Ameliorative effect of *Cassia auriculata* L. leaf extract on glycemic control and atherogenic lipid status in alloxan-induced diabetic rabbits

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Received 3 February 2009; revised 20 July 2009

Oral administration of aqueous leaf extract of *Cassia auriculata* L. (100, 200, 400 and 600 mg/kg body wt daily for 21 days) to alloxan-induced mild diabetic (MD) and severe diabetic (SD) rabbits produced dose dependent fall in fasting blood glucose up to 400 mg/kg dose from day 3 to day 21. Further, a significant elevation in the levels of insulin and reduction in glycosylated haemoglobin (HbA1c) was observed in both MD and SD rabbits when treated with 400 mg/kg dose of the extract. The significant decrease in serum levels of triglycerides (TG), total cholesterol (TC) and low-density lipoprotein-cholesterol (LDL-C) with a concomitant increase in high-density lipoprotein-cholesterol (HDL-C) was exhibited by MD as well as SD rabbits following treatment with the extract. Atherogenic indices (TG/HDL-C, TC/HDL-C and LDL-C/HDL-C) were also significantly reduced in both diabetic models of rabbits fed with the extract. Effect of the extract at 400 mg/kg dose was comparable to that of glibenclamide (600 µg/kg), a reference antidiabetic drug. Thus, the present study demonstrated that aqueous leaf extract of *C. auriculata* can be a possible candidate for antidiabetic drug.

**Keywords**: Alloxan, Atherogenic indices, *Cassia auriculata*, Hyperglycemia, Lipid profile

Diabetes mellitus is a metabolic syndrome, characterized by a loss of glucose homeostasis resulting in impaired metabolism of glucose and other energy-yielding fuels such as lipids and proteins\(^1\). The disease is very often associated with a marked increase in well known parameters of cardiovascular risk including hypertriglyceridemia, hypercholesterolemia and low level of high-density lipoprotein-cholesterol (HDL-C)\(^2,3\). Hence, there is a need to search a medication for lowering glucose as well as modifying atherogenic lipid profile.

Though different types of oral hypoglycemic agents along with insulin are available for the treatment of diabetes mellitus, healers heavily relied upon medicinal plants and herbs to treat diabetes. Actually more than 1200 plants have been described to be experimentally or ethnopharmacologically used in the treatment of diabetes\(^4,5\).

*C. auriculata* is a shrub that is used as ‘Avarai Panchaga Choornam’ (mixture of five parts of the shrub i.e. roots, leaves, flowers, bark and unripe fruits) which establishes good control on sugar levels\(^6\). Pari and Latha showed the antihyperglycemic and hypolipidemic activity of aqueous extract of *C. auriculata* flowers in experimental diabetes using the rat model\(^7\). The antihyperglycemic effect of hydro-ethanolic and ethanolic extract of *C. auriculata* leaves was also reported in experimentally-induced diabetic rats\(^8,9\). In addition, *C. auriculata* has been shown to have antiviral, antispasmodic and antipyretic activity\(^10,11\).

In this paper, we have demonstrated the effect of aqueous extract of *C. auriculata* leaves on glycemic control and atherogenic lipid profile in alloxan-induced diabetic rabbits. The study was performed in different diabetic models of rabbits having different intensities of hyperglycemia (mild and severe).

**Materials and Methods**

**Plant material**—The leaves of *Cassia auriculata* were obtained from Nagercoil, India. The plant was authenticated by Dr. Sudhanshu Shekher Dash, a taxonomist in the Botanical Garden of Indian Republic (BGIR), Noida, India, where a voucher specimen was submitted for reference (Voucher specimen no.1605).

**Preparation of plant extract**—The leaves of *C. auriculata* were dried in shade and powdered in an electric grinder. The leaf powder (100 g) was

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suspended in cold distilled water (500 ml). It was allowed to stand overnight and then filtered. The whole procedure was carried out at 4°C (extract prepared at room temperature did not produce marked anti-hyperglycemic effect). The filtrate was lyophilized. The yield of lyophilized aqueous extract was approximately 10 g/100 g (10% w/w) of C. auriculata leaves.

**Phytochemical screening**—The methods as described by Harborne\textsuperscript{12} and Ikhiri et al.\textsuperscript{13} were used to screen the aqueous extract of C. auriculata leaves for its chemical constituents.

