

Decoction of ginger (*Zingiber officinale* Roscoe var *Roscoe*) attenuate the oxidation stress in hyperlipidemia rat (*Rattus norvegicus*)

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Decoction of ginger (*Zingiber officinale Roscoe var Roscoe*) attenuate the oxidation stress in hyperlipidemia rat (*Rattus norvegicus*)

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Abstract

Based on UNICEF data, overweight and obesity increased across all age and income groups. Overweight has a risk of developing hyperlipidemia and caused increase free radical and lead to oxidative stress. It's result in cell damage and impacts on several diseases among others diabetes mellitus. Many Medicinal Plant have biochemical compound which is an important concern because of the potential to reduce free radicals. One of them is ginger (*Zingiber officinale Roscoe var Roscoe*). Medicinal plant generally is usually used with various extraction method to obtain the required chemical content. Information of ginger for reduce free radical with maceration extraction method has been reported in previous studies but little information with decoction extraction method. This study aims to determine the reduction of free radicals with MDA (malondialdehyde) as a marker and increased antioxidant activity with SOD (superoxide dismutase) as a marker after administration of ginger. This research was used the true experimental with the randomized posttest only control group design. The groups of experimental were divided into three which G1: control normal G2: High Fat Diet (HFD), G3: HFD + *Zingiber officinale Roscoe var Roscoe* (ZO) 5 g/day. The period of study was 6 weeks. The level of liver MDA and SOD were investigated by spectrophotometer UV Vis. Based on ANAVA showed that HFD rat increase of body fat weight and MDA ($p < 0.05$) and reduced of total antioxidant SOD ($p < 0.05$). The conclusion of this research decoction of *Zingiber officinale Roscoe var Roscoe* could attenuate of lipid peroxidation induce by HFD.

Keywords: Hyperlipidemia; MDA; SOD; Antioxidant; Free-radical

1. Introduction

Obesity, the mayor health burden of the 21st century because of health disorder including diabetes mellitus [1, 2]. Diabetes mellitus one of most serious metabolic diseases that cause morbidity and mortality in human [3]. Overweight is defined as body mass index (BMI) ≥ 25 kg/m² and greater than or equal to 30 kg/m² for the obesity. Approximately about 39 million people under the age of 5 are obesity in 2020 [4, 5]. BMI has certain limitation, because of does not distinguish the difference between lean mass and fat nor does it identify fat distribution. Based on recent research obesity related risk factor depend not on the excess body weight per se, but rather on body fat distribution such as abdominal adiposity, or visceral adiposity. Visceral fat induced oxidant and pro inflammatory states and abdominal fat is significant risk factor for obesity related diseases [1]. ROS from superoxide formation due to obesity induced oxidative stress [6]. Superoxide (O₂⁻), hydrogen peroxide (H₂O₂) and hydroxyl radical (OH[•]) are reactive oxygen species (ROS). Mitochondria is the most important site where intracellular ROS are produced, ROS may also be produced by plasma membrane and system such as endoplasmic reticulum (ER), lysosome, peroxisome and by cytosolic enzymes. ROS have many biological effects good if at low concentration but in high level they may damage DNA, lipid and protein that lead to cell death [7].

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ROS is one of particular interest because it has a vital role in physiological and pathological process. In general source of ROS comes from exogenous and endogenous induction. Exogenous induction varies, among others exposure to engineered nanoparticles, chemotherapeutics and microbial infection, radiation, cigarettes, pollution, drug, and xenobiotic [8, 9]. Endogenous induction via normal cell metabolism and respiration, however if the ROS become very high it will have a toxic effect on human life. Increasing of ROS leads to cell thorough oxidation of DNA, protein, lipid, that cause cell dead by apoptosis and necrosis [10]. Oxidation of DNA will form 8-oxoguanine, lipid peroxidation became Malondialdehyde (MDA) and protein change to protein carbonylation and will form hydroxy amino acid. MDA can also bond with Guanine and form MDA-DNA (M1Dg) [9].

