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The Effect of Bay Leaf Extract (*Syzygium polianthum*) Against Cholesterol Regulation in Hypercholesterolemic Rats

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ABSTRACT

Cholesterol is an important precursor that plays a role in the production and regulation of various sterol group compounds, especially steroid hormone compounds, androgen hormone compounds, cortisol compounds, and estrogen compounds. This herb is known to have the effect of improving glucose regulation by increasing glucose intake into cells and tissues. Its ability to improve cell and tissue metabolism is mediated by the content of secondary metabolite compounds. This ability is believed to have the potential to improve cholesterol regulation. Bay leaf extract is effective in lowering cholesterol levels through regulation of Acetyl CoA production.

1. Introduction

Cholesterol is an important precursor that plays a role in the production and regulation of various sterol group compounds, especially steroid hormone compounds, androgen hormone compounds, cortisol compounds, and estrogen compounds. People are more familiar with cholesterol as a bad precursor compound that causes various health problems, related to blockage of blood vessels, which leads to various cardiovascular disorders. Cholesterol is not always bad and useless, cholesterol deficiency results in a decrease in various hormones such as a decrease in sex hormones and a decrease in stress regulation hormones. Cholesterol reduction is believed to have an adverse effect on sexual function and manage stress in individuals. Cholesterol balance and regulation are of key importance in achieving homeostasis and

optimization of physiological functions.¹⁻⁴

The liver is a vital organ in cholesterol metabolism. Cholesterol is produced from the precursor acetyl co-a, in turn, by the role of HMG CoA reductase it is converted into mevalonate, squalene, and cholesterol. Cholesterol is then distributed to various tissues by binding to lipoproteins so that it can dissolve in the bloodstream, better known as low-density lipoprotein (LDL). LDL is a complex compound capable of depositing itself in tissues and blood vessels which will cause various health problems and problems. Control of cholesterol production is essential, in order to prevent LDL buildup in tissues. The regulation of acetyl co-a is an important point in the regulation of cholesterol production.⁵⁻⁸

Acetyl CoA is a precursor to the metabolism of glucose and can also be produced from protein or fat

through various other routes. The accumulation of glucose and various energy sources, in the form of protein or fat, causes an increase in the production of acetyl CoA. Optimizing the process of acetyl CoA metabolism is an important effort to pay attention to and further exploration efforts are needed in order to optimize and regulate cholesterol.

Bay leaf is one of the native plants of Indonesia which has been used as a spice in cooking and has been commonly used in various lives of Indonesian people. This herb is known to have the effect of improving glucose regulation by increasing glucose intake in cells and tissues. Its ability to improve cell and tissue metabolism is mediated by the content of secondary metabolite compounds. This ability is believed to have the potential to improve cholesterol regulation.

This study is the first study to explore the potential of bay leaves in blood cholesterol regulation by looking at the ability of bay leaf extract to reduce cholesterol levels and assess the levels of acetyl CoA compounds in liver tissue.

2. Methods

Animal model

A total of 30 white rats (*Rattus norvegicus*) Wistar strains were obtained from the Eureka Research Laboratory (Palembang, Indonesia) weighing between 200 – and 250 grams. All experimental animals were kept in cages under controlled conditions of 12 hours of the light-dark cycle, temperature $22 \pm 1^\circ\text{C}$ and humidity 40-60%, and given ad libitum food. The research treatment and procedures have received approval from the medical research ethics committee of the Faculty of Medicine, Sriwijaya University (No.187/kptfkunsri-rsmh/2020).

Bay leaf extraction preparation

Bay leaf *Simplicia* was obtained from the Tawangmangu Herbal Research Center, Karanganyar, Indonesia. The process of extracting bay leaves is carried out by maceration in which 500 grams of *simplicia* are macerated with 96% ethanol for 72

hours. Next, do the separation between the pulp and the macerate. The macerate was then evaporated using a rotary evaporator (Shimadzu) in order to obtain a thick extract, bay leaf extract (ES).

Animal model hypercholesterolemia

After 1 week of adaptation, the mice were randomly divided into the following five groups, each containing 6 animals: Normal control group, hypercholesterolemia group (negative control), Hypercholesterolemia + ES (50 mg/kg), Hypercholesterolemia + ES (100 mg/kg) and Hypercholesterolemia + ES (200mg/kg). Hypercholesterolemia induction was done by giving a high-fat diet with diet containing 50% fat. Hypercholesterolemia induction was carried out for 60 days. In the treatment group, the extract of bay leaf was carried out for 14 days. The mice were anesthetized by injecting 10% Chloral Hydrate (3.5 ml/kg) intraperitoneally. Rats were sacrificed by intraperitoneal injection of 10% chlorine hydrate, then blood serum was taken through the orbital vein. The serum was then centrifuged at a speed of 10.000 rpm for 10 minutes, the temperature was 25°C and the supernatant was stored at 20°C for analysis of cholesterol levels by the spectrophotometer method (Biorad). Part of the serum was used for testing Acetyl CoA by the ELISA method.

