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# The numbers of Lymphocytes and Capillaries in Silica-exposed Lung Tissues of Balb/c Mice *(Musmusculus)* treated with *Moringaoleifera* leaves extract

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**ABSTRACT**

This study was conducted to describe the effect of *M. oleifera* leaves extract (MLE) on lymphocytes and capillaries numbers in silica-exposed lung tissues of Balb/c mice (*Musmusculus*). This was an experimental study with randomized posttest-only control group design using 30 male Balb/c strain mice (*Musmusculus*), 8-10 weeks, weighing 20-30g, which were divided into five groups. The groups were negative control, positive control, treatment group with silica exposure and treated with MLE 2 mg/20g BW, 5 mg/20g BW, and 8 mg/20g BW. Positive control and treatment group were treated with suspension of silica particles intratracheally at concentration 2.5 mg which were dissolved in 60 µL saline solution. After 90 days, mice were sacrificed. The variables were MLE, silica exposure, lymphocytes and capillaries number. Results showed that the treatment groups showed lower numbers of lymphocytes and higher numbers of capillaries than the positive control group. The numbers of capillaries in lung tissues of mice treated with MLE at all doses were higher than those of positive control group. Meanwhile, the numbers of lymphocytes in treatment groups treated with MLE at all doses were lower than those of positive control group. However, there was no statistically significant difference (One Way ANOVA, $p>0.05$). In conclusion, administration of MLE potentially decrease the lymphocytes numbers and increase the numbers of capillaries in silica-exposed lung tissues of mice. *M. oleifera* leaves are potential in preventing diseases.

***Key words:*** *Lymphocytes, Lung, M. oleifera, Capillaries, Silica, Mice*

## Introduction

Silica particle is one of dust components widely distributed on earth's surface that potentially lead to lung problems. Studies in some areas exposed to dust containing silica have been conducted (Lin *et al.*, 2011). Silica particles may lead to lung damage. The damage can result from direct cytotoxicity of silica and ROS generated from the reactive surface of silica. Lung damage can also result from phagocytosis process. This process involves the recruitment of inflammatory cells, the release of inflammatory mediators, fibrogenic factors, and production of ROS (Castranova, 2004). Exposure of silica could

increase lymphocytes in lung tissues of animal models (Abdelaziz *et al.*, 2016).

Moringa leaves contain high vitamin C (Singh *et al.*, 2012). Moreover, it also contains microminerals which are necessary for various enzymes (Moyo *et al.*, 2011). Moringa leaves contains flavonoid compounds. Flavonoid is potential antiinflammatory and plays role in proliferation of capillaries. The benefit of moringa that widely studied includes the use of its leaves to treat other diseases such as hypertension, allergy, fever, malnutrition, diabetes and asthma (Kasolo *et al.*, 2010). However, the use of moringa leaves to prevent diseases induced by exposure of air pollution is limited. The study aimed to investigate the effect of *M. oleifera* leaves on lymphocytes and capillaries numbers in lung tissues of mice.

## Materials and Methods

### Plant Material

The leaves of *M. oleifera* were obtained from Sokon Village, Kupang East Nusa Tenggara. The plant identification was did in biosystematics laboratory of Department of Biology, Faculty of Science and Technology, Airlangga University.

### Animal Models

Thirthty male Balb/c strain mice *(Musmusculus),* 20-30 grams, 8-10 weeks, were obtained from biochemistry laboratory, Faculty of Medicine, Airlangga University. Research procedures were approved by Ethics Committee of Health Study, Faculty of Public Health, Airlangga University, No.544-KEPK. Mice were divided into five groups. The groups were negative control, positive control, treatment group with silica exposure and treated with *M. oleifera* leaves extract 2 mg/20 g BW, 5 mg/20 g BW, and 8 mg/20 g BW. The extract was administered via oral 10 days before and 90 days after silica exposure. Mice were sacrificed by cervical dislocation. Right lung was put into neutral buffered formalin (10%).

