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Comparison between Oral Ethanol Vegetable Fern Leaves (*Diplazium esculentum*) Extract and Simvastatin to Improve Lipid Profile and Decrease F2-isoprostane Level in Wistar Male Rats (*Rattus norvegicus*) With Dyslipidemia

Marlene Irawan[1], Anak Agung Gde Budhiarta[2], Ida Sri Iswari[3]

[1, 2, 3]Post Graduate Program in Biomedical Science, Concentration in Anti-Aging Medicine, Medical Faculty Udayana University, Bali, Indonesia
Email: *irawan.marlene[at]gmail.com*

Abstract: ***Background*: In dyslipidemia, lipid profile and lipid peroxidation increases. Lipid peroxidation was measured by F2-isoprostane level. Vegetable fern contains flavonoid and other constituent. This research aims to prove administration of oral ethanol vegetable fern leaves extract improves lipid profile and decrease F2-isoprostane level in male Wistar rats compared to simvastatin. *Methods*: This study used experimental pre test – post test control group design, 14 wistar rats, males, 2,5-3 months old, weighing 140-200 grams, divided into 2 groups. Simvastatin group was given simvastatin 0,36 mg, whereas vegetable fern groupwas given oral ethanol vegetable fern leaves extract 100 mg, by intragastric force-feeding, once a day, for 21 days. Blood serum has been used to measure lipid profile and F2-isoprostane level. *Results*: There was a decrease in total cholesterol level after intervention, not only in simvastatin group (221.45 mg/dL vs 191.61 mg/dL; p=0.001) but also vegetable fern group (225.24 mg/dL vs 196.54 mg/dL; p=0.002). LDLalso decreased in both groups (135.67 mg/dL vs 86.69 mg/dL; p<0.001 and 140.56 mg/dL vs 98.86 mg/dL; p<0.001). HDL increased in both groups (52.69 mg/dL vs 77.20 mg/dL; p<0.001 and 51.73 mg/dL vs 68.95 mg/dL; p<0.001). Triglyceride level decreased in both groups (165.40 mg/dL vs 138.58 mg/dL; p=0.002and164.78 mg/dL vs 143.66 mg/dL; p=0.003). F2-isoprostane level decreased in simvastatin group not significantly (11.87 ng/L vs 11.27 ng/L; p=0.420), but decreased significantly in vegetable fern group (12.18 ng/L vs 4.05 ng/L; p<0.001). Comparison between two groups after intervention showed no significant differences regarding delta total cholesterol, delta LDL, and delta triglyceride (p>0.05). *Conclusion*: As conclusion, administration of oral ethanol vegetable fern leaves extract improved lipid profile level in wistar male rats with dyslipidemianot as good as simvastatin but decreased F2-isoprostane better than simvastatin.***

Keywords: Dyslipidemia, Vegetable fern leaves, Lipid profile, F2-isoprostane

1. Introduction

Dyslipidemia is a metabolic disorder characterized by high level of total cholesterol, high LDL cholesterol level, low HDL cholesterol level, and high triglyceride level. Dyslipidemia is one of the risk factors for atherosclerosis, leading to cardiovascular disease. Nowadays, cardiovascular disease has become a leading cause of death worldwide.[1,2]

The prevalence of dyslipidemia increases simultaneously with aging and it also accelerates aging. Therefore, prevention and better control are needed to deal with dyslipidemia problem.[3]

In dyslipidemia, not only lipid profile, but also lipid peroxidation increase.[4] Lipid peroxidation is a marker of oxidative stress. For decades, screening and treatment for atherosclerosis has focused in improving cholesterol level. Whereas, control of lipid peroxidation process is also needed to prevent atherosclerosis.[5,6] High F2-isoprostane level was founded as indicator of lipid peroxidation in patients with high LDL level.[6]

Statin still exists as the first line of dyslipidemia therapy. Besides having the ability to improve lipid profile, statin also has potential as an antioxidant. However, patient compliance is not satisfying and side effects like myopathy and rhabdomyolysis are exist.[1,7]

Vegetable fern (*Diplazium esculentum*) is well-known as medicinal plant, easily found in Indonesia, and has antioxidant effect.[8] Phytochemical screening for ethanol extracts of *Diplazium esculentum*was conducted at analytic laboratory Udayana University. The result showed that extracts contained of vitamin C1056,55 mg/100 g, phenol 1274,1 mg/100g, flavonoid 6496,21 mg/100g, and tannin 3305,14 mg/100g. Saponin and terpenoid were also found positive qualitatively.

