

Full Length Research Paper

Antihyperglycemic and antihyperlipidemic effects of *Clitoria ternatea* Linn. in alloxan-induced diabetic rats

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This study aims to investigate the therapeutic effects of *Clitoria ternatea* Linn. leaves and flowers extract on alloxan-induced diabetic rats. The effect of aqueous extract of *C. ternatea* leaves and flowers on serum glucose, glycosylated hemoglobin, insulin, total cholesterol, triglycerides, HDL-cholesterol, protein, urea, creatinine were examined in control and extract treated diabetic rats. Glycogen was examined both in the liver and skeletal muscles of control and extract treated diabetic rats, whereas, the activity of glycolytic enzyme glucokinase and gluconeogenic enzyme glucose-6-phosphatase was examined in the liver. Oral administration of aqueous extract of *C. ternatea* leaves (400 mg/kg body weight) and flowers (400 mg/kg body weight) for 84 days significantly reduced serum glucose, glycosylated hemoglobin, total cholesterol, triglycerides, urea, creatinine and the activity of gluconeogenic enzyme glucose-6-phosphatase, but increased serum insulin, HDL-cholesterol, protein, liver and skeletal muscle glycogen content and the activity of glycolytic enzyme glucokinase. For all the above biochemical parameters investigated, *C. ternatea* leaves treated rat showed a little better activity than *C. ternatea* flowers treated diabetic rats. The present investigation suggests that *C. ternatea* leaves and flowers extract exhibit antihyperglycaemic and antihyperlipidaemic effects and consequently may alleviate liver and renal damage associated with alloxan-induced diabetes mellitus in rats.

Key words: Alloxan, diabetes mellitus, *Clitoria ternatea*, enzymes, hemoglobin.

INTRODUCTION

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion or insulin action (Jayakar and Suresh, 2003). Diabetes mellitus is also associated with an increased risk for developing premature atherosclerosis due to independent risk factors such as hypertriglyceridemia and hypertension (Schwartz et al., 1993). It is also characterized by polyuria, albuminuria, renal enlargement and an increase in serum creatinine value (Sassy-Prigent et al., 1995). Insulin therapy and oral hypoglycemic agents offer effective glycemic control; yet, their shortcomings limit their usage (Anuradha et al., 2004). Plants are reputed in the indigenous systems of medicine

for the treatment of various diseases (Arise et al., 2009), the available literature shows that there are more than 800 plant species showing hypoglycemic activity (Marles and Farnsworth, 1995). The world health organization has also recommended the evaluation of the effectiveness of plants in conditions where we lack safe modern drugs (World Health Organization, 1980). Phytochemicals isolated from plant sources are used for the prevention and treatment of cancer, heart disease, diabetes mellitus and high blood pressure (Waltner-Law et al., 2002).

Clitoria ternatea belonging to the family Fabaceae is a perennial twining herb, found in Indo-China, Philippines and Madagascar, since the flowers of the plant resemble a conch shell; it is commonly called "Shankpushpi" (Kulkarni et al., 1988). *C. ternatea* is reported to be a good "Medhya" (toning the brain) drug mainly used in the treatment of "Masasika" roga (mental illness), but it is also said to be useful in hectic fever, severe bronchitis, asthma and remedy for snakebite and scorpion sting (Chopra et al., 1982).

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The root is used by tribal to induce abortion while its paste is applied for curing abdominal swellings, sore throat, mucus disorders and fever (Devi et al., 2003). A preliminary study using fresh flowers of *C. ternatea* showed hypoglycemic and hypolipidemic effects (Rajathi and Daisy, 2000). Therefore, the present study was undertaken to evaluate the effectiveness of *C. ternatea* flowers (CTF) and leaves (CTL) in alloxan-induced diabetic rats for its antihyperglycemic and antihyperlipidemic effects.

MATERIALS AND METHODS

Plant Material

Leaf and flower of *C. ternatea* were collected from Thirumayam, Pudukkottai district, and Tamilnadu, India. They were carefully identified and authenticated by Dr. Annie Xavier, Professor of Botany, Holy Cross College, Tiruchirappali – 620 002. Shade dried leaves and flowers of *C. ternatea* were powdered and boiled in water (100 g/l distilled water). The decoction was filtered through nitrocellulose filter and the filtrates were evaporated to dryness under reduced pressure and at a lower temperature in a rotary evaporator. The dried residues were stored in airtight containers for further use.

