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Polyherbal Extract (Soy, Brown Rice and Coconut Water) Improves Endothelial Damage in Diabetes Mellitus Through Modulation of Oxidative Stress and Endothelial Progenitor Cells

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Abstract

This study aims to investigate the effects of poliherbal extract (soybeans, brown rice and coconut water) in repairing endothelial damage in diabetes mellitus through oxidative stress modulation and endothelial progenitor cells. A total of thirty Wistar rats will be divided into five study groups (n= 6 each), including the control group (without any treatment), the diabetic mouse rats group, the diabetic mouse rats administered various dosage poliherbal extracts (0.067 grams/200 grams body weight 0.135 gram/200 gram of body weight, or 0.270 gram/200 gram body weight). Giving poliherbal was done for 28 days. MDA and SOD levels in serum were analyzed by spectrophotometric technique.

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The amount of circulating endothelial cells and endothelial progenitor cells were analyzed by flow cytometry. The MDA levels was increased in the diabetes mellitus group compared to the control group. Poliherbal lowered levels of MDA than diabetes mellitus group. There was a decrease in SOD levels in the diabetes mellitus group compared to controls. Provision of poliherbal extract increased levels of SOD compared to diabetes mellitus group. The number of circulating endothelial cells increased in the diabetes mellitus group compared with the control group, this increase can be suppressed by all groups given the poliherbal extract. There was an increase in the number of endothelial progenitor cells in the diabetes mellitus group compared with the control group. The higher the dose of the extract given the lower the number of endothelial progenitor cells. It was concluded that the administration of poliherbal extract could inhibit oxidative stress and endothelial damage in diabetic mellitus rats through modulation of SOD antioxidants as well as modulation of endothelial progenitor cells.

***Key words*:** endothelial cells; herbs; hyperglycemia; oxidative stress; vascular complications.

1. Introduction

Diabetes mellitus is a multifactorial disease characterized by hyperglycemia that is still a major health problem as its incidence increases worldwide [1]. The primary target of hyperglycemia is endothelial cells, which will trigger endothelial dysfunction and accelerated atherosclerosis. This process is associated with inflammation involving various mediators, including reactive oxygen compounds, chemokines, and pro-inflammatory cytokines [2]. Endothelial dysfunction refers to the inability of endothelial cells to regulate vascular homeostasis [3,4]. Hyperglycaemia leads to endothelial dysfunction through four mechanisms, namely activation of protein kinase C (PKC), activation of hexosamin path, activation of polyol pathway, and formation of Advanced Glycation End Products (AGEs). These four pathways will trigger the formation of reactive oxygen compounds. In addition to these pathways, the reactive oxygen compounds are also produced through mitochondrial respiratory chains, uncoupled eNOS, NADPH oxidase, and xantin oxidase [5-7]. Furthermore, the production of reactive oxygen compounds will trigger oxidative stress.

Lipid peroxidation is a complex process due to the reaction of polyunsaturated fatty acids constituents of cell membrane phospholipids with reactive oxyacid compounds, forming hydroperoxides. Lipid peroxidation mediated by reactive oxygen compounds has three main components of the reaction, namely initiation, propagation and termination reactions. Previous studies have shown that in diabetic melitus mice found an increase in lipid peroxidation [8,9]. Lipid peroxidation was also increased in diabetic blood with complications and without complications compared to the control group [10]. There was a positive correlation between MDA levels and blood sugar levels in diabetic patients [11]. In addition, increased lipid peroxidation in diebetes is accompanied by increased circulating endothelial cells as endothelial markers that escape from the vascular to circulation [12].

Circulating endothelial cells (CECs) are mature endothelial cells with a diameter of about 15-50 μm and are found in the blood. This cell is believed to be the endothelial cell released after damage to the vascular intima. Given that endothelial cells are anchorage, CECs reflect anoic- ized endothelial cells (detachment-induced cell death) [13]. Endothelial progenitor cells (EPCs) are subset of progenitor cells derived from bone marrow and

mobilized in the circulation. These cells can differentiate into mature endothelial cells, and persist at the site of injury to play a role in vascular repair and tissue regeneration [14]. In diabetes mellitus was found an increase in the number of CECs, decreasing the number of EPCs as well as decreasing the ability of EPC proliferation, adhesion, and angiogenic [12, 15-17].

