

Effectiveness Of Antihypercholesterolemia Ethanol Extract Of Bay Leaf (*Syzygium polyanthum*) In Male Rats With Diabetes Mellitus Strain Wistar

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ABSTRACT

Introduction: Cholesterol was one part of fat as one of the nutrients that the body needs and a high calories producer. Plants made from natural ingredients, for example, bay leaves contained high flavonoids and hypolipidemic as well as antioxidants that could inhibit oxidative stress. **Objective:** This study aimed to determine the effectiveness of the ethanol extract of bay leaves (*Syzygium polyanthum*) against male white rats wistar strain diabetes mellitus with hypercholesterolemia. **Method:** The experimental animals used male wistar rats induced with streptozotocin at a dose of 30mg/kg BW. The test animals were divided into six groups, each group consisted of 5 rats. Group (I) as a normal control were given standard feed and 0.5% Na CMC, group (II) was given 0.5% Na CMC as a negative control, group (III) was given simvastatin as a positive control, groups (IV) to (VI) were given ethanol extract of bay leaves with a certain dose namely 500mg/kg BW; 750mg/kg BW, and 1g/kg BW. The data obtained were analyzed using One Way Anova statistical test with 95% confidence level. **Results:** The results of the analysis showed that the p value < 0.005, which means that the ethanolic extract of bay leaf has an effect on cholesterol levels in diabetes mellitus male rats strain Wistar. The effective dose that gives the optimum effect is the ethanolic extract of bay leaf 1g/kgBW. **Conclusion:** The ethanol extract of bay leaf 1g/kg BW was able to provide optimum antihypercholesterol effects in diabetes mellitus male rats strain Wistar.

ABSTRAK

Latar belakang: Kolesterol merupakan salah satu bagian dari lemak sebagai salah satu zat gizi yang dibutuhkan tubuh dan penghasil kalori yang tinggi. Tanaman dari bahan alam salah satu contohnya yaitu daun salam memiliki kandungan flavonoid yang tinggi dan bersifat hipolipidemia serta antioksidan yang dapat menghambat *stress oksidative*. **Tujuan:** Penelitian ini bertujuan untuk mengetahui efektivitas ekstrak etanol daun salam (*Syzygium polyanthum*) terhadap tikus putih jantan galur wistar diabetes mellitus dengan hiperkolesterolemia. **Metode:** Hewan coba menggunakan tikus wistar jantan yang diinduksi streptozotocin dengan dosis 30mg/kg BB. Hewan uji dibagi menjadi enam kelompok, masing-masing kelompok terdiri dari 5 ekor tikus. Kelompok (I) sebagai kontrol normal diberi pakan standar dan 0,5% Na CMC, kelompok (II) diberi 0,5% Na CMC sebagai kontrol negatif, kelompok (III) diberi simvastatin sebagai kontrol positif, kelompok (IV) untuk (VI) diberi ekstrak etanol daun salam dengan dosis tertentu yaitu 500mg/kg BB; 750mg/kg BB, dan 1g/kg BB. Data yang diperoleh dianalisis menggunakan uji statistik *One Way Anova* dengan tingkat kepercayaan 95%. **Hasil :** Hasil analisis menunjukkan bahwa nilai p value < 0,005 yang artinya ekstrak etanol daun salam berpengaruh terhadap kadar kolesterol pada tikus diabetes melitus jantan galur Wistar. Dosis efektif yang memberikan efek optimum adalah ekstrak etanol daun salam 1g/kgBB. **Kesimpulan:** Ekstrak etanol daun salam 1g/kg BB mampu memberikan efek antihiperkolesterol yang optimal pada tikus diabetes melitus jantan galur Wistar.

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Introduction:

Along with the development of the times and technology, modern society tends to ignore health. Many people ignored the right diet, even in the era of increasingly sophisticated technology, many people preferred instant food without thinking about the consequences of consuming fast food. Diabetes mellitus (DM) and heart disease are diseases that are most often associated with unhealthy eating patterns, namely by consuming fast food that contains a lot of cholesterol and fat. Lifestyle changes with unhealthy eating patterns are increasing so that many people are at risk of developing diabetes and hypercholesterolemia due to increased levels of fat and cholesterol in the blood (Kementrian kesehatan republik indonesia, 2020).

