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Indian Journal of Traditional Knowledge
Vol. 14(3), July 2015, pp.376-381

Antihyperglycemic activity of *Coccinia grandis* (L.) Voigt in streptozotocin induced diabetic rats

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Received 21 August 2014; revised 12 March 2015

Coccinia grandis (L.) Voigt (Cucurbitaceae) has been popularly used in traditional Ayurvedic medicine for the treatment of diabetes mellitus in Sri Lanka**.** The present study was to investigate the long term effect of aqueous leaf extract of *C. grandis* on serum/blood glycemic parameters and regenerative potential of islet cells in the pancreas of streptozotocin induced diabetic rats. Diabetes was induced in rats by injecting them with streptozotocin (65 mg/kg, ip). Group one and two served as the healthy untreated, diabetic untreated control rats and received distilled water. Group three and four were diabetic rats, received the optimum effective dose of *C. grandis* (0.75 gm/kg) and glibenclamide (0.50 mg/kg) daily for 30 days. There was a statistically significant decrease in the percentage of glycosylated hemoglobin together with a concomitant increase in the concentrations of serum insulin and C-peptide in plant extract and glibenclamide treated diabetic rats ($p<0.05$). The β-cell regeneration in *C. grandis* extract treated diabetic rats was noted through an increase in the percentage of insulin secreting β-cells and an increase in islet profile diameter ($p<0.05$). The findings of the present investigation helps to scrutinize the therapeutic benefits of the *C. grandis* extract in the management of diabetes mellitus in traditional medicine.

Keywords: Antidiabetic mechanisms, Biosynthesis of insulin, β-cell regeneration, *Coccinia grandis*, Streptozotocin induced diabetic rats

IPC Int. Cl.[8]: A61K 36/00, A01D 16/02, A01D 20/94

Coccinia grandis (L.) Voigt (Cucurbitaceae) is an edible perennial climber distributed in tropical Asia, commonly found in Sri Lanka, India and Pakistan. The leaf extract of *C. grandis* has been widely used as an adjuvant therapy in Sri Lankan Ayurvedic medicine for the treatment of diabetes mellitus[1]. Other therapeutic properties of leaves of the plant include hepatoprotective, antioxidative and anti-inflammatory[2,3]. Despite the presence of limited scientific evidence, Sri Lankans are well convinced about its antidiabetic effects. Ajay reported the acute hypoglycemic activity of the alcoholic extract of *C. grandis* in normoglycemic and streptozotocin induced diabetic rats[4]. The effect of methanolic leaf extract on liver enzymes and lipid profile was reported in streptozotocin induced diabetic rats[5]. Furthermore, the hypolipidemic effect of the aqueous leaf extract of *C. grandis* was reported in alloxan induced diabetic rats[6]. The preliminary investigations by our group confirmed that the aqueous leaf extract of *C. grandis* at a dose of 0.75 gm/kg was found to be optimum effective dose in diabetic rats and toxicologically safe as a potential antihyperglycemic agent in rats[7].

The present study aims to investigate the effect *C. grandis* (0.75 gm/kg) on antihyperglycemic activity, histology of the pancreas through histopathology and immune-histochemistry in streptozotocin induced diabetic rats.

Materials and methods

Chemicals and instruments

All chemicals were of analytical grade and used without any purification. UV visible spectrophotometer (Gallenkamp PLC, UK) and microplate reader (Mindray, China) were used for spectrophotometric and enzyme linked immune-sorbent assay (ELISA) measurements, respectively. Olympus CX 21(Japan) microscope was used in the assessment of histopathology and immunohistochemistry of pancreatic tissues.

Plant material

Leaves of *C. grandis* were collected during May-June 2013 from the Southern region of Sri Lanka. Botanical identity was confirmed by

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comparing authentic samples at the National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka. A voucher specimen was preserved at the Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka (Attanayake/2011/03).

