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Leaf Extract Microencapsulation of *Stevia rebaudiana* Bert Using Inulin-Chitosan as Anti-Diabetes Diet

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Abstract

Diabetes mellitus is a collection of symptoms that arise in someone who has increased blood glucose levels. The Stevia plant (*Stevia rebaudiana* Bert) contains a compound of diterpene glycosides as steviosida and rebaudiosida A. Purified extract of steviosida and rebaudiosida A is widely used as a sweetener for low calorie food and beverage products or as a sugar substitute for diabetics and has the effect of lowering blood sugar levels. This study aims to determine the antidiabetic effect of microencapsulated preparations of Stevia leaf extract (Stevia rebaudiana) with a combination of inulin chitosan encapsulation. Antidiabetic mellitus test was carried out in vivo using test animals of male white rats of Wistar strain. The inducing compound that can cause the condition of diabetes mellitus test animals is Aloxan with a dose of 150 mg / kg Body weight of rats. given intraperitoneally for one day, then the mice were allowed to stand for 3 days to reach a state of diabetes mellitus. Blood glucose levels of test animals were measured on days 1 (initial), 5 (induction) and 12 (treatment) to determine the initial blood glucose levels, after induction of alloxan and after administration of test compounds both CMC Na 0.5% , glibenclamide, and preparations microencapsulation of Stevia leaf extract at a dose of 100; 300; and 700 mg / kg body weight. The results were obtained after 7 days of treatment, it was seen that blood glucose levels in the CMC group remained high, while the Glibenclamid administration group, and the three dosages of microencapsulation preparations of Stevia leaf extract could reduce blood glucose levels. This can be seen from the statistical test that there is a significant difference ($p < 0.05$) between the CMC group and the Glibenclamide group, and microencapsulation preparations of Stevia leaf extract. Microencapsulation preparations of Stevia leaf extract can reduce blood glucose levels with an effective dose of 100 mg / kg body weight.

Keywords: stevia leaf extract, microencapsulation, anti-diabetes

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1. Introduction

Diabetes Mellitus is a disease when the body cannot produce insulin [1]. Insulin hormone deficiency causes interference with biochemical processes in the body, which is a decrease in glucose uptake into cells and an increase in glucose from the liver to the circulation [2]. This disease is the most common metabolic syndrome in the whole world with an incidence of 1-8%. Long-term manifestations of diabetes can cause several microvascular complications and macrovascular [3]. There are 2 types of DM disease, namely DM type 1, a condition wherein the deficiency of insulin production by the pancreas, this condition can only be treated with insulin. Type 2 diabetes that occurs due to the body's inability to respond appropriately to the activity of insulin produced by the pancreas (insulin resistance), so that no normal glucose levels in the blood are reached [4].

Stevia leaf (Stevia rebaudiana Bertoni) is a plant that has been used lately. The main content of stevia leaves are steviol derivatives, especially steviosid (4-15%), rebausid A (2-4%) and C (1-2%) and dulcocide A (0.4-0.7%) [5]. Many people use it as a natural sweetener. The sweet taste of stevia leaves obtained from the substance ofpenpenic glycosides called steviol contained from stevia leaves [6]. Stevia is a low-calorie sweetener that is safe for consumption by people with diabetes mellitus and is believed to be able to reduce blood glucose levels because it contains steviosida, rebaudiosida and a number of antioxidants [5]. Synthetic antioxidant compounds and naturally able to control glucose levels in the blood and prevent complications of diabetes [7]. Stevioside compounds in stevia can inhibit the absorption of glucose in the intestine and the formation of glucose in the liver by changing the activity of enzymes that play a role in the synthesis of glucose, so that glucose levels in the blood plasma are reduced [8].

This study aims to determine the anti-diabetic effect of microencapsulated preparations of Stevia leaf extract (*Stevia rebaudiana*). Microencapsulation needs to be done to maintain the active components in the stevia leaf extract and to cover any deficiencies of stevia. The usefulness of this technique includes controlling the release of active compounds from medicinal substances, making the active compounds safer, protecting substances that are sensitive to their environment [9], protecting the effects of undesirable effects due to the effects of light, moisture and oxygen [10].

