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Administration of Solo Garlic Extract (*Allium sativum Var. Solo Garlic*) Reduced Low Density Lipoprotein and Increased Superoxide Dismutase than Control in Male Wistar Rats (*Rattus norvegicus*) Fed with High Fat Diet

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ABSTRACT

Introduction: Obesity increases the release of ROS and cause oxidative stress. Solo Garlic Extract (Allium sativum Var. Solo Garlic) has secondary metabolites such as flavonoids, ajoene, organosulfur, allicin, phenol tannins and other compounds which are known to have antioxidant, anti-inflammatory activity and can improve blood lipid profiles. This study aims to prove the effect of solo garlic extract on LDL and SOD level in male Wistar rats fed with high-fat diet. ***Methods:*** This was true experimental research using post-test only control group design. The subjects were 30 male Wistar rats aged 2-3 months, weighing 150-200 grams that were randomly divided into 3 groups. The normal group (N) was given standard diet, the control group (C) was given high-fat diet and 1 cc of placebo, and the treatment group (T) was given high-fat diet and 0.2 gram in 1 cc of solo garlic extract, given once each day for 28 days. The blood was taken to examine LDL and SOD level after 28 days. Data was analyzed using One Way Anova. ***Results:*** The results showed that there was a significant difference in LDL: The N group (20.45 ug/mL), the C group (37.32 ug/mL) and The T group (24.44 ug/mL). The LDL in the T group was lower than that of the C group ($p<0.001$) and the LDL in the T group was higher than the N group ($p<0.001$). There was a significant difference in SOD between the N group (0.88 ng/mL), the C group (0, 89 ng/mL) and the T group (1.99 ng/mL). The SOD of the T group was higher than that of the C group ($p<0.001$) and the N group ($p<0.001$). ***Conclusion:*** Administration of 0.20 gram in 1cc/day of solo garlic extract reduced LDL level and increased SOD enzyme in male Wistar rats (Rattus norvegicus) fed with high-fat diet.

Keywords: solo garlic; low density lipoprotein; superoxide dismutase; high-fat diet; dyslipidemia

INTRODUCTION

Many degenerative disorders are caused by the accumulation of cellular damage over time and age. Oxidative stress caused by free radicals is one possible cause (Ngestiningsih et al., 2019). Oxidative stress can cause damage to biomolecules such as lipids, proteins, DNA and is involved in the pathogenesis of many diseases. Superoxide Dismutase (SOD) enzymes can be used as biomarkers to measure the level of oxidative stress in an organism (Muralidharan et al., 2017). The increased release of ROS and the occurrence of oxidative stress can be affected by obesity and the accumulation of increased lipid profiles (Susantiningsih, 2015). Inflammation obesity is often associated with increased oxidative stress. In particular, leptin, an adipocyte-derived hormone, is increased in obese individuals and can cause oxidative stress (Huang et al., 2015).

The occurrence of obesity, dyslipidemia, atherosclerosis, and cardiovascular disease is affected by a high-fat diet (Sari et al., 2017).

An unhealthy lifestyle can increase the risk of cardiovascular disease. One of the causes of the many factors that cause cardiovascular disease is dyslipidemia. Dyslipidemia is a disorder of lipid metabolism characterized by increased or decreased levels of lipid fractions in plasma (Perkeni, 2021). WHO reports that every year 17.9 million die from cardiovascular disease. Coronary heart disease (CHD) is the number one cause of death in the world, which is due to blockage or narrowing of the coronary arteries that form plaque. CHD increases every year and ranks as the highest cause of death in Indonesia, especially at productive ages (Kemenkes, 2013).

Natural ingredients are expected to prevent and treat dyslipidemia and obesity safely or at least provide minimal side effects. Garlic (Allium sativum L.) is herb with complex action used as a medicine for cardiovascular disease, headaches, and cancer. Garlic contains active compounds including Allicin, a number of antioxidants, including vitamin C, germanium, and organosulfur compounds (Mahr, 2016).

