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Expression of Catalase and Malondialdehyde Levels in Silicon Dioxyde-exposed Lung Tissue of Mice Treated with *Moringa oleifera* Leaves Extract

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ABSTRACT

Silica particle such as silicon dioxide (SiO_2), is considered as a hazardous and cytotoxic particle. Silica particle exposure leads to oxidative stress in lung tissue. *Moringa oleifera* is a plant with potential antioxidant compounds. Therefore the aim of this study was to analyze the effect of *M. oleifera* leaves extract (MLE) on expression of catalase enzyme and malondialdehyde (MDA) levels in lung tissue of mice exposed to silica particles. This study was an experimental study with randomized posttest-only control group design using 30 male Balb/c strain mice, 8-10 weeks of age, 20-30 g body weight (BW), which were randomly divided into five groups. Group 1 was the negative control group, group 2 was exposed to SiO_2 particle and set as the positive control group, group 3 was treated with MLE 2 mg/20 g BW, group 4 was treated with MLE 5 mg/20 g BW, and group 5 was treated with MLE 8 mg/20 g BW. After 90 days, mice were sacrificed by cervical dislocation and the lung tissues were examined. Study results showed that expression of catalase in lung tissues of MLE-treated group was higher than that of positive control group, but not statistically significant. There was a significant difference of MDA level in lung tissue among groups. MDA level of groups treated with MLE 2 mg/20 g BW and 5 mg/20 g BW was lower than that of control group, while group treated with MLE 8 mg/20 g BW showed higher MDA level than control group (One Way ANOVA, $p<0.05$). It is concluded that administration of MLE indicates to prevent SiO_2 induced-oxidative stress in lung tissue of Balb/c mice.

Keywords: Antioxidant, Catalase, Malondialdehyde, *Moringa oleifera*, Silicon dioxyde

INTRODUCTION

Background

All Environmental pollution highly influences public health. One of the factors which may lead to health problems is air pollution due to dust particles. Many activities could contribute to air pollution, such as transportation, road construction, drilling, *etc*. One of the hazardous dust components is silica particle. Silica dust could be found in material of road construction, foundry, cement, coal, glass, and ceramic industry, as well as gold, sand, iron, and any minerals mining[(1)].

Silica particle such as silicon dioxide (SiO_2), is considered as a hazardous and cytotoxic particle. Human could be exposed to silica through various ways, one of them is inhalation. The lungs are the most impacted organ due to exposure of inhaled silica particle. Studies in some areas exposed to silica dust has been conducted. The foundry workers in Samsun, Turkey, developed symptoms such as phlegm (20.46%), cough (14.98%), breathlessness (8.06%), and wheezing (2.01%). A study of foundry workers in Taiwan has reported that lung function is correlated to the level of silica dust[(2)]. Prolonged silica exposure could lead to silicosis. Silicosis has been reported in dental supply factories workers, located in the States of Michigan and New York, i.e five cases[(3)], and over 69.1% among agate workers at Shakarpur[(4)]. A retrospective study conducted in a clinic located in Edinburgh, Scotland, has reported six cases of silicosis in stonemasons[(5)].

Prolonged or repeated exposure to silica may cause lung tissue damage. Silica dust that passes into the lung causes various responses in lung tissue, such as inflammation response and oxidative stress. Oxidative stress may

result from ROS generated at the silica particle surface, phagocytosis process, as well as inflammation process. Oxidative stress, arising as a result of an *imbalance* between antioxidant in the body and ROS production. Therefore, materials that could increase antioxidant status in the body are required.

One of natural plant materials that frequently consumed by people is *Moringa oleifera* leaves (*Moringa oleifera*, Indonesia common name is *kelor*). It is frequently consumed as vegetable, or as additive in food and beverages. Many studies has reported that *M. oleifera* leaves contain a variety of compounds with its health benefits. *M. oleifera* leaves contain various active compounds as potential antioxidant[6-8]. A study in Mexico performed by Valdez-Solana *et al.* (2015) has reported that *M. oleifera* leaves contain phenolic acids (gallic and chlorogenic acids) and flavonoid (rutin, luteolin, quercetin, apigenin and kaempferol)[9]. *M. oleifera* leaves also contain microminerals such as Cu, Zn and Mn, as well as some vitamins[10].

