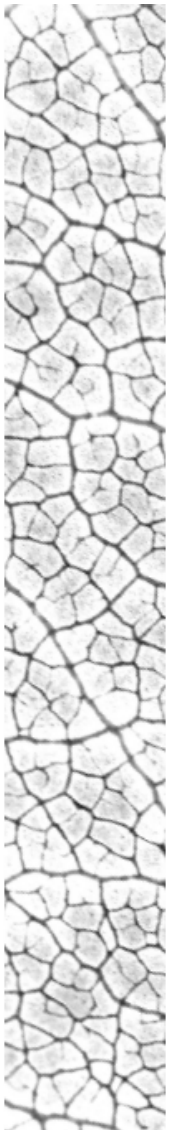


EurAsian Journal of BioSciences
Eurasia J Biosci 13, 231-238 (2019)

Fresh and roasted Canarium nut (*Canarium vulgare*) altering the lipid profile of hypercholesterolemic rats (*Rattus norvegicus*)

Meitycorfrida Mailoa [1*], Tri Dewanti Widyaningsih [2], Widya Dwi Rukmi Putri [2], Harijono [2]

[1] Department Agricultural Technology, Faculty of Agricultural, Pattimura University, Ambon 97233, INDONESIA
[2] Department Agricultural of Technology, Faculty of Agricultural Technology, Brawijaya University, Malang 65145, East Java, INDONESIA
*Corresponding author: meitymailoa@gmail.com

Abstract
Hypercholesterolemia is a disorder of cholesterol metabolism, caused by cholesterol level in the blood exceeding the normal limit. This study aimed to evaluate the effects of fresh and roasted canarium nut treatment (*Canarium vulgare* L.) on lowering cholesterol, low-density lipoprotein (LDL), and triglyceride levels, while increasing high-density lipoprotein (HDL) levels and repairing aortic tissue histopathology. This study used 24 male Wistar rats (*Rattus norvegicus*). The experimental animals were treated with induced hypercholesterolemia for 9 weeks. The treatment was conducted for 4 weeks, with the provision of 0.9, 1.8 or2.7 g fresh and roasted canarium nut. The design used in this study was a completely randomized design (CRD) followed by Tukey test on treatments which differed significantly. Fresh and roasted canarium nut treatment reduced cholesterol, LDL, and triglycerides and increased HDL in hypercholesterolemic rats. Fresh and roasted canarium nut treatment reduced endothelial damage in aortic histopathology. Fresh canarium nut therapy and roasted canarium nut treatment show potential in curing hypercholesterolemia and minimizing endothelial dysfunction.

Keywords: Cholesterol, LDL, HDL, Triglycerides, Histopathology

Mailoa M, Widyaningsih TD, Rukmi Putri WD, Harijono (2019) Fresh and roasted Canarium nut (*Canarium vulgare*) altering the lipid profile of hypercholesterolemic rats (*Rattus norvegicus*). Eurasia J Biosci 13: 231-238.

INTRODUCTION

Canarium nut is a tropical plant of the family Burseraceae, genus *Canarium*, and its 100 species mostly grow in lowland moist forests of Melanesia (Doke and Guha 2015, Rabbani et al. 2014, Safdari et al. 2013). Canarium nut (*Canarium harveyi*) grows in Indonesia, Papua New Guinea, the Solomon Islands, and Vanuatu (Kennedy and Clarke 2004). In Indonesia, canarium nut commonly grows in eastern areas such as Sulawesi, Maluku, and North Maluku. Canarium nut (*C. vulgare* L.) is high in unsaturated fatty acids and protein. Fresh canarium nut contains 2174.1–2618.0 mg/g linolenic acid, 136.8–142.6 mg/g linoleic acid, 8152.6–9295.2 mg/g oleic acid, and 8.2–9.7% protein, while sand-roasted canarium nut contains 3791.9–5106.0 mg/g linolenic acid, 182.7–252.2 mg/g linoleic acid, 10980.9–12824.2 mg/g oleic acid and 12.1–13.7% protein (Şen et al. 2014, Şenol et al. 2015, Thomson and Evans 2006).

Hypercholesterolemia is a disorder of cholesterol metabolism, caused by the cholesterol level in the blood exceeding the normal limit. The disease is also caused by the excess production of free radical oxygen and oxidative stress (Mailoa 2015). High LDL concentrations affect the development of LDL oxidation in hypercholesterolemia. Pet food which contains meat, liver, brain, and viscera causes an excess of total cholesterol in the blood (Shakirin et al. 2012). Research into the African canarium nut suggests that the unsaturated fatty acids in canarium nut can be used to lower cholesterol (Schlesinger 2011). Various research results show that unsaturated fatty acids and proteins can lower blood cholesterol levels (Ayoade et al. 2012, Wang et al. 2004). The results of the study of Shakirin et al. (2015) also concluded that there was a significant decrease in cholesterol in guinea pigs after being given a walnut oil.

This study aimed to compare the effect of fresh and sand-roasted canarium nut (*C. vulgare* L.) treatment on lowering cholesterol, low-density lipoprotein (LDL), and triglyceride levels, raising the level of high-density lipoprotein (HDL) and repairing the aortic tissue histopathology picture in hypercholesterolemic rats.

