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HYPOGLYCAEMIC EFFECT OF *Cassia auriculata* Linn. FLOWERS ON STREPTOZOTOCIN INDUCED MALE ALBINO RATS

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ABSTRACT

Diabetes mellitus is the most common endocrine disorder that impairs glucose homeostasis resulting in severe diabetic complications including retinopathy, angiopathy, nephropathy, neuropathy and causing neurological disorders due to perturbation in utilization of glucose. *Cassia auriculata* is used extensively in the indigenous system of medicine as an anti-diabetic agent. The current investigation focuses on the serum insulin augmentation, anti-hyperglycemic and anti-hyperlipidemic property of a ethanolic extracts of *Cassia auriculata* flowers on streptozotocin induced diabetic rats. *Cassia auriculata* Linn. has been claimed to possess antidiabetic property by many investigators. The results showed that it has significant antihyperglycemic effect in experimental model of diabetic rats.

Key words: *Cassia auriculata*, streptozotocin (STZ), Biochemical evaluation, Glibenclamide.

INTRODUCTION

Diabetes mellitus is a metabolic disorder initially characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (Barcelo and Rajpathak,2001). Without enough insulin, the cells of the body cannot absorb sufficient glucose from the blood; hence blood glucose levels increase, which is termed as hyperglycemia. If the glucose level in the blood remains high over a long period of time, this can result in long term damage to organs, such as the kidneys, liver, eyes, nerves, heart and blood vessels. Complications in some of these organs can lead to death (Pari and Saravanan, 2004).

The pancreas plays a primary role in the metabolism of glucose by secreting the hormones insulin and glucagon. The islets of Langerhans secrete insulin and glucagon directly into the blood. Insulin is a protein that is essential for proper regulation of glucose and for maintenance of proper blood glucose levels (Worthley, 2003).

WHO classification of diabetes introduced in 1980 and revised in 1985 was based on clinical characteristics. The two most common types of diabetes were insulin-dependent diabetes mellitus (IDDM) or (type I) and non-insulin-dependent diabetes mellitus (NIDDM) or (type II). WHO classification also recognized malnutrition-related diabetes mellitus and gestational diabetes. Malnutrition-related diabetes was omitted from the new classification because its etiology is uncertain, and it is unclear whether it is a separate type of diabetes (Holt, 2004; Tiwari and Rao, 2002).

There are more then 1200 plants species worldwide that are used in the treatment of diabetes mellitus and a substantial number of plants have shown effective hypoglycemic activity after laboratory testing (Eddouks *et al*., 2005). A multitude of herbs spices and other plant materials have been described of the treatment of diabetes throughout the world (Marles and Fransworth, 1995; Kesari *et al*., 2005 and 2006). The medicinal plants provide a useful source of oral hypoglycemic compounds for the development of new pharmaceutical leads as well as a dietary supplement to existing therapies (Bailey and Day, 1989). India has about 45,000 plant species and

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many of them have medicinal properties. Out of a large number of herbal drugs stated to possess anti-diabetic activity in the ayurvedic system of medicine of India.

The present study was carried out in rats to test the efficacy of *Cassia auriculata* on serum insulin, hyperglycemia and serum lipid profile changes associated with diabetes. *Cassia auriculata* has been extensively used in Ayurvedic and Siddha practice in Indian subcontinent. This work represents the study of antidiabetic potential of *Cassia auriculata* flower-extract in streptozotocin induced diabetic rats.

RESEARCH METHODOLOGY

Animals

Male albino rats weighing about 120-180g were obtained from the Indian Institute of science Banglore. The animals were housed in polypropylene cages and maintained in controlled temperature with 12 hours period of light or dark and fed with standard rat feed and water were provided *ad libitum*.

Chemicals

TBA, 2,4-DNPH, Streptozotocin, Reduced Glutathione were purchased from sigma chemicals Mumbai. All other reagent and chemicals used in this study was of analytical grade with high purity.

Collection of plant Material

The fresh flowers of *Cassia auriculata* were collected from Mannargudi, Thiruvarur District, Tamil Nadu, South India. During January – February – 2012. The plant was identified by Dr.S. John Britto, the Director, the Rapinet herbarium and the centre for Molecular Systematic, St.Joseph's College, Tiruchirappalli and a voucher specimen was deposited in the Rapinet herbarium of St.Joseph's College, Tiruchirappali (Voucher No.SV001/2012).

