Effect of *Parkia speciosa* Hassk Peels Extract on Total Cholesterol Levels of Hypercholesterolemia Rats

Joni Tandi[1], Tien Wahyu Handayani[1], Maria Tandebia[1], Jessica Anastasia Wijaya[1]

[1]Lecturer, Department of Pharmaceutical, Sekolah Tinggi Ilmu Farmasi Pelita Mas, Palu 94111, Central Sulawesi, Indonesia

Abstract

Background: This research aimed to examine the secondary metabolites in the ethanol extract of *Parkia speciosa* Hassk peels and determine the effect of the *P. speciosa* Hassk peels on the reduction in total cholesterol levels of male hypercholesterolemia white rats.

Methods: Thirty white male rats were divided into six treatment groups (n=5): Group I: normal control (0.5% CMC Na suspension), Group II: negative control (high cholesterol feed), Group III: positive control (suspension simvastatin), Group IV, V, VI: hypercholesterolemia rat received *P. speciosa* Hassk shell at dose of 300, 400, and 500 mg/kg BW orally for 14 days. The total cholesterol levels were measured on days 0, 14, 28, 35.

Results: Quantitative test showed that the ethanol extract of *P. speciosa* peels contained 0.21% alkaloids (w/w), 8.34% flavonoids (w/w), 4.21% tannins (w/w) and 0.18% saponins (w/w). The ethanol extract of *P. speciosa* peels at a dose of 300 mg/kg BW was able to reduce total cholesterol levels in hypercholesterolemia white mice (52 mg/dL).

Conclusion: The ethanol extract of *P. speciosa* peels can be considered as an effective treatment for hyperlipidemia disease.

Keywords*: Parkia speciosa Hassk shell; hypercholesterolemia; high cholesterol feed.*

Introduction

Hypercholesterolemia is indicated by an increase in total cholesterol, especially LDL, and followed by a decrease in blood HDL levels. Hypercholesterolemia is considered a high risk factor for coronary heart disease if an increase of cholesterol level reach to ≥ 240 mg/dL.[1] *Parkia speciosa* Hassk is a plant that contains alkaloids, polyphenols, flavonoids, saponins and tannins. This plant has the benefit to prevent and overcome several diseases such as anemia, high blood pressure, diabetes, cholesterol, constipation, reducing the activity of free radicals, and serves to capture superoxide anion and superoxide lipid radicals. *Parkia speciosa* pells has several nutrient such as phosphorus, vitamin C, vitamin A, protein, carbohydrate, mineral and iron content.[2,3]

Angelina et al.[3] reported that the ethanol extract of *Parkia speciosa* peel exhibited antioxidant activity and contained several metabolite compounds such as phenolic, flavonoid and tannin contents. Previous research by Tandi et al.[4] showed that the ethanol extract of purple eggplant peels at dose of 50 mg/kg BW was effective to reduce total cholesterol levels of 140.8 mg/dL. Tandi[5] reported the effects of ethanol extract of pumpkin seeds could reduce total cholesterol levels at dose 360 mg/kg BW.

Based on the above explanation, researchers are interested in conducting further research on the effectiveness of *Parkia speciosa* peels ethanol extract on reducing cholesterol levels of male white rats (*Rattus norvegicus*) hypercholesterolemia with different dose.

Corresponding Author :
Joni Tandi
jonitandi757@yahoo.com

Materials and Method

Preparation of *Parkia speciosa* peels extract

The extraction *Parkia speciosa* peel extract was carried out by maceration method using 96% ethanol. 1000 grams of powdered samples was extracted with 6 L ethanol for 3x24 hours in dark place while occasionally stirring. The vessels used were 4 maceration vessels. The maserate obtained was then evaporated using a Rotary Vaccum Evaporator at 60°C and continued with thickening carried out using a waterbath at 60°C.

Preparation of simvastatin suspension

Ten mg of simvastatin tablet were crushed. 1.8 mg of simvastatin were put into mortar by adding 0.5% Na CMC suspension and crushed until homogeneous.

High cholesterol induction

The component of high cholesterol feed was pig oil and quail egg yolks. The lard were melted by heating. The eggs are separated from the yolk and the egg whites, taken the yolk and mixed with pig oil until homogeneous, given for 14 days orally.

Experimental design and treatment

Thirty white male rats were divided into six treatment groups (n=5): Group I: normal control (0.5% CMC Na suspension), Group II: negative control (high cholesterol feed), Group III: positive control (suspension simvastatin), Group IV, V, VI: hypercholesterolemia rat received *P. speciosa* Hassk shell at dose of 300, 400, and 500 mg/kg BW orally for 14 days. The total cholesterol levels were measured on days 0, 14, 28, 35.

