

Scurrula atropurpurea increases nitric oxide and decreases malondialdehyde in hypertensive rats

Nour Athiroh AS* and Erna Sulistyowati**

ABSTRACT

BACKGROUND

Hypertension is the most prevalent chronic disease and has an impact on one billion people. Production of superoxide radicals and endothelial dysfunction are involved in hypertension. *Scurrula atropurpurea* (BL.) Dans. is a tea plant parasite. This study aimed to evaluate the role of *Scurrula atropurpurea* (BL.) Dans. on nitric oxide (NO) as a marker of endothelial dysfunction and malondialdehyde (MDA) as a marker of oxidative stress in hypertensive rats.

METHODS

This study subjected rats to deoxycorticosterone acetate (DOCA)-induced hypertension. The experimental groups consisting of the control group and 3 hypertension groups receiving *Scurulla atropurpurea* extract at a dosage of 50; 100; and 200 mg/KgBW. Scavenging activity of *Scurrula atropurpurea* (BL.) extract was analyzed by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The levels of arterial nitric oxide (NO) and pulmonary malondialdehyde (MDA) were analyzed by spectrophotometry. ANOVA and a post hoc test were applied to find the difference of arterial NO and pulmonary MDA levels between groups.

RESULTS

The level of arterial NO was significantly decreased in the hypertension groups as compared with the control group, while the level of pulmonary MDA was significantly increased ($p < 0.05$). *Scurulla atropurpurea* significantly increases the NO level at a dosage of 200 mg/KgBW, compared with the hypertension groups ($p < 0.001$). *Scurulla atropurpurea* significantly decreases pulmonary MDA level at a dosage of 100 and 200 mg/KgBW compared with the hypertension groups ($p < 0.05$).

CONCLUSION

Scurulla atropurpurea extract increases arterial NO and decreases pulmonary MDA in hypertensive rats, thus playing an important role in endothelial dysfunction and oxidative stress.

Key words: Mistletoe, arterial NO, pulmonary MDA, hypertension, rats

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Benalu teh meningkatkan kadar nitrit oksida dan menurunkan kadar malondialdehid pada tikus hipertensi

ABSTRAK

LATAR BELAKANG

Hipertensi merupakan penyakit kronik yang paling kerap ditemukan dan memberikan pengaruh kepada sekitar satu milyar individu. Hipertensi berhubungan dengan peningkatan produksi radikal superoksida dan disfungsi endotel. *Scurrula atropurpurea* (BL.) Dans. merupakan tanaman parasitik yang menyerang teh. penelitian ini bertujuan untuk mengevaluasi peranan benalu teh terhadap disfungsi endotel yang diukur menggunakan kadar nitrit oksida (NO) dan stres oksidatif diuukur menggunakan kadar malondialdehid (MDA) pada tikus model hipertensi.

METODE

Subyek penelitian adalah tikus model hipertensi yang diinduksi oleh deoxycorticosterone acetate (DOCA). Kelompok perlakuan terdiri atas kelompok kontrol, kelompok tikus hipertensi, kelompok ekstrak benalu teh dosis 50; 100; dan 200 mg/kgBB. Analisis aktivitas scavenging dari benalu teh dilakukan dengan metode DPPH. Analisis kadar NO arteri dan kadar MDA paru dilakukan dengan metode kolorimetrik dengan spektrofotometer. Uji ANOVA dan Post Hoc dilakukan untuk melihat perbedaan kadar NO arteri dan MDA paru pada berbagai kelompok perlakuan.

HASIL

Terdapat penurunan kadar NO arteri dan peningkatan kadar MDA paru secara bermakna pada tikus hipertensi dibandingkan kontrol ($p < 0,05$). Ekstrak benalu teh dosis 200 mg/kgBB dapat meningkatkan kadar NO arteri secara bermakna dibandingkan tikus hipertensi ($p < 0,001$). Ekstrak benalu teh dosis 100 dan 200 mg/kgBB dapat menurunkan kadar MDA paru secara bermakna dibandingkan tikus hipertensi ($p < 0,05$).

KESIMPULAN

Ekstrak benalu teh meningkatkan kadar NO arteri dan menurunkan kadar MDA paru pada tikus hipertensi. Jadi berperan penting pada disfungsi endotel dan stress oksidatif.