Obesity has a lot of bad effect among others lipid/lipoprotein abnormalities including elevated cholesterol, triglycerides, apolipoprotein-B and lower of level high density lipoprotein (HDL) [11]. The prevalence of obesity is increasing in all age groups due to many factors including environmental, individual factor such as food marketing, effort is needed to reduced it through life style changes like improve physical activity, reduced calorie intake and long terms treatment [12].

20
Ginger (*Zingiber officinale Roscoe var Roscoe*) is known and widely used spice and condiment, many study showed that ginger have antioxidant potential as well as medicinal application [13, 14]. In recent years, studies showed that ROS became important factor in the development of chronic deceases. Many plant including spices have phytochemical compound that can inhibit ROS via antioxidant activity. Antioxidant activity in spices can decrease lipid peroxidase, thorough free radical scavenger, antioxidant endogen stimulation, and increase antioxidant activity [15]. Based on research about ethanol extract of ginger showed that ginger could increase SOD and decrease MDA in cisplatin induced oxidative stress in rat [16]. Ginger also increased erythroid-2-related factor 2 (Nrf2) gene expression and decrease nuclear factor kappa (NFkB) in animal testis [14]. Nrf2 have to regulate antioxidant molecule and enzyme and isolated in cytoplasm in general condition [17]. Ginger has multiple bioactive compounds including 6-gingerol, 6-shogaol, gingerdions, gingerdiol, paradols, 6-dehydrogingerols, 12-gingerol, and etc. that influenced biological activities. The mayor active compound are 6-shogaol and 6-gingerol and have the beneficial effect among others as an antioxidant [18]. Based on recent research showed that ginger can inhibit apoptosis and oxidative stress in PD model (6-OHDHA-induced PC12 cells). Reduction of stress oxidation sees thorough enhancement of antioxidant activity such as catalase, SOD, and another important marker [17].

Ginger was used in a traditional medical field with many kinds of extraction method such as maceration, Soxhlet, as well as sonication by using ethanol as the solvent [19]. Sarmoko and colleagues used maceration of red ginger for the treatment of colon cancer. They used ethanol for extraction the chemical compound of red ginger [20]. Maceration with ethanol 70% of finger was used in study of hypercholesterolemia and hyperglycemia for reduced oxidant and induce antioxidant activity [21]. Maceration with n-hexane of ginger has also been reported as antibacterial activity [22].

The information of ginger as antioxidant activity in many previous research generally used maceration extraction method with ethanol or methanol as a solvent. This method is known as non-green chemistry method for extraction. To overcome this problem, the decoction [23] is introduced as the simple and more green chemistry method for extraction by using only water as the solvent. There is a little information studied with decoction extraction method especially for ginger. There for this study aims to determine the effect of *Zingiber officinale Roscoe var Roscoe* on oxidant-antioxidant status in rat hyperlipidemia given by the decoction method.

25 2. Material and methods

26 2.1. Research design

27
This research was used the true experimental design with the randomized posttest only control group design. The research was conducted at the PAU Nutritional Food University of Gadjah Mada Yogyakarta. Eight-teen of male Wistar rats (*Rattus norvegicus*) age of 3 months, weight 180-250 g was used in this research. Rats were housed one per cage and divided into three groups consist of G1 as normal control, G2 as hyperlipidemia and G3 as hyperlipidemia + addition of *Zingiber officinale Roscoe var Roscoe* (ZO) 5 g/200 g/day, respectively. The composition of the high fat diet (HFD) for hyperlipidemia condition was 60 % of Comfeed PAR-s, 27,8% of wheat, 2% of cholesterol, 0,2% of colic acid, and 10 % lard. It was given for 2 weeks before treatment with ginger (ZO). *Zingiber officinale Roscoe var Roscoe* was obtained from local market in Malang and then made in to powder. The extraction of ginger powder was done by the decoction method according to Mahmudati [2]. *Zingiber officinale Roscoe var Roscoe* administration was carried out for 28 days. Marker of hyperlipidemia was determined as a triglycerides number by using triglycerides assay kit (DyaSis, Germany).

