Antihyperglycemic Activity of Hydroalcoholic Extract of *Cassia auriculata* Linn. (Ceasalpiniaceae) Aerial Parts in Streptozotocin Induced Diabetic Rats

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**ABSTRACT**

**Background:** *Cassia auriculata* Linn. (Ceasalpiniaceae) is a potential folklore medicinal plant traditionally used for the treatment of diabetes. The objective of the present study was to evaluate the antihyperglycemic activity of hydroalcoholic extract of aerial parts of *Cassia auriculata* L. (HACA) in streptozotocin induced diabetes in rats, to focus on its possible mode of action and to identify the possible phytoconstituents responsible for the proposed activity.

**Materials and Methods:** Experimental diabetes was induced in Wistar rats by single intraperitoneal injection of streptozotocin (65 mg kg⁻¹). Diabetic rats were divided in six groups (n = 6) and treated with variable doses of HACA (100, 200 and 400 mg kg⁻¹) for 4 weeks. At the end of study, blood glucose, plasma insulin, glycosylated hemoglobin and lipid profile were determined. In addition, glycogen content of liver, skeletal muscle and in vitro intestinal glucose absorption were also evaluated. The activities of liver and kidney functional markers were measured. HACA was also subjected to *in vitro* α-amylase, α-glucosidase inhibition assay along with qualitative and quantitative phytochemical analysis.

**Results:** Daily oral administration of HACA for 28 days to diabetic rats produced significant decrease in fasting blood glucose, glycosylated hemoglobin, intestinal glucose absorption along with the corrections of diabetic dyslipidemia compared to untreated diabetic rats. Further, significant improvement was observed in glycogen content of liver and skeletal muscle in HACA treated diabetic rats. There was significant decrease in the activities of liver and renal functional markers in diabetic treated rats compared to untreated diabetic rats indicating the protective role of HACA against liver and kidney damage and its non-toxic property. HACA showed prominent inhibitory effect against α-amylase and α-glucosidase enzyme in the *in vitro* tests. Phytochemical analysis of HACA revealed the presence of gallic acid and quercetin in HACA.

**Conclusion:** The results of our study demonstrate antihyperglycemic potential of aerial parts of *Cassia auriculata* L. justifying its use in the indigenous system of medicine. Hence this plant may be considered as one of the potential sources for the isolation of new oral antihypoglycemic agent(s).

**Key words:** *Cassia auriculata*, streptozotocin, antihyperglycemic, quercetin, gallic acid


**INTRODUCTION**

Diabetes is defined as a state in which the homeostasis of carbohydrate and lipid metabolism is improperly regulated by the pancreatic hormone, insulin, ultimately resulting in increased blood glucose level. It is the world’s largest endemic disorder and is one of the major killers in recent times (Bhat et al., 2011). The disease is a degenerative ailment, affecting at least 15 million people and eventually results into complications which include hypertension, atherosclerosis and microcirculatory disorders such as neuropathy, nephropathy and retinopathy (Ibeh and Ezeaja, 2011). It is associated with reduced quality of life and increased risk factors for mortality and morbidity (Altan, 2003).

Currently available therapy for diabetes includes insulin and various oral hypoglycemic agents such as sulfonylureas, metformin, glucosidase inhibitors, troglitazones and GLP-1 agonists. These drugs are used as monotherapy or in combination to achieve better glycemic control. These synthetic hypoglycemic agents and insulin can produce serious side effects and they are not suitable for use during pregnancy (Bandawane et al., 2011). Therefore, the search for safer, specific and effective hypoglycemic agents has continued to be an important area of investigation (Klein et al., 2007). World Health Organization (WHO) has recommended the
evaluation of traditional plant treatments for diabetes as they are effective, non-toxic and are considered to be excellent candidates for oral therapy (Shokeen et al., 2008). The ethnobotanical information reports that about 800 plants may possess antidiabetic potential (Patil et al., 2011). Many indigenous Indian medicinal plants have been found to be useful to manage diabetes successfully which are easily available and have very low side effects (Naskar et al., 2011).

*Cassia auriculata* Linn. (Family: Caesalpiniaeeae) is a tall, branched, bushy shrub growing wild throughout forest along roadside and in waterlands (Wadekar et al., 2011). Traditionally this plant has been used in ayurvedic medicine as ‘Avarai Panchanga Chooran’ and as constituents of ‘kalpa herbal tea’ Indian herbal formulation used in treatment of diabetes to control blood sugar level (Pari and Latha, 2002).

Different components of aerial parts of *Cassia auriculata* L. especially leaves (Gupta et al., 2009), flowers (Vijayaraj et al., 2013; Hakkim et al., 2007) and stem (Uma Devi et al., 2006) have been reported to possess antihyperglycemic activity however, till date no study has been carried out to investigate the mechanism of action and phytoconstituent responsible for antihyperglycemic activity of *Cassia auriculata* aerial parts. Therefore, the present work was undertaken to find out antihyperglycemic activity of hydroalcoholic extract of aerial parts of *Cassia auriculata* L. (HACA) in streptozotocin (STZ) induced diabetic rats with the aim to focus on possible mode of action and to identify possible phytoconstituents responsible for proposed activity.

**MATERIALS AND METHODS**

**Collection and authentication of plant material:** The aerial parts of *Cassia auriculata* Linn. were collected from the Vite region of Maharashtra state, India in the month of August 2012. The plant was identified and authenticated by Botanical Survey of India, Pune and a voucher specimen (V. No. CAAAAM 5) was deposited in the herbarium for future reference. The aerial parts were dried in shade and subjected to size reduction to a coarse powder by using dry grinder.

**Preparation of hydroalcoholic extract:** Air dried aerial parts of *Cassia auriculata* were ground to coarse powder. The 100 g of the coarse powder was extracted in methanol: water (70:30) for 72 h at room temperature with intermittent shaking. The extracts were filtered using absorbent cotton wool and filter paper (Whatman No. A-1). Filtrates were collected and evaporated on rota evaporator at 40°C to obtain a yield of 21% w/w. The obtained hydroalcoholic extract of aerial parts of *Cassia auriculata* (HACA) was preserved in refrigerator till further use.

