ANALYSIS OF PHYTOCHEMICAL SUBSTANCE AND THE EFFECT OF *CANG SALAK* TEA (CST) DIET TO LIPID PROFILE ON HYPERLIPIDEMIC RAT

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Abstrak

Kulit salak sibetan (*Salacca zalacca*) dan kayu secang (Caesalpinia sappan L.) dikombinasikan menjadi produk Teh *Cang Salak* (CST). Teh ini diharapkan dapat bermanfaat untuk penyakit yang berhubungan dengan dislipidemia. Tujuan penelitian ini untuk menguji metabolit sekunder dalam CST, dan mengetahui pengaruh pemberian CST terhadap profil lipid kadar kolesterol total (TC), trigliserida (TG), HDL, dan LDL. Tikus wistar dibagi menjadi tiga kelompok yaitu kelompok kontrol (K), kelompok tikus hipelipidemia yang diberikan CST (P1), dan kelompok tikus hiperlipidemia tanpa pemberian CST. Kelompok P1 diberikan CST sebanyak 1,2 mL perhari selama 4 minggu. CST mengandung alkaloids, flavanoids, tannins, phenol, dan saponine. Kadar flavonoid sebesar 81,026 ppm; tannin 525,706 ppm, IC50 177,689 ppm , dan phenol 622,7844 ppm. Terdapat perbedaan yang signifikan profil lipid dari K dan P1 dengan P2. Pemberian CST pada tikus hiperlipidemia dapat menurunkan total kolesterol dan menormalkan kadar TG, HDL, dan LDL. Kandungan aktif pada CST berperan dalam metabolisme lipid, sehingga dapat memperbaiki profil lipid tikus hiperlipidemia.

Kata kunci: kulit salak, Caesalpinia sappan L., hiperlipidemia, profil lipid

Abstract

The bark of Sibetan snakefruit (Salacca zalacca) and sappan wood (Caesalpinia sappan L.) are combined into the product of *Cang Salak* Tea (CST). This tea is expected to be useful for diseases associated with dyslipidemia. The purpose of this study was to examine the secondary metabolites in CST and to determine the effect of CST treatment on the lipid profile of total cholesterol (TC), triglyceride (TG), HDL, and LDL level. Wistar rats were divided into three groups: control group (K), hyperlipidemic rats group given CST (P1), and hyperlipidemic rat group without CST treatment. Group P1 was given CST as much as 1.2 mL per day for 4 weeks. CST contains alkaloid, flavanoid, tannin, phenol, and saponine. Flavonoid level about 81.026 ppm; tannin 525.706 ppm, IC50 177.689 ppm, and phenol 622.7844 ppm. There were significant differences in the lipid profile of K and P1 with P2. CST treatment to hyperlipidemic rats can reduce total cholesterol and normalize level of TG, HDL, and LDL. The active substance in CST has role in lipid metabolism, so it can improve the lipid profile of hyperlipidemic rats.

Keywords : salacca bark, sappan wood, hyperlipidemia, lipid profile

INTRODUCTION

Dyslipidemia or hyperlipidemia is caused by disruption of lipid metabolism due to the interaction of genetic and environmental factors (Nelson, 2013). Hyperlipidemia is characterized by the increasing of total cholesterol (TC) level, triglycerides, and low density lipoprotein (LDL) along with the low level of high density lipoprotein (HDL), it is also one risk factor of atherosclerosis (Singh & Nain, 2019). The improvement of hyperlipidemia along with the changing of people life style, food consumption pattern and owns major role in increasing some of

degenerative disease such heart attack, hypertension and diabetes mellitus (Nelson, 2013).

Based on the recent report of WHO showed that hyperlipidemia significantly related with more than a half of ischemic heart disease globally. According to statistic data of WHO, prevalence of hyperlipidemia for adult aged 25 years old in Indonesia about 36% (33.1% for men and 38.2% for women). The research with small population showed that prevalence of hyperlipidemia to all of ethnic groups in Indonesia in the range of 9.0% and 25% (Lin et al., 2018).

The best treatment of hyperlipidemia can be done through three ways, namely nutritional therapy, physical activity, and medication. Pharmacological therapy of dyslipidemia can be used for primary and secondary prevention of cardiovascular disease (Verma, 2017; Zodda et al., 2018). However, there are many side effects caused by these drugs, such as hyperuricemia, diarrhea, nausea, gastric irritation and liver function abnormalities. Nutritional therapy is considered the most effective therapy, especially for the beginning of hyperlipidemia cases. Nutritional interventions used in the therapy of hyperlipidemia include decreasing energy and fat intake and functional foods selection that can create a positive effect (Kelly, 2011).

