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ORIGINAL ARTICLE

IMPACT OF *Pimenta dioica* LEAF EXTRACT ON CERTAIN BLOOD PARAMETERS IN STZ INDUCED DIABETIC WISTAR RATS

K. Yogalakshmi and *J. Vaidhe*

Department of Zoology, Faculty of Science, Annamalai University, Annamalai nager - 608 002, Tamil nadu, India

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**ABSTRACT**

The present study was designed to investigate the certain blood parameters in STZ induced diabetic rats. The methanolic leaf extract of *Pimenta dioica* at the dose of 75 and 150 mg/kg of body weight was administered orally once a day to the diabetic induced group for 45 days. Glibenclamide (0.6 μg/kg of body weight) was used as references drug. In the present study body weight was significantly (P<0.05) decreased in diabetic rats when compared with that of the normal control rats. In diabetic rats there was a significant increase in the level of plasma glucose and significant decrease in the plasma insulin. Oral administration of *Pimenta dioica* (75 mg and 150 mg/kg.bw) and glibenclamide (0.6 μg/kg. bw) to diabetic rats significantly (P<0.05) increased the body weight and plasma insulin and markedly decreased the plasma glucose level when compared with that of the diabetic control rats. The diabetic rats showed a significant decrease in the level of hemoglobin and significant (P<0.05) increase in the level of HbAlc. Whereas the levels of plasma urea, plasma uric acid and creatinine significantly increased in the diabetic control group when compared with that of the normal control group. The level of hemoglobin after the administration of *Pimenta dioica* (75 mg and 150 mg/kg.bw) and glibenclamide (0.6 μg/kg.bw) were significantly increased in the diabetic rats. The level of plasma urea, uric acid and creatinine after orally administering *Pimenta dioica* (75 mg and 150 mg/kg.bw) significantly decreased in the diabetic control group. Thus the present study suggested significant blood parameters potential in the methanolic leaf extracts of *P. dioica*.

**Keywords:** *Pimenta dioica*, Glibenclamide, blood parameters, methanolic leaf extract.

1. INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by high blood glucose level resulting from defects in insulin secretion, insulin action or both [Khan et al., 2009]. It is a chronic disorder that affects the metabolism of carbohydrates, fats, proteins and electrolytes in the body, leading to severe complications which are classified into acute, sub-acute and chronic [Rang et al., 1991]. Acute complications include hypoglycemia, diabetic ketoacidosis, hyperglycemic non-ketotic syndrome [Krentz and Natras, 1991] while sub-acute complications include thirst, polyuria, lack of energy, visual blurriness and weight loss [Kumar and Clark, 2002]. The management of diabetes is considered a global problem and a cure has yet to be discovered despite many great strides have been made in understanding the management of diabetes. Despite the presence of known antidiabetic medicine in the pharmaceutical market, diabetes and the related complications continued to be a major medical problem. The searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which take alternative and safe effect on diabetes mellitus. Concurrently, Phytochemicals identified from traditional medicinal plants are presenting exciting opportunities for the development of new drug therapies. Currently available drug regimes for the management of diabetes mellitus have certain drawbacks. Recently there has been increasing interest in the use of medicinal plants [Pul ok and Mukherjee 2002; Akuri and Krishnaraj, 2006]. In many countries traditional plants are used to control diabetes. Plants play a major role in the discovery of new therapeutic agents and have received much attention as sources of biologically active substances including antioxidants, hypoglycemic and hypolipidemic agents [Markes et al., 1995]. Many useful plants and herbs introduced in pharmacological and clinical trials have confirmed their blood sugar lowering effect. So it is essential to know about the pharmacological evaluation of various plants used in the traditional system of medicine [Gupta et al., 2007]. Spices and herbs have been added to foods since ancient times, not only as flavouring agents, but also as folk medicines and food preservatives [Beucha, 1994; Nakatani, 1994; Culter, 1995]. *Pimenta dioica* is one...
such plant used as a flavouring agent which was also later diagnosed for its antioxidant, antidiabetic, anti-inflammatory and anti-microbial properties. The whole plant exhibit medicinal values. 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. The existences of antioxidants are beneficial in preventing disease complexes such as cardiovascular, diabetes, cancer, rheumatoid arthritis, inflammatory bowel pancreatitis, hematologic and neurogenetic diseases [Irshed and Chaudhari,2002; Craig,1997]. In this context, the present study was aimed to investigate the anti-diabetic activity of the methanolic leaf extract of *Pimenta dioica* in streptozotocin induced diabetic rats.

