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Efficacy of aqueous extract of *Stevia rebaudiana* bertoni leaves in rats with streptpzotocin induced diabetes mellitus

M.B. Rashed, M. Mostofa, M.A. Hossain and F. Yasmin
Department of Pharmacology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

Abstract

Medicinal plants are becoming very popular for the treatment of different diseases all over the world. Some medicinal plants are being used for the treatment of diabetes. The present study investigated the effects of Stevia (*Stevia rebaudiana* Bertoni) leaves in terms of their hypoglycemic activity and effects of Glimepiride (800μg/kg) considered as a standard drug. The effects of aqueous extracts of Stevia (*Stevia rebaudiana* Bertoni) leaves was evaluated in the Streptozotocin (STZ; 55 mg/kg b. wt., as single intraperitoneal injection) induced diabetic rats and for this, test extracts were administered orally once a day, after 15 days of STZ-dosing, at 1ml/kg, 2ml/kg and 3ml/kg b. wt. dose levels for 3 weeks. Changes in the blood glucose levels and body weights were measured and the results were compared statistically with control group by using Student's unpaired t-test. The aqueous extracts of Stevia (*Stevia rebaudiana* Bertoni) leaves, like Glimepiride although less, showed significant ($p<0.05$) hypoglycemic effect and extracts at 3ml/kg b. wt. showed more potent effects. The extracts also nonsignificantly ($p>0.05$) reduced body weight, as compared to the significant ($p<0.001$) increasing body weight effect of Glimepiride. From this experiment it was observed that Stevia (*Stevia rebaudiana* Bertoni) leaves extracts had hypoglycemic and body weight reducing effect. Detail study is needed for definite conclusion.

Keywords: *Stevia rebaudiana* Bertoni, Glimepiride, Hypoglycemic effect, Body weight, Diabetic rat

Introduction

The worldwide prevalence of diabetes is increasing at such a rapid pace that the World Health Organization (WHO) has identified diabetes as an epidemic condition (King and Rewwers, 1991.). An estimate by WHO says that there will be about 250 million cases of diabetes mellitus throughout the world by 2025 (Friedman, 2002). Bangladesh ranks tenth among the diabetic populations of 40 countries in 2000 but its incidence is so much that in 2030 it will occupy seventh position. Statistical data in Bangladesh showed the gradual increase in the number of diabetic patients. When the Diabetic Association of Bangladesh (DAB) was founded in 1956, only 39 patients were registered, but by 31st December, 1985, the figure was increased to 49,510 (Karim *et al.*, 1986), and 28th August, 1992 about 1,12,295 diabetic patients registered in Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic (BIRDEM, 1992). Ethnobotanical studies of traditional herbal remedies used for diabetes around the world have been identified more than 1,200 species of plants with hypoglycemic activity (Marles and Farnsworth, 1995). Herbal agents like Stevia (*Stevia rebaudiana* Bertoni) leaf is known to have hypoglycemic properties (Jain and Vyas, 1974; ICMR, 1987). The study of such agents might offer a natural key to unlock a diabetologist's pharmacy for future.

The most common endocrine disorder, representing a global health problem, is Diabetes mellitus. It is a heterogeneous syndrome rather than a single disease entity in the human and pet animals; which have hyperglycemia as the hallmark (Zimmet, 1997). This systemic illness results due to a relative or absolute deficiency of insulin action on the blood sugar. Regarding its treatment; a suitable drug is yet to be available which can permanently cure this disease. Insulin has been used successfully in insulin- dependent diabetes mellitus. But it can not be given orally, daily intake through injection is obviously troublesome and hypoglycemic reactions as an adverse effect may occur in any diabetic patient treated with it. Again insulin resistance is another drawback for patients taking it for a long period (Larner, 2001). On the other hand, oral hypoglycemic agents such as glimepiride, glibenclamide etc also have some adverse effects.

A multitude of traditional medicinal plants are being used throughout the world for a range of diabetic presentations (Bailey and Day, 1989). It is an herbaceous perennial plant native to subtropical and tropical rainforest areas of South America (Brazil, Venezuela, Colombia and Paraguay). The leaves are used traditionally in various regions of the world including China, Japan, Korea, Taiwan, Thailand, Malaysia and Paraguay. The leaves have been known to contain 100 useful alkaloids among other pharmacologically active compounds. It has been using for prevention of diabetes and its anti-diabetic effect has been evaluated in diabetic animals in many countries and significant antihyperglycemic activity of the leaf extracts has been reported. However, its effects have not yet been investigated in Bangladesh.

In this study the effect of aqueous extracts of *Stevia rebaudiana* Bertoni leaves on fasting blood glucose (FBG) and body weight were compared to glimepiride in streptozotocin induced diabetic rats.

Materials and Methods

Young Stevia plants were collected from BRAC nursery at Joydevpur, Gazipur. These were planted in tobs and were reared for about three months on the roof of the Building-2, Faculty of Veterinary science, Bangladesh Agricultural University, Mymensingh.

