HYPOGLYCEMIC POTENTIAL OF *BOUGAINVILLEA SPECTABILIS* ROOT BARK IN NORMAL AND ALLOXAN-INDUCED DIABETIC RATS

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Summary

Background and the purpose of study: Bougainvillea -spectabilis is a part of various herbal formulations for the treatment of diabetes. The present study was aimed to study the hypoglycemic activity of *Bougainvillea spectabilis* root bark extract *in vivo* in albno Wistar rats.
Methods: Bougainvillea spectabilis root bark extracted with ethanol and administered to both

normal and alloxan induced diabetic rats. The blood glucose levels were measured at 0-6 hours, and on 0-7 days after oral administration of EtOH extract at doses of 50, 100 and 200 mg/kg/day.
Results: Bougainvillea spectabilis root bark exhibited significant hypoglycemic activity at different doses and intervals. Highest hypoglycemic activity was observed with root bark extract at 100 mg/kg/day after 7 days. It was found to be 12.5% more potent than standard oral hypoglycemic drug, glibenclamide 0.2 mg/kg.
Major conclusion: Permanent hyperglycemia in alloxan induced diabetic rats was reversed when treated up to a week with EtOH extract of *B. spectabilis* root bark. This study provides first scientific evidence that the EtOH extract of root bark of *Bougainvillea spectabilis* have anti-diabetic efficacy.

Key words
Blood glucose level, *Bougainvillea spectabilis*, Glibenclamide.

Introduction

Diabetes is a lifelong (chronic) metabolic disorder which is caused by deficiency of insulin, or ineffectiveness of insulin produced (1). Type-1 diabetes may be due to deficiency of insulin secretion-due to destruction of pancreatic β cells and the type-2 diabetes may be due to insulin resistance- a defect in the tissue response to insulin and impaired insulin release- slow basal secretion of insulin after meal (2).

Premature illness and death due to diabetes has reached epidemic proportions worldwide (3). World Health Organization estimates that developing countries will be most affected by this epidemic in the present 21st century (4). The global figure of patients with

diabetes is set to rise from the current estimate of 150 million to 220 million in 2010, and 300 million in 2025 (5-6) by root bark extract. The increasing prevalence of diabetes has already imposed a huge burden on health-care systems and this will continue to increase in the future (7-8).

Various oral hypoglycemic agents like sulfonylureas, biguanides, α-glucosidase inhibitors, thiazolidinediones, diphenylalanine derivatives, glinides and insulin formulations are used currently for the treatment of diabetes (9-12). In developing countries, these pharmaceuticals are expensive and not easily accessible. Hence, the WHO recommended for continue research for alternative antidiabetic agents from plants and other resources (13). With the advancement of analytical techniques; screening of natural products is now rapid and more fruitful. The discovery of lead compounds may serve as a tool to better understood targets and pathways in the disease process.

Herbs may be a potential source to treat this metabolic disorder (14-16). *Bougainvillea spectabilis* wild (Family: *Nyctaginaceae*) is reported for the treatment of diabetes is reported in herbal formulations (17). *Bougainvillea spectabilis* commonly known as Bougainvillea, Great Bougainvillea (in Indian languages: Booganbel, Cherei, Baganbilas, Booganvel, Bouganvila, Kagithala Puvvu).

Biological activities like hypoglycemic (18-20), cholesterol lowering effect (21), antibacterial (22), nematicidal (23), antifeedant and insecticidal (24), antiviral (25) and anti-inflammatory activities (26) were reported of *B. spectabilis*. Phytoconstituents like flavonoids, phenolic compounds, antiviral (27), ribosome inactivating protein (28), amylase inhibitors (29), oxidase (30) and pinitol (31) have been isolated from *B. spectabilis*. Antihyperclycemic agent- pinitol (32) isolated from leaves mimicked us to study antidiabetic activity in root bark of *B. spectabilis*.

Materials and methods

Collection of Root Bark

Root bark (1.5 kg) of *B. spectabilis* Wild (*Nyctaginaceae*), collected from village - Charkhari, District- Mahoba (Uttar Pradesh), India in October 2009. The plant was identified by plant taxonomist Dr. A.K. Sharma, Department of Botany, Multanimal Modi (P.G.) College, Modinagar, Ghaziabad (U.P.), India. Voucher specimens (MMCM/02/015) were deposited in the Herbarium of the Department of Botany, Multanimal Modi (P.G.) College, Modinagar, Ghaziabad (U.P.), India, for future reference.

