Evaluation of Hypoglycemic and Hypolipidemic Activity of Methanolic Extract of Coccus hirsutus (L.) Diels Leaves in Streptozotocin-Induced Diabetes Mellitus Rats

P. Palsamy and R. Malathi
Department of Biochemistry, Thanthai Hans Roever College, Perambalur, Tamil Nadu, 621 212, South India

Abstract: The aim of this study was to investigate the hypoglycemic and hypolipidemic activity of methanolic extract of Coccus hirsutus (L.) Diels leaves in streptozotocin-induced diabetes mellitus rats and the effect of the extract was compared with glibenclamide, a standard hypoglycemic drug. The plant extract (CLEt) was administered orally (500 mg kg⁻¹ body weight) and the effect of CLEt was estimated on the levels of hemoglobin, glycosylated hemoglobin, glucose, insulin, glucose-6-phosphatase, lipid peroxidation, triglycerides, cholesterol and phospholipids, in the plasma of STZ-induced (65 mg kg⁻¹ body weight) diabetic rats. The plasma lipoproteins levels were also measured. STZ-induced diabetic rats showed significant (p<0.05) increase in the levels of glycosylated hemoglobin, glucose, glucose-6-phosphatase, cholesterol, phospholipids, triglycerides and lipid peroxidation and significant (p<0.05) decrease in insulin, hemoglobin, which were considerably restored to near normal in CLEt or glibenclamide treatment rats. The plasma lipoproteins (HDL-cholesterol and LDL-cholesterol) were altered significantly (p<0.05) in STZ-induced diabetic rats and these levels were also restored back to near normal by CLEt or glibenclamide treatment. These results suggest that CLEt possess an antidiabetic principle and may be useful for diabetes treatment.

Key words: Coccus hirsutus, hypoglycemic, hypolipidemic, medicinal plants, STZ-induced diabetes

INTRODUCTION

Diabetes mellitus is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced (Chakraborty and Rajagopalan, 2002). The pathogenesis of diabetes mellitus and its management by the oral administration of hypoglycemic agents has stimulated great interest in recent years. The currently available oral antihyperglycemic agents for clinical use-sulphonylureas and biguanides-have characteristic profile of side effects (Mariam et al., 1996). These include hypoglycemia at higher doses, liver problems, lacte acidosis and diarrhoea. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost (Venkatesh et al., 2003). Therefore, investigation on some active principles from traditional medicinal plants has become more important (Suba et al., 2004). The World Health Organization (WHO, 1980) has also recommended the evaluation of the effectiveness of plants in condition where we lack safe modern drugs (Upathaya and Pandy, 1984).

Corresponding Author: R. Malathi, Department of Biochemistry, Thanthai Hans Roever College, Perambalur, Tamil Nadu, 621 212, South India Tel: +914528275574
Coccus hirsutus (L.) Diels (Menispermaceae) is a widely growing plant found in the plains of India in dry localities. Indian tribes use various plant parts of this plant for a wide range of ailments, including constipation, kidney problems (Cairns, 1986). The roots of Coccus hirsutus have been mentioned as bitter, acrid, laxative, demulcent and antiperiodic in fever, tonic and diuretic. The plant has been reported to contain essential oil, β-sitosterol, gymnol (Merchant et al., 1962), glycosides, sterol and alkaloids (Das et al., 1964). The alkaloids reported to be present in the plant are shaheenine (Rasheed et al., 1991a), cohirsine (Ahmad et al., 1991), hirsutine (Rasheed et al., 1991b), jamaninine (Ahmad and Iqbal, 1993a), jamanine-N-oxide (Ahmad et al., 1987a), cohirsine (Ahmad et al., 1987b), corsinmine (Ahmad and Iqbal, 1992), haidinine (Ahmad and Iqbal, 1993b), D-trilobine, DL-coclaurine (Jagannadha Rao and Ramachandra, 1961), isostrilbined, (+)-syringaresinol and protoquercetol (Ahmad and Rasheed, 1986). The juice of leaves coagulates in water and forms mucilage, which is used externally as a cooling and soothing agent in prurigo, eczema and impetigo (Nadkarani, 1982). The anti-inflammatory and analgesic activities of the roots were also reported (Nayak and Singh, 1993). So far, limited information is available on hypoglycemic activity of Coccus hirsutus (Satyanarayana et al., 1994). Therefore, the present investigation is carried out to study the hypoglycemic and hypolipidemic effects of Coccus hirsutus in normal and STZ-induced diabetes mellitus rats. The results were compared with glibenclamide as a reference drug.

