Antihypercholesterolemia activities of red ginger extract 
(Zingiber officinale Roxb. var rubrum) on wistar rats

S Jovita Nirvana¹, T Widiyani¹², A Budiharjo¹²
¹ Department of Bioscience, Graduate Program, Sebelas Maret University. Jl. Ir Sutami No. 36A, Kentingan, Surakarta,57126, Central Java, Indonesia.
² Faculty of Mathematics and Natural Sciences, University Sebelas Maret. Jl. Ir Sutami No. 36A, Kentingan, Surakarta,57126, Central Java, Indonesia.

Email: jovitashanghnesy@student.uns.ac.id

Abstract. The purpose of this study was to determine the phenolic content of red ginger, determine the effect on lipid profile and body weight. Total phenolic levels were measured by using UV/Vis spectrophotometry with the Folin-Ciocalteau reagent, at absorbance 760 nm wavelength. Hypercholesterolemia rat lipid profile was conducted in vivo by using a kit from Dyasis. Data was analyzed statistically with Two Way ANOVA. Rats were divided into 5 treatment groups with different of red ginger extract (0, 200, 350, 500 mg/kg bw, and simvastatin 7.2 mg/kg bw). Before the treatment of red ginger extract or simvastatin, rats were induced a high fat diet for 28 days. The treatment of red ginger extract and simvastatin were carried out for 2 weeks. The phenolic content of red ginger extract is 5194.15 ± 264.1 µg/g. The results of in vivo test showed that red ginger extract had a significant effect on lipid profile and body weight changes in hyperlipidemia rats at dose 200 mg/kg bw. Doses 200 mg/kg bw is not significantly different (p≥0.05) to positive control group (simvastatin 7.2 mg/kg bw). Total cholesterol levels decreases 47.9%, LDL 32.7% and triglycerides 64.3% and HDL increases 25.8%.

1. Introduction

Hypercholesterolemia is the main cause of atherosclerosis, coronary heart, stroke and causes many deaths around the world [1]. Hypercholesterolemia is abnormal lipoprotein metabolism characterized by increased levels of triglycerides, plasma total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) [2]. In recent years, there have been many in vivo studies and clinical trials showing that phenolic compounds from plants have antioxidant activity that is beneficial in the prevention of hypercholesterolemia [3].

Phenolic compounds in this case reduce the risk of hypercholesterolemia by increasing the activity of antioxidant enzymes and reducing the formation of free radicals. Free radicals are molecules that have unpaired electrons. This compound is very reactive and high-energy, so it can attract electrons from other molecules [4]. Therefore, phenolic compounds are tasked with stabilizing free radicals by providing electrons to supplement the lack of electrons from free radicals, and inhibiting chain reactions. In addition, phenolic compounds with higher hydroxy groups will increase the ability to clean free radicals [5].

Red Ginger (Zingiber officinale Roxb. Var rubrum) contains flavonoids, and phenolics. They inhibit lipid peroxidation, increase antioxidant enzymes that regulate low density lipoprotein (LDL)
receptors and 3-hydroxy-3-methylglutaryl coenzyme-A receptors (HMG-CoA), which affect cholesterol absorption in the liver [13]. The purpose of this study was to determine the phenol content of red ginger extract, determine the effect of body weight and the effect of lipid profiles on the administration of red ginger extract.

2. Experimental

2.1 Materials

Materials used in this research includes rotary evaporator (RVO 400 SD Boeco Germany), waterbath (Grant), UV/Vis spectrophotometric (Perkin Elmer Lambda 25), vortex (Heidolph REAX top), measuring glass (Pyrex), aquades (General), and ethanol p.a (Merck).

