INTRODUCTION

Diabetes mellitus is a group of metabolic disorder characterized by hyperglycemia and alteration in the carbohydrate, fat and protein metabolism associated with absolute or relative deficiencies in insulin secretion or insulin action. In the present study antihyperlipidemic activity of Parmelia perlata. Ach. has been evaluated in alloxan induced diabetic albino rats. The aqueous extract of the selected plant was administered at dose levels of 200mg and 400mg/kg body weight for 60 days. After the experimental period the blood and tissue samples were collected and subjected to various biochemical and enzymic parameters. There were profound alteration in fasting blood glucose, serum insulin, glycooxygenated hemoglobin (HbA1C) and liver glycogen levels in alloxanized rats. Glucose-6-phosphate, glucokinase, and fructose 1,6-bisphosphate activity were also altered in diabetic rats. Administration of plant extract significantly (P<0.05) reduced the fasting blood glucose and HbA1C level and increased the level of plasma insulin. The activities of glucose metabolizing enzymes were also resumed to normal. There was a profound improvement in serum lipid profiles by reducing serum triglyceride, cholesterol, LDL, VLDL, free fatty acids, phospholipids and increasing the HDL level in a dose dependent manner. The effects of leaf extract were compared with standard drug glibenclamide (600μg/Kg bw). The results indicate that Parmelia perlata. Ach., Linn. could be a good natural source for developing an antidiabetic drug that can effectively maintained the blood glucose levels and lipid profile to near normal values.

Keywords: Parmelia perlata. Ach., Insulin, HbA1C, Glucose-6-phosphatase, Glucokinase, Cholesterol, Triglycerides, HDL, LDL.

MATERIAL AND METHODS

Preparation of plant extract

Parmelia perlata Ach. were obtained from places in and around Trichy identified and authenticated with the herbarium specimen of RAPINAT herbarium of St Joseph's College, Trichy, Tamilnadu, India. The collected drug materials were coarsely powdered with electrical blender

200 g of Parmelia perlata Ach. coarse powder was taken and extracted with water. To one part of the material six parts of water was added, boiled and reduced to one third and the filtrate was evaporated to dryness. Paste form of the extract obtained was subjected to pre-clinical screening. The percentage yield of extract was 46.

Experimental Animals

Healthy adult wistar strain of albino rats of either sex, weighing 150-200 g was used as experimental models. Animals were kept in well-ventilated cages and fed with standard rat chow pellet obtained form Sai Durga Food and Feeds, Bangalore, India and water ad libitum. Animals were maintained under standard laboratory conditions (Temperature 24-28ºC, relative humidity 60-70%). After obtaining necessary clearance from the committee (approval No: 790/03/ac/CPSEA), the studies were conducted according to the ethical guidelines of CPCSEA.

Alloxan induction

Diabetes mellitus was induced in normoglycemic albino rats kept under starvation for 16 hours to group II, III, IV and V. 150mg/kg body weight of alloxan monohydrate was dissolved in physiological saline and injected intraperitoneally (IP). This dose of alloxan produced persistent hyperglycemia after 4 days as revealed by determination of sugar levels by the analysis of blood and urine samples [5].

Experimental design

The rats were divided into five groups each comprising of six rats.
Group I: Normal control

Group II: Animals treated with alloxan monohydrate in normal saline at a dosage of 150mg/kg body weight IP.

Group III: Animals were treated as in Group II. After 4 days of alloxan induction, treated with Parmelia perlat Aeh. aqueous extract (PPAE) - 200mg/kg body weight, orally for 60days.

Group IV: Animals were treated as in Group II. After 4 days of alloxan induction, treated with Parmelia perlat Aeh. aqueous extract (PPAE) - 400mg/kg body weight orally for 60 days.

Group V: Animals were treated as in Group II. After 4 days of alloxan induction, treated with standard drug glibenclamide - 600μg/Kg body weight orally for 60 days.

Collection of blood and liver from the rat

At the end of the experimental period, animals were sacrificed by cervical decapitation. Blood was collected and serum was separated by centrifugation (for 15min at 2000rpm). The liver were dissected out and washed in ice-cold saline. Tissues were cut into small pieces and homogenized, in 0.1M phosphate buffer (pH 7.4). The homogenate was centrifuged and the supernatant was used for various biochemical and enzymatic analysis.

The parameters studied

Fasting blood glucose by folin-Wu's method [6], glycogen [7], plasma insulin [6], Glycylated hemoglobin (HbA1C) [9] and protein were analyzed in blood sample. Glucokinase [10], Glucose-6-phosphatase [11&12], fructose 1-6 bisphosphatase [13] in liver tissues. Serum lipid profiles evaluation includes cholesterol [14], triglyceride [15], phospholipids [16&12], free fatty acids [17], HDL [18], LDL [18], and VLDL [18].