**Animals**—Male albino rabbits (1.8-2.0 kg) were procured from Central Animal House, University College of Medical Sciences (UCMS), Delhi, India. The animals were maintained on standard diet containing 52% carbohydrate, 20.15% protein, 4.52% fat, 5.53% fibre, 1.14% calcium, 0.52% phosphorus and 7.2% ash (Hindustan Lever Ltd., Mumbai, India) and provided water ad libitum. They were housed at 22°±2°C under 12 h light/dark cycle. The animals were acclimatized to the laboratory conditions for one week before carrying out the experimental work. The animal experimental protocols for the present study were approved by Institutional Animal Ethical Committee (IAEC) of UCMS, Delhi. All experimental procedures were conducted in accordance to the ethical guidelines of International Association of the Study of Pain\textsuperscript{14}.

Induction of experimental diabetes—Experimental diabetes was induced in rabbits with alloxan (80 mg/kg body wt) dissolved in 0.1 M citrate buffer (pH 4.0), injected intravenously (iv) to overnight fasted animals through their marginal ear vein\textsuperscript{15}. The animals were monitored for plasma glucose levels at weekly intervals for a month. The rabbits with fasting blood glucose (FBG) values between 150 and 250 mg/dl were considered as mild diabetic (MD) and those with FBG values higher than 250 mg/dl as severe diabetic (SD). Based on the plasma glucose levels, stabilized diabetic rabbits were selected for the study.

Experimental design—The rabbits were divided into three groups (five animals in each group or subgroup). Group I served as normal control. Group II and III served as MD and SD, respectively. Both Group II and III were divided into six subgroups: Subgroup A- diabetic control rabbits; Subgroup B- diabetic rabbits given C. auriculata leaf extract (100 mg/kg) in aqueous solution; Subgroup C- diabetic rabbits given C. auriculata leaf extract (200 mg/kg) in aqueous solution; Subgroup D- diabetic rabbits given C. auriculata leaf extract (400 mg/kg) in aqueous solution; Subgroup E- diabetic rabbits given C. auriculata leaf extract (600 mg/kg) in aqueous solution; Subgroup F- diabetic rabbits given glibenclamide (600 µg/kg) in aqueous solution.

The treatment was given orally using an intragastric tube every morning for a period of 21 days. Fasting blood glucose (FBG) was estimated before the treatment (day 0) and after the treatment on day 3, 7, 14 and 21. Insulin, glycosylated haemoglobin (HbA1c) and lipid profile parameters were determined at the end of the experiment. Initial and final body weight was recorded.

**Sample collection**—Blood samples were withdrawn from marginal ear veins of overnight fasted animals. Blood was collected in tubes containing sodium fluoride and potassium oxalate. Plasma was separated and used for the estimation of glucose. The whole blood, collected in EDTA vials, was used to measure HbA1c and separated plasma was used for assay of insulin. Serum was separated by collecting the blood in plain vials for the determination of triglycerides (TG), total cholesterol (TC) and HDL-C.

**Analytical methods**—Blood glucose was estimated by glucose oxidase-peroxidase method\textsuperscript{16}. Plasma insulin was measured by enzyme-linked immunosorbant assay (ELISA), using kit supplied by Mercodia, Sweden. The assay of HbA1c was performed by the method as described by Goldstein et al.\textsuperscript{17}. TC was assayed as described by Fossati and Prencipe\textsuperscript{18}. TC was assayed as described by Allain et al.\textsuperscript{19}. HDL-C was determined according to the method of Burstein et al.\textsuperscript{20}. Low-density lipoprotein-cholesterol (LDL-C) was calculated by using the formula of Friedewald et al.\textsuperscript{21}.

**Statistical analysis**—Values are expressed as the mean ± SEM for five animals in each group. Statistical analysis was done by using repeated measure analysis of variance (ANOVA) and one-way ANOVA followed by Tukey’s multiple comparison test at 5% level of significance.

**Results**

Preliminary phytochemical screening of the C. auriculata aqueous leaf extract revealed the presence of alkaloids, flavonoids, saponins, tannins, cardiac glycosides and phenols.