2.2 The extract preparation (Zingiber officinale Roxb. var rubrum) uses the maceration method

Red ginger (Zingiber officinale Roxb. var rubrum) obtained from the Health Research and Development Agency Research and Development Center for Medicinal and Traditional Medicinal Plants (B2P2TOOT). 2 kg of red ginger powder soaked with 96% ethanol solvent in ratio 1: 5 (2 kg of powder: 10 L of 96% ethanol). Maceration was done in 3 x 24 hours. Every 24 hours the results of maceration were filtered using a glass funnel that has been given a flannel cloth or filter paper. The filtrate was collected in a separate container while the pulp was re-macerated with a new find. Remaceration was done in 3 times replication. Maceration results were evaporated using a rotary at 40 °C. Results of the evaporation then were heated on a waterbath at 50 °C.

2.2 Test the phenol levels of red ginger extract (Zingiber officinale Roxb. var rubrum).

1000 ppm gallic acid solution is made by dissolving 10 mg of gallic acid into 10 mL methanol as a stock solution. As much as 2.5 mL of stock solution was dissolved in methanol to 25 mL to make gallic acid solution with a concentration of 100 ppm, from this solution as much as 1 mL, 2 mL, 3 mL, 3 mL, and 5 mL into methanol up to 10 mL. 0.5 mL Folin-Ciocalteu reagent was added. Then added 1.5 mL of 20% sodium carbonate diluted with aquabidest until the volume reaches 10 mL. Then wait for more than two hours and move to the cuvette, still absorbing at a wavelength of 760 nm. Measurement of each ginger extract was done by adding 10 mg of ginger extract with 0.5 mL of Folin-Ciocalteu reagent and 7.5 mL watheq. incubated for 5 minutes. Subsequently 1.5 mL of 20% sodium carbonate was added to each solution, and diluted using aquabidest to the limit of the pumpkin marker 10 mL and incubated for more than 2 hours and transferred to the cuvette, still absorbing at 760 nm wavelength.

2.3 Grouping of test animals

In this study, test animals in the form of male white rats Galur Wistar with 25 animals, aged 11-12 weeks, weighing 150-200 grams. Obtained from Wistar rat breeding in Yogyakarta. Test animals were divided into 5 treatment groups. Test animals as research objects have received approval from the Ethics Committee of the University of Muhammadiyah Surakarta with document number EC No. 2388/A.1/KEPK-FKUMS/IX/2019.

2.4 Increased total cholesterol level

Test animals induce high fat diets that produce hypercholesterolemia rats. A high fat diet is administered orally with a composition of 1 mL of lard and 2.5 mL/200 grams of weight of quail egg yolk and drinking water containing Propylthiouracil (PTU) 2 mg/kg bw in all treatment groups. Rats were given a high fat diet for 28 days. Provision of propylthiouracil (PTU) aims to increase cholesterol levels by reducing the amount of thyroid hormone produced by the thyroid gland and blocking the conversion of thyroxine T3. The mechanism for reducing thyroid hormone cholesterol levels is, increasing LDL receptors on liver cells [6].
2.5 Extract treatment
Red ginger extract is given for 14 days (after animal hypercholesterolemia) given as much as 2 mL/200 grams bw morning and evening. The dosage of treatment is as follows:

- Group 1: Red ginger 0 mg/kg bw.
- Group 2: Simvastatin 7.2 mg/kg bw.
- Group 3: Red ginger extract of 200 mg/kg bw.
- Group 4: Red ginger extract of 350 mg/kg bw.
- Group 5: Red ginger extract of 500 mg/kg bw.

The treatment dosage of ginger extract refers to the modified [7].

2.6 Weight weighing and serum preparation
Weighing the wistar rat body weight was done on days 0, 28, and 42, by weighing each wistar rat using a scale. Whereas the blood sample was taken on days 0, 28 and 42. Before the blood was taken the rats were fasted for 12 hours, but were still given drinking water. Blood samples were taken as much as 1.5 mL from the eye vein. The sample was then put into a vacutainer vacuum tube containing EDTA then centrifuged at a speed of 3000 rpm for 10 minutes. Blood is separated from serum and plasma. The serum was separated from eppendorf tube and then stored in a refrigerator at 20 ºC before being analyzed.