MATERIALS AND METHODS

Plant Material

The fresh leaves of Coccus hirsutus (L.) Diels was collected during the month of February, 2006 from Perambalur, Tamilnadu, South India. The plant was identified at the herbarium in St. Josephs’ college and a voucher specimen (BCCH-70/06) has been kept in our laboratory for future reference. The leaves were shade dried, powdered and passed through a 40-mesh sieve and kept in a well-closed container for further extraction.

Preparation of Plant Extract

Five hundred grams of dried, powdered plant material was extracted with petroleum ether (60-80°C) using soxhlet apparatus to remove lipids. It was then filtered and filtrate was discarded. The residue was then extracted successively with methanol using soxhlet apparatus and the methanol was evaporated in a rotary evaporator at 40-50°C under reduced pressure. The residual extract was suspended in water for overnight and filtered. The filtrate was dried and was stored at 4°C until used. The yield of the extract was 16.73% (w/w).

Animals

Male wistar rats, 180-210 g, were used for this study. The rats were 11-12 weeks of age at the time of this study. They were housed in cages and provided feed and water ad libitum. After randomization into various groups, the rats were acclimatized for a period of 2-3 weeks in the new environment before initiation of experiment. The experiment were designed and conducted in accordance with the ethical norms approved by Ministry of Social Justice and Empowerment, Govt. of India and Institutional Animal Ethics Committee Guidelines.

Induction of Experimental Diabetes

The rats were fasted for 18 h and made hyperglycemic by a single intraperitoneal injection of STZ (Sigma, USA) dissolved in 0.05 M of citrate buffer (pH 4.5), at a dose of 65 mg kg⁻¹ body weight
(Ganda et al., 1976). The blood glucose levels of these rats were estimated 72 h after STZ-administration and moderately STZ-diabetic rats having glycosuria and moderate hyperglycemia (above 250 mg dL$^{-1}$) were selected for the experiment (Pari and Satheesh, 2004).

**Experimental Designs**

The method described by Pari and Satheesh (2004) was adopted. In the experiment a total of 30 rats (18 diabetic surviving rats and 12 normal rats) were used. The rats were divided into 5 groups after the induction of STZ-diabetes. In the experiment six rats were used in each group. Group 1: Normal rats; Group 2: Normal rats were given CLEt 500 mg kg$^{-1}$ body weight day$^{-1}$ in aqueous solution orally for 28 days; Group 3: Diabetic control; Group 4: Diabetic rats were given CLEt 500 mg kg$^{-1}$ body weight day$^{-1}$ in aqueous solution orally for 28 days; Group 5: Diabetic rats were given glibenclamide 600 μg kg$^{-1}$ body weight day$^{-1}$ in aqueous solution orally for 28 days for comparison (Pari and Uma, 1999).

**Sample Collection**

At the end of 28th day, the animals were deprived of food overnight and sacrificed by decapitation. Fasting blood sample was collected in two different fresh vials containing sodium fluoride and potassium oxalate (for glucose estimation) and EDTA and Lithium heparin (for lipid and enzyme studies) and was then centrifuged at 3000 g for 10 min to obtain plasma and was used for the determination of glucose and lipid profile of the rats.

**Biochemical Estimations**

Hemoglobin was estimated by cyanomethemoglobin method (Drabkin and Austin, 1932). Glycosylated hemoglobin was determined by the method of Bannor (1982). The protein content of plasma was determined by the method of Lowry et al. (1951). Plasma glucose level was determined by O-toluidine method (Sasaki et al., 1972). Plasma insulin was assayed by ELISA kit (Boehringer-Mannheim kit). Glucose-6-phosphatase activity was assayed by method of Koide and Oda (1959). The plasma LPO was assayed spectrophotometrically by the thiobarbituric acid reactive substances (TBARS) method (Walls et al., 1976). Plasma triglycerides and cholesterol levels were assayed by using commercial diagnostic kits (Ranbaxy Diagnostics, New Delhi, India). Plasma phospholipids levels were determined by the spectrophotometric method of Chen et al. (1956). VLDL and LDL-cholesterol were assayed by using commercial diagnostic kits (Ranbaxy Diagnostics, New Delhi, India). The LDL-cholesterol was calculated using the formula of Friedewald et al. (1972).

