Antidiabetic activity of stem bark of *Bauhinia variegata* in alloxan-induced hyperglycemic rats

Sir,

Diabetes mellitus, a chronic metabolic disorder of insulin deficiency or ineffectiveness, constitutes a global public health burden and predictions estimate that India, China, and United States will have the largest number of diabetic people by the year 2030.[1] The search for plant-based products for control of diabetes mellitus continues, and the World Health Organization (WHO) has also long back recommended herbal treatment of diabetes mellitus.[2] *Bauhinia variegata* Linn (Family: Fabaceae), vernacularly called Kachnara, is an herbaceous medicinal plant, found throughout India. The leaves of the many *Bauhinia* species are used in antidiabetic treatments by many populations of the world.[3] In India, stem bark is used as an antidiabetic in the Ayurvedic system of medicine.[4] In a recent *in-vitro* study, the ethanolic extract of *B. variegata* and its major constituent, roseoside, have demonstrated enhanced insulin release from the beta-cell lines INS-1.[5] In view of these facts, this work studied the influence of the stem bark of *B. variegata* on alloxan-induced hyperglycemia in rats.

The stem barks of *B. variegata* were collected from the campus of Jiwaji University, Gwalior, in Apr. 2010 and authenticated by a Taxonomist of the Institute. The dried stem barks were powdered and defatted with petroleum ether (60–80°C) in a Soxhlet apparatus. The marc was then subjected to cold extraction with ethanol and water (50:50) thrice. The resultant mixture was filtered, and the filtrate was then concentrated to dryness in a *Buchi* type rotary evaporator to get the hydro-alcoholic extract (BVBE). BVBE was subjected to preliminary phytochemical screening by standard conventional methods which revealed the presence of carbohydrates, volatile oils, phenolic compounds, flavonoids, and alkaloids.

Healthy adult Wistar albino rats (200–250 g) of either sex between 2 and 3 months of age, raised at Central Animal Facility of the Institute under standard housing conditions, were used. Animals were provided standard pellet diet (Ashirwad Brand, Chandigarh) and water *ad libitum*. The studies were approved by Institutional Animal Ethics Committee.

Rats were divided into different groups ($n = 5$) in each study. All normoglycemic and hyperglycemic rats orally received vehicle of the extract (distilled water) or BVBE (200 and 400 mg/kg) or metformin (500 mg/kg) as a reference standard for 7 days. In all group of animals, the blood collection was done from retro-orbital plexus under light ether anesthesia[6] and fasting serum glucose levels were estimated by a semi-auto analyzer using a Glucose Oxidase-Peroxidase glucose estimation kit (Span Diagnostic Ltd., Surat, India).[7]

In a normoglycemic study, the blood glucose was estimated as above on 0, 1, 2, 3, and 24 h, third day and seventh day after drugs administration. In the oral glucose tolerance test in normal rats, glucose (4 g/kg) was fed orally, 3 h after the last dose of extract/vehicle on day 7[8] and blood glucose was estimated at 0, 30, 60, and 120 min of glucose administration. The hyperglycemia was induced in fasted rats by single intravenous dose of alloxan monohydrate (CDH Chemicals, Mumbai, India) 65 mg/kg[9] and diabetes was confirmed 48 h after alloxan administration. In alloxan hyperglycemic rats, blood glucose was estimated at 0, 1, 2, 3, and 24 h, third day and seventh day after administration of drugs. All the data were analyzed with two-way ANOVA followed by the Bonferroni multiple comparison test. A value of $P < 0.05$ was considered significant in all cases.

Table 1: Effect of BVBE on the oral glucose tolerance test

Groups	Blood glucose (mg/dl)			
	0 min	30 min	60 min	120 min
Vehicle	117.60 ± 5.50	197.20 ± 6.19	175.20 ± 4.85	143.20 ± 4.93
BVBE 200	104.200 ± 3.53	168.000 ± 7.09*	145.400 ± 6.30*	113.80 ± 7.91*
BVBE 400	102.00 ± 4.02	155.00 ± 6.80@	124.60 ± 12.56@	110.00 ± 11.73#
Metformin	122.40 ± 5.68	198.00 ± 7.18@	122.00 ± 2.49@	95.06 ± 8.56@

Results are expressed as mean ± SEM (n = 5 rats), *$P < 0.05$, #$P < 0.01$, @$P < 0.001$ compared to vehicle, BVBE - Hydro-alcoholic extract of Bauhinia variegata

Table 2: Effect of BVBE on alloxan-induced hyperglycemia

Groups	Blood glucose (mg/dl)						
	0 h	1 h	2 h	3 h	24 h	Day 3	Day 7
Saline	101.20 ± 7.35	103.80 ± 1.56	98.42 ± 4.98	96.80 ± 4.89	107.34 ± 5.68	115.00 ± 2.28	107.74 ± 3.21
Alloxan + Vehicle	302.60 ± 9.29#	303.60 ± 6.95#	299.80 ± 9.18#	285.40 ± 6.31#	294.00 ± 9.54#	296.00 ± 8.23#	293.40 ± 9.44#
Alloxan + BVBE 200	290.00 ± 9.30	194.00 ± 3.03*	140.60 ± 11.19*	130.20 ± 8.66*	124.20 ± 6.06*	116.00 ± 4.46*	103.00 ± 2.37*
Alloxan + BVBE 400	287.80 ± 10.01	216.00 ± 8.42*	192.00 ± 7.75*	122.20 ± 5.52*	119.00 ± 5.55*	108.20 ± 3.73*	102.20 ± 3.81*
Alloxan + Metformin	308.80 ± 9.57	280.20 ± 5.70	261.80 ± 6.19@	252.20 ± 5.64@	236.80 ± 5.36*	226.60 ± 10.66*	132.20 ± 9.19*

