



Original Article

The Effect of The Combination of The Sesame Oil and Vitamin E To Improve Erythrocyte Membrane On Hypercholesterol Rats

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ABSTRACT

Objective: The purpose of this study was to determine the combination effect of sesame oil and vitamin E to the number of abnormal erythrocytes, and MDA levels by the amount of hemolysis. Hypercholesterolemia that is grown by erythrocyte membranes were decreased conditions that leads to atherosclerosis associated with oxidative stress which leads to lipid peroxidation. Sesame oil (SO) has special antioxidants that also contains 85% unsaturated fatty acid, where 40% consists of poly unsaturated fatty acid. Vitamin E is a chain breaker antioxidant that prevents the multiplication of free radical reactions in phospholipids.

Methods: This research using 6 treatment groups, 3 groups received an atherogenic diet, while the other 3 groups received an atherogenic diet and treated with a combination of sesame oil with the same dose, added with 0.02 ml of vitamin E.

Results: The results showed that the proportion of 1.2 ml SO and 0.02 ml of vitamin E, with the average amount (%) of hemolysis at 0.45% NaCl concentration was (5, 05 ± 0.129), while in the control (33,87–1,919). The number (%) of erythrocytes is not normal (25,75 ± 2,473) while in the control (80,27 ± 4,631). Erythrocyte MDA level (0.229 ± 0.039) µmol / ml, while in the control (0.443 ± 0.013) µmol / ml.

Conclusion: This research shows that the combination of oil and vitamin E had a significant decrease in the number of abnormal erythrocytes, the amount of hemolysis and MDA levels. The MDA reduction is followed by a decrease in the number of abnormal erythrocytes and the amount of hemolysis.

Key Words: Sesame oil, Antioxidant, Vitamin E, Hypercholesterolemia

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INTRODUCTION

Hypercholesterol diet has an important role with the occurrence of oxidative stress¹. Oxidative stress causes cell damage associated with free radicals and lipid peroxidation which includes damage to the cell structure of the membrane. The results of in vitro studies on normal erythrocytes incubated in hypercholesterolic conditions, gradually changed shape. Koter² showed that hypercholesterolaemia caused changes in the structure and fluidity of plasma membranes of erythrocytes, also impairing the function and structure of protein membrane plasma. Hypercholesterolemia lowers erythrocyte deformability that damages the hemorheological environment and leads to atherosclerosis. Atherosclerosis is illustrated by abnormalities of erythrocyte composition and

rheological function and increased oxidative stress³. One of the ROS sensitive tissues or organs are erythrocytes⁴.

Sesame oil, known to have antioxidant effects associated with the characteristics of its components⁵. Sesame oil contains approximately 0.3-0.5% sesamol, 1-4% sesamol and 0.5-1% sesamin⁶. In addition, sesame oil contains 85% unsaturated fatty acids (Unsaturated Fatty Acid). Vitamin E (D-α Tocopherol) is an antioxidant that prevents sustained chain reactions as a chain breaking antioxidant in tissues, erythrocytes and plasma. Vitamin E in the cell membrane will prevent the multiplication of free radical reactions on phospholipids. In erythrocyte membranes, vitamin E protects erythrocytes from hemolysis⁷. The effect of the combination of sesame oil and vitamin E with the consideration that sesame oil in the body is susceptible to oxidation due to the content of PUFA that trigger oxidative stress, is interesting to be

studied. The addition of vitamin E as an antioxidant is intended to prevent sustained free radical reactions. Intake of high-PUFA foodstuffs is associated with increased antioxidant requirements, especially vitamin E. The purpose of this study was to prove that the combination of sesame oil and vitamin E increased the resistance of erythrocyte membranes in hypercholesterolic mice by inhibition of lipid peroxidation.

RESEARCH METHODS

This research was conducted experimentally in laboratory using simple randomized design post test only control group design. This research was conducted in Pharmacology and Biomedical laboratory, Faculty of Medicine, Universitas Brawijaya Malang and also conducted on Clinical Pathology laboratory of dr. Saiful Anwar Public Hospital, Malang, Indonesia.

Sample Determination

In this study used *Rattus norvegicus* Strain Wistar rat as a research sample, where the two groups as control (negative control and positive control). In the negative control of mice get a standard diet, on the positive control of rats get atherogenic diet. The treatment group consisted of 6 groups, 3 groups received atherogenic diet and sesame oil treatment (dose 0.3, 0.6 and 1.2 ml), 3 group got atherogenic diet and combination treatment of sesame oil (dose 0.3, 0.6 and 1.2) plus vitamin E by 0.02 ml.