**Statistical Analysis**

The results were expressed as mean±SEM of the 6 rats per group and the statistical significance was evaluated by one-way analysis of variance (ANOVA) using the SPSS (version 10.0) program followed by least significant difference (LSD) test. Values were considered statistically significant when $p<0.05$.

**RESULTS**

The diabetic rats showed a significant ($p<0.05$) decrease in body weight and insulin and a significant ($p<0.05$) increase in plasma glucose. The administration of CLEt and glibenclamide to diabetic rats restored the plasma glucose level, insulin and body weight significantly ($p<0.05$). The administration of CLEt to normal rats depict much significant ($p<0.05$) effect on plasma glucose and insulin (Table 1).
Table 1: Changes in body weight, total hemoglobin, glycosylated hemoglobin, urine sugar, fasting plasma glucose and activity of glucose-6-phosphatase in normal and experimental groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Hemoglobin (g/dL)</th>
<th>Glycosylated hemoglobin (mg g⁻¹ Hb)</th>
<th>Urine sugar (mg dL⁻¹)</th>
<th>Fasting plasma glucose (mg dL⁻¹)</th>
<th>Plasma insulin (µIU mL⁻¹)</th>
<th>Glucose-6-phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>179.8±0.51</td>
<td>21.3±1.39</td>
<td>12.5±1.68</td>
<td>0.5±0.02</td>
<td>69.5±5.87</td>
<td>16.0±0.39</td>
<td>0.18±0.01</td>
</tr>
<tr>
<td>Normal + CLEI</td>
<td>185.5±1.04</td>
<td>22.1±1.41</td>
<td>13.0±1.34</td>
<td>0.2±0.01</td>
<td>63.5±0.52</td>
<td>19.4±0.57</td>
<td>0.18±0.01</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>204.6±1.57</td>
<td>17.9±1.74</td>
<td>6.0±1.87</td>
<td>0.6±0.04</td>
<td>258.1±1.45</td>
<td>4.2±0.16</td>
<td>0.25±0.031</td>
</tr>
<tr>
<td>Diabetic + CLEI</td>
<td>207.6±1.46</td>
<td>21.8±1.43</td>
<td>12.7±1.35</td>
<td>0.3±0.04</td>
<td>85.8±9.27</td>
<td>13.7±1.02</td>
<td>0.19±0.013</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide</td>
<td>189.3±1.23</td>
<td>20.1±1.40</td>
<td>11.8±1.30</td>
<td>0.3±0.04</td>
<td>92.4±1.45</td>
<td>8.9±0.22</td>
<td>0.23±0.025</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM of 6 rats from each group. Values are statistically significant at *: p<0.05; #: moles of inorganic phosphorus liberated mg⁻¹ protein; #: Normal + CLEI rats were compared with normal rats; #: Diabetic rats were compared with normal rats; #: CLEI treated diabetic rats were compared with diabetic rats; #: Gilbenclamide treated diabetic rats were compared with diabetic rats; #: Present #: Indicates more than 2% sugar.