Preparation of plant extract

The flower was dried and soaked with ethanol (70%) for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used. The *Cassia auriculata* extract was dissolved in distilled water just before oral administration.

Streptozotocin – Induced Diabetic rats

Diabetic was induced by the intra peritoneal injection of Streptozotocin (150mg/kg) dissolved in saline for 3 consecutive days. Diabetes was confirmed 2 days after the last streptozotocin dose administration by determining the blood glucose concentration, treatments were started after confirmation of diabetic in rats.

Experimental Design

In the present investigation, a total of 24 rats (18 diabetic surviving rats and 6 normal rats) were taken and divided into four groups of 6 rats each.

Group I : Normal animal received normal diet and water *ad libitum*.

Group II **:**Streptozotocin (150mg/kg bwt.) induced diabetic rats.

Group III:Diabetic rats given water soluble fraction of ethanolic extract of *Cassia auriculata* flower (500mg/kg b.wt).

Group IV:Diabetic rats given Glibenclamide (150 mg/ kg b.wt) in aqueous solution daily using an intragastric tube for 15 days.

RESULTS AND DISCUSSION

Nature has been a source of medicinal treatment for thousands of years, and plants-based systems continue to play an essential role in the primary health care of 80% of the world's underdeveloped and developing countries. *Cassia auriculata* is one such plant used for a long time and hence, the study was carried out on the effect of flower of this plant on glycaemic control in STZ-induced diabetic rats.

Effect of *Cassia auriculata* on plasma insulin, Hb, HbA_{1c} and glucose

The levels of plasma insulin, Hb HbA_{1c} and glucose. Plasma insulin and Hb decreased and HbA_{1c} and glucose increased significantly in STZ-diabetic rats and these values reversed towards normal on treatment with *Cassia auriculata* flower extract and glibenclamide.

Effect of *Cassia auriculata* on lipid profile

The levels of total cholesterol (TC) and triglyceride (TG) in the serum of normal and diabetic rats. The diabetic rats had elevated levels of TC and TG in the serum and on treatment with *Cassia auriculata* flower-extract and glibenclamide decreased these values towards normalcy.

The levels of LDL, VLDL and HDL in the serum of normal and diabetic rats . The diabetic rats had elevated levels of LDL and VLDL and decreased level of HDL and on treatment with *Cassia auriculata* flower-extract and glibenclamide reversed these levels towards normal.

Effect of *Cassia auriculata* on protein profile

The level of total protein in the serum of normal and diabetic rats. The diabetic rats had decreased levels of serum total protein. When compared with normal rats. Treatment with *Cassia auriculata* and glibenclamide brought these parameters towards normal.

Effect of *Cassia auriculata* on kidney function

The levels of urea and creatinine in the serum of normal and diabetic rats. Diabetic rats have decreased levels of urea and creatinine when compare with normal

rats. Treatment with *Cassia auriculata* flower extract and glibenclamide reversed these parameters towards normal.

Effect of *Cassia auriculata* on redox status

The level of MDA in the serum of normal and diabetic rats . Diabetic rats had elevated level of MDA in the serum. Treatment with *Cassia auriculata* flower extract and glibenclamide showed reversal of these parameters towards normalcy.

The flower-extract of *Cassia auriculata* contains a range of active pharmacological agents such as alkaloids, flavonoids, tannins, glycosides, triterpenoids and sterols. The increase in insulin level could be due to increased pancreatic secretion from existing β-cells. Alkaloids and flavonoids are known for their ability of beta cell expansion (Chakravarti *et al*., 1981), thereby increasing insulin level.

The increased blood glucose stimulates nonenzymatic proteins glycosylation namely, serum albumin, α-crystallin, collagen, low-density lipoprotein, hemoglobin etc. The first indication that a very simple chemical reaction between glucose and free amino groups on protein can lead to irreversible modification came with the characterization of glycosylated hemoglobin (Cohen and Wu, 1994). HbA_{1C} concentration is proportionately increased in diabetic patients with ambient hyperglycemia and reflects the extent as well as management of diabetic condition (Wolffenbuttel *et al*., 1996). Several reports have been made on glycosylation-induced structural and functional modification of hemoglobin (De Rosa *et al*., 1998, Kar and Chakraborti, 2001). Lower levels of total hemoglobin observed in diabetic rats might be due to the increased formation of HbA_{1C}. HbA_{1C} was found to increase in patients with diabetes mellitus to approximately 16% and the amount of increased insulin level was directly proportional to the fasting blood glucose levels.

