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Opinion Article

Effect of chronic administration of *Boerhaavia diffusa* Linn. leaf extract on experimental diabetes in rats

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Abstract

***Purpose*:** *The root and aerial parts of Boerhaavia diffusa Linn. (Nyctaginaceae) were used in Ayurveda for the treatment of diabetes. The present study is aimed at evaluating the antidiabetic activity of chloroform extract of Boerhaavia diffusa leaves on chronic administration in streptozotocin-induced non-insulin-dependent diabetes mellitus (NIDDM) model diabetic rats.*

Methods: *The blood glucose lowering activity of the leaf extract was studied in streptozotocin-induced (65 mg/kg, i.v.) NIDDM model diabetic rats after oral administration of the extract at daily doses of 50, 100 and 200 mg/kg body weight for four weeks and compared with glibenclamide. Blood samples were collected from the tail vein before and also at weekly intervals for four weeks from the first dose of drug administration and blood glucose was analyzed by glucose-oxidase method using a visible spectrophotometer.*

Results: *The leaf extract of B. diffusa produced dose-dependent reduction in blood glucose in streptozotocin-induced NIDDM rats comparable to that of glibenclamide. The results indicate that the reduction in blood glucose produced by the extract is probably through rejuvenation of pancreatic β-cells or through extrapancreatic action.*

Conclusion: *The chloroform extract of Boerhaavia diffusa has significant antidiabetic activity and this supports the traditional usage of the plant by Ayurvedic physicians for the control of diabetes.*

Key words: *Blood glucose, Boerhaavia diffusa, Diabetes mellitus Streptozotocin, Rats*

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Introduction

Diabetes mellitus, an endocrine disorder, is characterized by hyperglycemia and affects a large number of people worldwide. By the year 2010, the total number of people worldwide with diabetes is projected to reach 239 millions[1]. In modern medicine, no satisfactory effective therapy is still available to cure diabetes mellitus[2]. Currently available therapeutic options such as dietary modification, oral hypoglycemics and insulin have limitations of their own in treating non-insulin dependent diabetes mellitus (NIDDM)[3-4]. Therefore, the search for more effective and safer hypoglycemic agents has continued to be an area of active research. The World Health Organization has recommended the evaluation of the effectiveness of medicinal plants in condition where the conventional allopathic treatment of diabetes is not adequate[5-6].

In the indigenous Indian system of medicine (Ayurveda), many herbal medicines have been recommended for the treatment of diabetes or 'madhumeha' and some of them have been experimentally evaluated. *Boerhaavia diffusa* Linn. (Nyctaginaceae) is a small perennial creeping herb, commonly known as "Red hogweed" and distributed widely all over in India, and in many other countries. The root and the whole plant are used as an Ayurvedic medicine in India and Unani medicine in Arab countries for the treatment of diabetes, stress, dyspepsia, abdominal pain, inflammation, jaundice, enlargement of spleen, congestive heart failure and bacterial infections[7-11]. The plant is known to possess anti-inflammatory[12-13], anticonvulsant[14], antifibrinolytic[15], diuretic[16], hepatoprotective[17-20] and immunomodulatory[21-22] activities. It has also been reported to be useful in the treatment of elephantiasis, night blindness, corneal ulcers and nephritic syndrome[23-26].

Recently, the aqueous leaf extract of the plant has been studied for its antidiabetic effect in alloxan-induced diabetic rats[27-28]. The present investigation is aimed at evaluating the antidiabetic activity of the chloroform extract of the plant leaves on chronic treatment of streptozotocin-induced NIDDM model diabetic rats.

Experimental

Chemicals

Glibenclamide was a generous gift sample from Hoechst Pharmaceuticals, Mumbai, India. Glucose assay kit was obtained from the Diagnostic Division of Dr. Reddy's Laboratories, Hyderabad, while streptozotocin was purchased from Sigma-Aldrich, St. Louis, USA.

Plant Material and Extraction

Fresh leaves of *B. diffusa* were purchased from local traders in Visakhapatnam, India and shade dried for about two weeks. The leaves were botanically authenticated and a voucher specimen has been preserved for future reference. The dry leaf powder (3 kg) was extracted with chloroform in Soxhlet apparatus for 24 hr and the extract was evaporated to dryness under vacuum in a vacuum desiccator (268 g).

Animals

Adult male Sprague-Dawley rats (National Institute of Nutrition, Hyderabad) weighing 250-300 g were used in the study. They were divided into 6 groups of six each and were provided with standard pellet diet (Ratan Brothers, Hyderabad) and water *ad libitum*. All rats were acclimatized to the laboratory conditions for at least 10 days prior to the experiment and were maintained in a well-ventilated animal house with 12-hr light and dark cycle. As appropriate, the animals were fed with the standard pellet diet throughout the experiment, except for 12 hr prior to blood collection when only water was given.

The experimental protocol was been approved by the Institutional Animal Ethics Committee and by the animal regulatory body of the India government (Regd. No. 516/01/A/CPCSEA).

