Anticholesterol Activity of Okra Fruit Extract (*Abelmoschus esculentus* (L) Moench) and Its Nanoemulsion *in Vivo*

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ABSTRACT

Background: Okra fruit has phytosterol, pectin that has the potential as an anticholesterol. **Objective**: The purpose of this study was to obtain 70% (v/v) ethanol extract of okra fruit and its nanoparticles and to evaluate their anti-cholesterol activity in vivo. **Material and Methods**: Okra fruit was extracted by kinetic maceration and the produced extract was tested for phytochemical screening and anti-cholesterolemi activity in vivo. The nanoparticles was prepared using a cosolvention method. In this study, DDY white mice was divided into 5 groups, namely normal control, negative control, positive control (simvastatin), the extract (400 mg /kg BW), and the nanoparticles (~ 400 mg /kg BW) group. All groups except normal were fed for 14 days with high cholesterol diet. After 14 days, the induction of hypercholesterolemia was stopped and simvastatin was administrated to positive control and the test groups were treated with the extract and the nanoparticles for 7 days. Measurement of total cholesterol level was carried out by cholesterol stripe test method. **Results**: The nanoparticles used demonstrated particle size of 134.7 nm and zeta potential of -26.72. The results showed a decrease in total cholesterol levels in positive control, the extract and the nanoparticles group as many as 48.68%; 32.44% and 42.95%, respectively. **Conclusion**: The nanoparticles can increase the activity of the extract.

Key words: *Abelmoschus esculentus* L. Moench, Hypercholesterolemia, Nanoparticles, Okra fruits, Total cholesterol level.

INTRODUCTION

Cardiovascular diseases, especially coronary heart and blood vessels are known as the cause of deaths in Indonesia. Consuming foods which are rich in cholesterol give rise degenerative diseases including coronary heart disease (CHD) and cardiovascular disease. CHD results from high cholesterol diets such as fatty foods, coconut milk and various fried foods. Hypercholesterolemia is a disorders in fat metabolism characterized by high level of total cholesterol in the blood. The formation of fat clots in blood vessels emerges a risk of atherosclerosis.[1,2]

One of the therapies that can be used by people in Indonesia is herbal medicines. Soluble fibers found in okra (*Abelmoschus esculentus* (L.) Moench) can reduce cholesterol level thus lower the possibility of cardiovascular disease. Okra fruit contains pectin which can reduce high blood cholesterol by modifying the formation of bile in the intestine. The content of other substances found in okra are vitamin C, phytosterols, and flavonoids. Phytosterols in okra which can be found in plant cell membranes have a structure similar to cholesterol but they can dissolve in water. Hence, they are able to interact with cholesterol in blood vessels.[3,4]

In previous studies okra seeds given for 42 days in 2 different doses to mice suffering from hypercholesterolemia resulted in a decrease in serum LDL cholesterol by 40.50% and 53.63%.[5] It was also mentioned that the effect of okra extract on HMG-CoA activity in mice showed a significant decrease in hypercholesterolemia.[6] One of the weaknesses of extracts is its low solubility in water thus reduce the bioavailability of their active compounds. The application of nanotechnology provides a number of advantages. Particle size and surface characteristics of nanoparticles can be modified to control the release of active compounds during the delivery of active compound and to improve the penetration through biological membranes.[7] Therefore, it was the aim of this study to formulate okra fruit extract into nanoemulsion to obtain a better anti-cholesterol activity.

MATERIAL AND METHODS

Material

Okra fruit (*Abelmoschus esculentus* (L.) Moench), propylene glycol, simvastatin tablet, sodium carboxymethylcellulose. sucrose, capmul, glicerine.

Extraction

The extract was obtained by extracting the simplicia powder with kinetic maceration method using 70% (v/v) ethanol, the maserate obtained was then concentrated with a rotary evaporator until a thick extract was obtained.

Phytochemical screening

Phytochemical screening is a chemical compounds study of the simplicia and the extract of okra fruit including identification of flavonoid, steroid and triterpenoid, saponin and coumarin groups.[8,9]

Nanoemulsion extract preparation

Nanoemulsion of okra fruit extract was prepared by the cosolvent method. The extract was dissolved in cosolvent consisting of capmul, propylen glycol, glycerin and aquadest. The optimal formula contained 100 mg of 70% (v/v) ethanol extract of okra fruit, 1 ml capmul, 2.5 ml propylene glycol, 2 ml glycerin, and 10 ml distilled water.[10]

Anticholesterol study

In this study, DDY male mice divided into 5 groups, namely normal, negative, positive (simvastatin), the extract (400 mg/kg BW), and the nanoparticles (~ 400 mg/kg BW). All groups except normal group were given hypercholesterol-inducing diet for 14 days. Afterwards, simvastatin, the extract and the nanoparticles were given for 7 days. Measurement of total cholesterol levels was carried out by the cholesterol strip test method.

