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In vivo antidiabetic and antioxidant activities of *Coccinia grandis* leaf extract against streptozotocin induced diabetes in experimental rats

Shahid Iqbal Mohammed[1], Manoj Zumbarlal Chopda[2], Ravindra Himmatrao Patil[3], Kishor Sukhlal Vishwakarma[1], Vijay Laxminarayan Maheshwari[1*]

[1]*Department of Biochemsitry, School of Life Sciences, North Maharashtra University, Jalgaon-425001, Maharashtra, India*

[2]*Department of Zoology, Moolji Jaitha College, Jalgaon-425001, Maharashtra, India*

[3]*Department of Microbiology, R. C. Patel Arts, Science and Commerce College, Shirpur-425405, Maharashtra, India*

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ABSTRACT

Objective: To evaluate antidiabetic and antioxidative properties of *Coccinia grandis* leaf extract in experimental diabetic rats and to study the co-relation of the two *in vivo*.
Methods: Ethanolic extract, showing the highest activity in *in vitro* experiments, was prepared in saline and given orally to streptozotocin induced albino Wistar diabetic rats for 21 days. Biochemical parameters, histopathological analyses, liver and muscles glycogen and *in vivo* antioxidant activity in normal, diabetic control, standard (metformin) and treated animals were determined and compared.
Results: Treatment of experimental rats with diabetes induced by streptozotocin by ethanolic leaf extract (500 mg/kg) caused significant ($P < 0.001$) reduction in blood glucose (312–169 mg/100 mL), increase in body weight (181–210 g) and serum insulin (1.28–3.10 IU/dL). It also maintained lipid profile and liver and kidney functions within normal range compared with diabetic control rats and almost at par with metformin-a standard antidiabetic drug. The oxidative stress induced decline in glutathione and catalase in liver and kidney tissues showed up to 60% recovery as a function of treatment. Histopathology of pancreas showed marked restorative effect in diabetic rats.
Conclusions: The results suggest that *Coccinia grandis* ethanolic leaf extract has strong antidiabetic activity and can be meaningfully utilized in the management of diabetes.

1. Introduction

Diabetes is a chronic metabolic disorder characterized by impaired insulin secretion or its abnormal utilization by peripheral tissues or both[1]. According to Chan *et al.*[2] and Liu *et al.*[3] the population with diabetes may go up to 0.3 billion by the middle of the next decade contributed mainly by the Asian countries particularly, China and India (57 million in India). Diabetes is characterized by persistent hyperglycemia associated with abnormalities in carbohydrate, protein and lipid metabolisms due to failure of insulin secretion or cells becoming resistant to it[4]. Diabetes is basically classified in to two types, type I and type II. The former is a common disorder in children affecting more and more of them every year. It is generally due to self-destruction of insulin producing cells of pancreas by the immune system leading to its deficiency[5]. The latter does not depend on insulin and is caused by development of resistance by cells to insulin produced in the body resulting in apparent deficiency of it in affected individuals[6].

Present diabetes treatment strategies employ insulin and a range of antidiabetic agents like sulfonylureas, biguanides, glinides *etc*. Despite considerable progress in treatment of diabetes by these agents, there is a need of some new drugs to overcome the limitations of presently used strategies/compounds[7]. Harmless diabetes management is an imposing task before the scientific community[8]. Considering the negative effects of man-made drugs on one hand and natural medicine as low cost, safer and effective on the other hand, there is an enhanced focus on exploring indigenous

*Corresponding author: Vijay Laxminarayan Maheshwari, Department of Biochemistry, School of Life Sciences, North Maharashtra University-425001, Maharashtra, India.
Tel: +91 9422618769
Fax: +91 257 2258403
E-mail: vlmaheshwari@rediffmail.com

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medicinal plants with antidiabetic potential[9,10]. Hypoglycemic effects of several plants used to treat diabetes are already known, and the underlying mechanisms of the observed effects are also being worked out[11,12].