Determination of the total alkaloid

A total of 100 mg sample was added with 5 mL of 2N HCl. Then, it were washed with 10 ml of Cloroform 3 times in a separating funnel and discarded the Cloroform phase. The solution was neutralized by adding 0.1 n NaOH and then added with 5 mL of BCG solution and 5 mL of Phosphate Buffer. The solution was extracted with 5 mL Cloroform and stirred with a magnetic stirrer at 500 rpm for 15 min. The extraction was repeat with Cloroform 2 times. The evaporated chloroform phase was collected with nitrogen gas and added the chloroform to 10 mL. The absorption levels was measured at 470 nm.

Determination of total flavonoid

Briefly, 0.10 g of samples was added with 2 ml of HCl 4 N and heated for 2 h at 110°C. Then, samples were cooled and extracted with ether. Ether solvent was steamed, then dried with N_2. 0.3 ml of 5% sodium nitrite was added. After 5 min, samples were added with 0.6 ml of 10% aluminum chloride, left for 5 min. Samples were added with 2 ml of 1 M NaOH and distilled water. Samples were diluted 25 times and moved into the cuvette. Then, the absorption levels were measured at 510 nm.

Determination of total saponin

Ten hundred mg of sample was added with 2 ml of 25% H_2SO_4. Samples was incubated for 120 min at 110^0C. Then, samples were extracted with ether and dried the filtrate. 1 ml of water was added and extracted for 5 min. Samples were then added with 50 µl of anisaldehyde and left for 10 min. Samples were added with 2 ml of 50% sulfuric acid and heated at 60°C for 10 min. Then, samples were added with water to a volume of 10 ml. As much as 10x dilution and the absorption was measured at 435 nm.

Determination of total tannin

Ten hundred mg of samples were added with 10 ml of methanol for 20 h. Samples were evaporated and then added with aquadest up to 10 ml volume. Then, 1 ml sample solution was added with 0.1 ml of Folin Ciocalteu reagent and incubated for 5 min. Samples were then added with 2 ml of 20% Sodium Carbonate and incubated for 5 min. Samples were stirred with aquadest up to a volume of 10 ml and diluted 5 times. After incubating for 30 min at room temperature, the absorbance was measured at 760 nm.

Data Analysis

Data were statistically analyzed using One Way ANOVA. Post Hoc Least Significant Difference (LSD) test was performed to determine significant differences between treatments ($p < 0.05$). Data analysis was done by SPSS v.23 for Windows.

Results and Discussion

A preliminary test was carried out to determine the secondary metabolite compounds from the peels of *Parkia speciosa*. Determination of the total quinine equivalent alkaloid content obtained by the regression equation Y = 1.88853e-004 - 3.95444e-004 and the correlation coefficient (r2) = 0.99926 (Table 1). Based on the regression equation, total alkaloid level of the ethanol extract of *P. speciosa* peels was 0.21% (w/w). The total levels of equivalent flavonoids quercetin was obtained from the regression equation Y = 0.00404347 x- 0.00637338 and the correlation coefficient (r2) = 0.99786. Based on the regression equation, total flavonoid levels of the ethanol extract of *P. speciosa* peels was 8.34% (b/b). The total saponin content obtained by the regression equation y = 9.44156e-004 - 0.00380439 and the correlation coefficient (r2) = 0.99959. Based on the regression equation, total saponin content was calculated in the sample to obtain the total saponin content the ethanol extract of *P. speciosa* peels was 0.18% (w/w). The total tannin content equivalent obtained regression equationy = 0.0116490 x + 2.92199e-004 and the correlation coefficient (r2) = 0.99996. Total tannin content of the ethanol extract of *P. speciosa* peels was 4.21% (b/b).

Table 1: Determination of total flavonoid, alkaloid, saponin, and tannin

Parameter	Result	Unit	Regression Equation	Method
Total Flavonoid quercetin	8.34	% b/b	y = 0.00404347 x- 0.00637338 Correlation Coefficient r2 = 0.99786	UV-Vis
Total Quinine Alkaloids	0.21	% b/b	Y = 1.88853e-004 - 3.95444e-004 Correlation Coefficient r2 = 0.99926	UV-Vis
Total Saponins	0.18	% b/b	y = 9.44156e-004 - 0.00380439 Correlation Coefficient r2 = 0.99959	UV-Vis
Total Tannin	4.21	% b/b	y = 0.0116490 x + 2,92199e-004 Correlation Coefficient r2 = 0.99996	UV-Vis

The results showed that all animals before treatment (day-0) have a normal total cholesterol levels (Table 2) range of 10-54 mg/dL.[6] The total cholesterol levels on the day-14 had a significant difference between normal control and all treatment groups (Figure 1). It was indicated that there was an effect due to high-cholesterol feed containing 844 mg/dL of quail eggs and pig oil containing 25% saturated fat, which could increase triglyceride levels and affect the viscosity of red blood cells and reduce HDL.[7]

Table 2: Total cholesterol levels after treatment with the ethanol extract of *P. speciosa* peels

