RESEARCH ARTICLE

Stevia rebaudiana Bertoni Leaves Extract as a Nutraceutical with Hypoglycemic Activity in Diabetic Rats

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Abstract

BACKGROUND: The prevalence of diabetes mellitus is growing every year, including in Indonesia. Medicinal herbs were used empirically for lowering blood glucose. One of potential herb to have hypoglycemic activity is Stevia. *Stevia rebaudiana* Bertoni leaves contain stevoside, a natural, low-calorie sweetener that is 300 times sweeter than saccharose. In this study, we aimed to explore the hypoglycemic activity of *S. rebaudiana* Bertoni leaves extract in a rat model of type 2 diabetes mellitus (T2DM).

METHODS: Male Wistar rats were feed high-fat, high-carbohydrate feed and sugar solution for 74 days to induce a diabetic rat model. The animals were then divided into five groups consisting of a negative control group treated with 2% *Pulvis Gom Arabicum*; a positive control group treated with Metformin 45 mg/kg body weight (BW); and three test groups treated with aqueous extract of *S. rebaudiana* Bertoni leaves at doses of 3.125, 6.25 and 12.5 mg/kg BW for 36 days. Blood glucose was measured on days 14, 28 and 36.

RESULTS: The results showed that blood glucose levels over 36 days were significantly (p=0.043) lower in the group treated with *S. rebaudiana* Bertoni leaves extract. Further Newman-Keuls analysis suggested that the hypoglycemic activity of *S. rebaudiana* Bertoni leaves extract was dose-dependent.

CONCLUSION: Our results indicate that *S. rebaudiana* Bertoni leaves extract has a potential role as a hypoglycemic agent in the treatment of T2DM.

KEYWORDS: *Stevia rebaudiana* Bertoni, nutraceuticals, hypoglycemic, diabetic rats

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Introduction

Type 2 Diabetes Mellitus (T2DM) is metabolic disorder that punctuated by insulin resistance. Some factors may trigger T2DM such as consumption of high calorie intake, obesity, sedentary lifestyle, as well as genetic.(1,2) More than 80% of all patients with diabetes are T2DM. Epidemiological research in developed countries showed that due to high older population structure and high rates of obesity, the incidence and prevalence of T2DM are increasing. According to World Health Organization estimation, Indonesia has the fourth largest diabetic population in the world after India, China, and the United States.(3)

Indonesia is the third most bio-diverse country in Asia, after India and China, possessing rich resources of medicinal herbs with the potential to act as hypoglycemic agents. The extract of *Stevia rebaudiana* Bertoni (*S. rebaudiana*; *Asteraceae*), a herbaceous plant, has been used for many years in the treatment of diabetes among indigenous people of Paraguay and Brazil.(4,5)

S. rebaudiana Bertoni leaves contain stevioside, a natural, low-calorie sweetener that is 300 times sweeter than saccharose. A number of recent studies revealed that consumption of *S. rebaudiana* Bertoni can lower the plasma glucose level.(5-7) Aquatic extract of *S. rebaudiana* Bertoni can stimulate insulin secretion, sensitivity by acting directly on pancreatic beta cells, and showed antioxidant properties. (4,8,9) Furthermore, stevioside and steviol that extracted from *S. rebaudiana* Bertoni showed to increase expresion of glucose transporter type 4 (GLUT4) gene and protein, and also glucose uptake in cells.(10)

Previous studies started the extraction of stevioside from *S. rebaudiana* Bertoni leaves were using water- or alcohol-based extraction and followed by purification, filtration, and identification by high performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR), and mass spectrometry (MS).(11) Stevioside and other 20 compounds that isolated from *S. rebaudiana* Bertoni leaves were extracted from hot water.(12)

A beverage prepared from *S. rebaudiana* Bertoni leaves caused a 35% reduction in blood glucose in humans. This indicates that *S. rebaudiana* Bertoni leaves extract possesses blood-glucose-lowering properties and may be a potential treatment for diabetes mellitus.(13-15) The aim of this study was to determine the hypoglycemic activity of *S. rebaudiana* Bertoni leaves aqueous extract in a rat model of T2DM.

