Original Article

Effect Of Cassia Auriculata On Blood Glucose, Plasma Insulin And Hepatic Key Enzymes In Streptozotocin- Nicotinamide Induced Diabetes

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DOI: 10.47750/pnr.2022.13.04.296

Abstract

Cassia auriculata L. (Ceasalpiniaceae) has been used traditionally as antidiabetic and has been proven scientifically to possess high antioxidant activity and anticancer properties. In the present study, we have investigated of Tanner's cassia *Cassia auriculta* flower extract (CFEt) and leaf extract (CLEt) on hepatic key metabolic enzymes of carbohydrate metabolism in streptozotocin (STZ) induced diabetic rats. The CFEt (0.45g/kg) and CLEt (0.45 g/kg) were orally administered to diabetic rats for 45 days, after which activities of hexokinase, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase, fructose-1,6-bisphosphatase, sorbitol dehydrogenase in liver and glycogen content in liver and muscle were assayed. The activities of gluconeogeneic enzymes were significantly increased, whereas the activities of hexokinase, glucose-6-phosphate dehydrogenase and glycogen were significantly decreased in diabetic control rats. Both CFEt and CLEt were able to restore the altered enzyme activities to almost near normal levels. CFEt was more effective than CLEt. Our results indicate that administration of CFEt and CLEt to diabetic animals normalizes blood glucose and causes marked improvement of altered carbohydrate metabolic enzymes during diabetes. The CFEt administration showed more effective than CLEt and glibenclamide.

Key Words: *Cassia auriculata*, corbohydrate metabolic enzymes, diabetes mellitus, glucose, insulin

INTRODUCTION

Diabetes is a metabolic disease and its incidence is considered to be high all over the world (Devendra and Eisenbarth, 2003). Epidemiological studies and clinical studies strongly support the notion that hyperglycemia is the principal cause of complications. Effective blood glucose control is the key for preventing or reversing diabetic complications and improving quality of life in patients with diabetes (Zimmet et al. 2001). Thus, sustained reduction in hyperglycemia will decrease the risk of developing microvascular and macrovascular complications (Brownlee, 2001). Throughout the world many traditional plant treatments for diabetes exist. However, few have received scientific or medical scrutiny and the WHO has recommended accordingly that traditional plant treatment for diabetes warrant further evaluation (WHO, 1980). Preliminary report indicates blood glucose lowering effect of *Cassia auriculata* (Pari and Murugan, 2007).

Cassia auriculata L. (Ceasalpiniaceae) is a shrub that has attractive yellow flowers, commonly used for the treatment of skin disorders and body odour. It is a native plant present in different parts of India. Indigenous people use various parts of the plant for diabetes mellitus (Murugan, 2010). It is widely used in Ayurvedic medicine as a "Kalpa drug" which contains five parts of the shrub (roots, leaves, flowers, bark and unripe fruits) which are taken in equal quantity, dried and then powdered to give "Avarai Panchaga Choornam", for the control of sugar levels and reduction of symptoms such as polyuria and thirst in diabetes (Brahmachari and Augusti, 1961; Shrotri and Aiman, 1963). A literature survey showed that a decoction of leaves, flowers, and seeds of the *Cassia auriculata* mediate an antidiabetic effect (Shrotri and Aiman, 1963; Murugan, 2015a). Thus, the available reports show that very little work has been done with respect to *Cassia auriculata* flowers, other than its hypoglycemic effects. In our previous study, we have demonstrated the antidiabetic effect of *Cassia auriculata* flower extract (CFEt) in STZ induced diabetic rats [Murugan, 2015b]

MATERIALS AND METHODS

Chemicals

STZ 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 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 (Jain, 1968).

Induction of diabetes

Experimental procedure

In the experiment, a total of 30 rats (24 diabetic surviving rats, six normal rats) were used. The rats were divided in to 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 45 days.

Group 3: Diabetic rats given CFEt (0.45 g/kg body weight) in 1 ml of aqueous solution daily using an intragastric tube for 45 days.

Group 4: Diabetic rats given CLEt (0.45 g/kg body weight) in 1 ml of aqueous solution daily using an intragastric tube for 45days.

Group 5: Diabetic rats given glibenclamide (600 µg/ kg body weight) in 1 ml of aqueous solution daily using an intragastric tube for 45days.

