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The Effect of Stevia Sugar (Stevia rebaudiana Bertoni) on Blood Glucose Level of Alloxan-Induced Male Wistar Rat

Dicka Indo Putri Priyono[1], Bambang Wirjatmadi[2], Merryana Adriani[3]
[1]Master Student at Faculty of Public Health, Airlangga University, Indonesia
[2&3]Lecturer at Faculty of Public Health, Airlangga University, Indonesia

Abstract
Stevia Sugar is a natural sweetener, made from Stevia rebaudiana Bertoni leaves, that is low in calories and safe to be consumed by people with diabetes mellitus and is believed to be able to reduce blood glucose level by increasing insulin production and its sensitivity. The purpose of this study was to determine the effect of stevia sugar on blood glucose and insulin resistance in alloxan-induced male wistar rats. This research was conducted using a randomized design with Pre-Post Test with Control Group Design. Independent variable was stevia sugar, while the dependent variable were blood glucose and insulin resistance which were measured using HOMA-IR index. Subjects of this study were 25 male wistar rats divided randomly into 5 groups. The result was analyzed using Wilcoxon test (p <0.05) to see the difference of pre and post fasting blood glucose (FBG) levels, Kruskal-Wallis test (p <0.05) followed by Mann-Whitney test (p <0,05) to observe the difference of FBG levels between control and treatment groups, then continued by linear regression of dummy variable to see the most effective dose. Statistic analysis result showed that there was a difference of pre and post FBG levels (p <0,005), stevia sugar effectively decreased blood glucose level in treatment group (p = 0,009), positive control group significantly different from group P1 (stevia 2, 5 mg), P2 (stevia 3.75 mg) and P3 (stevia 5 mg). 3.75 mg stevia sugar was the most effective dose to reduce FBG levels by 20,900 mg / dl.

***Keywords** : Stevia Sugar, Blood Glucose Levels, Alloxan*

I. INTRODUCTION

Diabetes Mellitus (DM) is an endocrine metabolical disease characterized by blood glucose concentrations above normal (hyperglycemia) as a result of impaired insulin secretion, insulin receptor susceptibility reduction or both[1]. Diabetes Mellitus is a non-communicable disease which has become public health problem in the world, including Indonesia, and is estimated to be in 7th place causing death by 2030[2].

Indonesia is the seventh-ranked country in the world after China, India, USA, Brazil, Russia and Mexico with 8.5 million people suffering from diabetes mellitus[3]. Basic Health Research (Risnakes, 2013) result showed an increasing prevalence of diabetes mellitus in Indonesia in the last 5 years from 1.1% in 2007 to 2.1% in 2013[4].

Etiologically, diabetes mellitus is distinguished into 2 main types, DM type 1 caused by lack of insulin secretion by pancreas β cells[5] and DM Type 2 caused by insulin resistance and mass dysfunction of pancreas β cells as a result of insulin resistance[6]. Approximately 90-95% of the incidence of Diabetes Mellitus worldwide was dominated DM type 2[7].

One of the pillars of controlling diabetes mellitus is through diet. In diet therapy, diabetes mellitus patients are recommended to limit the consumption of carbohydrates and sugars in order to make blood glucose regulation mechanism easier[8]. In fact there are many people with diabetes mellitus who have difficulty in managing their diet, especially on sweet foods. Therefore, an alternative that can be used is substitute sugar with the low calories one. Nowadays has been found low calories natural sweetener which is stevia sugar.

Stevia Sugar is a natural sweetener derived from one of stevia species *Stevia rebaudiana* Bertoni which is believed to reduce blood glucose levels by increasing insulin secretion and its sensitivity[9]. The sweet taste from stevia comes from steviol glycoside compound[10].

Stevia is safe for people with diabetes mellitus because it contains zero calorie, zero carbohydrate and zero glycemic index[11]. A number of antioxidants contained in stevia are proven to reduce blood glucose levels by inhibiting ROS formation and DNA chain damage due to the formation of ROS and decrease insulin resistance through activation of insulin signaling pathway[12].

Since 2008, FDA (Food and Drug Administration) has allowed stevia to be used as food additives. FDA classifies stevia in the GRAS (Generally Recognize As Safe) category with an ADI (Acceptable Daily Intake) consumption limit of 4 mg / kg Body Weight / day[13].

Research that explores the effects of stevia sugar on blood glucose levels should be conducted continuously considering that stevia sugar has many benefits and is safe for people with diabetes mellitus and does not cause carcinogenic effects.

II. METHOD

This research was an experimental research with randomized design method which was done by pre-post test with control group design. Research was conducted at Laboratory of Faculty of Medicine Airlangga University Surabaya in June-July 2017.