RESULTS AND DISCUSSION

Extraction

885.25 g of okra simplicia were extracted by kinetic maceration using 40 L 70%(v/v) ethanol. After evaporation by a vacuum rotary evaporator, 88.26 g thick extract was obtained.

Phytochemical Screening

The simplicia of okra fruit and the extract contained flavonoids, steroids, triterpenoids, saponins and coumarin.

Nanoemulsion extract preparation

The generated nanoemulsion demonstrated particle size of 134.7 nm, polydispersity index of 0.512 and zeta potential of -26.72 mV (Figures 1 & 2).

Anticholesterol study

Measurement of body weight of mice was carried out during the study to investigate the influence of the treatment during 21 days. Measurement of body weight of mice on day 0 was carried out to determine the body weight of mice before the treatment to ensure the body weight of mice used in accordance with the criteria for experimental animals used (20-30 gr). Table 1 shows the average body weight of the mice used that meets the requirements (20.68 ± 0.65 - 24.94 ± 1.07 gr).

Measurement of body weight of mice on day 21st was carried out to determine whether there was a decrease in body weight of mice after being given treatment. The results showed that the average body weight of mice ranged from 28.18 ± 2.80 - 32.66 ± 2.22 gr. Based on the results of the statistical analysis, the normality test (Shapiro-Wilk test) showed that the difference in body weight on day 14th up to 21st was normally distributed. On day 21st there was a decrease in body weight in the
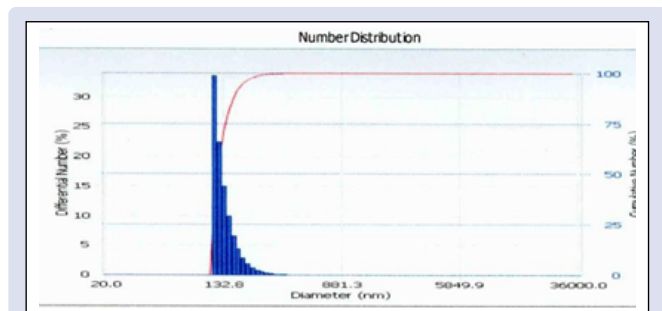

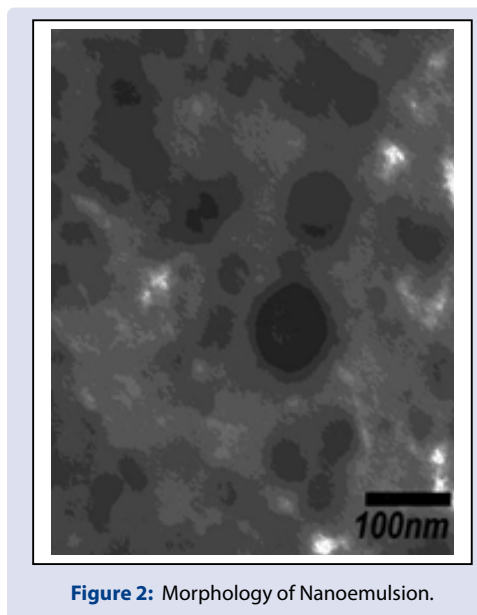**Figure 1:** Particle size of Nanoemulsion.

Figure 2: Morphology of Nanoemulsion.

extract and the nanoparticles group. There is a positive correlation between body weight with cholesterol, VLDL and LDL levels. However, positive group did not result in weight loss because statin therapy can increase calorie and fat intake,[11,12] thus weight loss caused by changes in lipid profile did not occur in this group.

Measurement of total cholesterol levels in mice

Measurement of total cholesterol levels on day 0 was carried out to find out the total cholesterol levels before treatment (initial conditions). The data showed that the average total cholesterol level of blood of mice under normal conditions in the range of 55.4 ± 6.58 mg/dL - 66.2 ± 3.03 mg/dL. The total cholesterol level on day 0 fulfilled the requirements for normal total blood cholesterol in mice (40-130 mg /dL). The total cholesterol level on day 0 among groups I, II, III, IV, and V was no significant difference (Table 1).

