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ANTI-HYPERCHOLESTEROLEMIC EFFECT OF ETHANOL FRUIT EXTRACT OF VANILLA (*Vanilla planifolia* Andrews) ON MALE MICE

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ABSTRACT
Hypercholesterolemia is a condition characterized by high levels of cholesterol in the blood. Vanilla is one among plants that has cholesterol-lowering substances, but research on this subject is still lacking. This study aimed to determine whether ethanol fruit extract vanilla (*Vanilla planifolia* Andrews) can be used as anti-hypercholesterolemia in mice induced by quail egg suspension. Male mice (n=20) were divided into five groups. Group-1 (negative control) was given only high-fat quail egg yolks suspension. Group-2 (positive control) was treated with simvastatin at the dose of 10 mg/kg. Whereas group-3, 4 dan 5 were given vanilla extract of 50, 100, dan 200 mg/kg body weight respectively. The results showed that ethanol fruit extract of vanilla at the dose of 200 mg/kg significantly reduced level of blood cholesterol compared with that of negative control. It is worth concluding that vanilla fruit extract potential to be used as anti-hypercholesterolemia.

KEYWORD: *Anti-hypercholesterolemia, Cholesterol, Hypercholestrolemia, Vanilin, Vanilla planifolia.*

I. INTRODUCTION

Globalization that echoed in the past two decades has recently changed people's lifestyles in most part of the world. People tend to eat fast food or often known as *junk food*. Foods that are high in fat and cholesterol can cause various diseases. One of them is high blood cholesterol levels in the body or also called hypercholesterolemia (Polychronopoulus *et al.*, 2005). Hypercholesterolemia Besides that, lack of exercise is a bad habit for people who can cause cardiovascular disease, such as coronary heart disease (Dalimartha, 2001). Foods that are high in cholesterol are found in the brain, tongue, liver, innards, egg yolk, shrimp, shellfish, beef, chicken meat, chicken skin, fatty milk, and used cooking oil (Apriadji, 2007).

High level of blood lipid is one of the risk factors for cardiovascular and metabolic diseases, such as atherosclerosis, coronary heart disease, and stroke (Riskesdas, 2013). Therefore, it is important to control plasma lipid levels so that the risk of the disease can be reduced .Decreasing total cholesterol and LDL levels can reduce deaths caused by coronary heart disease and total death (Dipiro, 2005).

Efforts that can be done to reduce cholesterol level in the blood that is reducing consumption of fat and excessive cholesterol. One plant that can reduce blood cholesterol levels is that which contains antioxidants in the form of flavonoids, tannins, and alkaloids. According to Jayashree *et al.*(2000) vanilla has antioxidants and the ability to prevent oxidative damage to membranes in mammalian tissues. Most of the components of vanilla contain vanillin whichacts as an antioxidant and is able to protect the membraneagainst lipid peroxidation and DNA to strands caused by reactive oxygen species (Salmah, 2014).

So far research on the benefits of vanilla as an anti hypercholesterolemia is still lacking. Current study aims to investigate vanilla extract can be used to reduce cholesterol levels in quail egg yolk-induced subjects. This research is a new effort to uncover other medical benefits of vanilla which is better known as food flavoring.

II. MATERIAL AND METHODS

Extraction of Vanilla Fruit

Fresh vanilla pods were obtained from a farmer in the District of Tanggamus, Lampung, Indonesia. The vanilla fruits were dried at 40°C for five days, and then chopped up to 0.2-0.5 cm. The pieces of vanilla fruit were macerated with 60% ethanol. Maceration was carried out for three days with solvent replacement every 24 hours at a temperature range of 20-30°C. Macerate then were evaporated using rotary evaporator at 60°C. The above extraction process is adopted from Setyaningsih (2006).

Experimental Design and Treatment

Twenty male mice aged 2-3 months with a body weight ranged 30-40 g were used as test subjects in this experiment. To make the test mice have high cholesterol levels, all test animals were induced with a suspension of quail egg yolks. Before inducement, the cholesterol level of each mouse is measured first as a baseline (Setiarto *et al.*,2018).

Mice (n=20) were divided into five groups. Group-1 (negative control) was given only high-fat quail egg yolks suspension. Group-2 (positive control) was treated with simvastatin at the dose of 10 mg/kg. Whereas group-3, 4 and 5 were given vanilla extract of 50, 100, and 200 mg/kg body weight respectively.

Blood Cholesterol Assessment

Blood sample were taken from tail-tip of mice. Total blood cholesterol were assessed using the *Easy Touch* GCU tool that has been previously calibrated. Cholesterol levels were measured on day 0, 15 and 30.

