# Antihypercholesterolemic Effect of Murbei (*Morus alba* L.) Leaves and Its Combination with Simvastatin in Rats Induced by Propyltiouracil and High Fat Diet

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***Abstract*—Empirically Murbei leaves are used to treat diseases such as hypercholesterolemia. Murbei leaves contain quercetin 3-(6-malonylglucoside) and rutin which shows a strong inhibitory effect on LDL oxidation. This study aimed to verify the effect of the ethanol extract of murbei leaves in lowering total cholesterol in hyperlipidemic rats and the effect of combination extract murbei with simvastatin to the hypocholesterolemic effect of simvastatin. Twenty four Wistar male rats were divided into 6 groups randomly, group I-VI were given high fat diet and PTU to induce hypercholesterolemia. Group I as positive control was treated simvastatin 3.6 mg/kgbw, group II as a negative control was given CMC Na, group III-V were given ethanol extract of murbei leaves orally at a dose of 0.4; 0.6; and 0.8 g/kgbw respectively, group VI was treated a combination of murbei extract 0.4 g/kg with simvastatin. Total cholesterol was measured using a spectrophotometer visible (λ = 500 nm) with CHOD-PAP method as many as 3 times, i.e on day 0 (baseline), 14 days after induced hypercholesterol-emia with PTU and high-fat-diet and 14 days after treatment of murbei extract with still be given PTU and a high-fat diet. The results showed that murbei leaves extract dose 0.4; 0.6 and 0.8 g/kg were able to reduce total cholesterol with percent decrease in cholesterol levels by 32.94 ± 10.07; 40.17 ± 4.61 and 45.71 ± 4.27% respectively. Ethanol extract of murbei 0.4 g/kg can increase hypocholesterolemic effect of simvastatin when used in combination (p< 0.05).**

***Keywords—Murbei (Morus alba L); antihypercholesterolemic effect; PT; high fat diet***

## I. INTRODUCTION

Hypercholesterolemia is a condition where there is excess cholesterol in the blood. High cholesterol levels can damage blood vessel walls and can lead to various diseases such as coronary heart disease and stroke due to atherosclerosis process. Forty percent of sudden death from a heart attack caused by hypercholesterolemia. In Indonesia, there are about 36 million people (18%) were suffering from hypercholesterolemia. Hypercholesterolemia is a health problem not only in developed countries but also in developing countries. Hypercholesterolemia is one of the causes of coronary heart disease (CHD). In developing countries, the tendency of changes in the diet of people who are dominated by foods high in fat and low in fiber, smoking and lack of physical activity is the cause of various diseases associated with cholesterol [3]. Consumption of foods that contain a lot of cholesterol causes atherosclerosis (fatty substances heap in the artery wall). Atherosclerosis and some conditions due to lack of fiber, such as hypertension and high cholesterol, it can lead to coronary heart disease [15].

In previous study showed that the water extract of Murbei able to reduce levels of cholesterol, triglycerides, and can inhibit the development of atherosclerosis in rabbits fed a diet high in cholesterol [4]. Murbei leaf aqueous extract can reduce levels of triglycerides in rats [21]. Murbei leaves contain quercetin 3- (6-malonylglucoside) and rutin [10]. These flavonoids showed strong inhibitory effect on LDL oxidation [14]. The content of flavonoids from Murbei leaves could reduce levels of triglycerides, total cholesterol, and LDL in mice that had been induced triton WR-1339 [5].

Beside as antihiperkolesterol, Murbei leaves are also widely used as an anti-hyperglycemic [8] so that the people possible use combination of

Murbei and simvastatin as antihypercholesterol. In the previous study in pharmacokinetics have not been found data that showed murbei affecting effect of simvastatin, so it is possible that effects antihypercholesterol of simvastatin will increase when combined with Murbei leaves.

In addition to a diet high in saturated fat, hypercholesterolemia can be induced by antihipertiroid PTU (propylthiouracil). PTU will improve cholesterol levels by inhibiting the synthesis of thyroid hormone. Increased thyroid hormone can lower cholesterol levels by increasing the secretion of cholesterol to the bile and then discharged through the feces.

Based on these studies, it is necessary to prove the effect of the ethanol extract of Murbei leaf in lowering total cholesterol in rats induced hypercholesterolemia by using PTU and a diet high in fat, and the influence of combination extract of murbei leaf with simvastatin to the effects of hypocholesterol simvastatin alone.

## II. MATERIALS AND METHODS

### *A. Materials and Tools*

***Material:*** Murbei leaves, reagent kit to measure cholesterol levels (Cholesterol FS, Diasys), ethanol 90%, PTU to induce hypercholesterolemia, aqua (for drinking), fat goat, butter, quail eggs, duck egg yolk for a high-fat diet.