At the end of 45 days, the rats were deprived of food overnight and blood was collected in a tube containing potassium oxalate and sodium fluoride for the estimation of blood glucose, hemoglobin and glycosylated hemoglobin. Plasma was separated for the assay of insulin. Liver was dissected out, washed in ice-cold saline, patted dry and weighed.

ANALYTICAL METHODS

Blood glucose and plasma insulin

Blood glucose was estimated colorimetrically using commercial diagnostic kits (Sigma Diagnostics (I) Pvt Ltd, Baroda, India)\22\. Plasma insulin was assayed by ELISA using a Boehringer-Mannheim kit with an ES300 Boehringer analyzer (Mannheim, Germany). The test was carried out by the method of Du Vigneaud and Karr (1925).

Hemoglobin and glycosylated hemoglobin

Haemoglobin content in blood was estimated by the cyan-methaemoglobin method of Drabkin and Austin (1932). When blood is diluted with an alkaline solution of potassium cyanide and potassium ferricyanide, haemoglobin is oxidized to methaemoglobin, which then combines with cyanide to form cyanmethaemoglobin that is measured colorimetrically at 540nm. Glycosylated haemoglobin was estimated by the method of Sudhakar Nayak and Pattabiraman (1981) with modifications according to Bannon (1982).

GLYCOGEN

Tissue glycogen was extracted and estimated by the method of Morales et al. (1973). The alkali extract of the tissue was prepared by digesting 50 mg of fresh tissue with 3 ml of 30% potassium hydroxide solution in boiling water bath for 15 min. The tubes were cooled and mixed with 5 ml of absolute alcohol and a drop of 1 M ammonium acetate to precipitate glycogen and left in the freezer overnight for complete precipitation. Glycogen was collected by centrifugation at 2000 g for 20 min. The precipitate was dissolved in distilled water with the aid of heating and again the glycogen was reprecipitated with alcohol and 1 M ammonium acetate and centrifuged. The final precipitate was dissolved in saturated ammonium chloride solution and 4 ml of anthrone reagent was added by cooling the tubes in an ice bath. The tubes were shaken well, covered with marble caps and heated in a boiling water bath for 20 min. After cooling, the absorbance was read at 640nm against reagent blank treated in a similar manner. Standard glucose solution was also treated similarly. The glycogen content was calculated from the amount of glucose present in the sample and was expressed as mg/g tissue.

Activity of hepatic hexokinase

Hexokinase activity was assayed by the method of Brandstrup et al. (1957). The incubation mixture contained 1 ml of buffer, 0.1 ml of magnesium chloride, 0.1 ml of $NADP^+$, 0.5 ml of phenazine methosulphate, 0.4 ml of the dye solution and the requisite amount of the enzyme extract. The mixture was allowed to stand at room temperature for 10 min to permit the oxidation of endogenous materials. The reaction was initiated by the addition of 0.5 ml of glucose-6-phosphate. The absorbance was read at 640nm against water blank at one min intervals for 3-5 min in a UV spectrophotometer. The activity of the enzyme was calculated in units by multiplying the change in OD/min by the factor 6/17.6, which is the molar extinction co-efficient of the reduced co-enzyme.

Activity of hepatic glucose-6-phosphate dehydrogenase

Glucose-6-phosphate dehydrogenase was assayed by the method of Ellis and Kirkman (1961). Incubation mixture contains 0.7 ml of citrate buffer, 0.3 ml of substrate and 0.3 ml of tissue homogenate. The reaction mixture was incubated at 37°C for 1 h. Addition of 1 ml of 10% TCA to the reaction tubes terminated the reaction of the enzyme. The suspension was centrifuged and the phosphorus content of the supernatant was estimated by the method of Fiske and Subbarow (1925). The supernatant

was made upto known volume. To this 1 ml of ammonium molybdate was added followed by 0.4 ml of ANSA. The blue colour developed after 20 min was read at 680nm. The enzyme activity was estimated as μmoles of inorganic phosphorus liberated/min/mg protein.

Activity of hepatic glucose-6-phosphatase

Glucose-6-phosphatase was assayed according to the method of Koida and Oda (1959). The assay mixture in a final volume of 2 ml contained 1.2 ml of buffer, 0.1 ml of substrate, 0.25 ml of magnesium chloride, 0.1 ml of potassium chloride solution, 0.25 ml of EDTA solution and 0.1 ml of enzyme homogenate. The incubation was carried out at 37°C for 15 min. The reaction was terminated by the addition of 10% TCA. The suspension was centrifuged and the supernatant was used for phosphorus estimation by the method of Fiske and Subbarow (1925). The supernatant was made upto known volume. To this 1 ml of ammonium molybdate was added followed by the 0.4 ml of ANSA. The blue colour developed after 20 min was read at 680nm. Enzyme activity was expressed as μmoles of inorganic phosphorus liberated/hour/mg protein.

