PharmacologyOnLine December 30, 2016

Archives • 2016 • vol.3 • 15-21

THE STUDY OF RED GINGER RHIZOMES ETHANOL EXTRACT (*ZINGIBER OFFICINALE* ROSCOE VAR. *SUNTI* VAL.) ON HYPERLIPIDEMIC-INDUCED RATS

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Abstract

One of the main causes of cardiovascular disease is hyperlipidemia, an elevation of lipid in the blood. Lipids are major components of atherogenic plaques and many studies have proven that lipids disrupt the structure and function of blood vessels by forming lipid layers in the endothelial wall. Therefore, hyperlipidemia is a risk factor for atherosclerosis. This study aims to determine activity of red ginger rhizomes ethanol extract (RGREE) in hyperlipidemic rats model. Hypercholesterolemic model was conducted in vivo in male Sprague-Dawley rats which were induced by administration of high-cholesterol diet, cholic acid, and propylthiouracil (PTU). Activity of the extract was evaluated by measuring lipid profile according to enzymatic reaction through UV spectrophotometry methods compared to simvastatin. Aorta from each rat was isolated and stained by Hematoxyllin-Eosine for further observation under optical microscope. Group treated with RGREE 100 mg/kg bw decreased the total cholesterol and triglyceride level by 15.83% ± 6.46% and 37.98% ± 54.99% also increased the HDL level by 7.52% ± 22.43%. Group treated with RGREE 400 mg/kg b. w. decreased the total cholesterol and triglyceride level by 25.51% ± 18.22% and 54.54% ± 32.72%, increased the HDL level by 33.93% ± 42.61%. Simvastatin treated group showed that there were reduction in term of total cholesterol and triglyceride level by 17.81% ± 12.66% and 49.17% ± 40.37% respectively. RGREE decrease total cholesterol level significantly compared to simvastatin.

Key words: red ginger, hyperlipidemia, atherosclerosis, simvastatin

Introduction

Every 36 seconds, one person died because of cardiovascular disease. In 2002, cardiovascular disease played a role by a third against the deaths that occurred in the world, which is expected to become major cause of death in 2020[1]. One of the main causes of cardiovascular disease is hyperlipidemia, a condition characterized by elevations of one or more lipoproteins in the blood circulation, such as cholesterol, cholesterol esters, phospholipids, or triglycerides [2]. Hyperlipidemic conditions will trigger the formation of atherogenic plaques that cause cardiovascular disease. Chronic inflammation characterized by the formation of foam cell contained atherogenic plaques is the hallmark of atherosclerosis, in which further it will inhibit blood circulation [3]. Many studies proved that lipids disrupt the blood vessels structure and its functions due to the formation of atheroma that may trigger cardiovascular diseases such as coronary heart disease, cerebrovascular ischemia, and peripheral vascular disease (PVD) [4]. Therefore, the needs to develop lipid-lowering agents have risen. Red ginger is one of natural herbs that are potentially employed for lowering cholesterol levels. Red ginger is a widely grown plant in Indonesia and has been used as a traditional medicine for generations Red ginger contains flavonoids and polyphenols that can prevent the formation of free radicals, possess hypolipidemic effect, and suppress 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase enzyme activity that plays a role in lipid synthesis [5]. Therefore, this study was conducted to determine the effect of red ginger extract for ameliorating lipid profile in hyperlipidemic model of rats.

Methods

Plant material and preparation of extract

Fresh ginger rhizomes of red ginger were obtained from Subang, West Java, Indonesia, on January 2015. The rhizomes were identified in *Herbarium Bandungense*, School of Life Sciences and Technology, Bandung Institute of Technology, Indonesia. The rhizomes of red ginger were dried by oven in temperature of 30 ^{o}C – 50 ^{o}C and were extracted with ethanol 96% by reflux apparatus. Liquid extract was then separated through filter paper and the filtrate was concentrated by using rotary evaporator. The concentrated extract further will be referred as ethanolic extract of red ginger (RGREE) and it was stored at the cold room of 4 °C in a container protected from sunlight until analyzing time.

Chemicals

Ethanol 96%, Dragendorff reagent, Mayer reagent, Steasny reagent, Liebermann-Burchard reagent, Hematoxyllin-Eosine reagent, formalin buffer, cholesterol, cholic acid, propylthiouracil, Na CMC, Total Cholesterol Kit, Triglyceride Test Kit, HDL Test Kit from Sclavo Diagnostic®. All chemical reagents used were pharmaceutical grades.