People can choose natural therapies to control hyperlipidemia, one of those treatment used is by consuming functional foods. The functional properties of these foods are determined by the bioactive components contained in the meal, such as dietary fiber, antioxidants, prebiotics, and phytochemical groups. Some of the bioactive components in the foods are known to influence genetic factors that can improve someone's health. Bioactive ingredients in nutrients are important components provided in foods or dietary supplements. Various of bioactive ingredients have been successfully characterized as biomolecules and have function or capacity to run some of metabolic processes in the body which give benefit impact to our health. Otherwise, excessive intake of bioactive nutrition ingredients maybe causes various steps of the disease development or changing natural history of a disease (Bautista-García et al., 2017). The influence of food on genetic factors is under the study of nutrigenomics which is currently widely studied. Nutrigenomics studies the dynamics, regulation, and ways how a gene interacts with a compound or bioactive in a particular meal. Food can be converted into expressed genetic information which provides different metabolic profile that will impact on diet and health (Sales et al., 2014).

One of the natural functional foods that potentially used to lower down lipid profiles is *Cang Salak* Tea (CST). CST is an innovative herbal drink made from the bark of Sibetan snakefruit (*Salacca zalacca*) and secang wood (*Caesalpinia sappan* L.). The results of the phytochemical analysis showed that *Cang Salak* tea has active ingredients such as flavonoids, tannins, alkaloids, terpenoids, and phenols (Karta et al., 2019). Flavonoids and terpenoids have been known to contain antihypercholesterolemic activity. Flavonoids can down lower blood cholesterol levels by decreasing the absorption of cholesterol and bile acids in the small intestine and increasing excretion through feces. Flavonoids have role in the prevention and treatment of atherosclerosis and atherosclerosis-related disorders. It serves as antioxidant, hypocholesterolemic, and antidiabetic agent (Zeka et al., 2017). Flavonoids can improve the quality of the lipid profile which significantly can reduce cardiovascular disease in the diet of induced hyperlipidemic rats (Babandi et al., 2019).

The combination of snakefruit bark and sappan wood into tea product is expected to improve lipid profiles or as antihyperlipidemia. Salaccaaa zalacca contains alkaloids, terpenoids, flavonoids, and sitosterols which can be useful for reducing cholesterol, antidiabetic, inhibiting cancer growth, and antioxidants (Ridho et al., 2019). Salak bark contains ferulic acid and proline, cinnamic acid derivatives, arginine, and pterostilbene which are useful for diabetes therapy (Dhyanaputri et al., 2016). Secang wood contains phenolic

compounds such as xanthones, coumarins, chalcones, flavones, homoisoflavonoids, and brazilin. This content has benefit as an antioxidant, antibacterial, anti-inflammatory, anti-aging, hypoglycemic, and antiacne. This wood is also safe and does not cause acute toxicity (Dhyanaputri et al., 2016). Rats fed a hypercholesterolemic diet and given sappan wood extract can reduce total cholesterol and increase HDL levels (Lee et al., 2010), and also enable to reduce LDL levels (Wediasari et al., 2020). Both of these ingredients own ingredients that can improve lipid profiles and act as antihyperlipidemia. Therefore, the combination of these ingredients into *Cang Salak* tea needs further testing by in vivo testing using rats, then checked at the value of the lipid profile..

The value of lipid profile can be used to analysis the ablity of CST as anti-hyperlipidemia. The existence of hyperlipidemia can be seen from metabolic disorders manifested by increasing levels of plasma total cholesterol (TC), triglycerides (TG), and low density lipoprotein cholesterol (LDL-C), accompanied by decreasing levels of high density lipoprotein cholesterol (HDL-C) (Ramchoun et al., 2020).

Regarding those fact, the research conducted to study about the content of phytochemical on CST and the influence of giving CST toward lipid profile to the rats. The result of this research showed that CST has potential as herbal treatment for hypercholesterolemic patients to anticipate the side effects of chemical drugs consumtion.

MATERIALS AND METHODS

Powder making of sappan wood

Sappan wood used derive from Tenganan Village, Karangasem Regency. About 200 grams of sappan wood cut in small part and heated in the oven for four hours every day and processed to be powder while water level test also done at the same time. drying process will be stopped if its water level below ±8%.

Powder making of Sibetan snakefruit bark (Salacca zalacca var.)

Bark of snakefruit used was bark of Sibetan snakefruit (*Salacca zalacca* var.) which obtained from at the fostered place for the manufacture of diversified snakefruit processed products, namely Agro Abian Salak, Sibetan Village, Karangasem. The bark of the snakefruit was cut into small pieces and weighed as much as 300 grams, then baked for 4 hours every day for 4 days at a temperature of 40^0C. Then the bark is blended and sifted and water level test also conducted. Drying in the oven was stopped until the water level content was below ±8%.