Both *in vitro* and *in vivo* studies have demonstrated that *M. oleifera* leaves possess antioxidant[11], inflammatory and immunomodulatory[12], hypolipidemic, hepatoprotective[12], hypoglycemic[13], antibacterial, wound healing[14] and anticancer characteristic[15]. Nevertheless, the role of *M. oleifera* leaves to treat the impact of silica exposure has never been studied.

Purpose

This study aimed to analyze the effect of *M. oleifera* leaves extract on expression of catalase and MDA levels in lung tissue of mice exposed to silicon dioxyde (SiO_2) particles.

METHODS

Plant Material

Moringa oleifera plant was obtained from *Moringa oleifera* plantation in Sokon Village, Kupang, East Nusa Tenggara, Indonesia. The plant was identified in laboratory of Department of Biology, Faculty of Science and Technology, Airlangga University.

Laboratory Animal

Healthy male Balb/c strain mice *(Mus musculus)*, 8-10 weeks of age, weighing 20-30 grams, were obtained from biochemistry laboratory in Faculty of Medicine, Airlangga University. Mice were raised in Laboratory Animal Care Unit of biochemistry laboratory, Faculty of Medicine, Airlangga University. Ethics approval certification was obtained from Ethics Committee of Health Study, Faculty of Public Health, Airlangga University, No. 544-KEPK. Mice were fed pellets and water *ad libitum.* They were acclimatized for 1 week. A total of 30 mice were randomly divided into five groups of six each. The groups consisted of negative control group (without silica exposure and administration of MLE), positive control group (with silica exposure and administration of MLE), treatment group with silica exposure and treated with MLE 2 mg/20 g body weight (BW), treatment group with silica exposure and treated with MLE 5 mg/20 g BW, and treatment group with silica exposure and treated with MLE 8 mg/20 g BW. The extract was administered via oral gavage over 10 days before and 90 days after silica exposure. In the end of the treatment period, mice were sacrificed by cervical dislocation.

Extraction of *Moringa oleifera* leaves

M. oleifera leaves were extracted using maceration technique with ethanol 96% as a solvent. Extraction was performed in Phytochemistry Laboratory, Faculty of Pharmacy, Airlangga University. Identification of compounds contained in extract was done in Testing Service Unit, Faculty of Pharmacy, Airlangga University. Ethanol extract of *M. oleifera* leaves was diluted in CMC-Na 0.5% to obtain the desired concentration for administration via oral gavage to the mice.

Silicon dioxyde (SiO_2) Particle Exposure

In this study, 1-5μm silica particles SiO_2 (Sigma Aldrich) 80% were used. Silica was administered intratracheally, with SiO_2 concentration of 2.5 mg in 60μl NaCl 0.9%.

Measurement of catalase expression

Expression of catalase in lung tissue was measured using immunohistochemical methods with Anti-Catalase antibody [EPR1928Y](ab76110). Expression of catalase was observed under light microscope at a

magnification of 400x with 10 field of view regions. Measurement of catalase expression was performed in Electron Microscope and Integrated Medical Laboratory Unit, Faculty of Medicine, Airlangga University.

Measurement of MDA level

MDA level in lung tissue was measured using Enzyme-linked immunosorbent assay (ELISA) and the absorbance was read at 450 nm with a microplate reader. Mouse Malondialdehyde ELISA Kit (Cat. No E0625Mo) was used. Measurement of MDA level was performed in Immunology Laboratory, Faculty of Veterinary Medicine, Airlangga University.

Data and Statistical Analysis

The numerical data were expressed as mean and standard deviation[16]. One-Sample Kolmogorov Test was used to test the normality of data. Data from each group were compared using One-Way ANOVA with 95% confidence interval and Post-Hoc analysis with LSD method.

RESULTS

Body weight

At the initial of the study, Balb/c mice *(Mus musculus)*weight was recorded to ascertain that mice used in the study were from homogenous population. Mean and standard deviation of Balb/c mice *(Mus musculus)* weight are displayed in Table 1. The study involved Balb/c mice *(Mus musculus)*, weighing 20-30 g. Statistical analysis showed that no significant difference in mice weight between groups (One Way ANOVA, $p>0.05$). Therefore, indicating that samples consist of mice from homogenous weight population.

