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Anti-hypercholesterol effects of microencapsulation of leaf extract *Stevia rebaudiana*

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Abstract

Several studies have proven that *Stevia rebaudiana* Bertoni leaf extract has been shown to be able to lower cholesterol. Furthermore, microencapsulation was developed to protect the content in the plant. This study aims to determine the effect of microencapsulated preparations of leaf extract of *Stevia rebaudiana* Bertoni on cholesterol in male Wistar rats. The encapsulants used are inulin and chitosan. This study was conducted with the aim of making hypercholesterol rats induced by alloxan at a dose of 150 mg/KgBW rats intraperitoneally. Hypercholesterolemic rats were given microencapsulated *Stevia rebaudiana* Bertoni leaf extract at different dose levels (100, 300, 700 and 1000 mg/kgBW) for 8 weeks; control mice were fed a basal diet during this period. The results of the study concluded that the microencapsulation of the leaf extract of *Stevia rebaudiana* Bertoni had an antihypercholesterolemic effect on male Wistar strain rats.

Keywords: Leaf extract of *Stevia rebaudiana* Bertoni; Antihypercholesterolemia; Microencapsulation

1 Introduction

Hyperlipidemia is a condition that combines various genetic disorders. This happens with an increase in lipid levels in the human body. A more objective definition is to describe hyperlipidemia as low-density lipoprotein (LDL), total cholesterol or triglyceride levels, or lipoprotein levels greater than the 90th percentile compared to the general population, or HDL levels less than the 10th percentile when compared with the general general population [1]. Based on WHO data in 2008, approximately 7.4% of the Indonesian population aged more than 25 years were recorded to have cholesterol levels above (> 6.2 mmol/L)[2]. One of the causes of this is the current diet of people who tend to consume foods that contain cholesterol, high eating intensity, obesity and smoking and the habit of consuming excessive junk food so that it makes blood cholesterol levels highly difficult to control. Hypertriglyceridemia is a major risk factor for cardiovascular disease [3].

Stevia rebaudiana Bertoni *(family Asteraceae)* is better known as stevia, honey leaf, sweet weed, or Paraguayan sweet herb. This plant is a natural ingredient, non-toxic, safe to use and non-calorie which is efficacious as a hypolipidemic. The content of glycosides including *rebaudiosides* (A, B, C, D, E), *stevioside*, *steviolbioside*, and *dulcoside* A are able to provide these benefits[4]. In addition to its hypolipidemic effect, stevia also has antihyperglycemic, antihypertensive, antidiarrheal, diuretic, antioxidant, and immunomodulatory properties. Although it is a low-calorie sweetener and dietary supplement for food, the content of stevioside is often used in the treatment of hypertension and hyperglycemia[5]. Based on research by Kitanaka et al, *stevioside* compounds and related compounds are also reported to have antitumor activity [6] .

Stevia rebaudiana Bertoni leaves contain various substances such as alkaloids, tannins and flavonoids. Flavonoids are very easily oxidized by light, air and heating so they are not stable in storage[7]. One alternative to improve the

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formulation is through the encapsulation process. This process is a way of inserting substances into a matrix to increase stability and application[8].

2 Methods

2.1 Tools and Materials

The tools used are analytical balance, rotary evaporator (Heidolph), beaker glass (pyrex), chocolate vial, test tube (pyrex), mortar and pestle, 24 mesh sieve, funnel, stopwatch, Moisture Meter (Shimadzu), Scanning Electron Microscope (Jeol JSM 6510 LA), measuring pipette, mouse probe, test tube rack. The ingredients used are *Stevia rebaudiana* Bertoni leaves, inulin, chitosan, and other chemicals.