Received: September 2018
Accepted: April 2019
Printed: May 2019

MATERIALS AND METHODS

Materials

The material used in this study was black canarium nut (*C. vulgare* Leenh.) taken from Lilibooy, West Leihitu, Central Maluku. It was divided into fresh canarium nut and roasted canarium nut. The roasted canarium nut was roasted with sand at 80±2 °C to produce dried canarium nut with a 1:2 ratio of canarium nut and sand. Other materials used in this study were Milk PAP feed, lard, quail egg yolk, a DiaSys reagent kit, physiological NaCl, formalin, alcohol, paraffin, xylol solution, hematoxylin and eosin (HE), and distilled water. Male Wistar rats (*Rattus norvegicus*) weighing approximately 200 g were used as the experimental animals in this study and were obtained from the Laboratory of Zoology, Faculty of Mathematics and Sciences, Pattimura University. Rats were divided into eight groups: the first group was the control group (KN), which consisted of three rats fed with a standard feed (PAP milk), and the other seven groups consisted of three rats each fed with various hypercholesterolemia dietary feeds, i.e., PAP milk mixed with lard and a quail egg yolk.

Induction of Hypercholesterolemia

Hypercholesterolemia was induced by mixing of lard and 1g of boiled quail egg yolk with distilled water and heating at100 °C until the volume of the mixture reached 2 mL. This was fed to the rats by a nasogastric tube 1h before the standard feeding. This treatment was performed for 9 weeks, and the rat lipid profile was tested every 3 weeks.

Canarium nut Treatment

Fresh canarium nutand roasted canarium nut werecut into small pieces resembling pellets and were given to rats with hypercholesterolemia. The rats were divided into eight groups : one group was fed with a regular feed (PAP milk), and the other seven groups were given atherogenic diet feed in the form of PAP milk mixed with lard and quail egg fat(yolk), and distilled water, administered via a nasogastric tube. Each group consisted of three rats. The fat profile of each experimental animal was measured every 3 weeks for 9 weeks.

The atherogenic diets were as follows: KS1 (0.9g fresh canarium nut therapy), KS2 (1.8g fresh canarium nut treatment), KS3 (2.7g fresh canarium nut treatment), KK1 (0.9g roasted canarium nut treatment), KK2 (1.8g roasted canarium nut treatment), and KK3 (2.7g roasted canarium nut treatment), while the standard feed was given in the form of 20–30g PAP milk feed/rat for 4 weeks; water was provided ad libitum.

Serum Sampling

Pre-examination serum was taken from the tail vein, while post-examination serum was taken from the heart. Blood was collected in an Eppendorf tube, placed in a 45° tilted position, precipitated at room temperature for ±3.5 h, and centrifuged for 15 minutes at 3000 rpm.

Examination of Cholesterol, LDL, HDL, and Triglycerides

Measurements of the lipid profile (cholesterol, LDL, HDL, and triglycerides) were made using a Biosystems kit. Measurements were performed using the following procedures: a 10mL sample was taken, turned into a 10mL standard solution, pipetted into a test tube, incubated for 10 min at 37 °C, and then placed on a spectrophotometer at 546 nm for examination (Wang et al. 2004).

Aorta Histology

After the rats had been treated with canarium nuts for 4 weeks, they were operated on to remove their aorta. The aortic tissue was washed with physiological NaCl and fixed with 4% formalin for 18 h. Dehydration (drying) was performed by using 30%, 50%, 70%, 80% and 90% alcohol rinse. Inside the tool, there was paraffin (top), xylol solution (middle), and alcohol (bottom); the process took 24 h. The tissue was placed in a block, and paraffin solution was added, before being cooled for ±15min until it hardened. Bleaching was done using HE. Samples were observed under a microscope (Olympus BX57) and photographed (Seneviratne 2011).

Statistical Analysis

This research employed a completely randomized design with three replications. Data from the research were then analyzed with ANOVA based on the design employed, followed by Tukey test on any distinguished treatments.

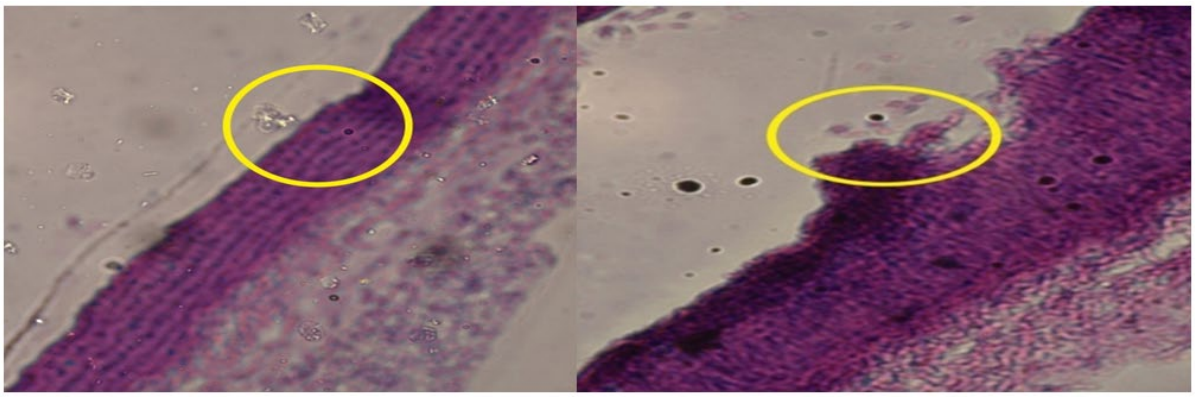
RESULTS AND DISCUSSION

Fat Profile for 9 Weeks (Pre-treatment)

After 9 weeks of atherogenic diet administration, there was an increase in cholesterol levels compared to the control group without atherogenic diet (KN). In the control group without atherogenic diet (KN), the blood cholesterol levels were 107.33mg/dL in the third week, increasing to 116.33mg/dL by the ninth week. Meanwhile, in treatment groups 1–7, which were fed with atherogenic diets, the cholesterol levels were 103.66–121.00mg/dL in the third week, increasing to 217.33–247.00mg/dL in the ninth week. The same pattern occurred for the levels of LDL and triglycerides, while HDL levels decreased (**Table 1**). After 9 weeks of atherogenic diet administration, a rat was selected from the atherogenic diet group and the control group. Necropsy was performed to remove the aorta toassess differences after staining with HE. The results presented in **Fig. 1**.