Tea mixture combination

The product formulation was made with a ratio of 1:1 sappan wood and salak bark powder, 100 grams of sappan wood powder mixed with 100 grams of salak bark powder.

A total of 1500 mL of mineral water was prepared and boiled, after boiling both of bark powder were put into boiling water (±1000C) and allowed to keep for 10 minutes. The evaporation of the solvent reaches a half volume (±250 mL) was carried out with a rotary evaporator at a temperature of 40^0C with a vacuum pump of 150 and the rpm is 200 for ±45 minutes. After obtaining half the volume, then filtered out. This concentrated solution will be used to test the effect of its giving on rats through qualitative and quantitative phytochemical tests.

Phytochemical Qualitative Tests

Screening alkaloid (Mayer Test)

A total of 1 mL of *Cang Salak* tea solution was dissolved with HCl, added with Mayer's reagent. The white precipitate indicates the existence of flavonoid compounds.

Flavonoid Screening (Shindo Test)

About 1.3 mL *Cang Salak* tea mixed with 0.5 grams magnesium and boiled for 5 minutes. If the color change from orange to red, it indicates the presence of flavonoids.

Screening Tanin Screening (Wohler Test)

A total of 1.6 mL of *Cang Salak* tea was dripped with lead acetate solution. The presence of a white sediment indicates the presence of tannin.

Phenol Screening

2 mL of the sample was taken and pipetted, added with a few drops of $FeCl_3$. The presence of a greenish color indicates the presence of high- or low-level content.

Saponin Screening (Frothing Test)

About 10 mL filtrate was taken and added with 5 mL of distilled water, then shaken vigorously until form foam. As much as 3 drops of olive oil were added to the foam, then shaken again and observed for the formation of emulsion.

Quantitative Test

Flavonoid Level Test

A total of 2 ml extracted sample added with ethanol about 2 ml of 2% $AlCl_2$, incubated for 30 minutes, the observation done one the absorbance of wavelength of 415 nm, with quercetin as the standard.

Tannin Level

About 5 mL *Cang Salak* tea reacted with folin denis reagent and saturated Na2CO3 (5%), mixture incubation for 60 minutes, check the color absorption with a spectrophotometer at λ 725 nm by using the standard curve of tannic acid.

Antioxidant capacity (IC_{50})

The standard curves making for gallic acid and ascorbic acid using various concentrations (0-100 mg/L). The treatment was carried out by weighing 1 mL of the sample, diluted with 99.9% methanol until the volume reach 5 ml in a measuring flask, vortexing, centrifuging 3,000 rpm for 15 minutes. The standard and supernatant were pipetted 0.5, then 3.5 ml of 0.1 mM DPPH was added (in 99.9% methanol solvent) in a tube test, continued to be vortexed. The incubation done on 25^{o}C for 30 minutes to allow DPPH to react with the hydrogen atom donated by antioxidant sample, the level of absorbance was measured at λ 517 nm. The antioxidant capacity was calculated by using the linear regression equation y = ax + b. It would be converted to obtain the inhibitor concentration (IC_{50}) value.

The total level of phenol

About 0.4 ml pipetted sample was put in test tube, 0.4 ml of folin–ciocalteu reagent added, vortexed until homogeneous and allowed to stand for 6 minutes before adding 4.2 ml of 5% sodium carbonate solution. The sample was allowed to stand for 90 minutes at room temperature before its color absorption at a wavelength of 760 nm. Standard curves were made by dissolving gallic acid in distilled water with various concentrations of 10-100 mgL-1. The total phenol calculation using the regression equation y = ax + b.

Experimental animal

The study used wistar rats (Rattus norvegicus) with the criteria of male rats, 2-3 months old, healthy with normal activities, and weight about 150-200 grams. Rats are characterized with long body with smaller head, thick and short ears with fine hair, red eyes, and the tail never longer than its body. Rats were obtained and kept at the Animal Laboratory Unit (ALU) Department of Pharmacology and Therapy, Faculty of Medicine, Udayana University. The rats were adapted for 7 days and tested for its cholesterol levels before treatment given.

Animal testing

The research used six rats for each group and divided into three different groups treatment:

Group I Normal Control Rats (K):

The rats were given a standard diet orally and water orally ad libitum during the research. The same treatment applied, the control group was also given water through a probe for 8 weeks (according to the treatment of diet with rich in fat to groups P1 and P2). Additional water was also given once a day around 1.2 ml via probe (according to the treatment of *Cang Salak* tea in group II) for 4 weeks.