### Extraction of *M. oleifera* leaves

*M. oleifera* leaves were extracted in Phytochemistry Laboratory, Faculty of Pharmacy, Airlangga University using maceration technique with 96% ethanol as a solvent.

### Silica Particle Exposure

This study used silica particles $SiO_2$ (Sigma Aldrich) 80%, size 1-5µm. Itswere sterilized at 200 $^oC$ for 2 hours, then weighed as 2.5 mg and suspended in 60 µL saline solution (Moore *et al.*, 2013).

### Measurement of lymphocytes and capillaries numbers

Measurement of lymphocytes and capillaries numbers was performed with hematoxylin-eosin staining.

### Statistical Analysis

The data obtained are presented as mean ± standard deviation (SD). Comparison of data between groups was done using One-way ANOVA and Brown-Forsythe (CI 95%).

## Results and Discussion

### The Numbers of Lymphocytes

The numbers of lymphocytes of treatment groups treated with ethanol extract of *M. oleifera* leaves at dose 2 mg/20 g BW, 5 mg/20 g BW and 8 mg/20 g BW were lower than those of positive control group. But the result showed no significant difference of lymphocytes numbers between groups ($p>0.05$).

Silica exposure may cause inflammatory response in lung tissues. Inflammatory process will keep continuing until antigen removal. If the causative agent of inflammation cannot be removed and exposure is repeated, chronic inflammation may occur. The study results showed that the mean numbers of lymphocytes of positive control group exposed to silica was higher than that of negative control group. Proinflammatory transcription factors could be activated by particle exposure (vrevik *et al.*, 2015). Phagocytosis process of silica particles may generate ROS. Generation of oxidants by silica particles results in the increased expression of inflammatory cytokines (Pandey and Agarwal, 2012).

**Table 1.** Mean and Standard Deviation of Lymphocytes Numbers Score in Lung Tissue of Balb/c Mice *(Musmusculus)*

| Kelompok | Lymphocytes Numbers Score |
|---|---|
| Negative Control | 1.17 ±0.41 |
| Positive Control | 1.83 ±0.98 |
| MLE 2 mg | 1.67 ±1.03 |
| MLE 5 mg | 1.67 ±0.82 |
| MLE 8 mg | 1.50 ±0.84 |
| Test | *One Way ANOVA* |
| *p value* | 0.71 |

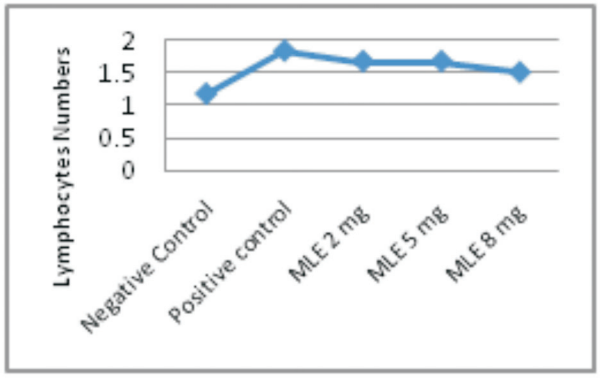


**Fig. 1.** Lymphocytes Numbers in Lung Tissue of Balb/c Mice *(Musmusculus)*

The study results showed that the numbers of lymphocytes of groups treated with *M. oleifera* leaves extract were lower than those of positive control group. Decreased numbers of lymphocytes presumably results from antioxidant activities of *M. oleifera* leaves which can inhibit ROS production, accordingly ROS-induced inflammatory process can also be inhibited. Furthermore, it also may be due to various potential compounds with anti-inflammatory properties in *M. oleifera* leaves (Saini *et al*., 2016). Other study found that phenolic content in ethyl acetate fraction of *Moringa oleifera* exhibited potent anti-inflammatory activity. An *in vitro* study of human macrophages showed that ethyl acetate fraction of *Moringa oleifera* could inhibit cytokines (IL-8) production (Kooltheat *et al*., 2014).

