Endothelial Protective and Antioxidant Effect of Polysaccharide Peptide of *Ganoderma Lucidum* in STEMI and NSTEMI Patients with Diabetes as a Risk Factor

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Abstract: Diabetes induced increases of *Reactive Oxygens Species* (ROS) Production. Oxidative stress and endothelial dysfunction are induced by high free radical. In Previous studies has been found that PsP of *Ganoderma Lucidum* has no toxicity in animal model and can be act as an antioxidants and antiendothelial dysfunction. This study was purposed to prove the effect of PsP of *Ganoderma Lucidum* as antioxidant and protective endothelial cell in STEMI and NSTEMI patients with diabetes. This clinical trial study conducted to 50 patients that diagnosed STEMI, NSTEMI and Diabetes, with pre- and post-test control group design. The parameters are MDA, SOD, CEC, and EPC counts. Patients divided into two group randomized, one group were given PsP *Ganoderma Lucidum* 750mg/day in 3 divided dose, and another group were given placebo for 90days. Normally distributed data will be analyzed by Dependent T-test and abnormally distributed data will be analyze by Wilcoxon test. The result are in PsP group patiens MDA level significantly decreased with P=0,030, SOD significantly increased with P=0,000, EPC count significantly increased with P=0,016, CEC count significantly decreased with P= 0,020. It concluded PsP of *Ganoderma Lucidum* act as antioxidant and protective endothelial cell against pathogenesis of atherosclerosis in STEMI and NSTEMI patients with diabetes.

1 INTRODUCTION

The pathophysiology of vascular damage in diabetes is complex and involves abnormalities in endothelial cells, vascular smooth muscle cells, and platelet function. Hyperglycemia reduces the availability of endothelial nitric oxide (NO) and vascular function through several mechanisms, especially an increase in the number of reactive oxygen species (ROS) derived from mitochondria and cytoplasm. ROS is not only the final effector but also the triggering factors of each of the following pathways involved in blood vessel damage caused by hyperglycemia: polyol pathway, formation of glycation end products (AGEs), de-novosynthesis of diacylglycerol (DAG), which leads to the activation of isoform protein-kinase C (PKC) and the hexosamine pathway (Bonora & DeFronzo, 2018).

Knowledge of the factors involved in the process of atherosclerosis, the main markers of antioxidants such as SOD or markers for oxidative stress such as MDA will help in the treatment of this disease. Pathological processes in endothelial due to oxidative stress using CEC (circular endothelial cells) which prove the existence of endothelial injury and EPC (endothelial progenitor cells) that improve vascular repair. The second marker representing vascular and endothelial integrity is important in the pathophysiology of cardiovascular disease (Rajendran *et al.*, 2013).

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Treatment using natural ingredients has long been used, one of which is the administration of polysaccharide peptides (PSP) derived from *Ganoderma Lucidum* extract. *Ganoderma Lucidum* is an oriental mushroom, has a history in China, Japan and other Asian countries as an ingredient that adds to health and longevity. This fungus is dark and large. In China, *Ganoderma Lucidum* is called Lingzhi, while in Japan it is called Reishi or Mannentake. Lingzhi contain bioactive compound, namely β-glucan has been proven to have an effect on controlling blood sugar, immune system modulation, hepatoprotective and many other effects

Molecular weight, conformation, and degree of branching are influenced β-glucan properties. The different structure of β-glucan can affect its antioxidant activity. This antioxidant activity can also be influenced by sources and extraction methods. The molecular weight of β-glucan extracted from barley with warm water is 40,000-100,000 Da, while oligomers made from β-glucan macromolecules with enzymatic degradation by lichenase have a molecular weight of around 2,000 Da. The smaller molecular size, inferred, can result in a reduction in hydroxyl radical uptake activity or a reduction in activity of antioxidant. The reduced molecular weight of about 1/20 - 1/50 can cause a reduction in antioxidant activity by about half from before. This finding shows that β-glucan still carries out hydroxyl radicals at various molecular sizes, even with different potentials (Kofuji *et al.*, 2012).

β-1,3 / 1,6-D-Glucan contained in PsP has been analyzed by the Complex Carbohydrate Research Center-University of Georgia, USA and the analysis shows that PsP has a large molecular weight (> 3,755,000 Da). β-1,3 / 1,6-D-Glucan in PsP is also known to have complex triple helix solution conformation and branching. Large molecular weight, triple helix formation and complex branching shows the potential of β-glucan in PsP as a powerful immunomodulator and antioxidant. The molecular weight of β-glucan in PsP extracted from *Ganoderma Lucidum* can be compared with the molecular weight of β-glucan taken from other sources. The molecular weight greater than the molecular weight of β-glucan from other sources shows the potential of β-glucan in PsP as a stronger antioxidant compared to other sources (HE *et al.*, 2010).