Polyphenol component like phenol and flavonoid has antioxidant effect and potent inhibitor of LDL oxidation.[9]Flavonoid, tannin, and vitamin C also have ability to improve lipid profile.[10,11]

Therefore, this research was conducted to prove the effects of vegetable fern leaves in improving lipid profile (total cholesterol, LDL cholesterol, high density lipoprotein cholesterol, and triglyceride) and decreasing F2-isoprostane level in blood of male Wistar rats with dyslipidemia compared to simvastatin.

2. Methods and Material

2.1 Experimental Design

This study was a true experimental randomized pretest-posttest control group design. Subjects were 14 male Wistar rats (*Rattus norvegicus*), aged 2,5-3 months old, body weight 140-200 grams, dyslipidemia with total cholesterol level ≥ 200 mg/dL, divided into two groups. Simvastatin group was given standard food and simvastatin 0,36 mgequals to 20 mg dosage for human diluted in 2 cc aquadest, whereas vegetable fern group was given standard food and oral ethanol vegetable fern leaves extract 100 mg diluted in 2 cc aquadest, given by intragastric force-feeding, once a day, for 21 days. Before and after 21 days of treatment, lipid profile, and F2-isoprostane level in blood was measured.

2.2 Plant Collection and Extract Preparation

Leaves of *Diplazium esculentum* were collected in Mambang village, East Selemadeg, Bali, Indonesia. Identification was done in Eka Karya Conservation Botanic Garden Bali. Collected leaves was sorted and rinsed, cut into 2 cm x 2 cm. All pieces were dried and aired. After that, leaves were mashed using blender and sifted using mesh sieve. Extraction was conducted with maceration methods using ethanol 96% for 3 days. Filtrate and dregs were separated. Liquid extract was evaporated in 40-50^{o}C using evaporator rotatory. Further evaporation on water heater in 70^{o}C was needed. Final result is thick extract.[12]

2.3 Lipid Profile and F2-isoprostane Test

Blood serum was used for all tests. Total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride level were measured using enzymatic-colorimetric quantitative assay. Triglyceride level was measured using Glycerol-3-phosphateoxidase- peroxidase aminoantipyrinephenol (GPO-PAP) method. Meanwhile, F2-isoprostane level was measured using enzyme-linked immunosorbent assay (ELISA).

2.4 Statistical Analysis

Statistical analysis was performed with SPSS version 25. Experimental result data were expressed as mean ± standard deviation. The effects of intervention were analysed using paired T test. Comparison of lipid profile and F2-isoprostane level between groups were analysed using independent T test. The delta between groups was also compared using independent T test. P value less than 0.05 was considered as statistically significant.

3. Results

Comparison of Total Cholesterol Level in Both Groups
The effect of intervention to the mean of total cholesterol level in each group was analysed using paired T test. Whereas, the comparison of cholesterol total level between two groups was analysed using independent T test. The decrease (delta) of total cholesterol was also analysed using independent T test. The results were shown in Table 1.

Table 1 showed that the mean of total cholesterol level in simvastatin group before intervention was 221.45±10.52 mg/dL and in vegetable fern groupwas225.24±6.78 mg/dL After intervention, total cholesterol level in simvastatin group was 191.61±5.67 mg/dL and 196.54±10.66 mg/dL in vegetable fern group. Paired T test for each group showed a significant reduction in total cholesterol level, either insimvastatin group (p=0.001) or in vegetable fern group (p=0.001). Independent T test showed no significant differences of total cholesterol before intervention (p=0.532), after intervention (p=0.302), and also the reduction level (delta) between two groups (p=0.880).

