

ORIGINAL ARTICLE

Antihyperglycaemic effect of *Cardiospermum halicacabum* Linn. leaf extract on STZ-induced diabetic rats

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Summary

The present study was designed to investigate the antihyperglycaemic effect of ethanolic extract of *Cardiospermum halicacabum* Linn. (Sapindaceae) leaves on normal and streptozotocin (STZ) diabetic rats. Diabetes was induced into male albino Wistar rats by intraperitoneal administration of STZ. The *Cardiospermum halicacabum* leaf extract (CHE) was administered orally at three different doses to normal and STZ-diabetic rats for 45 days. The diabetic rats showed an increase in levels of blood glucose and glycosylated haemoglobin (HbA_{1c}) and a decrease in the levels of insulin and haemoglobin (Hb). In addition, diabetic rats showed a significant reduction in the activity of glucokinase and an elevation in the activities of gluconeogenic enzymes such as glucose-6-phosphatase and fructose-1, 6-bisphosphatase. Treatment with CHE significantly decreased plasma glucose and HbA_{1c}, and increased the levels of insulin and Hb. CHE administration to diabetic rats reversed these enzyme activities in a significant manner. Thus, the results show that CHE possesses an antihyperglycaemic activity and provide evidence for its traditional usage in the control of diabetes. The 200 mg dose of the extract produced a better effect than 50 or 100 mg doses.

Keywords: *Cardiospermum halicacabum* – antihyperglycaemia – streptozotocin – insulin – carbohydrate metabolizing enzymes

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycaemia resulting from

defects in insulin secretion, insulin action, or both. The chronic hyperglycaemia of diabetes is associated with the long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (ADA 2007). The number of people affected with diabetes worldwide is projected to be 366 million by year 2030 (Wild et al. 2004). The disease has become a real public health problem in the developing countries, where its prevalence is increasing steadily and adequate treatment is often expensive or unavailable (Djrolo et al. 1998). Alternative strategies to the current modern pharmacological therapy of diabetes mellitus are urgently needed

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(WHO 2002), because of the inability of existing modern therapies to control all the pathological aspects of the disorder, as well as the enormous cost and poor availability of the modern therapies for many rural populations in developing countries. Plants used in traditional medicine to treat diabetes mellitus represent a valuable alternative for the control of this disease (Ueda et al. 2002, Ganesan et al. 2007).

The plant *Cardiospermum halicacabum* Linn. (Sapindaceae) is an annual or sometimes perennial climber, commonly found as a weed throughout India. The tender, young shoots are used as a vegetable, fodder, diuretic, stomachic, and rubefacient. It is used in rheumatism, lumbago, nervous diseases, and as a demulcent in orchitis and in dropsy. In Sri Lanka, it is used for the treatment of skeletal fractures. The juice of the herb is used to cure ear-ache and to reduce hardened tumours. It exhibits significant analgesic, anti-inflammatory and vaso-depressant activity, which is transient in nature. *In vitro* studies have revealed its antispasmodic and curative actions confirming the use of the herb in Ayurvedic medicine (Anonymous 1992). The leaves of this plant mixed with castor oil are administered internally to treat rheumatism and to check lumbago. Two glasses of a 12 h maceration of aerial parts of the plant are consumed or used for bathing in the treatment of hyperthermia, and in some areas water extract of the seeds is used (Newinger and Basu 1969).

No detailed investigations have been carried out to define the antihyperglycaemic activities of *Cardiospermum halicacabum* and thus, the present investigation sets out to study the antihyperglycaemic activity of *Cardiospermum halicacabum* leaf extract and other related biochemical parameters in normal and streptozotocin induced diabetic rats. The effect produced by the drug was compared with that of glibenclamide, a standard drug.

MATERIALS AND METHODS

Animals

Male albino Wistar rats (weighing 180-200 g, 9 weeks old) were procured from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, and maintained in an air-conditioned room (25 ± 1 °C) with a 12 h light/12 h dark cycle. Feed and water were provided *ad libitum*. The study was conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH, 1985) and the experimental study was approved by the Ethical Committee of Rajah Muthiah Medical

College and Hospital (Reg No.160/1999/CPCSEA), Annamalai University, Annamalaiagar.