Preparation of Hypercholesterolic Mice

Diet to make animals try to become hypercholesterol is by giving atherogenic diet consisting of: cholesterol crystal, cholesterol acid, lard, flour and Confeed PAR-S.

Provision of Sesame Oil and Vitamin E

How to administer sesame oil and vitamin E is to be inserted into the stomach of a mouse by injection through sonde. The doses of sesame are 0.3 ml; 0.6 ml; 1.2 ml /day respectively. Meanwhile the dose of vitamin E is 0.02 ml /day.

Inspection Technique

Amount (%) abnormal erythrocyte counted with microscope with 1000x magnification. The amount of hemolysis (%), measured by the Osmotic Fragility Test (OFT) test was erythrocytes exposed in vitro with NaCl solution with various concentrations (1.0%, 0.85%, 0.75%, 0.65%, 0.60%, 0.55%, 0.50%, 0.45%, 0.40%, 0.35%, 0.30%, 0.20%, 0.1%), erythrocyte MDA levels were measured by Tio Barbituric Acid (TBA) test.

RESULT

A. Amount (%) Abnormal Erythrocyte, Amount (%) Hemolysis and MDA Level

Statistic test on the mean amount (%) abnormal erythrocytes can be seen on table 1. There was a significant difference ($p > 0.05$) between the sesame oil group and the combination of sesame oil + vitamin E at various doses.

Table 1: Average Amount (%) Abnormal Erythrocyte

Variabel Group	Normal (x ± sd)	H (x ± sd)	H+SO1 (x ± sd)	H+SO2 (x ± sd)	H+SO3 (x ± sd)	H+SO1+VitE (x ± sd)	H+SO2+ Vit E (x ± sd)	HK+SO3+Vit E (x ± sd)
	n=4							
% Erythrocyte Abnormal	14.42 ± .819	80.27 ± 4.631	69.25 ± .356	46.20 ± 6.573	37.35 ± .435	66.70 ± 5.363	43.15 ± 4.582	25.75 ± 2.473

*Description: H: Hypercholesterol, SO : Sesame Oil

Statistic test on the mean Amount (%) Hemolysis there was a significant difference ($p > 0.05$) between the

Sesame Oil group and the Combination of Sesame Oil + Vitamin E at various doses.

Table 2. Mean of MDA Erythrocyte (μ mol/ml)

Variabel Group	Normal (x ± sd)	H (x ± sd)	H + SO1 (x ± sd)	H+SO2 (x ± sd)	H+SO3 (x ± sd)	H+SO1+VitE (x ± sd)	H+SO2+ Vit E (x ± sd)	HK+SO3+Vit E (x ± sd)
	n=4							
MDA Levels	0,129 ± 0,01	0,443 ± 0,013	0,385 ± 0,020	0,307 ± 0,010	0,26 ± 0,083	0,364 ± 0,025	0,284 ± 0,01	0,229 ± 0,039

Description: H: Hypercholesterol, SO : Sesame Oil

B. Erythrocyte morphology

treatment group. The results of rat blood smear in each group are shown in the following figure:

This research revealed that there were differences of erythrocyte morphology in the control group and the

