

Antidiabetic and Hypolipidemic Effect of *Cassia auriculata* in Alloxan Induced Diabetic Rats

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Abstract: Several reports have shown the various effects of *Cassia auriculata* flower extract such as hypoglycemic, hypolipidemic, antioxidant and hypotensive effects. The present study was conducted to investigate the antidiabetic and hypolipidemic efficacy of various parts (root, stem, leaves and flowers) of *Cassia auriculata* on alloxan-induced diabetic rats. In the *in vivo* experiments, alloxan induced diabetic wistar strains of male albino rats were randomized into 7 groups. It includes 1) Control 2) Diabetic control 3) Diabetic +250 mg kg⁻¹ b.w. of CA root extract 4) Diabetic+250 mg kg⁻¹ b.w. of CA stem extract 5) Diabetic+250 mg kg⁻¹ b.w. of CA leaf extract 6) Diabetic +250 mg kg⁻¹ b.w. of CA flower extract 7). Diabetic+100 mg kg⁻¹ Tolbutamide. The groups were treated with the plant extracts and drug along with a control for a period of 28 days. Serum and plasma from the experimented animals were collected and assayed for glucose tolerance (acute and chronic study), insulin, triglycerides and cholesterol. Results showed that there is a significant level of reduction in the serum glucose and triglycerides and cholesterol and increase in the plasma insulin levels for the flower and leaf extracts of *Cassia auriculata* when compared to root and stem extracts. From this study, it is confirmed that the leaves and flowers of *Cassia auriculata* has anti diabetic and anti lipidemic effect and these could be used in diabetic and CHD (Coronary Heart Disease) management.

Key words: *Cassia auriculata*, diabetes, hypolipidemic, alloxan, tolbutamide

INTRODUCTION

Diabetes mellitus is a metabolic disorder in which the body does not produce or properly use insulin. It causes disturbances in carbohydrate, protein, and lipid metabolism and complications such as retinopathy, microangiopathy and nephropathy (Rameshkumar *et al.*, 2004). In practical terms, diabetes mellitus is a condition in which cells are starving in the sea of glucose. During diabetes, a profound alteration in the concentration and composition of lipid occurs. The global figure of people with diabetes set rise from the current estimate of 150 to 220 million in 2010 and 300 million in 2025 (Paul Zimmer and Alberti, 2001).

Despite the great strides that have been made in the understanding and management of diabetes, the disease and disease related complications are increasing unabated (Tiwari and Madhusudana Rao, 2002). In spite of the presence of known antidiabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease (Bhattaram *et al.*, 2002). Many traditional plant treatments for diabetes are used throughout the world and there is an increasing demand by patients to use the natural products with antidiabetic activity (Swanston *et al.*, 1990).

Herbal drugs are prescribed widely even when their biologically active compounds are unknown, because of their effectiveness, minimal side effects in clinical experience and relatively low cost (Valiathan, 1998).

There are several medicinal plants which are used in the treatment of diabetes, among these herbal resources *Cassia auriculata* is selected for the present study. The plant *Cassia auriculata* is a fast growing branched tall, evergreen Indian shrub with reddish brown branches and vivid yellow flowers. It is a common plant in Madhya Pradesh, Western Penninsula, India, Burma, Ceylon and it belongs to the family of Fabaceae. *Cassia auriculata* has been widely used in traditional medicine and a potent adjunct in the treatment of rheumatism, conjunctivitis and diabetes (Joshi, 2000). Seeds are used in ophthalmia and dysentery (Kirtikar and Basu, 1988; Vaidya, 1998). Dried flowers and leaves of *Cassia auriculata* are being used for medicinal treatment (Sawhney *et al.*, 1978). The bark is astringent and tonic. The leaves and fruits are antihelmentic. The root is used in the treatment of skin diseases, leprosy, tumors, asthma and urethrorrhoea (Ran Osu Enterprises, www.nccsl.lk/ranosu/medicinal-plants).