A. Experimental Animal

The experimental animals of this study were 25 2-3 months old male wistar rats (*Rattus norvegicus*) weighing 100-200 gram divided into 5 groups: (1) negative control group, (2) positive control group (DM rats without stevia sugar), (3) DM rats with 2.5 mg / day stevia, (4) DM rats with 3.75 mg / day stevia, (5) DM rats with 5 mg / day stevia. Determination of alloxan dose until it caused DM condition was referred to research by Prameswari *et al* (2014), which was 125 mg / kg Body Weight. Alloxan was injected intraperitoneally with a single dose and the hyperglycemic effect would appear after 72 hours[14].

B. Blood Glucose Level Examination

Blood glucose level examination of rat was measured using glucose meter. Blood was taken through lateral vein (tip of the tail) by inserting rat into a jar of alcohol ether, an then after rat lost the consciousness, it was ejected from the jar then performed a fixation on its neck and body. After rat was fixated, then disinfected using alcohol swab, and waited until dry then slashed the tip of rat tail using razor. Examined the blood using glucose meter.

B. Data Analysis

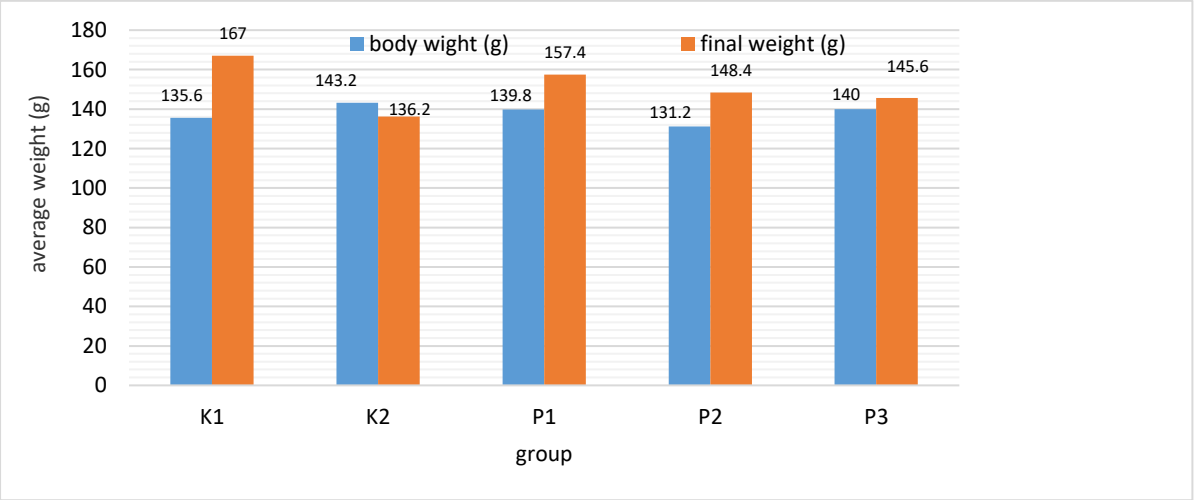
The research data result was analyzed using Wilcoxon test ($p < 0.05$) to see difference of pre and post FBG levels, Kruskal-Wallis test ($p < 0,05$) followed by Mann-Whitney test ($p < 0.05$) to identify the difference in FBG levels between control and treatment groups, and then continued by a linear regression of dummy variable to see which dose was most effective to lowered blood glucose levels.

III. RESULTS

Obtained data from this research were pre and post body weight, fasting blood glucose level in beginning, pre and post. Research data result could be describe as following.

A. Body Weight

Experimental animal characteristic based on body weight showed on Figure 1.



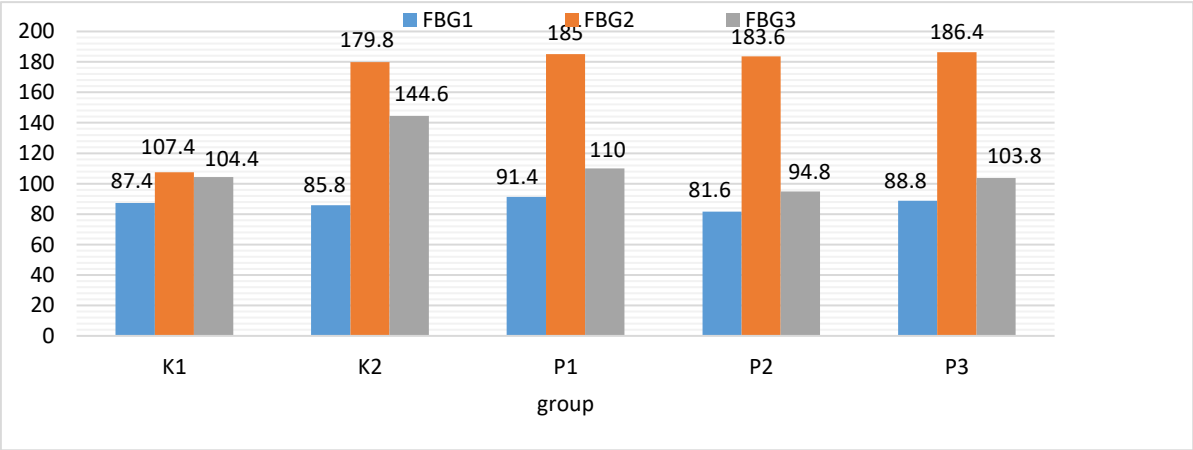
Note: (K1) normal diet, (K2) control DM, (P1) DM with 2.5 mg stevia, (P2) DM with 3.75 mg stevia, (P3) DM with 5 mg stevia.

