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Research Article
The Effect of Kersen Juice on Lipid Profile of Spargue Dawley Rats: A Randomized Controlled Trial

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Abstract

Background and Objective: Vitamin C is an antioxidant that has been linked to cholesterol metabolism and it's ability to reduce level of cholesterol, triglycerides and low density lipoprotein (LDL) in patients with hyperlipidemia. Kersen is a fruit that is easily found in Indonesia but fail to utilize it fully. Kersen is rich in vitamin C and contains flavonoids, phenols, niacin and beta-carotene which act as antioxidant agents. The aim of this study was to analyze the beneficial effects of kersen juice on lipid profile of Spargue Dawley rats. **Materials and Methods:** This study use a randomized controlled trial (RCT) method. Twenty five adult male Sprague-Dawley rats were divided into five groups. The first two groups were controls: i). Group 1 = no high fat diet (HFD) without kersen juice, ii). Group 2 = HFD without kersen juice. The three treatment groups are: i). Group 3 = HFD+0.9 mL/200 g body weight (BW) of kersen juice, ii) Group 4 = HFD+1.8 mL/200 g BW of kersen juice and iii) Group 5 = HFD+3.6 mL/200 g BW. All groups were treated for 2 weeks. Blood was drawn to examine the lipid profile before and after treatment was given. Mann-Whitney test was performed to determine the significant differences $p<0.05$ between groups. **Results:** The increased level of high density lipoprotein (HDL) was significantly different ($p<0.05$) with the treatment given. No differences were found between groups of treatment on the level of cholesterol, triglycerides and LDL respectively $p>0.116$, $p>0.383$ and $p>0.052$. **Conclusion:** It was concluded that the administration of kersen juice can significantly increase HDL up to 2.68 mg dL^{-1} but not significantly change the total cholesterol, tryglicerids and LDL in rats with HFD in this study.

Key words: Kersen juice, antioxidant, high fat diet, lipid profile, HDL

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Coronary heart disease (CHD) is the leading cause of death in the world. In United Kingdom, based on the epidemiological data from British Heart Foundation, there are 94,000 deaths from CHD in the UK each year[1]. Household Health Survey (Survey), suggests that CHD ranks on 3rd leading cause of death in Indonesia[2,3].

CHD is caused by the interruption of blood flow to the myocardium because there is a narrowing of blood vessels or coronary artery atherosclerosis. One of the causes of atherosclerosis is dyslipidemia[4,5]. Dyslipidemia is an abnormality in blood lipid, lipoprotein metabolism abnormalities and dysfunction of endothelial tissue that develops into atherosclerosis[6,7]. In general, the condition of dyslipidemia can be characterized by increasing levels of total cholesterol, low density lipoprotein-cholesterol (LDL-C), lipoprotein a (Lp a), triglycerides and a decrease in high density lipoprotein-cholesterol (HDL-C)[8].

Antioxidants in foods by the Panel on Dietary Antioxidants and Related Compounds of the Food and Nutrition Board is defined as "a substance contained in foods which may significantly reduce $p<0.05$ the adverse effects of reactive oxygen species , reactive nitrogen species , or both on normal human physiological function." Results of epidemiological studies indicate that the consumption of antioxidants can reduce the risk of CHD by 20-40%[9]. Antioxidants consisting of vitamin C, vitamin E, the minerals of selenium, zinc and copper, as well as some phytochemicals (such as polyphenols), work by blocking the oxidative stress from free radicals and repair damage to the endothelial dyslipidemia, as well as protecting LDL and very low density lipoprotein (VLDL) especially in the oxidation reaction. Endothelial tissue repaired in dyslipidemia causes a decrease in total cholesterol, triglycerides and LDL-C as well as having a good effect for patients with cardiovascular disease[10].

Vitamin C is one of the antioxidants that has been linked to cholesterol metabolism. The deficiency of vitamin C may increase synthesis of cholesterol[11]. Some studies reported that Vitamin C reduces levels of cholesterol, triglycerides and LDL-C in patients with high blood lipid levels.In addition, vitamin C can also raise levels of HDL-C[12,13]. Recommendation of vitamin C intake for healthy people is 60 mg day^{-1}. Vitamin C may be absorbed maximum at 0-120 mg day^{-1} [14].

