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

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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 $1{,}5x10^5$ 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 ± 2.082 cells, P1 group was 46 ± 2,517 cells, and P2 group was 82.33 ± 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

I. Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis,* is the leading cause of deathn 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.[1, 2] The DM condition can increase the frequency and severity of the infection, due to abnormalities in cell-mediated immunity and phagocyte function associated with hyperglicemia, including damaged vascularization.[3]
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.[4-6] Adaptive immune system dysfunction and decreased macrophage phagocytosis activity will increase the risk of DM infections, including *M. tuberculosis* infection.[7-11]

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.[12] The mechanism of immunostimulant is stimulating the immune system by phagocytosis.[13-15] Immunostimulant can be obtained from a variety of natural materials, one of which is *Hibiscus rosa-sinensis L.* This plant is 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 $1{,}5x10^5$ 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/H/FK-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^{o}C, blended becomes a homogenous powder,[35] 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 vaccum evaporator at 40^{o}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-27294^T (Sigma) strain were suspended aseptically into tube containing 5ml RPMI containing 10% HI-PHS and ± 6-8 bead glass, then homogenous vortex. Supernatant was equivalent 7,5 x 10^5 per ml (S) *M. tuberculosis* then suspended at 3,8ml RPMI (S1) to obtain 1,5 x 10^5 CFU/ml *M. tuberculosis.* As much of 200µl (1,5 x 10^5 CFU) *M. tuberculosis* was injected to the animals.

Animals Treatment

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 $1,5x10^5$ 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 l. The Animals Group

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 *H. rosa-sinensis L.* extract	9	9	9

Blood glucose evaluation was performed on the fifth day after injection of STZ. The animals were catagorized as having diabetes when their blood glucose levels are ≥ 250mg/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.

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.[16] The numberic 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).

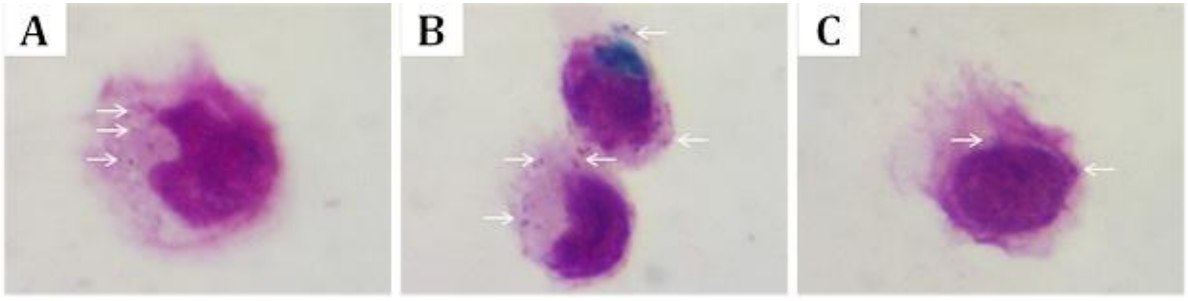

Figure 1. Peritoneal macrophage from group K; (B) Peritoneal macrophage from group P1; (C) Peritoneal macrophage from group P2. Arrows indicate the presence of *M. tuberculosis*.

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

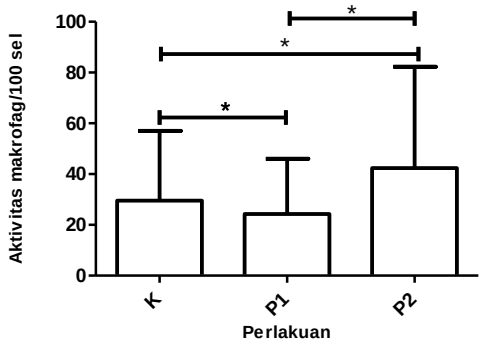

Figure 2. The mean of macrophage phagocytosis activity.

The largest macrophage activity was seen in group P2, and the lowest was P1 group. 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, chemotaxic disorders, phagocytosis, and antigen presenting to *M. tuberculosis* occur; monocyte chemotaxis does not occur in DM patients.[17]

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

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.[19] The addition of this extract shows that there is a significant increase in phagocytic activity in cases of TB-DM.[24, 25] *H. rosa-sinensis L.* extract proved able to inhibit bacterial growth with concentration 1000 µg/mL, and able to show eradication of *M. tuberculosis* and *Escherichia coli*.[20] 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.[21] 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.[22, 23] 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.

References

[1.] International Diabetes Federation. Diabetes and Tuberculosis. 2013. http://www.idf.org/diabetes/5e/diabetes-and-tuberculosis

[2.] World Health Organization. Global Tuberculosis Control. Geneva: *World Health Organization.* 2010.

[3.] Jeon CY, Murray MB. Diabetes Mellitus Increases The Risk of Active Tuberculosis: A Systematic Review of 13 Observational Studies. *PLoS Med*. 2008;15(7):e152.

[4.] Zhang X, Goncalves R, Mosser DM. The Isolation and Characterization of Murine Macrophages. *Curr Protoc Immunol.* 2008; Chapter 14: Unit–14.1.

[5.] Wang C, Yu X, Cao Q, Wang Y, Zheng G, Tan TK. *et al*. Characterization of Murine Macrophages from Bone Marrow, Spleen and Peritoneum. *BMC Immunol.* 2013; 14: 6.