Coccinia grandis (L.) Voigt (Cucurbitaceae) (*C. grandis*), locally known as "parval" or "tindora", is a unique tropical plant growing abundantly and widely all over India. It is a fast growing perennial climbing shrub with white flowers. It grows several meters long and forms dense mat that readily cover shrubs and small trees. Various plant parts like roots, leaves and fruits possess medicinal properties useful in wound healing, treatment of ulcer, jaundice, and diabetes. The leaves have hypoglycemic and antioxidant properties and are also used to treat infective hepatitis[13]. The paper describes a comprehensive study on antioxidant and antidiabetic properties of ethanolic leaf extract in streptozotocin (STZ) mediated diabetes in experimental rats.

2. Materials and methods

2.1. Collection of plant materials

The fresh and fully mature leaves of *C. grandis* were collected from Eklagna village near Jalgaon [20° 58.6' 54.3'' N, 075° 27' 09.5'' E (elevation: 199 m)], Maharashtra during January 2014. The plant species was identified and authenticated by Dr. J Jayanthi from Botanical Survey of India, Pune and a voucher specimen (MSMI-1) was deposited in the School of Life Sciences, North Maharashtra University, Jalgaon.

2.2. Preparation of leaf extract

The collected leaves of *C. grandis* were finely powdered using a mixer (Philips, India) after washing and shade drying (21 days, room temperature) and kept in airtight plastic boxes. The powdered material is packed in cellulosic filter paper to make thimble and was extracted with hexane (69 °C), ethyl acetate (48 °C) and ethanol (78 °C) (30 g per 300 mL of each) in Soxhlet apparatus for 8 h. The extracts were vacuum dried in a rotary evaporator under reduced pressure (Buchi R-215, Switzerland) and stored in a refrigerator in dehydrated conditions.

2.3. Chemicals and apparatus

STZ was purchased from Hi-media, India. Total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoproteins (LDL), very low lipoprotein (VLDL), bilirubin, aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), serum urea, uric acid, serum creatinine were analyzed using standard kits from Erba Diagnostic, (Mannheim GmbH, Germany) by an auto analyzer (Erba Mannheim, Chem-5 plus V2, Germany).

2.4. In vitro antidiabetic activity

2.4.1. α-Amylase inhibition assay

The assay of amylase inhibitory activity was based on modified method of Oboh *et al.*[14]. The activity was determined by mixing 100 μL of test extract with 200 μL of α amylase and 100 μL of 2 mmol/L phosphate buffer (pH-6.9) for 20 min and followed by addition of 100 μL 1% starch solution. Optical density was recorded at 540 nm in a UV-Visible spectrophotometer (Shimadzu, Japan) with acarbose as standard. Mixture without extract was used as a blank and mixture without extract and α amylase replaced by equal amount of phosphate buffer was taken as a control.

2.4.2. Non-enzymatic glycosylation of hemoglobin assay

In vitro antidiabetic activity of *C. grandis* leaf extracts was investigated by estimating degree of non-enzymatic glycosylation of hemoglobin spectrophotometrically at 520 nm[15]. α Tocopherol was used as standard.

2.4.3. Glucose uptake by yeast cells

The experiment was performed as per Daksha *et al.*[15] and Harish *et al.*[16] and metronidazole was used as standard drug. Per cent inhibition was calculated from following formula and IC_{50} values were determined using statistical software Stats Direct 2.8.0.

$$\text{Inhibition of α-amylase (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.5. Experimental animals

Healthy albino Wistar rats, 8 weeks old of either sex, were used for the study. The weight of rats ranged from 180 to 200 g. They were purchased from National Bio Sciences, Pune, Maharashtra (1091/PO/07/CPCSEA), India. Rats were housed in a registered animal house of Moolji Jaitha College, Jalgaon, India in polypropylene cages (6 rats per cage) lined with husk in standard environmental conditions of temperature (25 ± 2) °C and dark/light cycle of 12/12 h with 40%–60% relative humidity. The animals were allowed to feed on a standard diet available commercially (Amrut Agro Ltd., Sangli) and water was provided *ad libitum* during the experiment. The study was approved by the Institutional Animal Ethics Committee (IAEC/16/CPCSEA/MJC/14-15) and was carried out in accordance with the current guidelines of Organization for Economic Co-operation and Development (OECD) and Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India for the care of laboratory animals.