Table 1. The average of cholesterol, LDL, HDL, triglycerides on white rats for 9 weeks pre-canarium nut treatment (mg/dL)

TIME	GROUP	KN	1 (KP)	2 (KS1)	3(KS2)	4(KS3)	5(KK1)	6(KK2)	7(KK3)
Third Week	Cholesterol	107.33±4.16	121±1.63	104.33±3.22	106.33±1.15	108.66±1.15	106.66±3.51	103.66±1.53	105±3.61
	LDL	56.66±5.85	51.66±2.36	52±3	53.66±3.78	63.66±4.16	53.33±3.51	61.33±3.05	63±2.64
	HDL	43.66±3.52	42.33±3.29	41±1	44.66±4.04	45±3.61	49±3.61	48.66±3.21	44.33±3.05
	Triglycerides	65.33±8.51	93.33±3.39	84.33±4.93	79.66±1.53	74.66±0.57	72±9.84	90.66±7.02	65±7.94
Sixth Week	Cholesterol	112±4.58	200±3	207.33±2.08	207.66±5.03	206.33±1.53	206.33±3.78	206±3.61	207.3±3.78
	LDL	63.33±15.27	94±5.29	109±1.73	112.66±5.86	106.66±1.53	109±3.61	108.66±1.15	104±1.73
	HDL	44.66±3.05	37.66±2.52	35.33±0.57	33.66±2.08	34.33±1.15	33.33±1.53	37.33±0.57	33.33±1.53
	Triglycerides	76.33±8.51	110.3±15.6	130.33±18.9	152.33±9.29	168.66±3.22	155.66±6.02	159.33±1.53	138±12.76
Ninth Week	Cholesterol	116.33±3.51	223.3±11.5	240±10	235.33±4.50	247±2.64	217.33±2.52	219.33±1.15	227.6±5.86
	LDL	64±17.35	110±10	124±1.73	119±1	118.66±2.08	120±5	121.66±8.02	120±1
	HDL	46±1	34.66±3.22	31.66±1.53	34.33±2.08	32.66±1.15	30.33±1.53	31.33±2.08	35±1.73
	Triglycerides	86.33±11.85	155.6±4.04	170±10	167.66±4.93	180±1	183.6±14.57	160.66±5.13	180±15



A B

Fig. 1. Aorta histopathology structure of treated rats after HE staining; A (normal rat), B (hypercholesterolemia rat)

The consumption of lard and fat from quail eggs was a trigger factor for high cholesterol, LDL, and triglyceride levels and decreased HDL. Lard contains high cholesterol (130mg) compared to quail eggs (71mg) (Chris et al. 2015). A high intake of cholesterol leads to increased levels of total cholesterol and increased LDL due to uncompensated HDL being brought back to the liver (Mailoa 2015). Hypercholesterolemia is a condition caused by an excess of blood cholesterol. The limit of normal blood cholesterol levels is below 200mg/dL, LDL under 130mg/dL, HDL above 40mg/dL, and triglyceride below 200mg/dL (Chris et al. 2015).

Hypercholesterolemia is a condition caused by the excess production of free radical oxygen and oxidative stress. Hypercholesterolemia, especially the LDL fraction, is an essential factor in the formation of atherosclerosis. An increase in plasma LDL is associated with susceptibility to the development of atherosclerosis. Atherosclerosis comes from the Greek “athera” which means gruel, and “sclerosis” means hardening. Atherosclerosis is the formation of spots like gruel, consisting of cholesterol fat accumulated in the tunica intima layer of the blood vessel lumen (Mailoa 2015). It results in thickening of the blood vessel wall and loss of elasticity of the arteries, accompanied by changes in the media and degeneration of the intima layer (NIH 2014). An atherogenic diet is a high-cholesterol feed given to rats for 9 weeks and has been proven to cause a state of hypercholesterolemia. This triggers oxidative stress in rat endothelial cells (Mailoa 2015). Oxidative stress is the result of an imbalance between the production of oxygen and the natural antioxidant defense system (Orford 2005). The histopathological image of the aorta in **Fig. 1** was obtained by using HE staining and shows differences in endothelial cells in the tunica intima. The histopathological image of the aorta of an average rat (A) shows a layer of neat and smooth endothelial cells, while the image of a hypercholesterolemic rat (B) shows the presence of endothelial damage, with endothelial cells forming a layer of rough and irregular tunica intima until the endothelial cells are released into the lumen. Endothelial cells are a type of cell that forms a tissue called the endothelium, which separates blood vessels and the lymphatic system throughout the body. Endothelial cells have a flat surface with a cell nucleus. Vascular endothelium is a multifunctional organ system in active metabolism and the physiological response of cell components, i.e., regulating blood flow depending on the metabolic state (Platat et al. 2015). LDL oxidation is also harmful to the endothelium because it causes endothelial dysfunction. Endothelial cell damage is a significant stimulus for the occurrence of atherosclerosis (Pena et al. 2014). As shown in the rat aorta histopathological image (**Fig. 1**) and by the increased blood cholesterol, LDL, and triglyceride levels and decreased HDL (**Table 1**), it was proven that rats fed a

