Original article:

Beneficial role of Stevia rebaudiana dry extract on the blood glucose reduction in the chronic diabetes mellitus

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Abstract

Introduction: Prolonged hyperglycemia may lead to several detrimental microvascular or macrovascular complications in the chronic diabetes mellitus. Thus, achieving normal glycemic condition remains the most important strategy in the diabetes mellitus. Stevia rebaudiana plays an important role on the blood glucose reduction, however, its role in the chronic diabetes mellitus has not been fully elucidated yet. Method: Stevia rebaudiana dry extract (400 mg/kg BW) were given daily by oral gavage for 84 days in rat with diabetes. The measurement of blood glucose concentration were done monthly during the study. Urine output was analyzed on the day 1 and 84. Additionally, lipid profile were measured on the day 84. Results: Compared to the normal rat, significant hyperglycemia were observed in the diabetic rat. Blood glucose concentration tends to decrease in the rat received Stevia rebaudiana extract (St), however, significant difference was achieved on the day 84 of the study confronted to the diabetic rats. Moreover, lower concentration of plasma cholesterol concentration and urine output reduction was significantly observed in the St rat compared to the D rat on the day 84. **Discussion:** Daily intake of Stevia rebaudiana extract on its therapeutic doses may play beneficial role in the chronic diabetes mellitus by gradually reduced blood glucose concentration, urine output and plasma cholesterol concentration. However, further cellular analysis were required to explicate the exact

Keyword: Stevia rebaudiana, diabetes mellitus, hyperglycemia, hyperlipidemia, urine output

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Introduction:

Persistent hyperglycemia due to either insulin deficiency or insulin resistance has become the main culprit of several diabetic complications including diabetic heart and kidney.1 Hyperglycemia and glucotoxicity may stimulate advanced glycation end products (AGE) that contributes in the fibrosis, cardiac stiffness and impaired diastolic relaxation.² Furthermore, intraluminal glucose accumulation in the tubulus proximal of the kidney along with the AGE accumulation result in the hyperreabsorption which further contributes to the renal injury.3 Therefore, chronic hyperglycemia directly elicits specific complications through the cell injury due to impaired glucose metabolism. Thus, targeting euglycemia still remain the most important strategy in the diabetes mellitus in concomitant with multiple risk factor treatment including blood pressure, cholesterol, body weight and smoking.⁴

Stevia rebaudiana, from the Asteraceae family, is a nutrient-rich plants that has a sweeter along with a bitter taste compared to the ordinary sugar. Steviol glycosides, including stevioside and rebaudioside, play important roles in the bitter but sweet taste of Stevia. Steviol glycosides has been reported to be safe and not related to any teratogenic, mutagenic or carcinogenic risks.⁵ Accumulating evidences have shown that the active compound of Stevia rebaudiana including stevioside and minor glycoside (dulcoside A, rebaudioside B, C, D, steviolbioside) possess an anti-diabetic as well as an insulinomimetic properties by regulating GLUT4 receptors, ^{6,7} increasing insulin

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concentration, stimulating PPAR γ^8 and enhancing anti-oxidant pathway.^{7,9,10}

It is not only the stevioside that exerts an antidiabetic property but also its metabolite, steviol, that actively increases the uptake of the glucose in the brain and myocardium. We have reported that prolonged use of liquid stevia sweetener (equivalent with stevioside 40 mg/kg BW) contributed to the blood glucose reduction in the diabetes mellitus. However, different results were found in the clinical setting. Eight-week treatment of 2% stevia tea or three days treatment of 1 g stevia did not reduce blood glucose concentration in the diabetic patient 14,15,16 This study clarified the long-term use of Stevia rebaudiana extract in the chronic diabetes mellitus.

Methods and Materials:

Chronic diabetes mellitus animal model

Male Sprague-Dawley rats with the age of 8 to 10 weeks were induced by a diabetic agent, streptozotocin (STZ; Sigma-Aldrich Inc., USA). In brief, STZ powder was dissolved in the pH 4.5 citrate buffer in accordance with the previous reference.17 Single injection of fresh STZ solution was administered with dose of 40 mg/kg body weight (BW) to the rats through the intraperitoneal route with sterile tuberculin syringe.¹⁵ Random blood glucose concentration were measured from the rat tail with glucose strip and glucose meter (Nesco Inc., UK). Diabetes mellitus was defined as blood glucose concentration higher than 250 mg/dl. To mimic the chronic diabetes mellitus, the experimental protocol was done for 84 days.