Kata kunci: Benalu teh, NO arteri, MDA paru, tekanan darah tinggi, tikus

INTRODUCTION

Hypertension is the end result of a complex interaction between genetic and environmental factors affecting the physiological systems regulating blood pressure. Throughout the world hypertension is the most frequently encountered chronic disease and affects around one billion individuals.⁽¹⁾ Death resulting from hypertension may be caused by cerebrovascular and cardiovascular complications, such as stroke, end-stage renal disease, congestive heart failure, myocardial infarction, and cardiac standstill.⁽²⁾

Various lines of evidence reveal the involvement of reactive oxygen species and oxidative stress in hypertension and the development of its complications. Hypertension is associated with increased production of superoxide radicals and endothelial dysfunction.⁽³⁾ Superoxide radicals have a negative effect on endothelial function by reacting directly with nitric oxide (NO), such that they decrease NO bioavailability. In addition, peroxynitrites as the products of the reaction of superoxide radicals with NO, also have negative effects on endothelial cells.^(4,5) Hydroxyl radicals

produced by the decomposition of hydroperoxynitrites may trigger lipid peroxidation, marked by increased malondialdehyde (MDA) levels.

For the treatment of hypertension and its complications, many drugs of plant origin have been developed, comprising digitoxin from *Digitalis purpurea*, reserpine from *Rauwolfia serpentina*, aspirin from *Salix alba*, tetramethylpyrazine from *Jathropa podagrica*, and tetrandrine from *Stephenia tetradra*.⁽²⁾ *Scurrula atropurpurea* (BL.) Dans. is a parasitic plant attacking tea plants and therefore known as the tea parasite. In Indonesia, especially on the island of Java, the stems and leaves of this plant have been traditionally used, among others for the treatment of cancers.⁽⁶⁾ No studies have been found for evaluating the potential of the tea parasite for the treatment of hypertension. Therefore the objective of the present study was to evaluate the effect of tea parasite leaf extract (TPLE) on the levels of NO as marker of endothelial dysfunction and on the levels of MDA as a marker of oxidative stress in a rat model of hypertension.

METHODS

Study design

This was an laboratory experimental study conducted from February to July 2012.

Preparation of rat hypertension models

The study subjects were Wistar rats aged 3-5 months and weighing 250-300 grams. The rats were injected subcutaneously with deoxycorticosterone acetate (DOCA) 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 the weights of the rats were then determined.⁽⁷⁾ The treatment groups consisted of the control group, the group of non-TPLE hypertensive rats, three groups of hypertensive rats receiving TPLE at dosages of 50, 100, and 200 mg/kgBW. The rats

were assigned randomly into the groups, with each group containing five rats.

Preparation tea parasite crude extract

Botanical determination of the leaves of the tea parasite was done at the Indonesian Scientific Institute (LIPI) at Purwodadi, Pasuruan, East Java. The leaves were washed, left to dry in an oven at 40-60°C, then ground into a powder. One hundred milligrams of tea parasite leaf powder was steeped in methanol in an erlenmeyer flask of 1 L capacity. The mixture was shaken for 30 minutes to distribute the powder uniformly in the methanol. Subsequently the mixture was left to stand overnight until a precipitate was formed. The supernatant, being a mixture of methanol and the active constituents, was subjected to evaporation. The extract was labelled and stored in a freezer.⁽⁸⁾ The TPLE was administered daily by the oral route using a catheter, this being continued for 6 weeks.

Determination of antioxidant activity

The antioxidant activity of the extract was analyzed by a modification of the DPPH free scavenging activity method.^(9,10) The sample was dissolved in methanol (at concentrations of 10-100 ppm), reacted with 0.2 mM DPPH, and incubated for 30 minutes at room temperature. Absorbance was measured at 515 nm. Antioxidant activity was calculated as a percentage of inhibition of DPPH (percentage of scavenging effect), i.e. % inhibition = [1 - (absorbance of sample / absorbance of blank)] x 100%. The IC₅₀ is the concentration of the sample required for yielding 50% inhibition.

Determination of arterial NO and pulmonary MDA

The specimens used for the analysis of NO levels were arterial tissues from the tails of the rats. The method used was the Griess reaction. The analysis was performed according to the procedural instructions included in the kit. NO concentrations were expressed in μmol .⁽²⁾

The method used for determining pulmonary MDA levels was developed at the Pharmacology Laboratory, Faculty of Medicine, Brawijaya University, Malang, East Java. One hundred milligrams of tissue was homogenized with 2 ml of phosphate buffer at pH 7.4. Then 50 ml of 0.1% Triton-X was added and the mixture was vortexed. Thereafter, 250 ml of 1 N HCL was added to the mixture, which was again vortexed. The mixture was heated to 100^o C for 30 minutes, then centrifuged at 3000 rpm for 10 minutes until a colloid precipitate formed. The absorbance was read with a spectrophotometer at a wavelength (λ) of 532 nm. The MDA concentration was expressed in ng/200 mg.

