

## STUDY OF SUPEROXIDE DISMUTASE AND MALONDIALDEHYDE CONCENTRATIONS IN MICE AFTER ADMINISTRATION OF METHANOLIC EXTRACT of *Scurrula atropurpurea* (BL.)

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### ABSTRACT

The aim of this research was to investigate the potential effect of methanolic extract of *Scurrula atropurpurea* (MESA) in reducing oxidative stress through observation of superoxide dismutase (SOD) activity and malondialdehyde (MDA) concentration in mice. In this research, 25 male mice were divided into four groups with five replications. Group I (K1, without MESA) was considered as a control group, while Group II (K2), III (K3), and IV (K4) were given 1000, 2000, and 4000 mg MESA/kg BW, respectively. After 14 days, SOD activity and MDA concentration were measured spectrophotometrically. The SOD activity did not significantly increased ( $P>0.05$ ) in group K1, K2, K3, and K4 with values were  $19.469\pm 2.072$ ,  $17.4\pm 1.373$ ,  $20.53\pm 3.411$ , and  $23.87\pm 2.528$  U/ml, respectively. In contrast, MDA concentration was decreased ( $P>0.05$ ) with values of  $492.750\pm 19.418$ ,  $492.750\pm 19.418$ ,  $457.125\pm 30.095$ ,  $454.625\pm 19.080$ , and  $475.875\pm 37.659$  ng/mL in groups K1, K2, K3, and K4, respectively. It can be concluded that the administration of MESA tend to increase SOD activity and decrease MDA concentration in this study, thus might be able to reduce oxidative stress in mice.

Key words: acute toxicity, MESA, mice, oxidative stress

### ABSTRAK

Penelitian ini bertujuan mengetahui efek ekstrak *Scurrula atropurpurea* (MESA) terhadap penurunan stres oksidatif melalui observasi aktivitas superoxide dismutase (SOD) dan konsentrasi malondialdehid (MDA) pada mencit. Dalam penelitian ini digunakan 25 mencit jantan yang dibagi ke dalam empat kelompok dengan lima ulangan. Kelompok I (K1, tanpa MESA) merupakan kelompok kontrol, sedangkan Kelompok II (K2), III (K3), dan IV (K4) masing-masing diberikan 1.000, 2.000, dan 4.000 mg MESA/kg bobot badan. Setelah 14 hari, aktivitas SOD dan konsentrasi MDA diukur menggunakan spektrofotometri. Aktivitas SOD meningkat secara tidak signifikan ( $P>0,05$ ) pada K1; K2; K3; dan K4 masing-masing sebesar  $19,469\pm 2,072$ ;  $17,4\pm 1,373$ ;  $20,53\pm 3,411$ , dan  $23,87\pm 2,528$  U/ml. Sebaliknya, konsentrasi MDA menurun secara tidak signifikan ( $P>0,05$ ) masing-masing sebesar  $492,750\pm 19,418$ ;  $492,750\pm 19,418$ ;  $457,125\pm 30,095$ ;  $454,625\pm 19,080$ ; dan  $475,875\pm 37,659$  ng/ml pada K1; K2; K3; dan K4. Dapat disimpulkan bahwa pemberian MESA cenderung meningkatkan aktivitas SOD dan menurunkan konsentrasi MDA sehingga diduga mampu mengurangi stress oksidatif pada mencit.

Kata kunci: toksisitas akut, MESA, mencit, stres oksidatif

### INTRODUCTION

Tea parasite *Scurrula atropurpurea* BL. Danser (*S. atropurpurea* BL. Danser) plant is a hemiparasite plant which contains chlorophyll, thus allows it to assimilate by itself and only absorbs water and other organic and inorganic compound from tea plant it resides on (Murphy *et al.*, 2003; Athiroh and Permatasari, 2012). In general, tea parasite plant of Laranthaceae family have anti-cancer and anti-inflammatory potential (Kawamura and Pamudji, 2005), however, there no many reports available on its anti-hypertension effect.

In previous in vivo research, tea parasite (*S. atropurpurea* BL. Danser) able to improve superoxide dismutase (SOD) activity and decrease malondialdehyde (MDA) concentration in deoxycorticosterone acetate (DOCA)-salt induced hypertensive rat (Kawamura and Pamudji, 2005; Athiroh and Sulistyowati, 2013; Mustofa *et al.*, 2013; Athiroh *et al.*, 2014a). Thus, the tea parasite plant can reduce oxidative stress in hypertension model rat. In in vitro research, crude extract of tea parasite (*Scurrulla arena*) decreases artery vessel contractility in the rat which is pre-contracted by norepinephrine (NE) with endothelial

wall remained intact. In denuded endothelial cell condition, the decreasing of artery vessel contractility was not significant (Athiroh, 2009a; Athiroh, 2009b; Athiroh *et al.*, 2014b).

