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Effect Oral Administration of Betel Water (*Peperomia pellucida*) Ethanol Extract on Lipid Profile and F2 Isoprostane Levels in Dyslipidemic Male Rats (*Rattus norvegicus*)

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Abstract: ***Background: Dyslipidemia is the major risk factor for atherosclerosis. Dyslipidemia followed by increased level of lipid peroxidation as a marker of oxidative stress, measured by F2-Isoprostane level. Betel water (Peperomia pellucida) contains flavonoid and other antioxidant. This study aims to prove effect oral administration of betel water ethanol extract on lipid profile and F2-Isoprostane level in dyslipidemic male rats. Methods: Experimental randomized pretest posttest control group design was used in this study. Samples were 24 male white rats aged 2.5-3 months, weighing 180-220, which were given a high fat diet to achieve dyslipidemic state (total cholesterol levels 200mg/dL). Samples then divided into two groups. Control group was given simvastatin 0.36 mg/day + distilled water as placebo, whereas treatment group was given simvastatin 0.36 mg/day + betel water ethanol extract at a dose of 60 mg/day for 42 days. Lipid profile and F2-isoprostanes level were measured using blood serum before and after 42 days of treatment. Results: The results showed that there was a significant difference on the mean reduction value of total cholesterol, where in control group the result was 21.68±7.12 mg/dL, while in treatment group it was 8.46±5.28mg/dL (p<0.001), simvastatin alone gives better result. There was a significant difference on the mean reduction value of triglycerides, where in control group the result was 27.55±7.29 mg/dL, while in treatment group it was 32.07±4.79mg/dL (p=0.021), simvastatin+betel water ethanol extract give better result. There was a significant difference on the mean reduction value of LDL, where in control group the result was 20.90±8.78 mg/dL, while in treatment group it was 13.03±5.61mg/dL (p=0.016), simvastatin alone give better result. There was no significant difference on the mean increasement value of HDL, where in control group the result was 4.73 ± 3.60 mg/dL, while in treatment group it was 6.69 ± 1.44mg/dL (p = 0.094), simvastatin+betel water ethanol extract give better result, but not statistically significant. There was a significant difference on the mean reduction value of F2-Isoprostane, where in control group the results was 3.05±1.52 ng/dL, while in treatment group it was 4.45±1.43 ng/dL (p=0.030), simvastatin+betel water ethanol extract give better result. Conclusion: As conclusion, combination of betel water ethanol extract and simvastatin decreased total cholesterol and LDL in dyslipidemic male rats not as potent as simvastatin alone, but decreased triglycerides and F2-Isoprostane level better than simvastatin alone. Meanwhile, HDL in both groups were not increase significantly.***

Keywords: Betel Water Ethanol Extract, Dyslipidemia, F2-Isoprostane

1. Introduction

Dyslipidemia is a major risk factor for atherosclerosis formation, furthermore it will lead to coronary heart disease. It is characterized by overproduction of total cholesterol, LDL cholesterol, triglyceride and low in HDL cholesterol, which cause decreased level of antioxidant enzyme and lead to oxidative stress, marked with increased of lipid peroxidation.[1] Oxidative stress can be seen through F2-isoprostanes as a biomarker of lipid peroxidation. F2-isoprostanes are a unique series of prostaglandin-like compounds formed in vivo via a non-enzymatic mechanism involving the free radical-initiated peroxidation of arachidonic acid.[2] High levels of F2-isoprostanes are found in many human diseases such as coronary heart disease, obesity, cancer, and even genetic disorders.[20]

The ongoing demographic transition, combined with epidemiological and nutritional transitions, is contributing to the continued shift of the cardiovascular diseases (CVDs) burden from developed to developing countries. In Indonesia, CVDs are the leading cause of both morbidity and mortality, responsible for a third of all deaths in Indonesia.[3]

Statin is the most commonly prescribed medication for dyslipidemia. Statins inhibit HMG-CoA, which is a rate limiting step in cholesterol biosynthesis. Statin therapy has been shown to be effective in lowering low density lipoprotein cholesterol (LDL-C) levels 20-50%, as well as lowering triglyceride levels 10-20% and causing a possible rise in serum high density lipoprotein cholesterol (HDL-C) levels (5-10%).[19] Besides improving lipid profile, it can also cause some serious side effects including rhabdomyolisis, hepatotoxicity, and renal toxicity. Rhabdomyolysis is the most severe musculoskeletal form observed, with a rise in creatine kinase greater than 10 times the upper limit of normal with associated features including myoglobinuria, renal impairment and serum electrolyte abnormalities.Statins have multiple drug interactions, primarily those which interact with the cytochrome p450 enzymes in the liver.[4]

