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Research Articles

The Effect of Superoxide Dismutase (SOD) Supplementation Towards Plasma Levels of Malondialdehyde (MDA), Total Cholesterol and LDL Cholesterol in the Elderly

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Abstract

Background: Several various physiological functions in elderly people are diminished due to cell or tissue damage. One of the probable causes is oxidative stress yielded by free radicals. Oxidative stress (ROS) induce lipid peroxidation in the endothelial cell membrane, which generates atherosclerotic plaque. In a state of oxidative stress, the MDA level will increase. The purpose of this study is to determine the effect of SOD supplementation towards MDA, total cholesterol and LDL cholesterol plasma levels in the elderly.

Methods: This study was an open-label randomized control trial. Subjects were elders people aged > 60 years institutionalized at Social Rehabilitation Unit Pucang Gading Semarang, Indonesia. The treatment group consisted of 16 people, received SOD (Glisodin®) 1 capsule (250 IU) 1 hour before meals and daily elderly exercise (*senam lansia*) scheduled for 8 weeks. The control group consisted of 15 people, received a placebo, and daily elderly exercise. Plasma MDA levels were examined using TBARS method, while total cholesterol and LDL cholesterol were examined using CHOD-PAP method.

Results: This study shows a trend of reduction of plasma MDA levels in the treatment group compared to the control group (p = 0.062). A significant reduction of total cholesterol and LDL cholesterol levels in the treatment group were found (before 190.00 and 131.47 g/dl, after 182.27 and 121.93 g/dl, p = 0.005 and 0.001).

Conclusion: The SOD supplementation significantly reduces Total Cholesterol and LDL level, but not MDA level in the elderly.

Keywords: SOD; MDA; Total Cholesterol; LDL Cholesterol
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INTRODUCTION

Several various physiological functions in elderly people are diminished due to cell or tissue damage. Cellular damage accumulated over the years along with increasing age, resulted in many degenerative diseases.(1) One of the probable causes is oxidative stress yielded by free radicals. Free radicals are highly reactive, causing biochemical changes and damages in various components of living cells, such as proteins, lipids, carbohydrates and nucleic acids.

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Free radical attacks against lipid component in the cell membrane will cause lipid peroxidation reaction, producing toxic substances to the cells such as malondialdehyde (MDA).(1,2) Malondialdehyde (MDA) is one of the compound products of lipid peroxidation reaction used as a marker for oxidative stress state. In a state of oxidative stress, MDA serum levels will increase significantly.(3)

Reactive Oxygen Species (ROS) can induce lipid peroxidation in the cell wall, including endothelial cells, thus causing injury to these cells (endothelial injury). Injured endothelial cells are marked by increased NO response, followed by an immunologic response. In

addition, the continuous mild ROS can accelerate senescence cells. Constantly, this process will shorten endothelial cell telomeres while triggering the emergence of Advanced Glycation End (AGE) products. AGE accumulated by the age will activate NAD(P)H oxidase which will increase the production of superoxide anion while activating monocytes into macrophages. This process continues and finally induces endothelial dysfunction that leads to thrombosis.[4] NAD(P)H oxidases are not increased due to aging only, but also it occurs in the pathophysiologic state causing cardiovascular diseases such as hypercholesterolemia, hypertension and diabetes.[5] Macrophages and fat, especially low-density lipoprotein (LDL), accumulate in the area of injury where LDL is oxidized and eaten by macrophages or the macrophages themselves are also oxidized to produce foam cells, which can develop into atherosclerotic plaques.[4]

Lipid peroxide is formed from the process of lipid peroxidation due to the attack by free radicals. Biological membranes and lipoproteins are sensitive to lipid peroxide. It will decrease the stability of the cell membrane, inducing oxidation of the thiol groups by the enzyme in the membrane and releasing product breakdown (such as malondialdehyde [MDA]) which will cause cell damage. LDL oxidation by free radicals can cause tissue damage in blood vessels.[6]