Activity of hepatic fructose-1, 6-bisphosphatase

Fructose-1,6-bisphosphatase was assayed by the method of Gancedo and Gancedo (1971). To 1.6 ml of triethanolamine buffer, 0.1 ml of NADH was added followed by addition of 1 ml of tissue homogenate. The mixture was incubated for 30 min at 25°C till the extinction was constant. Then added 0.3 ml D-Fructose and mixed to start the reaction. The extinction was read at 60 sec intervals for 5-8 min (till the absorbance was stabilized) at 365nm. A system devoid of the enzyme served as control. The enzyme activity was calculated by multiplying 882 with the change in extinction (882 x change in extinction). The enzyme activity was expressed as one unit of sorbitol dehydrogenase = the amount of enzyme that produces a change in absorbance of 0.01/min/g protein.

Activity of hepatic sorbitol dehydrogenase

Sorbitol dehydrogenase, (SDH; EC. 1.1.1.14) was assayed by the method of Ulrich (1974). The dried residue of lipid was dissolved in 5 ml of $CHCl_3$: MeOH mixture (2:1 v/v) and transferred into a centrifuge tube, 2 ml of 0.1 M potassium chloride was added, shaken well and centrifuged. The upper aqueous layer containing gangliosides was discarded. The chloroform layer was mixed with 1 ml of chloroform-methanol-potassium chloride mixture (1:10:10 v/v) and then centrifuged. This washing was repeated thrice and each time, the upper layer was discarded. The lower layer was made up to 5 ml and used for the analysis of total cholesterol, triglycerides, free fatty acids and phospholipids and vitamin E. The serum was also treated similarly for the estimation of lipids. The dry defatted tissues were used for the estimation of tissue glycoproteins.

STATISTICAL ANALYSIS

Values were expressed as the mean ± SD and the significance of the differences between mean values were determined by one way analysis of variance (ANOVA) coupled with Duncan's Multiple Range Test (DMRT), taking $p<0.05$ as significant (Duncan, 1957).

RESULTS

Changes in body weight, blood glucose and plasma insulin

The changes in body weight and food intake in normal and experimental rats are presented in table 1. The body weights in CFEt, CLEt treated group significantly improved at the end of the experimental period when compared with diabetic control group. Table 1 also shows the level of blood glucose and insulin in control and experimental animals. The level of blood glucose was significantly increased where as the plasma insulin was significantly decreased in STZ diabetic control rats. The CFEt showed better effect than CLEt and glibenclamide.

Oral Glucose Tolerance Test (OGTT)

Table 2 shows the blood glucose levels of normal and experimental rats after oral administration of glucose (2 g/kg body weight). In diabetic rats, the blood glucose levels reached peak at 60 min. Although the glucose levels started to decline in CFEt, CLEt and glibenclamide treated rats (90 and 120 min), the levels of glucose remained higher even after 120 min intervals in diabetic control rats. Normal rats treated with CFEt and CLEt showed a significant decrease in the glucose level at 120 min when compared to its 30 and 60 min blood glucose levels.

Hemoglobin and glycosylated hemoglobin

Table 3 shows the levels of haemoglobin, HbA_1C and urine sugar of normal and experimental rats. Haemeoglobin levels were decreased with significant increase in HbA_1C levels in diabetic control rats. Administration of CFEt, CLEt and glibenclamide to diabetic rats significantly increased the haemoglobin and significantly decreased the HbA_1C levels. In diabetic control rats, urine sugar was more than 2%, but, in the case of rats treated with CFEt and CLEt, there was no urine sugar. These effects were compared with glibenclamide treated rats, which showed traces of sugar in their urine.

Table 4 also shows the changes in the level of liver and muscle glycogen of control and experimental rats. There was a significant reduction in the liver and muscle glycogen in diabetic rats as compared to CFEt and CLEt treated rats. Treatment with CFEt, CLEt and glibenclamide significantly increased the hepatic and skeletal muscle glycogen in diabetic rats.