Table 2. Changes of Wistarrats cholesterol level during the canarium nut treatment

Week	KN	KP	KS1	KS2	KS3	KK1	KK2	KK3
0	116.33 ±3.51	223.3 ± 11.5	240 ± 10	235.33 ± 4.50	247 ± 2.64	217.33 ±2.52	219.33 ± 1.15	227.6 ± 5.86
1	122.33 ± 5.51 efg	215.66 ± 4.04 a	215.66 ± 5.13 a	209.33 ± 6.66 a	211± 5.56 a	199.66 ± 6.81 ab	210 ± 6 a	209± 3.61a
2	120 ± 4.36 efgh	199.66 ± 1.53 ab	184.33 ± 29.77 abc	148 ± 17.08 cde	137.66 ± 7.51 def	180.66 ± 2.08 abc	170 ± 15.39 bcd	106.33 ± 4.04 fghi
3	123.66 ± 3.22 efg	164.33± 47.28 bcd	95.66 ± 2.08 ghi	87.33± 9.45 ghi	91.66± 3.78 ghi	94.33 ± 6.43 ghi	101± 3 fghi	81.66 ± 5.13 hi
4	119.33 ± 9.51 efgh	136.66 ±14.05 def	81.33 ± 6.66 hi	80.33 ± 4.73 i	86.66 ± 3.05 ghi	78.33± 3.51 i	85.66 ± 6.03 ghi	79.66 ± 4.73 i

Notes:
KN =Normal rats, normal diet
KP =Hypercholesterolemic rats, normal diet
KS1 =Hypercholesterolemic rats, fresh canarium nut treatment 0.9 g
KS2 =Hypercholesterolemic rats, fresh canarium nut treatment 1.8 g
KS3 =Hypercholesterolemic rats, fresh canarium nut treatment 2.7 g
KK1 =Hypercholesterolemic rats, dry canarium nut treatment 0.9 g
KK2 =Hypercholesterolemic rats, dry canarium nut treatment 1.8 g
KK3 =Hypercholesterolemic rats, dry canarium nut treatment 2.7 g

Table 3. Changes of Wistar rat LDL level during the canarium nut treatment

Week	KN	KP	KS1	KS2	KS3	KK1	KK2	KK3
0	64±17.35	110±10	124 ±1.73	119±1	118.66±2.08	120±5	121.66±8.02	120±1
1	66±17.35 ghij	111.33±6.11 ab	103.66±1.53 abcd	101.33±1.53 abcde	99.66±1.53 abcdef	114.66±4.51 a	106.33±4.16 abc	102.66±2.08 abcd
2	74.66±17.24 defghij	103.33± 12.58 abcd	91.66±5.68 abcdefg	88 ± 3 abcdefg	80 ± 5 cdefgh	87.66±2.08 abcdefg	87.66±3.05 abcdefg	83.66±4.16 bcdefgh
3	71±20.07 fghij	54.33±3.05 cdefgh	47.66±1.53 ij	74 ± 6 defghij	79.33±1.53 cdefgh	81.33±3.51 bcdefgh	80±7.81cdefgh	80.66±24.01 hij
4	77±17.52 cdefghi	71.66±3.78 cdefgh	45.33±4.51 j	73.66±10.26 defghij	74±3.61 defghij	78.66± 2.52 cdefgh	80.66±17.09 efghij	76±3.61 defghi

Table 4. Changes of Wistar rat triglyceride level during the canarium nut treatment

Week	KN	KP	KS1	KS2	KS3	KK1	KK2	KK3
0	86.33±11.85	155.6±4.04	170±10	167.66±4.93	180±1	183.6±14.57	160.66±5.13	180±15
1	88.33±9.07 ghi	175.66±14.01 a	158.66±1.53 ab	154.66±0.57 ab	160 ± 1 ab	173.66±6.11 a	148±2.65 abc	152.66±3.22 ab
2	91.66±3.05 ghi	152.33±4.73 ab	90.33±1.53 ghi	132±10.58 bcdef	132.33±7.37 bcde	131.66±59.18 bcdef	125.66±8.33 bcdefg	112±4.36 cdefgh
3	93.33±3.05 efghi	141.66±3.05 abc	84±7.94 hi	91±9.64 ghi	97.66±2.52 defghi	95.33±8.14 defghi	87.33±2.52 ghi	79.66±4.04 hi
4	90 ± 5 ghi	133±12.53 bcd	60.66±8.02 i	79.66±5.68 hi	94±3.61defghi	92.66±4.73 fghi	83±3.61hi	76±3.61 hi

Table 5. Changes of Wistar rat HDL level during the canarium nut treatment

Week	KN	KP	KS1	KS2	KS3	KK1	KK2	KK3
0	46±1	34.66±3.22	31.66±1.53	34.33±2.08	32.66±1.15	30.33±1.53	31.33±2.08	35±1.73
1	45.33±0.57 ab	32±2.65 b	40±2.65 ab	41 ± 1 ab	42.33±2.08 ab	40.7±1 ab	40.8±1ab	40.66±0.57 ab
2	46.33±1.15 ab	36.33±1.53 b	40.6±3.21 ab	42±1.73 ab	42.33±2.52 ab	42 ± 1 ab	42±2.65 ab	42.33±0.57 ab
3	47 ± 1 ab	39±1 b	41±1.01 ab	43 ± 2 ab	43 ± 1 ab	43±1.73 ab	43 ± 2 ab	45 ± 2 ab
4	48±5.29 ab	40.66±2.08 ab	42±1.73 ab	43.66±2.08 ab	43 ± 2 ab	43.66±1.53 ab	46±3.61 ab	46±1.73 ab

9-week atherogenic diet developed hypercholesterolemia and endothelial cell damage.

