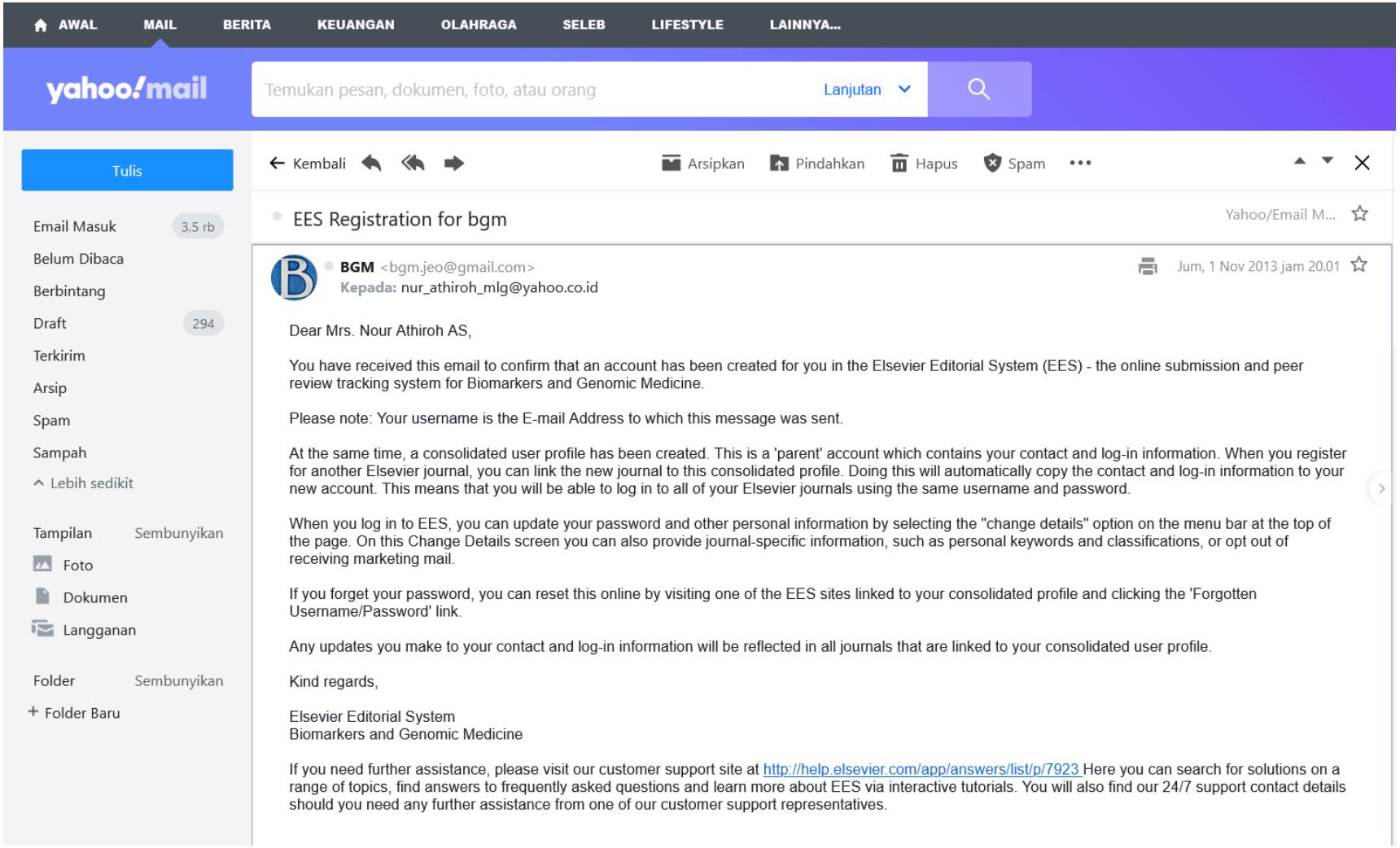
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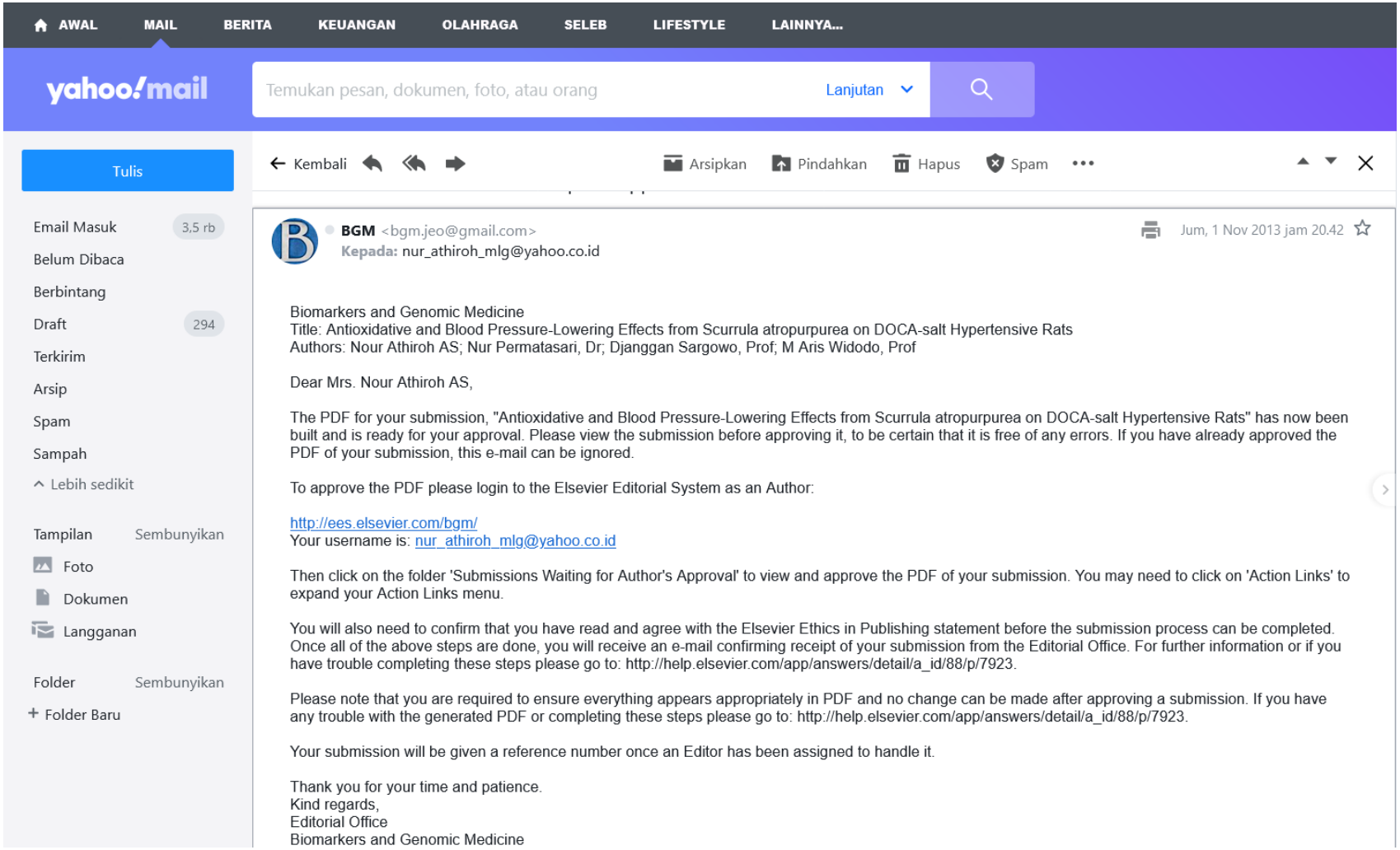
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Authors: Nour Athiroh AS; Nur Permatasari, Dr; Djanggan Sargowo, Prof; M Aris Widodo, Prof
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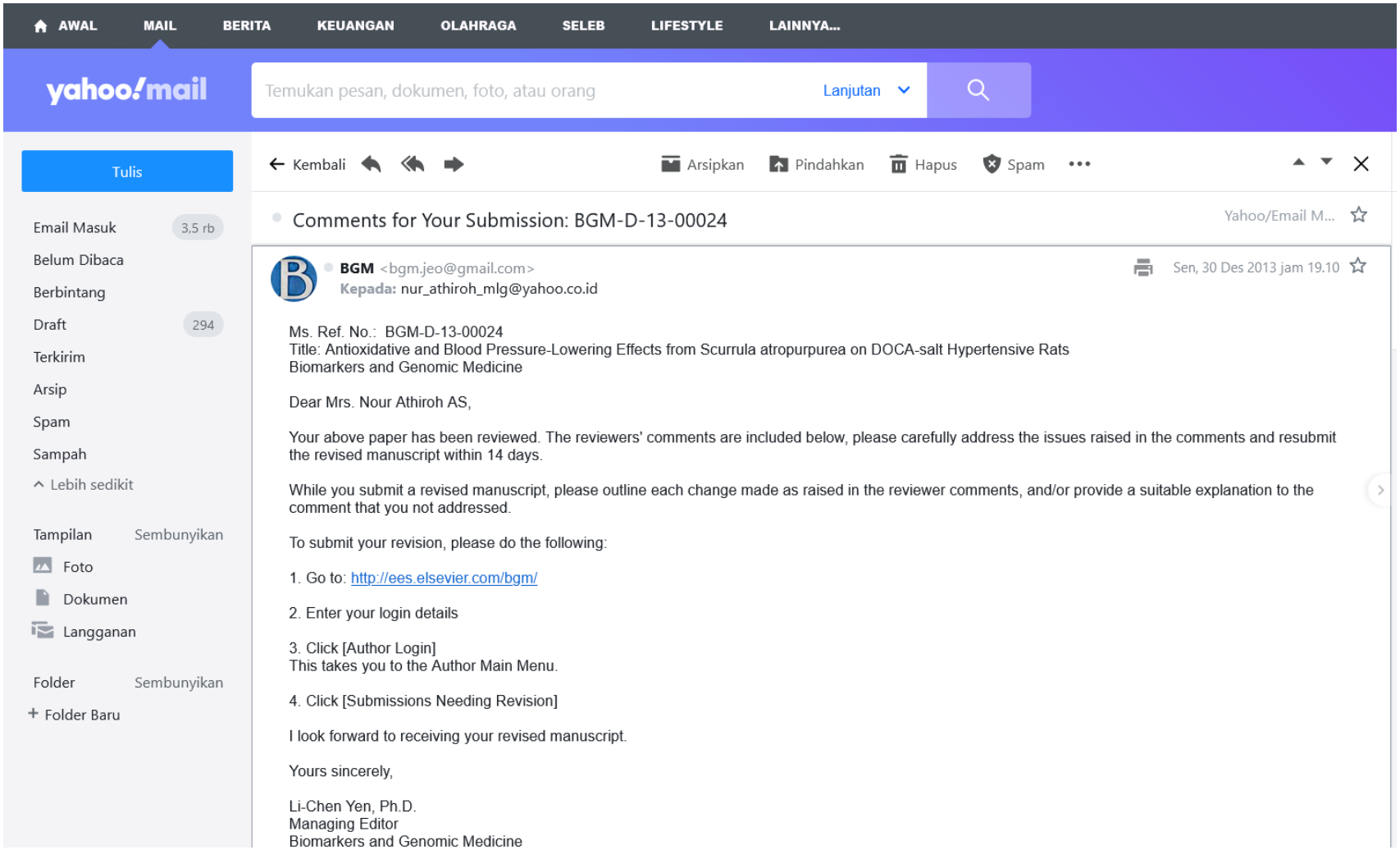
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Reviewers' comments:

Reviewer #1:
The autors identified the effects of Scurrula atropurpurea extract(MESA) on blood pressure in a rat model. The findings are new, but the impact of this manuscript is limited by their data present. A major revision is needed.
1. As a minor point, several typing errors are noticed. In addition, certain writing error may compromise the readers. For example,the authors describe "The administration of DOCA salt affected the MDA levels, as shown in Fig. 1." in Result section, however, Fig 1 is not a MDA data.
2. Blood pressure-lowering effect is one major find in this study, but the authors did not provide the dection method and experimental detail (such as time point)for blood pressure tests.
3. As a major point, the authors noly tested the effects of MESA in HR rat. The effects (such as blood pressure and SOD) of MESA on normal rat should also be performed.
4. Please check the data statistics or labeling. For example, in Fig.3, dose the HR-MESA50 group significantly different form Sham group? or HR group?

Reviewer #2:
The authors have investigated whether methanolic extract of Scurrula atropurpurea Dans. (MESA) is able to diminisihed oxidative stress in hypertensive rats. The authors have shown that the significantly increased systolic blood pressure in DOCA salt induced hypertension rats, and the administration of MESA200 significantly decreased the levels of blood malondialdehyde (MDA) compared to the hypertensive control groups. The superoxide dismutase (SOD) level was significantly decreased in hypertension rats compared to sham group. The authors should comment on the following points:

1. For their in vivo study, the author should clarify "does MESA200 produce any toxic effects or any other side effects in vivo?"
2. For their in vivo study, the author should clarify "does the treatment of MESA have any effects on the kidney damage of DOCA salt induced hypertension rats ?"
3. In the result part, the author should provide more molecular evidences such as performing western blot analysis to confirm that MESA suppresses the oxidative stress in DOCA salt induced hypertension rats.
4. In figure 1, the author should explain why MESA50, MESA100 and MESA200 did not show the dose dependent effect to reduce systolic blood pressure in DOCA salt induced hypertension rats.
Minor points

In abstract; line 8; the author should explain what does MESA mean?
In introduction part, line 18; the author should provide the full name of SOD means?
In page 3, line 2; the author should provide more references.
In figures, what does HR mean?

One more suggestion is that the English grammar of this manuscript should be improved by consulting an official language editing service, in particular in the

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Antioxidative and Blood Pressure-Lowering Effects from *Scurrula atropurpurea* on DOCA-salt Hypertensive Rats

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ABSTRACT

This study aimed to investigate whether methanolic extract of *Scurrula atropurpurea* (BL.) Dans (MESA) (untuk menjawab reviewer #2, abstrak line 8). able to diminisihed oxidative stress in hypertensive rats. This study subjected rats to DOCA-induced hypertension. The experimental groups consisting of the control group and three hypertension groups receiving *Scurulla atropurpurea* extract at a dosage of 50; 100; and 200 mg/KgBW. The levels of blood malondialdehyde (MDA) and superoxide dismutase (SOD) were analyzed by spectrophotometry. There was significantly ($p < 0.05$) increased systolic blood pressure in hypertension rats compared to sham group. Compared to its hypertensive group, the administration of MESA (significantly decreased systolic blood pressure, but not able to reach the level in sham group. There were significantly ($p < 0.05$) increased MDA levels in hypertension rats compared to sham group. The administration of $MESA_{200}$ significantly ($p < 0.05$) decreased the MDA levels compared to hypertensive groups. The SOD level were significantly ($p < 0.05$) decreased in hypertension rats compared to sham group. The administration of $MESA_{50}$ elevated the SOD levels to reach level in sham group. The SOD levels in $MESA_{100}$ and $MESA_{200}$ were higher significantly ($p < 0.05$) compared to sham group. In conclusion, *Scurulla atropurpurea* able to modulates superoxide dismutase, diminished oxidative stress, and decreased sistolic blood pressure in DOCA-salt hypertensive rats.

Key words: mistletoe, antioxidant, blood MDA, hypertension, rats.

INTRODUCTION

Hypertension is a clinical common vascular related disease, with high mortality and disability (more references ??? reviewer#2 : In page 3, line 2). Additionally, it is also an independent risk factor for stroke, coronary heart disease, heart failure, renal insufficiency, peripheral vascular diseases, early death, and many other major diseases [1]. Hypertension is the end result of a complex interaction between genetic and environmental factors affecting the physiological systems regulating blood pressure [2]. There are about 1 billion hypertensive patients in the world, and around 30% of the population died from cardiovascular and cerebrovascular events, in which 62% of acute stroke events and 49% of cardiovascular events were directly caused by hypertension. [3, 4].

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 [5]. Superoxide radicals have a negative effect on endothelial function by reacting directly with nitric oxide (NO), such that they decrease NO bioavailabily. In addition, peroxynitrites as the products of the reaction of superoxide radicals with NO, also have negative effects on endothelial cells [6, 7]. Hydroxyl radicals produced by the decomposition of hydroperoxynitrites may trigger lipid peroxidation, marked by increased malondialdehyde (MDA) levels. In other side, superoxide dismutase (reviewer#2 : In introduction, lin2 18) (SOD) plays an important role in scavenging superoxide anion which are formed during the early stages of oxidative stress, and in preventing aging [8]. SOD catalyzes the conversion of superoxide to hydrogen peroxide plus dioxygen. SOD can be classified into three groups, Cu/Zn SOD, Mn SOD, and Fe SOD, by the metals that they contain at their active sites. Cu/Zn SOD is usually found in the cytoplasm of eukaryotic cells and Mn SOD in mitochondria, whereas prokaryotic cells contain Fe SOD and Mn SOD [9].

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 *Jathropha 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 [10]. This study aimed to investigate whether methanolic extract of *Scurrula atropurpurea* (BL.) Dans. able to diminisihed oxidative stress in hypertensive rats.

MATERIAL AND METHODS

Eksperimen ini terdiri dari lima kelompok perlakuan antara lain: I. K(-), tidak dipapar DOCA-garam maupun MESA. II. K (+) dipapar DOCA-garam namun tanpa MESA. III-V. DOCA-garam + MESA (50, 100, 200mg/KgBB). Pemberian MESA secara sonde. Masing-masing kelompok diulang 5 kali (mohon rancangan ini dimasukkan tujuan riset kami KURATIF treatment untuk menjawab reviewer# 1 point 3).

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^{0}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 mnutes 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 [11]. The methanolic extract of *Scurrula atropurpurea* (MESA) was administered daily by the oral route using a catheter, this being continued for 6 weeks.

Animals

The study subjects were Wistar rats aged 3-5 months and weighing 250-300 grams. 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 the weights of the rats was then determined [12]. The treatment groups consisted of the control group, the group of non-MESA hypertensive rats, three groups of hypertensive rats receiving MESA at dosages of 50, 100, and 200 mg/kgBW. The rats were assigned randomly into the groups, with each group containing five rats.

Tikus dimasukkan dalam holder beberapa menit sebelum memulai pengukuran (3-5 menit). Dilepas lubang ekor dari holder kosong dengan cara mengendorkannya. Posisikan kepala lubang ekor sesuai dengan panjang tikus. Ambil tikus dengan memegang ekornya, letakkan di meja depan holder. Secara alami tikus akan menuju ke holder. Masukkan ekor ke lubang ekor dan kencangkan. Tekanan darah tikus diukur dengan alat *Blood Pressure Analyzer* merk IITC [15]. (reviewer #1 point 2)

Tissue sampling

At the end of the treatment, the animals in all groups were anesthetized; their blood was drawn by cardiac puncture and heparinized. Blood samples were centrifuged at a speed of 4000 g (4 min, 4°C) to obtain the plasma. All samples were stored at −80°C until analyzed.

Lipid peroxidation analysis

Plasma levels of lipid peroxides were determined as thiobarbituric acid reactive substance (TBARS) according to the method of Ohkawa et al [13], based on the reaction of lipid peroxides with thiobarbituric acid (TBA) at 95ºC. In the TBA test reaction, lipid peroxides and TBA react to form a pink pigment with an absorption maximum at 532 nm. The reaction was performed at pH 2-3 at 95ºC for 15 min. The sample was mixed with 2.5 volumes of 10% (w/v) trichloroacetic acid to precipitate the protein. The precipitate was pelleted by centrifugation and an aliquot of supernatant was reacted with 0.67% TBA in a boiling water-bath for 15 min. After cooling, the absorbance was read at 532 nm. Arbitrary values obtained were compared with a series of standard solutions (1,1,3,3 tetramethoxypropane). Results were expressed as nanomole per milliliter.

Superoxide dismutase analysis

Superoxide dismutase (SOD) was assayed by measuring the inhibition of the formation of blue colored formazan at 560 nm according to the technique of Kakkar et al [14]. The

inhibition by SOD of reduction of NBT to blue-colored chromogen in the presence of PMS and NADH was measured at 560 nm. One unit of enzyme activity was defined as enzyme concentration required to inhibit the absorbance at 560 nm of chromogen production by 50% in 1 min under assay conditions, and expressed as specific activity in unit of SOD min−1 mg−1 of protein.

Ethics

Animal care and experimental procedures were approved by the Institutional Animal Ethics Committee of 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 with SPSS 15.0 statistical package. Post Hoc test was used if the ANOVA was significant. Probability values of $p<0.05$ were considered statistically significant.

RESULTS

Effect of MESA on systolic blood pressure

The administration of DOCA salt affected the MDA levels, (harusnya blood pressure; reviewer #1 point 1) as shown in Fig. 1. There was significantly ($p < 0.05$) increased systolic blood pressure in hypertension rats compared to sham group. Compared to its hypertensive group, the administration of MESA significantly decreased systolic blood pressure, but not able to reach the level in sham group.

Effect of MESA on malondialdehyde level

The administration of DOCA salt affected the MDA levels, as shown in Fig. 2. There were significantly ($p < 0.05$) increased MDA levels in hypertension rats compared to sham group. The administration of $MESA_{200}$ significantly ($p < 0.05$) decreased the MDA levels compared to hypertensive groups.

Effect of MESA on superoxide dismutase level

The administration of DOCA salt affected the SOD levels, as shown in Fig. 3. The SOD level were significantly ($p < 0.05$) decreased in hypertension rats compared to sham group. The administration of $MESA_{50}$ elevated the SOD levels to reach level in sham group. The SOD levels in $MESA_{100}$ and $MESA_{200}$ were higher significantly ($p < 0.05$) compared to sham group.

DISCUSSION

Some species of Loranthaceae from China have been used as medicinal materials for the treatment of hypertension [15]. Various compounds have been found in Loranthaceaeous plants and some of them have been identified with hypotensive properties [10, 16]. In addition, *Taxillus theifer* (Hayata) H. S. Kiu (*Scurrula ritozanensis*), a Loranthaceaeous plant endemic to Taiwan [17], has been used as an anti-hypertensive agent in Formosan folk medicine. The DOCA-salt treatment induced systemic arterial hypertension, proteinuria, kidney hypertrophy, and impaired kidney function, as reported for this model [18]. This study revealed that DOCA-salt treatment significantly ($p < 0.05$) increased systolic blood pressure as marker of hypertension rats compared to sham group. The administration of MESA significantly decreased systolic blood pressure, but can not reach the level in sham group.

