either sex weighing 25-30 g were divided into groups of ten animals each. Hexobarbital sleeping time was determined in each group two days before and 30 minutes after CCl_4 administration. Each group, therefore, served as its own control. Two groups served as controls, one group received CCl_4 orally in doses of 0.2 ml/100 g body weight along with an equal quantity of liquid paraffin and another group received only propylene glycol. Other groups received drugs orally in the doses of 300 mg/kg one hour prior to the CCl_4 injection (0.2 ml/100 g with equal quantity of liquid paraffin). All drugs were dissolved in propylene glycol. Half an hour after CCl_4 administration, Hexobarbital in doses of 40 mg/kg was given intraperitoneally and the animals were watched for onset of sleep. The time at which loss of righting reflex occurred was taken as the starting time for sleep and the end point of sleep was noted when the animals gained the righting reflex. Difference in these two readings was taken as duration of sleep.

2. *Effect of drugs on carbon tetrachloride-induced liver toxicity in albino rats*

   Adult healthy albino rats of either sex weighing 120-150 g were divided into groups of ten animals each. Two groups served as controls. Carbon tetrachloride was given orally to all groups (except the blank control) in doses of 0.2 ml/100 g with an equal quantity of liquid paraffin. The drugs were dissolved in propylene glycol and given orally in doses of 300 mg/kg one hour prior to the CCl_4 administration. The blank control group received propylene glycol only.

Forty eight hours after CCl_4 treatment, the animals were counted in order to calculate the percent mortality, the remaining animals were sacrificed. The liver was weighed and its volume was measured in a measuring cylinder by the displacement method and standard error of the mean was calculated. Paraffin sections (5 μ thick) were prepared from a piece of the liver tissue and stained with haematoxylin and eosin and studied at 100 x and 450 x magnifications. The criteria used for histological assessment of liver damage were: (i) diffuse necrosis; (ii) hydropic degeneration; (iii) fatty degeneration; (iv) inflammatory infiltration in the liver, and (v) regenerative activity.

3. *Acute toxicity*

The approximate LD_{50} was determined in albino mice according to the method of Smith (1960).

The plants used are enumerated in Table 1. Known hepatoprotective agents e.g. ascorbic acid and Liv.52 (The Himalaya Drug Co.) were also included in the study.

Table 1

Sl. No.	Botanical Name	Sanskrit/ Hindi name	Extract and part used	LD_{50} in mice in mg/kg
1.	*Euphorbia neriifolia*	Sehund	Ethyl acetate extract of whole plant	1624 ± 36
2.	*Moringa oleifera (M. pterygosperma)*	Sehjan	Alcoholic extract of leaves	1850 ± 21
3.	*Cyperus rotundus*	Motha	Hexane extract of root tubers	2000 ± 30
4.	*Leucas cephalotes*	Moti Pati	Ethyl acetate extract of whole plant	1680 ± 21
5.	*Nymphaea stellata*	Neel Kamal	Alcoholic extract of defatted seeds	2260 ± 41
6.	*Nymphaea stellate*	Neel Kamal	Petroleum ether extract from defatted seeds	2010 ± 38
7.	*Withania somnifera*	Aswagandha	Alcoholic extract from defatted seeds	1750 ± 31

RESULTS

1. *Effects of drugs on hexobarbital sleeping time in CCl_4 treated mice*

The results of this study are summarized in Table 2. Hexobarbital in a dose of 40 mg/kg produced hypnosis of about 20 minutes duration. Carbon tetrachloride pretreatment markedly increased the duration of sleep. Liv.52, ascorbic acid, *Nymphaea stellata* (petroleum ether extract) and *Withania somnifera* pretreatment markedly reduced the prolonged hexobarbital sleeping time induced by CCl_4 and the results are statistically significant. The order of potency was found to be Liv.52 > *Nymphaea stellata* (petroleum ether extract) > ascorbic acid > *Withania somnifera. Nymphaea stellata* (alcoholic extract), *Euphorbia neriifolia, Moringa oleifera (M. pterygosperma), Cyperus rotundus* and *Leucas cephalotes* were inactive in this test.

Drugs effective in reducing the hexobarbital sleeping time also reduced the CCl_4 induced mortality. With CCl_4 the mortality rate was 50% whereas pretreatment with Liv.52, ascorbic acid, *Withania somnifera* and *Nymphaea stellata* lowered the mortality rate. Remaining drugs had no significant effect on the mortality rate.

