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Research Article

Potential Role of *Carica papaya* Leaves in Regulating Cholesterol as Inhibitor HMG COA Reductase in the Liver on Wistar Rats Hyperlipidemia

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ABSTRACT

Objectives: Cardiovascular disease is the leading cause of mortality worldwide. One of the main risk factors for cardiovascular diseases is hyperlipidemia. The aim of this study was to determine the inhibition of HMG-CoA Reductase enzyme activity of Carica papaya (*Carica papaya* L.) ethanol extract to decrease cholesterol levels in the blood. Methods: This study was performed on 24 male Wistar rats aged two months weighing 200-250 g randomly divided into 6 groups, that was group 1 (negative control group), group 2 (the positive control group), group 3 (Simvastatin 0.9 mg /kg), and group 4-6 (extract at a dose of 50, 100, and 200 mg/kg). All groups, except group 1, received drinking water containing 25% D-Fructose for 21 days. On day 21, each group was measured for total cholesterol, LDL, HDL and triglycerides levels in the blood. The rats were sacrificed and its liver was isolated for HMG CoA reductase activity measurement. Results: The results showed that papaya leaves extract at a dose of 200 mg/kg decreased the levels of total cholesterol, triglycerides, LDL cholesterol and increased HDL cholesterol. The IC50 of ethanol extract of papaya leaves as HMG CoA reductase inhibitor was 243 mg/kg. Conclusions: Based on these results it could be concluded that the ethanol extract of papaya leaves (*Carica papaya* L.) had antihyperlipidemic activity, one of its mechanism action was inhibiting the activity of HMG CoA reductase, the enzyme that plays a role in the synthesis of endogenous cholesterol.

Keywords: Antihyperlipidemic, Cholesterol, *Carica papaya* L., Fructose, HMG CoA reductase.

INTRODUCTION

Cardiovascular disease is the leading cause of death worldwide. Based on data from the World Health Organization (WHO) reported that hyperlipidemia is a major risk factor for cardiovascular disease. An estimated 17.5 million people died from cardiovascular disease in 2012, with a percentage of 31% of all deaths in the world. An estimated 7.4 million were due to coronary heart disease and 6.7 million were due to stroke. The initial stage of coronary heart disease (CHD) is hyperlipidemia[1-2].

Hyperlipidemia is an increase in total cholesterol, low-density lipoprotein cholesterol (LDL), or triglycerides, and decreased high-density lipoprotein cholesterol (HDL), or a combination of both in the blood. Hyperlipidemia and hypertension as risk factors for cardiovascular disease are related. A person who is overweight and hyperlipidemia have a great risk of developing hypertension. Hypertension is a common cardiovascular disease, which means a persistent rise in blood pressure. Hypertensive patients had a systolic blood pressure greater than or equal to 140 mmHg or diastolic blood pressure over 90 mmHg, or both[3].

Hypertension and hypertriglyceridemia is an important component of the metabolic syndrome. Therefore, the increasing prevalence of these components causes a metabolic disease. The relationship between blood pressure, central obesity, and high levels of insulin observed in adults have also been detected in children and adolescents. The increasing prevalence of hyperlipidemia will be associated with an increased prevalence of hypertension, and it can raise cardiovascular risk factors. Therefore, cardiovascular disease can be prevented by lowering hyperlipidemia as a primary risk factor[4].

Humans consume fructose in the diet mainly from fresh fruits and vegetables about 16-20 g per day, since thousands of years ago. Fructose is widely used as a sweetener by the food and beverage industry such as soft drinks, pastries, cookies, gums, jelly, dessert in the form of high fructose corn syrup (HFCS). Fructose has the sweetest taste amongst other types of carbohydrates, even 1.7 times sweeter than sucrose at a relatively cheap price. Fructose intake over 25% of energy consumption per day (approximately 85 g fructose) causes hypertriglyceridemia. Along with the increase in consumption of foods and beverages containing HFCS, an increase in the prevalence of various symptoms of metabolic syndrome such as dyslipidemia, central obesity, hypertension, hyperuricemia, and diabetes mellitus type II. In a previous study reported that mice fed a diet containing high fructose (66%) increase in blood pressure within 15 days. Provision of fructose per day by 25% in the drinking

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Tabel 1: The active compound contained in the leaves of dried and extract *Carica papaya.*

No.	Active compounds	Results in Dried leaves	Dried extract
1	Alkaloid	+	+
2	Flavonoid	+	+
3	Kuinon	+	+
4	Tanin	+	+
5	Saponin	-	-
6	Steroid	+	+
7	Triterpenoid	+	+

(+) = detected; (-) = not detected

Tabel 2: Results of Papaya Leaves Extract characterization (*Carica papaya* L.).