Poliherbal extract (soybeans, brown rice and coconut water) is an extract of pharmacological effects, such as repairing radiation-induced hematopoietic cells, triggering the differentiation of radiation-induced lymphocytes [18,19]. Until now, to the knowledge of researchers there has been no research that evaluates the potential in suppressing oxidative stress and endothelial damage in diabetes mellitus. Therefore, this study aims to investigate the effects of poliherbal extract (soybeans, brown rice and coconut water) in repairing endothelial damage in diabetes mellitus through oxidative stress modulation and endothelial progenitor cells.

2. Material and Methods

2.1 Subjects

Thirty rats (*Rattus Norvegicus*) male, age 5 months, weight 200-250 gram divided into five groups (n = 5 per group), including control group (without any treatment), diabetic group, diabetes mellitus rats were treated with various dosage poliherbal extracts (0.067 grams/200 grams body weight; 0.135 gra/200 gram body weight or 0.270 gram/200 gram body weight). Provision of poliherbal begins since the diagnosis of diabetes mellitus and will be given for four weeks.

2.2 Diabetes mellitus model

Diabetes mellitus was induced by a single intraperitoneal injection of a freshly buffered (0.1 M citrate, pH 4.5) solution of streptozotocin at dose of 60 mg/kg body weight. Tail vein blood was collected 72 hours after streptozotocin administration to determine the fasting blood glucose level. Only rats with fasting blood glucose over 250 mg/dL were considered diabetic and included in the experiments [12, 18].

2.3 Polyherbal extraction

Soybeans and brown rice that have been washed are dried in oven vacuum temperature 40^{o}C. Furthermore, the material is mashed with communiting milk mesh size 60. The extraction process is done by water solvent with the ratio of materials and water at 1:10. The extraction was done for 2 hours at 50^{o}C. The extraction results were then evaporated with a freezer dryer for 24 hours at 60°C.

2.4Tissue sampling

At the end of the treatment, the animals in all groups were anesthetized; the blood samples were then drawn by cardiac puncture and heparinized. Blood samples were centrifuged at a speed of 4000g (4 minutes, 4^{o}C) to obtain the plasma. The aorta and tail artery were collected, weighed, and rinsed with physiological saline. All samples were stored at -80^{o}C until analyzed.

2.5 Isolation of PBMCs

All necessary materials are removed from the refrigerator and left to room temperature. Prepared a 15 ml centrifuge tube and filled with Ficoll-Hipaque d = 1.077 g/mL (1: 1) with an amount comparable to a blood sample. The blood samples in the EDTA vacutainer to be tested, slowly reversed so homogeneously then mixed with 1: 1 with PBS/HBSS, then taken with a micropipette and gently coated on a 15 mL centrifuge tube wall filled with Ficoll-Hipaque, layer, then centrifuged the room temperature at a speed of 1600 rpm for 30 minutes. After the centrifuge will be separated into 5 layers, namely plasma, PBMC cells, Ficoll-Hipaque, granulocytes and red blood cells. The formed PBMC ring is taken slowly using a micropipette and placed in a new 15 ml centrifuge bottle. The PBMC solution was then washed with 10 ml PBS/HBSS and centrifuged at room temperature at 1200 rpm for 10 min. The supernatant was discarded and the formed cell pellet was washed again with PBS / HBSS and centrifuged back at room temperature of 1200 rpm for 10 min, performed twice. After a centrifuge there will be pellets (PBMCs cells) at the bottom of the tube.

2.6 Flow cytometry analysis

Cell pellets are washed with staining buffer cells once and then centrifuged at 2500 rpm, 3 min, 4oC. The supernatant is discarded and the pellet cells are formed ready for distaining with surface marker cell antibodies (5 µL per 1:10 diluted sample in staining buffer cells). Diluted antibodies were then taken as much as 50 µl and mixed with cell pellets and homogenized. Cell pellets that have been given antibodies are incubated for 20 minutes in the dark at room temperature. After incubation, 500 uL of buffered staining cells were added and then homogenized and then continued with intracellular staining.