Group II Hyperlipidemic Rats with Cang Salak tea treatment (P1):

The rats were induced by hyperlipemia with high-fat diet containing 3 grams of pork oil/200 grams of the rats weight per day, 2 grams of duck egg yolk/200 grams of weight per day, 3 grams of cholesterol/200 grams of

each weight of rat per day, and 2 grams of folic acid. /200 grams of rat weight per day for 8 weeks through a probe. If the condition of hyperlipidemia was achieved, the rats were given infusion of *Cang Salak* tea once a day about 1.2 ml for 4 weeks through probe. This group was also given a standard diet orally and drinking water was given orally ad libitum during the study.

Group III Hyperlipidemic Rats (P2):
The hyperlipemia were induced to rats with high-fat diet of 3 grams fork oil of 200 grams of every rat weight per day. Other treatment was 2 grams of duck egg yolk per 200 grams of the rat weight every day about 8 weeks by using probe. About 1.2 ml water also given for 4 weeks (the same treatment of *Cang Salak* tea to the second group). group III got standard diet per oral and water per oral ad libitum along the research.

Lipid profile inspection

The lipid profile check was carried out at the Bali Provincial Health Laboratory. The total cholesterol inspection was performed at first week (after adaptation) and the ninth week (8 weeks after hyperlipidemia induction). The cholesterol check was done after the hyperlipidemic conditions formed at the eleventh week (2 weeks after *Cang Salak* tea treatment) and complete lipid profile examination (total cholesterol, TG, HDL, and LDL) at thirteenth week (4 weeks after treatment of *Cang Salak* tea).

The rats were fasted for 12 hours and then anesthetized with ketamine 40 mg/kg and silacin 5 mg/kg via muscle intramuscular. The rat blood was sucked through retro-orbitally using microhematocrit device about 2.5 ml. The blood kept to stand for 30 minutes, then centrifuged at 3000 rpm for 15 minutes. Blood serum on the surface of the tube, was taken and transferred to an Eppendorf tube, stored at the temperature of -20^{o}C before measuring the lipid profile.

Total cholesterol level was measured enzymatically by the CHOD-PAD method. HDL cholesterol levels are measured as the total cholesterol levels after precipitation with precipitating reagents. Triglyceride levels content are measured enzymatically as cholesterol did. LDL cholesterol levels are calculated by the formula: LDL cholesterol levels = [total cholesterol levels] - [HDL cholesterol] - [VLDL cholesterol].

Statistical analysis
All data were analyzed to figure out the normality value and homogeneity of the data. The accepted condition will be continued with t-test to find out the differences between pre-treatment and post treatment on lipid profile in each group. The anova test was carried out on the profiles of total cholesterol, triglycerides, HDL, and LDL to see whether or not about differences due to treatment, as well as the LSD test to analyze the level of significant differences between each treatment.
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RESULTS AND DISCUSSION

The result of research covers the data of phytochemical content *Cang Salak* tea product, the average of lipid profile to each treatment and the result of statictis analysis test to each lipid profile.

Table 1. Phytochemical qualitative test results of *Cang Salak* tea (CST)

Parameter	Reactor	Results
Alkaloids	Mayer Test	++
Flavanoids	Shindo Test	+++
Tannins	Wohler Test	++
Phenol	$FeCl_3$	+++
Saponine	Tes Frothing	+

Note: (+++) very highly present, (++) highly present, (+) present.

Table 2. Phytochemical quantitative test results of *Cang Salak* tea (CST)

Parameter	Results (ppm)
Flavanoid	81.026
Tannin	525.706
IC_{50}	177.689
Phenol	622.7844

Table 3. Profiles Lipid (Total Cholesterol, Tryglyseride, HDL, LDL)

Treatment groups	Profiles lipid ±S.D (mg/dL)				
	Total Cholesterol		TG	HDL	LDL
	Pre	Post			
K	57±6,831	58±1,033	56±0,816	27,3±3,712	19,1±4,376
P1	70±6,066	56±2,066	43±1,225	28,8±0,820	19,0±2,834
P2	74±11,645	67±2,881	59±1,169	22,5±1,157	25,6±1,777

