The Effect of Cang Salak Tea Diet on Apolipoprotein C3 (ApoC3) Gene Expression on Hyperlipidemic Rats Model

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ABSTRACT

Cardiovascular disease (CVD) is the leading cause of global mortality and disability. Hyperlipidemia is a major risk factor for CVD that can be controlled through medical therapy, appropriate nutrition, and lifestyle. This study aimed to identify the cang salak tea diet's effect on the ApoC3 gene expression in a hyperlipidemia rat. 18 male Wistar rats were divided equally into three groups. A high-fat-diet-induced two groups of rats, and one group was the control. Once hyperlipidemia had been achieved, one of the two groups was treated with the cang salak tea, and one group was given a standard diet for four weeks. Authenticated rat and liver tissue were collected as a source of RNA isolation. Isolated RNA was used as a reaction template for the relative quantitation qPCR using β-actin as the housekeeping gene. The ApoC3 gene was specifically amplified with a Tm value of 82.73°C, Cq 17-19, and produced a sigmoid curve. The relative expression level of the ApoC3 gene in hyperlipidemia rats fed with the cang salak tea diet was 0.46 times significantly lower than the control (1.17) and P2 (1.32) groups. These results indicate that the cang salak tea has antihyperlipidemic properties to reduce CVD risk.

1. Introduction

Cardiovascular disease (CVD) is the primary cause of mortality and disability globally (Roth *et al.* 2020). CVD is also responsible for 37% of deaths in Indonesia. Among the types of CVD, stroke is the leading cause of death, followed by coronary heart disease and diabetes (Chow *et al.* 2017). Coronary heart disease and stroke are estimated to cause the death of more than 470,000 people annually in Indonesia (Hussain *et al.* 2016). The screening results in the population aged 40 years and over showed that 29.2% of the people in Indonesia had a high risk of developing CVD (Maharani *et al.* 2019). There are two types of CVD risk factors: modifiable risk factors and non-modifiable risk factors. Modifiable risk factors include blood pressure, body mass index (BMI), lipid profile, obesity, body mass index, and lifestyle, such as smoking habits (Anggraini and Adelin 2020).

Increasing blood lipid levels or hyperlipidemia is one of the primary risk factors for CVD (Nelson 2013). In hyperlipidemia, there are an increase in total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), and a reduction in serum high-density lipoprotein cholesterol (HDL-C) concentrations (Hedayatnia *et al.* 2020). Abnormalities in the lipid profile strongly correlate with the risk of coronary artery disease (Ramo *et al.* 2019). In atherosclerosis, there is a very high level of endothelial injury resulting from chronic inflammation related to lipids (Avci *et al.* 2012). Lipid deposits on the endothelium lead to intima thickening and trigger an adverse inflammatory reaction (Wei *et al.* 2021).

Hyperlipidemia control strategies can be applied through medical therapy, nutrition, and lifestyle modification (Nouh *et al.* 2019). Consuming foods or beverages that can help improve lipid profiles is one strategy to reduce CVD risk factors. One such beverage product is cang salak tea. Cang Salak Tea is a product made in a 1:1 ratio of sappan wood and Salacca bark powder (*Caesalpinia sappan* L.). The phytochemical analysis results showed that the cang salak tea has active ingredients such as flavonoids, alkaloids, tannins, phenols, and saponins (Karta *et al.* 2021). Flavonoids can prevent cardiovascular disease because they are anti-atherogenic, antithrombotic, and antioxidant. *In vitro* and *in vivo* studies have indicated that flavonoids can detain the activity

of various inflammatory mediators and obstruct the overactivity of immune cells. It is the potential to be developed as a new anti-inflammatory drug (Ciumărnean *et al.* 2020).

Recent research reports showed that cang salak tea could significantly improve the lipid profile of Wistar rats induced by hyperlipidemia. Hyperlipidemia rats treated with cang salak tea indicated a decrease in total cholesterol, LDL, and triglycerides and an increase in HDL (Karta *et al.* 2021). The mechanism of cang salak tea in improving lipid profile is based on the active content of tea and its role in body metabolism. The active ingredients in cang salak tea can modify fat metabolism pathways that improve lipid profiles. The modifications can be done by regulating the gene expression that manages fat metabolism in the body. A molecular study on cang salak tea's effect on the expression of protein-coding genes related to fat metabolism needs to be conducted. One of the body proteins related to fat metabolism is Apolipoprotein (ApoC).

