Abstract. – Background and Objectives: Herbal medicines have good curative effect on certain diseases especially for diabetes mellitus which needs continuous medication throughout the life. Present day allopathic medicines are costlier and having more side effects which could cause severe damages to the vital organs. Hence, finding a suitable herbal medicine for diabetes mellitus is very important in the current situation. In this present study, the fruit extract of Helicteres isora was used to evaluate the antihyperlipidemic activity in streptozotocin induced diabetic rats.

Material and Methods: Powdered fruits of Helicteres isora were extracted in ethanol and the crude extract was used for the treatment of diabetic rats. Streptozotocin was used to induce the diabetic condition in wistar rats. For the treatment, the drug glibenclamide also used to treat the diabetic rats to compare the efficacy of the herbal extract. After 45 days of treatment, the animals were sacrificed and lipid profiles were estimated in the serum and liver.

Results: The serum and liver lipid levels were abnormal in streptozotocin induced diabetic rats than in the control rats. Total cholesterol, triglycerides, phospholipids, LDL and VLDL were elevated and the HDL level was significantly decreased in diabetic rats. After treated with Helicteres isora fruit extract (HiFE), the lipid levels of diabetic rats were restored to near normal level.

Discussion: HiFE has the potential of antihyperlipidemic activity which was proved by the above results. It is suggested that HiFE may have the similar action mechanism of glibenclamide.

Key Words: Antihyperlipidemic activity, Helicteres isora, Diabetes mellitus, Streptozotocin.

Introduction

Diabetes mellitus is described as a glucose imbalance and impaired insulin secretion or action and the diabetic condition damages vital organs like the eyes, kidneys, nerves, heart, and blood vessels. Diabetes mellitus is the main disorder which causes most of the disabilities and death in the world. Sedentary life style, changing food habits, stress, obesity, genetical alterations and environmental factors are the main causes for diabetes mellitus. Hyperglycemia is a symptom of diabetes mellitus which causes glycation of proteins which leads to functional and morphological changes in eyes, kidneys, nerves and arteries.

In diabetic condition, age increases with serious complications which often results in death and the resources like medicines, diets and physical training are extensively used all over the world. Generally, lipids are useful as energy reserves, insulating material to maintain the temperature and many other functions in the body. However lipids have more vital roles in the body, they also produce adverse effects in the human body.

Even though various well-known conventional medicines are available in the market, herbal medicines are used successfully. Traditional treatments have been recommended to treat diabetes mellitus along with conventional system of medicine. Many herbal plants are having the active compounds like glycosides, alkaloids, terpenoids, flavonoids etc. are the potential hyperglycemic agents. The scientific data over the mode of action of herbal plant drugs or herbal formulation used for treating diabetes is scanty.
Preparation of Plant Extract

The ethanolic extract of Helicteres isora fruits was prepared according to the method of Hossain et al\textsuperscript{17}. 500 g of fresh Helicteres isora fruit powder was soaked in 1500 ml of 95% ethanol overnight. After filtration, the residue obtained was again re-suspended in equal volume of 95% ethanol for 48 hours and filtered again. The above two filtrates were mixed and the solvent was evaporated in a rotavapor at 40-50°C under the reduced pressure. The final concentrated extract obtained was stored at 0-4°C until used. A known volume of the ethanolic residual extract was suspended in distilled water and was orally administered to the animals by gastric intubation using a force-feeding needle during the experimental period.

Induction of Diabetes

Diabetes mellitus was induced in overnight fasted wistar rats by a single intraperitonial injection of streptozotocin (50 mg/kg body weight) in freshly prepared citrate buffer (pH 4.5).

Experimental Design

A total number of 30 rats (18 diabetic rats: 12 normal rats) were used and the rats were divided into 5 groups of six each. Group I served as control animals. Group II animals were treated with Helicteres isora fruit extract (HiFE) 300 mg/kg alone for the period of 45 days. Group III animals were treated with single intraperitoneal injection of streptozotocin (50 mg/kg body weight) after overnight fast for 12 hours. Determining the blood glucose concentration on 3 days and 5 days after streptozotocin treatment were assessed for diabetic condition. The rats with blood glucose level above 250 mg/dl were selected for the experimental study. Group IV animals were received HiFE (300 mg/kg) once daily for 45 days after the diabetic state was assessed. Group V animals were received glibenclamide (600 µg/kg body weight) once daily for 45 days after the diabetic state was assessed.