The survey of literature reveals that *cassia auriculata* flowers has antidiabetic activity (Pari and Latha, 2002) and the various extracts of whole

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plant powder (Panchang) have significant pharmacological activity towards lowering of blood glucose, managing dyslipidemia and other cardiovascular risk associated with type II diabetes (Juvekar and Halade, 2006) but there is no scientific report for the comparative analysis of various parts (stem, leaves, root and flowers) of *Cassia auriculata* for its antidiabetic activity. Hence, the present study was undertaken in the department of biochemistry, Dr. NGP. Arts and science college, Coimbatore, Tamilnadu, India in the year 2006 (January to April) to evaluate the blood glucose and lipid-lowering properties of various parts of *cassia auriculata* on alloxan induced experimental diabetic rats. The efficacy was compared with a standard hypoglycemic drug tolbutamide.

MATERIALS AND METHODS

Plant material: The stem, leaves, root and flowers of *Cassia auriculata* was collected from the local areas of Coimbatore district, Tamilnadu, India, identified and authenticated from botanist Dr. Aurumugaswamy, Department of Botany, Kongunadu college of arts and science, Coimbatore and the voucher specimens of *Cassia auriculata* is preserved in the Department of Biochemistry, Dr. NGP Arts and Science College, Coimbatore.

Preparation of plant extract: The collected plant parts of stem, leaves, root and flowers were cleaned and washed well with water. Then 50 g of selected plant parts were dried under shade at 25°C for 5 days in the absence of sunlight and grounded well to fine powder.

The powdered plant parts (nearly 30 g) were successfully extracted with boiling water using soxhlet extractor. The extraction was continued for 10 h. These extracts are then cooled and filtered using Whatmann No 1 filter paper. The filtrate was centrifuged at 10,000 rpm at room temperature (25°C) and the sediment was discarded. The supernatant was concentrated upto 100 mL on rotavapour under reduced pressure. The concentrated crude extract was lyophilized into powder (5 g) and used for the study. Extracts prepared by using fresh plant parts as well as air-dried plant parts showed the same activity.

Chemicals: Alloxan was obtained from Himedia Laboratory Limited; Mumbai, India. Chemically alloxan is 2, 4, 5, 6 tetra oxo hexahydro pyrimidine. Alloxan was one of the most widely used chemical diabetogens. Alloxan destroys the beta cells of pancreas, so insulin production was inhibited and it can be administered virtually through all routes.

Tolbutamide is a sulfonylurea oral hypoglycemic drug sold under the brand name Orinase. This drug may be used in the management of type II diabetes if diet alone is not effective. Tolbutamide stimulates the secretion of insulin by the pancreas. Since the pancreas must synthesize insulin in order for this drug to work (From Wikipedia, the free encyclopedia Tolbutamide).

Tolbutamide powder supplied by the Hoechst Fedco Pharma, Ltd., Bombay, was used. It was administered in the doses of 100 mg kg⁻¹. b.w. The dosage range used here is found to be optimum from the various pilot experiments which are not included in this report. All other reagents used were of analytical grade.

Experimental animals: The Wistar strains of male albino rats weighing between 100-150 g were obtained for the present study, from Kerala Agricultural University, Trissur, India. The animals were housed in larger spacious cages and they were fed with commercial pelleted rat chow marketed by Hindustan Lever Ltd. Bangalore India under the trade name Gold Mohur Rat Feed and had free access to water *ad libitum*. The animals were well acclimatized to standard environmental conditions of temperature (22°C±5°C) and humidity (55±5%) and 12 h light dark cycles throughout the experimental period. The animals used in the present study were approved by the institutional Ethical Committee constituted as per the directions of the committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) under Ministry of Animal Welfare Division, Government of India, New Delhi, India.

Preparation of standard drug: Tolbutamide was used as the reference drug for evaluating the antidiabetic activity. Tolbutamide powder supplied by the Hoechst Fedco Pharma, Ltd., Bombay, was powdered and made into suspension in distilled water using Tween-80 as a suspending agent. The strength of suspension was prepared as per the kg of body weight (100 mg kg⁻¹ b.w./day)

ASSESSMENT OF HYPOGLYCEMIC ACTIVITY

Experimental induction of diabetes in rats: The overnight fasted rats were injected intraperitoneally with alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg kg⁻¹ body wt. (Katsumata *et al.*, 1999). The control rats received the same amount of saline solution.