Allicin is useful as an anti-dyslipidemia. HMG CoA reductase, which is involved in making cholesterol, can be inhibited by allicin. By blocking HMG CoA reductase and other enzymes, this substance can reduce the fat fraction. (Pramitasari et.al, 2018). The content of flavonoids in solo garlic extract is an antioxidant compound that can increase the activity of the lipoprotein lipase enzyme. The enzyme lipoprotein lipase has an important role in lipid metabolism to hydrolyze triglycerides into fats and glycerol. (Brouwer et al., 2018).

Solo garlic is more beneficial because the level of oil and substances it contains is higher, compared to multibulb garlic (Barat et al., 2014). Previous studies have proven multibulb garlic extract can improve total cholesterol, triglyceride, LDL and HDL significantly in rats fed with high-fat diet (Pramitasari, 2012). Another study conducted by Shafey also proved that garlic effectively acts as a natural antioxidant in preventing oxidative stress which occurs due to decreased SOD enzymes (Shafey, 2013). Several studies have been carried out using multibulb garlic or ordinary garlic for various diseases, but studies conducted using solo garlic have not been studied further regarding the effectiveness of the compounds in experimental animals in hypercholesterolemia caused by a high-fat diet through an LDL and Superoxide Dismutase (SOD) indicator.

METHODS

Study Design and Experimental Animals

This study was true experimental research with post-test only control group design. Research and production of solo garlic extract was carried out at the Laboratory of Animal Unit, Pharmacology Section, Faculty of Medicine, Udayana University, Bali. While the examination of LDL and SOD was carried out at the Biochemistry Unit of Udayana University, Bali. The sample needed in this experiment was 27 male Wistar rats (n=9), 2-3 months, weighing 150-200 grams. To anticipate drop out, 10% of total sample were added, with the total amount to 30 rats divided into 3 groups: normal, control and treatment group (n=10). The study protocol has been approved by the ethics commission of Faculty of Medicine, Udayana University, Bali, (B/35/UN14.2.9/PT.01.04/2023).

Experimental Animal Treatment

Experimental animals were given food in the form of pellets and drink ad libitum. Rats were randomly divided into 3 groups: normal group was given standard diet, control group was fed with high fat diet (1% cholesterol, 5% yolk, 10% lard, 1% cooking oil, up to 100%s standard diet) and 1cc distilled water as placebo once a day, and treatment group was fed with high fat diet and 0.20 gram in 1cc/day of solo garlic extract. On the 29th day, LDL and SOD level were examined with ELISA Kit. The data was analyzed using One Way Anova with Post Hoc LSD test.

LDL and SOD Level Examination

SOD measurements were carried out using the SOD Kit while LDL using the LDL Kit with the ELISA method.

RESULTS

Normality test on body weight, LDL and SOD level was done using Saphiro-Wilk test. The test showed that the data was normally distributed ($p>0.05$).

Homogeneity test on body weight, LDL and SOD level was done using Levene's test. The test showed that the data was homogeny ($p>0.05$).

Based on One Way Anova test, it shows that the p value <0.001, meaning that the mean body weight, LDL and SOD in the three groups was significantly different ($p<0.05$).

It was further tested with post hoc Least Significant Difference (LSD) test because the data in each group was homogeny ($p>0.05$).

The results of data analysis showed that the mean weight of the treatment group was lower than the control group ($p<0.001$), but higher than the normal group ($p<0.001$). The mean LDL level in the treatment group was lower than the control group ($p<0.001$) but higher than the normal group ($p<0.001$). The mean SOD level in the treatment group was higher than the control group ($p<0.001$) and the normal group ($p<0.001$), but the SOD level in the control group was not different from the normal group (p 0.643).