2.6. Acute oral toxicity study

It was performed as per OECD guidelines 425 of 2001[17]. *C. grandis* ethanolic leaf extract (CGELE) (100–1 000 mg/kg) was given orally, by gavage to different groups, each group having six animals. After administration, animals were observed for changes in body weight, acclimatization, behavior and mortality for 7 days.

2.7. Induction of diabetes in Wistar albino rats

Rats were fasted overnight before inducing diabetes by a single intra peritoneal (*i.p.*) injection of STZ (Hi-Media, Mumbai, India) at a dose of 50 mg/kg body weight. It was prepared fresh in ice cold

citrate buffer (0.1 mol/L, pH 4.5). These animals were given sterile glucose solution (20%) for 24 h to counter the initial STZ induced mortality due to acute hypoglycemia. Diabetic rats were identified after 96 h of STZ administration by measuring the glucose levels in vein blood by a digital glucometer (one touch select, Johnson and Johnson, USA) based on glucose oxidase peroxidase method. Albino Wistar rats with glucose level above 250 mg/dL of blood were separated and used in study as diabetic animals[17,18].

2.8. Experimental design

The animals were divided into six groups with 6 animals in each group. Plant extract was prepared in saline and fed daily to the experimental animals by an oral gavage feeding needle.

Group A (normal control) and B (diabetic control) rats were fed with saline alone. Group C animals were fed with standard, metformin (oral hypoglycemic agent) prepared in saline at a dose of 10 mg/kg body weight. Group D, E and F rats were fed with 50, 250 and 500 mg/kg body weight of CGELE prepared in saline. Blood was drawn from the tail vein after 0, 7, 14, and 21 days for the determination of glucose using a digital glucometer (One touch select, Johnson and Johnson, USA).

2.9. Sample collection

After 21 days, rats were fasted overnight. On the next day, mild anesthesia was given to rats, samples of blood were collected from retro orbital plexus in fresh vials for measurement of various biochemical parameters from serum and they were sacrificed.

2.10. Biochemical parameters

Biochemical parameters of serum such as insulin level, TC, TG, HDL, LDL, VLDL, total protein, bilirubin, AST, ALT, ALP, serum urea, serum creatinine and uric acid were determined by an auto analyzer (Erba Mannheim, Chem-5 plus V2, Germany) using kits available commercially according to instructions of manufacturer (Erba Diagnostic Mannheim GmbH, Germany). Insulin level in serum was determined using rat insulin enzyme linked immunosorbent assay (ELISA) kit (DRG International, NJ, USA). Glycogen content in liver and kidney tissue was estimated by a method described in Irudayaraj *et al.*[25].

2.11. Histopathological study

The excised pancreas was washed in ice-cold saline, dried, weighed accurately, rinsed with normal saline and fixed in 10% formalin solution. After embedding in paraffin, 10 μm thick sections of the tissues were cut and stained with hematoxylin-eosin for histopathological study.

2.12. In vivo antioxidant activity

In vivo antioxidant activity in liver and kidney tissues of normal, diabetic control and treated rats were determined by measuring superoxide dismutase (SOD)[19] and catalase activities and level of reduced glutathione[20].

2.13. Statistical analysis

Results of the study were expressed as mean ± SEM using GraphPad Prism® 6 and IC_{50} values were calculated using statistical software Stats Direct 2.8.0.

3. Results

3.1. Yield of leaf extracts

The % yield of extract residues in ethanol, ethyl acetate and hexane were 9.10, 5.80 and 4.30 g, respectively. The residues of three solvents were semi solid, sticky and blackish green in appearance.

3.2. In vitro antidiabetic activity

The *C. grandis* leaf extracts in three solvents were rapidly screened for *in vitro* antidiabetic activity by their ability to inhibit enzyme α amylase, non-enzymatic glycosylation of hemoglobin and glucose uptake by yeast cells. The IC_{50} values of three extracts and standards (acarbose, α tocopherol and metronidazole, respectively) are given in Table 1. All three extracts showed a dose dependent inhibition but the IC_{50} value was the lowest for the ethanolic extract indicating that antidiabetic principle was primarily getting partitioned in ethanol and therefore, ethanolic leaf extract (CGELE) was used for all further *in vivo* experiments.