After one hour of alloxan administration, animals were given fed *ad libitum* and 1 mL of (100 mg mL⁻¹) glucose i.e., combats ensuring severe hyperglycemia. After 72 h of

the alloxan injection, the animals were tested for evidence of diabetes by estimating their blood glucose level by using glucose estimation kit. The blood glucose level more than 150 mg/100 mL of blood was criteria.

Experimental design: The rats were segregated into 7 groups with minimum of 8 rats in each group.

Group I: Normal control rats

Group II: Diabetic control rats

Group III: Diabetic rats treated with stem extract of CA

Group IV: Diabetic rats treated with leaf extract of CA

Group V: Diabetic rats treated with root extract of CA

Group VI: Diabetic rats treated with flower extract of CA

Group VII: Diabetic rats administered with tolbutamide (150 mg kg⁻¹ b.w.) in aqueous solution orally for 28 days.

Treatment protocol: Test extracts (250 mg kg⁻¹ b.w.), standard drug Tolbutamide (150 mg kg⁻¹ b.w.) and control (2 mL saline) were administered orally, every 24 h for a period of 28 days. The experimental rats were carefully monitored everyday; no sign of toxicity was noticed on the behavior and general health of the rats when exposed to the plant extract. Animals described as fasted were deprived of food for at least 12 h but allowed free access to drinking water. The blood samples were obtained through the tail vein puncturing with hypodermic needle under light ether anesthesia. 0.2 mL of blood was withdrawn at interval of initial, 1, 3 and 5th h of administration of single dose (for acute study) and at the end of 7, 14, 21 and 28th days (prolonged study). At the end of the experimental period, the fasted rats were then sacrificed by cervical decapitation. Blood was collected with EDTA as anticoagulant and centrifuged at 3000 rpm for 15 min to separate plasma. The blood without EDTA was centrifuged at 6000 rpm for 5 min for serum separation.

Estimation of blood glucose: The blood glucose was measured in all the groups by using glucose enzyme reagent system, manufactured by Span Diagnostic Private Ltd., Surat, India. The system uses glucose oxidase method for estimating glucose in blood.

Estimation of plasma insulin: Plasma insulin level was assayed by Enzyme Linked Immuno Sorbent Assay kit using human insulin as standard.

Estimation of total cholesterol and triglycerides: Total cholesterol were estimated by CHOD-PAP method and triglycerides by GPO-TRINDER method.

Statistical analysis: Data obtained was subjected to One Way ANOVA followed by student's 't' test to determine the statistical significance of the blood parameters.

RESULTS

Glucose is a substrate and an indispensable energy supplier, which supports cellular function. Glucose measurements are used in the diagnosis and monitoring of carbohydrate metabolism disorders including diabetes mellitus, neonatal hypoglycemia, idiopathic hypoglycemia and pancreatic islet carcinoma (Virella Lopes and Virella, 2003). In recent years, various plant extracts have been claimed to be useful for the treatment of diabetes mellitus. (Shukla *et al.*, 2000). Over 150 plant extracts and some of the active principle including flavonoids are known to be used for the treatment of diabetes (Bailey, 1989; Choi *et al.*, 1991).

Alloxan, a beta cytotoxin, induces chemical diabetes (alloxan diabetes) in a wide variety of animal species by damaging the insulin secreting pancreatic-cell, resulting in a decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues (Omamoto *et al.*, 1981). The mean fasting glucose levels for group I to group VII are indicated in Table 1 (for acute study) and Table 2 (for prolonged study).

Results in Table 1 shows that there was an elevation in blood glucose levels in alloxan treated diabetic rats when compared with normal rats. The intraperitoneal injection of alloxan in Wister rats produced hyperglycemia, impaired glucose tolerance and insulin resistance. Among the administration of the extracts of various plant parts of cassia auriculata, only the flower and leaf extracts and tolbutamide tends to bring the fasting blood glucose level towards the normal in the acute study. There is no significant level of reduction in fasting blood glucose level was noticed for the aqueous extracts of root and stem of cassia auriculata.