No.	Characterization of extract	Results (%)
1	Water content	8.5
2	Total ash	10.1
3	Water-soluble extract content	61.3
4	Ethanol-soluble extract content	79.8

water for 21 days significantly increases hyperlipidemia in rats[5-9].

The increasing prevalence of hyperlipidemia each year in Indonesia shows that existing therapies have not been able to control hyperlipidemia. This is an opportunity to find new drugs that are safer and more effective in treating hyperlipidemia, one of which is the use of natural medicine in Indonesia, namely papaya (*Carica papaya* L.).

Papaya is a fruit crop, grown in moist fertile soil and stagnant water. Papaya plant parts used as medicine are fruit, leaves, seeds, roots. Previous studies reported that papaya (*Carica papaya* L.) has several pharmacological activities including antioxidant, anticancer, anti-inflammatory, treatment for dengue, anti-diabetic, healing wounds, antifertility, and anticoagulants, and is used for the treatment of colon cancer, arthritis, and prostate cancer prevention. *Carica papaya* (*Carica papaya* L.) contain alkaloids carpainin, carpain, pseudocarpain, vitamin C and E, choline, and carposide. *Carica papaya* also contains minerals such as potassium, calcium, magnesium, copper, iron, zinc, and manganese. In addition, papaya also contains the alkaloid compounds include carica xanthine, violaxanthin, papain, saponins, flavonoids, and tannins[10-12].

The compounds in papaya leaves suspected to have activity as anti-hyperlipidemia is a flavonoid. A previous study, Leopoldini, et al. (2010) reported that flavonoids work as statins, namely as HMG CoA reductase inhibitor. Based on this background, led to the hypothesis that the ethanol extract of the leaves of papaya (*Carica papaya* L.) has an influence on the activity of HMG-CoA reductase enzyme that can lower cholesterol levels. Therefore, this study aimed to determine the effect of papaya leaves on the activity of HMG-CoA reductase in the liver, and total cholesterol, LDL, HDL, and triglycerides level in the blood.

MATERIALS AND METHODS

Plant materials

The *Carica papaya* L. leaves were obtained from Plantation Manoko, Lembang, Bandung, West Java, Indonesia. Plants have been determined in the Laboratory of Biology, Faculty of Science, Padjadjaran University, Bandung, West Java, Indonesia.

Extraction and Phytochemistry analysis

The *Carica papaya* L. leaves were extracted using 96% ethanol by maceration for 3 days. The filtrate was filtered, and concentrated using a rotary evaporator at 60 ° C and dried using a water bath at 60 ° C. Phytochemical analysis carried out on botanicals and extracts of papaya leaves (*Carica papaya* L.). Analysis of phytochemistry aimed to determine the class of secondary metabolites contained in papaya leaves (*Carica papaya* L.) such as alkaloids, flavonoids, tannins, saponins, steroids/triterpenoids, and quinones.

Animals model hyperlipidemia and treatment

This study conducted on Wistar male rats 2 months old weighing 200-250 g were obtained from D'Wistar (provider of test animals), Majalaya, Bandung, West Java, Indonesia. Animals were acclimatized for 25 days in a cage with standard feed and drinking water ad libitum, and 12 hours of dark and light cycle was maintained. All procedures were performed on animals during the study was approved by the Ethics Committee of the Faculty of Medicine, Padjadjaran University, Bandung, West Java, Indonesia (No: 249 / UN6.C1.3.2 / KEPK / PN / 2016).

The animals were randomly divided into 6 groups (n = 4) consists of a group 1 (normal group received a drug carrier), group 2 (positive control group received a drug carrier), group 3 (received simvastatin 0.9 mg/kg), 4-6 group (received 3 doses of papaya leaves extract variation of 50, 100, 200 mg/kg). All group of treatment (except the normal group) received 25% D-Fructose in drinking water in conjunction with the administration of the test drug orally for 21 days.

Blood collection and biochemistry analysis

Blood sampling performed on day 22. Before the blood was taken, on day 21, the mice were fasted for ± 12 hours but still given water to drink. After blood is drawn, allowed to stand for 30 minutes, then centrifuged at 3000 rpm for 5 minutes. Serum was separated for testing of total cholesterol, triglycerides, HDL, and LDL. Total cholesterol and triglycerides were determined enzymatically using a reagent kit Proline®, while measurement of HDL and LDL cholesterol levels using a reagent kit Sekisui®. The color change in the sample was measured using a MicroLab 300®.

