

POTENTIAL OF PECTIN IN LEMON'S AND BALI ORANGE'S PEEL AS ANTIDISLIPIDEMIA AND ANTIOXIDANT IN HYPERCHOLESTEROLEMIA RATS (*Rattus novergicus*)

Noer Kumala Indahsari¹, Aristanto¹, Rio¹

¹Departments of Biochemistry, Faculty of Medicine, University of Wijaya Kusuma, Surabaya, Indonesia

Correspondence Email: noerkumala04176@gmail.com

Abstract: *Dyslipidemia is a lipid metabolism disorder characterized by an increase or decrease in plasma lipid fractions. Lemon's and Bali Orange's Peels, which are usually discarded, can be used because they contain pectin compounds. The presence of pectin compounds contained in the Lemon and Bali oranges Peels is expected to potentially reduce the risk factors for cardiovascular disease. This research method was conducted with pure experimental, namely One Group Pre-Test-Post Test Design. With 5 groups of white rats, the first was a positive control group which was only given normal food, the second was a negative control group which was given hypercholesterolemia and the third group was the treatment group who were given Lemon orange peel extract 10%, Bali orange peels extract 10% and a mixture Lemon and Bali orange peel extract 10%. The results of this study were obtained that the administration of Lemon orange peel ethanol extract and Balinese orange peel can effectively reduce total cholesterol levels, from 181.87 mg / dL to 69.29 mg / dL, down around 61.91%, HDL levels are almost the same, levels LDL decreased from 56.76 mg / dL to 45.71 mg / dl, meaning around 19.47%, Triglyceride levels decreased from 192.50 mg / dL to 77.83 mg / dL, which was around 59.57%, levels MDA decreased from 8.19 mg / dL to 6.37 mg / dL, which is down about 22.22% in white rats (*Rattus novergicus*) made hypercholesteroalmia. Ethanol extract of Lemon and Bali orange peels containing pectin can effectively reduce total cholesterol, LDL, HDL, Tryglyceryde, and Malodialdehyd (MDA) Levels*

Keywords: Hypercholesterolamia, Pectin, Antidislipidemia, and Antioxidant

INTRODUCTION

Degenerative diseases such as hypertension, diabetes mellitus, and coronary heart disease can be the cause of death and are ranked highest. The main risk factor causing the disease is a state of high cholesterol in the blood (hypercholesterolemia) which is a sign of abnormalities in lipid metabolism (dyslipidemia). If there is a condition that causes increased cholesterol synthesis in the liver, then LDL which is rich in cholesterol also increases, LDL will bind to the scavenger receptor, an intermediate receptor for collecting cholesterol in macrophages, skin and blood vessels, this will cause accumulation of cholesterol in macrophage cells, skin and blood vessels so that it triggers atherosclerosis and coronary heart disease.¹⁻³

One alternative to overcome the dyslipidemia is to consume fibrous foods. A study showed that many fiber components contained in fruits and vegetables can reduce cholesterol levels in the blood. One component of fiber (fiber) that can dissolve is pectin.⁴

Pectin is known as an anticholesterol because it can bind bile acids which is the end result of cholesterol metabolism. The more bile acids that bind with pectin and are excreted outside the body, the more cholesterol is metabolized, so that eventually cholesterol decreases so that this potential is expected to be antidiislipidemia. In addition, pectin can also absorb excess water in the intestine, soften feces, and binds and removes toxins from the intestines. Flavonoid compounds found in lemons and Bali can also ward off free radicals so that they can be used as antioxidants.^{2,3,5}

So that researchers are interested to know the extent of the potential of pectin and flavonoids in skin extracts of Lemon Orange and Grapefruit as antidiislipidemia and antioxidants in white rats (*Rattus novergicus*) made by hypercholesterolemia through total cholesterol, LDL, HDL, triglyceride and MDA

RESEARCH METHOD

The tools used in this study were spectrophotometers, blenders, evaporators, ovens, and analytical balance. The materials used in this study were Lemon Orange Peel, Bali Orange Peel, White Rat (*Rattus novergicus*), 90% alcohol, cholesterol reagent, LDL reagent, HDL reagent, triglyceride reagent and MDA reagent.

Preparation of Lemon and Bali Oranges Peels are: 1) Lemon peel sliced into small pieces and dried 2-4 days, then blend; 2) Then weigh 1 kg macerated with 70% ethanol for 24 hours and filtered; 3) the residue is macerated again until the filtrate is clear; 4) Filtrate / maserate is collected together, concentrated with a vacuum evaporator (temperature 30-40 ° C, pressure 75mmHg). In the same way done for the extraction of Bali Orange Peel

The rats used in this study had the criteria of male, age 2-3 months, body weight 150-250 grams. The white rats were divided into five treatment groups, as follows: 1). KP groups were only given normal food and aquadest for 7 days; 2). KN, KA, KB, KC groups were given aquadest for 14 days and given a diet of 2.5g / 200 gBB on 8-21 days; 3). KA group was given 10% Lemon Orange peel ethanol extract on 22-30 days; 4). The KB group was given 10% extract of Bali orange peel on 22-30 days; 5). The KC group was given 10% 10 ethanol extract of Lemon and Bali Oranges Peels on 22-30 days.

