Effect of *Cassia Auriculata* Flowers on Blood Sugar Levels, Serum and Tissue Lipids in Streptozotocin Diabetic Rats

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ABSTRACT

Aim of the study: The main aim was to demonstrate the effects of *Cassia auriculata* flowers on blood glucose and lipid levels in experimental diabetic rats.

Methodology: Aqueous extract of *Cassia auriculata* flowers was administered orally and different doses of the extract on blood glucose, haemoglobin, glycosylated haemoglobin, serum and tissue lipids, hexokinase and glucose-6-phosphatase in streptozotocin-induced diabetic rats were studied. Glibenclamide was used as standard reference drug.

Results: *Cassia auriculata* flower extract (CFEt), at doses of 0.15, 0.30 and 0.45 g/kg body weight for 30 days, suppressed the elevated blood glucose and lipid levels in diabetic rats. *Cassia auriculata* at 0.45 g/kg was found to be comparable to glibenclamide.

Conclusion: Our findings indicate that the *Cassia auriculata* flowers possess antihyperlipidaemic effect in addition to antidiabetic activity.

Keywords: Blood glucose, *Cassia auriculata*, Carbohydrate enzymes, Insulin, Lipids

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INTRODUCTION

Diabetes mellitus is characterised by hyperglycaemia together with biochemical alterations of glucose and lipid metabolism[1]. Liver is an insulin dependent tissue, which plays a pivotal role in glucose and lipid homeostasis and is severely affected during diabetes[2]. Liver participates in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids and triglycerides. During diabetes a profound alteration in the concentration and composition of lipid occurs[3]. Decreased glycolysis, impeded glycogenesis and increased gluconeogenesis are some of the changes of glucose metabolism in the diabetic liver[4]. Many traditional plant treatments for diabetes mellitus are used throughout the world[5]. Few of the traditional plant treatments for diabetes have received scientific scrutiny, and the World Health Organisation has recommended that this area warrants attention[6].

This paper describes the study of *Cassia auriculata* L. (Cesalpinaceae, common name: Tanner's Cassia) a common plant in Asia, has been widely used in traditional medicine as a cure for rheumatism, conjunctivitis and diabetes[7]. In addition, *Cassia auriculata* has been widely used in Ayurvedic medicine as 'Avarai Panchaga Choornam' and the main constituent of Kalpa herbal tea, has come under extensive study in the light of its antidiabetic effects. We have recently reported the antiperoxidative effect of *Cassia auriculata* flowers in streptozotocin diabetic rats[8]. This study was thus initiated with the aim of evaluating the effects of an aqueous extract of *Cassia auriculata* flowers on the blood glucose level, serum and tissue lipids in streptozotocin diabetic rats.

MATERIALS AND METHODS

Animals

All the experiments were carried out with male Wistar rats aged seven to eight weeks (180-200 g), obtained from the Central Animal House, Rajah Muthiah Medical College, Annamalai University, India. The animals were housed in polypropylene cages and provided with water and standard pellet diet (Karnataka Agro Food Corporation Limited, Bangalore, India) ad libitum. The animals used in the present study were approved by the ethical committee, Annamalai University.

Chemicals

Streptozotocin was obtained from Himedia Laboratory Limited, Mumbai, India. All other reagents used were of analytical grade.

Plant Material

Cassia auriculata flowers were collected freshly from Neyveli, Cuddalore District, Tamil Nadu, India. The

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Table I. Blood glucose, plasma insulin, total haemoglobin, glycosylated haemoglobin, changes in body weight and urine sugar of normal and experimental animals.

