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Effect of Erytin Emsa Inhibition Endothelial Dysfunction in Diabetes Mellitus Rats

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Abstract *This study aims to determine the effect of administering the polyherbal EMSA Eritin on inhibiting endothelial dysfunction which is characterized by a decrease in the production of Endothelial progenitor cells (EPC), circulating endothelial cells (CEC), malondiadedhyde (MDA), and Superoxide dismutase (SOD). This study used a Randomized Post Test Control Group design which was divided into 5 groups (negative control, positive control, EMSA Eritin 1, EMSA Eritin 2, and EMSA Eritin 3). The intervention was provided for 28 days. Data were analyzed using anova with a confidence level of 95%. Data were analyzed using the SPSS version 21.0 program. The results showed that administration of the EMSA Eritin polyherbal had an effect on suppressing the production of EPC, CEC, MDA, and SOD. Polyherbal EMSA Eritin can inhibit endothelial dysfunction involving oxidative stress, endothelial damage which is characterized by a decrease in the production of EPC, CEC, MDA, and SOD.*

Keywords - *Endothelial progenitor cells*, *Circulating endothelial cells*, *malondiadedhyde*, dan *Superoxide dismutase*

I. INTRODUCTION

Diabetes mellitus (DM), or simply called diabetes, is a chronic metabolic disorder caused by the pancreas not producing enough insulin or the body being unable to use the insulin it produces effectively. Insulin is a hormone that regulates the balance of blood sugar levels. As a result, there is an increase in glucose concentration in the blood (hyperglycemia) (PUSDATIN, 2014). Diabetes Mellitus (DM) according to the American Diabetes Association (ADA) is a group of metabolic diseases characterized by hyperglycemia that occurs due to abnormalities in insulin secretion, insulin action or both (Ndraha, 2014). The definition of DM according to the 2015 Consensus on the Management and Prevention of Type 2 Diabetes Mellitus in Indonesia, DM is a group of metabolic diseases characterized by hyperglycemia which occurs due to abnormalities in insulin secretion, insulin action or both (Soelistijo, et all, 2015).

Cardiovascular disease is the main complication of type 1 and type 2 diabetes mellitus and individuals with diabetes mellitus have a 2-4-fold increased risk of cardiovascular disease (Wu et al, 2005). Diabetic macroangiopathy primarily refers to an accelerated form of atherosclerosis. This then affects the coronary blood vessels, cerebral blood vessels and other blood vessels (Bonnefont-Rousselot, 2002).

The pathomechanism underlying vascular abnormalities in individuals with diabetes mellitus is based on oxidative stress. Various studies have proven the existence of oxidative stress in vascular diabetes mellitus,

in the form of increased levels of malondialdehyde (MDA), decreased levels of enzymatic and non-enzymatic antioxidants (Nuttal et al, 1999; Lestari, 1999; Barbagallo et al, 1999; Kowluru et al, 2001). Furthermore, oxidative stress in diabetes mellitus is thought to trigger LDL oxidation leading to the development of atherosclerosis. LDL can be oxidized by metal ions, reactive nitrogen compounds, lipoxygenase, and myeloperoxidase. In vitro oxidation by metal ions occurs in three phases: an initial phase called the lag phase (consumption of endogenous antioxidants), a propagation phase (rapid oxidation of unsaturated fatty acids to form lipid hydroperoxides), and a decomposition phase (hydroperoxides will be converted into reactive aldehydes, namely malondialdehyde (MDA) and 4-hydroxynonenal). NO is a free radical produced by endothelial cells. NO will react with superoxide radicals to form peroxynitrite and will then be decomposed to form hydroxyl radicals as an LDL oxidation agent (Mertens & Holvoet, 2001).