In order to produce tea parasite phytopharmaca, research should be followed by a toxicity test on oxidative stress. Previous research has proven that tea parasite did not demonstrate toxicity effects on rat in subchronic administration in which blood biochemical and liver histopathology abnormality were not observed (Athiroh and Sulistyowati, 2015). According to National Agency of Drug and Food Control (BPOM) (2014), in order to produce standardized herbal product needs to test the respective product in 2-3 different species of animal. Therefore, the study to evaluate the effect of acute toxicity of methanolic extract of *Scurrula atropurpurea* (MESA) in mice is needed. Toxicity test of tea parasite aimed to investigate tea parasite toxicity in animal model before clinical trial is conducted on hypertensive patients (Athiroh, 2010). Stress oxidative in mice after administration of MESA in this study was observed through the evaluation of SOD activity and MDA concentration.

## MATERIALS AND METHODS

### Extraction of *Scurrula atropurpurea* leaves

*Scurrula atropurpurea* leaves were obtained from Blitar Tea Plantation, East Java. Identification and determination process were done in Balai Materia Medika, Batu, East Java. Extraction process was done after tea parasite leaves were oven-dried at 40-60° C and ground into small particles using blender. As much as 50 g of tea parasite powder were soaked in 800 ml of methanol, and shaken until well-mixed for  $\pm 30$  minutes. Afterwards, the solution was macerated over night until the powder precipitated. Maceration was repeated three times and followed by evaporation. The samples were then labeled and stored in the freezer (Eno *et al.*, 2004; Kawamura and Pamudji, 2005; Mustofa *et al.*, 2013; Athiroh *et al.*, 2014a; BPOM, 2014).

### Toxicity Test in Mice

Male mice were fasted for 3-4 hours before treatment; however they have access to drinking water. After fasted, the mice were weighted and given the MESA according to their treatment group by using gastric gavage as much as 1 ml/100 g BW. Feed were given 1-2 hours after treatment. In cases the dosage was not possible to be given at once, the MESA was administered in several attempts within 24 hours. Behavior observation was conducted every day for at least 14 days. The mice were weighted for at least two times a week. They were observed individually in the first 30 minutes after the administration of MESA and periodically every hour during 24 hours, and then followed by once a day for 14 days. After 14 days, the mice were dissected and SOD and MDA were analyzed (BPOM, 2014; Athiroh and Sulistyowati, 2015).

### Research Plan

The research was designed by experimental laboratory method using post test only control group design. The proposal of this research has been accepted by the Ethic Committee of Faculty of Medicine, Brawijaya University, with the number 369/ EC/KEPK/06/2015.

In this research 25 male mice were divided into four groups with 5 mice per group. Group I (K1, without MESA) was a control group, while Group II (K2), III (K3), and IV (K4) were given 1000, 2000, and 4000 mg/kg BW MESA, respectively for 14 days.

### Lipid Peroxidase Concentration Measurement (MDA/TBARS)

MDA concentration was conducted followed thiobarbituric acid technique by Philpot (1963) and Niehaus and Samuelsson (1968). As much as 0.5 mL serum was taken and added with TCA 40%. Afterward 200  $\mu$ l 1N HCl were added following by 0.5 mL distilled water and 100  $\mu$ l natrium thiosulfate (Nathio). The solution was heated in 100° C for 25 minutes. It was then centrifuged at 3000 rpm. Absorbance was read by using spectrophotometer (UV 1700 Pharma

Sec) and UV Vis (Shimadzu) in 532 nm wavelength (Athiroh and Sulistyowati, 2013).

### SOD Activity Analysis

As much as 100  $\mu$ l serum was transfer into a test tube and added with 100  $\mu$ l of 100 mM EDTA and 500  $\mu$ l buffer. Afterwards, 100  $\mu$ l NBT 25 U, 100  $\mu$ l xanthine 25 U, 100  $\mu$ l xanthine oxidase 1 U, and 1 mL phosphate buffered saline (PBS) were added. The solution was mixed using vortex and incubated at 39° C for 30 minutes. The tube then centrifuged at 3000 rpm and the supernatant was collected. Phosphate buffer was added into the supernatant up to 3.3 mL and the mixture was then measured by UV spectrophotometer (1700 Pharma Sec) and UV-Vis spectrophotometer (Shimadzu) at 580 nm wavelength (Athiroh and Sulistyowati, 2013; Mustofa *et al.*, 2013; Athiroh *et al.*, 2014a).

### Data Analysis

Data were analyzed by ANOVA followed by least significant difference (LSD) test.