2.2 Manufacturing of microencapsulated preparations

Microencapsulation of the leaf extract of *Stevia rebaudiana* Bertoni was made by emulsion using encapsulation of inulin: chitosan 25:75 w/w. previously, chitosan was dissolved in 1% acetic acid, then 1% Tween 80 was added as an emulsifier. Inulin was put into the chitosan solution and homogenized using a homogenizer. Then mixed at a speed of 5000 rpm for 5 minutes. Furthermore, spray drying was carried out with a feed rate of 15 ml/min and a temperature of 120°C. Furthermore, the analysis of the physical parameters of the microcapsules was carried out, namely the microencapsulated yield, water content, solubility, flow rate, levels of unencapsulated and encapsulated extracts.[7] .

2.3 Microencapsulation characteristic test

2.3.1 Microencapsulation efficiency (%EE)

The microencapsulation efficiency of *Stevia rebaudiana* Bertoni leaf extract was evaluated by comparing the weight of the material after processing it with the material before processing. [9].

2.3.2 Relative humidity

The moisture content of the microcapsules was evaluated using a moisture analyzer. A number of microcapsules were placed on an aluminum container and then measured at a temperature of 105 0C. The water content is determined based on the levels listed on the tool[10].

2.3.3 Flow speed

The flow rate test was carried out with 10 grams of microcapsule inserted into a closed funnel. Then the cover at the end of the funnel was opened and the microencapsulated was allowed to flow until there was nothing left in the funnel, then the flow time was recorded.[10].

2.3.4 Scanning Electron Miscroscopy (SEM)

The sample was placed in an aluminum sample holder with a thickness of 10 nm. The sample was then observed at various magnifications of the SEM device (Phenom pro-X, Netherlands). Voltage set at 5 kV and current 12 mA [10].

2.4 Anticholesterol Test

The adapted test animals were grouped into 6 treatment groups, their blood was taken through the eye vein and the total cholesterol level was measured initially as normal data (day 1). After that, all test animals were made to increase their cholesterol by induction of alloxan at a dose of 150 mg/KgBW rats intraperitoneally. Then on the third day after induction, blood was taken and total cholesterol levels were measured (5th day). Animals were fasted overnight prior to blood collection. Each group was given treatment once a day for 7 days. Then the blood was taken through the eye vein and the total cholesterol level was measured on the 12th day after treatment. Of the 5 groups that were induced, there was 1 group of test animals given CMC Na 0.1% orally as a negative control. Another group received simvastatin 0.9 mg/kgBW rats orally to lower cholesterol, this group acted as a positive control. The remaining four groups received microencapsulated *Stevia rebaudiana Bertoni* leaf extract with daily oral doses of 100, 300, 700 and 1000 mg/kgBW via the oral route. All groups were given feed and aquadest during treatment.[6]

2.5 Data analysis

The cholesterol data obtained was then calculated by the percentage reduction. Statistical analysis was carried out with SPSS version 16.0 with a 95% confidence level.

3 Results and discussion

Microcapsules of extract of the leaves of *Stevia rebaudiana* Bertoni with inulin chitosan were produced in the form of powder, smooth, and yellowish white in color. The microcapsules formed are homogeneous alloys where there is no visible difference between the constituent components, both in shape and color, because all components have been mixed evenly. The spray dry method is a dehydration process in encapsulation which is suitable for heat sensitive compounds. This dehydration process improves microbiological stability due to a decrease in water content and water activity in the final product, which in turn can prevent chemical and biological degradation [11].

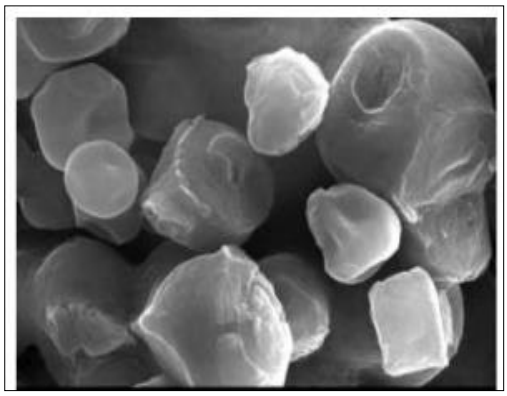

Figure 1 Microencapsulated Micrograph of 2000x magnification

The SEM results in Figure 1 show that the resulting microcapsules showed a spherical microcapsule morphology but not perfectly spherical or concave on the surface. The obtained microcapsules have various shapes with smooth microcapsules surface. The results of determining the EE value show the results of 29.30±3.76%. This shows the percentage of the total encapsulated active substance compared to the amount of the initial active substance. Encapsulation efficiency is influenced by several factors, including the nature of the coating material (viscosity and solubility), the ratio of the core to the coating, and the inlet air temperature.[12].