Extraction

Root bark of *B. spectabilis* was air dried in shade and pulverized in electric grinder. Powdered root bark (1.5 kg) was soaked in ethanol (70%), placed on a mechanical shaker for 24 hours. The extract was filtered and concentrated at 45˚C. The weight of crude extract obtained was 150.2g (≈10%, w/w, yield).

Phytochemical Investigations

Phytochemical studies of EtOH extract of root bark *B. spectabilis* carried out for the presence of alkaloids with Dragendroff's reagent, flavonoids with metallic magnesium and HCl, tannins with Ferric chloride reagent, , anthraquinones with Borntrager's test, saponins with ability to form suds, reducing sugars with Fehling's reagent, Cardiac glycosides with Liberman's test and Killer killiani test, triterpenes and steroids with sulphuric acid reagent according to standard methods (33-34).

Experimental animals

Albino Wistar rats (**150-200g**) of age 8-12 weeks were procured from Institutional Animal House (Reg. No. 1044/c/07/CPCEA), for the present study. The animals kept under standard conditions (25 °C, 12 hours light and 12 hours dark cycle, 60% humidity) and fed with rodent diet and water *ad libitum*, and acclimatized to the laboratory conditions for 6 days.

Blood glucose level determination
Blood glucose concentrations (mg/100ml) were determined using an Accu-Check active (Roche Diagnostic GmbH, Germany), based on the glucose oxidase method. Blood samples collected from the tip of tail at the defined time intervals.

Acute toxicity studies
Alcoholic extract was tested in albino Wistar rats for their acute and short- term toxicity (if any). For determining acute toxicity of a single oral administration of extract, the OECD guidelines (OECD/OCDE 2001, 423, Annex 2c) were followed (35). Stepwise doses of *B. spectabilis* root bark extract from 300mg/kg body weight up to the dose 5000 mg/kg b.w. were administered. Rats were observed continuously for the initial 4 hours and intermittently for the next six hours and then again at 24 hours and 48 hours following extract administration. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsion. No considerable signs of toxicity were observed in albino Wistar rats. On the basis of above acute toxicity study, doses 50, 100 and 200mg/kg were selected for examination of hypoglycemic activity.

Induction of Diabetes
Diabetes was induced in rats by a single intraperitoneal injection of alloxan monohydrate (CDH, Bombay) in normal saline (120 mg/kg) after overnight fasting for 12 hours. The fasting blood glucose level was measured after 48 hours of alloxan injection. The rats with effective and permanent elevated plasma glucose levels (above 300mg/100ml) were selected for the study.

Effect of extracts on normal and glucose-loaded rats (NG-OGTT)
Oral glucose tolerance test performed after overnight fasting (16 hours) on normal rats. Vehicle (distilled water), EtOH extract of root bark at three different doses (50, 100 and 200 mg/kg) and standard as glibenclamide (Daonil® Sanofi Aventis Pharma. Ltd. Mumbai,

India) (0.2 mg/kg) administered to six different groups of rats (n=6). Glucose (4 g/kg) was fed 60 min after treatment. Blood was withdrawn from the tip of tail at 0, 30, 60, 90, 120, 240 and 360 min from normal (control) and experimental animals and determined blood glucose level.

Measurement of blood glucose level in diabetic rats up to 6 hours

Hypoglycemic activity of *B. spectabilis* root bark extract performed on overnight fasted (16 hours) diabetic rats. Distilled water, root bark extract at three doses (50, 100 and 200 mg/kg) and glibenclamide (0.2 mg/kg) administered orally using gastric gavage needle to six different groups of normal rats. Blood was withdrawn at 0, 30, 60, 120, 240, and 360 min. from the tip of tail of diabetic control and experimental animals.

Measurement of blood glucose level in diabetic rats up to 7 days

Animals were divided randomly in four groups of 6 rats each. After overnight fasting diabetic rats treated orally with vehicle, EtOH extract of *B. spectabilis* root bark at three different doses (50, 100 and 200 mg/kg/day) and glibenclamide 0.2 mg/kg daily up to 7 days. Blood samples were collected from the tip of tail daily at the defined time intervals from control and experimental animals.

