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The editing of this proceeding has been carried out by **Siswoyo & B. Kuswandi** with assisted by the Scientific Committee of ISOSTECH'14.

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Foreword by Organising Committee

Assalamu'alaikum Wr. Wb.

Distinguished guests and delegates

On behalf of the organizing committee, I am deeply grateful to your present in the International Seminar on Science & Technology 2014 (**ISOSTECH '14**) that already held in Universitas Jember, Jember Indonesia on thirsday, 23 October 2014.

The **ISOSTECH '14** is jointly seminar between University of Jember (UNEJ), Indonesia and Universiti Sains Islam Malalaysia (USIM), it was arranged with substantive elements such as seminar pertaining to current advance on science and technology together with posters.

The seminar was provide an excellent platform for knowledge exchange between the academicians, researchers, scientists and engineers working in areas of mathematic and basic sciences, agricultural and food Technology, health sciences and enggineering as well as information technology. In addition, it provides an opportunity for the participants from Indonesia, Malaysia and Philiphine to share research findings, to establish networking and to encourage academic and student exchange and other participation in this exciting seminar.

We also would like to express our deep appreciation to the all organising committee members and steering committee, especially Dr. Zulfikar, on behalf of Rector, as Vice Rector of UNEJ who officially opens this seminar. Last but not least our appreciation to all participants especially delegate from USIM, IIU Malaysia and San Carlos University, Philiphines. We convey our great gratitude for your scientific speech and contribution. We do hope that all these research results are useful for further research progress and development in these fields.

Enjoy the conference proceeding and hope it will give inpiration on your research projects.

Wassalamu'alaikum Wr. Wb.

Mrs. L. Wulandary
Chairperson
University of Jember

Preface

The first International Seminar On Science & Technology 2014 (**ISOSTECH '14**), took place in University of Jember, Jember East Java Indonesia on 23 October 2014. This first seminar series is focused on all aspects related to recent advance in science and technology.

This proceeding contains papers that have been presented at **ISOSTECH '14** as plenary lectures, invited, oral and poster presentations. About 100 participants attended the conference, with 4 plenary lectures, 35 oral and 24 poster presentations. The proceeding of **ISOSTECH '14** has been published in electronic form as *.pdf file for simple and easy publication and to avoid heavy book of proceeding. We hope that this publication can be easily read, handled and transferred to other form. Furthermore, this paperless proceeding can be fruitful for all participants of the conference.

My sincerely thanks go to all the members of Scientific Committee for their valuable help in the review of the submitted papers, and also to the authors for their collaborative attitude. A special mention must go to **Mrs. L Wulandary**, our Conference Chairperson, who has put in a terrific amount of effort not only in general conference matter but also in the assembly of the papers for this proceeding. Finally, I congratulate the authors of all papers for producing the new and novel idea for research on mathematic and basic sciences, agricultural and food Technology, health sciences and enggineering as well as information technology.

Jember, October 2014

Siswoyo & B. Kuswandi
Editors

SCIENTIFIC COMMITTEE

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Lestyo Wulandari
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Expression of TLR-2 of Mice Infected by Mycobacterium Tuberculosis by Administration of Methanol Extract of Graftophyllum Pictum L. Griff

Atik Kurniawati

Faculty of Dentistry
University of Jember
Jl. Kalimantan 37, Jember 68121, Indonesia

Abstract - Tuberculosis is a chronic infectious disease caused by Mycobacterium tuberculosis. Toll-Like Receptor-2 (TLR-2) signaling plays a role in the body's defense system against Mycobacterium tuberculosis. Daun Ungu (Graptophyllum pictum L. Griff) is a traditional herbal plants which has medicinal properties for infectious and inflammatory diseases. This study aimed at analyzing the expression of TLR-2 of mice with the administration of methanol extract of Daun Ungu (Graptophyllum pictum L. Griff). The method used was an experimental laboratory, thirty male mice aged 8-12 weeks were randomly allocated into 5 groups; 2 control groups (K0, K1), 3 treatment groups (P1, P2, P3). K1, P1, P2 and P3 were infected using Mycobacterium tuberculosis at a dose of 108 ml and left for 4 weeks. At the fifth week groups P1, P2 and P3 were administered with methanol extract of Graptophyllum pictum L. Griff (EMDU) at a dose 1,703 mg, 3.406 mg, 6.812 mg. The administration of 0.2 ml was conducted every other day for 2 weeks. Examination of the expression of TLR-2 was carried out using immunohistochemical techniques. The data were analyzed using ANOVA and correlation analysis. The results showed that the EMDU in mice infected with Mycobacterium tuberculosis by immunohistochemical technique was succeed that the EMDU could increase the expression of TLR-2. The results of the correlation test showed a positive correlation between dose EMDU with TLR-2 expression. The conclusion of this study is that the EMDU can increase expression of TLR-2.

Keywords : TLR-2, Mycobacterium tuberculosis, EMDU.

