

Effectiveness of Garlic Extract (*Allium Sativum* Linn) to the Limited Cell Limfosit Cells (*Mus Musculus*) which is Inociated Egg Yolks

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Abstract: *Essential oils in garlic extract contain diallyl disulfide compounds, which are capable of suppressing the inflammatory response of endothelial injury by inhibiting the formation of free radicals that cause atherosclerosis. This study aims to prove the effect of garlic extract on the number of lymphocytes as a marker of inflammation in mice (*Mus Musculus*). The method of this research is True Experimental with One Group Pretest-postest design. Samples of 25 mice were adapted for 7 days, then divided into three groups, without treatment, given 0.5 ml of egg yolks until day 14, and given 0.5 ml yolk until day 14, then on day 15 was given garlic extract 0,009 g until day 21, then analyzed amount of lymphocyte. The results showed that the average number of first group, second group, third group lymphocytes before being given egg yolk was 2096 / mm³, 5,333 / mm³, 2,117 / mm³ of blood. Tests with the Independent Sample T Test test in the second and third groups showed significant differences between groups ($p = 0.00$, $p < 0.05$). The conclusion of garlic extract at doses of 0.009 g proved to decrease the number of lymphocytes per mouse tail given egg yolk.*

Keywords: Garlic extract, number of lymphocytes

1. Introduction

Atherosclerosis is a disease that causes the accumulation of lipids and fibrous tissue in the blood vessels, thus progressively narrowing the lumen of the blood vessels. [1]. Atherosclerosis begins when a white blood cell called a monocyte, transfers from the bloodstream into the artery wall and is converted into cells that collect fatty substances. This fat-filled monocyte will accumulate, and cause patches of thickening in the inner lining of the arteries. Any thickening area or so-called plaque, atherosclerosis or atheroma filled with a soft material such as cheese, contains a number of fatty materials, especially cholesterol, smooth muscle cells and connective tissue cells. Atheromes can be spread in medium arteries and large arteries, but they usually form in branches, probably because turbulence in this area causes injury to artery walls, making it easier to form atheroma [2].

In people with fatty deposits atherosclerosis is found throughout the tunica. Cholesterol and triglycerides in the blood are encased in a fat transporting protein called lipoprotein. High density lipoproteins or high density lipoproteins, HDLs carry excess fat out of cells to be described, and are known to be protective against atherosclerosis. However, LDL and very low-density lipoprotein lipoprotein, VLDL carries fat to the body cells, including arterial endothelial cells, cholesterol oxidation and triglycerides leading to the formation of free radicals that are known to damage endothelial cells [3].

The function of endothelial cells is to regulate adult leukocytes, inflammation, and maintain a balance between thrombosis and fibrinolysis, so that if there is damage to endothelial cells it will spur the process of inflammation that will activate lymphocyte cells as one of the markers. [3].

Garlic has long been known to have many positive effects for the human body, such as antioxidants, antibacterial, anti-carcinogenic, reducing platelet aggregation, and anti hyperlipidemia. Some experts have examined the effect of garlic on blood lipid levels. Garlic has the privilege of lowering total cholesterol, LDL, triglycerides, and raising HDL levels. Previous research on experimental animals, proving that garlic juice can raise HDL cholesterol [4]. Essential oil of garlic with diallyl disulphide or DADS can lower cholesterol. Garlic extract contains Methylallyl trisulfide which acts as a prevention of blood coagulation and clotting prevention that clogs the heart and brain blood vessels [5].

Based on these facts need to be examined the influence of garlic extract on the number of lymphocytes in mice or *Mus musculus* given egg yolks.

2. Research Methods

2.1 Tools and Test Materials

The tools used in the study were animal cages, analytical scales, EDTA anticoagulant tubes, gastric sonde, and disposable injections. Mice test material that meets the criteria, egg yolk separated from white, garlic essence

2.2 Procedur Test Material Preparation

- Preparation of quail egg yolk: Egg yolks are separated from white, given as much as 0.5 ml per head.
- Preparation of garlic juice: garlic juice weighed as much as 0.009 g dissolved in 0.3 ml aquadest.

