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EVALUATION OF THE PROPHYLACTIC HEPATO-PROTECTIVE ACTIVITY OF THREE HERBAL MEDICINAL PREPARATIONS AGAINST CCl_4-INDUCED LIVER DAMAGE IN RATS

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ABSTRACT

'Livotrit' is a compound Ayurvedic formulation comprising of extracts of certain medicinal herbs based on the prescriptions of ancient Ayurvedic text, for the treatment of liver disorders. The aerial parts of the herb, Phyllanthus niruri have also been utilised for this purpose in certain traditional systems of medicine and folklore practices. The present paper describes our observations on these drugs when tested for their prophylactic efficacy by applying a battery of functional, biochemical and histological parameters, against CCl_4-induced liver damage in rats. Animals receiving CCl_4 alone showed very marked increase in SGOT, SGPT and alkaline phosphatase activity and a decrease in serum triglycerides associated with a marked increase in the liver triglycerides and prolongation of hexobarbitone sleep. All these effects of CCl_4-induced hepatotoxicity were antagonised by prophylactic treatment with 'Livotrit' ($P < 0.02$). Phyllanthus niruri did not exhibit significant hepato-protective activity.

INTRODUCTION

It is generally accepted that there could be no specific drug treatment for a variety of liver disorders. The only drugs that are used, if at all, are corticosteroids or immunosuppressive agents, at times in very high doses. However, several alternative systems of medicine and certain folklore practices offer a number of herbal drugs for the treatment of liver disorders. Most of these are compound preparations containing extracts of a number of herbs and certain drugs of mineral origin. Though, only a few of these compound preparations have been experimentally evaluated under controlled conditions, in laboratory animals, their clinical use has grown remarkably in the recent past. It is evident that the practitioners of modern medicine are eager to employ these preparations, though with a certain amount of scepticism in the absence of any specific contemporary drug therapy. Vogel (1976) studied a number of plant extracts in various laboratory models of liver damage and found that, of all the herbs that he studied the seeds of the milk thistle, Silybum marianum were found to contain a flavanolignan called silymarin which showed a specific hepato-protective activity in the true sense of the term. Vogel (1976) also suggested that, carbon tetrachloride (in a single oral dose of 0.15 to 0.25 ml/kg) induced acute liver damage in rats may be used as a simple experimental model for quickly screening the herbal drugs for their hepatoprotective efficacy. The present paper describes our observations on the three herbo-mineral formulations claimed to be beneficial in the treatment of a variety of liver disorders in man, studied by using this model of liver injury in rats. Two compound Ayurvedic formulations ('Livotrit' syrup and 'Livotrit' tablets) and the fresh plant homogenate of the aerial parts of Phyllanthus niruri were investigated. The present paper describes these experiments.

MATERIALS AND METHODS:

ANIMALS : Thirty male albino rats (Haffkine Institute, Bombay) weighing around 200 g (± 10g.); were randomly distributed into five equal groups. These were housed group-wise in box-type aluminium cages (16" x 9" x 6") with SS grill top, at 27 ± 1°C. Pelleted rat feed (Lipton, India) and clean tap water was supplied ad lib. All the animals were acclaimatised to the laboratory environment for a period of one week before commencement of the experiment. The hexobarbitone (Na-salt, 100mg/kg, I.P.) sleep time of each rat was noted and those animals whose sleep-time was outside ± 2 S.D. of the mean of all the animals, were replaced with those showing a normal sleep response. This was done with the view of attaining an homogenous group of animals with a comparable basal liver function. One week later the animals received drug treatment as follows:

Gr. I	:	60% sucrose syrup
Gr. II	:	60% sucrose syrup
Gr. III	:	'Livotrit' syrup. (Batch No. 1419 of July 1985)
Gr. IV	:	Fresh plant homogenate of Phyllanthus niruri (0.5g/ml)
Gr. V	:	"Livotrit" tablets (Powdered and homogenised with 5% gum accacia in water to give a suspension of the strength 250mg/ml)

All the aforementioned treatments were administered orally by force feeding (intra-gastric tube) in a constant volume of 1 ml per 100 g. body weight of the animal. All treatments were administered once daily for five days. Twentyfour hours after the last dose of pretreatment, carbon tetra-chloride mixed in liquid paraffin (1:1) was administered orally in a dose of 0.25 ml/kg to each animal except those of the control group (Gr.I). This group received only liquid paraffin in a similar way. Then onwards the animals were fasted till the next 24 hrs, at the end of which following investigations were carried out.

