LAPORAN PENELITIAN

"THE EFFECT OF PACITAN'S SWEET ORANGE'S (*Citrus sinensis (*L*)* Osbeck*)* PEEL POWDER ON THE LIPID PROFILE OF MALE DYSLIPIDEMIA RATS *Rattus novergicus*"

Oleh:

Rinza Rahmawati S.

Baterun Kunsah

Rahma Widyastuti

LEMBAGA PENELITIAN DAN PENGABDIAN

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THE EFFECT OF PACITAN'S SWEET ORANGE'S (*Citrus sinensis (L)* Osbeck*)* PEEL POWDER ON THE LIPID PROFILE OF MALE DYSLIPIDEMIA RATS *Rattus novergicus*

Rinza Rahmawati Samsudin[1], Baterun Kunsah[1], Rahma Widyastuti[1]
Fakultas Ilmu Kesehatan
Universitas Muhammadiyah Surabaya
rinza_rahmawati@yahoo.com

ABSTRACT

INTRODUCTION Dyslipidemia is a lipid metabolism disorder characterized by the increasing TC, LDL cholesterol, triacylglycerol TG above normal values and decrease of HDL in the blood. Increasing LDL-C and triacylglycerol have a correlation with poor cardiovascular circumstances and increased lipid absorption may lead to conditions of dyslipidemia. Orange peel is often considered a waste that is not useful. The purpose of this study wanted to know the effect of giving orange peel to the mice dyslipidemia. **METHOD** This study was an experimental study, using a randomized post test only control group design using twenty-seven white rats (*Rattus norvegicus*) Wistar strains. This study lasted for eight weeks. Mice dyslipidemia is made by giving high fat feed to all groups of K1, K2 and K3. Each group consists of 9 white rats divided randomly. The composition of high-fat feed is a modification of the formula Nutrient requirements of laboratory animals. **RESULTS** The mean of body weight of K1, K2 and K3 groups tended to increase. Increased body weight of mice began to occur at week 2, after the K1, K2, and K3 groups were given a high-fat diet. Levels of TC, LDL-C and TG in K2 and K3 group treated with sweet peel leaf extract of pacitan with dosage of CMC-Na 1% dose 500 mg / kg BW and CMC-Na 1% dose 750 mg / kg BW Lower than the K1 group. Decreased levels of TC, LDL-C and TG occurring in groups of K2 and K3 significantly. **DISCUSSION**. The compound d-limonene of Pacitanese orange may affect PPAR. PPAR (Peroxisome proliferator-activated receptors) is a transcription factor of super family nuclear receptors. PPAR circulating with its ligand may affect lipid metabolism The compound d-limonene of Pacitanese orange may activiting PPAR. Activating PPAR can affect the total cholesterol levels in the blood. The mechanism of PPAR in affecting total cholesterol is by inhibiting NPC1L1 activity in the intestinal wall. NP1C1 is a transporter in charge of bringing cholesterol into the blood. The decrease of LDL-C level may occur due to hesperidin content in the peel of Pacitanese sweet orange where the mechanism of action of hesperidin is estimated by inhibition of reductase HMGCoA enzyme activity.

Keyword : Dyslipidermia, TC, LDL-C, TG, HDL-C, d-limonene, Pacitanese orange

INTRODUCTION

Dyslipidemia is a lipid metabolism disorder characterized by the increasing total cholesterol, Low Density Lipoprotein (LDL) cholesterol, triacylglycerol (TG) above normal values and decrease of cholesterol levels of high density lipoprotein (HDL) in the blood. LDL is a lipoprotein that serves to transport cholesterol from the liver to the peripheral tissues. Meanwhile, HDL is a lipoprotein that functions to bring cholesterol from the peripheral tissue back to the liver. Thus, HDL works in preventing the buildup of cholesterol in the body. Increasing LDL-C and triacylglycerol have a correlation with poor cardiovascular circumstances[1].