Preparation of the aqueous plant extract

The leaves were cut into small pieces, dried at 40˚C until a constant weight was reached and coarsely ground. Powdered plant material (50.00 gm) was dissolved in 400.0 mL of distilled water, refluxed for 4 hrs to yield the dose of 0.75 gm/kg. The mixture was strained through cheese-cloth and the final volume was adjusted to 50.0 mL.

Animals

Healthy Wistar albino rats 200 ± 25 gm body weights were used to carry out experiments. They were housed in standard environmental conditions at the animal house of Faculty of Medicine, University of Ruhuna, Sri Lanka (Temperature 25 ± 2˚C, relative humidity 55-65% and 12 hrs light/dark cycle). All protocols used in the study were approved by the Ethics Committee of Faculty of Medicine, University of Ruhuna, Sri Lanka guided by the Council for International Organization of Medical Sciences (CIOMS) international guiding principles of biomedical research involving animals.

Experimental group design

Streptozotocin (65 mg/kg, ip) was administered to rats to develop diabetes mellitus. Rats with fasting blood glucose concentration of 12.0 mmol/L or above were considered as hyperglycemic and used for the experiments[8]. Group one and two served as the untreated healthy, untreated streptozotocin diabetic control rats and received distilled water. Group three and four were streptozotocin induced diabetic rats, received the optimum effective dose of the extract *C. garndis* (0.75 gm/kg) daily for 30 days. The fifth group received glibenclamide (0.50 mg/kg) daily for 30 days which served as the positive control.

At the end of the study (on the 30th day), blood was collected by cardiac puncture and pancreas was excised from sacrificed rats. Serum was separated from blood of all rats for the estimation of biochemical parameters. Pancreatic tissue was used for the assessment of histopathology and immune-histochemistry assessments.

Blood/serum glycemic parameters

On the 30th day, blood was collected for the estimation of biochemical parameters. The percentage of glycosylated hemoglobin; HbA_{1C} and serum concentration of fructosamine were estimated in all rats using spectrophotometric enzyme assay kits. Furthermore, the concentration of serum insulin and C-peptide in all rats were estimated using enzyme linked immune-sorbent assay methods.

Histology of pancreas in diabetic rats

Paraffin embedded tissue blocks of the pancreas were used for detailed assessment of histopathology and immune-histochemistry. The sections of the pancreatic tissues were stained with hematoxylin and eosin for the light microscopic examination of histopathology changes of pancreatic tissue in all rats. Histopathology score was developed for the assessment of selected histological parameters of destruction of islet cells and regeneration of islet cells[9]. Immuno-histochemical staining was done to confirm the presence of insulin secreting cells in the islets of pancreas in all rats. Dako polyclonal guinea pig anti-insulin and Dako REAL^{TM} En Visison^{TM}/HRP, Rabbit/Mouse were used for immune-histochemical staining.

Islets were defined as small, average, and large with an islet diameter of ≤125 μm, 126-149 μm, and ≥150 μm respectively[15]. Four islets of each size in each rat (72 islets for each group) were chosen randomly. The percentage of insulin secreting β-cells in islets and islet diameter were estimated[10,11].

Statistical analysis

Results are expressed as mean ± SEM for biochemical estimations. The quantitative data were analyzed by ANOVA followed by Dunnett's multiple comparison tests. The Kruskal- Wallis test was used for the semi quantitative analysis of histopathology score values. Results were considered to be significant at $p<0.05$.

Results

Blood/serum glycemic parameters

Effect of plant extract on the percentage of HbA_{1C}, concentration of fructosamine, insulin and C-peptide in streptozotocin induced diabetic rats on the 30th day is shown in Table 1. The diabetic rats treated with the plant extract exhibited a remarkable glycemic control as evident by a reduction in the percentage of HbA_{1C}. The reduction in the percentage of HbA_{1C}, fructosamine was 33% and 34% in diabetic rats respectively. However, the glibenclamide treated diabetic rats demonstrated a fall of 40% and 43% in above parameters in diabetic rats. The concentrations of serum insulin and C-peptide were increased