I. Duration of hexobarbitone sleep:

Hexobarbitone (Na-salt) was dissolved in normal saline (10 mg/ml) and was injected intra-peritoneally in a dose of 100 mg/kg body weight (1ml/100g) of the animal. The duration of time between the loss and regain of wrighting reflex was measured for each animal. A painful stimulus was applied to the hind paw to evoke the wrighting reflex. All the animals were kept at a constant temperature (27°C ± 1°C) lying on their back during the sleep time measurements. Two hours later, all the animals were sacrificed under light ether anaesthesia. Blood samples were collected and the serum was separated by centrifugation and then stored in labelled vials at - 15°C till biochemical analysis. The entire liver tissue was dissected out and its weight and volume were noted. Around 1g. of the liver tissue was accurately weighed and homoginised in 10 ml normal saline (VirTis-23 homoginiser) centrifuged and the supernatants stored in glass vials at-15°C till determination of its triglycerides content. One lobe of liver tissue was fixed in 10% formal saline and then processed for microscopic examination. The following biochemical investigations were carried out on the serum samples, by employing Baker's 1.2.3 chemistry analyser and the reagent kits obtained from Boehringer Ingelheim (Ingo-test kits) of West Germany (through Kemwell Pvt. Ltd., Bombay).

II. Biochemical investigations:

a. SGOT - Ingotest kit 535271 for optimised kinetic UV test for the determination of GOT activity in serum.

b. SGPT - Ingotest kit 535371 for optimised kinetic UV test for the determination of GPT activity in serum.

c. Alkaline phosphatase activity - Ingotest kit 554051 for optimised standardised method for the determination of alkaline phosphatase activity in serum.

d. Liver and serum triglycerides: Ingotest kit 501071, 72 & 73 for enzymatic colour test for the determination of triglycerides and free glycerol in biological tissues.

All results were expressed as mean values for respective groups ± S.E.M. The data was analysed for statistical significance by applying student's single tail 't' test.

RESULTS:

Hexobarbitone sleep time: The duration of hexobarbitone sleep in the control group of animals (Gr. I) was 15.33 ± 2.16 min. The animals receiving carbon tetrachloride alone (Gr. II) showed a marked prolongation of the sleep time (68.25 ± 11.52 min). The animals receiving "Livotrit" syrup (Gr. III) and "Livotrit" tablets (Gr. V) showed a statistically significant antagonisum of the prolongation of sleep time induced by carbon tetrachloride (mean sleep time - Gr. III - 44.33 ± 6.04 min and Gr.V-36.80 ± 8.39 min) ($p < 0.025$ and $p < 0.005$ respectively). The duration of sleep time in animals receiving Phyllanthus niruri (Gr.IV-53.20 ± 5.39 min.) was not significantly different from that in Gr.II.($P < 0.10$). Two animals receiving Phyllanthus niruri died during hexobarbitone sleep and hence sleep times of only four animals could be recorded. These observations are presented as histograms in Fig. 1.

Biochemical parameters:

a. SGOT: A very marked rise in the GOT activity was observed in animals treated with CCl_4 as compared with that in the controls. (Control - Gr. I - 226.398 ± 10.872 units per litre, CCl_4- alone, Gr. II - 1009.188 ± 36.46 U/Lit.). Animals pretreated with 'Livotrit syrup' showed significant protection against this parameter of CCl_4-induced hepato-toxicity (Gr.III - 400.706 ± 117.02 U/Lit., $p < 0.005$). However, animals pretreated with 'Livotrit' tablets and Phyllanthus niruri did not show such a protective effect. (SGOT activity :- Gr.IV - 1439.33 ± 465.10 and Gr.V. - 1085.16 ± 269.04 U/Lit.). The values of SGOT activity showed very wide variation in these groups of animals. Thus only "Livotrit" syrup showed a significant hepato-protective effect when serum GOT activities were compared in various groups of animals. These results are expressed in Fig. 2.

b. SGPT: Here also, the CCl_4-induced marked rise in SGPT activity was significantly antagonised by pretreatment with only 'Livotrit' syrup. (SGPT Values - control, 45.56 ± 4.67; CCl_4-alone, 633.81 ± 117.61 and CCl_4 + Livotrit syrup, 184.37 ± 39.36 Units per litre, $p < 0.005$). The other two drugs, ie. 'Livotrit' tablets and Phyllanthus niruri did not exhibit such a protective action. (SGPT values - Gr.IV - 620.99 ± 90.42 and Gr.V - 744.23 ± 95.55 Units per litre). These results are presented in Fig. 3.

c. **Alkaline phosphatase activity:** The alkaline phosphatase activity in the serum was also found markedly raised on CCl_4 administration (control-228.71 ± 31.03 and CCl_4 - alone - 758.94 ± 47.32 Units/Lt.) Both, 'Livotrit' syrup and tablets showed small but significant reduction in the extent of rise in the serum of the enzyme. (AP activity - Gr. III - 606.06 ± 51.856 and Gr.V - 589-68 ± 80.80 units per litre). ($p < 0.01$). The alkaline phosphatase activity in the serum of animals receiving Phyllanthus niruri, did not show a significant difference from those in animals receiving CCl_4 alone. These results are presented in Fig. 4.

d. **Liver and Serum triglycerides:** The animals receiving carbon-tetrachloride alone showed a marked increase in the triglyceride content of the liver and a concommitant fall in their serum levels of triglycerides, as compared with the controls. (Liver Trig., Control - 4.36 ± 0.67, CCl_4 alone - 18.21 ± 1.37 mg./g.).

