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Antidiabetic Activity Test of Ethanol Extract of *Stevia Rebaudiana Bertoni* Leaf in Alloxan-Induced Male Mice

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Abstract

Diabetes mellitus is a metabolic disease with characteristics of hyperglycemia that occur due to abnormalities of insulin secretion, insulin action, or both of them. This study was to prove that the extract of *Stevia rebaudiana Bertoni* leaves has an activity to reduce blood sugar levels in diabetics at an effective dose.

The method carried out to achieve this goal was to make extracts by maceration of Stevia rebaudiana Bertoni leaf powder using 70% ethanol solvent for 5 days. Testing of antidiabetic activity used white mice as experimental animals. This activity was performed in 6 treatment groups, namely group I (normal control), group II (negative control), group III (leaf extract of Stevia rebaudiana Bertoni 200 mg/200 g of body weight), group IV (leaf extract of Stevia rebaudiana Bertoni 400 mg / 200 g of body weight), group V (leaf extract of Stevia rebaudiana Bertoni 600 mg / 200 g of body weight), and group VI (positive control given glibenclamide 2 mg / 200 g of boody weight). All treatment groups were given the treatment for 10 days. Measurement of blood sugar levels was done by using the Easy Touch Glucometer. The antidiabetic activity of Stevia rebaudiana Bertoni leaf extract was indicated by looking at the difference in the decrease in blood sugar levels and then analyzed by Anova test with a significance value of 0.05.

The best results in reducing blood sugar levels were in group III which showed the same antidiabetic activity as group VI. Conclusion: Stevia leaf extract has antidiabetic activity at a dose of 200 mg/200g body weight of mice.

Keywords: Antidiabetic, *Stevia Rebaudiana Bertoni* Leaf Extract, Blood Sugar

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia (Triplitt *et al*., 2008) [14]. Diabetes Mellitus is said to be chronic if it is characterized by disturbances in the metabolism of carbohydrates, proteins and fats followed by microvascular complications (Sukmono, 2009) [13]. An article by the Ministry of Health (2009) states that Indonesia has a prevalence of diabetes mellitus in 2030 reaching 21.3 million people and the 2007 Basic Health Research (Riskesdas) stated that in the age group 45-54 years, DM is the leading cause of death 2nd in urban areas and 6th in rural areas.

Patients with diabetes mellitus require continuous treatment for the rest of their lives. Therefore, therapy needed is an easy to obtain one and has an economical price as well. This causes researchers to try to find traditional medicines derived from nature that are easy to obtain to treat Diabetes Mellitus, in addition to treatment with synthetic drugs which have detrimental side effects for sufferers.

The Stevia plant is useful as a sugar substitute sweetener that is safe for consumption for people with DM. Compounds that are efficacious as sweeteners are steviol glycosides (which are widely found in Stevia plants are stevioside and rebaudioside). An article written by Riani and Isnawati (2011) [10] states that stevioside can be efficacious as an antihyperglycemic by increasing the insulin response mechanism, namely increasing insulin content in INS-1 cells and suppressing glucagon levels.

Increased blood sugar levels can cause histological changes in the islets of Langerhans in pancreatic tissue by directly forming reactive oxygen in pancreatic β cells which make up most of the endocrine cell mass of the islets of Langerhans (Farid *et al*., 2014) [6]. Stevia leaves contain flavonoid glycosides which are antioxidants that are thought to be able to regenerate damaged pancreatic cells as a result of the formation of reactive oxygen so that they can overcome insulin deficiency (Akrom *et al*., 2014) [1].

So far, the use of Stevia rebaudiana Bertoni leaves in Indonesia is known only as a low-calorie sweetener that is safe for

diabetes mellitus patients. Nevertheless, it has not been used as a treatment for lowering blood sugar levels. The stevia leaves studied were previously in powder form but in this study, they were used in the form of an extract, namely macerate. Stevia leaves can be utilized as an antidiabetic because they contain an active compound, namely stevioside (approximately 4-15%) which has an antihyperglycemic effect (Misra *et al*., 2011) [9]. Study by Kujur (2010) [8] showed that the administration of aqueous extract, ether extract and methanol extract in a single dose of 2 g/kg per day for 28 days can reduce blood sugar levels in diabetic rats. This stevioside compound in stevia leaves can dissolve in methanol, ethanol, spirit and distilled water (Ratnani and Anggraeni, 2005). So, if in this study the macerated extraction method used was 70% ethanol solvent, it is likely that the active substance of stevioside contained in the leaves of Stevia rebaudiana Bertoni can dissolve and be extracted perfectly to further provide an antidiabetic effect. The objective of this study was prove that Stevia rebaudiana Bertoni leaf extract has the activity of reducing blood sugar levels at effective dose of alloxan-induced diabetic mice.

