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Bekasam "Traditional Fermented Food" Reduces Blood Cholesterol in Wistar Rats Induced High Fat Diet.

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Abstract

Bekasam is an traditional Indonesian fermented food from fish product with a sour taste and it is a popular food in South Sumatera. Lactic acid bacteria (LAB) is the main microorganism found in fermented fish. LAB has been suggested to lower blood cholesterol through activity of bile salt hydrolase (BSH) that increasing the rate of bile excretion via deconjugate bile acids enzymatically and through inhibiting activity of HMG CoA reductase and decreasing cholesterol synthesis. An experimental study. Ten-week-old male Wistar rats (weight, 150-200 g) were randomized into six groups (6 rats/group). Group 1: normal group. Group 2: 6 rats were given high fat diet for 42 days and aquadest per oral for 14 days (28^{th}- 42^{nd} days). Group 3: 6 rats were given high fat diet and simvastatin 2 mg/kgBW . Group 4: 6 rats were given high fat diet and suspension of bekasam 10 mg/kgBW/mL . Group 5: 6 rats were given high fat diet and suspension of bekasam 100 mg/kgBW/mL . Group 6: 6 rats were given high fat diet and bekasam 1000 mg/kgBW/mL. LDL level, HDL level, Bile acid level and HMG CoA reductase were assayed with ELISA. Bekasam doses 100 mg/kgBW/mL was more decrease level of bile acid, HMG CoA reductase and LDL level than simvastatin group, as positif control. Bekasam doses 100 mg/kgBW/mL was more increase level of HDL level than simvastatin group, as positif control. Bekasam has a potention to reduce cholesterol level via inhibit cholesterol synthesis and inhibit absorption of fat in intestine.

Keywords: Bekasam-cholesterol-HMG CoA reductase-Fermented food

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INTRODUCTION

Cholesterol is a basic block for body tissues, elevated blood cholesterol is a well-known major risk factor for coronary heart disease. WHO has predicted that, by 2030, cardiovascular diseases will remain the leading causes of death, approximately 23.6 million people around the world. The risk of heart attack is three times higher in those with hypercholestrolemia, compared to those who have normal blood lipid profiles. [1]

Bekasam is an traditional Indonesian fermented food from fish product with a sour taste and it is a popular food in South Sumatera. The production of bekasam involves fermentation process of freshwater fish, supplemented by salt and rice. [2] Some fermentation products have a potention to reduce blood cholesterol. Lactic acid bacteria (LAB) is the main microorganism found in fermented fish. LAB causes changes in taste, smell and texture that improved preservation of the product. [3]

LAB has been suggested to lower blood cholesterol through activity of bile salt hydrolase (BSH) that increasing the rate of bile excretion via deconjugate bile acids enzymatically. Cholesterol being a precusor of bile acid, converts its molecules to bile acid replacing those lost during excretion leading to a reduction in serum cholesterol. This mechanism could be operated in the control of serum cholesterol levels by conversion of deconjugated bile acids into secondary bile acids by LAB. [4, 5] LAB proposed to lower blood cholesterol through inhibiting activity of HMG CoA reductase and decreasing cholesterol synthesis. HMG CoA (3-hydroxy-3-methyl glutaryl-CoA) reductase is a major regulatory enzyme in cholesterol biosynthesis. [6-8] The aim of the study is to determine the effect of bekasam on HDL, LDL level in serum, HMG-CoA reductase level in hepar and level of bile acid in intestine.

MATERIALS AND METHODS

Ten-week-old male Wistar rats (weight, 150-200 g) were obtained from Pra-Clinical Laboratory of Sriwijaya University, Palembang, Indonesia, with approval of the Ethics Committee of Faculty of Medicine Sriwijaya University, Palembang, Indonesia. Rats were placed in separate cages with food and water provided *ad libitum*, and room temperature of 22℃. They were remained in auditory and olfactory contact with the other rats until use.