Relative humidity is a parameter that determines the quality of microcapsules. Low water content can prevent the growth of microbes that damage the microencapsulation. The results obtained for the moisture content were 8.56%±2.51. The microencapsulated flow rate is influenced by the shape of the microencapsulated, surface conditions, humidity. The results of the 5.25 gram/s±5.12 flow rate test show that the microencapsulated has a flow rate between 4-10 grams/s in the easy flowing category.

Data on total cholesterol results and treatment of male Wistar strain rats with microencapsulated *Stevia rebaudiana* Bertoni leaf extract can be seen in table 1.The treatment data showed that there were significant differences from all treatment groups, thus indicating that the microencapsulation of the leaf extract of *Stevia rebaudiana* Bertoni was able to provide antihypercholesterol activity.

Table 1 Average Total Cholesterol Levels mg/dL , % Increase, and % Decrease in All Treatment Groups

Groups	Total Cholesterol Level (mg/dL)±SD			% increase	%reduction
	H1	H5	H12		
Simvastatin	87 ± 23.02	265.4 ± 37.89	64.2 ± 18.35	227.92 ± 117,08	75.19 ± 9.16
CMC Na	79.8 ± 32.8	271.2 ± 42.13	335.2 ± 120.69	280.31 ± 137.29	75.19 ± 37.14
Stevia 100	87.8 ± 26.36	269.2 ± 35.09	121.2 ± 47.59	226.17 ± 88.68	53.24 ± 24.36
Stevia 300	88.6 ± 33.98	272.8 ±34.05	132.8 ± 70.51	228.00 ±72.82	50.96 ± 25.61
Stevia 700	93.4 ± 21.65	314.2 ± 51.20	101.2 ± 37.43	241.25 ± 31.47	66.96 ± 13.15
Stevia 1000	22.4 ± 22.45	93.3 ± 93.32	76.1 ±76.17	154.54 ± 154.55	26.33 ±26.33

This study used alloxan as an inducer. There is a strong relationship between Diabetes Mellitus (DM) with a lipid profile or an increase in cholesterol levels to be one reason. Alloxan as a diabetogenic agent is very effective as a trigger for cardiovascular complications. When DM occurs, the insulin secretion process will be inhibited, and then the lipase and lipoprotein lipase enzymes will be disrupted. The role of these two hormones is very large in lipids, so that in the presence of inhibited insulin, fatty acids and glycerol as a result of the hydrolysis of triglycerides become unbalanced with their storage in the tissues. This causes cholesterol levels to increase in DM patients [13].

Based on the research, it was shown that the microencapsulation of *Stevia rebaudiana* Bertoni leaf extract was able to reduce cholesterol levels by 53.24%, 50.97%, 66.96% and 26.33% with doses of 100, 300, 700 and 1000 mg/KgBW, respectively. However, when compared with Simvastatin positive control, the percentage of cholesterol reduction was still below it. The full graph can be seen in Figure 2 and 3.

Stevia rebaudiana Bertoni leaf extract is thought to contain *stevioside* which is able to lower cholesterol levels because it can increase bile excretion by preventing reabsorption of the small intestine through impaired bile micelle formation. With an increase in bile acid excretion so that cholesterol can activate cholesterol 7α-hydroxylase by converting liver cholesterol into bile acids and decreasing cholesterol[14]. The process of increasing HMG-CoA can stimulate liver cholesterogenesis.