Stevia rebaudiana treatment

Dried Stevia rebaudiana leaves were taken from PT. Agro Jabar Bandung Jawa Barat and then extracted into dry extract at the Bogor Agricultural University Jawa Barat. Stevia rebaudiana dry extract was dissolved in the distilled water and freshly given at the evening to the experimental rat (400 mg/kg BB for 84 days).

Chronic protocol treatment

Rats with diabetic conditions were randomly divided into the diabetic rat without treatment (D group; n = 5) and the diabetic group received Stevia rebaudiana (St group; n = 5). Normal rats were received solvent and treated as the negative control (N group; n = 5). During 84 days, experimental animals may freely access the food and water. All research protocols were done in accordance with the ethical animal protocol of Faculty of Medicine, Universitas Islam Negeri Syarif Hidayatullah Jakarta and the recommendation of the Declaration of Helsinki.

Blood glucose and lipid profile

The measurement of blood glucose concentration was done on the day 1, 28, 56 and 84 of the

study. Plasma lipid profile including total cholesterol, HDL and triglyceride concentration was measured on the day 84 of the study using kit (Sclavo Diagnostics International, Siena, Italy) in accordance with the company protocol.

Urine output

Twenty four hours urine were collected from the rats on the day 1 and 84 of the study. In brief, rats were housed at the metabolic cage for 24 hours. All urine collected during 24 hours were measured and centrifuged at the 5000 rpm for 15 minutes.

Statistical analysis

All data were shown numerically as means and standard of deviation (SD). Data were analyzed using one way analysis of variance (ANOVA) or Kruskal-Wallis, wherever applicable. Probability value less than 0.05 was defined as significant.

Results

Chronic model of diabetes mellitus

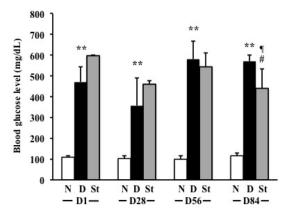


Figure 1. Blood glucose concentration during the protocols. N = normal rat; D = diabetic rat; St = diabetic rat with Stevia rebaudiana dry extract. **p<0.01 compared to the N group, #p<0.05 compared to the D group in the same time of treatment, ¶p<0.05 compared to the St group on the day 1.

Hyperglycemia (blood glucose concentration > 250 mg/dl) was achieved in the rats received STZ injection. Diabetic rats were divided into the D group and the St group. As depicted in the Fig. 1, persistent hyperglycemia was significantly observed in the diabetic rat compared to the normal rat during the study with the blood glucose concentration of between 300 to 600 mg/dl in the average.

Stevia rebaudiana role on hyperglycemia

The concentration of blood glucose concentration in the St group vs. the D group from the day 1, 28, 56 and 84 were 598.00 ± 4.00 vs. 471.33 ± 71.39 mg/dl, 461.50 ± 14.48 vs. 357.00 ± 133.42 mg/dl, 543.50 ± 67.46 vs. 579.67 ± 88.96 mg/dl, and 441.75 ± 92.64 vs. 569.00 ± 33.00 mg/

dl, respectively. Blood glucose concentration tended to increase in the D group and remained above 500 mg/dl after the day 56 of the study. In contrast, blood glucose concentration gradually decreased in the St group during the study (Fig. 1). However, significant difference of blood glucose concentration (p<0.05) between the St and the D group was reported only in the day 84 of the study along with the lowest blood glucose concentration in the St group (Fig. 1). Conclusively, we found that daily decoction of 400 mg/kg BW Stevia rebaudiana dry extract gave beneficial role in the chronic diabetes mellitus by reducing blood glucose concentration only after 84 days of usage. Stevia rebaudiana and lipid profile

As depicted in the Table 1, significant hypercholesterolemia and hypertriglyceridemia were noticed in the group of diabetic rat compared to the group of normal rat on the day 84 of the study. Additionally, significant low concentration of total plasma cholesterol and triglyceride were noticed in the St group when confronted to the D group. Stevia rebaudiana did not give any effect on HDL concentration as shown by the non-significant difference observed in the HDL concentration among groups. These result have shown us that daily decoction of Stevia rebaudiana may improve lipid profiles in the chronic diabetes mellitus specifically through cholesterol and triglyceride regulation but not through the HDL.

Stevia rebaudiana and urine output

Hyperglycemia may further elicits diuresis process in the kidney tubules through the removal of excessive glucose from the tubules. Therefore, urine volume was one of the most important indicator of glycemic control in the chronic diabetes mellitus.