1. Introduction

World Health Organization (WHO) states that there are 22 countries with high prevalence of tuberculosis. Most patients are in Asia (55%) followed by Africa (30%), Middle East (7%), Europe (4%) and America (3%). Thus, most parts of the world are still not free from tuberculosis. In Indonesia, the incidence of tuberculosis is the fifth rank in the world, after India, China, South Africa and Nigeria. According to the *Household Health* survey held by the Ministry of Health in 2001 that every year 583,000 new tuberculosis patients have been found of which 50% are patients with the category of positive *Acid-Fast Bacillus* (AFB) meant as transmitters [1],[2].

Tuberculosis is an infectious disease that is highly dependent on the immune response. Severity of tuberculosis disease is mainly influenced by the host immune response. Various theories state that innate immunity is a leading immune response, particularly the most potent macrophages against *Mycobacterium tuberculosis*. Macrophages are professional phagocytic cells with main function to destroy immunogen and as *Antigen Precenting Cells* (APC) through several receptors recognizing microbes associated with its function to stimulate cell migration to the site of infection and stimulate the production of microbial substances. Pathogen recognition accuracy is affected by receptor that influences the attachment of pathogens on APC. Allegedly receptor that activates macrophages to stimulate innate immunity one of which is the *Toll-Like Receptor* (TLR) that will affect the transcription factors of *Nuclear Factor Kappa Betha* (NFkB), then stimulates the phagocytic activity and cytokine production. One of the 10 TLRs, TLR-2, is considered to be the key mediator in innate immunity against *Mycobacterium tuberculosis*. In tuberculosis, it is assumed to occur the suppression of TLR-2 which results in a decrease in the phagocytic function and affects the proinflammatory cytokines TNF-α and IFN-γ and anti-inflammatory cytokine TGF-β. Changes in cytokine levels affect the level of damage to lung tissue [3],[4].

Daun ungu (*Graptophyllum pictum*) is one of Indonesian traditional medicinal plants. It is included in the list of 66 medicinal crops established by the Decree of the Minister of Agriculture No. 511 / Kpts / PD.310 / 9/2006. Indonesian people use this plant to treat swelling, ulcers, hemorrhoids and to help menstruation process [5], and is also used to cure bleeding cough [6]. Tuberculosis is a chronic infectious disease that of which bleeding cough is one of the clinical symptoms. Several studies have been conducted to mention that the ethanol extract of *Daun*

Ungu (Graptophyllum pictum (L) Griff) has activity against *Mycobacterium tuberculosis H37Rv* antimikobakterial *in vitro* [7].

Based on the data from scientific reports about the capabilities of the *phytopharmaca* activity of *Daun Ungu* and its long term use in the midst of society without causing any side effects, thus the researchers want to observe further about whether the methanol extract of *Daun Ungu* affects the expression of TLR-2 in the lung tissue of mice infected with *Mycobacterium tuberculosis*. The goal of this study is to analyze the increased expression of TLR-2 in lung tissue of mice infected with *Mycobacterium tuberculosis* by administration of methanol extract of *Daun Ungu* (EMDU).

2. Methods

This study was an experimental study in mice (Mus muculus) Balb type / c to compare the groups of mice infected with *Mycobacterium tuberculosis* (M.tb), mice infected with M.tb accompaned with *Daun Ungu* methanol extract (EMDU) at a dose of 1,703 mg / kg / BW), 3.406 mg / kg / BW), and 6,812 mg / kg / WB) for 2 weeks, and the group of mice without any treatment (normal).Tuberculosis infection in animal models is due to the exposure (injection) of 60μL *of Mycobacterium tuberculosis H37Rv* with 10^8 per mL concentration by means of intra-tracheal [8]. TLR-2 protein expression was the expression of TLR-2 protein derived from the lung tissue of mice, examined using immunohistochemistry examination using monoclonal antibodies anti-TLR-2. Calculations performed on immunoreactive cells showed positive expression and appeared reddish-brown in the cytoplasm. Counted as many as 10 fields of views using a light microscope at a magnification of 400 times to take average value. Immunoreactive cell count average value entered as data [9],[10].

Preparation of Test Materials : *Griff L. pictum Graptophyllum* leaves taken from Traditional Medicine (OBTRA) Garden of Traditional Medicine Division of the Center of Health Development and Research (PUSLITBANGKES) Surabaya. The plant has been determined in Indonesian Institute of Sciences Plant Conservation Unit *Kebun Raya Purwodadi, Pasuruan*. Making EMDU was conducted by maceration using 80% of methanol. To determine the class of compounds contained in *Daun Ungu* phytochemical screening was carried out using *Thin Layer Chromatography* (TLC) method [11], [12]. Dose calculation based on the dose immunomodulatory of *Daun Ungu* was 0.2 ml infusion 10% /head / po / day [13].

Analysis of Data. Sequence analysis of the data: the Kolmogorov-Smirnov Normality Test: to determine the normal distribution of data (p> 0.05). Levene test conducted to determine the homogeneity of the data. If the variance of the data was homogeneous (p> 0.05) followed with ANOVA test and Duncan's test orderly. Path analysis was conducted to determine the correlation.

3. Results and Analysis of Research

Table of the mean, standard deviations and ANOVA test TLR-2 expression in lung tissue of mice.