On the 21st day blood was drawn through the lateral vein of the animal's tail trying to see its cholesterol level and on the 30th day its blood was taken through the heart to see its cholesterol, LDL, HDL, triglyceride and MDA levels. The data from this research were analyzed using SPSS 16 with a p-value of 0.05.

The study was approved by EC clearance committee of Medical Faculty Wijaya Kusuma Surabaya, East Java

RESULT AND DISCUSSION

The following is data from lipid profile research (cholesterol, LDL, HDL, triglycerides) and MDA after treatment of

Lemon and Bali oranges peels extract 10% in hypercholesterolemia white rats as seen bellow table 1.

Table 1 Cholesterol levels, LDL, HDL, Triglycerides and MDA after treatment with administration of Lemon and Bali orange peel extract to hypercholesterolemia rats

Variable	KN	KP	KA	KB	KC	p-value
Cholesterol	166.652±6.287	181.868±7.307	69.294±4.295	70.640±6.175	71.995±3.926	0,000
LDL	43.842±3.086	56.5160±2.866	45.576±1.393	45.392±5.165	46.185±5.967	0,000
HDL	32.906±2.222	32.796±1.763	31.162±2.360	30.546±1.825	31.895±3.407	0,400
Triglycerida	77.882±10.194	44.624±73.912	94.642±17.611	83.555±36.291	6.428±8.326	0,001
MDA	6.504±0.457	8.192±0.424	6.372±0.119	6.356±0.413	6.497±0.082	0,000

The table 1, line 1 shows the results of the measurement of total cholesterol levels when rats were treated with extracts of Lemon and Bali. KN is the Negative Control group which is only given normal food, while KP is the group that is given a fat diet of 2.5 g / 200 BB each for 7 days, KA is the group besides the fat diet and the treatment of Lemon peel extract 10%, KB is a group of mice that were given a diet of fat and the treatment of Bali orange peel extract 10% and KC was a group of rats that were given a fat diet of 2.5 g / 200 BB for 7 days and the treatment of a mixture of extracts of Lemon and Bali 10%.

Based on statistical analysis, the results of the determination of cholesterol levels are very significantly different with a p-value of less than 0.05 wich is 0,0000.

The Table 1 line 2 and 3 of the results of LDL and HDL levels in white rats after the treatment of ethanol extracts of Lemon and Bali oranges peels in the KA, KB and KC treatment groups. This treatment caused a significant decrease in LDL levels with a p-value of less than 0.005, that is 0.0000, even though the HDL level was not significantly different because the p-value was 0.4000.

The following are table 1 line 4 the results of the determination of triglyceride levels from white mice after being given ethanol extract 10% Lemon Orange (for KA), ethanol extract of Bali orange peel 10% (for KB) and ethanol extract extracts of Lemon and Bali orange peels 10%. KC).

Based on table 1, line 4 shows that the highest Triglyceride level results were obtained in the KC rat group which was 109.66 mg / dL, then KB was 102.20 mg / dL and the lowest was KA which was 77.83 mg / dL, whereas in KP group triglycerides levels are 192.50 mg / dl means the addition of extracts of lemon peel or grapefruit peel can effectively reduce triglyceride levels in white rats made hypercholesterolamia. This treatment caused a significant decrease in triglyceride levels with a p-value of less than 0.005, that is 0.0010.

Table 1 line 5 below is the result of determining MDA levels from white rats after being treated with 10% Lemon Orange Skin ethanol extract (for KA), 10% Bali Citrus Orange ethanol extract (for KB) and Lemon and Bali Skin Orange ethanol extract mixture 10%. (for KC).

Based on figure 5 shows that the lowest MDA level results were obtained in the KA rat group of 6.37 mg / dL, then KB and KC were almost the same, namely 6.44 and 6.51 mg / dL, whereas the initial group of KP groups The MDA is 8.19 mg / dL, which means that the addition of lemon peel extract and Bali peel can effectively reduce MDA levels in white rats that are made hypercholesterolemia. This treatment caused a significant decrease in MDA levels with a p-value of less than 0.005, that is 0.0000.