Changes in Fat Profile after Canarium nut Treatment

The ANOVA results for cholesterol, LDL, triglyceride, and HDL levels showed that there were differences between each treatment (P<0.05), so the research continued with the Tukey test. The *t*-test results for each parameter (cholesterol, LDL, triglyceride, and HDL) from the first week to the fourth week are presented in **Tables 2**–**5**.

Cholesterol

Cholesterol is metabolite that contains sterol fat, which can be found in the cell membrane and is circulated in blood plasma. High cholesterol can trigger several degenerative diseases. Some research has shown that unsaturated fatty acids and proteins can help to lower blood cholesterol (Ayoade et al. 2015, Frisca et al. 2008).

The data in this research show that treatment with either fresh or roasted canarium nuts can lower blood cholesterol. Similarly, other research has shown that there is a significant lowering of blood cholesterol after guinea pigs are given walnut oil (Wang and de Meija 2005). After 1week of treatment, the rat blood cholesterol level was lower than that of hypercholesterolemic rats that were not treated with canarium nuts (**Table 2**) and continued to decrease

during the 4-week treatment. Rats that were not treated with canarium nuts (positive control) also showed lowering of blood cholesterol, but it was slower than in rats treated with canarium nuts. After comparing the results for each amount of fresh canarium nuts, it was found that the treatments with 1.8 and 2.7 g were better than that with 0.9 g. Therefore, it is suggested that humans should consume 100 or 150 g of canarium nuts a day. On the other hand, for dry canarium nuts, it was found that 2.7 g was better, so it is suggested that humans should consume 150 g of canarium nuts a day.

LDL

LDL is synthesized in the liver and is transported by blood. Excess LDL in the blood is deposited on the inside of artery walls. LDL can turn into plaque and narrow the artery so that the artery becomes less flexible. This condition is known as atherosclerosis. Several types of research have shown that treatment with either fresh canarium nuts or roasted canarium nuts can lower LDL, and there is no significant difference between different amounts of walnuts given. This shows that both fresh and dry walnuts can break off the plaque in arteries, which is a significant factor in overcoming atherosclerosis (Coplo 2005).

Given that there was no significant difference between 0.9, 1.8, and 2.7 g of either fresh or roasted canarium nuts, it can be concluded that 50 g of canarium nuts a day –fresh or dry – could lower LDL.

Triglycerides

Triglycerides are neutral fats composed of glycerol and three fatty acid chains which are synthesized in the liver or small intestine. Triglycerides come from fat in foods or are formed inside the body from other energy sources such as carbohydrates. Triglyceridesare a kind of fat absorbed by the intestine after undergoing hydrolysis. Triglycerides then go into the blood plasma in two forms: as chylomicron, which comes from intestinal absorption, and VLDL (very low-density lipoprotein), which is formed by the liver with the help of insulin derived from the body. Triglycerides that are outside the liver but inside a tissue like a blood vessel, muscle, or fat tissue are hydrolyzed by the enzyme lipoprotein lipase. The hydrolysis residue is metabolized by the liver into LDL cholesterol. The data in **Table 2** show that during the 9 weeks of atherogenic feed treatment, there was an improvement in triglyceride levels as the LDL improved. The high deposit of triglyceride can be used by the liver to form VLDL and LDL; this is apparently a risk for increasing blood levels of LDL, which is a risk factor for atherosclerosis (Tsallissavrina et al. 2006).

The high level of triglycerides in the mice was likely caused by the feeds that contained high fat (pork contained 65 g of fat and quail eggs contained 11.1 of fat). However, the research showed that either fresh or dry canarium nut treatment could lower blood triglyceride levels.

HDL

HDL is a reliable and tiny lipoprotein particle that is synthesized in the liver or intestine. HDL is often called a “good” fat because it cleans excessive cholesterol inside the blood vessel wall and brings it back to the liver. Increased HDL may slow the process of atherosclerosis (Zou et al. 2005).

Although the Tukey test did not show any significant difference between the groups of rats during the 4-week treatment, there were visual differences in HDL level. In the fourth week of canarium nut treatment, all the groups of rats that were previously hypercholesterolemic (30.33–35 mg/dL) had stable HDL levels (42.00–46.00 mg/dL). All treatments with different amounts of feed showed an improvement in HDL. HDL particles are essential in reverse cholesterol transfer, which is a process of transporting excessive cholesterol from the peripheral tissues to the liver. This process is often seen as the primary mechanism of HDL in protecting the body from any atherosclerosis risks (Tjandrawinata 2011). A high level of cholesterol, LDL, and triglyceride, and a low level of HDL cholesterol are relevant factors that can cause cardiovascular diseases (Gonzalo et al. 2014). Omega 3 is not useful in lowering LDL level because, besides its capability to reduce LDL, it is also capable of lowering HDL (Casagrande 2015). The results of the research have shown that the lowering of cholesterol, LDL, and triglyceride in rats is followed by an improvement in HDL. This is caused by the nature of canarium nuts that contain not only omega 3 fatty acids but also omega 6 and omega 9 fatty acids. These three fatty acids work together to lower the blood cholesterol level.

