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Effect of *Phyllanthus niruri* Extract on Low Density Lipoprotein of Dyslipidemic White Rats (*Rattus norvegicus*)

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Abstract

Background: Dyslipidemia is a lipid metabolism disorder characterized by elevated levels of total cholesterol, Low Density Lipoprotein (LDL) and triglycerides and a decrease in high-density lipoprotein (HDL). Meniran contains tannins, saponins, alkaloids, rutin and quercetin, which have hypolipidemia effect.

Objective: This study aimed to observe the effect of meniran extract on the Low Density Lipoprotein (LDL) in Wistar rats, compared with simvastatin.

Methods: Thirty-five Wistar rats (*Rattus novergicus*) were divided into five groups, then all were induced with American Diet 2 (AD2) high fat modification diet for 60 days, except the control group I. Then during the next 60 days, groups I and II were not given any treatment, group III was given simvastatin 0.8 mg/200 g body weight (BW)/ day, group IV was given meniran extract 50 mg/200 g BW/day and group V was given meniran extract 100 mg/200 g BW/day.

Results: Meniran therapy 50 mg/200 g BW/day was better in lowering LDL level than meniran dose 100 mg/200 g BW/day. Meniran therapy, which was significantly ($p<0.05$) better than simvastatin, was meniran dose of 50 mg/200 g BW/day in lowering LDL.

Conclusion: Meniran therapy 50 mg/200 g BW/day can lower LDL level significantly. In this dose meniran decreased LDL better than simvastatin.

Keywords: *Phyllanthus niruri* extract; Lipid profile; Wistar rat; Dyslipidemia; Simvastatin

Introduction

Dyslipidemia is a disorder of fat metabolism marked by increase in one or more of the fraction of blood fats, such as cholesterol, cholesterol esters, phospholipids, and triglycerides. According to the World Health Organization (WHO), approximately 39% of the world population was suffering from hypercholesterolemia [1]. Thirty-four million Americans have dyslipidemia with total cholesterol over 240 mg/dl (American Heart Association) at a cost of US $400 billion. According to research by Kamso et al. severe dyslipidemia cases in Indonesia with total cholesterol levels ≥ 240 mg/dl are most prevalent in Jakarta and Padang (>56%) [2].

An alternative therapy with lesser side effects would be useful, one of which is the extract from meniran herb (*Phyllanthus niruri*). Meniran (*Phyllanthus niruri*) contains various active compounds such as flavonoids, lignin, tannins, alkaloids, saponins and terpenoids [3]. Meniran's active compounds in the extract that can reduce levels of cholesterol and lipids are saponins, and alkaloids [4].

In a previous study, Kahono concluded that meniran (*Phyllantus niruri*) significantly lowered triglycerides in *Rattus norvegicus* rat with induced dyslipidemia with a dose of 100 mg/200 g BW/day [5]. Khanna et al. revealed that meniran herbs can lower very low density lipoprotein (VLDL) and low density lipoprotein (LDL) levels in white rats at dose of 250 mg/kg in mice simultaneously fed cholesterol (25 mg/kg) [6].

Materials and Methods

Meniran extract preparation

Meniran extract was derived from the extraction of herbaceous meniran plant (*Phyllantus niruri*) leaf in Lembaga Penelitian dan Pengujian Terpadu (LPPT) Unit I, Universitas Gadjah Mada (UGM), Yogyakarta. Samples were obtained from

Merapi Farma, Palagan Street, Tentara Pelajar km 8.8, Sleman, Yogyakarta.

Meniran extract was obtained by the extraction from dried meniran leaves using 70% ethanol by maceration techniques in LPPT Unit I, UGM, Yogyakarta. Meniran leaves were made into simplicia by drying at room temperature. After drying, the material was made into powder until smooth and then filtered through a sieve to obtain a homogeneous powder. This homogeneous powder was extracted by maceration. Maceration was done by soaking and stirring the powder in liquid (ratio of 1 part powder and 9 parts fluid) for approximately 5 days. This made the active substances from the cell cavity dissolve as a result of different concentrations. Then the active substances went out of the cell. After that the solution was filtered, and the residue was added by fluid until the water became colorless. Finally, the liquid was concentrated by ethanol evaporator until exhausted [7].

