



The protective role of single bulb garlic (*Allium sativum* L.) towards foam cells and vascular cell adhesion molecules-1 (VCAM-1) on mice (*Mus musculus*) with high-fat diet

Isfatun Chasanah¹, Abdul Ghofur², Sri Rahayu Lestari^{2*}

¹Bachelor Program of Biology, Department of Biology, ²Department of Biology, Faculty of Mathematics and Natural Science, Universitas Negeri Malang, Malang, East Java, Indonesia

ABSTRACT

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Atherosclerosis is initiated by inflammatory response characterized by the accumulation of lipids in the arteries, causing blockage of blood vessels. The excessive of low-density lipoprotein (LDL) in the blood is a major factor in endothelial dysfunction and results in an inflammatory process. The inflammatory process in atherosclerosis is due to the expression of adhesion molecules, vascular cell adhesion molecules-1 (VCAM-1). This research aimed to evaluate the effect of single bulb garlic oil extract (SGBOE) against VCAM-1 expression in high-fat diet mice. Thirty male mice strain *Balb/C* were acclimatized for one week. Mice were divided into 6 groups (n=5): normal, high-fat diet (HFD), HFD + simvastatin, and HFD + SGBOE 12.5, 25, and 50 mg/kg BW respectively. HFD was given for 45 days. The SGBOE was given for four weeks orally. The aortic VCAM-1 expression was assayed using the immunohistochemical-flouresencemethod. The result of One-Way ANOVA showed that the treatment influenced significantly (p<0.05). The foam cells increase in the high-fat diet group compared than normal mice. Dose 12,5 mg/kg BW reduces VCAM-1 expression near the normal group. *Allicin* and *S-allyl cysteine* (SAC) compounds in single bulb garlic are antioxidant compounds that act to prevent oxidative stress and prevention mechanisms against lipoprotein modification.

ABSTRAK

Atherosklerosis disebabkan oleh respon inflamasi yang ditandai dengan akumulasi lipid pada arteri, dan menyebabkan penyempitan pada pembuluh darah. Kadar *low-density lipoprotein* (LDL) yang berlebih dalam darah merupakan faktor utama disfungsi endotel dan menyebabkan proses inflamasi berlangsung. Proses inflamasi pada atherosklerosis disebabkan oleh ekspresi molekul adhesi, yakni *vascular cell adhesion molecules-1* (VCAM-1). Penelitian ini bertujuan untuk mengevaluasi efek ekstrak minyak bawang tunggal (SGBOE) terhadap ekspresi VCAM-1 pada mencit diet lemak tinggi. Mencit jantan strain *Balb/C* diaklimatisasi selama satu minggu. Mencit kemudian dibagi ke dalam 6 kelompok (n=5), yaitu normal; diet lemak tinggi (HFD); HFD + simvastatin; HFD + SGBOE 12,5; 25, dan 50 mg/kg BB. Diet lemak tinggi diberikan selama 45 hari. SGBOE diberikan secara oral selama empat minggu. Ekspresi VCAM-1 pada aorta dianalisis menggunakan metode immunohistokimia fluoresen (IHK-F). Hasil *one-way* ANOVA menunjukkan pengaruh yang signifikan (p<0,05) pada perlakuan yang diberikan. *Foam cell* pada aorta mencit kelompok diet lemak tinggi mengalami peningkatan bila dibandingkan dengan kelompok normal. Dosis 12,5 mg/kg menurunkan ekspresi VCAM-1 mendekati kelompok normal. Komponen *Allicin* dan *S-allyl cysteine* (SAC) pada bawang tunggal merupakan antioksidan yang berperan mencegah stress oksidatif dan modifikasi lipoprotein.