The liver is an important organ that plays a pivotal role in glycolysis and gluconeogenesis. A partial or total deficiency of insulin causes derangement in carbohydrate metabolism that decreases activity of several key enzymes including glucokinase, phosphofructokinase and pyruvate kinase (Hikino *et al*., 1989), resulting in impaired peripheral glucose utilization and augmented hepatic glucose production. In our study, blood sugar level was increased in the liver of diabetic rats which may be due to a deficiency of insulin and treatment with *Cassia auriculata* flower extract and glibenclamide elevated the activity of glucose. *Cassia auriculata* flower extract administration increased insulin level which, in turn, activated glucokinase, thereby increasing the utilization of glucose leading to decreased blood sugar level.

Thus, the results showed a sequential metabolic correlation between increased glycolysis and decreased glycogenolysis stimulated by *Cassia auriculata* suggesting the possible biochemical mechanism via insulin secretion, through which glucose homeostasis is regulated.

STZ-diabetic rats showed increase in plasma cholesterol and triglyceride concentrations (Sachdewa and Khemani, 2003), which may contribute to the development and progression of micro- and macro-vascular complications. Further, the abnormal high concentration of serum lipids in the diabetic subjects is mainly due to increase in the mobilization of free fatty acids from fat deposits (Rhoads *et al*., 1976) since insulin is required for the inhibition of hormone sensitive lipase. Glucagon and other hormones enhance lipolysis. The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of uninhibited actions of lipolytic hormones on the fat deposits (Goodman and Gilman, 1985). Diabetic rats treated with *Cassia auriculata* flower extract and glibenclamide significantly decreased TC and TG towards normalcy.

The concentration of low density lipoprotein cholesterol (LDL) is one of the most important predictors of atherosclerosis and coronary heart disease (CHD) (Temme *et al*., 2002) and reduction in its level reduces the morbidity and mortality in patients with CHD. In diabetic rats, the rise in TC and TG is associated with the increase in LDL and VLDL and decrease in HDL. In our study, the diabetic rats treated with *Cassia auriculata* flower extract showed an elevation in HDL and reduction in LDL and VLDL as evidenced by decreased levels of TC and TG. Thus, *Cassia auriculata* flower extract could alleviate the risk of cardiovascular diseases.

Thus, the present study showed that *Cassia auriculata* flower extract is having antihyperlipidemic effect, which is evidenced by the decreased levels of TC, TG, LDL, VLDL and elevated level of HDL in diabetic rats. Literature has shown flavonoids, alkaloids to be the active hypoglycemic principle in many medicinal plants with blood glucose and lipids-lowering attributes (Oladele *et al*., 1995). The presence of these phytochemicals in the plant flower-extract, may account for the observed hypoglycemic and hypolipidemic effects of the extract.

Insulin generally has an anabolic effect on protein metabolism in that it stimulates protein synthesis and retards protein degradation (Murray, 2000). Previous report shows that protein synthesis is decreased in all tissues due to decreased production of ATP in absolute or relative deficiency of insulin (Chatterjee, 1994), which may be responsible for the decreased level of serum proteins in diabetic rats. Further, excess generation of reactive oxygen species (ROS) also has the ability, either directly or indirectly, to damage proteins, DNA and other cell biomolecules (Shacter, 2000).

Table 1. Effect of *Cassia auriculata* on biochemical analysis of control and treated rats

Groups	Plasma insulin (µU/ml)	Hb (mg/dl)	HbA1c (mg/gHb)	Glucose (mg/dl)	Cholesterol (mg/dl)
Group I	57.04 ± 0.21	12.62 ± 1.23	0.28 ± 0.03	81.03 ± 7.40	74.12 ± 1.48
Group II	26.15 ± 0.56	8.40 ± 1.03	0.84 ± 0.06	290.99 ± 23.23	97.62 ± 4.30
Group III	39.64 ± 0.72*	11.42 ± 1.12*	0.31 ± 0.03*	82.08 ± 10.11*	83.64 ± 2.15*
Group IV	56.06 ± 0.54	13.23 ± 1.32	0.27 ± 0.03	80.35 ± 6.32	90.62 ± 1.32