2.7 Malondialdehyde and SOD levels

Analysis of serum malondialdehyde and SOD levels was performed as the method employed in previous studies [12, 19].

2.8 Ethics

This research has passed the research ethic of institutional ethics committee of Faculty of Medicine Universitas Lambung Mangkurat, Banjarmasin, South Kalimantan, Indonesia.

2.9 Statistical analysis

Data was presented in the mean ± standard deviation and analyzed by ANOVA test. Statistical analysis using SPSS for Windows version 14.0 statistical package. The p value < 0.05 was statistically significant.

3. Results

Figure 1 shows MDA levels in various research groups. In this study, MDA levels increased in the group of diabetes mellitus than the control group. Polyherbal extract decreased MDA levels compared to diabetes

mellitus group.

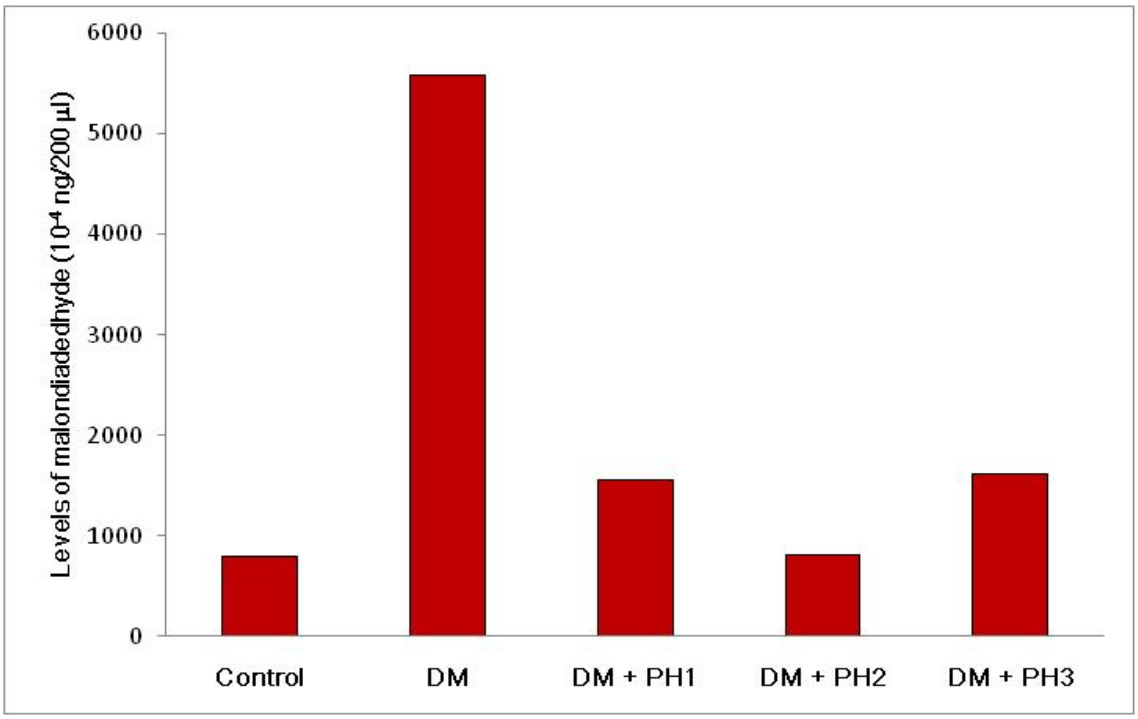

Figure 1: The levels of malondialdehyde in the all experimental groups. Note: DM: diabetic rats; DM + PH1: DM group treated with polyherbal extract of the first dose; DM + PH2: DM group treated with polyherbal extract of the second dose; DM + PH3: DM group treated with polyherbal extract of the third dose; ng: nanogram; µl: microliter.

For SOD levels, there was a decrease in the diabetes mellitus group compared to controls. Giving extract increased levels of SOD compared to diabetes mellitus group (Figure 2).