Keywords:

atherosclerosis,
single bulb garlic oil
extract, *VCAM-1*

INTRODUCTION

Cardiovascular diseases are the primary cause of death worldwide including in Indonesia. The number of people dying from cardiovascular diseases such as heart attacks and strokes is reported to increase every year from 16.7 million in 2010 to 17.7 million in 2015.¹ Furthermore, it is estimated that 7.4 million people died of coronary heart disease and 6.7 million of strokes.² In Indonesia, death caused by cardiovascular diseases, especially coronary heart disease and stroke are estimated to increase by 23.3 million in 2030.³

Atherosclerosis is the primary risk factor in the development of cardiovascular disease⁴ brain (ischemic stroke). It is caused by inflammation responses indicated by lipid accumulation in arterial blood vessels.^{5,6} Hypercholesterolemia due to lifestyle changes is the main factor of narrowing blood vessels progress. Hypercholesterolemia is an increase in cholesterol, especially the low-density lipoprotein (LDL) in the body.⁷ The LDL in the body oxidize to form the oxidized LDL (oxLDL), which is the main trigger of foam cell formation by macrophages. Foam cell formation is the main characteristic of atherosclerosis.^{7,8}

Atherosclerosis occurs due to endothelial dysfunction in blood vessels. Endothelial dysfunction caused by LDL accumulation in blood vessels, and then entering the blood vessels, causes oxidative stress in arterial endothelium and triggers inflammation response. Inflammation response produces adhesion molecules, such as vascular cell adhesion molecules-1 (VCAM-1), will be expressed on arterial endothelial walls.⁸

VCAM-1 stimulates monocyte attachment on arterial endothelial walls during inflammation, leading to macrophage differentiation.

Macrophage accumulation to phagocyte oxLDL will lead to the form of foam cells. Furthermore, proinflammatory cytokine was released in an excessive amount and trigger the proliferation of non-striated muscles which respond to the thickening of the arterial walls. The end proces is the formation of the fibrous cap, and in the further process, it could rupture.^{9,10}public health and eco-nomic perspectives. Cardiovascular disease is the leading cause of mortality worldwide, accounting for 17.5 million deaths per year; 6.7 million of these deaths are related to stroke 1 . Over 80% of strokes are classified as ischaemic, and approximately two thirds of ischaemic strokes result from one of three pathological states: athero sclerosis, lipohyalinosis (a small-vessel disease

Current atherosclerosis treatment is based on the maintain lipid levels with a combination of anti-inflammation therapy. The decline in lipid synthesis could prevent LDL modification into oxidized LDL¹¹, thus will less the possibility of arterial endothelial dysfunction. Arterial endothelial cells that do not experience dysfunction causes adhesion molecules, such as VCAM-1, will not be increased.¹²

Antioxidant activities play a role in preventing the occurrence of oxidative stress and prevention mechanism for lipoprotein modification. Garlic (*Allium sativum* L.) is a famous spice which is almost uses globally. Garlic contains high antioxidant due to allicin and S-allyl cysteine (SAC) contents.^{13,14} However, there is little information about the exact role of single bulb garlic to accomplish atherosclerosis. Single bulb garlic is a traditional medicine which is grown in extreme condition. Based on the description, research is needed to investigate the role of single bulb garlic (*A. sativum* L.) extract on the VCAM-1 expression on atherosclerosis mice model.

MATERIALS AND METHODS

Preparation of single bulb garlic oil extract

Single bulb garlic oil extract (SBGOE) was obtained from UPT Materia Medica, Batu, Malang, East Java. One kg fresh single bulb garlic was dissolved in 4.5 L hexane then evaporated. The final volume extract that obtained was 2 mL. SBGOE was stored in -4°C until ready to use.

Experimental animals

Experimental animals used were 3 months old Balb-C (*Mus musculus*) male mice strain with body weight was 30 ± 5 g obtained from Jember, East Java. The mice were adapted for a week and placed in an iron cage individually. The mice were gained free access to feed and drink. After that, the mice were divided into two major groups i.e. normal diet (ND) and high-fat diet (HFD). The HFD is given consisted of Higro-551 (300 g), corn (200 g), cooking oil waste (150 g), duck egg yolk (100 g), flour (50 g), and cholic acid (0.1 g). It was given daily for 45 days consecutively.¹⁵ because 20% of death in the world is caused by atherosclerosis diseases like stroke and myocardial infarct. One of animal models that was successful in atherosclerosis research was New Zealand white rabbit. The purpose of this preliminary research was to determine the atherogenic diet of white rats (*Rattus norvegicus* strain Wistar¹⁶). After that, HFD mice were divided into 6 groups (n=5): HFD, HFD + Simvastatin 1.04 mg/kg¹⁶, HFD + SBGOE 12.5, 25, and 50 mg/kg BW respectively. The SBGOE was administered orally once a day, whereas simvastatin (Simv) was administered orally once in two days. The SBGOE and Simv were given for four weeks. Protocol of the study was approved by ethical clearance from the

Research Ethics Committee, Universitas Brawijaya (Ref. 880-KEP-UB).

