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Article *in* Bangladesh Journal of Veterinary Medicine · April 2009

DOI: 10.3329/bjvm.v6i2.2338

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COMPARATIVE EFFICACY OF POWDERED FORM OF STEVIA (*STEVIA REBAUDIANA* BERTONI) LEAVES AND GLIMEPIRIDE IN INDUCED DIABETIC RATS

M. H. Sumon, M. Mostofa, M. S. Jahan, M. E. H. Kayesh[1] and M. A. Haque[1]

Department of Pharmacology, [1]Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202

ABSTRACT

Now a day, various medicinal plants are becoming popular for the treatment of different diseases. Some medicinal plants are being used for the treatment of diabetes all over the world. The study was conducted to investigate the effects of powdered form of Stevia (*Stevia rebaudiana* Bertoni) leaves on blood glucose concentration and body weight in Streptozotocin induced diabetic rats and for its efficacy study with a patent drug, Glimepiride. The effects of powdered form of Stevia leaves was evaluated in the Streptozotocin (STZ; 55 mg/kg body weight as single intraperitoneal injection) induced diabetic rats and for this, powdered form of Stevia leaves was orally administered at three different dose rates of 150 mg/kg, 200 mg/Kg and 250 mg/Kg body weight, respectively once a day for 3 weeks. Changes in the blood glucose levels and body weights were measured and the data obtained were compared with that of Glimepiride statistically by using Student's unpaired t-test. The powdered form of Stevia leaves produced significant ($p<0.01$ or $p<0.05$) hypoglycemic effects on Streptozotocin induced diabetic rats in comparison with that of the standard drug, Glimepiride. Powdered form of Stevia leaves at a dose rate of 250 mg/Kg decreased body weight significantly ($p<0.01$ or $p<0.05$) in STZ-induced diabetic rats. From this study, it was observed that powdered form of Stevia leaves possessed both hypoglycemic and body weight reducing effects.

Key words: *Stevia rebaudiana* Bertoni, glimepiride, streptozotocin, diabetic rat

INTRODUCTION

The most common endocrine disorder in human and pet animals is Diabetes mellitus, a heterogeneous syndrome rather than a single disease entity which have hyperglycemia as the hallmark (Zimmet, 1997). Diabetes mellitus is a major health problem not only in urban but also in the rural areas of Bangladesh. This systemic illness occurs as a result of relative or absolute deficiency of insulin action on blood sugar. Although insulin has been used successfully in insulin-dependent diabetes mellitus but a suitable drug is yet to be available which can cure the disease permanently. Even insulin can not be given orally and daily intake through injection is obviously troublesome and insulin resistance is another drawback for patients taking it for a long period of time (Larner, 2001). On the other hand, oral hypoglycemic agents such as Glimepiride, glibenclamide etc. have some adverse effects and these are unavailable in rural area also. Now a day, various medicinal plants are becoming very popular for the treatment of different diseases in our country as well as all over the world. Ethnobotanical studies of traditional herbal remedies used for diabetes around the world have identified more than 1,200 species of plants with hypoglycemic activity (Bailey and Day, 1989). *Stevia rebaudiana* Bertoni is one of them which are 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 used for the treatment of diabetes and its anti-diabetic effect has been evaluated in diabetic animals in many countries and significant hypoglycemic activities of powdered form of Stevia (*Stevia rebaudiana* Bertoni) leaves have been reported. However, its effects have not yet been investigated in Bangladesh. Therefore, the present research work was undertaken to study the comparative efficacy of powdered form of Stevia leaves and Glimepiride on blood glucose concentration and body weight in Streptozotocin induced diabetic rats.

MATERIALS AND METHODS

The experiment was performed in the Department of Pharmacology, Bangladesh Agricultural University, Mymensingh for a period of 6 months from April to September 2007.

Collection and acclimatization of rats

Thirty mixed albino rats, Long Evan's strain (*Ratus norvegicus*) aged between 2–3 months and weighing between 150 – 200 gm were collected from International Center of Diarrheal Disease Research, Bangladesh (ICDDR,B). These rats were divided into six groups containing five rats in each group 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 hour prior to drug administration allowing access only to water.

Induction of diabetes

After 18 hours of starvation, body weight and blood glucose level of each rat were measured, and rats of different groups (B-F) were rendered diabetic by intraperitoneal injection of STZ (Sigma) dissolved in 0.1M sodium citrate buffer pH 4.5, at a dose rate of 55 mg/kg body weight (Chattopadhyay, 1999). Blood sample were collected from the tail vein of rats on 15^{th} day of Streptozotocin injection to determine the blood glucose level for confirming diabetic condition. Diabetic rats with blood glucose level above 25.0 m mol/L were selected for the study.