Berdasarkan hasil penelitian oleh peneliti sebelumnya, LD50 dari benalu teh > 5 g/kg bb (Winarno, 2000). *Acute Toxicity Test* Lethality studies showed that the crude extract from the leaves of *V. album* (mistletoe) had an LD value of 417.5mg/kg. mice, i.p.. The high dose recipients were immobile and were lying on their abdomen. They were cold to touch with piloerection (Eno, et.al. 2004). (Untuk menjawab reviewer #2 point 1, bahwa MESA 200mg tidak menyebabkan toksik scara invivo).

Hasil penelitian oleh tim (payung) penelitian oleh Rahman (2013) Gambaran histopatologi epitel tubulus proksimal ginjal tikus wistar kelompok kontrol negatif dan kelompok kontrol positif, yang diamati dengan menggunakan mikroskop trinokular. Gambaran histopatologi ginjal yang diinduksi *Deoxycorticosterone acetate* (DOCA) dan garam (kontrol positif). Tampak gambaran glomerulosklerosis pada glomerulus serta pelebaran (dilatasi) tubulus proksimal serta banyak sel epitel tubulus proksimal nekrosis dengan perubahan bentuk inti sel (piknosis, karioreksis, kariolisis). Perbedaan efek pemberian ekstrak metanolik daun benalu teh (*Scurulla atropurpurea [Bl.] Danser*) dengan dosis 50mg/KgBB dan 100mg/KgBB mampu menurunkan jumlah nekrosis epitel tubulus proksimal ginjal tikus wistar (*Rattus norvegicus*) yang diinduksi *Deoxycorticosterone acetate* (DOCA) dan garam secara signifikan dibandingkan dengan kelompok kontrol positif dengan nilai signifikansi kedua kelompok *p* 0,00. Penurunan jumlah nekrosis epitel tubulus proksimal ginjal tikus wistar (*Rattus norvegicus*) yang diinduksi *Deoxycorticosterone acetate* (DOCA) dan garam paling banyak terjadi pada pemberian ekstrak metanolik daun benalu teh dosis 100mg/KgBB (5,25 ± 0,86) dengan nilai signifikansi *p* 0,00. Pemberian ekstrak metanolik daun benalu teh dosis 200mg/KgBB tidak memberikan hasil yang signifikan dibandingkan dengan kelompok kontrol positif dengan nilai signifikansi *p* 0,09, namun berbeda signifikan dengan kelompok pemberian ekstrak metanolik daun benalu teh dosis 50mg/KgBB dan 100mg/KgBB dengan nilai signifikansi *p* 0,00. (Untuk menjawab reviewer# 2 point 2).

Hasil penelitian oleh peneliti sebelumnya bahwa peredaman radikal bebas hasil ekstraksi benalu teh dengan berbagai sistem pelarut dari benalu teh dengan metode DPPH, diantaranya ekstrak n-heksan, ekstrak etil asetat, ekstrak metanol dan ekstrak air. Ekstrak metanol memiliki aktivitas peredaman radikal bebas yang paling besar dengan daya hambat sebesar 93,59 ppm. (Simanjuntak, *dkk.*, 2004) ((Untuk menjawab reviewer #2 point 3).

Oxidative stress in DOCA-salt-treated animals has been studied with a variety of stress markers. Subunits of the NADPH-oxidase were found to be markedly expressed, the oxidative stress-scavenging protein heme oxygenase-1 is up-regulated and also the urinary oxidative stress marker 8-isoprostane is often increased [19, 20]. Besides, increased amounts of the oxidative base modification 8-oxodG in the DOCAsalt-group. 8-oxodG is now widely used as a marker of hypertension in urine [21]. In this study, the increase in blood MDA indicates an increase in oxidative stress in DOCA-salt hypertensive rats. Depending on the levels of reactive oxygen compounds, various transcription factors sensitive to change in redox status will be activated and will coordinate intracellular biological response. Modest oxidative stress will induce Nrf2, a transcription factor implicated in the transactivation of genes that encode antioxidant enzymatic activity [22]. The level of superoxide dismutase significantly ($p < 0.05$) decreased in DOCA-salt hypertensive rats. This finding indicate that DOCA-salt hypertensive rats produces more superoxide radical. Oxidative stress has been implicated in the pathogenesis of Ang II–related hypertension [23, 24]. Ang II, through AT1R, stimulates NADPH oxidase, induces oxidative stress, and alters endothelial cell function [25].

The antioxidant compounds of *Scurrula atropurpurea* extract in our study may contains quercetin, quercetin-3-*O*-glucoside, quercitrin (a glycoside rhamnose of quercetin) and kaempferol. Quercetin exerts its antioxidant activity through scavenging reactive oxygen speces [26, 27]. Quercitrin also has a free radical scavenging activity [28]. Kaempferol has shown strong inhibitory/scavenging activity on reactive oxygen species generation with

numerous hydroxyl groups on their structures [28]. Moreover, it has been found to be a particularly potent blocker of extracellular reactive oxygen species production, and to inhibit the ascorbate-dependent NADH oxidase and superoxide anion production activities [29]. Therefore, the malondialdehyde-lowering effect and modulation of superoxide dismutase by *Scurrula atropurpurea* extract demonstrated in this study might have been associated with flavonol or phenolic compounds.

Conclusions

In conclusion, *Scurulla atropurpurea* able to modulates superoxide dismutase, diminished oxidative stress, and decreased sistolic blood pressure in DOCA-salt hypertensive rats.

Declaration of interest

The author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

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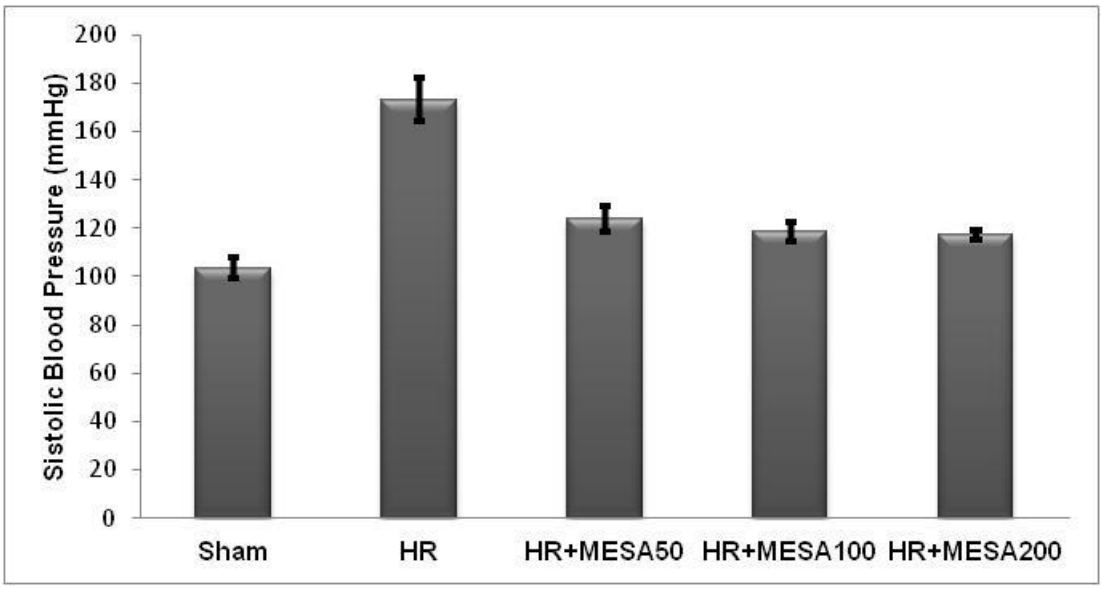
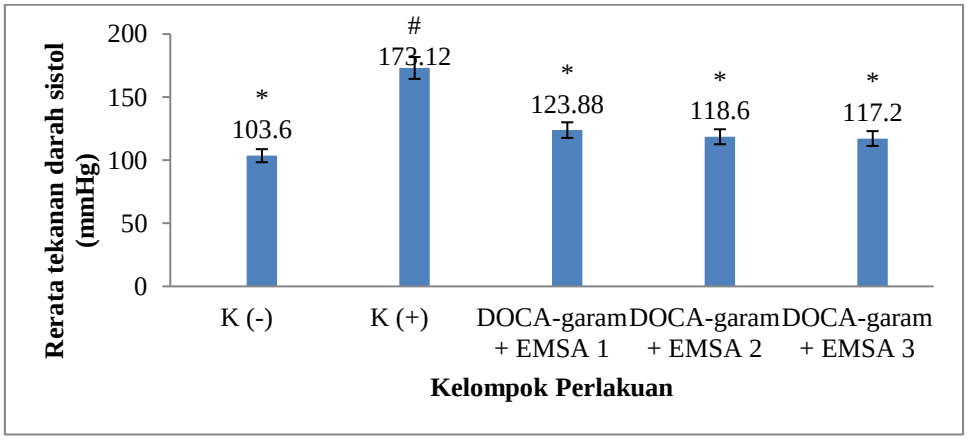


Figure 1. **Systolic blood pressure in DOCA-salt hypertensive with or without the administration of methanolic *Scurrula atropurpurea* extract rats compared to sham control group.** There was significantly ($p < 0.05$) increased systolic blood pressure in hypertension rats compared to sham group. Compared to its hypertensive group, the administration of MESA significantly decreased systolic blood pressure, but not able to reach the level in sham group. HR (hypertensive rats) untuk mnjawab reviewer#2: In figures, what does HR mean?

PAKE GAMBAR YANG INI SAJA PAK



Gambar 1. Efek EMSA (50, 100, 200 mg/KgBB) terhadap tekanan darah sistol pada tikus hipertensi paparan DOCA-garam

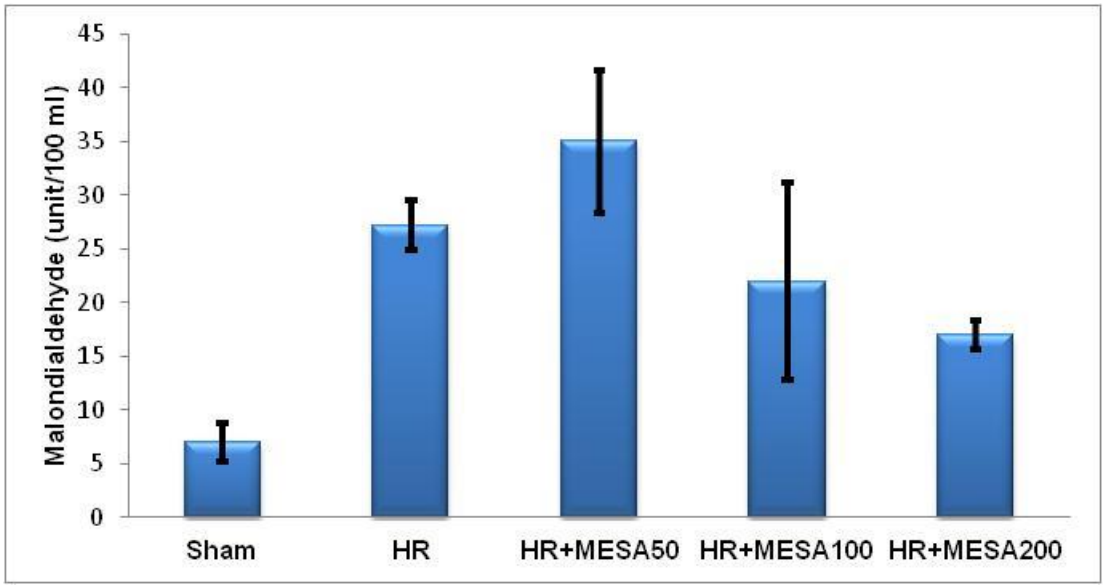
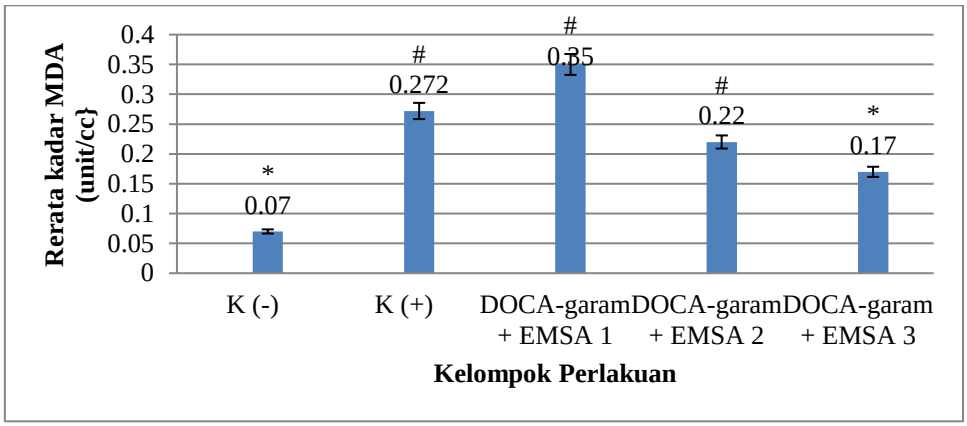


Figure 2. **Malondialdehyde in DOCA-salt hypertensive with or without the administration of methanolic *Scurrula atropurpurea* extract rats compared to sham control group.** There were significantly ($p < 0.05$) increased MDA levels in hypertension rats compared to sham group. The administration of $MESA_{200}$ significantly ($p < 0.05$) decreased the MDA levels compared to hypertensive groups.

PAKE GAMBAR YANG INI SAJA PAK



Gambar 2. Efek EMSA (50, 100, 200 mg/KgBB) terhadap kadar MDA pada tikus hipertensi paparan DOCA-garam

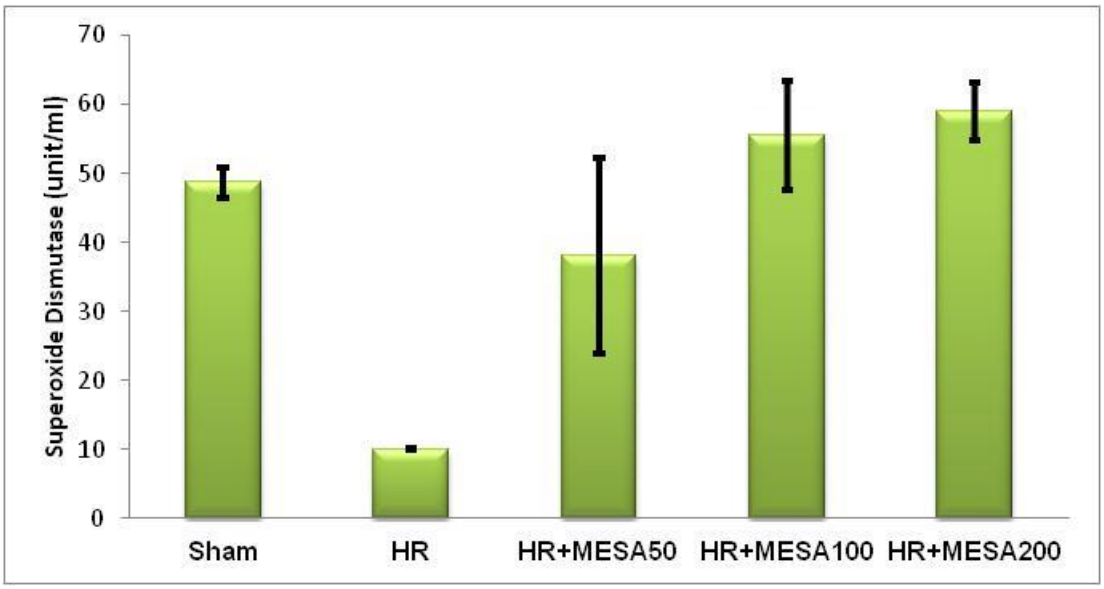
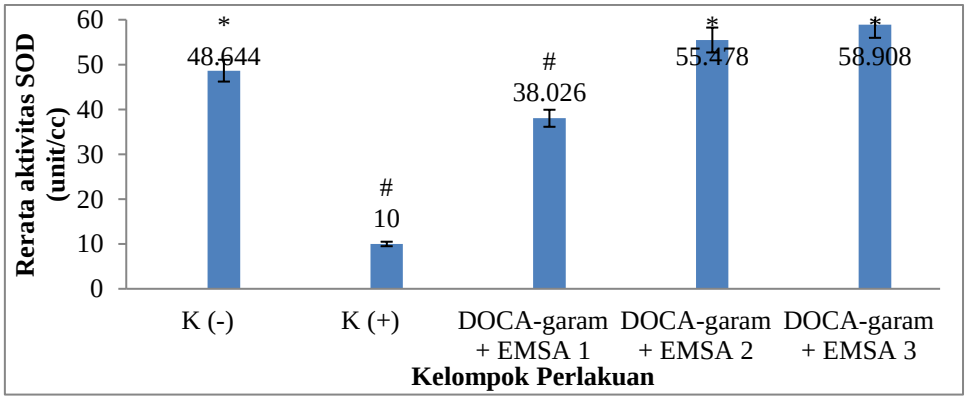


Figure 3. **The level of superoxide dismutase in DOCA-salt hypertensive with or without the administration of methanolic *Scurrula atropurpurea* extract rats compared to sham control group.** The SOD level were significantly ($p < 0.05$) decreased in hypertension rats compared to sham group. The administration of $MESA_{50}$ elevated the SOD levels to reach level in sham group (seharusnya HR group : reviewer #1 point 4). The SOD levels in $MESA_{100}$ and $MESA_{200}$ were higher significantly ($p < 0.05$) compared to sham group (seharusnya HR group : reviewer 1 point 4).

PAKE GAMBAR YANG INI SAJA PAK



Gambar 3. Efek EMSA (50, 100, 200 mg/KgBB) terhadap aktivitas SOD pada tikus hipertensi paparan DOCA-garam

Dear Chief Editor

Biomarker and Genomic Medicine

We submit our revision manuscript. We hope this manuscript accepted for publish in Biomarker and Genomic Medicine. Answer for reviewer question below.

Reviewer #1:

The autors identified the effects of Scurrula atropurpurea extract(MESA) on blood pressure in a rat model. The findings are new, but the impact of this manuscript is limited by their data present. A major revision is needed.

1. As a minor point, several typing errors are noticed. In addition, certain writing error may compromise the readers. For example,the authors describe "The administration of DOCA salt affected the MDA levels, as shown in Fig. 1." in Result section, however, Fig 1 is not a MDA data. We have revise this part in the text,

2. Blood pressure-lowering effect is one major find in this study, but the authors did not provide the dection method and experimental detail (such as time point)for blood pressure tests. We add the method to measured systolic blood pressure.

3. As a major point, the authors noly tested the effects of MESA in HR rat. The effects (such as blood pressure and SOD) of MESA on normal rat should also be performed. We can not performed this additional data

4. Please check the data statistics or labeling. For example, in Fig.3, dose the HR-MESA50 group significantly different form Sham group? or HR group? We have revise this this part in the figure.

Reviewer #2:

The authors have investigated whether methanolic extract of Scurrula atropurpurea Dans. (MESA) is able to diminisihed oxidative stress in hypertensive rats. The authors have shown that the significantly increased systolic blood pressure in DOCA salt induced hypertension rats, and the administration of MESA200 significantly decreased the levels of blood malondialdehyde (MDA) compared to the hypertensive control groups. The superoxide dismutase (SOD) level was significantly decreased in hypertension rats compared to sham group. The authors should comment on the following points:

1. For their in vivo study, the author should clarify "does MESA200 produce any toxic effects or any other side effects in vivo?"
2. For their in vivo study, the author should clarify "does the treatment of MESA have any effects on the kidney damage of DOCA salt induced hypertension rats ?"

4. In figure 1, the author should explain why MESA50, MESA100 and MESA200 did not show the dose dependent effect to reduce systolic blood pressure in DOCA salt induced hypertension rats.

For question number 1, 2, and 4 we add these statement in discussion: “This finding indicated there is no dose dependent effect maybe due to the ability of kidney restoration. Previous study showed that MESA reduce necrosis of renal proximal tubulus achieved at dose 50 and 100 mg/kg BW. At higher dose, there is no significantly compared with DOCA-salt treatment group. In other word, at 200 mg/kgBW may induces toxic effect on kidney.[20]

3. In the result part, the author should provide more molecular evidences such as performing western blot analysis to confirm that MESA suppresses the oxidative stress in DOCA salt induced hypertension rats.

Oxidative stress is a phenomenon that involves the evaluation of production, defense and oxidative damage markers. We can not performed additional data of oxidative stres marker because we provide not only oxidative damage marker but also antioxidant (SOD) level.

Minor points

In abstract; line 8; the author should explain what does MESA mean?
In introduction part, line 18; the author should provide the full name of SOD means?
In page 3, line 2; the author should provide more references.
In figures, what does HR mean?

We have correct this mistake in the text.

One more suggestion is that the English grammar of this manuscript should be improved by consulting an official language editing service, in particular in the abstract of this manuscript.

We have revised the abstract using online grammar check (www.reverso.com)

Thank you

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ABSTRACT

This study aimed to investigate whether a methanolic extract of *Scurrula atropurpurea* (BL.) Dans. (MESA) able to reduced oxidative stress and systolic blood pressure in DOCA salt- hypertensive rats (HR). Twenty male Wistar was divided into the control group and three HR groups who receiving the *Scurulla atropurpurea* extract at a dosage of 50; 100; and 200 mg/KgBW. Systolic blood pressure was recorded by tail cuff methods. The levels of serum malondialdehyde (MDA) and superoxide dismutase (SOD) were analyzed by colorimetric. Systolic blood pressure was increased significantly in the HR group compared to sham group ($P< 0.05$). The administration of MESA significantly decreased systolic blood pressure, but not able to reach the level in the sham group. The level of MDA was higher significantly in the HR group compared to sham group ($P< 0.05$). The administration of $MESA_{200}$ significantly decreased the MDA levels compared to HR groups ($P < 0.05$). The SOD level was significantly decreased in HR compared to the sham group ($P < 0.05$).The administration of $MESA_{50}$ elevated the SOD levels to reach level in the sham group. The SOD levels in $MESA_{100}$ and $MESA_{200}$ were higher significantly compared to sham group ($P < 0.05$). In conclusion, *Scurulla atropurpurea* able to modulate superoxide dismutase, diminished oxidative stress, and decreased systolic blood pressure in DOCA-salt hypertensive rats.

Key words: mistletoe; antioxidant; oxidative stress; high blood pressure; rats.