Measurement of total cholesterol levels on day 14th was carried out to determine whether the total cholesterol levels in group II, III, IV, and V increased after being given a food inducing hypercholesterolemia. The data in Table II shows that the average blood cholesterol level in mice groups II, III, IV, V, and VI ranged from 149.8 ± 8.98 mg/dL - 155.2 ± 8.84 mg/dL. However, there were no significant differences among the groups (Table 2).

Measurement of total cholesterol levels on day 21st shows a decrease in total cholesterol levels in mice group III, IV and V. There were significant differences in total cholesterol levels on day 21st among group I, II, III, IV and V, which means there are significant differences among the group. In this study, statistical analysis was performed on the difference in total blood cholesterol levels before and after treatment, which is between day 0 and day 14th and between day 14th and day 21st (Table 3).

The results of statistical tests show that there were significant differences between the extract and the nanoparticles group. Therebye, the nanparticles has the ability to lower total blood cholesterol levels better than the extract. This could be caused by the characteristics of nanoemulsion which has a very small particle size and low interface tension so that it can easily penetrate. In addition, nanoemulsion has long-term stability (without creaming, sedimentation, flocculation or coalescence) due to thermodynamic stability.[13,14]

Table 1: The Body Weight of Mice on Day 0.

Mice Number	Weight (gram)				
	I	II	III	IV	V
1	24.2	20.3	24.3	22.1	23.7
2	23.7	20.7	23.1	21.4	25.1
3	22.6	20.4	23.1	23.7	24.0
4	23.3	20.2	24.0	25.7	26.2
5	22.7	21.8	21.7	22.7	25.7
Average	23.3	20.68	23.24	23.12	24.94
SD	0.67	0.65	1.01	1.67	1.07

I : Normal group
II : Negative group
III : Positive group
IV : The extract group
V : The nanoemulsion group

Table 2: Cholesterol Level on Day 14th.

Mice Number	Cholesterol Level (mg/dL)				
	I	II	III	IV	V
1	61	148	151	158	163
2	66	145	163	143	152
3	58	155	145	145	142
4	69	165	147	161	163
5	59	163	150	142	146
Average	62.6	155.2	151.2	149.8	153.2
SD	4.72	8.84	7.01	8.98	9.63

I : Normal group
II : Negative group
III : Positive group
IV : The extract group
V : The nanoemulsion group

Table 3: Cholesterol Level on Day 21st.

Mice Number	Cholesterol Level (mg/dL)				
	I	II	III	IV	V
1	65	145	78	105	80
2	64	140	71	96	75
3	60	150	78	98	70
4	65	160	81	107	76
5	55	165	80	100	72
Average	71,8	152	77,6	101,2	74,6
SD	4.32	10.37	3.91	4.66	3.85

I : Normal group
II : Negative group
III : Positive group
IV : The extract group
V : The nanoemulsion group

DISCUSSION

In this study, okra fruit extracts and the nanoemulsion were tested for their anticholesterol activity. Compounds in okra fruit extract that act as anticholesterol agents are steroids (phytosterol) and pectin. For this reason, the selection of methods and solvents is a matter that must be considered. The method used for extracting in this study is maceration. Maceration was chosen because it is the simplest and safest method that prevents the destruction of compounds which are not stable on heating . The solvent used for maceration is 70% (v/v) ethanol. Steroids which are polar compounds can be extracted using 70% (v/v) ethanol. Phytochemical screening of the simplicia and the extract were carried out to determine the content of secondary metabolites found in okra fruit. The results displayed both the simplicia and the extract contained secondary metabolites including flavonoids, steroids, saponins and coumarin.