Aortic Histopathology of Rats after Canarium nut Treatment

Rats with hypercholesterolemia were fed with canarium nut for 4 weeks, and then four rats were taken as a representative sample for each of the four treatments negative control (KN), positive control (KP), fresh canarium nut (KS), and roasted canarium nut (KK). The aorta of each rat was removed for observation.

The results are shown in **Fig. 2**. Structure are shown in **Figs. 1** and **2**. They show that there was an improvement, where endothelial cells seemed to be neatly arranged, smooth and flat-shaped, just as a normal cell, in the fourth week after canarium nut therapy. This result indicates that the quality of the unsaturated fatty acids (omega 3, omega 6 and omega 9) and protein in canarium nut remained stable, although it had been through the soaking and drying process in the post-harvest treatment (Mailoa et al. 2016). Results showed that 4weeks was necessary for the canarium nut treatment to cure rats with hypercholesterolemia or atherosclerosis. Images of aorta histopathology in the

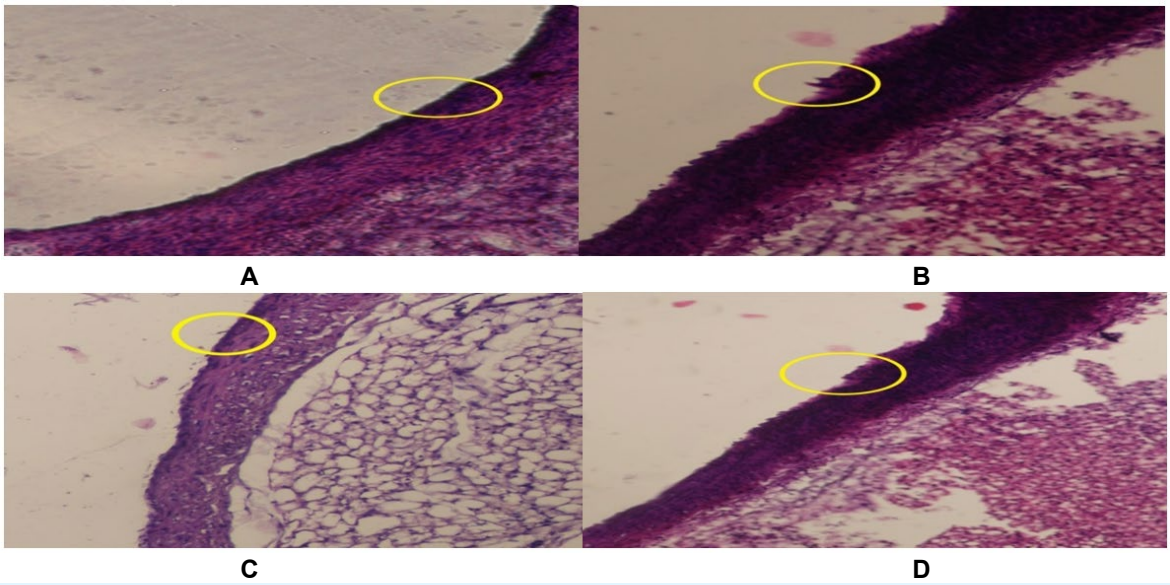


Fig. 2. Aorta histopathology structure of treated rats after HE staining; A (normal rat), B (hypercholesterolemia rat)

treatment group showed reduced endothelial damage. This is presumably caused by the unsaturated fatty acids and proteins contained in the canarium nut, which reduce endothelial damage by lowering cholesterol and LDL levels. Increased synthesis of bile acids can minimize the occurrence of LDL oxidation.

Soy protein can reduce cholesterol and triglycerides which circulate in the blood of an individual with hypercholesterolemia. This is presumably caused by the protein's ability to bind bile acids in the intestine and prevent reabsorption, thereby decreasing blood cholesterol (Ayoade et al. 2015). The protein given to experimental animals is hydrolyzed by protease enzymes in the digestive system, while the peptides released are biologically active; thus, these peptides which are bound to bile acids will lower blood cholesterol levels. Peptides alter intestinal cholesterol with bile acids and thus lower cholesterol levels (Huang et al. 2009). The mechanism for this involves the excess cholesterol being transferred back to the liver by the lipoprotein HDL, where it is processed into the gall bladder as bile acids. This causes an improvement in bile acid synthesis, and bile acids are then released into the small intestine to help digest fat from foods. After doing its job, some bile acids are wasted in feces, and some others are reabsorbed into the liver through the small intestine. However, the active peptides, which are the result of protein hydrolysis, bind bile acids so that they are not reabsorbed, and bile acids are also wasted through the large intestine, so there are fewer bile acids in the liver. In order to replace the missing bile acids, the liver takes cholesterol from the blood to produce bile acids, which lowers cholesterol and LDL levels in the blood (Doke and Guha 2015).