The study used 36 animals were randomized into six groups. Group 1: 6 rats as normal group. Group 2: 6 rats were given high fat diet for 42 days and aquadest per oral for 14 days (28^{th}- 42^{nd} days). Group 3: 6 rats were given high fat diet for 42 days and simvastatin 2 mg/kgBW per oral for 14 days (28^{th}- 42^{nd} days). Group 4: 6 rats were given high fat diet for 42 days nd suspension of bekasam 10 mg/kgBW/mL per oral for 14 days (28^{th}- 42^{nd} days). Group 5: 6 rats were given high fat diet for 42 days and suspension of bekasam 100 mg/kgBW/mL per oral for 14 days (28^{th}- 42^{nd} days). Group 6: 6 rats were given high fat diet for 42 days and bekasam 1000 mg/kgBW/mL per oral for 14 days (28^{th}- 42^{nd} days).

Seluang fish used in this experiment was obtained from Musi River, South Sumatera Province. Bekasam was prepared by a traditional method of fermentation. One kilograms of Seluang fish was washed and mixed in refined salt 8% w/w and mixed with rice for 250 grams, placed in screw capped glass jars. The mixture was incubated at room temperature (26 ± 2 °C) for two weeks.

Rats were anasthesized by ketamine in lethal doses. Blood was collected from orbita vein. The abdomen was rapidly excised. The Intestine (duodenum area) and hepar were evacuated to the tube. Each was washed 5 times with phosphate buffer saline (PBS).

Samples were homogenized, add 0,5 mL sample buffer and centrifuge 3.000 rpm for 20 minutes, at room temperature. Supernatan was collected. Solid phase sandwich ELISA (Rat Bile Acid ELISA Kit, Abcam; Rat HMG CoA Reductase ELISA kit, Abcam; Rat HDL ELISA kit, Abcam; Rat LDL ELISA Kit, Abcam) were used to analysis concentration of Bile Acid, HMG CoA Reductase, HDL and LDL.

The results are presented as mean ± SD. The mean concentration of HMG CoA Reductase, HDL, LDL and Bile acid were compared among groups by T test followed by pos hoc analysis with bonferroni test . A value of $p<0.05$ was considered statistically significant.

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RESULTS

Table 1 showed bekasam doses 100 mg/kgBW/mL was more decrease level of LDL in serum than aquadest group. Bekasam doses 100 mg/kgBW/mL significantly decreased level of LDL ($p<0,05$), compare with simvastatin group, as positif control.

Tabel 1: Efficacy of Bekasam on LDL Level in Serum

Group (n=6 each group)	LDL (ng/mL) Mean ± SD
HFD+aquadest	1380,75±82,76
HFD+simvastatin	1249,5±72,19 [b]
HFD+bekasam 10mg/kgBW/mL	1535±76,13 [a]
HFD+ bekasam 100 mg/kgBW/mL	673,25±41,74 [a,b]
HFD+ bekasam 1000 mg/kgBW/mL	1322±64,51 [a,b]

Unpaired t test, [a] $p<0,05$ vs HFD+simvastatin; [b] $p< 0,05$ vs HFD+aquadest; significance level was determined by one way ANOVA followed by bonferroni pos-hoc test

Table 2 showed bekasam doses 100 mg/kgBW/mL was more increase level of HDL in serum than aquadest group. Bekasam doses 100 mg/kgBW/mL significantly increased level of HDL ($p<0,05$), compare with simvastatin group, as positif control.

Tabel 2: Efficacy of Bekasam on HDL Level in Serum

Group (n=6 each group)	HDL (ng/mL) Mean ± SD
HFD+aquadest	755,75±52,16
HFD+simvastatin	974,5±42,13 [b]
HFD+bekasam 10mg/kgBW/mL	910±56,09 [a]
HFD+ bekasam 100 mg/kgBW/mL	1173,25±71,14 [a,b]
HFD+ bekasam 1000 mg/kgBW/mL	609,5±34,14 [a,b]

Unpaired t test, [a] $p<0,05$ vs HFD+simvastatin; [b] $p< 0,05$ vs HFD+aquadest; significance level was determined by one way ANOVA followed by bonferroni pos-hoc test

Table 3 showed bekasam doses 100 mg/kgBW/mL was more decrease level of HMG CoA reductase in hepar than aquadest group. Bekasam doses 100 mg/kgBW/mL significantly decreased HMG CoA reductase level of ($p<0,05$), compare with simvastatin group, as positif control.

