EFFECTS OF “CIRHEP10” ON BIOCHEMICAL PARAMETERS BASED ON SERUM AND HEART IN ALCOHOL INDUCED LIVER CIRRHOSIS

Jency Blesson¹*, Sibi Narayanan¹ and V. G. Smitha²

¹Department of Research and Development, Bipha Drug Laboratories Pvt. Ltd., Kottayam-686 001, Kerala, India.
²Department of Biochemistry, School of Medical Education, Mahatma Gandhi University, Kottayam-685 587, Kerala, India.

ABSTRACT
The aim of the study was to investigate the hepatoprotective activity of the polyherbal formulation on alcohol induced liver cirrhosis albino rats. The groups of animals were administered with CIRHEP10 at different doses. The effects of CIRHEP10 on preventing the changes in the metabolism of lipids in alcohol induced liver cirrhosis was investigated. Further, the drug was also studied for its effect of decreasing the concentration of cholesterol and triglycerides in serum and heart. The results demonstrated that CIRHEP10 significantly reduces the values in level of GOT, GPT and alkaline phosphatase. The treatment with drug decreases the level of total cholesterol, free fatty acid, TAG, ALP, AST and ALT activity very significantly. Thus it significantly reduced the deleterious effect caused by the administration of alcohol. The drug also showed significant increase in total protein content and A/G ratio. All our findings suggest that CIRHEP10 could protect the liver cells from alcohol induced liver damages.

KEYWORDS: CIRHEP10, Liver cirrhosis, Serum, Heart, Alcohol.

INTRODUCTION
The liver plays vital role in the maintenance and performance of the body. In today’s world liver diseases become a global health problem, lacking helpful curative approach. Given the
high incidence and prevalence of hepatitis B, hepatitis C, and fatty liver disease, hepatic cirrhosis is a common condition in India. Generally, Indian patients first seek help from conventional medicine, and in many cases liver transplantation is suggested to them. Actually, India would require up to 20,000 liver transplants per year (Kedarisetty et al., 2014). However, currently just 200–300 transplants per annum are possible within the framework of the Indian medical system. Liver transplants are complex procedures; they require sophisticated infrastructures, expert medical teams, preservation of the transplant organs, expensive drugs, and prolonged stays in ICUs, all of which add significantly to the overall costs. A liver transplant requires about 50,000 USD, plus a lifelong commitment to immunosuppressants, costing about 2,500 USD per month, which is unmanageable for most Indian patients (Aggarwal & Goel, 2015).

As to our observation, since the only curative yet unaffordable treatment option is liver transplantation in conventional medicine. In India, Complementary and Alternative Medicine (CAM) treatments are often sought out instead, especially Ayurveda, the most established traditional Whole Medical System in South Asia with a well developed infrastructure, recognized by the World Health Organization (Rastogi & Rastogi, 2012). There are so many plants that are used as a hepatoprotective agent in traditional medicine systems. It is, therefore, necessary to assess the scientific basis for the reported hepatoprotective activity of herbal drugs in the form of PHF.

**Ayurveda and Liver cirrhosis**

Due to high prevalence of chronic hepatic diseases in South Asia, Traditional Indian Medicine (Ayurveda) has generated extensive empirical knowledge in their treatment over several centuries. In addition to observations of successfully treated cases in Ayurveda clinics, preliminary scientific findings suggest that an exploration of traditional Ayurvedic literature and commonly implemented Ayurvedic treatment modalities might be worthwhile in this field. In Ayurveda, the liver is principally governed by pitta dosha (Holikatti, 2015). Its main predominant element is fire, so dysfunction is usually expressed in pitta symptoms such as inflammation, burning, acidity or infection. Substances that are harmful to the liver can be recognized by their heating nature as well as by their toxic properties. Ayurveda has many wonderful herbs for cleaning the liver and boosting its function (Manish et al., 2015; Saper et al., 2008; Saper et al., 2004). There are several herbs with specific actions on the liver including promoting the flow of bile as well as detoxifying the liver and reducing pitta.
Plants contain many phyto-constituents, but sometimes the individual phyto-constituent may not be enough to achieve the desired therapeutic effect (Dhiman & Chawla, 2005). Therefore, the polyherbal formulation (PHF) was prepared by Bipha in order to enhance the therapeutic effectiveness and improve the bioavailability.