Biochemical Parameters

Glucose content was estimated by O-Toluidine method\textsuperscript{18}. Total cholesterol was estimated by the method of Parekh and Jung\textsuperscript{19}. Phospholipids were estimated by the method of Zilversmit and Davis\textsuperscript{20} and triglycerides were estimated by the method of Foster and Dunn\textsuperscript{21}. HDL was estimated by the method of Gidez and Webb\textsuperscript{22}. VLDL and LDL were calculated by using the formula of Friedewald et al\textsuperscript{23}.

Materials and Methods

Experimental Animals

Male albino wistar rats, weighing 150-200 g were used for the present study. The animals were obtained from Central Animal House, Rajah Muthiah Institute of Health Sciences, Annamalai University, Tamil Nadu, India and were maintained in an air-conditioned experimental room at 12 hour light: dark cycles. The animals were randomized into experimental and control groups and were housed 4 or 5 in a polypropylene cage. Standard pellets were used as a basal diet during the experimental period. The control and experimental animals were provided with purified drinking water ad libitum. The animals were maintained in accordance with the “CPCSEA guidelines for laboratory Animal Facility” (Committee for the Purpose of Control and Supervision on Experiments on Animals) and the approval number is 451/09.10.2007.

Chemicals

Streptozotocin was purchased from Sigma-Aldrich Chemicals Pvt Ltd, Bangalore, India. All other chemicals and reagents used were of analytical grade.

Plant Material

The fruits of Helicteres isora were purchased from local traditional market, Chidambaram, Tamil Nadu, India and were botanically authenticated by the botanists (Department of Botany of the Annamalai University). The fruits of Helicteres isora were shade dried for 15 days and powdered.
VLDL = TG/S and LDL was calculated by using the formula:
\[
LDL = \text{Total Cholesterol} - (\text{HDL} + \text{VLDL})
\]

Atherogenic Index (AI)²⁴ = \[
\frac{\text{LDL-cholesterol}}{\text{HDL-cholesterol}}
\]

Coronary Risk Index (CRI)²⁵ = \[
\frac{\text{Total cholesterol}}{\text{HDL-cholesterol}}
\]

### Statistical Analysis

Values were expressed as mean ± S.D. The data were statistically analyzed using ANOVA followed by Duncan Multiple Range Test (SPSS 11.5) and considered statistically significant if the p-value was less than 0.05.

### Results

Table I shows the serum total cholesterol, triglycerides, HDL and LDL levels of control and streptozotocin induced diabetic rats. Total cholesterol, triglycerides and LDL were significantly increased in streptozotocin induced diabetic rats when compared with controls. HDL level was decreased in streptozotocin induced diabetic rats when compared with control rats. The status of total cholesterol, triglycerides, HDL and LDL levels were restored in streptozotocin treated rats after treatment with *Helicteres isora* fruit extract and glibenclamide. *Helicteres isora* fruit extract has the restoring capability like that of glibenclamide.

Table II shows the serum phospholipids, VLDL, atherogenic index (AI) and coronary risk index (CRI) of normal and streptozotocin induced diabetic rats. Serum phospholipids, VLDL, atherogenic index and coronary risk index of streptozotocin treated rats were significantly increased when compared with control rats. The status of serum phospholipids, VLDL, atherogenic index and coronary risk index of streptozotocin induced diabetic rats were restored as like that of controls after treatment with *Helicteres isora* fruit extract and glibenclamide.

Table III shows the liver total cholesterol, triglycerides and phospholipids of control and streptozotocin induced diabetic rats. Liver total cholesterol, triglycerides and phospholipids were significantly increased in diabetic rats and were restored in diabetic rats treated with *Helicteres isora* fruit extract and glibenclamide.

### Discussion

Diabetes mellitus, a third leading cause of death²⁶. Prolonged hyperglycemia ends with severe complications. Hyperglycemia generates more oxidative stress. Free radicals react with lipids and causing lipid peroxidation. Manimegalai et al²⁷ had reported that the increased level of oxidative stress will increases the hyperlipidemia in animals.

In the present study, antihyperlipidemic activity of *Helicteres isora* fruit extract (HiFE) has been evaluated in normal and streptozotocin induced diabetic rats.