**2. MATERIALS AND METHODS**

**Chemicals**

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

**Plant material**

*Pimenta dioica* was collected from Kumuli, Kerala State, India.

**Preparation of extract**

The *Pimenta dioica* leaves were dried at room temperature and then were powdered using dry grinder and passed through sieve. Hundred grams of *Pimenta dioica* leaves were packed in a Soxhlet apparatus and extracted with methanol. The methanol extracts were concentrated on a rotary evaporator.

**Experimental animals**

Male Wistar albino rats (150-200 g) were procured from Rajah Muthiah Medical College and Hospital, Annamalai University, Chidambaram, Tamilnadu, India and were housed in polycarbonate cages in an animal room with 12 hours day – night cycle. The animals were allowed free access to tap water and standard laboratory rat food. The animal treatment and protocol employed were approved by the TAEQ, Annamalai University, Annamalai Nagar, India (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) [Bhandaranyake,2002]. The rats were allowed to drink 5% glucose solution overnight 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

1. Normal untreated rats
2. Diabetic control rats (STZ 55 mg/kg of body weight),
3. Diabetic rats treated with methanolic extract of *Pimenta dioica* leaves (75 mg/kg of body weight)
4. Diabetic rats treated with methanolic extract of *Pimenta dioica* leaves (150 mg/kg of body weight)
5. Diabetic rats treated with standard drug, glibenclamide (0.6 μg/kg of body weight).

**Analytical procedures**

The estimation of blood glucose was carried out by the method O-toluidine using the modified reagent of [Sasaki et al., 1972]. The estimation of hemoglobin was done by the method of [Dubin and Austin,1932]. The glycosylated hemoglobin in the blood was estimated by the protocol of [Sudhakar Nayak and Patil,1981]. The plasma insulin was assayed by ELISA method (Enzyme Linked Immunosorbant Assay) using Boehringer Mannheim Kit (Boehringer analyzer, ES 300). Urea in the plasma was estimated by using the diagnostic kit based on the method of [Fawcett and Scott,1960]. Uric acid was estimated by the method adapted by Caraway [1955]. Creatinine in the plasma was estimated by using the diagnostic kit based on the protocol of Tietz [1987] using [Jaffe,1886] colour reaction.

**Statistical Analysis**

All 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.

**3. RESULTS**

In the present study, the changes in the body weight, plasma glucose and plasma insulin in control and experimental animals were presented in Table 1. Body weight was significantly (P<0.05) decreased in diabetic rats when compared with that of the normal control rats. In diabetic rats there was a significant increase in the level of plasma glucose and significant decrease in the plasma insulin. Oral administration of *Pimenta dioica* (75 mg and 150 mg/kg,bw) and glibenclamide to diabetic rats significantly (P<0.05) increased the body weight and plasma insulin and markedly decreased the plasma glucose level when compared with that of the diabetic control rats.

The level of hemoglobin, glycosylated hemoglobin (HbAl), urea, uric acid and creatinine in normal control and experimental animals are shown in Table 2. The diabetic rats showed a significant decrease in the level of hemoglobin and significant (P<0.05) increase in the level of HbAl. Whereas the levels of plasma urea, plasma uric acid and creatinine significantly increased in the diabetic control.
The level of hemoglobin after the administration of *Pimenta dioica* (75mg and 150 mg/kg, bw) and glibenclamide were significantly increased in the diabetic rats. The level of plasma urea, uric acid and creatinine after orally administering *Pimenta dioica* (75mg and 150 mg/kg, bw) significantly decreased in the diabetic control group.