ApoC is a family of apolipoproteins found on the surface of chylomicrons molecules, very-low-density lipoproteins (VLDL), and high-density lipoproteins (HDL). One of the ApoC protein family, ApoC3, is associated with an increased incidence of cardiovascular disease (Taskinen and Borén 2016). The ApoC3 protein is a crucial regulator of triglyceride (TG) metabolism and an independent predictor of coronary heart disease risk. ApoC3 also causes atherosclerotic lesion formation and several other pathological processes involved in atherosclerosis (Luo and Peng 2016). The increase in ApoC3 concentration of VLDL is a solid and independent prediction of coronary heart disease, even more than the function of TG as a predictor of coronary heart disease (Chan *et al.* 2008).

ApoC3 gene expression of the cang salak tea's effect on hyperlipidemia rats is important to be studied. The impact of cang salak tea in modulating the gene expression related to fat metabolism can be identified. This is the beginning stage of a nutrigenomic study on cang salak tea in its development as an antioxidant drink with anti-hyperlipidemia activity. This nutrigenomic research strengthens the results of previous studies showing the potential of cang salak tea in improving lipid profiles. ApoC3 can then be developed as a genomic protein marker to determine the risk of cardiovascular disease. This research also opens the development of natural products using ApoC3 as a target for hyperlipidemia therapy.

2. Materials and Methods

The research design regarding the effect of the cang salak tea diet on apolipoprotein C3 (ApoC3) gene expression in the hyperlipidemic rats model is depicted in Figure 1.

2.1. Materials and Instruments

This study involved a high-fat diet (pork oil, duck's egg yolk, synthetic cholesterol, and folic acid). The other materials were ketamine, xylazine, ApoC3 gene forward and reverse primer, β-actin gene forward and reverse primer, Direct-zol™ RNA Miniprep Plus (Zymo Research), The SensiFAST™ SYBR® Hi-ROX One-Step Kit (Meridian Bioscience), and 70% alcohol. The instruments included ABI-StepOne Real-Time PCR System, BioSafety Cabinet (Esco AC2-458), Microcentrifus (Eppendorf 5424 R), and Rotary Evaporator (Rotavapor BUCHI R.300).

2.2. The Research Subject

This study used 18 Wistar rats (*Rattus norvegicus*) complying with the criteria of male rats aged 2-3 months old having healthy and regular activities. The body weight of the rats was approximately 150-200 grams. The rats were reared in controlled conditions for their type of feed, ambient temperature, and appropriate lighting. The ethical committee of the Faculty of Veterinary Medicine, Udayana University, has approved the research subject of using rats in this study under certificate No. 39/UN14.2.9/PT.01.04/2020.

2.3. Preparation of Test Materials

200 g of sappanwood and 300 g of the bark of snake fruit were mashed and dried at 40°C for 4 hours/day until the moisture content was below ±8%. The product formulation was made by mixing 100 g of sappanwood powder and 100 g of the bark of salak powder (1:1) (Karta *et al.* 2021). The mixture was put into 1,500 ml of mineral water, heated at 100°C, and then allowed to stand for 10 minutes. The cang salak tea fluid was evaporated to a volume of 250 ml. It used a rotary evaporator at a temperature of 40°C at a vacuum pump of 150 and 200 rpm for ±45 minutes. The concentrated liquid was stowed in a dark bottle, stored in a cooler, and used as research test material.