Lipids derived from food will undergo digestion process in the intestine into free fatty acids, triacylglycerol, phospholipids and cholesterol. In the free fatty acid intestine, triacylglycerol, phospholipids and cholesterol are processed and absorbed into the bloodstream in the form of chylomicrons. High levels of lipids in the diet will cause cholesterol absorption increasing during digestion in the intestine. Increased lipid absorption may lead to conditions of dyslipidemia[2].

Indonesia is a country that has a diversity of plants. One of the typical Indonesian fruit is the Pacitanese sweet orange (*Citus sinensis (L.) Osbeck*). Pacitanese orange is a type of sweet orange that goes into taxonomy citrus sinensis. In addition to have a sweet taste of chemical compounds in Pacitanese orange potentially as an alternative treatment of dyslipidemia. In general, the composition of orange contains multivitamins, especially vitamin C, water soluble fiber (pectin), flavonoids include hesperidin and d-limonene[345].

D-limonene is a chemical compound that has a fairly low toxicity. In a study has been done proposed that d-limonene compounds do not cause mutagenic, carcinogenic, or nephrotoxic that are at risk to humans. In the citrus sinensis group is d-limonene. The d-limonene compound of Pacitanese orange may affect PPAR. PPAR (Peroxisome proliferator-activated receptors) is a transcription factor of super family nuclear receptors. PPAR circulating with its ligand may affect lipid metabolism[5]. The mechanism of PPAR in affecting total cholesterol is by inhibiting NPC1L1 activity in the intestinal wall. NP1C1 is a transporter in charge of bringing cholesterol into the blood[6].

A study presented at the American Heart Association (AHA) in Las Vegas states that a flavonoid in oranges known as hesperidine proven to effectively improve blood vessel function and help reduce the risk of heart disease. Hesperidin is a specific flavonoid found in sweet oranges and lemons that the hesperidin content can reach > 14% net weight[4].

Hesperidin is most common found in young and green oranges, the concentration increases during storage. The distribution of hesperidin in the citrus fruits is spread over each layer and in large quantities can be isolated from the skin. In the ripe oranges, high concentrations of hesperidin can be found on the inner layers of orange rind and segmented membranes, whereas in lower concentrations can be found in juice and seed vesicles[4] . The mechanism of hesperidin is estimated by three pathways: inhibition of HMGCoA reductase and ACAT enzyme activity, expression stimulation and transcription of LDL receptor gene, and inhibition of apoprotein B secretion by hepatocyte cells[478].

The high potency possessed by Pacitanese orange sweet peel, it will be very useful for the community if experimental research is done, especially about the effect of giving Pacitanese sweet orange to lipid profile.

MATERIAL AND METHOD

Experimental Animal

This research is a pure experimental study, using randomized post test only control group design using twenty seven Strain Wistar white rats (*Rattus norvegicus*) obtained from experimental animal unit of Biochemistry Department Laboratory of Medicine Faculty, Airlangga University Surabaya. Criteria of experimental animal used in this study are male, 2-3 months old, 110 – 130 gram weight with healthy physical condition. The samples were then divided into three groups namely groups K1, K2 and K3. Each group consisted of 9 white rats divided randomly. During the research one rat occupied a cage so that it was required 27 Pacitanese orange. Every week the weight of mice was weighed, and then the data obtained were recorded. This study lasted for 8 weeks. The acclimatization period was done at the 1^{st} week with standard feeding and aquades *ad libitum.* followed by a high-fat diet started in the second week until the eighth week, then the mice were treated based on each group at 4^{th} week to 8^{th} week.
Group K1: high fat diet *ad libitum* + CMC-Na 1% + aquadest *ad libitum*
Group K2: high fat diet *ad libitum* + CMC-Na 1% dose 500 mg / kg BW + aquadest *ad libitum*
Group K3: high fat diet *ad libitum* + CMC-Na 1% dose 750 mg / kg BW + aquadest *ad libitum*
One day before the blood taking in the heart, the rats were fastened first.
Large amount of blood taking was about 3 – 5 ml per rat are intra cordially and then the rats were sacrificed and buried properly.