SOD is one of the antioxidant enzymes which catalyze dismutase reactions or disproportionation of superoxide into molecular oxygen and peroxide. Peroxide then undergoes catalytic reaction into water molecules by catalase and peroxidase. SOD plays an important role in protecting cells from toxic products resulted from the process of aerobic metabolism, and oxidative phosphorylation.[7]

Antioxidant supplementation has been used often by Western people. Many supplements have been developed over several years. Studies have been conducted and data were collected from the animal experiments to clinical studies.[8]

Since 2000, the melon extract with naturally fortified SOD has been developed as a food supplement. Due to the low pH and high proteolytic activity in the digestive system, however, the SOD will undergo structural changes and will become inactive, thus a single administration of SOD is considered less effective. At present, the compound used for SOD coating processes that are often studied is gliadin, which is produced from wheat. Some studies claim that wheat gliadin protects the SOD from degradation process in the stomach.[8]

Based on the previous studies, consumption of SOD supplements may provide a beneficial effect towards the diseases triggered by oxidative stress such as cardiovascular disease, cancer and infections, such as Feline Immunodeficiency Virus (FIV) that is homologous with Human Immunodeficiency Virus (HIV) virus. SOD oral supplementation may also indicate a significant increase in quality of life.[9]

Plasma cholesterol, especially the oxidized cholesterol, contributes to generate free radicals on the endothelial cells of blood vessel walls. LDL oxidized into OxLDL stimulates the formation of superoxide anion ($O_2^{\cdot -}$) causing cell wall apoptosis in the vascular.[10,11]

Based on the above explanation, this study aims to compare the plasma MDA levels, total plasma cholesterol levels and plasma LDL cholesterol levels in the SOD group compared to the control group/placebo.

METHODS

Research design

This study was an open-label randomized control trial of experimental research.

Study subjects

Subjects for this study were elders aged > 60 years institutionalized at Social Rehabilitation Unit Pucang Gading Semarang. Physically healthy, able to perform activities of daily life (ADLs) independently based on the Barthel index, able to communicate, willing to participate in the study and signed informed consent.

From a total of 115 elderly populations in the orphanage, there were 31 people who met the inclusion criteria. From samples that met the inclusion criteria, measurements of weight, height, Body Mass Index (BMI) and blood pressure were conducted.

Intervention

Thirty-one persons who met the inclusion criteria were randomly divided into two groups: treatment group (n=16) which was administered with SOD supplementation (Glisodin^R) and control group (n=15) which was administered with placebo. Both groups received their relevant treatment one capsule daily, one hour before breakfast for 8 weeks and given daily elderly exercise (*senam lansia*) for 0.5 – 1 hour.

Laboratory analysis

Plasma MDA levels were measured by using Thiobarbituric Acid Reactive Substances (TBARS) method at the Biochemistry laboratory, Faculty of Medicine Diponegoro University. Total plasma cholesterol levels and plasma LDL cholesterol levels were determined by using Cholesterol Oxidase – Peroxsidase Aminoantypirin (CHOD-PAP) enzymatic methods in CITO clinical laboratory Semarang.

Statistical analysis

Data analysis was performed using Shappiro-Wilk test and paired t-test with SPSS software version 20.0. p value <0.05 was considered as significant.

Ethical clearance

This study was approved by medical research ethics committee (KEPK) Faculty of Medicine Diponegoro University Semarang

RESULTS

A total of 31 subjects with mean (±SD) age 71.42(±6.2) years were recruited for this study. Baseline characteristics of the study population were presented in table 1.