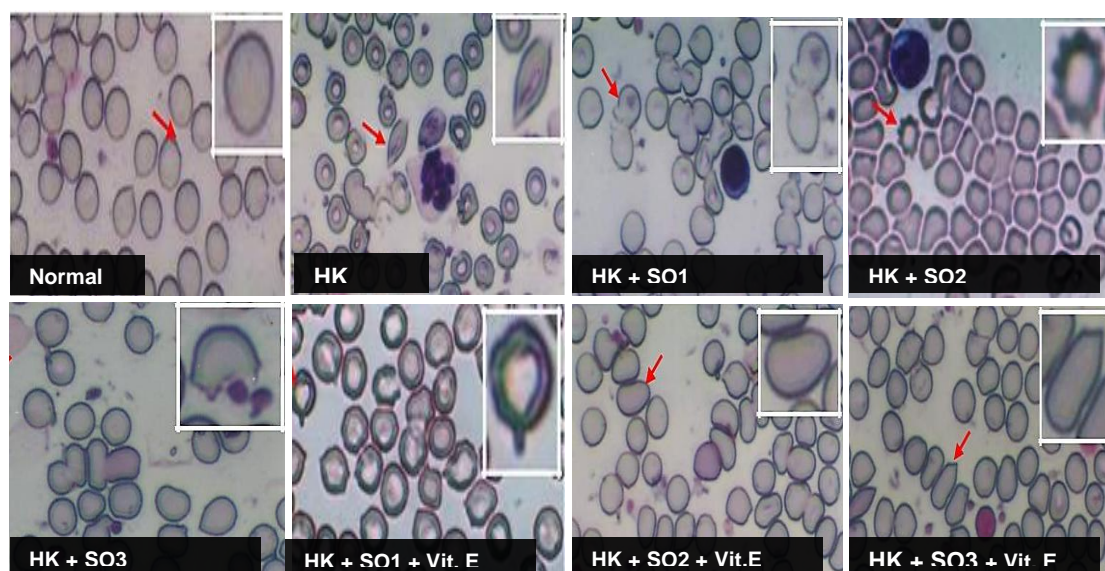


Figure 1: Histopathologic changes in erythrocytes following atherogenic diet, sesame oil and combination of sesame oil and vitamin E. Giemsa staining with 1000xmagnification.

The effect of sesame oil treatment and the combination of sesame oil + vitamin E on the decrease in abnormal erythrocyte count can be seen in the following figure:

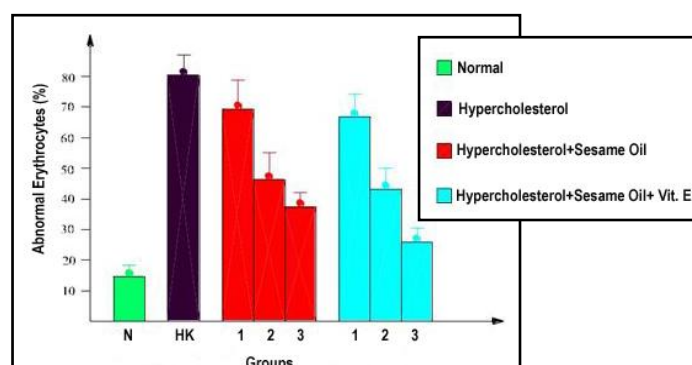


Figure 2. Amount (%) Abnormal Erythrocyte in normal and treated group.

The best treatment to hypercholesterolemia is on group 8 that was a combination of sesame oil and vitamin E. This

results are also give effect towards the erythrocyte MDA levels. This results can be seen of Figure 3.

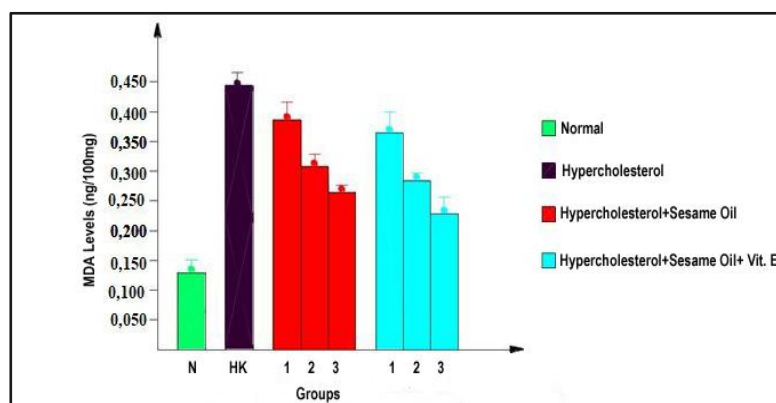


Figure 3. Erythrocyte MDA Levels in normal and treated group.

Treated groups that received an atherogenic diet had resilience lower than the group who got standard diet (normal). From the result of statistical analysis to amount of hemolysis in the control and treatment group was found to be different which is significant ($p > 0.05$). From Duncan test can be known at concentration NaCl

how in each group began to occur hemolysis. The best treatment is in the combination treatment of sesame oil dose 3 and Vitamin E (group 8). The group has resistance higher against osmotic lysis than other treatments (Figure 4).