Table 1-Effect of the extract of *Coccinia grandis* on some glycemic parameters in streptozotocin induced diabetic rats for 30 days

Treatment	Glycosylated haemoglobin (%)	Fructosamine (µmol/L)	Insulin (µIU/mL)	C-peptide (ng/mL)
Healthy control rats	4.86±0.10	221.88±3.10	14.23±0.44	9.53±0.80
Diabetic control rats	9.00±0.09	405.39±2.78	6.23±0.09	5.75± 0.80
C. grandis (0.75 gm/kg)	6.00±0.07*	268.20±3.11*	10.86±0.11*	8.80±0.40*
Glibenclamide (0.50 mg/kg)	5.38±0.06*	230.08±0.99*	11.75±0.20*	6.00±0.60*

The values are expressed as mean ± SEM (n=6/group). * Statistically different from diabetic control at $p < 0.05$ (ANOVA followed by Dunnett's test). HbA_{1C}: glycosylated hemoglobin

Table 2- Semi-quantitative analysis of pancreatic tissue on histopathological parameters in streptozotocin induced diabetic rats for 30 days

Treatment	Percentage area of insulin secreting cells in islets (%)			Diameter of islets (µm)		
	small	average	large	small	average	large
Healthy control rats	86.17±3.54	72.00±3.90	78.33±7.53	86.80±1.32	138.50±5.57	173.16±8.97
Diabetic control rats	9.17±0.91	7.50±1.23	6.83±0.87	32.34±1.55	127.43±2.70	153.05±0.37
C. grandis (0.75 gm/kg)	84.33±4.05*	67.17±5.73*	74.83±3.76*	68.14±2.42*	134.68±1.60*	177.56±8.29*
Glibenclamide(0.50mg/kg)	33.33±2.34*	10.00±0.15*	7.17±1.42*	36.10±3.31	128.38±1.99	154.08±5.88

0-none, 1-mild, 2-moderate, 3-severeNA: not applicable * Statistically different from diabetic control at $p < 0.05$ (Kruskal-Wallis test)

Table 3-Quantitative analysis of immune staining of insulin secreting cells and mean diameter of islets

Treatment	Destruction of islet cells	Regeneration of islet cells
Healthy control rats	0	N/A
Diabetic control rats	3	0
C. grandis (0.75 gm/kg)	0*	2*
Glibenclamide (0.50 mg/kg)	2*	0

The values are expressed as mean ± SEM (n=6/group).
*Statistically different from diabetic control at $p < 0.05$ (ANOVA followed by Dunnett's test).

significantly by 74%, 53% in plant extract treated diabetic rats respectively ($p<0.05$).

Assessment of histopathology and immunohisto-chemistry of pancreatic tissues

As shown in Table 2 and Fig. 1, the streptozotocin induced untreated diabetic rats showed an extensive destruction of islet cells as compared with the sections of pancreas from healthy control rats (score value of 3 vs 0). Further, there was a definite reduction in number of islets in diabetic rats, than the number in healthy rats. However, hemorrhages were not observed and acinar cells were intact in pancreatic tissues of streptozotocin induced diabetic control rats. Further severe inflammatory cell infiltrations in islets were also seen in streptozotocin induced untreated diabetic control rats. Immuno-histochemical staining with anti-insulin antibody confirmed the marked reduction (less than 10%) in insulin secreting cells in small, average and large size islets in diabetic control rats (Table 3, Fig. 2). The mean diameter of islets was reduced in small (63%), average (8%), large (13%) islets in diabetic control rats as compared with the normal control rats. The sections from *C. grandis* extract treated diabetic rats revealed a statistically significant score value for the regeneration of islet cells with some hyperplastic islets as compared to diabetic untreated group (score value of 2 vs 0, $p< 0.05$). The number of islets was increased in plant treated diabetic rats when compared to diabetic control rats. Further, the C. *grandis* extract produced a significant increase in mean profile diameter in small (111%), average (6%) and large (16%) size islets as compared with streptozotocin induced diabetic control rats.