Statistical analysis

All the results were expressed as mean ± S.E.M. The data were statistically analyzed by one – way analysis of variance (ANOVA) between plant extract treated groups and disease control group. P values <0.05 were considered as significant.

RESULTS

The effect of PPAE on blood glucose, glycylated haemoglobin (HbA1C) serum insulin and liver glycogen were shown in Table: 1. Administration of alloxan (150 mg/kg; b.w; IP) showed an elevated level of fasting blood glucose and glycylated haemoglobin (HbA1C) and reduction in serum insulin and liver glycogen compared to normal rats, which might be due to the destruction of beta-cells. Oral administration of aqueous extract of Parmelia perlat Aeh (200 mg/kg; b.w and 400 mg/kg; b.w) for 60 days showed a significant reduction (P < 0.05) in blood glucose and HbA1C levels, when compared with diabetic control animals. Serum insulin and glycogen levels were also restored to near normal.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Blood Glucose (mg/dl)</th>
<th>Serum insulin (μU/ml)</th>
<th>HbA1C (%)</th>
<th>Glycogen (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>86.4 ± 9.20</td>
<td>6.4 ± 2.61</td>
<td>2.6 ± 0.21</td>
<td>465.8 ± 891</td>
</tr>
<tr>
<td>Diabetic</td>
<td>270.4 ± 21.24*</td>
<td>4.3 ± 0.93*</td>
<td>6.6 ± 1.41</td>
<td>11.7 ± 0.91*</td>
</tr>
<tr>
<td>Diabetic + PP treated (200mg/Kg)</td>
<td>211.7 ± 18.26**</td>
<td>4.9 ± 0.74**</td>
<td>5.9 ± 1.62</td>
<td>227.5 ± 5.05**</td>
</tr>
<tr>
<td>Diabetic + PP treated (400mg/Kg)</td>
<td>101.2 ± 12.73**</td>
<td>6.1 ±0.97 **</td>
<td>2.9 ± 0.55</td>
<td>37.5 ± 7.25**</td>
</tr>
<tr>
<td>Diabetic + Gilbenclamide (600μg/Kg )</td>
<td>83.4 ± 7.85</td>
<td>5.7 ± 0.64</td>
<td>3.1 ± 0.76</td>
<td>41.3 ± 8.05</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM (n=6)

Inter-group comparison

*P < 0.05 statistically significant when Group II and Group I were compared

**P < 0.05 statistically significant when Group III & IV were compared with Group II.

The activity of glycolytic and gluconeogenic enzymes in control and PPAE treated rats were shown in Table: 2. Alloxanized group II rats showed marked alteration in the activity of glucokinase, Fructose – 1 – 6 bisphosphatase and glucose – 6 – phosphatase. Treatment with PPAE at a dose level of 200mg/Kg bw and 500mg/Kg bw increases the activity of glucokinase activity and reduces the activity of Fructose – 1 – 6 bisphosphatase and glucose – 6 – phosphatase. The effect was found to be higher in higher dose (400mg/Kg) than the lower dose (200mg/Kg).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Glucokinase (μ moles of Glu-6-PO4 formed/ min/ mgprotein)</th>
<th>Fructose – 1- 6- bisphosphatase (μ moles of PO4 liberated/ min/mg protein)</th>
<th>Glucose –6 – phosphatase (μmoles of Pi liberated/ min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>136.7 ± 20.26</td>
<td>26.4 ± 5.61</td>
<td>7.7 ± 1.21</td>
</tr>
<tr>
<td>Diabetic</td>
<td>96.2 ± 12.05*</td>
<td>124.7 ± 11.23*</td>
<td>15.4 ± 2.24*</td>
</tr>
<tr>
<td>Diabetic + PPAE treated (200mg/Kg)</td>
<td>102.7 ± 17.24**</td>
<td>99.7 ± 8.52**</td>
<td>12.7 ± 2.11**</td>
</tr>
<tr>
<td>Diabetic + PPAE treated (400mg/Kg)</td>
<td>128.5 ± 16.05**</td>
<td>33.7 ± 8.11**</td>
<td>9.7 ± 1.66**</td>
</tr>
<tr>
<td>Diabetic + Gilbenclamide (600μg/Kg )</td>
<td>134.6 ± 18.65</td>
<td>32.4 ± 6.62</td>
<td>7.1 ± 1.67</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM (n=6)

Inter-group comparison

*P < 0.05 statistically significant when Group II and Group I were compared

**P < 0.05 statistically significant when Group III & IV were compared with Group II.

Natural products in the Management of Cancer, Diabetes and Viral infections

Brindha et al.