Phytochemical screening of rhizome extract

The red ginger rhizomes ethanol extract (RGREE) was subjected to pass preliminary phytochemical screening for secondary metabolites.

Animals

Male Sprague-Dawley rats with age of 2-3 months and had approximately 140-200 gram in weight were utilized in this study. They are provided by NA-DFC (National Agency of Drug and Food Control) of Indonesia. All methods conducted have been approved by Animal Ethics Committee of School of Pharmacy ITB with the document number of 01/KEPHP-ITB/04-2015.

Protocols

All rats were acclimated for 7 days before the experiment. Animal laboratories received high-fat diet and along with that, they were given cholic acid 0.1%, cholesterol 100 mg/kg and propylthiouracil 2 mg/kg (suspended on Na CMC 0.3%) for a week before treatment and 2 weeks during treatment. However, the control group was given a standard chow animal laboratory. Induction was carried out for 7 weeks and it was followed by the treatment for the next 2 weeks. There were 4 groups in this experiment including the control group which received vehicle solution only (CMC Na 0.75%) during the periods, RGREE treated group with a dose of 100 mg/kg bw, RGREE treated group with a dose of 400 mg/kg bw, and simvastatin treated group with a dose of 25 mg/kg bw All dosage forms were given once daily after induction.

Biochemistry measurements

Total cholesterol, triglyceride and HDL levels were measured before (which was referred as T0) and after induction (T7), as well as 2 weeks after treatment (T9). Blood was collected from each rat and was centrifuged for 10 minutes, 10 000 rpm. Pellet cell was withdrawn and serum was transferred into a new tube and then stored in the refrigerator -20 °C before analyzing time.

ISSN: 1827-8620

Upon thawing, serum was incubated in 37 °C for 10 minutes. Total cholesterol and triglyceride levels were measured by adding 5 µl serum with 500 µl reagent from Sclavo Diagnostic® and incubated for 10 minutes. Absorbance levels were quantified by spectrophotometer Photometer 5010 V5+, λ 546 nm. For HDL levels measurement, mixture of 50 µl serum and 5µl HDL reagents from Sclavo Diagnostic® were centrifuged for 15 minutes, 3000 rpm. Taking 50 µl supernatant and adding 500 µl total cholesterol reagent, the solution was incubated in 37 °C for 10 minutes before it underwent measurement. Absorbance levels were quantified by spectrophotometer Photometer 5010 V5+, λ 546 nm.

Aorta histology

In the end of the experiment periods, all rats were sacrificed in a chamber allowing CO_2 to flow inside. Upon death confirmation, the heart and aorta were isolated and immersed on 10% formalin buffer. The aorta were sliced by 5 µm thickness, colored with Hematoxylline-Eosine and observed by microscope.

Statistical analysis

Statistical analyses were conducted by Statistical Package for the Social Sciences (SPSS®) software, Version 16.0 for Windows®. Lipid profiles were presented as mean ± standard deviation (SD). Lipid profiles comparison and its change were calculated by independent sample T-test method. Mean aorta wall thickness were calculated by one way ANOVA method.

Results

The yield of RGREE was 13.74 % There were three secondary metabolites found in RGREE (Table 1). Total cholesterol (Table 2), triglyceride (Table 3), and HDL (Table 4) levels showed significant differences compared to control group after 2 weeks treatment with RGREE. The aorta wall thickness (Table 5 and Figure 1) also showed a significant decrease in RGREE and simvastatin treatments.

Discussion

Phytochemical screening of RGREE clearly indicated flavonoid, phenol, and steroid/ triterpenoid compounds existence. This result suggested that the antihyperlipidemic effect of RGREE may be related to its flavonoid constituents [6].