Histopathological analysis of aorta

The mice were sacrificed using cervical dislocation after 4 weeks. The aorta was collected then wash three times in phosphate buffer saline (PBS). The aorta was fixed in formalin 10%. The aorta was stained using hematoxylin-eosin (HE) staining and observed using microscope Olympus CX21. The aorta slices were observed in 400x magnification to evaluate the existence of the foam cell, which is the characteristic of atherosclerosis. Next, the preparations were stained using fluorescent immunohistochemistry (F-IHC) method with isothiocyanate (FITC) staining. The aorta was observed using fluorescence (FSX-100) microscope connected with Olympus Fluoview Software and calculated the VCAM-1 expression (intensity/mm²).

Statistical analysis

VCAM-1 expression was represented as mean \pm standard deviation (SD). The data obtained were analyzed using One-way ANOVA followed by Duncan multiple range test (DMRT) as poc hoc test. One-way ANOVA was performed using SPSS 16.0 for Windows. A p value <0.05 was considered significant.

RESULTS

Comparison of foam cell existence in aorta's mice with/without SBGOE treatment

The aorta od mice was marked with non-striated muscle cells that were pink in color in the preparations with dark cell nuclear. Atherosclerosis mice had aorta with non-striated muscle cells containing foam cells. The characteristic

of foam cells is white (colorless) due to fat content with cell nuclear located on edge. Mice aorta stained using HE

indicated a different amount of foam cell existence (FIGURE 1).