Both, 'Livotrit' syrup and 'tablets' showed significant protective effect against the CCl_4-induced fatty infilteration of the liver. 'Livotrit' tablets showed a much more pronounced protective effect as compared with the syrup. (Liver Trig. Gr.III - 13.25 ± 0.72 and Gr. V - 7.70 ± 0.88 mg./g.) (p 0.05, p 0.001). Animals pretreated with Phyllanthus niruri did not show any significant difference, in their liver triglycerides content (14.10 ± 1.34). Both, 'Livotrit' syrup and tablets, also antagonised the fall in serum triglycerides caused by CCl_4. These results are presented in Fig. 5 and 6.

e. **Liver Weight and Volume :-** Table I shows the liver weights and volumes in all the five groups of animals. A small but significant increase in the liver weight and volume was evident in animals treated with CCl_4-alone when compared with those in the controls. None of the three drugs were found to significantly affect the increase in weight and volume of liver induced by CCl_4.

f. **Mortality:** Two animals each in Group II and IV (i.e. animals receiving CCl_4-alone and CCl_4 + Phyllanthus niruri) died during the hexobarbitone-induced sleep. Blood samples of these animals could not therefore be collected for bio-chemical analysts.

Histological observations:

Fatty infilteration was the single, most marked histopathological change observed in animals treated with CCl_4. The extent of fat infilteration was quantitated by measuring the area occupied by fact globules in a given area in 6 different fields of view per liver tissue section observed at a fixed magnification. This was then expressed as percent of total area of view. Animals treated with CCl_4-alone showed 31.58 ± 2.95 % fat infilteration. Animals receiving pretreatment with 'Livotrit' syrup, 'Livotrit' tablets and Phyllanthus niruri showed fat infilteration to the extent of 8.22 ± 2.08, 5.40 ± 1.1 and 17.85 ± 4.7% respectively. Thus animals pretreated with both, 'Livotrit' syrup and tablets showed significant reduction in the area of fatty infilteration whereas those receiving Phyllanthus niruri did not exhibit a significant protective effect. Histological index of fat infilteration thus co-related very well with the biochemical determination of liver triglycerides.

DISCUSSION:

In the present experiments, two compound ayurvedic formulations namely 'Livotrit' syrup and 'Livotrit' tablets as well as the fresh plant homogenate of Phyllanthus niruri were evaluated for their hepatoprotective activity. Acute liver damage induced by a single oral dose of carbon-tetrachloride in rats was used as the experimental model which simulates the condition of acute viral hepatitis in man (Rege et al, 1985). A single dose of carbon-tetrachloride given orally is known to cause marked fatty infilteration of the liver tissue leading to its raised triglycerides content (Lombardi, 1966). Several other hepatotoxins have also been shown to induce a similar accumulationof triglycerides in the liver (Farber, 1967 and Roheim, 1965). Concomitant to this, the serum levels of triglycerides have been found reduced (Helmberg et al, 1962 and Lombardi and Recknagel, 1962). Our observations on the triglycerides content of liver and serum subsequent to carbon tetrachloride administration are in conformity with the above workers. This parameter of liver damage has been found to be most susceptible and sensitive in evaluating the hepatoprotective activity of drugs (Vogel, 1977 and Antweiler, 1976). These authors have reported inhibitory effect of silymarin on carbon tetrachloride and ethionine induced fat accumulation of liver. Shaligram et al (Pers. Comun.) have also observed a similar effect of Livotrit syrup and some other herbal preparations on the accumulation of triglycerides in liver of rats subsequent to CCl_4 intoxication. The present observations therefore substantiate the claims of the ancient ayurvedic literature indicating the use of such combinations in a variety of liver disorders. The two compound preparations namely 'Livotrit' syrup and 'Livotrit'

tablets significantly differ from each other with respect to their contents (refer table II and III). The tablet contains certain drugs of mineral origin in addition to the herbal constituents. In the present experiments, marked difference is seen in the activity of the two preparations. The syrup showed hepatoprotective activity on all the biochemical parameters studied whereas the tablet showed a more pronounced effect on the fat accumulation in liver caused by CCl_4 witdout affecting the raised transaminase levels. This selectivity of action needs to be further investigated with respect to Arogyavardhini Rasa, the mineral constituent of the preparation.

The histological quantitation of fatty infilteration attempted in the present experiments correlates very well with the biochemical parameters of the determination of liver triglycerides. It is therefore proposed that for a quick screening of hepatoprotective activity of herbal drugs, the biochemical determinations of liver triglycerides is a much simpler and accurate parameter as compared with the semi-quantitative technique of microscopic examination. Similar inference was also drawn by Chaudhari (1983).

To conclude therefore, the present experiments offer experimental data to substantiate the claims of efficacy of two different compound formulations described in ayurvedic texts for the treatment of liver disorders. Further studies employing graded doses of these drugs and other parameters of lipid metabolism and cell membrane stabilisation will elucidate the differences in their modes of action.

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