2.7 Determination of plasma total cholesterol levels
Measurement of plasma total cholesterol by the CHOD-PAP enzymatic colorimetric and following the procedure from the DiaSys (Diagnostic System International) kit. Blood serum was taken as much as 10 μL, added cholesterol reagents FS diasys 1000 μL then vortex. The solution was incubated at 25 ºC for 20 minutes. Furthermore, it was measured using a microlab 300 spectrophotometer with a wavelength of 500 nm.

\[
\text{Total cholesterol mg/dl} = \frac{\Delta \text{serum absorbance}}{\Delta \text{standard absorbance}} \times \text{standard absorbance}
\]  

2.8 Determination of triglycerides
Measurement of plasma triglycerides by the enzymatic colorimetric Glycerol-3-Phosphatase-Oxidase-Paminophenazone (GPO-PAP). 10 μL of blood serum was added to the FS 1000 triglyceride reagent then vortexed, and incubated at 25 ºC for 10 minutes. Furthermore, it was measured using a microlab 300 spectrophotometer with a wavelength of 500 nm.

\[
\text{Triglycerides} \text{ mg/dl} = \frac{\Delta \text{serum absorbance}}{\Delta \text{standard concentration}} \times \text{standard concentration}
\]

2.9 High density lipoprotein (HDL)
Measurement of plasma HDL by the CHOD-PAP enzymatic and procedure from the DiaSys (Diagnostic System International) kit. 100 μL of serum sample and 250 μL of HDL precipitant reagent were inserted into the eppendorf tube, incubated at room temperature for 10 minutes. Subsequently centrifuged for 10 minutes at 4000 rpm, 100 μL supernatant was taken and 1000 μl cholesterol FS reagent was incubated at room temperature for 20 minutes and the absorbance was read at 500 nm.

\[
\text{Cholesterol mg/dl} = \frac{\Delta \text{serum absorbance}}{\Delta \text{serum absorbance dl}} \times \text{standard concentration}
\]

\[
\text{Cholesterol HDL} = \text{cholesterol total} - \text{supernatant cholesterol}
\]

2.10 Low density lipoprotein (LDL)
Measurement of plasma LDL by the CHOD-PAP enzymatic and the procedure from the DiaSys (Diagnostic System International) kit. 100 μl blood serum and 1000 μl precipitant LDL reagent were inserted into the ependorf tube. Incubated at room temperature for 10 minutes, centrifuged at 2500 rpm for 15 minutes. 100 μL supernatant was added to 1000 μL cholesterol FS reagent, incubated for 10 minutes at 20-25 ºC and the absorbance was read at 500 nm.
Cholesterol mg/dl = \frac{\Delta \text{Cholesterol mg}}{\text{Cholesterol mg/dl}} \times \text{standard concentration} \tag{5}

\text{Cholesterol LDL} = \text{total cholesterol} - \text{supernatant cholesterol} \tag{6}

2.11 Statistical data analysis

The quantitative data collected were analyzed by Two Way ANOVA statistical analysis. Significant differences were obtained at p <0.05. The results are expressed as means and standard deviations (SD).

3. Results and Discussion

3.1 Result

Measurement of total phenol levels was carried out using gallic acid as a standard. Determination of total phenol levels of red ginger extract was carried out based on gallic acid standard curves. The use of gallic acid as a standard that is, because gallic acid is a derivative of hydrobenzoic which is a simple phenol acid which is pure and stable. Absorbance of five gallic acid concentrations was measured at a wavelength of 760 nm. The results are then used to form a regression curve and obtain a regression equation to measure phenolic levels of red ginger. Based on the regression curve, shows the gallic acid regression equation at concentrations of 10, 20, 30, 40, and 50 ppm is \( y = 0.123272 \times + 0.049 \), which is the standard solution of phenol compounds is linear between absorbance and concentration with the correlation coefficient (r) is 0.996. In the absorbance measurement shown by the correlation coefficient (r) of 0.996, this value (r) approaches the number 1 which shows that the regression equation is linear. Gallic acid standard curves are presented in Figure 1. Ginger extract solution was measured at a wavelength of 760 nm.