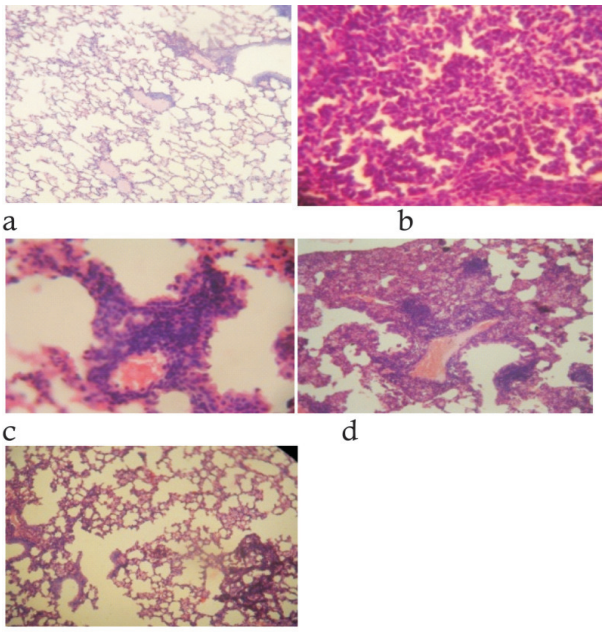


**Fig. 2**. Hematoxylin-eosin Staining of Lymphocytes in Lung Tissue of Balb/c Mice *(Musmusculus)* (a) Negative Control (b) Positive control (c) MLE 2 mg (d) MLE 5 mg (e) MLE 8 mg

Identification of compounds in ethanol extract of *M. oleifera* also reveals the presence of saponin. Besides its antioxidant activity, saponin also has anti-inflammatory acivity (Moghimipour and Handali, 2015). Other study showed that saponin found in legumes was beneficial for health as an antiiflammatory agent (Singh *et al.*, 2017). Triterpenoid-saponins from *Ilex pubescens* roots also showed anti-inflammatory activities (Wu *et al.*, 2017). It suggests that saponin content in *M. oleifera* leaves is likely to have anti-inflammatory activities.

**The numbers of capillaries**

The numbers of capillaries of groups treated with ethanol extract of Moringa leaves at dose 2 mg/20 g BW, 5 mg/20 g BW and 8 mg/20 g BW were higher than those of positive control group. But the result showed no significant difference of capillaries numbers between groups ($p>0.05$).

The study results showed that the mean numbers of capillaries in silica-exposed lung tissues of mice treated with ethanol extract of *M. oleifera* extract were higher than that of positive control. Increased numbers of capillaries indicates the presence of body's defense mechanism. Inflammatory process promotes inflammatory cell infiltration to the injured sites. Proliferation of blood vessels is promoted by mediators such as VEGF and FGF (Mura *et al*., 2004).

Increased mean numbers of capillaries of groups treated with ethanol extract of *M. oleifera* leaves indicates that administration of the extract has effect on generation of capillaries. It is likely that ethanol extract of Moringa leaves contains compounds with its effect on generation of capillaries. However, further study is required to prove this.

**Table 2.** Mean and Standard Deviation of Capillaries Numbers in Lung Tissue of Balb/c Mice *(Musmusculus)*

| Group | Capillaries Numbers |
|---|---|
| Negative Control | 8.33 ±5.99 |
| Positive Control | 8.67 ±3.26 |
| MLE 2 mg | 9.50 ±8.55 |
| MLE 5 mg | 12.83 ±4.87 |
| MLE 8 mg | 11.00 ±2.97 |
| Test | Brown-Forsythe |
| *p value* | 0.62 |