Collection and preparation of Stevia leaves powder

Young Stevia plants were collected from BRAC nursery at Joydevpur, Gazipur. After that these were planted in tobs and were kept for about three months on the roof of the Building-2, Faculty of Veterinary science, Bangladesh Agricultural University, Mymensingh. Fresh Stevia leaves were obtained from that garden.

Fresh Stevia leaves collected from the garden were oven dried. Then dried leaves were ground with the help of a Grinder machine, and from that ground sample 1 gm was mixed with 100 ml of distilled water. Everyday new preparation was prepared following the previous techniques.

Preparation of aqueous solution of Glimepiride (Amaryl®)

Tablets Amaryl® was collected from K.R. Market, BAU, Mymensingh. Each tablet contained 1.0 mg Glimepiride. Glimepiride was dissolved in adequate amount of distilled water to make a solution with the concentration of 800 µg/ml.

Effect on diabetic rats

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 powdered form of Stevia leaves orally at a dose rate of 150 mg/kg, 200 mg/kg and 250 mg/kg body weight respectively for 21 consecutive days. Rats of group F were treated with Glimepiride orally at a dose rate of 0.8 mg/kg body weight for 21 consecutive days.

Determination of blood glucose level and body weight

Blood sample were collected from the tail vein of rats on 15th day of Streptozotocin injection i.e., at day 0 (pre-treatment) and also at day 7, 14 and 21 during treatment with Stevia and glimepiide to determine the blood glucose level of individual animals. Estimation of blood glucose was done 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 (Steel and Torre, 1960).

RESULTS AND DISCUSSION

The effects of powdered form of Stevia leaves at three different dosages on blood glucose concentration and body weight in Streptozotocin induced diabetic rats were studied and these effects were compared with that of Glimepiride.

Effects of Stevia leaves and Glimepiride (Amaryl®) on blood glucose concentration in Streptozotocin induced diabetic rat

A significant reduction ($p < 0.05$) in blood glucose 4.55, 6.93, and 20.26 % was observed on the 7th day with the powdered form of Stevia leaves at 200 mg/kg, 250 mg/kg and Glimepiride at 800 µg/kg of body weight, respectively; 1.46, 6.29, 12.43 and 27.10 % was observed on the 14th day and 2.85, 8.98, 15.76 and 40.67 % was observed on the 21st day with the powdered form of Stevia leaves at 150 mg/kg, 200 mg/kg, 250 mg/kg and Glimepiride at 800 µg/kg of body weight, respectively. The mean blood glucose concentration of control, Stevia leaves powder and Glimepiride treated rats on the 7th, 14th and 21st day are presented in Table 1.

Table 1. Effect of powdered form of Stevia leaves and Glimepiride (Amaryl®) on blood glucose (m mol/L, mean±SD) in normal and STZ treated diabetic rats

Groups	Drug, dose and route (/kg bwt orally)	Pre-treatment	Post-treatment		
		Day 0	Day 7	Day 14	Day 21
A	Normal control	5.54±.43	5.57±0.57^{b} 0.54%^{c}	5.62±0.65^{b} 1.44%^{c}	5.71±0.35^{b} 3.10%^{c}
B	Diabetic control	26.10±0.49#	26.45±0.43^{b}# 1.34% ^{c}	26.89±0.59^{b}# 3.03%^{c}	27.14±0.61^{b}# 4%^{c}
C	Powdered form of Stevia leaves (150 mg)	26.00±0.41 NS	25.88±0.61^{a}NS 0.46% ^{c}	25.62±0.80^{a}* 1.46%^{c}	25.26±0.92^{a}** 2.85%^{c}
D	Powdered form of Stevia leaves (200 mg)	26.40±0.35 NS	25.20±0.53^{a}* 4.55%^{c}	24.74±0.69^{a}** 6.29%^{c}	24.03±0.71^{a}*** 8.98%^{c}
E	Powdered form of Stevia leaves (250 mg)	26.71±0.54 NS	24.86±0.56^{a}** 6.93% ^{c}	23.39±0.33^{a}*** 12.43%^{c}	22.50±0.91^{a}*** 15.76%^{c}
F	Amaryl® (Glimepiride) (800 µg)	26.65±.51 NS	21.25±0.39^{a} 20.26%^{c}***	19.44±0.48^{a} 27.10%^{c}***	15.96±0.43^{a} 40.67%^{c}***

Values are mean blood glucose changes (± S.D.M.) of five animals, NS = Non Significant, * =Significant at 5%, ** = Significant at 1%, ***/# = Significant at 0.1%, %^{c}= Changes in blood glucose level in percentage at every 7 days from day 0, a = decrease in blood glucose level, b= increase in blood glucose level.

