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# Comparison between Oral Simvastatin and the Addition of Mung Bean Sprout (Vigna radiata) Extract to Improve Lipid Profile and Decrease F2-isoprostane Level in Wistar Male Rats (*Rattus norvegicus*) with Dyslipidemia

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**Abstract:** ***Background*: One of the main factors causing atherosclerosis and oxidative stress in the body is dyslipidemia. Mung bean sprouts extract (Vigna radiata) is known to contain various components and antioxidants that play a role in improving lipid profiles and F2 isoprostane. This study examined total cholesterol, triglyceride, HDL, and LDL levels as an indicator of dyslipidemia and F2 isoprostane as an indicator of oxidative stress. *Methods*: Experimental randomized pretest posttest control group design was used in this study. The research's samples were 16 male white rats aged 2-3 months, weighing 180-200 grams which were given a high cholesterol diet to achieve dyslipidemic state (total cholesterol level ≥200mg/dl). Samples were divided into two groups. The control group was given simvastatin 0.36g/200g BW and 1 ml distilled water as a placebo. The treatment group was given simvastatin 0.36g/200g BW and mung bean sprouts extract 200mg/200g BW for 42 days. Lipid profile and F2 isoprostane were measured using blood serum. *Results*: The results showed that there was no significant difference on the mean reduction value of total cholesterol, where in the control group the result was 13.01 ± 8.61 mg / dl while in the treatment group it was 9.31 ± 6.81 (p = 0.358). There was no significant difference on the mean reduction value of triglycerides where in the control group the result was 22.5 ± 5.91 while in the treatment group it was 29.08 ± 8.32 (p = 0.092). There was no significant difference on the mean reduction value of LDL where in the control group the result was 12.01 ± 6.02 while in the treatment group it was 7.71 ± 6.04 (p = 0.176). There was no significant difference on the mean increasement value of HDL where in the control group the result was 3.51 ± 4.59 while in the treatment group it was 4.21 ± 1.50 (p = 0.687). There was a significant difference on the mean reduction value of F2 isoprostane where in the control group the result was 2.07 ± 0.45 while in the treatment group it was 4.95 ± 0.46 (p <0.001). *Conclusion*: It can be concluded that the addition of mung bean sprouts extract does not improve lipid profile significantly but decreased F2 isoprostane level significantly in dyslipidemic male Wistar strain white rats (Rattus norvegicus) that received oral simvastatin.***

**Keyword:** Mung Bean Sprouts Extract, Simvastatin, Dyslipidemia, Lipid Profile, F2 isoprostane

## 1. Introduction

Dyslipidemia has the potential to cause atherosclerosis and coronary heart disease. It is characterized by high level of total cholesterol, LDL cholesterol, triglyseride, and low level of HDL cholesterol.[1] Dyslipidemia also causes a decrease in antioxidant enzymes and an increase in lipid peroxidation which in turn causes a state of oxidative stress to atherosclerosis. This oxidative stress state can be seen through the F2 isoprostane indicator, which is the result of lipid peroxidation from cell membranes in the body. F2-isoprostane is a prostaglandin-like component which is formed from the catalysis of free radical peroxidation of essential fatty acids (primarily arachidonic acid) without the command or direct action of the cyclooxygenase enzyme.[2]

Cardiovascular disease is a complex and multifactorial problem that requires holistic attention including healthy lifestyle and the use of chemical drugs that can lower cholesterol levels.[3]

Statin is the first line of dyslipidemia therapy. Besides improving lipid profile, it can cause various side effects including rhabdomyolysis, increased plasma creatinine kinase level, myopathy and kidney failure.[4] Liver function must also be considered when using statin because of its metabolism by cytochrome P450 in the liver.[5] In a study by Scheffer et al (2013) showed that simvastatin had no effect on oxidative stress in patients at high risk of developing cardiovascular disease.[6] Because statin can cause various side effects and has no effect on oxidative stress condition, it is necessary to consider the addition of other treatments that can lower lipid profile and act as antioxidant to improve oxidative stress condition.[3]

Mung bean sprouts is known as sources of antioxidant and play a role in regulating lipid metabolism.[3] Phytocytosterols in mung bean sprouts can improve lipid profile through inhibition of cholesterol absorption by intestinal cells. Phytosterols also inhibit the uptake of biliary cholesterol and dietary cholesterol and reduce the level of cholesterol esterification in intestinal cells and increase the removal of cholesterol from the body through transintestinal cholesterol excretion (TICE).[7]

Saponin in mung bean sprouts can bind to cholesterol and reduce cholesterol absorption resulting in a decrease in the accumulation of plasma and liver cholesterol. 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 with 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 decrease.[8]