Experimental induction of diabetes

The animals were made diabetic by an intraperitoneal injection of streptozotocin (STZ, 40 mg/kg body weight, between 8:00 AM to 9:00 AM) in a freshly prepared citrate buffer (0.1M, pH 4.5) after an overnight fast. STZ injected animals were given 20% glucose solution for 24 h to prevent initial drug-induced hypoglycaemic mortality. The animals exhibited massive glycosuria (determined by Benedict's qualitative test, Benedict 1911) and hyperglycaemia within a few days. Diabetes was confirmed by measuring the fasting blood glucose concentration (Trinder 1969), 96 h after induction. Albino rats with a blood glucose level above 220 mg/dl were considered diabetic and were used in the experiment.

Plant material

Leaves of *Cardiospermum halicacabum* were collected from the local areas, Jeyankondam, Ariyalur district, Tamil Nadu, India. The plant was botanically identified and authenticated in the Department of Botany, Annamalai University, Annamalaiagar, Chidambaram, Tamil Nadu, India and a voucher specimen was deposited at the herbarium of botany.

Preparation of plant extract

The plant leaf was shade dried at room temperature (32 ± 2 °C) and the dried leaf was ground into fine powder using a pulverizer. The powdered part was sieved and kept in deep freezer until use. 100 g of dry fine powder was suspended in 300 ml of ethanol for 72 h. The extract was filtered using a muslin cloth and concentrated at 40 ± 5 °C.

Experimental design

The animals were randomly divided into five groups of six animals each. The extract was suspended in 2% gum acacia vehicle solution and fed by intubation.

Group I: Normal (2% gum acacia)

Group II: Normal + CHE (200 mg/kg body wt.) in 2% gum acacia

Group III: Diabetic rats (2% gum acacia)

Group IV: Diabetic + CHE (50 mg/kg body wt.) in 2% gum acacia

Group V: Diabetic + CHE (100 mg/kg body wt.) in 2% gum acacia

Group VI: Diabetic + CHE (200 mg/kg body wt.) in 2% gum acacia

Group VII: Diabetic + glibenclamide (600 µg/kg body wt.) in 2% gum acacia

After 45 days, the animals were fasted for 12 h, anaesthetized between 8:00 am to 9:00 am using ketamine (24 mg/kg b.wt, intramuscular injection), and sacrificed by decapitation. Blood was collected

in tubes with a mixture of potassium oxalate and sodium fluoride (1:3) for the estimation of blood glucose and ethylenediaminetetraacetic acid (EDTA) for the estimation of haemoglobin, and glycosylated haemoglobin. Tissues such as liver and kidney were collected and stored at 4 °C for the measurement of various enzyme activities.

Chemicals

Streptozotocin was obtained from Sigma-Aldrich Company (St. Louis, Missouri, USA). All other chemicals used were of analytical grade obtained from E. Merck, Mumbai and HIMEDIA, Mumbai, India.

Biochemical estimations

Blood glucose was estimated by using the reagent kit method of Trinder (1969). The insulin in the rat plasma was measured by the method of Burgi et al (1988). Haemoglobin (Hb) and glycosylated Hb (Hb_{A1c}) were estimated by the methods of Drabkin and Austin (1932), and Sudhakar and Pattabiraman (1981), respectively. The activities of hexokinase, glucose-6-phosphatase, and fructose-1-6-bisphosphatase were assayed by the methods of Brandstrup et al (1957), Koide and Oda (1992), and Gancedo and Gancedo (1971), respectively.