Data analysis

Data are expressed as means±S.E.M. The results obtained were analyzed using GraphPad instat version 5 software using Student's t test for paired data and one way ANOVA using Dunnett’s Multiple Comparison Test. A difference in the mean values of $P<0.05$ considered significant.

Results

Phytochemistry of extracts

Root bark of *B. spectabilis* showed the presence of cardiac glycosides, saponins, alkaloids, glycosides, steroids and tannins.

Effect of extract on glucose tolerance test in normal rats

Alloxan treatment produced a significant increase in the blood glucose levels as compared to the vehicle treated rats. There was no significant change in the blood glucose level of vehicle treated rats. Normal overnight fasted rats loaded with glucose (4 g/kg) orally, showed significant increase in blood glucose (*P<0.05)* after 30 min. Root bark extract of *B. spectabilis* at the dose of 100 mg/kg reduced the blood glucose level significantly from after 30 min which was comparable to the glibenclamide (0.2 mg/kg) treatment (*P<0.05)*(Table 1).

Effect of extract on fasting blood glucose levels in diabetic rats after 6 hours

Vehicle (10 ml/kg) oral administration did not change the level of basal blood glucose significantly. In diabetic rats, glibenclamide (0.2 mg/kg) was found to be highly effective and decreased blood glucose level more than doses of the extract used. After 6 hours, glibenclamide (0.2 mg/kg) significantly decreased the blood glucose from 313.9±4.10 to 201.9±4.12 mg/dl (*P<0.05).* As compare to control, the oral administration of root bark extract of *B. spectabilis* (50, 100 & 200 mg/kg) induced a significant decrease of blood glucose in alloxan induced diabetic rats (P<0.05) (n=6). Root bark extract at the dose of 100 mg/kg was found to be most effective of the doses tested. The potency of these extract was similar to glibenclamide. The hypoglycemic activity of root bark extract persists until 6 hours (Table 2).

Effects of root bark extract on fasting blood glucose levels in diabetic rats after 7 days

Blood glucose levels were reduced significantly (*P<0.05)* after administration of extracts orally for 7 days. The oral daily administration of vehicle (10 ml/kg/day) did not any significant effect in diabetic rats. Further, treatment with root bark extract at the dose of 100 mg/kg/day, p.o. significantly reversed the permanent

hyperglycemia. The highest anti-hyperglycemic effect was observed by the EtOH extract of root bark at 100 mg/kg. The EtOH extract of root bark was 12.5% more potent than standard oral hypoglycemic drug, glibenclamide 0.2 mg/kg b.w. (Table 3).

Acute oral toxicity of B. spectabilis extracts

The LD_{50} for *B. spectabilis* root bark extract was found to be >5000mg/kg (p.o.) in Albino Wistar rats. Morbidity and sign of toxicity was not observed in any of the normal rats tested with root bark extract.

Table 1. Antihyperglycemic effect of *B. spectabilis* extracts in glucose loaded normal hyperglycemic rats.

Treatment	Mean blood glucose concentration ±SEM (mg/dl)						
	0 min	30 min	60 min#	90 min	120 min	240 min	360 min
Control	93.27±1.22	101.5±1.12	98.47±1.30	161.1±1.99	135.3±1.24	111.2±1.25	98.97±1.73
Glib. (0.2 mg/kg)	95.93±1.57	81.26±1.72***	60.15±1.39***	97.94±1.76***	83.15±1.69***	62.98±2.07***	75.17±1.49***
R.B.E.(50 mg/kg)	94.79±2.32	99.83±1.05	92.16±1.33	152.1±5.31	141.1±6.14	103.1±3.19	97.69±3.99
R.B.E.(100 mg/kg)	95.91±1.48	94.99±2.66	90.28±2.10	132.1±2.27***	123.0±3.72	98.26±2.88	93.45±1.23
R.B.E.(200 mg/kg)	97.67±2.27	98.81±1.42	95.97±2.49**	148.8±2.45	139.5±6.92	104.5±2.69	99.53±3.19

SEM - Standard error of the mean; N=6, # Glucose load (4g/kg), *p<0.05, significantly different compared to control, **p<0.01, significantly different compared to control, ***p<0.001, significantly different compared to control, Glib. - Glibenclamide, R.B.E. - Root bark extract of *Bougainvillea spectabilis*

Table 2. Antihyperglycemic effect of *B. spectabilis* extracts in diabetic rats up to 6 hours.