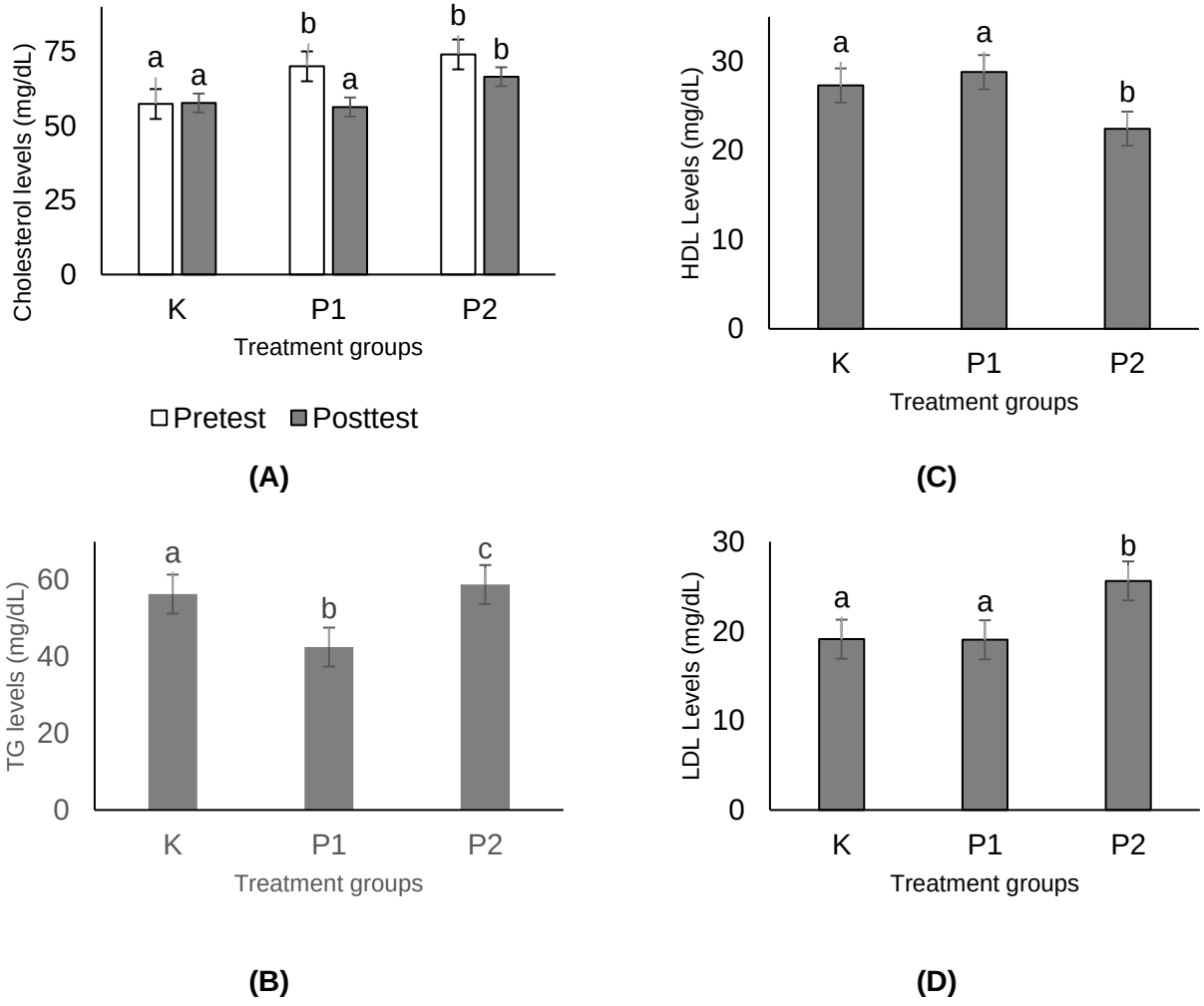

Figure 1. Effect Cang Salak Tea (CST) on lipid profile in wistar rats. **(A)** Total Cholesterol (TC). **(B)** Triglyceride (TG). **(C)** High density lipoprotein-cholesterol (HDL-C). **(D)** Low-density lipoprotein-cholesterol (LDL-C). Pretest dan postest at TC according to T-Test analysis. Mean values followed by the same letters are not significantly different at $p < 0.05$ according to LSD test. K= control normal wistar rats, P1 = hyperlipidemia wistar rats with CST, P2 = hyperlipidemia wistar rats without CST.

Phytochemical Content of CST

Refering Table 1 shows that CST is qualitatively positive containing secondary metabolites of alkaloids, flavanoids, tannins, phenols, and saponines. These compounds may have function to reduce LDL levels and prevent cardiovascular disease by running different metabolic scheme. This ability obtained through the increasing mechanism of reverse cholesterol transport, inhibiting intestinal cholesterol absorption, accelerating cholesterol excretion in the liver, and reducing cholesterol synthesis (Islam et al., 2021). Quantitatively, CST contains flavonoids, tannins, IC_{50}, and phenol, namely 81.026 ppm; 525,706 ppm; 177,689 ppm; and 622.7844 ppm. Each of these contents contribute to the ability of CST to improve the rats lipid profile.