Preparation of Orange Peel Powder

Washing orange pacitan with the flowing water. Separate the orange peel from the fruit and then cut into small pieces. Input the orange peel into the dryer at a temperature of forty degrees celsius for eight hours. The dried orange peel is ground until smooth into powder. Orange peel powder that has been formed can be used in the experiments of mice subjected to dyslipidemia given daily in dosage of CMC-Na 1% dose 500 mg / kg BW and CMC-Na 1% dose 750 mg / kg BW

High-Fat Feed

In this study, the dyslipidemia rats were made by giving high-fat feed to all groups of K1, K2 and K3. The composition of high-fat feed is a modification of the formula *Nutrient requirements of laboratory animals* such as fish meal, soybean meal, rice bran, *karak* (dry rice), green beans, corn, rice flour, wheat flour, *gaplek* (dry cassava), mineral, quail egg and pork oil. The high-fat diet was administered for seven weeks, starting from 2^{nd} week to 8^{th} week aiming to improve the lipid profile which included total cholesterol, LDL-C, HDL-C and TG levels.

Lipid Profile Check

The measurement of total cholesterol levels was determined by enzymatic colorimetric with CHOD-PAP (Cholesterol Oxidase - Aminophenezone) method. Serum triacylglycerol levels can be enzymatically and colorimetrically examined using the GPO-PAP (Glycerol Phosphate Oxidase-Aminophenazone) method.

Statistic Method

Data obtained from the study were analyzed using one-way Anova. Normally distributed and homogeneous data were continued using *Post-Hoc Multiple Comparison item Turkey*. If the value was $P < 0.05$, it can be concluded statistically that there was a difference among of significant data variances. Data analysis used SPSS Statistic Program Version 23.

RESULTS

The Influence of High Fat Feeding on Mouse Weight

Based on the data analysis, it can be concluded that the average weight results of K1, K2 and K3 group rats tend to increase (table 1).

Table 1 Mean ± std.deviation

Variable	Mean ± std.deviation (gram)		
	K1	K2	K3
BBM1 (gram)	108.89 ± 1.900	108.00 ± 2.915	107.11 ± 2.147
BBM2 (gram)	118.33 ± 2.000	116.22 ± 4.206	113.89 ± 1.900
BBM3 (gram)	127.56 ± 3.941	124.67 ± 5.000	121.00 ± 2.000
BBM4 (gram)	138.33 ± 4.796	132.22 ± 6.241	128.56 ± 1.236
BBM5 (gram)	145.67 ± 3.808	139.56 ± 7.350	135.89 ± 2.522
BBM6 (gram)	155.78 ± 4.764	148.67 ± 6.385	142.89 ± 4.106
BBM7 (gram)	167,78 ± 3.833	157.78 ± 5.263	150.44 ± 4.503
BBM8 (gram)	177.78 ± 4.055	167.33 ± 3.162	162.67 ± 2.500
BBM9 (gram)	185.08 ± 4.153	175.33 ± 3.000	169.67 ± 1.803

Mean ± SE (n = 9 for each group)

*Significant at P< 0.05

The increase of body weight of the rats began to occur at 2nd week, after the K1, K2, and K3 groups were given a high-fat diet (Figure 1). The results of this study showed that high-fat feeding successfully increased the weight of the rats. The pork and quail eggs are foods that contain high levels of fatty acid and cholesterol when compared to the other animal oils.