**Experimental animals:** Wistar rats of either sex (180-220 g) were procured from National Institute of Bioscience, Chaturshruni, Pune. Animals were housed in standard polypropylene cages (32.5×21×14 cm) lined with raw husk (renewed after 48 h). The animal house was maintained on 12 h light/dark cycle at approximately 22±2°C, relative humidity 60-70% and the animals were provided with standard laboratory diet (Nutrivet Life Sciences, Pune) and water *ad libitum*. The animals were randomly assigned to different groups and a minimum period of 7 days was allowed for adaptation. The animals described as fasting were deprived of food for 24 h before experimentation but allowed free access to water throughout. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Modern College of Pharmacy in accordance with the regulations of CPCSEA (884/ac/05/CPCSEA).

**Chemicals:** Streptozotocin (STZ) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade.

**Preliminary phytochemical study:** Hydroalcoholic extract of aerial parts of *Cassia auriculata* (HACA) was screened for the presence of various phytoconstituents like alkaloids, glycosides, flavonoids, tannins, carbohydrates, amino acids and proteins (Khandelwal, 2006).

**Determination of total phenolic content:** Total phenolic content was determined using KMnO₄ colorimetric assay. All the determinations were carried out in triplicate and mean values were calculated and expressed as mg quercetin equivalents (Khandelwal, 2006).

**Determination of total tannin content:** Total tannin content was determined by hide powder test according to the WHO procedure. Weight difference between tanned and untanned hide powder was used for quantitative determination of tannins (WHO, 1998).

**Determination of total flavonoid content:** Total flavonoid content was measured by means of aluminium chloride assay (Elbenny et al., 2011) with slight modification. An aliquot (1 mL) of HACA (1 mg mL⁻¹) or standard solutions of quercetin (10-50 µg mL⁻¹) was added to a 10 mL volumetric flask containing 4 mL of 50% solution of methanol. To the flask, 0.3 mL of 5% NaNO₂ was added. After 5 min, 0.3 mL of 10% AlCl₃ was added. At the sixth min, 2 mL of NaOH (1 M) solution was added and then the total volume was made up to 10 mL with distilled water. The solution was well mixed and absorbance was measured against reagent blank at
510 nm. The total flavonoid content was determined from the calibration curve and expressed as mg quercetin equivalents. All the determinations were carried out in triplicate and the mean values were calculated.

**High performance thin layer chromatography (HPTLC) analysis of HACA:** HPTLC chromatography was performed on 20×10 cm aluminum Lichrosphere HPTLC plates precoated with 200 μm layers of silica gel 60F254 (E. Merck, Germany). HACA (10 μL) and standard quercetin (600 ng band⁻¹) and gallic acid (800 ng band⁻¹) were applied as bands 6 mm wide and 10 mm apart by means of Camag Linomat V sample applicator (Muttenz, Switzerland) equipped with a 100-μL syringe. The constant application rate was 160 nL sec⁻¹. Linear ascending development with toluene: Ethyl acetate: Formic acid (5:4:1, v/v/v) as mobile phase was performed in a twin-trough glass chamber 20×10 cm (Camag) previously saturated with mobile phase for 15 min at room temperature (25±2°C) and relative humidity 60±5%. The development distance was 8 cm (development time 10 min) and amount of mobile phase used was 20 mL. HPTLC analysis was performed at 270 nm in reflectance mode with a Camag TLC scanner III operated by WinCATS software (Version 1.2.0). The slit dimensions were 5×0.45 mm and the scanning speed was 20 mm sec⁻¹ (Hussain et al., 2012).

**In vitro α-amylase inhibitory activity:** α-amylase inhibitory assay was carried out according to the method described by Ranilla et al. (2010) with slight modifications. HACA was dissolved in dimethylsulfoxide (DMSO) in order to obtain concentrations of 10, 20, 40, 60, 80 and 100 μg mL⁻¹. A total of 250 μL of sample and 125 μL of 0.02 M sodium phosphate buffer (pH 6.9 with 6 mM NaCl) containing 1 mL of α-amylase solution (0.5 mg mL⁻¹) was incubated at 25°C for 10 min. After preincubation, 250 μL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 6 mM NaCl) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 0.5 mL of dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted using 5 mL of distilled water and absorbance was measured at 540 nm using spectrophotometer (Jasco V600, Germany). Acarbose was used as the positive control. The reference sample included all other reagents and the enzyme with the exception of the test sample.

\[
\text{Inhibition (\%) = } \frac{\text{Abs}_{\text{ref}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{ref}}} \times 100
\]

**In vitro α-glucosidase inhibitory activity:** α-glucosidase inhibitory activity was assessed according to the method described by Oboh et al. (2012). The α-glucosidase inhibitory activity was expressed as percentage inhibition.

**Acute toxicity study:** Acute toxicity study was performed according to OECD guidelines No. 423 (OECD, 2001). After dosing, the animals were observed for changes in behavioral, neurological, autonomic profiles and finally recording mortality up to 24 h till 14 days (Mali et al., 2013).

**Experimental induction of diabetes:** Rats were fasted overnight before being injected intraperitoneally with a single dose of freshly prepared solution of streptozotocin (STZ, 65 mg kg⁻¹) in ice cold citrophosphate buffer (pH 4.3). STZ was first weighed individually for each animal according to their weight and administered within 5 min to prevent its degradation. Since STZ is capable of producing fatal hypoglycemia as a result of massive insulin release, rats were treated with 5% glucose solution for 24 h. Normal control rats received an equivalent volume of citrophosphate buffer. After 3 days of STZ administration, fasting blood glucose levels of each rat were determined. Rats showing fasting blood glucose more than 200 mg dL⁻¹ were considered diabetic and used for the study (Juvekar and Bandawane, 2009). Treatment commenced on 7th day of STZ administration (Yankuzo et al., 2011) was considered as the 1st day of study.