Vitamin C, vitamin E and flavonoid in mung bean sprouts also acts as an antioxidant. Vitamin C inhibits lipid peroxidation by deactivating lipoxygenase mechanisms and binding free radicals and reactive oxygen species (ROS).[9] Vitamin E prevent the formation of free radicals.[10] Vitamin E can also react with free radicals so that further stable vitamin E radicals will be formed. Furthermore, further oxidation will occur to form quinones which can be excreted from the body.[11] Meanwhile, flavonoid can prevent cell damage caused by oxidative stress in two ways, including donating hydrogen to neutralize the toxic effects of free radicals or by increasing expression of endogenous antioxidant genes.[12]

Therefore, this research was conducted to prove the effects of adding mung bean sprouts 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. The research's samples were 16 male Wistar rats, aged 2-3 months old, weighing 180-200 grams which were given a high cholesterol diet for 30 days to achieve dyslipidemic state (total cholesterol level ≥200mg/dl). Samples were divided into two groups. The control group was given simvastatin 0.36g/200g BW and 1 ml distilled water as a placebo. The treatment group was given simvastatin 0.36g/200g BW and mung bean sprouts extract 200mg/200g BW, both are given once a day using intragastric force feeding for 42 days. Lipid profile and F2 isoprostane level were measured using blood serum before and after 42 days of treatment.

**Extract Preparation**
A total of 1000 grams of mung beans were soaked in 2% alginate solution at room temperature for 8 hours. Furthermore, the mung beans are washed, drained and then covered with a cloth and germinated at room temperature for 24 hours. During germination, watering is done every 4 hours.

Blanching is done with 3 heating variations, those were boiling at 90°C for about 5 minutes, steaming at 90°C for about 5 minutes and roasting for about 10 minutes. After blanching, it is then drained, cooled and prepared for drying

Drying was carried out for 6 hours at 50$^{o}$C in a cabinet dryer, then milled with a diskmill and 80 mesh sieved.[18] The dose of mung bean sprouts extract (Vigna radiata) used in this study was based on previous study by Maris et al, 200 mg/200 grams body weigth.[19]

**Lipid Profile and F2-isoprostane 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 immunoassay (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 significant statistically.

## 3. Results

**Comparison of Total Cholesterol Level in Both Groups**
The mean of total cholesterol level in control group before intervention was 210,04±7,45 mg/dL and in treatment group was 210,03±5,64 mg/dL as seen on table 1. Total cholesterol level in control group after intervention was 197,02±1,82 mg/dL and 200,71±1,79 mg/dL in treatment group. There was a significant reduction in total cholesterol level, either in control group (p=0.004) or in treatment group (p=0.006) that showed by paired T test for each group. Independent T test showed no significant difference of total cholesterol before intervention (p=0.998), and a significant difference after intervention (p=0.001). There was no significant difference (p=0.358) in the reduction level between two groups.

**Comparison of Trygliseride Level in Both Groups**
The mean of trygliseride level before intervention in control group was 175,59±3,71 mg/dL and in treatment group was 174,88±2,84 mg/dL as shown on table 1. Trygliseride level after intervention in control group was 153,04±3,41 mg/dL and 145,79±6,47 mg/dL in treatment group. Paired T test was conducted for each group and showed that there was a significant reduction in trygliseride level both in control group (p<0,001) and treatment group (p<0,001). There was no significant difference of trygliseride before intervention (p=0.672), and a significant difference after intervention (p=0.014) that showed by independent T test. The reduction level between two groups showed no significant difference (p=0.092).

**Comparison of LDL Cholesterol Level in Both Groups**
The mean of LDL cholesterol level before intervention in control group was 134,64±6,14 mg/dL and in treatment group was 134,78±4,78 mg/dL as shown on table 1. LDL cholesterol level after intervention in control group was 122,63±2,41 mg/dL and 127,06±2,09 mg/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=0.009). Independent T test showed no significant difference of LDL cholesterol before intervention (p=0,962), and a significant difference after intervention (p=0,001). There was no significant difference (p=0,176) in the reduction level between two groups.

**Comparison of HDL Cholesterol Level in Both Groups**
The mean of HDL cholesterol level before intervention in control group was 40,28±4,05 mg/dL and in treatment group was 40,28±0,95 mg/dL as shown on table 1. HDL cholesterol level after intervention in control group was 43,79±1,23 mg/dL and 44,49±0,89 mg/dL in treatment group. Paired T test was conducted for each group and showed no significant difference of HDL cholesterol level in control group (p=0,067), and a significant increasement of HDL cholesterol in treatment group (p=0.009). Independent T test showed no significant difference of HDL cholesterol both before (p=1,000) and after intervention (p=0,209). There was no significant difference (p=0,687) in the reduction level between two groups.