2. Methods

2.1 Tools and Materials

The tools used were 500 ml Erlenmeyer vessel, 500 ml beaker glass, glass funnel, flannelette, aluminum foil, "Mettler A 30" analytical scale with an accuracy of 0.0001 gram, reaction tube, measuring cup with an accuracy of 0.1 ml, evaporator, oven, 100 ml measuring flask, dropping pipette, porcelain cup, spatula, stir bar, gastric tube, 1 ml syringe. The materials used were stevia leaves (Stevia rebaudiana Bertoni), male white Wistar rats aged 2-2.5 months with body weight between 80-150 grams obtained from the Pharmacology Laboratory of Setia Budi University, Surakarta, alloxan and glibenclamide.

2.2 Research Procedures

2.2.1 Preparation of Extracts

The method to be used is to make the extract by maceration of stevia leaf powder using 70% ethanol solvent for 5 days. Maserate is concentrated to form a thick extract. The thick extract was made into a suspension with 0.5% CMC solvent to facilitate giving it to experimental animals.

2.2.2 Identification of the Chemical Content of Stevia Leaf Extract

Identification of chemical content in stevia leaf extract was performed to determine the chemical content in stevia leaf extract. To identify, a qualitative test was carried out using a color reaction to the content of alkaloids, flavonoids, saponins and tannins.

Identification of alkaloids was carried out by preparing 500 mg of ethanol extract of avocado peel then adding 1 ml of 2 M HCL and 9 ml of aquadest. The material is then heated for 2 minutes then cooled and filtered. The filtrate was divided into 3 parts, each of which was added with Dragendroff, Wagner and Mayer reagent (Hanani *et al*., 2015) [12]. In the addition of the Mayer and Wagner reactions, the results are positive if the addition of Dragendroff reagent is positive, which is indicated by the presence of orange to red precipitates (Soerya *et al*., 2005).

Identification of flavonoids was done by dissolving 500 mg of the extract in 10 ml of hot water and then heating it for 5 minutes. Then the filtrate was filtered and added a little Mg metal powder, 1 ml concentrated HCl and 1 ml amyl alcohol then shaken. Positive results for flavonoids showed red/yellow/orange color on the amyl alcohol layer (Ismiyarto, 2009) [7].

Identification of saponins was performed by weighing 500 mg of avocado peel extract and then adding 5 ml of aquadest and heating for 5 minutes. The result is then filtered using filter paper then the filtrate is cooled and shaken vigorously. A positive result in the saponin test is if a foam is formed as high as ± 1 cm and is stable after standing for 15 minutes (Biringan, 2021) [3].

The third identification, the identification of tannins, was carried out by weighing 500 mg of avocado peel extract. Then the avocado peel was dissolved with 10 ml of aquadest, filtered using filter paper then the filtrate was added with 3 drops of 1% FeCl3. The tannin test shows a positive result if a dark blue or greenish-black color is formed (Rotta *et al*., 2014).

2.2.3 Ethics Approval

The Health Research Ethics Committee, Dr. Moewardi General Hospital, School of Medicine Sebelas Maret University approved this study (Reference number: 786/VIII/HREC/2017, Date: 11th of August 2017).

2.2.4 Diabetic Activity Testing

Antidiabetic activity testing was carried out using white rats as experimental animals. Tests for antidiabetic activity were carried out in 6 treatment groups, namely group I (normal control that was not given alloxan induction and was given aquadest), group II (negative control were given alloxan 180 mg/kg body weight and aquadest), group III (induction with alloxan 180 mg /kg BW and Stevia rebaudiana Bertoni leaf extract 200 mg/200 g BW), group IV (induced by alloxan 180 mg/kg BW and Stevia rebaudiana leaf extract Bertoni 400 mg/200 g BW), group V (induced by alloxan 180 mg /kgBW and Stevia rebaudiana Bertoni leaf extract 600 mg/kgBW), and group VI (positive control induced by alloxan 180 mg/kg BW and glibenclamide 10mg/kg BW). All treatment groups were given this treatment for 10 days and blood sugar levels were measured on days 0, 5, 8, 11. Blood glucose levels were measured using the Easy Touch Glucometer. The anti-diabetic activity of Stevia rebaudiana Bertoni leaf extract was shown by looking at the difference in blood glucose level reduction between blood sugar levels on days 0, 5, 8 and 11 and then analyzed with the ANOVA test.

2.2.5 Results Analysis

The data obtained was processed using SPSS for Windows Release 17.0. While the normality test of data distribution used Shapiro-Wilk. The normal data distribution was carried out by the One Way Anova test to find out whether there was a difference or not in the treatment group. Meanwhile, for abnormal data distribution, the Kruskal-Wallis test was carried out, followed by the Mann-Whitney test.

The effectiveness of antidiabetic was demonstrated by looking at the difference in blood glucose levels reduction between the pretest and blood glucose measurements on days 3, 7 and 10.