Cholesterol is a sterol useful in cell membrane integrity and precursor for steroid hormones. Total cholesterol level is increased in diabetic rats when compared with control rats. Diabetes mellitus leads with impaired carbohydrate metabolism and increased lipolysis causes accumulation of acetyl Co A. Increased availability of acetyl Co A leads to synthesis of cholesterol and causes hyperlipidemia²⁸. The increased total cholesterol level was

### Table I. Effect of *Helicteres isora* fruit extract on serum total cholesterol, triglycerides, HDL and LDL levels of control and diabetic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>78.2 ± 4.2a</td>
<td>69.7 ± 3.4a</td>
<td>41.3 ± 3.8a-d</td>
<td>22.8 ± 5.8a</td>
</tr>
<tr>
<td>Control + Extract (300 mg/kg)</td>
<td>77.1 ± 4.5a</td>
<td>63.2 ± 2.9a</td>
<td>43.3 ± 3.9a</td>
<td>21.2 ± 1.1a</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>137.9 ± 9.1c</td>
<td>164.1 ± 9.3c</td>
<td>30.1 ± 3.3a</td>
<td>74.9 ± 9.2c</td>
</tr>
<tr>
<td>Diabetic + Extract (300 mg/kg)</td>
<td>107.2 ± 6.4b</td>
<td>114.5 ± 4.9b</td>
<td>35.6 ± 4.0b</td>
<td>48.6 ± 8.7b</td>
</tr>
<tr>
<td>Diabetic + Drug (600 µg/kg)</td>
<td>101.2 ± 10.1b</td>
<td>108.7 ± 4.6b</td>
<td>37.0 ± 4.9b-c</td>
<td>42.4 ± 11.5b</td>
</tr>
</tbody>
</table>
restored in the diabetic rats treated with *Helicteres isora* fruit extract (HiFE) and glibenclamide. Insulin has the significant role in lipid metabolism. Insulin deficiency is associated with hypercholesterolemia due to metabolic abnormalities\textsuperscript{28}. Serum lipids increased by lipolysis due to insulin deficiency in diabetic rats\textsuperscript{29}. Usually, insulin increases the lipogenesis and decreases lipolysis and ketogenesis. However in diabetic condition, insulin deficiency reverses the above said role in lipid metabolism. Triglycerides (triacylglycerols) are neutral fats, major energy reserve for the body stored at adipose tissue. Diabetic condition increases the lipolysis and produces more free fatty acids (FFA). Increased release of FFA increases the production of ketone bodies and triglycerides synthesis. In the present investigation, triglycerides are increased significantly. Normally, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. But in diabetic state, lipoprotein lipase is not activated due to insulin deficiency, resulting in hypertriglyceridemia\textsuperscript{30}. The increased level of triglycerides was well maintained in diabetic rats after treated with HiFE and glibenclamide. This result has coincided with the report by Sharma et al\textsuperscript{31}.

The abnormal high concentration of serum lipids in diabetes is mainly due to the increased release of FFA from the peripheral tissues. Lipolysis is also enhanced by glucagon and catecholamines\textsuperscript{32}.

The elevated serum phospholipid levels result in elevation of lipoproteins\textsuperscript{33}. Phospholipids, cholesterol and triglycerides are formed as lipoproteins in the liver\textsuperscript{34}. Lipoproteins have the major role in the occurrence of premature atherosclerosis in diabetic patients\textsuperscript{35}. LDL has a significant role in atherosclerosis and other related diseases. In the present study LDL level was increased in streptozotocin induced diabetes mellitus. LDL transports cholesterol from liver to other peripheral tissues. LDL is formed from VLDL-cholesterol. VLDL level also increased in the present study. It denotes the increased production of LDL cholesterol. The effective control of glycemic imbalance will reduce the VLDL and triglyceride levels\textsuperscript{36}. HDL cholesterol exerts a role in the prevention of atherosclerosis by transporting the cholesterol from peripheral tissues to liver for excretion. HDL decrease was observed in the present study in diabetic rats which will increase the chances of atherosclerosis. HDL decrease was restored in the treated diabetic rats shows the potential of alleviating capacity of HiFE. The raised plasma total cholesterol and LDL concentrations are having negative correlation with plasma HDL\textsuperscript{37}. Increase in HDL