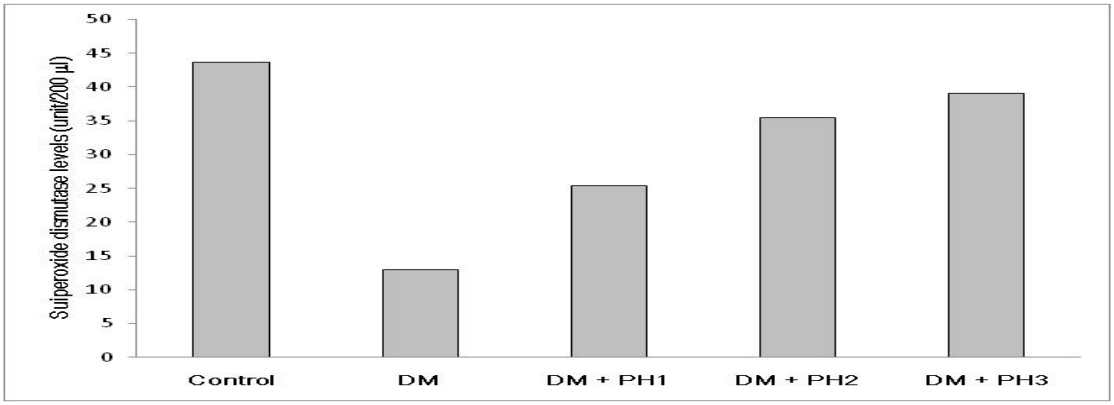

Figure 2: The levels of superoxide dismutase in the all experimental groups. Note: DM: diabetic rats; DM + PH1: DM group treated with polyherbal extract of the first dose; DM + PH2: DM group treated with polyherbal extract of the second dose; DM + PH3: DM group treated with polyherbal extract of the third dose; µl: microliter.

Figure 3 presents the number of circulating endothelial cells in all study groups. The number of circulating endothelial cells increased in the group of diabetes mellitus compared to the control group. All the groups given the extract decreased the amount of circulating endothelial cells compared to the diabetes mellitus group. The higher the dose extract is given the lower the amount of circulating endothelial cells.

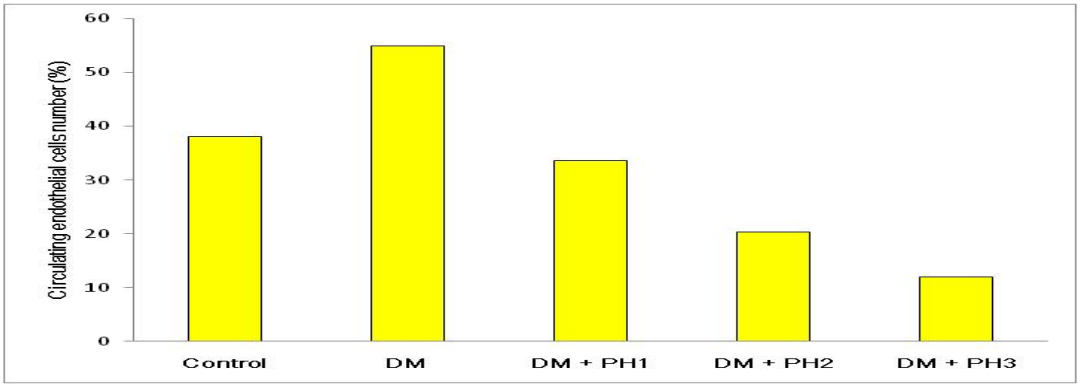

Figure 3: The circulating endothelial cells number in the control group and experimental group. Note: DM: diabetic rats; DM + PH1: DM group treated with polyherbal extract of the first dose; DM + PH2: DM group treated with polyherbal extract of the second dose; DM + PH3: DM group treated with polyherbal extract of the third dose; %: percentage.

The number of endothelial progenitor cells in various groups can be seen in Figure 4. There was an increase in the number of endothelial progenitor cells in the diabetes mellitus group compared with the control group. In the group given the extract there was a decrease in the number of endothelial progenitor cells compared to the diabates melitus group.

