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Evaluation of Hypoglycemic and Hypolipidemic Activity of Methanolic Extract of *Cocculus 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 *Cocculus 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: *Cocculus 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, lactic 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 Pandey, 1984).

Corresponding Author: R. Malathi, Department of Biochemistry, Thanthai Hans Roever College, Perambalur, Tamil Nadu, 621 212, South India Tel: +914328275574

Cocculus 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 (Caius, 1986). The roots of *Cocculus 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, ginnol (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), cohirsinine (Ahmad *et al.*, 1991), hirsutine (Rasheed *et al.*, 1991b), jamtinine (Ahmad and Iqbal, 1993a), jamitine-N-oxide (Ahmad *et al.*, 1987a), cohirsine (Ahmad *et al.*, 1987b), corsitinine (Ahmad and Iqbal, 1992), haiderine (Ahmad and Iqbal, 1993b), D-trilobine, DL-coclaurine (Jagannadha Rao and Ramachandra, 1961), isotrilobine, (+)-syringaresinol and protoquercitol (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 Singhai, 1993). So far, limited information is available on hypoglycemic activity of *Cocculus hirsutus* (Satyanarayana *et al.*, 1994). Therefore, the present investigation is carried out to study the hypoglycemic and hypolipidemic effects of *Cocculus 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 *Cocculus 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-7/06) 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 gram 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 0.05M 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 Bannon (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

Groups	Body weight (g)		Hemoglobin (g dL ⁻¹)	Glycosylated hemoglobin (mg g ⁻¹ Hb)	Urine sugar	Fasting plasma glucose (mg dL ⁻¹)	Plasma insulin (μU mL ⁻¹)	Glucose-6-phosphatase [#]
	Initial	Final						
Normal	179.83±06.51	213.57±8.39	12.55±1.68	0.328±0.02	Nil	69.36±3.87	16.08±0.39	0.184±0.014
Normal + CLEt	185.50±11.04	221.81±17.41 ^{ab}	13.01±0.34 ^a	0.296±0.01 ^a	Nil	63.08±6.52 ^{ab}	19.41±0.57 ^a	0.180±0.010 ^a
Diabetic control	204.61±15.73	178.93±11.46 ^{ab}	09.83±1.77 ^{ab}	0.648±0.54 ^{ab}	+++	258.13±12.45 ^{ab}	4.25±0.16 ^{ab}	0.258±0.031 ^{ab}
Diabetic + CLEt	207.16±16.46	218.70±12.18 ^{bc}	12.76±1.33 ^{bc}	0.327±0.04 ^{bc}	+	85.88±9.27 ^{bc}	13.71±1.02 ^{bc}	0.198±0.013 ^{bc}
Diabetic + Glibenclamide	189.33±12.13	201.52±14.09 ^{bd}	11.85±1.30 ^{bd}	0.341±0.05 ^{bd}	+	92.46±15.48 ^{bd}	8.93±0.22 ^{bd}	0.230±0.025 ^{bd}

Values are given as mean±SEM of 6 rats from each group; Values are statistically significant at *: p<0.05; #: μmoles of inorganic phosphorus liberated min⁻¹ mg⁻¹ protein; ^a: Normal + CLEt rats were compared with normal rats; ^b: Diabetic rats were compared with normal rats; ^c: CLEt treated diabetic rats were compared with diabetic rats; ^d: Glibenclamide 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

Groups	LPO	Triglycerides	Total cholesterol	Phospholipids	LDL-cholesterol	HDL-cholesterol
		(mg dL ⁻¹)				
Normal	23.8±4.6	80.2±4.7	84.8±5.6	79.4±8.1	40.8±3.7	19.6±1.8
Normal + CLEt	20.3±3.7 ^{ab}	76.8±3.9 ^{ab}	79.4±8.4 ^{ab}	72.8±3.8 ^{ab}	36.3±2.9 ^{ab}	20.9±5.2 ^a
Diabetic control	38.9±6.1 ^{ab}	148.1±5.8 ^{ab}	203.8±8.8 ^{ab}	148.6±10.9 ^{ab}	108.6±4.8 ^{ab}	15.7±8.3 ^{ab}
Diabetic + CLEt	29.2±2.9 ^{bc}	96.3±8.2 ^{bc}	91.5±5.3 ^{bc}	88.3±4.6 ^{bc}	46.1±2.6 ^{bc}	18.5±3.7 ^{bc}
Diabetic + Glibenclamide	30.7±5.8 ^{bd}	102.6±7.9 ^{bd}	96.7±7.3 ^{bd}	94.9±5.8 ^{bd}	50.3±5.1 ^{bd}	17.8±4.2 ^{bd}

Values are given as mean±SEM of 6 rats from each group; Values are statistically significant at *: p<0.05; #: μmoles of MDA liberated mg⁻¹ protein; ^a: Normal + CLEt rats were compared with normal rats; ^b: Diabetic rats were compared with normal rats; ^c: CLEt treated diabetic rats were compared with diabetic rats; ^d: Glibenclamide 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 CLEt 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 CLEt, 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 CLEt 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 CLEt 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 CLEt 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 (Szkudelski, 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 diabetes mellitus rats (Ravi *et al.*, 2004; Rajasekaran *et al.*, 2005; Sekar *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 glucagons 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 *Cocculus 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 *Cocculus hirsutus* (L.) Diels 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-induced enhancement in glucose autooxidation, protein glycation and subsequent oxidative degradation of glycated 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 lipoyxygenase-derived peroxides. Low levels of lipoyxygenase-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, CLEt treated STZ-diabetic rats had significantly lowered TBARS levels when compared with diabetic control. The ability of CLEt to reduce the TBARS levels further confirmed the antioxidant property of the plant.

Thus, the antihyperglycemic activity of CLEt 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 CLEt 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.

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