[6.] Italiani P. and Boraschi D. From Monocytes to M1/M2 Macrophages: Phenotypical vs. Functional Differentiation. *Front Immunol.* 2014; 17(5): 514.

[7.] Wang W, Wang J, Dong SF, Liu CH, Italiani P, Sun SH. *et al*. Immunomodulatory Activity of Andrographolide on Macrophage Activation and Specific Antibody Response. *Acta Pharmacol Sin.* 2010. 31(2): 191-201.

[8.] Vega VL, Charles W, Alexander LEC. Rescuing of Deficient Killing and Phagocytic Activities of Macrophages Derived from Non-Obese Diabetic Mice by Treatment with Geldanamycin or Heat Shock: Potential Clinical Implications. *Cell Stress Chaperones*. 2011; 16(5): 573-81.

[9.] Espinoza-Jiménez A, Peón AN, Terrazas LI. Alternatively Activated Macrophages in Types 1 and 2 Diabetes. *Mediators Inflamm*. 2012;815953.

[10.] Liu HF, Zhang HJ, Hu QX, Liu XY, Wang ZQ, Fan JY. *et al.* Alteredpolarization, Morphology, and Impairedinnate Immunity Germane to Resident Peritoneal Macrophages in Mice with Long-Term Type 2 Diabetes. *J Biomed Biotechnol*. 2012; 867023.

[11.] Parsa R, Andresen P, Gillett A, Mia S, Zhang XM, Mayans S. *et al.* Adoptive Transfer of Immunomodulatory M2 Macrophages Prevents Type 1 Diabetes in NOD Mice. *Diabetes.* 2012; 61(11): 2881-92.

[12.] Baratawidjaja KG. Imunologi Dasar. Jakarta. *FKUI Press.* 1996.

[13.] Orsi RO. Immunomodulatory Action on Macrophage Activation. *J Venom Anim Toxin*. 2000; 6(2): 205-19.

[14.] Price SA, dan Wilson L M. Patofisiologi Konsep Klinis Proses-proses Penyakit. Edisi 6. Jakarta: *EGC.* 2005.

[15.] Petrunov B, Nenkov P, Shekerdjisky R. The Role of Immunostimulants Inimmunotherapy and Immunoprophylaxis. Bulgaria: *BulBio-NCIPD Natsim Ltd.* 2007.

[16.] Okoli CO, Akah PA, Onuoha NJ, dan Okoye TC. *Acanthus montanus*: An Experimental Evaluation of The Antimicrobial, Anti-Inflammatory and Immunological Properties of A Traditional Remedy for Furuncles. *BMC Complemen Altern Med.* 2008; 8: 27.

[17.] Petrunov B., Nenkov P., and Shekerdjisky R. 2007. The Role of Immunostimulants in Immunotherapy and Immunoprophylaxis. Bulgaria. National Center of Infectious and Parasitic Disease. BulBio-NCIPD Natsim Ltd.

[18.] Sanusi S., 2004. Diabetes Mellitus dan Tuberkulosis Paru. J. Med Nus. 25: 1-5.

[19.] Tukiran, Suyatno dan Hidayati N. Skrining Fitokimia pada Beberapa Ekstrak dari Tumbuhan Bugenvil (*Bougainvillea Glabra),* Bunga Sepatu (*Hibiscus rosa-sinesis* L.) dan Daun Ungu (*Graptophylum pictum* Griff.). *Prosiding SemNas Kimia Unesa.* 2014.

[20.] Kairupan CP, Fatimawali, dan Lolo WA. Uji Hambat Ekstrak Etanol Daun Kembang Sepatu (*Hibiscus rosa-sinesis* L.) terhadap Pertumbuhan Bakteri *Escherichia coli. Pharmacon.* 2014;3(2):93-8.

[21.] Rojib dan Sukarjati, Pengaruh Ekstrak Etanol Bunga, Daun dan Akar Kembang Sepatu (*Hibiscus rosa sinesis*) Terhadap Berat, Volume dan Diameter Tubulus Seminiferus Mencit (*Mus Musculus*). *STIGMA.* 2016;9(1):26-31.

[22.] Mamun A, Islam S, Alam AHMK, Rahman MAA, dan Rashid M. Effects of Ethanolic Extract of *Hibiscus rosa-sinensis* Leaves on Alloxan-Induced Diabetes with Dyslipidemia in Rats. *Bangladesh Pharm J.* 2013.16(1):27-31.

[23.] Sachdewa A, Nigam R, dan Khemani LD. Hypoglycemic Effect of *Hibiscus rosa sinesis L.* Leaf Extract in Glucose and Streptozotocin Induced Hyperglycemic Rats. *Indian J Expt Biol*. 2001; 39: 284-6.

[24.] Antari Arlita Leniseptaria, Indah Saraswati, David Pakaya, and Aryoko Widodo. Macrophage Activity Test of Pulmonary Tuberculosis Patients With Diabetes Mellitus (TB-DM). *Journal of Biomedicine and Translational Research.* 2016. 02: 16-21.

[25.] Antari LA, Saraswati I, and Pakaya D. Comparative Studies Evaluating Macrophage Activity of Pulmonary Tuberculosis Patients With and Without Diabetes Mellitus (TB-DM and Non TB-DM). *Bangladesh Journal of Medical Science.* 2018; 17: 107-111.