Meniran extracts were given in two doses to two different groups: 50 mg/200 g body weight/day and 100 mg/200 g body weight/day. The meniran extract was given orally sonded to animal models every day in the morning.

Preparation of simvastatin

Simvastatin was obtained from a local pharmacy and the dilution carried out in the Laboratory of Pharmacology, Faculty of Medicine, Public Health and Nursing, UGM.

The dose used for humans was 40 mg/day, after being converted to white rats it weighed 200 grams, dose of simvastatin in rats=0.018 × 40 mg/200 g body weight of rat/day=0.72 mg/200 g body weight of rat/day ≈ 0.8 mg/200 g body weight rat/day. The conversion factor per ratio of human body weight to rat body weight is 0.018 to 1.

Provision and adaptation animals model

The study has been approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Universities Gadjah Mada (Ref: KE/FK/434/EC). The animal models used in this experiment were thirty-five Wistar rats, which were obtained from LPPT UGM with the following inclusion criteria. Those rats were divided randomly into five cages. Rats were fed in a cage, with each cage containing one group of mice consisting of 7 rats, with cage size at (length × width × height)=(40 cm × 30 cm × 20 cm). Rats in each group underwent an adaptation for one week before the blood sampling to check the levels of LDL. During the adaptation period, mice were given AD2 feed ad libitum and distilled water, with lighting for 12 hours. Room temperature ranged between 21°C -25°C and humidity range 67%-81%. After the adaptation period, the rats were fasted for 12 hours to take blood samples.

Blood sampling procedure

Before the blood samples were drawn, rats were fasted for 12 hours. At the time of blood sampling, rats were anesthetized with ketamine 0.2 mg/ml that was given as 0.3 ml per rat. Anesthetic effects appeared 15 minutes after the administration of anesthesia. Once the animal models were anesthetized, they were held by hand to perform blood sampling by inserting a capillary tube with a 45° angle carefully into the medial canthus and rotated to break bulbar conjunctiva by reaching the orbital bone and rupturing the orbital sinus. Blood was taken as much as 1-3 ml through the orbital plexus media, and samples were collected in containers that were properly labeled. Subsequently, the samples were sent to the LPPT UGM laboratory for LDL level measurement.

Dyslipidemia induction

Dyslipidemia induction was done by providing as much as 10% lard in American Diet 2 (AD2) standard food to groups II, III, IV, and V. Those rats were divided randomly. The first group was fed with standard food AD2. Induction of dyslipidemia is intended to increase LDL. After 60 days of induction, LDL level was checked.

LDL level measurement

LDL examination was conducted by direct method. In the direct method, the first step was precipitating reagent deposition. After being incubated for 10 minutes at 15°C -25°C, sample was centrifuged for 15 minutes at 4000 rotations per minute (rpm). The supernatant was taken and mixed with the reaction solution. Then the sample was incubated for 10 minutes at 15°C-25°C and the absorbance was measured [8].

Data analysis

Statistical evaluation was done by using the SPSS statistical program. Data were presented as mean ± standard deviation. First, the data were tested with the Shapiro-Wilk test for normality distribution test. If the distribution was normal, data was analyzed using One Way ANOVA with a significance level of 95%. If the data from One Way ANOVA was significant ($p<0.05$), the Post Hoc Test was conducted using Tukey HSD, followed by Paired T Test to assess changes in the pre and post-test in the same group. If the data were not normally distributed, transformation was performed. If the data were still not normal, analysis was continued with non-parametric Kruskal-Wallis tests to assess the significance of differences between groups and Wilcoxon tests to assess the significance of changes in pre and post-test in the same group.