### 4. DISCUSSION

The loss of body weights observed in STZ induced diabetic rat group (after a period of 30 days) may be due to muscle wasting and loss of tissue proteins upon the induction of diabetes with STZ [Wanston et al., 1990; Chatterjee and Sinde, 2002]. Earlier [Sathikakumar et al., 2006; Kaleem et al., 2005] reported 20% and 52% reduction in the FGB levels of diabetic rats treated with aqueous extracts of *Piper betel* leaves and *Piper nigrum* seeds respectively for 30 days. Glycosylated hemoglobin is used as a marker for estimating the degree of protein glycation in diabetes mellitus. HbA1c was found to increase in patients with diabetes mellitus and the amount of increase is directly proportional to the fasting blood glucose level [Al-yassin and Ibrahim, 1981]. In diabetic condition, the excess glucose present in the blood reacts with hemoglobin to form HbA1c [Koeng et al., 1976]. Estimation of HbA1c has been known to be particularly useful in monitoring the effectiveness of therapy in diabetes. The observed increase in the levels of HbA1c in diabetic control group rats was due to the presence of excessive amounts of blood glucose. During diabetes as the excess of glucose present in blood reacts with Hb to form HbA1c, the total Hb level was observed to be decreased in diabetic rats [Saudek et al., 2006]. The decrease in body weight with diabetes mellitus has been attributed to the gluconeogenesis i.e., catabolism of proteins and fats, which is associated with the characteristic loss of body weight due to increased muscle wasting and loss of tissue proteins [Shinvaiker et al., 2004; Shinvaiker et al., 2006] However, in diabetic state, lipoprotein lipase is not activated due to insulin resistance deficiency, resulting in hyperglycemia and insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities [Jankel et al., 2008]. Administration of *Acorus calamus* methanol extract to diabetic rats showed a significant decrease in the fasting blood glucose level and an increase in the serum insulin levels and may be due to the presence of saponins, glycosides and sequiterpenoids which possesses hypoglycemic property [Parab and Mengi, 2002; Campos et al., 2009; Gengaith et al., 2011].

The diabetic hyperglycemia induces elevation of the plasma levels of urea, uric acid and creatinine which are considered as significant markers in renal function [Almald, 1988]. Catabolism of the protein and nucleic acids results in the formation of urea. In diabetic condition the amino acids breakdown result in an increased production of urea [Chattopadhyay and Bandyopadhyay, 2005]. Diabetes mellitus impairs the normal capacity of the liver to synthesize glycogen. The regulation of glycogen metabolism in vivo occurs by the enzymes glycogen synthase and glycogen phosphorylase. The reduced glycogen store in the diabetic rats has been attributed to the reduced activity of glycogen synthase and increased activity of glycogen phosphorylase. This is probably due to lack of insulin in the diabetic state, which results in the inactivation of the
glycogen synthase systems [Shinwalla, 2006]. Glucose is transported out of the liver to increase the blood glucose concentration. Normally insulin inhibits the hepatic glucose production by suppressing glucose-6-phosphatase and fructose-1,6-bisphosphatase enzyme activities [Chandramohan et al., 2008]. Since lipid abnormalities accompanying with premature atherosclerosis are the major causes of cardiovascular diseases in diabetic patients, ideal treatment for diabetes, in addition to glycemic control, should have a favorable effect on lipid profile. Cardiovascular diseases are listed as the cause of death in 65% of people suffering from diabetes [Kesari et al., 2007]. The liver is regarded as the central metabolic organ in the body, with an important role in glucose and lipid homeostasis [Eidi and Eidi, 2009]. The present study thus showed that the methanolic extract of Pimenta dioica leaf extract has potent antidiabetic properties in STZ induced diabetic rats which could be supplemented to such animals in different proportions depending on the body weight.

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Free radical scavenging activity of methanolic leaf extract of *Pimenta dioica* on streptozotocin-induced diabetic rats

K. Yogalakshmi*, J. Vaidehi

1Department of Zoology, Annamalai University, Annamalai nager-608 002, Tamilnadu, India.