**Experimental groups:** A total of 30 rats (6 normal; 24 diabetic) were used to accommodate the designated study. They were divided into five different groups consisting of six animals in each group as follows:

**Group 1:** Normal control rats administered cold citrophosphate buffer (pH 4.3)
**Group 2:** Diabetic control rats i.e., rats treated with STZ (65 mg kg⁻¹ i.p.)
**Group 3:** Diabetic rats treated with 100 mg kg⁻¹ of HACA
**Group 4:** Diabetic rats treated with 200 mg kg⁻¹ of HACA
**Group 5:** Diabetic rats treated with 400 mg kg⁻¹ of HACA
**Group 6:** Diabetic rats treated with 5 mg kg⁻¹ glimepiride (standard oral hypoglycemic drug)
**Group 7:** Normoglycemic rats administered 400 mg kg⁻¹ of HACA
Fasting blood glucose: Fasting blood glucose was determined on 28th day of study period using glucometer (Accu check, Germany).

Oral glucose tolerance test (OGTT): Oral glucose tolerance test was performed in overnight fasted (18 h) diabetic rats at the end of 28th day of study. Glucose (3 g kg⁻¹) was fed 30 min after the administration of extract or standard drug. Blood glucose was determined at 0, 30, 60, 90 and 120 min of glucose administration (Bandawane et al., 2013).

Body weight, food and fluid intake: During 28 days study period body weight, food and fluid intake of animals were recorded.

Biochemical parameters: Glycosylated hemoglobin (HbAlc%) was determined in EDTA-blood samples obtained at the end of the 28th day study using commercial kit (Crest Biosystems, Goa, India). Blood samples were centrifuged at 7000 rpm for 15 min at 4°C to separate the serum. Insulin concentrations were determined through a radio immunoassay procedure, using insulin kit (Coral, India) according to manufacturer's instructions. Serum total protein was measured using commercial kit (Autozyme, India).

Glycogen content of liver and skeletal muscle: Glycogen content of liver and skeletal muscle was estimated according to method described by Das and Barman (2012).

Intestinal glucose absorption by estimation of glucose uptake: The effect of HACA on intestinal glucose absorption was studied by method described by Das et al. (2001) with slight modification. At the end of 28th day an intestinal loop of 8 cm from the pyloric end was made in rats of all groups. D-glucose 2.5 mg mL⁻¹ was given in the loop by tuberculin syringe. The animals were sacrificed after 15 min and the intact loop was excised and weighed before and after draining the contents of the loop. After constant dilution, the drained fluid was estimated for glucose content using glucose oxidase method. The absorption was expressed in terms of mg/g dry weight/h. Dry weight of the intestinal segment was measured after dehydrating the loop in ethyl alcohol for 24 h and then drying in hot air oven at 110-120°C for 2 h.

Lipid profile: Serum triglycerides (TG), Total Cholesterol (TC) and High Density Lipoprotein (HDL) levels were estimated using standard kits (Autozyme Diagnostics, India). Very Low Density Lipoproteins (VLDL) and Low Density Lipoproteins (LDL) levels were calculated using Friedewald formula (Friedewald et al., 1972):

\[
\text{VLDL} = \frac{TG}{5} \\
\text{LDL} = \text{T-CH} - (\text{HDL-CH} + \text{VLDL-CH})
\]

In vivo antioxidant activity
Estimation of reduced glutathione (GSH): GSH activity was studied by method described by Kaur et al. (2006). The optical density of yellow color was read immediately at 412. The unknown GSH concentration was obtained by extrapolation from the standard curve.

Estimation of malondialdehyde (MDA) lipid peroxidation: MDA activity was studied by method described by Kumar et al. (2011). Briefly, the homogenate was incubated with 15% trichloroacetic acid (TCA), 0.38% thiobarbituric acid (TBA) and 5 N hydrochloric acid (HCl) at 95°C for 15 min. The mixture was cooled, centrifuged and the absorbance of the supernatant was measured at 532 nm against appropriate blank.

Estimation of liver catalase (CAT): Catalase activity was studied by method described by Sahreen et al. (2011). The sample readings were taken by placing 1 mL of phosphate buffer and 5 µL of tissue homogenate in the reference cuvette and test cuvette. Hydrogen peroxide (10 µL) was then added in the test cuvette in the spectrophotometer. Reading was taken at 240 nm, 1 min after placing the cuvettes in the spectrophotometer.

Estimation of superoxide dismutase (SOD): SOD activity was measured according to method of Marklund (1985). Assay mixture consisted of 2.95 mL Tris-HCl buffer, 25 µL of pyrogallol and 0.05 mL of tissue homogenate in total volume of 3 mL. The difference between the optical densities obtained at 1.30 and 3.30 min was determined and expressed as U mg⁻¹ protein.

Liver function parameters: Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) and Alkaline Phosphatase (ALP) were analyzed by using commercial kits (Crest Biosystems, Goa, India).

Histopathology study of pancreas, liver and kidney: At the end of study, animals were sacrificed, pancreas, liver and kidney were isolated for histopathological estimation (Luna, 1960).

Statistical analysis of data: All the data are presented as Mean±SEM of measurements made on six animals in
each group. Statistical analysis was performed with one-way analysis of variance (ANOVA) followed by Dunnet's multiple test for comparison using Graph Pad Instat (version-3) software. A value of p<0.05 was considered to be statistically significant compared with the respective control.

RESULTS

Qualitative phytochemical screening: During preliminary phytochemical investigation HACA showed presence of flavonoids, phenolic compounds, tannins and alkaloids.

Quantitative phytochemical analysis: The total phenolic content of HACA was found to be 235.00 mg quercetin equivalent/g of extract. Total tannin content of HACA was found to be 17% w/w. Total flavonoid content of HACA was found to be 102.02 mg quercetin equivalent/g of extract.