Hypercholesterolemia was an increase in cholesterol levels in the blood that exceeds normal limits. The normal level of cholesterol or fat in the blood was 200 mg/dL (Wirawan, 2018). Hypercholesterolemia is a condition that occurs where cholesterol levels and or triglyceride levels in the blood increase beyond normal limits. Hypercholesterolemia had a correlation with hyperglycemia that occurs due to a decrease in insulin production which results in the work of several enzymes to carry out fat metabolism, namely the enzyme lipoprotein lipase and hormone sensitive lipase which hydrolyzes triglycerides and tissue was not inhibited. Fat levels in the blood circulation eventually increased and fat levels in adipose tissue decreased. Hyperglycemia was also associated with changes in triglyceride and total cholesterol transport (Baxter et al., 2018).

Diabetes Mellitus (DM) is a disease that occurs because there was interference by insulin and glucagon in the endocrine hormones of the pancreas. Lack of insulin caused arteriosclerosis, heart attacks, strokes, and other vascular diseases. Excess insulin caused fat synthesis and storage, increased glucose transport into liver cells, excessed citrate and isotate ions (Classification, 2014) Storage of fat in adipose cells inhibited

the action of hormone-sensitive lipases and increased transport into fat cells.

Bay leaf was a plant that was easy to find and had properties as a lowering of fat and sugar levels in the blood. Bay leaves had chemical content, namely tannins, flavonoids, and 0.05% essential oil. Bay leaves also contain secondary metabolites that have many pharmacological activities in overcoming various diseases. Based on research, bay leaves contain alkaloids, saponins, steroids, phenolics, and flavonoids. The methanol extract of bay leaves contains a lot of flavonoids and phenols (Pramesti, 2018). The purpose of this study was to determine the effectiveness of the ethanolic extract of bay leaves as anti hypercholesterolemia in Wistar male rats.

Methods:

This research was conducted at the laboratory of the University of dr. Soebandi. This study aimed to determine the effectiveness of the ethanolic extract of bay leaves as anti hypercholesterolemia in Wistar male rats. The bay leaves used were obtained from the Jember Regency, East Java Province. Plant determination has been carried out at UAD (Yogyakarta) with number 020/Lab.Bio/B/I/2021, aiming to show that the test material was bay leaf (*Syzygium polyanthum*) (Wight) Walp from the Myrtaceae tribe. This research had obtained ethical approval with the results of ethically appropriate information, namely the number 041/SDS/KEPK/IV/2021 from KEPK Stikes dr Soebandi.

Tools and materials in this study were analytical balance (Precisa), mouse tube (Lion star), thick cloth gloves (Nippon), injection syringe (Terumo), sonde (Bertamed), mask (KF 94), handsoon (KF 94), biscuit jar (Hermetico), glass-tight jar (Hermetico), food container and drinking rat (Navo), husk, porcelain cup (Navo), bioanalyzer, easy touch tool (Onemed), bay leaf ethanol 96%, wistar male rat weighing 200-300g, simvastatin (Kimia Farma), streptozotocin (Sigma-Aldrich), pig oil (Pork-Oil), aquades, CMC Na (Kimia Jaya).

In this study, experimental animals in the form of male wistar rats were adapted for 14 days and fasted for 16 hours prior to the experiment. Rats were placed in cages where each cage contains 3 rats and has been provided with food and drink as intended so that rats did not feel stressed and minimized the possibility of rats fighting and stress. Test animals were grouped into 6 groups randomly. One group contains 5 rats. Distribution groups as follows;

Group I : As a normal control, they were given standard feed

Group 2: As a negative control, 0.5% CMC Na suspension was given orally

Group 3: As a positive control, they were given simvastatin 0.9 mg/kg BW suspension.

Group 4: As the test group, ethanol extract of bay leaves was given at a dose of 500 mg/day. kg BW of rats orally.

Group 5: As the test group, ethanol extract of bay leaves was given at a dose of 750 mg/kg BW rats orally.

Group 6: As the test group, ethanol extract of bay leaves was given at a dose of 1g/kg BW rats orally.

Stage 2

The 6 groups were the normal control group which was only given standard feed, the group negative control given Na CMC 0.5% positive control group given simvastatin. suspension 0.9 mg/kg BW. and 3 treatment groups were fed a high-cholesterol diet (duck egg yolk) for 4 weeks and followed by i.p. (Intraperitoneal) administration of streptozotocin, after the 3 treatment groups were given ethanolic extract of bay leaves at a dose of 500 mg/kg BW, 750 mg/kgBW and 1g/kgBW for 2 weeks. The normal control group was used in comparison with the treatment group and the negative control group. Negative control group used to ensure an increase in total cholesterol levels due to high feeding cholesterol and streptozotocin.

