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In Vivo Antihypercholesterolemic Effects of Caricapubescens Seeds Hypercholesterolemic-induced Rats

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Abstract

Background: Carica is a typical plant originating from the Dieng Plateau. Carica processed products leave waste in the form of Carica seeds which have not been utilized. Based on the previous study, Carica seeds contain tannin, flavonoids, and alkaloids, so in this study, Carica seeds extract was used in the antihyperlipidemic study.

Objective: To analyze the reduction of total cholesterol and secondary metabolites in the ethanol extract of Carica seeds using thin-layer chromatography.

Method: Extraction was carried out by the maceration method using 96% ethanol. Antihypercholesterolemic study used 6 groups of rats Wistar strains consisting of normal group, positive control, negative control, treatment 1 (200 mg / KgBB), treatment 2 (400 mg / KgBB), treatment 3 (600 mg / KgBB). Total cholesterol level tests were carried out by using a biosystem with diasysic reagents. Statistical tests were carried out with one way ANOVA followed by LSD post hoc tests.

Result: Carica seed extract has flavonoids and alkaloids. The percentage decrease in cholesterol levels in treatments 1, 2 and 3 was 15% ± 11,619, 24% ± 8,813 and 21% ± 19,471 with a significance value in the one way ANOVA test was 0,000.

Conclusion: The dosage variations of Carica seed extract Affects comparable to simvastatin in reducing total cholesterol levels.

Keyword: Total cholesterol, *CaricaPubescens*Lenneet Koch, Carica seeds

BACKGROUND

Hypercholesterolemic is a state of increased plasma lipid levels, including an increase in LDL (*Low Density Lipoprotein),* an increase in total cholesterol, an increase in triglycerides, and a decrease in HDL (*High Density Lipoprotein).* Hypercholesterolemic is a major health problem in developed countries, including Indonesia (Bieber et al, 2015) . The formation of atherosclerosis can be caused by an increase in LDL levels and total cholesterol, which is followed by a decrease in HdDL. Atherosclerosis that forms in the coronary arteries is a clinical manifestation of ischemic heart disease (Murwarni et al. 2013). The occurrence of cardiovascular disease can be minimized by reducing the process of forming atherosclerosis (Ismail et al. 2010) (Ismawati et al. 2016) .

The results of a survey analysis in 13 large cities in Indonesia prove that hypercholesterolemic is a risk factor for CHD (coronary heart disease). The modern lifestyle, make people tend to choose the instant way of doing everything, including food. An example is the consumption of *fast food,* contain high fat, but low fiber (Dunn et al. 2012). Prevention of the occurrence of hypercholesterolemic can be done by weight control, whichisthe regulation of a balanced diet and regular exercise. The use of hypolipidemic drugs, for example, simvastatin can reduce the activity of the enzyme 3-hydroxy-3-methyl-glutaril-coenzyme A (HMG-CoA) reductase. HMG-CoA acts as a catalyst for cholesterol formation. Side effects of statins, when used long term,are myopathy (Thompson et al. 2016). Based on these descriptions, testing herbal medicines as cholesterol-lowering drugs is still being developed.

The karika plant (*Caricapubescens*L) is a flora that grows typical of the Dieng Plateau. Karika belongs to the Caricaceae family, so it looks like papaya. The fruit is smaller than papaya, and rarely consumed raw. Arika fruit contains sap, which can cause itching. In the Diengarea ,karika fruit is better known as processed sweets. The seeds are clustered, black and solid. Other parts of the karika plant, including seeds and leaves, have been not used (Laily et al. 2012, Minarno et al. 2015).

The content of flavonoids karika fruit is known higher than papaya. The high flavonoids are comparable to their antioxidant activity (Mu'awwanah&Ulfah. 2017). The research on papaya seed juice showed that papaya seeds have anti-hypercholesterolemic activity (Adeneye et al, 2009). This activity was caused by secondary metabolic content of flavonoids, saponins, and tannins found in papaya seeds (Minarno et al, 2015). Papaya seeds have been studied as having anti-bacterial and larvicidal activities (Supono et al. 2015, Novalina et al. 2013). The advantage of using natural ingredients is that it contains several active substances (secondary metabolites) that have various synergistic pharmacological activities. The advantage of this research is to increase the economic value of karika seeds which were originally only as a waste to be an alternative medicine for hypercholesterola disease.

METHODOLOGY

The main material of this research is karika seed extract. Karika seeds are obtained from 'CaricaGemilang' at Wonosobo. Karika was determined in Biosystematics Ecology Laboratory Department of Biology, Faculty of Science, University of Diponegoro.