HPTLC analysis of hydroalcoholic extract: The overlay spectra of HACA with gallic acid and HACA with quercetin are shown in Fig. 1a and b, respectively. Gallic acid and quercetin were resolved at Rf 0.93 and 0.91, respectively. The spectra clearly reveal that HACA contains both quercetin and gallic acid.

Acute toxicity study: Acute toxicity study revealed the non-toxic nature of HACA. There was no lethality or any toxic reaction in animals at a single large dose of 5000 mg kg⁻¹. No mortality was recorded within the 14 days of observation.

Oral glucose tolerance test (OGTT) in diabetic rats: The effect of HACA on oral glucose tolerance test (OGTT) is shown in Fig. 2. Blood glucose level of normal and diabetic rats increased significantly (p<0.01) at 30 min after glucose administration. HACA at all dose levels significantly (p<0.01) reduced the increase in blood glucose at 90 min in glucose loaded rats compared with diabetic control rats which show elevation in blood glucose throughout the total measurement period (120 min).

Biochemical parameters: Treatment with HACA for 4 weeks exhibited a significant (p<0.01) decrease in fasting blood glucose in STZ diabetic rats as compared to diabetic control. There was significant (p<0.01) decrease in fasting blood glucose level in HACA (400 mg kg⁻¹) as compared to diabetic control group as shown in Table 1. The standard oral hypoglycemic drug glibenclamide showed 69.41% reduction in blood glucose level as compared to diabetic control group.

As evident from result in Table 1 treatment with HACA and glibenclamide for 28 days significantly (p<0.01) decreased glycosylated haemoglobin level.

A significant (p<0.01) increase was noted in the glycogen content of liver and skeletal muscle and intestinal glucose absorption as compared to diabetic control which showed a significant reduction in (p<0.01) glycogen content and (p<0.05) intestinal glucose absorption as compared to normal control which is indicated in Table 1.

Significant decrease (p<0.01) in serum total protein was observed in HACA (400 mg kg⁻¹) treated groups as compared to diabetic control at the end of study period.

However, there was no significant (p>0.05) increase in plasma insulin level of treated rats as compared to diabetic control rats as shown in Table 1.

Body weight, food intake and water intake: Table 2 shows effect of 28 days treatment of HACA on body weight, food intake and water intake. Body weight of streptozotocin induced diabetic control rats was found to be significantly (p<0.05) less compared to normal control rats. After 4 weeks of treatment with HACA body weight significantly (p<0.05) increased compared to diabetic control groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental groups</th>
</tr>
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<tbody>
<tr>
<td>Blood glucose level (mg dl⁻¹)</td>
<td>NC: Normal control</td>
</tr>
<tr>
<td></td>
<td>DC: Diabetic control</td>
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<tr>
<td>HbA1c (%)</td>
<td>DC + HACA (100 mg kg⁻¹)</td>
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<tr>
<td></td>
<td>DC + HACA (200 mg kg⁻¹)</td>
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<td></td>
<td>DC + HACA (400 mg kg⁻¹)</td>
</tr>
<tr>
<td></td>
<td>DC + GL (5 mg kg⁻¹)</td>
</tr>
<tr>
<td></td>
<td>NC + HACA (400 mg kg⁻¹)</td>
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<tr>
<td>Liver glycogen (mg 100 g⁻¹)</td>
<td>167.20 ± 16.23</td>
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<td>183.56 ± 59.82</td>
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<td></td>
<td>242.60 ± 57.72*</td>
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<td></td>
<td>194.74 ± 14.22</td>
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<td></td>
<td>169.95 ± 16.72</td>
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<tr>
<td>Skeletal muscle glycogen (mg 100 g⁻¹)</td>
<td>23.85 ± 1.42</td>
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<td>39.58 ± 1.35**</td>
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<td>17.44 ± 0.97**</td>
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<td></td>
<td>23.14 ± 0.54**</td>
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<td></td>
<td>24.32 ± 1.15**</td>
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<tr>
<td></td>
<td>26.39 ± 1.459</td>
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<tr>
<td>Int. Glucose absorption (mg g⁻¹ dry wt⁻¹)</td>
<td>144.24 ± 14.22</td>
</tr>
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<td></td>
<td>169.95 ± 16.72</td>
</tr>
<tr>
<td>Serum total protein (g dl⁻¹)</td>
<td>7.61 ± 0.69</td>
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<td>3.29 ± 0.67**</td>
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<td></td>
<td>4.90 ± 0.25</td>
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<td>6.77 ± 0.47**</td>
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<td></td>
<td>7.70 ± 0.45</td>
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<tr>
<td>Serum insulin (mg dl⁻¹)</td>
<td>14.55 ± 1.26**</td>
</tr>
<tr>
<td></td>
<td>2.13 ± 0.05**</td>
</tr>
<tr>
<td></td>
<td>3.56 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>7.70 ± 0.30**</td>
</tr>
</tbody>
</table>

NC: Normal control, DC: Diabetic control, HACA: Hydroalcoholic extract of Cuscuta areniculata aerial parts, GL: Glibenclamide, STZ: Streptozotocin, n = 6, values are Mean ± SEM, *p<0.05, **p<0.01 as compared to normal control group, *p<0.05, **p<0.01 as compared to Diabetic control data analysed by one way ANOVA followed by Dunnet’s Multiple Range Test for comparison.
Food intake was significantly high in diabetic control rats as compared to normal control. At the end of 28 days of treatment food intake of treated groups significantly (p<0.01) decreased as compared to diabetic control.

Significant decrease (p<0.01) in water intake was observed in treated groups as compared to diabetic control at the end of study period.