On chronic administration, (Table 2) the effect of CA flower Et (133.77±3.167) and leaf Et (134.33± 2.280) causes a fall in fasting blood sugar of rats. The fall is evident even in the 1st week and goes on progressively increasing till at the end of 4 weeks and the fall in the fasting blood sugar was nearly equal to that of reference drug Tolbutamide (126.60±2.555). The anti-diabetic activity of root and stem is not significant in prolonged study also. These findings clearly established that the anti diabetic efficacy of the flower and leaf extract of CA are almost equal and both exhibited more potent antidiabetic activity by reducing the blood glucose level significantly than all other root

Table 1: Effect of *Cassia auriculata* on blood glucose level of alloxan induced diabetic rats after single dose

Group (n)	Dose	Initial	1 h	3 h	5 h
Normal control	2 mL saline	97.62 ± 0.296	98.33 ± 0.304	98.74 ± 0.279	98.95 ± 0.482
Diabetic control	2 mL saline	179 ± 2.749*a	186 ± 3.109*a	189 ± 3.498*a	193 ± 2.941*a
Diabetic+CA Stem Et	250 mg kg ⁻¹ b.w.	177 ± 4.220b	176 ± 3.419b	175.7 ± 4.226b	173.7 ± 4.326b
Diabetic+CA leave Et	250 mg kg ⁻¹ b.w.	178.3 ± 4.156	171.2 ± 3.697*b	166.6 ± 3.22*b	154.8 ± 3.818*b
Diabetic+CA Root Et	250 mg kg ⁻¹ b.w.	175.9 ± 3.784b	175.2 ± 3.128b	174.9 ± 3.157b	173.4 ± 3.401b
Diabetic+CA Flower Et	250 mg kg ⁻¹ b.w.	178.5 ± 3.043	171.2 ± 3.572*b	169.1 ± 3.409*b	151.8 ± 3.769*b
Tolbutamide	100 mg kg ⁻¹ b.w.	168.2 ± 3.824	161.5 ± 4.352*c	156.0 ± 3.909*c	149.31 ± 2.539*c

Values are given as mean ± SD (*p < 0.05 significant vs control) n-Number of animals in each group (eight rats per group). Values are statistically significant at p < 0.05; Statistical significance was compared within the groups as follows: Diabetic control rats were compared with normal control rats. b. CA extract treated diabetic rats were compared with diabetic control rats. c. Tolbutamide treated diabetic rats were compared with diabetic control rats

Table 2: Effect of *Cassia auriculata* on blood glucose level of alloxan induced diabetic rats after prolonged treatment

Group (n)	Initial	7th day	14th day	21st day	28th day
Normal control 2 mL saline	97.62 ± 0.296	88.56 ± 0.854	85.38 ± 1.276	84.37 ± 1.790	85.03 ± 0.207
Diabetic control 2 mL saline	179 ± 2.749	187.6 ± 3.987*a	209.00 ± 2.280*a	231.76 ± 1.914*a	244.63 ± 2.911*a
Diabetic+CA Stem Et 250 mg kg ⁻¹ b.w.	177 ± 4.220	196.8 ± 4.032	189.09 ± 1.405	184.90 ± 0.830	181 ± 3.409
Diabetic+CA leaf Et 250 mg kg ⁻¹ b.w.	178.3 ± 4.156	151.7 ± 5.034*b	134.33 ± 2.280*b	127.31 ± 3.167*b	108.00 ± 1.706*b
Diabetic+CA Root Et 250 mg kg ⁻¹ b.w.	175.9 ± 3.784b	172.9 ± 4.022b	170.33 ± 3.342b	168.20 ± 2.201b	168.97 ± 3.067b
Diabetic+CA Flower Et 250 mg kg ⁻¹ b.w.	178.5 ± 3.043	149.8 ± 4.269*b	133.77 ± 3.167*b	126.02 ± 1.929*b	102 ± 3.951*b
Tolbutamide (100 mg kg ⁻¹ b.w.)	168.2 ± 3.824*c	132.4 ± 3.824*c	126.60 ± 2.555*c	106.74 ± 2.748*c	99.21 ± 1.989*c