Phytochemical Test

Extraction. Karika seeds extract was extracted by the maceration method. The first step was 250g of seeds-dried powder in 1750 ml of 96% ethanol for 3 days and remaseration with 750 ml of solvent for 2 days. The solvent was evaporated under reduced pressured 78ºC using a rotarty evaporator for the elimination of ethanol, then thickened used a *water bath* temperature of 78ºC.

Phytochemical Test. The mobile phase used to identify flavonoid compounds were butanol: glacial acetic acid: water (4: 1: 5), and the mobile phase for identification of alkaloid compounds were ethyl acetate: n-hexane (9: 1) which have been saturated. The stationary phase used was silica gel GF 254. The standard that was used for flavonoids was quercetin and alkaloids with piperine. The observation of stains used UV lamps 254 and 366 nm.

Antihypercholesterol testing and statistical analysis

Experimental animals. The animals used were male Wistar strain rats aged 2-2.5 months, body weight 150-200 grams. Test animals were divided into 6 groups (1. Normal group, 2. Positive group, 3. Negative group, 4. Treatment group I, 5. Treatment group II, 6. Treatment group III).

Experimental hypercholesterolemic feed. hypercholesterolemic food was made by combining egg yolks with pork oil in a ratio of 5: 1 (Wahjuni et al.2016). Calculation of hypercholesterolemic feed is:

Volume of administration : 5 mL / rat

Egg yolk : $\frac{5}{6} x\ 5\ mL = 4{,}167\ mL \frac{5}{6} x\ 5\ mL = 4{,}167\ mL$

Pork oil : $\frac{1}{6} x\ 5\ mL = 0{,}83 \frac{1}{6} x\ 5\ mL = 0{,}83$

Experimental induction of hypercholesterolemic. Test animals that have been conditioned for 1 week were divided into 6 treatment groups, each comprising five rats. Measurement of total cholesterol levels was carried out 2 times, on the 7th day (after the induction process), and on the 16th day

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after the therapeutic process. During the therapy process, the test animals were still given BR II feed and ad libitum water.

Classifying test animals as follows :

1) Group 1 (normal control): rats were given BR II feed and ad libitum water days 1-14
2) Group 2 (positive control): rats induced hypercholesterolemicfeed about 5 ml/ 200 g.bwrats on day 1. On days 8-14 Imvastastin therapy was given a dose of 0.252 mg/gbw of rats, BR II feed, and ad libitum water.
3) Group 3 (negative control): rats induced hypercholesterolemic feeds as much as 5 ml / 200 g.bw, BR II feed, ad libitum water at 1-14.
4) Group 4 (extract dosage of 200 mg/kgbw): rats induced hypercholesterolemic feeds as much as 5ml / 200 g.bw, BR II feed, ad libitum water on day 1. On the day 8-14, the extract therapy was given a dose of 200 mg/kg BW of rats.
5) Group 5 (extract dose 400 mg/kgbw): rat induced hypercholesterolemic feed 5 ml / 200 g.bw, BR II feed, water ad libitum on day. On days 8-14 extract therapy was given a dose of 400 mg/kgbwof rats.
6) Group 6 (extract dose 600 mg/kgbw): rat induced hypercholesterolemicfeed 5 ml / 200 g.bw, BR II feed, water ad libitum on day 1. On days 8-14 extract therapy was given a dose of 600 mg/kgbwof rats.

Preparation of blood samples for analysis. The rat was anesthetized with ether and blood was drawn through a retro-orbital plexus and then inserted into a tube that had been given EDTA. Blood is centrifuged for 15 minutes at 3000 rpm. Then the blood plasma is taken using a micro pipette and stored with ependorf. Blood plasma was pipetted using 0.01 mL micropipette inserted into a tube. An additional 1 mL ELITech cholesterol reagent solution was added and then the total plasma cholesterol level was read in a biosystem tool.

*Statistical Analysis.*The data obtained are a percentage decrease in total cholesterol levels analyzed descriptively using tables and analyzed statistically with SPSS. Calculation of percent reduction in cholesterol levels:

$$\text{Percent decrease in cholesterol levels} = \frac{pretest - post\ test}{pretest} \frac{pretest - post\ test}{pretest}$$

Information; pretest cholesterol level (7th day cholesterol level), posttest cholesterol level (16th day cholesterol level).

RESULTS AND DISCUSSION

Plant Determination

The first step in the research is plant determination. The determination was carried out at the Biosystematic Ecology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Diponegoro University, Semarang. Karika or carica types of mini papaya plants that grow typical in the Dieng Plateau. The fruit is shaped like a chocolate fruit (cocoa), but the color and texture are similar to papaya. The flesh is fragrant, yellow, and sour. Karika is rarely eaten directly, because of the sap and sour taste. The seeds huddle in the middle, large amounts, and black.