Expression of catalase

Measurement of catalase expression was performed using immunohistochemical method with Anti-Catalase antibody. Means of catalase expression in negative control group and positive control group were 2.77±1.50 and 2.15±1.49, respectively. Means of catalase expression in lung tissue of mice in both treatment groups MLE 2 mg/20 g BW and MLE 5 mg/20 g BW were higher than positive control group (Table 1). Expressions of catalase in lung tissue of Balb/c mice *(Mus musculus)* treat with MLE tended to increase although statistical analysis showed that there was no significant difference between treatment groups (One Way ANOVA, $p>0.05$). Expressions of catalase on immunohistochemical examination are shown in Figure 1.

Malondialdehyde level

Measurement of MDA level is an indirectly assessment of free radical activity, thus what can be measured are products of the damage produced by free radicals, not directly the free radical compounds. In the study, MDA level in lung tissue was measured as the end-product of lipid peroxidation. The result showed the significant difference of MDA levels between groups ($p<0.05$). The result of Post-Hoc analysis with LSD method is presented in Table 1. It shows a significant difference of MDA levels between negative and positive control group ($p<0.05$), negative control group and MLE 8 mg/20 g BW group ($p<0.05$), positive control group and MLE 2 mg/20 g BW ($p<0.05$), positive control group and MLE 5 mg/20 g BW ($p<0.05$), positive control group and MLE 8 mg/20 g BW ($p<0.05$). MDA level of positive control group was higher than that of negative control group. The lowest MDA level was found in MLE 5 mg/20 g BW group, 0.3024±0.0328 nmol/ml, indicating that the dose level is able to decrease MDA. The results of linear regression showed that MDA contents was influenced by catalase expression ($p<0,05$).

Table 1. Body Weight, Expression of Catalase and MDA Levels in Lung Tissue of Balb/c mice *(Mus musculus)*

Group	Body Weight	Catalase	MDA
Negative control	25.17±1.72	2.77±1.50	0.3101^{a} ±0.0138
Positive control	25.33±1.21	2.15±1.49	0.3412^{b} ±0.0192
MLE 2 mg/20 g BW	25.67±1.03	3.08±1.96	0.3088^{a} ±0.0183
MLE 5 mg/20 g BW	25.33±0.82	4.77±2.19	0.3024^{a} ±0.0328
MLE 8 mg/20 g BW	25.00±1.41	2.40±0.79	0.3748^{c} ±0.0150

Data are presented as the means±SD, n= 6. Body weights are expressed in gram, catalase expressed in the number cells expressing catalase antibody and MDA levels are expressed in nmol/ml tissue. [abc] The same superscript indicates no significant difference between groups according One way ANOVA test followed by Pos Hoc LSD test.