Ayurvedic medicine recognize that balancing liver function is pivotal to ensuring overall health. For dealing with liver problems, Ayurvedic medicines primarily enhance liver detoxification processes and help to protect against further damage to the liver (Patel, 2001). Based on traditional use, herbs are selected and combined for their ability to help to promote balance within the body and to nourish liver and related functions, including digestion and bile acid secretion. For treating liver complaints and herbal decoction consists of multiple herbs that individually have a tremendous variety of properties is commonly used. The majority of ayurvedic herbs work on multiple biochemical pathways capable of influencing several organ system simultaneously (Palbag et al., 2014). The benefit of a herbal decoction is to nourish the body as a whole by supporting various organ systems, yet its main function is to support the liver (Manvar et al., 2012). CIRHEP 10 is a hepatoprotective drug manufactured by Bipha Drug Laboratories Pvt. Ltd., Kottayam, Kerala.

MATERIALS AND METHODS

MATERIALS

Chemicals
The various general chemicals employed for different extraction procedures, separation procedures and estimation procedures were of analytical reagent grade (AR/analar) supplied by various companies like British drug house (Glaxo Laboratories) chemical division, India, E. merck, Sigma diagnostic (India) Pvt. Ltd.

Experimental animals
Adult male albino rats (Sprague dawley strain) 6-8 months old weighing 150-180g were purchased from the animal house of Veterinary College, Mannuthy, Thrissur. The rats were divided into four groups containing 6 rats in each group so that the total weight were more or less the same for different groups.

Diet for experimental rats
The rats were fed stock laboratory diet (Gold Mohur rat feed supplied by Brooke Bond Lipton, India Ltd.) and their daily requirements were measured. It was observed that 10g of
feed was required for each rat per day. The details of the given diet for each group and along with the same, the dose of alcohol administered to each group and the type of treatment are also described below.

METHODS

Collection of blood and tissues
The rats were sacrificed by decapitation after overnight fasting. Blood was collected in centrifuge tubes and were allow to clot. After this the sample were centrifuged at 3000 g for 10 minutes and clear serum was separated and used for the estimation of enzyme activity. The heart tissue was removed to ice cold containers.

Extraction of lipids from tissue
1g of tissue was homogenized with 3.4g of anhydrous sodium sulphate in a mortar. It was heated at 65ºC for two hours, with 5 ml of ethanol ether mixture (3:1). The filtrate was collected. Then the process was twice repeated with chloroform ethanol mixture (1:1) and the filtrate was added to the earlier filtrate. The final volume was made to 20 ml with chloroform ethanol mixture.

  a) Extraction of cholesterol and HDL cholesterol
4 ml of lipid extract was evaporated to dryness in a tube. It was dissolved in 2 ml of glacial acetic acid. 0.05 ml of this solution was used for the estimation.

  b) Extraction of triglycerides
2 ml of lipid extract was evaporated to dryness in a tube. It was dissolved in 0.5 ml of isopropanol, 0.1 ml of it was taken as test solution for TAG assay.

  c) Extraction of FFA
‘n’ grams tissue homogenized with S volume of extraction solvent chloroform-heptanone-methanol (5:5:1). The extract was centrifuged and the supernatant was used for the estimation.

Extraction of enzymes of liver
  a) Extraction of ALP
Accurately weighed 100 mg liver tissue was ground in a mortar with pestle under cold condition. A 10% homogenate was prepared by adding 9 volumes i.e., 0.9 ml of sodium
carbonate – sodium bicarbonate – sodium buffer (pH 10). Mixed well and centrifuged at 2000 g, the supernatant was used for the assay of enzyme.

b) **Extraction of AST and ALT**

Accurately weighed 100 mg liver tissue was ground in a mortar with pestle under cold condition. A 10% homogenate was prepared by adding 9 volumes i.e., 0.9 ml of phosphate buffer (pH 7.4), mixed well and centrifuged at 2000 g, the supernatant was used for the assay of enzyme.

**ESTIMATIONS**

**Estimation of Total Cholesterol**

Total cholesterol was estimated by Enzymatic method (Zlatkis *et al.*, 1953).

**Estimation of HDL Cholesterol**

HDL cholesterol was estimated by Phosphotungstic acid (PTA) precipitation and enzymatic method (Russell *et al.*, 2001).

**Estimation of LDL Cholesterol**

LDL cholesterol can be determined by the formula

\[
LDL = \text{Total cholesterol} - (\text{HDL cholesterol} + \frac{\text{TG}}{5})
\]

**Estimation of Triglyceride**

Triglyceride was estimated by GPO method (Rellan-Alvarez *et al.*, 2006).

**Estimation of free fatty acid**

The free fatty acids were estimated according to the procedure of Falholf et al. (Falholf *et al.*, 1973).

**Estimation of Total protein**

Total protein was estimated by Biuret method (Lowry *et al.*, 1951).