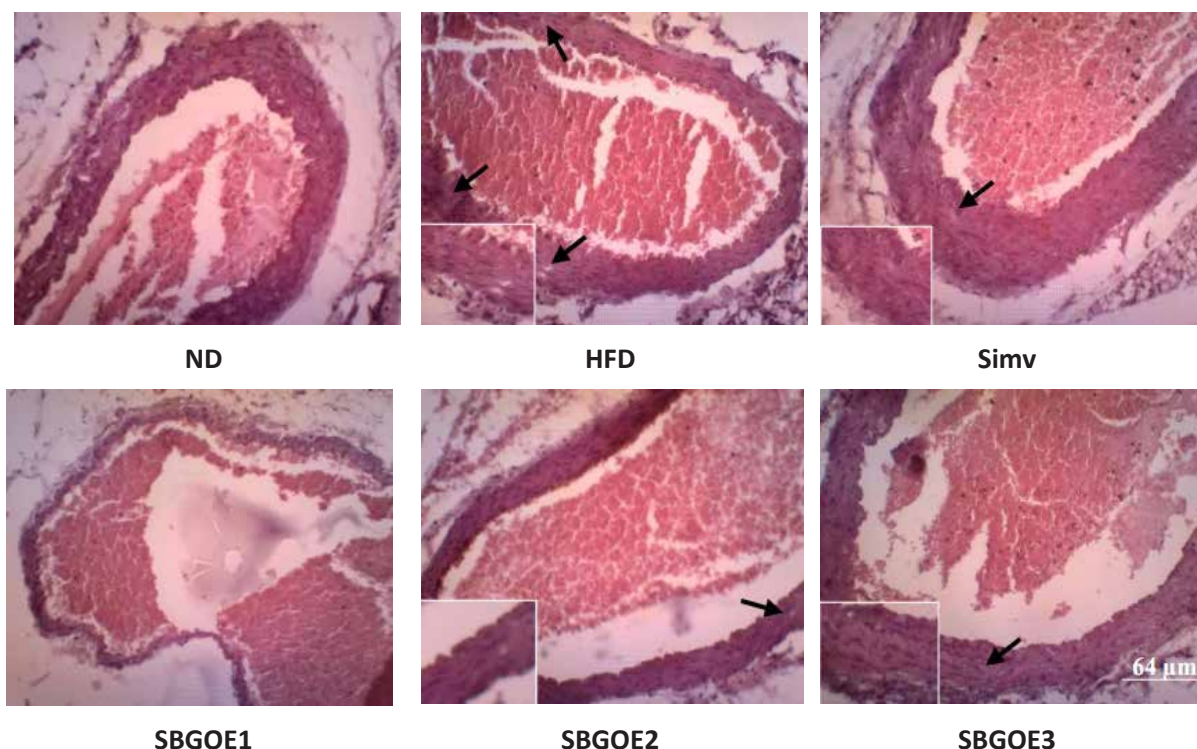


FIGURE 1. The existence of foam cells in aorta's mice stained with HE (100x magnification). The arrows indicate the existence of a foam cell. ND = Normal Diet, HFD = High-Fat Diet, Simv = Simvastatin 1,04 mg/kg BW, SBGOE1 = SBGOE dose 12.5 mg/kg BW, SBGOE2 = SBGOE dose 25 mg/kg BW, and SBGOE3 = SBGOE dose 50 mg/kg BW

The result this study indicated that HFD treatment had more foam cells compared to other treatments. The ND at dose of 25, 50 mg/kg BW showed the existence of foam cell in small numbers. The decrease in the existence of foam cells compared to HFD indicated a decrease in cholesterol formation and inflammation. The SBGOE treatment decrease the foam cell number, which was the characteristic of atherosclerosis when compared to the HFD group.

Comparison of VCAM-1 expression

in aorta's mice with/without SBGOE treatment

Our result showed that VCAM-1 expression increased significantly in HFD mice compared to normal diet ($p < 0.05$). Interestingly, SBGOE treatment declined VCAM-1 expression in HFD mice significantly in a dose-dependent manner (FIGURE 2 and 3). Surprisingly, VCAM-1 expression was decreased significantly in SBGOE dose 25 and 50 mg/kg BW below the normal group (FIGURE 3).