2.3 Research procedure

Group one consisting of 5 mice after through adaptation then on the 7th day taken blood then analyzed amount of

lymphocyte to mengetahui lymphocyte number of mice early without treatment. The second group consisting of 10 mice after 7 days adaptation, on day 8 was isolated yellow quail eggs 0,5 ml per day, as well as feed CP511 and drinking water ad libitum until day 14, then the blood was taken to analyze the number of lymphocytes on day 14. The third group consisting of 10 mice after 7 days adaptation, on day 8 was inoculated 0,5 ml yolk quail per day, and feed CP511 and drinking water ad libitum until day 14, then at 15th day was given 0.009 g garlic extract dissolved in 0.3 ml of aquadest, CP511 feed and drinking water ad libitum until 21st day, then blood was taken to analyze the number of lymphocytes on the 21st day.

2.4 Data analysis technique

Data analysis in this study to compare changes in the number of lymphocytes in 2 treatment groups before and after being given garlic extract, using SPSS. Data is normally distributed, Homogeneous and scaled interval or ratio, using Parametric statistic test, if not used Non-Parametric Test For normality test data used smirnov kolmogrof test.

3. Result and Discussion

In this study the results of the number of lymphocytes in mice who were given egg yolks and given egg yolk and garlic extract are in the table below

Table 1: Number of lymphocytes / mm³ of blood before and after inoculation of garlic extract

Sample code	number of lymphocytes / mm ³ of blood before inoculation of garlic extract	number of lymphocytes / mm ³ of blood after inoculation of garlic extract
A	5828	1735
B	6663	3140
C	5359	1310
D	5056	3101
E	4928	892
F	8245	724
G	4100	1520
H	4302	2014
I	4807	3650
J	4045	3088
Mean	5.333	2.117

Based on the results of normality test data with Kolmogrov Smirnov obtained results $p = 0.471$ at $\alpha = 0.05$, meaning the data is normally distributed. To know the result of research about Effect of Bawang Putih (*Allium sativum* Linn) on Lymphocyte Mice (*Mus musculus*) which given Yellow Egg, conducted data processing with T Test Paired test, that there is influence of garlic extract $p = 0,000 < \alpha = 0,05$, meaning H_0 is rejected, and H_1 accepted. Conclusion there is effect of garlic extract to change of lymphocyte count.

The first group consisting of 5 mice after adaptation then on the 7th day was taken blood for the analysis of lymphocyte count, the first group functioned to know the number of initial lymphocytes in mice without treatment, the average number of first group lymphocyte count was 2.086 / mm³ blood. The second group consisting of 10 mice after 7 days adaptation, on the 8th day was given 0.5 quail yolk quail per

day, until day 14, the average number of lymphocyte group of the second group was 5,333 / mm³ blood. The third group consisting of 10 mice after 7 days adaptation, on day 8 was given 0 quail yolk quail per day, until day 14, then on the 15th day was garlic extract 0,009 g dissolved in 0,3 ml aquadest, CP511 feed and drinking water ad libitum up to 21 days, obtained the average number of lymphocytes group three is 2,117 / mm³ blood

Provision of egg yolk in mice greatly affect the metabolism of blood cholesterol levels. Egg yolks rich in cholesterol and triglycerides are decomposed by gastric lipase enzymes, having previously been emulsified by bile salts. The decomposition results in the form of free fatty acids and two monoglycerides in the form of micelles in the small intestine. By the small intestine epithelium, free fatty acids and monoglycerides are synthesized again into triglycerides and phospholipids, then merged with kilomikrons, transported to the liver and tissues. The speed of cholesterol synthesis in the body will decrease as more cholesterol is absorbed. So that spur the process of inflammation [4].