CST is herbal tea that can help to manage the lipid metabolism processes and multi-target interventions in cholesterol absorption, cholesterol synthesis, distribution and excretion. The research of alkaloid shows it has anti-hyperglycemic and anti-hyperlipidemic effects (Zhang et al., 2018). Saponin own the potential to increase lipid peroxidation and superoxide dismutase activity, inhibit pancreatic lipase or modulate adipogenesis and appetite (Marrelli et al., 2016). Saponins also prevents cholesterol absorption by interfering enterohepatic circulation and increasing fecal cholesterol excretion. The tannins reported to have the ability to reduce cholesterol levels by increasing the excretion of fecal bile acids (El-newary, 2016). Tannin also increases nitric oxide synthesis to relaxes blood vessel segments (Baharvand-ahmadi et al., 2015).

CST contains high compound of phenolic. Polyphenol in phenolic compounds has strong potential to change dyslipidemia or to reduce oxidative and inflammatory conditions. It can inhibite the absorption of lipid in the intestine; stimulates efflux of cholesterol from atheroma and excretion of cholesterol through the gallbladder or small intestine; and obstruct de novo lipid synthesis in the liver (Bayir et al., 2019). One type of phenolic compound in CST is ferulic acid, which is found in the bark of salak. This compound has the ability to modulate the level of lipid peroxidation, antioxidant status, and lipid profile (Sudheer et al., 2005). The results showed that the use of ferulic acid could increase the activity of CAT (catalase), T-SOD (total superoxide dismutase), and HDL levels, but the content of MDA (malondialdehyde), total cholesterol, and LDL levels decreased (Chen et al., 2020). Tannin is also phenolic compounds that can prevent cognitive disorders, neurodegenerative disorders, metabolic syndrome, type 2 of diabetes, and dyslipidemia or obesity (Fraga-corral et al., 2021).

The antioxidant activity of IC_{50} from CST belong to moderate category. It is based on the antioxidant activity category, namely strong (IC_{50} <50 ppm), active (IC_{50} 50-100 ppm), moderate (IC_{50} 101-250 ppm), weak (IC_{50} 250-500 ppm), and inactive (IC_{50} > 500 ppm) (Muchtaromah et al., 2020). CST is included in the medium category since its manufacturing process uses tea stew, not using the extraction. The activity of antioxidant in CST is caused by the exitence of flavonoids, alkaloids, and brazilin. Antioxidants in CST assist to stabilize free radicals by complementing the electron deficiency of free radicals and inhibiting the reaction of free radical formation. Excessive fatty acid oxidation will increase the amount of cholesterol in the blood.

One of the mechanisms in hampering the cholesterol formation of is by inhibiting cholesterol synthesis through the enzyme 3-hydroxy-3-methylglutaryl Coenzym A (HMG CoA) reductase, and retardation the absorption of cholesterol mediated by lipase enzymes. The HMG-CoA reductase enzyme can be inhibited by antioxidants to reduce the cholesterol synthesis. Alkaloid and flavonoid also have inhibitory properties to this enzyme (Rahmania et al., 2017). Retardation of this enzyme would be an effective way to lower plasma cholesterol in human body. Polyphenols provide phenol hydrogen atoms to free radicals and inhibit the oxidation of lipid and protein. Flavonoid can decrease apo B secretion in hepatocytes by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A

(HMG CoA) (Baharvand-ahmadi et al., 2015).

The mixture of CST consists of cinnamic acid derivatives. This type of compound can provide anti-obesity and antihypertensive effects through inhibition of lipid digestive enzymes and angiotensin converting enzyme (ACE). In addition, derivation of this compound can protect the aorta and aortic arch and avoid vasoconstriction by increasing diameter and hepatic steatosis and renal toxicity index (Mnafgui et al., 2015).

The influence of CST to total cholesterol

According to the results of the study, the average cholesterol level of rats during 7 days adaptation before high fat induction was carried out, namely in the K treatment of 46±4.721 mg/dL; P1 treatment was 50±3.830 mg/dL, and P2 was 52±13,810 mg/dL. The inspection of total cholesterol levels was again carried out at the beginning of the ninth week. The cholesterol levels of rats in P1 and P2 increased after given a high-fat diet for eight weeks to 70±6.066 mg/dL (40%) and 74±1.645 mg/dL (42%). The control also increase in cholesterol levels about 57±6.831 mg/dL (24%), which indicates increased growth in rats. Normal cholesterol levels in Rattus norvegicus rats are 10-54 mg/dL (Smith & Mangkoewidjojo, 1998). Based on the cholesterol levels, the rats in each treatment were above normal levels or had hyperlipidemia.

The CST probe were given after the rats got hyperlipidemia condition, all group including P1, control and P2 were watered probes for 4 weeks. After 4 weeks, total cholesterol levels of P1 and P2 changed. The mean total cholesterol in P1 was 56±2.066 mg/dL or decreased by 20%, while P2 was 67±2.881 mg/dL or decreased by 9.5%. The graph of changes in total cholesterol levels can be seen on Figure 2.