2.4. Induction of Hyperlipidemia Rats Model

18 male Wistar rats were randomly divided into three groups, namely P1, P2, and control groups (6 individuals/group). P1 and P2 Groups were induced with a high-fat diet containing 3 grams of pork oil, 2 grams of duck's egg yolk, 3 grams of cholesterol, and 2 grams of folic acid/200 grams of rat body weight/day via gastric probe for eight weeks until a hyperlipidemia description was formed. The control group was given a standard diet containing water (13%), protein (19-21%), fat (5%), fiber (5%), ash (5%), calcium (0.9%), phosphorus (0.6%), and aflatoxin (50 ppb).
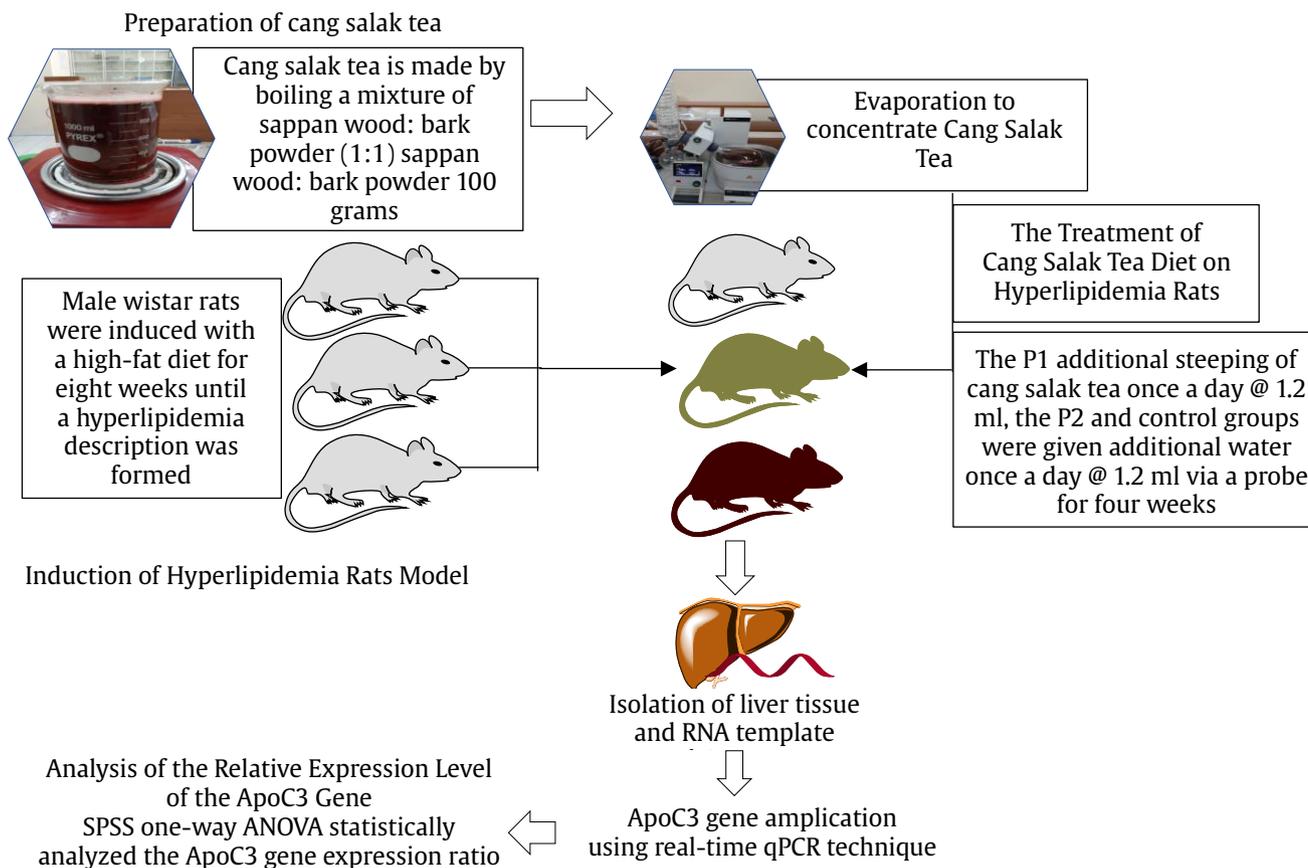
Figure 1. The research design regarding the effect of cang salak tea diet on apolipoprotein C3 (ApoC3) gene expression in hyperlipidemic rats model

2.5. The Treatment of Cang Salak Tea Diet on Hyperlipidemia Rats

Along with the treatment, the three groups of rats were given a standard diet orally and water orally ad libitum. The P1 group was administered with additional steeping of cang salak tea once a day @ 1.2 ml for four weeks through a gastric probe. Meanwhile, the P2 and control groups were given additional water once a day @ 1.2 ml via a probe for four weeks.

2.6. Isolation of Liver Tissue and RNA Template

After four weeks treated with cang salak tea, the rats were euthanized, and liver tissue was collected as a source of RNA isolation. RNA was purified according to the Direct-zol™ RNA Miniprep Plus procedure (Zymo research). The RNA was then used as a template for the qPCR reactions of the ApoC3 and β-actin genes.

2.7. ApoC3 Gene Amplication Using Real-Time qPCR Technique

Real-time qPCR of the ApoC3 gene was carried out with the procedure of The SensiFAST™ SYBR®

Hi-ROX One-Step Kit (Meridian Bioscience) kit. The master mix for the qPCR reaction was prepared for 20 µl, containing 10 µl of SensiFAST™ SYBR® Lo-ROX One-Step mix, 0.8 µl of Forward primer 10 µM, 0.8µl of Reverse primer 10 µM, 0.2 µl of Reverse transcriptase, 0.4 µl of RNase Inhibitor, 3.8 µl of H_2O and 4 µl of RNA template. The primer pair used in the qPCR reaction of the ApoC3 gene and the β-actin gene has the following sequence:

ApoC3 gene	Forward	5'-TCCTTGCTGCTGGGCTCTAT-3'
	Revers	5'-GCATGCTGCTTAGTGCATCCT-3'
β-actin gene	Forward	5'-AGGCCAACCGTGAAAAGATG-3'
	Revers	5'-ACCAGAGGCATACAGGGACAA-3'

The qPCR reaction used the ABI-StepOne Real-Time PCR System machine, with the following reaction settings shown in Table 1 below:

Table 1. The qPCR reaction setting of ApoC3 and β-actin gene

Reaction	Temperature	Time	Cycle
Reverse transcription	45°C	10 min	1
Polymerase activation	95°C	2 min	1
Denaturation	95°C	5 s	
Annealing	60°C	10 s	40
Extension	72°C	5 s	

2.8. Analysis of the Relative Expression Level of the ApoC3 Gene

The analysis of the ApoC3 gene expression applied the comparative quantitation qPCR method. The study used mathematical calculations to measure the relative quantitative level of the ApoC3 gene expression using the β-actin gene as the housekeeping gene. The data were normalized, and then the CT values were calculated for all real-time qPCR reactions. The following is the analysis procedure:

- The average value of the CT ApoC3 and β-actin genes in the control group, P1 and P2
- Calculation ▲CT (CT ApoC3-CT gene β-actin gene)
- The average calculation ▲CT control
- Calculation ▲▲CT (CT ApoC3 gene-▲CT control)
- Calculation $2^{-▲▲Cq}$

SPSS One-way ANOVA statistically analyzed the ApoC3 gene expression ratio with a 95% confidence level to see whether the relative expression value of each treatment group was significantly different. In relative gene quantification, the relative expression level >1 indicates an increase in expression; On the other hand, if the change in expression level is <1, it means a decrease in expression. Furthermore, the effect of giving cang salak tea on the ApoC3 gene expression in hyperlipidemia rats was analyzed descriptively.

3. Results

3.1. Amplification of ApoC3 Gene Using qPCR

The results of the amplification of the ApoC3 and β-actin genes using the qPCR technique are shown in Figure 2.

Figure 2 shows that the qPCR technique successfully amplified the ApoC3 gene from the liver tissue of Wistar rats. The primer pair can recognize the ApoC3 gene; amplification reactions can occur. The reaction results were indicated by forming a sigmoid curve that indicates DNA products' presence. In this reaction, the ApoC3 gene was amplified using RNA from liver tissue as a template. The response was carried out by the one-step qPCR method. The RNA by the enzyme reverse transcriptase is converted into DNA and directly
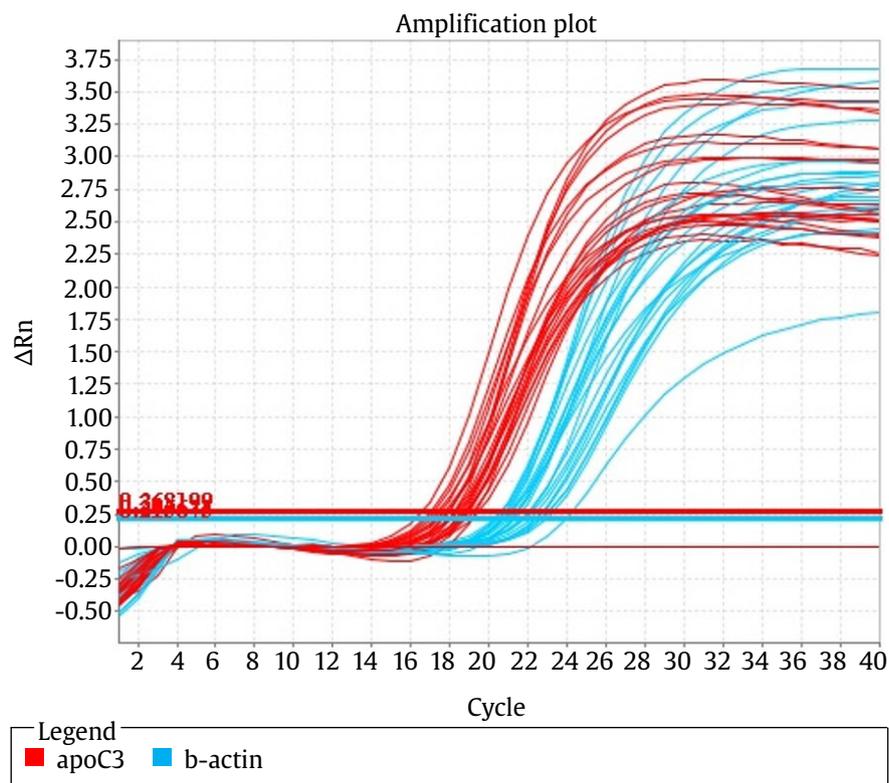

Figure 2. Amplification curve of ApoC3 e and β-actin genes with qPCR technique

used as a template for amplification reactions. The PCR product shows the RNA from the ApoC3 gene found in liver tissue.

The amplification data of ApoC3 and β-actin genes were analyzed using a melt curve to evaluate the specificity of the reaction. The results of the melt curve analysis are shown in Figure 3.