In this study, simvastatin was chosen as a standar reference because simvastatin is an inhibitor of the HMG-CoA enzyme. The enzyme catalyzes the formation of mevalonates from HMG CoA which is the initial stage of cholesterol formation. Where one of the okra fruit extracts that can provide cholesterol-reducing effects is pectin. Weight measurement and determination of total blood cholesterol levels of mice were performed on day 0th, 14th, and 21st respectively. In this study, the anti-cholesterol activity of the extract and the nanoemulsion were compared. On day 14th, the average growth rate of mice is 1 gram / day. The growth rate of animals is influenced by species, individuals, sex, age, feeding, and diet consumed.[11] In the negative, positive, low, moderate, and high doses there was a significant increase in body weight on days 0-14. Weight gain can be caused by an increase in the amount of fat deposited in adipose tissue, especially those under the skin and the abdominal cavity.[12]

The anticholesterol activity test consists of 5 groups: group I is used as a normal control without receiving any treatment in order to find out whether there were other effects that could influence the total blood cholesterol levels. However, until the end of the experiment mice showed a normal total blood cholesterol level with an average level of 40-130 mg / dL. Group II was used as a negative control induced by high cholesterol diet given orally without any other treatment. On day 14th, the mice in group II experienced an increase in body weight and in total cholesterol levels with an average level of 155.2 mg / dL and only experienced a slight decrease on day 21st with an average level of 152 mg / dL. Hence, after the treatment of high cholesterol diet for 14 days mice were in hypercholesterolemia condition.

Group IV and V were used as test groups induced hypercholesterolemic diets for 14 days before treatment, after hypercholesterolemia, on day 15th the mice were treated with both the extract at a dose of 400 mg / kgBW and the nanoemulsion at a dose which was equal to 400 mg /kg BW of the extract. The extract and the nanoemulsion lowered the total cholesterol level with an average level of 101.2 mg/dL and 74.6 mg /dL, respectively On day 21st. This demonstrated both the extract and the nanoemulsion could reduce the total cholesterol level. The nanoemulsion was considered to deliver the active compunds in the body and permeate through biological membranes easier compared to the extract alone.
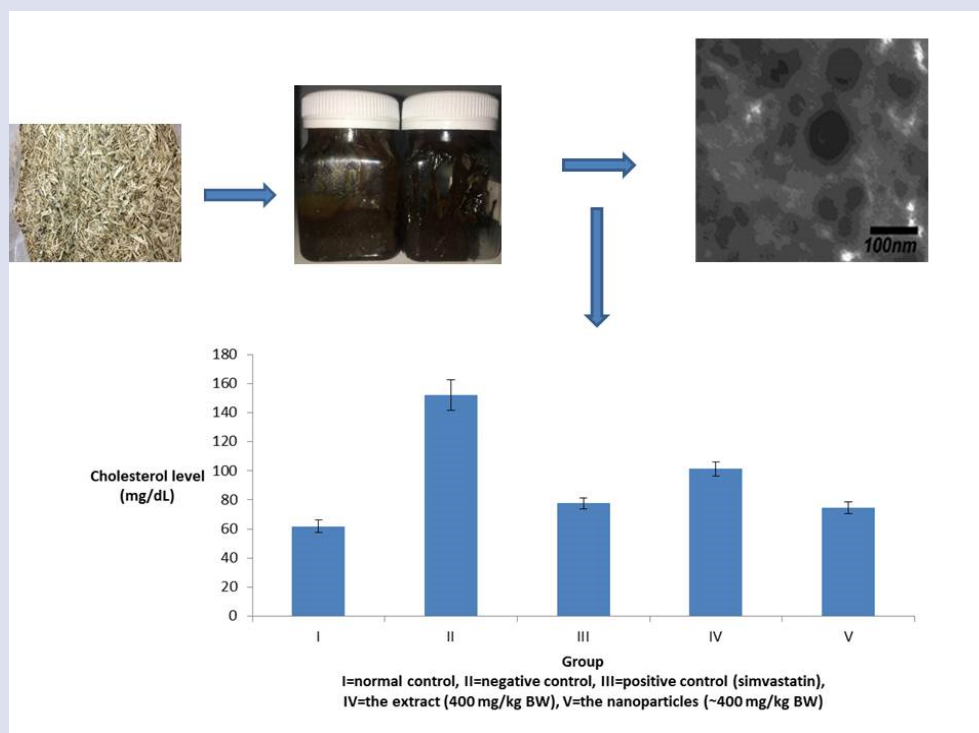
CONCLUSION

The nanoemulsion formed by mixing propylene glycol, capmul and glycerine could entrapped the extract and reduced the total blood cholesterol level lower compared to the extract alone in hypercholesterolemic mice. Accordingly, this formulation could be developed for its pharmaceutical dosage form with effective therapheutic effect.

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GRAPHICAL ABSTRACT

Cholesterol level (mg/dL)

Group

I=normal control, II=negative control, III=positive control (simvastatin), IV=the extract (400 mg/kg BW), V=the nanoparticles (~400 mg/kg BW)

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