***Tools:*** Spectrophotometer UV / Vis (Stardust FC 15), cuvette, animal scales, centrifuge, electric balance, micropipette, Eppendorf, vortex, syringe injection, glassware, holder rats.

*Animal testing:* white male Wistar rats aged 2-3 months, weighing 150-250 g.

### *B. The course of Study*

#### *1. Making Ethanol Extract of Murbei (Morus alba L.) leaf*

Murbei leaves washed clean using water subsequently dried until dry and was made powder until smooth. A total of 1 kg of Murbei leaf powder was extracted by maceration for 5 days with ethanol 90% as much as 7 liters, then filtered and the filtrate obtained then evaporated to dryness using the evaporator.

#### *2. Preparation of hypercholesterolemia in rats*

Cholesterol levels improved with the administration of PTU and high-fat feed in rats. PTU 100 mg dissolved in 8 mL of distilled water, so that 1 ml contains 12.5 mg of PTU. PTU given dose is 12.5 mg / day divided in 2 doses [2]. High-fat feed was made by heating the fat goat, butter and cooking oil, after melt then duck eggs yolk and quail eggs put little by little. Standard feed was entered and was stirred until blended. High-fat feed and PTU given every day.

Table 1. Composition of high-fat feed

| Amount | Ingredients |
|---|---|
| 1 kg | goat fat |
| 250 g | butter |
| 100 mL | cooking oil |
| 2 | duck eggs yolk |
| 20 | quail eggs |
| 5 kg | standart fat |

#### *3. Treatment in Animal Testing*

A total of 24 male rats were divided into 6 groups randomly, group I-VI were fed a high-fat. Group I-VI were given PTU to induce hypercholesterolemia. Group I as positive control were given simvastatin at a dose of 3.6 mg/kg body weight of rats, the group II as a negative control were given CMC Na, group III-V were treated the ethanol extract of Murbei leaves orally at a dose of 0.4; 0.6; and 0.8 g/kg respectively, group VI treated with a combination of Murbei extract 0.4 g/kg and simvastatin.

Sampling was performed three times in total cholesterol, namely cholesterol (baseline), 14 days after induced hypercholesterolemia (pretest) and 14 days after treatment ethanol extract of Murbei (posttest) with PTU and still be given a high-fat diet.

Treatment for 2 weeks were carried out after the rats cholesterol level increased significantly, high-cholesterol diet was still given, and then observed by measuring total cholesterol.

#### *4. Determination of Total Cholesterol Levels*

Cholesterol measurements performed on day 0,14 and 28. Determination of total cholesterol using the reagent kit (Cholesterol FS from Diasys) with CHOD-PAP method (enzymatic photometric test). Cholesterol FS contains several components (Table 2).

Table 2. Composition of cholesterol reagent kit [6]

| Ingredients | Amount |
|---|---|
| Good's buffer pH 6,7 | 50 mmol/L |
| Phenol | 5 mmol/L |
| 4-Aminoantipyrine | 0,3 mmol/L |
| Cholesterol esterase (CHE) | ≥ 200 U/L |
| Cholesterol oxydase (CHO) | ≥ 50 U/L |
| Peroxidase (POD) | ≥ 3 U/L |

Standard : 200 mg/dL (5,2 mmol/L)

The reaction mechanism of the method CHOD-PAP based on cholesterol esters are hydrolyzed to free cholesterol with cholesterol esterase. Free cholesterol is then oxidized by cholesterol oxidase to form cholesterol-3-one and $H_2O_2$. Colorimetric indicator is quinonimin which has an absorption maximum at 500 nm is formed on the 4-

aminoantipirin and phenol by the catalytic reaction of peroxidase[1] [6].

Total cholesterol measurement procedure was done with a blood sample in 1 mL from rat tail vein, then centrifuged 20 minutes at 12,000 rpm to take the serum. Before reading the data in total cholesterol, previously prepared blank, standard and sample (serum) (Table 3)

Table 3. Composition blanks, samples, and standards

| | **Blank** | **Sample/ standart** |
|---|---|---|
| Sample/ standart | - | 10 µL |
| Aquadest | 10 µL | - |
| Reagen | 1000 µL | 1000 µL |
| Mixed and incubated for 20 minutes at $20^0$-$25^0$C or for 10 minutes at $37^0$C, then read the absorbance at 500 nm against the reagent blank no more than 60 minutes. | | |

*5. Analysis of Data*

The data obtained (cholesterol baseline/on day 0), cholesterol levels after induction PTU and a diet high in fat / on day 14) and cholesterol levels after treated with Murbei extract were analyzed distribution and homogeneity of data using a statistical test of Shapiro Wilk and Test of homogeneity of variance. Data were normally distributed and homogeneous, then followed by parametric test one-way ANOVA , and then post hoc test with level of confidence 95%.