From a study conducted by Rasmussen et al (2016) showed that simvastatin did not influence markers of oxidative stress that relate to intracellular oxidative stress (DNA and RNA oxidation), lipid peroxidation or plasma concentrations of antioxidant vitamins. [5]

Considering the various side effects caused by statin, also it has no effect on oxidative stress, we should consider additional therapy that can lowering lipid profile and act as an antioxidant to improve oxidative stress.[6]

Betel water is known rich in antioxidants and also play a role in regulating lipid metabolism. Phytochemical screening for ethanol extract of *Peperomia pellucida* was conducted at analytic laboratory Udayana University. The result showed that extract contained flavonoid 5.502, 50 mg/100g, tanin 451, 56 mg/100g, and saponin was also found positive qualitatively.

Flavonoids in betel water work as HMG-CoA reductase inhibitor, this will cause reduction in cholesterol synthesis. HMG-CoA reductase inhibitors work by inhibiting the synthesis of cholesterol in the liver. Flavonoids can also prevent injury caused by free radicals. It stabilise the reactive oxygen species by reacting with the reactive compound of the radical. Flavonoids may also have preventive action against atherosclerosis.[7]

Saponin in betel water extract can bind to cholesterol and reduce cholesterol absorption, resulting in a decrease of plasma and liver cholesterol accumulation. In addition, saponin can reduce blood cholesterol and LDL level by increasing bile acid synthesis. Saponin prevent the absorption of bile acids, as a result, bile acids will be excreted through feces, and as a compensation for the loss of bile acids, blood cholesterol will be changed by the liver to form bile acids, so that cholesterol levels in the blood will decrease.[8]

Meanwhile, Tannin in betel water extract have the ability to increase the excretion of bile acids, so they can help lower cholesterol levels. The antihyperlipidemic activity of tannin is through the mechanism of inhibiting cholesterol biosynthesis and reducing cholesterol absorption from the food consumed.It also capable to precipitate protein and amino acids derived from food, so that it can inhibit the absorption of lipid.[9]

Therefore, this research was conducted to prove the effects of adding betel water ethanol extract orally in improving lipid profile (total cholesterol, LDL, HDL, and triglyceride) and decreasing F2-isoprostane level in blood of male Wistar rats with dyslipidemia that received simvastatin.

2. Methods and Material

Experimental Design

This study was a true experimental randomized pretest posttest control group design. Samples were 24 male Wistar rats aged 2, 5-3 months old, weighing 180-220 grams which were given a high cholesterol diet for 28 days to achieve dyslipidemic state (total cholesterol level 200mg/dL). Samples were divided into two groups. Control group which was given simvastatin 0.36 mg/day + 1 ml distilled water as placebo. Treatment group which was given simvastatin 0.36 mg/day + betel water ethanol extract at a dose of 60 mg/day, both are given once a day using intragastric force feeding for 42 days. Lipid profile and F2-isoprostanes level were measured using blood serum before and after 42 days of treatment.

Extract Preparation

A total of 1000 grams of betel water leaves were selected, washed, cut into small pieces, then air dried. Dried simplicia were mashed using blender then shifted. Extraction by maceration method using 96% ethanol solvent, powder: solvent ratio = 1:9. Maceration process will be done in three days.

The filtrate and dregs were separated using Whatman filtration paper, liquid extract was obtained. Evaporation process at temperature of 40-50° celsius using a rotary evaporator. Liquid will begin to separate with extract. Evaporation of the extract on a water bath with a temperature of <70° celsius. Thick liquid extract was ready to be used.[10] The dose of betel water ethanol extract (*Peperomia pellucida*) in this study was based on previous research by Mazroatul et al, 300 mg/1000 grams body weight.[11]

Lipid Profile and F2-Isoprostanes Test

Total cholesterol, LDL, and HDL cholesterol levels were measured using enzymatic colorimetric quantitative assay method. Triglyceride level was measured using Glycerol 3 phosphate oxidase peroxidase aminoantipyrine phenol (GPO-PAP) method. Enzyme linked immunosorbent assay (ELISA) method was used to measure F2 isoprostane level.