Carbohydrate metabolic enzymes

Table 5 shows the activity of hexokinase, glucose-6-phosphatase and fructose-1,6-bisphosphatase in the liver of normal control and experimental rats. A significant decrease in the activity of hexokinase was observed in the liver of diabetic rats, whereas the activity of hepatic glucose-6-phosphatase, fructose-1,6-bisphosphatase and sorbitol dehydrogenase were significantly increased when compared with normal rats. Oral CFEt, CLET and glibenclamide to diabetic rats resulted a significant increase in hexokinase activity and a decrease in gluconeogenic enzymes activity in the liver of diabetic rats.

Histopathological observations

Histopathological studies (compared to normal Fig. 4 A) demonstrate fatty infiltration and islet shrinkage in pancreas of STZ diabetic rats (Fig. 4B) and these changes were markedly reduced in diabetic rats treated with CFEt and CLEt (Fig. 4C and 4D).

DISCUSSION

Global estimates suggest that three fourth of the world population cannot afford the products of allopathic medicine and thus, have to rely upon the use of traditional medicines, which are largely derived from plants (Hu et al. 2003). For the study of antidiabetic agents, STZ-induced hyperglycemia in rodents is considered to be a good preliminary screening diabetic model (Ivorra et al. 1989) and is widely used. STZ is a potent methylating agent for DNA and acts as NO donor in pancreatic cells (Spinas, 1999). The present study was undertaken to assess the antihyperglycemic property of *Cassia auriculata*, which has been reported in Ayurveda to be useful in treatment of diabetes mellitus (Murugan and Pari, 2007).

CFEt and CLEt might enhance glucose utilization since it significantly reduces blood glucose in diabetic rats. From the data obtained with the oral glucose tolerance test, it is clear that blood glucose levels reached a peak and returned to near normal values after 120 min in both normal and treated rats (0.45 g/kg body weight of CFEt and CLEt). Elevated blood glucose levels remained high even after 120 min in diabetic control rats. CFEt and CLEt administration effectively prevented the increase in blood glucose without causing a hypoglycemic state, and this effect may be due to the restoration of the delayed insulin response.

Rasch (1980) reported that the rise in body weight was far less in the poorly controlled diabetic rats as compared to well-controlled diabetic rats. Similar observation was made in this study. Loss of body weight may be due to excessive breakdown of tissue proteins during diabetes (Chatterjee and Shinde, 2002). The daily administration of CFEt and CLEt to STZ diabetic rats for 6 weeks caused a statistically significant increase in the body weight when compared with diabetic control rats

Glycosylated haemoglobin was found to be increased over a long period of time in diabetes (Bunn et al. 1978; Bunn et al. 1979). Therefore, measurement of HbA_1C is supposed to be very sensitive index for glycemic control. In uncontrolled or poorly controlled diabetes there is increased glycosylation of a number of proteins including haemoglobin and α-crystalline of the lens. During long-term diabetes the glycosylated form of haemoglobin has altered affinity for oxygen and this may be a factor in tissue anoxia (Bunn et al. 1979).

In diabetes, the glycation and subsequent browning (glycoxidation) reactions are enhanced by elevated glucose levels and there is some evidence that glycation itself may induce the formation of oxygen derived free radicals (Inouye et al. 1998). Studies show that HbA_1C comprises 3.4 to 5.8% of total haemoglobin in normal red cells, but is elevated in patients with diabetes mellitus (Trevilli et al. 1971). The levels of HbA_1C are monitored as a reliable index of glycemic control in diabetics (Koening et al. 1978). The levels of HbA_1C follow a similar pattern in the current study, they were elevated in diabetic control rats and the amount of this increase is directly proportional to the fasting blood glucose level (Jackson et al. 1979). Koening et al. (1978) also reported a 16% increase in the level of HbA_1C in diabetic patients.

The level of total haemoglobin was found to be decreased in diabetic control group and this may be due to increased formation of HbA_1C. This was well correlated with earlier reports that showed a decrease in the level of haemoglobin in experimental diabetic rats (Chandalia and Krishnaswamy, 2002; Venkateswaran and Pari, 2002). The increase in the level of haemoglobin in animals given CFEt and CLEt may be due to the decreased level of blood glucose. CFEt and CLEt exhibited significant reduction in HbA_1C in diabetic rats. The extract was not only effective in lowering blood glucose level but also caused significant increase in plasma insulin (Fig. 12 and 13), which could correct other metabolic alterations. In this context, several medicinal plants were also reported to have the capacity of decreasing the level of HbA_1C in diabetic rats (Latha and Pari, 2003).