Table 1. Baseline characteristics of study population

Variable	Treatment group (n = 16)	Control group (n = 15)	Total (n = 31)
Age (years)			
60 - 69	6 (37.5%)	5 (33.3%)	11 (35.5%)
70 - 79	8 (50%)	10 (66.7%)	18 (58.1%)
80 - 89	2 (12.5%)	-	2 (6.5%)
Gender			
Male	3 (18.8%)	7 (46.7%)	10 (32.3%)
Female	13 (81.3%)	8 (53.3%)	21 (67.7%)
BMI groups			
Under/normal weight (BMI <25)	15 (93.8%)	12 (80%)	27 (87.1%)
Overweight/obese (BMI ≥25)	1 (6.3%)	3 (20%)	4 (12.9%)
Blood pressure			
Hipertension	10 (62.5%)	5 (33.3%)	15 (48.4%)
Normal	6 (37.5)	10 (66.7%)	16 (51.6%)

BMI, Body Mass Index

In the treatment group (given SOD for 8 weeks) there was a trend of decrease in plasma MDA levels, but it was not significant (table 2). The differences between treatment and control group were not differently significant.

Increased plasma MDA levels may have resulted from continuous exposure to free radicals continuously, such as free radicals in metabolism products, chronic inflammation, and cigarette smoke. Aging process has similar characteristic with mild chronic inflammation

Table 2. Table 1. Mean MDA level (ngr/dl) in treatment and control groups

	Before (ngr/dl)	After (ngr/dl)	p*	δ	p**
Treatment	13.44 ± 1.30	12.63 ± .94	.062	.81	0.59
Control	13.05 ± 1.57	13.08 ± .95	.968	.03	

*paired t-test
**independent t-test

There was a decrease in plasma cholesterol levels after administration of SOD for 8 weeks. After being analyzed the decline was significant (p value 0.005). However the differences between treatment and control group were not statistically significant (table 3).

There was a decrease in plasma LDL Cholesterol levels after administration of SOD for 8 weeks (table 4). After being analyzed the decline was significant (p value 0.005). However the differences between treatment and control group were not statistically significant.

increase MDA levels. Cigarette smoke also can increase lipid peroxidation which in turn increase the levels of plasma MDA.[2, 6,12]

Bose et al. found that by administering of tomatoes for 45 days, there was an increase in the levels of antioxidant enzymes (superoxidedismutase (SOD), glutathione peroxidase (GSH-Px), glutathionereductase (GR), reduced glutathione (GSH)) and decreased levels of lipid peroxidation (measured by the levels of plasma malondialdehyde (MDA)). One important role of the

Table 3. Mean Total Cholesterol level (gr/dl) in treatment and control group

	Before (gr/dl)	After (gr/dl)	p*	δ	p**
Treatment	190.00 ± 42.86	182.27 ± 46.41	**.005**	7.73	0.42
Control	218.13 ± 50.29	226.13 ± 74.40	.503	8	

*paired t-test: significant p value is bolded
**independent t-test

DISCUSSION

This study found a trend of reduction of plasma MDA levels in the treatment group, although it was not statistically significant. Reduction in plasma cholesterol levels, total cholesterol and LDL cholesterol levels, were also found after SOD administration for 8 weeks and it was statistically significant (p value of 0.005 and 0.001). No reduction was found in neither plasma cholesterol levels, total cholesterol nor LDL cholesterol levels, in the control group despite the exercise prescription.

intracellular antioxidant is as a free radical scavenger by changing harmful superoxide ions into stable hydrogen peroxide. In the oxidative stress state, all biomedical substances may be attacked by free radicals, but lipids are more vulnerable. Human cells are rich sources of polyunsaturated fatty acids (PUFAs), so it can be easily attacked by free radicals by a process known as lipid peroxidation to form lipid peroxides. This is a continuous chain reaction that is very destructive.[13,14]

Table 4. Mean LDL Cholesterol level (gr/dl) in treatment and control group.