INTRODUCTION

Hypertension is a clinical common vascular related disease, with high mortality and disability. Additionally, it is also an independent risk factor for stroke, coronary heart disease, heart failure, renal insufficiency, peripheral vascular diseases, early death, and many other major diseases.[1] Hypertension is the end result of a complex interaction between genetic and environmental factors affecting the physiological systems regulating blood pressure.[2] There are about 1 billion hypertensive patients in the world, and around 30% of the population died from cardiovascular and cerebrovascular events, in which 62% of acute stroke events and 49% of cardiovascular events were directly caused by hypertension.[3,4]

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.[5] Superoxide radicals have a negative effect on endothelial function by reacting directly with nitric oxide (NO), such that they decrease NO bioavailabily. In addition, peroxynitrites as the products of the reaction of superoxide radicals with NO, also have negative effects on endothelial cells.[6,7] Hydroxyl radicals produced by the decomposition of hydroperoxynitrites may trigger lipid peroxidation, marked by increased malondialdehyde (MDA) levels. In other side, superoxide dismutase (SOD) plays an important role in scavenging superoxide anion which are formed during the early stages of oxidative stress, and in preventing aging.[8] SOD catalyzes the conversion of superoxide to hydrogen peroxide plus dioxygen. SOD can be classified into three groups, Cu/Zn SOD, Mn SOD, and Fe SOD, by the metals that they contain at their active sites. Cu/Zn SOD is usually found in the cytoplasm of eukaryotic cells and Mn SOD in mitochondria, whereas prokaryotic cells contain Fe SOD and Mn SOD.[9]

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 *Jathropha podagrica*, and tetrandrine from *Stephenia tetradra*.[2,10,11] *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.[12] This study aimed to investigate whether methanolic extract of *Scurrula atropurpurea* (BL.) Dans. able to diminisihed oxidative stress in hypertensive rats.

MATERIAL AND METHODS

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^{0}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 mnutes 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.[13] The methanolic extract of *Scurrula atropurpurea* (MESA) was administered daily by the oral route using a catheter, this being continued for 6 weeks.

Animals

The study subjects were twenty male Wistar rats aged 3-5 months and weighing 250-300 grams. 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 the weights of the rats was then determined.[14] The treatment groups consisted of the control group, the group of non-MESA hypertensive rats (HR), three groups of hypertensive rats receiving MESA at dosages of 50, 100, and 200 mg/kgBW. The rats were assigned randomly into the groups, with each group containing five rats.

Blood pressure measurement

Systolic blood pressure was recorded in the end of study by tail cuff methods (IITC, Non-Invasive Blood Pressure Instrument) according previous study.[15]

Tissue sampling

At the end of the treatment, the animals in all groups were anesthetized; their blood was drawn by cardiac puncture and heparinized. Blood samples were centrifuged at a speed of 4000 g (4 min, 4°C) to obtain the plasma. All samples were stored at −80°C until analyzed.

Lipid peroxidation analysis

Plasma levels of lipid peroxides were determined as thiobarbituric acid reactive substance (TBARS) according to the method of Ohkawa et al.,[16] based on the reaction of lipid peroxides with thiobarbituric acid (TBA) at 95°C. In the TBA test reaction, lipid peroxides and TBA react to form a pink pigment with an absorption maximum at 532 nm. The reaction was performed at pH 2-3 at 95°C for 15 min. The sample was mixed with 2.5 volumes of 10% (w/v) trichloroacetic acid to precipitate the protein. The precipitate was pelleted by centrifugation and an aliquot of supernatant was reacted with 0.67% TBA in a boiling water-bath for 15 min. After cooling, the absorbance was read at 532 nm. Arbitrary values obtained were compared with a series of standard solutions (1,1,3,3 tetramethoxypropane). Results were expressed as nanomole per milliliter.

Superoxide dismutase analysis

Superoxide dismutase (SOD) was assayed by measuring the inhibition of the formation of blue colored formazan at 560 nm according to the technique of Kakkar et al.[17] The inhibition by SOD of reduction of NBT to blue-colored chromogen in the presence of PMS and NADH was measured at 560 nm. One unit of enzyme activity was defined as enzyme concentration required to inhibit the absorbance at 560 nm of chromogen production by 50% in 1 min under assay conditions, and expressed as specific activity in unit of SOD min−1 mg−1 of protein.

Ethics

Animal care and experimental procedures were approved by the Institutional Animal Ethics Committee of 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 with SPSS 15.0 statistical package. Post Hoc test was used if the ANOVA was significant. Probability values of $p<0.05$ were considered statistically significant.

RESULTS

Effect of MESA on systolic blood pressure

The administration of DOCA salt affected the systolic blood pressure levels, as shown in Fig. 1. There was significantly ($P < 0.05$) increased systolic blood pressure in hypertension rats compared to sham group. Compared to its hypertensive group, the administration of MESA significantly decreased systolic blood pressure, but not able to reach the level in sham group.

Effect of MESA on malondialdehyde level

The administration of DOCA salt affected the MDA levels, as shown in Fig. 2. There were significantly ($P < 0.05$) increased MDA levels in hypertension rats compared to sham group. The administration of $MESA_{200}$ significantly ($P < 0.05$) decreased the MDA levels compared to hypertensive groups.

Effect of MESA on superoxide dismutase level

The administration of DOCA salt affected the serum SOD levels, as shown in Fig. 3. The serum SOD level were significantly ($P < 0.05$) decreased in hypertension rats compared to sham group. The administration of $MESA_{50}$ elevated the SOD levels to reach level in sham group. The SOD levels in $MESA_{100}$ and $MESA_{200}$ were higher significantly ($P < 0.05$) compared to sham group.

DISCUSSION

Some species of Loranthaceae from China have been used as medicinal materials for the treatment of hypertension.[16] Various compounds have been found in Loranthaceaeous plants and some of them have been identified with hypotensive properties.[12,19] In addition, *Taxillus theifer* (Hayata) H. S. Kiu (*Scurrula ritozanensis*), a Loranthaceaeous plant endemic to Taiwan,[20] has been used as an anti-hypertensive agent in Formosan folk medicine. The DOCA-salt treatment induced systemic arterial hypertension, proteinuria, kidney hypertrophy, and impaired kidney function, as reported for this model.[21] This study revealed that DOCA-salt treatment significantly ($p < 0.05$) increased systolic blood pressure as marker of hypertension rats compared to sham group. The administration of MESA significantly decreased systolic blood pressure, but can not reach the level in sham group. This finding indicated there is no dose dependent effect maybe due to the ability of kidney restoration. Previous study showed that MESA reduce necrosis of renal proximal tubulus achieved at dose 50 and 100 mg/kg BW. At higher dose, there is no significantly compared with DOCA-salt treatment group. In other word, at 200 mg/kgBW may induces toxic effect on kidney.[22]

Oxidative stress in DOCA-salt-treated animals has been studied with a variety of stress markers. Subunits of the NADPH-oxidase were found to be markedly expressed, the oxidative stress-scavenging protein heme oxygenase-1 is up-regulated and also the urinary oxidative stress marker 8-isoprostane is often increased.[23,24] Besides, increased amounts of the oxidative base modification 8-oxodG in the DOCAsalt-group. 8-oxodG is now widely used as a marker of hypertension in urine.[25] In this study, the increase in blood MDA indicates an increase in oxidative stress in DOCA-salt hypertensive rats. Depending on the levels of reactive oxygen compounds, various transcription factors sensitive to change in redox status will be activated and will coordinate intracellular biological response. Modest oxidative stress will induce Nrf2, a transcription factor implicated in the transactivation of genes that encode antioxidant enzymatic activity.[26] The

level of superoxide dismutase significantly ($p < 0.05$) decreased in DOCA-salt hypertensive rats. This finding indicate that DOCA-salt hypertensive rats produces more superoxide radical. Oxidative stress has been implicated in the pathogenesis of Ang II–related hypertension.[27,28] Ang II, through AT1R, stimulates NADPH oxidase, induces oxidative stress, and alters endothelial cell function.[29]

The antioxidant compounds of *Scurrula atropurpurea* extract in our study may contains quercetin, quercetin-3-*O*-glucoside, quercitrin (a glycoside rhamnose of quercetin) and kaempferol. Quercetin exerts its antioxidant activity through scavenging reactive oxygen speces.[30,31] Quercitrin also has a free radical scavenging activity.[32] Kaempferol has shown strong inhibitory/scavenging activity on reactive oxygen species generation with numerous hydroxyl groups on their structures.[32] Moreover, it has been found to be a particularly potent blocker of extracellular reactive oxygen species production, and to inhibit the ascorbate-dependent NADH oxidase and superoxide anion production activities.[33] Therefore, the malondialdehyde-lowering effect and modulation of superoxide dismutase by *Scurrula atropurpurea* extract demonstrated in this study might have been associated with flavonol or phenolic compounds.

Conclusions

In conclusion, *Scurulla atropurpurea* able to modulates superoxide dismutase, diminished oxidative stress, and decreased sistolic blood pressure in DOCA-salt hypertensive rats.

Declaration of interest

The author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

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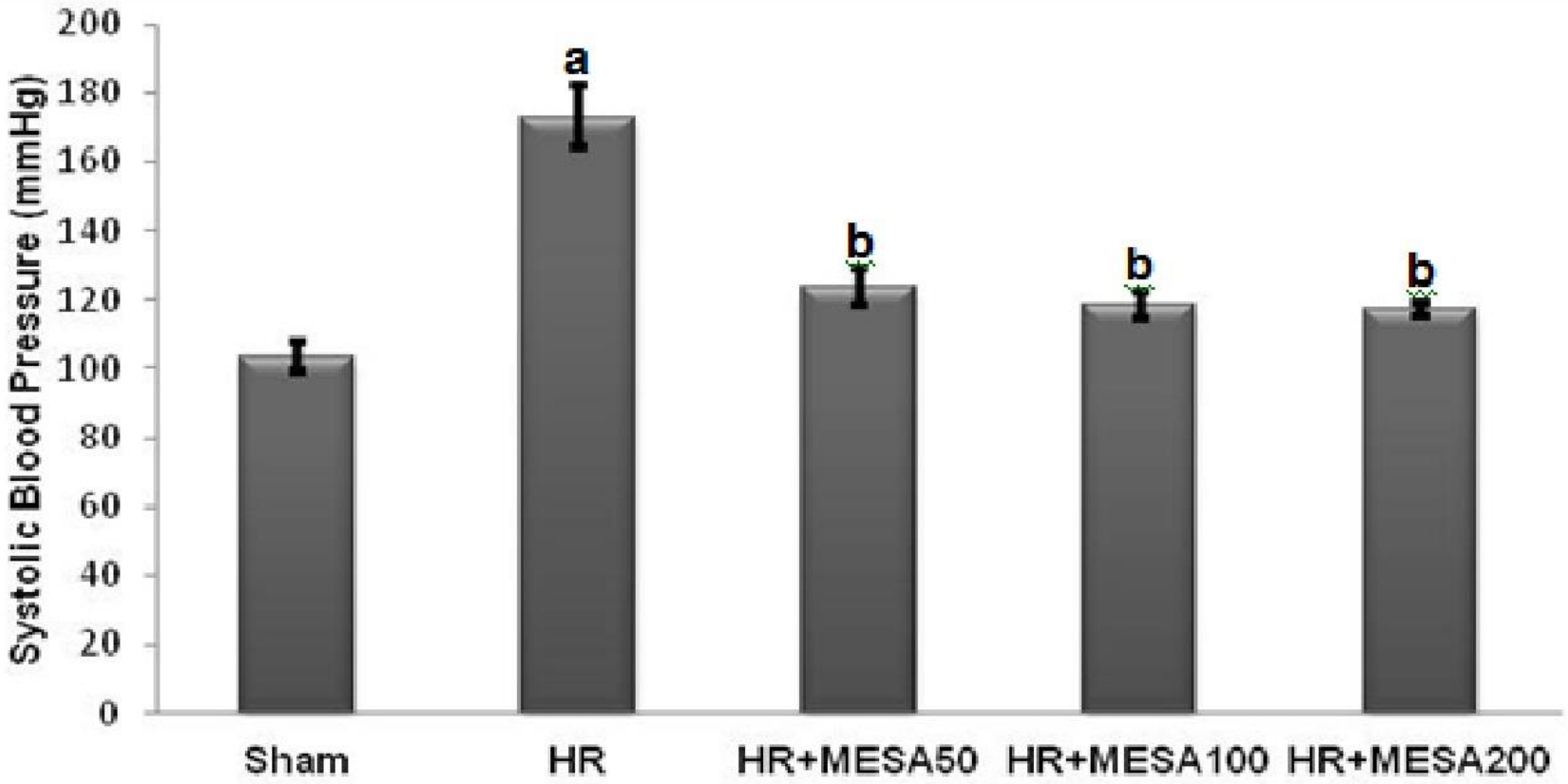
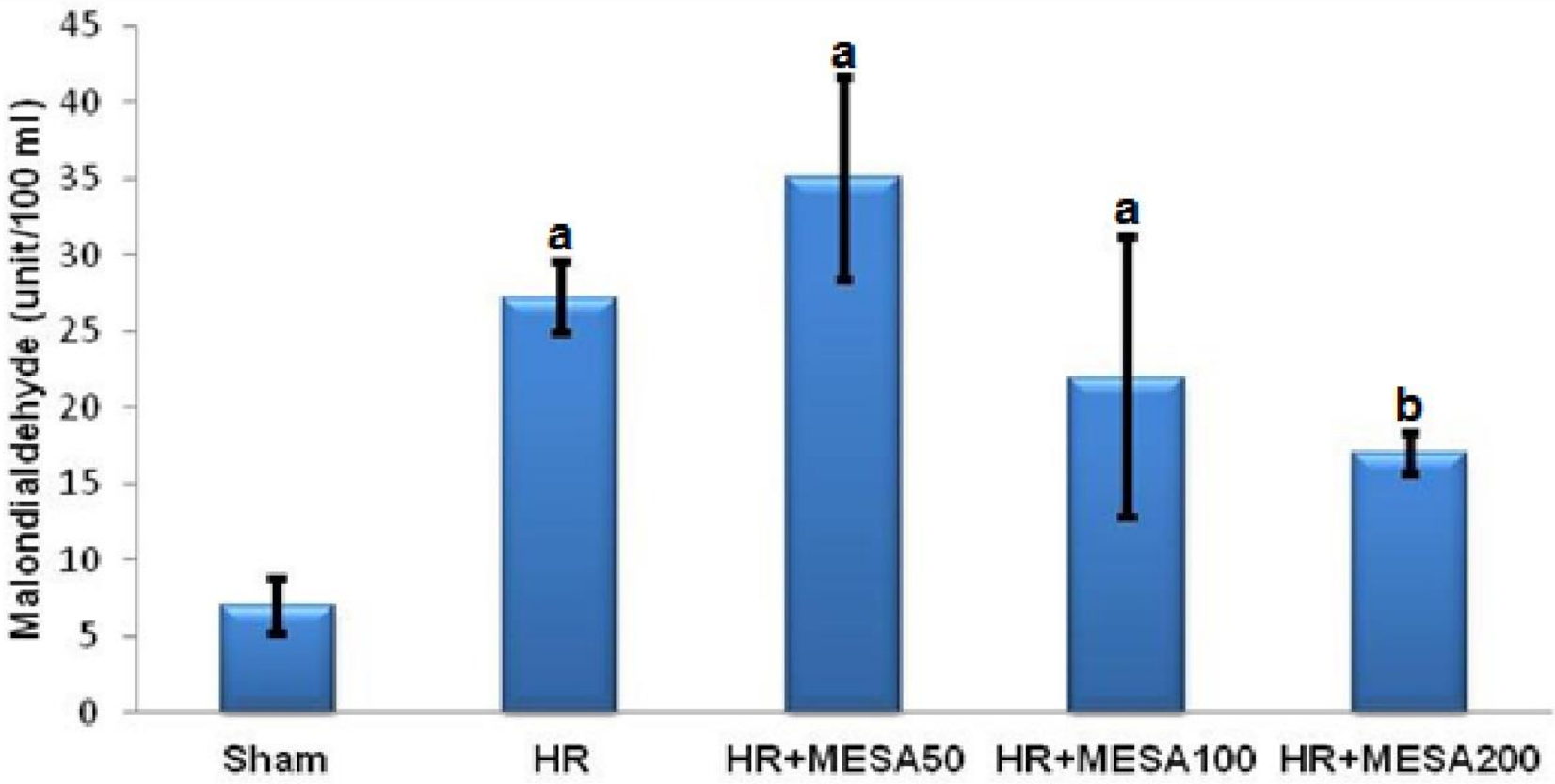
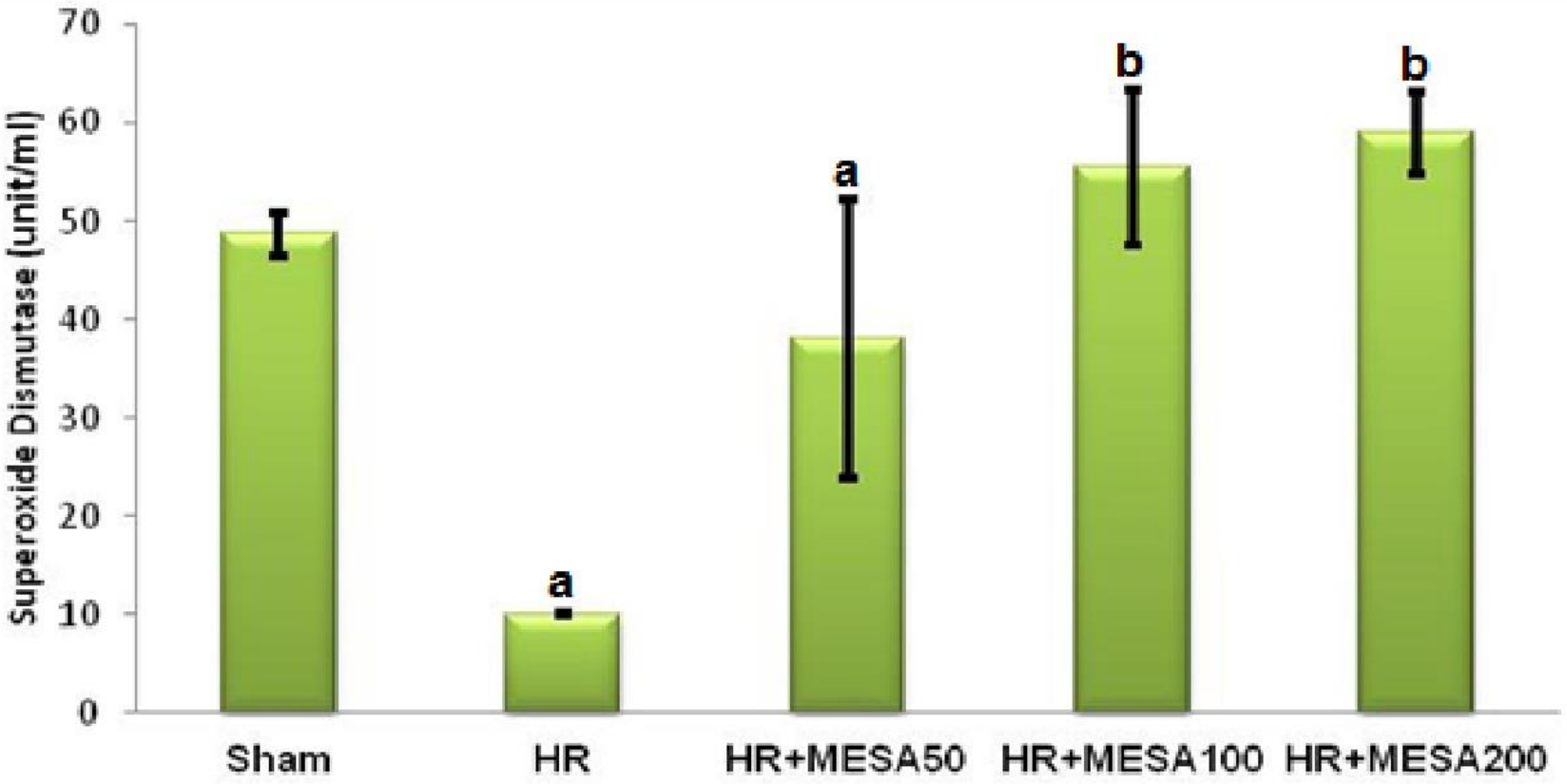
Figure 1. **Systolic blood pressure in DOCA-salt hypertensive with or without the administration of methanolic *Scurrula atropurpurea* extract rats compared to sham control group.** There was significantly ($P < 0.05$) increased systolic blood pressure in hypertension rats compared to sham group. Compared to its hypertensive group, the administration of MESA significantly ($P < 0.05$) decreased systolic blood pressure, but not able to reach the level in sham group. [a] $P < 0.05$ in comparison with sham group; [b] $P < 0.05$ in comparison with DOCA-salt hypertensive group. HR: hypertensive rats; MESA: methanolic extract of *Scurrula atropurpurea*.

Figure 2. **Malondialdehyde in DOCA-salt hypertensive with or without the administration of methanolic *Scurrula atropurpurea* extract rats compared to sham control group.** There were significantly ($P < 0.05$) increased MDA levels in hypertension rats compared to sham group. The administration of $MESA_{200}$ significantly ($P < 0.05$) decreased the MDA levels compared to hypertensive groups. [a] $P< 0.05$ in comparison with sham group; [b] $P< 0.05$ in comparison with DOCA-salt hypertensive group. HR: hypertensive rats; MESA: methanolic extract of *Scurrula atropurpurea*.

Figure 3. **The level of serum superoxide dismutase in DOCA-salt hypertensive with or without the administration of methanolic *Scurrula atropurpurea* extract rats compared to sham control group.** The serum SOD level were significantly ($P < 0.05$) decreased in hypertension rats compared to sham group. The administration of $MESA_{50}$ elevated the SOD levels to reach level in sham group. The SOD levels in $MESA_{100}$ and $MESA_{200}$ were higher significantly ($P < 0.05$) compared to HR group. [a] $P< 0.05$ in comparison with sham group; [b] $P< 0.05$ in comparison with DOCA-salt hypertensive group. HR: hypertensive rats; MESA: methanolic extract of *Scurrula atropurpurea*.