2. Experimental section

2.1. Tools and Materials used

The tools used are spray dryer (Buchi Mini Spray Dryer B-29-), rotary vacuum evaporator (Buchi R-114), Scanning Electron Microscope (JEOL JSM-5310LV-20 kV), analytical balance, homogenizer (Ultra Turrax ® T50 Basic), spectrophotometer (UV-Vis Mini Shimadzu U-1240), rotary-evaporator, cabinet drying, grinder, analytical balance (Mettler H80), spatula, cuvette, centrifuge, and glassware. The materials used in this study consisted of stevia leaves obtained from Solo. Inulin, chitosan, 1% acetic acid, Tween 80 1%, male rats of Wistar strain, ethanol, alloxan monohydrate, glucose, sucrose, saccharin, aquabides, and the GOD-PAP enzyme.

2.2. Microencapsulation of Stevia Leaf Extract

The microencapsulation of Stevia leaf extract made an emulsion system with an inulin: chitosan encapsulation ratio of 25: 75b / b. Chitosan is dissolved in 1% acetic acid and then

Tween 80 1% emulsifiers are added. Furthermore, inulin is put into chitosan solution and homogenized using Homogenizer Ultra Turrax ® T50 Basic with a speed of 5000 rpm for 5 minutes. Furthermore, the microencapsulation process of stevia leaf extract is carried out using the spray drying method at a feed rate of 15 ml / min and inlet temperature of 120°C.

2.3. Measurement of Blood Sugar Levels

Rats that had been fasting for 12 hours and had been measured fasting blood sugar levels, were then given a treatment feed (microencapsulation of stevia leaf extract) dose of 100; 300 and 700 mg/kg body weight with the administration of drinking by ad libitum (unlimited). Furthermore, rats were measured their blood sugar levels after eating [11]. As a positive control, Glibenklamid was given a dose of 0.45 mg/kg Body Weight for 6 days.

3. Results and Discussion

This study aims to determine the antidiabetic effect of microencapsulated preparations of Stevia leaf extract (*Stevia rebaudiana*). Antidiabetic mellitus test was carried out in vivo using test animals of male white rats of Wistar strain. The selection of male white rats is intended to avoid the existence of a hormonal cycle (estrous cycle) which is likely to affect the metabolic processes in the body that can affect the results of research. The inducing compound that can cause the condition of the diabetes mellitus test animal is Aloxan with a dose of 150 mg / kg Body Weight of rats. Alloxan solution in aquabidest was given intraperitoneally for one day, then the rats were allowed to stand for 3 days to reach a state of dissolved diabetes mellitus.

Alloxan is selectively toxic to pancreatic beta cells that produce insulin due to the accumulation of alloxan specifically through glucose transporters, namely GLUT2 [12]. Its diabetogenic effect is antagonistic to glutathione which reacts with the SH group. Aloxan reacts by destroying the essential substances in the pancreatic beta cells, causing the reduction of insulin-carrying granules in the pancreatic beta cells [13]. Cytotoxic action of alloxan is mediated by free radicals. The toxic action of alloxan on beta cells is initiated by free radicals formed by redox reactions. Alloxan and its reduction product, dialuric acid, form a redox cycle with superoxide radical formation. This radical undergoes dismutase into hydrogen peroxide. Hydroxyl radicals with high reactivity formed by the Fenton reaction. The action of free radicals with high stimulation increases the concentration of calcium cytosol which causes rapid destruction of beta cells [12].

Blood glucose levels of test animals were measured on days 1 (initial), 5 (induction) and 12 (treatment) to determine the initial blood glucose levels, after induction of alloxan and after administration of test compounds both 0.5% CMC Na, glibenclamide, and preparations microencapsulation of Stevia leaf extract with three doses. Mean blood glucose levels, percent increase and percent decrease in all treatment groups can be seen in table 1.

