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The Effects of Giving Coconut Milk on Aortic Wall Thickness of Rats (Rattus norvegicus strain Wistar) Fed with High Fat Diet (HFD)

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ABSTRACT

Atherosclerosis is a chronic disease due to fat accumulation in the blood vessel walls. Atherosclerosis is the leading cause of cardiovascular disease. Indonesian people's habit of consuming coconut milk is suspected of causing dyslipidemia, a risk factor for atherosclerosis, by increasing low-density lipoprotein (LDL). However, other studies suggest that coconut milk can increase high-density lipoprotein (HDL), an antioxidant that prevents atherosclerosis. Because of the different findings, this study aims to determine the effects of coconut milk administration on the aortic wall thickness of rats (Rattus norvegicus strain Wistar) fed a high-fat diet (HFD). This true experimental study used samples of 28 male Wistar rats divided into seven groups. These groups consisted of the normal group (N), the negative control group (Kn), and five treatment groups that received coconut milk at various doses, including 2, 5, 3.75, 5, 7. 5, and 10 ml/kgBW/day. The results obtained were then analyzed using SPSS. The statistical tests used are the independent t-test, one-way ANOVA, LSD post hoc, Pearson correlation test, and regression test. Based on this study, the results showed that giving HFD for 45 days could cause the aortic wall to thicken. Meanwhile, giving an HFD accompanied by coconut milk for 45 days can prevent the thickening of the aortic wall, with the maximum effective dose that can be given being 9.75 ml/kg BW/day.
Keywords: aortic wall thickening; coconut milk; high-fat diet

INTRODUCTION

Atherosclerosis is a chronic disease of the cardiovascular system that occurs as a result of fat accumulating on the walls of blood vessels so that the lumen narrows progressively (Kumar, Abbas, and Aster 2013; Herrington et al. 2016; American Heart Association 2020). In 2018, 1.017.290 Indonesians suffered from cardiovascular disease (Badan Penelitian dan Pengembangan Kesehatan 2019). In 2015, as many as 31% of deaths worldwide occurred due to atherosclerosis (Kim et al. 2019).

One of the risk factors for atherosclerosis is dyslipidemia, a disorder of the blood lipid profile characterized by increased levels of total cholesterol, LDL cholesterol, and triglycerides (TGA) or decreased HDL levels (Kumar, Abbas, and Aster 2013). Endothelium damage brought on by high cholesterol levels can cause increased LDL in the blood to enter the tunica intima. When LDL in the tunica intima reacts with reactive oxidative species (ROS), it produces oxidized LDL, also known as ox-LDL. Macrophages will phagocytize oxyLDL and produce foam cells as a result. High cholesterol also causes the breakdown of nitric oxide (NO). Foam cells and smooth muscle cells thicken the tunica intima, while reduced NO permanently damages the endothelium (Kumar, Abbas, and Aster 2013; Hao and Friedman 2014).

Regularly consuming high saturated fat foods can lead to elevated blood cholesterol levels. Blood LDL levels may rise due to the body's high-fat content (Sacks et al. 2017). One of the foods with high levels of saturated fat is coconut milk (Gani 2015; Wong, Choo, and Chew 2020). Coconut milk is the result of the juice of grated coconut, which is liquid and white (Wulandari, Lestari, and Alfiani 2017). Research conducted by Eyres et al. (2016) states that consuming coconut milk or other processed coconut products increases blood LDL levels. Elevated LDL levels can raise the risk of atherosclerosis because oxidized LDL can harm endothelial cells, promoting the growth of foam cells and macrophages and thickening the aortic wall. However, according to several other studies, medium-chain fatty acids (MCFA), particularly lauric acid, which accounts for up to 49.2% of the saturated fat in coconut milk, are the main component. Since it can be transported and metabolized in the liver via the portal vein, this lauric acid does not lead to fat accumulation. Lowering body fat can raise HDL levels while lowering LDL levels. Polyphenols, which have antioxidant properties, are also present in coconut milk. By preventing endothelial dysfunction and

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foam cell formation brought on by the rise in HDL levels and antioxidant effect, the atherosclerotic process can be inhibited (McCarty and DiNicolantonio 2016; Schönfeld and Wojtczak 2016; Liu et al. 2017).