Statistical analysis

Statistical evaluation was done using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) by using the statistical package of social sciences (SPSS) 10.0 for Windows. The significance level was $2\alpha=0.05$

RESULTS

Table 1 shows the effect of the 45 day oral administration of CHE at three different doses (50, 100, 200 mg/kg b.w.) on body weight and blood glucose levels in normal and STZ-diabetic rats. Diabetic rats showed a decreased body weight and elevated blood glucose level. Oral administration of CHE and glibenclamide in diabetic rats showed an improvement in body weight and decreased blood glucose level.

Table 2 shows the effect of CHE on the levels of plasma insulin, Hb, and Hb_{A1c}. Plasma insulin and Hb decreased, and Hb_{A1c} increased significantly in diabetic control rats. The administration of CHE and glibenclamide in diabetic rats increased plasma insulin and Hb significantly, and decreased Hb_{A1c}.

Table 3 shows the effect of oral administration of CHE on carbohydrate metabolic enzymes in the liver of normal and STZ-diabetic rats. Diabetic rats showed decreased activity of glucokinase and

increased activity of glucose-6-phosphatase, and fructose-1,6-bisphosphatase in the liver. Administration of CHE and glibenclamide reversed these enzyme activities towards normalcy.

In comparing CHE treatment at three different doses of CHE, we found that the 200 mg dose showed a better effect than the other two doses in all the above parameters.

DISCUSSION

Streptozotocin selectively destroys the pancreatic insulin secreting β -cells, leaving less active cells and resulting in a diabetic state (Kamtchouing et al. 1998). STZ-induced diabetes is characterized by severe loss in body weight (Chen and Ianvzzo 1982), and this reduction is due to loss or degradation of structural proteins, as the structural proteins are known to contribute to body weight. In our study, a significant weight loss was observed in the diabetic group and significant improvement in weight was observed in the groups treated with CHE. This may be due to the ability of CHE to reduce hyperglycaemia.

The fundamental mechanism underlying hyperglycaemia in diabetes mellitus involves the over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues (Latner 1958), and studies have shown that the level of blood glucose was elevated in STZ-induced diabetic rats. Hence, in the present study, we observed an increased level of blood glucose. Oral administration of CHE resulted in a significant reduction in blood glucose and 200mg/kg body weight dose exhibited maximum reduction when compared to the other two doses.

Flavonoids are one of the most numerous and widespread groups of phenolic compounds in higher plants (Carini et al. 2001). Some of them, due to their phenolic structure are known to be involved in the healing process of free radical mediated diseases including diabetes (Czinner et al. 2000). The plant leaf possesses several flavonoids such as apigenin, pinitol and luteolin (TEAEMP 1999), which are reported as the antidiabetic principles. Apigenin, a component of CHE, was also isolated from *Myrcia multiflora* leaves (Matsuda et al. 2002), and found to possess an inhibitory effect on the aldose reductase enzyme. This enzyme is known to play a key role in the polyol pathway, by catalyzing the reduction of the glucose to sorbitol, which under normal conditions cannot diffuse out of cell membranes. Because of the intracellular accumulation of sorbitol, the chronic complications (such as neuropathy, retinopathy and cataracts) of diabetes can occur. Apigenin and luteolin were shown to possess antihyperglycaemic (Matsuda et al. 1995, Asgary et al. 2002) and antioxidant activity (Romanova et al. 2001).

Table1. Effect of alcoholic leaf extract of *Cardiospermum halicacabum* leaf extract (CHE) on the body weight and glucose in the normal and STZ-diabetic rats