Table 2. Effect of *Cassia auriculata* on lipoprotein profile of control and treated rats

Groups	Triglyceride (mg/dl)	LDL-Cholesterol (mg/dl)	VLDL-Cholesterol (mg/dl)	HDL-Cholesterol (mg/dl)	Protein (gm/dl)
Group I	43.35 ± 3.26	38 ± 5.68	42.01 ± 4.18	27.3 ± 0.65	7.12 ± 1.11
Group II	63.80 ± 1.62	90.32 ± 7.21	73.06 ± 10.40	19.41 ± 0.52	4.39 ± 0.78
Group III	54.12 ± 2.54*	38.30 ± 5.3*	38 ± 4.42*	26.12 ± 0.61*	6.95 ± 0.92*
Group IV	57.64 ± 1.70	37 ± 5.41	41.04 ± 3.08	29.4 ± 0.24	6.31 ± 0.71

Table 3. Effect of *Cassia auriculata* on renal markers and lipid peroxidative marker of control and treated rats

Groups	Urea (mg/dl)	Creatinine (mg/dl)	MDA (nmol/L)
Group I	25.3 ± 2.4	0.68 ± 0.64	5.21 ± 0.81
Group II	38.6 ± 4.10	1.78 ± 0.07	11.62 ± 0.67
Group III	27.41 ± 3.2*	0.75 ± 0.06*	6.51 ± 1.10*
Group IV	26.41 ± 3.8	0.67 ± 0.51	4.45 ± 0.94

The present study showed the elevated level of serum total proteins may be related with increased level of plasma insulin in diabetic rats treated with *Cassia auriculata* flower extract and glibenclamide.

The diabetic hyperglycemia induces elevation of the serum levels of urea and creatinine which are considered as significant markers of renal function (Almdal and Vilstrup., 1988). Urea is the major nitrogen containing metabolic product of protein metabolism. Creatinine is endogeneously produced and released into body fluids and its clearance is measured as an indicator of glomerular fitration rate (Burtis and Ashwood., 1996; Perone *et al*., 1992). Treatment with *Cassia auriculata* flower extract reversed these parameters to near normal level which could be due to decreased disturbances of protein and nucleic acid metabolisms as evidenced by improved glycemic control.

Free radicals may play an important role in the causation of diabetic complications and alterations in the endogenous free radical scavenging defense mechanisms may lead to ineffective scavenging of reactive oxygen species, resulting in oxidative damage and tissue injury. In this connection, especially medicinal plants have come under serious scrutiny. *Cassia auriculata* flower is widely distributed in India, and has been used as a remedy against various complaints in traditional medicine worldwide since ancient times.

The increased free radicals produced may react with polyunsaturated fatty acids in cell membrane leading to lipid peroxidation and it will in turn result in the elevated free radicals production (Levy *et al*., 1999). In this study, the concentration of serum MDA was significantly increased in the of diabetic rats as reported earlier in clinical and experimental diabetes (Sundaram *et al*., 1996). The increased concentration of lipid peroxidative markers suggests that an increase in oxygen free radicals, either by increased production or decreased destruction (Kakkar *et al*., 1995). The levels of lipid peroxidative markers in *Cassia auriculata* flower extract treated rats decreased significantly, which might be due to the presence of phytochemicals.

From this study, I conclude that the ethanolic extract of *Cassia auriculata* flower-extract possesses good glycemic control along with the antihyperlipidemic properties in STZ-diabetic rats. Further studies are warranted in the isolation and characterization of the active principle(s) and its molecular mechanism of action. Our study justifies the traditional use of *Cassia auriculata* flower in diabssetes management.

REFERENCES

Almdal JP, Vilstrup H. Strict insulin therapy normalizes organ nitrogen contents and the capacity of urea nitrogen synthesis in experimental diabetes in rats. *Diabetologia,* 31, 1988, 114-118.

Burtis CA, Ashwood ER. Enzymes. Teitz Fundamentals of Clinical Chemistry, 4th ed. NB Saunders Company, *Philadelphia*, USA. 1996, 312-335.

Chakravarti BK, Gupta S, Gambir SS, Gode KD. Pancreatic betacell regeneration in rats by (−) epicatechin. *Lancet*, 2, 1981, 759–760.