### Table II. Effect of *Helicteres isora* fruit extract on serum phospholipids, VLDL, atherogenic index and coronary risk index of control and diabetic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Phospholipids (mg/dl)</th>
<th>VLDL (mg/dl)</th>
<th>Atherogenic index</th>
<th>Coronary risk index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>118.1 ± 6.2\textsuperscript{b}</td>
<td>14.0 ± 0.5\textsuperscript{b}</td>
<td>0.56 ± 0.1\textsuperscript{a}</td>
<td>1.90 ± 0.20\textsuperscript{a}</td>
</tr>
<tr>
<td>Control + Extract (300 mg/kg)</td>
<td>107.0 ± 7.6\textsuperscript{a}</td>
<td>12.6 ± 0.50\textsuperscript{a}</td>
<td>0.49 ± 0.05\textsuperscript{a}</td>
<td>1.78 ± 0.06\textsuperscript{a}</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>1674 ± 7.8\textsuperscript{c}</td>
<td>32.8 ± 1.8\textsuperscript{d}</td>
<td>2.51 ± 0.4\textsuperscript{a}</td>
<td>4.60 ± 0.49\textsuperscript{a}</td>
</tr>
<tr>
<td>Diabetic + Extract (300 mg/kg)</td>
<td>144.8 ± 9.2\textsuperscript{d}</td>
<td>22.9 ± 1.0\textsuperscript{e}</td>
<td>1.39 ± 0.3\textsuperscript{b}</td>
<td>3.04 ± 0.47\textsuperscript{b}</td>
</tr>
<tr>
<td>Diabetic + Drug (600 µg/kg)</td>
<td>128.0 ± 5.7\textsuperscript{c}</td>
<td>21.7 ± 0.9\textsuperscript{c}</td>
<td>1.18 ± 0.4\textsuperscript{b}</td>
<td>2.78 ± 0.48\textsuperscript{b}</td>
</tr>
</tbody>
</table>

### Table III. Effect of *Helicteres isora* fruit extract on liver total cholesterol, triglycerides and phospholipids levels of control and diabetic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total cholesterol (mg/g)</th>
<th>Triglycerides (mg/g)</th>
<th>Phospholipids (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.6 ± 0.7\textsuperscript{a}</td>
<td>17.8 ± 1.5\textsuperscript{c}</td>
<td>23.9 ± 2.0\textsuperscript{b}</td>
</tr>
<tr>
<td>Control + Extract (300 mg/kg)</td>
<td>4.6 ± 0.4\textsuperscript{a}</td>
<td>17.2 ± 1.0\textsuperscript{c}</td>
<td>21.8 ± 2.9\textsuperscript{c}</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>9.5 ± 1.2\textsuperscript{c}</td>
<td>42.4 ± 1.7\textsuperscript{d}</td>
<td>48.8 ± 3.4\textsuperscript{d}</td>
</tr>
<tr>
<td>Diabetic + Extract (300 mg/kg)</td>
<td>7.1 ± 0.8\textsuperscript{b}</td>
<td>29.0 ± 2.9\textsuperscript{c}</td>
<td>31.3 ± 2.9\textsuperscript{b}</td>
</tr>
<tr>
<td>Diabetic + Drug (600 µg/kg)</td>
<td>6.3 ± 0.5\textsuperscript{b}</td>
<td>24.9 ± 3.8\textsuperscript{b}</td>
<td>30.3 ± 2.8\textsuperscript{b}</td>
</tr>
</tbody>
</table>
cholesterol is associated with a decrease in coronary risk38.

The coronary risk is well established by the elevated levels of total cholesterol and especially LDL cholesterol39. Atherogenic index (AI) and elevated levels of total cholesterol and especially to elucidate the mechanism of action.

on to separate the active compound in HiFE and induced diabetic rats. Further findings are going lipidemic condition occurring in streptozotocin has the active principle to counteract the hyper-

well documented that the fruit of Heliceres isora has the active principle to counteract the hyper-

lipidemic condition to its normal level as compared with glibenclamide.

In conclusion, from the present findings, it is well documented that the fruit of Heliceres isora has the active principle to counteract the hyperlipidemic condition occurring in streptozotocin induced diabetic rats. Further findings are going on to separate the active compound in HiFE and to elucidate the mechanism of action.

References


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