Figure 1 . Fruit and seeds of the karika plant (*Caricapubescens*L.)

The karika plant classification is kingdom Plantae, Spermatophyta division, Caricales class, Caricaceae family, genus Carica, species *Caricapubescens*L., and regional names carica, karika, papaya Dieng. The results of the determination show that the sample used is true karika fruit (*Caricapubescens*L.)

Phytochemical screening

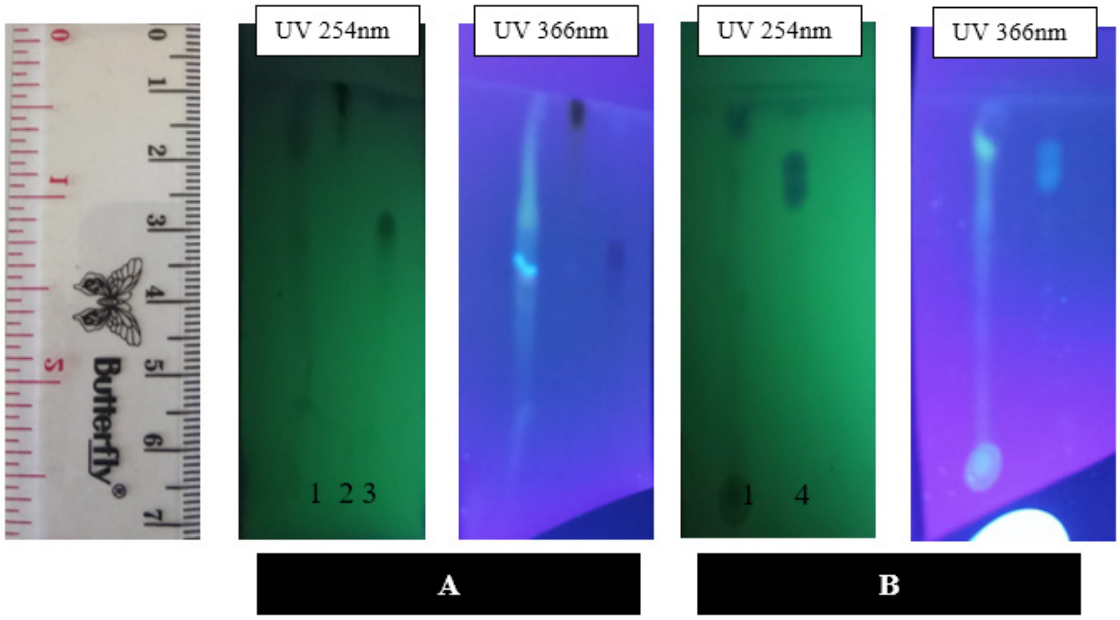

Figure 2. Chromatogram pattern ofkarika seed ethanol extract (EBK).
A.Flavonoid identification. Silica silent phase GF 254 nm. BAA mobile phase(4: 1: 5)
B. Alkaloid Identification. N hexane mobile phase: ethyl acetate (1: 9)
Note: 1. Samples, 2. Quercetin, 3. Routine, and 4. Piperin

Phytochemical identification of karika seed extract used Thin Layer Chromatography. The separation with Thin Layer matography (TLC) based on different levels of eluent polarity to obtain a solvent capable of providing good separation and suitable dye stains. Spots on the TLC plate were monitored under UV light 254 nm and UV 36 6 nm. Comparative standards used are quercetin and routine for flavonoid comparison and piperin for alkaloid comparison. The identification of flavonoids in karika seeds using the mobile phase of butanol: acetic acid: water = 4: 1: 5. The mobile phase of n-hexane: ethyl acetate 1: 9 ratio is used for the identification of alkaloids. Silica GF 254nm as a stationary phase.

Flavonoid identification results prove that the positive karika seed ethanol extract contains

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quercetin and routine glycosides, based on the Rf value of the sample close to or equal to the comparative Rf. The positive stains of quercetin and black routine were observed under UV 254 nm, with the Rf value of quercetin 0.87 and routine 0.65. On observation with UV 366 nm quercetin fluorescent bluish yellow. The identification of the karika seed extract alkaloid showed an rf value close to the comparison of 0.8, blackish brown based on observations under UV light of 254 nm. Different Rf values between the alkaloid standard and the sample are possible because of the different types of alkaloids between the standard and sample. The literature describes the typical alkaloids in the genus Caricaas are carpain group.