Methods

Plant Material

S. rebaudiana Bertoni leaves were collected from Manoko Lembang, Jawa Barat, Indonesia. Plants were identified in the Plant Taxonomy Laboratory, Department of Biology, Faculty of Natural Sciences, Universitas Padjadjaran, with the identification number 560/HB/II/2016. The result of the identification process indicated that the plant samples used in this study were *S. rebaudiana* Bertoni species from the *Asteraceae* family. *S. rebaudiana* Bertoni leaves were cleaned and dried at room temperature then chopped into small slices. The small leaf slices were extracted with distilled water and freeze dried. The freeze-dried *S. rebaudiana* Bertoni leaves was stored at 4°C until used in assays.

Diabetic Rat Model Preparation

Male Wistar rats 2-3 months old and weighing approximately 150-250 g were used in the model. All rats were acclimatized to the new environment for 1 week prior to treatment, housed under standard laboratory conditions with a 12:12 h light/dark cycle, fed in pellet feed and water *ad libitum*. Rats were divided into two groups; normal group and diabetic rats groups. Normal rat group were fed with commercially available rat standard pellet diet and diabetic rat groups were fed with high-fat, high-carbohydrate pellet diet that feed 20 g/rat/day and 10% sugar solution for 74 days to induce T2DM (without aqueous extract of *S. rebaudiana* Bertoni leaves). A fasting blood glucose level of ≥110 mg/dL was the criterion for T2DM in high fat and high fructose diet fed rat.(16,17) Blood glucose levels were measured by using glucometer strips.

Hypoglycemic Activity of *S. rebaudiana* Bertoni leaves Extract in Diabetic Rats

Diabetic rats were divided into five groups (each group consisting of five rats) as follows: the negative control group was treated with 2% *Pulvis Gom Arabicum* (PGA), the positive control group was treated with Metformin 45 mg/kg body weight (BW) in 2% PGA. The three test groups were treated with freeze-dried aqueous extract of *S. rebaudiana* Bertoni leaves in 2% PGA at doses of 3.125 mg/kg BW (Group 1), 6.25 mg/kg BW (Group 2), and 12.5 mg/kg BW (Group 3) once a day for 36 days. During treatment with the aqueous extract of *S. rebaudiana* Bertoni leaves, all the animal groups were fed high-fat, high-carbohydrate feed and 10% sugar solution. Blood samples were taken by cutting the tip of the rat's tail, and blood glucose levels were measured on days 0, 14, 28 and 36 after treatment with aqueous extract of *S. rebaudiana* Bertoni leaves. Before blood sampling, the rats were fasted for 8-12 hours, to eliminate interference of consumed food with blood glucose levels. BW was also measured on day 0, 14, 28 and 42 after treatment.

All procedures involving animal subjects were performed in accordance with the institutional and/or national research ethical standards and approved by Universitas Padjadjaran Medical Research Ethics Committee number 1150/UN6C1.3.2/KEPK/PN/2016.

Statistical Analysis

The data were presented in mean±SD. The statistical significance were analyzed using paired Student's t-tests.

Results

T2DM Rat Model Induction

The rat model of T2DM was induced by high-fat, high-carbohydrate feed and 10% sugar solution. Fasting blood

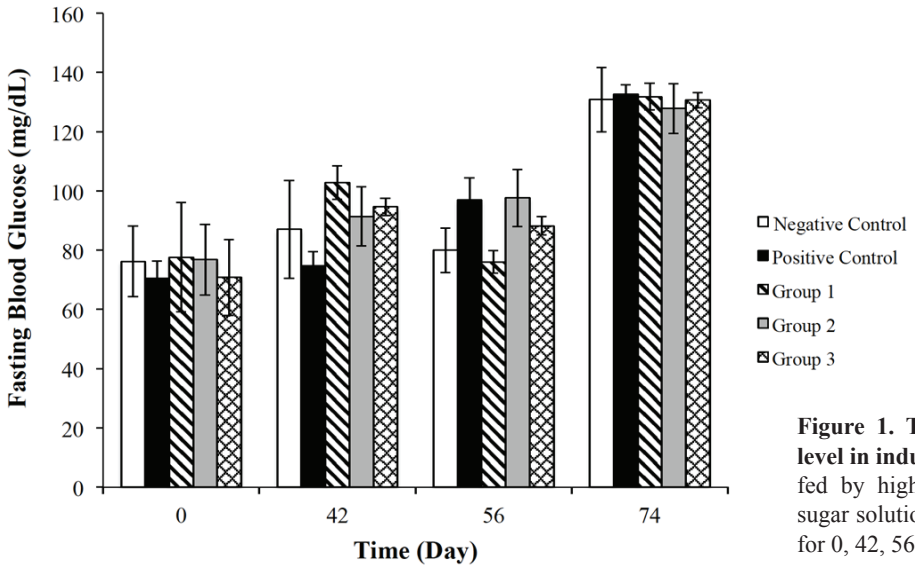

Figure 1. The average of fasting blood glucose level in induction of T2DM. All the rat groups were fed by high fat-high carbohydrate feed and 10% sugar solution until blood glucose level ≥110 mg/dL for 0, 42, 56 and 74 days.

glucose was increased slightly at days 42 and 56 and met the criteria for T2DM (≥110 mg/dL) at day 74 (Figure 1). At day 74, the T2DM rat model had an average fasting blood glucose level was 129.8±5.9 mg/dL.