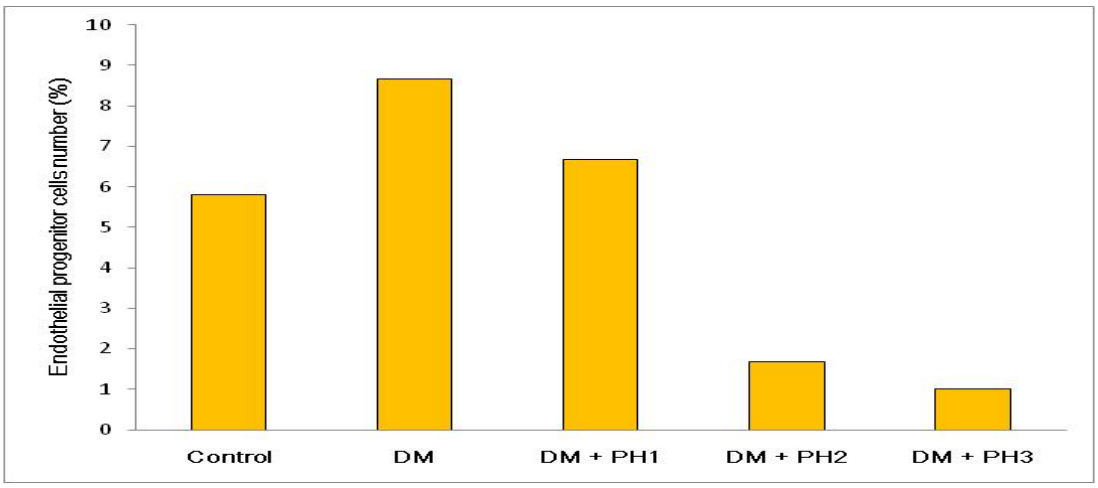

Figure 4: The endothelial progenitior cells number in the control group and experimental group. Note: DM: diabetic rats; DM + PH1: DM group treated with polyherbal extract of the first dose; DM + PH2: DM group treated with polyherbal extract of the second dose; DM + PH3: DM group treated with polyherbal extract of the third dose; %: percentage.

The higher the dose of the extract given the lower the number of endothelial progenitor cells.

4. Discussion

In this study, for the diabetes mellitus group found an increase in oxidative stress characterized by elevated MDA levels compared to the control group. Increased oxidative stress is based on low levels of SOD antioxidants in the diabetes mellitus group than in the control group. This is consistent with earlier findings that there is an increase in oxidative stress in diabetes mellitus. The mechanisms of formation of reactive oxygen compounds in diabetes mellitus include activation of protein kinase C, activation of hexosamine path, activation of polyol pathway, and the formation of Advanced Glycation End Products (AGEs), mitochondrial respiration chains, uncoupled eNOS, NADPH oxidase, and xantin oxidase [5 -7]. Giving extracts can suppress oxidative stress in the diabetes mellitus group, which is characterized by decreased levels of MDA. In this study, poliherbal extracts can supplement SOD production as an endogenous antioxidant. The researchers suspect that the poliherbal extract acts on the Nrf-2 signal as an endogenous antioxidant transcription factor. Brown rice is one of the poliherbal components that is scavenging reactive oxygen compounds [20].

In this study found an increase in the number of circulating endothelial cells increased in the group of diabetes mellitus than the control group. This suggests that in diabetes mellitus there is an increase in endothelial damage. One of the mechanisms of endothelial damage is through oxidative stress, which in this study is evidenced by increased lipid peroxidation. To inhibit this endothelial damage, there is compensation through the production of endothelial progenitor cells (the number of endothelial progenitor cells in the diabetes mellitus group is higher than the control group). Administration of poliherbal extracts suppresses the amount of circulating endothelial cells compared to the diabetes mellitus group. The higher the dose extract is given the lower the amount of circulating endothelial cells. This change also suppresses endothelial progenitor cells, when the higher the dose of the extract is given the lower the number of endothelial progenitor cells. This study extends previous findings that genistein as a poliherbal constituent can regenerate and trigger the proliferation of hematopoetic stem cells. In addition, the active ingredients of coconut water ie kinetin and riboside can inhibit senescence of endothelial cells through cell proliferation capacity [21- 23].

It was concluded that administration of poliherbal extract (soybeans, brown rice and coconut water) could inhibit oxidative stress and endothelial damage in diabetic mouse through modulation of SOD antioxidants as well as modulation of endothelial progenitor cells.

5. Conflict of interest statements

All authors acknowledge and declare that there is no conflict of interest in the research or publication of this article.

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