

Therapy of Cirrhosis of Liver and Liver Damage with Indigenous Drugs - Experimental and Clinical Studies*

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Cirrhosis of the liver¹² is quite a common condition in paediatric practice in our country and its therapy has been an intricate combination of experimentation, trial and error.

COELHO⁴ mentions, "Among affections of childhood that carry a high mortality rate, cirrhosis of liver is the one. I wonder how many of us and how often, are able to diagnose a cirrhotic liver early. Enlargement of the liver which may even be firm is quite common in the first three years of life".

ACHAR¹ divided palpable livers into various groups and also found it not easy to say whether a particular liver in a group was cirrhotic or not. PAREKH¹⁷ mentions that early manifestations of cirrhosis of the liver are vague and the condition may be called "latent cirrhosis".

Once the disease has developed and its diagnosis confirmed, known methods of therapy do not seem to offer help and in established cases the disease marches on to its inevitable fatal end². One repeatedly feels extremely disappointed to see the poor results with modern lines of treatment. This and reports of encouraging results with Liv.52 therapy by others along with results of the experimental works actuated the authors to study the effects of this new drug in the established cases.

It is a known fact that no substance is known to convert interstitial tissue into a parenchymatous one and hence, once cirrhosis of liver has developed and progressed, search for its cure is futile. All that could be hoped for, is to get functionally the best out of the remaining healthy or less affected liver parenchymatous cells.

Liv.52 is a complex combination of indigenous drugs claimed to be effective in cirrhosis of the liver. Its various constituents are: *Capparis spinosa*, *Cichorium intybus*, *Solanum nigrum*, *Cassia occidentalis*, *Terminalia arjuna*, *Achillea millefolium*, *Tamarix gallica* and *Mandur Bhasma* (prepared in juices of various hepatic stimulants and each tablet contains 324 mg of the compound).

The following observations have been based on a study of 59 cases of cirrhosis of the liver observed and treated by the authors.

The disease is definitely less common in Parsis and Muslims²⁶ and proportionately more common among Hindus. It is met with most commonly between the ages of 6 months and three years, with a maximum incidence between one and three years. In this series 3 per cent, were observed at the age of six months, 17 per cent, at one year and 63 per cent between one and two years, 14 per cent between two and three years and 3 per cent at subsequent age periods.

The dietetic history of the children varied from breast milk without supplements to artificial feeding and in older infants food such as rice, dal, chappatis and vegetables, little milk and no fruits. The

history of inadequate feeding after the stoppage of breast feeds or feeding with a high carbohydrate diet with too little milk or high fat, or low carbohydrate diet or marked loss of weight six months after weaning, was quite common. Radhakrishna Rao²¹ in Bombay found cirrhosis of liver more common in vegetarian families at the weaning time, when the child was weaned from the milk of the mother and put on protein free cereal diet. Disturbance of nutrition with or without signs of vitamin deficiency was present in 90 per cent of cases. We have come across a family where four consecutive children suffered from typical cirrhosis of liver and three of them died of the classical picture of cirrhosis of the liver and the fourth — symptomless, showed a typical picture of early cirrhosis of the liver on liver biopsy. We also have come across two other families where three children suffered from cirrhosis of the liver. All the children in each group were of the same parents and were on breast feeds. Sarma^{23,24} of Madras has reported three sets of twins with cirrhosis. It is possible that hereditary, familial and genetic factors may be responsible for this condition. Anticipation of disease in cases with family history has been found in this series in five cases. Routine interrogation and examination for other known aetiological factors did not yield information of material value.

The liver resists many forms of stress best when its stores of protein and carbohydrate are ample and its efficiency is impaired when it is laden with fat. Experimentally high fat and low protein diet produced fatty changes in the liver in dogs. In Jamaica (West Indies) fatty changes in the liver were found more frequently in the areas where cirrhosis was more common. Vitamin B₁ and its relation to choline deficiency and vitamin C and its relation to the adrenal cortex, may have some bearing.

We entirely agree with Merchant¹³ that one cannot explain all the cases of infantile cirrhosis of the liver on the basis of the dietetic factor.