![Figure 1. Total Curve Standard for Red Ginger Phenol (Zingiber officinale Roxb. var rubrum).](image)

Red ginger extract has a significant effect on wistar rat body weight changes at an extract dose of 200 mg/kg BW which is equivalent to positive control (simvastatin 7.2 mg/kg bw) (p≥0.05). Giving extract of 200 mg/kg bw for two weeks showed a higher percentage of decline compared to the other groups, namely 22.5%. The effect of red ginger extract on body weight is presented in Table 1.

| Table 1. Effect of ginger extract (Zingiber officinale Roxb. var rubrum) on body weight. |
### Body Weight (gram)

| Time    | Control          | Simvastatin 7.2 mg/kg bw | Red ginger 200 mg/kg bw | Red ginger 350 mg/kg bw | Red ginger 500 mg/kg bw |
|---------|------------------|---------------------------|-------------------------|-------------------------|-------------------------|
| Day 0   | 187.6±9.55<sup>a</sup> | 189.0±7.90<sup>a</sup>   | 183.0±9.56<sup>a</sup>  | 184.8±10.4<sup>a</sup>  | 185.6±8.20<sup>a</sup>  |
| Day 28  | 229.2±8.16<sup>ab</sup> | 218.6±13.9<sup>a</sup>   | 233.6±6.06<sup>b</sup>  | 224.4±6.45<sup>ab</sup> | 229.2±1.48<sup>ab</sup>  |
| Day 42  | 228.2±5.21<sup>c</sup> | 170.8±2.28<sup>a</sup>   | 174.4±3.11<sup>a</sup>  | 174.6±7.76<sup>a</sup>  | 181.0±2.54<sup>a</sup>  |
| Percentage (%) | 0.4% | 21.9% | 22.5% | 22.1% | 21.0% |

Note: The same letters behind the numbers in each column show no significance difference.

Red ginger extract had a significant effect on changes in the lipid profile of hypercholesterol rats at an extract dose of 200 mg/kg bw which was equivalent to positive control (simvastatin 7.2 mg/kg bw) (p≥0.05). The effect of red ginger extract on the lipid profile is presented in Table 2.

#### Table 2. Effect of ginger extract (*Zingiber officinale* Roxb. var rubrum) on lipid profile at day 42.

| Treatment group | Cholesterol | LDL | HDL | Triglyceride |
|-----------------|-------------|-----|-----|--------------|
| KN              | 73.1isera<sup>b</sup> | 34.0isera<sup>b</sup> | 27.3isera<sup>a</sup> | 78.1isera<sup>b</sup> |
| KP              | 52.4isera<sup>a</sup> | 22.2isera<sup>a</sup> | 36.0isera<sup>b</sup> | 31.8isera<sup>a</sup> |
| JA              | 48.8isera<sup>a</sup> | 22.6isera<sup>a</sup> | 35.5isera<sup>b</sup> | 31.5isera<sup>a</sup> |
| JB              | 51.8isera<sup>a</sup> | 25.8isera<sup>a</sup> | 33.3isera<sup>b</sup> | 38.9isera<sup>a</sup> |
| JC              | 64.5isera<sup>ab</sup> | 22.9isera<sup>a</sup> | 34.6isera<sup>ab</sup> | 34.5isera<sup>a</sup> |

Note: The same letters behind the numbers in each column show no significance difference.

Red ginger extract has a significant effect on changes in hypercholesterolemia lipid profile at an extract dose of 200 mg/kg body weight which is equivalent to positive control (simvastatin 7.2 mg/kg bw (p≥0.05). The percentage effects of red ginger extract on the lipid profile in Table 3.