Groups	Body weight (g) Initial	Final	Fasting Blood Glucose (mg/dl)	Plasma insulin (μU/ml)	Haemoglobin (g/dl)	Glycosylated haemoglobin (mg/gHb)	Urine sugar[A]
Normal	196 ± 10.40	208 ± 9.80	97.50 ± 8.04[a]	16.03 ± 1.04[a]	12.85 ± 0.72[a]	0.22 ± 0.01[a]	Nil
Diabetic control	201 ± 15.70	151 ± 13.66[***]	232.00 ± 15.40[b]	4.35 ± 0.95[b]	5.60 ± 0.45[b]	0.81 ± 0.07[b]	+ + +
Diabetic + *Cassia auriculata* (0.15g/kg)	193 ± 17.70	198 ± 15.33[***]	216.66 ± 20.80[b]	4.90 ± 0.41[b]	6.91 ± 0.61[c]	0.68 ± 0.03[c]	+ +
Diabetic + *Cassia auriculata* (0.30g/kg)	198 ± 18.30	208 ± 10.32[***]	158.60 ± 14.20[c]	7.05 ± 0.64[c]	9.54 ± 0.93[d]	0.48 ± 0.04[d]	+
Diabetic + *Cassia auriculata* (0.45g/kg)	202 ± 19.68	214 ± 12.72[***]	113.3 ± 10.30[ad]	14.16 ± 0.67[d]	11.5 ± 0.91[e]	0.37 ± 0.04[e]	NIL
Diabetic + Glibenclamide (600 μg/kg)	195 ± 11.80	206 ± 13.43[***]	124.6 ± 10.32[d]	12.70 ± 0.65[e]	10.36 ±1.01[d]	0.47 ± 0.04[d]	TRACE

Values are given as mean ± S.D for six rats in each group.
Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).
Duncan procedure, Range for the level 2.89, 3.03, 3.13, 3.20, 3.25.
Diabetic control was compared with normal, [***] $p<0.001$.
Experimental groups were compared with diabetic control, [***] $p<0.001$.
A - Indicates 0.25% sugar and (+ + +) indicates more than 1% sugar.

Table II. Changes in levels of cholesterol, free fatty acids, triglycerides and phospholipids in serum of normal and experimental animals.

Groups	Cholesterol (mg/100 ml)	Free fatty acids (mg/100 ml)	Triglycerides (mg/100 ml)	Phospholipids (mg/100 ml)
Normal	74.00 ± 1.49[a]	69.43 ± 4.06[a]	44.53 ± 3.36[a]	80.25 ± 1.57[a]
Diabetic control	98.66 ± 4.03[b]	83.86 ± 6.67[b]	62.83 ± 1.50[b]	98.75 ± 4.28[b]
Diabetic + *Cassia auriculata* (0.45 g/kg)	83.46 ± 2.18[c]	75.06 ± 1.55[c]	53.93 ± 2.70[c]	85.50 ± 2.86[c]
Diabetic + Glibenclamide (600 μg/kg)	90.26 ± 1.37[d]	78.51 ± 0.87[d]	58.46 ± 1.70[d]	90.00 ± 2.12[d]

Values are given as mean ± S.D for six rats in each group.
Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).
Duncan procedure, Range for the level 2.95, 3.09, 3.20.

plant was identified and authenticated at the Herbarium of Botany Directorate in Annamalai University. A voucher specimen (No.231) was deposited in the Botany Department of Annamalai University.

Preparation of plant extract

Five hundred g of *Cassia auriculata* flowers were extracted with 1,500 ml of water by the method of continuous hot extraction at 60ºC for six hours and evaporated. The residual extract was dissolved in water and used in the study[9].

Induction of experimental diabetes

A freshly prepared solution of streptozotocin (45 mg/kg i.p) in 0.1 M citrate buffer, pH 4.5 was injected intraperitoneally in a volume of 1 ml/kg. After 48 hours of streptozotocin administration, rats with moderate diabetes having glycosuria and hyperglycaemia (i.e. with a blood glucose of 200-300 mg/dl) were taken for the experiment[10].

Experimental procedure

In the experiment, a total of 36 rats (30 diabetic surviving rats, six normal rats) were used. The rats were divided into six groups of six rats each.

Group 1: Normal untreated rats.

Group 2: Diabetic control rats given 1 ml of aqueous solution daily using an intragastric tube for 30 days.

Group 3: Diabetic rats given CFEt (0.15 g/kg body weight) in 1ml of aqueous solution daily using an intragastric tube for 30 days.

Group 4: Diabetic rats given CFEt (0.30 g/kg body weight) in 1 ml of aqueous solution daily using an intragastric tube for 30 days.

Group 5: Diabetic rats given CFEt (0.45 g/kg body weight) in 1 ml of aqueous solution daily using an intragastric tube for 30 days.

Group 6: Diabetic rats given glibenclamide (600 μg/kg body weight)[11] in 1 ml of aqueous solution daily using an intragastric tube for 30 days.

