EFFICACY OF METHANOL LEAF EXTRACT OF \textit{PIMENTO DIOICA} (MYRTACEA) ON THE BLOOD GLUCOSE LEVEL AND LIPID PROFILE OF STREPTOZOTOCIN INDUCED DIABETIC ALBINO RATS

K. Yogalakshmi\textsuperscript{1*} and J. Vaidehi\textsuperscript{2}

\textsuperscript{1,2}Department of Zoology, Faculty of Science, Annamalai University, Annamalai Nagar-608002, Tamil Nadu, India.

ABSTRACT

The present study was designed to investigate the antidiabetic and antihyperlipidemic activity of the methanolic extract of the leaves of \textit{Pimento dioica} in streptozotocin (STZ) induced diabetic rats. Diabetes was induced in male albino wistar rats by single intraperitoneal injection of STZ. Three days after STZ induction, the diabetic rats were treated orally with \textit{P.dioica} at the doses of 75mg/kg and 150 mg/kg of body weight daily for 45 days. Glibenclamide (0.6mg/kg of body weight) was used as reference drug. Blood glucose estimation was performed every week of the study. At the end of the study period, animals were sacrificed for the measurement of fasting blood glucose, total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL). Significant reduction (P<0.005) in fasting blood glucose levels was observed with increasing treatment durations. Thus the present study suggested significant antidiabetic and antihyperlipidemic potential in the methanolic leaf extracts of \textit{P. dioica}.

KEY WORDS: Antidiabetic, antihyperlipidemic, \textit{Pimento dioica}, glibenclamide.

INTRODUCTION

Diabetes is a disorder of metabolism based on the way the body uses digested food for energy. The digestive tract breaks down carbohydrates, sugars and starches found in many foods into glucose, a form of sugar that enters the bloodstream. With the help of the hormone
insulin, cells throughout the body absorb glucose and use it for energy. Diabetes mellitus with its devastating consequences has assumed epidemic proportion in many countries of the world. There are an estimated 143 million people worldwide with diabetes, which is almost five times more than the estimation during ten years ago. This number will probably double by 2030.\(^1\) Diabetes mellitus is a major cause of morbidity such as blindness, kidney failure, lower extremity amputation, cardiovascular disease and premature mortality.\(^2\) Despite the presence of known antidiabetic medicines in the pharmaceutical market, diabetes and the related complications continued to be a major medical problem. There are two main categories of this disease i.e. Type 1 (Insulin dependent diabetes mellitus) and Type 2 (Non-insulin dependent diabetes mellitus). Type 1 diabetes represents a heterogenous and polygenic disorder, with a number of non-HLA loci contributing to disease susceptibility.\(^3\) Though this form of diabetes accounts for 5 to 10% of all cases yet there is no identified agent substantially capable of preventing this type of disease.\(^4\)

Type 2 diabetes mellitus is more common and results from a combination of defects in insulin secretion and action, either of which may predominate. People with Type-2 diabetes are not dependent on exogenous insulin, but may require it for the control of blood glucose levels if this is not achieved with diet alone or with oral hypoglycemic agents. This type of diabetes accounts for 90 to 95% of all diabetic patients.\(^5\) Treatment of Type2 diabetes is complicated by several factors inherent to the disease process, typically insulin resistance, hyper insulinemia, impaired insulin secretion, reduced insulin-mediated glucose uptake and its utilization.\(^6-5\) All forms of diabetes are characterized by chronic hyperglycemia and the development of diabetes-specific cardiovascular pathology in retina, renal glomerulus and peripheral nerve. As a consequence of its microvascular pathology, diabetes is a leading cause of blindness, stage renal disease and a variety of debilitating neuropathies. Antihyperglycemic effects of various plants are attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibiting the intestinal absorption of glucose or the facilitation of metabolites in insulin dependent processes.\(^7\) More than 800 plant species having hyperglycemic activity have been available in literature.\(^8\) Diabetes mellitus is often linked with abnormal lipid metabolism and dyslipidemia and hyperlipidemia that are recognized complications of diabetes mellitus characterized by increased levels of cholesterol, triglycerides and phospholipids and alterations in lipoprotein composition.\(^9\) It has been reported that abdominal obesity, impaired postprandial lipid metabolism and insulin resistance are all inter related risk markers for
coronary heart diseases.\textsuperscript{[10]} Impairment in insulin sensitivity due to high concentration of lipids in the cells is responsible for the elevated cardiovascular risk in diabetes mellitus.\textsuperscript{[11-12]} Membrane fluidity is known to be dependent on the molar ratio of cholesterol to phospholipids.\textsuperscript{[13]} The liver participates in oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol and phospholipids and in the secretion of specific classes of plasma lipoproteins. Erythrocyte membranes and liver cells showed marked alterations in the concentration of lipids during diabetes.\textsuperscript{[14-15]}