Statistical Analysis

SPSS was used to perform statistical analysis. All data were expressed as mean ± standard deviation. Paired T test was used to analyse the effect of intervention. Independent T test was used to compare lipid profile and F2 isoprostane level between groups. Independent T test was also used to compare the reduction or increasement level between groups. P <0.05 was considered as statistically significant.

3. Results

Comparison of Total Cholesterol Level in Both Groups

The mean of total cholesterol level in control group before intervention was 215, 76±4, 18 mg/dL and in treatment group was 215, 02±3, 38mg/dL as seen on table 1. Total cholesterol level in control group after intervention was 194, 08±3, 81 mg/dL and 206, 56±3, 96mg/dL in treatment group. There was a significant reduction in total cholesterol level, either in control group (p<0, 001) or in treatment group (p=0, 044) that showed by paired T test for each group. Independent T test showed no significant difference of total cholesterol before intervention (p=0, 636), and a significant difference after intervention (p<0, 001). There was a significant difference (p<0, 001) in the reduction level (delta) between two groups, but the reduction level was bigger in control group. It shows that simvastatin alone works better in reducing total cholesterol.

Comparison of Triglyceride Level in Both Groups

The mean of triglyceride level in control group before intervention was 165, 71±10, 10 mg/dL and in treatment group was 165, 57±3, 53 mg/dL as seen on table 1. Triglyceride level in control group after intervention was

138, 16±6, 42mg/dL and 133, 50±3, 32mg/dL in treatment group. Paired T test was conducted for each group and showed that there was a significant reduction in triglyceride level, both in control group (p<0, 001) and treatment group (p<0, 001). There was no significant difference of triglyceride before intervention (p=0, 966), and a significant difference after intervention (p=0, 036) that showed by independent T test. The reduction level (delta) between two groups showed a significant difference (p=0, 021). The reduction level was bigger in treatment group. It shows that simvastatin+betel water ethanol extract work better in reducing triglyceride.

Comparison of LDL Cholesterol Level in Both Groups

The mean of LDL cholesterol level in control group before intervention was 142, 06±5, 04 mg/dL and in treatment group was 141, 44±3, 79mg/dL as seen on table 1. LDL cholesterol level in control group after intervention was 121, 16±5, 06mg/dL and 128, 41±3, 25mg/dL in treatment group. There was a significant reduction showed by paired T test for LDL cholesterol level, both in control group (p<0, 001) and treatment group (p<0, 001). Independent T test showed no significant difference of LDL cholesterol before intervention (p=0, 737), and a significant difference after intervention (p<0, 001). There was a significant difference (p=0, 016) in the reduction level (delta) between two groups. The reduction level was bigger in control group. It shows that simvastatin alone works better in reducing LDL.

The mean of HDL cholesterol level in control group before intervention was 40, 56±1, 63 mg/dL and in treatment group was 40, 46±1, 25mg/dL as seen on table 1. HDL cholesterol level in control group after intervention was 45, 29±2, 54mg/dL and 47, 15±1, 40mg/dL in treatment group. Paired T test were conducted for each group and showed a significant increasement of HDL cholesterol level, both in control group (p=0, 001) and treatment group (p<0, 001). Independent T test showed no significant difference of HDL cholesterol before intervention (p=0, 867), and a significant difference after intervention (p=0, 037). There was no significant difference (p=0, 094) in the increasement level (delta) between two groups. It shows that both groups cannot increase HDL significantly.

Comparison of F2-Isoprostanes Level in Both Groups

The mean of F2-Isoprostanes level in control group before intervention was 11, 89±1, 21 ng/dL and in treatment group was 11, 11±0, 82ng/dL as seen on table 1. F2-Isoprostanes level in control group after intervention was 8, 83±1, 25ng/dL and 6, 66±1, 11ng/dL in treatment group. There was a significant reduction in F2-Isoprostanes level showed by paired T test, both in control group (p<0, 001) and treatment group (p<0, 001). Independent T test was conducted and showed no significant difference of F2-Isoprostanes before intervention (p=0, 080), and a significant difference after intervention (p<0, 001). There was a significant difference (p=0, 030) in the reduction level (delta) between two groups. The reduction level was bigger in treatment group. It shows that simvastatin+betel water ethanol extract work better in reducing F2-Isoprostanes.