Referring to Figure 3, the specificity of the qPCR reaction of ApoC3 and β-actin genes in this experiment was indicated by the formation of a single peak on the analysis using the melting curve. The melting curve in the amplification reaction of the ApoC3 gene has a tm value of 82.73°C, while the β-actin gene has a tm value of 81.54°C

In the relative qPCR quantification method, the Cq value quantifies ApoC3 gene expression toward the β-actin gene as an internal control. Cq qPCR values for each sample according to the treatment group are shown in Table 2.

Based on Table 2, the Cq values for the amplification reaction of the ApoC3 gene with the minor average were in the control group (17.12±1.04), P2 (17.37±0.91), and Pl (19.14± 2.31). The Cq value and the shape of the sigmoid curve indicate that the ApoC3 gene in this experiment was successfully amplified. Referring to Table 2, the amplification result of the β-actin gene in this experiment had a CT score of 21-22. These results indicate that the expression of the β-actin gene in rat liver tissue is stable enough to be used as an internal control.

3.2. Expression Analysis of ApoC3 Gene using Relative Quantitation qPCR Method

The Cq values from the ApoC3 and β-actin genes' expression using the relative quantitation qPCR method were transferred from the ABI-StepOne Real-Time PCR System software to Microsoft Excel to calculate relative expression levels using mathematical formulas. It is intended to count the differences in expression levels of target genes. The analysis result of the relative expression levels of the ApoC3 gene is shown in Table 3.
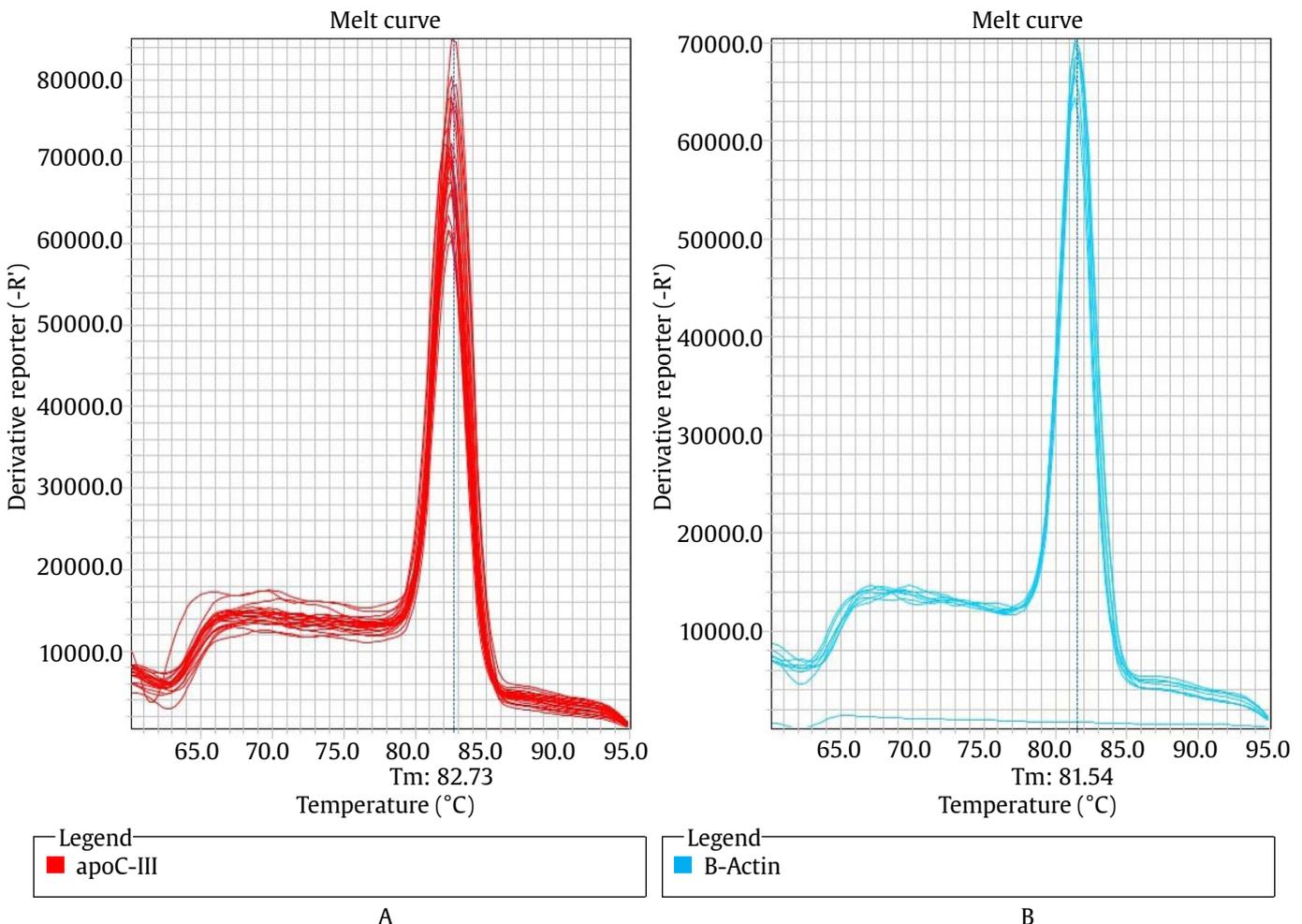