Discussion

The effect of aqueous leaf extract of *C. grandis* (0.75 gm/kg) on serum/blood glycemic parameters, serum lipid parameters and regenerative potential of islet cells were investigated in streptozotocin induced diabetic rats. To explore the mechanisms by which the extract exerts the antihyperglycemic activity, the study was focused mainly on the possibility that *C. grandis* extract might induce islet cell regeneration and biosynthesis of insulin in diabetic rats.

Streptozotocin causes rapid destruction of pancreatic β-cells which was confirmed with the decreased percentage of insulin secreting β-cells in the pancreas of diabetic control rats.

Hot water, crude, leaf extract of *C. grandis* was used for the experiments as this method of extraction results in many of the important phytochemicals such as polyphenol compounds and flavonoids which are known to responsible for antidiabetic activity[12]. Oral administration was selected to simulate the human

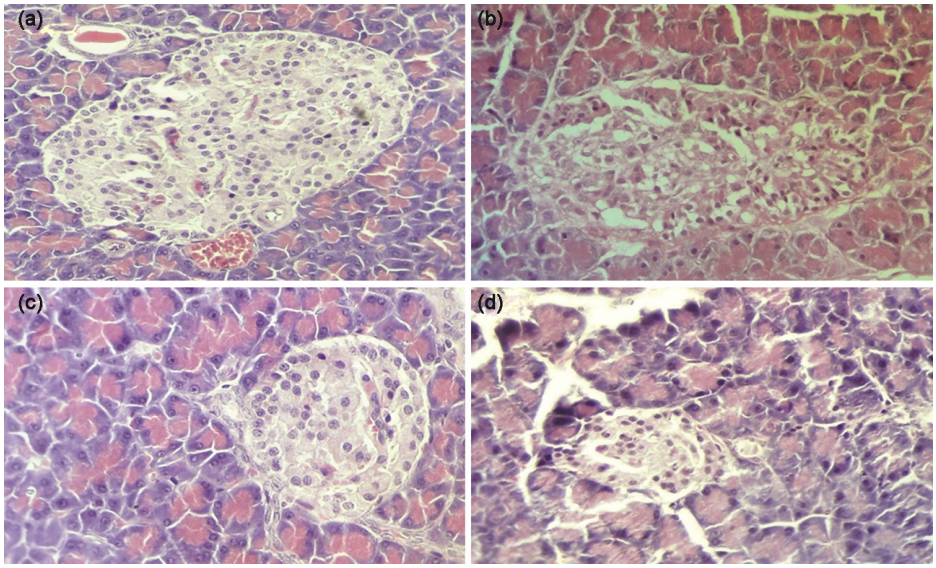

Fig. 1- Photomicrographs of pancreatic tissues, stained with hematoxylin and eosin after 30 days of treatment (x 400); (a) Healthy control rats, islets of Langerhans with normal islet cell population; (b) Diabetic control rats, a islet with few preserved islet cells, fibrosis and infiltration by inflammatory cells; (c) *Coccinia grandis* treated (0.75 gm/kg) diabetic rats, restoration of pancreatic islet cells with prominent hyperplastic islets; (d) Glibenclamide treated (0.50 mg/kg) diabetic rats, reduced number of islet cells.