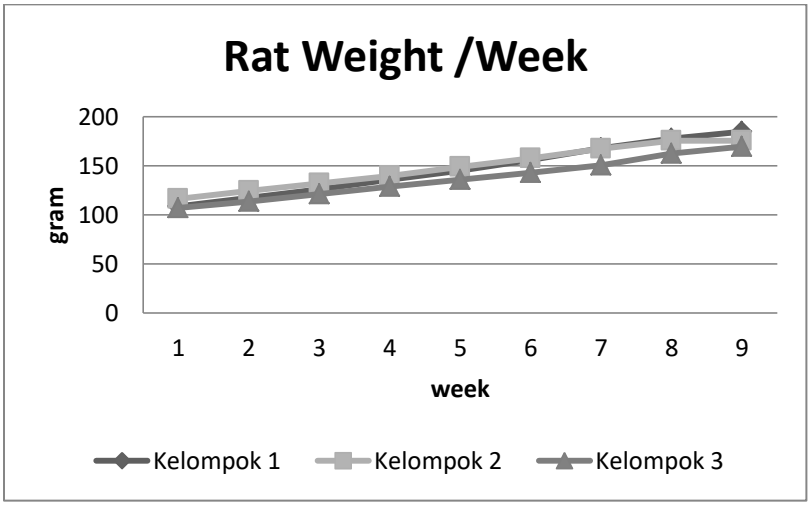

Figure 1 Rat Weight Mean/week

Effect of giving Pacitanese sweet orange peel extract on lipid profile

Lipid profile parameters tested in this study were TC, LDL-C, HDL-C and TG. The average and SD results of the rat lipid profiles including total TC, LDL-C, HDL-C and TG levels in the K1, K2, and K3 groups are summarized in table 2.

Table 2. Mean ± SD

Variable	Group		
	K1 Mean ± SD	K2 Mean ± SD	K3 Mean ± SD
TC (mg/dL)	86.33 ± 6.964	69.33 ± 9.487*	58.11 ± 10.191*
LDL-C (mg/dL)	31.56 ± 5.833	22.33 ± 2.828*	15.67 ± 2.179*
HDL-C (mg/dL)	30.78 ± 2.224	41.44 ± 5.199	46.56 ± 6.425
TG (mg/dL)	196.67 ± 14.089	182.67 ± 6.910*	149.78 ± 7.629*

Mean ± SE (n = 9 for each group)
*Significant at P< 0.05

TC concentration in K2 and K3 group treated with extract of Pacitanese sweet orange peel with dosage of CMC-Na 1% dose 500 mg / kg BW and CMC-Na 1% dose 750 mg / kg BW lower than K1 group . The decrease of TC levels significantly occurred in groups of K2 and K3. Levels of LDL-C in the K2 and K3 groups treated with extract of Pacitanese sweet orange leaf were found to be lower in LDL-C than in the K1 group. The decrease of LDL-C level significantly occurred in groups of K2 and K3. On HDL-C examination, the K2 and K3 groups were higher than in the K1 group. The levels of HDL-C in the group K2 and K3 were not significantly higher than in the K1 group.

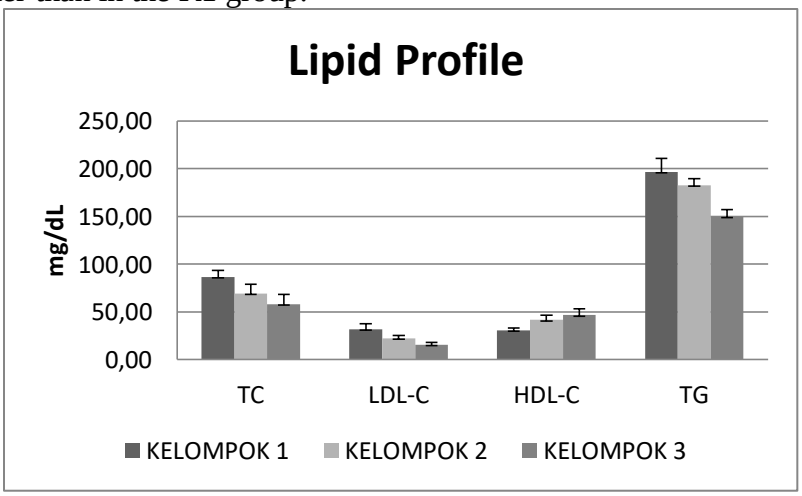

Figure 2 Lipid Profile

DISCUSSION

The increase of body weight of rats in this study is in line with the assertion that the type of food consumed can affect weight[9]. The occurrence of dyslipidemia in white rats was determined on the 15th day after high fat-induced feeding, which was characterized by increased

