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Effect of an Indigenous Drug on I.C.G. (Indocyanine Green): Clearance and Autoradiographic patterns in Albino Rats with Experimentally-induced Hepatotoxicity

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In spite of the rapid advances in modern drug therapy, the treatment of liver disorders still confronts the practitioner with a formidable task. Certain indigenous plants from India have been claimed to have hepatic restorative and protective effects (Kirtikar and Basu, 1933).

Liv.52 is a proprietary combination (The Himalaya Drug Co.), containing the following plant principles, *Capparis spinosa, Cichorium intybus, Solanum nigrum, Cassia occidentalis, Terminalia arjuna, Achillea millefolium* and *Tamarix gallica,* and is being used in India for various hepatic ailments. It has shown promising results, both in early experimental work (Joglekar *et al.,* 1963; Karandikar *et al.,* 1963) and in preliminary clinical studies reported (Sheth *et al.,* 1960; Patrao, 1957; Sule *et al.,* 1956, 1968).

MATERIALS AND METHODS

The aim of this project was to study the short-term protective and curative ability of these plant products against a battery of hepatotoxins, using I.C.G. clearance and autoradiography of the liver with triated thymidine.

One hundred and seventy five albino Sprague Dawley male rats of an average weight range of 180-300 g were used. The hepatotoxins employed in various groups of rats were carbon tetrachloride, 0.2 ml/100 g orally, thioacetamide, 200 mg/kg i.m. and allyl alcohol (0.7% w/v), 0.4 ml/100 g orally.

Indocyanine Green (I.C.G.) clearance in a group of rats served as a control for the whole series. The effect of the hepatotoxin alone on I.C.G. clearance was determined to know the amount of hepatic dysfunction induced. Indocyanine Green Rats clearance was estimated 46 hours after the dose of carbon tetrachloride and allyl alcohol, whereas it was observed only 30 hours after thioacetamide, since the maximum effect of thioacetamide is produced by that time (Gupta, 1956).

The plant product was administered in the form of a suspension in water using gum acacia as the suspending agent. Each rat received Liv.52 in 2 ml as one dose. The effect of two such doses of Liv.52 on I.C.G. clearance was studied in separate groups.

In the preventive schedule each rat was given two doses – one 24 hours prior, the other three hours prior to the challenge dose of the hepatotoxin.

In the curative schedule, one dose was given immediately after and the other, five hours after the hepatotoxin was administered. Indocyanine Green clearance was studied after 46 hours of carbon tetrachloride or allyl alcohol administration and after 30 hours in the thioacetamide groups.

All the rats received tritiated thymidine, 1 µg/g, i.p. 4 hours prior to I.C.G. studies. The clearance studies were done by the method described by *Baumgartner et al.* (1967), disappearance rates (P.D.R.) calculated.

Rats were anaesthetised with nembutal 50 mg/kg i.p. The jugular vein and the carotid artery were cannulated. Two units of heparin was injected intravenously. Arterial blood (0.5 ml) was collected to serve as the blank. A freshly diluted aqueous solution of I.C.G. (5 mg/ml) was given intravenously in the dose of 1 mg/100 g. Arterial blood samples were collected 2, 3, 4, 5, 9 and 13 minutes later. 0.05 ml of blood was suspended in 1.95 ml of normal saline (1:40 dilution). After centrifugation, the values of I.C.G. were read at 800 mµ on a Beckmann Du Spectrophotometer. Optical density was plotted against time in minute on a semilog paper (3 cycles) and the half time was derived by extrapolation. The disappearance constant (K) was calculated using the formula

$$K = \frac{0.693}{t/2.}$$

The P.D.R. values were derived by multiplying K by 100.

Histology and autoradiography of the liver sections were studied to know the mitotic activity and the number of labelled hepatocytes, as described by Leevy *et al.*, 1959.

RESULTS: See Tables I to V.

Table 1: Showing the effect of Liv.52 on carbon tetrachloride-induced liver damage. Prior Liv.52 administration reduced the damage caused by CCl_4 and subsequent administration caused the liver to recover to control level

	Treatment	No. of animals	P.D.R.	Compared with CCl4 alone	Compared with control
1.	Control	8	18.48 ± 0.87		
2.	Liv.52 only	11	16.18 ± 0.83		
3.	CCl_4	7	9.30 ±0.48		t=8.91 p<0.001
4.	Liv.52 (Preventive) + CCl_4	9	16.75 ± 0.43	t=7.46 p<0.001	t=4.65 p<0.001
5.	CCl_4 + Liv.52 (Curative)	12	16.28 ± 0.82	t=6.07 p<0.001	t=1.78 p<0.05

Table 2: Showing the effect of Liv.52 on thioacetamide-induced liver damage. Prior Liv.52 administration reduced the extent of damage and subsequent administration caused significant recovery

	Treatment	No. of animals	P.D.R.	Compared with TA* only	Compared with control
1.	Control	8	18.48 ± 0.87		
2.	Liv.52 only	11	16.18 ± 0.83		
3.	TA	10	6.61 ± 1.01		t=12.83 p<0.001
4.	Liv.52 (Preventive) + TA	9	12.35 ± 0.65	t=7.042 p<0.001	t=5.78 p<0.001
5.	TA + Liv.52 (Curative)	9	13.31 ± 0.91	t=6.21 p<0.001	t=3.91 p<0.005

