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Hypoglycemic activity of *Bougainvillea spectabilis* stem bark in normal and alloxan-induced diabetic rats

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ABSTRACT

Objective: *Bougainvillea spectabilis* (*B. spectabilis*) is one of the main constituent of various herbal formulations available for diabetes. The aim of present study was to screen hypoglycemic potential of *B. spectabilis* stem bark extracts in albno rats (Wistar strain). **Methods:** The EtOH extracts (100, 250 and 500 mg/kg/day) of the *B. spectabilis* were administered to both normal and alloxan induced diabetic rats at defined time intervals. Blood glucose levels were measured at 0, 0.5, 1, 2, 4, 6 h, and on 0, 1, 3, 5, 7th day after oral administration of extracts. Of the doses test, highest anti-hyperglycemic effect was observed by the extract of stem bark at 250 mg/kg after a week treatment. **Results:** *B. spectabilis* stem bark extract exhibited significant hypoglycemic activity at different doses and intervals. Stem bark extract was found to be 22.2% more potent than standard oral hypoglycemic drug, glibenclamide 0.2 mg/kg. **Conclusion:** Treatment of alloxan induced diabetic rats up to a week with stem bark extract reversed the permanent hyperglycemia. Hence, *B. spectabilis* stem bark alcoholic extract exhibited potent hypoglycemic activity.

1. Introduction

Diabetes is a group of metabolic disorders characterized by increased blood glucose level resulting from defects in insulin secretion, insulin action, or both. The chronic diabetes is associated with long-term damage, dysfunction and failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels. Diabetes broadly classified in to two categories, type I (Insulin dependent or Juvenil) and type II diabetes (non insulin dependent or diabetes mellitus). Type II diabetes is much more prevalent which may be due to combination of resistance to insulin action and an inadequate compensatory insulin secretion. The incidence of type 2 diabetes mellitus is increasing worldwide. It may results from the interaction between a genetic predisposition and behavioral and environmental risk factors[1-4].

Diabetes is major causes of premature illness and death worldwide. The prevalence of diabetes is increasing in epidemic proportions[5]. World Health Organization predicted that developing countries like India will bear the brunt of this epidemic in the present century[6]. India has more than 50.8 million people with diabetes and projected to increase to 87 million by year 2030[7-9]. In developing countries allopathic medicines are expensive and not easily accessible. Herbal drugs are more popular due to lesser side effects and natural origin, hence explored for the discovery of potentially useful antihyperglycemic[10-15]. Therefore, the WHO recommended for continue research for antidiabetic leads from plants and other resources[16].

Bougainvillea spectabilis (*B. spectabilis*) may be a potential candidate for above objective as it is a part of various herbal formulations for diabetes[17]. *B. spectabilis* Wild commonly known as bougainvillea, great bougainvillea (family: Nyctaginaceae) (local Indian names: booganbel, cherei, baganbilas, booganvel, bouganvila, kagithala puvvu).

It possess various biological activities like hypoglycemic[18], cholesterol lowering effect[19], antibacterial[20], nematicidal[21], antifeedant and insecticidal[22], antiviral[23]

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and anti-inflammatory activities[24] were reported of *B. spectabilis*. Antidiabetic compound pinitol[25] isolation from leaves mimicked to study antidiabetic activity in stem bark of *B. spectabilis*.

2. Materials and methods

2.1. Collection and extraction of plant material

Stem (1.3 kg) bark of *B. spectabilis* Wild (Family-Nyctaginaceae), were collected in October 2009 from Charkhari, Mohaba, U.P., India. Plant identification done by the plant taxonomist Dr. A.K. Sharma, department of botany, Multanimal Modi (P.G.) College, Modinagar, Ghaziabad (U.P.), India. Voucher specimen (MMCM/02/013) was deposited in the herbarium of the department of botany, Multanimal Modi (P.G.) College, Modinagar, Ghaziabad (U.P.), India, for reference purpose. Stem bark of *B. spectabilis* was dried in shade and powdered in electric grinder. Powdered stem bark was soaked in ethanol (70%), placed on a mechanical shaker for 24 h, filtered. The ethanolic extract was concentrated at 45 ℃ and crude extract weighed about 132.5 g (=10%, w/w).