**Estimation of Albumin**

Albumin was estimated by BCG Dye binding method (Bartholomew & Delaney, 1966).
**Estimation of A/G ratio**
Protein was estimated by biuret method and albumin by BCG method by subtracting albumin from total protein, globulin fraction was found out. Then the albumin to globulin ratio was found out by dividing albumin value by globulin value.

**Assay of Alkaline phosphatase**
ALP was estimated by King and Amstrong method (King & Armstrong, 1980).

**Assay of ALT (GPT) EC 2.6.1.2**
Alanine transaminase (glutamate pyruvate transaminase) activity was estimated by a method using 2,4 Dinitrophenyl hydroxide (2,4 DNPH) (Patterson & Lazarrow, 1955).

**Assay of AST (GOT) EC 2.6.1.1**
Aspartate transaminase in liver was determined according to the procedure of Reitman and Frankel method (Reitman & Frenkel, 1957).

**Statistical Analysis**
The data given in the Tables are the average of the value from six rats indicated in each case ± SD. Statistical significance was calculated using students ‘t’ test.

**RESULTS AND DISCUSSION**

**Total cholesterol level**
Cirrhotic rats showed a highly significant size in total cholesterol level in their serum and heart as compared to normal rats (P<0.001). Rats treated with drug (both preventive and curative) showed highly significant reduction (P<0.001) in total cholesterol as compared to curative fed rats. Results are shown in Table 2 and Figure 1.

**Concentration of HDL cholesterol**
There are highly significant size in the concentration of HDL cholesterol in curative group as compared to normal groups of rats in their serum and heart (P<0.001). Both preventive as well as curative groups showed highly significant size (P<0.001) in HDL-Cholesterol serum and heart as compared to the cirrhotic groups. However the values are slightly above normal for these groups. Results are shown in Table 3 and Figure 2.
Non-cholesterol level
The cirrhotic rats showed highly significant size (P<0.001) in the non-cholesterol in their serum and heart as compared to normal group. Rats treated with drug (preventive and curative) showed highly significant decrease as compared to the cirrhotic groups. Results are shown in Table 4 and Figure 3.

Triacylglycerol level
Cirrhotic rats which were on normal diet showed very significant rate (P<0.001) in triacylglycerol levels in their serum by heart, when compared to normal rats. The treated groups (Preventive and curative) showed highly significant reduction in triacylglycerol values as compared to alcohol fed rats. Results are shown in Table 5 and Figure 4.

Free fatty acid level
Cirrhotic rats exhibited highly significant (P<0.001) free fatty acid levels in their serum when compared to normal rats. The groups treated with drug (Preventive and curative) showed highly significant (P<0.001) decrease in free fatty acid level and the groups showed comparable decrease in serum free fatty acid. Results are shown in Table 6 and Figure 5.

Concentration of total protein and A/G ratio
The concentration of total protein and A/G ratio in serum are significantly decreased (P<0.01, P<0.001) respectively) in the case of cirrhotic group when compared to normal rats. The preventive groups as well as curative group showed significant increase for total proteins (P<0.001) and A/G ratio (P<0.001) in serum as compared to alcohol fed rats. The values in the treated groups are near to normal. Results are shown in Table 7 and Figure 6.

Alkaline phosphatase level
Alcohol fed rats, which were on normal diet showed very significant rise (P<0.001) in alkaline phosphatase level in serum when compared to normal rats. Both preventive and curative groups showed highly significant decrease (P<0.001) in serum ALP activity whereas it showed significant decrease (P<0.001) in serum as compared to alcohol fed rats. Results are shown in Table 8 and Figure 7.

Glutamate pyruvate transaminase activity
The cirrhotic rats, which were on normal diet showed highly significant rise (P<0.001) in glutamate pyruvate transaminase activity in the serum when compared to normal rats. The
treated (Preventive & curative) groups showed highly significant decrease (P<0.001) in glutamate pyruvate transaminase level in serum. Both the group showed closely comparable decrease in the activity of the enzyme in serum. Results are shown in Table 9 and Figure 8.

**Glutamate oxaloacetate transaminase activity**

The cirrhotic rats which were on normal diet showed highly significant rise (P<0.001) in their activity of glutamate oxaloacetate transaminase in the serum as compared to normal diets. The groups treated with drug (Preventive and Curative) showed highly significant decrease (P<0.001) in AST activity in serum as compared to cirrhotic group. Hence the preventive group showed more significant decrease as compared to curative group. Results are shown in Table 10 and Figure 9.

Treatment with alcohol resulted in liver injury as evidenced by enhanced level of GPT (Table 9) staeotosis in liver was indicated by elevated levels of triglycerides (Table 4 & 5) and fibrosis was indicated by elevated levels of hydroxyproline as reported from the earlier studies in this lab.