Data analysis

To examine the differences in arterial NO and pulmonary MDA, the data were tested with ANOVA and a post hoc test. A value $p < 0.05$ was considered to indicate a significant difference. The statistical analysis was done using SPSS version 13.

Ethical clearance

This study was approved by the Committee on Research Ethics, Faculty of Medicine,

Brawijaya University, Malang.

RESULTS

On the basis of DPPH radical scavenging activity, TPLE was a strong antioxidant with an IC_{50} of 11.64%.

Figure 1 presents arterial NO levels in the treatment groups. Post hoc testing found a significant reduction in arterial NO in the non-TPLE hypertension group, as compared with the control group ($p < 0.001$). To raise arterial NO, TPLE was administered daily for 6 weeks. Administration of TPLE at a dosage of 200 mg/kgBW was able to significantly increase arterial NO, as compared with non-TPLE hypertensive rats ($p < 0.001$), although the increase did not attain the levels in control rats.

In Figure 2 are presented the pulmonary MDA concentrations in the treatment groups. Post hoc testing found a significant increase in pulmonary MDA concentration in rats with non-TPLE hypertension as compared with control rats ($p < 0.05$). Administration of TPLE at the dosages of 100 and 200 mg/kgBW was able to significantly reduce MDA levels in comparison with non-TPLE hypertensive rats, although not attaining the levels in control rats.

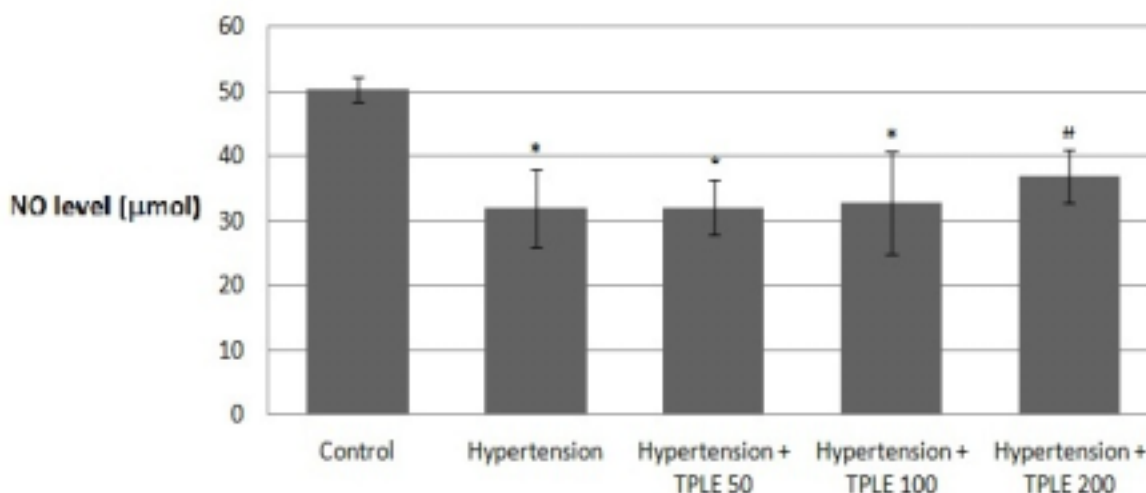


Figure 1. NO concentration (μmol) in the treatment groups. Data are presented as mean \pm standard deviation. Dosage unit of TPLE is mg/kgBW; *significantly different in comparison with control group ($p < 0.05$); # significantly different in comparison with non-TPLE hypertension group ($p < 0.05$)