2.2. Phytochemical studies

Phytochemical investigations of EtOH extract of stem bark was carried out for the presence of flavonoids with metallic magnesium and HCl, tannins with Ferric chloride reagent, alkaloids with Dragendroff's reagent, Cardiac glycosides with Liberman's test and Killer killiani test, anthraquinones with Borntrager's test, saponins with ability to form foam, reducing sugars with Fehling's reagent, triterpenes and steroids with sulphuric acid reagent according to standard methods reported[26-27].

2.3. Experimental animals

Male albino Wistar rats (150-200 g) of age 8-12 weeks old were obtained from animal house (Reg. No. 1044/c/07/CPCEA), I.T.S Paramedical College (Pharmacy), Murad Nagar, Ghaziabad, Uttar Prasesh, India, for antihyperglycemic activity of extract. The animals were housed under standard conditions (25 ℃, 12 h light and 12 h dark cycle, 60% humidity), fed with rodent diet, water ad libitum, and acclimatized to the laboratory conditions for 6 days.

2.4. Estimation of blood glucose levels

Blood glucose concentration (mg/100 mL) was estimated using an Accu-Check active (Roche Diagnostic GmbH, Germany), based on the glucose oxidase method. Blood samples were withdrawn from the tip of tail at the defined time intervals.

2.5. Acute toxicity studies

B. spectabilis stem bark extract was tested for their acute and short- term toxicity (if any) in albino Wistar rats. For determining the acute toxicity of a single oral administration of the herbal drug, the OECD guidelines (OECD/OCDE 2001, 423, Annex 2c) were followed[28]. Stepwise doses of extracts administered from 300 mg/kg to 5 000 mg/kg b.w. orally. Rats were kept under observation continuously for the initial 4 h and intermittently for the next 6, 24, 48 h following drug administration. Parameters like grooming, hyperactivity, and sedation, loss of righting reflex, respiratory rate and convulsion were observed. No considerable signs of toxicity were observed in any of tested albino Wistar rats. On the basis of above acute toxicity study, doses 100, 250 and 500 mg/kg of extracts were selected for present study.

2.6. Induction of diabetes

Diabetes was induced in overnight fasted (12 h) rats by a single intraperitoneal injection of alloxan monohydrate (CDH, Bombay) in normal saline (120 mg/kg). The fasting blood glucose level was estimated after 48 h of injection. The rats with effective and permanent elevated plasma glucose levels (≥ 300 mg/100 mL) were selected for present study.

2.7. Effect of extracts on normal and glucose-loaded rats (NG-OGTT)

Oral glucose tolerance test was performed after overnight fasting (16 h) of normal rats. Vehicle (distilled water), EtOH extracts of the *B. spectabilis* stem bark (100, 250 and 500 mg/kg) and standard as glibenclamide (Daonil® Sanofi Aventis Pharma. Ltd. Mumbai, India) (0.2 mg/kg) were administered to six different groups of rats (n=6). Glucose (4 g/kg) was fed to normal rats after 60 min of treatment. Blood was withdrawn from the tip of tail at 0, 30, 60, 90, 120, 240 and 360 min from normal control and experimental rats and blood glucose level was measured.

2.8. Measurement of blood glucose level in diabetic rats up to 6 h

The hypoglycemic effect of *B. spectabilis* extract was carried on overnight fasted (16 h) diabetic rats. Distilled water, *B. spectabilis* stem bark extract (100, 250 and 500 mg/kg) and glibenclamide (0.2 mg/kg) were administered orally using gastric gavage needle to six different groups of diabetic rats. Blood samples were withdrawn at 0, 30, 60, 120, 240, and 360 min from the tip of tail of experimental animals and blood glucose level was measured.

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

Experimental animals were divided randomly in six groups of 6 rats each. Overnight fasted diabetic rats were treated orally with vehicle, EtOH extracts of *B. spectabilis* bark at doses of 100, 250 and 500 mg/kg/day and glibenclamide 0.2 mg/kg daily up to 7 days. Blood samples were collected at the defined time intervals from control and experimental animals. The effect of extract on body weight was also monitored up to 7 days.

2.10. Statistical analysis

Observed data are represented as means±S.E.M. Results are 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 $P<0.05$ were considered significant statistically.

3. Results

3.1. Phytochemistry of extracts

The EtOH extract of *B. spectabilis* stem bark showed the presence of cardiac glycosides, flavonoids, saponins, alkaloids, glycosides, steroids and tannins.