Treatment	Mean blood glucose concentration ±SEM (mg/dl)					
	0 min	30 min	60 min	120 min	240 min	360 min
Diabetic Control	321.3±2.65	324.1±6.19	319.9±3.68	311.5±2.57	316.6±2.17	312.3±2.99
Glib. (0.2 mg/kg)	313.9±4.10	297.3±4.66**	284.3±1.79***	261.2±6.33***	241.9±3.91***	201.9±4.12***
R.B.E.(50 mg/kg)	319.9±3.24	321.2±3.95	307.7±3.96	269.1±3.88***	269.3±2.60***	229.7±4.69***
R.B.E.(100 mg/kg)	313.7±2.08	310.6±3.93	283.3±5.66*	265.6±3.74***	252.1±5.21***	211.3±4.43***
R.B.E.(200 mg/kg)	309.6±6.23	321.1±4.63	297.9±2.78	281.3±4.91**	263.5±8.29***	236.2±1.97***

SEM: Standard error of the mean; N=6, *p<0.05, significantly different compared to control, **p<0.01, significantly different compared to control. ***p<0.001, significantly different compared to control, Glib.- Glibenclamide, R.B.E. - Root bark extract of *Bougainvillea spectabilis*

Table 3. Antihyperglycemic effect of *B. spectabilis* extract in diabetic rats up to 7 days.

Treatment[#]	Mean blood glucose concentration ±SEM (mg/dl)				
	0 day	**1st day**	**3rd day**	**5th day**	**7th day**
Control	93.91.24	93.9±4.23	96.1±4.70	94.9±2.31	96.9±3.65
Diabetic control	325.9±3.60	319.2±1.59	321.7±1.99	318.9±3.11	322.4±2.35
Glib. (0.2 mg/kg)	323.7±4.21	295.3±9.68	223.6±11.74***	169.9±3.47***	93.6±1.01***
R.B.E. (50 mg/kg)	316.9±7.61	289.6±6.24*	236.3±4.65***	171.9±5.22***	87.2±4.23***
R.B.E. (100 mg/kg)	310.2±7.02	271.8±6.99**	229.1±3.32***	170.6±6.15***	81.9±2.23***
R.B.E. (200 mg/kg)	311.3±4.67	285.7±6.32	242.1±4.52***	175.7±5.96***	90.5±4.19***

#mg/kg/day for 7 days. S.E.M - Standard error of the mean; N=6, *p<0.05, significantly different compared to diabetic control. **p<0.01, significantly different compared to diabetic control, ***p<0.001, significantly different compared to diabetic control. Glib. – Glibenclamide, R.B.E. - Root bark extract of *Bougainvillea spectabilis*

Discussion

Diabetes was induced in albino Wistar rats by single intraperitoneal injection of alloxan monohydrate. Rats that manifest a high glucose level (>200 mg/dl) may have either type 1 or type 2 diabetes depending on the extent of pancreatic destruction (36). The EtOH extract of *B. spectabilis* root bark explicitly exhibited significant potent anti-hyperglycemic activity in diabetic rats, which significant, as compared to the control (alloxan treated) as well as glibenclamide treated group. However, there was no such effect observed with the extract in normal rats (data not shown).

Out of the various doses tested, 100 mg/kg of root bark extract was found to be the most effective. The comparable effect of the extract with glibenclamide may suggest similar mode of action, since alloxan monohydrate permanently destroys the pancreatic β-cells and the root bark extract lowered blood glucose level in alloxanised rats, indicating that the extract possesses extrapancreatic effects.

Conclusion

On the basis of data obtained, it can be concluded that ethanolic extract of root bark of *Bougainvillea spectabilis Wild*. have potential anti-hyperglycemic property. Permanent hyperglycemia induced by alloxan was reversed by root bark extract treatment up to week. Phytochemical screening showed the presence of flavonoids, cardiac glycosides, saponins and tannins in *B. spectabilis* root bark extract. Many reports suggested the hypoglycemic properties of flavonoids isolated from various plant sources (37-39) which may be the reason for antidiabetic properties of *B. spectabilis* root bark.

Acknowledgements

Our sincere thank to Mr. Harikesh Kalonia, University Institute of Pharmaceutical sciences, Punjab University, India. for his excellent technical support and I.T.S. Paramedical College (Pharmacy), Delhi-Meerut Road, Murad Nagar, Ghaziabad, Uttar Pradesh, India. for providing necessary facilities for carrying out the research work successfully.

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