2.2. Plant and Animals

Ginger (*Zingiber officinale Roscoe var Roscoe*) was obtained from the local market in Malang, East Java Indonesia. Rhizomes from the ginger were used in this research that done harvesting about 8 months. The experimental unit in this study was 3 months age of male *Rattus norvegicus* originating from inter university center (PAU) Nutrition Food UGM Yogyakarta.

2.3. Determination of MDA Level

MDA level was measured according to Wuryastuti and co-workers with the following steps. [24] Exactly 750 µL of phosphoric acid was mixed with 13 mL as well as liver tissue as sample in a tube. The mixture was added with 250 µL 40 mM thiobarbituric acid (TBA), followed by 45 mL of double distilled water and then mixed and covered. The mixture was heated in a water bath for 60 minutes up to 100 °C and cooled to 30 °C. The column Sep-Park C₁₈ was prepared for inserting the mixture contained sample. The column was added with 5 mL of methanol and double distilled water, respectively. The sample was inserted into the column and discarded. TBA were eluted from the column with 4 mL methanol and collected in cuvette tube. Sample was measured by Spectrophotometer UV-Vis at 532 nm wavelength and 1,1,3,3-tetraethoxypropane was used as standard.

2.4. Determination of SOD Level

Determination of liver SOD level was used SOD assay protocol in Superoxide dismutase activity assay kit by Dyasys, Germany (catalog K355-100). The SOD level was measured by using WST-1 method. First, 200 µL of sample solution was added to sample well and blank 2 well. Second, 20 µL H₂O to each blank 1 and 3 well. Third, 200 µL of WST working solution was added to each well and followed by addition of 20 µL of dilution buffer to blank 2 and 3 well. Fourth, 20 µL of enzyme working solution was added to each sample and blank 1 well, and then mixed thoroughly. All the well was incubation plate at 37 °C for 20 minutes. The absorbance was read at 450 nm by using microplate reader and calculated the SOD activity.

3. Results

3.1. Hyperlipidaemia condition

Hyperlipidemia is known as an abnormal condition of the lipid which found in the blood [25]. The level of hyperlipidemia is easily counted as the present of total triglycerides or cholesterol in blood. In this study, we used the white rats (*Rattus norvegicus*) which given with high fat diet (HFD) as mentioned before for two weeks. The level of triglycerides was counted as the marker of hyperlipidemia and shown in the Table 1. HFD rats showed the higher TG level than the normal rats.

Table 1 Triglyceride (TG) level in Normal rats and HFD rats

Treatment	Mean TG+ SD (n=6)
Normal rats	75.42±3.19
HFD rats	151.50±3.59

3.2. MDA and SOD Level of Liver Tissue after Treatment with ginger extract

Malondialdehydes (MDA) and Superoxide Dismutase (SOD) was measured from the rat's liver tissue as mentioned before. The result of MDA is shown in the Figure 1. The HFD rats was treated with addition of 5 g ginger/day for 28 days which selected from the previous study by Mahmudati [2]. The data showed that treatment with addition of ginger powder 5 g/day decreased the MDA level compared to HFD rats only. It concluded that antioxidant inside the ginger decreased ROS which causes lipid peroxidation. Reduction in lipid peroxidation was measured using the MDA marker. Meanwhile, the SOD level increased with the same treatment. The number of SOD increased from 29.51±3.67 % to be 58.13±13.35 %.