Research on Peptide Polysaccharide (PsP) of *Ganoderma Lucidum* has been started since 2013 which aims to find out the effect of PsP administration on Rattus novergicus strain Wistar rats with a high-fat diet and diabetes mellitus. The results showed that PsP is a chronic anti-inflammatory and antioxidant in Rattus novergicus strain Wistar rats (Sargowo *et al.*, 2018). In 2014, an acute and sub-chronic PsP toxicity study was conducted. In this test, observations of blood chemical parameters and histopathology of organs including kidney, lung, heart, aorta, and liver in Rattus novergicus strain Wistar rats.

The results showed that 5 variations of PsP dose did not have a toxic effect with a certain dose in Rattus novergicus strain Wistar rats (Wihastuti *et al.*, 2016). In 2015 a study was conducted to determine the effect of giving PSP as a chronic anti-inflammatory, antioxidant, antilipid, and endothelial antidisfunction in stable Angina Pectoris patients and at high risk of heart disease based on Framingham scores. The results showed that PsP has an anti-inflammatory, antioxidant, antilipid, and endothelial anti-inflammatory function so that it can play a role as a secondary preventive for Angina Pectoris patients. Stable and primary preventive for patients at high risk of heart disease based on Fraingham scores (Sargowo *et al.*, 2018).

Based on the results of the study, the administration of PsP is thought to be beneficial for patients with STEMI and NSTEMI. Therefore, further research is needed on the potential of β-1,3 / 1,6-D-glucans from mycelia *Ganoderma Lucidum* extract as an antioxidant and endothelial protection in STEMI and NSTEMI patients with diabetes.

2 METHODS

The study was true experimental using double blinds randomized perspective with pre-test and post-test design. All patients who participated in this study were patients who came from the cardiology outpatient unit of the Saiful Anwar Malang Hospital and Lavalette Malang Hospital which had agreed to participate in this study and had signed informed consent. Following are the inclusion, exclusion, and drop out criteria in this study.

2.1 Study Subject

The study group consisted of 50 patients with STEMI and NSTEMI. They are admitted to CVCU or heart poly of Saiful Anwar Hospital Malang and signed the informed consent. Patients who drinking alcohol a day more than 50g of ethanol, has acute hepatitis, kidney failure, hematological abnormalities and bleeding, anaphylactic disorder, using steroid, have transplant of grafting organ, sepsis, drug abuse in previous year, have low moderate risk according

Framingham cardiovascular risk score criteria according to ATP III, rheumatological abnormalities, neoplasm less than 5 years, severe disease and severe comorbidity were excluded from the study. All protocols in this study were agreed with informed consent and the Ethics Committee of the Medical Faculty of Brawijaya University, Malang Indonesia (171 / EC / KEPK / 07/2018)

2.1 Collection of Specimens and Biochemical Analysis.

Venous blood sample were collected from all the patient, 20 ml of blood was collected from each patient. The blood sample was centrifuges at 1000 rpm for 15 minutes at 2-8 ° C. Measurement of SOD and MDA in humans by taking pretest and posttest blood preparations then using the ELISA kit method. SOD uses an ELISA kit that is applied in quantitative in vitro determination of SOD concentrations in humans in serum, plasma, and other body fluids. A detectable dose of SOD of at least 37.5 pg / mL. MDA is measured using ELISA with the ELISA competitive method. MDA in the sample will compete with MDA on the plate, with the aim that the two compete to bind antibodies that are specific to MDA. The amount of CEC was measured by the flowcytometry method of blood plasma samples with anti-CD146 antibody markers labeled Phycoerythrin and anti-CD45 labeled FITC. EPC counts were measured by flowcytometry from blood plasma samples with Phycoerythrin labeled anti-CD133 antibody markers and FITC labeled anti-CD34

All data are expressed of mean ± SD. Paired T test was used to estimate the differences between groups. If the data are abnormally distributed, Wilcoxon test will be used to analyze. SPSS 25.0 was used for statistical analyze and the statistically significant was set at $p<0,05$.

3 RESULTS AND DISCUSSION

In this study there were five patient who dropped out caused by allergic reactions. In the extract group there was significant decrease in MDA and CEC and there was significant increase in SOD and EPC. Anthropometric results and characteristics of research subjects can be seen in table 1.