Chatterjee MN, Rana Shinde. Text Book of Medical Biochemistry. Metabolism of carbohydrate. Jaypee Brothers Medical Publishers Pvt Ltd, New Delhi, India. 1994, 421.

Cohen MP, Wu V. Purification of glycated hemoglobin. *Methods Enzymol*, 1994, 65– 75.

De Rosa MC, Sanna MT, Messana I, Castagnola M, Galtieri A, Tellone E, Scatena R, Bolta M, Giardina B. Glycated human hemoglobin (HbA1c): functional characteristics and molecular modeling studies. *Biophys Chemist*, 72, 1998, 323–335.

Goodman LS, Gilman A. *The pharmacological Basis of Therapeutics*, 7th ed, Mc Millan, New York, 1985, 490.

Hikino H, Kobayashi M, Suzuki Y, Konno C. Mechanisms of hypoglycemic activity of aconitan A, a glycan from *Aconitum carmichaeli* roots. *J Ethnopharmacol*, 25, 1989, 295-304.

Holt RIG. Diagnosis, epidemiology and pathogenesis of diabetes mellitus: an update for psychiatrists. *British journal of Psychiatry*, 184 (suppl.47), 2004, s 55- s 63

Hymete A. Phytochemical investigation of the fruit of *Lagenaria breviflora* Robert. MSc thesis, University of IFE, ILE-IFE, Nigeria, 1986, 54-67.

Kakkar R, Kalra J, Mantha SV, Prasad K. Lipid peroxidation and activity of antioxidant enzymes in diabetic rats. *Mol Cell Biochem*, 151, 1995, 113-119.

Kar M, Chakraborti AS. Effect of glycosylation on iron-mediated free radical reactions of hemoglobin. *Curr Sci*, 80, 2001, 770– 773.

Levy U, Zaltzber H, Ben-Amotz A, Kanter Y, Aviram M.β-Carotene affects antioxidants status in non-insulin-dependent diabetes mellitus. *Pathophysiol*. 6, 1999, 157-161.

Murray RK, Granner DK, Mayesa PA *et al*., Harper's Biochemistry, 25th ed, Appleton and Lange, Stamford, Connecticut. 2000.

Oladele SB, Ayo JO, Adaudi AO. Medicinal and physiological properties of flavonoids, coumarin derivatives and anthraquinones of plant origin. *West Afr J Pharmacol Drug Res*, 11, 1995, 134– 144.

Pari L and Saravanan R. Antidiabetic effect of diasulin, a herbal drug, on blood glucose, plasma insulin and hepatic enzymes of glucose metabolism in hyperglycaemic rats. *Diabetes, Obesity and Metabolism*, 6, 2004, 286–292.

Perone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: New insights into old concepts. *Clin Chem*, 38, 1992, 1933-1953.

Rhoads GG, Gulbrandse CL, Kagan A. Serum lipoproteins and coronary artery diseases in a population study of Hawaii, a Japanese man. *N Eng J Med*, 294, 1976, 293-298.

Sachdewa A, Khemani LD. Effect of *Hibiscus rosa sinensis Linn*. ethanol flower extract on blood glucose and lipid profile in streptozotocin induced diabetes in rats. *J Ethnopharmacol*, 89, 2003, 61–66.

Shacter E (2000). Quantification and significance of protein oxidation in biological samples. *Drug Metab Rev*.; 32: 307–326.

Sundaram RK, Bhaskar A, Vijayalingam S, Viswanatthan M, Moha R, Shanmugasundaram KR. Antioxidant status and lipid peroxidation in type II diabetes mellitus with and without complications. *Clin Sci*, 90, 1996, 255-260.

Temme EH, Van HPG, Schouten EG, Kesteloot H. Effect of a plant sterol-enriched spread on serum lipids and lipoproteins in mildly hypercholesterolaemic subjects. *Acta Cardiol*, 57, 2002, 111–115.

Tiwari AK and Rao JM. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Current Science*, 83, 2002, 30-38

Wolffenbuttel BH, Giordino D, Founds HW, Bucala R. Long-term assessment of glucose control by hemoglobin-AGE measurement. *Lancet*, 347, 1996, 513– 515.

Worthley LIG. *The Australian short course on intensive care medicine*, Handbook, Gillingham printers, South Australia, 2003, 31-55.