Himsworth and Glynn^{7,8,9,34} brought forward evidence that besides acute massive necrosis and its sequelae of post-necrotic scarring and nodular hyperplasia, dietetic injury also produced independently an insidiously developing diffuse hepatic cirrhosis. They showed that acute massive necrosis was related to a protein, especially cysteine deficiency, and to tocopherol deficiency, and post-necrotic scarring and nodular hyperplasia to fatty infiltration of liver due to a low protein and high fat diet. They brought evidence that the lesion could be prevented by giving the amino acids containing sulphhydryl groups viz methionine and cysteine.

Trowel *et al.*³¹ found that in kwashiorkor a starchy diet deficient in proteins led to fatty liver but follow-up studies have not shown the development of cirrhosis of the liver. Higginson *et al.*,^{6,10} Sheth and Warerkar²⁷ and Sheth²⁶ studied liver in malnutrition and also cirrhosis of the liver. The survival rate in recent years has improved probably due to a high protein diet therapy and medical dietetic regime as could be seen from the comparison of the data of Ratnoff and Patex²² and Patek^{18,19,19a}. There may be other factors also in the management which may be responsible for this.

The aetiological role of infective hepatitis in cirrhosis of the liver is not quite certain. Parekh¹⁷ found it in 10 per cent, Ratnoff and Patek²² in 6.5 per cent., Howard and Watson¹⁰ in 17 per cent., and Sherlock²⁸ in 33 per cent of cases. Hepatocellular failure was the cause of death in 30.6 per cent in the series by Douglas and Snell⁵, 36.2 per cent by Ratnoff and Patek²², 13.2 per cent by Kinare by and Purandare¹¹ and 45.5 per cent by Shah and Shah²⁵. It seems that cirrhosis of the liver is the end result of a sequence of events the earlier stages of which are preventable or curable and if diagnosed earlier, a patient can be helped. Cirrhosis seems to be the result of a variety of factors causing repeated or continued liver damage singly or in combination with drugs, chemicals, bacteria, viruses, protozoa, toxic agents and dietary deficiency.

MATERIAL AND METHODS

Fifty nine cases showing cirrhosis of the liver clinically in its various stages were selected for study and clinical diagnosis was made and later confirmed, in the majority of cases, both biochemically and histopathologically. The cases were divided into early, intermediate and late cirrhosis. Thirty eight cases were treated on the usual lines and twenty-one were treated in addition with Liv.52. One to two tablets three times a day for over a period of 6 weeks to 6 months were administered.

In the first series 68 per cent expired, 15 per cent were partially relieved but not cured and the disease continued to progress slowly, and 17 per cent could not be treated.

In the second series of 21 patients six were in the early stage of cirrhosis, five in the intermediate stage and ten in the late stage. In the early and intermediate group anorexia, nausea, fever, constipation, jaundice, ascites, oedema and abdominal distension were relieved in most of the cases, but the advanced group of 10 cases did not show any response and expired. The improved group showed returning of liver function to normal as evidence by increase in total proteins and improvement in serum alkaline phosphates. Cephalin cholesterol and thymol turbidity test showed no marked improvement. Wahi³³ has studied improvement in structure and functions of the liver in disease.

When the patient showed improved in symptoms and liver function tests, decrease in size of the liver and a change in consistency of the liver, it was designated as marked improvement. When the patients showed improvement in symptoms and liver function tests but no change in size and consistency of the liver, it was designated as partial improvement. Four patients go completely cured and are progressing satisfactorily in growth five years after cessation of therapy. Functional improvement was noteworthy though histopathological changes did not revert completely to normal. Histopathological changes showed a definite decrease in fibrosis and a change in the architecture of the liver, indicating improvement in some cases. Seven patients showed partial improvement with return of appetite, clearance of ascites and oedema and complete freedom of movement.