Results

Rats in each group underwent an adaptation for one week before the blood sampling to check the levels of LDL. During the adaptation period, mice were given AD2 feed ad libitum and distilled water, with lighting for 12 hours (there was no treatment difference between each groups).

LDL levels between groups of 60 days of dyslipidemia induction showed no significant difference ($p=0.350$), while LDL levels between groups of 60 days of therapy showed a significant difference ($p=0.006$). It shows that treatment in this study make a difference significantly.

Table 1 shows the average rat's body weight after one week of adaptation (baseline).

Table 1: Average rats body weight after adaptation.

Average Rats Body Weight After Adaptation	
Group	**Body weight (grams)**
I	115.90 ± 10.17
II	155.66 ± 17.49
III	141.34 ± 22.97
IV	141.40 ± 17.99
V	146.74 ± 11.12
Group I-V: Group given AD2 feed ad libitum and distilled water with lighting for 12 hours for one week (adaptation).	

Table 2 shows the average rat body weight after 60 days of hyperlipidemic induction and after therapy.

Table 2: Average rats body weight before and after therapy.

Body Weight Comparison Before and After Therapy		
Group	**Before therapy (grams)**	**After therapy (grams)**
I	248.44 ± 13.27	274.72 ± 16.01
II	282.78 ± 24.81	316.16 ± 33.16
III	306.08 ± 46.34	343.40 ± 62.43
IV	311.44 ± 47.03	352.58 ± 60.84
V	310.52 ± 36.62	317.60 ± 46.60
Group I: Group given standard feed AD2 Group II: Group given a high-fat feed modification AD2 Group III: Group given a high-fat feed modification AD2+simvastatin therapy 0.8 mg/200 g body weight/day Group IV: Group given a high-fat feed+AD2 modification therapy meniran extract 50 mg/200 g body weight/day Group V: Group given a high-fat feed+AD2 modification therapy meniran extract 100 mg/200 g body weight/day		

Table 3 shows that LDL levels before and after induction were rise significantly in all group ($p<0.05$). After 60 days of dyslipidemia induction, group I had the lowest LDL levels, while group III had the highest LDL levels, but the difference was not significant.

Table 3: Comparison of LDL levels after adaptation (Baseline) and 60 days of dyslipidemia induction.

Comparison of LDL Levels After Adaptation (Baseline) and 60 Days of Dyslipidemia Induction			
Group	**After adaptation (baseline)**	**After 60 days of induction**	**P**
I	7.92 ± 1.53	34.3 ± 3.31	0
II	12 ± 2.47	36.94 ± 5.58	0.043
III	10.56 ± 1.86	39.26 ± 3.76	0
IV	9.06 ± 1.54	35.42 ± 5.53	0
V	9.18 ± 2.17	36.78 ± 3.95	0
P	0.017	0.35	-
Group I: Group given standard feed AD2 Group II-V: Group given a high-fat feed modification AD2			

Table 4 shows that roughly all LDL levels decreased after 60 days of therapy. The reductions of LDL cholesterol level were significant in all groups ($p<0.05$), except in group V.

Table 4: Comparison of LDL levels after 60 days of dyslipidemia induction and 60 days of therapy.

Comparison of LDL After 60 Days of Dyslipidemia Induction and 60 Days of Therapy			
Group	**After 60 days of induction**	**After 60 days of therapy**	**P**
I	34.3 ± 3.31	23.08 ± 2.51	0.003
II	36.94 ± 5.58	26.62 ± 4.83	0.043
III	39.26 ± 3.76	25.8 ± 3.17	0.002
IV	35.42 ± 5.53	19.7 ± 4.10	0.002
V	36.78 ± 3.95	30.68 ± 5.13	0.066
P	0.35	0.006	-
Group I: Group given standard feed AD2 Group II: Group given a high-fat feed modification AD2 Group III: Group given a high-fat feed modification AD2+simvastatin therapy 0.8 mg/200 g body weight/day Group IV: Group given a high-fat feed+AD2 modification therapy meniran extract 50 mg/200 g body weight/day Group V: Group given a high-fat feed+AD2 modification therapy meniran extract 100 mg/200 g body weight/day			