In accordance with the analysis of one-way anova, the application of lemon and Bali peel extracts showed an influence

on all treatment variables both for KN, KP and the treatment of KA, KB, KC. For the provision of high-fat diets in this research proves that the research carried out can increase LDL and triglyceride levels.

Based on the results of statistical analysis using Post Hoc shows that there is a significant difference between KN and KP. this shows that the administration of fat diets between the normal group and the hypercholesterolemia group was successful because the body's acceptance of rats towards high cholesterol foods caused an increase in blood cholesterol levels. The body's response if there is food that is high in cholesterol enters the body, the food containing cholesterol will enter the intestine that passes through the small intestine mucosa, whereas in the intestine there is also cholesterol, if excess cholesterol occurs in the body then the body is unable to absorb cholesterol again so that it increases in blood vessels.⁶

The body's response if there is food that is high in cholesterol or triglyceride enters the body, the food containing triglyceride will enter the intestine which passes through the intestinal mucosa and is absorbed in the form of free fatty acids, in the intestine fatty acids will be converted again into triglycerides, if excess triglycerides occur in the body, the body is not able to absorb triglycerides anymore so it increases in blood vessels.⁷

Based on statistics, there is a significant difference between the high-fat diet group (KP) and the treatment group of lemon and balinese peel extract both KA, KB, and KC. shows that this research has succeeded in reducing blood cholesterol, triglyceride and MDA levels.

The existence of pectin content in the lemon and Bali Oranges Peels is very influential in reducing lipid and mda profiles because the similarity of the structure of pectin with cholesterol causes the body's absorption of cholesterol, LDL, triglycerides and pectin can also be antioxidants to ward off free radicals.

Pectin are complex branched polysaccharides that are rich in galactoside residues that can be extracted from apple peels and orange peels. Recent research shows that plants containing pectin are effective against various types of cancer. Pectin is able to induce apoptosis in cancer cells without having a bad effect on normal cells, which makes it a good candidate in drug research. This means that pectin contains antioxidants and can reduce MDA levels.^{4,5,10}

There is other research that explains the natural antioxidant and anti-inflammatory properties. The product, often recognized in traditional treatment systems, represents a therapeutic modality for reducing or preventing the uncontrolled an inflammatory process which in turn has the potential to improve or prevent chronic inflammation sequelae disease. We have investigated the antioxidant and anti-inflammatory effects of honokiol (HNK) and modified citrus pectin (MCP) in vitro and check whether the combination of MCP: HNK has a synergistic effect on antioxidants and anti-inflammatory property. Although HNK and MCP induce an increase in dose-dependent antioxidant activity, the latter has consistency higher antioxidant effect. The combination of MCP: HNK (9: 1) induces a synergistic effect on antioxidant activity this combination is far more effective than individual compounds. Insert monocytes, lipopolysaccharides- (LPS-) synthesis of tumor- α (TNF- α)-induced tumor necrosis is significantly inhibited by HNK and MCP: combination of CNN in dose dependence synergistic ways and effects clearly demonstrated by the TNF-inhibitory combination.¹¹

Kalamansi has the colour of a tangerine with a very thin green or orange coloured peel. Despite its appearance and aroma, the taste of the fruit itself is quite sour, though the peel is sweet. This kind of fruit have active ingredients that are essential for health such as vitamin C, flavonoids, carotenoids, limonids, and

minerals. In vivo studies in mice fed a high-fat diet and high cholesterol resulted in decreased lipids after grapefruit. The top benefits of kalamansi juice include its ability to boost the immune system, soothe acidity in the stomach, aid in weight loss, stimulate growth and repair, bleach the skin, detoxify the body, lower cholesterol, manage diabetes and treat respiratory infections, among others. Some studies have found that this juice can help lower our cholesterol levels, which is an important step towards weight loss and avoiding metabolic syndrome. This is because flavonoids in citrus fruits contain antioxidants that have antihypertensive properties and anti- Hypercholesterolemia.

Based on statistical data analysis using the One Way Anova test, each total cholesterol, HDL, LDL, Triglyceride and MDA levels obtained p-value <0.05, indicating that there was a significant effect on the administration of ethanol extracts of Lemon orange peel and Bali orange peel can reduce total cholesterol, HDL-LDL levels, triglyceride levels, and MDA levels effectively, which means it can be an antidislipidemia and antioxidant in hypercholesterolemia rats.