Table 2: Levels of plasma LPO, triglycerides, total cholesterol, phospholipids, LDL-cholesterol and HDL-cholesterol in normal and experimental groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>LPO (µM)</th>
<th>Triglycerides (mg dL⁻¹)</th>
<th>Total cholesterol (mg dL⁻¹)</th>
<th>Phospholipids (mg dL⁻¹)</th>
<th>LDL-cholesterol (mg dL⁻¹)</th>
<th>HDL-cholesterol (mg dL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>23.8±4.6</td>
<td>80.2±4.7</td>
<td>84.8±5.6</td>
<td>79.4±8.1</td>
<td>40.8±3.7</td>
<td>19.8±1.8</td>
</tr>
<tr>
<td>Normal + CLEI</td>
<td>20.3±3.7</td>
<td>76.8±3.9</td>
<td>79.4±4.4</td>
<td>72.8±3.8</td>
<td>56.3±5.2</td>
<td>20.9±5.2</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>38.9±6.1</td>
<td>148.1±5.8</td>
<td>203.8±8.4</td>
<td>148.6±10.9</td>
<td>108.6±4.8</td>
<td>15.7±8.3</td>
</tr>
<tr>
<td>Diabetic CLEI</td>
<td>29.2±2.9</td>
<td>96.3±8.2</td>
<td>91.5±5.3</td>
<td>88.3±4.6</td>
<td>46.1±2.6</td>
<td>18.5±3.7</td>
</tr>
<tr>
<td>Diabetic Glibenclamide</td>
<td>30.7±5.8</td>
<td>102.6±7.9</td>
<td>96.7±7.9</td>
<td>94.9±5.8</td>
<td>50.3±5.1</td>
<td>17.8±4.2</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM of 6 rats from each group. Values are statistically significant at *: p<0.05; #: moles of MDA liberated mg⁻¹ protein; #: Normal + CLEI rats were compared with normal rats; #: Diabetic rats were compared with normal rats; #: CLEI treated diabetic rats were compared with diabetic rats; #: Gilbenclamide treated diabetic rats were compared with diabetic rats.

The diabetic control rats showed a significant (p<0.05) decrease in the total hemoglobin level and significant (p<0.05) increase in glycosylated hemoglobin level. The administration of CLEI and glibenclamide to diabetic rats restored the changes in the total hemoglobin and glycosylated hemoglobin level to near normal (Table 1). In case of normal rats with CLEI, the levels of total hemoglobin and glycosylated hemoglobin remains unchanged.

An increase in the activities of glucose-6-phosphatase was observed in diabetic control rats. The changes in the glucose-6-phosphatase activity are significantly (p<0.05) reversed by the administration of CLEI and glibenclamide (Table 1).

A significant (p<0.05) increase in the levels of plasma lipids was observed in diabetic control rats, which were restored, to near normal by the administration of CLEI or glibenclamide. The levels of LDL were significantly (p<0.05) increased, whereas the HDL-cholesterol was markedly (p<0.05) decreased in diabetic rats (Table 2). The diabetic rats treated with CLEI or glibenclamide for 28 days, LDL level was reduced significantly (p<0.05), whereas HDL-cholesterol was significantly (p<0.05) increased.

**DISCUSSION**

Streptozotocin, a β-cell cytotoxin, induces chemical diabetes in a wide variety of animal species by damaging the β-cells of the pancreas, leaving less β-active cells and resulting in a diabetic state. STZ-induced experimental diabetes is a valuable experimental model to study the activity of hypoglycemic agents (Szkucelski, 2001). Further, STZ-diabetic animals may exhibit most of the
diabetic complications, namely, myocardial, nervous, vas deferens, gastrointestinal, kidney and urinary bladder dysfunction, through oxidative stress. Several studies have documented the hypoglycemic and hypolipidemic activity of various medicinal plants extracts in STZ-induced diabetic mellitus rats (Ravi et al., 2004; Rajasekaran et al., 2005; Selvar et al., 2005). Glibenclamide, a hypoglycemic drug, is more effective in moderate diabetic rats than in severe diabetic rats. The hypoglycemic effect of glibenclamide results has been shown from the stimulation of insulin release from the residual β-cells and inhibition of glucagon secretion. Thus, we used glibenclamide as a reference drug for this study (Sharma et al., 1997).

The phytochemical analysis of individual solvent free extract of Coccidi hirsutus revealed the presence of relatively more number of active ingredients (alkaloids, phenols, glycosides, flavonoids and carbohydrates) in methanolic extract and hence methanolic extract was used in this study (data not shown). The dose of 500 mg kg\(^{-1}\) body weight of CLEt was fixed after assessment of hypoglycemic activity in 4 groups of rats with different doses of CLEt ranges from 250, 500, 750 and 1000 mg kg\(^{-1}\) body weight (data not shown), because the dose of 500 mg kg\(^{-1}\) body weight of CLEt was found to have maximum hypoglycemic activity.