Systolic Blood Pressure (mmHg)
200
180
160
140
120
100
80
60
40
20
0
a
b
b
b
Sham
HR
HR+MESA50
HR+MESA100
HR+MESA200


Malondialdehyde (unit/100 ml)
45
40
35
30
25
20
15
10
5
0
a
a
a
b
Sham
HR
HR+MESA50
HR+MESA100
HR+MESA200


Superoxide Dismutase (unit/ml)
70
60
50
40
30
20
10
0
a
a
b
b
Sham
HR
HR+MESA50
HR+MESA100
HR+MESA200

ABSTRACT

This study aimed to investigate whether a methanolic extract of *Scurrula atropurpurea* (BL.) Dans. (MESA) able to reduced oxidative stress and systolic blood pressure in DOCA salt- hypertensive rats (HR). Twenty male Wistar was divided into the control group and three HR groups who receiving the *Scurulla atropurpurea* extract at a dosage of 50; 100; and 200 mg/KgBW. Systolic blood pressure was recorded by tail cuff methods. The levels of serum malondialdehyde (MDA) and superoxide dismutase (SOD) were analyzed by colorimetric. Systolic blood pressure was increased significantly in the HR group compared to sham group ($P< 0.05$). The administration of MESA significantly decreased systolic blood pressure, but not able to reach the level in the sham group. The level of MDA was higher significantly in the HR group compared to sham group ($P< 0.05$). The administration of $MESA_{200}$ significantly decreased the MDA levels compared to HR groups ($P < 0.05$). The SOD level was significantly decreased in HR compared to the sham group ($P < 0.05$).The administration of $MESA_{50}$ elevated the SOD levels to reach level in the sham group. The SOD levels in $MESA_{100}$ and $MESA_{200}$ were higher significantly compared to sham group ($P < 0.05$). In conclusion, *Scurulla atropurpurea* able to modulate superoxide dismutase, diminished oxidative stress, and decreased systolic blood pressure in DOCA-salt hypertensive rats.

Key words: mistletoe; antioxidant; oxidative stress; high blood pressure; rats.

INTRODUCTION

Hypertension is a clinical common vascular related disease, with high mortality and disability. Additionally, it is also an independent risk factor for stroke, coronary heart disease, heart failure, renal insufficiency, peripheral vascular diseases, early death, and many other major diseases.[1] Hypertension is the misregulation of a complex interaction between genetic and life style factors affecting the physiological systems regulating blood pressure.[2] There are about 1 billion hypertensive patients in the world, and around 30% of the population died from cardiovascular and cerebrovascular events, in which 62% of acute stroke events and 49% of cardiovascular events were directly caused by hypertension.[3,4]

Consistence evidence reveal the involvement of reactive oxygen species and oxidative stress in hypertension and its complications. Hypertension is associated with increased production of superoxide radicals which have a negative effect on endothelial function. These effects based on the reaction between superoxide and nitric oxide (NO) to decrease NO bioavailabily. Besids, peroxynitrites as the products of this reaction, also have detrimental effects on endothelial cells.[5-7] Hydroxyl radicals as product decomposition of hydroperoxynitrites may trigger lipid peroxidation, as measured by increased malondialdehyde (MDA) levels. In other side, superoxide dismutase (SOD) plays an important role in scavenging superoxide anion which are formed during the early stages of oxidative stress, and in preventing aging.[8] SOD catalyzes the conversion of superoxide to hydrogen peroxide plus dioxygen. SOD can be classified into three groups, Cu/Zn SOD, Mn SOD, and Fe SOD, by the metals that they contain at their active sites. Cu/Zn SOD is usually found in the cytoplasm of eukaryotic cells and Mn SOD in mitochondria, whereas prokaryotic cells contain Fe SOD and Mn SOD.[9]

For the combat of hypertension and its complications, many drugs of herbal origin have been developed, including digitoxin from *Digitalis purpurea*, reserpine from *Rauwolfia*

serpentina, aspirin from *Salix alba*, tetramethylpyrazine from *Jathropha podagrica*, and tetrandrine from *Stephenia tetradra*.[2,10,11] *Scurrula atropurpurea* (BL.) Dans. is a parasitic in tea plants. In Indonesia, especially on the island of Java, the stems and leaves of this vegetation have been traditionally used for the treatment of cancers.[12] This study aimed to investigate whether methanolic extract of *Scurrula atropurpurea* (BL.) Dans. able to diminisihed oxidative stress in hypertensive rats.

MATERIAL AND METHODS

Preparation tea parasite crude extract

Biological determination of the leaves of *Scurrula atropurpurea* was done at the Indonesian Scientific Institute (LIPI) at Purwodadi, Pasuruan, East Java. The methanolic extract of *Scurrula atropurpurea* (MESA) was obtained from sequential step. The leaves were washed, left to dry in an oven at 40-60^{0}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. 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.[13] This extract was administered daily by the oral route using a catheter, this being continued for 6 weeks.

Animals

Twenty five male Wistar rats, aged 3-5 months, and weighing 250-300 grams were involved in this study. The rats were divided into five groups (n=5 each) consisted of the control group, the group of non-MESA hypertensive rats (HR), and three groups of hypertensive rats receiving MESA at dosages of 50, 100, and 200 mg/kgBW. Hypertensive rats performed by 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 the weights of the rats was then determined.[14]

Blood pressure measurement

Systolic blood pressure was recorded in the end of study by tail cuff methods (IITC, Non-Invasive Blood Pressure Instrument) according previous study.[15]

Tissue sampling

At the end of the treatment, the animals in all groups were anesthetized; their blood was drawn by cardiac puncture and heparinized. Blood samples were centrifuged at a speed of 4000 g (4 min, 4°C) to obtain the plasma. All samples were stored at −80°C until analyzed.

Lipid peroxidation analysis

Plasma levels of lipid peroxides were determined as thiobarbituric acid reactive substance (TBARS) according to the method of Ohkawa et al.,[16] based on the reaction of lipid peroxides with thiobarbituric acid (TBA) at 95°C. In the TBA test reaction, lipid peroxides and TBA react to form a pink pigment with an absorption maximum at 532 nm. The reaction was performed at pH 2-3 at 95°C for 15 min. The sample was mixed with 2.5 volumes of 10% (w/v) trichloroacetic acid to precipitate the protein. The precipitate was pelleted by centrifugation and an aliquot of supernatant was reacted with 0.67% TBA in a boiling water-bath for 15 min. After cooling, the absorbance was read at 532 nm. Arbitrary values obtained were compared with a series of standard solutions (1,1,3,3 tetramethoxypropane). Results were expressed as nanomole per milliliter.

Superoxide dismutase analysis

Superoxide dismutase (SOD) was assayed by measuring the inhibition of the formation of blue colored formazan at 560 nm according to the technique of Kakkar et al.[17] The inhibition by SOD of reduction of NBT to blue-colored chromogen in the presence of PMS and NADH was measured at 560 nm. One unit of enzyme activity was defined as enzyme concentration required to inhibit the absorbance at 560 nm of chromogen production by 50% in 1 min under assay conditions, and expressed as specific activity in unit of SOD min−1 mg−1 of protein.

Ethics

Animal care and experimental procedures were approved by the Institutional Animal Ethics Committee of 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 with SPSS 15.0 statistical package. Post Hoc test was used if the ANOVA was significant. Probability values of $p<0.05$ were considered statistically significant.

RESULTS

Effect of MESA on systolic blood pressure

The administration of DOCA salt affected the systolic blood pressure levels, as shown in Fig. 1. There was significantly ($P < 0.05$) increased systolic blood pressure in hypertension rats compared to sham group. Compared to its hypertensive group, the administration of MESA significantly decreased systolic blood pressure, but not able to reach the level in sham group.

Effect of MESA on malondialdehyde level

The administration of DOCA salt affected the MDA levels, as shown in Fig. 2. There were significantly ($P < 0.05$) increased MDA levels in hypertension rats compared to sham group. The administration of $MESA_{200}$ significantly ($P < 0.05$) decreased the MDA levels compared to hypertensive groups.

Effect of MESA on superoxide dismutase level

The administration of DOCA salt affected the serum SOD levels, as shown in Fig. 3. The serum SOD level were significantly ($P < 0.05$) decreased in hypertension rats compared to sham group. The administration of $MESA_{50}$ elevated the SOD levels to reach level in sham group. The SOD levels in $MESA_{100}$ and $MESA_{200}$ were higher significantly ($P < 0.05$) compared to sham group.

DISCUSSION

Some species of Loranthaceae from China have been used as medicinal materials for the treatment of hypertension.[16] Various compounds have been found in Loranthaceaeous plants and some of them have been identified with hypotensive properties.[12,19] In addition, *Taxillus theifer* (Hayata) H. S. Kiu (*Scurrula ritozanensis*), a Loranthaceaeous plant endemic to Taiwan,[20] has been used as an anti-hypertensive agent in Formosan folk medicine. The DOCA-salt treatment induced systemic arterial hypertension, proteinuria, kidney hypertrophy, and impaired kidney function, as reported for this model.[21] This study revealed that DOCA-salt treatment significantly ($p < 0.05$) increased systolic blood pressure as marker of hypertension rats compared to sham group. The administration of MESA significantly decreased systolic blood pressure, but can not reach the level in sham group. This finding indicated there is no dose dependent effect maybe due to the ability of kidney restoration. Previous study showed that MESA reduce necrosis of renal proximal tubulus achieved at dose 50 and 100 mg/kg BW. At higher dose, there is no significantly compared with DOCA-salt treatment group. In other word, at 200 mg/kgBW may induces toxic effect on kidney.[22]

Oxidative stress in DOCA-salt-treated animals has been studied with a variety of stress markers. Subunits of the NADPH-oxidase were found to be markedly expressed, the oxidative stress-scavenging protein heme oxygenase-1 is up-regulated and also the urinary oxidative stress marker 8-isoprostane is often increased.[23,24] Besides, increased amounts of the oxidative base modification 8-oxodG in the DOCAsalt-group. 8-oxodG is now widely used as a marker of hypertension in urine.[25] In this study, the increase in blood MDA indicates an increase in oxidative stress in DOCA-salt hypertensive rats. Depending on the levels of reactive oxygen compounds, various transcription factors sensitive to change in redox status will be activated and will coordinate intracellular biological response. Modest oxidative stress will induce Nrf2, a transcription factor implicated in the transactivation of genes that encode antioxidant enzymatic activity.[26] The

level of superoxide dismutase significantly ($p < 0.05$) decreased in DOCA-salt hypertensive rats. This finding indicate that DOCA-salt hypertensive rats produces more superoxide radical. Oxidative stress has been implicated in the pathogenesis of Ang II–related hypertension.[27,28] Ang II, through AT1R, stimulates NADPH oxidase, induces oxidative stress, and alters endothelial cell function.[29]

The antioxidant compounds of *Scurrula atropurpurea* extract in our study may contains quercetin, quercetin-3-*O*-glucoside, quercitrin (a glycoside rhamnose of quercetin) and kaempferol. Quercetin exerts its antioxidant activity through scavenging reactive oxygen speces.[30,31] Quercitrin also has a free radical scavenging activity.[32] Kaempferol has shown strong inhibitory/scavenging activity on reactive oxygen species generation with numerous hydroxyl groups on their structures.[32] Moreover, it has been found to be a particularly potent blocker of extracellular reactive oxygen species production, and to inhibit the ascorbate-dependent NADH oxidase and superoxide anion production activities.[33] Therefore, the malondialdehyde-lowering effect and modulation of superoxide dismutase by *Scurrula atropurpurea* extract demonstrated in this study might have been associated with flavonol or phenolic compounds.

Conclusions

In conclusion, *Scurulla atropurpurea* able to modulates superoxide dismutase, diminished oxidative stress, and decreased sistolic blood pressure in DOCA-salt hypertensive rats.