Kersen (*Muntingia calabura*) is a fruit that is still underutilized[15]. A study showed that kersen contains vitamin C which is quite high at approximately of 80.5 mg[16]. Besides, kersen also contains flavonoids, phenols, niacin and beta-carotene which act as antioxidant agents[17,18]. So far, the research on the effect of kersen on the condition of a high-fat diet has not been reported yet. Therefore, the purpose of this study was to determine the effect of kersen juice on lipid profile Spargue Dawley rats fed a high-fat diet.

MATERIALS AND METHODS

Standard feed (BR II) (Comfeed, state, Conutry), lard, duck egg yolk, cholesterol, triglycerides, LDL and HDL kit from diagnostic system (Diasys) kits GmbH (Germany) were used during study.

The preparation of Kersen Juice: Kersen used in this study has the following characteristics: the color is red, clean and undamaged. Preparation of kersen juice was according to the standard with the form in analytical grade.The fruit obtained is then washed thoroughly. Further more, kersen fruit is smoothed by means of a blender without water addition and subsequently, the kersen juice obtained is filtered to separate the juice from the pulp using filter[19].

Studies in animals: This study was carried out on September-November, 2012 in Laboratory of Pharmacology Faculty of Medicine Universitas Gadjah Mada. During this study, 25 Spargue Dawley rats with 2-month-old male, weight of 140-230 g were obtained from the Laboratory of Pharmacology, Faculty of Medicine, Gadjah Mada University. Before treatment, the rats were adapted for 7 days by providing a standard diet BR II Japfa Comfeed and drink *ad libithum*. Rats are individually caged with a controlled temperature room and 12 h lighting for 8 weeks. Ethical clearence of this study was obtained from the Ethics Committee of Faculty of Medicine. Once adapted, the rats were divided into 5 groups, each group consisted of 5 mices (Fig. 1).

Each treatment group received a standard diet and high-fat diet (HFD) as much as 15 g day^{-1}. The provision of HFD with the composition of 1 kg standard diet mixed with 50 g of pork-fat and duck egg yolks 100 g and the duck egg yolk was added to 2 mL day^{-1} per oral[20]. The HFD was provisioned for 5 weeks. Kersen juices were given for 2 weeks per oral. Body weight (BW) was measured every 3 days, while the left-over of the food were measured daily. Blood was drawn at week 5th after the administration of HFD as a pre-test and week 7th after the administration of kersen as a post-test. Blood was drawn by orbital sinus for lipid profile analysis.