Below are quoted the results of experiments³ carried out by one of us¹⁶ on the protective effect of Liv.52 against liver damage resulting from varying doses of carbon tetrachloride. Studies on the serum albumin, serum proteins, serum bilirubin, serum transaminase activity (using a pyruvate standard) and the effect on the histopathology of the liver were carried out. In this study mice were selected as test animals; varying doses of carbon tetrachloride were used and the effects of liver damage at varying intervals of 24 hours, five days or three or more weeks noted. In all eight experiments were carried out using twenty eight groups of ten mice each. Liv.52 in doses of 100, 200 and 300 mg per Kg of body weight was used. The first problem was to find suitable doses of carbon tetrachloride which would produce a suitable degree of central lobular necrosis. For this gingly oil solution of carbon tetrachloride was used. The required dose was contained in 0.1 cc. of the solution. The study was carried out in each experiment with a proper control group.

In the first experiment 4 groups of 10 mice were used. The first group received no carbon tetrachloride and served as a control; the second group received 0.02 cc/100 gm body weight; the third group 0.04 cc/100 gm body weight; and the fourth group 0.80 cc/100 gm body weight. The animals were sacrificed after 24 hours. Heart blood was taken and two pieces of liver were taken. One was preserved in 10 per cent formal saline the other in 95 per cent alcohol. The blood sample was examine for serum bilirubin, serum transaminase and electrophoretic studies of serum proteins. The formalin preserved material was sent to the Normal Institute of Pathology for the assessment of necrosis and the alcohol preserved material for the determination of glycogen using the Venning method and expressed as mg of glycogen per gram of liver tissue. The result of the experiment showed that there was severe damage with 0.80 cc/100 gm of carbon tetrachloride and almost

negligible damage with 0.02 cc/100 gm of carbon tetrachloride. The results seemed to indicate that a single dose of 0.50 cc/100 gm of body weight would be suitable for future experiments.

In the second experiment four groups of 10 mice each were used. The first group did not receive any carbon tetrachloride and served as a control. The second, third and fourth groups received 0.04 cc/100 gm of body weight of carbon tetrachloride every day for 5 days and the animals were sacrificed on the sixth day. The tests as in experiment 1 were performed except the serum bilirubin and transaminase activity determinations. The results indicated that the dose of 0.40 cc/100 gm had produced very extensive damage whilst the damage produced by 0.01 cc./100 gm was negligible. So for future experiments 0.02 cc/100 gm dose was decided upon.

In the third experiment four groups of 10 mice each were used. The first group did not receive any carbon tetrachloride and served as a control. The second, third and fourth groups received 0.02 cc/100 gm, 0.04 cc/100 gm and 0.08 cc/100 gm of body weight of carbon tetrachloride twice a week for three weeks. Autopsy material was taken and tests were performed as in the previous experiment. The results indicated that 0.80 cc/100 gm produced moderately severe damage and moderate damage with the dose of 0.40 cc and 0.02 cc respectively. The dose of 0.4 cc/100 gm of body weight was chosen as suitable for future experiments. By this a clear correlation was noticed between the degree of central lobular necrosis and the content of glycogen of the liver. Serum protein picture was often not numerically related to the extent of the morphological damage.

In the fourth experiment four groups of ten mice were used. The first group received 0.5 cc of water intragastrically every day for 15 days and 0.50 cc/100 gm of body weight carbon tetrachloride on the fifteenth day and served as a control. The second, third and fourth groups received 100, 200, 300 mg. Kg body weight Liv.52 respectively intragastrically every day for 15 days and 0.50 cc/100 gm of carbon tetrachloride on the fifteenth day. The animals were killed on the sixteenth day. The autopsy material and experiments were performed as in the second experiment. Results showed that whilst Liv.52 in the doses used in this experiment did not produce any demonstrable protection against central lobular necrosis by carbon tetrachloride, it had prevented the deglycogenation of the peripheral part of the lobule and had stimulated the same part of the liver lobule to synthesise serum albumin. All the three groups receiving Liv.52 showed a more normal albumin globulin ratio mainly due to the rise in the absolute concentration of albumin present.