Most of the plants contain glycosides, alkaloids, terpenoids, flavonoids, cartenoids, etc., that are frequently implicated as having antidiabetic effect.\textsuperscript{[16]} Berries of \textit{Pimenta dioica} (L.) Merril (fam:Myrtaceae) are commonly known as allspice in culinary. It takes its name from the aroma of dried berries, which smells like the combination of spices, especially cinnamon, cloves, ginger and nutmeg. Allspice owes its characteristic odour due to the presence of essential oil in the pericarp of the seeds. The plant Allspice and its characteristics are well mentioned in Wealth of India. The dried leaves contain 0.7 to 2.9 \% of oil which is called pimento oil. Like berry oil it contains eugenol as its main constituent but has an inferior odour and flavour. Phytochemistry and pharmacology of berries were well reported in literature.\textsuperscript{[17-18]} Hence, the present study was conducted to explore the antidiabetic and antihyperglycemic effect of the methanolic leaves extract of the \textit{Pimento dioica} in streptozotocin-induced diabetic rats.

**MATERIALS AND METHODS**

**Chemical**

Streptozotocin (STZ) was purchased from Sigma –Chemical Co. Bangalore. All other chemicals and reagents used for this study were of analytical grade.

**Plant material**

\textit{Pimento dioica} was collected from Kumuli, Kerala State, India.

**Preparation of extract**

The \textit{Pimento dioica} leaves were dried at room temperature and then were powdered using dry grinder and passed through sieve. Hundred grams of \textit{Pimento dioica} were packed in a soxhlet apparatus and extracted with methanol. The methanol extracts were concentrated in a rotary evaporator.
Experimental animal
Male Wistar albino rats (180-220 g) were procured from Rajah Muthiah Medical College and Hospital, Annamalai University, Chidambaram, India and were housed in polycarbonate cages in an animal room with 12 hr day–night cycle. The animals were allowed free access to tapwater and standard laboratory rat food. All the animal experimentation was approved by Institutional Animal Ethical Committee (Registration Number -1084/2014/CPCSEA).

Induction of experimental diabetes
Diabetes was induced in rats by intraperitoneal (I.P.) injection of streptozotocin (STZ) at a dose of 55 mg/kg b.w dissolved in 0.1 M cold citrate buffer (pH = 4.5). The rats were allowed to drink 5% glucose solution over night to overcome the drug-induced hypoglycemia. The blood glucose values above 250 mg/dl on the third day after streptozotocin injection were considered as diabetic rats. Then the treatment was started on the fifth day after streptozotocin injection and it was considered as the first day of treatment.

Experimental design
All animals were randomly divided into five groups with six animals in each group
I. Normal untreated rats
II. Diabetic rats (55mg/kg/bw)
III. Diabetic rats treated with methanolic extract of *Pimento dioica* leaves (75 mg/kg of body weight) for 45 days
IV. Diabetic rats treated with methanolic extract of *Pimento dioica* leaves (150 mg/kg of body weight) 45 days
V. Diabetic rats treated with standard drug, glibenclamide (0.6mg /kg of body weight).