Declaration of interest

The author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

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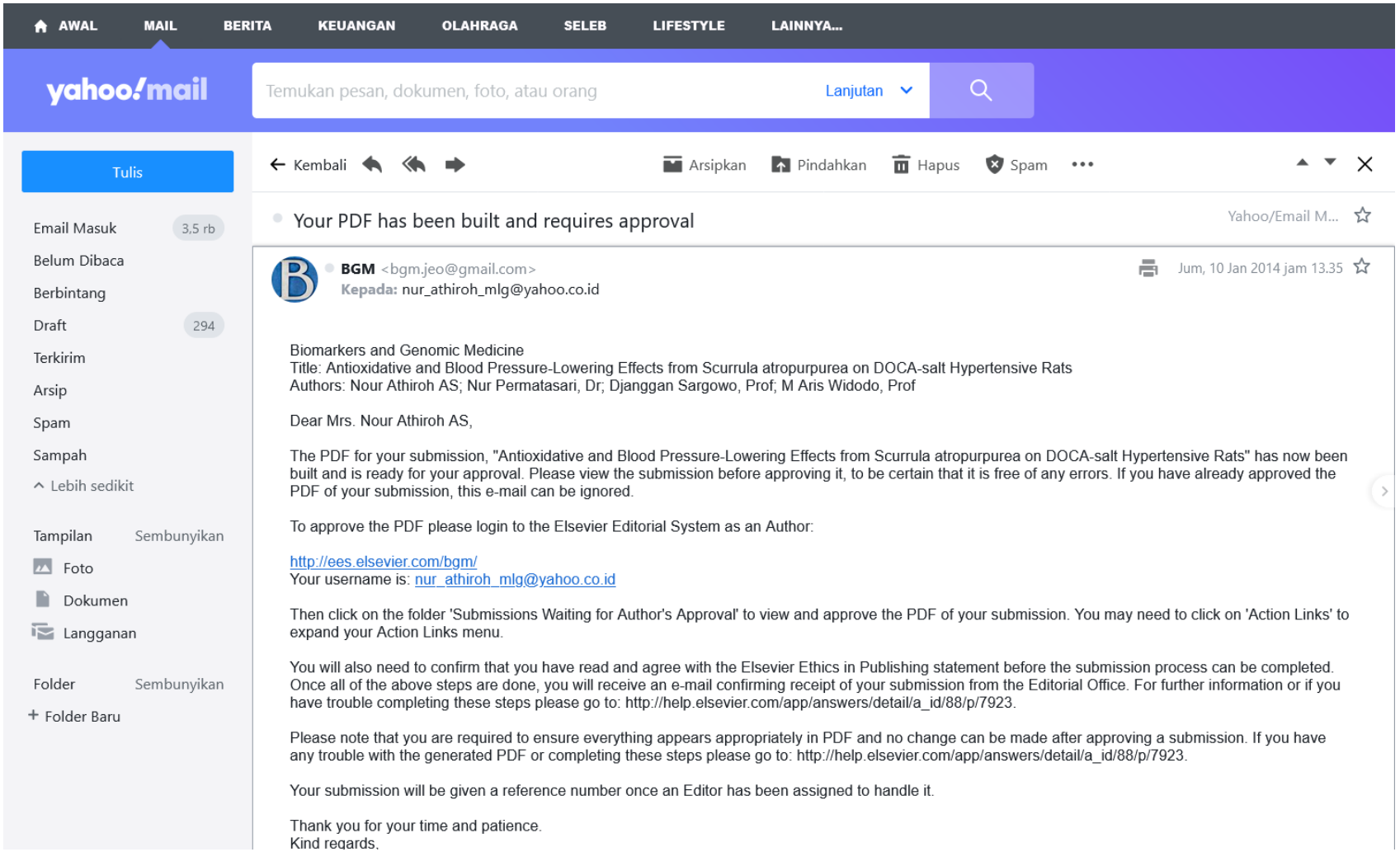
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Title: Antioxidative and Blood Pressure-Lowering Effects from Scurrula atropurpurea on DOCA-salt Hypertensive Rats
Authors: Nour Athiroh AS; Nur Permatasari, Dr; Djanggan Sargowo, Prof; M Aris Widodo, Prof
Dear Mrs. Nour Athiroh AS,
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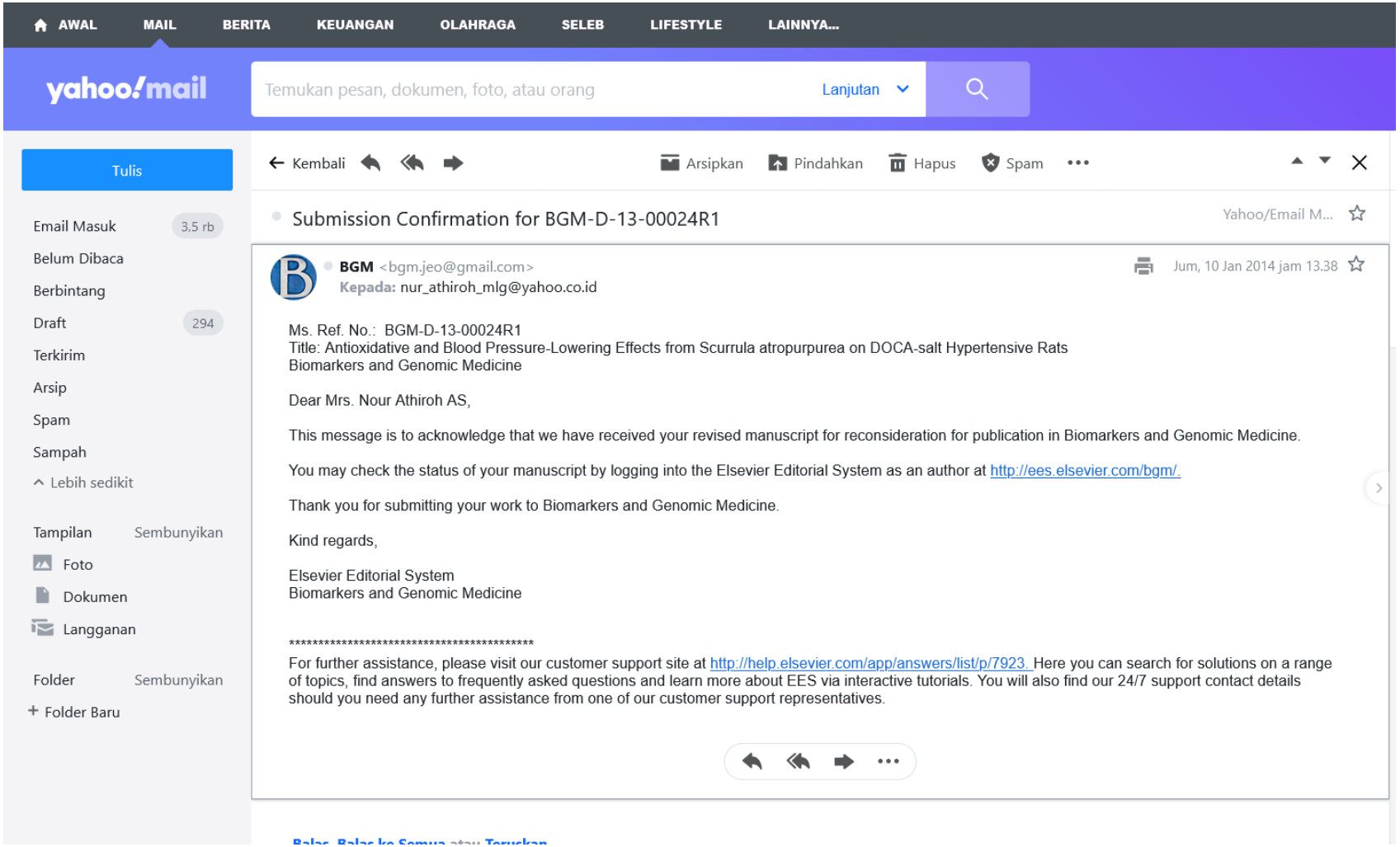
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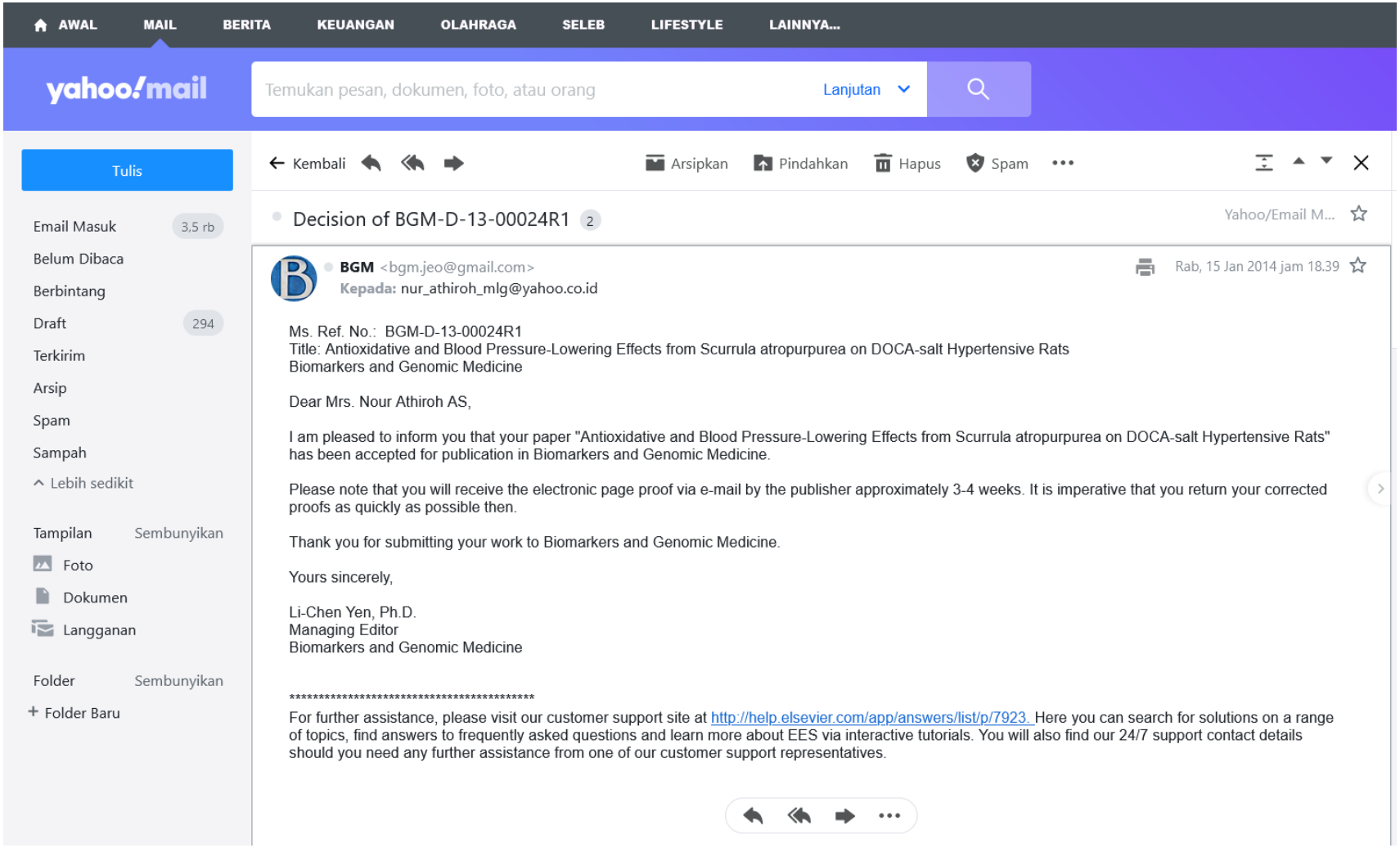
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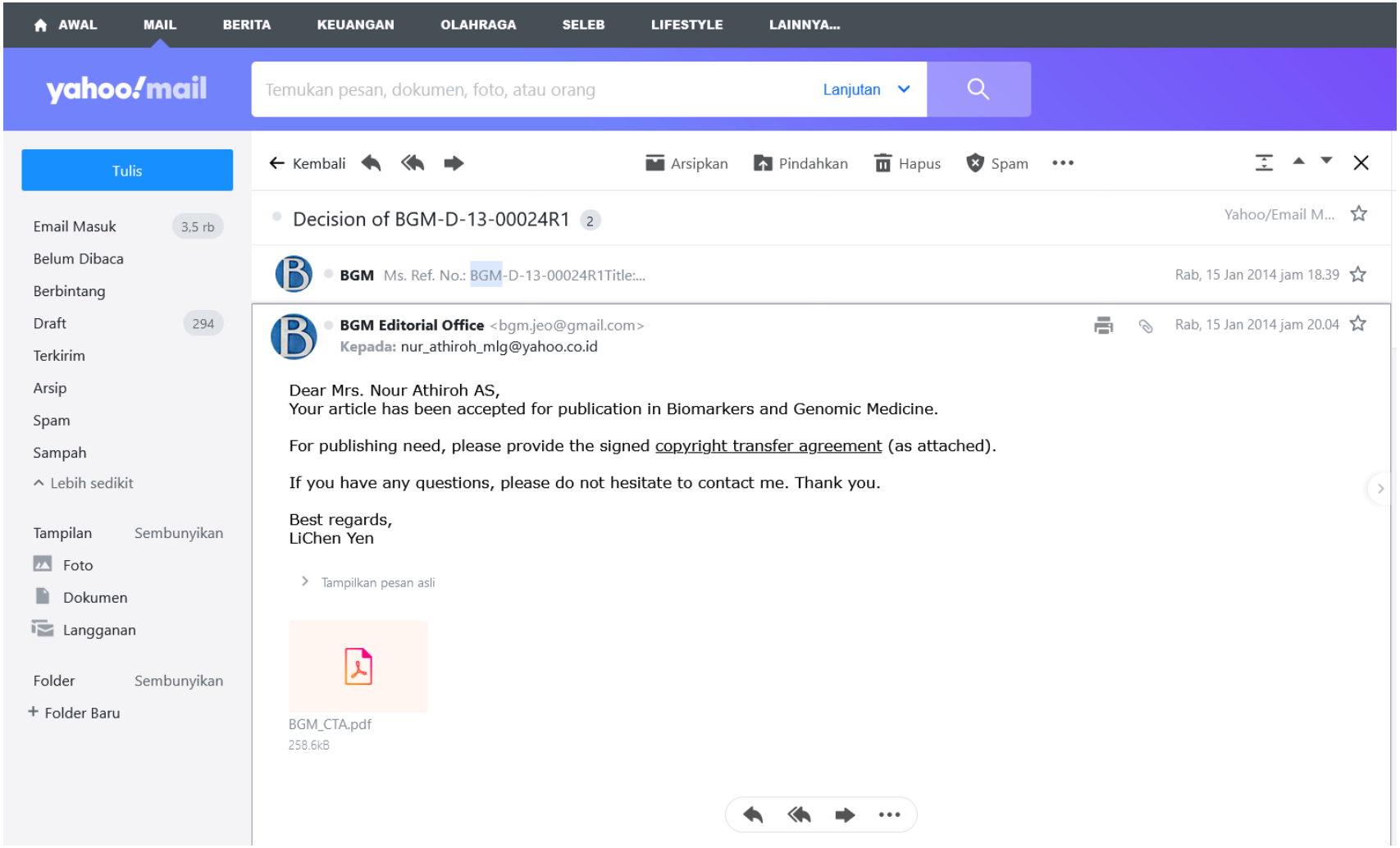
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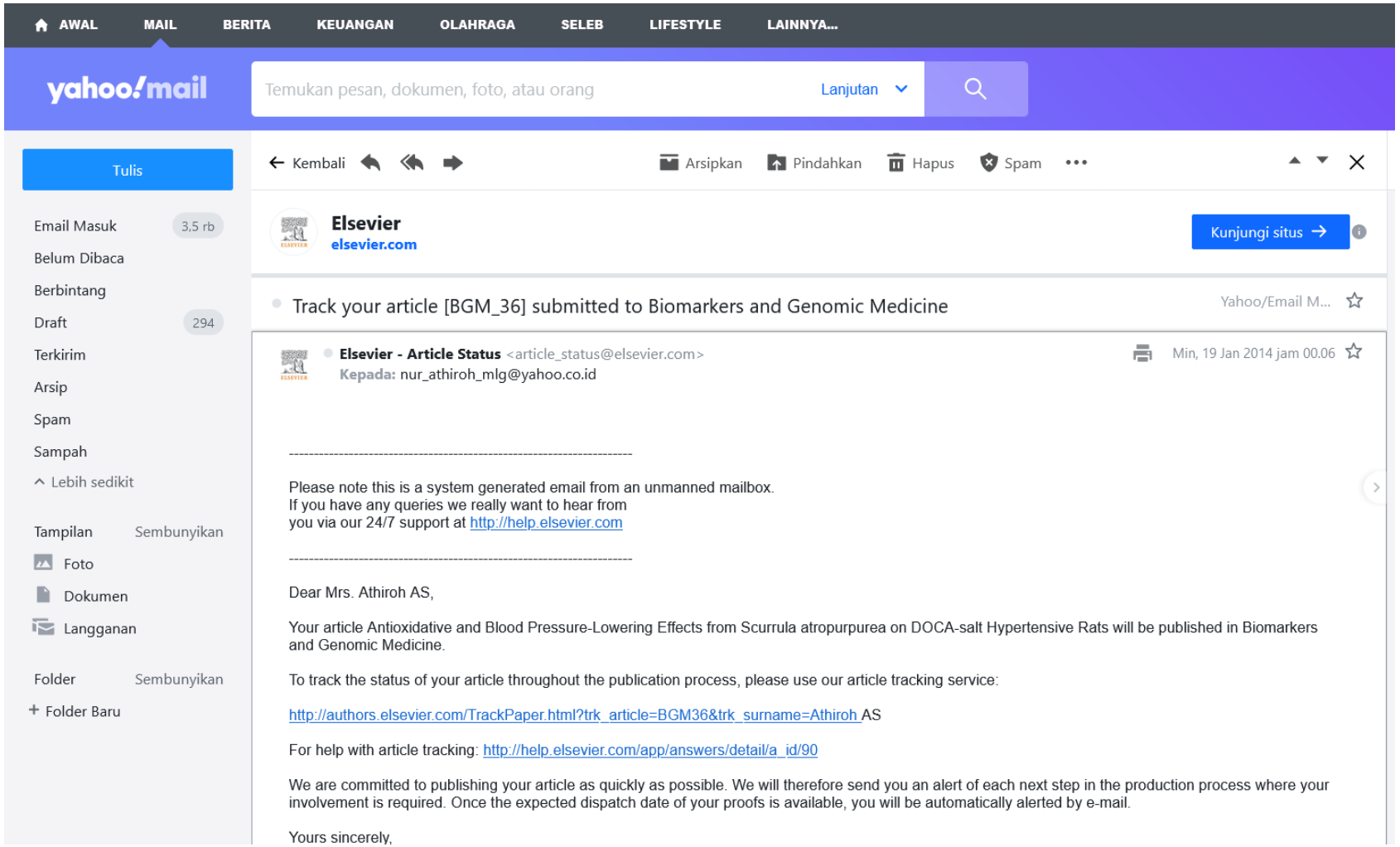
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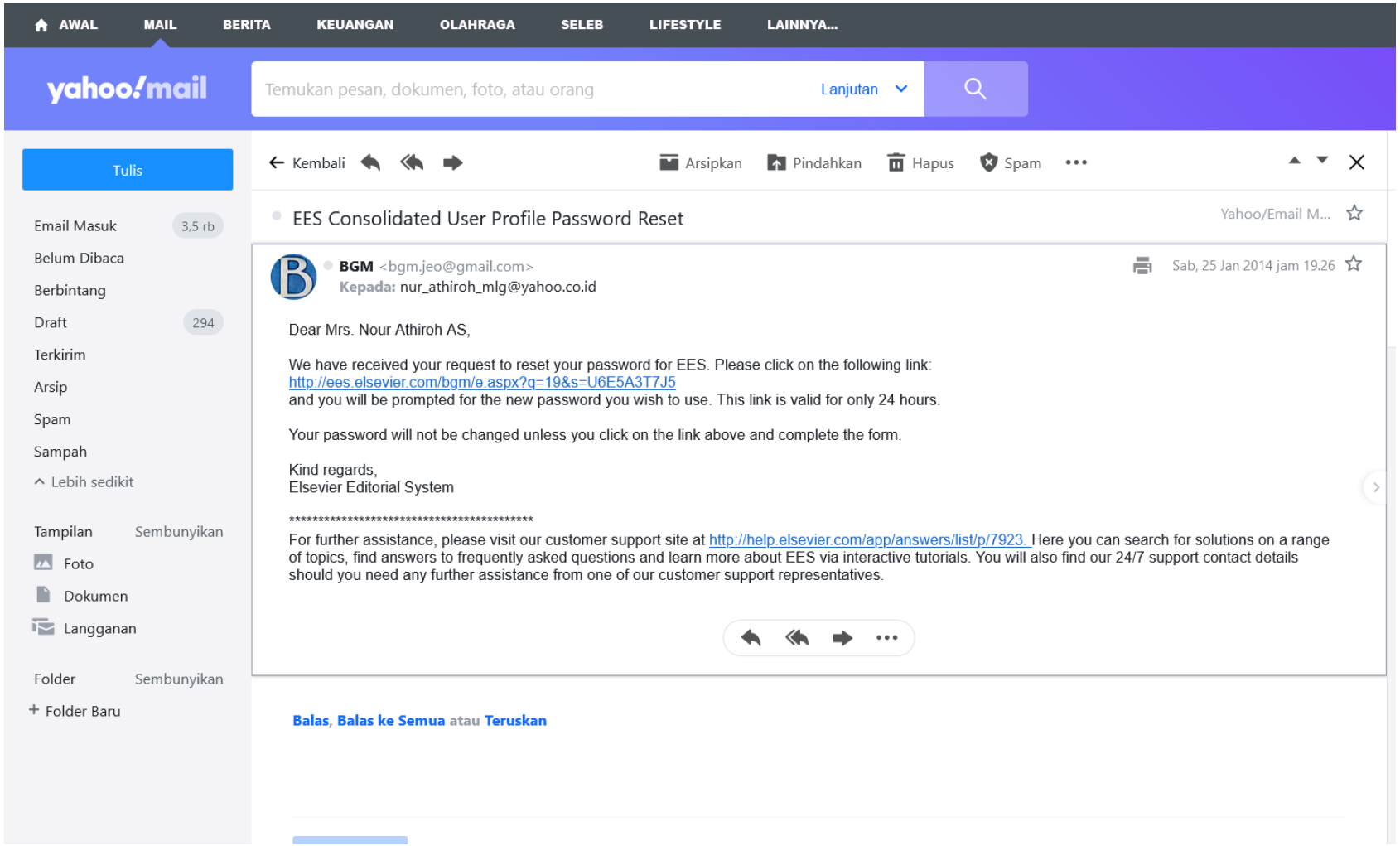
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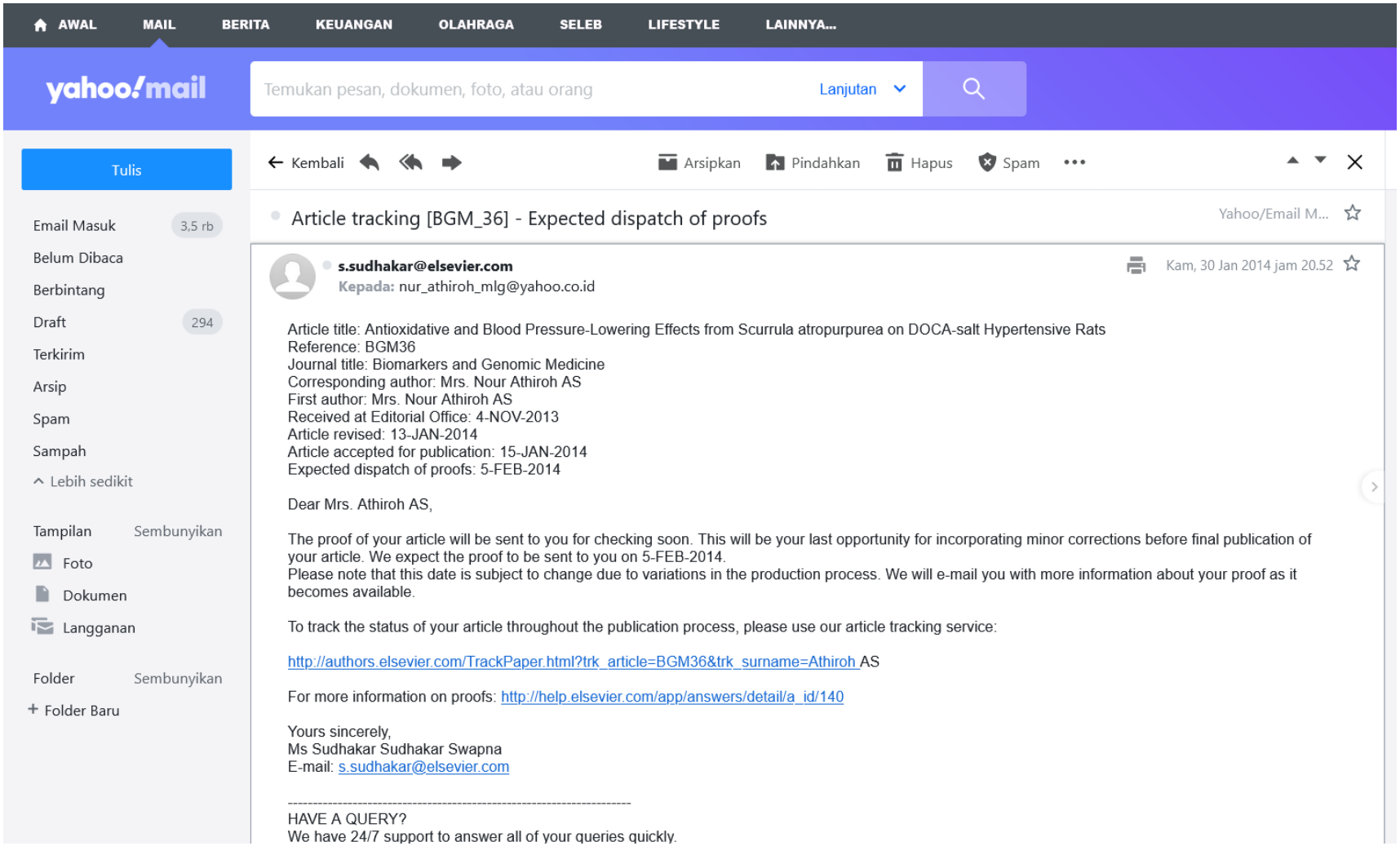
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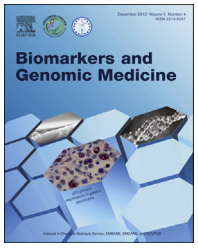

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Antioxidative and blood pressure-lowering effects of *Scurrula atropurpurea* on deoxycorticosterone acetate–salt hypertensive rats

Nour Athiroh [a,*], Nur Permatasari [b], Djanggan Sargowo [c], M. Aris Widodo [b]

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KEYWORDS
antioxidant;
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Abstract This study aimed to investigate whether a methanolic extract of *Scurrula atropurpurea* (BL.) Dans. (MESA) was able to reduced oxidative stress and systolic blood pressure (SBP) in deoxycorticosterone acetate–salt hypertensive rats. Twenty-five male Wistar were divided into the control group and four hypertensive groups that received the MESA at a doses of 50 mg/kg, 100 mg/kg, or 200 mg/kg bodyweight, or received no MESA. SBP was recorded by tail cuff methods. The levels of serum malondialdehyde (MDA) and superoxide dismutase (SOD) were analyzed by colorimetry. SBP was increased significantly in the hypertensive group compared to the sham group ($p < 0.05$). Administration of MESA significantly decreased SBP, but not to reach the level of the sham group. The level of MDA was significantly higher in the hypertensive group compared to the sham group ($p < 0.05$). Administration of $MESA_{200}$ significantly decreased the MDA levels compared to HR groups ($p < 0.05$). The SOD level was significantly decreased in HR compared to the sham group ($p < 0.05$). Administration of $MESA_{50}$ elevated the SOD levels to reach the level in the sham group. The SOD levels in $MESA_{100}$ and $MESA_{200}$ were significantly higher compared to the sham group ($p < 0.05$). In conclusion,

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Scurulla atropurpurea is able to modulate SOD, diminish oxidative stress, and decrease SBP in deoxycorticosterone acetate—salt hypertensive rats.
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Introduction

Hypertension is a clinical common vascular related disease, with high mortality and disability. It is also an independent risk factor for stroke, coronary heart disease, heart failure, renal insufficiency, peripheral vascular diseases, early death, and many other major diseases.[1] Hypertension is the misregulation of a complex interaction between genetic and lifestyle factors affecting the physiological systems regulating blood pressure.[2] There are about 1 billion hypertensive patients in the world, and around 30% of the population die from cardiovascular and cerebrovascular events, in which 62% of acute stroke events and 49% of cardiovascular events are directly caused by hypertension.[3,4]

Consistent evidence reveals the involvement of reactive oxygen species (ROS) and oxidative stress in hypertension and its complications. Hypertension is associated with an increased production of superoxide radicals that have a negative effect on endothelial function. These effects are based on the reaction between superoxide and nitric oxide (NO) to decrease NO bioavailability. Peroxynitrites as the products of this reaction, also have detrimental effects on endothelial cells.[5–7] Hydroxyl radicals as product decomposition of hydroperoxynitrites may trigger lipid peroxidation, as measured by increased malondialdehyde (MDA) levels. Superoxide dismutase (SOD) plays an important role in scavenging superoxide anions that are formed during the early stages of oxidative stress, and in preventing aging.[8] SOD catalyzes the conversion of superoxide to hydrogen peroxide plus dioxygen. SOD can be classified into three groups, Cu/Zn SOD, Mn SOD, and Fe SOD, by the metals they contain at their active sites. Cu/Zn SOD is usually found in the cytoplasm of eukaryotic cells and Mn SOD in mitochondria, whereas prokaryotic cells contain Fe SOD and Mn SOD.[9]

For the decrease of hypertension and its complications, many drugs of herbal origin have been produced, including tetramethylpyrazine from *Jatropha podagrica*, tetrandrine from *Stephenia tetrandra*, digitoxin from *Digitalis purpurea*, reserpine from *Rauwolfia serpentina*, and aspirin from *Salix alba*.[2,10,11] *Scurrula atropurpurea* (BL.) Dans. is a parasite of tea plants. In Indonesia, especially on the island of Java, the stems and leaves of this vegetation have been traditionally used for the treatment of cancers.[12] This study aimed to investigate whether methanolic extract of *Scurrula atropurpurea* (BL.) Dans. (MESA) is able to diminish oxidative stress in hypertensive rats.

Material and methods

Preparation of tea parasite crude extract

Scurrula atropurpurea was determined biologically at the Indonesian Scientific Institute (LIPI) at Purwodadi, Pasuruan, East Java. MESA was obtained through several steps. The leaves were washed, dried in an oven at 40—60 °C, then ground into a powder. A 100 mg portion of this powder was steeped in methanol in a 1 L Erlenmeyer flask. The mixture was shaken for 30 minutes to distribute the powder homogenously in the methanol. To collect the precipitate, the mixture was left to stand overnight. The upper layer know and supernatant, being a mixture of methanol and the active constituents, was subjected to evaporation. The extract was then labeled and stored in a freezer.[13,14] This extract was administered daily by oral gavage for 6 weeks.

Animals

Twenty-five male Wistar rats, aged 3—5 months, and weighing 250—300 g were involved in this study. The rats were divided into five groups ($n = 5$ each): a control group, a group of non-MESA hypertensive rats, and three groups of hypertensive rats receiving MESA at dosages of 50 mg/kg, 100 mg/kg, or 200 mg/kg body weight. Hypertensive rats were generated by injecting subcutaneously with deoxycorticosterone acetate (DOCA; Sigma Aldrich, Pte Ltd., Singapore) at a dosage of 10 mg/kg body weight, twice 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.[15]

Blood pressure measurement

Systolic blood pressure (SBP) was recorded at the end of the study by tail cuff methods (IITC, Non-Invasive Blood Pressure Instrument) according to a previous study.[16]

Tissue sampling

At the end of the treatment, the animals in all groups were anesthetized; their blood was drawn by cardiac puncture and heparinized. Blood samples were centrifuged at 4000 × *g* (4 minutes, 4 °C) to obtain the plasma. All samples were stored at −80 °C until analysis.

Lipid peroxidation analysis

Plasma levels of lipid peroxide were determined as thiobarbituric acid (TBA) reactive substance according to the method of Ohkawa et al,[17] based on the reaction of lipid peroxides with TBA at 95 °C. In the TBA test reaction, lipid peroxides and TBA react to form a pink pigment with an absorption maximum at 532 nm. The reaction was performed at pH 2—3 at 95 °C for 15 minutes. The sample was mixed with 2.5 volumes of 10% (w/v) trichloroacetic acid to precipitate the protein. The precipitate was pelleted by centrifugation and an aliquot of supernatant was reacted

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with 0.67% TBA in a boiling water-bath for 15 minutes. After cooling, the absorbance was read at 532 nm. Arbitrary values obtained were compared with a series of standard solutions (1,1,3,3 tetramethoxypropane). Results are expressed as μM.

SOD analysis

SOD was assayed by measuring the inhibition of the formation of blue-colored formazan at 560 nm according to the technique of Kakkar et al.[18] The inhibition by SOD of reduction of nitro blue tetrazolium to blue-colored chromogen in the presence of phenazine methosulfate and nicotinamide adenine dinucleotide was measured at 560 nm. One unit of enzyme activity was defined as enzyme concentration required to inhibit the absorbance at 560 nm of chromogen production by 50% in 1 minute under assay conditions, and expressed as specific activity in units of SOD/minute/mg of protein.

Ethics

Animal care and experimental procedures were approved by the Institutional Animal Ethics Committee of University of Brawijaya, Malang, East Java, Indonesia.