Table 2: Effect of some indigenous drugs on hexobarbital sleeping time in CCl_4 treated albino mice					
Group No.	Pretreatment	Sleeping time in min ± SE		*'p'* value	% Mortality at 48 hours
		Before CCl_4	After CCl_4		
I	Normal (Propylene glycol)	20 ± 3.5	22 ± 2.8*	–	0
II	Propylene glycol	25 ± 4.0	124 ± 18.2	<0.001	50
III	Liv.52	25 ± 3.0	30 ± 6.1	<0.01	0
IV	Ascorbic acid	22 ±4.2	48 ± 4.2	<0.01	20
V	*N. stellate* (Alcoholic)	26 ± 5.4	117 ±19.3	>0.05	20
VI	*N. stellate (Pet. eth. extract)*	24 ± 3.8	47 ± 7.0	<0.01	0
VII	*W. somnifera*	21 ± 4.3	49 ± 6.9	<0.01	0
VIII	*E. neriifolia*	28 ± 5.4	115 ± 23.0	>0.05	50
IX	*M. oleifera*	26 ± 6.2	85 ± 11.5	>0.05	70
X	*C. rotundus*	24 ± 3.6	90 ± 14.2	>0.05	50
XI	*L. cephalotes*	23 ± 2.8	105 ± 21.0	>0.05	60
*Not treated with CCl_4. All drugs were administered in doses of 30 mg/100 g body wt. orally.					

2. *Effect of drugs on carbon tetrachloride-induced mortality and liver toxicity in albino rats*

(a) Effect on mortality: Before the animals were sacrificed for assessing the effect of indigenous drugs on CCl_4-induced changes in liver weight, volume and histological appearance, 48 hours mortality was estimated. It was observed that Liv.52, ascorbic acid, *Nymphaea stellata* and *Withania somnifera* protected animals from CCl_4-induced mortality.

(b) *Effect of drugs on liver weight and volume*

Results are summarized in Table 3. Mean liver weight in untreated (control) animals was found to be about 2 g/100 g body weight. Carbon tetrachloride treatment markedly increased the weight to about 3.5 g/100 g of body weight. Pretreatment of CCl_4-treated animals with Liv.52 ascorbic acid or *Nymphaea stellate* (alcoholic or petroleum ether extract) prevented the increase of liver weight induced by CCl_4 administration and the results were statistically significant.

Table 3: Effect of some indigenous drugs on CCl_4 induced changes in liver weight and volume of albino mice					
Pretreatment	Weight (gm/100 g body eight ± SE)	'*p*' value	Volume (ml/100 g body weight ± SE)	*'p'* value	Mortality (48 hours)
Black control (Normal)	2.12 ± 0.10	–	2.25 ± 0.12	–	0
Control (CCl_4)	3.48 ± 0.21	<0.001	4.20 ± 0.21	<0.001	60
Liv.52	2.25 ± 0.16	<0.01	2.36 ± 0.14	<0.01	10
Ascorbic acid	2.29 ± 0.16	<0.01	3.10 ± 0.11	<0.01	10
N. stellata (Alcoholic extract)	3.12 ± 0.20	>0.05	3.80 ± 0.40	>0.05	40
N. stellata (Pet. eth. extract)	2.51 ± 0.23	<0.01	2.85 ± 0.31	<0.01	20
W. somnifera	2.81 ± 0.12	<0.01	3.05 ±0.30	< 0.01	20
M. oleifera	3.40 ± 0.30	>0.05	4.10 ± 0.28	>0.05	60
E. neriifolia	3.80 ± 0.41	>0.05	4.4 ± 0.20	>0.05	50
L. cephalotes	4.00 ± 0.32	>0.05	4.60 ± 0.19	>0.05	40
All drugs were given orally in doses of 30 mg/100 g body weight followed by 0.2 ml/100 g body weight of CCl_4.					

The liver volume (vol/100 g body weight) in control animals was found to be 2.25 ml whereas in CCl_4-treated animals it was 4.2 ml. In Liv.52 pretreated rats the liver volume was similar to the normal control group. Ascorbic acid, *Nymphaea stellate* and *Withania somnifera* pretreatment also prevented CCl_4-induced increase in liver volume.

(c) *Effect on liver histology*

Histological assessment of liver injury: Carbon tetrachloride produced diffuse necrosis of the liver parenchyma, which was more marked in the layers of cells surrounding the central vein. Other ares showed hydropic acid fatty degeneration. These changes were similar to the observations reported by other (Joglekar *et al.*, 1963; Karandikar *et al.,* 1963). Liv.52, ascorbic acid, *Nymphaea stellata* and *Withania somnifera* protected the liver from the hepatotoxic effects of CCl_4. Results are summarized in Table 4.