We conclude that CFEt and CLEt has beneficial effects on glucose concentration as well as sequential metabolic correlation between increased glycolysis, decreased gluconeogenesis, increased hydrogen shuttle reactions. It suggests the possible biochemical mechanisms through which CFEt and CLEt regulates glucose homeostasis in diabetic condition. The CFEt administration showed more effective than CLEt and glibenclamide.

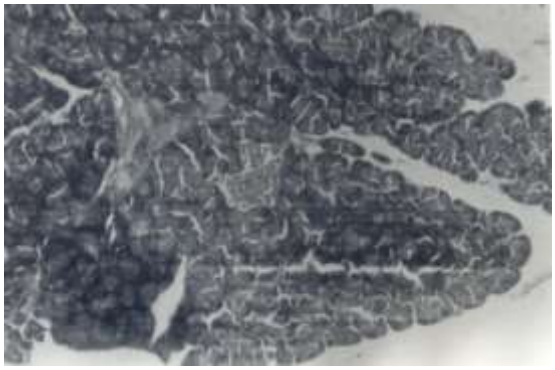

Figure 1 A. Normal rats pancreas H&E x 20
Pancreas showing β-islets (→)

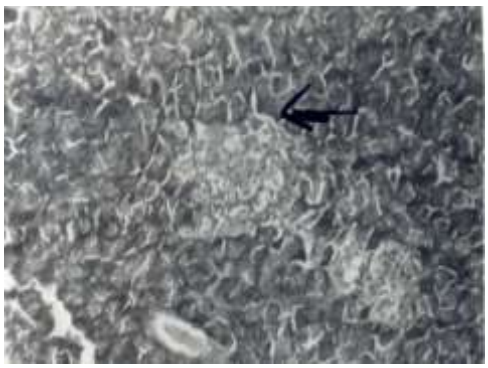

Figure 1 B. Diabetic control rats pancreas H&E x **20**
Fatty infiltration of islet cells and shrinkage (→)

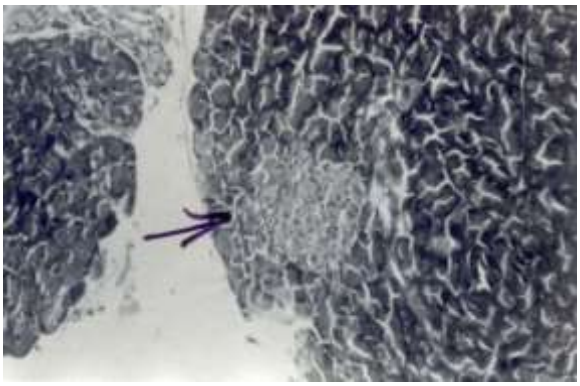

Figure 1 C. Diabetic + CFEt (0.45 g) treated rats pancreas H&E x 20
Normal appearance of islets (→)

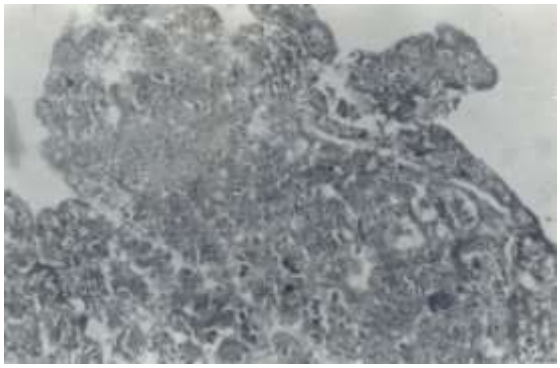

Figure 1 D. Diabetic + CLEt (0.45g) treated rats pancreas H&E x 20
Parenchymal inflammation and necrosis

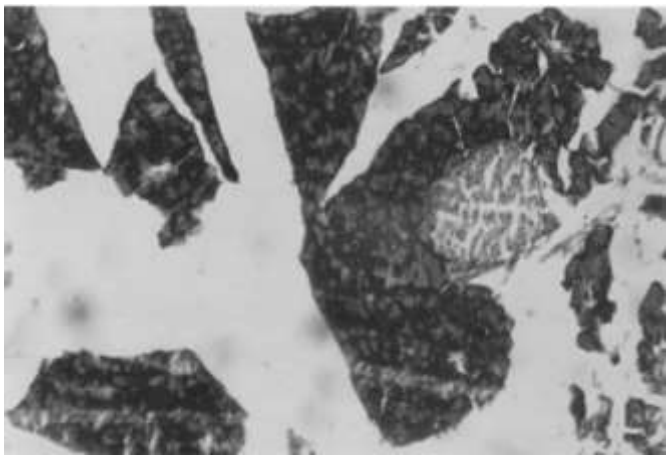

Figure 1 E. Diabetic + glibenclamide treated rat pancreas. Preservation of islet cells in the pancreas.