Figure 1. Average Body Weight of Experimental Animal

Based on Figure 1 ascertainable that there is body weight reduction occurred to DM control group (K2), while group K1, P1, P2, and P3 experience body weight excalation.

B. Fasting blood glucose Level

Experimental animal characteristic based on fasting blood glucose showed on Figure 2.



Note: 1) fasting blood glucose level examination in beginning, (FBG 2) fasting blood sugar level examination pre (after alloxan induction), (FBG 3) fasting blood glucose level examination post (after being given stevia for 28 days).

Figure2. Average value of FBG levels in beginning, pre and post treatment

Based on Figure 2 found that distribution of average number of FBG 3 level in P1, P2 and P3 group are decreasing next to normal. FBG level reduction most occurred to the group of DM that was given 3,75 mg stevia per day (P2).

To pointed out the difference of FBG levels pre test and post test, it was necessary to do non-parametric statistical test using Wilcoxon Sign Rank Test which can be seen in Table 1.

Table 1. The difference of FBG levels pre and post test

Group	FBG Average Level (mg/dL)	p-value
FBG pre	168.44	0.000
FBG	111.52	

Table 1 shows that p-value = 0.000 ($p<\alpha$), which means FBG average level pre test is different with FBG average level post test.

Furthermore, to test the difference of fasting blood glucose after treated with stevia sugar between control and treatmen group, Kruskal Wallis test was conducted in which the result is showed in Table 2.

Table 2. The difference of fasting blood glucose after treated with stevia sugar between control and treatment group

Dependent Variable	Group	Mean ± SD	p-value
FBG levels	K1	104.40 ± 15.47	0.009
	K2	144.60 ± 8.36	
	P1	110.00 ± 11.47	
	P2	94.80 ± 11.69	
	P3	103.80 ± 20.11	

Table 2 shows that blood glucose has p-value = 0.009 ($p<\alpha$), which means there is a difference in blood glucose level after being given stevia sugar between control and treatment group.

To identified which groups were different, it was necessary to perform Mann Whitney U test which result is laid out in Table 3.

Table 3. The difference of Blood Glucose Levels in each group

Group	K1	K2	P1	P2	P3
K1	-	0.008	0.548	0.310	0.841
K2	0.008	-	0.008	0.008	0.008
P1	0.548	0.008	-	0.095	0.841
P2	0.310	0.008	0.095	-	0.310
P3	0.841	0.008	0.841	0.310	-

Table 3 showed that blood glucose level was different, with $p < \alpha$ (0.05), between K1 and K2 group, K2 and P1 group, K2 and P2 group, K2 and P3 group.

In order to identify the most effective dose of stevia sugar for decreasing blood glucose level, statistical test using linear regression of dummy variable was conducted, with test result shown in Figure 3.

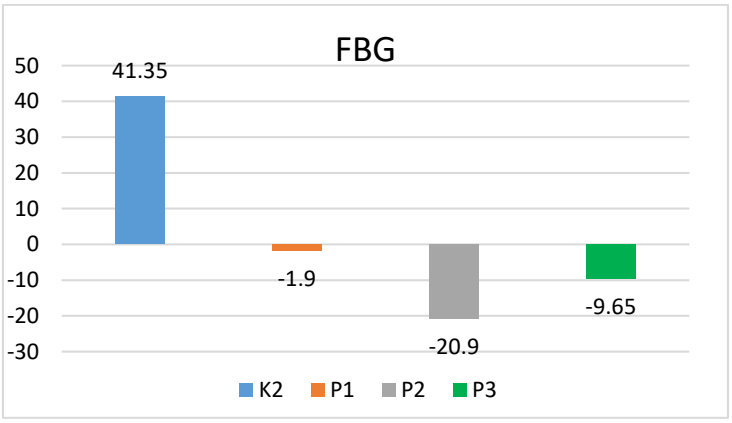


Figure 3. The most effective dose of stevia sugar for decreasing blood glucose level

Based on Figure 3 has been identified that 3.75 stevia sugar is able to reduce blood glucose level by 20.9, which means the most effective dose of stevia sugar for reducing blood glucose level is 3.75 mg/day.