The results showed that high-fat, high-carbohydrate feed and 10% sugar solution produced a T2DM rat model similar to a sedentary lifestyle as a factor for T2DM in humans. Fasting blood glucose levels were increased significantly in all groups at day 74 compared with day 0 ($p \leq 0.05$) (Table 1). The BWs of T2DM model rats were measured every 2 weeks following induction of T2DM. The BWs of all groups were increased by induction with high-fat, high-carbohydrate feeding and 10% sugar solution.

Hypoglycemic Activity of *S. rebaudiana* Bertoni leaves

Evaluation of the hypoglycemic activity of freeze-dried *S. rebaudiana* Bertoni leaves extract was carried out to determine the potential of the extract to lower blood glucose levels effectively. The mean fasting blood glucose levels of rats during the treatment were calculated (Figure 2, Table 2). Day 0 refers to day 0 of *S. rebaudiana* Bertoni leaves extract-treatment, which is day 74 after induction. Treatment with *S. rebaudiana* Bertoni leaves extract for 5 weeks decreased fasting blood glucose levels in a time- and dose-dependent manner. The higher the dose of *S. rebaudiana* Bertoni leaves extract, the higher the observed blood-glucose-lowering activity.

Table 1. The *p*-value of student's t-test on fasting blood glucose levels of high fat, high and 10% sucrose-induced rat in day 74 compared to day 0.

Group	*p*- value
C (-)	**0.0001**
C (+)	**0.0000**
Group 1	**0.0002**
Group 2	**0.0001**
Group 3	**0.0240**

In the positive control group (Metformin) and the test group (*S. rebaudiana* Bertoni leaves aqueous extract), at several dose levels, the mean fasting blood glucose level was decreased by 43.33% and 38.6%, respectively. Statistical analysis using analysis of variance showed a significant difference with each treatment in the parameter measured at α=0.05. This means that each treatment had a significant blood-glucose-lowering effect at the 95% confidence level (Table 3). Treatment with *S. rebaudiana* Bertoni leaves extract significantly decreased fasting blood glucose levels at the 36th day in the Metformin group (p=0.042) and Group 3 (p=0.043) rats according to analysis by Student's t-test (Figure 2).

BW increases in the T2DM rat model after induction and treatment with *S. rebaudiana* Bertoni leaves extract are shown in Figure 3. From the graph shown, it can be observed that after a total 116 days of high-calorie sugar induction and treatment with *S. rebaudiana* Bertoni leaves extract, all animals in three test groups and two control groups had gained BW.

Discussion

In all groups of rats, T2DM was induced by high-fat, high-carbohydrate feeding and 10% sugar solution, mimicking the sedentary lifestyle that stimulates T2DM development