Measurement of lipid profile
Measurement of serum lipid profile such as triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDLc), very low density lipoprotein cholesterol (VLDLc) and high density lipoprotein cholesterol (HDLc) were measured bio-chemically.

Statistical analysis
All biochemical data are expressed as mean ± S.E Statistical analysis was performed using one-way ANOVA followed by Tukey’s multiple tests using SPSS (version 18) computer software. In all cases, P-value of less than 0.05 was considered to be significant.
RESULTS

Effect of methanolic extract of *Pimento dioica* on serum glucose levels in diabetic rats was depicted in Table 1. In animals treated with streptozotocin (55 mg/kg b.w) (Group II), a significant increase in serum glucose level was observed on 1\(^{st}\) week, 2\(^{nd}\) week, 3\(^{rd}\) week, and 4\(^{th}\) week respectively when compared with normal rats (Group I). Group III and Group IV that received *Pimento dioica* leaf extract showed decrease in the serum glucose level when compared with diabetic control rats. After the oral administration of glibenclamide (0.6 mg/kg b.w) in diabetic rats, (Group V) a significant reduction in serum glucose level was observed on the 1\(^{st}\) week, 2\(^{nd}\) week, 3\(^{rd}\) week, and 4\(^{th}\) week when compared with diabetic control rats (Group II).

The lipid profiles in control and experimental rats are presented in Table 2. The diabetic control rats (Group II) showed significant increase in serum triglycerides, total cholesterol, very low density lipoproteins (VLDL) and low density lipoproteins (LDL) while increase in high density lipoproteins (HDL) when compared with the normals (Group I). The methanolic extract of *Pimento dioica* (Group III and IV) showed significant decrease (p<0.05) in total cholesterol, LDL, VLDL, triglycerides and significant increase (p<0.05) in HDL when compared with diabetic control group (Group II). All these effects were prominently observed on the 4\(^{th}\) week. Standard glibenclamide (Group III) also reduced triglycerides, total cholesterol, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and increased high density lipoproteins (HDL) when compared with the normals (Group I). The present experimental result indicated that methanolic extracts exhibited potent blood glucose lowering properties in STZ diabetic rats.

**Table 1:** Effect of *P.dioica* on fasting serum glucose (mg/dl) in STZ induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>1(^{st}) Week</th>
<th>2(^{nd}) Week</th>
<th>3(^{rd}) Week</th>
<th>4(^{th}) Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>85.0±1.6</td>
<td>86.7±2.4</td>
<td>82.8±2.6</td>
<td>88.03±2.6</td>
</tr>
<tr>
<td>II</td>
<td>STZ control</td>
<td>293.6±8.20</td>
<td>295.10±2.3</td>
<td>270.60±2.4</td>
<td>300.2±2.6</td>
</tr>
<tr>
<td>III</td>
<td>Diabetic + <em>P.dioica</em> (75 mg/kg b.w)</td>
<td>252.28±6.50</td>
<td>226.86±4.30</td>
<td>215.86±4.30</td>
<td>196.58±3.23</td>
</tr>
<tr>
<td>IV</td>
<td>Diabetic + <em>P.dioica</em> (150 mg/kg b.w)</td>
<td>154.88±6.48</td>
<td>138.56±3.20</td>
<td>121.56±3.20</td>
<td>109.88±2.28</td>
</tr>
<tr>
<td>V</td>
<td>Diabetic+ glibenclamide (0.6mg/kg b.w)</td>
<td>120.77±5.88</td>
<td>112.50±8.56</td>
<td>106.50±8.80</td>
<td>101.30±3.29</td>
</tr>
</tbody>
</table>