Tabel 3: Efficacy of Bekasam on HMG CoA Reduktase Level in Hepar

Group (n=6 each group)	HMGCOAR (pg/mL) Mean ± SD
HFD+aquadest	874,5±42,14
HFD+simvastatin	748,86±42,29 [b]
HFD+bekasam 10mg/kgBW/mL	1016,25±56,23 [a]
HFD+ bekasam 100 mg/kgBW/mL	146,36±11,24 [a,b]
HFD+ bekasam 1000 mg/kgBW/mL	815,75±54,51 [a,b]

Unpaired t test, [a] $p<0,05$ vs HFD+simvastatin; [b] $p< 0,05$ vs HFD+aquadest; significance level was determined by one way ANOVA followed by bonferroni pos-hoc test

Table 4 showed bekasam doses 100 mg/kgBW/mL was more decrease level of bile acid in doudenum than aquadest group. Bekasam doses 100 mg/kgBW/mL significantly decreased bile acid level of ($p<0,05$), compare with simvastatin group, as positif control.

Tabel 4: Efficacy of Bekasam on Bile Acid Level in Intestine

Group (n=6 each group)	Bile acid (pg/mL) Mean ± SD
HFD+aquadest	1130,75±62,14
HFD+simvastatin	999,5±52,29 [b]
HFD+bekasam 10mg/kgBW/mL	1285±56,23 [a]
HFD+ bekasam 100 mg/kgBW/mL	415,13±19,14 [a,b]
HFD+ bekasam 1000 mg/kgBW/mL	1065,75±64,51 [a,b]

Unpaired t test, [a] $p<0,05$ vs HFD+simvastatin; [b] $p< 0,05$ vs HFD+aquadest; significance level was determined by one way ANOVA followed by bonferroni pos-hoc test

DISCUSSION

Bekasam can inhibit activity of HMG CoA reductase, decrease LDL level and increase HDL level. Biosynthesis of cholesterol is directly regulated by the cholesterol levels present. High fat diet leads to a net decrease in endogenous production. The main regulatory mechanism is the sensing of intracellular cholesterol in the endoplasmic reticulum by the protein SREBP (sterol regulatory element-binding protein 1 and 2). In the presence of cholesterol, SREBP is bound to two other protein; SCAP (SREBP cleavage-activating protein) and INSIG-1. When cholesterol levels fall, INSIG-1 dissociates from the SREBP-SCAP complex, which allows the complex to migrate to the golgi apparatus. SREBP is cleaved by S1P and S2P, two enzymes that are activated by SCAP when cholesterol levels are low. [9] The cleaved SREBP then migrates to the nucleus, and acts as a transcription factor to bind to the sterol regulatory element (SRE), which stimulates the transcription of many genes. Among these are LDL receptor and HMG CoA reductase. [10] LDL particles are the major blood cholesterol carriers. LDL molecule shells contain just one molecule of apolipoprotein B100, recognized by LDL receptors in pheripheral tissue. A cell with abundant cholesterol will have its LDL receptor synthesis blocked, to prevent new cholesterol in LDL molecules from being taken up. Conversely, LDL receptor synthesis procceds when a cell is deficient in cholesterol. When this process becomes unregulated, LDL molecules without receptors begin to appear in the blood. These LDL molecules are oxidized and taken up by macrophages, which become engorged and form foam cells. These foam cells often become trapped in the walls of blood vessels and contribute to atherosclerotic plaque formation. [11, 12] HDL particles are thought to transport cholesterol back to the liver, either for excretion or for other tissues that synthesize hormones. Large numbers of HDL particles correlates with better health outcomes. [13]

Bekasam decreased bile acid level in intestine. Cholesterol is oxidized by the liver into a variety of bile acids. These, in turn, are conjugated with glycine, taurine, glucoronic acid or sulphate. A mixture of conjugated and non-conjugated bile acids, along with cholesterol itself, is excreted from the liver into the bile. The excretion and reabsorption of bile acids forms the basis of the enterohepatic circulation, which is essential for the digestion and absorption of dietary fats. [14]

CONCLUSION

Bekasam has a potention to reduce cholesterol level via inhibit cholesterol synthesis and inhibit absorption of fat in intestine.

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