IV. DISCUSSION

This study showed that pre and post blood glucose levels were significantly different (p = 0.000). One of the cause may be while in blood glucose levels examination pre, blood glucose levels had elevated due to the provision of alloxan. Aloxan causes blood glucose levels increase because alloxan is selectively toxic to pancreas beta cells that produce insulin because of the accumulation of alloxan in particular through glucose transporters, GLUT 2. The high concentration of alloxan has no effect on other experimental tissues[15]. Alloxan reacts by weakening the essential substance within pancreas beta cells, causing insulin-carrying granules reduction in pancreas beta cells[16]. Insulin levels decrease in the blood causes blood glucose levels increase[17].

On late fasting blood glucose level measurement (FBG 3), there was changes in rat blood glucose levels, where there was decrease in some treatment groups. The highest blood glucose levels decrease occurred in treatment group 2 (P2), which was diabetic rats given 3.75 mg / day stevia sugar. This means the treatment of stevia sugar for 28 days can reduce glucose levels in rats with hyperglycemia after alloxan induction.

This study result suit a research by Djas *et al* (1986)[18], Badawi *et al* (2005) and Fatimah (2015)[19][20] which showed that sweetener within stevia leaves (*Stevia rebaudiana* Bertoni) was able to lower blood glucose level in diabetic animals. The sweet taste derived from stevia sugar is a diterpene glycoside which has zero negative effect on blood glucose level. Stevia contains zero calories and zero glycemic index, making sweetener consumption of stevia sugar does not become a burden for blood glucose regulating mechanism[8].

Steviosida active compounds contained in stevia sugar is believed to reduce blood glucose level by improving pancreas beta cells function and increase insulin sensitivity, and lowering insulin resistance. Steviosida is also able to inhibit glucose absorption in liver by altering the activity of some important enzymes that involved in glucose synthesis process, and reduce the glucose accumulation in blood plasma[9].

Rebaudiosida A has an insulinotropic effect but does not cause excessive stimulation of insulin release in people with blood glucose levels next to normal, preventing hypoglycemia to occur due to too much insulin stimulation[20].

Stevia sugar contains several natural antioxidants, which are also believed to stabilize blood glucose level. In hyperglycemia conditions occur oxidative stress elevation and endogenous antioxidants reduction (produced by the body), therefore the body requires exogenous antioxidants. Antioxidant intake is a protective form against diabetes mellitus progression by inhibiting peroxidate reaction that damages pancreas beta cells. Natural antioxidants present in stevia sugar are isosteviol and polyphenol compounds (flavonoids, phenols, tannins).

Flavonoids can play a role in pancreas tissue destruction that caused by DNA alkylation from drug induction to improve morphology of rat pancreas. Flavonoids are reported to have antidiabetic activity which is capable of regenerating cells on Langerhans island. Flavonoids are characterized as hypoglycemic by increasing glycogenesis (the process of glycogen formation from glucose) resulting in no elevation of glucose level in blood[12]. Flavonoids can prevent complication or progression of diabetes mellitus by clearing excessive free-radical compounds, breaking the chain of free-radical reactions, binding metal ions (chelating) and blocking polyol pathways by inhibiting aldose reductase enzymes. Flavonoids also have an inhibitory effect on alpha glucosidate enzyme through hydroxylation bond and substitution on β ring. This inhibitory principle is similar to acarbose which has been used as a medicine for diabetes mellitus treatment, by inhibiting the hydrolysis of carbohydrates, disaccharides and glucose absorption as well as inhibiting the metabolism of sucrose into glucose and fructose[21].

Beside flavonoids, tannins are reported to have the ability to reduce insulin resistance by inducing glucose transport through activation of insulin signaling pathways in adipocytes[6]. Insulin signals elevation can increase the number of activated receptors, causing a reduction of insulin resistance which is measurable by HOMA-IR index. An increase occurs in GLUT-4 translucent rate which increase the amount of GLUT-4 and glucose uptake by tissue cells as well. In these conditions, circulating glucose in blood decreases making blood glucose level lowers.

V. CONCLUSION

Based on the result of the study could be concluded that 3.75 mg stevia sugar extention effectively could decrease fasting blood glucose level in alloxan-induced male wistar rat.

VI. RECOMMENDATION

It is necessary to conduct further research about stevia sugar effect on insulin level and β cell of Langerhans island histopathological illustration in alloxan-induced male wistar rat.

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