Active phagocytes will secrete myeloperoxidase which produces reactive compounds including hypochlorous acid (HOCl), chloramine, tyrosyl radicals, and nitrogen dioxide (NO2). This compound will convert LDL into an atherogenic form which will be taken up by macrophages to form foam cells (Mertens & Holvoet, 2001). Macrophage cholesterol accumulation will trigger the formation of foam cells, which is a hallmark of the beginning of atherosclerosis. Cholesterol accumulation in macrophages results from an imbalance in cellular cholesterol flux, for example increased uptake of atherogenic lipoproteins and/or decreased cholesterol efflux from cells. Oxidized LDL will be taken up by macrophages at an increased rate through scavenger receptors, thereby triggering lipid-laden foam cells and accelerated atherosclerosis. Oxidative stress not only affects LDL lipids, but also cellular lipids found in arterial macrophages. Macrophages that experience lipid peroxidation will have an increased ability to oxidize LDL and uptake oxidized LDL (Kaplan et al, 2001). It is suspected that increased oxidative stress due to hyperglycemia will increase the possibility of LDL and cellular lipids to undergo lipid peroxidation, thereby increasing foam cell formation.

II. METHOD

This research is a laboratory experimental study using a Randomized Post Test Control Group design using experimental Wistar rats as research subjects. Wistar rats were divided into 5 groups, namely positive control (non-DM rats), negative control (DM rats), diabetes mellitus rats + first dose of EMSA Eritin, diabetes mellitus rats + second dose of EMSA Eritin and diabetes mellitus rats + third dose of EMSA Eritin. Treatment was carried out for 28 days. Group K-: Negative Control of non-DM mice who were not given treatment, Group K+: Positive Control of DM mice who were not given treatment. Group P1: DM mice + EMSA Erytrin first dose (0.067gr/ 200gr BW) given for 28 days. Group P2: DM mice + second dose of EMSA Erytrin (0.135gr/200gr BW) given for 28 days. Group P2: DM mice + EMSA Erytrin third dose (0.270gr/200gr BW) given for 28 days. The research was carried out at the Pharmacology Laboratory and Anatomy Pathology Laboratory, Faculty of Medicine, Brawijaya University, Malang. This research was carried out from June 2016 to August 2017. The experimental animals in this research were male Rattus norvegicus Wistar strain weighing 200-250 grams in healthy condition characterized by active, agile movements. Mice were kept in standard cages with a 12-hour light-dark cycle. Feed and drinking water were provided ad libitum. The cages are provided with rice husks and will be cleaned once a day to provide comfort and health for the test animals. According to WHO, the sample size taken was 5 individuals and the estimated drop-out was 10% (WHO, 2000), so this study used a sample size of 6 individuals, for each treatment group.

III. RESULT

In carrying out the analysis, the researcher first tested the normality of the data using the Shapiro-Wilk test and obtained that the data had a normal distribution. Next, bivariate analysis was carried out using the anova test. If significance is found, it will continue with the Post Hoc test. Next, the research results were analyzed using the SPSS program and described as follows:

Table 3.1 Baseline Kadar EPC, CEC, MDA, dan SOD

Baseline	Mean±SD
Non-DM	
Endothelial progenitor cells (EPC)	5,80± 4,43
Circulating endothelial cells (CEC)	38,00± 32,65
Malondiadedhyde (MDA)	800,40±207,57
Superoxide dismutase (SOD)	43,60±3,20
DM	
Endothelial progenitor cells (EPC)	8,80±2,95
Circulating endothelial cells (CEC)	55,00±7,64
Malondiadedhyde (MDA)	5580,80±1206,04
Superoxide dismutase (SOD)	13,00±2,55

Table 3.1 Number of Endothelial progenitor cells (EPC)

Group		**Endothelial progenitor cells (%)**	
		n	**Mean±SD**
Control	Non-DM	5	5,80± 4,43
	DM	5	8,80±2,95
Action	EMSA Eritin Dosis 1 (0,067gr/ 200gr BB)	5	6,80±4,02
	EMSA Eritin Dosis 2 (0,135gr/200grBB)	5	1,80±1,48
	EMSA Eritin Dosis 3 (0,270gr/200grBB)	5	1,00±0,70