In the fifth experiment, four groups of ten mice were used. The first group received 0.02 cc/100 gm of body weight of carbon tetrachloride and at the same time 0.05 cc. of water intragastrically every day for five days. The second, third and fourth groups received 0.02 cc/100 gm of body weight of carbon tetrachloride in each group and 100, 200, 300 mg Liv.52 per kg body weight respectively intragastrically for five days. All the animals were sacrificed on the sixth day. Autopsy material was taken and experiments were performed as in the second experiment. The results were almost like the previous experiment.

In the sixth experiment, four groups of ten mice were used. The first group received 0.50 cc/100 gm of body weight of carbon tetrachloride twice a week for seven weeks and 0.5 cc. of normal saline by mouth every day for the same period and served as a control. The second, third and fourth groups received 0.05 cc of carbon tetrachloride per 100 gm. of body weight twice a week for seven weeks and 100, 200, 300 mg Liv.52 per kg of body weight respectively every day for the same period. All the animals were sacrificed at the end of the seven weeks and the tissues examine as in the previous experiment. Serum protein in the control group was very disturbed. The results showed that Liv.52 in the doses used here whilst it did not protect the central lobular necrosis of carbon tetrachloride poisoning it did somehow stimulate the remainder of the liver lobule to greater biochemical activity.

In the seventh experiment tests for acute toxicity were carried out. Two groups of ten mice each were used. Group one received no treatment and served as a control group. Group two received 2 gm. per kg of Liv.52 as a single dose by the subcutaneous route. All animals lived for six weeks in both the groups showing that acute toxicity of Liv.52 is negligible. All the animals were sacrificed after one week had elapsed and they showed no signs upon careful microscopical examination. In the eighth experiment, chronic toxicity tests of Liv.52 were performed. Two groups of ten mice each were used. The first group received once 1 cc. of normal saline daily and the second group 1 gm. Liv.52 per kg of body weight every day for 8 weeks. All the animals were sacrificed after 8 weeks. At no time did any of the animals show any distress, none died and at autopsy examination of the organs nothing suggesting damage was discovered.

By way of summary we can say that in experiments four, five and six we have been able to show quite considerable biochemical protection but no significant protection against central lobular necrosis. The pathologist's report all indicate that there was no reduction in the extent of the area of the central part of the lobules which shows necrosis, but the remaining liver parenchyma definitely showed less deglycogenation in those animals which had been receiving the drug for 15 days prior to the dose of carbon tetrachloride. We have seen that the maximum protective action was exerted by the doses of 200 mg per kg of body weight. In just the same way we seen that there was less reduction in the serum albumin concentration in those animals which has been pre-treated with the drug. A daily dose of 200 mg per kg of the drug to mice gave almost complete protection against the fall in serum albumin obtained with a dose of 0.05 cc of carbon tetrachloride per 100 gm of body weight given on the fifteenth day. In no dose level were we able to show any alteration in the degree of fatty infiltration of the liver, neither were we able to detect any significant difference in the levels of serum transaminase. The whole picture in fact adds up to the fact that the drug has not protected cells of the central part of liver lobule against damage, but rather has protected and stimulated the more peripheral parenchymatous cells to greater biochemical activity. Experiments seven and eight show that it has no acute or chronic toxicity.

Mathur¹⁴ from Bhopal has reported clinical response to the drug in 8 cases and has found it to be very effective in 7 cases without any toxic and side effects or complications. He has found improvement in size and consistency of the liver and the degree of ascites and severity of jaundice. Sule and Sathe^{29,30} from Poona have reported treatment of 14 cases of cirrhosis of which six showed good response and two fair response. They observed definite improvement in liver function tests, total proteins, albumin-globulin ratio, haematological picture and decrease in thymol turbidity. Patrao²⁰ reported marked improvement in three out of six cases of cirrhosis of the liver. K.J. Vyas³² from Bhavnagar has reported results of 50 cases of which nine were cured and 24 were relieved.

In this series when Liv.52 (2 tablets t.d.s.) were added to therapy the preliminary results have been far more encouraging than those in the control series. We feel this drug needs further controlled double blind studies to establish its place in the therapeutics.

In conclusion we feel that in the earlier and less advanced stages, cirrhosis of the liver is either preventable or curable and that it is not very difficult to diagnose the disease at an earlier period when the patient could be helped.

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