Values are given as mean ± SD (*p < 0.05 significant vs control), n-Number of animals in each group (eight rats per group). Values are statistically significant at p < 0.05; Statistical significance was compared within the groups as follows: Diabetic control rats were compared with normal control rats. b. CA extract treated diabetic rats were compared with diabetic control rats. c. Tolbutamide treated diabetic rats were compared with diabetic control rats

Table 3: Changes in levels of serum cholesterol, triglycerides and plasma insulin in normal and experimental animals

Group (n)	Cholesterol (mg/100 mL)	Triglycerides (mg/100 mL)	Insulin (µU mL)
Normal control	72.80 ± 4.40	73.00 ± 6.07	14.18 ± 0.74
Diabetic control	121.10 ± 6.33*a	154.58 ± 4.87*a	3.45 ± 0.38*a
Diabetic+CA Leave Et	89.32 ± 3.36*b	97.10 ± 2.60*b	9.05 ± 0.64*b
Diabetic+CA Flower Et	96.27 ± 4.80*b	89.42 ± 3.63*b	11.18 ± 0.74*b
Diabetic+Tolbutamide	88.40 ± 7.43*c	81.90 ± 7.19*c	12.18 ± 0.41*c

Values are given as mean ± SD (*p < 0.05 significant vs control) n-Number of animals in each group (eight rats per group). Values are statistically significant at p < 0.05; Statistical significance was compared within the groups as follows: Diabetic control rats were compared with normal control rats. b. CA extract treated diabetic rats were compared with diabetic control rats. c. Tolbutamide treated diabetic rats were compared with diabetic control rats

and stem extracts. Hence, for further biochemical evaluation, the flower and leaf extract alone was taken.

Triglycerides are a group of lipids absorbed from the diet and produced endogenously from carbohydrates. Hyperlipidemia, a common feature of diabetes, is evidenced by the increased serum cholesterol, triglycerides and decreased plasma insulin in diabetic rats when compared to normal control rats. Table 3 shows the levels of serum cholesterol, triglycerides and plasma insulin in control and experimental groups of rats.

A significant increase in the levels of cholesterol, and triglycerides were observed in diabetic rats when compared to normal control groups. Treatment with the flower and leaf extracts of CA and tolbutamide to diabetic rats resulted in a significant decrease (p < 0.05) in the levels of cholesterol and triglycerides when compared to diabetic control rats.

Results showed that the plasma insulin level decreased significantly in alloxan diabetic rats when compared to normal rats. Treatment with CA increases the level of insulin. These effects were compared with tolbutamide (Table 3).

DISCUSSION

Diabetes mellitus is one of the most common chronic disease and is associated with hyperlipidemia and co-morbidities such as obesity, hypertension. Hyperlipidemia is a metabolic complication of both clinical and experimental diabetes (Bierman *et al.*, 1975). In this study, we have observed that aqueous extracts of Flower and leaves of *Cassia auriculata* decreases blood glucose in alloxan diabetic rats. The reason may be flowers and leaves contain more constituents such as alkaloids, steroids and tannins. As per literature, alkaloids, steroids and tannins are known to reduce blood glucose level in diabetic condition (Satyanarayana *et al.*, 2002). In general, there is very little biological knowledge on the specific modes of action in the treatment of diabetes, but most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc., that are frequently implicated as having antidiabetic effects (Loew and Kaszkin, 2002).

Hence the more potent antidiabetic activity of *Cassia auriculata* leaves and flower extracts may be due

to nature of more alkaloids, sterols or tannins present in them. Like the plant extract, Tolbutamide also produced significant reduction in blood glucose levels of alloxan diabetic rats.

The possible mechanism of action of extract could be correlated with the reminiscent effect of the hypoglycemic sulphonylureas, Tolbutamide, that promote insulin secretion by closure of K⁺-ATP channels, membrane depolarization and stimulation of Ca²⁺ influx, an initial key step in insulin secretion. In this context, number of other plants has also been reported to have antihyperglycemic and insulin stimulatory effects (Venkateswaran and Pari 2002; Latha and Pari, 2003).