## RESULTS AND DISCUSSION

The activity of SOD and MDA concentration after administration of MESA in mice was presented in Table 1. Table 1 showed that the administration of MESA increase ( $P > 0.05$ ) the average of SOD activity in K0, K1, K2, and K4 with values of  $19.469 \pm 2.072$ ,  $17.4 \pm 1.73$ ,  $20.53 \pm 3.411$ , and  $23.87 \pm 2.528$  U/ml, respectively. In contrast, the MDA concentration was decreased in groups given different MESA concentration with values were  $492.750 \pm 19.418$ ,  $492.750 \pm 19.418$ ,  $457.125 \pm 30.095$ ,  $454.625 \pm 19.080$ , and  $475.875 \pm 37.659$  ng/ml ( $P > 0.05$ ) in K1, K2, K3, and K4, respectively.

The increasing of SOD activity reduces the oxidative stress due to the decreasing of superoxide anion ( $O_2^*$ ) (de Moura *et al.*, 2005) and reduction of free radical formation, thus preventing lipid peroxidation (lowering MDA). Oxidative stress is known to have an important role in the development of human disease. For the last three decades, it has been known that free radical does not have important roles in the pathogenesis of various diseases. However, recently many studies have been proven that free radical and related oxidant are associated in the development of various diseases. In normal cellular metabolism, radical superoxide plays important roles as a terminator of lipid peroxidation, signaling molecule, and control NO concentration which function in controlling homeostasis.

A healthy cell stays in a stable but with vulnerable condition, a small bad stimulus that affect cell may cause fatal injury to the cell. Oxidative stress significantly contributes to various diseases associated with blood circulation disorder and energy formation (Ortiz and Garvin, 2001). The involvement of lipid peroxidation can be known by MDA basic reaction with thiobarbituric acid (TBA) which forms a compound called MDA-TBA and absorbs light with

**Table 1.** SOD activity and MDA concentration in mice given different doses of MESA

No	Treatment	MDA (ng/ml)	SOD (U/ml)
1	Control	492.750±19.418 <sup>a</sup>	19.469±2.072 <sup>b</sup>
2	1000 mg MESA/kg BW	457.125±30.095 <sup>a</sup>	17.400±1.373 <sup>b</sup>
3	2000 mg MESA/kg BW	454.625±19.080 <sup>a</sup>	20.525±3.411 <sup>b</sup>
4	4000 mg MESA/kg BW	475.875±37.659 <sup>a</sup>	23.872±2.528 <sup>b</sup>

<sup>a, b</sup>The same superscripts in the same column showed a non-significant

500-600 nm wave length. This colored compound concentration can be measured based on its color absorbance and compared it with standard liquid color absorbance with known concentration by using spectrophotometer (Ortiz and Garvin, 2001).

MDA is one of the products of major degradation and lipid peroxidation. Lipid interaction with free radical will increase lipid peroxidation by the cell membrane, which will then induce atherogenesis process. In individual with coronary heart disease, endothelial SOD activity suffers marked decrease, which can cause endothelial dysfunction. Administration of anti-oxidant can improve oxidative stress through elevating SOD and depressing MDA (Halliwell and Gutteridge, 2010).

Experimental researches which supports antioxidant's role in degenerative diseases have been conducted by Vaziri and Rodriguez (2006) who studied oxidative stress in hypertensive animal model. Antioxidant administration in animal model can prevent oxidative stress by correcting inflammatory and hypertensive condition through elevating the activity of vascular nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) (Redon *et al.*, 2003). Flavonoid compound can function as natural antioxidant, which protect biological system and inhibit cellular oxidation through reduction-catching active oxygen and free radical especially superoxide (Murphy *et al.*, 2003; Athiroh *et al.*, 2014b).

Flavonoid inhibits NADPH oxidase, reduces plasma ET-1, and depresses p22 gene (phox). Thus there is a possibility of tea parasite plant able to lower MDA concentration and increase SOD. The most important function of SOD is to protect blood vessel wall from bioactive nitric oxide (NO). SOD lowers O<sub>2</sub><sup>-</sup>, its potential is faster than other antioxidant such as vitamin C or E. As such, SOD has the main effect of protecting NO from reacting with O<sub>2</sub><sup>-</sup> (Ortiz and Garvin, 2001). The higher SOD concentration by consuming tea parasite results to the more optimal protection against free radical in each cell and body organ. Generally, other antioxidant nutrition (such as vitamin and mineral) improve the lack of daily needs, however, SOD act in strengthening internal system by activating and optimizing natural immune system.

Flavonoid free radical capture mechanism is initiated by hydrogen release, which will cause radical reactive flavonoid. Afterwards, radical flavonoid will bind free radical which decrease, or even eliminate, its reactivity. The diminishing or vanishing O<sub>2</sub><sup>\*</sup> reactivity lower its ability in reacting with NO and make ONOO, and thus lowering endothelial damage (Suharto, 2008).

## CONCLUSION

It can be concluded that the administration of MESA tend to increase SOD activity and decrease MDA concentration in this study, thus might be able to reduce oxidative stress in mice

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