HMG CoA reductase activity assay

Tests carried out on liver homogenates isolated from test animals. Animals were sacrificed, and the liver is isolated and cleaned using 0.9% NaCl, then dried with tissue paper and weighed. Liver cut into small pieces to make 20% liver homogenates in phosphate buffer pH 7.4 and centrifuged at 8000 rpm. The supernatant was separated from the residue in the sample (homogenates). 50 mL of liver homogenates were mixed with 260 mL of phosphate buffer pH 7.4, 42 mL of 50 mM Na 2 EDTA solution, 60 mL solution of dithiothreitol (DTT) 100 mM (purchased from Sigma), 50 mL of 2.16 M KCl solution, and 12 mL of

Tabel 3: The average value of total cholesterol and triglycerides at day 0 and day 21.

Group	Mean of (mg/dL) ± SD (standard deviation)					
	Total cholesterol level		Difference (%)	Triglicerides level		Difference (%)
	t0	T21		t0	t21	
1	50.0±14.0	57.0±2.0*$^{\alpha}$	14	72.5±12.3	64.6±4.9*$^{\alpha}$	-11
2	44.0±9.2	156.0±2.1	256	68.4±9.8	132.0±3.6	93
3	42.0±7.6	53.6±3.9*	28	70.0±6.2	75.6±4.4*	8
4	42.8±11.3	66.4±4.3*	55	65.8±7.9	136.9±3.0	108
5	49.7±8.2	72.2±1.6*	45	66.3±10.5	138.8±10.4	109
6	47.5±8.6	57.8±4.5*$^{\alpha}$	22	63.7±16.9	85.4±2.7*$^{\alpha}$	34

and 6 (received papaya leaves extract dose of 50, 100, and 200 mg/kg bw respectively).
* = indicated significantly different compare to positive control group (group 2).
$^{\alpha}$ = indicated significantly different compare to control group standar drug (group 2) (P>0.05).

Tabel 4: The average value of total cholesterol HDL and LDL at day 0 and day 21.

Group	Mean of (mg/dL) ± SD (standard deviation)					
	HDL level		Difference (%)	LDL level		Difference (%)
	t0	t21		t0	t21	
1	14.4±4.0	14.6±2.2*$^{\alpha}$	1	19.6±4.8	28.7±1.0*$^{\alpha}$	46
2	15.0±2.6	3.8±1.1	-75	19.60±1.5	129.3±0.8	560
3	13.1±2.1	15.1±2.8*	15	23.6±1.5	28.3±0.5*	20
4	16.6±4.7	18.4±4.0*$^{\alpha}$	11	23.6±5.1	23.1±1.6*	-2
5	15.0±4.5	22.7±2.1*	52	18.7±5.9	24.9±1.6*	33
6	9.6±3.7	20.4±3.7*$^{\alpha}$	112	20.5±5.0	23.8±1.2*	16

Group 1 and 2 (received 1% Na-CMC), group 3 (received Simvastatin 0.9 mg/kg bw), group 4,5 and 6 (received papaya leaves extract dose of 50, 100, and 200 mg/kg bw respectively).
* = indicated significantly different compare to positive control group (group 2).
$^{\alpha}$ = indicated significantly different compare to control group standar drug (group 2) (P>0.05).

HMG CoA substrate solution (purchased from Sigma) is then mixed using a vortex for 10 seconds and incubated at 37 ° C for 30 minutes. After an incubation period, then added 55 mL of 1.105 mM NADPH (purchased from Sigma) and remixed using a vortex for 10 seconds and then incubated for 10 minutes. The absorbance of the sample measured at a wavelength of 340 nm using a MicroLab 300®.

Statistical analysis

The data were analyzed statistically (one way ANOVA) with a significance value of p <0.05.

RESULTS

Extraction of papaya leaves by maceration produces a yield of 6.24%. Results of phytochemical screening on dried leaves and the ethanol extract of papaya leaves can be seen in Table 1. The results of measurements on the dried extract of papaya include moisture content, total ash, water-soluble extract content, and the levels of soluble extract ethanol, can be seen in Table 2.