Therefore, the present study was carried out by giving 500 mg kg\(^{-1}\) body weight of CLEt. In this present study, CLEt exhibited hypoglycemic activity in normoglycemic rats. Likewise, CLEt produce significant plasma glucose lowering effect in STZ-induced diabetic rats after the administration of extract. The significant hypoglycemic effect of CLEt on normal rats may indicative that the chemical constituents of Coccidi hirsutus (L.) Dills exerts their effect either by increasing the pancreatic secretion from the β-cells or its release from bound insulin. In STZ-induced diabetic rats, CLEt probably act by stimulating the few surviving β-cells to release more insulin rather than by aiding the regeneration of necrotic β-cells of the pancreas, since STZ administered at a dose of 65 mg kg\(^{-1}\) body weight should have caused severe destruction of β-cells of the pancreas (Sharma et al., 1997).

Moreover, during diabetes the excess glucose present in circulation reacts with hemoglobin to form glycosylated hemoglobin. Therefore, the total hemoglobin level is decreased in STZ-induced diabetic rats and the glycosylated hemoglobin was significantly increased in diabetic rats. Administering CLEt tends to normalize the total hemoglobin and glycosylated hemoglobin in STZ-induced diabetic rats, by reducing plasma glucose levels (Sheela and Augusti, 1992).

The body weight was decreased in STZ-diabetic rats. Administration of CLEt increases the body weight in STZ-diabetic rats. The ability of CLEt to protect massive body weight loss seems to be due to its ability to reduce hyperglycemia.

It is known that in diabetes the levels of hepatic glucose metabolic enzymes, especially glucose-6-phosphatase and is adversely affected (Shieh et al., 2004) and the process of gluconeogenesis is much more favoured than glycolysis. It is therefore expected that a potent antidiabetic agent would decreased the flux through the gluconeogenic pathway by reducing the activity of glucose-6-phosphatase. In this study, CLEt significantly decreased the activity of glucose-6-phosphatase in STZ-diabetic rats. The extract-induced reduction in enzyme activity may lead to a decrease in flux through the gluconeogenic pathway and thus, place a lower demand on pancreatic insulin than in the STZ-diabetic rats.

Clinical and experimental evidences suggest that free-radical-mediated oxidative processes are involved in the pathogenesis of diabetic complications. An increase in the production of free radicals can result in hyperglycemia-enhanced enhancement in glucose autooxidation, protein glycation and subsequent oxidative degradation of glycerated proteins. There is growing evidence that oxidative stress is implicated in cardiac dysfunction, leading to heart failure in diabetes (Somogyi et al., 2005). It has been reported that over 75% of early deaths in diabetes are related to coronary artery disease caused by abnormal lipid metabolism, which often leads to altered lipid profile of the victim. In this study, CLEt administered for 28 days altered significantly the lipid profile of the STZ-diabetic rats when compared with controls. STZ-diabetic rats had significantly higher plasma triglycerides, LDL-cholesterol and total cholesterol levels when compared to normal. This result is consistent with the
findings of Ravi et al. (2005), who reported elevated levels of total cholesterol, LDL-cholesterol and HDL-cholesterol in STZ-diabetic rats. In their study, these biochemical indices were significantly reduced in diabetic rats following administration of the extract.

LPO is one of the characteristic features of chronic diabetes. The increased free radicals produced may react with polyunsaturated fatty acids in cell membranes leading to LPO. LPO will in turn, result in the elevated production of free radicals. Insulin secretion is also closely associated with lipoygenase-derived peroxides. Low levels of lipoygenase-derived peroxides stimulate the secretion of insulin, but when the concentration of endogenous peroxides increases, it may initiate uncontrolled LPO leading to cellular infiltration and islet cell damage in diabetes (Metz, 1984). In agreement with previous studies that have used the TBARS assay as an index of LPO (Kakkar et al., 1998), we found an increase in TBARS level in STZ-diabetic rats. In this study, Cleet treated STZ-diabetic rats had significantly lowered TBARS levels when compared with diabetic control. The ability of Cleet to reduce the TBARS levels further confirmed the antioxidant property of the plant.

Thus, the antihyperglycemic activity of Cleet may be due to the presence of above-said alkaloids. This was evidenced in the root alkaloids of Cocculus hirsutus in diabetic rats (Satyanarayana et al., 1994). Therefore, it can be concluded that the Cleet can prevent the rise in plasma glucose and lipid concentrations that occur in STZ-induced diabetic rats. Further, we have been designed our study on antioxidant potential of this extract in STZ-induced diabetes mellitus rat.

REFERENCES