Reducing cholesterol and LDL can also be triggered by unsaturated fatty acids. Oleic acids will hamper the process of LDL oxidation, and the negative impact will be even stronger with the help of linoleic and linolenic acid. These unsaturated fatty acids can lower oxidized LDL in the blood vessel intima, which can cause atherosclerosis. It can also lower plasma LDL and improve plasma HDL, making it anti-atherosclerotic because it brings cholesterol from tissues to the liver, it is transformed into bile acids. The bile acids are excreted into the intestine so that cholesterol and LDL are lowered (Huang et al. 2009). Also, fresh walnuts contain 2.46% squalene that can lower cholesterol in the blood (Thomson and Evans 2006). Research into squalene in shark liver oil concluded that the benefit of squalene is a lowering of free radical oxidative damage as it is an antioxidant (Huang et al. 2009). Besides squalene, vitamin E (tocopherol) in walnuts also contributes to healing hypercholesterolemia in mice. Fresh walnuts contain 163.2–168.4 µg/g of tocopherol and dry walnuts contain143.3–165.3 µg/g of tocopherol (Mailoa et al. 2016). Vitamin E can be found in the phospholipid bilayer of the cell membrane and is very useful in the prevention of lipid peroxidation (Traber and Stevens 2011). Vitamin E can maintain the oxidation balance (Oford 2005).

In a study with *C. schweinfurthii*, it was found that vitamin E could be derived from methanol extract and hexane extract and could be used as an antioxidant to cure cancer, diabetes, hypertension, and other cardiovascular diseases (Frisca et al. 2008). Also, this Maluku walnut also contains phenol, while walnuts from Sangihe, Minahasa, and Ternate contain 7.4–8.8 mg/g of phenol (Djarkasih et al. 2011). Phenol is a compound with the potential to prevent oxidative stress (Tekkeşin et al. 2014). A study in Africa showed that African walnuts (*C. schweinfurthii*), which are rich in nutrients,

can be used to treat humans (Maduelosi and Angaye 2015).

CONCLUSIONS

This study showed that 1.8 g of roasted canarium nut and doses of fresh canarium nut could improve the lipid profiles of hypercholesterolemic rats by decreasing the level of cholesterol, LDL, and triglyceride and increasing the level of HDL. Furthermore, this also minimizes endothelial dysfunction.

REFERENCES

Ayoade GW, Amoo IA, Akpambang VOE (2015) Physicochemical and fatty acid composition of crude and refined oils of African canarium. International Journal of Scince and Technology, 4(5): 1-5.

Ayoade GW, Isiaka, A, Kunk A, Eka AE (2015) Phytochemical composition and antioxsidative potential of purple canary (Canarium schweinfurthie) fruit. The Pharma Innovation Journal, 4(1): 49-52.

Casagrande F. da Silva, Angerten VG, Ulbrich AZ, Maranhao RC, de Carvalho T, da Silva EI (2015) Omega-3 fatty acids improve lipid metabolism and inflammatory markers in patients with heart failure undergoing exercise training. International Journal of Food and Nutritional Sciences, 4(3): 29-35.

Chris IO, Kukwa DT, Lapshak LJ, Ogakwu AS, Ekalu I (2015) Comparative study of total cholesterol and total protein levels of chicken an quail eggs produced in Benue State, Nigeria. International Journal Current Research in Chemistry and Pharmaceutical Sciences, 2(10): 74-8.

Coplo A (2005) LDL cholesterol: bad cholesterol or bad science, Journal of American Physicians and Surgeons, 10(3): 83-9.

Djarkasih GSS, Nurali EJN, Sumual MF, Lalujan LE (2011) Analysis of bioactive compound in canarium nut (Canarium indicum L.). Research Final Report on Tropical Plant Curriculum Project. Retrieved from http://seafast.ipb.ac.id/tpc-project/wp-content/uploads/2014/02/RSC-Final-Report-Kenari.pdf

Doke S, Guha M (2015) Nutriotinal, Physico-Chemical and Functional Properties of Ready-to-use Chikpea and Soybean Flour. International Journal of Food and Nutritional Sciences, 2(5): 72-7.

Frisca T, Caroline, Sardjono and Sandra F (2008) Endhotelial progenitor cell, stem cell division, Stem Cell and Cancer Institute, Kalbe Pharmaceutical Company Jakarta, Indonesian, CDK 161, 35(2): 1-4.

Gonzalo DCD, Cortes VL, Orbe J, Paramo JA, Badimon L (2014) High triglyceride low HDL cholesterol lipid profile is associated with a dysregulated gene expression in mononuclear leukocyte from hypercholesterolemic patiens. International Journal of Cardiologi, 178: 102-4. https://doi.org/10.1016/j.ijcard.2014.10.126

Huang ZR, Lin YK, Fang JY (2009) Biological and pharmacological activities of squalene and related compounds: potential uses in cosmetic dermatology. Moleculles Journal, 14(1): 540-54. https://doi.org/10.3390/molecules14010540

Kennedy J, Clarke W (2004) Cultivated landscapes of the Southwest Pasific resources management in Asia-Pasific. Canberra: Research School of Pasific and Asian Studies, Australian National University.

Maduelosi NJ, Angaye SS (2015) Characterization of african elemi (Canarium schweinfurthii). International Journal of Advanced Research in Chemical Science, 2(11): 34-6.

Mailoa M (2015) GC-MS analysis on canary (Canarium vulgare Leenh) kernel fresh (Analisa GCMS pada biji kenari segar). Prosiding Seminar Agroindustri dan Lokakarya Nasional FKPT-TPI Program Studi TIP. Trunojoyo University, Indonesia. 2-3 September 2015.

Mailoa M, Harijono, Widyaningsih TD, WidyaDwi RP (2016) Effect soaking and drying on nutritional composition and color canary (Canarium vulgare Leenh) kernel in Moluccas (Efek perendaman dan pengeringan terhadap kandungan gizi dan warna biji kenari di Maluku), Research Report, Brawijaya University, Indonesia.