The number of EPCs in the non-DM control group averaged 5.80 ± 4.43, in the DM control group the average was 8.80 ± 2.95, in the EMSA Eritin Dose 1 group the average was 6.80 ± 4.02 , EMSA Eritin Dose 2 average 1.80 ± 1.48, EMSA Eritin Dose 3 average 1.00 ± 0.70 (Table 4.3; Figure 1)

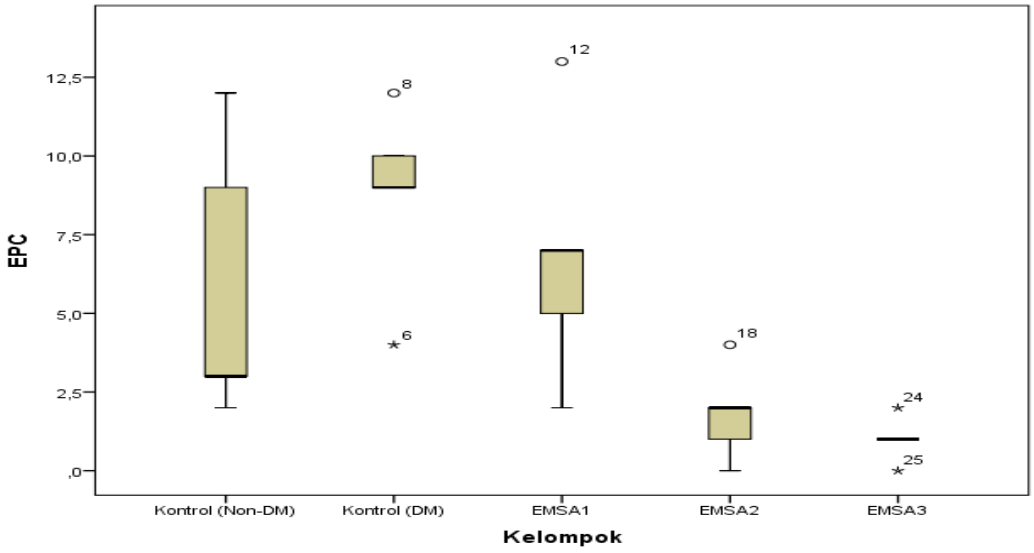

Figure 1. Number of Endothelial progenitor cells (EPC)

The statistical test results showed that there was no difference in the number of EPCs between the Non-DM control group and the other groups (DM control p=0.549; EMSA Eritin Dose 1=0.985; EMSA Eritin Dose 2 p=0.277; and EMSA Eritin Dose 3 p=0.138). Meanwhile, in the DM control group, there was a difference in EPC levels in the EMSA Eritin Dose 2 (p=0.014) and EMSA Eritin Dose 3 (p=0.006) treatment groups, while there was no difference found in the EMSA Eritin Dose 1 (p=0.839) so it could be concluded that EMSA Eritin Doses 2 and 3 have an effect on suppressing EPC production. The more effective dose was found at EMSA Eritin Dose 3 which was statistically different from dose 1 (p=0.114) (Table 3.2).

Table 3.2 Differences in the Number of Endothelial Progenitor Cells (EPC) According to Groups

Group		**Mean±SD Different progenitor Endothelial cells (%)**	**p**
Control Non-DM vs	Control DM	-3,00±1,94	0,549
-	EMSA Eritin Dosis 1	-1,00±1,94	0,985
	EMSA Eritin Dosis 2	4,00±1,94	0,277
	EMSA Eritin Dosis 3	4,80±1,94	0,138
Control DM vs	EMSA Eritin Dosis 1	2,00±1,94	0,839
	EMSA Eritin Dosis 2	7,00±1,94	**0,014**
	EMSA Eritin Dosis 3	7,80±1,94	**0,006**
EMSA Eritin Dosis 1 vs EMSA Eritin Dosis 2		5,00±1,94	0,114
EMSA Eritin Dosis 1 vs EMSA Eritin Dosis 3		5,80±1,94	**0,051**
EMSA Eritin Dosis 2 vs EMSA Eritin Dosis 3		0,80±1,94	0,994