Discussion

Our data shows that *Phyllanthus niruri* causes a decrease in the serum level of LDL in induced hyperlipemic rats. After 60 days of therapy, the group which received meniran therapy dose 50 mg/200 g body weight/day experienced a significant reduction in LDL. This decrease is parallel with study by Dominiczak which found flavonoids have a protective effect on LDL cholesterol to not be oxidized [9]. Sattanathan et al. stated that Rutin compounds can inhibit lipid peroxidation [10]. According to research by Sattanathan et al., the active compound supplement for 60 days in humans can reduce LDL levels significantly [10]. Odbayar et al. stated that Rutin decreases the activity of HMG-CoA reductase inhibitors and the activity of acyl CoA: cholesteryl acyl transferase (ACAT) thus decreasing hepatic lipogenesis by the liver [11]. Rutin also increases fecal excretion of sterols resulting in reduction of fat absorption. Sudoyo et al. also stated that the decrease in cholesterol synthesis in the liver will also lead to a decrease in the synthesis of Apo B-100 and causing LDL cholesterol in the blood to be drawn to the heart [12]. Nonetheless, meniran therapy dose 100 mg/200 g body weight/day had the smallest decline in LDL level, which was statistically insignificant.

Compared with the control group, the greater reduction in LDL level was found in meniran dose 50 mg/200 g body weight/day group, and both of them are significant.

The simvastatin treatment group experienced a significant reduction in LDL levels. This result can be due to simvastatin which is a drug that inhibits the HMG-CoA reductase enzyme which is important in the biosynthesis of cholesterol. Due to reduction of cholesterol synthesis, LDL receptors take up more cholesterol from the blood so that cholesterol levels in the blood are reduced. Statins have been shown to decrease the concentration of LDL and triglycerides and slightly raise HDL [13,14].

Compared with the group that was given simvastatin therapy, a greater reduction in LDL level was found in the meniran treatment group receiving 50 mg/200 g body weight/day, and both of them are significant. When we compared using ANOVA, meniran dose 50 mg/200 g body weight/day can lower LDL cholesterol significantly (p=0.03) compared to the simvastatin therapy group. Meniran dose 100 mg/200 g body weight/day lowered LDL levels but it was statistically insignificant (p=0.066). This insignificant reduction is probably caused by the excessive amount of high-fat feeding that cannot be exactly controlled by ad libitum way of feeding. Tuminah et al. stated that animal fats contain much saturated fatty acids that can increase total cholesterol and LDL levels, and at the same time improve blood HDL cholesterol levels [15]. Trans unsaturated fatty acid contained in animal fat products not only increase LDL cholesterol levels but simultaneously also reduce levels of HDL cholesterol. Further, Dewi explained that the excessive intake of saturated fat causes the insulin sensitivity impairment, so that the blood glucose levels rise. Triglycerides and VLDL particles bind on the position where lipase enzymes work, causing the enzyme to be no longer able to clean the fat [16]. Beside the excessive amount of high-fat feeding, the insignificant result from the meniran treatment group dose 100 mg/200 g body weight/day could also be caused by the antagonistic nature of the active compound meniran at a certain higher doses [17]. Further research is needed to clarify and confirm these important results with more controlled parameters and future clinical human trials.

Conclusion

Meniran therapy lowers LDL level in hyperlipidemic rats significantly. Meniran extract therapy dose 50 mg/200 g body weight/day gives reduction effects in LDL levels better than meniran dose of 100 mg/200 g body weight/day significantly in Wistar rats. Compared with simvastatin, meniran dose 50 mg/200 g body weight/day gives better effect in significantly lowering LDL levels in hyperlipidemia induced rat model.

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