The rats assigned to groups I to V were rendered diabetic by injecting streptozotocin (65 mg/kg, i.v.; dissolved in 0.1 *M* citrate buffer; pH 4.5) after a baseline blood glucose estimation was done. After 2 to 3 days, the condition of diabetes was assessed by periodic examination of blood glucose using glucose-assay sticks. Only animals with stable blood glucose above 300 mg/dl were selected for the study.

B. diffusa extract, in the form of a suspension in 0.1% sodium CMC at daily doses of 50, 100 and 200 mg/kg body weight, were administered orally to the rats in groups I, II & III, respectively for 4 weeks. Rats in group IV received a similar treatment with glibenclamide (25 µg/kg) daily for 4 weeks while the rats in groups V and VI received 3 ml of 0.1% sodium carboxymethylcellulose and were used as diabetic and normal controls, respectively.

Collection of Blood and Analytical Procedure

Blood samples were collected before and also at weekly intervals for 4 weeks from the from the tail vein of the rats from the first dose of drug administration. Glucose-Oxidase assay method[29-30] was used to determine the blood glucose.

Data and Statistical Analysis

Data are expressed as means ± standard error of means of at least triplicate determinations. Statistical analysis was carried out using one-way analysis of variance (ANOVA) and Dunnett's *t*-test. At 95% confidence interval p values less than 0.05 were considered to be significant.

Results and Discussion

The present study was conducted to study the antidiabetic activity of *B. diffusa* in rats to provide scientific evidence for its traditional usage in the control of diabetes. From the results obtained, it is obvious that chronic administration of *B. diffusa* extract to streptozotocin-induced NIDDM rats produced blood glucose reduction in a dose-dependent manner. To determine whether there was a statistically significant difference in hypoglycemia achieved by the three doses (50, 100 and 200 mg/kg, p.o.) chronically administered, the data were compared by using one-way analysis of variance and the individual groups were compared with control group using Dunnet's *t*-test. The extract of *B. diffusa* produced a significant reduction in blood glucose after 1

Table 1: Percentage blood glucose reduction produced by *B. diffusa* after chronic oral administration in streptozotocin-induced NIDDM model rats

	Percent Blood Glucose Reduction (n=6)			
Rats	Week 1	Week 2	Week 3	Week 4
Normal Control	-9.65 ± 1.20	4.05 ± 0.84	7.58 ± 2.59	-4.36 ± 1.64
Diabetic Control	-10.35 ± 9.81	-15.31 ± 16.58	-18.93 ± 10.02	-20.33 ± 19.35
Treated with 50 mg/kg of extract	4.34 ± 2.43	9.56 ± 4.66	13.82 ± 8.57*	16.30 ± 4.56
Treated with 100 mg/kg of extract	10.32 ± 8.08*	18.60 ± 9.89*	24.51 ± 8.24***	29.98 ± 6.94*
Treated with 200 mg/kg of extract	25.37 ± 10.50*	28.32 ± 8.68**	33.04 ± 7.13***	38.63 ± 9.46**
Treated with 25 µg/kg glibenclamide	66.20 ± 9.58***	60.60 ± 7.93***	65.74 ± 8.22***	59.01 ± 9.89***

*Values are means ± S.E.M; n = number of animals; *p<0.05; **p<0.01; ***p<0.001.*

week of treatment and increased furthermore at the end of 4th week by 30% ($p<0.01$) and 38.6% ($p<0.001$) at doses of 100 and 200 mg/kg body weight, respectively although the lowest dose (50 mg/kg) tested could produced significant change only after 3 weeks of treatment (Table 1). Glibenclamide (25 µg/kg, administered orally, p.o.) produced a significant reduction ($p< 001$) compared to diabetic control at the end of 1st week (66.2%). The results obtained in this study are in good agreement with those observed by previous investigators in alloxan-induced diabetic rats[27-28].

Streptozotocin, a betacytotoxin induces a "chemical diabetes" in a wide variety of animal species through a massive destruction of β-cells of the islets of Langerhans and resulting in reduced synthesis and release of insulin[32]. It is well established that sulphonylureas produce hypoglycemia by increasing the secretion of insulin from pancreas and by increasing the glycogen deposition in the liver[33,34]. These compounds are active in mild streptozotocin-induced diabetes whereas they are inactive in intense streptozotocin diabetes (nearly all β-cells have been destroyed). Since our results showed that glibenclamide reduced blood glucose levels in hyperglycemic animals, the state of diabetes is not severe[35,36]. Streptozotocin-treated animals receiving the leaf extract of *B. diffusa* showed rapid normalization of blood glucose levels in comparison to control and this could be due to the possibility that some β-cells were still active and were acted upon by *B. diffusa* to exert their insulin releasing effect. This suggests that the mode of action of the active ingredients of *B. diffusa* is probably mediated by an enhanced secretion of insulin, like sulphonylureas. Earlier investigations [27-28] also revealed an insulin releasing mechanism by the aqueous leaf extract of *B. diffusa* and lend support for the results observed in this study. However, the possibility of enhanced tissue glucose utilization by *B. diffusa* cannot be ruled out.

Further work on fractionation, purification, identification of active principle(s) and detailed mechanistic evaluation on the leaves of *B. diffusa* is yet to be carried out.

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