**Lipid profile:** The protective effect of HACA on lipid profile has been shown in Fig. 3. There was a significant
Table 2: Effect of HACA on body weight, food and water intake in STZ diabetic rats

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Body weight (g)</th>
<th>Food intake (g 24 h⁻¹)</th>
<th>Water intake (ml 24h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
</tr>
<tr>
<td>Normal control</td>
<td>189.16 ± 8.78</td>
<td>197.00 ± 8.45</td>
<td>14.50 ± 0.77</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>183.16 ± 7.11</td>
<td>139.16 ± 4.527</td>
<td>21.00 ± 1.75</td>
</tr>
<tr>
<td>DC + HACA (100 mg kg⁻¹)</td>
<td>175.00 ± 8.169</td>
<td>178.33 ± 6.878</td>
<td>25.91 ± 0.436</td>
</tr>
<tr>
<td>DC + HACA (200 mg kg⁻¹)</td>
<td>164.33 ± 6.76</td>
<td>183.16 ± 6.466</td>
<td>26.33 ± 1.815</td>
</tr>
<tr>
<td>DC + HACA (400 mg kg⁻¹)</td>
<td>195.16 ± 5.05</td>
<td>201.33 ± 5.12</td>
<td>31.66 ± 2.24</td>
</tr>
<tr>
<td>DC + GL (5 mg kg⁻¹)</td>
<td>164.33 ± 5.42</td>
<td>168.16 ± 7.46</td>
<td>18.33 ± 0.90</td>
</tr>
<tr>
<td>NC + HACA (400 mg kg⁻¹)</td>
<td>188.00 ± 7.03</td>
<td>191.50 ± 5.69</td>
<td>14.45 ± 0.74</td>
</tr>
</tbody>
</table>

NC: Normal control; DC: Diabetic control; HACA: Hydroalcoholic extract of Cassia auriculata aerial parts; GL: Glibenclamide; STZ: Streptozotocin. n = 6. Values are Mean ± SEM. *p<0.05, **p<0.01 as compared to normal control group. *p<0.05, **p<0.01 as compared to diabetic control group. Data analysed by one way ANOVA followed by Dunnet’s multiple range Test for comparison.

Table 3: Effect of HACA on liver superoxide dismutase (SOD), catalase, glutathione (GSH) and malondialdehyde (MDA) of STZ diabetic rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DC</th>
<th>DC + HACA (100 mg kg⁻¹)</th>
<th>DC + HACA (200 mg kg⁻¹)</th>
<th>DC + HACA (400 mg kg⁻¹)</th>
<th>DC + GL (5 mg kg⁻¹)</th>
<th>NC + HACA (400 mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver SOD</td>
<td>15.170 ± 0.727**</td>
<td>14.336 ± 0.316**</td>
<td>10.590 ± 0.641***</td>
<td>11.596 ± 0.320**</td>
<td>12.113 ± 0.483**</td>
<td>13.498 ± 0.420**</td>
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<td>(U mg⁻¹ protein)</td>
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<tr>
<td>Liver catalase</td>
<td>75.91 ± 0.676**</td>
<td>43.680 ± 1.417**</td>
<td>51.160 ± 0.745**</td>
<td>55.560 ± 1.151**</td>
<td>66.091 ± 0.543**</td>
<td>71.023 ± 0.782**</td>
</tr>
<tr>
<td>(U mg⁻¹ protein)</td>
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<tr>
<td>Liver GSH</td>
<td>48.786 ± 2.469**</td>
<td>13.630 ± 1.072**</td>
<td>24.091 ± 0.869**</td>
<td>31.330 ± 0.650**</td>
<td>36.650 ± 1.158**</td>
<td>38.580 ± 1.545**</td>
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<td>(nmol mg⁻¹ protein)</td>
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<tr>
<td>Liver MDA</td>
<td>199.230 ± 0.944**</td>
<td>448.910 ± 12.12**</td>
<td>339.031 ± 7.600**</td>
<td>227.043 ± 3.856**</td>
<td>214.270 ± 3.745**</td>
<td>225.420 ± 3.978**</td>
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<tr>
<td>(nmol mg⁻¹ protein)</td>
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NC: Normal control; DC: Diabetic control; HACA: Hydroalcoholic extract of Cassia auriculata aerial parts; GL: Glibenclamide; SOD: Superoxide dismutase, GSH: Glutathione, MDA: Malondialdehyde, STZ: Streptozotocin, n = 6. Values are Mean ± SEM. *p<0.05, **p<0.01 as compared to normal control group. *p<0.05, **p<0.01 as compared to diabetic control group. Data analysed by one way ANOVA followed by Dunnet’s multiple range Test for comparison.

Fig. 2: Effect of HACA on oral glucose tolerance test (OGTT) in STZ diabetic rats. NC: Normal control; DC: Diabetic control; HACA: Hydroalcoholic extract of Cassia auriculata aerial parts; GL: Glibenclamide; STZ: Streptozotocin; n=6. Values are Mean ± SEM. *p<0.05, **p<0.01 as compared to normal control group. *p<0.05, **p<0.01 as compared to diabetic control group. Data analysed by one way ANOVA followed by Dunnet’s Multiple range Test for comparison.

(p<0.01) decrease in T-CH, TG, LDL-CH, VLDL-CH and significant (p<0.01) elevation in serum HDL-CH in diabetic rats when compared to normal rats. HACA (400 mg kg⁻¹) treated diabetic rats decreased T-CH by 26.02%, TG by 61.52%, LDL-CH by 90.61% and VLDL-CH by 61.56%. Whereas, HACA (400 mg kg⁻¹) treated group showed a significant (p<0.01) increase in HDL-CH as compared to diabetic control group. However, lipid profile remains unchanged in HACA treated normoglycemic rats.

Enzymatic and non-enzymatic biomarkers of liver oxidative stress: As shown in Table 3, diabetes resulted in significant decrease in antioxidant enzymes like GSH,
catalase and SOD. Moreover, the levels of MDA were significantly increased. HACA exhibited improvements in antioxidant enzymatic activity compared to diabetic control group and nearly normalized the levels of SOD, MDA, catalase and GSH.