3.3. Acute oral toxicity study

The acute oral toxicity profile of CGELE, in rat model as per OECD guidelines, did not produce any signs of toxicity or deaths in experimental animals (no observed adverse effect level) up to a dose of 1 000 mg/kg body weight indicating minimal or no chance of toxicity of the extract at the likely therapeutic doses in human which are lower by several orders of magnitude than no observed adverse effect level.

3.4. Effect of CGELE on blood glucose level, serum insulin and body weight

Intra peritoneal administration of STZ at a dose of 50 mg/kg body weight of animal caused an increase in level of glucose in blood [(312.70 ± 2.05) mg/dL] as compared to normal rats [(118.60 ± 1.63) mg/dL]. CGELE, when administered orally, at concentrations of 50, 250 and 500 mg/kg body weight for 21 days, caused a marked decline in blood glucose level to (265.80 ± 1.51), (193.90 ± 1.30) and (169.60 ± 0.70) mg/dL, respectively (Table 2). Intra peritoneal injection of STZ caused significant decrease in serum insulin in comparison to control (Group B verses Group A, Table 2). A dose dependent increase in insulin level was visible (compare Groups D, E and F with group B, Table 2) as a function of treatment and, at 500 mg/kg body weight dose, it was almost at 75% of the standard drug, metformin (compare Groups F and C, Table 2). Similarly, a dose dependent recovery in body weight of diabetic rats was observed as a result of treatment at the doses studied for 21 days as compared to untreated rats (Table 2).

Table 1
In vitro antidiabetic activity of *C. grandis* leaf extracts in three different solvents showing IC_{50} values in comparison to standard compounds.

Extract	IC_{50} (μg/mL)		
	α Amylase inhibition assay	Non-enzymatic glycosylation of hemoglobin	Inhibition of glucose uptake in yeast cells
Ethanolic leaf extract	78.47 ± 0.18	58.33 ± 0.26	68.28 ± 0.13
Ethyl acetate leaf extract	94.65 ± 0.15	158.56 ± 0.22	81.89 ± 0.36
Hexane leaf extract	110.27 ± 0.04	256.86 ± 0.29	84.88 ± 0.24
Standard drugs	35.24 ± 0.24	44.85 ± 0.12	37.84 ± 0.12

The standard drugs for α amylase inhibition assay, non-enzymatic glycosylation of hemoglobin assay and inhibition of glucose uptake in yeast cells assay were acarbose, α tocopherol and metronidazole, respectively. Values are given as mean ± SEM of three independent experiments.

Table 2
Effect of CGEL extract on body weight, serum glucose and serum insulin in experimental diabetic animals after 21 days of treatment.

Group	Description	Body weight (g)	Serum glucose (mg/dL)	Serum insulin (IU/dL)
A	Normal control	220.33 ± 1.37	118.60 ± 0.63	4.71 ± 0.05
B	Diabetic rats	181.30 ± 1.01	312.70 ± 2.05	1.28 ± 0.05
C	B + Metformin (10 mg/kg)	216.90 ± 1.43^{c}	164.80 ± 0.55^{b}	4.15 ± 0.01^{b}
D	B + CGELE (50 mg/kg)	186.80 ± 0.46	265.80 ± 1.51^{b}	1.96 ± 0.10^{a}
E	B + CGELE (250 mg/kg)	198.40 ± 0.87^{b}	193.90 ± 1.30^{b}	2.09 ± 0.02^{b}
F	B + CGELE (500 mg/kg)	210.40 ± 1.77^{c}	169.60 ± 0.70^{b}	3.10 ± 0.08^{b}

Values are given as mean ± SEM (n = 6). ^{a}: $P < 0.05$, ^{b}: $P < 0.01$ and ^{c}: $P < 0.001$ *vs.* Group B.

3.5. Effect of CGELE on lipid profile

Lipid profiles of control and experimental rats are shown in Table 3. Induction of diabetes, by STZ, was accompanied by marked increase in the parameters of lipid profile (TC, TG, LDL and VLDL) except HDL which showed an opposite trend. The trend was observed to be reversed in treated rats fed orally with CGELE. It not only showed a dose dependent decline in the levels of TC, TG, LDL and VLDL but also a significant increase in HDL as compared to STZ induced diabetic control rats.

