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## 7 The Effect of *Hibiscus rosa-sinensis* L. Extract on Improvement of Macrophage Phagocytosis Activity

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### 11 ABSTRACT

**Background:** Diabetes mellitus (DM) is a risk factor of tuberculosis, that hyperglycemia conditions causing impaired immune responses including phagocyte function so that it can facilitate *M. tuberculosis* infection. Therefore, it takes therapy to improve the immune system, one of them by using *Hibiscus rosa-sinensis* L. extract as immunostimulant.

**Aim:** To analyze the effect of extract *H. rosa-sinensis* L. on macrophage phagocytosis activity in animal TB-DM model.

**Method:** The research was an experimental used 9 male Wistar rats, 8 weeks-old, weight 200-230grams, divided into 3 treatment groups i.e. K (control), P1 (positive TB-DM), P2 (positive TB-DM treated with *H. rosa-sinensis* L. extract), and treated for 21 days. Animal TB-DM model was prepared with the administration of 100mg/kg nicotinamide (NA) and 65mg/kg streptozotocin (STZ), and injected with  $1.5 \times 10^5$  CFU *M. tuberculosis*. Macrophage activity test was performed using intraperitoneal fluid that staining with Giemsa. Phagocytosis activity was determined based on the number of macrophage cells in 100 cells. The data were obtained from that test, then analyzed using *Kruskal Wallis*.

**Result:** The macrophage activity test showed that the mean macrophage activity in group K was  $57 \pm 2.082$  cells, P1 group was  $46 \pm 2.517$  cells, and P2 group was  $82.33 \pm 2.404$  cells. The result of statistic analysis showed that *H. rosa-sinensis* L. extract was able to increase macrophage activity significantly ( $P = 0.027$ ).

**Conclusion:** *H. rosa-sinensis* L. extract increase the activity of peritoneal macrophages phagocytosis activity in animal TB-DM model.

**Keywords:** *Hibiscus rosa-sinensis* L., TB-DM, macrophage

### 10 I. Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis*, is the leading cause of death among bacterial infectious diseases in the world. Diabetes Mellitus (DM) is a metabolic disorder caused by a defect of secretion

and even insulin action, is one of the risk factors for TB inflammation. Diabetics have impaired immune response, so it can facilitate *M. tuberculosis* infection. Diabetic patient's have 2-3 times higher risk of developing TB disease than non-diabetic patients.<sup>1,2</sup> The DM condition can increase the frequency and severity of the infection, due to abnormalities in cell-mediated immunity and phagocyte function associated with hyperglycemia, including damaged vascularization.<sup>3</sup>

Macrophages are the phagocyte cells that play a major role in inflammation and body defense. Macrophages differentiate from monocytes and are distributed in various tissues, organs, and intraperitoneal. Intraperitoneal macrophages are the most mature macrophages, the largest in size, and have high phagocytic capacity and complete receptor.<sup>4,6</sup> Adaptive immune system dysfunction and decreased macrophage phagocytosis activity will increase the risk of DM infections, including *M. tuberculosis* infection.<sup>7-11</sup>

Treatment of TB-DM cases, one of them emphasizes the return of immune system abnormalities functions, which can be done by using immunostimulant that can stimulate the immune system.<sup>12</sup> The mechanism of immunostimulant is stimulating the immune system by phagocytosis.<sup>13-15</sup> Immunostimulant can be obtained from a variety of natural materials, one of which is *Hibiscus rosa-sinensis L.* This plant Malvaceae from East Asia and is widely grown as a plant in the tropics and subtropics. This study is aimed to analyze the effect of extract *H. rosa-sinensis L.* on macrophage phagocytosis activity in animal TB-DM model.

## II. Methods

This laboratory works were performed in Microbiology Laboratory of Faculty of Medicine Diponegoro University on June-November 2017. The experimental animals used were male Wistar rats, 8 weeks old, weight 200-230grams, in good health, activity, and normal behavior. The animals were grouped into 3 treatment groups, control (K): group of normal control rats; P1: TB-DM positive group; P2: TB-DM group treated with 250mg/kg *H. rosa-sinensis L.* extract. TB-DM model was performed with the administration of 100 mg/kg nicotinamide (NA) and 65mg/kg streptozotocin (STZ), and injected with  $1,5 \times 10^5$  CFU *M. tuberculosis*. Animals care were tried for 21 days.