This study was conducted to see the effect of ethanolic extract of bay leaves on cholesterol levels, by measuring the blood

levels of male white rats before induction measured at the beginning of the study, after being fed a high-cholesterol diet (such as duck egg yolk) on the 31st day and continued induction streptozotocin, after giving ethanol extract of bay leaves on the 38th and 45th days to see short and long term effects.

Ethanolic extract of bay leaves was made from a total of 100.0065 grams of simplicia powder, was then added with 500 mL of ethanol solvent and macerated for 24 hours. The macerate was separated by filtration, this filtering process was repeated 2 times, using the same type and amount of solvent. All the macerate was collected, then evaporated using a rotary evaporator at a temperature below $\pm 50^{\circ}\text{C}$ to obtain a thick extract.

Before testing, each animal was weighed and fasted for ± 16 hours with constant given to drink (ad libitum), then measured the blood glucose levels of rats through the tail vein of rats, after Intra-peritoneal induction of streptozotocin was given at a dose of 30 mg/kg BW.

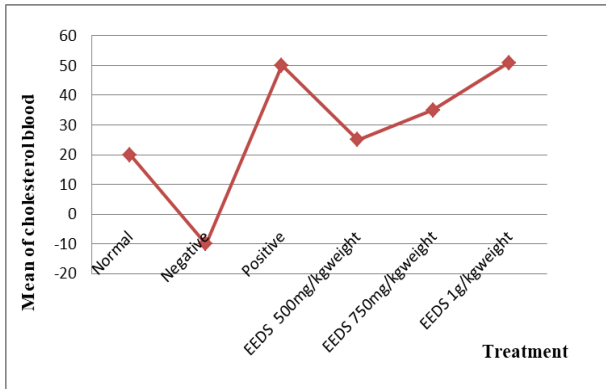
The parameters observed in this study were to compare cholesterol levels before and after treatment of ethanolic extract of bay leaves in rats that had been given streptozotocin by ip, i.e. on day 0, 31, 38 and 45.



Pictures: rats and sample's blood

Results:

From the results of observations carried out for 45 days using ethanol extract of bay leaf which was divided into three doses, namely with details of ethanol extract of bay leaf 500 mg/kg BW, 750 mg/kg BW, 1g/kg BW and giving high cholesterol feed treatment gave results. as follows;



Graph. The Relationship of Blood Cholesterol Levels With Treatment In Experimental Animals

From the graph, the X-axis is the treatment group consisting of group 1, namely normal control with standard feeding, group 2, negative control with 0.5% CMC Na suspension, group 3, positive control with simvastatin suspension 0.9 mg/kgBB, group 4 is ethanolic extract of bay leaves (EEDS) at a dose of 500mg/kgBW, group 5 was ethanolic extract of bay leaves (EEDS) at a dose of 750 mg/kgBW, group 6 is ethanolic extract of bay leaves (EEDS) at a dose of 1g/kgBW. While the Y axis is the difference between the initial and final cholesterol levels after administration of ethanol extract of bay leaves.

Statistical data analysis using One Way Anova related to the relationship between rat blood cholesterol levels and the treatment group could be seen in the table below:

Table 1. Statistical data analysis of rat blood cholesterol levels against the treatment group

	Sum of Square	df	Mean Square	F	Sig
Between Groups	11052,651	5	2210,530	72,13	,002
Within Groups	551,619	18	30,645		
Total	11604,269	23			

The table above shows that the p value < 0.005 so that the administration of bay leaf ethanol extract can have an effect on the blood cholesterol levels of rats.

Discussion:

Syzygium polyanthum leaves can be used not only as a spice for cooking purposes, but can also be used as medicine. Both root and fruit extracts have the ability to neutralize the effects of too much alcohol consumption. In addition, *Syzygium polyanthum* leaf extract was usually used to stop diarrhea, gastritis, diabetes mellitus, itching, astringent, and scabies (Hariningtias & Setiarso, 2021). Bay leaves have chemical content, namely tannins, flavonoids, and 0.05% essential oil. Bay leaves also contain secondary metabolites that have many pharmacological activities in overcoming various diseases. Based on research, bay leaves contained alkaloids, saponins, steroids, phenolics, flavonoids. Bay leaf methanol extract contains a lot of flavonoids and phenols (Dari et al., 2019). Phytochemical tests were carried out using several solvents according to the tests carried out so that the phytochemical content will be identified. The ethanol extract was made by maceration (Verawati et al., 2017). Maceration can prevent the decomposition of secondary metabolites that are not heat-resistant (Zanaria et al., 2019).