3.2. Effect of extract on body weight in diabetic rats

There was a significant ($P<0.05$) alteration in the body weight of the alloxan treated rats as compared to the control group. Oral administration of EtOH extract of stem bark up to 7 days, at doses of 100, 250 and 500 mg/kg, (p.o.), attenuated the change in the body weight ($P<0.05$) of the alloxan treated rats significantly comparable to the glibenclamide (Table 1).

3.3. Effect of extract on glucose tolerance test

There was no significant change in the blood glucose level of vehicle treated normal rats. Overnight fasted normal rats loaded with glucose (4 g/kg) orally, showed significant increase in blood glucose ($P<0.05$) after 30 min. Stem bark extract at the doses of 100, 250 and 500 mg/kg respectively, reduced the blood glucose level significantly ($P<0.05$) from

Table 1.
Effect of *B. spectabilis* extracts on body weight in diabetic rats ($n=6$).

Treatment#	Dose(mg/kg)	Body weight of rats in different groups (g)		Relative weight gain (%)
		Initial	7th day	
Control	–	173.2±2.68	173.6±4.04	0.23
Diabetic control	–	181.3±4.26	189.1±3.17*	4.30
Glibenclamide	0.2	163.4±3.52	164.8±3.27*	0.85
S.B. Ext.	100	177.4±4.63	180.3±3.94**	1.63
S.B. Ext.	250	162.5±2.49	164.1±2.78**	0.98
S.B. Ext.	500	171.6±3.99	174.3±5.62*	1.57

#mg/kg/day for 7 days. Values are expressed as mean±SEM; *$P<0.05$, significantly different compared to Initial, **$P<0.001$, significantly different compared to Initial, S.B. Ext. – Stem bark extract of *B. spectabilis*.

Table 2.
Antihyperglycemic effect of *B. spectabilis* extracts in glucose loaded normal hyperglycemic rats ($n=6$).

Treatment	Mean blood glucose concentration ± SEM (mg/dL)						
	0 min	30 min	60 min#	90 min	120 min	240 min	360 min
Control	98.40±2.51	100.30±1.89	99.70±1.63	161.30±2.06	136.80±1.63	111.20±1.84	100.20±1.39
Glib. (0.2 mg/kg)	96.90±1.34	84.60±1.79***	63.50±2.97***	94.40±1.82***	81.30±2.06***	65.30±1.56***	74.80±1.66***
S.B. Ext. (100 mg/kg)	92.80±4.86	96.30±1.99**	92.83±2.81	146.60±2.65	136.70±5.04	106.10±3.84	92.80±2.67
S.B. Ext. (250 mg/kg)	96.50±3.21	93.70±2.74**	89.80±1.68*	124.40±2.77**	116.40±4.43*	96.20±1.89**	89.40±2.69
S.B. Ext. (500 mg/kg)	94.70±2.96	98.58±2.08	90.40±1.79	132.60±2.93*	130.70±3.91	97.60±1.22	93.60±4.71

SEM – Standard error of the mean; #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, S.B. Ext. – Stem bark extract of *B. spectabilis*.

Table 3.
Antihyperglycemic effect of B. spectabilis extracts in diabetic rats up to 6 h ($n=6$).

Treatment	Mean blood glucose concentration ± SEM (mg/dL)					
	0 min	30 min	60 min	120 min	240 min	360 min
Diabetic control	306.70±6.98	310.90±4.65	315.60±2.57	313.70±3.49	316.40±3.19	308.40±3.67
Glib. (0.2 mg/kg)	311.20±3.47	299.30±5.09**	279.70±3.81***	254.70±5.39***	243.90±6.33***	203.30±5.81***
S.B.Ext.(100 mg/kg)	319.90±4.89	313.80±5.77	287.60±3.58*	267.80±4.53***	240.20±5.17***	211.30±5.73***
S.B.Ext.(250 mg/kg)	316.50±5.36	310.60±3.78	275.70±6.35*	253.90±4.29***	235.90±4.59**	206.20±4.12***
S.B.Ext.(500 mg/kg)	319.80±3.62	321.70±4.69	307.60±5.29	268.50±4.63**	260.80±4.39**	230.90±4.56***

SEM: Standard error of the mean; *$P<0.05$, significantly different compared to control, **$P<0.01$, significantly different compared to control. ***$P<0.001$, significantly different compared to control, Glib.– Glibenclamide, S.B. Ext. – Stem bark extract of *B. spectabilis*.

Table 4.
Antihyperglycemic effect of *B. spectabilis* extracts in diabetic rats up to 7 days (n=6).