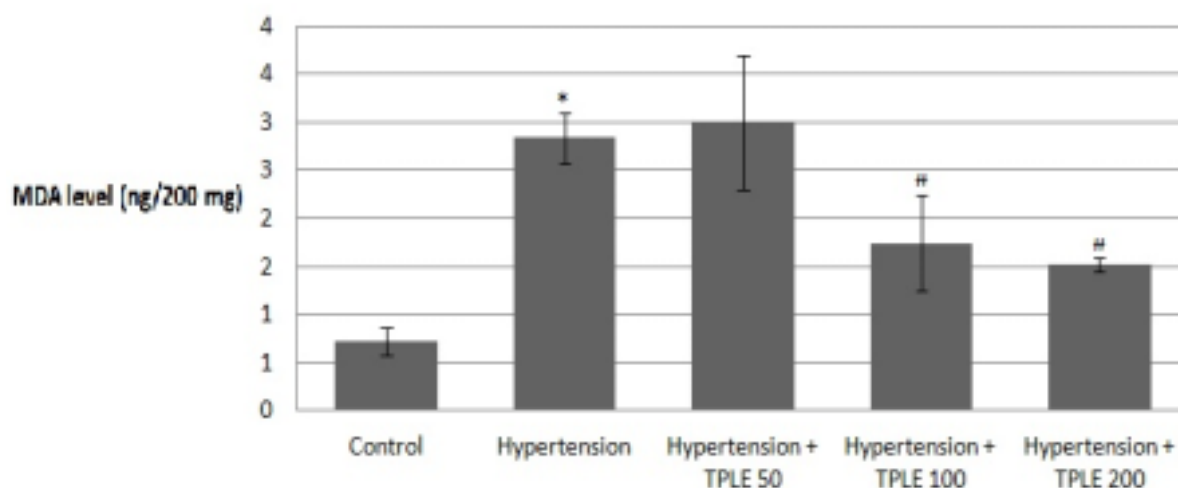


Figure 2. MDA concentration (ng/200 mg) in the treatment groups. Data are presented as mean \pm standard deviation. Dosage unit of TPLE is mg/kgBW; *Significantly different in comparison with control group ($p < 0.05$); # significantly different in comparison with the non-TPLE hypertension group ($p < 0.05$)

DISCUSSION

The present study demonstrates that in the rat model of hypertension there is a significantly reduced arterial NO concentration and a significantly increased pulmonary MDA concentration, in comparison with the control group. The reduction in arterial NO level indicates the occurrence of endothelial dysfunction in hypertension. The increase in pulmonary MDA indicates an increase in oxidative stress in hypertension. Endothelial dysfunction and pulmonary oxidative stress in hypertension may be modulated by the administration of TPLE.

The reduced arterial NO in hypertension may be due to decreased expression of endothelial nitric oxide synthase (eNOS) or by decreased NO bioavailability.⁽¹¹⁾ The latter may be the result of a reaction between NO and superoxide radicals to form peroxynitrites.⁽¹²⁾ Subsequently peroxynitrites may trigger the formation of hydroxyl radicals leading to lipid peroxidation, which is marked by an increase in MDA levels.

Administration of crude TPLE at a dosage of 200 mg/KgBW is able to significantly increase arterial NO concentration, as

compared with the group of non-TPLE hypertensive rats, but is not able to attain the NO levels in the control rats. The ability of TPLE to modulate NO 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, gallic acid, and epigallocatechin).⁽⁶⁾ Quercetin diffuses directly into endothelial cells and increases NO production.⁽¹³⁾ Catechin increases eNOS phosphorylation and NO bioavailability by inhibition of NADPH oxidase.⁽¹⁴⁾

In the present study, pulmonary oxidative stress increased significantly in comparison with the controls. This indicates pulmonary involvement in the rat model of hypertension induced by DOCA. In the vascular system, one of the mechanisms for increases in reactive oxygen compounds is the high activity of NADPH oxidase.⁽¹⁵⁾ We suppose that this mechanism is also at work in the lungs.

Administration of TPLE at dosages of 100 and 200 mg/KgBW is capable of significantly decreasing pulmonary MDA levels in comparison with the group of non-TPLE

hypertensive rats, but cannot attain the MDA levels in the control rats. This indicates that TPLE has antioxidant properties and inhibits pulmonary oxidative stress. Based on its DPPH radical scavenging activity, TPLE is a strong antioxidant with an IC_{50} value of 11.64%. Quercetin has a high scavenging ability for DPPH radicals⁽¹⁶⁾ and may also modulate endogenous antioxidants.⁽¹⁷⁾ The ability to inhibit oxidative stress is also found in rutin and catechin.^(18,19)


In the pathomechanism of hypertension, the antioxidant properties of TPLE may inhibit oxidative stress at several points. First, TPLE scavenges superoxide radicals such that the latter do not react with NO to form peroxynitrites. Second, TPLE scavenges hydroxyl radicals formed as a result of decomposition of hydroperoxynitrites. One limitation of this study is that no high performance liquid chromatography (HPLC) analysis was performed to determine the active constituents present in *Scurrula atropurpurea* such that its antihypertensive action may be revealed in detail.

CONCLUSION

TPLE increases arterial NO and decreases pulmonary MDA in hypertensive rats, thus playing an important role in endothelial dysfunction and oxidative stress.

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