Values are expressed as mean±S.E (n=6) significantly different at p<0.005 when compared with control groups.
Table 2: Effect of *P. dioica* on lipid profile (mg/dl) activity in STZ induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Total Cholesterol</th>
<th>Triglycerides</th>
<th>HDL</th>
<th>LDL</th>
<th>VLDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>86.34±2.10</td>
<td>69.30±0.41</td>
<td>36.90±1.10</td>
<td>39.53±2.10</td>
<td>17.20±1.45</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>140.33±1.20</td>
<td>132.20±1.20</td>
<td>30.96±0.85</td>
<td>94.50±3.20</td>
<td>37.90±2.32</td>
</tr>
<tr>
<td>III</td>
<td>Diabetic + <em>P. dioica</em> (75mg/kg b.w)</td>
<td>112.14±2.10</td>
<td>92.32±3.20</td>
<td>43.18±1.32</td>
<td>46.69±3.21</td>
<td>20.50±2.27</td>
</tr>
<tr>
<td>IV</td>
<td>Diabetic + <em>P. dioica</em> (150mg/kg b.w)</td>
<td>104.18±2.31</td>
<td>90.68±2.20</td>
<td>49.26±1.38</td>
<td>49.43±1.90</td>
<td>18.90±2.30</td>
</tr>
<tr>
<td>V</td>
<td>Diabetic + glibenclamide (0.6mg/kg b.w)</td>
<td>110.10±3.20</td>
<td>89.58±1.31</td>
<td>39.25±1.28</td>
<td>42.50±2.90</td>
<td>21.67±1.89</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E (n=6) significant different at <0.05 when compared with control group.

**DISCUSSION**

Diabetes mellitus is probably the fastest growing metabolic disease in the world and as knowledge of the multifactorial heterogenous nature of the disease increase so does the need for more challenging and appropriate therapies. Diabetes mellitus is mainly manifested by hyperglycemia and hyperlipidemia, which contribute directly to atherosclerosis at later stages.[19] In the present study, an increase in the serum glucose concentration was observed accompanied by a marked reduction in plasma lipids and altered lipid and lipoprotein patterns in the plasma, in streptozotocin induced diabetic rats. In recent years, considerable interest has been directed towards the investigation of plasma lipids (total cholesterol, triglycerides, phospholipids) in diabetes mellitus due to the fact that abnormal lipid levels lead to the development of coronary artery disease in diabetic patients. Cholesterol and phospholipids constitute among two third of the total plasma lipids whereas free fatty acids (FFA) are metabolically more active. Increase in plasma and tissue cholesterol and phospholipids have been reported in diabetic rats.[14-15] Diabetes is associated with profound alterations in the serum lipids and lipoprotein profile with increased risk of coronary heart disease.[20]

Non-insulin-dependent (Type II, NIDDM) diabetes is characterized by mature onset, by varying basal insulin levels and a frequent association with obesity. The levels of serum lipids are usually raised in diabetes mellitus, and such elevation represents a risk factor for coronary heart diseases. This was observed in diabetic animals in the study, where serum TC and TG levels were significantly elevated in comparison to control. It has been observed...
that the abnormally high concentration of serum lipids is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots.[21] The elevated blood glucose concentration was accompanied by increase in total cholesterol, triglyceride, LDL, VLDL and decrease in HDL cholesterol in streptozotocin induced diabetic rats as compared to the control animals. Thus, the altered lipid and lipoprotein pattern observed in diabetic rats could be due to a defect in insulin secretion and/or action. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic rats. Accumulation of cholesterol and phospholipids in the liver due to elevated plasma free fatty acids has been reported in diabetic rats. In the present study, the methanolic extract of the leaves of Pimento dioica had significantly decreased the total cholesterol, triglyceride, VLDL, and LDL with a concomitant increase in HDL which is having a protective function for the heart when compared with that of diabetic control group.

ACKNOWLEDGEMENT

The authors would like to acknowledge university grants commision (UGC, NON SAP) and Annamalai University for financial and infrastructure facilities respectively.

REFERENCES