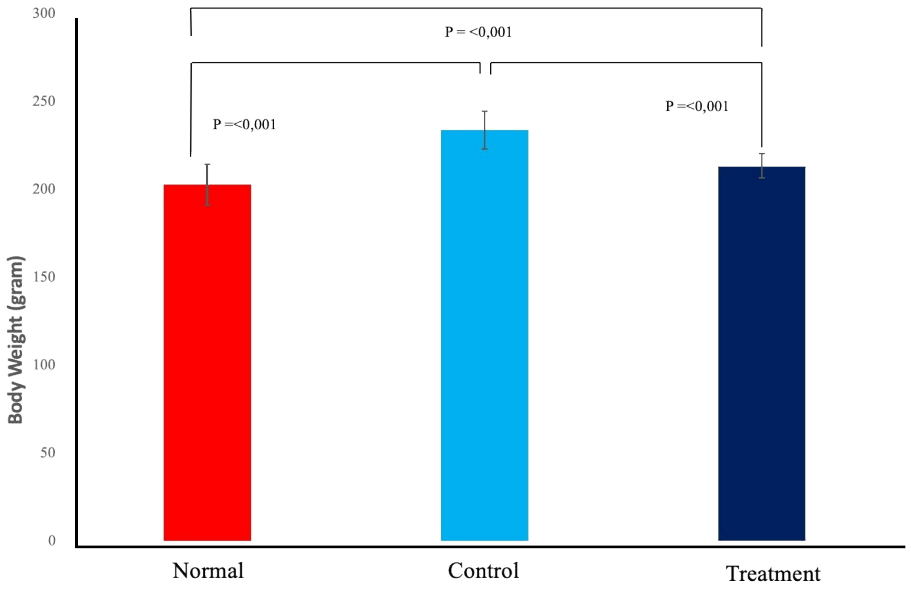

FIGURE 1: Comparison on Body Weight between Groups.

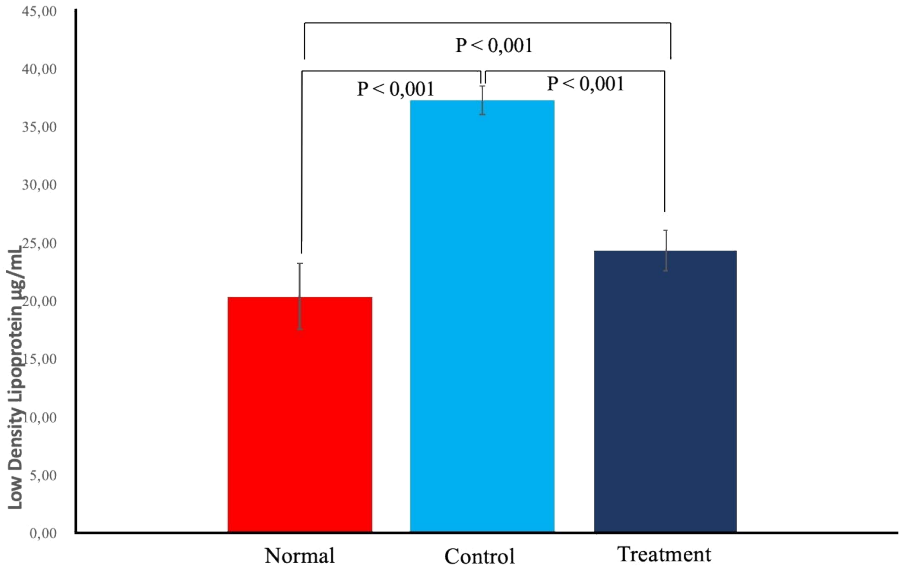

FIGURE 2: Comparison on LDL between Groups.

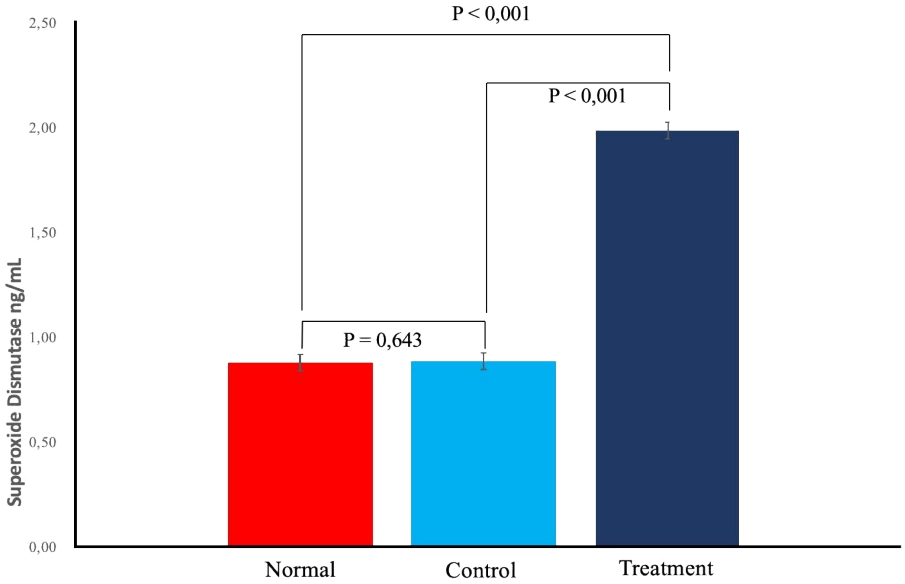

FIGURE 3: Comparison on SOD between Groups.