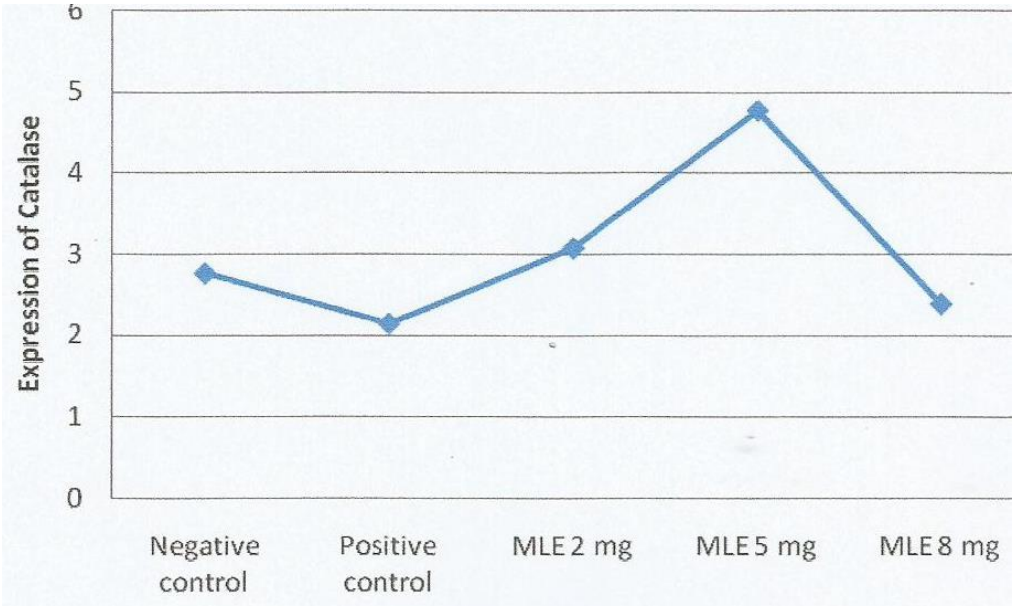

Figure 1. Expression of Catalase in Lung Tissue of Balb/c mice *(Mus musculus)*

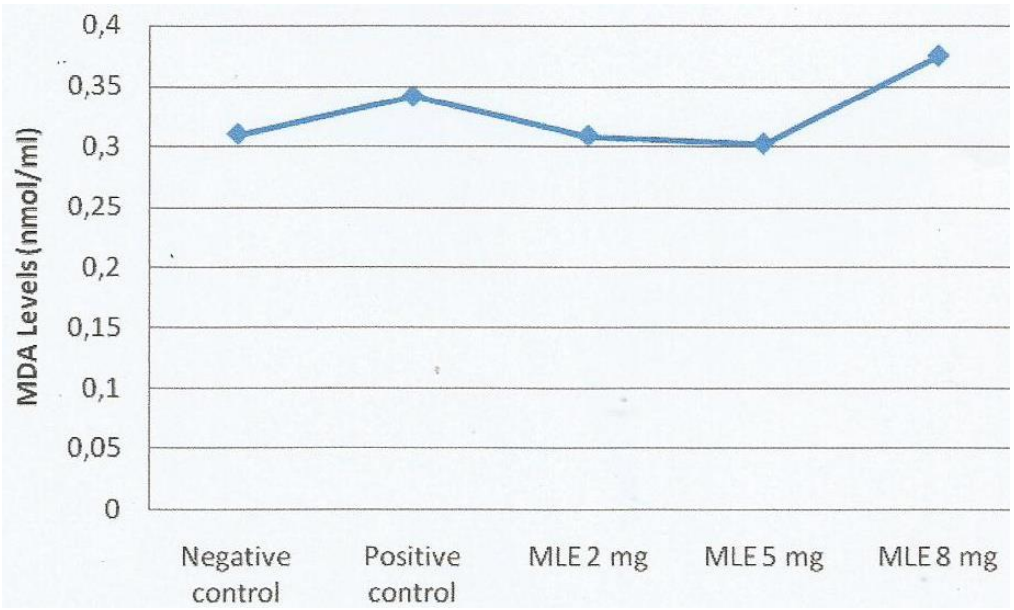

Figure 2. MDA Levels in Lung Tissue of Balb/c mice *(Mus musculus)*

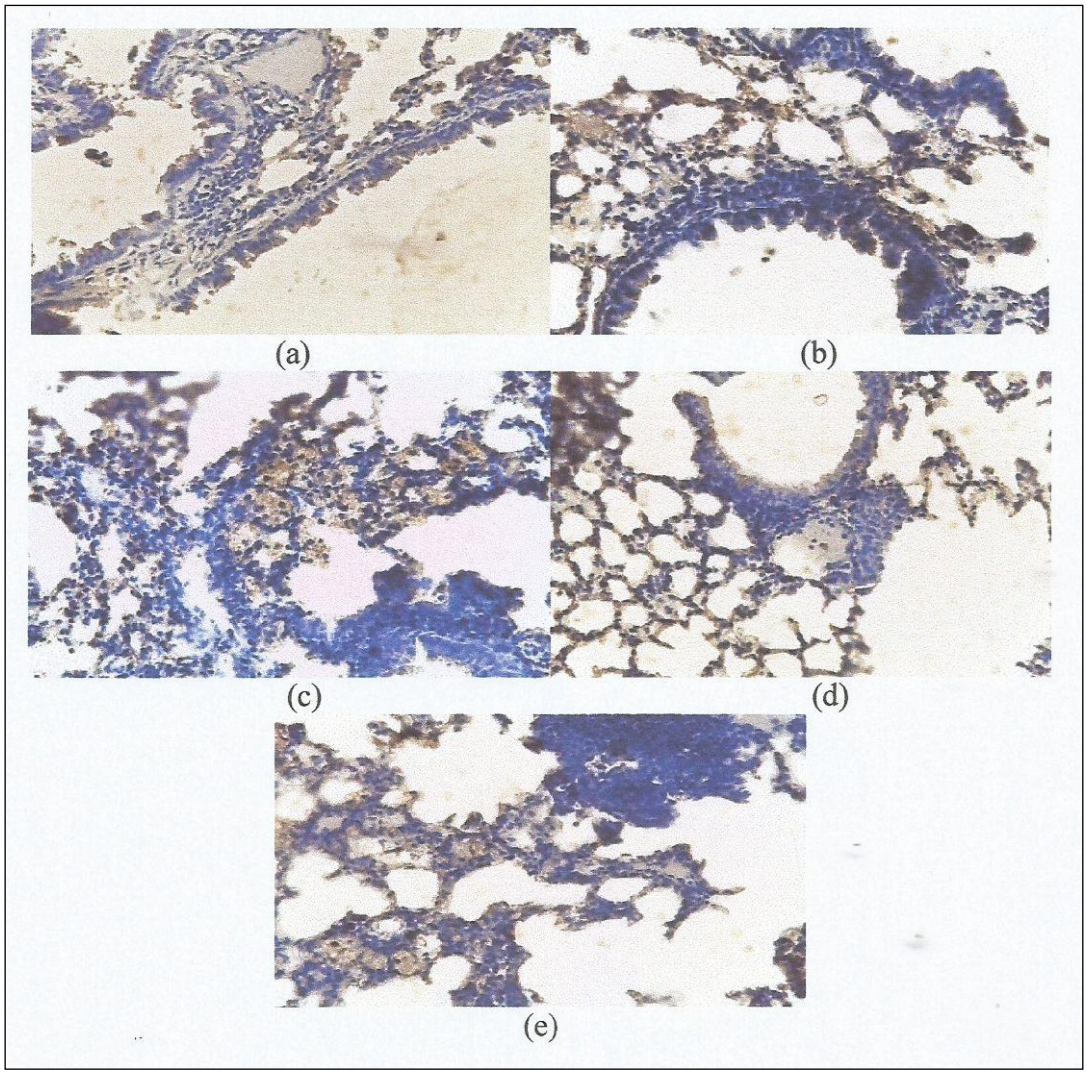

Figure 3. Expression of catalase in lung tissue of Balb/c mice *(Mus musculus)* by Imunohistochemical assay, 400x. Cells expressing catalase show a brown color. (a) Catalase expression in lung tissue of normal group. (b) Silica-treated group showing catalase expression decreasing. (c) MLE 2 mg/20 BW-treated group showing catalase expression increasing. (d) MLE 5 mg/20 BW-treated group showing catalase expression increasing. (e) MLE 8 mg/20 BW-treated group showing catalase expression decreasing.

DISCUSSION

Expression of Catalase

Catalase is endogenous antioxidant enzyme which catalyzes H_2O_2 into H_2O and O_2. The study result showed that silica-exposed groups had decreased catalase expression (Table 1). This is apparently due to silica could impair antioxidant system in the body[17]. Accordingly, it may impair the expression of antioxidant enzymes. Body has defense system to protect the body against ROS-induced oxidative stress via antioxidant system that consists of three line defense antioxidants. First, preventive antioxidants, i.e endogenous enzymes and minerals such as SOD, catalase, GPx, glutathione reductase, Se, Mn, Cu. Second, radical scavenger, such as glutathione, vitamin C, albumin, vitamin E, carotenoid, flavonoid. Beta-carotene scavenges singlet oxygen, vitamin C interacts with radicals such as O_2, OH. GSH acts as scavenger of free radicals such as O_2, OH, lipid hydroperoxide. Third, a group of enzymes which repairs damaged DNA, protein and lipids, and also biomolecular and cell membrane damage[18].