**Effect of HACA on *in vitro* α-amylase and α-glucosidase inhibition assay:** The effect of HACA on α-amylase and α-glucosidase is depicted in Fig. 4a and b, respectively. The highest inhibitory effects of HACA and acarbose were 61.45 and 60.07, respectively at 100 μg mL⁻¹ which was the highest concentration tested. The α-amylase inhibition property of HACA increased with increase in concentration which was more than the standard acarbose. The EC₅₀ showed that HACA (75.41 μg mL⁻¹) higher inhibitory property than acarbose (72.46 μg mL⁻¹).

The highest inhibitory effects of HACA and acarbose on α-glucosidase were 50.19 and 52.53, respectively at 100 μg mL⁻¹ which was the highest concentration tested. The α-glucosidase inhibition property of HACA increased with increase in concentration which was more than the standard acarbose. The EC₅₀ showed that HACA (95.05 μg mL⁻¹) has inhibitory property which is comparable to standard acarbose (85.05 μg mL⁻¹).

**Liver function parameters:** The effect of HACA on the hepatic function markers such as SGOT, SGPT and ALP in different experimental groups of rats is shown in Fig. 5. In comparison with normal control group, the level of SGOT, SGPT and ALP were increased significantly in diabetic untreated groups and after treatment with HACA for 28 days the level of SGOT, SGPT and ALP were significantly decreased to normal value as compared to diabetic rats.

**Histopathology study of pancreas:** The histopathological changes of pancreas of diabetic rat are shown in Fig. 6 which indicates severe necrotic changes of pancreatic islets, nuclear changes with dilatation and congestion of blood vessels. Treatment with HACA did not show restoration of architecture of pancreas that was earlier affected with STZ.

**Histopathology study of liver:** Histopathological sections of liver of diabetic rats showed hepatocellular injury pronounced in loss of the normal architecture of the liver, there was severe fibrosis and leucocytic infiltration around the portal veins which appeared congested with blood with dilatation in blood vessels. The treatment with HACA showed improvement in
Fig. 4(a-b): Effect of HACA on in vitro, (a) α-amylase and (b) α-glucosidase inhibition assay. HACA: Hydroalcoholic extract of Cassia auriculata aerial parts.

Fig. 5: Effect of HACA on liver function parameters in STZ diabetic rats. NC: Normal control, DC: Diabetic control, HACA: Hydroalcoholic extract of Cassia auriculata aerial parts, GL: Glibenclamide, SGOT: Serum Glutamic Oxaloacetate Transaminase, SGPT: Serum Glutamic Pyruvate Transaminase, ALP: Alkaline Phosphatase, STZ: Streptozotocin, n=6. Values are Mean±SEM, *p<0.05, **p<0.01 as compared to normal control group, *p<0.05, **p<0.01 as compared to Diabetic control group. Data analysed by one way ANOVA followed by Dunnett’s Multiple range Test for comparison.

Histopathology study of kidney: The histological changes in the renal specimen of normal and diabetic animals are shown in Fig. 8. Diabetic glomeruli showed some areas of mesangial matrix expansion, mesangial...
Fig. 6(a-g): Histopathology of pancreas sections of STZ diabetic rats treated with HACA. Pancreas sections were stained with haematoxylin–eosin and observed under 40X magnification of digital microscope. NC: Normal control, DC: Diabetic control, HACA: Hydroalcoholic extract of Cassia auriculata aerial parts, GL: Glibenclamide, STZ: Streptozotocin (a) Normal control with typical histological structure of rat pancreas, (b) Diabetic control group showing necrosis of pancreatic islet cells (*), dilation of blood vessel (~). (c) DC+HACA (100 mg kg⁻¹), (d) DC+HACA (200 mg kg⁻¹), (e) D+HACA (400 mg kg⁻¹), (f) DC+GL (5 mg kg⁻¹) and (g) NC+HACA (400 mg kg⁻¹). All HACA treated group did not show significant improvement in pancreas histopathology as compared to DC.

cell proliferation and thickening of glomerular basement membrane. Treatment with HACA lead to regeneration of tissues that were earlier affected with STZ.

DISCUSSION
The currently available drug regimens for management of diabetes mellitus have certain drawbacks (Grover et al., 2000) therefore need for more rigorous
Fig. 7(a-g): Histopathology of liver sections of STZ diabetic rats treated with HACA. Liver sections were stained with haematoxylin-eosin and observed under 40X magnification of digital microscope. NC: Normal control, DC: Diabetic control, HACA: Hydroalcoholic extract of Cassia auriculata aerial parts, GL: Glibenclamide, STZ: Streptozotocin, (a) Normal control with typical histological structure of rat liver, (b) Diabetic control group showing loss of normal architecture with distended central vein (→) (c) DC+HACA (100 mg kg⁻¹), (d) DC+HACA (200 mg kg⁻¹), (e) DC+HACA (400 mg kg⁻¹), (f) DC+GL (5 mg kg⁻¹) and (g) NC+HACA (400 mg kg⁻¹). All HACA treated group showed significant improvement in liver histopathology as compared to DC.

Clinical and scientific research is strongly advocated for larger acceptances and visibility of herbal and traditional medicine. Traditional herbal medicines have a long history of use and are generally considered to be safer than synthetic drugs (Puranik et al., 2011). In the present study, anti-hyperglycemic activity of hydroalcoholic extract of aerial part of Cassia auriculata L. (HACA) was evaluated by using STZ induced diabetes in rats as the animal model.