REFERENCES

1. Bannon P. Effect of pH on the elimination of the labile fraction of glycosylated haemoglobin. Clin Chem 1982; 28: 2183.
2. Brahmachari HB, Augusti KT. Hypoglycaemic agents from indigenous plants. J. Pharm. Pharmacol. 1961, 13: 381-385.
3. Brandstrup N, Kirk JE, Bruni C. Determination of hexokinase in tissues. J Gerontol 1957; 12: 166-171.
4. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001; 414: 813-820.
5. Bunn HF, Shapiro R, McManns M, Garrick L, MacDonald HH, Gallop PM, Gabby KH. Structural heterogenicity of human haemoglobin. A due to non-enzymic glycosylation. J Biol Chem 1979; 254: 3892-3898.
6. Bunn JF, Gabbay KJ, Gallop MP. The glycosylation of haemoglobin: relevance to diabetes mellitus. Science 1978; 200: 21-27.
7. Chandalia HB, Krishnaswamy PP. Glycated haemoglobin. Curr Sci 2002; 83: 1522-1531.
8. Chatterjee MN, Shinde R. Text book of Medical Biochemistry, 5th ed. Jaypee Brothers, Medical Publishers (P) Ltd, New Delhi 2002: 317.
9. Devendra D, Eisenbarth GS. Immunologic endocrine disorders. J Allergy Clin Immunol 2003; 111: S624-S636.
10. Drabkin DL, Austin JM. Spectrophotometric constants for common haemoglobin derivatives in human, dog and rabbit blood. J Biol Chem 1932; 98: 719-733.
11. Du Vigneaud V, Karr WG. Carbohydrates utilisation rate of disappearance of D-glucose from the blood. J Biol Chem 1925; 66: 281-300.
12. Duncan BD. Multiple range tests for correlated and heteroscedastic means. Biometrics 1957; 13: 359-364.
13. Ellis HA, Kirkman HN. A Colorimetric method for assay of erythrocyte glucose-6-phosphate dehydrogenase. Proc Soc Exp Biol Med 1961; 106: 607-609.
14. Fiske CH, Subbarow J. The colorimetric determination of phosphorus. J Biol Chem 1925; 66: 375-400.
15. Gancedo JM, Gancedo C. Fructose-1, 6-bisphosphatase, phospho-fructokinase and glucose-6-phosphate dehydrogenase from fermenting and non-fermenting yeasts. Arch Microbiol 1971; 76: 132-138.
16. Hu X, Sato J, Oshida Y, Xu M, Bajotto G, Sato Y. Effect of Gosha-jinkigan (Chinese herbal medicine): Niu-Che-Sen-Qi-Wan) on insulin resistance in streptozotocin-induced diabetic rats. Diabetes Res Clin Pract 2003; 59: 103-111.
17. Inouye M, Hashimoto H, Mio T, Sumino K. Levels of lipid peroxidation product and glycated haemoglobin $A1_C$ in the erythrocytes of diabetic patients. Clin Chim Acta 1998; 276: 163-172.
18. Jackson RL, Hess RL, England JD. HaemoglobinA1c values in children with overt diabetes maintained in varying degrees of control. Diabetes care 1979; 2: 391-395.
19. Jain, S.R.: Planta. Antidiabetic activity of aqueous bark extract of Cassia glauca in streptozotocin-induced diabetic rats. Medica., 1: 43-47. (1968).
20. Koening RJ, Peterson CM , Jones R. Correlation of glucose regulation and haemoglobin A1C in diabetes mellitus. New Engl J Med 1978; 295: 417-420.
21. Koida H, Oda T. Pathological occurrence of glucose-6-phosphatase in liver disease. Clin Chim Acta 1959; 4: 554-561.
22. Latha M, Pari L. Preventive effects of *Cassia auriculata* L. flowers on brain lipid peroxidation in rats treated with streptozotocin. Mol Cell Biochem 2003; 243: 23-28.
23. Morales MA, Jabbagy AJ, Tenenzi HP. Mutations affecting accumulation of glycogen. Neurospora News Lett 1973; 20: 24-25.
24. Murugan P. Effect of *Cassia auriculata* l on plasma antioxidants in streptozotocinnicotinamide induced experimental diabetes. Inter J Inform Res Rev. 2015a: 02, 05; 6930-6934.
25. Murugan P. Preventive effects of *Cassia auriculata* on brain lipid peroxidation streptozotocin diabetic rats. Inter J Inform Res Rev. 2015b: 02, 05; 6924-6929.
26. Murugan P. Taner's Cassia (*Cassia auriculata* L) extract prevents hemoglobin glycation tail tendon collagen properties in experimental diabetic rats. J cell tissue res. 2010; 10 (1): 2109-2114.
27. Pari L, Murugan P. Influence of *Cassia auriculata* flowers on Insulin Receptors in Streptozotocin Induced Diabetic Rats: Studies on Insulin Binding to Erythrocytes. Afr J Biochem Res. 2007; 1 (7): 148-155.
28. Shrotri DS, Aiman R. The relationship of the post-absorptive state to the hypoglyceamic action. Ind. J. Med. Res.1960. 48: 162 – 168.
29. Spinas GA. The dual role of nitric oxide in islets □-cells. News Physiol Sci 1999; 14: 49-54. Diabetic rats by insulin treatment. Diabetologia 1980; 18: 413-416.
30. Sudhakar Nayak S, Pattabiraman T. A new colorimetric method for the estimation of glycosylated haemoglobin. Clin Chim Acta 1981; 109: 267-274.
31. Trevilli LA, Ranney HM, Lai H. Hemoglobin components in patients with diabetes mellitus. New Engl J Med 1971; 284: 353-357.
32. Ulrich HB. Methods of Enzymatic Analysis. 2nd edn, 1974: 569-573.
33. Venkateswaran S, Pari L. Effect of Coccinia indica leaf extract on plasma antioxidants in streptozotocin-induced diabetes in rats. Phytother Res 2002; 17: 605-608.
34. WHO (World Health Organization). Expert Committee on diabetes mellitus, Second Report of the WHO Expert Committee on Diabetes mellitus. Technical Report Series. Geneva 1980; 646: 66.
35. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. Nature 2001; 414: 782-787.