Comparison of HDL Cholesterol Level in Both Groups

Table 1. Lipid Profile and F2-Isoprostanes Level in Both Groups Before and After Intervention

Variable	Group	Pre Test Mean±SD	Post Test Mean±SD	Delta Mean±SD	P**
Total Cholesterol (mg/dL)	Control	215,76±4,18	194,08±3,81	21,68±7,12	<0,001
	Treatment	215,02±3,38	206,56±3,96	8,46±5,28	0,044
	P*	0,636	<0,001	<0,001	
Triglyceride (mg/dL)	Control	165,71±10,10	138,16±6,42	27,55±7,29	<0,001
	Treatment	165,57±3,53	133,50±3,32	32,07±4,79	<0,001
	P*	0,966	0,036	0,021	
LDL (mg/dL)	Control	142,06±5,04	121,16±5,06	20,90±8,78	<0,001
	Treatment	141,44±3,79	128,41±3,25	13,03±5,61	<0,001
	P*	0,737	<0,001	0,016	
HDL (mg/dL)	Control	40,56±1,63	45,29±2,54	4,73±3,60	0,001
	Treatment	40,46±1,25	47,15±1,40	6,69±1,44	<0,001
	P*	0,867	0,037	0,094	
F2-Isoprostane (ng/dL)	Control	11,89±1,21	8,83±1,25	3,05±1,52	<0,001
	Treatment	11,11±0,82	6,66±1,11	4,45±1,43	<0,001
	P*	0,080	<0,001	0,030	

*Independent T test; **Paired T test; SD (Standard Deviation)

4. Discussion

Effects of Betel Water Ethanol Extract on Lipid Profile and F2-Isoprostane Level

Flavonoids in betel water ethanol extract work by inhibiting the formation of cholesterol and act as an antioxidant. It works by inhibiting the absorption of bile acids and cholesterol in the small intestine as well as inhibiting the activity of HMG-CoA reductase enzyme which plays a role in cholesterol formation.[12]. Meanwhile, the antioxidant effect of flavonoids is achieved by three mechanisms: (1) by eliminating reactive oxygen species, (2) by preventing the production of reactive oxygen species, secondary to the interaction of flavonoids with enzymes that control the production of free radicals, or (3) by increasing the protection of antioxidant systems. Under oxidative stress, flavonoids additionally achieve lipid protection from theperoxidation process. Their effect on lipid oxidation is due to the interaction of flavonoids with nonpolar compounds in the hydrophobic portion of the membrane. In the hydrophobic region it blocks the access of oxidants, thus protecting the membrane structure. [13]

Betel water ethanol extract also contain tannins which have a pronounced anti-hypercholesterolemic effect by enhancing reverse cholesterol transport and also by reducing intestinal cholesterol absorption and increasing bile acid excretion.[14] The antioxidant property of tannins prevents the cholesterol oxidation, which is a precursor of plaque formation in vessels, thus prevents the body from cardiovascular diseases.[15]

Saponins in betel water ethanol extract possess a wide range of biological activities, one of them being the ability to inhibit cholesterol absorption and to decrease serum and liver cholesterol. The latter effect has important implications for human health, as it could help the battle against hypercholesterolemia and the cardiovascular problems, associated with it.[18] Saponins have been shown to influence nutrient digestion and absorption in a variety of ways.It has been determined that saponins form insoluble complexes with cholesterol and inhibit the availability of bile salts. These interactions may exert effects on micelle formation and thus, impair the absorption off at and fat-soluble compounds.[16]

Flavonoid and tannin were classified into phenolic and known for its antioxidant effect. Flavonoid inhibit myeloperoxidase (MPO) activity which is able to break protein, lipid, nucleic acid, and even LDL and HDL oxidation in artery. It can be a protective factor for cardiovascular disease.[17]

The result from this study showed that combination of simvastatin and ethanol extract of betel water can reduce triglyceride and F2-Isoprostanes level better than simvastatin alone. Whereas, simvastatin alone gives better result in reducing total cholesterol and LDL. Both groups cannot increase HDL significantly. This could be due to the ineffective dose of betel water ethanol extract or the lack of duration of the study.

5. Conclusion

As conclusion, combination of betel water ethanol extract and simvastatin decreased total cholesterol and LDL cholesterol in dyslipidemic male rats not as potent as simvastatin alone, but this combination decreased triglycerides and F2-Isoprostane level better than simvastatin alone. Meanwhile, HDL in both groups were not increase significantly. Hopefully, the further study with longer period of time can be conducted to know the effective dose of betel water ethanol extract to improve lipid profile and decrease F2-isoprostanes level significantly.

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