Muntiha M (2001) The technique of preparing histopathologi preparations of animal tissues with hemactosilin and eosin staining (Teknik pembuatan preparat histopatologi dari jaringan hewan dengan pewarnaan hemaktosilin dan eosin). Temu Teknis Fungsional Non Peneliti 2001: 156-63.

NIH (2014) How is high blood cholesterol diagnosed, National Heart Lung and Blood Institute. Retrieved from https://www.nhlbi.nih.gov/health-topics/high-blood-cholesterol

Orford JL (2005) Atherosclerosis clinical and research fellow in cardiovascular disease, Department of Internal Midicine, Brigham and Women's Hospital, Harvard Medical School.

Pena DG, Angulo J, Vallejo S, Coca CC, de Ancos B, Ferrer CFS, Peiro C, Moreno CS (2014) High cholesterol diet enciched with onion affects endothelium dependent relaxation and NADPH oxidase activity in mesenteric

microvessels from wistar rats. Nutrition and Metabolism Journal, 11(57): 1-11. https://doi.org/10.1186/1743-7075-11-57

Platat C, Habib H, Othman A, Al-Marzooqi S, Al-Bawardi A, Pathan JY, Hilary S, Al-Maqbali F, Souka U, Al-Hammadi S, Ibrahim W (2015) Safety and Protective Effect of Date (Phoenix dactylifera) Seed Extract Against Oxidative Damage in Rat. International Journal of Food and Nutritional Sciences, 4(4): 21-7.

Rabbani M, Bagherzadeh N, Rafiei H (2014) Calculating raw material and work-in-process inventories in MTO. MTS production, UCT Journal of Research in Science, Engineering and Technology, 2(3): 109-16.

Safdari M, Asadi Z (2013) Privatization to serve economic development.

Schlesinger DP (2011) Raw Food Diets in Companium Animals: A critical review Canadian Veterinary Journal, 52(1): 50-4.

Şen V, Ece A, Uluca Ü, Güneş A, Tan I, Tuncel T, ... Yıldırım B (2014) Evaluation of the Mean Platelet Volume in Children with Juvenile Idiopathic Arthritis. European Journal of General Medicine, 11(4): 262–7. https://doi.org/10.15197/sabad.1.11.83

Seneviratne KN, Kotuwegedara RT, Ekanayake S (2011) Serum cholesterol and triglyceride levels of rats fed with consumer selected coconut oil blends. International Food Research Journal, 18(4): 1303-8.

Şenol S, Yörük A, Kuru İ, Kıral A, Ersoy G (2015) Diffuse fatal infantile hemangioendothelioma in an infant liver: Case report. J Clin Exp Invest., 6(3): 312-4. https://doi.org/10.5799/ahinjs.01.2015.03.0540

Shakirin FH, Azlan A, Ismail A, Amom Z, Yuon LC (2012) Antiatherosclerotic effect of Canarium odontophyllum Miq. fruit parts in rabbits fed high cholesterol diet. Evidence-Based Complementary and Alternative Medicine: 1-10. https://doi.org/10.1155/2012/838604

Shakirin FH, Azrina A, Khoo A, Avira HE (2015) Protective effect of pulp and kernel oil from Canarium odontophyllum fruit in normal and hypercholesterolemic rabbits. International Food Research Journal, 22(4): 1318-26.

Tekkeşin N, Bekoz H, Tükenmez F (2014) The largest reference range study for hematological parameters from Turkey: A case control study. Journal of Clinical & Experimental Investigations/Klinik ve Deneysel Arastirmalar Dergisi, 5(4). https://doi.org/10.5799/ahinjs.01.2014.04.0455

Thomson LAJ, Evans B (2006) Island *Canarium indicum* var. indicum and C. harveyi (canarium nut) burseraseae (torchwood family). Species Profiles for Pacific Agroforestry 2006 (www.traditionaltree.org)

Tjandrawinata (2011) Dyslipidemia. Scientific Journal of Pharmaceutical Development and Medical Application: 1-68.

Traber MG, Stevens JF (2011) Vitamin C and E: beneficial effect from a mechanistic perspective. Free Radic. Biol. Med., 51(5): 1000-13. https://doi.org/10.1016/j.freeradbiomed.2011.05.017

Tsallissavrina I, Wahono D, Handayani D (2006) The influence of high carbohydrate diet administration in comparison with high fat diet toward triglyceride and HDL level in blood on *Rattus novergicus* strain wistar (Pengaruh pemberian diet tinggi karbohidrat dibandingkan diet tinggi lemak terhadap kadar trigliserida dan HDL darah pada *Rattus novergicus* galur wistar). Medical Journal Brawijaya, 12(2): 80-9. https://doi.org/10.21776/ub.jkb.2006.022.02.5

Wang W, de Meija EG (2005) A new frontier in soy bioactive peptides that may prevent age-related chronic diseases. Journal Food Sci. Food Safety, 4(4): 63-78. https://doi.org/10.1111/j.1541-4337.2005.tb00075.x

Wang Y, Jones PJH, Ausman LM, Lichtenstein AH (2004) Soy protein reduces triglyceride levela and triglyceride fatty acid fractional synthesis rate in hypercholesterolemic subjects. Atherosclerosis, 173: 269-75. https://doi.org/10.1016/j.atherosclerosis.2003.12.015

Zou Y, Lu Y, Wei D (2005) Hypocholesterolemic effects of a flavonoid-rich extract of Hypericum perforatum L. in rats fed a cholesterol-rich diet. J. Agritech and Food Chem., 53(7): 2462-6. https://doi.org/10.1021/jf048469r