Level of total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride at T21 compared to T0 for all treatment group can be seen in table 3 and 4. The activity of the HMG-CoA Reductase enzyme for all treatment group at T21 expressed in percentage of inhibition can be seen in figure 1.

Group 1 and 2 (received 1% Na-CMC), group 3 (received Simvastatin 0.9 mg/kg bw), group 4,5

DISCUSSION

Anti-hyperlipidemia activity

Statistical test results that the average level of total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol at T0 showed no significant difference (P> 0.05) (Table 3 and 4). It showed that the lipid profile in all treatment groups at T0 is equal. While the average level of lipid profile in T21 showed significant differences (P <0.05) are marked with (*), which indicates that there are differences in levels of lipid profile at t21.

Statistical test results Bonferonni comparing lipid profiles normal control group against the positive control group at day 21 showed significant differences (P <0.05), which means that the administration of 25% D-Fructose raising levels of total cholesterol, triglyceride, LDL cholesterol and lowering HDL cholesterol levels with a percentage increase of total cholesterol, triglyceride, LDL cholesterol levels were 64%, 51%, 74% and a decrease in HDL cholesterol level by 78%.

HMG CoA Reductase activity

Provision of papaya leaves extract on a group of 4-6 showed a decrease in the activity of the enzyme HMG-CoA Reductase with the percentage of inhibition were 17, 23, and 41% respectively (Figure 1). A decrease in NADPH absorbance is due to the inhibition of the activity of the enzyme HMG-CoA Reductase by the papaya extract in which the activity of this enzyme always requires NADPH as a cofactor. NADPH undergo oxidation-reduction reactions which will be converted into NADP + and produce a color when the enzyme HMG-CoA reductase actively change the HMG-CoA into mevalonic acid and then converted into cholesterol. Provision of papaya leaves extract for 21 days can inhibit the activity of

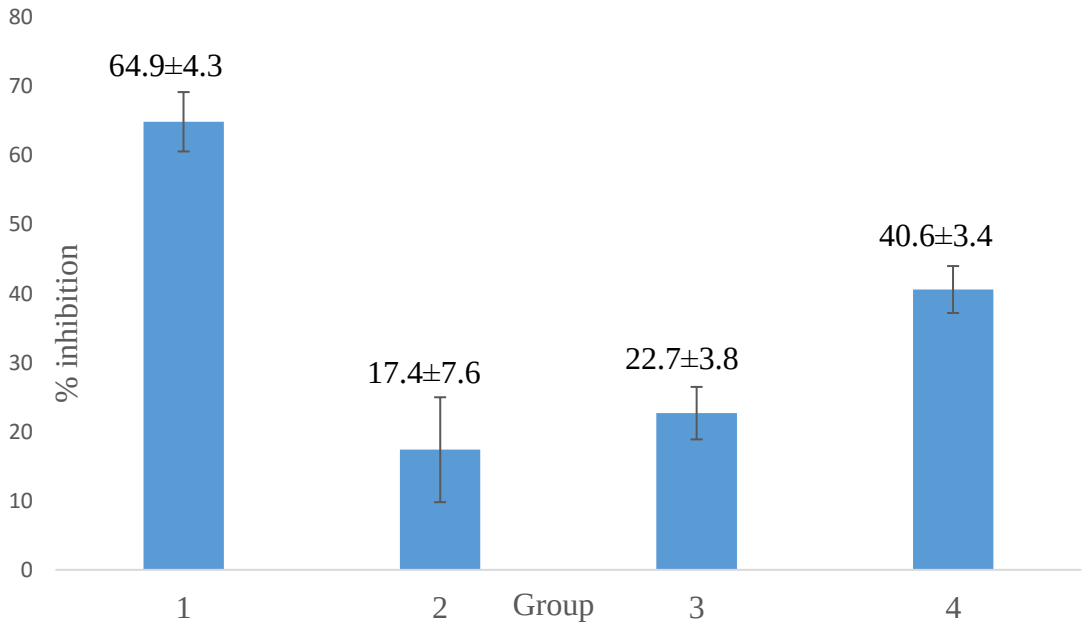

Figure 1: The percentage inhibition of HMG-CoA Reductase Activity for all treatment groups compared to control group.
Group 1 (received Simvastatin 0.9 mg/kg), group 2, 3, and 4 (received papaya leaves extract dose of 50, 100, and 200 mg/kg respectively)

the enzyme HMG-CoA reductase in the liver of test animals, so the use of NADPH by the enzymes will decrease, causing the formation of cholesterol also decreased.