Abstract
The present study was designed to investigate the free radical scavenging activity of *Pimenta dioica* on streptozotocin (STZ)-induced diabetic rats. The methanolic leaf extract of *Pimenta dioica* at the doses of 75 and 150 mg/ kg of body weight was administered orally once in a day to the diabetic induced group for 45 days. Glibenclamide (0.6 mg/kg of body weight) was used as reference drug. The antioxidant properties were assessed by estimating the liver and kidney catalase (CAT), thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), glutathione peroxidase (Gpx) and reduced glutathione (GSH). Antioxidant levels were significantly restored towards normal levels in *P. dioica* treated rats when compared with the STZ control. The results of the study indicate that the *Pimenta dioica* leaf methanolic extract exhibit promising antioxidant activity towards diabetic rats.

Key words: Free radical scavenging activity, *Pimenta dioica*, glibenclamide, catalase.

*Corresponding Author: K. Yogalakshmi*, Department of Zoology, Annamalai University, Annamalai nager-608 002, Tamilnadu, India.

1. Introduction

Diabetes mellitus (DM) is a chronic disease caused by inherited or acquired deficiency in the production of insulin by the pancreas or by the ineffectiveness of the produced insulin. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body systems and in particular the blood and nervous systems. It is one of the alarming worldwide health problems at present leading to microvascular (retinopathy, neuropathy and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications [1]. It is expected that about 366 million people are likely to be diabetic by the year 2030 [2]. Hyperglycemia is known to produce reactive oxygen species (ROS) which plays a central role in the complications of diabetes [3]. Diabetes is associated with oxidative stress, leading to an increased production of reactive oxygen species (ROS), including superoxide radical, hydrogen peroxide and hydroxyl radicals or reduction of antioxidant defense.
system. Implications of oxidative stress in the pathogenesis of diabetes is suggested not only by oxygen free radical generation but also due to non enzymatic protein glycosylation, auto oxidation of glucose, impaired antioxidant enzymes and formation of peroxides. Lipid peroxidation is a key marker of oxidative stress that results in extensive membrane damage and dysfunction [4].

Treatment of diabetes with sulphonylureas and biguanides is associated with adverse side effects. However, complementary medicine has grown in popularity in recent years owing to its minimal side effects and appropriate action. Dietary measures and traditional plant therapies as prescribed by Ayurvedic and other indigenous systems of medicine are used commonly in India. Many indigenous Indian medicinal plants have been found to be useful in the successful management of diabetes and some of them have been tested for their active ingredients. The World Health Organization (WHO) has also recommended the evaluation of the plant’s effectiveness and conditions against chronic ailment in place of chemically synthesized drugs. Despite the development of new drugs and their validation by scientific criteria, research still continues in scientific community around the world to evaluate antidiabetic activities of raw plant materials or isolated natural products without adverse effects.

*Pimenta dioica* (L.) Merril (Family: Myrtaceae) is 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 is mentioned in the Wealth of India [5]. The natives of Kerala and Mangalore use Allspice leaves as medicine for pain, arthritis, fever and stress. The drug has derived the name “Allspice” since its aroma resembles the aroma of spices such as clove, nutmeg and cinnamon [6]. In India, the leaves of *Pimenta* are used to flavor rice which gives it a typical aroma. Allspice is considered as a very important spice in the meat industry which utilizes the powder of the berries for the tenderizing of meat [7, 8].

In all the previously mentioned pathological conditions, oxidative stress is one of the causes, which trigged the momentum to explore the *P. dioica* leaf extract for in vitro antioxidant activity. Antioxidant and hepatoprotective activity in CCl₄ (Carbon Tetra Chloride) induced liver toxicity of all spice leaves had been reported earlier [9]. In the present study *Pimenta dioica* leaves were subjected to evaluate the antioxidant activities against diabetes.

2. Materials and Methods

**Chemicals**

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

**Plant material**

*Pimenta dioica* was collected from Kumuli, Kerala State, India.

**Preparation of extract**

The *Pimenta dioica* leaves were dried at room temperature and then were powdered using dry grinder and passed through sieve. Hundred grams of *Pimenta dioica* were packed in a soxhlet apparatus and extracted with methanol. The methanolic extracts were concentrated on a rotary evaporator.

