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Effect of *Scurrula atropurpurea* on nitric oxide, endothelial damage, and endothelial progenitor cells of DOCA-salt hypertensive rats

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ABSTRACT

Objective(s): To know whether *Scurrula atropurpurea is* able to modulate total plasma nitrate/nitrite levels, decrease endothelial damage, and increase endothelial progenitor cells (EPCs) in hypertensive rats.

Materials and Methods: The rats were divided in 5 groups: control (normotensive) group, Desoxy cortico sterone (DOCA)-salt hypertensive group, and three DOCA-salt hypertensive groups. All 5 groups received methanolic extract of *S. atropurpurea* (MESA) at a dosage of 50; 100; and 200 mg/KgBW. Serum nitric oxide (NO) was assayed by colorimetric. Circulating endothelial cells (CECs) and EPCs were assayed using flow cytometry.

Results: The administration of $MESA_{100}$ and $MESA_{200}$ elevated the total plasma nitrate/nitrite levels but cannot reach the level in control group. $MESA_{100}$ and $MESA_{200}$ also elevated the EPCs number compared with hypertensive group. The administration of MESA significantly ($P< 0.05$) decreased the CECs number compared to hypertensive groups.

Conclusion: Methanolic extract of *S. atropurpurea* is able to modulate total plasma nitrate/nitrite levels and diminish endothelial damage via increasing EPCs.

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Introduction

Hypertension is associated with increase of endothelial dysfunction which has been demonstrated in vessels from hypertensive humans and in many experimental models of hypertension (1). The endothelium plays a major role in the initiation of vascular remodeling. It serves as a sensor of hemodynamic and humoral variables and a transducer of signals to subjacent vascular smooth muscle cells (SMC). Subsequently, the alterations of SMC growth, migration, differentiation, death, and ECM modifications are responsible for the resulting vascular remodeling (2).

Circulating endothelial cells (CECs) are mature cells that are not associated with vessel walls, but are released from vascular wall and circulate within peripheral blood. The presence of CEC in the blood has been found to increased in response to various diseases (3). Indeed, the level of CECs has been recognized as a useful biomarker for vascular damage. The association between CECs and hypertension is unclear. Endothelial progenitor cells (EPCs) will repair the ischemic tissue due to neovascularization. The average lifespan of EPCs was recently reported to be shortened by oxidative stress and regulated by anti-oxidative mechanisms (8). The dysfunction of EPCs was clearly correlated with vascular injury in the case of various risk factors such as hypertension (9). Flow cytometry analysis did not show any effects of increasing blood pressure on the number of circulating EPCs, which had been measured by several markers (4).

Scurrula atropurpurea (BL.) Dans. is a parasitic tea plant. In Indonesia, especially on the island of Java, the stems and leaves of this plant have been empirically used for therapeutic applications. As far we know, there is no study to evaluate the effects of *S. atropurpurea* (BL.) Dans. on nitric oxide, endothelial damage, and endothelial progenitor cells in hypertensive rats. Therefore, this study aimed to investigate whether methanolic extract of *S. atropurpurea* (BL.) Dans. is able to modulate total

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plasma nitrate/nitrite levels, decrease endothelial damage, and increase endothelial progenitor cells in hypertensive rats.

Materials and Methods

Preparation tea parasite crude extract

Prior to the experiment, the characteristic of botanical determination of the leaves was performed at the Indonesian Scientific Institute (LIPI) at Purwodadi, Pasuruan, East Java. One hundred milligrams of dry leaf powder was steeped in methanol in a 1000 ml-erlenmeyer flask. The mixture was shaken for 30 min to distribute the powder homogenously in methanol. Subsequently, the mixture was left to stand overnight to precipitate. The supernatant; a mixture of methanol and the active constituents, was subjected to evaporation. The extract was labelled and stored in a freezer. The methanolic extract of *S. atropurpurea* (MESA) was administered daily by the oral gavage (2 ml) using a catheter for 6 weeks.

Animals

Twenty five male Wistar rats, aged 3-5 months and weighing 250-300 g. The rats were injected subcutaneously with deoxycorticosterone acetate (DOCA) (Sigma Aldrich, Pte Ltd. Singapore) at a dosage of 10 mg/KgBW, 2 times weekly for 6 weeks. The rats were given 2% NaCl instead of drinking water. The blood pressure and weights of the rats were then determined (5). The treatment groups consist of the control group, the group of non-MESA hypertensive rats, three groups of hypertensive rats were received MESA at dosages of 50, 100, and 200 mg/kgBW (6). The rats were assigned randomly into the groups, each group contained five rats. After 6 weeks of DOCA with or without MESA treatment, the blood pressure of the rats was determined.