High-fat diet induced hyperlipidemic rats were conducted by giving cholic acid and propylthiouracil. Cholic acid is able to increase lipid absorption and attenuate bile acids productions causing high lipid levels on blood circulation [3]. Propylthiouracil inhibits thyroid hormone by blocking iodine oxidation resulting in decrease of lipid metabolism. Propylthiouracil was given to ensure atherogenesis in Sprague-Dawley Rats [7]. The high-fat diet induced hyperlipidemia was successfully demonstrated, which was showed by elevations of mean total cholesterol levels by week 7 ($p<0.05$). Data showed that there is no noticeable reduction in terms of total cholesterol levels in positive control treated with Na CMC 0.3% and simvastatin 25 mg/kg. However, there were significant decrease in total cholesterol levels on group treated by RGREE 100 mg/kg and 400 mg/kg. The percentage of total cholesterol reduction in simvastatin group was higher than that of group treated by RGREE 100 mg/kg bw, even though it was not statistically significant.

Other lipid parameters were evaluated including triglyceride and HDL levels. It is utilized to indicate hyperlipidemia occurrence in patients [8]. A significant decrease on triglyceride levels was shown in rats treated with RGREE 400 mg/kg and simvastatin 25 mg/kg daily. On the contrary, there was no significant elevation on HDL levels of group treated with RGREE 100 mg/kg or 400 mg/kg compared to simvastatin. The mean HDL elevation data could not well represented because of failure in decreasing HDL levels induction. This failure was most likely caused by lack of pure cholesterol dose at induction or induction time needed should be extended. The presence of flavonoid and polyphenol in red ginger extract may contribute to reduction in the total cholesterol and triglyceride levels in both RGREE groups. Flavonoid and polyphenol compounds include quercetin, kaempferol, naringenin, epicatechin, catechin, fisetin, morin, and rutin [9]. Flavonoid and polyphenol could prevent formation of free radicals, possess hipocholesterolemic effect by suppressing HMG-CoA reductase activity [6]. Naringenin affects peroxisome proliferator-activated receptors receptor (PPAR). PPAR receptors inhibit lipogenesis genes regulated by Liver X Receptor α (LXRα), causing a decrease in the work of HMG-CoA reductase enzyme. Decreasing activity of HMG-CoA reductase enzyme leads to decrease the number of apoB containing lipoproteins. It results in reducing levels ofcholesterolester in blood [10][11]. Another RGREE compound that contributed in decreasing lipid in plasma was gingerol. Gingerol causes elevations in antioxidant activity when it is given to rats. Gingerol increases the number of glutathione (GSH) transferase and amplifies adiponectin activity. Increasing activity of GSH transferase leads to

inhibition of LDL oxidation enzyme thereby reducing the possibility of plaque formation in endothelial wall [12]. Aorta histology presents that there is no foam cells in tunica intima of aorta wall. Thickening of aorta wall was observed in a control, RGREE and simvastatin group. However, thicknesses of aorta wall in RGREE treated groups were thinner than that of control group. These results reveal that RGREE may prevent thickening of aorta wall that leads to atherogenic lesion formation. Plaque formation was attenuated by increasing activity of adiponectin. Elevation on adiponectin levels caused by 6-gingerol could attenuate endothelial inflammation and foam cell. Endothelium produces nitric oxide (NO) which is important for maintaining vascular wall from injury, inflammation, and thrombosis by preventing leukocytes adhesion to endothelium, inhibiting cell proliferation and platelet aggregation. When inflammations occurred, NO reacts to reactive oxygen species (ROS) and reduce its levels on blood vessels [13][14].

Conclusion

Red Ginger Rhizomes Ethanol Extract (RGREE) given by doses of 100 mg/kg and 400 mg/kg daily has ability to improve lipid profile including decreasing cholesterol and triglycerides levels, as well as increasing HDL levels on blood plasma. RGREE 400 mg/kg showed better activity to lowering cholesterol than that of lower dose and simvastatin group. Aorta histology revealed RGREE treatment with both dosage (100 mg/kg and 400 mg/kg daily) attenuated aorta wall thickening caused by high lipid in blood plasma.

Acknowledgments

This study was supported by Bandung Institute of Technology (ITB) on Riset Inovasi KK with the document number of: 1059.2-I1.C03-KU-2015.

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Table 1: Phytochemical screening of RGREE

Secondary metabolite	Result
Flavonoid	+
Tannin	-
Phenol	+
Quinone	-
Saponin	-
Alkaloid	-
Steroid/Triterpenoid	+

+: Detected; -: Undetected

Table 2: Measurement of total cholesterol levels.