**Table 1:** Lipid Profile and F2 isoprostane Level in Both Groups Before and After Intervention

| Variabel | Kelompok | Pre Test Rerata±SD | Post Test Rerata±SD | Delta Rerata±SD | P** |
|---|---|---|---|---|---|
| Kolesterol Total | Kontrol | 210,04±7,45 | 197,02±1,82 | 13,01±8,61 | 0,004 |
| | Perlakuan | 210,03±5,64 | 200,71±1,79 | 9,31±6,81 | 0,006 |
| | P* | 0,998 | 0,001 | 0,358 | |
| Trigeliserida | Kontrol | 175,59±3,71 | 153,04±3,41 | 22,5±5,91 | <0,001 |
| | Perlakuan | 174,88±2,84 | 145,79±6,47 | 29,08±8,32 | <0,001 |
| | P* | 0,672 | 0,014 | 0,092 | |
| LDL | Kontrol | 134,64±6,14 | 122,63±2,41 | 12,01±6,02 | 0,001 |
| | Perlakuan | 134,78±4,78 | 127,06±2,09 | 7,71±6,04 | 0,009 |
| | P* | 0,962 | 0,001 | 0,176 | |
| HDL | Kontrol | 40,28±4,05 | 43,79±1,23 | 3,51±4,59 | 0,067 |
| | Perlakuan | 40,28±0,95 | 44,49±0,89 | 4,21±1,50 | <0,001 |
| | P* | 1,000 | 0,209 | 0,687 | |
| F2 Isoprostan | Kontrol | 11,96±0,41 | 9,89±0,24 | 2,07±0,45 | <0,001 |
| | Perlakuan | 11,89±0,49 | 6,94±0,23 | 4,95±0,46 | <0,001 |
| | P* | 0,767 | <0,001 | <0,001 | |

Keterangan: * Uji *t-independent*; ** Uji *t-paired*

**Comparison of F2 isoprostane Level in Both Groups**
The mean of F2 isoprostane level in control group before intervention was 11,96±0,41 ng/L and in treatment group was 11,89±0,49 ng/L as shown on table 1. F2 isoprostane level after intervention in control group was 9,89±0,24 ng/L and 6,94±0,23 ng/L in treatment group. There was a significant reduction in F2 isoprostane level that 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 isoprostane before intervention (p=0,767), and a significant difference after intervention (p<0,001). There was a significant difference (p<0,001) in the reduction level between two groups.

## 4. Discussion

**Effects of Mung Bean Sprouts Extract on Lipid Profile and F2 isoprostane level**
Vitamin E in mung bean sprouts can inhibit the formation of cholesterol and act as an antioxidant. In its function to lower blood cholesterol levels, vitamin E inhibits the formation of 2,3 squalene oxides to form a relatively stable alpha tocopherol quinone, which causes inhibition of cholesterol formation.[13] Meanwhile, as an antioxidant, vitamin E prevents the formation of free radicals by preventing reactive oxiradicals in biological membranes.[10] The vitamin in mung bean sprouts which also plays a role in lowering cholesterol and as an antioxidant is vitamin C. Vitamin C will break down cholesterol to form bile acids which are then excreted through feces so that blood cholesterol levels can decrease.[13]

The saponin content in mung bean sprouts can lower cholesterol by reducing cholesterol absorption and inhibiting the activity of pancreatic lipase enzyme.[14] Mung beans contain aglycone isoflavones which can reduce LDL cholesterol level and increase HDL cholesterol. Isoflavones in mung beans are from genistein, glycitein, and daidzein. Due to their antioxidant properties, isoflavones can protect against oxidation and lower LDL cholesterol levels. In vivo, isoflavones are able to prevent LDL oxidation and inhibit the formation of atheroma in the artery. Daidzein and genistein can also increase the secretion of apoA-I from liver cells, which is the main component of HDL.[15]

Increased HDL level can also be caused by the protein content in mung beans that can induce adiponectin. Adiponectin is known to cause an increase in HDL level by increasing the ABCA1 pathway and the synthesis of ApoA-I. Adiponectin also inhibits the expression of the scavenger receptor class A-1 (SR-A) macrophages, thereby reducing the uptake of oxidized LDL and inhibit the formation of

foam cells. Adiponectin can also decrease triglyceride synthesis through its role in increasing insulin sensitivity in the liver. Increased insulin sensitivity can increase the excretion of lipoprotein lipases, thereby reducing LDL and triglyceride levels in the blood.[16]

The flavonoids in mung bean sprouts also play a role in 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.[17] Flavonoids also have high bioactivity as antioxidants which play a role in inhibiting cell damage due to oxidative stress through two mechanisms, by donating hydrogen to neutralize the toxic effects of free radicals, and by increasing the expression of endogenous antioxidant genes.[12]

In this study, there were no significant results on the mean reduction in total cholesterol, LDL, triglycerides, and the mean increase in HDL between the control and treatment groups. However, there was a significant difference in the mean reduction in F2 isoprostane level between the control and treatment groups. This could be due to the ineffective dose of mung bean sprouts extract in improving the lipid profile, or the lack of duration of the study.

## 5. Conclusion

It can be concluded that the addition of mung bean sprouts extract decreased F2 isoprostane level in dyslipidemic male Wistar strain white rats (Rattus norvegicus) that received oral simvastatin. Hopefully, the further study can be conducted to know the effect of mung bean sprouts extract to the pharmacokinetic and pharmakodinamic of simvastatin.

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