Table 1. Changes in body weight, blood glucose and plasma insulin in normal and experimental rats

Groups	Body weight (g)		Fasting Blood Glucose (mg/dl)	Plasma insulin (μU/ml)
	Initial	Final		
Normal	197.55 ± 5.54	222.77 ± 4.99	91.52 ± 7.14^{a}	12.32 ± 0.78^{a}
Diabetic control	210.12 ± 6.57	$175.44 \pm 7.24^{\dagger}$	282.52 ± 7.78^{b}	3.99 ± 0.36^{b}
Diabetic + CFEt (0.45 g/kg)	212.54 ± 5.24	219.55 ± 6.32*	105.22 ± 6.47^{e}	9.89 ± 0.42^{e}
Diabetic + CLEt (0.45 g/kg)	191.44 ± 6.56	207.77 ± 6.52*	122.12 ± 7.53^{f}	8.32 ± 0.41^{f}
Diabetic + Glibencalamide (600 μg/ kg)	197.44 ± 6.56	204.77 ± 6.12*	1228.21 ± 7.58^{f}	8.79 ± 0.39^{f}

Values are given as mean ± S.D for 6 rats in each group.
Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).
Diabetic control was compared with normal, † $p<0.001$.
Experimental groups were compared with diabetic control, * $p<0.001$.
+ + +, > 2% sugar, ++; -2% sugar; +, -1%

Table 2. Oral glucose tolerance test in normal and experimental rats

Groups	Blood Glucose Levels (mg/dl)				
	0 min	30 min	60 min	90 min	120 min
Normal	91.22 ± 5.55[a]	180.75 ± 6.48[a]	159.42 ± 8.47[a]	107.79 ± 5.25[a]	98.25 ± 5.54[a]
Diabetic control	277.56 ± 8.45[b]	327.32 ± 13.21[b]	370.42 ± 8.91[b]	340.55 ± 8.32[b]	311.22 ± 8.54[b]
Diabetic + CFEt (0.45 g/kg)	102.25 ± 4.58[c]	195.72 ± 8.45[c]	176.45 ± 5.14[c]	129.12 ± 5.54[c]	116.22 ± 5.54[c]
Diabetic + CLEt (0.45 g/kg)	114.21 ± 6.21[d]	217.21 ± 7.54[d]	192.45 ± 4.54[d]	136.15 ± 6.45[d]	127.58 ± 5.45[d]
Diabetic + Glibencalamide (600 µg/ kg)	119.25 ± 6.21[d]	225.05 ± 7.54[d]	192.87 ± 4.45[d]	140.42 ± 6.12[d]	127.58 ± 5.58[d]

Values are given as mean ± S.D for 6 rats in each group.
Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT).