Measurement of total plasma nitrate/nitrite levels

Total plasma nitrate/nitrite levels were determined indirectly as its metabolic products (nitrate + nitrite ions) spectrophotometrically using a test kit (Boeringher, USA) in which all the nitrate ions in serum were first reduced to nitrite ions by nitrate reductase followed by the reaction between nitrite ions and the Greiss reagent (0.1% naphthylethylenediamine dihydrochloride in distilled water and 1% sulfanilamide in 5% H3PO4) to form a blue color solution. Absorbance measurement was done at 540 nm. The levels of nitric oxide in all groups were determined by extrapolation from absorbance-concentration curve of the sodium nitrate standard solution (10–100 µM) (7).

Measurement of endothelial progenitor cells and circulating endothelial cells

Endothelial progenitor cells and circulating endothelial cells were isolated from peripheral blood according to previous studies with modification. Ten ml of venous blood with ethylenediaminetetraacetic acid (EDTA) was obtained by peripheral veinpuncture, stored at 4◦C to 10◦C, and processed within 6 hr after collection. In order to isolate mononuclear cells, we performed density-gradient centrifugation using Ficoll-Paque Plus (Amercontrol Pharmacia Biotech, Uppsala, Sweden). After that, the isolated cells were washed twice with PBS and resuspended in 20 ml of PBS supplemented with 0.5% of bovine serum albumin and 2 mM of EDTA. For endothelial progenitor cells measurement, $CD133_+$ cells in peripheral blood were evaluated by immunostaining with PE-conjugated CD133 monoclonal antibody (Biolegend) and detected by flow cytometry (BD FACS Calibur Flow Cytometer) (8). For circulating endothelial cells, $CD146_+$ cells in peripheral blood were evaluated by immunostaining with FITC-conjugated $CD146_+$ monoclonal antibody (Biolegend) and detected by flow cytometry (BD FACSCalibur Flow Cytometer) (9).

Ethics

The animal handling and details experimental procedures were evaluated and approved by the Ethics Committee of Faculty of Medicine, University of Brawijaya, Malang, East Java, Indonesia.

Statistical analysis

Data are presented as mean±SD and the differences between groups were analyzed using One-way ANOVA that was performed with SPSS 16.0 statistical package. *Post Hoc* test was used if probability values of $P<0.05$, were considered statistically significant.

Results

Effect of MESA on the total plasma nitrate/nitrite levels

The total plasma nitrate/nitrite levels were significantly ($P< 0.05$) decreased in DOCA-salt hypertensive rats compared to control group. The administration of $MESA_{100}$ and $MESA_{200}$ elevated the total plasma nitrate/nitrite levels but cannot reach the level in control group.

Effect of MESA on the endothelial progenitor cells number

The administration of DOCA salt affected the endothelial progenitor cells number, as shown in Figure 1. Endothelial progenitor cells level were significantly ($P<0.05$) decreased in DOCA-salt hypertensive rats compared to control group. The administration of $MESA_{100}$ or $MESA_{200}$ significantly ($P<0.05$) elevated the endothelial progenitor cell number compared with hypertensive group. The level of endothelial progenitor

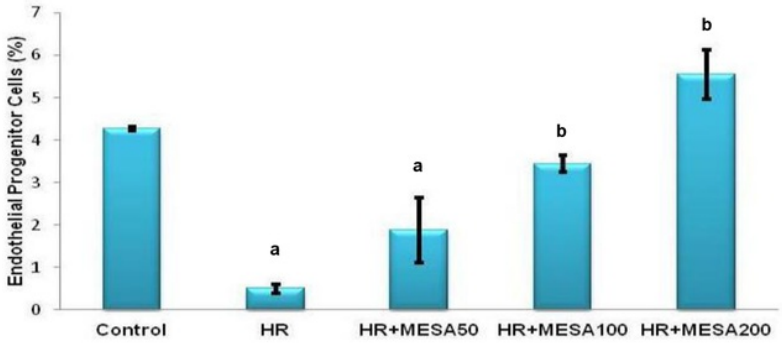

Figure 1. The number of endothelial progenitor cells (EPCs) in DOCA-salt hypertensive and the effects of methanolic *Scurrula atropurpurea* extract. Endothelial progenitor cells level were significantly ($P< 0.05$) decreased in DOCA-salt hypertensive rats compared to control group. The administration of $MESA_{100}$ or $MESA_{200}$ elevated the endothelial progenitor cells number; $MESA_{200}$ is able to reach the level in control group. [a] $P< 0.05$ in comparison with control group; [b] $P< 0.05$ in comparison with DOCA-salt hypertensive group

cells in $MESA_{200}$ is not significantly different than that of control group ($P> 0.05$).