*TA=Thioacetamide

Table 3: Showing the effect of Liv.52 on allyl alcohol-induced liver damage. Prior administration of Liv.52 completely prevented the damage caused by allyl alcohol and subsequent administration caused complete recovery

Treatment		No. of animals	P.D.R.	Compared with AA* only	Compared with control
1.	Control	8	18.48 ± 0.87		
2.	Liv.52 only	11	16.18 ± 0.83		
3.	AA	10	7.57 ± 1.01		t=12.62 p<0.001
4.	Liv.52 (Preventive) + AA	9	16.03 ± 1.1	t=5.67 p<0.001	t=1.71 p<0.10
5.	AA + Liv.52 (Curative)	9	15.56 ± 1.06	t=5.43 p<0.001	t=2.10 p<0.05

*AA=Allyl alcohol

Table 4: Showing the effect of Liv.52 on injury produced by the three toxic agents as judged by mitotic activity and autoradiography of liver cells. Prior administration of Liv.52 did not alter the response of the liver to a considerable extent, but subsequent administration caused increased mitotic activity, and increased labelled hepatocytes after carbon tetrachloride and allyl alcohol indicating increased repair process

Treatment		Mitoses/10^5 cells	Labelled hepatocytes/ 10^5 nuclei
1.	Control	2.8	30
2.	Liv.52	3.2	29
3.	CCl_4	210.0	8,000
4.	Liv.52 + CCl_4	215.0	8,500
5.	CCl4 + Liv.52	300.0	10,000
6.	Thioacetamide (TA)	185.0	6,500
7.	Liv.52 + TA	200.0	6,800
8.	TA + Liv.52	210.0	7,200
9.	Allyl alcohol (AA)	240.0	9,000
10.	Liv.52 + AA	252.0	10,200
11.	AA + Liv.52	230.0	13,300

Table 5: Showing PDR (percentage disappearance Rates of Indocyanine Green) in control rats and effect of Liv.52 on carbon tetrachloride, thioacetamide and allyl alcohol-induced impairment. Liv.52 reduced the damage caused by all agents and caused complete recovery in case of carbon tetrachloride and allyl alcohol-induced impairment

	Control	Carbon tetrachloride	Thioacetamide	Allyl alcohol
Control	18.48 ± 0.81	9.30* ± 0.48	6.61* ± 0.32	7.57* ± 1.01
Liv.52 (Preventive)	16.18 ± 0.83	14.15*$^{\#}$ ± 0.43	12.32 ± 0.65*$^{\#}$	16.03$^{\#}$ ±1.1
Liv.52 (Curative)	–	16.28$^{\#}$ ± 0.82	13.31*$^{\#}$ ± 0.97	15.56$^{\#}$ ±1.06

* = Significantly different from Control control
$^{\#}$ = Significantly different from respective drug control.

COMMENTS

A wide variety of agents such as antihistamines, phenothiazines, quinine, procaine and antioxidants (Rees *et al.,* 1962; Flume *et al.,* 1961) have been studied for their protective effects against experimentally-induced hepatotoxicity. Eger (1964) has used the allyl alcohol test for quantitative evaluation of the preventive and curative ability of various drugs. Drugs with curative effects will be decidedly better therapeutically.

Many indigenous plants of India have empirically been used in liver diseases and claimed to have beneficial results. Modern scientific appraisal of this claim must be done before discarding them, since powerful drugs like digitalis glycosides, Rauwolfia and Veratrum alkaloids have been derived from the plant kingdom. This work was undertaken as a possible preliminary screening procedure for determining the immediate effect of the plant products on disturbed hepatic function.

Indocyanine Green is a tricarbocyanine dye almost exclusively excreted by the liver. Plasma disappearance of this dye is used as a test of the excretory function of the liver. This test has been considered by many observers to be a simple and satisfactory measure of liver function (Fox *et al.,* 1957; Cooke *et al.* 1963; Hunton *et al.,* 1960; Bockus, 1965; Cherrick *et al.,* 1960; Reemstsma *et al.* 1960).

The administration of Liv.52 alone did not alter the P.D.R. values in comparison with the control.

Phagocyte disappearance rate (P.D.R.) of I.C.G. in rat treated with hepatotoxins are significantly depressed as compared with the control.

Rats pre-treated with Liv.52 and subsequently challenged with hepatotoxins show significantly higher P.D.R. values than those after receiving the hepatotoxin alone. Rats receiving Liv.52 after the hepatotoxins also show significantly better clearance rates. The effects are most marked against allyl alcohol and carbon tetrachloride (curative therapy), wherein the values approach those in the control group. This indicates preservation of hepatic excretory ability and some protective and restorative effect of Liv.52 on liver function.

An increased labelling of hepatocytes with H^3-thymidine is observed with Liv.52 when administered after the hepatotoxin. This reflects an increase in DNA synthesis prior to actual cell division Higher mitotic activity is also observed. It appears that this plant product further increases the normal regenerative processes of the liver in response to hepatotoxins.

SUMMARY

Indocyanine Green clearance has been used as a parameter to study hepatic function. Hepatic damage was produced by Thioacetamide, carbon tetrachloride and allyl alcohol administration to rats. All these three agents produced significant decrease in excretion of the dye. Liv.52 given before or after the damaging agents caused increased dye excretion in all cases which came to the level of the undamaged state, except in the case of thioacetamide.

Similarly, as judged by the increase in the number of cells in autoradiographis, Liv.52 stimulated regeneration in livers damaged by carbon tetrachloride and allyl alcohol.

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