Figure 2 indicates the improvement of cholesterol levels due to the treatment of high-fat diet at P1 and P2. The rats got hyperlipidemia after giving high-fat diet for 8 weeks. The next step, each treatment was not given a high-fat diet. It is needed to determine the effect of giving CST to reduce the hyperlipidemia. Giving CST to hyperlipidic rats (P1) for 2 weeks can reduce total cholesterol levels, and it continues to decrease close to total cholesterol levels in the control group (K) after giving tea for 4 weeks. On P2 also decreased due to cholesterol metabolism of the rats.

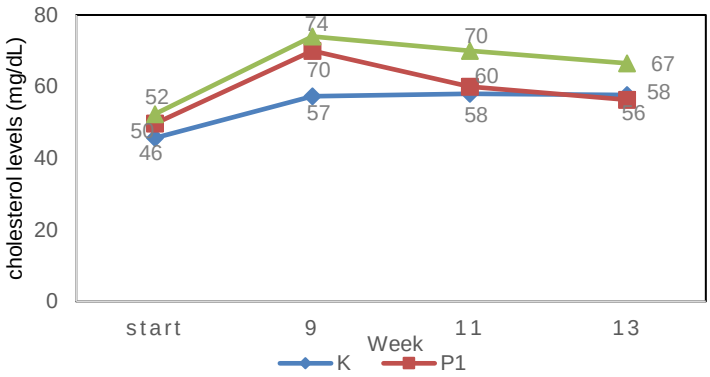

Figure 2. Total cholesterol levels of wistar rats at the beginning of adaptation, 9th week, 11th week, and 13th week.

Figure 1 (A) shows the graph of the statistical test results. Anova test showed significant differences in each treatment on total cholesterol levels of hyperlipidemic rats and the treatment of CST. Based on LSD test, it was obtained significant differences (p value > 0.05) in total cholesterol levels of hyperlipidemic rats in control (K) with P1 and P2, while P1 and P2 there were not significant differences (p value <0.05). Meanwhile, after getting CST treatmen on P1, there was significant distinct among K, P1 and P2. The letter on the different graphs indicate a significant difference. In determining the effect of CST treatment, some tests were conducted namely pre-test (hyperlipidemic rats) and post test (after CST treatment) with T-test. The result showed that there is significant differences in TC-levels at P1, while at K and P2 there is no significant differences. It shows that the effect of CST treatment can help reduce TC-levels in hyperlipidemic rats.

The reduction of total cholesterol by giving CST treatment is certainly related to the active substance in tea to lower down cholesterol by its mechanism. Secondary

metabolites in CST such as flavonoid can obstruct cholesterol synthesis by inhibiting HMG-CoA, thereby decreasing the secretion of apoliprotein B in hepatocytes and increasing the activity of Lechitin Acyl-CoA Cholesterol Acyl Transferase (LACAT) and lipoprotein lipase (LPL) enzymes (Stanely Mainzen Prince & Kannan, 2006). HMG-CoA reductase is enzyme that has role in cholesterol formation. Inhibition of this enzyme causes the effect of lowering cholesterol levels in the body.

The improvement of LCAT can lower free cholesterol levels in the blood. Flavonoid reduces cholesterol synthesis by inhibiting the activity of the enzyme acyl-CoA cholesterol acyl transferase (ACAT) in HepG2 cells which has function in reducing cholesterol esterification in the intestine, liver, and inhibiting the activity of the enzyme 3-hydroxy-3-methylglutaryl-CoA which set inhibition of cholesterol synthesis(Arief et al., 2012).

Saponin in CST can form insoluble complex bonds with cholesterol derived from food and bind to bile acids. The production of bile acid requires cholesterol as its raw material to increased bile acid secretion, the total cholesterol levels in the blood will decrease. It can be concluded that secondary metabolites in CST can detain the cholesterol synthesis process.

The effect of CST to Triglyceride Level, HDL, and LDL.

Figure 1 (B) shows significant distinction of triglyceride levels among K, P1, and P2 based on the results of statistical tests. Triglyceride is the most abundant type of lipid and found abundantly in adipocytes. The TG inspection was carried out at the thirteenth week after 4 weeks of treatment. There was great different between the level control of P1 and the LSD test. The point of TG level in control and P2 were higher than P1. It shows that CST can improve the point of TG level.