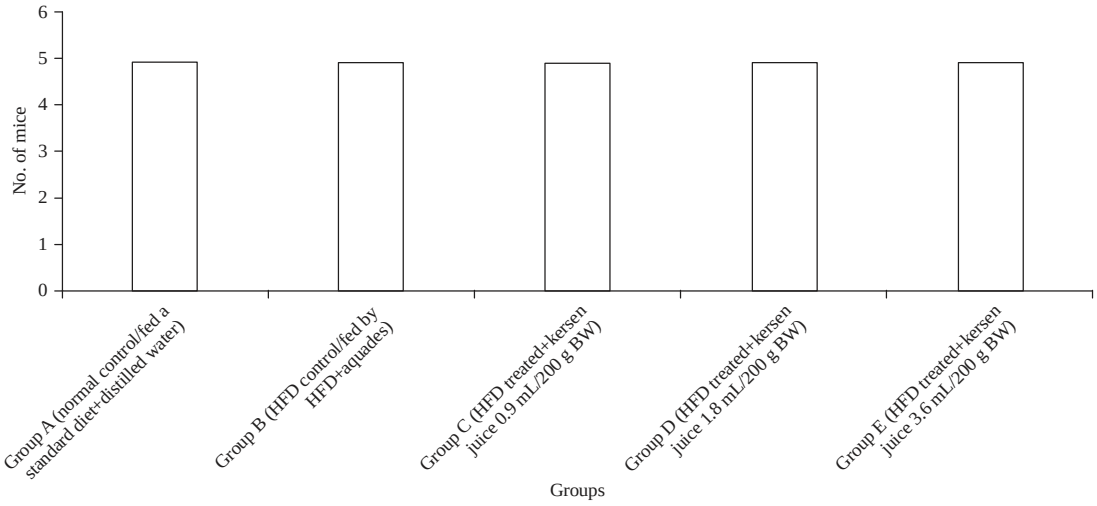

Fig. 1: Distribution of the treated groups of mice

Analysis of total cholesterol, triglycerides, LDL and HDL: Analysis of total cholesterol, triglycerides, LDL and HDL was conducted with CHOD-PAP method using diagnostic system kit (Diasys) Gesellschaft mint beschrankter Hattung GmbH (Diasys, Germany). Analysis of the lipid profile follows to the protocols contained in the kit.

Statistical analysis: The results of the analysis of total cholesterol, triglycerides, HDL and LDL were expressed as Mean±SD. The differences between treatments were evaluated by Kruskal-Wallis test. To determine the existence of significant differences between groups, we used Mann-Whitney test with $p<0.05$[21].

RESULTS AND DISCUSSION

In this study, administration of BR II Japfa Comfeed+egg yolks 100+50 g lard for 5 weeks cannot results any expected effects such as hyperlipidemia, that total cholesterol is more than 88 mg dL^{-1}. A previous study also suggested that the provision of yolk sonde for 28 days have not gotten yet hyperlipidemic mice[22,23]. This is due to the body homoestasis process to metabolize high fat by a factor that regulates the process of lipogenesis, lipolysis, lipid transport, heredity, nutrition, enzymes and hormonal[24,25]. The normal level of cholesterol in the blood of Sprague-Dawley rats is 47-88 mg dL^{-1}, with an average of 65 mg dL^{-1} [26]. This normal cholesterol level can be caused by lack of time in providing HFD. The duration of HFD also affect total cholesterol levels.

Previous study shows that HFDs interfere with the behavior and molecular circadian rhythms in mice[27]. The results of this study showed that the consumption of high-calorie diet changed the function of the mammalian circadian time. Besides, It was caused by the homeostasis of the body to store fat and lipogenesis in adipose tissue. This study analyzed the effects of HFD on 24 h profiles of leptin, glucose, insulin, free fatty acids (FFA) and corticosterone. The results showed that the five metabolic parameters can be changed by the animals fed a HFD.

Lipogenesis is closely related to the conversion of excess glucose. Fat in the intestine derived from the secretion of bile and fat from food called exogenous fat[28]. The presence of fat in the diet causes the suppression of lipogenesis in the liver and if there are more than 10% fat in the diet, the conversion of carbohydrates into fat in foods occurs slightly[24]. Determinants of fat depend on the adequacy of carbohydrate. The rate of lipogenesis occured more frequently in animals fed a high-carbohydrate diet compared to those fed a HFD because of the free fatty acids in the tissue[23,29]. Cholesterol from food intake is increased to 2%, as a consequence, it decrease the production of endogenous cholesterol. The cholesterol in egg yolk is 2630 mg/100 g. Cholesterol in the yolk is high and can decrease the production of endogenous cholesterol[30].

HFD led to increased acetyl-CoenzymeA (acetyl-CoA) which is the basic ingredient of cholesterol formation[31]. Cholesterol can be synthesized entirely in the body from acetyl-CoA through a complex trajectory. Cholesterol synthesis in the liver is regulated by dietary intake of cholesterol as a form of residual chylomicrons enriched cholesterol. In the tissue, the cholesterol balance is maintained by cholesterol-enhancing factor, that is synthesis, uptake via LDL receptor, cholesterylester hydrolysis with cholesterol-lowering factors