Table 1: Characteristics of research subjects and anthropometric examination results.

Variabel	Mean pretest	Mean Posttest		p- value
		PsP	Placebo	
SOD	2,8878±0,9315	3,4099± 0,1171	2,6807± 0,1930	0,000
MDA	20,8239±1,5969	16,7858± 2,1828	23,6827 ± 2,26851	0,03
CEC	88,1119±13,928	61,618± 9,5877	48,3465 ± 7,48505	0,02
EPC	101,4794±8, 116	93,7319±9,2121	101,1762±11,088	0,16

Table 2: The result of SOD, MDA, CEC, EPC.

	Sample pretest mean		Sample posttest mean		P Value
	PsP	Placebo	PsP	Placebo	
Gender	M: 53,78%				
Age (Years)	59,26				
Weight (kg)	58,35	63,8	58,12	63,84	0,743
Height (m)	1,55±0,07				
BMI	24.24	26.27	24.14	26.29	0.706
WC (cm)	87.5	93,92	88.85	94.08	0.482
Pulse	81.85	74.36	75.2	77.24	0.000
SBP (mmHg)	148.35	148,48	134.5	147,6	0.021
DBP (mmHg)	86.4	85.64	80	83.44	0.317
GDP	139,81	151,5	109,937	129,312	0,041
HBA1C	7,934	8,125	7,156	8,121	0,025

This study purposed to prove the potential of β-1,3 / 1,6-D-Glukan (Polysaccharide Peptide) from Miselia *Ganoderma Lucidum* as an antioxidant and endothelial protective in STEMI and NSTEMI patients with Diabetes as a risk factor. Data analysis showed that extract of *Ganoderma Lucidum* could reduce MDA levels significantly (p = 0.03), it can significantly increase SOD (p = 0.00), decrease in CEC levels significantly (p= 0,02), and significantly increase in SOD level (p = 0.016). This data shows that β-1,3 / 1,6-D-glucan (Polysaccharide Peptide) has an antioxidant effect and increases the potential of endothelial cells in carrying out angiogenesis even in oxidative stress conditions and it is associated with SOD improvement, especially MnSOD. β-1,3 / 1,6-D-Glucan (Polysaccharide Peptide) increases the expression of MnSOD and increases its antioxidant activity. This increase can then reduce free radicals in endothelial cells and increase cell resistance to oxidative stress.

SOD has enzymatic activity to scavenge on superoxide anion molecules (O2), through the mechanism of redox reactions. Under oxidized conditions, SOD will release excess electrons in superoxid anions, and release O2 molecules. Under reduced conditions, SOD will catalyze the reaction of superoxid anions with hydrogen cations (2H +) to form hydrogen peroxide (H2O2) which is more unreactive. Determination of the form of reduced or oxidized SOD, is determined by the amount of redox from metal ions that are conjugated with SOD. Furthermore, H2O2 will be catalyzed further by enzymes catalase and glutathione peroxidase (Fukai and Ushio-Fukai, 2011).

At high conditions of free radicals, there is an initial response to an increase in SOD production, specifically Cu / ZnSOD through the PI3K / Akt transmission pathway and NF-κB transcription factor in cells exposed to oxidants. However, in conditions of high free radicals that continue, causing problems in the process of SOD formation, especially in MnSOD and EC SOD. MnSOD is made on mitochondria, which require "mitochondrial import machines" to be used in the cytosol and carry out their functions. In high conditions of free radicals, there is a disturbance in the process of important mitochondrial, which can reduce the level of cytosolic MnSOD (Candas and Li, 2014). In addition, SOD scavenge metabolites, namely H2O2 can also be the amount of functional SOD2 protein, also can be displayed mRNA from SOD2. It can act as H2O2 affecting the amount of SOD2 protein in the post-translational process (Candas and Li, 2014).

Malondialdehyde (MDA) is a product of lipid peroxidation and one of the oxidant markers most often studied. MDA is involved in many disease such as cancer, Alzheimer's, diabetes and heart disease observed an increase in MDA which contribute to heart disease. Other research states that agents that decrease MDA and increase SOD provide benefits and are even considered as therapeutic agents for heart disease because they can reduce oxidative stress in the body (Kim, Yun and Kwon, 2016).

Endothelial dysfunction is characterized by a state of endothelial stiffness, pro inflammation, and pro thrombi nature. This occurs in most cardiovascular diseases, such as coronary disease, peripheral vascular disease, hypertension, chronic heart failure, diabetes, chronic kidney failure, and severe viral infections. High free radicals cause oxidative stress, and can cause endothelial dysfunction.