Table 1. Lipid profile and F2-isoprostane level before and after intervention in both groups

Variable	Group	Before intervention Mean±SD	After intevention Mean±SD	Delta Mean ±SD	P**
Total cholesterol (mg/dL)	Simvastatin	221.45±10.52	191.61±5.67	29.84±12.50	0.001
	Vegetable fern	225.24±11.53	196.54±10.66	28.71±14.85	0.002
	P*	0.532	0.302	0.880	
LDL cholesterol (mg/dL)	Simvastatin	135.67±7.44	86.69±8.09	49.98±14.60	<0.001
	Vegetable fern	140.56±11.74	98.86±10.49	41.70±12.53	<0.001
	P*	0.370	0.032	0.336	
HDL cholesterol (mg/dL)	Simvastatin	52.69±5.04	77.20±6.32	24.51±5.75	<0.001
	Vegetable fern	51.73±4.14	68.95±7.84	17.22±5.71	<0.001
	P*	0.702	0.051	0.035	
Triglyceride (mg/dL)	Simvastatin	165.40±12.73	138.58±7.28	26.82±13.69	0.002
	Vegetable fern	164.78±13.38	143.66±6.37	21.12±11.39	0.003
	P*	0.930	0.191	0.674	
F2-isoprostane (ng/L)	Simvastatin	11.87±1.09	11.27±1.94	0.61±1.85	0.420
	Vegetable fern	12.18±0.96	4.05±0.82	8.13±1.42	<0.001
	P*	0.587	<0.001	<0.001	

* Independent T test; ** Paired T test; SD (standard deviation)

Comparison of LDL Cholesterol Level in Both Groups
Table 1 showed that the mean of LDL cholesterol level in simvastatin group before intervention was 135.67±7.44 mg/dL and vegetable fern group was140.56±11.74 mg/dL. After intervention, LDL cholesterol level in simvastatin groupdecreased into 86.69±8.09mg/dL and 98.86±10.49 mg/dL in vegetable fern group. Paired T test for each group showed a significant reduction in LDLcholesterol level, either in simvastatin group ($p<0.001$) or in vegetable fern group ($p<0.001$). Independent T test showed no significant differences of LDL cholesterollevel before intervention ($p=0.532$). However,there was differences between two groups ($p=0.032$) after intervention. The reduction (delta) of LDL cholesterol level before and after intervention did not show significant difference in both groups ($p=0.336$).

Comparison of HDL Cholesterol Level in Both Groups
As shown in Table 1, the mean of HDL cholesterol level in simvastatin group before intervention was 52.69±5.04 mg/dL and 51.73±4.14 mg/dL in vegetable fern group. After intervention, HDL cholesterol level was 77.20±6.32mg/dL in simvastatin group and 68.95±7.84mg/dL in vegetable fern group. Paired T test for each group showed a significant increasement in HDL cholesterol level, either in simvastatin group ($p<0.001$) or in vegetable fern group($p<0.001$). Independent T test showed no significant differences of HDL cholesterol before intervention ($p=0.702$) and after intervention ($p=0.051$). However, there was significant difference for the increase (delta) after intervention in both groups ($p=0.035$).

Comparison of Triglyceride Level in Both Groups
Table 1showed the mean of triglyceride level in simvastatin group before intervention was 165.40±12.73 mg/dL and 164.78±13.38 mg/dL in vegetable fern group. After intervention, triglyceride level was 138.58±7.28mg/dL in simvastatin group and 143.66±6.37mg/dL in vegetable fern group. Paired T test for each group showed a significant reduction in triglyceride level, either in simvastatin group ($p=0.002$) or in vegetable fern group($p=0.003$). Independent T test showed no significant differences of triglyceride level before intervention ($p=0.930$), afterintervention ($p=0.191$), and the decrease level (delta) between two groups ($p=0.674$).

Comparison of F2-isoprostane Level in Both Groups
Table 1 showed the mean of F2-isoprostane level in simvastatin group before intervention was 11.87±1.09 ng/L and 12.18±0.96 ng/L in vegetable fern group. After intervention, F2-isoprostane level was 11.27±1.94 ng/L in simvastatin group and 4.05±0.82 ng/L in vegetable fern group. Paired T test for each group showed no significant reduction in simvastatin group ($p=0.587$) but there was significant reduction in vegetable fern group ($p<0.001$). Independent T test showed no significant differences of F2-isoprostane level before intervention ($p=0.587$), but there was differences of F2-isoprostane level ($p<0.001$) and the reduction (delta) of F2-isoprostane level after intervention in both groups ($p<0.001$).