The P1 treatment had lower TG level than the other treatments. It is closely related to the active substance in CST. Plasma triglyceride (TG) is important factor that can provide indication of health status related to lipid metabolism markers. Flavonoid in CST may generate positive effect on components of the metabolic syndrome due to their ability to modulate different gene expression and gut microbiota; several cytokines, enzymes and metabolites associated with inflammation, oxidative stress and metabolism are activated or inhibited on its turn causes decreasing of body weight, blood pressure, and glucose level (Neri-numa et al., 2020). The mechanism of favonoids in reducing triglycerides is by increasing the enzyme lipoprotein lipase (LPL) through the activation of PPAR (Peroxysome Proliferator Activated Enzyme) which is subfamily of intracellular isoform receptors (transcription factor fucntion) that induces peroxisome proliferation (Hayudanti et al., 2018).

PPAR takes part in transkription control of gen that codify involved protein in important metabolic steps of fat and energy homeostasis, such as fatty acid distributin and trapped by cells, intracellular binding and activation, and catabolism (Abranches et al., 2011). PPAR activation can affect fatty acid metabolism and its activation lowers lipid level, manage blood glucose and cholesterol level, and reduces lipid accumulation in heart cells even during a high-fat diet. PPRA ligand can decrease triglyceride-rich lipoproteins in serum through increased expression of gen involved in fatty acid-β-oxidation and decreased expression of apolipoprotein C-III gen. This activation causes the PPAR receptor to bind to the 9-cis retinoic acid receptor and to bind to the peroxisome proliferator response elements. This condition create reduction of triglycerides as well as the increasing of HDL cholesterol and apolipoprotein gen expression A-I and A-II (Grygiel-górniak, 2014). In hence, it causes the level of TG in the treatment with CST (P1) lower than the control and P2.

Figure 1 (C) shows difference average HDL level in each treatment. According to statistical analysis, there are significant differences among K, P1, and P2. The LSD test shows that K and P1 are significantly different from P2. K and P1 do not have any meaningful differences. HDL

level in K and P1 are higher than P2. Meanwhile, LDL level is in the contrary of HDL level. Figure 1 (D) shows difference meaning of the mean LDL level among K, P1 and P2, where P2 has higher level. Based on that point, it shows that CST can help the HDL level of rats in P1 are not much different from the control, likewise LDL level in P1 is lower than P2.

The substance of secondary metabolites in CST can help to control HDL levels in P1 rats by following the control. The flavonoid compounds in CST have mechanism to increase the number of HDL cholesterol. Flavonoid own potential to enhance HDL function through its effects on cellular antioxidant status and inflammation. Flavonoid can affect reverse cholesterol transport (RCT) and HDL function beyond modest HDL cholesterol concentrations by managing cellular cholesterol release from macrophages, expression and 1 hepatic paraoxonase activity (Millar et al., 2017). Furthermore, it can also increase the expression of ATP-binding cassette (ABCA1) and improve apolipoprotein A1 as the basic material to form HDL (Behrens et al., 2019). ABCA1 has function in the efflux of intracellular free cholesterol and phospholipid across the plasma membrane to combine with apolipoprotein, especially apolipoprotein AI (Apo AI), and form high-density lipoproteincholesterol (HDL-C) particles (Jacobo-albavera et al., 2021). ABCA1 takes part in the beginning step of reverse cholesterol transport (RCT) by regulating the movement of overcholesterol and phospholipids from peripheral tissues to the liver. ABCA1 is able to form new HDL particle through binding to apolipoprotein (apo) A-I and apoE (Ye et al., 2020).

CST contains ferulic acid that has potential to improve lipid profiles in the blood. Ferulic acid supplementation can drof down the level of TC, LDL-C, and TG. The mechanism is by inhibiting HMG-Co A reductase to control cholesterol synthesis and modulates lipogenic gen expression in the liver. Ferulic acid can hinder HMG-Co A reductase in decreasing TC and LDL-C. The effect of ferulic acid can reduce lipid peroxidation which causes reduction of malondialdehyde (MDA) level and LDL-C oxidation (Bumrungpert et al., 2018).

The active substance in CST can control the level of TG, HDL, and LDL in the body. The rats with P2 which got deficient lipid profile compared to control and P1, due to the absence of secondary metabolite supplementation. The lipid profiles between control and P1 rats were not significantly different which marked that CST treatment could normalize the lipid profile conditions of hyperlipidemic rats.

CONCLUSION

Cang Salak Tea (CST) contains alkaloid, flavanoid, tannin, phenol, and saponine. The Flavonoid level about 81.026 ppm; tannin 525.706 ppm, IC50 177.689 ppm, and phenol 622.7844 ppm. The CST treatment to hyperlipidemic rats can reduce total cholesterol and normalize level of TG, HDL, and LDL. The active substance in CST has role in lipid metabolism, so it can improve the lipid profile of hyperlipidemic rats.

In future research, it is necessary to investigate the effect of giving CST on other lipid metabolism diseases.

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