**Experimental animals**
Male Wistar albino rats (150-200 g) were procured from Rajah Muthiah Medical College and Hospital, Annamalai University, Chidambaram, Tamilnadu, India, and were housed in polycarbonate cages in an animal room with 12 hours day – night cycle. The animals were allowed free access to tap water and standard laboratory rat food. The animal treatment and protocol employed were approved by the TAEC, Annamalai University (Registration Number -1084/2014/CPCSEA)

**Induction of experimental diabetes**

Diabetes was induced in the 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) [10]. The rats were allowed to drink 5% glucose solution overnight 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
1. Normal untreated rats
2. Diabetic control rats (STZ) (55 mg/kg bw).
3. Diabetic rats treated with methanolic extract of *Pimenta dioica* leaves (75 mg/kg of body weight)
4. Diabetic rats treated with methanolic extract of *Pimenta dioica* leaves (150 mg/kg of body weight)
5. Diabetic rats treated with standard drug, glibenclamide (0.6 µg/kg of body weight).

**Estimation of antioxidant parameters**

Tissues (liver and kidney) were dissected out and washed immediately with ice cold saline to remove any blood. The antioxidant enzymes such as superoxide dismutase (SOD) [11], reduced glutathione (GSH) [12] catalase (CAT) [13], thiobarbituric acid reactive substances (TBARS) [14] and glutathione peroxidase (GPx) [15] activity were estimated in the liver and kidney.

**Statistical analysis**

All antioxidant 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) of computer software. In all cases, P-value of less than 0.05 was considered to be significant.

**3. Results**

The antioxidant enzymes such as TBARS, SOD, CAT, GSH, and GPx were analyzed in the liver and kidney of normal and STZ induced diabetic rats and treated with the methanolic leaf extracts of *Pimenta dioica* and the standard drug glibenclamide (Table 1). An increased level of TBARS was observed and the levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH) were significantly (p<0.05) reduced in STZ induced diabetic rats. These adverse changes were reversed to near normal values in the methanolic leaf extract of *P. dioica* treated rats on par with the results obtained during the administration of the standard drug, glibenclamide.

**4. Discussion**

Antioxidants are substances or nutrients which can prevent or slow down the oxidative damage to the body. When the body cells use oxygen, they naturally produce free radicals (by-products) which can cause damage [16].
Table 1. Effect of *P. dioica* on the liver and kidney antioxidant enzymes in STZ – induced diabetic rats

<table>
<thead>
<tr>
<th>Organs</th>
<th>Group</th>
<th>Treatment</th>
<th>TBARS</th>
<th>SOD</th>
<th>CAT</th>
<th>GSH</th>
<th>GPx</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>Normal control</td>
<td>3.90±0.10</td>
<td>112.90±2.28</td>
<td>32.55±0.90</td>
<td>241.28±3.40</td>
<td>34.50±0.81</td>
</tr>
<tr>
<td>Liver</td>
<td>II</td>
<td>Diabetic control</td>
<td>8.41±0.16</td>
<td>81.01±1.48</td>
<td>21.60±0.31</td>
<td>220.35±1.32</td>
<td>22.10±0.39</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>Diabetic + <em>P. dioica</em> extract (75 mg/kg b.w)</td>
<td>4.60±0.11</td>
<td>96.39±2.19</td>
<td>26.20±0.51</td>
<td>235.89±3.10</td>
<td>29.45±0.89</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>Diabetic + <em>P. dioica</em> extract (150 mg/kg b.w)</td>
<td>4.54±0.15</td>
<td>102.48±3.12</td>
<td>28.21±0.51</td>
<td>237.41±0.51</td>
<td>32.04±0.44</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>Diabetic + glibenclamide (0.6 mg/kg b.w)</td>
<td>4.15±0.09</td>
<td>109.69±3.64</td>
<td>31.47±0.51</td>
<td>240.33±2.31</td>
<td>33.01±0.55</td>
</tr>
<tr>
<td>Kidney</td>
<td>I</td>
<td>Normal control</td>
<td>5.13±0.10</td>
<td>146.69±2.01</td>
<td>28.81±0.55</td>
<td>334.98±5.71</td>
<td>32.60±0.89</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>Diabetic control</td>
<td>7.35±0.15</td>
<td>115.59±2.05</td>
<td>20.01±0.84</td>
<td>302.18±3.13</td>
<td>23.80±0.68</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>Diabetic + <em>P. dioica</em> extract (75 mg/kg b.w)</td>
<td>5.93±0.10</td>
<td>131.12±3.41</td>
<td>27.60±0.50</td>
<td>326.80±4.98</td>
<td>27.02±0.59</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>Diabetic + <em>P. dioica</em> extract (150 mg/kg b.w)</td>
<td>5.39±0.20</td>
<td>133.10±3.65</td>
<td>28.16±0.55</td>
<td>329.41±6.41</td>
<td>30.12±1.02</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>Diabetic + glibenclamide (0.6 mg/kg b.w)</td>
<td>5.27±0.15</td>
<td>144.81±2.90</td>
<td>28.61±0.51</td>
<td>331.65±3.41</td>
<td>31.42±1.15</td>
</tr>
</tbody>
</table>