In this study the provision of a high-fiber diet is one solution to reduce the risk of coronary heart disease through a decrease in blood cholesterol levels. Foods high in fiber, especially fiber that is soluble in water, are known to reduce cholesterol levels in the blood circulation. Pectin is a water-soluble fiber. Pectin can bind bile acids and inhibit its reabsorption so that cholesterol levels in the blood will eventually decrease, as well as LDL and triglyceride levels which are lipid profiles in white hypercholesterolemia rats.⁹

As an antioxidant, pectin was found to degrade levels of malondialdehyde (MDA) in hypercholesterolemia rats. This can be proven in the KA, KB and KC groups, their levels decreased significantly with p-values less than 0.05. MDA in the blood shows an indicator of free radicals that exist in the body, in the condition of

hypercholesterolemia, MDA levels increase due to cholesterol considered to be free radicals by the body, with the administration of extracts of Lemon and Bali peels containing pectin, so MDA levels are decreasing. Means the Lemon and Bali Oranges peels can function as antioxidants.¹²

Other researchers explain that secondary metabolites are usually found in plants that are useful in defense mechanisms against pathogens, radiation and antioxidant activity. This is the main concern in right because they are oxidative retarded lipid degradation and hence improve quality and nutritional value of foods such as nutritional sage score. These substances are produced through phenylthe propanoid pathway and the shikimic acid pathway. Flavonoids are a group of phenolic compounds and they can be categorized into six groups like flavone, flavanol, flavanon, flavan-3-ols, isoflavones and anthocyanidin compounds. Phenolic compounds have antitumor, antimicrobial properties and anti-inflammatory properties. Some flavonoids have high antioxidant activity compared to vitamins C, glutathione and beta carotene.¹³

CONCLUSION

Ethanol extract of lemon peel and Bali orange peel containing pectin can effectively reduce total cholesterol levels in white rats (*Rattus novergicus*) made hypercholesterolemia. Based on the One Way Anova test and Post Hoc the p-value <0.05 shows that there is a significant influence on the ethanol extract of Lemon and Bali oranges peels as antidislipidemia and antioxidants in hypercholesterolemia white rats.

Based on our research, it is recommended that research on other herbal extracts have the potential to reduce total cholesterol, HDL-LDL, triglycerides and MDA

REFERENCES

1. Samuel, *et al.*, 2019, Cholesterol and Atherosclerotic Cardiovascular Disease: A Lifelong Problem, Journal of the American Heart Association; 8:e012924
2. Wong A.P, *et al.*, 2016, Atherosclerosis and the Cholesterol Theory: Reappraisal, World Journal of Cardiovascular Diseases, 6, 391-409
3. Leticia F, *et al.*, 2017, Normal LDL-Cholesterol Level Are Associated with Subclinical Atherosclerosis in the Absence of Risk Factors, Journal of the American College of Cardiology, Vol.70, No 24, ISSN 0735-1097
4. Minzanova S.T., *et al.*, 2018, Biological Activity and Pharmacological Application of Pectic Polysaccharides: A Review, Journal Polymer, 10, 1407
5. Vanitha T and Khan M., 2019, Role of Pectin in Food Processing and Food Packaging, book.department@interhopen.com,
9. Efry T and Evi, 2019, Effect of Pectin on Reducing The Risk Of Coronary Heart Disease, Majority Vol 8, No 1, p 162-167
10. Salehi F., *et al.*, 2018, Oxidative DNA damage Induced by ROS-modulating agents with the ability to target DNA; A Comparison of the biological characteristic of citrus pectin and apple pectin, Scientific Report 2018-8:13902 | DOI:10.1038/s41598-018-32308-2
11. Cheppail R, *et al.*, 2017, Synergic Antioxidant and Antiinflammatory Effect between Modified Citrus Pectin Open Acces peer-review chapter, DOI:10.5772/intechopen.83677
6. Alamir, M.A, *et al.*, 2017, The Correlation of Dyslipidemia with the extent of Coronary Artery Disease in the Multiethnic Study of Artherosclerosis, Journal of Lipids, Volume 2018, Article ID 5607349, 9 pages, <https://doi.org/10.1155/2018/5607349>
7. Vicente A.B., *et al.*, 2018, Familial, Hypercholesterolemia: The Most Frequent Cholesterol Metabolism Disorder Caused Disease, International Journal of Molecular Sciences, 19, 3426 doi:10.3390/ijms19113426
8. Shaojie Zhao, *et al.*, 2018, Citrus oil Emulsions Stabillized by Citrus Pectin: The Influence mechanism of Citrus Variety and Acid treatment, Journal of Agricultural and Food Chemistry, p 1-41 and Honokiol, Hindawi, Evidence-Based Complementary and Alternative Medicine, Vol.2017, Article ID 8379843, 9 pages
12. Yadav A, *et al.*, 2016, Antioxidants and its functions in human body- A Review, Research in Environment and Life Sciences, 9(11) 1328-1331
13. Ravimannan N, and Nisansala A, 2017 Study on antioxidant activity in fruits and vegetables- A Review, International Journal of Advanced Research in Biological Sciences, 4(3):93-101