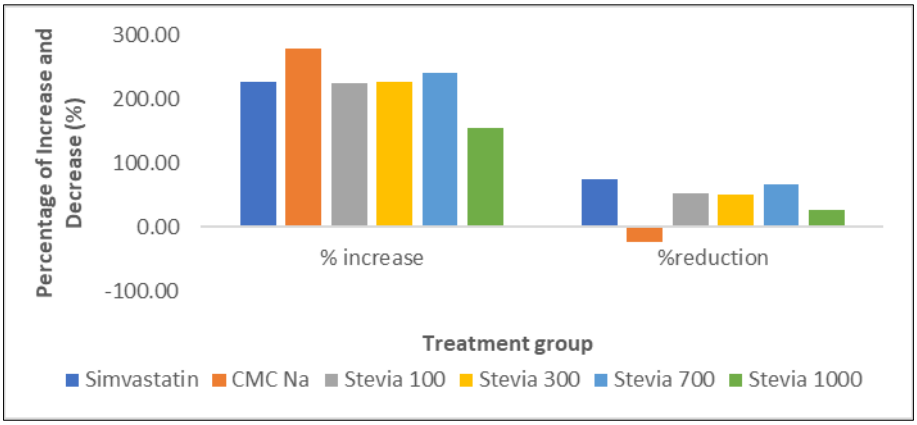

Figure 2 Graph of the percentage increase and decrease after treatment of Microencapsulated Compounds

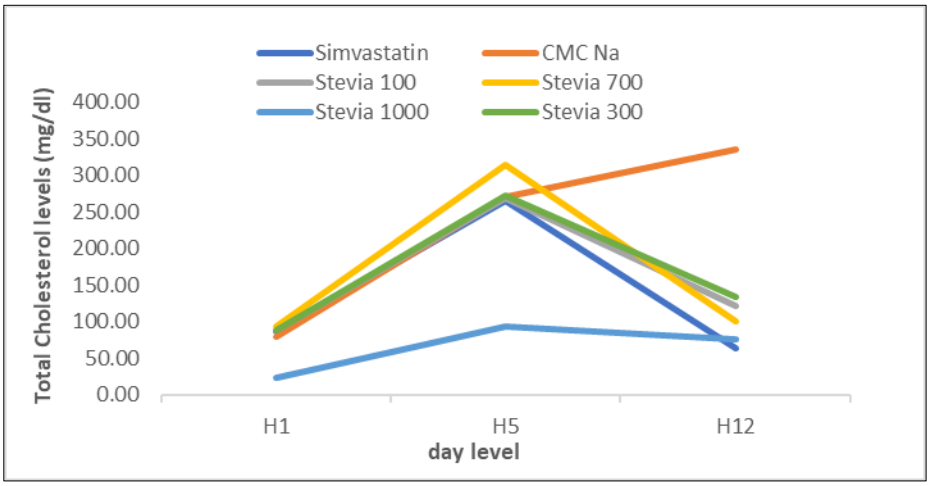

Figure 3 Graph of changes in cholesterol levels day to 0, 5 (after induction), and 12 (after treatment) of all groups

Several studies have shown that the antihypolipidemic properties of the leaf extract of *Stevia rebaudiana* Bertoni are due to the interaction of stevia with receptor activation activated by a peroxisome proliferator (PPARs). This peroxisome proliferator which functions in lipogenesis in activating lipoprotein lipase (LPL) gene expression and apo C-II in the process of absorption and esterification of liver free fatty acids, which is accompanied by increased mitochondrial free fatty acid oxidation[15]. The results showed that the microencapsulation of the leaf extract of *Stevia rebaudiana* Bertoni was able to reduce total cholesterol levels.

4 Conclusion

Based on the research conducted, it was shown that the microencapsulation of *Stevia rebaudiana* Bertoni leaf extract was able to reduce total cholesterol levels with an effective dose of 100 mg/kgBW.

Compliance with ethical standards

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Disclosure of conflict of interest

No Conflict of interest.

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