Figure 1 showed that the increase in percent inhibition in accordance with the increase in the dose administered. It showed that the higher dose of papaya leaves, the higher of the inhibitory effect on the enzyme.

Based on data obtained from the percentage of inhibition, obtained linear equations to calculate the IC50 (50% of inhibitory concentration) value of the test extract. The IC50 value indicates the concentration of test extracts to produce inhibition of HMG-CoA Reductase activity by 50 percent. Obtained linear equation Y = -5.134 0,227X with r = 0.972. R-value is the value correlativity between two variables, in this case, the variable is the test dose and the percentage inhibition (% inhibition). R values obtained from the linear regression equation was 0.972, showing a relationship (correlativity) between variables X (dose) to Y (% inhibition) has strong correlativity. The calculation result is obtained that the IC50 value of the ethanol extract of papaya is 243 mg/kg.

The same study using the method of induction 25% D-fructose for 21 days conducted by R. Borate, et al., 2011 showed an increase in total cholesterol, triglyceride, LDL cholesterol levels respectively 32%, 41%, 56% and a decrease in HDL cholesterol by 47%. They reported that dietary fructose leads to hyperlipidemia because fructose is metabolized to "glycerol-3-phosphate" and "acetyl CoA". Both metabolites are then used as a substrate for synthesis of glycerides, which contribute to the production of VLDL-TG in the liver[9].

The ethanol extract of leaves of papaya (*Carica papaya* L.) can reduce levels of total cholesterol, triglyceride, LDL cholesterol levels and raise HDL levels in the blood. This suggests that the compounds contained in the ethanol extract of *Carica papaya* (*Carica papaya* L.) leaves have activity as antihyperlipidemic. Flavonoids contained in papaya leaves suspected to have the same mechanism as statins in lowering cholesterol levels in the blood that is an inhibitor of the enzyme HMG Co-A reductase, an enzyme that plays a role in cholesterol synthesis in the liver[13].

Flavonoids in papaya leaves, especially quercetin, believed to have antihyperlipidemic activity by increasing LDL receptor expression. Our results showed that the ethanol extract of papaya better at lowering LDL cholesterol than others lipoprotein levels (table 3 and 4)[14-15].

Flavonoids (naringenin) in papaya leaves are also thought to have the activity in lowering TG with the mechanism of action induces the expression of peroxisome proliferator-activated receptor alpha (PPARs-α). PPARs are receptors that regulate gene transcription. As a result of the interaction of compounds with PPAR-α, an increase in fatty acid oxidation and synthesis of LPL (lipoprotein lipase) results in increased clearance of TG-rich lipoprotein[16-17].

Gogna N. (2015) reported that naringenin compound content in papaya leaves young age is greater than in the old papaya leaves. Our research used papaya leaves that are not too young nor too old so that the activity in lowering triglyceride levels only occur at doses of 3 (200 mg/kg) probably because the compound naringenin at doses of 1 and 2 is too small so that triglyceride levels decreased is not statistically significant.

This study is in line with other studies that reported a water extract of papaya leaves can reduce levels of lipid profile at doses of 400 mg/kg. Water extract of papaya in lowering lipid levels require higher doses than the ethanol extract.

When used water as a solvent so that the compound is extracted only the polar (water soluble), therefore fewer compounds extracted compared with ethanol as a solvent. Ethanol is the universal solvent that can dissolve the compounds that are polar, semi-polar and non-polar so that the compound is extracted by ethanol will be greater, and therefore the ethanol extract requires smaller doses as antihyperlipidemia[18].

The results of this study showed that the ethanol extract of papaya leaves inhibit the activity of the enzyme HMG-CoA Reductase. A compound thought to inhibit the enzyme HMG-CoA Reductase are flavonoids and tannins. As research conducted by Leopoldini (2010) computationally reported that the flavonoids act as a statin drug class (inhibitor of HMG-CoA Reductase activity). According to the research of Gyeong Min-Do, et al. (2011), tannin is an agent that can reduce the activity of the enzyme HMG-CoA Reductase. Both of these compounds contained in papaya extract (Table 1) which may play a role in inhibiting the activity of the enzyme HMG-CoA Reductase[13,19].

CONCLUSION

The present study has shown that the ethanol extract of papaya leaves (*Carica papaya* L.) had antihyperlipidemic activity, one of its mechanism of action was inhibiting the activity of HMG CoA reductase, the enzyme that plays a role in the synthesis of endogenous cholesterol.

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