Groups	Changes in body weight (g)		Changes in blood glucose (mg/dL)	
	0 th day	45 th days	0 th day	45 th days
Normal control (2% gum acacia)	180.19 ± 2.79	216.30 ± 5.21 ^a	66.54 ± 5.89	74.55 ± 4.34 ^a
Diabetic control	185.26 ± 2.89	154.16 ± 3.10 ^b	246.63 ± 9.45	290.52 ± 16.24 ^b
Normal + CHE (200 mg/kg body wt.)	190.19 ± 3.52	211.83 ± 4.75 ^a	72.48 ± 5.52	68.93 ± 3.94 ^a
Diabetes + CHE (50 mg/kg body wt.)	185.35 ± 3.49	192.46 ± 3.84 ^c	248.33 ± 16.24	204.03 ± 19.23 ^c
Diabetes + CHE (100 mg/kg body wt.)	179.34 ± 2.55	188.47 ± 5.07 ^d	250.73 ± 14.23	170.12 ± 9.87 ^d
Diabetes + CHE (200 mg/kg body wt.)	180.33 ± 3.38	192.32 ± 4.01 ^e	246.37 ± 12.39	138.45 ± 7.10 ^e
Diabetes + glibenclamide (600 µg/kg body wt.)	180.34 ± 2.84	200.31 ± 4.77 ^e	251.38 ± 17.48	98.50 ± 9.56 ^f

Values are means ± SD of 6 rats from each group

Values not sharing a common superscript differ significantly (DMRT)

Table 2. **Effect of alcoholic leaf extract of CHE on the insulin in the plasma, haemoglobin and glycosylated haemoglobin in the erythrocyte of normal and STZ-diabetic rats**

Groups	Insulin ($\mu\text{L}/\text{mL}$)	Haemoglobin (g/dL)	Glycosylated haemoglobin (mg/g of Hb)
Normal Control (2% gum acacia)	15.12 ± 1.40^a	14.92 ± 0.52^a	1.88 ± 0.12^a
Diabetic control	4.56 ± 0.39^b	6.71 ± 0.52^b	4.68 ± 0.30^b
Normal + CHE (200 mg/kg body wt.)	15.00 ± 1.27^a	14.77 ± 0.74^a	1.84 ± 0.12^a
Diabetes + CHE (50 mg/kg body wt.)	5.02 ± 0.42^c	8.11 ± 0.66^c	4.02 ± 0.23^c
Diabetes + CHE (100 mg/kg body wt.)	7.80 ± 0.66^d	9.02 ± 0.75^d	3.36 ± 0.28^d
Diabetes + CHE (200 mg/kg body wt.)	10.64 ± 0.71^e	11.00 ± 0.43^e	2.60 ± 0.21^e
Diabetes + glibenclamide (600 $\mu\text{g}/\text{kg}$ body wt.)	14.86 ± 1.26^a	13.22 ± 0.70^f	1.92 ± 0.12^a

Significance as per Table 1

Table 3. Effect of alcoholic leaf extract of CHE on the carbohydrate metabolic enzyme activities in the liver of normal and STZ-diabetic rats

Groups	Hexokinase (U*/h/ mg protein)	Glucose-6-phosphatase (U [#] /min/mg protein)	Fructose-1,6-bisphosphatase (U [*] /h/mg protein)
Normal control (2% gum acacia)	0.25 ± 0.02 ^a	0.14 ± 0.01 ^a	0.37 ± 0.02 ^a
Diabetic control	0.11 ± 0.01 ^b	0.35 ± 0.02 ^b	0.62 ± 0.04 ^b
Normal + CHE (200 mg/kg body wt.)	0.26 ± 0.02 ^{ac}	0.15 ± 0.01 ^a	0.36 ± 0.02 ^a
Diabetes + CHE (50 mg/kg body wt.)	0.13 ± 0.01 ^{bd}	0.30 ± 0.03 ^c	0.57 ± 0.04 ^c
Diabetes + CHE (100 mg/kg body wt.)	0.15 ± 0.01 ^d	0.26 ± 0.02 ^d	0.53 ± 0.02 ^{cd}
Diabetes + CHE (200 mg/kg body wt.)	0.19 ± 0.01 ^e	0.21 ± 0.02 ^c	0.49 ± 0.02 ^d
Diabetes + glibenclamide (600 µg/kg body wt.)	0.22 ± 0.02 ^c	0.16 ± 0.01 ^a	0.40 ± 0.03 ^a