Treatment	Mean Total Cholesterol Levels (mg/dl)			Percentage of reduction (%)
	To	T7	T9	
Control	64.4 ± 12.76	109.80 ± 19.74	89.3 ± 1.53	12.74 ± 12.90
RGREE 100 mg/kg	69.00 ± 12.88	116.25 ± 12.82	97.5 ± 8.85[a]	15.83 ± 6.46
RGREE 400 mg/kg	55.60 ± 9.45	112.40 ± 43.74	77.8 ± 7.40[a]	25.51 ± 18.22
Simvastatin 25 mg/kg	48.25 ± 9.11	104.75 ±19.16	84.75 ± 9.88	17.81 ± 12.66

T: time post-induction (day); [a]Significantly different compared to T7 ($p<0.05$). T7: after 7 weeks induction; T9: after 2 weeks treatment; Control: mice treated with Na CMC 0.3% daily; RGREE 100 mg/kg: mice treated with red ginger rhizomes ethanol extract with dose 100 mg/kg daily; RGREE 400 mg/kg: mice treated with red ginger rhizomes ethanol extract with dose 400 mg/kg daily; Simvastatin 25 mg/kg: mice treated with simvastatin with dose 25 mg/kg daily

Table 3: Mean triglyceride levels.

Treatment	Mean Triglyceride Levels (mg/dl)			Percentage of reduction (%)
	To	T7	T9	
Control	47.80 ± 16.95	212.00 ± 136.97	173.30 ± 137.00	32.68 ± 33.03
RGREE 100 mg/kg	51.00 ± 12.57	244.50 ± 144.33	101.75 ± 35.67	37.98 ± 54.99
RGREE 400 mg/kg	71.80 ± 36.64	216.80 ± 128.77	73.60 ± 25.62 [a]	54.54 ± 32.72
Simvastatin 25 mg/kg	64.50 ± 12.71	226.25 ± 107.38	82.75 ± 20.45 [a]	49.17 ± 40.37

T: time post-induction (day); [a]Significantly different compared to T7 ($p<0.05$) T7: after 7 weeks induction; T9: after 2 weeks treatment; Control: mice treated with Na CMC 0.3% daily; RGREE 100 mg/kg: mice treated with red ginger rhizomes ethanol extract with dose 100 mg/kg daily; RGREE 400 mg/kg: mice treated with red ginger rhizomes ethanol extract with dose 400 mg/kg daily; Simvastatin 25 mg/kg: mice treated with simvastatin with dose 25 mg/kg daily

Table 4: Mean HDL levels

Treatment	Mean HDL levels (mg/dl)			Increase Percentage (%)
	T0	T7	T9	
Control	56.60 ± 11.41	60.60 ± 9.45	65.67 ± 4.04	3.99 ± 11.54
RGREE 100 mg/kg	73.75 ± 25.13	63.50 ± 14.62	68.25 ± 22.19	7.52 ± 22.43
RGREE 400 mg/kg	54.40 ± 5.32	53.40 ± 5.73	72.00 ± 24.82	33.93 ± 42.61
Simvastatin 25 mg/kg	52.25 ± 2.87	50.00 ± 10.49	71.50 ± 12.92[a]	49.39 ± 48.74

T: time post-induction (day); [a]Significantly different compared to T7 ($p<0.05$). T7: after 7 weeks induction; T9: after 2 weeks treatment; Control: mice treated with Na CMC 0.3% daily; RGREE 100 mg/kg: mice treated with red ginger rhizomes ethanol extract with dose 100 mg/kg daily; RGREE 400 mg/kg: mice treated with red ginger rhizomes ethanol extract with dose 400 mg/kg daily; Simvastatin 25 mg/kg: mice treated with simvastatin with dose 25 mg/kg daily

Table 5: Mean aorta wall thickness

Treatment	Mean aorta wall thickness (µm)
Normal	56.77 ± 6.38
Control Na CMC 0.3%	67.03 ± 14.09
RGREE 100 mg/kg	59.40 ± 7.37
RGREE 400 mg/kg	45.67 ± 3.57[a]
Simvastatin 25 mg/kg	50.22 ± 2.53[a]

[a]Significantly different compared to Control group ($p<0.05$). Normal: without high-fat induction; Control: mice treated with Na CMC 0.3% daily; RGREE 100 mg/kg: mice treated with red ginger rhizomes ethanol extract with dose 100 mg/kg daily; RGREE 400 mg/kg: mice treated with red ginger rhizomes ethanol extract with dose 400 mg/kg daily; Simvastatin 25 mg/kg: mice treated with simvastatin with dose 25 mg/kg daily