Different results regarding how coconut milk affects LDL and HDL levels result in different results regarding how the atherosclerosis process, characterized by aortic thickening, is affected. The purpose of this study is to ascertain how coconut milk affects the thickness of the aortic wall in rats that have been induced by a high-fat diet (HFD),

METHODOLOGY

Materials

Coconut milk is made by squeezing the grated coconut purchased from Tanjung Market in Jember. The coconut used comes from plantations in Kacapiring, Jember. Pure coconut milk can be produced from grated coconut squeezed with a cloth.

The high-fat diet (HFD) was made from duck egg yolk and used cooking oil obtained from frying 450 grams of tofu in 1 liter of Sabrina brand palm cooking oil. Frying was carried out nine times (Jannah, Isdadiyanto, and Sitasiwi, 2020).

The experimental animals used were male Wistar rats obtained from rat farms in Malang. Wistar rats were around 2-3 months old and weighed 150–250 g. This research has obtained Ethical Clearance No. 1657/H25.1.11/KE/2022.

Methods

The posttest randomized control group design is the research design used in this true experimental study (Sugiyono 2021). Data were obtained from microscopic observation of aortic histopathology (Callista, Handayani, and Tritisari 2015).

After acclimatization, samples were randomly divided into seven groups with different treatments for 45 days.

Group 1: N, the normal control group, was given a standard feed; Group 2: Kn, the negative control group, was given HFD; Group 3: P1, the first treatment group, was given HFD and 2.5 ml/kg BW/day of coconut milk; Group 4: P2, second treatment group; experimental animals were given HFD and 3,75 ml/kg BW/day coconut milk; Group 5: P3, experimental animals were given HFD and 5 ml/kg BW/day coconut milk;mGroup 6: P4, test animals were given HFD and 7.5 ml/kg BW/day coconut milk; Group 7: P5 was given HFD and 10 ml/kg BW/day of coconut milk.

Coconut milk and HFD were given to the experimental animals through a gastric tube. The HFD given is 2.5 ml (200 g) of duck egg yolk and 1.5 ml (200 g) of used cooking oil (Jannah, Isdadiyanto, and Sitasiwi 2020).

After 45 days of treatment, the experimental animals were terminated using ketamine and xylazine. Then, the abdominal aorta was taken to make histological preparations with hematoxylin and eosin (HE) staining. An AmScope and an Olympus light microscope with 400x magnification were used to make the observations. Using Image Raster software, the thickness of the aortic wall was measured in eight viewing zones (Callista, Handayani, and Tritisari 2015).

SPSS, with a statistical significance level of 0.05 ($p = 0.05$), was used to analyze the data. An independent sample t-test was the analytical method used to evaluate the impact of HFD administration on aortic wall thickness. The means of each group were compared using one-way analysis of variance (ANOVA) and the LSD post-hoc test. A Pearson's correlation test was performed to determine the relationship between aortic wall thickness and the administration of coconut milk at different doses. A linear regression test was used to determine the maximum effective dose of coconut milk in preventing aortic wall thickening (Sugiyono 2021).

RESULT AND DISCUSSION

After 45 days of treatment, the rats were weighed to determine their gain. Table I shows the average increase in BW in each group. The highest average body weight (BW) change was found in the rats in the HFD (Kn) group, which was 51,50 gr. In the rats given coconut milk group, weight changes did not increase as much as in the group that was only given HFD. Administration of coconut milk at a dose of 10 ml/kg BW caused the lowest increase in BW, almost the same as in the normal group, namely 28,50 gr.

The variable examined in this study was the thickness of the aortic wall, which is the initial phase of atherosclerosis. The thickness of the aortic wall was measured by drawing a straight line from the tunica media's outermost line to the tunica intima's innermost line. The aortic wall was observed in eight visual fields in each rat with a magnification of 400x. In Figure 1, it is shown that the aortic wall is thicker compared to the other groups. In the treatment group given coconut milk, the aortic wall gets thinner, in line with the higher dose of coconut milk. The thinnest aortic walls were in the normal and P5 groups, who were given 10 ml/kg BW of coconut milk.