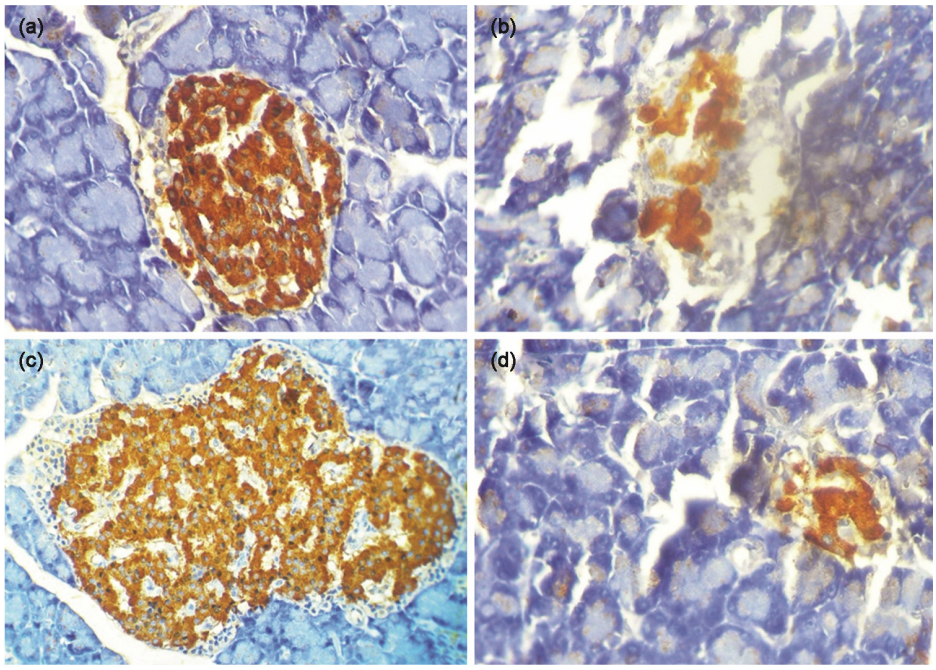

Fig. 2- Photomicrographs of insulin immunoreactivity in pancreatic islets after 30 days of treatment (x 400); (a) Healthy control rats, a normal islet composed predominantly of insulin secreting cells; (b) Diabetic control rats, marked reduction in the number of insulin secreting β-cells due to the destruction of islet cells by streptozotocin; (c) *Coccinia grandis* treated (0.75 gm/kg) rats, a hyperplastic islet with a marked increase in insulin secreting β-cells; (d) Glibenclamide treated (0.50 mg/kg) rats, mild increase in insulin secreting cells with no islet cell hyperplasia.

consumption of plant decoctions in traditional Ayurvedic medicine[13]. Glibenclamide was used as the reference drug in many animal experiments with chemically induced diabetes mellitus. It mediates the antidiabetogenic effect through the binding to the receptors on surface of pancreatic β-cells, there by the cell membrane creates an influx of calcium ions and a subsequent release of insulin[14]. It was observed that

treatment of *C. grandis* for 30 days reduced the fasting blood glucose concentration, acquiring good glycemic control suggesting its antihyperglycemic properties. The estimation of HbA_{1C} is a well-accepted biochemical parameter in the diagnosis and prognosis of diabetic state[15,16]. The diabetic rats fed with the plant extract for 30 days exhibited a remarkable glycemic control as demonstrated by a statistically significant reduction in the percentage of HbA_{1C} in diabetic rats. This may further confirm the potential long term antihyperglycemic activities of the extract of *C. grandis*. However, the reduction in the percentage of HbA_{1C} in glibenclamide treated diabetic rats was superior to the reduction in the plant extract treated diabetic rats at the end of study period (30^{th} day). Fructosamine, is a glycated protein which has a crucial role in the progression of many pathological conditions including diabetes mellitus[17]. It results from spontaneous non-enzymatic condensation of excess glucose present in blood and a number of plasma proteins[18,19]. The estimation of serum fructosamine is important in the screening of a claimed antidiabetic agent intervened for a sub-chronic period as it indicates the average blood glucose concentration over one/two weeks. A reduction in serum fructosamine was noted in plant extract treated diabetic rats in the present study. This could be due to decrease concentration of serum glucose as a result of antihyperglycemic effect of plant extract in diabetic rats.