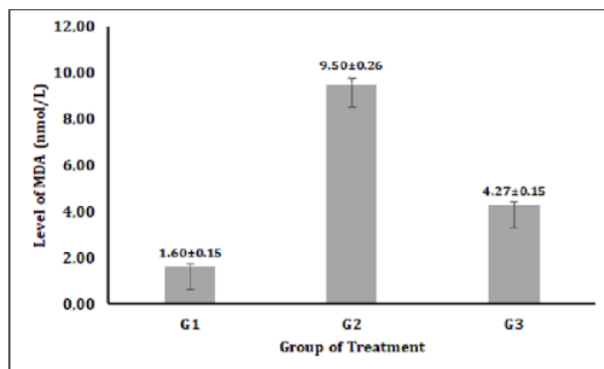


Figure 1 MDA level graph in liver tissue (note: G1= normal rats, G2= HFD rats, and G3= HFD rats+ addition of 5 g ginger/day)

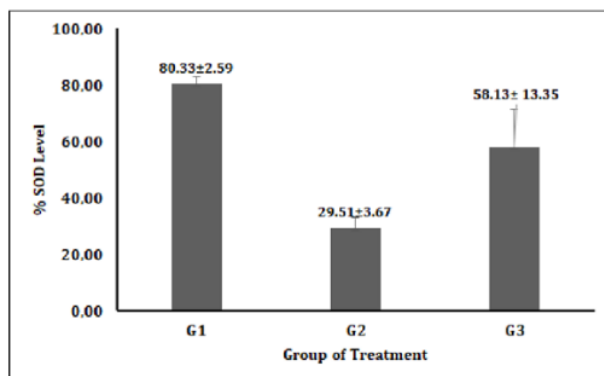


Figure 2 SOD level graph in liver tissue (note: G1= normal rats, G2= HFD rats, and G3= HFD rats+ addition of 5 g ginger/day)

4. Discussion

Based on ANAVA decoction extraction method of ginger (*Zingiber officinale Roscoe var Roscoe*) in this research can significantly reduce oxidative stress in hyperlipidemic rat. The decrease free radical in this study was mark by a decrease malondialdehydes (MDA) than followed by increased antioxidant activity thorough superoxide dismutase (SOD) markers. Information about ginger as an antioxidant is vast in previous research because of phytochemical compound such as volatile oils are generally composed of terpenoid and give a unique aromatic smell, and gingerol as the spicy component [26]. Gingerol in heat treatment or long storage can be transformed into corresponding shogaol and after hydrogenation can be transformed into paradol. Ginger also has another phenolic compound such as quercetin, gingerenone-A, and 6-dehydrogingerdione. Gingerol and shogaol has strong antioxidant activity [27].

The result of this research the same as previous research where 6-gingerols reduced ROS production by 18.5 % compared to control in C-elegants [28]. Based on research by Shekarforoush, ethanolic extract of ginger decrease ovarian tissue MDA in rat treatment with sodium metabisulfite (SBM) in high dose [29]. Combination of ginger with fish oil modulatory effect of antioxidant status like elevated in antioxidant capacity [30]. Administration of ginger in ethanol induced hepatotoxicity in male rat can decrease MDA level and increase SOD level compared to un treated ginger and further improve liver function [31].

Information in previous and this research showed that *Zingiber officinale Roscoe var Roscoe* can inhibit cell damage by regulatory in antioxidant status. Based on LC-MS data, the decoction of *Zingiber officinale Roscoe var Roscoe* have

phytochemical 6-gingerol and 6-shogaol (data not shown), Therefore decoction extraction method can be considered to inhibit oxidation stress and maintain of health. Decoction method is more practice than maceration or another type of extraction with chemical solution because of very simple and cheap.

5. Conclusion

Decoction of *Zingiber officinale Roscoe var Roscoe* 5 g/day can improve oxidant status in hyperlipidemia rats by decreased the MDA and increased SOD level in liver tissue.

7 Compliance with ethical standards

Acknowledgments

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7 Disclosure of conflict of interest

There is no conflict of interest from the authors.

Statement of ethical approval

An ethical clearance certificate which is a requirement for conducting this research has been obtained from the Health Research Ethics Committee of University of Muhammadiyah Malang (No. E.5.a/242/KEPK-UMM/2021).

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