Effects of Stevia leaves and Glimepiride (Amaryl®) on body weight in STZ induced diabetic rats

In case of body weight a reduction of 1.64, 1.15 and 1.44 % was observed on the 7th day; 2.94, 3.67 and 4.55 % was observed on the 14th day and 3.26, 5.01 and 8.68 % was observed on the 21st day with the powdered form of Stevia leaves at 150 mg/kg, 200 mg/kg, 250 mg/kg of body weight, respectively, that was nonsignificant ($p<0.05$) statistically, while a significant ($p< 0.001$) gain of 8.93, 10.25 and 12.00 % 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 leaves powder and Glimepiride treated rats on the 7th, 14th and 21st days are presented in Table 2.

The powdered form of Stevia leaves produced a reduction in the blood glucose concentration of diabetic rats. This finding got the support of Chang *et al.* (2005). However, it was observed that as hypoglycemic drug, Glimepiride (Amaryl®) was better, though powdered form of Stevia (*Stevia rebaudiana* Bertoni) leaves @ 250 mg/kg body weight showed very potent hypoglycemic efficacy, but comparatively less effective than Glimepiride.

It is known that sulphonylureas like Glimepiride, produce hypoglycemia by increasing the secretion of insulin from the pancreas 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) (Yallow *et al*., 1960; Grodsky *et al*., 1971). Since our results showed that Glimepiride reduce the blood glucose levels in hyperglycemic animals, so it can be postulated that the state of diabetes was not severe.

Table 2. Effects of powdered form of Stevia leaves and Glimepiride (Amaryl®) on body weight changes (%) in normal and STZ induced diabetic Rats

Groups	Drug, dose and route	Pre-treatment		Post-treatment	
		Day 0	Day 7	Day 14	Day 21
A	Normal control	142.25±5.54	142.40±5.63[b] 0.11%[c]	142.62±5.36[b] 0.26%[c]	142.92±5.52[b] 0.47%[c]
B	Diabetic control	130.00±7.39#	129.15±7.89[a] 0.65%[c]#	128.90±8.50[a] 0.85%[c]#	127.21±7.56[a] 2.15%[c]#
C	Powdered form of Stevia leaves 150mg/kg orally	131.15±8.46 NS	129.00±7.64[a]NS 1.64%[c]	127.30±8.28[a]NS 2.94%[c]	126.88±8.24[a]NS 3.26%[c]
D	Powdered form of Stevia leaves 200mg/kg orally	130.80±8.06 NS	129.30±8.25[a]NS 1.15%[c]	126.00±7.78[a]NS 3.67%[c]	124.25±7.28[a]NS 5.01%[c]
E	Powdered form of Stevia leaves 250 mg/kg orally	131.40±8.38 NS	129.51±7.72[a]NS 1.44%[c]	125.42±7.52[a]NS 4.55%[c]	120.00±8.47[a]NS 8.68%[c]
F	Amaryl® (Glimepiride) tablet 800 µg/kg orally	131.65±5.43 NS	143.40±5.67[b] 8.93%[c]***	145.15±5.51[b] 10.25%[c]***	147.45±5.62[b] 12.00%[c]***

Values represent the Mean ± Standard Deviation (SD) of body weight of five animals, NS = Non Significant, * =Significant at 5%, ** = Significant at 1%, ***/# = Significant at 0.1%, %[c]= Changes in body weight in percentage at every 7 days from day 0, a= decrease in body weight, b= increase in body weight.

Streptozotocin-treated animals receiving the powdered form of Stevia leaves 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 powdered form of Stevia leaves may act through this way too. Other probable mechanisms by which the powdered form of Stevia leaves 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.

Powdered form of Stevia leaves were found to have the body weight reducing effect on diabetic rats although not significant, whereas Glimepiride increases the body weight significantly. This finding is in conformity with that of Weitgasser *et al.* (2003). Body weight reducing effect of Stevia leaves powder may be due to inhibition of glucose from the intestine as well as induction of gluconeogenesis in the liver and muscles.

Our results have shown that the powdered form of Stevia leaves 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 studies are needed to determine 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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