Table 4: Effect of some indigenous drugs on CCl_4 induced histological changes in albino rats

Drugs	No. of rats treated	Number of rats showing histological changes				
		Diffuse necrosis	Hydropic degeneration	Fatty degeneration	Inflammatory infiltration	Regenerative activity
Blank control	10	X	X	X	X	X
CCl_4 (Control)	10	10	8	7	X	X
Liv.52	10	2	2	5	3	5
Ascorbic acid	10	2	3	4	4	6
N. stellate (Alcoholic)	10	4	4	9	3	X
N. stellate (pet. eth.)	10	2	3	6	4	4
W. somnifera	10	3	4	5	3	5
E. neriifolia	10	10	9	8	X	X
M. oleifera	10	10	8	7	X	X
C. rotundus	10	10	9	8	X	X
L. cephalotes	10	10	9	9	X	X

3. Approximate LD_{50} (oral) of the drugs are given in Table 1.

DISCUSSION

Up to the present time, the etiology and treatment of most liver diseases are not known. The liver is the commonest site affected during the toxic manifestation of many drugs. Liv.52 (an indigenous drug) has been shown to protect the liver against carbon tetrachloride-induced hepatotoxicity and ethanol-induced hepatic damage (Subbarao and Gupta, 1974; Subbarao, 1975; Patrao, 1957 and Sule *et al.*, 1956). The mechanism of this protective effect of Liv.52 remains to be elucidated. It definitely protects the liver from the hepatotoxic effect of CCl_4 by changing the histological appearance of the liver. Ascorbic acid also prevents experimentally induced portal cirrhosis of the liver. In our study Liv.52 and ascorbic acid were taken as controls for comparison. The results obtained are very interesting as they provide evidence in favour of the protective effects of Liv.52, Ascorbic acid, *Withania somnifera* (extract) and *Nymphaea stellata* (petroleum ether extract) against carbon tetrachloride-induced hepatotoxicity and mortality in mice and rats.

SUMMARY

- The etiology of many liver diseases is obscure and treatment is largely symptomatic.
- A number of drugs and drug combinations of the indigenous system of medicine are useful in liver diseases.
- The present study was carried out to evaluate hepato-protective activity of some indigenous plant materials against carbon tetrachloride-induced hepatotoxicity in rats and mice.
- Liv.52, ascorbic acid, *Nymphaea stellate* (petroleum ether extract) and *Withania somnifera* markedly reduced the prolongation of sleeping time and significantly prevented the increase in liver weight and liver volume induced by CCl_4.
- These drugs also prevented the necrosis induced by CCl_4 in the liver tissue and some regenerative changes were also noted in the groups treated with these drugs.
- CCl_4-induced mortality was also reduced by these drugs.

ACKNOWLEDGEMENTS

Authors are grateful to the Central Council for Research in Indian Medicine for financial help.

REFERENCES

1. BRODIE, B. B. (1956). Pathways of drug metabolism. J. Pharm. Pharmacol. *8*, 1-17.

2. JOGLEKAR, G. V., CHITLE, G. K. and BALWANI, J. H. (1963). Protection by indigenous drugs against hepatotoxic effects of CCl_4 in mice. Acta Pharmacol. et Toxicol. *20*, 73-79.

3. KARANDIKAR, S. M., JOGLEKAR, G. V., CHITLE, G. K. and BALWANI, J. H. (1963). Protection by indigenous drugs against hepatotoxic effects of CCl_4 – a long term study. Acta Pharmacol. et Toxicol. *20*, 274-280.

4. PATRAO, S. (1957). Observations in Liv.52. J. Ind. Med. Prof. *4,* 1878-1879.

5. SMITH, G. W. (1960). Pharmacological screening tests. Progress in Medicinal Chemistry. I, I, Butterworths, London.

6. SUBBARAO, V. V. (1975). Effect of an indigenous drug Liv.52 against alcohol-induced hepatic damage – a biochemical study. Paper read at 31st International Congress on alcoholism and drug dependence, February, Bangkok, Thailand.

7. SUBBARAO, V. V. and GUPTA, M. L. (1974). Anabolic and hepatoprotective actions of an indigenous drug Liv.52 Ind. J. Physiol. Pharmac. *18*, 226-227.

8. SULE, C. R., SATHE, P. M., KOSHY, M. C. and DESHPANDE, M. S. (1956). Ascites due to liver deficiency treated with an indigenous drug. The Ind. Practitioner, *9,* 357-362.