The optimum pancreatic β-cell function is essential for the regulation of intracellular glucose homeostasis[20]. Insulin and C-peptide are the products of enzymatic cleavage of proinsulin and secrete into the circulation in equimolar concentration. C-peptide promotes insulin action at low hormone concentration and vice versa, suggesting a modulatory effect by C-peptide on insulin signaling[21]. In addition, C-peptide has insulin-mimetic effects on its own by activating insulin receptors and increases the synthesis of glycogen and uptake of amino acids. The estimation of both concentration of C-peptide and insulin has been reported to be valuable indices of insulin secretion rather than insulin alone[22]. The increase in serum insulin and C-peptide concentration in plant extract treated rats corroborated the formation of functional islets, biosynthesis of insulin as evident through the assessment of histopathology and immunohistochemistry. The semi-quantitative assessment of pancreatic sections on haematoxylin and eosin stains clearly demonstrated the potency of plant extract to induce islet cell regeneration in diabetic rats.

Histomorphological evidence of restoration of islet cells and islet cell regeneration in plant treated diabetic rats in the present study led further investigation of morphometric assessment of pancreatic islets through advanced immune-histochemical studies. The pancreatic mechanism of the *C. grandis* extract was confirmed through immune-histochemical studies together with biochemical data obtained in the study. The extract of *C. grandis* showed β-cell regeneration as evident through an increase in the percentage of insulin secreting β-cells. The raise in the diameter of small, medium and large size islets was found in *C. grandis* treated streptozotocin induced diabetic rats. This may confirm that the size of the islet and the entire regenerative capacity of the pancreas were increased with the treatment of *C. grandis* extract in diabetic rats. This may be the probable rationale for the increase in the concentration of insulin and C-peptide in diabetic rats treated with the extract of *C. grandis*. The immune-histochemical findings are in agreement with glycemic parameters in plant extract treated diabetic rats.

Glibenclamide was used as the standard drug in the present study. It has been proposed that sulphonylureas produce the antihyperglycemic effects though secretion of insulin from pancreatic β-cells and enhancement of insulin action on target tissues. Glibenclamide is widely accepted as a standard drug in diabetic animal experiments associated with mild or moderate hyperglycemia in rats[23].

Phytochemical studies indicated the presence of polyphenol compounds, flavonoids and tannins in the *C. grandis* extract[24]. The antihyperglycemic, and antihyperlipidemic activities may be attributed mainly due to the presence of polyphenol compounds and flavonoids in aqueous leaf extract of *C. grandis*.

Medicinal plant extracts exert their antihyperglycemic effects through a variety of mechanisms *in vivo*. These include enhancement of secretion of insulin and/or sensitivity, biosynthesis of insulin, islet cell regeneration, glucose utilization in skeletal muscle and adipose tissue and inhibition of glucose absorption from intestine. Even though, islet cell regeneration is an important mechanism of antihyperglycemic activity of *C. grandis* extract, it is not rational to exclude other possible mechanisms in which plant extracts exert *in vivo*[25]. Future studies on these postulations are therefore warranted. In addition, structure elucidation of active compounds of

the extract of *C. grandis* and its antidiabetic effects are needed to be investigated in near future.

Conclusion

The results confirms that the aqueous leaf extract of *C. grandis* possess *in vivo* antidiabetic activity through increased biosynthesis of insulin probably by β-cell regeneration in the pancreas of streptozotocin induced diabetic rats. In addition, the plant extract exerts antihyperlipidemic activities diabetic rats.

This is the first ever study reported the detailed pancreatic mechanisms of *C. grandis in vivo.* The findings of the present investigation helps to scrutinize the therapeutic benefits of the *C. grandis* extract in the management of diabetes mellitus in traditional medicine.

Acknowledgement

The financial assistance given by University Grants Commission in Sri Lanka is greatly appreciated (UGC/ICD/CRF 2009/2/5). The authors wish to thank Dr D.A.B.N. Gunarathne of the Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Sri Lanka for the guidance given in statistical data analysis, Mrs. B.M.S. Malkanthie, Mr G.H.J.M. Priyashantha and Mr D.G.P. Pathmabandu, Mrs G.G.D.D. Gunawardane, Faculty of Medicine, University of Ruhuna, Sri Lanka for technical assistance.

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