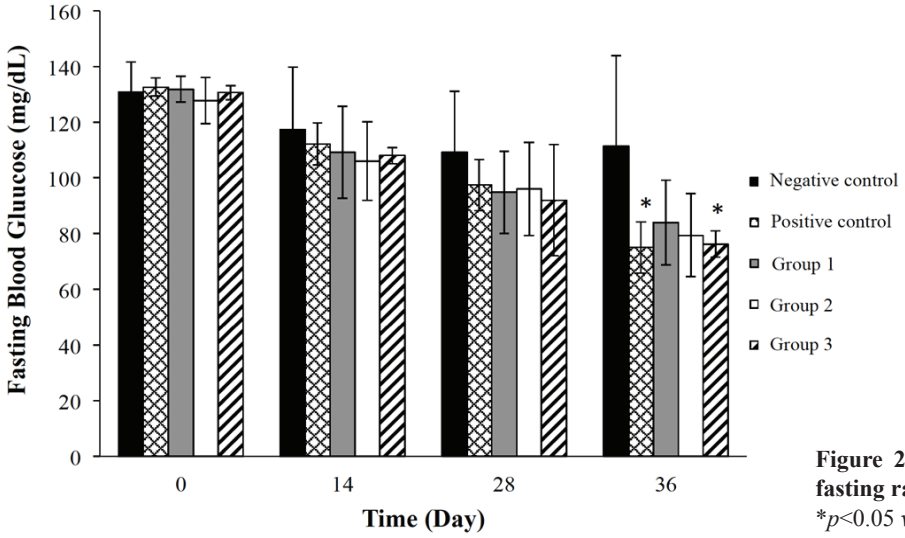

Figure 2. The average of blood glucose level fasting rats graph during 36 days of treatment. *$p<0.05$ *vs.* negative control at day 36.

in humans. In the present study, we did not use alloxan or streptozotocin, because they destroy the pancreatic β cells and mimic type 1 diabetes mellitus.(18-20) Although high fat and high carbohydrate feed induced T2DM showed some limitation, but these induction established suitable condition for obesity and T2DM in etiology, pathology and treatment.(21) Our previous study also using high fat diet-fed mouse in safrol-free nutmeg seed extract to establish T2DM mouse model.(22) One of limitation of present study that insulin level was not measured during the treatment and to confirm T2DM progression in diabetic rats.

High-fat and high-carbohydrate diets will increase the fat level in the blood, stimulate fat metabolism, and produce reactive oxygen species (ROS) in the blood and adipose cells. Increasing the production of ROS in adipose cells will disturb the balance of redox reactions. Furthermore, it will decrease the amount of antioxidant enzymes in the circulation, producing oxidative stress.(23,24) Hence, glucose uptake in muscle cells and fat cells will be inhibited and insulin secretion by pancreatic β cells will be decreased. Oxidative stress also directly affects vascular membranes and plays a pivotal role in the pathophysiology of T2DM and atherosclerosis.(25)

Table 2. The average of fasting glucose levels of rats during 36 days of treatment.

Group	The Average of Fasting Glucose Levels of Rats (mg/dL)			
	Day 0	Day 14	Day 28	Day 36
C(-)	126.2±10.92	117.4±22.45	109.2±21.80	111.4±32.42
C(+)	132.6±3.29	112.2±7.60	97.4±9.18	75±9.22
Group 1	131.8±4.55	109.2±16.57	94.8±14.79	84±15.25
Group 2	127.8±8.35	106±14.14	96±16.72	79.4±14.88
Group 3	130.6±2.51	108±2.92	92±19.99	76.2±14.14

Day 0: The blood glucose levels of rats before treated; C(-): Negative Control (PGA 2%); C(+): Positive Control (PGA 2% + Metformin 45 mg/g BW); Group 1: PGA 2% + stevia water extract with a dose of 3.125 mg/kg BW; Group 2: PGA 2% + stevia water extract with a dose of 6.25 mg/kg BW; Group 3: PGA 2% + stevia water extract with a dose of 12.5 mg/kg BW.

S. rebaudiana Bertoni as a herbal plant shows promising activity as a T2DM therapy or supplement. In the present study, we observed that freeze-dried *S. rebaudiana* Bertoni leaves extract decreased the blood glucose level in a T2DM rat model at a small dose (12.5 mg/kg BW). With high-fat, high-carbohydrate and 10% sugar solution feeding, the BWs of *S. rebaudiana* Bertoni leaves extract-treated rat groups was increased, but the blood glucose levels were decreased significantly. This result indicates that *S. rebaudiana* Bertoni may control blood glucose levels in a T2DM rat model.

In previous studies, 400 mg/kg BW of aquatic extract of *S. rebaudiana* Bertoni leaves was observed to lower fasting blood glucose, triglycerides, and hepatic parameters after 28 days of treatment.(8) A methanol extract of *S. rebaudiana* Bertoni root treatment in diabetic rats was observed to lower blood glucose levels, increase the hepatic glycogen content, and maintain BW and lipid-profile parameters in the near-normal range.(9) A study of *S. rebaudiana* Bertoni as a sweet herb (1 g *S. rebaudiana* Bertoni leaves powder) in T2DM subjects significantly lowered fasting and post-prandial blood glucose levels, triglycerides, and very low-density lipoprotein cholesterol (VLDL-C) levels.(15)

The sweet taste of *S. rebaudiana* Bertoni comes from stevioside which is a glycosidic diterpenes, that has 300 times sweeter than sucrose with zero calorie.(26,27)

Table 3. The *p*-value of Student's t- test on fasting blood glucose levels of diabetic rat after treatment with *S. rebaudiana* Bertoni extract in day 36 compare to negative control group.