In the present study, result of acute toxicity test revealed high margin of safety and absence of mortality at a single dose up to 5000 mg kg⁻¹ of body weight. Hence,
Fig. 8(a-g): Histopathology of kidney sections of STZ diabetic rats treated with HACA. Kidney sections were stained with haematoxylin-eosin and observed under 40X magnification of digital microscope. NC: Normal control, DC: Diabetic control, HACA: Hydroalcoholic extract of Cassia auriculata aerial parts, GL: Glibenclamide, STZ: Streptozotocin, (a) Normal control with typical histological structure of rat kidney, (b) Diabetic control group showing glomerular basement membrane thickening (→), tubular dilation (↑) and cell infiltration (↑), (c) DC+HACA (100 mg kg⁻¹), (d) DC+HACA (200 mg kg⁻¹), (e) DC+HACA (400 mg kg⁻¹), (f) DC+GL (5 mg kg⁻¹) and (g) NC+HACA (400 mg kg⁻¹). All HACA treated group showed significant improvement in kidney histopathology as compared to DC.

the minimum lethal dose (LD₅₀) could not be calculated and the choice of graded dose levels for the extract was as a result of high margin of safety which is also in agreement with previous reported studies involving different plant extracts (Tanko et al., 2008; Ghosal and Mandal, 2013).

Oral Glucose Tolerance Test (OGTT) is simple and widely accepted method for indirect assessment of in vivo peripheral insulin action and insulin resistance (Nain et al., 2012). Results of OGTT in the present study indicated that HACA showed decreased blood glucose level in STZ diabetic rats which may involve insulin like
action that increases utilization of glucose by peripheral tissues or due to increased glycosogenesis.

Pancreas is the primary organ involved in sensing the organism’s dietary and energetic states via glucose concentration in the blood and insulin is secreted in response to elevated blood glucose level (Eden, 2009). In the present study, STZ was used for induction of experimental diabetes as it selectively destroys insulin-producing β-cells of the pancreas by inducing high levels of DNA strand breaks in these cells (Adisakwattana et al., 2005). The present study showed that oral administration of HACA decreased the blood glucose level in STZ diabetic rats. However, blood glucose level was not altered in HACA treated normoglycemic rats which further strengthen its antidiabetogenic potential.

Blood glucose lowering potential of HACA might be due to activation of β-cells giving insulingenic effect through the stimulation of regeneration process and revitalization of the remaining beta cells or by absorption of glucose into the muscle and adipose tissues. To find out the possible mechanism by which HACA show antihyperglycemic effect, various biochemical parameters were studied amongst which serum insulin is an important one. It is evident from the results that there was non-significant increase in insulin even after treatment with HACA which demonstrates that the effectiveness of HACA to treat diabetic condition might rely on actions other than pancreatic β-cells insulin release. This result was in confirmation with the histopathological findings of pancreas which showed no change in β-cells of STZ damaged pancreatic architecture even after HACA treatment. From the above findings its clear that the antihyperglycemic activity of HACA is certainly not because of its insulin secretagogue property and there may be involvement of other possibilities like insulin mimetic action, regulation of post prandial glucose level, increase in sensitivity of target tissues or ability to increase peripheral glucose utilization.

Glycosylated haemoglobin (HbA1c), an excellent marker of overall glycemic control, was found to be increased in the diabetic control group. The increased HbA1c in diabetic patient is due to glycosylation of haemoglobin and the amount of increase is directly proportional to the fasting blood glucose levels (Babu et al., 2007). The increased HbA1c level is also associated with increased vascular complications of diabetes (Jain et al., 2010). In the present study, treatment with HACA showed a significant decrease in level of glycosylated haemoglobin which suggests its potential to control long term diabetic condition.

In diabetes mellitus, insulin deficiency causes an impairment in glycogen synthase activation in the liver and skeletal muscle due to the inability of insulin to phosphorylate insulin receptor substrate-1 (IRS-1), this causes a decreased activation of the enzyme phosphatidylinositol 3-kinase (PI-3K) leading to decreased expression and translocation of GLUT-4 glucose transporters (Das et al., 2001). HACA due to its insulin like action probably increased PI-3K activation, thus leading to stimulation of liver and skeletal muscle glycogen synthase. The increase in glycogen concentration in the skeletal muscle might also be due to increased expression and translocation of GLUT-4 glucose transporters as a result of increased activation of PI-3K, thus leading to increased peripheral glucose uptake. The increase in liver and skeletal muscle glycogen content caused by HACA may thus be due to its insulin mimetic response or due to increase synthesis of liver glycogen synthase enzyme.

Diabetic rats showed marked increase in food and water intake along with reduction in their body weight when compared to normal rats which could be due to poor glycemic control. This decrease in body weight is due to excessive catabolism of protein to provide amino acids for gluconeogenesis during insulin deficiency resulting in muscle wasting in diabetic untreated rats (Juvekar and Bandawane, 2009; Ramachandran et al., 2011). In this study, treatment with HACA in diabetic rats prevented the loss of body weight by increasing utilization of glucose by the body or by decreased gluconeogenesis. To further study the effect of HACA on gluconeogenesis, level of serum total protein was determined. Treatment of HACA significantly decreased serum total protein in STZ diabetic rats which is possibly due to reduced catabolism process involved in gluconeogenesis. The increased food and water intake in diabetic rats were reversed by the 28 days treatment of HACA which could be due to insulin like action or peripheral glucose utilization.

In vivo α-amylase and α-glucosidase inhibition are considered to be an effective strategy for the control of postprandial glucose level. So, to study the effect of HACA on postprandial glucose level α-amylase and α-glucosidase inhibition assay was carried out. α-amylase and α-glucosidase enzymes are key enzymes in the digestive system involved in the digestion of carbohydrates and exert anti-diabetic effects via decreasing the glucose absorption from intestinal lumen (Tarling et al., 2008; Li and Qu, 2012). Thus, potential of HACA to inhibit α-amylase and α-glucosidase contributes to the management of diabetes mellitus.