3.6. Effect of CGELE on liver functions tests

Table 4 shows the results of liver functions tests of the study. In STZ induced diabetic rats, there was significant increase in serum bilirubin, AST, ALT and ALP (Table 4) and significant decrease in protein (Table 5) compared to normal control rats. Treated groups (Groups D, E and F) showed a dose dependent decline in all four parameters as a function of treatment compared to untreated group (diabetic control, Group B). At the highest dose of 500 mg/kg body weight tried in the present study, decline in level of serum bilirubin and ALT was almost equal to standard drug metformin. The total serum protein showed dose dependent recovery and, at the highest dose, recovery was 95% of standard (Group C) and 85% of normal control (Group A, Table 4).

3.7. Effect of CGELE on kidney functions tests

The results of kidney functions tests of control and experimental animals are shown in Table 5. In STZ induced diabetic rats, there was a significant increase in the levels of serum urea, creatinine and uric acid, (70.08 ± 0.90), (1.21 ± 0.16) and (21.08 ± 0.23) mg/dL, respectively vis a vis normal control rats. Animals which were orally administered with CGELE showed a strong dose dependent decline in all the three parameters and, at the highest dose (500 mg/kg body weight), the recovery was same as that with the standard drug metformin (compare Groups B and F).

Table 3
Effect of CGELE on lipid profile of experimental diabetic animals after 21 days of treatment. mg/dL.

Group	Description	TC	TG	HDL	LDL	VLDL
A	Normal control	82.05 ± 0.81	104.20 ± 1.03	36.65 ± 0.69	24.73 ± 0.50	19.87 ± 0.80
B	Diabetic rats	197.81 ± 1.61	177.10 ± 1.29	16.03 ± 0.27	144.10 ± 0.11	35.14 ± 0.46
C	B + Metformin (10 mg/kg)	93.19 ± 0.87^{b}	88.42 ± 1.00^{b}	39.00 ± 0.63^{b}	34.66 ± 0.62^{c}	17.18 ± 0.22^{c}
D	B + CGELE (50 mg/kg)	177.61 ± 1.26^{b}	158.10 ± 1.73^{a}	21.83 ± 0.92^{a}	125.40 ± 1.01^{c}	30.20 ± 0.96
E	B + CGELE (250 mg/kg)	122.70 ± 0.75^{b}	120.50 ± 1.36^{b}	26.75 ± 0.69^{b}	73.17 ± 1.80^{c}	24.35 ± 0.63^{b}
F	B + CGELE (500 mg/kg)	95.39 ± 1.03^{b}	95.37 ± 1.19^{b}	32.95 ± 0.26^{b}	40.73 ± 1.47^{c}	18.04 ± 0.78^{c}

Values are given as mean ± SEM (n = 6); ^{a}: $P < 0.05$, ^{b}: $P < 0.01$, ^{c}: $P < 0.001$ *vs.* Group B.

Table 4
Effect of CGEL on liver functions of experimental diabetic animals after 21 days of treatment.

Group	Description	Bilirubin (mg/dL)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
A	Normal control	0.38 ± 0.05	48.74 ± 0.48	60.15 ± 0.94	120.60 ± 2.33
B	Diabetic rats	0.96 ± 0.01	105.2 ± 0.96	115.1 ± 1.92	203.50 ± 0.54
C	B + Metformin (10 mg/kg)	0.41 ± 0.02^{c}	60.73 ± 0.53^{c}	58.60 ± 0.50^{c}	132.30 ± 0.94^{c}
D	B + CGELE (50 mg/kg)	0.93 ± 0.02	97.36 ± 1.20^{b}	104.20 ± 1.25^{a}	194.30 ± 1.33
E	B + CGELE (250 mg/kg)	0.76 ± 0.15^{b}	96.75 ± 0.61^{b}	95.25 ± 0.98b	163.20 ± 1.16^{c}
F	B + CGELE (500 mg/kg)	0.44 ± 0.15^{c}	69.80 ± 0.51^{c}	59.31 ± 0.37^{c}	145.30 ± 1.36^{c}

Values are given as mean ± SEM (n = 6); ^{a}: $P < 0.05$, ^{b}: $P < 0.01$, ^{c}: $P < 0.001$ *vs.* Group B.