4. Discussion

Effects of Vegetable Fern Leaves Ethanol Extract on Lipid Profile
The results showed that generally vegetable fern leaves extract can improve lipid profile. Delta of total cholesterol level, LDL cholesterol level, and triglyceride level after intervention in both groups did not have significant differences. So, the effect of vegetable fern leaves extract administration was as good as simvastatin in lowering total cholesterol level, LDL cholesterol level, and triglyceride level. Meanwhile, vegetable fern leaves increased HDL cholesterol level significantly, but not as potent as simvastatin.

Vegetable fern can easily be found in Indonesia and well known as medicinal plant. Polyphenol as a secondary metaboliteswas found in vegetable fern as phenol and flavonoid. Polyphenol has protective effect for cardiovascular disease through LDL oxidation inhibition.[9]Flavonoid can improve lipid profile by decreasing HMG-CoA reductase activity, increasing lipoprotein plasma (LPL) activity, and increasing lecithin cholesterol acyltransferase (LCAT) activity. LPL decreases LDL cholesterol level and also increases triglyceride elimination.[10,13]LCAT facilitates cholesterol esterification and cholesterol movement from HDL surface into its core.

Vitamin C can facilitate cholesterol conversion into bile acid by modifying 7α-hydroxylase enzyme activity. Another meta-analysis also reported that vitamin C supplementation can lower LDL cholesterol and triglyceride level significantly.[11]

Another bioactive component like tannin can also decrease cholesterol level by increasing cholesterol metabolism into bile acid and improve its secretion through feces. Tannin also inhibit LDL oxidation.[14]

The result of this study was in line with research conducted by Junejo et al in 2017. The study showed that administration of oral ethanol vegetable fern leaves extract can improve lipid profile in diabetes induced wistar rats.[15]

Effects of Ethanol Vegetable Fern Leaves Extract on F2-isoprostane Level
This study showed that the reduction of F2-isoprostane level was not significant in simvastatin group. Whereas, there was significant reduction in vegetable fern leaves extract group. It proved that vegetable fern leaves extract was better in lowering F2-isoprostane level compared to simvastatin.

Isoprostane is the result of nonenzymatic peroxidation metabolite form arachidonate acid. The most common form is F2-isoprostane, which widely used as oxidative stress marker.[16]

Several clinical studies stated that there was antioxidant effect in statin. Potential mechanisms were increasement of nitrite oxide synthase. Another study also showed that statin decrease activity of NADPH oxidase. Study by Pignatelli et al showed lowering F2-isoprostane level in urine after atorvastatin administration.[17] However, another study

showed different result. Study by Scheffer et al stated that there was no significant reduction of F2-isoprostane level after simvastatin administration and antiatherogenic effect was not the focus of statin therapy.[18] This result was in line with our result.

On the other hand, vegetable fern leaves contained of flavonoid, phenol, tannin, and vitamin C can decrease F2-isoprostane level. 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.[10]

Vitamin C prevents damage from oxidative stress by downregulating reactive oxygen species (ROS)/reactive nitrogen species (RNS) production and also regenerating vitamin E. Vitamin C inhibit lipid peroxidation by inhibiting lipoxygenase, binding into free radical and ROS through reaction cycle process and metal ion chelation.[8]

Effect of Ethanol Vegetable Fern Leaves Extract in Anti-Aging Medicine

In Anti-Aging Medicine, antioxidant has an important role to supress free radical effect that contributed to aging.[19]

Dyslipidemia is known to accelerate vascular aging, proved by aorta stiffness, carotid wall thickness, and also endothelial function damage. Dyslipidemia also accelerates aging in hematopoietic stem cell, telomer shortening, and even lipid accumulation in hepar.[20,21]

The ability of ethanol vegetable fern leaves extract in improving lipid profile raises the hope as an alternative therapy for dyslipidemia and other degenerative disease related. Moreover, the antioxidant function can also give protective effect for cardiovascular disease. The expected final result is human being can age with a good quality of life.

5. Conclusion

As conclusion, administration of oral ethanol vegetable fern leaves (*Diplaziumesculentum*) extract improved lipid profile level in wistar male rats (*Rattus norvegicus*) with dyslipidemia not as potent as simvastatin but decreased F2-isoprostane better than simvastatin. Hopefully, the further study can be conducted to know the benefit of oral ethanol vegetable fern leaves (*Diplazium esculentum*) extractin preventing dyslipidemia among high fat diet groups. Moreover, there is also a potential for combination therapy between statin and vegetable fern.

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