Values are expressed as mean±S.E (n=6) and are significantly different at p<0.005 when compared with control groups.

Chronic hyperglycemia in diabetes leads to auto-oxidation of glucose, non-enzymatic protein glycosylation, impaired glutathione metabolism, alteration in
antioxidant enzymes and formation of lipid peroxides. The above events accelerate the production of free radicals and weaken the antioxidant defense system. Hence, attention has been given to naturally occurring antioxidants that counteract the deleterious effects of reactive antioxidants. The increase in oxygen free radicals in diabetes could be primarily due to an increase in the blood glucose levels, which upon auto-oxidation generate free radicals. The increased susceptibility of the tissues of the diabetic animals may be due to the activation of the lipid peroxidation system. The possible source of oxidative stress in diabetes includes shifts in redox balance resulting from altered carbohydrate and lipid metabolism and increased generation of reactive oxygen species [17]. The antioxidant activity of P. dioica in liver and kidney was studied in diabetic rats. After the induction of diabetes by STZ, significantly (P<0.005) decreased levels of SOD, CAT, GPx, reduced GSH and increased level of TBARS in liver and kidney were observed compared to normal control rats. These altered above antioxidant levels were reversed significantly (P<0.005) to near normal levels after the administration of P. dioica 75 and 150 mg/kg dose and glibenclamide 0.6μg/kg dose compared to diabetic control rats. It is well known that CAT, SOD and GPx play an important role as protective enzymes against free radical formation in the tissues [18]. These adverse change were reversed to near normal values in the methanolic extract of P. dioica leaf treated rats. Recent studies have clearly demonstrated the importance of medicinal plants in the treatment of experimental diabetes, where oxidative stress induced apoptosis or β -cell death occur [19, 20]. Oral administration of Asparagus racemosus (EEAR) showed significant hypoglycemic effects against STZ-induced diabetes in rats. The extract significantly lowered the levels of blood glucose and TBARS and significantly increased the levels of GSH, SOD and CAT [21, 22]. From the present study it could be concluded that the methanolic leaf extract of P. dioica possess potent antioxidant properties in STZ induced diabetic rats.

References


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

K. Yogalakshmi¹ and J. Vaidehi²

¹²Department 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 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 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 P. dioica.

KEY WORDS: Antidiabetic, antihyperlipidemic, 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 far 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.) Merrill (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 characterstics 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{Pimenta dioica} in streptozotocin-induced diabetic rats.

\textbf{MATERIALS AND METHODS}

\textbf{Chemical}

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

\textbf{Plant material}

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

\textbf{Preparation of extract}

The \textit{Pimenta dioica} leaves were dried at room temperature and then were powdered using dry grinder and passed through sieve. Hundred grams of \textit{Pimenta 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 tap water 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\textsuperscript{st} week, 2\textsuperscript{nd} week, 3\textsuperscript{rd} week, and 4\textsuperscript{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\textsuperscript{st} week, 2\textsuperscript{nd} week, 3\textsuperscript{rd} week, and 4\textsuperscript{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\textsuperscript{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\textsuperscript{st} Week</th>
<th>2\textsuperscript{nd} Week</th>
<th>3\textsuperscript{rd} Week</th>
<th>4\textsuperscript{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>88.0±2.6</td>
</tr>
<tr>
<td>II</td>
<td>STZ control</td>
<td>293.64±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. 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.

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**REFERENCES**