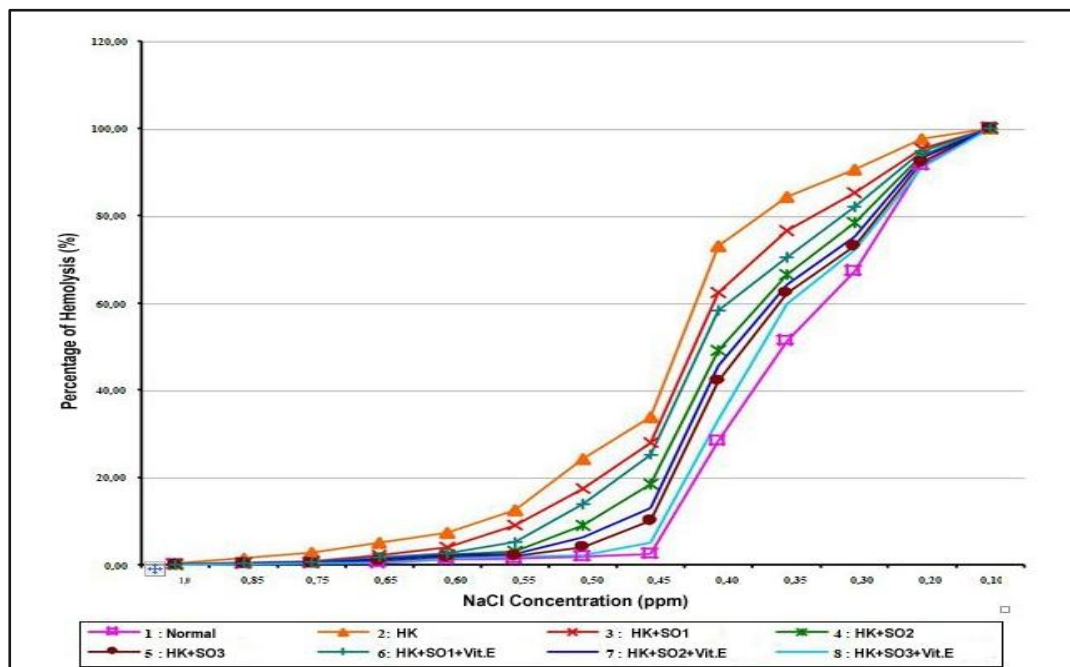


Figure: 4. Amount (%) Hemolysis At Various NaCl Concentrations From the figure shows that the best treatment is on group 8 is a combination of 3 dose sesame oils and vitamin E.

DISCUSSION

Treated group with atherogenic diets for 10 weeks, the mice examined their blood cholesterol levels, all the groups receiving the atherogenic diet had cholesterol levels exceeding the normal group cholesterol level of more than 50 mg/dl. The results of observation of qualitative erythrocyte morphology, observed by microscope (one field of vision) found that there was a difference of erythrocyte form in mice that received an atherogenic diet compared to the normal group (Figure 1). Normal blood smear all cells exhibit the same volume, shape and color⁸. in. Amount (%) of abnormal erythrocytes in the group treated with atherogenic diet for 10 weeks, on average exceeding the group that received the standard diet (normal group). In the treatment group, the best treatment was found in group 8 of 3 (1.2 ml) vitamin D (0.02 ml) combination of sesame oil, with amount (%) of abnormal erythrocytes ($25,75 \pm 2,473$) while in control ($80,27 \pm 4,6$).

The study by Manjunatha & Singh⁹, in vitro found that normal erythrocytes incubated in hypercholesterol conditions for half of hour, 1 hour and 1 and half hours gradually changed form from their normal form. Koter² suggests that hypercholesterolemia changes in the structure and fluidity of the erythrocyte membrane plasma that also impairs the function and structure of

protein membrane plasma. According to Keenoy³, hypercholesterolemia lowers erythrocyte deformability that damages the hemorheological environment and leads to atherosclerosis. Regression results showed that both the sesame oil treatment and the combined treatment of sesame oil + vitamin E both had an effect on the decrease in abnormal (%) amounts of erythrocytes, but on the combination of sesame oil + vitamin E, the effect was stronger.

Hypercholesterol causes changes in erythrocyte form through lipid peroxidation mechanism. Lipid peroxidation is initiated by free radical attack on the fatty acid hydrocarbon chain (PUFA)¹⁰. Erythrocytes can easily and reversibly change shape due to fluid and flexible membranes¹¹. The erythrocyte membrane fluidity is affected by the presence of the PUFA chain. PUFA damage tends to reduce membrane fluidity that ultimately affects membrane function. A number of peripheral cytoskeleton proteins attached to the internal surface of erythrocyte membranes and these proteins play an important role in maintaining biconcave shape and erythrocyte flexibility, if the membrane structure is damaged, it will affect the erythrocyte form¹².

The results of research from Myung Hwa¹² and Moriguchi¹³ for experimental animals (rats) reported that erythrocytes induced by a particular agent cause oxidative stress leading to lipid peroxidation of erythrocyte membrane PUFA, as well as high levels of

MDA and cause rigidity of the cell membranes decreased and erythrocyte deformability. The role of a combination of sesame oil and vitamin E is to increase the resistance of erythrocyte membranes by inhibition of lipid peroxidation characterized by a decrease in the number of stronger erythrocytes in the group.