Treatment#	Mean blood glucose concentration ± SEM (mg/dL)				
	0 day	1st day	3rd day	5th day	7th day
Control	94.80±3.27	96.80±2.13	96.30±3.33	98.80±2.52	96.60±2.69
Diabetic control	321.60±1.68	316.90±2.95	315.60±3.54	317.10±2.32	320.40±2.44
Glib. (0.2 mg/kg)	316.50±4.10	298.50±13.27	221.50±5.29***	174.50±9.34**	93.60±3.32***
S.B. Ext. (100 mg/kg)	310.60±7.66	279.40±6.05**	229.30±5.38**	159.70±3.92**	78.30±2.91***
S.B. Ext. (250 mg/kg)	315.20±5.56	276.70±6.59*	225.80±3.39***	148.30±6.36***	72.80±3.63***
S.B. Ext. (500 mg/kg)	312.90±5.84	284.60±4.17*	240.20±5.73**	170.90±6.28**	85.80±2.78***

#mg/kg/day for 7 days. S.E.M – Standard error of the mean; *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, S.B. Ext. – Stem bark extract of *B. spectabilis*.

after 30 min, comparable to glibenclamide (0.2 mg/kg) (Table 2).

3.4. Effects of extract in diabetic rats up to 6 h treatment

Oral administration of vehicle (10 mL/kg) did not change the level of basal blood glucose significantly. Glibenclamide (0.2 mg/kg) was found to be highly effective and decreased blood glucose level more than doses of the extracts used in diabetic rats. After 6 h, glibenclamide (0.2 mg/kg) significantly reduced the blood glucose from 311.20±3.47 to 203.30±5.81 mg/dL (P<0.05). As compare to control, the oral administration of EtOH extracts of stem bark of *B. spectabilis* (100, 250 & 500 mg/kg) induced a significant decrease of blood glucose in diabetic rats (P<0.05) (n=6). Stem bark extract at the dose of 250 mg/kg was found to be most effective of the doses tested. The potency of these extracts was similar to glibenclamide and hypoglycemic effect persists until 6 h (Table 3).

3.5. Effects of extract in diabetic rats up to 7 days treatment

Oral administration of vehicle (10 mL/kg/day) did not any significant effect on the level of blood glucose in diabetic rats. Glucose level in blood was reduced significantly (P<0.05) after oral administration of extracts up to 7 days. Further, treatment with stem bark extract at doses 100, 250 & 300 mg/kg/day, p.o. reversed the permanent hyperglycemia significantly in alloxan induced diabetic rats. Highest antihyperglycemic effect was observed by the EtOH extract of stem bark at 250 mg/kg/day and found to be 22.2% more potent than glibenclamide 0.2 mg/kg b.w. (Table 4).

3.6. Acute oral toxicity of extract

LD50 for *B. spectabilis* stem bark extract were found to be >5 000mg/kg (p.o.) in albino Wister rats. No morbidity and sign of toxicity was observed in any of the normal rats tested with extracts.

4. Discussion

In normal and subdiabetic rats, the extent of improvement of glucose tolerance by the EtOH extract of *B. spectabilis* stem bark is comparable to that produced by glibenclamide. The EtOH extract of the stem bark explicitly exhibited significant potent antihyperglycemic activity in diabetic rats, which significant, as compared to the control (alloxan treated) as well as glibenclamide treated group. However, there was no hypoglycemic activity observed with stem bark extract in normal rats (data not shown). The change in body weight of diabetic rats is also attenuated by EtOH extracts similar to glibenclamide.

Of the doses tested, 250 mg/kg of stem bark extracts was found to be the most effective. The comparable effect of the extract with glibenclamide may suggest similar mode of action, since alloxan permanently destroys the pancreatic β-cells and the extract lowered blood glucose level in alloxan induced diabetic rats, indicating that the extract possesses extrapancreatic effects.

Statistical analysis of observations suggests that alcoholic extracts of stem bark of *B. spectabilis* Wild. have potential anti-hyperglycemic properties. Treatment up to week with these extracts reversed the permanent hyperglycemia. Phytochemical screening showed the presence of cardiac glycosides, flavonoids, saponins and tannins in the stem bark extracts of *B. spectabilis*. Many researchers documented the hypoglycemic properties of flavonoids[29-33]. This may be the reason for antidiabetic properties of *B. spectabilis* bark.

Conflict of interest statement

We declare that we have no conflict of interest.

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