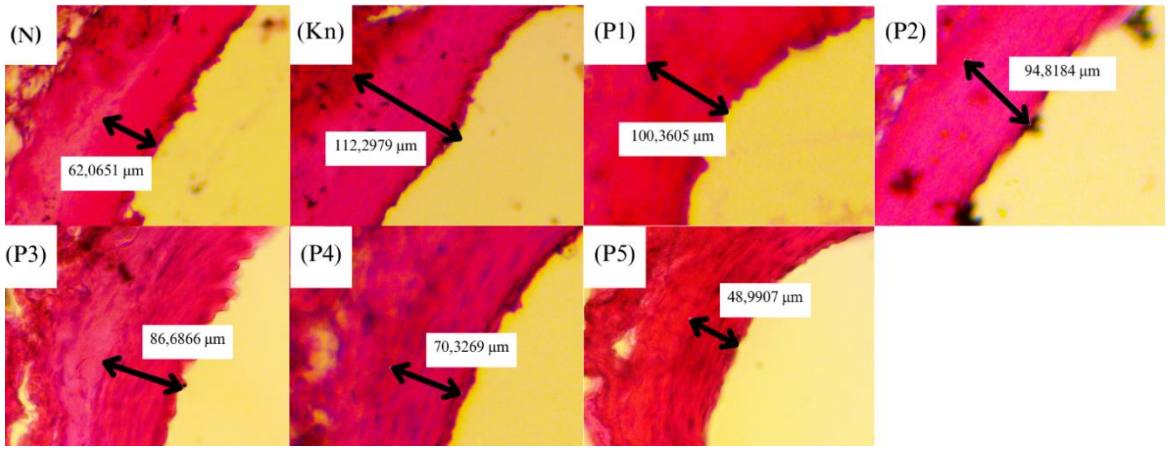

Figure 1. Histopathological picture of the aortic wall of rats with hematoxylin-eosin staining at 400x magnification. N: normal group (given standard feed), Kn: negative control group (given HFD); P1: treatment group 1 (given HFD and coconut milk 2,5 ml/kg BW), P2: treatment group 2 (given HFD and coconut milk 3,75 ml/kg BW), P3: treatment group 3 (given HFD and coconut milk 5 ml/kg BW), P4: treatment group 4 (given HFD and coconut milk 7,5 ml/kg BW), P5: treatment group 5 (given HFD and coconut milk 10 ml/kg BW)

Table I. Mean body weight

Group	Mean ± Standard deviation (gr)
N	27,25 ± 2,217
Kn	51,50 ± 5,000
P1	44,25 ± 5,965
P2	37,75 ± 2,062
P3	32,50 ± 3,109
P4	30,00 ± 5,099
P5	28,50 ± 2,887

Then, the mean in each group was calculated from the measurements of the aortic wall thickness. Table II shows each group's mean aortic wall thickness in Wistar rats.

Figure 2 illustrates that the negative control group (Kn) had the thickest aortic wall compared to the other groups. Demonstrates that administering 2.5 ml/200 g of duck egg yolk and 1.5 ml/200 g of used cooking oil can thicken the aortic wall. According to Jannah, Isdadiyanto, and Sitasiwi's (2020) research, giving rats duck egg yolk and repeatedly cooking oil can result in dyslipidemia in rats. The atherogenic index of plasma (AIP) has been shown to rise after the administration of trans oils, such as used cooking oil (Supiyani et al., 2021).

According to Jannah, Isdadiyanto, and Sitasiwi (2020), the frying process generates ROS, which can harm the endothelium. Research conducted by Xian et al. (2012) states that the antioxidant properties of vitamin E contained in the oil are susceptible to heating, so repeated heating of the oil can decrease the antioxidant properties of cooking oil. Under oxidative conditions, nitric oxide (NO) bioavailability was decreased, leading to smooth muscle proliferation, permanent damage to endothelial cells, and inhibition of vasodilator activity. Additionally, the endothelium's damage enables monocytes to enter the tunica intima. Also, the presence of endothelium damage allows monocytes to enter the tunica intima. In time, the monocytes will mature into macrophages. After that, foam cells developed as a result of macrophages phagocytosing oxidized LDL. The accumulation of smooth muscle cells and foam cells in the tunica intima causes the aortic wall to thicken (Kumar, Abbas, and Aster 2013; Hao and Friedman 2014; Di Pietro et al. 2020; Jannah, Isdadiyanto, and Sitasiwi 2020; Wei et al. 2021).