Enzyme activity in the body is highly influenced by enzyme cofactors, one of them is micronutrient *i.e.* selenium (Se). Se plays role in neutralizing process of ROS. Se acts as enzyme cofactor of GPx. A study involved patients with silicosis revealed reduced serum Se concentration[19]. Other study involved patients with silicosis also found different selenium level compared with control participants. Patients with silicosis were found to have a lower serum selenium concentration compared to healthy control[17].

The results showed that administration of *M. oleifera* leaves extract increased expression of catalase (Figure 1). This is due to *M. oleifera* leaves extract contains various compounds that could stimulate endogenous antioxidants. The results of identification of compounds in ethanol extract of *M. oleifera* leaves using thin layer chromatography revealed the presence of polyphenol, flavonoid, saponin, saturated sapogenin and unsaturated steroid compounds.

Table 1 showed that group treated with ethanol extract of MLE at dose 5 mg/20 g BW had the highest increase of catalase expression compared to those at dose 2 mg/20 g BW and 8 mg/20 g BW. *M. oleifera* leaves contain various bioactive compounds and nutrients which possess a lot of health benefits, e.g its function as antioxidant[20]. *M. oleifera* leaves are also the source of mineral and protein-rich food, and vitamin A, B, C and E[21]. A study of supplementation *M. oleifera* leaves powder could significantly improve serum GPx and SOD in postmenopausal women, and also decrease marker of oxidative stress i.e. MDA[22]. *M. oleifera* leaves extract also could restore antioxidant status in mice fed with high-fat diet[23].

The findings of identification of compounds in *M. oleifera* leaves extract revealed the presence of flavonoid. Flavonoid found in ethanol extract of Moringa leaves possess antioxidant activity[24]. In addition, the presence of saponin was also shown in *M. oleifera* leaves extract. Saponin is bioactive compound with potential antioxidant capacity. A study conducted by Muhammad *et al.* (2017) showed that triterpenoidsaponins from the stems and bark of *Jaffrea xerocarpa*exhibited antioxidant activity in DPPH assay[25]. Other study also showed that administration of flavonoid, quercetin, could improve catalase activity in the bleomycin-treated lung[26].

Malondialdehyde Levels

Measurement of MDA level is an indirectly assessment of free radical activity, thus what can be measured are products of the damage produced by free radicals, not directly the free radical compounds. In the study, MDA level in lung tissue was measured as the end-product of lipid peroxidation. The result showed the significant difference of MDA levels between groups ($p<0.05$). The result of Post-Hoc analysis with LSD method is presented in Table 1. It shows a significant difference of MDA levels between negative and positive control group ($p<0.05$), negative control group and MLE 8 mg/20 g BW group ($p<0.05$), positive control group and MLE 2 mg/20 g BW ($p<0.05$), positive control group and MLE 5 mg/20 g BW ($p<0.05$), positive control group and MLE 8 mg/20 g BW ($p<0.05$). MDA level of positive control group was higher than that of negative control group. The lowest MDA level was found in MLE 5 mg/20 g BW group, 0.3024±0.0328 nmol/ml, indicating that the dose level is able to decrease MDA.

Exposure of silica particle could cause any changes in the body, particularly the lungs. It results in some effects such as ROS formation and antioxidant imbalance in the body. Generation of oxidants by silica particles and by silica-activated cells results in the increased lipid peroxidation, cell and lung injury[19]. The present study did not perform directly measurement of ROS. Instead, MDA level as a product of lipid peroxidation by ROS was measured. The results showed that silica-exposed mice group had higher MDA level than control group. It indicated that exposure of silica particle leads to lipid peroxidation. It is similar to the study in humans. A study conducted in foundry plants which frequently exposed to metal dust and silica dust showed that among foundry workers, plasma MDA levels of exposure group were higher than that of in control group[27]. Administration of intratracheal silica to female Balb/c mice also elevated serum H_2O_2 level[28]. This indicated that silica could increase ROS in both animals and humans.