Induction of diabetes in rats

Male adult wistar strain albino rats (100 - 150 g) were used for the test. Ethical approval was obtained from CPCSEA (Committee for the purpose of control and supervision on experiments on animals), institutional ethical review committee number is 585/05/A/CPCSEA. The animals were obtained from Tamilnadu veterinary and animal science University, Chennai, India. The animals were fed with a standard feed (Sai Durga feeds and foods, Bangalore, India) and water *ad libitum*. The animals described as fasted were deprived of food for 16 h but were allowed free access to water. After randomization into various groups, the rats were acclimated to the laboratory conditions of temperature and photoperiod for a period of 1 - 2 weeks before initiation of the experiment. Diabetes mellitus was induced in a batch of normoglycemic albino rats starved for 16 h, by injecting intraperitoneally 150 mg/kg body weight of alloxan monohydrate dissolved in physiological saline. Alloxan monohydrate was obtained from Sigma chemical company, St. Louis, Mo, USA. All the other chemicals used were of analytical grade and were purchased from commercial sources. Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution intraperitoneally after 6 h. For the next 24 h, the rats were kept on 5% glucose solution in their cages to prevent hypoglycemia, as revealed by the determination of blood glucose levels.

Experimental design

After 7 days of alloxan injection, rats with blood glucose > 300 mg/dl were considered as diabetic and included in the study. They were divided into different groups, with 5 rats in each group. Aqueous extracts of CTL and CTF ranging from 50 mg/kg body weight to 500 mg/kg body weight at an interval of 50 mg/kg body weight were administered to the animals and blood glucose was estimated at the end of 5 h after the oral administration of the extract. The lowest dose that brought about the maximum antihyperglycemic effect for each plant part was given through oral intubations for the repeated administration. It was observed that the dose was the same for CTL and CTF (400 mg/kg body weight).

1 week after induction of diabetes in albino rats, the fasting blood glucose levels of rats were measured, rats with blood glucose > 300 mg/dl were included in the study. They were divided into 4 groups with 10 rats in each group. The selected doses of the plant extracts (the dosage arrived at after preliminary study) were given everyday till completion of the experiment (84 days), whereas control and diabetic control group was given distilled water everyday through oral intubations.

Group 1: Control rats given only distilled water.

Group 2: Diabetic control rats given only distilled water.

Group 3: Diabetic rats treated orally with CTL (400 mg/kg body weight) in aqueous solution for 84 days.

Group 4: Diabetic rats treated orally with CTF (400 mg/kg body weight) in aqueous solution for 84 days.

At the end of the experiment, animals from each group were sacrificed by cervical dislocation for biochemical and histological studies. Blood was collected from the heart and allowed to clot and the serum was separated by centrifugation at 3500 rpm for 10 min. Serum was assayed either immediately or stored at -20°C. Tissues like liver, skeletal muscle and pancreas were collected. Liver and skeletal muscles were collected for biochemical estimations and pancreas was used for histological studies.

Biochemical assays

Serum glucose (Reddy's laboratories, Hyderabad, India), glycosylated hemoglobin (Bio Systems, Costa Brava, Spain), insulin (Radioimmunoassay kit, Diasorin, Italy), total cholesterol (Beacon Diagnostics, Kabilpore, India), triglycerides (Bio Systems, Costa Brava, Spain), HDL-cholesterol (Beacon Diagnostics, Kabilpore, India), creatinine (Reddy's laboratories, Hyderabad, India) were estimated using a commercial diagnostic kit. Liver and skeletal muscle glycogen were estimated by the method described by Plummer (1987). Glucokinase and glucose -6-phosphatase were assayed by the method of Brandstrup et al. (1957) and Baginsky et al. (1992). Serum protein and urea were estimated by the method described by Lowry et al. (1951) and Fawcett and Scott, (1960).

Statistical analysis

All the grouped data was statistically evaluated via the statistical package for social sciences version 7.5. Hypothesis testing method included one-way analysis of various (ANOVA) followed by least significant difference test. P-values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as mean \pm SD for 10 animals in each group.

RESULTS

A significant increase in blood glucose, glycosylated hemoglobin and a decrease in serum insulin were observed in diabetic control rats when compared to control rats (Table 1). Administration of CTL and CTF to diabetic rats significantly decreased the level of blood glucose and glycosylated hemoglobin, increased serum insulin to near control level. Table 1 depicts the levels of total cholesterol, triglyceride and HDL-cholesterol in control and alloxan-induced diabetic rats. The level of cholesterol and triglyceride increased in diabetic animals when compared to control animals. After CTL and CTF treatment, the higher level of cholesterol and triglyceride, decreased to

Table 1. Effect of treatment with CTL (400 mg/kg) and CTF (400 mg/kg) on serum parameters of control and alloxan diabetic rats.