Table1: Lipid profile and 24 hours urine output among the groups

Measurement	1st day	84 th day
Plasma cholesterol concentration (mg/dl)		
N		72.03±28.91
D		380.51±189.76*
St		103.39±34.40#
HDL concentration (mg/dl)		
N		77.99±62.60
D		82.81±63.52
St		111.50±80.28

Measurement	1 st day	84 th day
Triglycerides concentration (mg/dl)		
N		94.69±50.03
D		254.89±79.18*
St		105.12±22.31#
24 hours urine output		
(ml) N	5.75±1.26	7.00±2.00
D	82.33±22.90**	71.33±5.03**
St	91.00±5.57	43.00±11.49##,¶¶

N = normal rats; D = diabetic rats; St = the diabetic rat with Stevia rebaudiana. *p<0.05 and **p<0.01 confronted to the N group; *p<0.05 and **p<0.01 confronted to the D group and *p<0.01 confronted to the St group on the day 1.

As summarized in the Table 1, 24 hours urine output was significantly higher in the D group than in the N group (p<0,01) on the day 1 of the study. Diuresis process remained high in the D group compared to the N group on the day 84. Interestingly, on the day 84 of the study, significant reduction of urine output was observed in the St group confronted to the D group along with significant reduction of blood glucose concentration. Additionally, significant reduction of urine output was observed between the St group day 1 and St group day 84. These results suggest us that supplementation of Stevia rebaudiana extract may reduce the diuresis process in the chronic diabetes mellitus by reducing the blood glucose concentration.

Discussion:

Through these studies, we concluded several key findings including: (1) daily decoction of Stevia rebaudiana dry extract for 400 mg/kg BW gave beneficial role in the chronic diabetes mellitus by reducing blood glucose concentration started on the day 84 of the study along with significant reduction of 24 hours urine output; (2) Stevia rebaudiana improved lipid profile including cholesterol and triglyceride on the day 84 of the study.

Persistent hyperglycemia has been reported to be the main culprit of several diabetic complications including diabetic cardiomyopathy and diabetic nephropathy. 1-3 Additionally, hyperglycemia can directly activate several apoptotic pathways that play pivotal role in the development of diabetic complications. The excessive amounts of AGE due to glycosylated glucose may also stimulate diabetic either macrovascular and microvascular complication. In conclusion, controlling blood glucose concentration and achieving euglycemic condition remain the most important approach on

the diabetes mellitus treatment.4

Several evidences have shown that Stevia rebaudiana plays essential roles in the management of diabetes mellitus. Furthermore, we have reported that supplementation of stevioside-contained liquid sweetener for 84 days in the therapeutic dose significantly reduced the blood glucose concentration.¹³ In this study, we confirmed also that daily decoction of Stevia rebaudiana as a dryextract not as a sweetener also gave beneficial role in the chronic diabetes mellitus by reducing blood glucose concentration with subsequent reduction of urine output on the day 84 of the study. These results may be mediated through the properties of Stevia rebaudiana as an anti-oxidant and also as an insulin-mimetic agents in the pancreas islets.^{6-8,12} Not only the stevioside but also the steviol glycoside metabolite gave pivotal role in the reduction of blood glucose concentration. The beneficial role of Stevia rebaudiana on the lipid profile in the chronic diabetes still a controversy. One clinical trial reported that daily supplementation of Stevia rebaudiana extract did not give any beneficial effect on the lipid profile of diabetic patients. However, evidence have shown that supplementation of Stevia rebaudiana improved lipid and carnitine profile in the high-fed rats.¹⁷ This result have explained the mechanism of Stevia rebaudiana as a liver protector in the lipid accumulation of the obese mice.¹⁸ Another clinical trial have shown the beneficial role of Stevia rebaudiana supplementation on the lipid profile in the non-insulin dependent diabetic patient. ¹⁹ Our result have supported the beneficial role of Stevia rebaudiana extract on lipid profile at least through the regulation of plasma cholesterol and triglyceride. In conclusion, through this study and the previous study, we have shown that daily decoction of Stevia rebaudiana either as a dry extract or as a liquid sweetener gave beneficial role in the chronic diabetes mellitus management as a supplemental therapy.

Limitations and Problems:

Limitations and problems of this study include the various dose given to the rat.

Ethical Approval:

This research proposal was accepted by the Ethics Committee of Universitas Islam Negeri Syarif Hidayatullah Jakarta

Conflict of interest: None declared

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We acknowledge all staffs that already supported this research.

Author's Contributions:

Data gathering and idea owner of this study: FRS, DA, SA

Study design: FRS, HH Data gathering: CA, RJW

Data analysis and consultation: FRS, HH, Writing

and submitting manuscript: FRS

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