Figure 3. The melting curve of amplified ApoC3 gene (A) and β-actin gene (B)

Based on Table 3, ApoC3 gene expression of P1 Rats was 0.46 times lower than in the control group in this treatment. The P2 group of rats showed a higher ApoC3 gene expression, 1.32 times higher than the control group.

3.3. Statistical Analysis of the Relative Expression Level of the ApoC3 Gene

The results of statistical tests on the average value of the relative expression level of the ApoC3 gene in each treatment showed that the data were normally distributed (P>0.05) and homogeneous (P<0.05). The results of the one-way ANOVA and LSD tests to determine differences in the relative expression

levels of the ApoC3 gene in each treatment group are shown in Table 4 and 5.

Based on the one-way ANOVA, there were differences in the average value of the relative expression level of the ApoC3 gene in each treatment group (P<0.05) (Table 4). The group of hyperlipidemia rats fed with the cang salak tea diet (P1) showed significantly lower ApoC3 gene expression levels than the control group and P2 (P<0.05). Meanwhile, the relative expression level of the ApoC3 gene in the P2 group with control did not show a significant difference (P>0.0.5) (Table 5). These results illustrate that the cang salak tea diet significantly reduces the ApoC3 expression gene in hyperlipidemic Wistar rats.

4. Discussion

The expression level of the ApoC3 gene was analyzed from the Cq value of the ApoC3. The gene normalized by Cq of the β-actin gene as the internal control. The cang salak tea diet treatment significantly decreased the ApoC3 gene expression in relative gene quantification. The change in relative expression level (fold change), which is >1, indicates the increase of expression level; on the other hand, if the change in expression level is <1, it means a decrease in expression. The value of 1 is the agreed quantity to determine the target gene (Zhang *et al.* 2015).

As an internal control, the β-actin gene was amplified using the same tissue source. Based on the curve in Figure 2, the β-actin gene in this experiment was successfully detected, as indicated by the formation of the sigmoid curve in the amplification plot. The β-actin gene encodes cytoskeleton structural protein, which is widely used to normalize gene expression in experiments (Pohjanvirta *et al.* 2006). Using the β-actin gene as a housekeeping gene from rat liver tissue showed good stability of expression, which was not different from other commonly used genes such as the GAPDH gene (Chen *et al.* 2006). The expression of the β-actin gene was also

Table 2. The average of Cq ApoC3 and β-Actin genes

Treatment group	The average Cq ApoC3 gene	The average Cq β-Actin genes
Control	17.12±1.04	21.20±0.41
P1	19.14±2.31	22.00±1.81
P2	17.37±0.91	21.76±0.46

P1 (hyperlipidemia rat group with the cang salak tea treatment)
P2 (hyperlipidemia rat group without the cang salak tea treatment)

Table 3. Expression analysis of ApoC3 gene using relative quantitation qPCR method

Treatment group	Level of relative expression of ApoC3 gene
Control	1.17±0.67
P1	0.46±0.19
P2	1.32±0.54

P1 (hyperlipidemia rat group with the cang salak tea treatment)
P2 (hyperlipidemia rat group without the cang salak tea treatment)

Table 4. One-way ANOVA test results on the relative expression level of the ApoC3 gene between treatment groups

	Sum of Squares	df	Mean square	F	Sig.
Between groups	2.502	2	1.251	4.819	0.24
Within groups	3.894	15	260		
Total	6.396	17			

Table 5. LSD test results on the relative expression level of the ApoC3 gene between treatment groups

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower bound	Upper bound
Control	P1	.070833'	.29416	.029	.0813	1.3353
P1	P2	-.14500	.29416	.629	-.7720	.4820
	Control	-.70833	.29416	.029	-1.3353	-.0813
	P2	-.85333	.29416	.011	-1.4803	-.2263

P1 (hyperlipidemia rat group with the cang salak tea treatment), P2 (hyperlipidemia rat group without the cang salak tea treatment)

reported to be the most stable in tissues infected by the nervous necrosis virus, more stable than the expression of other housekeeping genes such as the EF1α, GAPDH, and ARP genes (Krishnan *et al.* 2019).