Table 3. Effect of CFEt and CFLt on the levels of haemoglobin, glycosylated haemoglobin and urine sugar in normal and experimental rats

Groups	Total haemoglobin (g/dl)	Glycosylated haemoglobin (mg/g Hb)	Urine sugar
Normal	12.54 ± 0.66[a]	0.27 ± 0.03[a]	Nil
Diabetic control	8.45 ± 0.32[b]	0.73 ± 0.04[b]	+++
Diabetic + CFEt (0.45 g/kg)	11.32 ± 0.47[d]	0.36 ± 0.03[e]	Nil
Diabetic + CLEt (0.45 g/kg)	9.58 ± 1.11[e]	0.40 ± 0.03[f]	Trace
Diabetic + Glibencalamide (600 µg/ kg)	9.96 ± 1.13[e]	0.42 ± 0.03[f]	Trace

Values are given as mean ± S.D from six rats in each group.
Values not sharing a common superscript letter differ significantly at p <0.05 (DMRT).

Table 4. Effect of CFEt and CFLt on the levels of liver and muscle glycogen in normal and experimental rats

Groups	Glycogen (mg/g tissue)	
	Liver	*Muscle*
Normal	35.32 ± 1.56[a]	6.51 ± 0.23[a]
Diabetic control	22.43 ± 1.47[b]	3.56 ± 0.16[b]
Diabetic + CFEt (0.45 g/kg)	30.32 ± 1.42[c]	5.71 ± 0.22[c]
Diabetic + CLEt (0.45 g/kg)	27.69 ± 1.42[d]	5.13 ± 0.14[d]
Diabetic + Glibencalamide (600 µg/ kg)	26.51 ± 1.42[d]	5.01 ± 0.13[d]

Values are given as mean ± SD from 6 rats in each group.
Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT).

Table 5. Effect of CFEt and CFLt on the activities of hepatic hexokinase, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase, fructose-1, 6- bisphosphatase and sorbitol dehydrogenase in normal and experimental rats

Groups	Normal	Diabetic control	Diabetic + CFEt (0.45 g/kg))	Diabetic + CLEt (0.45 g/kg)	Diabetic+ Glibencalamide (600 µg/ kg)
Hexokinase (units[1]/g protein)	149.71 ± 8.35[a]	108.39 ± 6.39[b]	137.15 ±5.74[c]	125.61 ± 6.47[d]	125.72 ± 6.47[d]
Glucose-6-phosphate dehydrogenase (x 10^{-4} mIU / mg protein)	4.53 ± 0.12[a]	2.18 ± 0.13[b]	3.72 ± 0.19[c]	3.37 ± 0.11[d]	3.37 ± 0.12[d]
Glucose- 6-phosphatase (units[2]/ mg protein)	0.18 ± 0.01[a]	0.27 ± 0.01[b]	0.19 ± 0.01[c]	0.21 ± 0.01[d]	0.20 ± 0.01[d]

Fructose-1,6-bisphosphatase (units[3]/ mg protein)	0.37 ± 0.03^{a}	0.57 ± 0.03^{b}	0.39 ± 0.02^{c}	0.44 ± 0.02^{d}	0.44 ± 0.02^{d}
Sorbitol dehydrogenase (units[4]/ g protein)	4.31 ± 0.29^{a}	8.59 ± 0.55^{b}	5.54 ± 0.33^{c}	6.25 ± 0.39^{d}	6.38 ± 0.41^{d}

Values are given as mean ± SD from 6 rats in each group.
Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT).

[1] - μmoles of glucose phosphorylated/min

[2] - μmoles of Pi liberated/min

[3] - μmoles of Pi liberated/hour

[4] - 1 unit of sorbitol dehydrogenase = the amount of enzyme that produces a change in absorbance of 0.01/min