Available online at www.journalijmrr.com

INTERNATIONAL JOURNAL OF MODERN RESEARCH AND REVIEWS

ISSN: 2347-8314

Int. J. Modn. Res. Revs.
Volume 3, Issue 8, pp 739-743, August, 2015

ORIGINAL ARTICLE

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

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Article History: *Received 20th July ,2015, Accepted 16th August, 2015, Published 17th August ,2015*

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 administrated orally once in a day to the diabetic induced group for 45 days. Glibenclamide (0.6 mg/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 (0.6µg/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 $HbAl_c$. 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 *Pimento dioica* (75mg and 150 mg/kg.bw) and glibenclamide (0.6µg/kg.bw) 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. 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 [Knentz and Nattras,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 certaindrawbacks.Recentlythere has been increasing interest in the use of medicinal plants [Pul ok and Mukherjee 2002; Alluri 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 [Marles 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; Cutler,1995]. *Pimento dioica* is one

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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, hematological and neurodegenerative diseases [Irshed and Chaudhuri,2002; Craig,1997]. In this context, the present study was aimed to investigate the antidiabetic 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 diocia was collected from Kumuli, Kerala State, India.

Preparation of extract

The *Pimenta dioca* leaves were dried at room temperature and then were powdered using dry grinder and passed through sieve. Hundred grams of *Pimento dioca* 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 TAEC, 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) [Bandaranayake ,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 diocia* leaves (75 mg/kg of body weight)
4. Diabetic rats treated with methanolic extract of *Pimenta diocia* 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 [Drabkin and Austin,1932]. The glycosylated hemoglobin in the blood was estimated by the protocol of [Sudhakar Nayak and Pattabiraman1981]. The plasma insulin was assayed by ELISA method (Enzyme Linked Immunosorbant Assay) using Boehringer Manheim 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,18861] 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 ($HbAl_c$), 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 $HbAl_c$. Whereas the levels of plasma urea, plasma uric acid and creatinine significantly increased in the diabetic control

Table 1: Effect of *Pimenta dioica* on body weight, plasma glucose and plasma insulin in normal control and diabetic rats.

Groups	Body weight (g) Initial (0 day)	Final (45days)	Plasma glucose (mg/dl)	Plasma Insulin (µU/ml)
Control	182.89±4.10[c]	210.03±3.81[a]	86.71±4.50[d]	15.80±1.08[a]
Diabetic	184.0±4.20[b]	140.45±4.66[e]	287.78±11.45[a]	7.40±0.31[e]
Diabetic + *P.dioica*(M)/75	178.51±3.30[d]	190.09±4.40[c]	135.24±5.84[b]	14.21±0.70[d]
Diabetic+*P.dioica*(M)/150	172.25±4.21[e]	187.51±2.30[d]	105.23±8.81[c]	15.31±1.20[b]
Glibenclamide (0.6µg)	185.48±4.50[a]	206.00±3.80[b]	97.54±6.80[e]	15.08±1.15[c]

Values are given as mean ± SD (n=6 rats) significantly different at P<0.05 when compared with control group

Table 2: Effect of *Pimenta dioica* on haemoglobin, glycosylated haemoglobin, urea, uric acid and creatinine in normal control and diabetic rats

Groups	Hemoglobin (g/dl)	Glycosylated hemoglobin (H_bA_1C) %	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
Control	13.06±1.02[a]	0.42±0.01[e]	24.90±1.80[e]	1.29±0.08[e]	0.43±0.030[e]
Diabetic	9.08±0.46[e]	1.38±0.10[a]	44.41±3.40[a]	2.29±0.19[a]	0.82±0.04[a]
Diabetic + *P.dioica* (M)/75	11.01±0.84[d]	0.49±0.03[b]	31.71±2.91[b]	1.79±0.13[b]	0.52±0.08[b]
Diabetic+*P.dioica* (M)/150	12.04±1.06[c]	0.47±0.05[c]	30.33±2.35[c]	1.71±0.14[c]	0.49±0.11[d]
Glibenclamide (0.6µg)	12.75±1.13[b]	0.45±0.03[d]	29.17±2.21[d]	1.64±0.01[d]	0.45±0.12[c]

Values are given as mean ± SD (n=6 rats), significantly different at P<0.05 when compared with control group

group when compared with that of the normal control group. 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 Shinde,2002]. Earlier [Santhakumar et al., 2006; Kaleem et al., 2005] reported 26% and 52% reduction in the FBG 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 [Koenig et al., 1976]. Estimation of $HbAl_c$ has been known to be particularly useful in monitoring the effectiveness of therapy in diabetes. The observed increase in the levels of $HbAl_c$ 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 $HbAl_c$ 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 [Shirwaikar et al., 2004; Shirwaikar et al., 2006] However, in diabetic state, lipoprotein lipase is not activated due to insulin resistance deficiency, resulting in hypertriglyceridemia and insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities [Jarald et al., 2008]. Administration of *Acorus calamus* methanolic 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; Gengaihi 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 [Almdal ,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 the lack of insulin in the diabetic state, which results in the inactivation of the

glycogen synthetase systems [Shirwaikar,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 % people suffering from diabetes [Kesari etal., 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 upon the body weight.

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