	Before (gr/dl)	After(gr/dl)	p*	δ	p**
Treatment	131.47 ± 35.80	121.93 ± 33.11	**.001**	9.54	0.99
Control	149.75 ± 44.43	148.88 ± 57.05	.934	.87	

*paired t-test: significant p value is bolded
**independent t-test

In this study, SOD supplementation caused a slight trend of decrease in levels of plasma MDA, but it was not statistically significant. This could occur due to the volatile MDA so if is not examined immediately after sampling, the actual levels of MDA would have changed. Moreover, it could be caused by a small number of samples included in this study.

After SOD supplementation for 8 weeks, there were changes in total plasma cholesterol levels in both groups in comparison with pre-treatment levels. But no difference was found between SOD and control group. The sample of this study were institutionalized at Social Rehabilitation Unit Pucang Gading Semarang, were given the same amount and types of food and did routine elderly exercise every morning.

Oliveras-Lópezetal found that there were significant improvements in lipid profiles, including a decrease in total cholesterol levels and an increase in HDL levels after daily consumption of virgin olive oil daily for 6 weeks in 62 people aged 65-96 years. Park and Kim reported that triglyceride levels, total plasma cholesterol and plasma LDL cholesterol in Korean elders decreased significantly after the administration of Spirulina (a cyanobacterium/blue-green algae, which serves as a food supplement that contains essential fatty acids, vitamin B, iron, manganese). Pourghassem-Gargari et al. (2009) reported that the administration of *Nigella sativa*/cumin in hyperlipidemic rabbits can lower total cholesterol, triglycerides and LDL cholesterol and lipid peroxide (MDA) levels significantly (p <0.05). Hasan et al. (2011) suggested that *U. lactuca* (green algae) extract intra gastric in hypercholesterolemic rats caused a decrease in total serum lipids, triglycerides, total cholesterol, LDL cholesterol and VLDL cholesterol where the levels of HDL-cholesterol clearly increased by approximately 180%.[17–19]

This is in line with the research theory by Paolo Mondola stating that Cu, Zn-SOD inhibits the activity of HMG Co-A reductase in the liver cells of rats. HMG Co-A reductase is an enzyme that plays an important role in the biosynthesis of cholesterol and other large molecules through the production of mevalonic acid pathway. Cu, Zn-SOD inhibits HMG Co-A reductase by binding to the hepatocyte cells and undergoes internalization by absorptive endocytosis. Inhibitory effect of Cu, Zn-SOD is mediated by phospholipid membrane pathways that are activated by the bond between the receptor and the membrane of Cu, Zn-SOD surface. Inhibition of HMG Co-A reductase will lead to reduced cholesterol synthesis. Inhibition of cholesterol synthesis will lead to activation of LDL Receptor Pathway accompanied by an increasing amount of LDL cholesterol which binds to a receptor of hepatocyte cell surface, thereby lowering LDL cholesterol in blood. LDL cholesterol is a major lipoprotein of cholesterol carrier in the body and becomes an important independent risk factor in the formation of atherosclerosis.[20] On aging, there is also an increased risk of atherosclerosis in relation with the increased fatty streak in the intima layer of blood vessels.[20][21][22]

In this research we found that SOD supplementation is beneficial in lowering serum LDL levels and we did not find any complaint from the subject during and after the treatment. We found there was no side-effect of SOD supplementation for the elderly, so that SOD supplementation is safe to use for the elderly.[23][24]

Our study limitation is the lack data on food intake history and exercise compliance. Food intake from all of the residents was controlled by nursing personnel every day, however the compliance was still low.

CONCLUSION

In this study, Glisodin® supplementation reduced Total Cholesterol and LDL level, but not MDA level in the elderly. Further research needs to be done on SOD supplementation in the elderly with a longer period time and a larger number of samples so it can be known whether the SOD can be used as a supplementation for statins in reducing total plasma cholesterol and plasma LDL cholesterol levels. However further research on the effects of SOD supplementation on the lipid profile, such as triglycerides and plasma HDL, requires carrying out at long-term in an effort to determine the effects of the prevention of cardiovascular disease. Its effect towards the cognitive status in the elderly is also required.

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