#### Table 3. Lipid profile change (%).

| Treatment group | Cholesterol | LDL | HDL | Triglyceride |
|-----------------|-------------|-----|-----|--------------|
| KN              | -25.3       | -2.6 | -4.9 | -15.8        |
| KP              | -42.2       | -31.7 | +28.1 | -61.6        |
| JA              | -47.9       | -32.7 | +25.8 | -64.3        |
| JB              | -43.4       | -31.4 | +27.1 | -62.5        |
| JC              | -28.5       | -30.4 | +22.7 | -57.5        |

Note: The (-) sign is decreasing, the (+) sign is increasing.

KN: Negative control
KP: Simvastatin 7.2 mg/kg bw
JA: Red ginger 200 mg/kg bw
JB: Red ginger 350 mg/kg bw
JC: Red ginger 500 mg/kg bw

3.2 Discussion

The results of this study, the ethanol extract of *Zingiber officinale* Roxb. var rubrum has a higher total phenolic content of 5.19 mg/GAE/g compared to other Zingiberaceae plants such as *Curcuma aeroginosa* [8], *Kaemferia pulchra* [8], and *Boesenbergia pandurata* [9]. However, some plants such
as Curcuma longa [10], Zingiber zerumbet, [11] and Curcuma xanthorrhiza [8] have higher total phenolic levels compared to Zingiber officinale Roxb. var. rubrum.

Whereas as in this study to induce hypercholesterolemia using 1 mL of lard and 2.5 mL/200 grams bw of quail egg yolk and drinking water that already contains propylthiouracil (PTU) 2 mg / kg bw. This induction was able to increase body weight in all treatment groups on day 28. These results are consistent with a previous report by [12], which indicates that excess intake of fatty foods can greatly increase body weight. After 42 days the control group showed a weight loss of 0.4%. Whereas in the positive group giving simvastatin 7.2 mg/kg bw and red ginger extract 200, 350, and 500 mg/kg bw decreased by a percentage of 21.9%; 22.5%; 22.1% and 21.0%.

In the lipid profile test the control group showed a change in lipid profile, but the value of the percentage change in lipid profile was smaller compared with the treatment of simvastatin and red ginger extract with a percentage decrease in cholesterol levels of 23.3%, LDL 2.6%, triglycerides 15.8%, although a decrease in cholesterol, LDL and triglyceride levels but no increase in HDL. This shows that there is still an increase in the accumulation of fat in the liver resulting in an increase in the amount of acetyl CoA in the liver cells that produce cholesterol. Increased plasma cholesterol causes a decrease in liver LDL receptor activity resulting in decreased LDL absorption which causes plasma LDL to increase.

Whereas in the treatment group simvastatin 7.2 mg/kg bw (positive control) was able to provide changes in lipid profile levels of hypercholesterolemia rats which was able to reduce total cholesterol levels by a percentage of 42.2%, 31.7% LDL levels, and triglyceride levels 61.6% and able to increase HDL levels 28.1%. The mechanism of statins in inhibiting cholesterol formation by inhibiting the HMG-CoA reductase enzyme in the liver, a rate limiting enzyme in the cholesterol biosynthetic pathway. As a result, the conversion of HMG-CoA to mevalonate is inhibited and results in a reduction in cholesterol levels that are produced. This reduction activates a transcription factor called the sterol regulatory element binding protein (SREBP), which will be transported from the endoplasmic reticulum to golgi. LDL receptor transcription will be activated and will affect the increase in LDL receptor absorption there by reducing plasma LDL levels [13].