Here, the specificity of the qPCR reaction of ApoC3 and β-actin genes was indicated by the formation of a single peak on the analysis using the melting curve. The Tm value indicated the temperature when 50% of the ApoC3 and β-actin dsDNA genes dissociate into ssDNA. The amplification results analysis with the melting curve aims to evaluate the specificity of the qPCR reaction (Downey 2014). The formation of a single peak indicates that the qPCR response of the ApoC3 gene from the liver tissue of Wistar rats has progressed specifically. Besides the sigmoid curve, the amplification results of the ApoC3 gene were also known from the threshold cycle (Cq) value in each reaction. Cq is a cycle when the fluorescence intensity increases above the signal detection limit, proportional to the initial amount of DNA template. The formation of a sigmoid curve illustrates the increase of fluorescence intensity. Providing that more amplification products are generated, a more significant accumulation of fluorescent could be observed (Kralik and Ricchi 2017). The smaller Cq value indicated an increase in fluorescence intensity, which occurred at the beginning of the reaction cycle, proportional to the amount of product produced.

The treatment of cang salak tea affected the expression of the ApoC3 gene in hyperlipidemic rats. The downregulation of the expression level of ApoC3 will improve the lipid profile in cases of hyperlipidemia. These results confirm previous research that cang salak tea can improve the lipid profile of hyperlipidemic rats. Cang salak tea significantly reduced cholesterol levels, LDL, TG, and HDL. Cang salak tea contains alkaloids, tannins, phenols, and saponins (Karta *et al.* 2021). Flavonoids have antihypercholesterolemic activity by decreasing blood cholesterol levels by absorbing cholesterol and bile acids in the small intestine and increasing their excretion through faeces. This activity causes liver cells to increase the formation of bile acids from cholesterol and will reduce fat because it is converted into energy (Zeka *et al.* 2017). Flavonoids have antioxidant effects since they reduce LDL oxidation, increasing lipid profile. Another positive effect of flavonoids on the cardiovascular system

is vasodilation and regulating apoptotic processes in the endothelium (Ciumărnean *et al.* 2020).

The ApoC3 gene is 3367 bp in size, consisting of 4 exons encoding 99 amino acid glycoproteins synthesized mainly in the liver and at a lower level in the intestine (Verrijken et al. 2013). The ApoC3 gene indicates a common origin with ApoA-I and other ApoC genes and ApoA and ApoE. The ApoC3 gene belongs to the important multigene group ApoA5-ApoA4-ApoC1-ApoA1 on human chromosome 11q23.3 (Pranavchand and Reddy 2017). ApoA4 and ApoA5 genes are transcribed in the same direction on those gene clusters, while ApoC3 is from the opposite direction (Halley *et al.* 2014). ApoC3 gene expression is controlled by enhancers located at nucleotides 590-790 in the upstream region of the ApoC3 gene. Several factors regulate genes, such as glucose, insulin, and cytokines associated with diabetes (Kan *et al.* 2000; Valladolid-Acebes *et al.* 2021).

Various studies have analyzed the ApoC3 gene and its relation to some parameters of cardiovascular disease. The ApoC3 gene was historically considered the most muscular candidate gene locus associated with plasma TG concentrations (Huff and Hegele 2013). Analysis of ApoC3 gene polymorphisms using the PCR-RFLP technique showed a relationship to the improvement of cardiovascular disease. CVD patients with hyperlipidemia were reported to have a higher alleles ratio of 3238 G than CVD patients without hyperlipidemia. The ApoC3 3238 G allele contributes to the increased risk of CVD since it affects TG and VLDL-C metabolism (Cui *et al.* 2014).

Polymorphisms in the ApoC3 gene are linked with non-alcoholic fatty liver disease (NAFLD), hypertriglyceridaemic, and insulin resistance. The study of polymorphisms in the T-455C ApoC3 gene in the Egyptian population was associated with NAFLD but did not affect hematologic parameters, liver, and lipid profiles (Youssef *et al.* 2017). The Sst I polymorphism of the ApoC3 gene in DM patients with hypertriglyceridemia is also related to serum levels of various lipoprotein fractions, including TG. About 26.33% of DM patients have polymorphisms in the Sst I ApoC3 gene. Polymorphic genes were found in 35.33% of these cases, while only 17.33% were in the control group. The average cholesterol level in the group with Sst I polymorphism was significantly higher than with only the S1 allele

(Kalita *et al.* 2018). In patients with intracerebral haemorrhage (ICH), genetic variation in the ApoC3 gene 3238 G allele was significantly associated with increasing TG and VLDL plasma. For the patients with ICH, the genotype frequency of the ApoC3 3238 GG and ApoC3 3238 G alleles was higher than the control group (Jiang *et al.* 2015).