Oral administration of aqueous leaf extract of C. auriculata produced dose dependant fall in
FBG up to 400 mg/kg dose in both MD and SD rabbits (Table 1). However, significant reduction in FBG was observed with 200 and 400 mg/kg dose of the extract from day 3 to day 21 in both models of diabetic rabbits when compared with day 0. Glibenclamide-treated MD and SD rabbits also showed significant fall in FBG at each day interval. On the other hand, MD and SD controls maintained the marked hyperglycemia throughout the experimental period. In MD as well as SD rabbits, 100 mg/kg dose of the extract did not produce significant reduction in FBG and the FBG levels in rabbits treated with 600 mg/kg dose of the extract were comparable to that of 400 mg/kg (Table 1). On the basis of these findings, further biochemical studies were carried out with 400 mg/kg dose of the extract.

A significant decrease in the levels of insulin and increase in HbA1c levels were observed in both MD and SD rabbits as compared to normal rabbits (Table 2). Administration of the extract and glibenclamide to MD and SD rabbits significantly increased insulin levels and decreased HbA1c. Alloxan-induced MD and SD rabbits showed significant reduction in body weight compared with normal. However, treatment with the extract and glibenclamide significantly recovered the body weight loss in MD as well as SD rabbits.

The levels of TG, TC and LDL-C were significantly increased in both MD and SD rabbits when compared with day 0. Glibenclamide-treated MD and SD rabbits also showed significantly decreased in both MD and SD animals. Alloxan-induced MD and SD rabbits showed significant reduction in body weight compared with normal. However, treatment with the extract and glibenclamide significantly recovered the body weight loss in MD as well as SD rabbits.

The levels of TG, TC and LDL-C were significantly increased, where as HDL-C was significantly decreased in both MD and SD animals compared with normal (Fig. 1). Following supplementation with the extract, these changes were significantly reversed in MD as well as SD animals. Effect of the extract was comparable to that of glibenclamide, however HDL-C was not significantly increased by glibenclamide in SD rabbits.

Atherogenic indices (TG/HDL-C, TC/HDL-C and LDL-C/HDL-C) were found to be significantly higher in MD as well as SD rabbits compared to normal group (Fig. 2). A significant decrease in these indices was observed following treatment with the extract and glibenclamide in both diabetic models of rabbits.

**Discussion**

Abnormal increase in blood glucose in diabetes mellitus is associated with dyslipidemia in both clinical and experimental diabetes. It was evident in the present study as alloxan-induced diabetic rabbits exhibited hyperglycemia with hypertriglyceridemia, hypercholesterolemia and low level of HDL. Alloxan, a beta cytotoxin, induces ‘chemical diabetes’ through selective destruction of pancreatic β-cells which results in a decrease of insulin secretion. Major classes of oral hypoglycemic agents currently available for the treatment of diabetes include sulphonylureas, biguanides, thiazolidinediones, α-glucosidase inhibitors and so on. Glibenclamide-used as the reference antidiabetic drug in this study-is a member of sulphonylureas. It has been proposed that sulphonylureas produce their hypoglycemic effect primarily through increased release of insulin from pancreatic β-cells. Thus, any

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**Table 1—Changes in the levels of fasting blood glucose in normal, mild and severe diabetic rabbits**

[Values are mean ± SEM of 5 animals in each group]