Test antihypercholesterol activity

The research method of anti-hypercholesterolemic test activities has been approved by the UNISSULA Semarang Medical Research Medical Commission, with certificate number: No.380 / VII / 2019 / Bioethics Commission. The hypercholesterol-inducing agents used high cholesterol feed (hypercholesterolemic feed). The feed was made from a mixture of pork oil: quail yellow with a ratio of 1: 5 (Wahjuni et al. 2016). Cholesterol content in 100 grams of pork is 97 grams and cholesterol in the yolk quail is 844 mg/dl has been shown to increase rat cholesterol levels. The results of the study (Table. 1) showed that all groups except the normal control group experienced significantly increase after induction of hypercholesterolemic feed. The results of the pretest showed that ratsthat were not given hypercholesterolemic feed did not experience increasing total cholesterol levels This is consistent with Rattusnorvegicus clinical data which explains that the total cholesterol level in normal mice is 54-74 mg/dl.

Table 1. Results of measurement of cholesterol levels (mg / dL ± SE) after induction of induction and after therapy

Treatment	Pretest (mg / dl)	Post test (mg / dl)
Normal control	71.75 ± 4,323	74.75 ± 3,897
Negative control	83.5 ± 3,500	98.75 ± 10,353
Positive control	160.25 ± 7,462	118.00 ± 12,865
EBK 200mg / kg	120.25 ± 10,280	101.00 ± 7.874
EBK 400 mg / kg	117.50 ± 4,153	88.50 ± 5.766
EBK 600 mg / kg	107.00 ± 21,943	82.25 ± 9.364

Note : EBK = karika seed extract

After the therapy period from days 7-14, there was a statistically significant decrease in total cholesterol levels in all groups, except the normal and negative control groups. The increase in the normal group was still in accordance with the range of normal total cholesterol in mice. The increase in the total cholesterol level of the negative control group was due to the absence of drug therapy given. So that mice experience hypercholesterolemia. This showed the success of the method, that the induction of high-fat feed in the control group that was not given therapy for days 7 to 14 experienced an increase in cholesterol levels.

The percentage reduction in cholesterol levels was followed by statistical analysis using the One-Way ANOVA, to know the significant differences between treatment groups. The results showed there were significant differences ($p < 0.05$) between treatments. Statistical testing was continued with the *Post hoc* test, to find out which groups had significant differences in experimental. The results of the average percentage reduction in cholesterol levels showed significantly ($p<0.05$) between the

variations in the dose group of karika seed extract with the normal and negative groups. Karika seed extract dose of 200, 400, and 600 mg/kgbw statistically there was no significant difference with the positive control group (p> 0.05). This showed that the secondary metabolites of karika seeds have the ability to reduce cholesterol levels (Table 2). Its effects were also comparable with statin drugs commonly used for the treatment of hypercholesterolemic.

Table 2. The average percentage reduction in cholesterol levels after therapy

Treatment	Average% decrease in cholesterol levels
Normal control [a]	-6±4.573[c.d.e.f]
Negative control [b]	-18.00±15.019[c.d.e.f]
Positive control [c]	26.00±9.018[a.b]
EBK 200mg / kg [d]	15.00±11.619[a.b]
EBK 400 mg / kg [e]	24.00±8.813[a.b]
EBK 600 mg / kg [f]	21.00±19.471[a.b]

Note: Significance results are based on One Way Anova statistical test results followed by Post Hoc Test. The index shows significant differences between groups

(p <0.05)

The percentage decrease in EBK cholesterol levels of 600 mg/kgbw can only reduce by 21% compared to EBK 400 mg/kg bw (24%). This often happens in the development of new drug candidates, where there is an optimum dose that is the effect of the maximum pharmacological response at certain doses (Purwati et al. 2016). Based on these data, a dose of EBK 400 mg/kgbw was more effective as a decrease in total cholesterol than EBK 600 mg/kgbw. This dose, when converted into human doses, results in high doses. Future studies are focused on finding the effective dose and safety profile of karika seeds. So that karika seeds can be developed as useful candidates for herbal medicines, but with cheap raw materials, and are easy to obtain.

The content of the alkaloid, quercetin, and rutinesekonder metabolites in karika seeds were thought to have cholesterol-lowering activity. The results of the literature explain that berberine(alkaloidsisokuinolon) can reduce levels of co lesterol LDL, cholesterol in total. Its mechanism of action is almost similar to statins (Derosa et al. 2014). Quercetin has been used as a treatment for dyslipidemic patients by increasing HDL levels from 1.29 mmol / l to 2.91 mmol / l. The mechanism of action of flavonoids is to reduce cholesterol synthesis in the function of the ACAT enzyme (*acyl-CoA cholesterol acyl transferase)* on HepG2 cells and the CoA enzyme (3-hydroxy-3-methyl-glutaril) (Li et al. 2016).

CONCLUSION

The antihypercholesterol effectiveness of karika seed extract at a dose of 400 mg/kgbw is better than the variation of doses of 200 and 600 mg/kgbw and it is not significantly different from the positive control group.

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