In this study, we have also observed an increase in the concentration of total cholesterol, triglycerides, in alloxan untreated diabetic rats. Hyperlipidemia is a recognized consequence of diabetes mellitus (Pushparaj and Tan, 2000; Pepato *et al.*, 2003; Sharma *et al.*, 2003). Diabetes induced hyperlipidemia is attributable to excess mobilization of fat from the adipose due to the under utilization of glucose (Krishnakumar *et al.*, 2000). The ability of aqueous extracts of stem, leaves, root and flowers of *Cassia auriculata* to reduce the levels of plasma lipids in diabetic rats has never been studied before. In the present investigation, the *C. auriculata* leaves and flowers extract feeding resulted in significant reduction in triglycerides and a non-significant reduction was noticed for stem and root extracts.

Regarding the mechanism of action, the leaves and flower extracts of the plant extracts may enhance activity of enzymes involved in bile acid synthesis and its excretion and this may have caused decrease in serum cholesterol and triglycerides (Sethupathy *et al.*, 2002). The decrease in serum TG level is an important finding because recent studies show that triglycerides are independently related with coronary heart disease (El hazmi and Warsy, 2001; Bainton *et al.*, 1992).

Most of the hypolipidemic drugs do not decrease serum triglycerides level, but *C. auriculata* leaves and flower extract lowered it significantly. Since under normal condition, insulin activates the enzyme lipoprotein lipase and hydrolysis triglycerides (Frayn, 1993). Plant extract reduces triglycerides in serum of alloxan-induced diabetic rats and may prevent the progression of CHD. Accumulation of triglycerides is one of the risk factors in Coronary Heart Disease (CHD).

Insulin also plays an important role in the metabolism of lipids apart from its regulation of carbohydrate metabolism. Insulin is potent inhibitor of lipolysis. Since it inhibits the activity of the hormone sensitive lipases in adipose tissue and suppresses the release of free fatty acids (Locci *et al.*, 1994). During diabetes, enhanced

activity of this lipase enzyme increases lipolysis and releases more free fatty acids in to the circulation (Agardh *et al.*, 1999). Increased fatty acids concentration also increases the β -oxidation of fatty acids by increasing the activity of HMG-CoA reductase, producing more acetyl CoA and cholesterol during diabetes (Goodman and Gilman, 1985).

In normal condition, insulin increases the receptor-mediated removal of LDL-cholesterol and decreased activity of insulin during diabetes causes hypercholesterolemia. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic rats (Bopanna *et al.*, 1997). It can be seen from the Table 3 that chronic administration with aqueous extracts of leaves and flowers of *C. auriculata* reduced cholesterol level and increased the plasma insulin levels significantly. When compared with flower extract (96.27 ± 4.80), leaves extracts reduced the cholesterol level (89.32 ± 3.36) more effectively, which is almost equal to that of tolbutamide (88.40 ± 7.43).

As per the literature the ethanol and aqueous extract of *Cassia auriculata* (entire plant powder) has significant pharmacological activity towards lowering of blood glucose, managing dyslipidemia and other cardiovascular risk associated with type II diabetes (Juvekar and Halade, 2006). The results of the present study confirms that the leaves and flower extracts of the selected plant has cardio protective potential along with antidiabetic effects.

CONCLUSIONS

These results demonstrate the strong hypoglycemic and hypolipidemic impacts of *C. auriculata* leaf and flower extracts and the absence of these effects in stem and root extracts. The World Health Organization has recommended and encouraged the use of alternative therapy especially in countries where access to the conventional treatment of diabetes is not adequate (WHO, 1980). The present study provides the benefits of using the leaf and flower extracts of *C. auriculata* in the prevention and treatment of diabetes. There is a close association between ischaemic cardiovascular disease and hyperlipidemia (Assimann *et al.*, 1999; Jacobson, 2001).

The beneficial effect of lowering elevated serum triglycerides and cholesterol level in the prevention of coronary heart disease is well established (Scandinavian Simvastatin Survival Study, 1994). In the present study the possible lipid lowering potential of *C. auriculata* leaves and flower extracts has been explored on serum biochemical parameters.

So it may be concluded from the results of our present study that *Cassia auriculata* leaves and flowers possess beneficial effect on the hyperglycemia associated with hyperlipidemia.

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