Above statement is further supported by the effect of HACA on in vivo intestinal glucose absorption in STZ diabetic rats. In the present study, treatment with HACA significantly decreased intestinal glucose absorption which confirms that it exerts antihyperglycemic activity through its control on postprandial glucose level.
Hyperlipidemia is one of the major cardiovascular risk factors. It has been demonstrated that insulin deficiency in diabetes mellitus leads to a variety of derangements in metabolic and regulatory processes, which in turn leads to accumulation of lipids especially TG and T-CH in diabetic patients (Ashok Kumar et al., 2012). Further, in diabetic state, there is inactivation of the lipoprotein lipase by which free fatty acids are converted into phospholipids and cholesterol, which are finally discharged into blood, causing an elevation of serum phospholipid level (Pushparaj et al., 2007). In the present study, markedly increased levels of TG, T-CH, VLDL-CH, LDL-CH and decreased level of HDL-CH in STZ diabetic rats contributes to the pathogenesis of diabetes and its related complications such as atherogenesis and coronary artery disease (Noh et al., 2006; Jia et al., 2010; Rosario and Prabhakar, 2006). Potential of HACA to reduce T-CH, TG, LDL-CH, VLDL-CH and elevate HDL-CH level in STZ diabetic rats can thus prove to be helpful in improving lipid metabolism which will in turn help to prevent diabetic complications. The effect of HACA on lipid parameters clearly reflects that anti-hyperglycemic action of HACA could be due to its consequence of an improved lipid metabolism apart from the direct effect on glucose homeostasis.

Hyperglycemia induced oxidative stress plays a major role in generation of free radicals (Ramachandran et al., 2011b). To focus on the antioxidant potential of HACA, its effect on key antioxidant enzymes of liver viz., SOD, MDA, CAT and GSH was evaluated. Increased lipid peroxidation (MDA) under diabetic conditions can be due to increased oxidative stress in the cell as a result of depletion of antioxidant scavenger systems. In association with the changes in lipid peroxidation, the diabetic liver tissues showed decreased activities of key antioxidants SOD, CAT and GSH, which play an important role in scavenging the toxic intermediate of incomplete oxidation (Murugan and Pari, 2006). The natural cellular antioxidant enzyme SOD plays a pivotal role in oxygen defense metabolism by intercepting and reducing superoxide to water and molecular oxygen. CAT is known to be involved in detoxification of high hydrogen peroxide concentration (Kumar et al., 2012) and GSH protects cellular proteins against oxidation through glutathione redox cycle and also directly detoxifies reactive oxygen species (Yu, 1994) and thus maintains functional as well as structural integrity of cell and organelle membranes. In the present study, increased level of liver MDA and decreased levels of hepatic SOD, CAT and GSH were restored to normal level by 28 days treatment of HACA which can be attributed to strong antioxidant potential of drug. The restoration of liver SOD, CAT, MDA and GSH contents in STZ diabetic rats thus reflects that besides antihyperglycemic activity, HACA also possess antioxidant potential which will assist in preventing diabetes induced oxidative stress and associated complications.

Indigenous plant extracts used for treatment of diabetes mellitus often leads to liver and kidney toxicity as the treatment continues for longer period (Fatima et al., 2010). To evaluate the effect of HACA on major organ system, liver and kidney function parameters in STZ diabetic rats as well as normoglycemic rats was carried out. SGOT, SGPT and ALP are reliable markers of liver function (Kisetti et al., 2010). Elevation of SGOT, SGPT and ALP in STZ diabetic rats is mainly due to leakage of these enzymes from the liver cytosol into the blood stream which gave an indication of the hepatotoxic effect of streptozotocin (Ohaeri, 2001). Treatment of STZ diabetic rats with HACA caused reduction in the activity of these enzymes and consequently alleviated the damage caused by streptozotocin while treatment of normoglycemic rats with HACA maintained the levels of serum SGOT, SGPT and ALP thereby showing its non-toxic nature.

Further, histopathological examination of liver sections of STZ diabetic rats showed marked hepatocellular necrosis, fatty changes and extensive vacuolization of nuclei and disordered liver structure. Treatment with HACA restored the normal architecture of liver tissue in STZ diabetic rats thereby exhibiting protective role in hepatic damage. No alterations were found in the liver histopathology of normoglycemic rats indicating its non toxic nature. Microscopic examination of kidney sections of STZ diabetic rats showed glomerular basement membrane thickening, cell proliferation, mesangial matrix accumulation and tubular dilatation which is due to hyperglycemia induced oxidative stress. Treatment with HACA effectively reduced all these changes and restored the normal structure of kidney tissues in STZ diabetic rats due to its protective action while the kidney tissues of normoglycemic rats treated with HACA showed no detrimental effect which may be due to its non toxic nature. The protective effect of HACA on liver and kidney functioning in diabetic rats is partly due to its antioxidant activity.

To identify the phytoconstituents responsible for the proposed activity, preliminary phytochemical analysis of HACA was carried out. Qualitative phytochemical study of HACA showed presence of flavonoids and tannins which was quantified using total tannins, total flavonoids and total phenolic assays. The results of quantitative analysis indicated that HACA contains higher flavonoid contents than tannin content. HPTLC analysis of HACA confirms presence of quercetin and gallic acid. Both quercetin and gallic acid have been reported to possess
antihyperglycemic and antioxidant activity (Sajeeth et al., 2010). Thus, HACA possess antihyperglycemic activity
due to its major flavonoid quercetin along with tamin
component gallic acid. The antihyperglycemic activity of
HACA is potentiated by its antihyperlipidemic and
antioxidant action.

CONCLUSION

The results of the present study show that HACA
possess antihyperglycemic activity along with
antihyperlipidemic and antioxidant effect which is due
to its active principles such as quercetin (flavonoid) and
gallic acid (tamin). HACA possess antihyperglycemic
activity which is due to its extrapancreatic mechanisms
like decreased glycogenolysis and enhanced glycosynthesis
by the liver and/or enhanced transport of blood glucose
to peripheral tissues and enzyme inhibitory action.
Antihyperlipidemic and antioxidant potential of HACA
along with its remedial effect on liver and kidney
supports its long term use not only for better control of
blood glucose but also for prevention of diabetes related
complications. Thus Cassia auriculata L seems to have a
promising value for the development of potent
phytomedicine for diabetes.

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