Groups	Mean	Standard Deviation	Anova
Control (-)	$4,083^a$	1,59	F =38.57
Control (+)	$6,00^{a,b}$	0,71	p= 0,000
P1 / (*M.tb*+D1)	$7,83^b$	1,69	
P2 / (*M. tb*+D2)	$15,17^c$	3,28	
P3 / (*M. tb*+D3)	$15,42^c$	2,27	

Note : varied *superscript* shows the present of significant differences (p<0,05)

Test Results of ANOVA shows a significant difference (p = 0.000). This means that the increased expression of TLR-2 is caused by EMDU administration. The results of the Duncan test demonstrate that expression of TLR-2 increased along with increasing dose EMDU but increased expression of TLR-2 seems real (significant) in the P2 group (dose of 3.406 mg / kg) and P3 (dose of 6,812 mg / kg) when compared to control (+). Correlation between EMDU with TLR-2 is positive and significant (r = 1.487, p = 0.000). This means that the higher the dose EMDU will be followed by the higher expression of TLR-2.

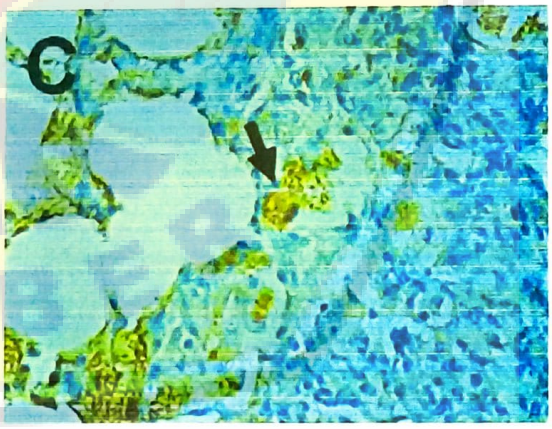

Figure 2.1 Expression of of TLR-2 lung tissue of mice infected with *Mycobacterium tuberculosis* and treated with EMDU. Brown macrophage cytoplasm (mark →) indicates a positive result. Immunohistochemical staining. 400X magnification.

4. Discussion

The results of this study demostrates that the methanol extract of *Daun Ungu* (EMDU) may

increase the expression of TLR-2 in groups P1, P2 and P3, likely due to the effects of flavonoids of EMDU which enhance the activity of immunomodulating on alveolar macrophage cell with co-stimulation of *Mycobacterium tuberculosis* leading to increased TLR expression-2.This mechanism occurs due to *Daun Ungu*'s flavonoids. Flavonoids identified from the methanol extract of *Daun Ungu* (EMDU) i.e. *quercetin, kaempherol* and *myrecetin flavonols* [6], [13]. *Kaempherol flavonol* is able to bind to the estrogen receptor (ER) on the surface of macrophages [14]. Therefore, administering EMDU is expected to result bonds between flavonols and estrogen receptors on macrophages. It will result in a transduction signal delivery. It is known that the *quercetin flavonol* can activate TLR-2 and NF-kβ [15], [16]. Increased activation of the NF-kβ will increase the TLR-2 gene transcription as well. Expression of TLR-2 increased with increasing dose EMDU. This is consistent with the results of the research conducted by Kiat Lim *et al.*, (2013), that the *flavonoid quercetin* is able to increase the production of cytokines IL-1β through increased expression of TLR-2 on monocytes [15]. Based on Duncan test, increased expression of TLR-2 in this study is evident (significant) in the positive group (K1) with P2 group (dose 3.406 mg / kg/BW) and P3 (dose of 6,812 mg / kg/BW). It shows that EMDU is able to increase the expression of TLR-2 in mice infected with *Mycobacterium tuberculosis* significantly at dose 2 (3.406 mg / kg/BW) and dose 3 (6,812 mg / kg/BW), whereas at dose 1 (1.703 mg / kg/BW) increased expression of TLR-2 is not significant. This likely occurs because EMDU at dose 1 has low affinity. Affinity is a measure of the ability of drugs to bind to the receptor [17], [18]. Due to the low EMDU quantity of dose 1, the ability to provide therapeutic effects has not been optimized so that the expression of TLR-2 is not increased significantly.

The increased expression of TLR-2 becomes advantageous. The TLR-2 in patients with tuberculosis serves to increase the maturation of dendritic cells to enhance the ability to perform phagocytosis against *Mycobacterium tuberculosis* [19], [20]. TLR-2 has a role as introductory mediator of *Mycobacterium tuberculosis* and initiates innate immune response against infectious disease. *Mycobacterium tuberculosis* recognized by TLR-2 will activate macrophages and dendritic cells. According to research from Takeda and Akira (2004), the expression of TLR-2 will lead to immature dendritic cells located in the periphery becomes mature and subsequently heads to limfonoduli, it will initiate an adaptive response activation which then produces a signal for transcription of NF-kβ which is the main regulators of the immune response [21]. Based on the results, it can be concluded that the methanol extract of the *Graftophyllum pictum L. Griff* leaves is able to increase the expression of TLR-2 in lung tissue of mice infected with *Mycobacterium tuberculosis*.

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