## III. RESULT AND DISCUSSION

*A. Extraction of Murbei Leaves*

Maceration of 1 Kg of Murbei leaf using ethanol 90% as much as 7 liters obtained yield 10.57% with 105.7 g sticky extract.

*B. Induction hypercholesterolemia and treatment extract Murbei in rats*

Cholesterol levels increased after 2 weeks the rats were given PTU and a high-fat feed. cholesterol level was tested statistic with paired t test between baseline cholesterol with cholesterol levels 2 weeks after administration of PTU and high-fat feeding (the pretest). P value <0.05 in paired t test showed that cholesterol levels after 2 weeks of high fat feeding and PTU increased significantly. Normal cholesterol levels in rats is 10-50 mg/dL [12].

High-fat diet and PTU were used to make rats become hypercholesterolemia. A diet of high-fat and fatty acids can reduce the formation of LDL receptors, so that the levels of cholesterol in the blood is high [7].

Treatment with ethanol extract of Murbei leaves was conducted for 2 weeks after the condition of the rats underwent hypercholesterolemia or cholesterol test animals increased significantly. During treatment with the extract, the administration of high-fat feed and PTU still being done to see that the decrease in cholesterol levels was caused by the extract and simvastatin instead of impact discontinuation induced hypercholesterolemia in rats.

Cholesterol levels were measured on days 0, 14, and 28. Pretest value measured after the rats given PTU and high-fat feed. Postest value derived from cholesterol levels after rats given ethanol extract of Murbei leaves for 2 weeks (Table 4)

Positive control group showed a decrease in total cholesterol due to administration of simvastatin. Simvastatin works by inhibiting the first enzymatic step in the manufacture of cholesterol with inhibition of HMG CoA reductase, so the formation of cholesterol is inhibited [8]. HMG CoA reductase is the microsomal enzyme that catalyzes the limiting step in reaction velocity of cholesterol synthesis [11].

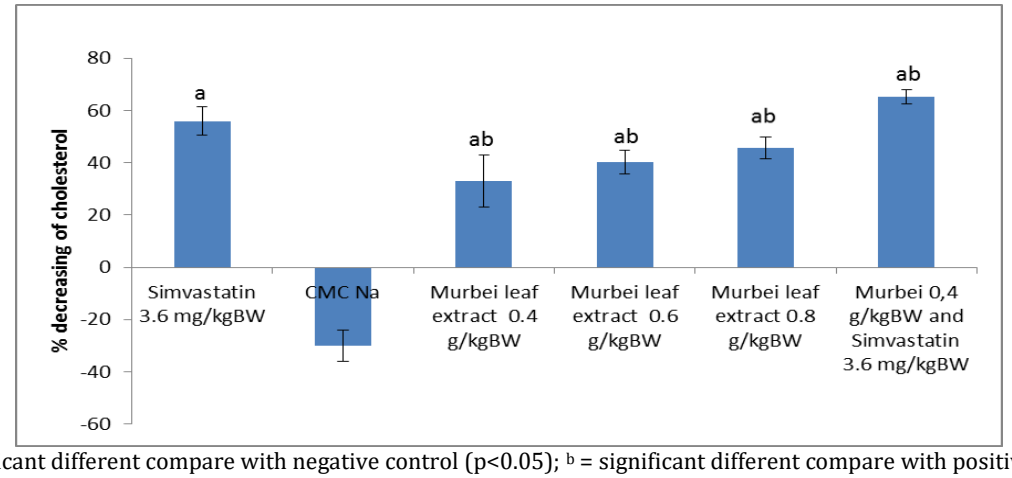
Results of t test all treatment groups had a value of $p < 0.05$. It showed significant difference between total cholesterol levels of rats when hypercholesterolemia and after being given a Murbei leaf extract for 2 weeks. The results showed extract dose of 0.4; 0.6 and 0.8 g/kg and the combination of extracts 0.4 g/kg + simvastatin able to lower total cholesterol levels This means that the smallest dose of 0.4 g/kg has been able to lower total cholesterol levels. While in the second week after the treatment is based on post hoc test shows that the simvastatin group and the combination have significant differences, it suggesting that combination of Murbei leaf extract with simvastatin had higher activity in lowering cholesterol levels compared to the use of simvastatin alone. Thus, it can be concluded that the ethanol extract of Murbei leaves affect antihipercholesterolemia effect of simvastatin when used simultaneously.