The study results showed that administration of MLE 2 mg/20 g BW and 5 mg/20 g BW could significantly decrease MDA level in lung tissue of mice. It proves that *M. oleifera* leaves possess antioxidant activity. These results were supported by *in vitro* study of *M. Oleifera*leaves extract through DPPH assay which showed its high antioxidant activity expressed as IC_{50}: 49.30µg/mL[29]. Other study involved Wistar rats treated with swimming test and aqueous extract of *M. Oleifera* leaves showed increased activity of antioxidant enzymes and decreased blood concentration of MDA[30]. There was a negative correlation between serum MDA and total antioxidant status and GPx in red blood cell of Wistar rats treated with Moringa leaves. Serum MDA was observed to be significantly lower in rats fed with Moringa leaves than that of in control group. Total antioxidant status was also significantly higher in treatment group[31]. Various compounds in *M.oleifera* leaves extract are potential antioxidants in preventing oxidative stress or silica-induced lipid peroxidation. Vitamin content in *M. oleifera* leaves possibly plays role in reducing ROS. Saxena and Singh (2012) showed in their study that administration of vitamin C and vitamin E may recover histology of testis and epididymis of albino rats exposed to silica intraperitoneally[32].

Many natural antioxidants possess ability to inhibit oxidative stress through various mechanisms. Phenolic compounds in plants could be the source of natural antioxidants[33]. A study of ant-plant (*Myrmecodiapendans*) administration which contains antioxidants such as polyphenol, flavonoid, and tannin could increase serum SOD level and decrease serum MDA level of rats exposed to Pb-acetate[34]. Identification result of ethanol extract of *M. oleifera* leaves also found the presence of saponin. This compound also works as potential antioxidant. A study

of administration of saponin extracted from the root of *Garcinia kola* (Bitter kola) in diabetic animals model showed that saponin could decrease MDA level and increase SOD and catalase activity[35].

A study of saponin fraction extracted from stem bark of *Erythropheleum suaveolens* showed that it may inhibit lipid peroxidation and prevent free radical-induced damage[36]. Singh *et al*. (2017) reported that saponins in pulses were found to be beneficial in promoting health, which worked as antioxidant[37]. Other study showed that aqueous extract of *M. oleifera* leaves could inhibit lipid peroxidation in testis tissue by forming a complex with Fe^{2+}, thus preventing the initiation of lipid peroxidation[38]. Thus, saponins present in *M. oleifera* leaves may possibly decrease MDA level.

Flavonoid possess antioxidant activity through some mechanisms, such as scavenging free radicals, chelating metal, suppressing enzyme related to free radical formation, and stimulating internal antioxidant enzymes[39]. However, high dose of flavonoid molecules could possibly act as pro-oxidant[40]. The study results showed that administration of MLE at dose 8 mg/20 g BW to mice increased the MDA levels (Figure 2). This finding is in accordance with an *in vitro* study that showed administration of *M. oleifera* leaves extracted in ethanol 80% decreased oxidative stress, but administration of the extract at high dose (2000-3000 mg/l) could induce cytotoxicity[41]. Increase of MDA level could be caused by many factors. Environmental factor and prolonged treatment may influence the increase of MDA level. In addition, compounds in *M. oleifera* could possibly act as pro-oxidant.

In certain condition, flavonoid could act as pro-oxidant, which promotes oxidation in other components[42]. Pro-oxidant and antioxidant effect of flavonoid depends on its concentration[42],[43]. This is in accordance with a study by Shobot and Hadacek (2011) that found flavonoid antioxidant (myricetin) may also act as pro-oxidant. Pro-oxidant could occur due to oxidation process by phenolic flavonoid radicals, inhibition of mitochondrial respiration, absorption inhibition of low-molecular-weight antioxidants[43]. Pro-oxidant effect could also be beneficial[42]. The use of appropriate dose is crucial to obtain optimal benefit.

CONCLUSION

Ethanol extract of *M. oleifera* leaves 2 mg/20 g BW and 5 mg/20 g BW could inhibit oxidative stress in lung tissue of mice (*Mus musculus*) exposed to silicon dioxyde particle, which demonstrated by increased expression of catalase, and decreased MDA levels in lung tissue. These indicate that ethanol extract of *M. oleifera* leaves contains various compounds that work as potential antioxidant, such as phenolic, flavonoid, and saponin compounds. MDA levels affected by catalase expression.

Further study related to the effect of *M. oleifera* leaves extract on other antioxidant enzymes such as, SOD and GPx is needed to analyze the mechanism of ethanol extract of *M. oleifera* leaves in preventing oxidative stress-induced lung damage.

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