Solvents work by diffusing through plant cell walls to dissolve components in the cell and extracting solutions from within the cell to diffuse out. Stirring aids the diffusion process and ensures that the accumulated solvent was dispersed around the particle surface. Repeated maceration (remaceration) was more effective than single maceration, this happens because there may be many active compounds left in the first maceration process (Septiana, 2018). The macerate (the result of maceration) was evaporated at low pressure to achieve the required consistency, which was no more than 50°C (Jannah, 2021).

Hypercholesterolemia was an increase in cholesterol levels in the blood that exceeds the normal limit. The normal level of cholesterol or fat in the blood was 200 mg/dL ceride levels in the blood increased beyond normal limits (Wirawan, 2018). Hypercholesterolemia has a correlation with hyperglycemia that occurred due to a decrease

in insulin production which results in the work of several enzymes to carry out fat metabolism, namely the enzyme lipoprotein lipase and hormone sensitive lipase which hydrolyzed triglycerides and tissue was not inhibited. Fat levels in the blood circulation eventually increase and fat levels in adipose tissue decrease. Hyperglycemia was also associated with changes in triglyceride and total cholesterol transport (Baxter et al., 2018). Measurement of cholesterol levels was carried out on days 0, 31, 38 and 45. Giving ethanol extract of bay leaves for 2 weeks gave significant results on blood cholesterol levels of rats with the administration of several doses in the study. Therefore, bay leaf could be used as an environmentally friendly herbal plant compared to the use of synthetic drugs which have an impact on harmful side effects of drugs.

Before testing, each animal was weighed and fasted for ± 16 hours with constant given to drink (*ad libitum*). then measured the blood glucose levels of rats through the tail vein of rats, after intra-peritoneal induction of streptozotocin was given at a dose of 30 mg/kg BW. Streptozotocin or 2-deoxy-2-[3-(methyl-3-nitrosourea)-D-glucopyranose] was obtained from *Streptomyces achromogenes*. Streptozotocin had a glucose analog structure with the addition of N-acetyl glucosamine and was toxic to pancreatic beta cells. Streptozotocin works by penetrating from pancreatic beta cells via the glucose transporter GLUT 1. Streptozotocin was able to alkylate DNA through the group nitrosourea which can cause changes in pancreatic beta cells and could also cause cell damage by the mechanism of increasing guanylyl cyclase activity and cGMP formation. Normal rat blood glucose levels ranged from 62.8 mg/dL – 176 mg/dL. Glucose level measurement blood was measured with a glucometer. The next step was to measure cholesterol levels at the beginning after *i.p.* administration of streptozotocin, which was intended to compare baseline cholesterol (Saputra et al., 2018).

The graph 1 at result study showed that the administration of ethanolic extract of bay leaves at a dose of 1g/kg BW was more effective in treating hypercholesterolemia in white male Wistar strain rats with diabetes mellitus with simvastatin as a positive control. Based on research conducted by (Kementrian kesehatan republik indonesia, 2020) (Silalahi, 2017) bay leaf fraction with several solvents hexane, ethyl acetate and water ethanol provided anticholesterol activity with an effective dose of 200 mg/kg BW because bay leaves contained several chemical compounds, namely flavonoids which were hypolipidemic and antioxidants that could inhibited oxidative stress so that cholesterol levels decreased. Flavonoids, and the fabric content of bay leaves such as tannins and polyphenols were able to reduce LDL (Low Density Lipoprotein) and triglycerides so that LDL that accumulates on the walls of blood vessels could be inhibited, besides that flavonoids and tannins were able to inhibit the work of the HMG Co-A reductase enzyme (Ward et al., 2019).

Simvastatin was an antilipemic compound derived from mevnic acid which had a mechanism of action by inhibiting 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase which has a function as a catalyst in the formation of cholesterol. HMG-CoA reductase was responsible for the conversion of HMG-CoA to mevalonic acid. Inhibition of HMG-CoA reductase caused a decrease in cholesterol synthesis and an increased in the number of Low Density Lipoprotein (LDL) receptors present in the cell membranes of the liver and extrahepatic tissues, thereby causing a lot of LDL to be lost in the plasma. Simvastatin tended to reduced triglyceride counts and increase High Density Lipoprotein (HDL) cholesterol (Ward et al., 2019). Giving ethanol extract of bay leaves to male white rats of wistar strain at a dose of 1g/kgBW was comparable to the administration of simvastatin, the flavonoid content contained in bay leaves provided the same mechanism of action as simvastatin to treat hypercholesterolemia.

Conclusion:

From the results of research conducted that the ethanol extract of bay leaf 1g/kg BW was able to provide optimum anti hypercholesterolemia effects in diabetes mellitus male rats strain Wistar.

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