Treatment of animals with CIRHEP10 produced a significant liver protective effect. This is based on the observation that administration of CIRHEP10 caused significant decrease in the degree of staeotosis and fibrosis. Staeotosis was considerably reduced as evidently by a significant reduction in the triglycerides and free fatty acid in heart and serum.

Chronic adminstration of alcohol for three months significantly increase the biochemical parameters like total cholesterol, HDL cholesterol, LDL cholesterol, triacylglycerol, free fatty acid and total protein on the development of cirrhosis and staeotosis. Other enzymes like ALP, ALT and AST were also increased in liver very significantly.

The treatment with drug decreases the level of total cholesterol, free fatty acid, TAG, ALP, AST and ALT activity very significantly. Thus it significantly reduced the deleterious effect caused by the administration of alcohol.

Serum transaminase and alkaline phosphatase was significantly reduced on treatment with drug but remains at elevated levels in cirrhotic condition. It has been reported that as cirrhotic conditions the activity of these enzymes significantly increased as a result of disrupted liver function.
A significant decrease in the total protein content and a decrease in A/G ratio is characteristic of liver injury. This has been attributed to the defective transport of metabolites and protein due to the decreased mechanical stability of liver structure under conditions of hepatic injury. The fibrotic state is associated with disorganization of cells and extracellular matrix and the delicate balance parenchyma and stroma is disrupted.

Treatment of animals with the drug significantly increased the total protein content and A:G ratio suggesting mechanical stabilisation of hepatic cells and providing free transport of proteins between the parenchyma cells and blood stream. This may also suggest regeneration of hepatic cells.

Table 1: Grouping and type of treatment.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal rat feed. This forms the normal control group</td>
</tr>
<tr>
<td>II</td>
<td>Normal rat feed + 8.4 ml of 20% v/v of Alcohol for 3 months and this forms the cirrhotic group</td>
</tr>
<tr>
<td>III</td>
<td>Normal rat feed + 8.4 ml (4ml/100g body weight) of 20% v/v of alcohol for 3 months + 0.5 ml of the drug CIRHEP10 and this forms the preventive group</td>
</tr>
<tr>
<td>IV</td>
<td>Normal rat feed + 8.4 ml (4ml/100 g body weight) 20% v/v of alcohol for 3 months and then fed 0.5 ml of drug CIRHEP10 for another one month. At this time no alcohol was given and this forms the curative group</td>
</tr>
</tbody>
</table>

Table 2: ‘t’ value for Total cholesterol levels in serum and heart.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t</td>
<td>P&lt;</td>
</tr>
<tr>
<td>I&amp;II</td>
<td>39.01</td>
<td>0.001</td>
</tr>
<tr>
<td>II&amp;III</td>
<td>12.45</td>
<td>0.001</td>
</tr>
<tr>
<td>II&amp;IV</td>
<td>7.92</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 3: ‘t’ value for HDL cholesterol levels in serum and heart.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T</td>
<td>P&lt;</td>
</tr>
<tr>
<td>I&amp;II</td>
<td>7.15</td>
<td>0.001</td>
</tr>
<tr>
<td>II&amp;III</td>
<td>9.45</td>
<td>0.001</td>
</tr>
<tr>
<td>II&amp;IV</td>
<td>9.69</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 4: t-value for LDL cholesterol in serum and heart.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t</td>
<td>P&lt;</td>
</tr>
<tr>
<td>I&amp;II</td>
<td>24.01</td>
<td>0.001</td>
</tr>
<tr>
<td>II&amp;III</td>
<td>16.80</td>
<td>0.001</td>
</tr>
<tr>
<td>II&amp;IV</td>
<td>15.94</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Table 5: t-value for Triacylglycerol levels in serum and heart.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum T</th>
<th>P&lt;</th>
<th>Heart t</th>
<th>P&lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>I&amp;II</td>
<td>35.12</td>
<td>0.001</td>
<td>70.37</td>
<td>0.001</td>
</tr>
<tr>
<td>II&amp;III</td>
<td>17.60</td>
<td>0.001</td>
<td>18.41</td>
<td>0.001</td>
</tr>
<tr>
<td>II&amp;IV</td>
<td>8.07</td>
<td>0.001</td>
<td>12.03</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 6: 't' value for free fatty acid level in serum.