Statistical analysis

Data are presented as mean ± standard deviation and the differences between groups were analyzed using one-way analysis of variance (ANOVA) with SPSS version 15.0 (SPSS Inc. Chicago, IL, USA). A *post-hoc* test was used if the ANOVA was significant. Probability values of $p < 0.05$ were considered statistically significant.

Results

Effect of MESA on SBP

The administration of DOCA–salt affected the SBP levels, as shown in Fig. 1. There was significantly ($p < 0.05$) increased SBP in hypertensive rats compared to the sham group. Compared to the hypertensive group, the administration of MESA significantly decreased SBP, but not to the level of the sham group.

Effect of MESA on MDA level

The administration of DOCA–salt affected the MDA levels, as shown in Fig. 2. There were significantly ($p < 0.05$) increased MDA levels in hypertensive rats compared to the sham group. The administration of $MESA_{200}$ significantly ($p < 0.05$) decreased the MDA levels compared to hypertensive groups.

Effect of MESA on SOD level

The administration of DOCA–salt affected the serum SOD levels, as shown in Fig. 3. The serum SOD levels were significantly ($p < 0.05$) decreased in hypertensive rats compared to the sham group. The administration of $MESA_{50}$ elevated the SOD levels to reach the level observed in the sham group. The SOD levels in $MESA_{100}$ and $MESA_{200}$ were significantly higher ($p < 0.05$) compared to the sham group.

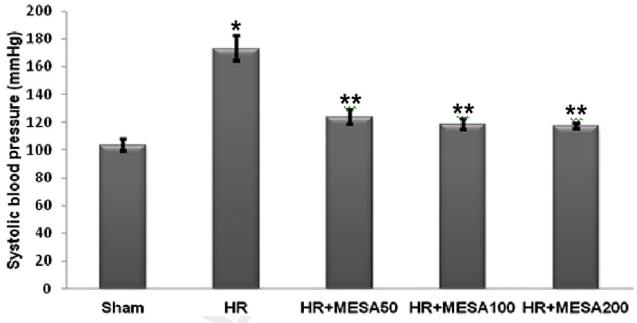

Figure 1 Systolic blood pressure in deoxycorticosterone acetate–salt hypertensive rats (HR) with or without the administration of methanolic *Scurrula atropurpurea* extract (MESA) compared to the sham control group. There is significantly ($p < 0.05$) increased systolic blood pressure in HR compared to the sham group. Compared to its hypertensive group, the administration of MESA significantly ($p < 0.05$) decreased systolic blood pressure, but is not able to reach the level in the sham group. * $p < 0.05$ in comparison with sham group. ** $p < 0.05$ in comparison with deoxycorticosterone acetate–salt hypertensive group.

Discussion

Some species of Loranthaceae from China have been used as medicinal materials for the treatment of hypertension.[17,19] Various compounds have been found in Loranthaceaeous plants and some of them have been identified with hypotensive properties.[12,20] In addition, *Taxillus theifer* (Hayata) H. S. Kiu (*Scurrula ritozanensis*), a Loranthaceaeous plant endemic to Taiwan,[21] has been used

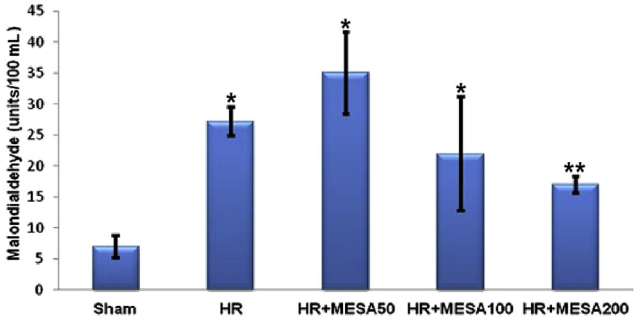

Figure 2 Malondialdehyde in deoxycorticosterone acetate–salt hypertensive rats (HR) with or without the administration of methanolic *Scurrula atropurpurea* extract (MESA) compared to the sham control group. There are significantly ($p < 0.05$) increased MDA levels in HR compared to the sham group. The administration of $MESA_{200}$ significantly ($p < 0.05$) decreased the malondialdehyde levels compared to HR groups. * $p < 0.05$ in comparison with sham group. ** $p < 0.05$ in comparison with deoxycorticosterone acetate–salt hypertensive group.

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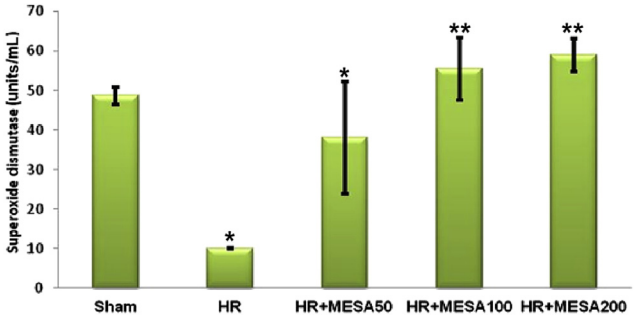

Figure 3 The level of serum superoxide dismutase in deoxycorticosterone acetate—salt hypertensive rats (HR) with or without the administration of methanolic *Scurrula atropurpurea* extract (MESA) compared to the sham control group. The serum SOD level are significantly ($p < 0.05$) decreased in HR compared to the sham group. The administration of $MESA_{50}$ elevated the SOD levels to reach the level in the sham group. The SOD levels in $MESA_{100}$ and $MESA_{200}$ were higher significantly ($p < 0.05$) compared to HR group. * $p < 0.05$ in comparison with sham group. ** $p < 0.05$ in comparison with deoxycorticosterone acetate—salt hypertensive group.

as an antihypertensive agent in Formosan folk medicine. The DOCA—salt treatment induced systemic arterial hypertension, proteinuria, kidney hypertrophy, and impaired kidney function, as reported for this model.[22] This study revealed that DOCA—salt treatment significantly ($p < 0.05$) increased SBP as a marker of hypertensive rats compared to the sham group. The administration of MESA significantly decreased SBP, but this did not reach the level of the sham group. This finding indicates there is no dose dependent effect possibly due to the ability of kidney restoration. A previous study showed that MESA reduces necrosis of the renal proximal tubules achieved at doses of 50 mg/kg and 100 mg/kg body weight. At a higher dose, there is no significant difference compared with the DOCA—salt treatment group, that is, MESA at 200 mg/kg body weight may induce a toxic effect on kidney.[23]

Oxidative stress in DOCA—salt-treated animals has been studied with a variety of stress markers. Subunits of the nicotinamide adenine dinucleotide phosphate oxidase were found to be markedly expressed, the oxidative stress-scavenging protein heme oxygenase-1 is upregulated and also the urinary oxidative stress marker 8-isoprostane is often increased.[24,25] Additionally, there were increased amounts of the oxidative base modification 7,8-dihydro-8-oxo-guanine (which is now widely used as a marker of hypertension in urine) in the DOCA—salt group.[26] In this study, the increase in blood MDA indicates an increase in oxidative stress in DOCA—salt hypertensive rats. Depending on the levels of reactive oxygen compounds, various transcription factors sensitive to change in redox status will be activated and will coordinate intracellular biological response. Modest oxidative stress will induce Nrf2, a transcription factor implicated in the transactivation of genes that encode antioxidant enzymatic activity.[27] The level of SOD significantly ($p < 0.05$) decreased in DOCA—salt hypertensive rats, indicating that they produce more superoxide radical. Oxidative stress has been implicated in the pathogenesis of Ang II—related hypertension.[28,29] Ang II, through AT1R, stimulates nicotinamide adenine dinucleotide phosphate oxidase, induces oxidative stress, and alters endothelial cell function.[30]

The antioxidant compounds of *Scurrula atropurpurea* extract in our study may include quercetin, quercetin-3-*O*-glucoside, quercitrin (a glycoside rhamnose of quercetin), and kaempferol. Quercetin exerts its antioxidant activity through scavenging reactive oxygen speces.[31,32] Quercitrin also has a free radical scavenging activity.[33] Kaempferol has shown a strong inhibitory/scavenging activity on ROS generation with numerous hydroxyl groups on their structures.[33] Moreover, it has been found to be a particularly potent blocker of extracellular ROS production, and to inhibit the ascorbate-dependent NADH oxidase and superoxide anion production activities.[34] Therefore, the MDA-lowering effect and modulation of SOD by *Scurrula atropurpurea* extract demonstrated in this study might have been associated with flavonol or phenolic compounds.

In conclusion, *Scurrula atropurpurea* is able to modulate SOD, diminish oxidative stress, and decrease SBP in DOCA—salt hypertensive rats.

Conflicts of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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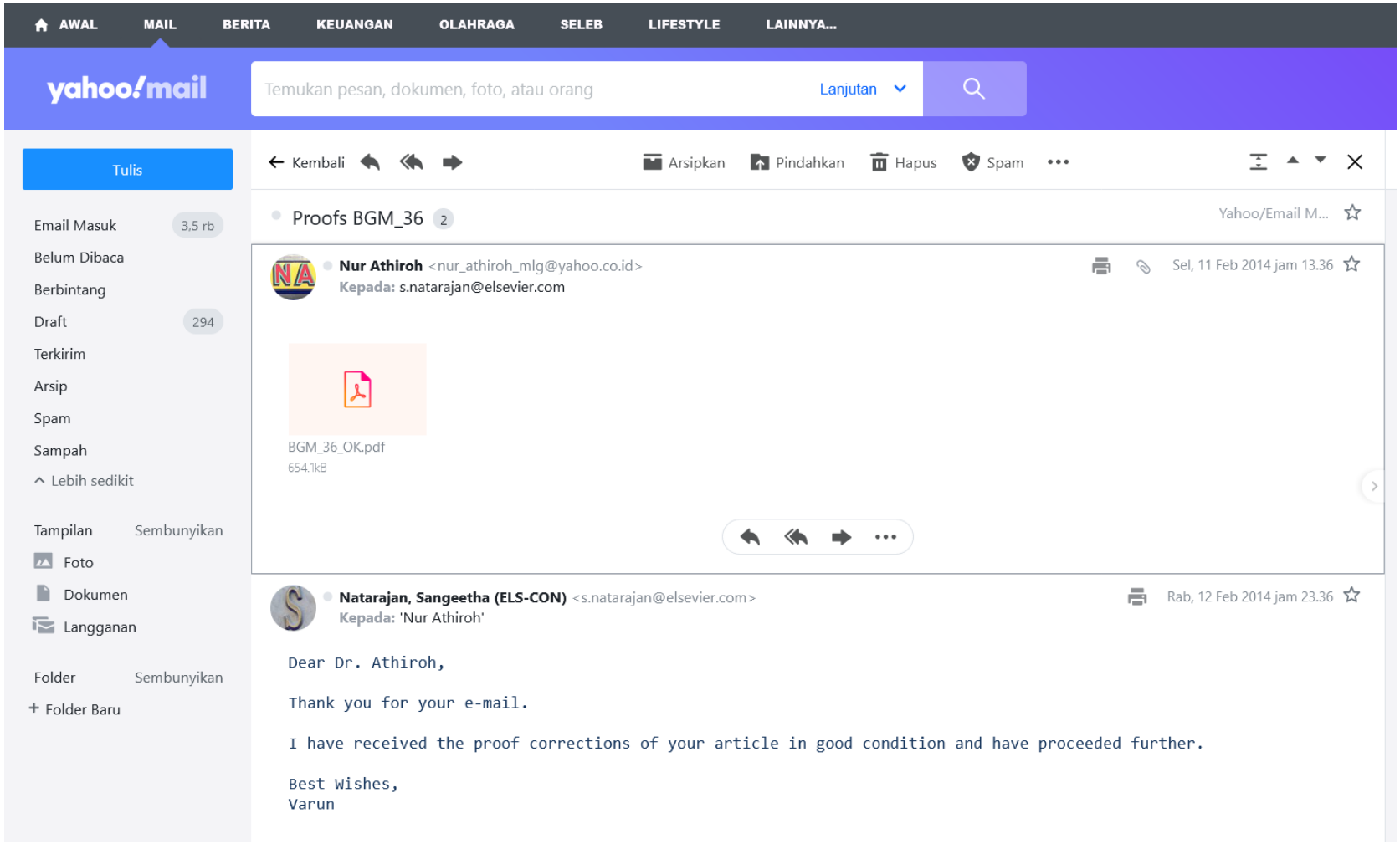
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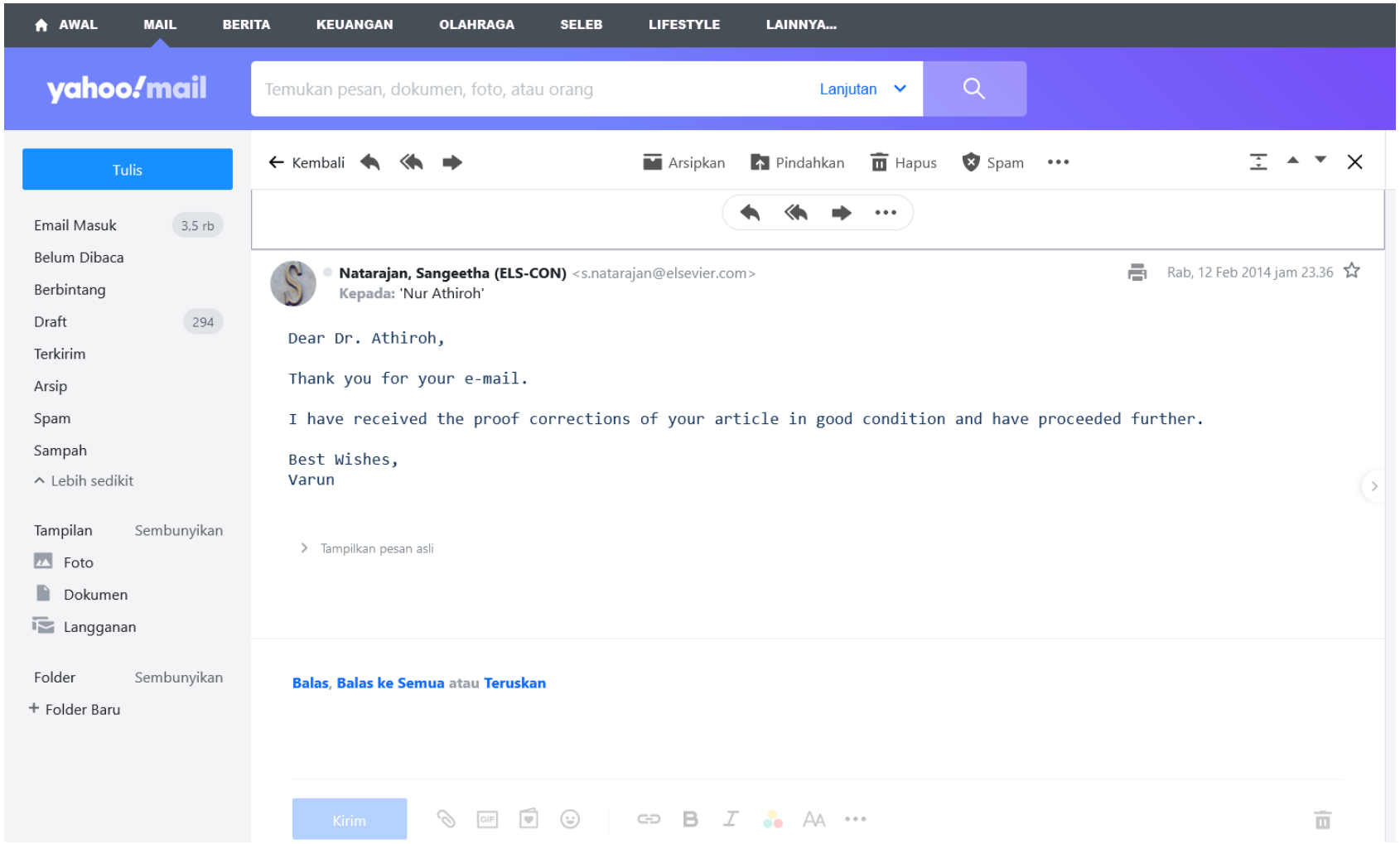
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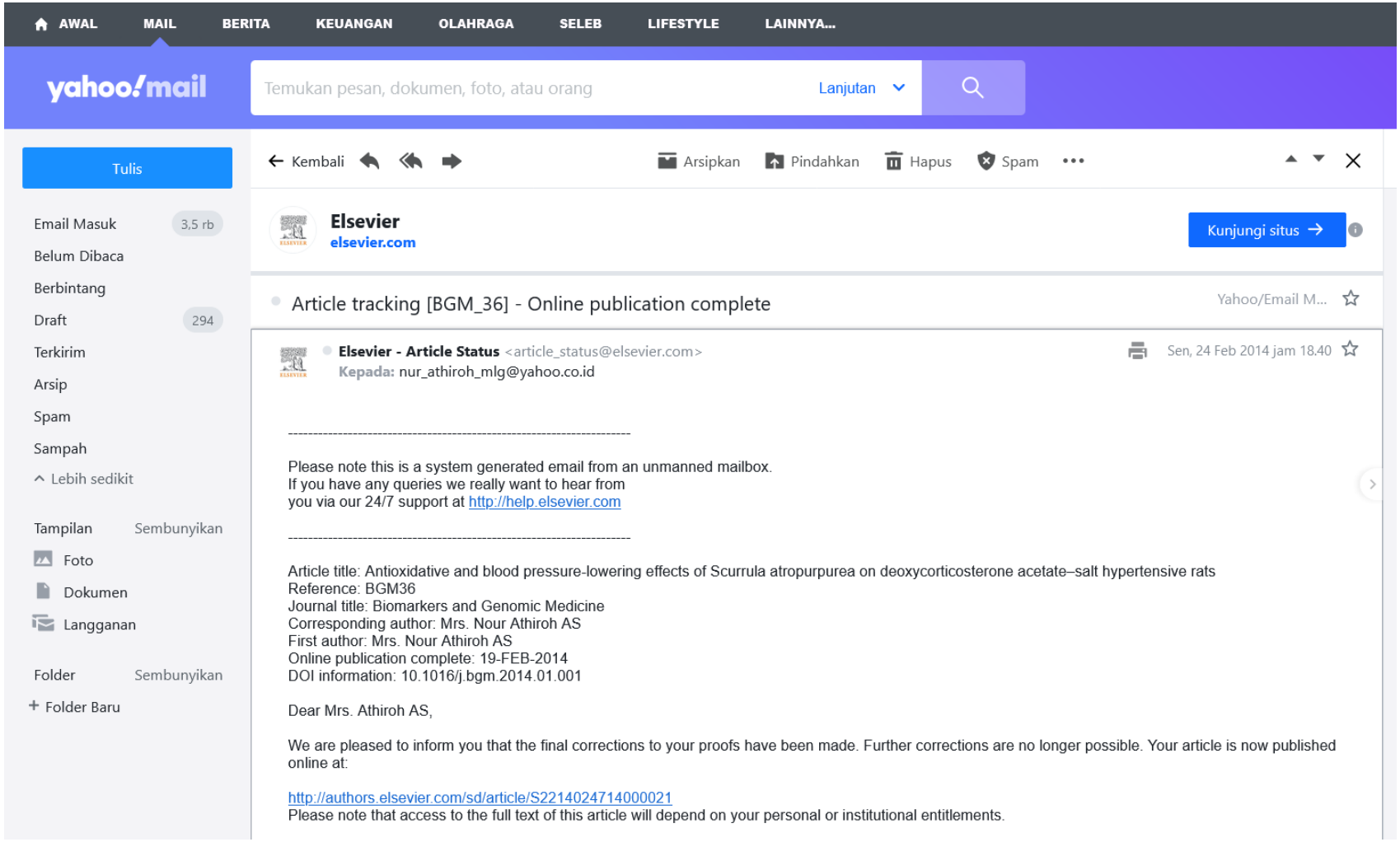
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ABSTRACT

This study purposed to examine whether methanolic extract of *Scurrula atropurpurea* (BL.) Dans. able to modulate total plasma nitrate/nitrite levels, decrease endothelial damage, and increase endothelial progenitor cells in hypertensive rats. The experimental research groups composed of the control (normotensive), DOCA-salt of hypertension rats group, and and three DOCA-salt hypertensive groups receiving methanolic extract of *Scurulla atropurpurea* (MESA) at a dosage of 50; 100; and 200 mg/KgBW respectively. The total plasma nitrate/nitrite levels was analyzed by colorimetric method. The number of circulating endothelial cells (CECs-CD146) and endothelial progenitor cells (EPCs-CD133) were analyzed using flow cytometry. ANOVA and a post hoc test were applied to find the difference of total plasma nitrate/nitrite levels, CECs-CD146, and EPCs-CD133 numbers between groups. The total plasma nitrate/nitrite levels and EPCs-CD133 number were significantly ($P < 0.05$) decreased in DOCA-salt of hypertension rats group compared to control group. The administration of $MESA_{100}$ and ESA_{200} elevated the total plasma nitrate/nitrite levels but can not reach the level in control group. The administration of $MESA_{100}$ and $MESA_{200}$ significantly elevated the EPC-CD133 number ($P < 0.05$). Otherwise the administration of DOCA-salt affected the CEC-CD146 number. There were significantly ($P < 0.05$) increased CECs-CD146 number in hypertensive rats compared to control group. The administration of MESA significantly ($P < 0.05$) decreased the CECs-CD146 number compared to hypertensive groups. Methanolic extract of *Scurrula atropurpurea* able to modulate total plasma nitrate/nitrite levels, preserves endothelial damage via increasing endothelial progenitor cells.

Key words: endothelial damage; endothelial progenitor cells; hypertensive; mistletoe; **total plasma nitrate/nitrite levels**.

RUNNING TITLE:

***Scurrula atropurpurea* (BL.) Dans. on total plasma nitrate/nitrite levels, Endothelial Damage, and Endothelial Progenitor Cells of Hypertensive Rats**

INTRODUCTION

Hypertension is the most frequently encountered chronic disease and affects around one milliard individuals [1]. Hypertension is associated with increased of endothelial dysfunction which has been demonstrated in vessels from hypertensive humans and in many experimental models of hypertension [2, 3]. 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 [3]. There are two types of experimental hypertension exist in relation to NO. In a normal situation, vasoconstrictor influences are opposed by NO production. In one type of hypertension, an augmented production of vasoconstrictor factors could lead to an increased synthesis of NO to act as a protective mechanism. In another form of hypertension, with a decrease in NO production, the vasoconstrictor activity in the vascular wall would be unopposed, leading to an increase in blood pressure [4].