At the end of 30 days, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in two different tubes (i.e.,) one with anticoagulant- potassium oxalate and sodium fluoride for plasma and another without

Table III. Changes in levels of cholesterol, free fatty acids, triglycerides and phospholipids in liver of normal and experimental animals.

Groups	Cholesterol (mg/100 g wet tissue)	Free fatty acids (mg/100 g wet tissue)	Triglycerides (mg/100 g wet tissue)	Phospholipids (g/100 g wet tissue)
Normal	329.04 ± 2.88[a]	607.70 ± 30.68[a]	347.88 ± 13.04[a]	1.66 ± 0.11[a]
Diabetic control	512.70 ± 5.88[b]	915.22 ± 50.27[b]	621.35 ± 8.40[b]	2.54 ± 0.08[b]
Diabetic + *Cassia auriculata* (0.45 g/kg)	420.14 ± 4.40[c]	774.09 ± 46.86[c]	442.98 ± 13.05[c]	2.02 ± 0.05[c]
Diabetic + Glibenclamide (600 μg/kg)	441.98 ± 5.36[d]	806.67 ± 25.30[c]	530.19 ± 11.70[d]	2.29 ± 0.10[d]

Values are given as mean ± S.D for six rats in each group.
Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).
Duncan procedure, Range for the level 2.95, 3.09, 3.20.

Table IV. Changes in activities of hexokinase and glucose-6-phosphatase in liver of normal and experimental animals.

Groups	Hexokinase (units[A]/g protein)	Glucose- 6-phosphatase (units[B]/mg protein)
Normal	146.66 ± 6.09[a]	0.168 ± 0.013[a]
Diabetic control	107.48 ± 5.74[b]	0.242 ± 0.023[b]
Diabetic + *Cassia auriculata* (0.45 g/kg)	128.70 ± 9.44[c]	0.186 ± 0.011[ac]
Diabetic + Glibenclamide (600 μg/kg)	123.20 ± 5.40[c]	0.200 ± 0.008[c]

Values are given as mean ± S.D for six rats in each group.
Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).
Duncan procedure, Range for the level 2.95, 3.09, 3.20.
A - μ moles of glucose phosphosylated/min.
B - μ moles of Pi liberated/min.

anticoagulant for serum separation. Plasma and serum were separated by centrifugation.

Liver was immediately dissected out, washed in ice cold saline, patted dry and weighed.

Analytical Procedure

Fasting blood glucose was estimated by O-toluidine method (Sasaki et al)[12]. Plasma insulin level was assayed by Enzyme Linked Immunosorbent Assay (ELISA) kit, using human insulin as standard. Haemoglobin was estimated by the method of Drabkin and Austin[13] and glycosylated haemoglobin by the method of Sudhakar Nayak and Pattabiraman[14]. Lipids were extracted from serum and tissues by the method of Folch et al[15]. Total cholesterol and triglycerides were estimated by the method of Zlatkis et al[16] and Foster and Dunn[17] respectively. Free fatty acids and phospholipids were analysed by the method of Falholt et al[18] and Zilversmit et al[19].

Hexokinase and glucose-6-phosphatase were assayed by the method of Brandstrup et al[20] and Koida and Oda[21].

Statistical analysis

All values were expressed as the mean obtained from a number of experiment (n). Data from all the tables of normal animals, diabetic control animals, reference drug treated and CFEt treated animals were compared by ANOVA followed by Duncan's Multiple Range Test (DMRT)[22].

RESULTS

Blood glucose and Plasma insulin

Table I shows the levels of blood glucose, plasma insulin, total haemoglobin, glycosylated haemoglobin, changes in body weight and urine sugar of normal and experimental rats. There was a significant elevation in blood glucose and glycosylated haemoglobin levels, while the plasma insulin and total haemoglobin levels decreased significantly in streptozotocin diabetic rats when compared with normal rats. Administration of CFEt and glibenclamide tends to bring the parameters significantly towards the normal. The effect of CFEt at a dose of 0.45 g/kg body weight was more highly significant than 0.15 and 0.30 g/kg body weight and therefore the dose was used for further biochemical studies.

In diabetic rats, the urine sugar was (+++) but in the case of CFEt treated rats at a dose of 0.15 and 0.30 g/kg body weight showed decreased urine sugar (++) and (+) respectively. CFEt at a dose of 0.45 g/kg body weight, showed urine sugar as seen in normal rats. These effects were compared with glibenclamide.

