

Macassar fruit extract (*Brucea javanica* (L.) merr) increased the level of superoxide dismutase (SOD) but had no effect on the level of malondialdehyde (mda) in paraquat-treated male swiss Webster mice

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ABSTRACT

Introduction: Paraquat exposure causes aging because it induces oxidative stress marked by decreased level of SOD and increased MDA serum level. Macassar fruit contains bioactive compounds such as vitamin C, flavonoids, tannins and polyphenol that have antioxidant activity. The purpose of this study was to prove that Macassar fruit extract increased the level of Superoxide Dismutase (SOD) enzyme and reduce the level of Malodialdehyd (MDA) in male Swiss Webster mice treated by paraquat.

Methods: A randomized pretest-posttest control group study was conducted using 14 male mice which were 2-3 months old, healthy and had 25-30 gram in weight. They divided into 2 groups namely P0 (control) and P1 (Treatment). Both groups

were treated by paraquat but only group P1 received 20 mg Macassar fruit extract while the P0 only got 1 cc placebo for 14 days.

Results: Our result showed that the level of SOD was increased in P1 group from 17.18±1.69 U/ml to 67.56±3.65 U/ml (p<0.01) while no change was observed in P0 group 16.97 ±1.45U/ml to 17.07±1.89 U/ml (p>0.05). However, no effect on MDA level was observed as the level of MDA tended to slightly decrease in both groups.

Conclusion: It can be concluded that Macassar fruit extract 20 mg/kgBB significantly increased the level of SOD while have no effect on MDA level in male mice Swiss Webster treated by paraquat.

Keywords: Macassar fruit, Oxidative stress, SOD, MDA, Paraquat toxicity

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INTRODUCTION

One factor that caused aging was the accumulation of oxidative damaged caused by free radicals. Free radicals were reactive molecules with one or more unpaired electrons in their outer orbit.¹ The source of free radicals could be from the body's metabolic processes (internal) or external sources. One of the external sources of free radical is herbicides or paraquat.²

Paraquat was an herbicide that was used to control weeds in agricultural and non-agricultural areas. Paraquat had been used in more than 120 countries and it was currently pesticides with the 3rd highest sales in the world.³ The active ingredients contained in paraquat were classified as class II (moderately hazardous) according to the Classification of Pesticides by Hazard WHO.⁴

Paraquat that was accumulated in the lungs would experience a redox reaction that caused oxidative stress. This oxidative stress would form Reactive

Oxygen Species (ROS) which would cause acute lung disease. In addition, excessive ROS formation causes lipid oxidation, protein oxidation and DNA damage.⁵

Malondialdehyde (MDA) was formed from damaged membrane cell due to ROS in oxidative stress. The high level of MDA was influenced by level of lipid peroxidation, which indirectly showed the high number of free radicals and showed the presence of oxidation processes in cell membrane.⁶ In contrast, high antioxidant level was usually followed by the decreasing of MDA levels.⁷

Naturally, the body had endogenous antioxidants or enzymatic antioxidants to fight free radicals that potentially interfere with the balance of body functions. The enzyme of superoxide dismutase (SOD) was the first defense against the activity of reactive oxygen compounds (ROS). In oxydative stress occurred decreasing of enzymatic system and glutathione peroxidase. If production of free radicals

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Submitted: 21 August 2019

Accepted: 14 October 2019

Published: 31 October 2019

exceeded the ability of endogenous antioxidants to neutralize it, it can potentially damage the cell. Therefore, the body needed a supply of antioxidants from outside the body which were better known as exogenous antioxidants such as vitamin E, vitamin C, green vegetables and fruits.⁸

Antioxidants were compounds that free in nature and able to prevent cellular damage due to reactivity toward free radical. Exogenous antioxidants are abundant in nature, one example of a plant that had the potential as an exogenous antioxidant in Indonesia is the Macassar fruit (*Brucea javanica*). The results of phytochemical analysis found in Macassar fruit (*Brucea javanica*) contained flavonoid, tannin, saponin, anthraquinone and anthocyanoside compounds.⁹ Tannin, alkaloid and flavonoid compounds had been known as antioxidant compounds that could reduce the levels of free radicals in the body (Widiyantoro et al., 2010).¹⁰ Therefore, it has potential use as antioxidant food, especially its extract. However, the evidence that linked between Macassar fruit consumption with reduced oxidative state as well as physiological improvement in anti-oxidant defense is still lacking. Therefore, this study aimed to evaluate the effect of Macassar fruit extract consumption with systemic oxidative state that measured as the level of MDA and SOD.