Table 1: Levels of total cholesterol, triglycerides, LDL and HDL

Levels	Time	Group A	Group B	Group C	Group D	Group E	p-values
TC	Pre test	59.68±8.58	78.36±16.09	68.68±6.21	75.14±9.74	56.25±20.73	0.107
	Post test	51.80±13.97	65.90±2.37	58.33±1.80	63.40±5.87	50.50±12.30	0.116
TG	Pre test	65.52±32.30	137.22±18.50	153.53±71.49	159.04±44.17	132.10± 64.92	0.089
	Post test	62.54±38.25	92.72±29.22	61.85±3.58	73.50±13.98	71.13±22.81	0.383
LDL	Pre test	41.68±6.39	54.78±8.52	38.68±8.01	42.52±9.37	38.63±15.47	0.143
	Post test	34.32±10.20	47.70±3.32	41.85±4.02	47.30±7.65	35.38±8.57	0.052
HDL	Pre test	24.26±9.63	32.92±6.44	27.18±7.69	27.76±4.64	25.13±11.08	0.569
	Post test	21.56±4.68[a]	29.50±3.40[b]	27.38±1.52 [b]	30.44±4.90[b]	20.70±5.53[ab]	0.021

Values are expressed as Mean±SD, [abc]Different notation indicates $p<0.05$ using Mann-Whitney test, Total cholesterol (TC), Triglyceride (TG), Low density lipoprotein (LDL) and High density lipoprotein (HDL), Normal control (A), HFD control (B), HFD treated with 0.9 mL day^{-1} kersen juice (C), HFD treated with 1.8 mL day^{-1} kersen juice (D) and HFD treated with 3.6 mL day^{-1} kersen juice (E)

(steroid synthesis), cholesterylester formation and transport of cholesterol via HDL. The activity of ACAT (Acyl-CoA Cholesterol Acyl Transferase) enzyme is known to be quite high compared to LCAT (Lechitin Cholesterol Asiltransferase) in rat liver cholesteryl, allowing excretion cholesterol esters in nascent VLDL[23].

The provision of HFD cause the free fatty acid levels in the blood increase and more fatty acids drawn to the liver to be esterified form triglycerides to be carried by VLDL to adipose tissue. This incident led to lipogenesis in the liver and is hampered because the main source of triglycerides are derived from exogenous fat[23].

Enzymes and hormones play an important role in lipogenesis. Complex enzymes of fatty acid synthase and acetyl-CoA carboxylase are essential enzyme in lipogenesis, because both enzymes work adaptively with the physiological needs of the body. Acetyl-CoA will increase in a state of satiety and will be inhibited in the presence of long-chain acyl-CoA molecules, because if the acyl-CoA accumulated and not enough esterified, this compound will reduce the synthesis of new fatty acids. This enzyme will decrease in the consumption of high-fat, fasting state and diabetes. The insulin hormone stimulates lipogenesis while epinephrine and glucagon prevent lipogenesis[23]. The existence of these hormones affects the balance of fats in the body.

In the administration of HFD, free fatty acid levels in the blood will increase and there will be more fatty acids drawn to the liver. The high levels of free fatty acids in plasma will proportionally increase with the conversion of free fatty acids into ketone bodies[25]. This process is called ketogenesis which may occur due to the presence of fatty acid oxidation in the liver at high speed. Ketogenesis is regarded as a mechanism that allows the liver to oxidize fatty acids with increasing numbers. The result of this process is the ketones body formation, namely acetoacetate, 3-hydroxybutyrate and acetone which can be used as energy by extrahepatic tissues or excreted in the urine and the lungs. Non-pathological form of ketosis was observed in a state of HFD[23]. Giving a HFD is allegedly to produce many ketones and this study (analysis ketones in the urine) was not conducted.

It is known that the administration of HFD in this study can cause a hyperlipidemic condition in rats due to a homeostatic of rats by pressing lipogenesis, improve the ketogenesis process, the use of exogenous cholesterol for synthesis of steroid compounds. The results of the esterification of fatty liver were carried by VLDL heading into body tissues to be oxidized and to adipose tissue for storage processes. Hyperlipidemia is a measure of cholesterol levels before and after given a HFD[32].