Parameters	Control	Diabetic control	Diabetic ± CTL	Diabetic ± CTF
Glucose (mg/dl)	74.8 ± 3.1	361.0 ± 10.4	102.4 ± 4.8 *	107.6 ± 4.9*
Glycosylated hemoglobin (%)	2.44 ± 0.29	4.86 ± 0.68	2.81 ± 0.76 *	2.92 ± 0.38*
Insulin (μU/ml)	38.6 ± 4.5	8.2 ± 1.3	30.6 ± 2.1 *	29.3 ± 2.2 *
Cholesterol (mg/dl)	90.0 ± 4.3	200.0 ± 7.0	106.0 ± 5.3 *	107.0 ± 5.9 *
Triglyceride (mg/dl)	68.0 ± 3.2	15.3 ± 9.8	84.9 ± 4.8 *	87.0 ± 3.7 *
HDL-cholesterol (mg/dl)	60.4 ± 3.3	15.0 ± 2.8	40.2 ± 4.1 *	39.7 ± 5.2 *
Protein (g/dl)	7.80 ± 0.83	3.00 ± 0.39	5.64 ± 0.50 *	5.40 ± 0.41 *
Urea (mg/dl)	32.9 ± 2.2	90.2 ± 7.3	44.2 ± 4.7 *	44.9 ± 3.9 *
Creatinine (mg/dl)	0.40 ± 0.03	4.30 ± 1.00	1.50 ± 0.36 *	1.70 ± 0.02 *

Values are given as mean ± SD for groups of ten animals each. Values are statistically significant at *P < 0.05. CTL and CTF treated diabetic rats were compared with diabetic rats.

Table 2. Effect of treatment with CTL (400 mg/kg) and CTF (400 mg/kg) treated diabetic rats on liver glycogen, skeletal muscle glycogen, glucokinase, glucose-6-phosphatase activities of control and alloxan diabetic rats.

Parameters	Control	Diabetic control	Diabetic±CTL	Diabetic±CTF
Liver glycogen (mg/g)	49.0 ± 1.3	9.0 ± 2.5	35.0 ± 1.0 *	34.0 ± 1.9 *
Skeletal muscle glycogen (mg/g)	9.8 ± 1.8	1.8 ± 0.6	6.2 ± 0.4 *	5.8 ± 0.5 *
Glucokinase (μ mol of glucose-6-Po ⁴ formed /min/mg protein)	207.5 ± 6.4	115.4 ± 8.9	163.7 ± 4.2 *	161.0 ± 3.2 *
Glucose-6-phosphatase (μ mol of Pi liberated/min/mg protein)	0.150 ± 0.021	0.252 ± 0.028	0.199 ± 0.006 *	0.201 ± 0.036 *

Values are given as mean±SD for groups of ten animals each. Values are statistically significant at *P < 0.05. CTL and CTF treated diabetic rats were compared with diabetic rats.

near control. The level of HDL-cholesterol in serum of diabetic animals decreased. These lowered levels of HDL-cholesterol were restored significantly in CTL and CTF treated diabetic rats.

The level of protein in serum reduced in diabetic control animals when compared to control animals. The lowered level of protein after CTL and CTF treatment, increased to near control. Urea and creatinine levels were significantly elevated in alloxan-induced diabetic rats when compared to control animals. Oral administration of CTL and CTF for 84 days significantly lowered urea and creatinine levels in alloxan-induced diabetic rats. Table 2 shows the content of liver glycogen and skeletal muscle glycogen in control and diabetic rats. The content of liver glycogen and skeletal muscle glycogen reduced in diabetic animals when compared to control animals. The lowered content of liver glycogen and skeletal muscle glycogen, increased to near control after CTL and CTF treatment. Table 2 represents the activities of glucokinase and glucose -6- phosphatase in the liver of control and alloxan-induced diabetic rats. The activity of glucokinase decreased in the liver of diabetic control animals, whereas, the activity of glucose -6- phosphatase was found to be increased in the liver of diabetic animals. Oral administration of CTL and CTF for 84 days resulted in an increase in the activity of glucokinase and a decrease in

the activity of glucose-6-phosphatase enzyme in the liver of diabetic animals.

DISCUSSION

The currently available drug regimens for management of diabetes mellitus have certain drawbacks and therefore, there is a need to find safer and more effective antidiabetic drugs (Grover et al., 2002; Rajagopal and Sasikala, 2008). Diabetes mellitus of long duration is associated with several complications such as atherosclerosis, myocardial infarction, nephropathy etc. These complications have long been assumed to be related to chronically elevated glucose level in blood (Alarcon-Aguilara et al., 2000). Alloxan causes a massive reduction in insulin release by the destruction of β-cells of the islets of langerhans and thereby induces hyperglycemia (Siyem et al., 2002). Daily administration of aqueous extracts of CTL and CTF for 84 days had resulted in a decrease in blood glucose level in alloxan-induced diabetic rats. The possible hypoglycemic mechanism of CTL and CTF may be through potentiation of pancreatic secretion of insulin from β-cell of islets or due to enhanced transport of blood glucose to the peripheral tissues.