Glimepiride (Amiryl®), Aventis Pharma, Dhaka, Bangladesh and Streptozotocin (Sigma Chemical Company, USA) were used as standard / control drug and diabetes inducers, respectively. All other reagents used were of analytical grade. A total number of 30 albino rats of either sex (120-140 g) obtained from ICDDR'B, Dhaka and used in the study. They were divided into six groups (A to E) of five rats each and were fed with standard rat diet and water ad libitum. They were kept in cages and maintained in well-ventilated room under conditions of natural light and dark cycle for a period of three weeks to acclimatize them prior to experimental uses. Animals were fasted for 18 h prior to drug administration allowing access only to water. After fasting for 18 h, rats of group (B-E) were rendered diabetic by injecting a freshly prepared solution of streptozotocin (55 mg/kg i.p.) after a base-line glucose estimation. After 2 weeks, blood glucose content was measured by using Accu-Check Advantage blood glucose system (strip method) using a blood sample from the tail vein of rats. When the condition of diabetes was established animals with blood glucose levels above 25.0 mmol/L were selected for the study. Fresh Stevia leaves collected from the garden were dried under shade for several days, groaned with Grinder machine to coarse powder. One grow of leaves samples were mixed with 10 ml distilled water, allowed to stay for whole night, and sieved to separate from coarse particles and was used for experiment purpose. Everyday new extract was prepared following the previous techniques. Glimepiride was dissolved in adequate amount of distilled water to make a solution with the concentration 800 μg/ml. Group A served as a nondiabetic control while groups B to E were rendered diabetic. Group B served as diabetic control. Group C, D and E were treated with the aqueous extract of *Stevia* leaves at 1 ml/kg, 2 ml/kg and 3 ml/kg, respectively for 21 consecutive days.. The *Stevia* leaves were freeze dried first and then dissolved in distilled water. Group F were rendered with diabetes and treated with glimepiride (0.05 mg/kg, p.o.) for consecutive 21 days. The reference drugs and aqueous-extracts were administered orally to the rats.

Blood samples were collected from tail vein of each rat of a group at day 0, 7, 14 and 21 of treatment. The samples were analyzed for blood glucose content by using Accu-Check Advantage blood glucose system (strip method). Body weights were also taken of each rat of a group with electric balance on the same day.

Statistical Analysis: Data was expressed as mean ± standard deviation of means. Statistical analysis was made by using Student's unpaired *t*-test and the values were considered statistically significant when $P < 0.05$. Statistical calculations and the graphs were prepared using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com).

Results and Discussion

The effects of the extracts of *Stevia rebaudiana* Bertoni on the fasting blood sugar and body weight were investigated in the control and streptozotocin-induced diabetic rats using glimepiride as standard antidiabetic agents. To determine whether there was a statistically significant difference in hypoglycemia and body weight achieved by the extracts on Day 0, 7, 14 and 21 day was compared with the control group, using Student's unpaired *t*-test.

A significant reduction ($p < 0.05$) in blood glucose 1.74, 4.21, 11.61 and 14.83 % was observed on the 7th day; 1.94, 13.03, 19.66 and 24.71 % was observed on the 14th day and 10.00, 20.31, 29.96 and 40.68 % was observed on the 21st day with the extracts of *Stevia rebaudiana* Bertoni at 1 ml/kg, 2 ml/kg, 3 ml/kg and glimepiride at 800 µg/kg of body weight, respectively. The mean blood glucose concentration of control, *Stevia rebaudiana* Bertoni extract and glimepiride treated rats on 7 days interval are shown in Table. 1.

Table 1. Blood glucose level changes affected by *Stevia rebaudiana* Bertoni after oral administration in streptozotocin-induced diabetic rats

Groups	Drug, dose and route (/kg b. wt. orally)	Pre-treatment	Post-treatment		
		Day 0	Day 7	Day 14	Day 21
A	Normal control	5.49±0.63	5.52±0.77 0.55%^{b}	5.57±0.85 1.46%^{b}	5.65±0.55 2.91%^{b}
B	Diabetic control	26.00±0.69$^{\#}$	26.10±0.54$^{\#}$ 0.38%^{b}	26.90±0.79$^{\#}$ 3.46%^{b}	27.63±0.81$^{\#}$ 6.27%^{b}
C	Aqueous extract of Stevia leaves @ 1ml	25.80±0.58^{NS}	25.35±0.76* 1.74%^{a}	25.30±0.5** 1.94%^{a}	23.22±0.51*** 10.00%^{a}
D	Aqueous extract of Stevia leaves @ 2ml	26.10±0.61^{NS}	25.00±0.81** 4.21%^{a}	22.70±1.00*** 13.03%^{a}	20.80±0.72*** 20.31%^{a}
E	Aqueous extract of Stevia leaves @ 3ml	26.70±0.55^{NS}	23.60±0.63*** 11.61%^{a}	21.45±0.89*** 19.66%^{a}	18.70±0.57*** 29.96%^{a}
F	Glimepiride solution @ 800 µg	26.30±0.71^{NS}	22.4±0.59*** 14.83%^{a}	19.80±0.68*** 24.71%^{a}	15.60±0.63*** 40.68%^{a}

Values are mean blood glucose changes (± S.D.M.) of five animals. Difference significance from control at corresponding intervals: $^{NS}p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***/\#}p < 0.001$. Difference in percent from control at corresponding intervals: a = decrease, b = increase.