When compared with the treatment of red ginger extract treatment showed that red ginger extract significantly affected the changes in hypercholesterolemia lipid profile at extract doses of 200 mg/kg bw and 350 mg/kg bw which were equivalent to positive control of simvastatin dose 7.2 mg/kg bw (p<0.05). Percentage reduction of 200mg / kg bw dose is plasma total cholesterol level of 47.9%, LDL 32.7% and triglyceride 64.3% and HDL increase of 25.8%. While the 350 mg/kg bw extract dose with a decrease in plasma total cholesterol levels was 43.4%, LDL 31.4%, and triglycerides 62.5% and increase in HDL 27.1%. These results indicate that the dose of 200 mg/kg bw showed the highest percentage reduction in plasma total cholesterol, LDL, and triglyceride levels compared to other treatment groups. But it has a lower HDL percentage increase compared to the 7.2 mg/kg bw simvastatin treatment. When compared with the treatment of red ginger extract dose of 350 mg /kg bw the percentage of HDL 200 mg/kg bw is smaller than the dose of 350 mg/kg bw. Percentage of red ginger extract on changes in lipid profile.

As is well known that red ginger extract contains phenolic compounds as antioxidant agents. Phenolic compounds have been shown to provide protection against metabolic disorders such as hypercholesterolemia. Hypercholesterolemia is the result of increased cholesterol levels and increased production of free radicals [14]. Phenolic compounds in this case reduce the risk of hypercholesterolemia by increasing the activity of antioxidant enzymes and reducing the formation of free radicals. In addition, antioxidant activity can accumulate superoxide anions and hydroxy radicals, able to inhibit lipid peroxidation, and regulate low density lipoprotein (LDL) receptors [7].

The mechanism proposed in various studies so far is that ginger extract is able to inhibit the HMG-CoA enzyme in the liver (an enzyme that limits the rate of conversion of HMG-CoA to mevalonic acid). Enzyme inhibition leads to a reduction in cholesterol in the liver. However, the mechanism is better than both HMG-CoA reductase and LDL receptors, where HDL receptors act by increasing LDL absorption there by reducing plasma LDL levels [15].
4. Conclusion

Based on the results and discussion that has been submitted, it can be concluded that the red ginger extract has a total phenol level of 5194.15 ± 264.1 μg/g, and has a significant influence on changes in lipid profile and changes in body weight of hypercholesterolemia rats.

Acknowledgments

The writer would like to thank the University of Sebelas Maret especially the facilities provided by the integrated central laboratory so that the research can be carried out.

Reference

[1] Balamurugan G, and Shantha A 2010 J. Pharm. Bioallied. Sci 2(4) 350-355
[2] Elly P, Wheni E R, and Cikra INHS 2018 JDMS. 17(5) 55-59.
[3] Babak B A, Mahmoud R K, Mohammad M Z, and Mahmoud B 2015 Der. Pharmacia. Lettre 7(12) 81-88
[4] Geetika P, Chirag S, Riyaz A H V, Pooja B, and Sibi Res J. Med. Plant. 9(6) 300-306
[5] Mathew S, Abraham T E, and Zakaria Z A 2015 JFST 52(9) 5790-5798
[6] Belal P, Rahim H, and Hassan M 2017 VRF. 8(3) 185-193.
[7] Sunarti, Edy F, Urip H, Delzuzar, Tri W, and Lokot DL 2017 Univ. med. 36(3) 228 - 235
[8] Alafiatayo A A, Ahmad S A, and Maziah M 2014 Afr. J. Tradit. Complement. Altern. Med 11(3) 7-13
[9] Aweng E R, Karunakaran T and Zakia K 2018 J. Pharm. Bio, and Chem. Sci 9(1) 962 - 971
[10] Array E J, Tonfack D F, Kinge K F, Kinge E E, and Womeni, H M 2019 J. Food. R. 3(1) 86 - 90
[11] Vipada K, and Yingyong P 2012 JDSA. 7 89-96
[12] Patrick, Ambrose T, Faith P, Samson H A, and Umaru 2015 JBAH. 5 (18) 123-127
[13] Meor F R M A S, Intan N S, Johnson S, Shariful H, and Subashini C T 2017 IJEM. 15(2) E 43319
[14] Sushma D, and Randhir S 2017 Phcog. J. 9(6) 807-814
[15] Salah M E S, and Reham A M 2016 JPCBS. 3(4) 561-572