Groups	*p*- value		
	Day 14	Day 28	Day 36
C (+)	0.6368	0.297	0.0422*
Group 1	0.5295	0.2564	0.1256
Group 2	0.3648	0.314	0.0798
Group 3	0.3802	0.2297	0.0431*

*significant $p<0.05$.

Mechanistically, *S. rebaudiana* Bertoni lowers blood glucose levels by increasing insulin secretion and decreasing glucagon secretion in α-pancreatic cells. Stevioside, which is the major compound of *S. rebaudiana* Bertoni, has the effect of stimulating insulin secretion and increasing insulin sensitivity by acting directly on pancreatic beta cells. (28,29) Another study showed that *S. rebaudiana* Bertoni extract increased the expression of peroxisome proliferator-activated receptor-γ (PPARγ) and insulin mRNA.(8) PPARγ plays an important role in maintaining glucose metabolism by stimulating glucokinase and GLUT2 in the pancreas and liver.(30) By increasing the expression of PPARγ and GLUT4, stevioside will enhance the glucose uptake into the cells, thereby glucose in blood were metabolized and resulting in lowering blood glucose level.(30)

The finding that the aqueous extract of *S. rebaudiana* Bertoni could increase the BW of a rat animal model in this study could be very valuable to the supplementary use of *S. rebaudiana* Bertoni, since most diabetic patients struggle with significant BW loss due to strict limitation of the blood glucose level. In contrast, another study that used birds as an animal model has proven that *S. rebaudiana* Bertoni leaves used as a poultry food supplement could contribute to increasing the abdominal fat content. The mechanism by which *S. rebaudiana* Bertoni acts to increase broiler BW is by it component stevioside increasing glucose intake from the vascular blood, which is later converted to fat and stored in the abdomen of test animals.(31)

The insulin resistance caused high blood glucose level in diabetic subjects can decrease BW through gluconeogenesis in liver which are producing endogenous glucose from fat. This condition showed a metabolic disturbance.(32) *S. rebaudiana* Bertoni consumption increasing insulin secretion, increase insulin sensitivity, and decreasing glucagon secretion in α-pancreatic cells. Therefore, gluconeogenesis is decreased, lipolysis is decreased or stops. Hence, *S. rebaudiana* Bertoni consumption will improve the metabolism and homeostatic BW can be arisen. However, further studies on how *S. rebaudiana* Bertoni could increase BW in a rat model should be performed to clarify these results.

Conclusion

Aqueous extract of *S. rebaudiana* Bertoni leaves at doses of 3.125 mg/kg BW, 6.25 mg/kg BW and 12.5 mg/kg BW can lower blood glucose levels significantly. The percentage decrease in blood glucose levels was correlated with the given dose, and the greatest decrease in the blood was obtained from a dose of 12.5 mg/kg BW of 38.6% in a T2DM rat model. Based on the analysis, stevia extract has potential dose-dependent hypoglycemic activity.

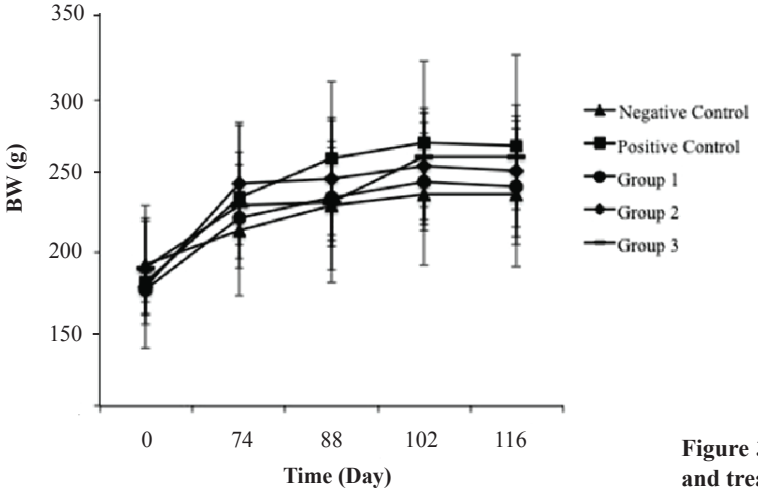

Figure 3. Increasing BW in T2DM-rats model after induction and treatment of *S. rebaudiana* Bertoni extract.

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