Total blood cholesterol levels (mg/dL)							
Day	**Normal Control**	**Negative Control**	**Positive Control**	**300 mg/kg BW**	**400 mg/kg BW**	**500 mg/kg BW**	**P value**
0	35 ± 9.2	45 ± 2.3	36 ± 8.6	39 ± 12	43 ± 13.1	44 ± 9.2	0.464
14	41 ± 5.8	150 ± 16.5	152 ± 20	160 ± 30.4	150 ± 17.5	171 ± 27.3	0.000
28	51 ± 7.3	165 ± 48.3	84 ±28.5	146 ± 60.4	118 ± 30.6	110 ± 27.4	0.004
35	50 ± 10.2	110 ± 38.2	47 ±23.2	52 ± 31.7	71 ± 19.2	87 ± 35	0.023

On day-28th day, treatment with *P. speciosa* peels extract could not reduce the total cholesterol of hypercolesterolemia mice. However, on day-35, the total cholesterol of hypercolesterolemia mice was decreased after *P. speciosa* peels extract treatment. Only dose of 300 mg/kg BW of *P. speciosa* peels extract could reduce the total cholesterol of hypercolesterolemia mice compared to other dose treatment. The decreasing of total cholesterol in hypercolesterolemia mice was also found in simvastatin group treatment. This study also showed that *P. speciosa* peels extract at dose 300 mg/kg BW exhibited the same effect as simvastatin group to decrease total cholesterol reach to normal levels. Simvastatin is a drug which act as HMGCo-A inhibitors and the most effective drug for reducing cholesterol levels.[8,9]

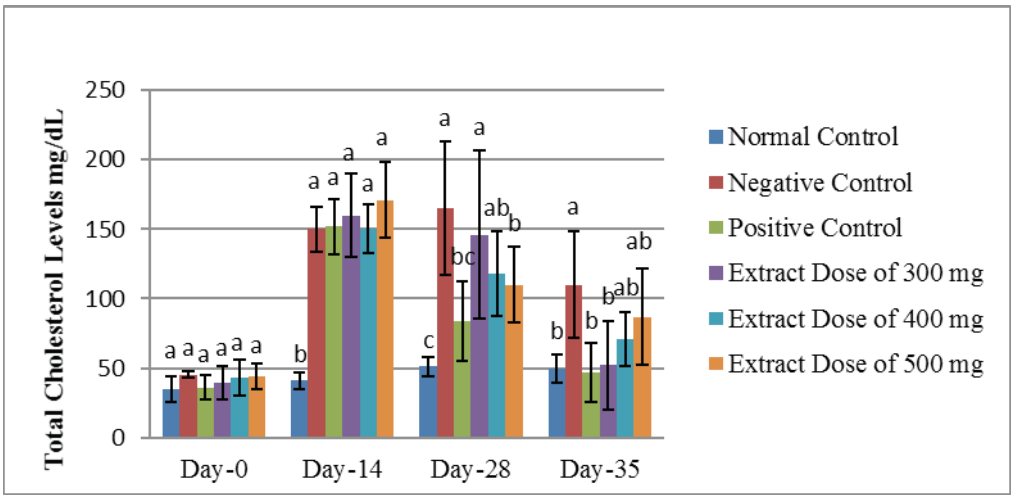

Figure 1: The total cholesterol levels after *P. speciosa* peels extract treatment

Alkaloid compounds could prevent the increase in levels of total cholesterol, triglycerides, LDL, and atherogenic indices, and also significantly increase HDL. Flavonoid compounds and tannins act as antioxidants by reducing triglycerides and LDL, so it can inhibit the accumulation of LDL in the blood vessel wall. Saponins can affect the biosynthesis of cholesterol in the liver.

Previous study showed that the ethanol extract of purple eggplant skin at a dose of 50 mg/kg BB with an average reduction of 140.8 mg/dL to reduce total cholesterol levels,[10] then ethanol extract of yellow pumpkin seeds at a dose of 360 mg/kg body weight with an average average reduction of 159 mg/dL to reduce total cholesterol in male white rats[11] and ethanol extract of kenikir leaves (*Cosmos caudatus*) dose of 400 mg /

kg BW with an average decrease of 209.9 mg / dL to reduce total cholesterol levels.[12] Based on current study, the peels of *Parkia speciosa* Hassk was more effective to reduce total cholesterol levels with an average reduction of 52 mg/dL.

Conclusion

In conclusion, the ethanol extract of *Parkia speciosa* Hassk peels contains several secondary metabolites include 0.21% alkaloids (w/w), 8.34% flavonoids (w/w), 4.21% tannins (w/w) and 0.18% saponins (w/w). The administration of 300 mg/kg BW of *Parkia speciosa* peels extract was an effective dose to reduce total cholesterol levels in hypercholesterolemia rats.

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