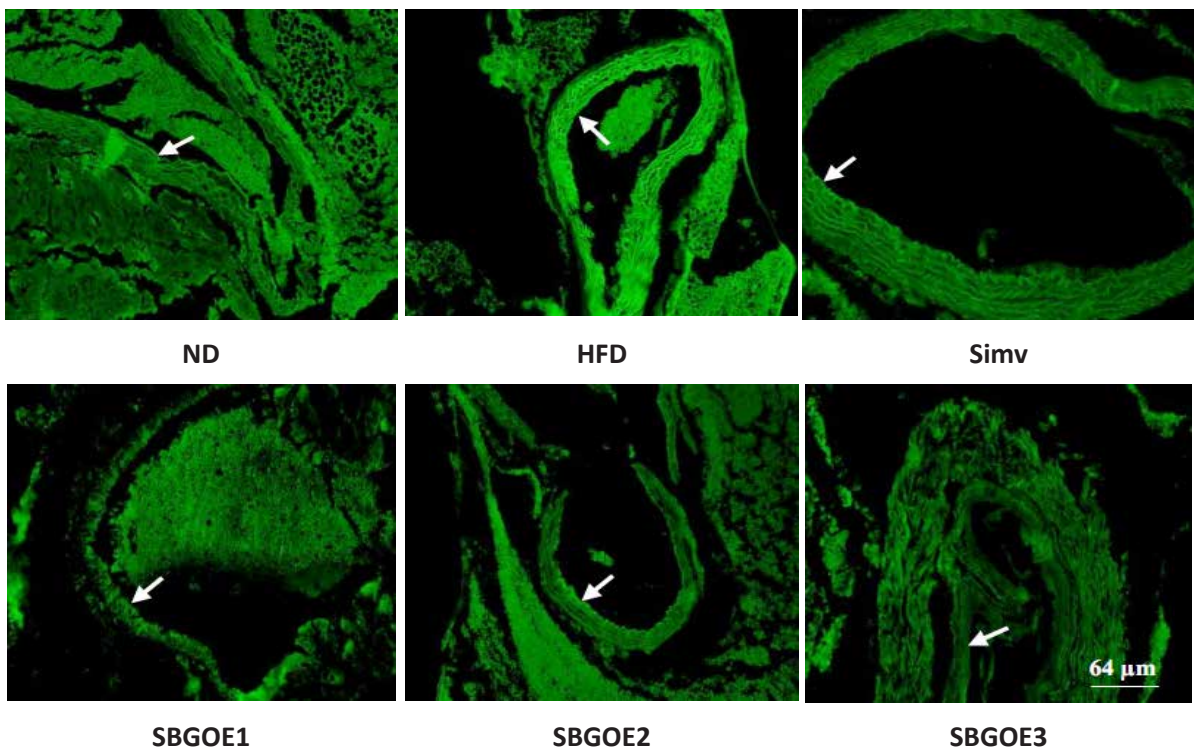


FIGURE 2. VCAM-1 expression with/without SBGOE treatment for four weeks and simvastatin as a positive control. Fluorescent IHC of mice aorta with FITC staining (FSX-100 microscope) with a magnification of 100x. VCAM-1 expressed on the arterial endothelial walls indicated by an arrow mark.

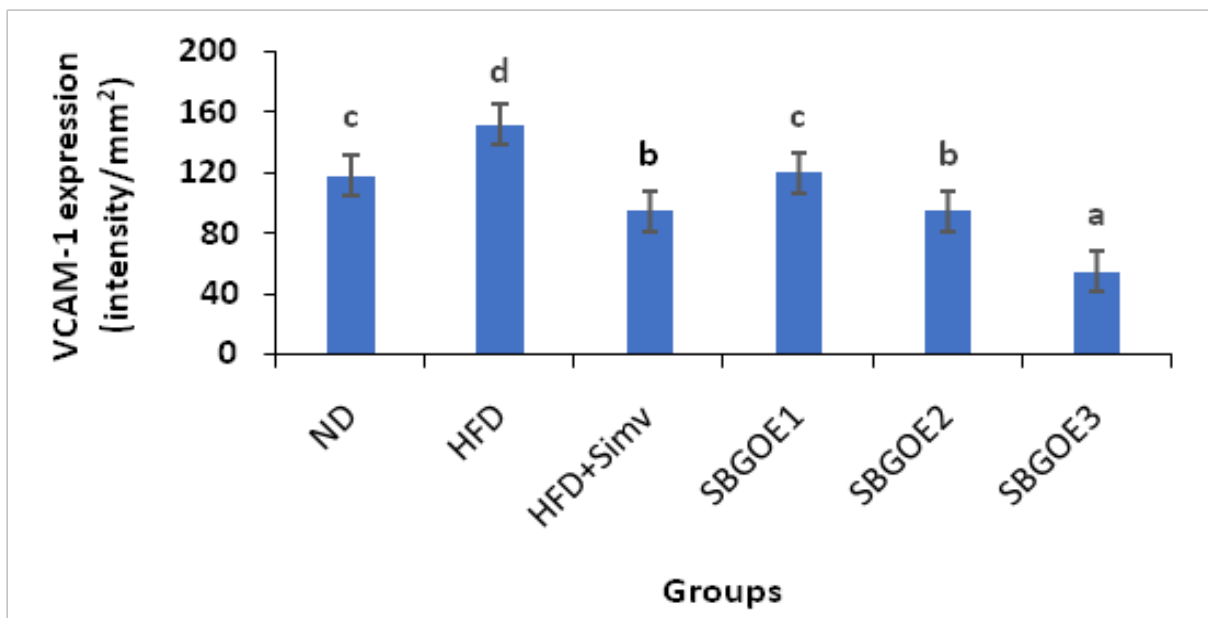


FIGURE 3. Histogram of VCAM-1 expression. The different letter indicated the significant different between groups based on DMRT as pos hoc test ($p < 0.05$). ND = Normal Diet, HFD = High-Fat Diet, Simv = Simvastatin 1,04 mg/kg BW, SBGOE1 = SBGOE dose 12.5 mg/kg BW, SBGOE2 = SBGOE dose 25 mg/kg BW, and SBGOE3 = SBGOE dose 50 mg/kg BW.