Data Analysis

Data were analyzed using One-Way ANOVA with α = 5% as the level of significance. LSD test was used in post hoc test at the level of significance α = 5%.

III. RESULTS AND DISCUSSION

Total blood cholesterol levels of mice assessed before and after treatment are presented in table 1. There is no statistical difference in total blood cholesterol levels among mice groups. On day 15, all groups showed a higher level of blood cholesterol compared to negative control group. On day 30, except for negative control, all mice group showed decrease of cholesterol levels, especially in mice treated with the highest dosage of vanilla extract (200 mg/kg).

Table1: Total blood cholesterol levels of mice before and after treatment.

Treatment	Total blood cholesterol in test mice expressed as mg/dl (mean ± SD)		
	Baseline	Day 15	Day 30
Negative control	143.00±5.48^{a}	144.00±4.90^{a}	148.50±7.94^{a}
Positive control	133.50±21.40^{a}	173.50±18.52^{b}	133.30±24.10^{ab}
Vanilla 50 mg/kg	132.30±22.00^{a}	165.00±27.40^{b}	122.00±12.88^{ab}
Vanilla 100 mg/kg	125.00±16.67^{a}	174.75±18.21^{b}	125.50±14.39^{ab}
Vanilla 200 mg/kg	124.25±16.98^{a}	152.80±24.00^{*b}	111.00±1.27^{b}
Values in the same column followed by the same superscript is not different by LSD post hoc test (α=0.05).			

As can be seen in Table 1, quail egg yolks suspension is effectively inducing total blood cholesterol levels in mice. It is because quail egg yolk is containing a very high cholesterol level, up to 3600 mg in 10 grams of yolk (Septianggi *et al.*, 2003). This shows that mice experience hypercholesterolemia because cholesterol levels in mice are between 26 - 82 mg/dl. The highest increase in cholesterol levels occurred in the positive control group and P2 groups as much as 40 mg/dl and 49.75 mg/dl. Cholesterol itself is an essential component of the structural membranes of all brain cells and nerves, cholesterol is also an important component in cell membranes, precursors of bile acids and steroids (Cheng and Hardy, 2004). Cholesterol in the body is obtained from two sources, namely from food (exogenous) and synthesis in the body (endogenous).

According to Hartoyo (2002), there are 3 factors that cause cholesterol levels in the body to increase namely a diet that contains too much cholesterol and fat, excretion of cholesterol into the colon through too little bile acid and too much cholesterol production in the liver.

Based on the total cholesterol on day 30 presented in Table1, it is found that the effective decrease was the vanilla 100 mg/kg group with a decrease in total cholesterol level of 49.25 mg/dl, followed by the vanilla 50 mg/kg group which is able to reduce cholesterol levels by 43 mg/dl. The most effective treatment group used vanilla fruit ethanol extract was 4.2 mg per day of ethanol extract of vanilla fruit. According to Da'i (1998) that vanilla fruit extract contains vanillin compounds which have antioxidant activity by hydroxy groups in aromatic nuclei, this has also been proven in research on the antioxidant properties of curcumin and its analogues with various approaches that have proven the role of hydroxy groups for reducing properties and radical catcher (Rianto, 1998) on curcumin and its analogues.

This decrease is assumed that the presence of antioxidant activity in vanilla fruit extract can react with free radicals through direct capture of oxygen free radicals and inhibit enzymes that cause free radicals such as cyclooxygenase and lipoxygenase to form.The average measurement results of total blood cholesterol levels of mice were then tested for homogeneity to determine the variance in the data, and the significance value showed ($p > 0.05$).Furthermore, the data is further tested by BNT test with a significance level of 5%.

In the control group using the drug simvastatin 0.039 mg/day also experienced a decrease in total cholesterol levels in mice. This is because simvastatin is a drug from the statin class that can inhibit and reduce the activity of enzymes that play a role in cholesterol biosynthesis. The three stages are the formation of HMG-CoA

(Hydroxymethylglutaryl-CoA) from acetyl-CoA, turning HMG-CoA into skualene (McKee and McKee,2003).

The results of this study are ethanol extract of vanilla (*Vanilla planifolia* Andrews) fruit which has antihypercholesterolemic activity which can influence the reduction of total cholesterol levels of male mice (*Mus musculus* L.) induced by quail egg yolk.

IV. CONCLUSION

It is worth concluding that vanilla fruit extract potential to be used as anti-hypercholesterolemia.

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