Int J Pharm Pharm Sci, Vol 6, Suppl 1, 43-46
The effect of PPAE on serum total cholesterol, triglyceride (TGL), phospholipids (PL) and free fatty acid (FFA) were summarized in Table 3. Animals treated with test drug showed a profound (P<0.05) decrease in the levels of serum cholesterol, TGL, PL and FFA.

Table 4 shows the lipoprotein profiles of normal, disease control and plant treated group of animals. The levels of VLDL and LDL were elevated markedly in group II diabetic animals. On treatment with plant drug, the above parameters brought back to near normal. HDL, that was considered as good cholesterol was decreased in untreated group II of animals and on treatment with Parmelia perlata Ach aqueous extract it was found that HDL – good cholesterol was elevated significantly (P<0.05) and it was dose dependent.

### Table 3: Serum lipid profiles of untreated and PPAE treated rats for 60 days

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Cholesterol (mg/dl)</th>
<th>TGL (mg/dl)</th>
<th>PL (mg/dl)</th>
<th>FFA (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>60.8 ± 7.42</td>
<td>68.4 ± 6.81</td>
<td>58.2 ± 5.14</td>
<td>44.4 ± 7.52</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>141.5 ± 23.94 *</td>
<td>168.6 ± 22.56 *</td>
<td>122.3 ± 14.81 *</td>
<td>110.7 ± 19.32 *</td>
</tr>
<tr>
<td>III</td>
<td>Diabetic + PPAE treated (200 mg/Kg bw)</td>
<td>122.4 ± 17.25 **</td>
<td>121.7 ± 19.02 **</td>
<td>111.6 ± 16.42 **</td>
<td>96.5 ± 9.28 **</td>
</tr>
<tr>
<td>IV</td>
<td>Diabetic + PPAE treated (400 mg/Kg bw)</td>
<td>79.5 ± 9.44 **</td>
<td>72.1 ± 6.53 **</td>
<td>61.2 ± 5.05 **</td>
<td>48.7 ± 6.71 **</td>
</tr>
<tr>
<td>V</td>
<td>Diabetic + Glibenclamide treated (600 μg/Kg bw)</td>
<td>59.8 ± 9.20</td>
<td>64.7 ± 7.22</td>
<td>62.3 ± 9.57</td>
<td>47.6 ± 8.62</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM (n=6)

**Inter-group comparison**

*P < 0.05 statistically significant when Group II and Group I were compared.

**P < 0.05 statistically significant when Group III, IV were compared with Group II.

**P < 0.05 statistically significant when Group III, IV were compared with Group II.

### Table 4: Serum lipoprotein profile of untreated and treated rats after oral administration of PPAE for 60 days

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>HDL-Cholesterol (mg/dl)</th>
<th>LDL-Cholesterol (mg/dl)</th>
<th>VLDL-Cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>61.5 ± 6.42</td>
<td>41.5 ± 5.01</td>
<td>13.9 ± 1.14</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>22.5 ± 6.04 *</td>
<td>69.3 ± 9.52 *</td>
<td>30.3 ± 3.01 *</td>
</tr>
<tr>
<td>III</td>
<td>Diabetic + PPAE treated (200 mg/Kg bw)</td>
<td>29.6 ± 5.22 **</td>
<td>55.3 ± 7.26 **</td>
<td>24.2 ± 3.25 **</td>
</tr>
<tr>
<td>IV</td>
<td>Diabetic + PPAE treated (400 mg/Kg bw)</td>
<td>50.7 ± 7.41 **</td>
<td>43.1 ± 5.51 **</td>
<td>14.4 ± 2.05 **</td>
</tr>
<tr>
<td>V</td>
<td>Diabetic + Glibenclamide treated (600 μg/Kg bw)</td>
<td>52.5 ± 6.38</td>
<td>44.7 ± 6.54</td>
<td>17.2 ± 4.37</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM (n=6)

**Inter-group comparison**

*P < 0.05 statistically significant when Group II and Group I were compared.