Results are expressed as mean ± SEM (n = 5 rats), #$P < 0.001$ compared to saline, @$P < 0.01$, *$P < 0.001$ compared to vehicle, BVBE - Hydro-alcoholic extract of Bauhinia variegata

In an acute toxicity study, BVBE was found to be safe upto the level of 2000 mg/kg. Data analysis revealed that BVBE (200 and 400 mg/kg) and metformin did not influence blood glucose in normal rats ($P > 0.05$) [data not shown], suggesting that BVBE, *per se,* has no hypoglycemic effect. Further, BVBE (200 and 400 mg/kg) and metformin have normalized the impaired glucose tolerance ($P < 0.01$–0.001) with observable reduction in glucose levels from 60 to 120 min after glucose load [Table 1]. This indicates the efficacy of the BVBE to suppress the elevated blood glucose levels. Alloxan injection induced significant hyperglycemia ($P < 0.001$) (glucose > 250 mg/dl) in 48 h of administration. BVBE ($P < 0.001$) and metformin ($P < 0.01$ to $P < 0.001$) treatment significantly reduced the blood glucose levels in hyperglycemic animals [Table 2]. The results revealed the maintenance of blood sugar levels in diabetic rats during the 7 days administration of BVBE throughout the study period. The glucose levels reduced with single dose on day 1 and decreased further after subsequent doses. The antihyperglycemic effect of BVBE was comparable to metformin. Metformin reduces both fasting and postprandial hyperglycemia by promoting insulin-mediated peripheral glucose utilization and metabolism in adipose tissues and skeletal muscles through up-regulation of glucose transporters, provided that some endogenous insulin is present. Although the precise mechanism of the hypoglycemic action of BVBE remains speculative, the extract may be acting like metformin, and/or improving insulin action at the cellular level. In previous studies, antidiabetic plants exhibited antihyperglycemic effects by enhancing the action of insulin or by increasing the glucose metabolism or glucose homeostasis in diabetic animals.[10] A similar mechanism may be accounted for the antihyperglycemic effect of BVBE. Previously, leaves' extract of *B. variegata* exhibited hypoglycemic effects due to the presence of insulin-like proteins.[11] However, no such hypoglycemic effect was observed with the bark extract, rather exhibited antihyperglycemic effect indicating the absence of insulin-like proteins in the barks. It is possible that one or more observed constituents may be mediating the antihyperglycemic effect of BVBE. In conclusion, BVBE exhibited significant antihyperglycemic effects which may be attributed to increased glucose metabolism.

Parveen Kumar, Shailesh Baraiya[1], S. N. Gaidhani[2], M. D. Gupta, Manish M. Wanjari

National Research Institute for Ayurveda-Siddha Human Resource Development, Gwalior 474 009, [1]School of Studies (Biochemistry), Jiwaji University, Gwalior 474 011, Madhya Pradesh, [2]Central Council for Research in Ayurveda and Siddha, New Delhi 110 058, India

Author for correspondence:
Manish M. Wanjari, National Research Institute for Ayurveda-Siddha Human Resource Development, (Under C.C.R.A.S, Department of AYUSH, Ministry of Health and Family Welfare) Aamkho, Gwalior 474 009, Madhya Pradesh, India.
E-mail: wmanish@yahoo.com

REFERENCES

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047-53.
2. WHO. World Health Organization Expert Committee on Diabetes Mellitus. Second report, Technical report series: Geneva; 1980.p. 646.
3. Cechinel Filho V. Chemical composition and biological potential of plants from the genus *Bauhinia*. Phytother Res 2009;23:1347-54.
4. Col Herber D. In: Useful plants of India. 2nd ed. Dehradun: Allied Book Center; 1991. p.75.
5. Frankish N, de Sousa Menezes F, Mills C, Sheridan H. Enhancement of insulin release from the beta-cell line INS-1 by an ethanolic extract of *Bauhinia variegata* and its major constituent roseoside. Planta Med 2010;76:995-7.
6. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother 2010;1:87-93.
7. Trinder P. Determination of blood glucose using an oxidase-peroxidase system

with a non-carcinogenic chromogen. J Clin Pathol 1969;22:158-61.
8. Zanatta L, de Sousa E, Cazarolli LH, Junior AC, Pizzolatti MG, Szpoganicz B, *et al*. Effect of crude extract and fractions from *Vitex megapotamica* leaves on hyperglycemia in alloxan-diabetic rats. J Ethnopharmacol 2007;109:151-5.
9. Frode TS, Medeiros YS. Animal models to test drugs with potential antidiabetic activity. J Ethnopharmacol 2008;115:173-83.
10. Zhang XF, Tan BK. Anti-diabetic property of ethanolic extract of *Andrographis paniculata* in streptozotocin diabetic rats. Acta Pharmacol Sin 2000;21:1157-64.
11. Azevedo CR, Maciel FM, Silva LB, Ferreira AT, da Cunha M, Machado OL, *et al*. Isolation and intracellular localization of insulin-like proteins from leaves of *Bauhinia variegata*. Braz J Med Biol Res 2006;39:1435-44.

Access this article online	
Quick Response Code:	Website: www.jpharmacol.com
	DOI: 10.4103/0976-500X.92518