Glycosylated hemoglobin is produced through glycosylation of hemoglobin. Glycosylated hemoglobin is formed

progressively and irreversibly over a period of time and is stable till the life of the RBC and is unaffected by diet, insulin or exercise on the day of testing. Therefore glycosylated hemoglobin can be used as an excellent marker of overall glycemic control. Since it is formed slowly and does not dissociate easily, it reflects the real blood glucose level (Kameswararao et al., 2003). In this study, the diabetic rats had higher levels of glycosylated hemoglobin, the significant decrease of glycosylated hemoglobin in alloxan-induced diabetic rats due to CTL and CTF therapy indicates that the overall blood glucose level is controlled which must be due to improvement in insulin secretion.

Serum insulin level of diabetic animals treated with the extracts of CTL and CTF increased when compared to the diabetic control animals. Administration of aqueous extract of CTL and CTF increased the serum insulin level in alloxan-induced diabetic rats suggesting its possible action by increasing insulin release. This result is in agreement with other previous studies on *Gymnema sylvestre* (Shanmugasundaram et al., 1990), *Momordica charantia* (Cakici et al., 1994), *Encostemma littorale* (Maroo et al., 2002).

Insulin deficiency or insulin resistance is associated with hypercholesterolemia and hypertriglyceridemia (Sharma et al., 1996). The abnormally high concentration of serum lipids in diabetes mellitus is mainly due to an increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. The marked hyperlipidaemia that characterises the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots (Al-Shamaony et al., 2004). Administration of CTL and CTF to diabetic rats brought down serum cholesterol, triglycerides and increased HDL-cholesterol level.

Renal disease is one of the most common and severe complications of diabetes. Insulin is a physiological factor, which plays an important role in the maintenance of protein balance, since it not only stimulates the uptake of amino acids and protein synthesis, but also inhibits protein degradation (Pathak and Dhawan, 1988). In addition, significant elevations in serum creatinine and urea levels indicate impaired renal function of diabetic animals. Aqueous extract of CTL and CTF increased the total protein and lowered the serum urea and creatinine levels by enhancing the renal function that is generally impaired in diabetic rats. This result is in agreement with a previous study (Daisy et al., 2007).

Glycogen content of skeletal muscles and liver markedly decreases in diabetes. The decrease in glycogen content of liver and skeletal muscle observed in the present study is probably due to the lack of insulin in the diabetic state. This prevention of glycogen depletion in the liver and muscles might possibly be due to stimulation of insulin release (Chakrabarti et al., 2003).

Administration of CTL and CTF to diabetic animals increased the activity of glucokinase in liver. The extract-

induced decrease in the concentration of blood glucose in alloxan-treated rats may be the result of increased glycolysis. This is in agreement with the previous studies on *Catharanthus roseus* (Singh et al., 2001), *Tinospora cordifolia* (Prince and Menon, 2000), *Gymnema sylvestre* (Shanmugasundaram et al., 1983), the activity of gluconeogenic enzyme glucose-6-phosphatase is enhanced during diabetes (Vijayvargia et al., 2000). During the extract-induced hypoglycemia, the blood glucose was reduced and there was an increase in liver glycogen content. This may be due to mobilization of blood glucose towards liver glycogen reserve. The activity of glucose-6-phosphatase was inhibited after administration of the extracts suggests that glucose-6-phosphate is not utilized for the synthesis of glucose in the glycogenic pathway, but may be used as a substrate for glycogenesis or in the HMP pathway. In this context a number of plants have been reported that decreased the activity of glucose-6-phosphatase in the liver of diabetic rats (Sachdewa and Khemani, 2003).

The results of the present investigation clearly indicate that the leaf and flower extract of *C. ternatea* have hypoglycemic effect on alloxan-induced diabetic rats. The extracts were highly effective in managing the complications associated with diabetes mellitus, such as hypercholesterolemia, hypertriglyceridemia and impaired renal function. Therefore, *C. ternatea* leaf and flower extracts show therapeutic action against the development and progression of diabetic complications mentioned above. Further studies are in progress to isolate the active principle(s) and elucidate the exact mechanism of action of *C. ternatea* leaves and flowers.

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