IV. DISCUSSION

Effect of EMSA Eritin polyherbal administration on the production of Endothelial progenitor cells (EPC). In this study, it was found that the number of EPCs increased in the diabetes mellitus group compared to the control group. This can be a reference that there is increased endothelial damage in diabetes mellitus. One of the mechanisms of endothelial damage is through oxidative stress, which in this study has been proven through increased lipid peroxidation. (Widlansky & Gutterman, 2011).

Based on the results of statistical tests, it was found that EMSA Eritin Doses 2 and 3 had an effect on suppressing EPC production. The more effective dose was found at EMSA Eritin Dose 3. Administration of the polyherbal extract suppressed the number of EPCs, when a larger dose of the extract was given the number of EPCs was reduced. This research continues previous findings where genistein as a polyherbal element can regenerate and trigger the proliferation of hematopoietic stem cells. In addition, the active elements of coconut water are kinetin and riboside which can inhibit the aging of endothelial cells through cell proliferation capacity.

The role of EPCs in human disease is complicated by the fact that so many different definitions of EPCs have been used. In many cases, circulating EPC concentrations have been quantified and correlated to disease state in an attempt to serve as biomarkers for disease detection or staging. In some cases, a functional role of EPCs has been described when cells are infused as a reparative therapy. Defects in EPC function have also been identified in some diabetic patients and potential therapies to restore some aspects of EPC function. Although there is ambiguity in fully characterizing EPC, many clinical trials have been conducted in patients with heart disease, diabetes, peripheral arterial disease, lung disease, and cancer in which putative EPC has been examined as a biomarker or used as a cell therapy to treat human subjects (Yoder, 2012).

The effect of administering EMSA Eritin polyherbal on the production of Circulating Endothelial Cells (CEC). In this study, an increase in the number of CECs was found in the diabetes mellitus group compared to the control group. This can be a reference that there is increased endothelial damage in diabetes mellitus. One of the mechanisms of endothelial damage is through oxidative stress, which in this study has been proven through increased lipid peroxidation (Schmidt, 2007).

About 30 years ago circulating endothelial cells (CEC) were first observed in peripheral blood. Since then CEC has been established as a reliable indicator of vascular injury and damage and more sophisticated detection techniques, such as immunomagnetic isolation and fluorescence-activated cell sorting (FACS), have become available. Yet even today there remains controversy regarding the best approach to isolate and quantify these cells. Recent evidence also reveals interesting interactions between CECs and healthy endothelium in vitro although the relevance of these findings for human vascular disease in vivo remains unclear. CEC is considered a sensitive and specific marker of endothelial damage and is a potential mediator in vascular disease (Erdbruegger, Dhaygude, Haubitz, Woywodt, 2010).

Based on the results of statistical tests, it was found that EMSA Eritin Doses 2 and 3 had an effect on suppressing the production of Circulating Endothelial Cells (CEC). The more effective doses were found in EMSA Eritin Doses 2 and Doses 3.

Administration of polyherbal extracts suppressed the number of CECs compared to the diabetes mellitus group. The higher the dose of extract given, the lower the number of CECs. This research continues previous findings where genistein as a polyherbal element can regenerate and trigger the proliferation of hematopoietic stem cells. In addition, the active elements of coconut water are kinetin and riboside which can inhibit the aging of endothelial cells through cell proliferation capacity.

V. CONCLUSION

Endothelial progenitor cells (EPC) production increased in the DM group and administration of EMSA Eritin polyherbal doses 2 and 3 had an effect on suppressing EPC production. The production of Circulating Endothelial Cells (CEC) increased in the DM group and the administration of EMSA Eritin polyherbal had an effect on suppressing the production of Circulating Endothelial Cells (CEC). The more effective doses were found in EMSA Eritin Doses 2 and Doses 3.

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