3. Results and Discussion

3.1 Preparation of Stevia Leaf Ethanol Extract

The extraction process used in this extraction was the

maceration method with the aim that the active substances extracted were more optimal.
The yield of stevia leaf ethanol extract can be seen in Table 1.

Table 1: Yield of stevia leaf ethanol extract

Powder weight (g)	Extract weight (g)	Yield (%)
500	100	10

3.2 Identification of Chemical Content of Powder and Ethanol Extract of Stevia Leaves

Identification of the chemical content in the powder and ethanol extract of stevia leaves was carried out to determine the chemical content in the powder and stevia leaf extract by conducting qualitative tests using color reactions to determine the content of alkaloids, flavonoids, saponins and tannins. Identification results can be seen in Table 2.

Table 2: Identification results of the chemical content of the powder and ethanol extract of stevia leaves

S. No	Chemical Content	References	Results	
			Powder	Extract
1	Alkaloids	Mayer: white/yellow precipitate Dragendorf: Brown/black precipitate (Robinson 1991)	Mayer: White precipitate (+)	Dragendorf: Brown precipitate (+)
2	Flavonoids	Red, yellow or orange on the amyl alcohol layer (Depkes 1995)	Yellow on the amyl alcohol layer (+)	Yellow on the amyl alcohol layer (+)
3	Saponins	Stable foam for more than 10 minutes 1-10 cm high (Depkes 1995)	Stable foam is formed (+)	Stable foam is formed (+)
4	Tannins	Red brown/purple ring (Robinson 1991)	Purple ring (+)	Purple ring (+)

Based on the results of qualitative identification of the chemical content of the ethanol extract of stevia leaves, it can be seen that the chemical constituents such as alkaloids, flavonoids, saponins, and tannins were tested positive because there was a conformity between the observations and the literature used.

3.3 Diabetic Activity Testing and Result Analysis

Research on the activity of the ethanol extract of stevia leaves aims to determine the effect of reducing blood sugar levels from the extract and determine the most effective dose for reducing blood sugar levels in male white mice induced by alloxan. Examination of blood sugar levels was carried out by the glucose test method using the Easy Touch glucometer. Measurement of blood sugar levels using blood taken through the tail vein. Blood is taken and dripped into a glucometer and then the blood sugar results are read. Continuous taking can cause mice to become stressed and can cause death. Because of this, blood sampling was carried out only 3 times and the time span between each collection was far enough to reduce these constraints.
Measurement of blood sugar levels on day 0 (T0) aims to determine the normal blood sugar levels of white mice before being affected by alloxan and treatment with ethanol extract of stevia leaves. Measurement of blood sugar levels on day 5 (T1) aims to determine the increase in blood sugar levels after being induced by alloxan at a dose of 180 kg/BW of mice. Measurement of blood sugar levels on day 8 (T2) and day 11 (T3) aims to determine the decrease in blood sugar levels of white mice after being treated with ethanol extract of stevia leaves. The results of the average blood sugar levels on day 0, day 5, day 8 and day 11 after oral treatment with stevia leaf ethanol extract can be seen in Table 3.

Table 3: Average blood sugar levels of male white mice

Groups	Average total cholesterol level (mg/dL)					
	T0 (Day-0)	T1 (Day-5)	T2 (Day-8)	T3 (Day-11)	Increase (T1-T0)	Decrease (T3-T1)
I	111,4 ± 26,5	-	93,8 ± 8,1	76,2 ± 9,0	-	
II	110,8 ± 20,7	202,4 ± 20,0	112,0 ± 13,6	210,8 ± 10,9	91,6 ± 20,2	-8,4 ± 28,4
III	124,0 ± 19,6	193,0 ± 12,8	55,2 ± 7,8	63,0 ± 21,6	69,0 ± 20,6	130,0 ± 30,2
IV	123,2 ± 15,1	218,2 ± 9,7	111,0 ± 22,1	88,4 ± 6,5	95,0 ± 19,3	129,8 ± 8,2
V	119,8 ± 20,6	205,8 ± 31,8	111,2 ± 52,6	86,2 ± 21,1	86,0 ± 28,4	119,6 ± 43,1
VI	99,6 ± 12,4	206,0 ± 13,8	103,0 ± 4,6	94,4 ± 23,1	106,4 ± 21,3	111,6 ± 20,3