Cardiovascular risk factors are associated with an increase in ROS sources such as NADPH oxidase, xanthine oxidase, cyclooxygenase and mitochondria. Free radicals can disrupt the balance of nitric oxide, damage the endothelium, and if it occurs in a long time, will affect the permeability of blood vessels, causing toxic substances easily enter the tissues. Oxidative stress that occurs can also cause increased proliferation of vascular smooth muscle cells, increase in metalloproteinase matrix and be involved in the formation of atherosclerotic plaque (Alessio *et al.*, 2013).

Factors that can induce endothelial cell release from the vascular wall include mechanical injury to the vascular wall, after arterial or venous blood sampling, and acute plaque rupture. ROS activity together with prolonged inflammation that disrupts the integrity of integrins and cadherins can also cause endothelial cell release. In this study, the results obtained by giving PsP of *Ganoderma Lucidum*, a significant decrease in the number of CEC compared with placebo. This indicates that PsP *Ganoderma Lucidum* can improve endothelial dysfunction that occurs in the process of atherosclerosis. CEC itself is known that can be induced through various processes that occur in atherosclerosis, such as persistent inflammation and high free radical conditions (Rajendran *et al.*, 2013).

Protease enzymes and pro-inflammatory cytokines produced will disrupt the integrity of integrin and cadherin proteins that function for endothelial cell adhesion (Schmidt, Manca and Hoefer, 2015). The number of CECs in addition to indicating endothelial dysfunction, also gives an idea of the severity of the atherosclerotic process. Sugimoto et al. reported that there was a positive

correlation between the number of CECs and Carotid Plaque Score indicating the severity of atherosclerosis(Sugimoto *et al.*, 2015).

Inversely related to CEC, EPC has a negative correlation with the progression of atherosclerosis by having a role in regenerating damaged vascular endothelium. EPC is a derivative of stem cells in the bone marrow, which are able to stimulate proliferating and migrating. Proliferation and migration of EPC depend on cytokines and growth factors that result from vascular endothelial damage processes, such as VEGF, IGF-1, SDF-1α, to erythropoietin. EPC in circulation is divided into 2 pathways of differentiation, namely early EPC (also called angiogenic EPC) and late EPC (also called out-growth EPC). Early EPC plays a role in the process of angiogenesis, whereas late EPC, which can differentiate to form mature endothelial cells, plays a role in the process of vascular endothelial regeneration. This difference is supported by the expression of adhesion molecule markers in late EPC, such as VE-cadherin and von Willebrand Factor (Shantsila, Watson and Lip, 2007). In this study, the results obtained by giving PsP of *Ganoderma Lucidum* increased the number of EPC compared to the placebo group significantly.

These results indicate that PsP of *Ganoderma Lucidum* can increase the process of endothelial cell regeneration, which is damaged in the development of the atherosclerotic process. This can be thought to be caused by the anti-oxidant properties of PsP *Ganoderma Lucidum*. Lin et al. reported that increased free radical production correlates with a decrease in the amount of EPC through the induction of apoptosis from EPC. Similar results were obtained in studies from Fiorito et al. which illustrates the increase in the amount of EPC in the administration of vitamins C and E as anti-oxidants. In addition, an increase in the amount of EPC due to the administration of this vitamin is also induced by a decrease in TNF-α levels which indicates the presence of anti-inflammatory factors that play a role in increasing the amount of EPC (Fiorito *et al.*, 2008).

TNF-α is a marker of inflammation, and various studies have described its relationship with EPC. Research by Chen et al. gave the results that the incubation of EPC with TNF-α which described the pro-inflammatory environmental conditions caused a decrease in EPC proliferation and migration and induced apoptosis of EPC. In addition, exposure with TNF-α reduces the ability of EPC adhesion by decreasing the expression of VEGFR and SDF-1 proteins that play a role in adhesion with sub endothelium (Chen et al., 2011). Other studies from Koshikawa et al. reported that hsCRP levels were negatively correlated with the amount of EPC in circulation. The hsCRP protein is one of the inflammatory markers produced more in chronic diseases such as diabetes and atherosclerosis (Koshikawa *et al.*, 2010). From these studies it can be concluded that inflammatory conditions reduce EPC activity in endothelial regeneration.

4 CONCLUSIONS

From this study, it can be concluded that PsP of *Ganoderma Lucidum* can be act as antioxidant and endothelial protection in STEMI and NTEMI patients with diabetes as a risk factor.

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