Table 1. Average Blood Glucose Levels ± until (mg/dl), Percentage of Increase and Percentage of Decrease in All Treatment Groups

Group	Blood Glucose Levels ± until (mg/dl)			% Increase	% Decrease
	Initial	Induction	Treatment		
CMC Na 0,5 %	103.75±10	323.25±107*	415.25±135	208.35±82	-29.80±15[b]
Glibenclamide 0.63 mg / kg Body Weight of rats	83.50±37	296.50±94*	83.75±23	310.24±236	71.16±7[a]
Microencapsulation of Stevia leaf extract of 100 mg / kg Body Weight	129.50±77	392.25±229*	104.25±53	207.22±114	70.81±9[a]
Microencapsulation of Stevia leaf extract of 300 mg / kg Body Weight	153.75±74	240.25±39*	85.50±12	78.74±63	64.22±3[a]
Microencapsulation of Stevia leaf extract of 700 mg / kg Body Weight	124.50±52	270.50±98*	79.75±27	155.23±157	67.22±17[a]

Description:
*: *paired sample t test kg early vs induction which showed significantly different values* ($p<0.05$)
a: significantly different ($p <0.05$) against the negative group with the Mann-Whitney test
b: significantly different ($p <0.05$) against the positive group with the Mann-Whitney test

Based on table 1, blood glucose levels in all treatment groups showed an increase of more than 50 percent after alloxan induction. After 7 days of treatment, it was seen that the blood glucose level in the CMC group remained high, while the Glibenclamid administration group, and the three dosages of microencapsulation preparations of Stevia leaf extract could reduce blood glucose levels. This can be seen from the statistical test that there is a significant difference between the CMC group and the Glibenclamide group, and microencapsulation preparations of Stevia leaf extract at a dose of 100; 300; and 700 mg / kg body weight. In addition, the comparison between the Glibenclamide group and microencapsulation preparations of Stevia leaf extracts of the three doses showed insignificant differences. This means that the antidiabetic effect of mellitus microencapsulation of Stevia leaf extracts in three doses is comparable to Glibenklamid. Based on this it is proved that the microencapsulation preparation of Stevia leaf extract can reduce blood glucose levels with an effective dose of 100 mg / kg Body Weight or equivalent to 1120 mg / 70 kg human body weight.