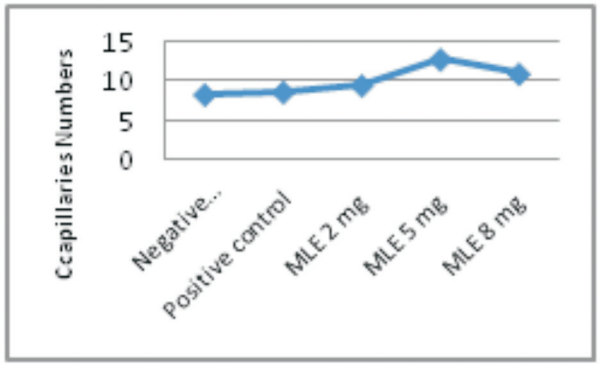


**Fig. 3.** Capillaries Numbers in Lung Tissue of Balb/c Mice *(Musmusculus)*

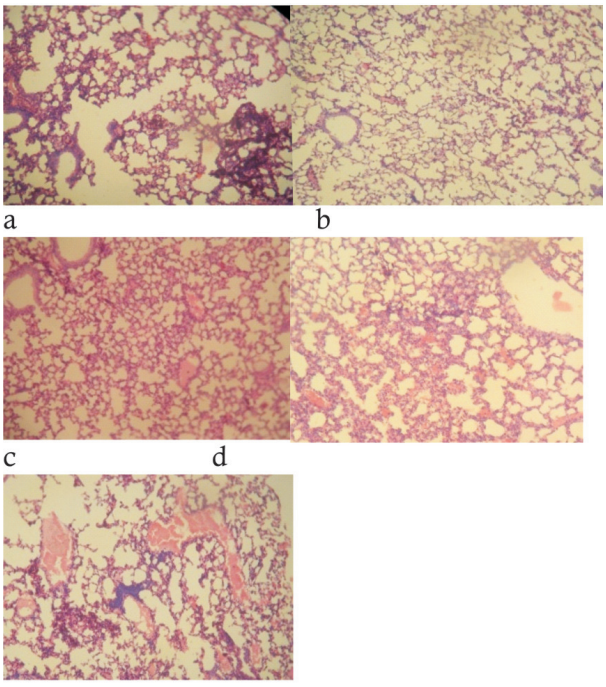


**Fig. 4.** Hematoxylin-eosin Staining of Capillaries in Lung Tissue of Balb/c Mice *(Musmusculus)* (a) Negative Control (b) Positive control (c) MLE 2 mg (d) MLE 5 mg (e) MLE 8 mg

Flavonoid compound have anti-inflammatory activities that could shorten inflammatory period, therefore proliferative phase may occur early that stimulates generation of new capillaries. During proliferative phase, it is likely that macrophages M2-polarization act as key players with its regenerative properties characterized by production of VEGF that promotes the process of wound healing (Sica *et al.*, 2015). Flavonoid content in *M. oleifera* leaves extract apparently increase secretion of VEGF. It is in accordance with other study that revealed quercetin could improve skin wound healing (Gopalakrishnan *et al*., 2016). A study of flavonoid fraction isolated from the stem bark of *Buteamonosperma* (Lam) in albino wistar rats also showed the significant wound healing activity (Muralidhar *et al.*, 2013).

*M. oleifera* leaves possess potential compounds for health. A study in South Africa showed that total phenolic and flavonoid contents in *M. oleifera* leaves were higher than other vegetables such as broccoli, cabbage, spinach, cauliflower, and peas (Pakade *et al*., 2013). Other study revealed that Moringa leaves extract possessed wound healing activity (Mehra *et al*., 2017). Increased numbers of capillaries promotes better supply of oxygen and nutrition, accordingly results in good repair of the injured tissues.

## Conclusions

Administration of ethanol extract of *M. oleifera*leaves is potential to decrease the numbers of lymphocytes and increase the numbers of capillaries in silica-exposed lung tissues of mice. In further study, it is recommended to use different concentration of silica and method of silica administration. *M. oleifera* leaves are potential in preventing diseases.

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