Decrease in cholesterol levels can occur due to the flavonoids in Murbei leaves. Previous study showed that ethanol extract of Murbei leaves contain quercetin 3-(6-malonylglucoside) and the rutin. They were the dominant component in Murbei leaves [10]. These flavonoids showed strong inhibitory effect on LDL oxidation at concentrations of 250 and 500 ng/mL [14]. Flavonoids are antioxidants that can lower cholesterol in the blood and is able to inhibit the synthesis of cholesterol by inhibiting HMG-CoA reductase [4].

The levels of LDL in the blood is considered important in relation to the formation of plaque in the arteries [17]. Oxy-LDL is the cholesterol that has been oxidized by free radicals that can settle on vessel walls and cause atherosclerosis [20]. Lipoprotein LDL is the largest carrier cholesterol around 70% of total cholesterol. LDL contain cholesterol and triglycerides by 50% and 10% [19].

Table 4. Total cholesterol level before and after treatment of ethanol extract of murbei leaves

| Groups | Animal test | Total cholesterol (mg/dL) | | |
|---|---|---|---|---|
| | | **On day 0** ***Baseline*** | **On day 14 (2 weeks after induction of PTU and high fat diet)** | **On day 28 (2 weeks after treatment of extract)** |
| Positive control (simvastatin) | 1 | 51 | 100 | 50 |
| | 2 | 53 | 114 | 53 |
| | 3 | 50 | 108 | 46 |
| | 4 | 50 | 107 | 40 |
| | x̄±SD | 51.00±1.41 | 107.25±5.74 | 47.25±5.62 |
| Negative control (CMC Na) | 1 | 72 | 117 | 160 |
| | 2 | 69 | 114 | 149 |
| | 3 | 58 | 105 | 137 |
| | 4 | 61 | 104 | 127 |
| | x̄±SD | 65.00±6.58 | 110.00±6.48 | 143.25±14.34 |
| Extract 0.4 g/kgBW | 1 | 54 | 107 | 67 |
| | 3 | 55 | 131 | 75 |
| | 4 | 51 | 105 | 71 |
| | 5 | 61 | 104 | 84 |
| | x̄±SD | 55.25±4.19 | 117.75±12.89 | 74.25±7.27 |
| Extract 0.6 g/kgBW | 1 | 74 | 118 | 65 |
| | 2 | 51 | 109 | 69 |
| | 4 | 69 | 109 | 70 |
| | 5 | 68 | 127 | 72 |
| | x̄±SD | 65.50±10.02 | 115.75±8.62 | 69.00±2.94 |
| Extract 0.8 g/kgBW | 2 | 55 | 103 | 52 |
| | 3 | 60 | 109 | 62 |
| | 4 | 47 | 95 | 56 |
| | 5 | 50 | 118 | 60 |
| | x̄±SD | 53.00±5.72 | 106.25±9.71 | 57.5±4.43 |
| Extract 0.4 g/kgBW + Simvastatin | 1 | 44 | 107 | 41 |
| | 2 | 41 | 110 | 39 |
| | 3 | 42 | 130 | 43 |
| | 4 | 48 | 127 | 41 |
| | x̄±SD | 43.75±3.10 | 118.50±11.68 | 41.00±1.63 |




[a] = significant different compare with negative control ($p<0.05$); [b] = significant different compare with positive control ($p<0.05$)

Figure 1. Percentage decreasing of cholesterol level after treatment of ethanol extract of murbei leaves for 2 weeks

The inhibition of oxidation of LDL will inhibit the deposition of cholesterol in the blood vessels and will lower cholesterol levels in the blood. Murbei leaf extract water 0.5% and 1% were able to lower serum cholesterol and triglycerides and suppress the development of atherosclerosis in rabbits [4]. This result may be associated with a preventive effect by anthocyanins against LDL oxidation in the arterial wall and supports the use of water extract of Murbei leaves to reduce the incidence of atherosclerosis and CHD. Hypercholesterolemic in the long term is one of the risk factors for hypertension. It need additional drug treatment for hypertension that occurs. Aside from being antihypercholesterolemia Murbei leaf also has

potential as an antihypertensive [13], antioxidants [10], antihyperglycemic[8][16]. So the use simvastatin together with the ethanol extract of Murbei leaves is likely to help overcome these problems, but more research needs to be done.

## IV. CONCLUSION

Based on the research that has been done can be concluded that ethanol extract Murbei leaf doses of 0.4; 0.6 and 0.8 g/kg were able to lower total cholesterol levels in Wistar rats as many as 30.52 ± 10.25; 38.40 ± 5.62 and 44.45 ± 4.65% respectively and the ethanol extract of Murbei leaves can increase antihipercholesterolemia effect of simvastatin when used simultaneously.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