rat body weight and total cholesterol levels greater than 54 mg / dL with normal values of 10 – 54 mg / dL[10].

The results of this study is in line with the research conducted by Santiago found that lipid profile values given high-fat diet have a tendency of higher lipid profile values[11]. Lipid profile of K2 and K3 groups tends to be lower than K1

In this study the administration of high-fat diet began to be administered in the second week aimed at increasing the lipid profile which includes total cholesterol, LDL-C, HDL-C and TG levels. The results showed that the high-fat feeding (Table 2) successfully increased levels of total cholesterol significantly.

Lipids derived from food will undergo digestion process in the intestine into free fatty acids, triacylglycerol, phospholipids and cholesterol. In the free fatty acid intestine, triacylglycerol, phospholipids and cholesterol are processed and absorbed into the bloodstream in the form of chylomicrons. High levels of lipids in the diet will cause cholesterol absorption during digestion in the intestine to increase. Increased lipid absorption can lead to a condition of dyslipidemia that is characterized by elevated total cholesterol, LDL-C and triacylglycerol levels and decreased levels of HDL-C in the blood[2].

The decrease of LDL-C level may occur due to hesperidin content in the peel of Pacitanese sweet orange where the mechanism of action of hesperidin is estimated by inhibition of reductase HMGCoA enzyme activity[4]. The activity of reductase HMG-CoA inhibited by hesperidine causes the cholesterol synthesis of the liver to be reduced. The decrease in the amount of cholesterol in the liver stimulates the formation of more LDLR. LDLR is a receptor that functions to enter LDL-C in the blood into the liver, the more formation of LDLR can cause LDL-C in the blood to be reduced[2].

The result of literature study has been done is known that in the peel of sweet orange there is d-limonen compound which can affect to lipid profile. The d-limonene compound of an orange peel can affect PPAR. PPAR (Peroxisome proliferator-activated receptors) is a transcription factor of super family nuclear receptors. PPAR circulating with its ligand may affect lipid metabolism[5]. Activating PPAR can affect the total cholesterol levels in the blood. The mechanism of PPAR in affecting total cholesterol is by inhibiting NPC1L1 activity in the intestinal wall. NP1C1 is a transporter in charge of bringing cholesterol into the blood[6].

In orange peel there is also a water soluble fiber that is pectin. The mechanism of action pectin in lowering cholesterol is by binding to cholesterol contained in the digestive system, thus preventing it to be absorbed into the bloodstream. The higher the viscosity of pectin, it will be more effective in absorbing cholesterol. High viscosity pectin will lower cholesterol levels by increasing the excretion of bile acids and neutral sterols. Pectin which has a high viscosity will play a role in forming mycers and bile acids with low diffusion rate through the bolus to bind cholesterol to the gastrointestinal tract[12]. From the results of research conducted by Mattes, a food technology expert proves that pectin can lower cholesterol levels in the blood. By consuming at least 6 grams of pectin per day will be able to reduce blood cholesterol levels up to 13% within 2 weeks[13].

CONCLUSION

Administration of Pacitanese sweet orange peel extract with dose of CMC-Na 1% dose 500 mg / kg BW and CMC-Na 1% dose 750 mg / kg BW for six weeks on white male wistar rats can significantly decrease total cholesterol, LDL-C, and TG, however, cannot increase HDL-C levels

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