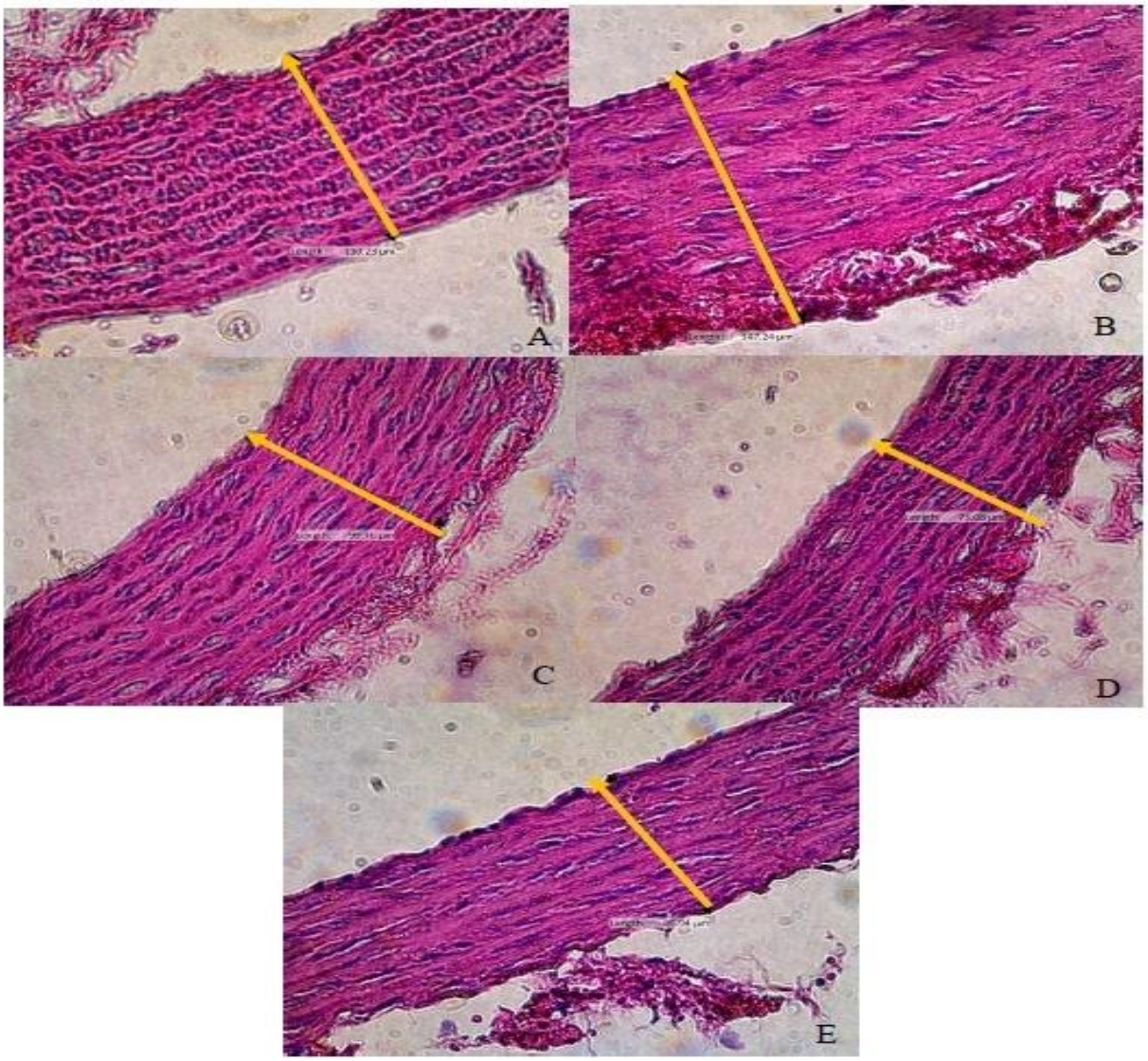

Figure 1: Cross-sectional aorta wall histology. (A) Normal; (B) Control; (C) RGREE 100 mg/kg bb; (D) RGREE 400 mg/kg bb; (E) Simvastatin 25 mg/kg; Magnified up to 400 times.