U* = µmoles of glucose phosphorylated

U[#] = µmoles of inorganic phosphorus liberated

U^{*} = µmoles of inorganic phosphorus liberated

Significance as per Table 1

STZ causes a massive reduction in insulin release, by the destruction of the beta cells of the islets of Langerhans and thereby induces hyperglycaemia (Schein et al. 1973). In CHE treated diabetic rats, the significant elevation of plasma insulin may be due to the stimulation of insulin secretion from the existing β -cells of the pancreas. The decrease in blood glucose level of diabetic rats treated with CHE might be due to elevated secretion of insulin, which in turn, increases the utilization of glucose by the tissues. The standard drug glibenclamide showed a better reduction when compared to leaf extract treated rats. CHE treated normal rats showed similar blood glucose levels to that of the normal rats. In uncontrolled or poorly controlled diabetes, there is an increased glycosylation of a number of proteins including haemoglobin (Alberti et al. 1982). During diabetes, the excess glucose present in the blood reacts with haemoglobin to form glycosylated haemoglobin. The rate of glycation is proportional to the concentration of blood glucose (Paulsen 1973). HbA_{1C} consists of 3.4% to 5.8% of total haemoglobin in normal human red cells but it is increased in patients with overt diabetes mellitus (Koeing et al. 1976). It was found to increase in diabetic patients up to 16% (Gabbay 1976), and the level of HbA_{1C} is monitored as a reliable index of glycaemic control in diabetes. In our study, the diabetic rats have showed higher levels of glycosylated haemoglobin compared to control rats indicating their poor glycaemic control (Chatterjee and Shinde 2000).

Diabetic rats treated with CHE showed a significant decrease in the glycosylated Hb levels that might be due to the antihyperglycaemic effect of CHE. In diabetic animals total haemoglobin levels were found to be low when compared to normal rats, as the Hb synthesis might also be depressed. Thus CHE treated animals showed improved levels of Hb because of its glucose lowering effect.

Diabetes mellitus is characterized by a reduced capacity of the β -cells of the pancreas to release sufficient insulin to induce the activity of glucose metabolizing enzymes whether the cells are destroyed as in type 1 diabetes (IDDM) or intact as in type 2 diabetes (NIDDM) (Chatterjee and Shinde 2000). Insulin increases hepatic glycolysis by increasing the activity and amount of several key enzymes including glucokinase, phosphofructokinase and pyruvate kinase (Murray et al. 2000). One of the key enzymes in the catabolism of glucose is glucokinase, which phosphorylates glucose to glucose-6-phosphate. In our study, glucokinase activity was decreased significantly in the liver of diabetic rats as reported earlier (Grover et al. 2000). Treatment with CHE and glibenclamide increase insulin secretion, which, in turn, activates the glucokinase, thereby increasing utilization of

glucose and thus, the increased utilization leads to decreased blood sugar level.

Glucose-6-phosphatase plays an important role in the homeostasis of blood glucose (Berg et al. 2001). However, the activity of glucose 6-phosphatase was known to be inhibiting under hyperinsulinaemic and hyperglycaemic conditions (Guignot and Mithieux 1999). Fructose-1,6-bisphosphatase is one of the key enzymes of the gluconeogenic pathway. It is present in liver and kidney but absent in heart, muscle and smooth muscle. The activities of the gluconeogenic enzymes such as glucose-6-phosphatase and fructose-1,6-bisphosphatase, increased significantly in the liver and kidney of diabetic rats (Baquer et al. 1998), which may be due to insulin deficiency. In the diabetic rats treated with CHE and glibenclamide the activities of these enzymes were significantly lower, which might be due to increased secretion of insulin.

In conclusion, in this study *Cardiospermum halicacabum* leaf extract is shown to exhibit an anti-hyperglycaemic activity. However, the effect of the crude extract is weaker than that caused by the standard drug glibenclamide. Several of the compounds found in this plant have been reported as possessing antidiabetic activity. Those investigated include flavonoids, such as luteolin and apigenin which are known constituents of *Cardiospermum halicacabum* (TEAEMP 1999), and which have well documented antidiabetic activity (Matsuda et al. 1995, Asgary et al. 2002).

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