Table 5
Effect of CGELE on kidney functions of experimental diabetic animals after 21 days of treatment.

Group	Description	Total protein (g/dL)	Serum urea (mg/dL)	Serum creatinine (mg/dL)	Uric acid (mg/dL)
A	Normal control	8.54 ± 0.23	36.69 ± 0.51	0.84 ± 0.05	8.68 ± 0.19
B	Diabetic rats	4.70 ± 0.25	70.08 ± 0.90	1.21 ± 0.16	21.08 ± 0.23
C	B + Metformin (10 mg/kg)	7.29 ± 0.26[a]	37.06 ± 0.19[b]	0.86 ± 0.01	8.48 ± 0.43[c]
D	B + CGELE (50 mg/kg)	5.54 ± 0.42	66.10 ± 0.20	0.14 ± 0.02	16.16 ± 0.60[b]
E	B + CGELE (250 mg/kg)	6.03 ± 0.04	52.90 ± 0.91[b]	0.88 ± 0.08	11.02 ± 0.34[c]
F	B + CGELE (500 mg/kg)	7.45 ± 0.33[a]	35.54 ± 0.64[b]	0.91 ± 0.04	9.17 ± 0.13[c]

Values are given as mean ± SEM (n = 6); [a]: $P < 0.05$, [b]: $P < 0.01$, [c]: $P < 0.001$ *vs.* Group B.

3.8. Effect of CGELE on skeleton muscles and liver glycogen of diabetic rats

The skeletal muscle and liver glycogen content in experimental diabetic rats was found to be (1.92 ± 0.05) and (6.38 ± 0.17) mg/100 mg fresh weight, respectively as compared to (8.57 ± 0.41) and (18.92 ± 0.24) mg/100 mg fresh weight, respectively in normal rats (Table 6). It showed significant dose dependent improvement as a function of treatment in both the tissues.

Table 6
Effect of CGELE on liver and muscles glycogen contents in experimental diabetic animals after 21 days of treatment. mg/100 mg fresh weight.

Group	Description	Glycogen content	
		Liver	Muscle
A	Normal control	18.92 ± 0.24	8.57 ± 0.41
B	Diabetic rats	6.38 ± 0.17	1.92 ± 0.05
C	B + Metformin (10 mg/kg)	18.55 ± 8.18[c]	7.77 ± 0.41[c]
D	B + CGELE (50 mg/kg)	8.18 ± 0.02	2.35 ± 0.21
E	B + CGELE (250 mg/kg)	12.59 ± 0.28[b]	4.52 ± 0.35[a]
F	B + CGELE (500 mg/kg)	16.89 ± 0.32[c]	5.93 ± 0.24[b]

Values are given as mean ± SEM (n = 6); [a]: $P < 0.05$, [b]: $P < 0.01$, [c]: $P < 0.001$ *vs.* Group B.

3.9. In vivo antioxidant activity

The renal and hepato-protective effects of CGELE in treated animals was evaluated as a function of SOD and catalase activities and level of reduced glutathione (Table 7). As anticipated, induction of diabetes in experimental animals resulted in almost three-fold drop in the activity of SOD, catalase and level of reduced glutathione in both the tissues as compared to the control except in kidneys were the activity of catalase showed an eight-fold drop. A dose dependent recovery in the level of all the three parameters was recorded as a function of treatment with CGELE. The range of recovery in the activity of SOD and catalase and level of reduced glutathione was between 40% and 130% as that of metformin - the standard oral hypoglycemic agent, at the highest dose of extract studied (500 mg/kg body weight).

3.10. Histopathology

The results of histopathological examination of pancreas of treated and control group of animals are shown in Figure 1. Whereas, the pancreas and islet appeared normal in control group (Figure 1 A). Reduction in their size, damage, necrosis and granulation were observed in the pancreas of diabetic rats (Figure 1 B). Treatment with metformin (10 mg/kg) and CGELE (500 mg/kg body weight) restored necrosis and degranulation of cells and also increased the population and size of islets (Figure 1C, D).