METHODS

A randomized pretest-posttest control group study was conducted at Faculty of Medicine, Udayana University, Jl. PB Sudirman, Denpasar, Bali using 14 Swiss Webster mice (*Mus musculus*), aged 2-3 months, healthy, 25-30 grams of weight. The mice then divided into 2 groups with each group consisted of 7 mice. One group as the control group was given paraquat treatment + 1 cc placebo (P0) for 7 days while the other group (P1) was given paraquat + Macassar fruit extract of 20 mg/day (P1) for 14 days.

Protocol SOD and MDA Assay

Blood was collected from each mouse and then left for a maximum 1 hour. The blood then centrifuged at 3000 rpm for 10 minutes. 20 μ L of sample solution was added to each sample and blank 2 followed by 200 μ L H₂O for each blank 1. A total of 200 μ L WST Working Solution was added for each blank. Then, as much as 20 μ L Dilution Buffer was added for blank 2 and blank 3. A total of 20 μ L Enzyme Working Solution was added for each sample and blank 1 by using the Multiple Channel pipette. Then it was mixed by gently rocking the

microplate. Then, the sample was incubated at 37°C for 20 minutes. Spectrophotometer was used to read the absorbance and determining the SOD or MDA concentration.

Protocol MDA Assay by TBARS Method.

1 ml of blood plasma was taken and stored in a test tube followed by addition of 1 ml 20% cold TCA and centrifugation at 3000 rpm for 10 minutes. Then the supernatant was taken and put into another test tube which contained 2 ml of 0.67% TBA. All samples then incubated in the water bath at 100°C for 10 minutes and cooled afterward. Spectrophotometer was used to assess the MDA concentration by taking 1 ml of sample solution into cuvette tube and measure it in the device with a wavelength of 530 nm.

Data obtained from the measurement results of serum levels of SOD and MDA of mice would be analyzed by paired t-test using the Statistical Program for Social Science (SPSS) software for Windows version 24.

RESULTS

Prior to the experiment, a phytochemical analysis was carried out to identify the content of the Macassar fruit extract. The results showed that the extract contained flavanoids (898.55mg/100gr QE), phenols (515.93mg/100gr QE), tannins (28187.31mg/100gr QE), antioxidant capacity of 2,407.9mg/100gr QE), vitamin C (5443,2990 mg / 100gr QE) (Yulainti, 2018).¹¹

Regarding the animal study, it was showed that the level of SOD and MDA in P0 and P1 were almost similar with P1 has a slightly higher mean SOD level (17.18 ± 1.69 U / ml). However, the level of SOD was increased significantly in P1 group after treatment, increasing to 67.56 ± 3.65 U / ml ($p < 0.001$). In terms of blood MDA level, both group showed similar trend toward lower MDA level. It appeared that the mean MDA level was higher in P1 group compared to P0. However, statistical analysis showed that the difference was not statistically significant (Table 1).

DISCUSSION

Paraquat exposure reduced SOD enzyme activity and increased serum MDA levels

Paraquat is a pro-oxidant substance that commonly found in the environment due to its usage as herbicide. The main mechanism for paraquat toxicity is through the redox cycle (oxidation-reduction) which results in oxidative stress.

Table 1. Level of SOD and MDA between groups

Variable	Group	Average Value		T	P
		Pre Test	Post Test		
SOD (U/ml)	Control (P0)	16.97±1.45	17.07±1.89	-0.103	0.922
	Treatment (P1)	17.18±1.69	67.56±3.65	-39.6	0.000
MDA (mg/dL)	Control (P0)	4.59±0.16	4.52±0.10	0.959	0.374
	Treatment (P1)	4.59±0.19	4.34±0.15	2.209	0.069

p = significancy at < 0.05

Through the active transport carrier, paraquat molecules transverse the cell and enters the mitochondrial matrix. In the mitochondrial matrix, paraquat experiences the redox cycle, paraquat-kations reduced by NADPH into a radically reduced form. Furthermore, reduced radicals react with oxygen molecules to form superoxide anions. The superoxide anion produces hydrogen peroxide which then further transformed into hydroxyl radicals due to its interaction with Fe^{2+} .⁵