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td></td>
<td>4.31 ± 0.09</td>
<td>4.38 ± 0.12</td>
<td>4.20 ± 0.11</td>
<td>4.33 ± 0.09</td>
<td>4.46 ± 0.12</td>
</tr>
<tr>
<td>Mild diabetic</td>
<td></td>
<td>10.72 ± 0.72</td>
<td>10.41 ± 0.53</td>
<td>10.42 ± 0.79</td>
<td>10.92 ± 0.95</td>
<td>11.19 ± 0.87</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>10.26 ± 0.37</td>
<td>8.81 ± 0.43*</td>
<td>7.56 ± 0.54*</td>
<td>6.40 ± 0.52*</td>
<td>4.84 ± 0.38*</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>600 μg</td>
<td>10.93 ± 0.38</td>
<td>9.48 ± 0.40*</td>
<td>8.48 ± 0.39*</td>
<td>7.30 ± 0.52*</td>
<td>6.29 ± 0.41*</td>
</tr>
<tr>
<td>C. auriculata</td>
<td>200 mg</td>
<td>10.47 ± 0.95</td>
<td>8.67 ± 0.68*</td>
<td>7.68 ± 0.81*</td>
<td>6.58 ± 0.80*</td>
<td>5.08 ± 0.53*</td>
</tr>
<tr>
<td>C. auriculata</td>
<td>400 mg</td>
<td>10.54 ± 0.67</td>
<td>8.63 ± 0.54*</td>
<td>7.94 ± 0.75*</td>
<td>6.69 ± 0.76*</td>
<td>4.92 ± 0.62*</td>
</tr>
<tr>
<td>C. auriculata</td>
<td>600 mg</td>
<td>15.63 ± 0.72</td>
<td>14.97 ± 0.64</td>
<td>15.41 ± 1.17</td>
<td>15.30 ± 1.15</td>
<td>15.99 ± 0.87</td>
</tr>
<tr>
<td>Severe diabetic</td>
<td></td>
<td>16.24 ± 0.63</td>
<td>14.43 ± 0.86*</td>
<td>12.98 ± 1.08*</td>
<td>11.13 ± 0.85*</td>
<td>10.01 ± 0.69*</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>16.33 ± 0.60</td>
<td>14.40 ± 0.60*</td>
<td>13.20 ± 0.80*</td>
<td>11.72 ± 0.74*</td>
<td>10.49 ± 0.63*</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>600 μg</td>
<td>16.28 ± 0.56</td>
<td>13.94 ± 0.54*</td>
<td>11.82 ± 0.36*</td>
<td>9.92 ± 0.33*</td>
<td>8.24 ± 0.53*</td>
</tr>
<tr>
<td>C. auriculata</td>
<td>200 mg</td>
<td>16.21 ± 0.49</td>
<td>13.88 ± 0.53*</td>
<td>11.35 ± 0.58*</td>
<td>9.66 ± 0.67*</td>
<td>8.11 ± 0.71*</td>
</tr>
</tbody>
</table>

*P <0.001 compared to day 0
plant secondary metabolite or chemical constituent which is capable of affecting the insulin secretion from pancreatic β-cells will be a good mimicker of sulphonylureas. Due to least or no side-effects of plant based drugs, these can be better candidates for the treatment of diabetes mellitus because synthetic drugs are known to have undesirable side-effects.

In investigation of antihyperglycemic activity of *C. auriculata* aqueous leaf extract, the extract was found to be significantly effective in lowering blood glucose from day 3 to 21 in alloxan-induced MD as well as SD rabbits. The antihyperglycemic activity of *C. auriculata* leaf extract could be due to its insulinogenic action as increased levels of insulin were found in MD as well as SD rabbits following treatment with the extract. Therefore, the extract was able to potentiate the release of insulin from pancreatic islets similar to that observed after glibenclamide administration. A number of plants have been reported to exhibit glycemic control through insulin releasing stimulatory effect.

During diabetes, the excess of glucose present in the blood reacts with Hb to form HbA1c and the amount of increase in HbA1c is found to be directly proportional to the FBG level. Hence, estimation of HbA1c is a well accepted parameter used in the management and prognosis of the disease. In the present study, administration of extract for 3 weeks tended to significantly bring down the elevated levels of HbA1c in both MD and SD rabbits. This might have been due to improved glycemic control produced by the extract.

### Table 2—Effect of aqueous extract of *C. auriculata* leaves (400 mg/kg) on insulin, HbA1c and body weight after 21 days of treatment in mild and severe diabetic rabbits

[Values are mean ± SEM of 5 animals in each group.]

<table>
<thead>
<tr>
<th>Group</th>
<th>Insulin (µU/ml)</th>
<th>HbA1c (%)</th>
<th>Body weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
</tr>
<tr>
<td>Normal control</td>
<td>14.32 ± 0.69</td>
<td>3.13 ± 0.13</td>
<td>1.92 ± 0.03</td>
</tr>
<tr>
<td>Mild diabetic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.09 ± 0.48</td>
<td>6.33 ± 0.19</td>
<td>1.71 ± 0.04</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>12.51 ± 0.83*</td>
<td>3.99 ± 0.14*</td>
<td>1.73 ± 0.06</td>
</tr>
<tr>
<td><em>C. auriculata</em></td>
<td>12.94 ± 0.59*</td>
<td>3.74 ± 0.17*</td>
<td>1.76 ± 0.03</td>
</tr>
<tr>
<td>Severe diabetic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.59 ± 0.29+</td>
<td>8.10 ± 0.20+</td>
<td>1.63 ± 0.06</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>8.76 ± 0.68*</td>
<td>5.82 ± 0.23*</td>
<td>1.68 ± 0.09</td>
</tr>
<tr>
<td><em>C. auriculata</em></td>
<td>10.28 ± 0.61*</td>
<td>5.26 ± 0.21*</td>
<td>1.63 ± 0.05</td>
</tr>
</tbody>
</table>

+*P* <0.001 compared to normal control; *P* <0.001 compared to diabetic control.