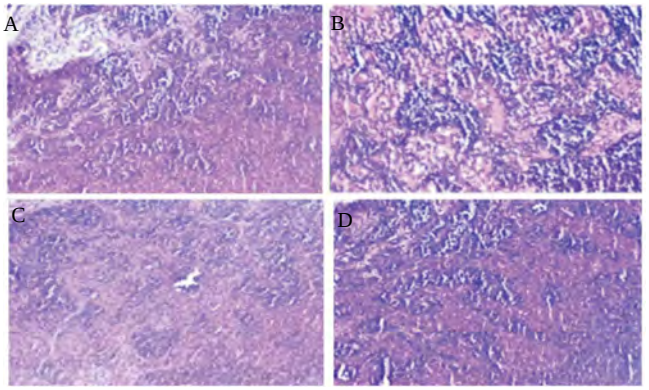

Figure 1. Histopathology of pancreas in experimental rats after 21 days of treatment (10 ×).
A: Normal rats treated with vehicle alone; B: Diabetic rats treated with vehicle alone; C: Diabetic rats treated with standard drug metformin (10 mg/kg); D: Diabetic rats treated with CGELE (500 mg/kg body weight).

4. Discussion

The aim of the present investigation was to comprehensively evaluate the antidiabetic and antioxidative potential of *C. grandis* - an indigenous medicinal plant. Of the leaf extracts in three solvents, ethanol, ethyl acetate and hexane, the former was chosen based on its superior ability to inhibit enzyme amylase, non-enzymatic glycosylation of hemoglobin and glucose uptake by yeast cells *in vitro*. The level of glycosylated hemoglobin

Table 7
In vivo antioxidant activity of CGELE in liver and kidney tissues experimental diabetic animals. IU/g fresh weight.

Group	Description	SOD		Reduced glutathione		Catalase	
		Liver	Kidney	Liver	Kidney	Liver	Kidney
A	Normal control	13.21 ± 0.33	26.4 ± 0.36	73.69 ± 0.90	132.70 ± 1.30	20.76 ± 0.60	70.77 ± 0.53
B	Diabetic rats	5.68 ± 0.29	9.3 ± 0.28	21.36 ± 0.61	45.20 ± 0.55	7.25 ± 0.06	9.73 ± 0.15
C	B + Metformin (10 mg/kg)	12.33 ± 0.63[b]	21.3 ± 0.74[b]	68.36 ± 0.78[b]	78.20 ± 2.77[b]	20.87 ± 0.51[b]	61.01 ± 0.89[c]
D	B + CGELE (50 mg/kg)	5.51 ± 0.47	10.4 ± 0.32	26.26 ± 0.46	84.90 ± 2.06[b]	12.12 ± 0.58[b]	10.65 ± 0.27
E	B + CGELE (250 mg/kg)	5.73 ± 0.46	12.3 ± 0.36[a]	39.14 ± 0.34[a]	100.4 ± 1.73[b]	16.63 ± 0.33[b]	13.41 ± 0.56
F	B + CGELE (500 mg/kg)	7.51 ± 0.46	16.9 ± 0.43[b]	49.65 ± 0.32[a]	103.8 ± 1.60[b]	18.07 ± 0.83[b]	23.86 ± 0.40[c]

Values are given as mean ± SEM (n = 6); [a]: $P < 0.05$, [b]: $P < 0.01$, [c]: $P < 0.001$ *vs.* Group B.

is a measure of long term control of diabetes. Its level in red blood counts was found to be increased in persons with poorly controlled diabetes. The mode of transport of glucose across cell membrane of yeast can be used as an *in vitro* rapid screening model for hypoglycemic effect of different compounds/ medicinal plant extracts. It is well known that glucose transport in yeast cells takes place by facilitated diffusion, based on its concentration gradient across the membrane. It means that lower the extracellular level of glucose, slower will be the rate of uptake[21,22]. Substances that inhibit enzyme amylase (and glucosidase) reduce the availability of glucose in small intestine and thereby, limit its uptake in post prandial state. In all the three parameters, the ethanolic leaf extract of *C. grandis* showed better results compared to ethyl acetate and hexane extract in our study. It is an indicator that principal antidiabetic agent(s) in *C. grandis* may be getting preferentially partitioned in ethanol.