DISCUSSION

The SBGOE improved atherosclerosis through reducing the VCAM-1 expression. The inhibition mechanism may be caused by allicin, SAC and allyl disulfide in the single bulb garlic which acts as antioxidant.^{13,17,18} Allicin as antioxidant plays a role to prevent oxidative stress and a prevention mechanism towards LDL modification.¹⁹ SAC block the activation of nuclear factor kappa beta (NF- κ B), the transcription factor which played a pivotal role in the initiation of the inflammation process. Antioxidant activities prevent the formation of free radicals, such as anion superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), peroxy radical (ONOO \cdot) or (LOO \cdot), thus prevent the increase of oxidative stress.¹³

Antioxidant plays a role as an inhibitor for oxidation by reacting with reactive free radicals to form a relatively stable unreactive free radical that protect the dangerous effect to cells.²⁰ Antioxidant work through donating one of its electrons to oxidant compounds. Hence the compounds activities disrupt the reactive free radicals.²¹ Allicin and SAC in single bulb garlic is an exogenous antioxidant since it is obtained from outside the body. Exogenous antioxidant works by cutting the chain oxidation reaction from free radicals or acting as scavenger so that the free radicals will not react with cellular components.²⁰

Inflammation response occurred in atherosclerosis will cause adhesion molecules, such as VCAM-1 overexpressed. VCAM-1 plays a role in the attachment of monocyte on arterial endothelial cell walls. Receptors in monocyte bind to VCAM-1 and enter the sub-endothelial layer or tunica intima artery. Then the monocytes differentiate into a macrophage. The macrophages in atherosclerosis lesion produce excessive ROS (such as O_2^-) that induces LDL oxidation to oxidized LDL. Macrophages phagocyte the oxidized LDL into foam cells through scavenger receptors.^{22,23}

Furthermore, foam cells continue to form fatty streaks, then develop and continues with the proliferation of non-striated muscles from tunica media artery to tunica intima to form fibrous plaque and results in the hardening of blood vessel walls.¹⁰ public health and economic perspectives. Cardiovascular disease is the leading cause of mortality worldwide, accounting for 17.5 million deaths per year; 6.7 million of these deaths are related to stroke. Over 80% of strokes are classified as ischaemic, and approximately two thirds of ischaemic strokes result from one of three pathological states: atherosclerosis, lipohyalinosis (a small-vessel disease

The SBGOE acts in dose dependent-manner to decrease the VCAM-1 expression. Our result indicates that the decrease of VCAM-1 expression is following by reduces the inflammation. Furthermore, the reduction of VCAM-1 expression indicated the prevention of LDL into oxidized LDL, which also complied with the decline of foam cells as the main atherosclerosis characteristics. The prevention of cholesterol formation also caused a decrease in the number of VCAM-1. Active compounds in single bulb garlic suppress lipogenic and cholesterologenic enzymes, such as malate enzyme, fatty acid synthase, glucose 6-phosphate dehydrogenase and 3-hydroxy-3-methyl-glutaryl-CoA (HMG CoA) reductase.²⁴

HMG CoA enzyme plays a role in changing 3-hydroxy-3-methyl-glutaryl-CoA (HMG CoA) into mevalonate. The inhibition of the formation occurred due to allicin that is a competitive inhibitor of the enzyme. Allicin plays a role in the inhibition of thiolase enzyme. Thiolase enzyme is an enzyme played a role in the conversion of 2-acetyl-CoA into acetoacetyl-CoA.²⁵ The inhibition causes the formation of acetyl-CoA, as the source of all carbon acid in cholesterol, which has implications for reducing cholesterol synthesis. The decline of the cholesterol formed prevent LDL accumulation and causes endothelium dysfunction.^{24,25}

CONCLUSION

In conclusion, SBGOE reduces the VCAM-1 expression on dose-dependent manner in mice with a high-fat diet. The SBGOE at dose of 12,5 mg/kg reduces VCAM-1 expression near the normal group.

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