The resistance of erythrocyte membranes to lipid peroxidation can be determined by the percentage of hemolysis. Figure 4 shows that the erythrocytes exposed to a 0.45% NaCl concentration, in the normal group only some erythrocytes are lysed, but in the hypercholesterol group nearly 40% have lysis. According to Phaniendra et al¹⁴ that one of the free radical products of hydroxyl radicals, is a highly reactive molecule and can react with proteins, nucleic acids, lipids and other molecules and change the structure of cells / tissues. Erythrocytes are highly sensitive to oxidative damage, lipid peroxidation that results in the destruction of erythrocyte lysis. From the results of regression test, showed that both sesame oil treatment and combination treatment of sesame oil and vitamin E had significant effect on the decrease in hemolysis, except in the 0.1% NaCl concentration, did not show significant result because the concentration was very hypertonic, so that the sesame oil treatment as well as the combination treatment of sesame oil and vitamin E causes 100% hemolysis.

In hypercholesterolic rats, high oxidant products cause lipid peroxidation in erythrocyte membranes resulting in hemolysis (decreased erythrocyte membrane resistance) the mechanism is that as described above that in conditions of hypercholesterolemia, erythrocyte changes (abnormal, unformed biconcave), decreased fluidity, deformability decreased. The test results with an osmotic fragmented (Osmotic Fragility Test) cell test when exposed to osmotic pressure is slightly lower than normal pressure because the cell has a little extra volume to hold extra water.

Normal erythrocytes in the form of biconcave discs can absorb water and swell to within the limits of endurance possessed, but cells whose surface area is relatively smaller than its contents will be broken by the amount of water⁸. Spherocytes and other cells with membrane damage will rupture in a slightly hypotonic solution. Hypercholesterolemia also occurs abnormalities in the structure so that no longer bind closely to other proteins that in normal circumstances interact, causing weak membrane. Abnormalities of certain proteins, for example, ankyrin also causes weak membranes. Erythrocytes break very easily in hypotonic solutions, their osmotic power (resistance) decreases¹⁵. Conversely, if erythrocytes are very difficult to experience hemolysis, it is said to increase resistance. The role of a combination of sesame oil + vitamin E is to increase the resistance of erythrocyte membranes by inhibition of lipid peroxidation characterized by a decrease in the number of hemolysis stronger in the treated group.

In the group with an atherogenic diet, MDA levels were higher than those in standard diet. This is due to the ingredients contained in the high cholesterol atherogenic

diet causing high oxidant products. In accordance with the opinion of Ibrahim et al¹, that the hypercholesterol diet plays an important role in the occurrence of lipid oxidation, the continuation of this reaction is the breakdown of the fatty acid chain into Malondialdehyde compounds (MDA), 9-hydroxy-nonenal, ethane, pentane. Then the higher cholesterol levels will be followed by MDA levels. From the results of regression tests, showed that both the sesame oil treatment and the combination of sesame oil and vitamin E had significant effect on the decrease in MDA levels. The combined treatment of sesame oil and vitamin E had a stronger effect in lowering MDA levels compared with sesame oil treatment alone. The results of the correlation test showed that MDA levels were strongly correlated with abnormal amounts of erythrocytes and number hemolysis. The direction of positive correlation, indicating that the higher the level of MDA, the number of abnormal erythrocytes and the number hemolysis is also higher.

The role of a combination of sesame oil and vitamin E is to increase the resistance of erythrocyte membranes by inhibition of lipid peroxidation characterized by a decrease in MDA levels that are stronger in the group. From the above discussion it can be concluded that the atherogenic diet causes high oxidative products, causing oxidative stress leading to lipid peroxidation and high levels of MDA, in erythrocytes erythrocyte changes are indicated by high percentage of abnormal erythrocytes and high percentage of hemolysis. The role of sesame oil and vitamin E is to inhibit lipid peroxidation / increase the resistance of erythrocyte membranes, due to the lignans component possessed by sesame oils that have antioxidant activity.

CONCLUSION

The combination of sesame oil and vitamin E increases the resistance of erythrocyte membranes in hypercholesterolic mice by inhibition of lipid peroxidation. There was a decrease in abnormal (%) amounts of abnormal erythrocytes and significant decrease in (%) hemolysis in hypercholesterolic mice treated with a combination of sesame oil and vitamin E. In further development, it is necessary to investigate the determination of the effective dose of sesame oil and vitamin E for patients with hypercholesterolemia with more practical packaging, for example, made in capsule form. Further research is needed on the resistance of erythrocyte membranes by using other parameters such as MCV (Mean Cell Volume) or RDW (Red Cell Distribution Width)

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest

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