Based on the Table 1, there are significant difference between groups on the variables of HDL ($p = 0.021$). In this study, it was found that the variables of TC and TG declined in all groups although the decrease was not statistically significant. Cholesterol level listed in the table showed non-significant decrease. The ability of Kersen Juice to reduce total cholesterol levels are showed in a previous study regarding changes in cholesterol level in mice *(Musmusculus*) hypercholesterolemic male, after administration of kersen extract (*Muntingia calabura*)[33]. The authors stated that kersen fruit is able to lower cholesterol levels in mice.The result of this study is not consistent from the previous study because it has difference in the rat's hyperlipidemic condition. The HFD given for five weeks was not yet resulted in hyperlipidemic condition.

The ability of Kersen juice (*Muntingia calabura*) in lowering total cholesterol is allegedly due to the high content of vitamin C[16], in which vitamin C is a water soluble antioxidant that can prevent oxidation[34]. Kersen extracts (*Muntingia calabura* contained the high antioxidant compounds in large quantities which are not only vitamin C but also phenolic compounds, saponins, tannins and flavonoids[35]. These results are consistent with research that explains that vitamin C has the effect of cholesterol-hipocholesterolemia through activities of hydroxylase 7α-hydroxylation reactions that increase cholesterol to bile acids changes so as to increase the excretion of cholesterol.

Increased excretion of cholesterol causes a decrease in the amount of cholesterol in the blood[36,37]. In addition to increasing the excretion of cholesterol and vitamin C also helps increase level of HDL and serves as a laxative to increase faecal disposal and lower the re-absorption of bile acids that can be converted into cholesterol[38]. This beneficial effect on kersen juice to increase level of HDL is also demonstrated in this study and so this fruit is proven to be a good source of vitamin C and other antioxidants.

There has been a report that stated vitamin C deficiency can lead to decreased production of bile salts and increased blood cholesterol levels[39]. Restricted intake of vitamin C in the diet resulted in a decrease in the activity of cholesterol 7α-hydroxylase, which is associated with decreased synthesis of bile acids and bile acid half-life extension, so that it may inhibit the excretion of bile acids.

The increase in bile acid synthesis can be performed with high dose of ascorbic acid consumption. Consumption of high dose of vitamin C increases the Bile Acid sulphation through the formation of *Ascorbate sulfate*[39]. Bile Acid Sulphationis an eliminator of cholesterol mechanism by increasing bile acid solubility, decreasing absorption of bile acids in the intestine and increasing its excretion in the feces and urine[40].

The increase of energy intake or dietary fat will lead to increasing lipogenesis activity that leads to the increasing number of free fatty acid formations. The free fatty acids will move from fat tissues to the liver. Free fatty acids in the liver with glycerol forms triglycerides. The higher fat intake the higher triglycerol synthesis in the liver and higher level of triglycerides in the blood were observed[41]. HFD and high carbohydrates can lead to an increasing level of blood triglyceride. Saturated fatty acids are precursors of triglycerides formation[42]. Triglyceride is the major lipid deposit in adipose tissue[43]. Consumption of food rich in cholesterol and saturated fatty acids can suppress the formation of LDL receptors, thereby increasing the amount of cholesterol circulating in the blood[44].

Kersen juice (*Muntingia calabura*) contains vitamin C, which serves as an exogenous antioxidant[45,46]. Antioxidants are compounds that may neutralize free radicals, so that the unpaired atoms changed into pairs and stable[47]. Mechanism of action of antioxidants on free radical compounds is by reducing the formation of new free radicals by breaking the chain reaction, which are more stable[48].

CONCLUSION

The administration of kersen juice was significantly increase HDL, but not significantly change the total cholesterol, tryglicerids and LDL in rats with HFD. This benefit from kersen juice that can increase HDL, may become a promising nutritional therapy for low HDL or hyperlipidemic patients.

SIGNIFICANCE STATEMENT

This study discovers the benefits from kersen juice that can increase HDL level, which may become a promising nutritional therapy for low HDL or hyperlipidemic patients. This study will help the researchers to uncover the critical areas of hyperlipidemic/dyslipidemic that many researchers were not able to explore. Thus, a new theory on nutritional therapy for patients with hyperlipidemic/dyslipidemic will soon be arrived at the realm of science.

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