<table>
<thead>
<tr>
<th>Groups</th>
<th>T</th>
<th>P&lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>I&amp;II</td>
<td>20.27</td>
<td>0.001</td>
</tr>
<tr>
<td>II&amp;III</td>
<td>15.55</td>
<td>0.001</td>
</tr>
<tr>
<td>II&amp;IV</td>
<td>10.66</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 7: t-values for Total protein, A/G ratio in serum.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total protein t</th>
<th>P&lt;</th>
<th>A/G ratio t</th>
<th>P&lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>I&amp;II</td>
<td>3.90</td>
<td>0.001</td>
<td>21.25</td>
<td>0.001</td>
</tr>
<tr>
<td>II&amp;III</td>
<td>3.53</td>
<td>0.001</td>
<td>6.92</td>
<td>0.001</td>
</tr>
<tr>
<td>II&amp;IV</td>
<td>3.49</td>
<td>0.001</td>
<td>4.97</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 8: t-value for Alkaline phosphatase activity in serum.

<table>
<thead>
<tr>
<th>Groups</th>
<th>T</th>
<th>P&lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>I&amp;II</td>
<td>10.9</td>
<td>0.001</td>
</tr>
<tr>
<td>II&amp;III</td>
<td>12.38</td>
<td>0.001</td>
</tr>
<tr>
<td>II&amp;IV</td>
<td>7.17</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 9: t-value for Glutamate pyruvate transaminase (GPT/ALT) activity in serum

<table>
<thead>
<tr>
<th>Groups</th>
<th>T</th>
<th>P&lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>I&amp;II</td>
<td>25.72</td>
<td>0.001</td>
</tr>
<tr>
<td>II&amp;III</td>
<td>7.91</td>
<td>0.001</td>
</tr>
<tr>
<td>II&amp;IV</td>
<td>4.61</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 10: t-value for Glutamate oxaloacetate transaminase activity (GOT/AST) in serum.

<table>
<thead>
<tr>
<th>Groups</th>
<th>T</th>
<th>P&lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>I&amp;II</td>
<td>19.28</td>
<td>0.001</td>
</tr>
<tr>
<td>II&amp;III</td>
<td>10.31</td>
<td>0.001</td>
</tr>
<tr>
<td>II&amp;IV</td>
<td>8.70</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Figure 1: Total cholesterol levels in serum (mg/100ml) and heart (mg/100g wet tissue)
Values are mean of six rats ± SD.

Figure 2: HDL cholesterol levels in serum (mg/dl) and heart (mg/100g wet tissue)
Values are mean of six rats ± SD.

Figure 3: LDL cholesterol in serum (mg/dl) and heart (mg/100mg wet tissue) Values are mean of six rats ± SD.
Figure 4: Triacylglycerol levels in serum (mg/dl) and heart (mg/100g wet tissue) Values are mean of six rats ± SD.

Figure 5: Free fatty acid level in serum (mg/dl) Values are mean of six rats ± SD.

Figure 6: Total protein, A/G ratio in serum (mg/100ml) Values are mean of six rats ± SD.
Figure 7: Alkaline phosphatase activity in serum (IU/L) Values are mean of six rats ± SD.

Figure 8: Glutamate pyruvate transaminase (GPT/ALT) activity in serum (IU/L) Values are mean of six rats ± SD.

Figure 9: Glutamate oxaloacetate transaminase activity (GOT/AST) in serum (IU/L) Values are mean of six rats ± SD.
CONCLUSION
Alcohol induced liver cirrhosis induced changes in the metabolism of lipids in serum and heart. Treatment with drug showed a decrease in the concentration of cholesterol and triglycerides in serum and heart. GOT, GPT and alkaline phosphatase serum and heart were increased and showed significant reduction in their values. The total protein and A/G ratio was decreased in cirrhosis. Treatment with the drug showed significant increase in total protein content and A/G ratio. From the above observations, it can be concluded that CIRHEP10 may be effective in preventing the changes in the metabolism of lipids in alcohol induced liver cirrhosis.

ACKNOWLEDGEMENTS
The authors would like to gratefully acknowledge the School of Medical Education, MG University, Kottayam. We also express sincere thanks to Bipha Drug Laboratories Pvt. Ltd., Kottayam for assisting in the completion of this study.

REFERENCES
1. Kedarisetty, CK; Anand, L; Khanam, A; Kumar, A; Rastogi, A; Maiwall, R; Sarin, SK. Growth factors enhance liver regeneration in acute-on-chronic liver failure. Hepatol Int., 2014; 8: 514-525.
5. Manish, VP; Kalapi, BP; Shivenarain, G; Andreas, M; Elmar, S; Christian SK. A Complex Multiherbal Regimen based on ayurveda medicine for the management of hepatic cirrhosis complicated by ascites: Nonrandomized, uncontrolled, single group, open-label observational clinical study. Evidence Based Complementary and Alternative Medicine, 2015; 1-12.