Enzyme-linked immunosorbent assays (ELISA) acetyl CoA

Acetyl CoA levels were checked with Rat ELISA Acetyl CoA (Cloud Clone), based on the manufacturer's protocols. Briefly, 50 μl of standard diluent or serum samples were added to the well which had been coated with anti-acetyl CoA and incubated at 37°C for 30 minutes. After the plates were washed, 100 μl of the biotinylated antibody solution was added and incubated for 30 minutes at 37°C . After three washing, 50 μl of avidin-peroxidase complex solution were added and incubated for 15 minutes at 37°C . After washing, 50 μl of tetramethylbenzidine color solution was added and incubated in the dark for 15 minutes

at 37°C. Finally, 50 ul stop solution was added to stop the reaction, and the optical density (OD) was measured using an ELISA reader (Biorad), a wavelength of 450 nm.

Phytochemical test

The bay leaf extract was analyzed for phytochemical screening which included tannins, alkaloids, flavonoids, quinones, saponins, and steroids/triterpenoids. The ethyl acetate fraction was separated using TLC as a stationary phase in the form of silica gel GF254 and the mobile phase in the form of n-hexane: chloroform: ethyl acetate (2: 5: 5).

Statistical analysis

All data were presented as mean \pm standard deviation and all statistical analyzes were performed with the SPSS 25 (IBM) program. One-way ANOVA followed by post hc analysis was carried out to assess the difference in mean expression levels of each protein. P <0.05 was determined as an indication that there was a significant difference in mean levels.

3. Results and Discussion

Table 1 shows the efficacy of bay leaf extract on

Acetyl CoA levels. Bay leaf extract showed efficacy in lowering

Acetyl CoA levels. Acetyl CoA is an important raw material in cholesterol production. Acetyl CoA is the result of the metabolic process of various glucose, protein, and fat compounds. The ability of bay leaf extract to improve metabolism and glucose intake into cells is believed to be the key to the success of bay leaf extract in preventing the accumulation of acetyl CoA and preventing beta-oxidation which in turn will reduce cholesterol levels more functionally and not only focus on HMG CoA reductase inhibition.⁹⁻¹³

Table 2 shows the efficacy of bay leaf extract in lowering cholesterol levels. Bay leaf extract can effectively reduce cholesterol levels. The ability of bay leaf extract to optimize metabolism with the ability to regulate Acetyl CoA is effective in lowering cholesterol levels, as shown in Table 2.

Table 3 shows the secondary metabolite content of bay leaf extract. Bay leaf extract is rich in flavonoids. The dominant flavonoids in bay leaf extract are believed to be responsible for the effects of metabolic regulation via acetyl CoA regulation.

Table 1. Efficacy of Bay leaf extract against acetyl CoA

Group	Level of Acetyl CoA (pg/mL)	P-Value*
Normal Control	167.56 +/- 12.43	0.00
Hypercholesterolemia	564.55 +/- 43.12	-
Syzygium 50 mg/kgBW	498.23 +/- 34.23	0.00
Syzygium 100 mg/kgBW	398.33 +/- 23.56	0.00
Syzygium 200 mg/kgBW	256.56 +/- 12.12	0.00

* Independent T-test VS Hypercholesterolemia, p <0.05

Table 2. Efficacy of Bay leaf extract on cholesterol levels

Group	Level Cholesterol (mg/dL) +/- SD	P-value*
Normal control	124.34 +/- 9.32	0.00
Hypercholesterolemia	342.54 +/- 22.24	-
Syzygium 50 mg/kgBW	278.23 +/- 21.21	0.00
Syzygium 100 mg/kgBW	213.21 +/- 17.12	0.00
Syzygium 200 mg/kgBW	176.56 +/- 12.11	0.00

* Independent T-test VS Hypercholesterolemia, p <0.05

Table 3. Phytochemical test

<i>Material Test</i>	<i>Saponins</i>	<i>Alkaloids</i>	<i>Triterpenoids</i>	<i>Steroids</i>	<i>Flavonoids</i>
<i>ES</i>	+	-	++	++	+++

4. Conclusion

Bay leaf extract is effective in lowering cholesterol levels through the regulation of Acetyl CoA production.

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