In body weight a reduction of 0.68, 0.72 and 1.44 % was observed on the 7th day; 0.72, 2.24 and 3.79 % was observed on the 14th day and 3.24, 5.25 and 9.89 % was observed on the 21st day with the extracts of *Stevia rebaudiana* Bertoni at 1 ml/kg, 2 ml/kg, 3 ml/kg of body weight, respectively, that was nonsignificant ($p < 0.05$) statistically, while a significant ($p < 0.001$) gain of 10.47, 11.86 and 13.69 % was observed on the 7th, 14th and 21st day with glimepiride at 800 µg/kg of body weight, respectively. The mean body weight of control and *Stevia rebaudiana* Bertoni extract and glimepiride treated rats on 7 days intervals are shown in Table 2.

The aqueous extract of *Stevia rebaudiana* Bertoni produced reduction in blood glucose of diabetic rats. However, it was not found to be, nevertheless, better. Streptozotocin, a beta-cytotoxin, induces chemical diabetes through damage of insulin secreting cell (Elsner *et al.,* 2002). It is known that sulphonylureas like glimepiride, result hypoglycemia by increasing the secretion of insulin from the pancreas (Yallow *et al.*, 1960; Grodsky *et al.,* 1971) and these compounds are active in mild streptozotocin-induced diabetes whereas they are inactive in intense streptozotocin diabetes (nearly all b-cells have been destroyed). Since our results showed that glimepiride reduce the blood glucose levels in hyperglycemic animals, so it can be postulated that the state of diabetes is not severe. Streptozotocin-treated animals receiving the extracts of *Stevia rebaudiana* Bertoni showed rapid reduction of blood glucose levels in comparison to the control. This suggests that the mode of action of the active constituent (s) of *Stevia rebaudiana* Bertoni is probably mediated by an enhanced secretion of insulin from b-cells, like sulphonyl ureas. No histological studies were carried out to prove this. Again glimepiride enhance glucose metabolism and improved tissue sensitivity to the action of insulin (Clark and Matthews, 1996). The extracts of *Stevia rebaudiana* Bertoni may act through this way all. Other probable mechanisms by which the extracts of *Stevia rebaudiana* Bertoni lowered blood glucose levels in diabetic rats might be by increasing glycogenesis, inhibiting gluconeogenesis in the liver and muscles, or inhibiting the absorption of glucose from the intestine. The extract of *Stevia rebaudiana* Bertoni also reduced the body weight of diabetic rats. It may be due to inhibition of glucose absorption from the intestine.

Table 2. Body weight changes affected by *Stevia rebaudiana* Bertoni after oral administration in streptozotocin-induced diabetic rats

Groups	Drug, dose and route (/kg b. wt.)	Pre-treatment	Post-treatment		
		Day 0	Day 7	Day 14	Day 21
A	Normal control	136.20±5.24	136.36±5.33 0.12%^{b}	136.61±5.06 0.30%^{b}	136.92±5.22 0.53%^{b}
B	Diabetic control	124.00±7.11$^{\#}$	123.30±7.59$^{\#}$ 0.56%^{a}	122.50±8.20$^{\#}$ 1.21%^{a}	121.57±7.26$^{\#}$ 1.96%^{a}
C	Aqueous extract of Stevia leaves @ 1ml	125.15±8.16^{NS}	126.00±7.34^{NS} 0.68%^{a}	124.25±7.98^{NS} 0.72%^{a}	121.10±7.94^{NS} 3.24%^{a}
D	Aqueous extract of Stevia leaves @ 2ml	124.80±7.76^{NS}	123.90±7.95^{NS} 0.72%^{a}	122.00±7.48^{NS} 2.24%^{a}	118.25±6.98^{NS} 5.25%^{a}
E	Aqueous extract of Stevia leaves @ 3ml	125.40±8.08^{NS}	123.60±7.42^{NS} 1.44%^{a}	120.65±7.22^{NS} 3.79%^{a}	113.00±8.17^{NS} 9.89%^{a}
F	Glimepiride solution @ 800 µg	125.65±5.13^{NS}	138.80±5.37*** 10.47%^{b}	140.55±4.67*** 11.86%^{b}	142.85±4.96 *** 13.69%^{b}

Values are mean percent blood glucose reduction (± S.E.M.) of five animals. Difference significance from control at corresponding intervals: $^{NS}p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***/\#}p < 0.001$. Difference in percent from control at corresponding intervals: a = decrease, b = increase.

Our results have shown that the aqueous extract of *Stevia rebaudiana* Bertoni possesses blood glucose and body weight reducing effect in streptozotocin-induced hyperglycemic rats. The medicinal value of this plant was trialed in Bangladesh for first time and there was also lack of related works. Further detailed studies are needed to elucidate the mechanism of action of *Stevia rebaudiana* Bertoni leaves, the constituent and effective dose to elucidate the body weight reducing effect, and to observe side effects and adverse effects of it for taking as an antidiabetic drug in human being.

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