Streptozotocin is a β-cell specific toxin. It induces diabetes by (i) selectively destroying insulin producing cells of pancreas and (ii) disturbing the redox balance by over producing reactive oxygen species[23]. The histopathological study of pancreas showed remarkable improvement in the number of islets, shrinkage and necrosis of β-cells as a function of treatment in STZ induced diabetic animals as reported earlier[24,25].

The CGELE at a dose of 500 mg/kg body weight showed significant recovery in level of glucose and insulin in serum and body weight in STZ induced diabetes in experimental animals. This could be due to a combination of factors like diminished absorption in intestine, improved uptake of glucose by peripheral tissues and up regulation of glycolytic and glycogenic processes[22]. Also the extract may be facilitating insulin release from remnant or regenerated pancreatic β-cells[25,26]. Diabetes induced by STZ is accompanied by decline in body weight because of loss of protein and muscle wasting primarily[27,28]. A smart recovery in body weight of diabetic rats at the highest dose of treatment, almost comparable to recovery observed with standard drug metformin, correlates well and supports the antidiabetic effect of the extract. It also signifies protective effect of extract in regulating muscle wasting[25].

Diabetes induced by STZ resulted in hypertriglyceridemia and hypercholesteremia in experimental animals leading to secondary complications associated with it[29]. Significant reduction in the total and LDL cholesterol, triglycerides and increase in level of HDL cholesterol as a function of treatment by CGELE over a period of 21 days proved its potential to reduce the incidences of lipid born complications associated with diabetes. STZ induced hypertriglyceridemia and hypercholesteremia are a result of inhibition/down regulation of lipoprotein lipase due to insulin deficiency[30]. Restoration of almost normal level in lipid profile by administration of CGELE suggests that it may have insulin like activity.

Experimental diabetes is characterized by specific changes in renal metabolism, enhanced specific proteolysis and muted protein synthesis[31]. The serum protein level was found to be significantly improved in diabetic rats as a function of treatment with CGELE at the dose of 500 mg/kg body weight. Remarkable decline in the urea, creatinine and uric acid levels in serum of diabetic animals as a functions of treatment are indicators of improved kidney health and prevention of progression of renal damage due to hyperglycemia. Similarly, the markers of liver functions, AST, ALT and ALP levels, showed statistically significant improvement, almost at par with the standard drug metformin in our study. It should be pointed here that the levels of three enzymes go up in STZ induced diabetes due to hepatotoxic effects of the latter.

Glucose is primarily stored in the form of glycogen mainly in liver and is regulated by the level of insulin which promotes glycogen storage by up regulating glycogen synthase and down regulating glycogen phosphorylase. Apparently, insulin deficiency in STZ induced diabetic rats resulted in marked reduction in both liver and muscles glycogen content in our study. These animals, when treated with CGELE, showed a dose dependent recovery in its content in both the tissues, probably due to improved insulin synthesis/secretion or both, which correlates well with other results in the study.

A natural consequences of chronically elevated glucose level in STZ induced diabetes is oxidative stress. It not only compliments diabetes but is also associated with insulin resistance[32,33]. The underlying mechanism of oxidative stress mediated insulin resistance or impaired insulin secretion at molecular level is not known but chronic hyperglycemia and elevated fatty acids result in over production of reactive oxygen and nitrogen species than what could be handled by endogenous antioxidant network resulting in redox imbalance[34]. The redox imbalance in body triggers stress sensitive signaling pathways and expression of genes, products of which are responsible for cellular damages[34]. In our study, marked recovery in the level of SOD, reduced glutathione and catalase - three important components of endogenous antioxidant system, in both liver and kidney was recorded. It suggests strong antioxidant property of the CGELE.

The excellent antidiabetic activity of CGELE thus observed in our study apparently seems to be a result of multiple and mutually complimentary activities present in the extract. It was found to be nontoxic to experimental animals up to 1 000 mg/ kg body weight dose indicating minimal or no chance of toxicity at the likely therapeutic dose in humans. It is a comprehensive study of an indigenous plant extract, establishing its antidiabetic potential which can be gainfully utilized in future diabetes management strategies.

Conflict of interest statement

We declare that we have no conflict of interest.

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