DISCUSSION

The results showed that there was an average increase in LDL levels in the control group (37.32 ug/L) compared to the normal group (20.45 ug/L) which was only given a standard diet. This refers to previous studies where rats induced by high-fat diets experienced dyslipidemia (Alaydrus *et al*., 2020). Research by Harsa used 5% egg yolk to induce dyslipidemia where rats induced by a high-fat diet for 28 days experienced an increase in HDL, LDL and triglycerides (Harsa, 2014).

The recommended cholesterol intake per day is less than 200 mg (3.6 mg after being converted to the rat dose). Consumption of food high in cholesterol and saturated fatty acids reduces the number and activity of LDL receptors, thereby reducing the regulatory control of the HMG-CoA reductase enzyme (Naufalina & Nuryanto, 2014). Feed 552 contains higher fat compared to feed 594, meaning this feed increases body weight which also causes an increase in blood cholesterol level of rats given a high-fat diet and affects the increase in the amount of fat deposited in adipose tissue. This is one of the factors causing high levels of LDL in the treatment group (Nurmawati, 2016).

The results of the comparison showed that the administration of single garlic in the treatment group could reduce LDL levels ($p<0.001$). The control group that was given the placebo effect experienced an increase in LDL levels with the mean difference between the control and treatment groups=12.88 ug/L. Even so, the cholesterol level of the treatment group could not return to normal. This proves that giving a solo garlic extract can reduce LDL levels. This is in accordance with research conducted by Pramitasari *et al*. where the use of garlic extract 0.20 g/head/day has been shown to be effective in reducing LDL levels (Pramitasari *et.al*, 2018). According to the study described by Carolyn *et al.* stated that solo garlic can significantly reduce cholesterol, LDL and triglyceride levels in blood serum (Carolyn *et al*., 2021).

Administration of garlic extract showed a significant increase in SOD levels ($p<0.001$) in the treatment group (1.99 ug/L).

The SOD level of the treatment group was higher than the control and normal groups, but the normal group was not significantly different from the treatment group (p=0.643). Flavonoid content in solo garlic extract acts as an antioxidant compound that can increase the activity of the lipoprotein lipase enzyme. The enzyme lipoprotein lipase has an important role in lipid metabolism to hydrolyze triglycerides into fatty acids and glycerol (Brouwer *et al*., 2018). This is in line with research conducted by Shafey that garlic effectively acts as a natural antioxidant in preventing oxidative stress that occurs due to increased endogenous enzymes, namely SOD (Shafey, 2013).

Single garlic extract is thought to have antioxidant capacity, anti-inflammation and reduce LDL levels of the active components in it, namely Allicin, Flavonoids, organosulfur, Phenolic compounds and others. Allicin is responsible for the inhibition of the HMG CoA enzyme and the thiolase enzyme. Allicin is also able to increase the antioxidant activity of SOD so that levels of lipid peroxide which act to trigger oxidative stress will decrease. Increased levels of SOD are expected to form the immune system from Reactive Oxidation Stress to avoid the risk of the body experiencing various diseases. In line with the principles of Anti-Aging Medicine to prevent, inhibit and slow down aging, the potential of solo garlic which is rich in antioxidants needs to be explored more deeply to prevent, inhibit dyslipidemia and other diseases that can be caused.

CONCLUSION

The administration of 0.20 grams in 1 cc/day of solo garlic extract (*Allium sativum Linn var Solo Garlic*) lowered LDL level and increased SOD level in male Wistar rats fed with high fat diet.

CONFLICT OF INTEREST

All researchers declare that there is no conflict of interest related to this article.

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AUTHOR'S CONTRIBUTION

All authors contribute equally in compiling this research article.

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