Serum and tissue lipids

The effect of CFEt on serum and tissue lipids of

normal and experimental rats is summarised in Table II and III respectively. A marked increase in the frequency of cholesterol, free fatty acids, triglycerides and phospholipids were observed in diabetic control rats. Treatment with CFEt significantly reduced the lipid levels.

Hepatic hexokinase and glucose-6-phosphatase

The activities of carbohydrate enzymes are represented in Table IV. Activity of hexokinase in liver decreased markedly while the glucose-6-phosphatase activity increased significantly in diabetic control rats. Treatment with CFEt in diabetic rats increased the hexokinase activity and decreased the glucose-6-phosphatase activity.

DISCUSSION

Streptozotocin is well known for its selective pancreatic islet β-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals. It interferes with cellular metabolic oxidative mechanisms[23]. Intraperitoneal administration of streptozotocin (45 mg/kg) effectively induced diabetes in normal rats as reflected by glycosuria, hyperglycaemia, polyphagia, polydipsia and body weight loss when compared with normal rats[24]. In our present study we have observed that an aqueous extract of *Cassia auriculata* flower can reverse these effects. The possible mechanism by which CFEt brings about its antihyperglycemic action may be by potentiation of pancreatic secretion of insulin from β-cell of islets or due to enhanced transport of blood glucose to peripheral tissue. This was clearly evidenced by the increased level of insulin in diabetic rats treated with CFEt. In this context a number of other plants have also been reported to have antihyperglycemic and insulin-release stimulatory effect[25,26].

We have observed a decrease in total haemoglobin during diabetes and this may be due to the formation of glycosylated haemoglobin. Increase in the level of haemoglobin in animals given CFEt may be due to decreased level of blood glucose and glycosylated haemoglobin.

CFEt administration to streptozotocin dosed animals reversed the weight loss. The ability of CFEt to recover body weight loss seems to be due to its antihyperglycemic effect.

Excess of fatty acids in serum produced by the streptozotocin-induced diabetes promotes conversion of excess fatty acids into phospholipids and cholesterol in liver. These two substances along with excess triglycerides formed at the same time in liver may be discharged into blood in the form of lipoproteins[27]. The abnormal high concentration of serum lipids in the diabetic subject is due, mainly to increase in the mobilisation of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in streptozotocin diabetic rats[28,29] and significant increase observed in our experiment was in accordance to these studies. The marked hyperlipidaemia that characterise the diabetic state may therefore, be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots[30].

The antihyperlipidaemic effect of CFEt may be due to the down regulation of NADPH and NADH, a cofactor in the fat metabolism. Higher activity of glucose-6-phosphatase provides H+ which binds with NADP+ in the form of NADPH and is helpful in the synthesis of fats from carbohydrates. When glycolysis slows down because of cellular activity, the pentose phosphate pathway still remain active in liver to breakdown glucose that continuously provides NADPH which converts acetyl radicals into long fatty acid chains. CFEt may be capable of oxidising NADPH. Enhanced hexokinase activity in CFEt treated rats suggests greater uptake of glucose from blood by the liver cells.

Activities of enzymes suggest that enhanced lipid metabolism during diabetes is shifted towards carbohydrate metabolism and it enhances the utilisation of glucose at the peripheral sites. One of the possible actions of CFEt may be due to its inhibition of endogenous synthesis of lipids.

Metabolic aberration in streptozotocin diabetic rats suggest a high turnover of triglycerides and phospholipids. CFEt may antagonise the metabolic aberration and thereby restore the normal metabolism by tilting the balance from high lipids to high carbohydrate turnover. Alteration of fatty acid composition by increased lipid levels contribute to lowering the resistance of tissues and higher rate of oxidative stress. Decreased activity of glucose-6-phosphatase through pentose phosphate shunt results in high reduced glutathione to oxidised glutathione ratio (GSH/GSSG)[27], which is coupled with conversion of NADPH to NADP. CFEt may produce high NADP+ which results in down regulation of lipogenesis and lower risk of the tissues for oxidative stress and high resistance for diabetes.

It can be concluded from the data that CFEt significantly reduces the levels of serum and tissue lipids, which are actively raised in streptozotocin diabetes rats. CFEt has beneficial effect on plasma insulin and hexokinase activity. Moreover

its antihyperlipidaemic effect could represent a protective mechanism against the development of atherosclerosis.

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