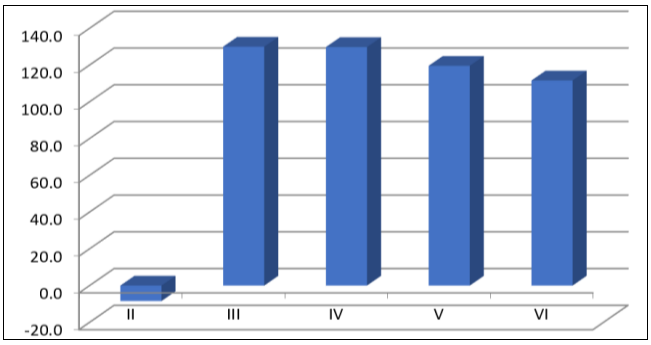

Fig 1: Graph of the average difference in the decrease in blood sugar levels of the test animals on T3-T1

Information:

Group I: Normal group, without treatment
Group II: Negative group, treated with 0.5% CMC
Group III: Positive group, given glibenclamide
Group IV: Test dose group I, stevia leaf ethanol extract dose of 200 mg/day 200 g BW
Group V: Test dose group II, stevia leaf ethanol extract dose of 400 mg/day 200 g BW
Group VI: Test dose group III, stevia leaf ethanol extract dose of 600 mg/day 200 g BW

Based on the graph, it can be seen that on the T3 examination (day 11), the blood sugar levels of the test animals showed a decrease. On the T1 examination (day 5), the blood sugar levels of the test animals increased because the mice were given alloxan induction treatment in all treatment groups, except the normal group. On the T3 examination (day 11), blood sugar levels showed a decrease in each group. In contrast to the negative group (CMC 0.5%) which only experienced a slight decrease because CMC administration did not contain an active substance that acts as an antidiabetic.

Based on Fig 1, it showed that the test dose group of stevia leaf ethanol extract was proven to reduce blood sugar levels in male white mice after being treated with alloxan induction. In the test dose group II (400 mg/200 g BW) and the test dose group III (600 mg/200 g BW) the results of a decrease in blood sugar levels were lower than the test dose group I (200 mg/200 g BW). The decrease in blood sugar levels in the test dose group I looked different from the other groups.

Data on the results of decreased blood sugar levels were analyzed by the One way Anova test which was previously carried out by the distribution test with the Shapiro-Wilk to see whether the data was normally distributed or not. The criterion for this test is the significance value (Asymp. Sig) of more than 0.05. Based on the results of the Shapiro-Wilk test, a significance value of 0.524 > 0.05 was obtained, which means that the data is normally distributed.

Furthermore, a sample homogeneity test was carried out with Kolmogorov Smirnov, and a significance value of 0.03 <0.05 was obtained. Therefore, it was stated that the data was not distributed homogeneously. The test was continued with the One way Anova test and the results obtained were 0.00 <0.05, which means that there was a significant difference in the decrease in blood sugar levels in each group.

The next step was the Post Hoc Test (Games-Howell) to find out there were significant differences in each group. Based on the test results, it was seen that there was no significant difference between the normal group and the alloxan-induced group. This happened because the normal treatment group was only given regular feed (BR II) and drinking water ad libitum, and the alloxan-induced treatment group only experienced a slight decrease because the giving of CMC did not contain an active substance that acts as an antidiabetic. Meanwhile, in the glibenclamide drug group there was no significant difference with the test dose groups I, II, and III (80 mg/200 g BW). This happened because the drug group was treated with glibenclamide which could lower the blood sugar levels of the test animals. The decrease in blood sugar levels in the test dose group I (200 mg/200 g BW) showed no significant difference to the test dose group II (400 mg/200 g BW) and the test dose group III (600 mg/200 g BW). The test dose group I showed the best decrease in blood sugar levels when compared to the test dose II and test dose III. This is due to the highest dose given so that the ability of the ethanol extract of stevia leaves has experienced saturation of its levels in its receptors in lowering blood sugar levels.

Giving stevia leaf ethanol extract can reduce blood sugar levels because the chemical compounds in stevia leaf extract such as flavonoids can act as antidiabetics. Stevioside, which is an abundant component of Stevia rebaudiana leaves, is used as a non-caloric sweetener in several countries. Chatsudthipong & Muanprasat (2008) explained that the sweet taste found in stevioside along with other compounds including rebaudioside, steviol and isosteviol (metabolic components of stevioside) show therapeutic benefits because these compounds have antihyperglycemic, antihypertensive, anti-inflammatory, antitumor, antidiarrheal, diuretic, and immunomodulatory effects. It should be noted that the effect on plasma glucose levels and blood pressure can be observed when the glucose levels and blood pressure are higher than normal. Steviol in the stevia plant can interact with drug transporters which act as modulators of the intended drug.

4. Conclusion

1. Stevia rebaudiana Bertoni leaf extract has anti-diabetic activity as seen from the decrease in blood sugar levels of alloxan-induced diabetic mice.
2. The best dose of Stevia rebaudiana Bertoni leaf extract which lowers blood sugar levels is 200 mg/200 g BW

5. Acknowledgment

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6. Conflict of Interest

There was no conflict of interest in the manuscript.

7. References

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