HbA1c = glycosylated haemoglobin.

**Fig. 1—Effect of aqueous extract of *C. auriculata* leaves (400 mg/kg) on serum lipid profile in mild and severe diabetic rabbits after 21 days of treatment.** [Values are mean ± SEM of 5 rabbits in each group. +*P* <0.01, ++*P* <0.001 compared to normal control; *P* <0.01, **P* <0.001 compared to diabetic control.]
The loss of body weight is one of the threats associated with diabetes mellitus. It was further observed in our study as alloxan-induced diabetic animals showed loss of body weight. Treatment with the extract showed sign of recovery in body weight in MD as well as SD rabbits.

The most common lipid and lipoprotein abnormalities in diabetes are hypertriglyceridemia, hypercholesterolemia and low level of HDL-C, which was also revealed in the present study. Repeated administration of aqueous extract of *C. auriculata* for 3 weeks led to significant reduction in TG, TC and LDL-C with a concomitant elevation in HDL-C in both MD and SD rabbits. Improvement in serum lipid profile following treatment with the extract suggested its role as a lipid-lowering agent.

Abnormal high concentration of serum lipids in diabetes is mainly due to increased mobilization of free fatty acids from peripheral depots which is in turn due to the activation of hormone sensitive lipase during insulin insufficiency. Dysfunction of lipoprotein lipase in insulin deficient state also contributes to hypertriglyceridemia due to impaired catabolism of triglyceride-rich particles. On the other hand, insulin increases receptor-mediated removal of LDL-C and hence decreased activity of insulin during diabetes leads to increased level of serum LDL-C and consequently hypercholesterolemia. Reduction in the production of HDL-C is due to impaired metabolism of TG-rich lipoproteins which provide a significant portion of HDL-C when metabolized by the enzyme lipoprotein lipase. As insulin deficiency in diabetes is responsible for derangement of lipid and lipoprotein metabolism, the significant control in the levels of serum lipids might have been due to improvement in insulin level upon administration of the extract.

Diabetic dyslipidemia has long been shown to have a strong relation with coronary heart disease (CHD). CHD is the most dangerous and life threatening complication of diabetes and the risk for CHD in diabetes increases two or more fold. Increased TG and TC levels and decreased HDL-C represent a displayed lipid profile known as atherogenic profile which leads to the development of CHD. As favourable effect on lipid profile was observed following treatment with extract, this indicated that extract might help to prevent the progression of cardiovascular diseases. In addition, several atherogenic indices such as TG/HDL-C, TC/HDL-C and LDL-C/HDL-C have been used to predict CHD risk. Among these indices, TG/HDL-C is a marker of small, dense LDL-C which is an atherogenic lipoprotein. Reduction of these indices in extract-fed MD and SD animals strongly supported the notion that dietary supplementation with aqueous extract of *C. auriculata* leaves may lead to reduction in the risk of developing heart diseases.

Phytochemical screening of *C. auriculata* aqueous leaf extract revealed the presence of biologically active ingredients such as alkaloids, flavonoids, saponins, tannins cardiac glycosides and phenols. These chemical compounds were speculated to account for the observed pharmacological effects of the extract.

In conclusion, the aqueous leaf extract of *C. auriculata* improved glycemic control in alloxan-induced MD as well as SD rabbits. As *C. auriculata* extract produced favourable lipid profile, this showed its therapeutic potential in preventing the progression of cardiovascular pathogenesis in diabetes. Further
studies are in progress to isolate active principle(s) and elucidate its exact mechanism of action, useful for developing potent herbal antidiabetic drug(s).

Acknowledgement
The authors thank the Department of Science and Technology, New Delhi (India) for financial assistance.

References
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