Ethical clearance was provided by Ethical Commission of Health Research (KEPK) Faculty of Medicine Diponegoro University and RSUP Dr. Kariadi Semarang (EC number 15/EC/H2K-RSDK/V/2017). Most of the laboratory works were performed in Department of Microbiology Central Laboratory Faculty of Medicine Diponegoro University.

### *H. rosa-sinensis L.* Extraction

*H. rosa-sinensis L.* that has been cleaned, cut into smaller pieces and dried at 40-60°C, blended becomes a homogenous powder,<sup>35</sup> then extracted by maseration method using 70% ethanol (maserate:solvent = 1:10). After 2-3 days, the maserate is filtered using a filter paper, then evaporated using a rotary vacuum evaporator at 40°C to obtain 100% thickened extract.

*H. rosa-sinensis L.* extract used was a 60µg/ml. The soluble extract in sterile aquadest is added to RPMI medium containing 10% Heat Inactivated Pooled Human Serum (HI-PHS), 50µl for each ml medium. The maximum volume that can be given to the animals is 5ml per oral each day.

### Preparation *M. tuberculosis* Suspension

An ose of platinum ( $10^6$  CFU/ml) of *M. tuberculosis* H37Rv ATCC 7294<sup>T</sup> (Sigma) strain were suspended aseptically into tube containing 5ml RPMI containing 10% HI-PHS and  $\pm$  6-8 bead glass, then homogenous vortex. Supernatant was equivalent  $7,5 \times 10^5$  per ml (S) *M. tuberculosis* then suspended at 3,8ml RPMI (S1) to obtain  $1,5 \times 10^5$  CFU/ml *M. tuberculosis*. As much of 200µl ( $1,5 \times 10^5$  CFU) *M. tuberculosis* was injected to the animals.

**Animals Treatment**

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The experimental animals used were male Wistar rats, 8 weeks old, weight 200-230 grams, in good health, activity, and normal behavior. The animals were grouped into 3 treatment groups, control (K): group of normal control rats; P1: TB-DM positive group; P2: TB-DM group treated with 250mg/kg *H. rosa-sinensis L.* extract. TB-DM model was performed with the administration of 100mg/kg nicotinamide (NA) and 65mg/kg streptozotocin (STZ), and injected with  $1.5 \times 10^5$  CFU *M. tuberculosis*. The maximum volume of diabetic agent that can be given intraperitoneally to the animals is 2-5ml. Animals care were tried for 21 days.

**Table I.** The Animals Group

3 Group of Animals	Week of Termination Group		
	1	2	3
K : Group of normal control	9	9	9
P1 : TB-DM positive group	9	9	9
P2 : TB-DM group treated with 250 mg/kg <i>H. rosa-sinensis L.</i> extract	9	9	9

Blood glucose evaluation was performed on the fifth day after injection of STZ. The animals were categorized as having diabetes when their blood glucose levels are  $\geq 250$ mg/dL (7,8mmol/L).

**Macrophages Activity Test**

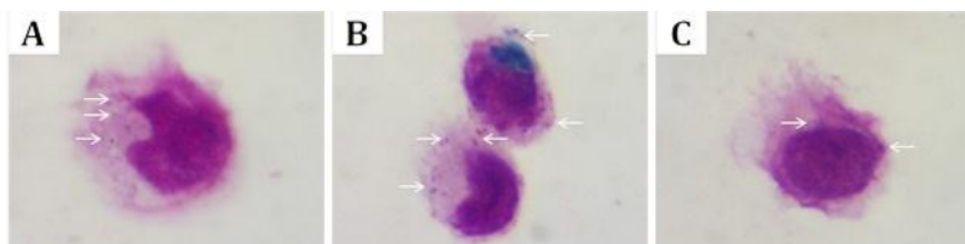
In termination process, intraperitoneal fluid is taken to see macrophages activity using Giemsa staining. Phagocytosis activity was determined based on the number of macrophage cells.