**P < 0.05 statistically significant when Group III, IV were compared with Group II.

### DISCUSSION

Diabetes mellitus is a chronic metabolic disorder that has risen dramatically over the past 2 decades. Although the prevalence of both type I and II diabetes mellitus are increasing world wide, the prevalence of type II diabetes is expected to rise rapidly. In spite of this, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs have paved way to increase the emphasis on the use of plant based medicines for a wide variety of human ailments and medications. In this present investigation, effect of aqueous extract of Parmelia perlata Ach. has been evaluated for its antidiabetic and antihyperlipidemic potential. Alloxan is a potent diabetogen that is reduced to dialuric acid which is then auto oxidized to alloxa resulting in the production of H2O2, O2, O2- and hydroxyl radicals and causes damages to the beta-cells of islets of langerhans [19]. This causes a profound decrease in insulin level and consequent increase in fasting blood glucose level in disease control animals (Group II) (Table 1). Administration of test drug for 60 days (Group III & IV) was found to regenerate the pancreatic beta-cells which results in the normal secretion of insulin. Insulin, the potent hypoglycemic hormone thereby reduces the blood glucose level significantly (P<0.05).

Glycosylated hemoglobin (HbA1C) is formed progressively and irreversibly when the blood glucose level is elevated. HbA1C increases over a period of time and does not dissociate easily, it reflects the real blood glucose level in diabetes hence HbA1C is used as an exceptional marker of overall glycemic control [20]. Group II animals showed elevated levels of HbA1C (P < 0.05). Due to insulin deficiency in diabetic rats the cells utilizes glycogen rather than glucose for their energy [21]. Hence the diabetic rats showed decreased level of liver glycogen compared to the normal rats (Table 1). Plant treated groups (III and IV) showed a marked increase (P < 0.05) in glycogen level and significant decrease in HbA1C levels. The depletion of glycogen in the liver tissue might be prevented by the stimulation of insulin release from β -cells that in turn activates the glycogen synthase system and thereby helps in the resumption of liver glycogen level. Reference drug also increased the glycogen level but not as effective as plant extract treatment.

Glucokinase is the key enzyme catalysing glucose phosphorylation in liver. Impairment of glucokinase activity suggests the impaired oxidation of glucose via glycolysis causes its accumulation resulting in hyperglycemia. Insulin increases hepatic glycogen by increasing the activity and amount of the key enzyme – glucokinase [22]. The diabetic rats showed decreased activity of glucokinase compared to normal rats. Groups (III, and IV) treated with the Parmelia perlata Ach. extract (200 mg/kg; b.w and 400 mg/kg; b.w) showed a significant increase (P < 0.05) in the activity of glucokinase. Glucose-6-phosphatase plays a vital role in the blood glucose homeostasis. When the blood glucose level falls, liver is releases glucose into the circulation rapidly, where it serves as a fuel for other tissues that are not dependent on insulin for energy.
lack the ability to make glucose [23]. Increased hepatic glucose output is a major cause of the fasting hyperglycemia that characterizes diabetes. Group III and IV animals that received 200 mg/kg; bw and 400 mg/kg; bw for 60 days showed a remarkable decrease in the activity of Glucose-6-phosphatase and Fructose – 1-6-bisphosphatase (Table: 3 & 4). It was observed that treatment with Parmelia perlata Ach showed a significant reduction (P < 0.05) in the tissue lipid profiles (TC and TGL) and lipoprotein (LDL, VLDL). The level of HDL-cholesterol was increased significantly in groups III and IV (Table: 3 & 4). This suggest that the aqueous extract of Parmelia perlata Ach is a potent antidiabetic agent.

The allxan induced diabetic rats showed a remarkable increase (P < 0.05) in the levels of lipid profiles such as total cholesterol (TC), Triglycerides (TGL) and lipoproteins such as LDL-cholesterol, VLDL-cholesterol (Table: 3 & 4). There was a marked reduction in HDL-cholesterol compared to normal control. Studies in human and animal models have demonstrated that the alteration of lipid profiles in conditions of diabetes represents a risk factor for cardiovascular diseases. Elevated levels of TC and LDL-cholesterol levels in diabetes are the cause of coronary heart disease. The high concentration of serum lipids in diabetes mellitus is essentially due to an increase in the mobilization of free fatty acids from the peripheral fat depots. The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots [24].

Breakdown of fatty acids for energy results in increased production of acetyl CoA which may take up the pathway of cholesterol biosynthesis. TGL is the source of energy during starvation. In diabetes mellitus as glucose is not taken up by the cells. The cells are under starvation and use of coronary heart disease. The high concentration of serum lipids in diabetes mellitus is essentially due to an increase in the mobilization of free fatty acids from the peripheral fat depots. The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots [24].

CONCLUSION

Many indigenous Indian medicinal plants are used as remedies against various diseases. In the present scenario herbal medicines and herbal research is coming to light. The present investigation evaluates the antidiabetic and antihyperlipidemic efficacy of Parmelia perlata Ach. Further investigations to identify the active principles are obviously needed together with a detailed evaluation on the mechanism of action of Parmelia perlata Ach in combating altered glycemic and lipid profile in diabetic condition.

REFERENCE