The inflammatory response of the endothelial vessels makes the endothelium express an inflammatory mediator such as Intercellular Adhesion Molecule (ICAM). Expression of ICAM occurs in endothelial and macrophages in the process of atherosclerosis formation. Increased ICAM will invite lymphocytes, leukocytes and other bioactive blood to the site of the lesion. The chemoattractant factor is targeted for the prevention of lymphocyte accumulation and atherogenesis using antibodies to the mediator [1].

According to research [6] found that the increase in lymphocyte count occurs when the activity is severe, and the increase is significant. The results of this study can increase the number of lymphocytes due to heavy activity in mice when the process of giving egg yolk and also garlic through sonde gastric. The presence of severe activity will trigger the release of lymphocytes from the lien to the bloodstream because of the stimulation of stress hormones such as cortisol and catecholamines. Increased leukocytes by the presence of an activity, in this case caused by several factors such as mediation from catecholamines, cortisol, demarginasi the inflammatory response, transmitre neuron, and peptide. The increased number of leukocytes after activity due to the large number of leukocytes that follow or enter the blood vessel wall or endothelium by seeping into the circulation of storage suddenly. Dermaginations are influenced by the hormone adrenaline which causes the decrease of attachment of leukocytes to the endothelium.

The bioactive components of garlic can lower serum cholesterol levels, which can protect against heart disease and atherosclerosis. The effects of lowering fat have been studied in various laboratories. The mechanism of action, among others, is to inhibit the dependence of cholesterol biosynthesis concentration at different stages of enzyme (14-alpha-demethylase, HMG CoA reductase). Feeding high cholesterol will cause hypercholesterolemia in the form of free cholesterol (FC), cholesterol ester (esters cholesterol = Ec) and total cholesterol. Cholesterol ester accumulates mainly in the intima layer of blood vessels, thereby increasing tissue cholesterol and formation of atheroma in the aorta. Garlic will inhibit hyperkholesterolemia

significantly, reduce tissue cholesterol and suppress the formation of atheroma in the aorta. It can be seen from the decrease of ester cholesterol level and the increasing of FC / EC ratio. So that will reduce the development of fat deposits [7].

The essential oil of garlic has been extensively studied containing the content of Diallyl Disulfide (DADS) to inhibit HMG CoA reductase which is one of the enzymes that play a role in the formation of cholesterol. So the administration of essential oils can inhibit the inflammatory reaction caused by the egg yolk diet. This inhibitory action will cause a decrease in the number of lymphocytes. In addition, Diallyl-disulphide (DADS) has an allyl chain which can easily be reduced to saturated propyl chains, thus lowering the levels of NADH and NADPH essential for triglyceride and cholesterol synthesis as a marker of inflammatory processes [7].

Allicin in garlic extract also has a binding nature of SH group that is a functional part of Ko-A necessary for cholesterol biosynthesis [7].

Effects on the cardiovascular system, According to clinical observations in 114 cases of hypertension and atherosclerosis that is thickening and hardening of artery walls, garlic conspicuously reduces systolic blood pressure by 0.5 to 2.7 kPa. The use of garlic extract orally may inhibit platelet collection induced by ADP and adrenaline. The use of oral garlic for a long time can prevent atherosclerosis [5].

Garlic has a fairly complete potential to prevent cardiovascular diseases, such as hyperlipidemia, hypertension, platelet aggregation, and blood fibrinolysis activity [1].

Based on the theory, the garlic is quite potential to decrease the progression of atherosclerosis. It was shown in the results of research showing that the application of garlic extract has an effect on the decrease in the number of lymphocytes and leukocytes in the group given the sari bawang putih after the giving of egg yolk for 7 days

4. Conclusion

From the results of research on the effectiveness of garlic extract on changes in the number of lymphocytes of mice given egg yolks can be concluded that:

- 1) The number of lymphocytes in mice treated with an average egg yolk is 5.337 / mm³ of blood.
- 2) The number of lymphocytes in mice treated with egg yolk then the average garlic extract is 2.136 / mm³ blood.

There were significant differences in the number of lymphocytes of mice before being given garlic extract and after being given garlic extract.

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