Effect of MESA on the circulating endothelial cells number

The administration of DOCA salt affected the circulating endothelial cell number, as shown in Figure 2. Circulating endothelial cell levels were significantly ($P<0.05$) increased in hypertensive rats compared to control group. The administration of MESA significantly ($P<0.05$) decreased the circulating endothelial cells number compared to hypertensive groups.

Discussion

In this study we found that total plasma nitrate/nitrite levels in hypertensive animals was lower than normotensive group. Previous studies also supported the lower NO bioavailability in hypertensive subjects (10). Higher production of reactive oxygen species which accompanied by low expression of endothelial NO synthase, and/or impaired L-arginine uptake are different mechanisms for decreased NO bioavailability in high blood pressure. The administration of $MESA_{100}$ and $MESA_{200}$ elevated the total plasma nitrate/nitrite levels but cannot reach the level in control group, maybe due to reactive oxygen species-lowering effect. The ability of MESA to modulate total plasma nitrate/nitrite levels is due to its active constituents, among others flavonol glycosides (quercetin and rutin), monoterpene glucosides (icariside B), lignan glycosides (aviculin), flavans (catechin, epicatechin, epicatechin-3-0-gallate, epigallocatechin-3-0-gallate, gallocatechin, and epigallocatechin). Quercetin diffuses directly into endothelial cells and increases NO production. Catechin increases eNOS phosphorylation and NO bioavailability by inhibition of NADPH oxidase (11).

In this study, there were significantly ($P<0.05$) increased circulating endothelial cells levels in hypertensive rats compared to control group. Endothelial progenitor cells level were significantly

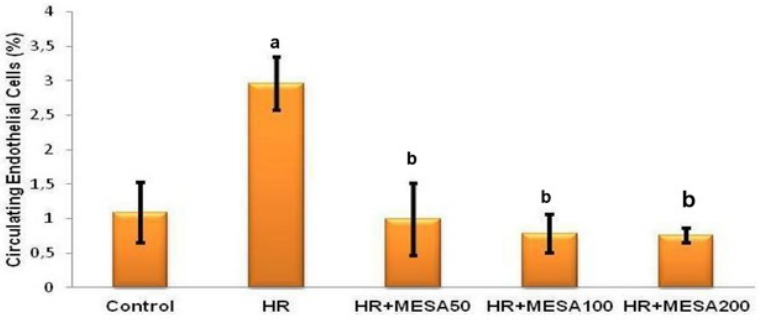

Figure 2. The number of circulating endothelial cells (CECs) in DOCA-salt hypertensive and the effects of methanolic *Scurrula atropurpurea* extract. There were significantly ($P<0.05$) increased circulating endothelial cells levels in hypertensive rats compared to control group. The administration of MESA significantly ($P<0.05$) decreased the circulating endothelial cells number compared to hypertensive groups.
[a] $P< 0.05$ in comparison with control group; [b] $P<0.05$ in comparison with DOCA-salt hypertensive group

(P<0.05) decreased in DOCA-salt hypertensive rats compared to control group. All doses administration of MESA significantly (P<0.05) decreased the circulating endothelial cells number compared to hypertensive groups. The administration of $MESA_{100}$ and $MESA_{200}$ elevated the endothelial progenitor cell number than that of hypertensive group (P<0.05). The administration of $MESA_{200}$ can reach the level in control group (P>0.05). This finding is consistent with previous studies that EPCs decreased in hypertensive rats (12). The bioactive compound from *S. atropurpurea* is able to diminish endothelial damage via increasing endothelial progenitor cells. Besides, total plasma nitrate/nitrite levels-increasing effect of *S. atropurpurea* may contribute to endothelial progenitor cells differentiation (13-15).

Conclusion

Methanolic extract of *S. atropurpurea* modulate the total plasma nitrate/nitrite levels and diminish endothelial damage via increasing endothelial progenitor cells.

Acknowledgment

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Declaration of interest

There is no coflict of interest.

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