To counter oxidative stress, the body has intracellular antioxidant enzymes or endogenous antioxidants, namely superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (Cat). SOD is one of the endogenous antioxidants that used to catalyze reaction of free radicals superoxide anion (O_2^-) to hydrogen peroxide and oxygen molecules.¹² Research had shown that oxidative stress caused by chronic renal failure significantly decreases CuZn SOD and Mn SOD.¹³ Therefore, it is directly linked both with systemic antioxidant defense status as well as pathological condition that the patients may have.

Malondialdehyde (MDA) is metabolite produces by lipid peroxidation and usually use as a biological biomarker for oxidative stress.¹³ The high level of MDA is influenced by levels of lipid peroxidation, which indirectly shows the high number of free radicals.⁶ High level of MDA indicates oxidation process in the cell membrane while high antioxidant status is usually followed by decrease level of MDA levels.⁷

Effect of Macassar fruit extract on SOD and MDA level

The results of phytochemical analysis carried out in the laboratory of the Faculty of Agricultural Technology, Udayana University showed that Macassar fruit extract (*Brucea javanica*) contained flavanoid, phenol, tannin, antioxidant capacity,

vitamin C. This active compound could potentially neutralize free radicals and prevented oxidative stress.¹¹

The body had intracellular antioxidant enzymes or endogenous antioxidants, namely superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (Cat). SOD was one of the endogenous antioxidants that used to catalyze free radicals dismutation of superoxide anion (O_2^-) to hydrogen peroxide and oxygen molecules.⁸ Research had shown that oxidative stress caused by chronic renal failure significantly decreases CuZn SOD and Mn SOD. It was because of antioxidant enzymes such as SOD play important role in binding to free radicals and turned them into harmless forms.¹³

This study showed consistent findings regarding the effect of Macassar fruit extract on systemic oxidative status. It showed that the extract could significantly increase the level of SOD. However, there was insignificant decreasing in MDA level, this could be caused by various factors between the frequency of exposure, absorption, dosage of drugs and differential in physiology in resisting counteracting free radicals. However, in theory, anti-oxidant in Macassar fruit extract should be capable enough to prevent lipid peroxidation.

Vitamin C and flavonoids in Macassar fruit could reduce the bad effects of free radicals, by inhibiting lipid peroxidation through the activation of peroxidase against hemoglobin, which was an endogenous antioxidant (enzymatic).¹² Peroxidase was useful to prevent accumulation of H_2O_2 , which was dangerous if it was together with O_2 because it could form a OH radical which was the most reactive and most dangerous free radical, which could damage cell membranes by causing disconnection of unsaturated fatty acids.¹⁴ In addition, flavonoid content was also known as a chain breaking antioxidants which was unsaturated in fatty acids and could break the chain of lipid peroxidation.⁸

Vitamin C could directly react with superoxide anions, hydroxyl radicals, singlet oxygen and lipid peroxide. As reducing agent, vitamin C would donate one electron to form semi dehydro-ascorbate which was not reactive and then experienced disproportionation reaction to form dehydro-ascorbate which was unstable. Ascorbate could directly capture oxygen free radicals, either with or without enzyme catalysts. Indirectly ascorbate could reduce free radical activity by changing reduced tocopherol.¹⁵

CONCLUSIONS

Based on the results of this study, it can be concluded that 20 mg Macassar could significantly increase the level of the superoxide dismutase (SOD) in Swiss Webster mice that had been induced by paraquat while have little effect toward blood MDA level.

CONFLICT OF INTEREST

All authors declared that there is no conflict of interest regarding this publication

AUTHOR CONTRIBUTION

All authors contributed equally in the writing of this article

FUNDING

This study was self-funded without any contribution from third party.

ETHIC APPROVAL

This study had been ethically approved by ethical commission of Faculty of Medicine Udayana University with approval letter number 388/KE-PH-Lit-2/VII/2018

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