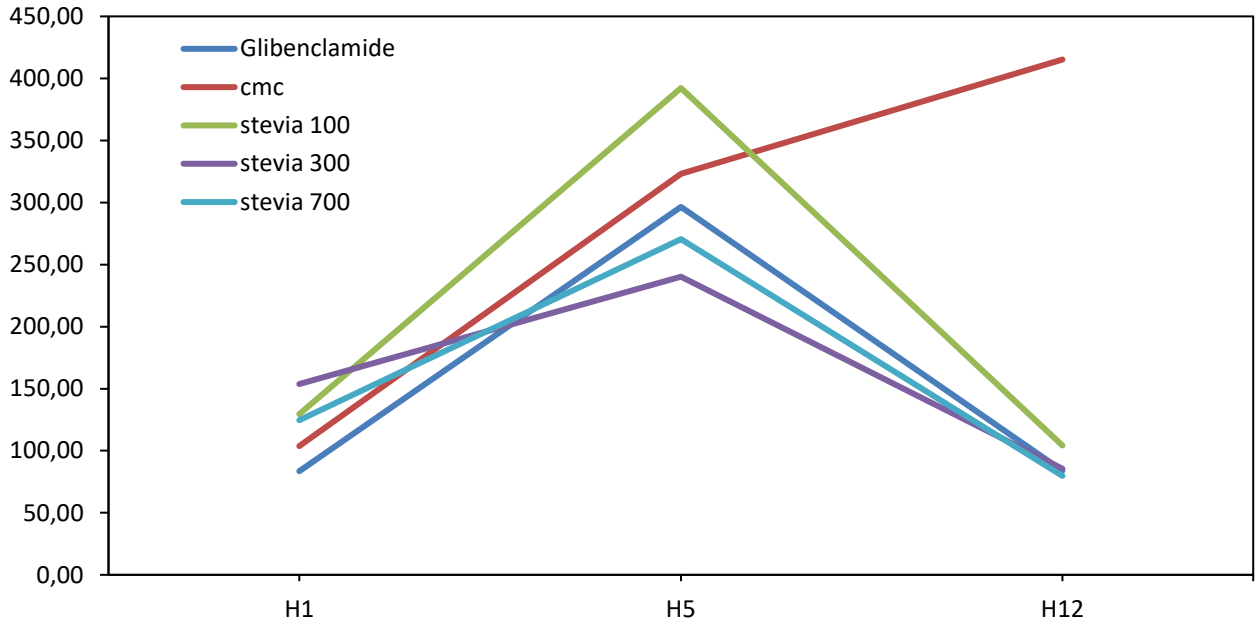

Figure 1. Graph of changes in blood glucose levels before, induction, and traetment

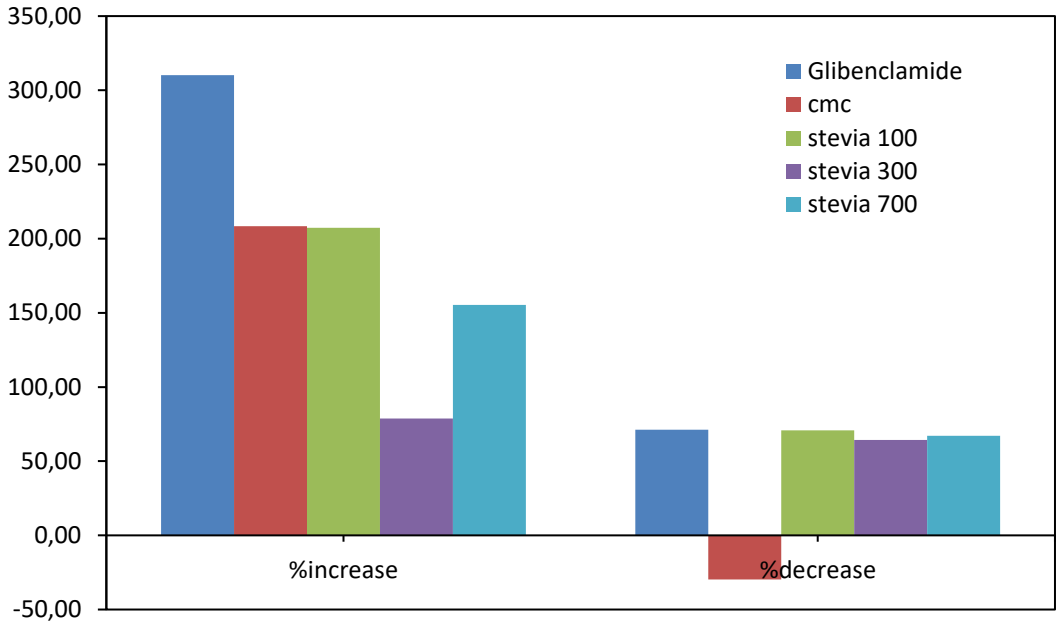

Figure 2. Diagram of % increase and % decrease in blood glucose levels

Microencapsulation preparations of Stevia leaf extract can increase percent reduction in blood glucose levels. Microencapsulation of Stevia leaf extract has been proven to reduce blood glucose levels by decreasing in the range of 60-80%. Microencapsulation is a process of encapsulating active ingredients designed to protect wrapped materials from factors that can reduce the quality of these ingredients [14]. Microencapsulation needs to be done to maintain the active components in the stevia leaf extract and to cover any deficiencies of stevia.

The mechanism of action of Stevia leaves in lowering blood glucose levels is due to the compounds of stevioside glycosides and steviol can stimulate insulin secretion through direct action on pancreatic beta cells and can repair damage to pancreatic beta cells, which leads to an increase in carbohydrate metabolism enzymes so as to form normal glucose levels [15]. Antidiabetic effects may be caused by steviosides which counteract glucotoxicity in cells β [16] or also by suppressing glucagon secretion by pancreatic α cells [17].

4. Conclusion

Microencapsulation preparations of Stevia leaf extract with a dose can reduce blood glucose levels with an effective dose of 100 mg / kgBB.

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Conflict of Interest

The authors declare there is no conflict of interest.

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