On the other hand, rats treated with coconut milk (groups P1, P2, P3, P4, and P5) had thinner aortic walls than the Kn group. Administration of 10 ml/kg BW/day of coconut milk in the P5 group

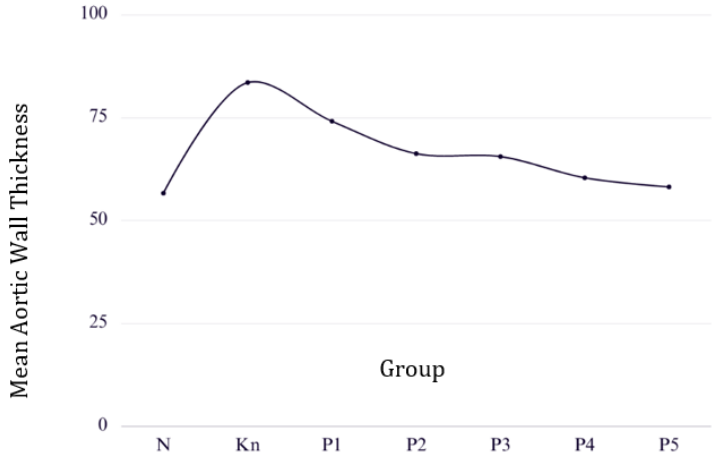

Figure 2: Average aortic wall thickness graph

Table II. Mean aortic wall thickness

Group	Mean ± Standard deviation (µm)
N	56,7211 ± 4,6412
Kn	83,5861 ± 14,436
P1	74,1832 ± 9,9255
P2	66,3197 ± 8,6548
P3	65,6036 ± 19,769
P4	60,4467 ± 1,1655
P5	58,2341 ± 2,0007

significantly prevented the thickening of the aortic wall ($p<0,05$) and had almost the same wall as the normal group. According to a study by Ejike, Nwankwo, and Ijeh (2010), consuming 0.2 ml of coconut milk per day did not increase the risk of cardiovascular disease. This is due to the MCFA in coconut milk having a distinct metabolism from other fats. According to Liu et al. (2017), MCFA can be taken directly to the other liver and eliminated as bile. These findings, however, differ from those of a study done by Neelakantan, Seah, and Van Dam (2020), which found that the saturated fat present in coconut milk can have a hypercholesterolemia effect when consumed, which is characterized by an increase in cholesterol and LDL cholesterol levels.

In the regression test, the numbers contained in the estimation parameters section are then entered into the equation to determine the maximum value (Xmax) of the dose with the first derivative In the regression test, the numbers contained in the estimation parameters section are then entered into the equation to determine the maximum value (Xmax) of the dose with the first derivative ($y' = 0$). Regression equation:

$$y = 0,277x^2 - 5,402x + 84,872$$

example, $y'=0$

$$0 = 0,554x - 5,402$$

$$x = 9,75 \text{ ml/kg BW/day}$$

The regression test results showed that the maximum effective dose of coconut milk in preventing the thickening of the aortic walls of rats was 9.75 ml/kg BW/day. The ability of coconut milk to prevent thickening of the aortic wall is because it contains antioxidants, namely polyphenols, which remain stable even though they have been heated (Karunasiri et al. 2020).

The administration of polyphenols can reduce ROS production and increase NO production in the aorta. Oxidative stress can be reduced due to increased enzymes that play a role in eliminating ROS, namely superoxide dismutase (SOD) and catalase (CAT). The SOD plays a role in the dismutation of superoxide radicals into hydrogen peroxide (H_2O_2), and the CAT works to decompose H_2O_2 into water and oxygen molecules (Cheng et al. 2017; Famurewa and Ejezie 2018).

Coconut milk has a 23.1% inhibitory effect on the thickening of the aortic wall. Coconut milk's effects follow other all-natural ingredients like neem leaf extract, which can prevent 88.5% of atherosclerosis (Jannah, Isdadiyanto, and Sitasiwi 2020). Simvastatin, a common drug frequently used to treat atherosclerosis, is within 89.80% of

this capability. Simvastatin functions similarly to coconut milk in regulating lipids, preventing platelet aggregation, stabilizing plaque, enhancing endothelial function, and reducing inflammation (Zhang et al., 2021).

CONCLUSION

According to the research that has been done, it can be concluded that giving coconut milk prevents the thickening of the aortic wall of rats due to HFD. Giving coconut milk at a higher dose causes increased prevention ability with an effective dose of 9.75 ml/kg BW/day.

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