The sequencing of the ApoC3 gene on several variants related to lipid level and BMI has also been reported. This study indicated that one of the ApoC3 variants, namely rs5128, can function as a marker for assessing genetic risk in dyslipidemia and obesity in a population in Arabia (Malalla *et al.* 2019). The other study using transgenic pigs as an animal model of hyperlipidemia showed that the expression of the ApoC3 gene was detected in the liver and intestines. Transgenic pigs presented significant improvement in plasma TG levels compared to the non-transgenic control group (Wei *et al.* 2012).

Various factors can influence ApoC3 expression in tissues. Plasma ApoC3 levels are a new risk factor for diet-induced obesity. The rats induced with a high-fat diet indicate plasma ApoC3 overexpression. The rats expressing ApoC3 had significantly higher adipose tissue mass and plasma leptin levels than those of the control rats. Meanwhile, basic adiposity lipolysis and *in vivo* lipolysis index were significantly decreased in ApoC3 rats compared to control rats (Raposo *et al.* 2015).

The dropping of the ApoC3 gene expression is important for improving lipid profiles and reducing the risk of cardiovascular disease. ApoC3 protein can trigger hypertriglyceridemia (HTG) through various mechanisms in fat metabolism. In addition, ApoC3 promotes a pro-inflammatory response in endothelial cells and increases LDL binding affinity to realize the accumulation of atherogenic lipoproteins in the arterial wall (Taskinen and Borén 2016). ApoC3 protein moves into plasma rapidly and is exchanged between TG and HDL. In plasma, ApoC3 protein decreases lipoprotein lipase activity and interferes with receptors for TG clearance (Huff and Hegele 2013).

Other studies have shown that the enhancement of plasma ApoC3 concentration correlated closely with hypertriglyceridemia in rats and humans. ApoC3 levels in humans with hypertriglyceridemia are associated with increased production and the decrease of the ApoC protein catabolism (Chan *et al.* 2008). Overexpression of ApoC3 in humans, either in cultured hepatocytes or in ApoC3 transgenic rats, stimulates the assembly and secretion of TG-rich VLDL under lipid-rich conditions (Qin *et al.* 2011). The enhancement of ApoC3 concentration from VLDL is a solid and independent prediction of coronary heart disease even more than the function of TG as a coronary heart disease predictor (Sacks *et al.* 2000). Moreover, LDL, containing ApoC3, produced by partial lipolysis in ApoC3-containing VLDL plasma, is highly predictive of coronary heart disease in patients with type 2 diabetes mellitus (Lee *et al.* 2003).

ApoC3 gene expression, which decreased significantly in hyperlipidemic rats, showed the potential of cang salak tea as a beverage with antihyperlipidemic properties to reduce CVD risk. The bioactive ingredients in cang salak tea allegedly modulate the expression mechanism resulting in the downregulation of ApoC3 gene expression. Bioactive components of the diet can modulate the expression of a gene by altering its chromatin structure (including DNA methylation and histone modification), non-coding RNA, activate transcription factors by cascade signal, or play a role as ligands for receptors in the nucleus (Mierziak *et al.* 2021). The molecular effect of drugs on the regulation of ApoC3 gene expression has been reported in a specific study. Treatment of hyperlipidemia-induced rats with nicotinic acid is proven to inhibit the activity of Peroxisome-proliferator activated receptor (PPAR) coactivator-1β (PGC-1β) and decrease ApoC3 expression. PGC-1β is a transcriptional coactivator that induces hypertriglyceridemia and stimulates ApoC3 expression by coactivating orphan nuclear receptor ERRα and recruiting chromatin-remodelling cofactors (Hernandez *et al.* 2010).

Analyzing the interaction between dietary components and gene regulation is a modern approach to designing appropriate dietary guidelines to affect health positively. Food is a source of primary energy and nutrients and factors that influence health, biochemical mechanisms, activation of biochemical pathways, and regulation of gene expression. The cang salak tea is rich in bioactive ingredients that can manage the molecular mechanism of a gene that improves the lipid profile, thereby reducing the risk of lipid-related diseases such as CVD. More in-depth studies in nutrigenetics and nutrigenomics need to be carried out to identify the basic molecular

mechanism that causes the downregulation of the ApoC3 gene after treatment with cang salak tea. The study is essential to explain the interactions or reactions that occur with the treatment of cang salak tea.

In conclusion, the ApoC3 gene from the liver tissue of hyperlipidemic Wistar rats was successfully amplified by the real-time (qPCR) method. The amplification reaction occurred explicitly with a single peak having a Tm of 82.73°C on the melt curve. ApoC3 gene expression on hyperlipidemic rats treated with cang salak tea was significantly lower (0.46) compared to the expression in control rats (1.17) and hyperlipidemic rats without cang salak tea treatment (1.32). The treatment of cang salak tea in hyperlipidemic rats reduces the ApoC3 gene expression. Hence, cang salak tea can reduce the risk of cardiovascular disease.

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