Circulating endothelial cells (CECs) are mature cells that are not associated with vessel walls, but are detached from the endothelium and circulate within peripheral blood. The number of CECs present in the blood has been found to increase in response to cardiovascular disease, vasculitis, infectious disease, and various cancers [5]. Indeed, the level of CECs has been recognized as a useful biomarker for vascular damage. The association between CECs and hypertension is unclear. Previous studies found higher numbers of CECs in lacunar stroke when compared to atherothrombotic or cardioembolic stroke, but these differences did not reach statistical significance. Lacunar infarcts are noncortical infarcts caused by the occlusion of a single penetrating branch of a large cerebral artery. The underlying mechanism is a ruptured microatheroma or lipohyalinosis

caused by arterial hypertension [6]. Beside, CECs also increased in pulmonary hypertension [7].

Endothelial progenitor cells (EPCs) play a significant role in neovascularization of ischemic tissue. 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 showed that there was no difference in the number of circulating EPCs, demonstrated by expression of EPC markers CD34, cKit, and vascular endothelial growth factor, between hypertensive and normotensive rats [10].

Scurrula atropurpurea (BL.) Dans. is a parasitic plant for tea plants. In Indonesia, especially on the island of Java, the stems and leaves of this plant have been empirically used, for the treatment of malignancy [11]. Some species of Loranthaceae from China have been used as medicinal materials for the treatment of hypertension [12]. Various compounds have been found in Loranthaceaeous plants have been identified with hypotensive properties [13]. In addition, (*Scurrula ritozanensis*), a Loranthaceaeous plant endemic to Taiwan [14], has been used as an anti-hypertensive agent in Formosan folk medicine. As far we know, there is no study to evaluate the effects of *Scurrula 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 *Scurrula atropurpurea* (BL.) Dans. able to modulate total plasma nitrate/nitrite levels, decrease endothelial damage, and increase endothelial progenitor cells in hypertensive rats.

MATERIAL AND METHODS

Preparation *Scurrula atropurpurea* 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 an erlenmeyer flask of 1 L capacity. The mixture was shaken for 30 minutes to distribute the powder homogenously in the methanol. Subsequently, the mixture was left to stand overnight until a precipitate. The supernatant, being a mixture of methanol and the active constituents, was subjected to evaporation. The extract was labelled and stored in a freezer [15; Athiroh & sulistywati (univ med); Athiroh, et al, (BGM)]. The methanolic extract of *Scurrula atropurpurea* (MESA) was administered daily by the oral gavage using a catheter, this being continued for 6 weeks. KALAU DI UNIV MED REFERENSI INI (Fard, *et al*., 2011), DI BGM REFERENSINYA Fard *et al*., 2011 ; DAN Athiroh & Sulis, BERARTI DI IJBMS INI REFERENSINYA Fard; Athiroh & Suli; Athiroh et al yang BGM???

Animals

To produce hypertension, it need DOCA obtained from Sigma Aldrich Pte Ltd. Singapore D7000. Rat were given DOCA dissolved in corn oil. Each group was injected subcutaneously at dose 10mg/Kg bw, twice weekly for 43 days, and drinking water is replaced 2% NaCl *ad libitum* (Badyal, *et al*., 2003). The blood pressure and the weights of the rats was then determined [16]. The treatment groups consisted of the control group, the group of non-MESA hypertensive rats, three groups of hypertensive rats receiving MESA at dosages of 50, 100, and 200 mg/kgBW [17]. The rats were assigned randomly into the groups, with each group containing five rats. After 6 weeks of DOCA with or without MESA treatment, the blood pressure of the rats was then determined

Measurement of total plasma nitrate/nitrite levels

Total plasma nitrate/nitrite levels was 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 540nm against the reagent blank in which the serum sample was replaced with de-ionized water. The levels of nitric oxide in the experimental animals and control were determined by extrapolation from absorbance-concentration curve of the sodium nitrate standard solution (10–100 μM) [18].

Measurement of endothelial progenitor cells and circulating endothelial cells

Endothelial progenitor cells and circulating endothelial cells were isolated from peripheral blood according previous studies with modification. Briefly, 10 ml of venous ethylenediaminetetraacetic acid (EDTA) blood was obtained by peripheral veinpuncture, stored at 4^{o}C to 10^{o}C, and processed within 6 hours after collection. Peripheral blood mononuclear cells were isolated by density-gradient centrifugation using Ficoll-Paque Plus (Amersham Pharmacia Biotech, Uppsala, Sweden). Isolated cells were washed twice with PBS and resuspended in 20 0mL of PBS supplemented with 0.5% of bovine serum albumin and 2 mM of EDTA. For measurement endothelial progenitor cells, CD133^{+} cells in peripheral blood were evaluated by immunostaining with PE-conjugated CD133 monoclonal antibody (Biolegend) and detected by flow cytometry (BD FACSCalibur Flow Cytometer) [19]. 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) [20].

Ethics

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

Statistical analysis

All data results are showed as mean ± SD for the experiment group. Data were analyzed using ANOVA was performed with SPSS 15.0 statistical package. Values of $P < 0.05$ were considered statistically significant by ANOVA. Post Hoc test was used if the ANOVA was significant.

RESULTS

Effect of ESA on the total plasma nitrate/nitrite levels

The **total plasma nitrate/nitrite levels** were significantly ($P < 0.05$) decreased in DOCA-salt of hypertension rats group compared to control group. The administration of $MESA_{100}$ and $MESA_{200}$ elevated the **total plasma nitrate/nitrite levels** but can not reach the level in normotensive group, as seen in Figure 1.

Endothelial progenitor cells level were significantly ($P < 0.05$) decreased in DOCA-salt of hypertension rats group compared to normotensive group.

Effect of ESA on the endothelial progenitor cells -CD133 number

The administration of DOCA salt affected the EPC-CD133 number, as shown in Fig. 2. The EPC-CD133 number 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 EPC-CD133 number compared with hypertensive group. The level of the EPC-CD133 number in $MESA_{200}$ is not significantly different than that control group ($P > 0.05$).

Effect of ESA on the circulating endothelial cells-CD146 number

The administration of DOCA salt affected the circulating endothelial cells number, as shown in Fig. 3. 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.

DISCUSSION

There are various models of experimental hypertension. Each model of such diseases show a specific aspect of such diseases in humans, and none of them is a full representation of the diseases [21]. Deoxycorticosterone acetate (DOCA)- salt is an agent commonly used to increase the blood pressure in experimental animals [22]. Previous studies showed that DOCA-salt (20 mg/kg, twice weekly) significantly induced hypertension in comparison with saline group at the end of 4 weeks of treatment [23]. In this study we found that DOCA-salt (10 mg/kg, twice weekly) significantly induced hypertension at the end of 6 weeks of treatment.

Nitric oxide (NO) has several effects on cardiovascular systems including modulation of synaptic signaling and regulation of blood pressure [24]. In this study, we found that total plasma nitrate/nitrite levels in hypertensive animals was lower than normotensive group. Hypertension is associated with cardiovascular abnormalities including endothelial dysfunction and it seems that reduced serum NO concentration in hypertensive group may be the result of endothelial dysfunction [25, 26]. Previous studies also supported the lower NO bioavailability in hypertensive subjects [27-29]. Higher production of reactive oxygen species, lower endothelial NO synthase expression, and/or impaired L-arginine uptake are possible mechanisms for reduced NO bioavailability in hypertensive subjects [25, 26]. The administration of $MESA_{100}$ and $MESA_{200}$ elevated the total plasma nitrate/nitrite levels but can not 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 [28]. Catechin increases eNOS phosphorylation and NO bioavailability by inhibition of NADPH oxidase [29].

Mesoderm-derived adult stem cells, such as cardiac-derived stem cells, mesenchymal stem cells, skeletal myoblasts, hematopoietic stem cells, and endothelial progenitor cells, represent a more suitable cell source for cell therapy intervention [30]. In recent years, it has been proposed that these cells can circulate to the site of injury, where they contribute to endothelial regeneration. Endothelial cells appear to regulate the trafficking and release of EPCs from bone marrow [31]. 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 ($P < 0.05$) 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 EPC-CD133 number than that 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 was decreased in hypertensive rats [32]. The bioactive compound from *Scurrula atropurpurea* is able to diminished endothelial damage via increasing endothelial progenitor cells. Besides, total plasma nitrate/nitrite levels--increasing effect of *Scurrula atropurpurea* may contributed to endothelial progenitor cells differentiation [33-35].

Conclusions

Methanolic extract of *Scurrula atropurpurea* able to modulate total plasma nitrate/nitrite levels-, preserves endothelial damage via increasing endothelial progenitor cells and decreasing circulating endothelial cells .

Declaration of interest

There is no coflict of interest regarding the publication of this article.

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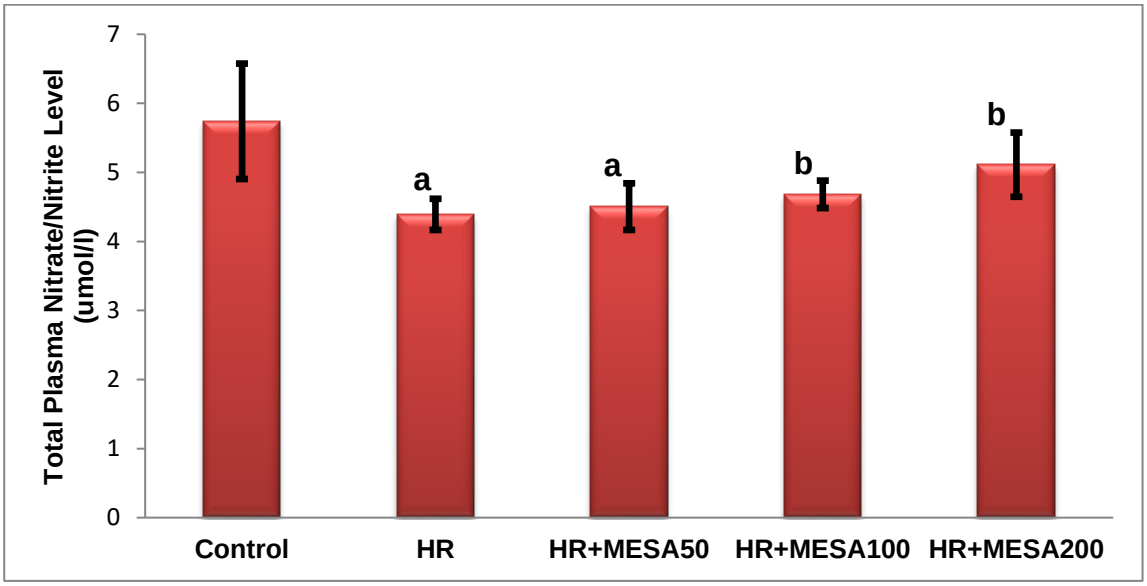


Figure 1. **The level of total plasma nitrate/nitrite in experiment group.** The **total plasma nitrate/nitrite** level were significantly ($P < 0.05$) decreased in DOCA-salt of hypertension rats group compared to control group. The administration of $MESA_{100}$ and $MESA_{200}$ elevated the **total plasma nitrate/nitrite levels**. [a] $P<0.05$ when compared to control group, [b] $P<0.05$ when compared to DOCA-salt of hypertension rats group.

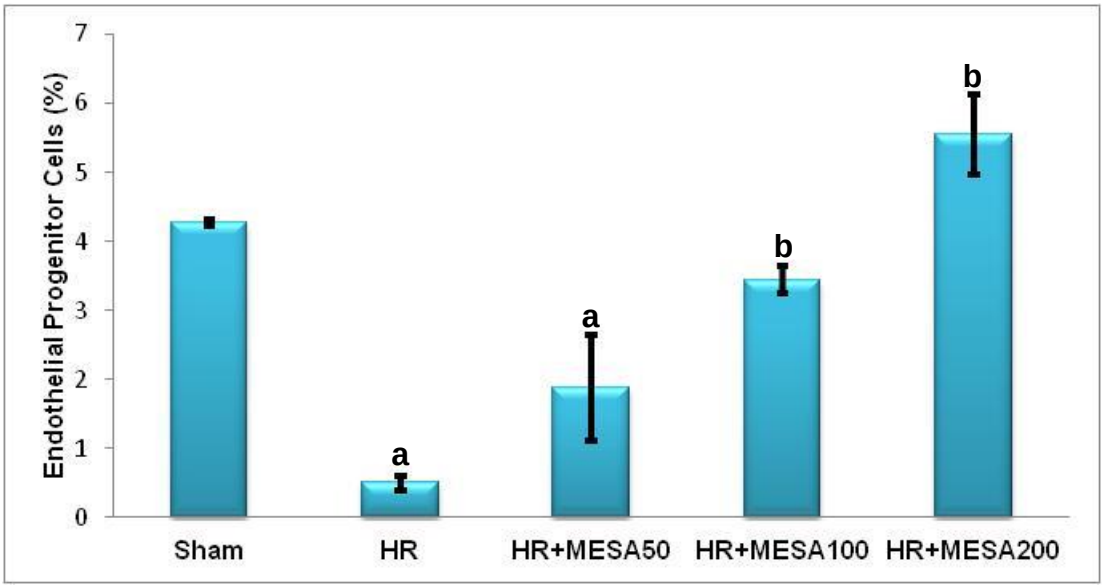


Figure 2. **The number of EPCs-CD133 in DOCA-salt hypertensive and the effects of methanolic *Scurrula atropurpurea* extract**. The number of EPCs-CD133 were significantly ($P < 0.05$) decreased in DOCA-salt of hypertension rats group compared to control group. The administration of $MESA_{100}$ and $MESA_{200}$ elevated the number of EPCs-CD133. [a] $P<0.05$ when compared to control group, [b] $P<0.05$ when compared to DOCA-salt of hypertension rats group.

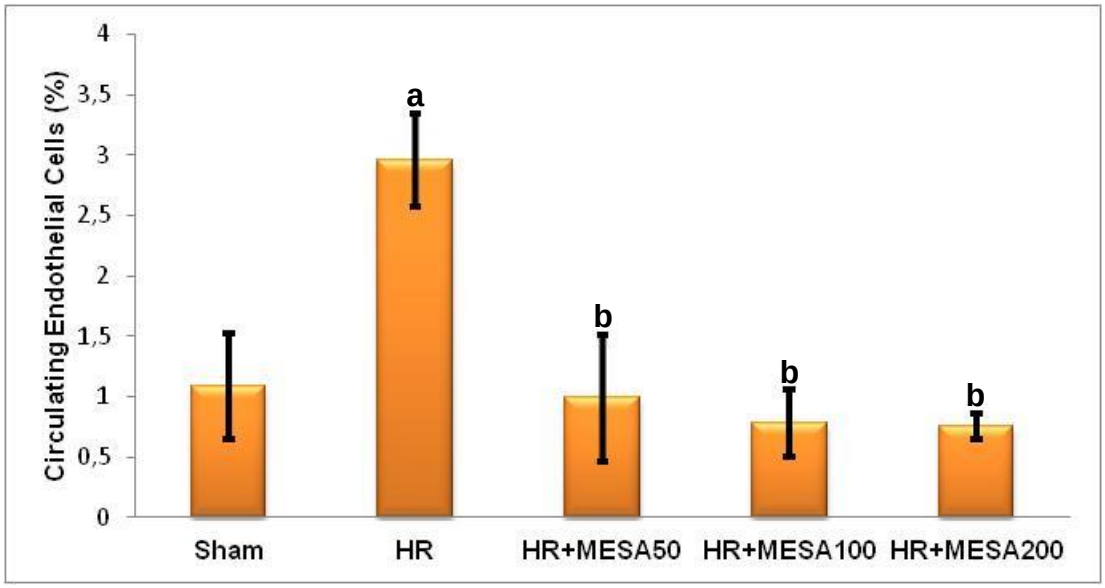
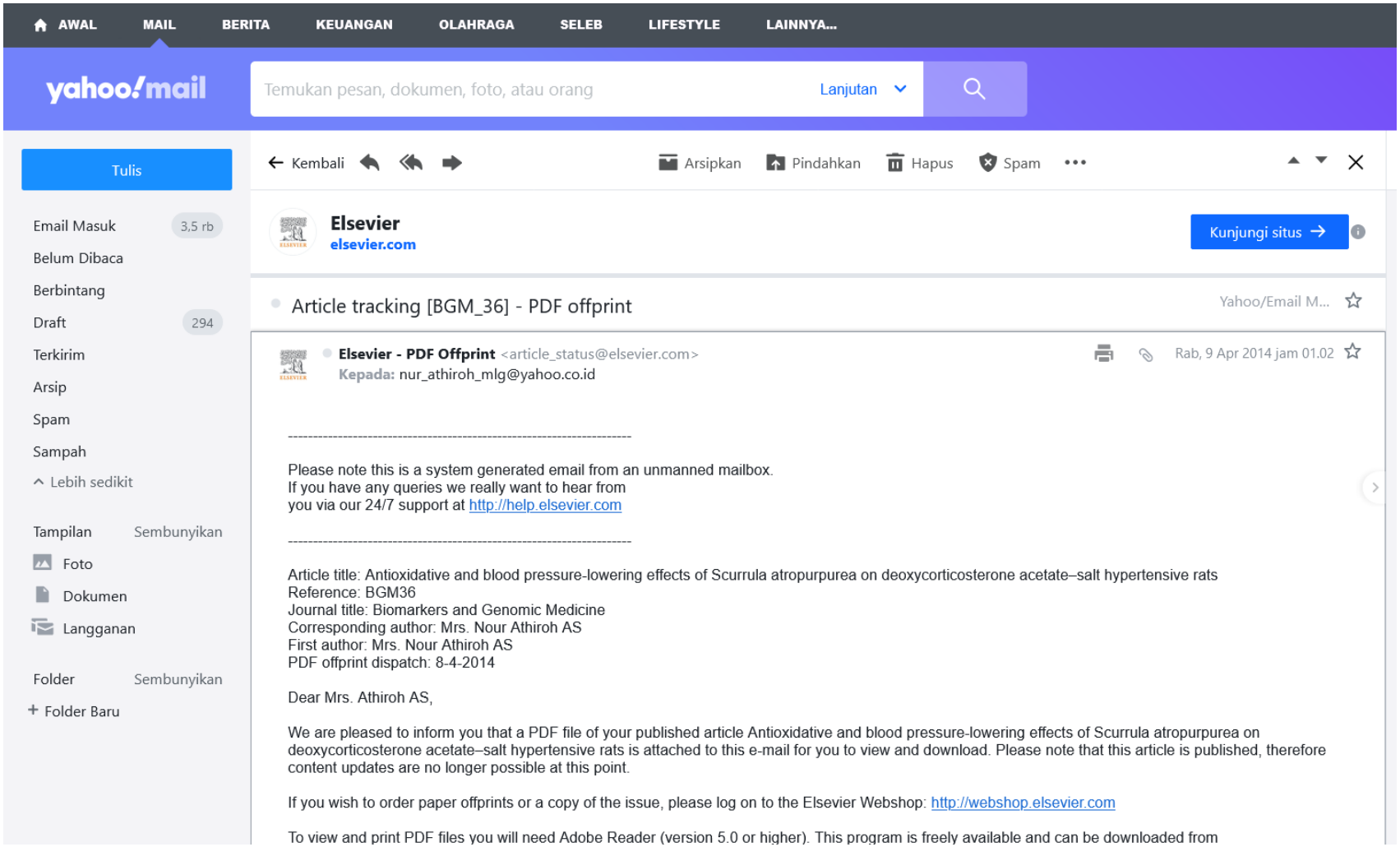