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**III. Data Analysis**

The data came from the result of the macrophage microscopic observation of the samples and the number of macrophage cells in 100cells.<sup>16</sup> The numeric data then analyzed using *Kruskal Wallis*.

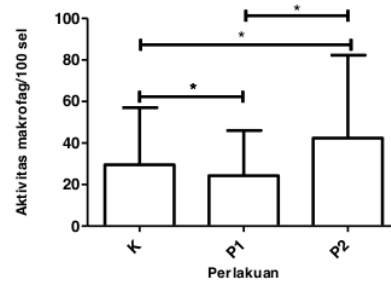
**Results**

The study result showed that macrophages from control group has a regular round shape, single nucleus and "horse-shoe" shape. Macrophages were obtained from P1 group has an irregular round shape. Macrophages from P2 group also has an irregular round shape with a microphili/ pseudopodies, single nucleus, and some macrophages indicate the presence of giant cells. Macrophage activation due to addition of *H. rosa-sinensis L.* extract is seen from the morphological view, which is macrophages were larger with wider pseudopodies than the others (Figure 1).



**Figure 1.** Peritoneal macrophage from group K; (B) Peritoneal macrophage from group P1; (C) Peritoneal macrophage from group P2. Arrows indicate the presence of *M. tuberculosis*.

The result of macrophage phagocytosis activity showed that the mean of macrophage activity in group K was  $57 \pm 2,082$  cells; group P1 was  $46 \pm 2,517$  cells; and group P2 was  $82,33 \pm 2,404$  cells as shown in Figure 2.



**Figure 2.** The mean of macrophage phagocytosis activity.

The largest macrophage activity was seen in group P2, and the lowest was P1. The macrophage activity P2 group significantly different both with control group (K) ( $p = 0,05$ ) and the P1 group ( $p = 0,05$ ). Macrophage activity between K group and P1 group also had significant differences ( $p = 0,05$ ). Statistical analysis showed that *H. rosa-sinensis* L. extract was able to significantly increase macrophage activity ( $P = 0,027$ ).

#### IV. Discussions

Differences in phagocytosis activity between groups of K and P1 indicated that immune cells have defects in DM/ hyperglycemia conditions, so their macrophages become less activated. In addition, chemotactic disorders, phagocytosis, and antigen presenting to *M. tuberculosis* occur; monocyte chemotaxis does not occur in DM patients.<sup>17</sup>

The disturbance of phagocytosis is also due to intrinsic defects of PMN.<sup>18</sup> This is accordance with the results of this study which the macrophage phagocytosis activity of TB-DM group is lower than healthy conditions.<sup>24,25</sup>

Significant increases in phagocytosis activity which added with *H. rosa-sinensis* L. are affected by the ability of the various active substances contained there in, *i.e.* steroids, alkaloids, phenolics, saponins, and tannins.<sup>19</sup> The addition of this extract shows that there is a significant increase in phagocytic activity in cases of TB-DM.<sup>24,25</sup> *H. rosa-sinensis* L. extract proved able to inhibit bacterial growth with concentration 1000  $\mu\text{g}/\text{mL}$ , and able to show eradication of *M. tuberculosis* and *Escherichia coli*.<sup>20</sup> This function can be obtained because it contains saponins, tannins, and phenols that act as antimicrobials. In addition, the ethanolic extract of *H. rosa-sinensis* L. gives an anabolic effect with marked weight gain and testicular weight in the experimental animal.<sup>21</sup> The ethanolic extract of *H. rosa-sinensis* L. can also decreased blood glucose levels, so that hyperglycemia conditions that affects to inflammation can be suppressed.<sup>22,23</sup> This may be possible because the antioxidant contained in the extract works to resemble the sulphonyl urea mechanism, so the extract is able to control the hyperglycemia, thus improve the abnormalities of immune systems.

#### V. Conclusions

*H. rosa-sinensis* L. extract significantly increased peritoneal phagocytosis macrophage activity of TB-DM animal model ( $P = 0,027$ ).

#### VI. Acknowledgement

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Disclaimer: all authors report no conflict of interest relevant to this article.

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