Figure 3. **The number of CECs-CD146 in DOCA-salt hypertensive and the effects of methanolic *Scurrula atropurpurea* extract.**. The number of CEC-CD146 were significantly ($P < 0.05$) increased in DOCA-salt of hypertension rats group compared to control group. The administration of $MESA_{50}$ decreased the number of CECs-CD146. [a] $P<0.05$ when compared to control group, [b] $P<0.05$ when compared to DOCA-salt of hypertension rats group.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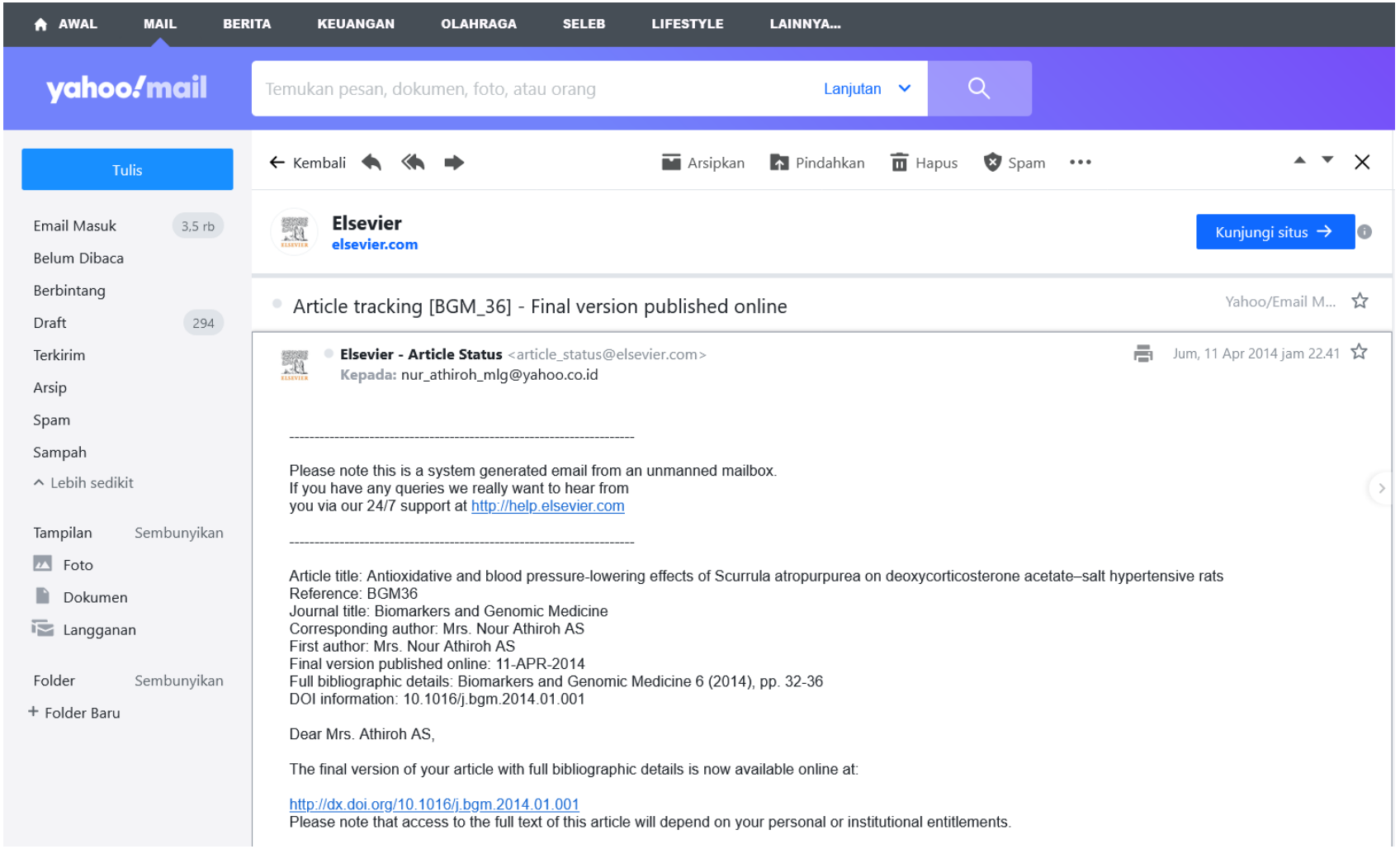
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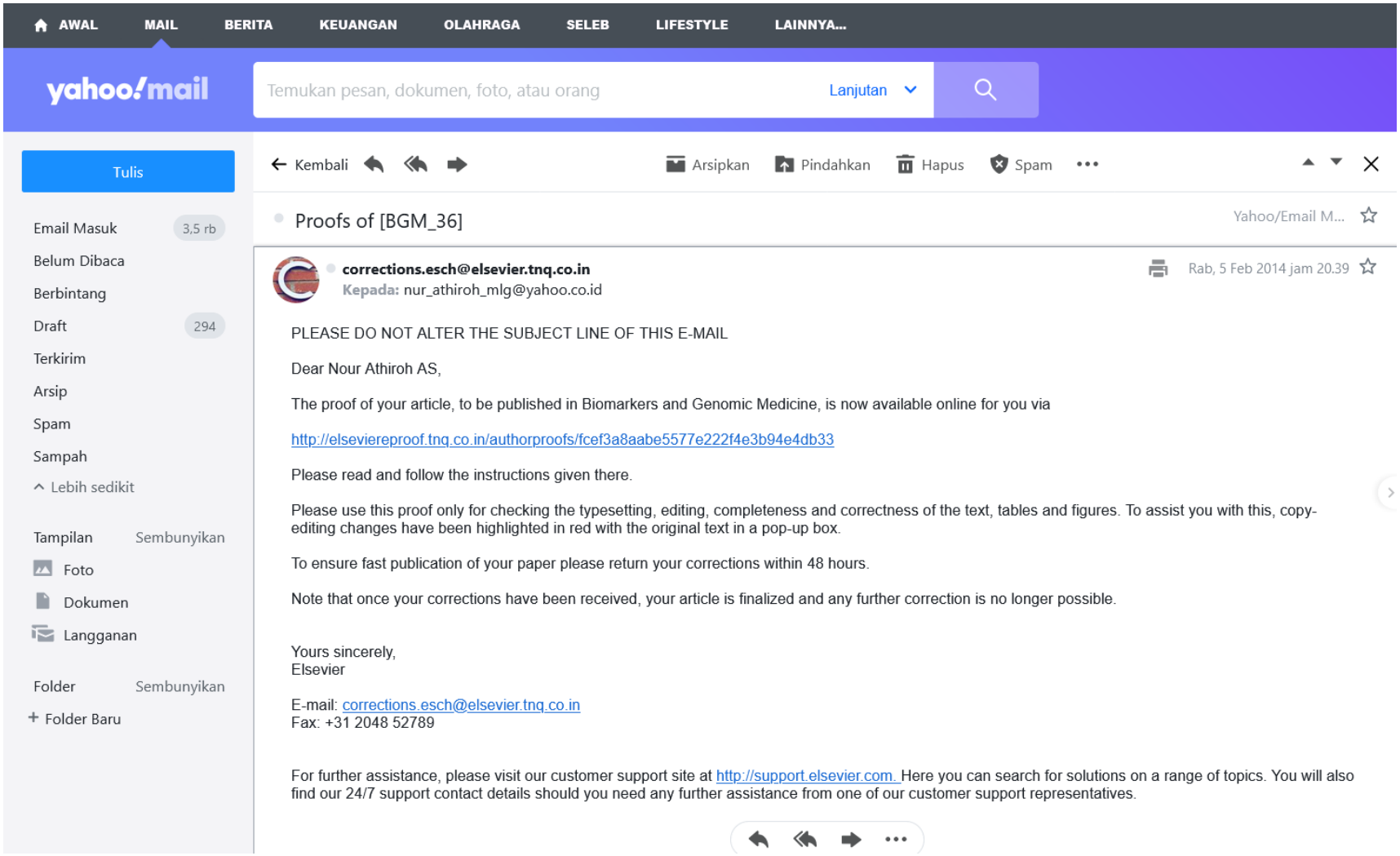
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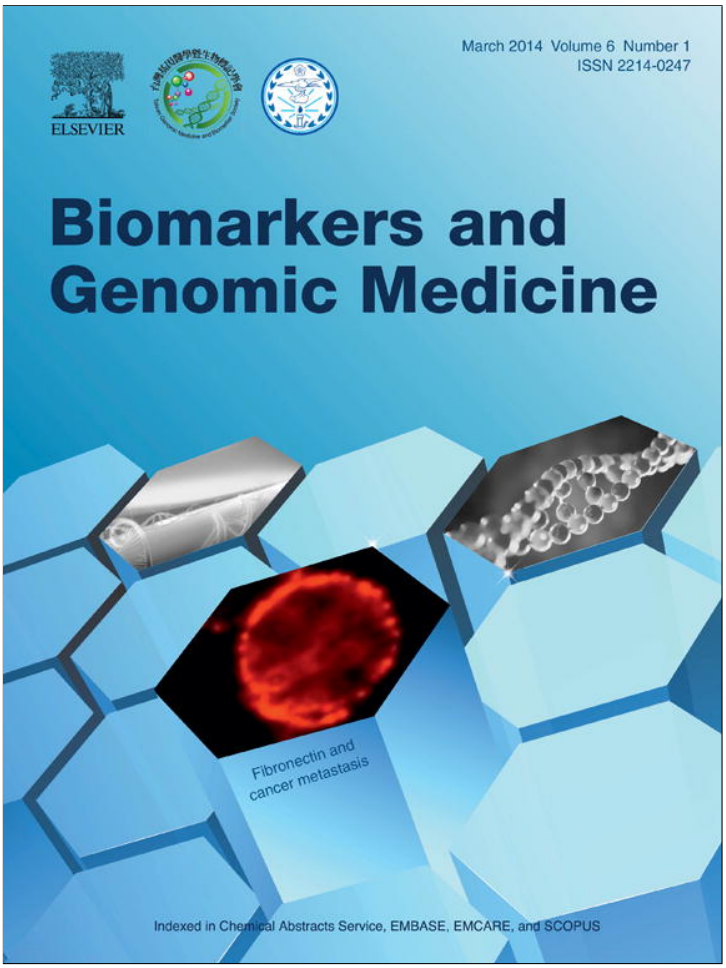

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ORIGINAL ARTICLE

Antioxidative and blood pressure-lowering effects of *Scurrula atropurpurea* on deoxycorticosterone acetate–salt hypertensive rats

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KEYWORDS
antioxidant;
high blood pressure;
oxidative stress;
rats

Abstract This study aimed to investigate whether a methanolic extract of *Scurrula atropurpurea* (BL.) Dans. (MESA) was able to reduced oxidative stress and systolic blood pressure (SBP) in deoxycorticosterone acetate–salt hypertensive rats. Twenty-five male Wistar were divided into the control group and four hypertensive groups that received the MESA at a doses of 50 mg/kg, 100 mg/kg, or 200 mg/kg bodyweight, or received no MESA. SBP was recorded by tail cuff methods. The levels of serum malondialdehyde (MDA) and superoxide dismutase (SOD) were analyzed by colorimetry. SBP was increased significantly in the hypertensive group compared to the sham group ($p < 0.05$). Administration of MESA significantly decreased SBP, but not to reach the level of the sham group. The level of MDA was significantly higher in the hypertensive group compared to the sham group ($p < 0.05$). Administration of $MESA_{200}$ significantly decreased the MDA levels compared to HR groups ($p < 0.05$). The SOD level was significantly decreased in HR compared to the sham group ($p < 0.05$). Administration of $MESA_{50}$ elevated the SOD levels to reach the level in the sham group. The SOD levels in $MESA_{100}$ and $MESA_{200}$ were significantly higher compared to the sham group ($p < 0.05$). In conclusion,

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Scurulla atropurpurea is able to modulate SOD, diminish oxidative stress, and decrease SBP in deoxycorticosterone acetate—salt hypertensive rats.
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Introduction

Hypertension is a clinical common vascular related disease, with high mortality and disability. It is also an independent risk factor for stroke, coronary heart disease, heart failure, renal insufficiency, peripheral vascular diseases, early death, and many other major diseases.[1] Hypertension is the misregulation of a complex interaction between genetic and lifestyle factors affecting the physiological systems regulating blood pressure.[2] There are about 1 billion hypertensive patients in the world, and around 30% of the population die from cardiovascular and cerebrovascular events, in which 62% of acute stroke events and 49% of cardiovascular events are directly caused by hypertension.[3,4]

Consistent evidence reveals the involvement of reactive oxygen species (ROS) and oxidative stress in hypertension and its complications. Hypertension is associated with an increased production of superoxide radicals that have a negative effect on endothelial function. These effects are based on the reaction between superoxide and nitric oxide (NO) to decrease NO bioavailability. Peroxynitrites as the products of this reaction, also have detrimental effects on endothelial cells.[5–7] Hydroxyl radicals as product decomposition of hydroperoxynitrites may trigger lipid peroxidation, as measured by increased malondialdehyde (MDA) levels. Superoxide dismutase (SOD) plays an important role in scavenging superoxide anions that are formed during the early stages of oxidative stress, and in preventing aging.[8] SOD catalyzes the conversion of superoxide to hydrogen peroxide plus dioxygen. SOD can be classified into three groups, Cu/Zn SOD, Mn SOD, and Fe SOD, by the metals they contain at their active sites. Cu/Zn SOD is usually found in the cytoplasm of eukaryotic cells and Mn SOD in mitochondria, whereas prokaryotic cells contain Fe SOD and Mn SOD.[9]

For the decrease of hypertension and its complications, many drugs of herbal origin have been produced, including tetramethylpyrazine from *Jathropha podagrica*, tetrandrine from *Stephania tetrandra*, digitoxin from *Digitalis purpurea*, reserpine from *Rauwolfia serpentina*, and aspirin from *Salix alba*.[2,10,11] *Scurrula atropurpurea* (BL.) Dans. is a parasite of tea plants. In Indonesia, especially on the island of Java, the stems and leaves of this vegetation have been traditionally used for the treatment of cancers.[12] This study aimed to investigate whether methanolic extract of *Scurrula atropurpurea* (BL.) Dans. (MESA) is able to diminish oxidative stress in hypertensive rats.

Materials and methods

Preparation of tea parasite crude extract

Scurrula atropurpurea was determined biologically at the Indonesian Scientific Institute (LIPI) at Purwodadi, Pasuruan, East Java. MESA was obtained through several steps. The leaves were washed, dried in an oven at 40—60°C, then ground into a powder. A 100 mg portion of this powder was steeped in methanol in a 1 L Erlenmeyer flask. The mixture was shaken for 30 minutes to distribute the powder homogenously in the methanol. To collect the precipitate, the mixture was left to stand overnight. The upper layer know and supernatant, being a mixture of methanol and the active constituents, was subjected to evaporation. The extract was then labeled and stored in a freezer.[13,14] This extract was administered daily by oral gavage for 6 weeks.

Animals

Twenty-five male Wistar rats, aged 3—5 months, and weighing 250—300 g were involved in this study. The rats were divided into five groups ($n = 5$ each): a control group, a group of non-MESA hypertensive rats, and three groups of hypertensive rats receiving MESA at dosages of 50 mg/kg, 100 mg/kg, or 200 mg/kg body weight. Hypertensive rats were generated by injecting subcutaneously with deoxycorticosterone acetate (DOCA; Sigma Aldrich, Pte Ltd., Singapore, Singapore) at a dosage of 10 mg/kg body weight, twice 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.[15]

Blood pressure measurement

Systolic blood pressure (SBP) was recorded at the end of the study by tail cuff methods (IITC Model 179, Non-Invasive Blood Pressure Instrument, Woodland Hills, USA) according to a previous study.[16]

Tissue sampling

At the end of the treatment, the animals in all groups were anesthetized; their blood was drawn by cardiac puncture and heparinized. Blood samples were centrifuged at 4000*g* (4 minutes, 4°C) to obtain the plasma. All samples were stored at −80°C until analysis.

MDA analysis

Plasma levels of MDA were determined as thiobarbituric acid (TBA) reactive substance according to the method of Ohkawa et al,[17] based on the reaction of lipid peroxides with TBA at 95 °C. In the TBA test reaction, lipid peroxides and TBA react to form a pink pigment with an absorption maximum at 532 nm. The reaction was performed at pH 2—3 at 95°C for 15 minutes. The sample was mixed with 2.5 volumes of 10% (w/v) trichloroacetic acid to precipitate the protein. The precipitate was pelleted by centrifugation and

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an aliquot of supernatant was reacted with 0.67% TBA in a boiling water-bath for 15 minutes. After cooling, the absorbance was read at 532 nm. Arbitrary values obtained were compared with a series of standard solutions (1,1,3,3 tetramethoxypropane). Results are expressed as units/100 ml.

SOD analysis

SOD was assayed by measuring the inhibition of the formation of blue-colored formazan at 560 nm according to the technique of Kakkar et al.[18] The inhibition by SOD of reduction of nitro blue tetrazolium to blue-colored chromogen in the presence of phenazine methosulfate and nicotinamide adenine dinucleotide was measured at 560 nm. One unit of enzyme activity was defined as enzyme concentration required to inhibit the absorbance at 560 nm of chromogen production by 50% in 1 minute under assay conditions, and expressed as specific activity in units of SOD/minute/mg of protein.

Ethics

Animal care and experimental procedures were approved by the Institutional Animal Ethics Committee of University of Brawijaya, Malang, East Java, Indonesia.

Statistical analysis

Data are presented as mean ± standard deviation and the differences between groups were analyzed using one-way analysis of variance (ANOVA) with SPSS version 15.0 (SPSS Inc. Chicago, IL, USA). A *post-hoc* test was used if the ANOVA was significant. Probability values of $p < 0.05$ were considered statistically significant.

Results

Effect of MESA on SBP

The administration of DOCA—salt affected the SBP levels, as shown in Fig. 1. There was significantly ($p < 0.05$) increased SBP in hypertensive rats compared to the sham group. Compared to the hypertensive group, the administration of MESA significantly decreased SBP, but not to the level of the sham group.

Effect of MESA on MDA level

The administration of DOCA—salt affected the MDA levels, as shown in Fig. 2. There were significantly ($p < 0.05$) increased MDA levels in hypertensive rats compared to the sham group. The administration of $MESA_{200}$ significantly ($p < 0.05$) decreased the MDA levels compared to hypertensive groups.

Effect of MESA on SOD level

The administration of DOCA—salt affected the serum SOD levels, as shown in Fig. 3. The serum SOD levels were significantly ($p < 0.05$) decreased in hypertensive rats compared to the sham group. The administration of $MESA_{50}$ elevated the SOD levels to reach the level observed in the sham group. The SOD levels in $MESA_{100}$ and $MESA_{200}$ were significantly higher ($p < 0.05$) compared to the sham group.

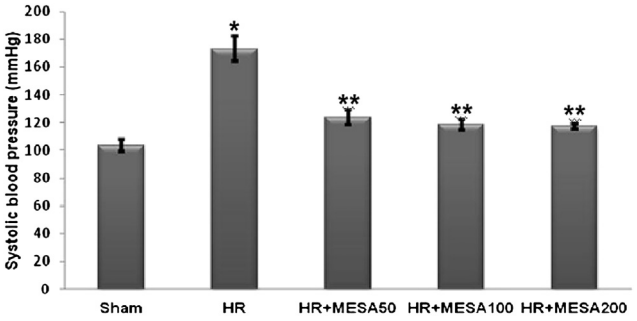

Figure 1 Systolic blood pressure in deoxycorticosterone acetate—salt hypertensive rats (HR) with or without the administration of methanolic *Scurrula atropurpurea* extract (MESA) compared to the sham control group. There is significantly ($p < 0.05$) increased systolic blood pressure in HR compared to the sham group. Compared to its hypertensive group, the administration of MESA significantly ($p < 0.05$) decreased systolic blood pressure, but is not able to reach the level in the sham group. * $p < 0.05$ in comparison with sham group. ** $p < 0.05$ in comparison with deoxycorticosterone acetate—salt hypertensive group.

Discussion

Some species of Loranthaceae from China have been used as medicinal materials for the treatment of hypertension.[17,19] Various compounds have been found in Loranthaceaeous plants and some of them have been identified with hypotensive properties.[12,20] In addition, *Taxillus theifer* (Hayata) H. S. Kiu (*Scurrula ritozanensis*), a

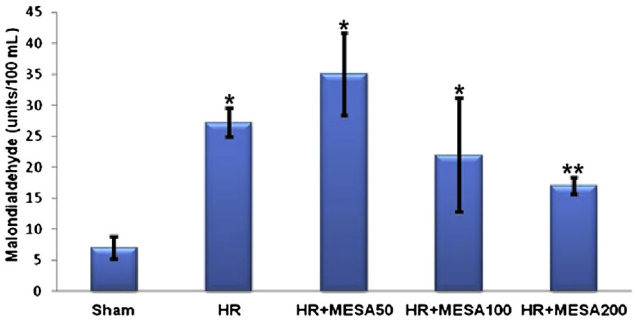

Figure 2 Malondialdehyde in deoxycorticosterone acetate—salt hypertensive rats (HR) with or without the administration of methanolic *Scurrula atropurpurea* extract (MESA) compared to the sham control group. There are significantly ($p < 0.05$) increased MDA levels in HR compared to the sham group. The administration of $MESA_{200}$ significantly ($p < 0.05$) decreased the malondialdehyde levels compared to HR groups. * $p < 0.05$ in comparison with sham group. ** $p < 0.05$ in comparison with deoxycorticosterone acetate—salt hypertensive group.

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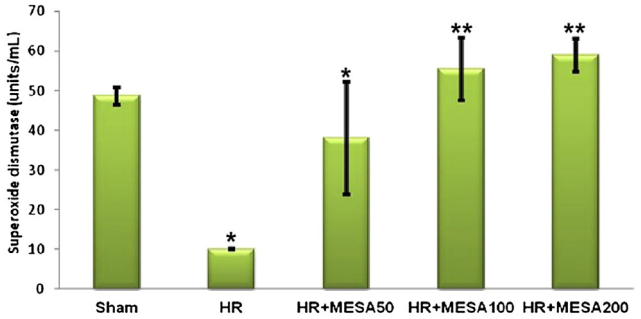

Figure 3 The level of serum superoxide dismutase in deoxycorticosterone acetate—salt hypertensive rats (HR) with or without the administration of methanolic *Scurrula atropurpurea* extract (MESA) compared to the sham control group. The serum SOD level are significantly ($p < 0.05$) decreased in HR compared to the sham group. The administration of $MESA_{50}$ elevated the SOD levels to reach the level in the sham group. The SOD levels in $MESA_{100}$ and $MESA_{200}$ were higher significantly ($p < 0.05$) compared to HR group. * $p < 0.05$ in comparison with sham group. ** $p < 0.05$ in comparison with deoxycorticosterone acetate—salt hypertensive group.

Loranthaceaeous plant endemic to Taiwan,[21] has been used as an antihypertensive agent in Formosan folk medicine. The DOCA—salt treatment induced systemic arterial hypertension, proteinuria, kidney hypertrophy, and impaired kidney function, as reported for this model.[22] This study revealed that DOCA—salt treatment significantly ($p < 0.05$) increased SBP as a marker of hypertensive rats compared to the sham group. The administration of MESA significantly decreased SBP, but this did not reach the level of the sham group. This finding indicates there is no dose dependent effect possibly due to the ability of kidney restoration. A previous study showed that MESA reduces necrosis of the renal proximal tubules achieved at doses of 50 mg/kg and 100 mg/kg body weight. At a higher dose, there is no significant difference compared with the DOCA—salt treatment group, that is, MESA at 200 mg/kg body weight may induce a toxic effect on kidney.[23]

Oxidative stress in DOCA—salt-treated animals has been studied with a variety of stress markers. Subunits of the nicotinamide adenine dinucleotide phosphate oxidase were found to be markedly expressed, the oxidative stress-scavenging protein heme oxygenase-1 is upregulated and also the urinary oxidative stress marker 8-isoprostane is often increased.[24,25] Additionally, there were increased amounts of the oxidative base modification 7,8-dihydro-8-oxo-guanine (which is now widely used as a marker of hypertension in urine) in the DOCA—salt group.[26] In this study, the increase in blood MDA indicates an increase in oxidative stress in DOCA—salt hypertensive rats. Depending on the levels of reactive oxygen compounds, various transcription factors sensitive to change in redox status will be activated and will coordinate intracellular biological response. Modest oxidative stress will induce Nrf2, a transcription factor implicated in the transactivation of genes that encode antioxidant enzymatic activity.[27] The level of SOD significantly ($p < 0.05$) decreased in DOCA—salt hypertensive rats, indicating that they produce more superoxide radical. Oxidative stress has been implicated in the pathogenesis of Ang II—related hypertension.[28,29] Ang II, through AT1R, stimulates nicotinamide adenine dinucleotide phosphate oxidase, induces oxidative stress, and alters endothelial cell function.[30]

The antioxidant compounds of *Scurrula atropurpurea* extract in our study may include quercetin, quercetin-3-*O*-glucoside, quercitrin (a glycoside rhamnose of quercetin), and kaempferol. Quercetin exerts its antioxidant activity through scavenging reactive oxygen speces.[31,32] Quercitrin also has a free radical scavenging activity.[33] Kaempferol has shown a strong inhibitory/scavenging activity on ROS generation with numerous hydroxyl groups on their structures.[33] Moreover, it has been found to be a particularly potent blocker of extracellular ROS production, and to inhibit the ascorbate-dependent NADH oxidase and superoxide anion production activities.[34] Therefore, the MDA-lowering effect and modulation of SOD by *Scurrula atropurpurea* extract demonstrated in this study might have been associated with flavonol or phenolic compounds.

In conclusion, *Scurrula atropurpurea* is able to modulate SOD, diminish oxidative stress, and decrease SBP in DOCA—salt hypertensive rats.

Conflicts of interest

The authors declare that there are no conflicts of interests regarding the publication of this article.

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