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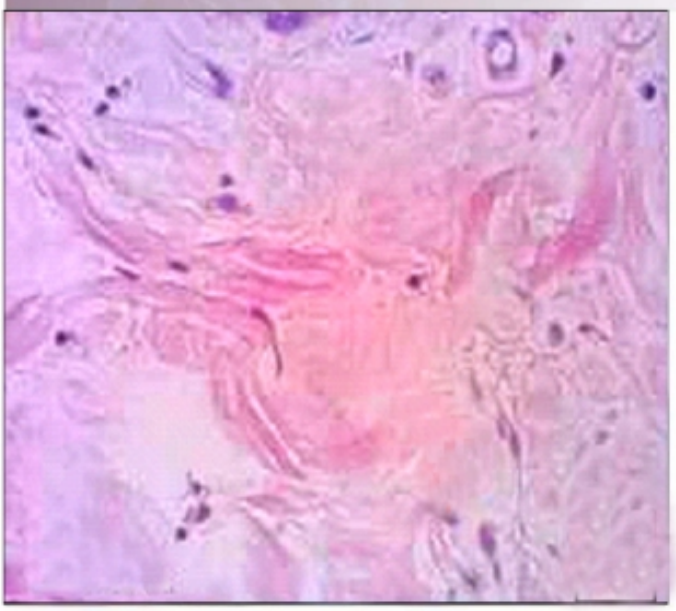

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Alamat Redaksi : Fakultas Kedokteran Hewan Universitas Airlangga
Kampus C Unair Jl. Mulyorejo Tel. (031) 5992785 – 5993016 Surabaya 60115
Fax (031) 5993015 E-mail : vetmed_ua@yahoo.com

Rekening : BNI Cabang Unair No Rek. 0112443027 (Hani Plumeriastuti)
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      Staropoli, I., J.M. Clement, M.P. Frenkiel, M. Hofnung and V. Deuble. 1996. Dengue-1 virus envelope glycoprotein gene expressed in recombinant baculovirus elicits virus neutralization antibody in mice and protects them from virus challenge. Am.J. Trop. Med. Hygi; 45: 159-167.
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DAFTAR ISI

Efek Ekstrak Kulit Manggis (*Garcinia mangostana* L.) pada Ekspresi dari TLR5 dan CD14 pada Mencit yang Diberi Vaksin Newcastle Disease

Effect of Mangosteen (*Garcinia mangostana* L.) Pericarp Extract on TLR5 and CD14 Expression in Immunized Mice Against Newcastle Disease Vaccine

[1]Demas Moch. Zain Fithronny, [2]Rochmah Kurnijasanti, [2]Fedik A. Rantam

[1]PPDH Fakultas Kedokteran Hewan Universitas Airlangga
[2]Fakultas Kedokteran Hewan Universitas Airlangga

Kampus C Unair, Mulyorejo Surabaya 60115
Tlp. 031-5992785 fax.0315993015
e-mail: vetunair@telkom.net

Abstract

Mangosteen pericarp has well-recognized for reducing the incidence of degenerative diseases including cancer, heart disease, inflammation, arthritis, and immune system. The main chemical substance of mangosteen pericarp that role in improving health is the xanthone which is belonged to phenolic acid that has been studied for its remarkable biological activities in recent years. The research was conducted to analyze the immunomodulatory effect of mangosteen pericarp extract (MPE), by measuring both the expression of TLR5 and CD14 in mice PBMCs. Animal used in the research were 36 female Balb/c mice, that randomly separated into six groups (T0, T1, T2, T3, T4, T5) with six animal each. T0 was negative control group, TI was administered with 40 mg/ml MPE, T2 until T4 was administered with 20 mgml, 40 mg/ml, 60 mg/ml respectively, and T5 was positive control group using Stimuno®. All groups were vaccinated by inactive velogenic type ND vaccine except for T1 group once and without booster. Blood collection has been done a week after vaccination. The result indicated that MPE could increase both the activity of TLR5 and CD14 with 40 mg/ml as optimal dose.

Keywords: mangosteen pericarp extract, immunomodulatory effect, mice, TLR5, CD14

Introduction

Immunomodulator is the substances that have a capability to interact with the immune system to upregulate or down-regulate specific aspect of the host response (Stanilove *et al*., 2005; Utoh-Nedosa *et al.,* 2009). Immunomodulators may include some bacterial product, lymphokines and plant derived substances. The effects of immunomodulator can be classified into three which are stimulation, suppression and restoration of the immune system. Unlike vaccine, most of immunomodulator agents are not real antigens but antigenomimetics or so called mitogens. Due to their actions as a non-specific and nonantigens properties, they do not stimulate the development of memory lymphocytes. Thus the effect of immunomodulator agents

towards specific immune system will be reduced after a short of period of time (Wagner, 1999).

The innate immune system enables the host to differentiate itself from invading microbes, discriminate among pathogens, and initiate a cascade of inflammatory molecules that influence formation of the acquired immune response as well as host survival. Important components of the innate immune system are CD14, an adaptor molecule, and a system of pathogen receptors named toll-like receptors (TLRs) (Cook *et al*., 2004).

CD14 is a multifunctional high-affinity receptor for endotoxins, lipopoly-saccharides and other bacterial wall components. It has been implicated in the development and maturation of the innate immune system (Guera *et al*., 2004; Bieli *et al*., 2007). Other studies have also shown that CD14 may function as a receptor for other bacterial products from *Pseudomonas* (Espevick *et al*., 1993), insoluble cell wall fragments from several Gram-positive bacteria (Gupta *et al*., 1994), mycobacterial lipoarabinomannan (Savedra *et al*., 1996), rhamnose-glucose polymer from *Streptococcus* (Soel *et al*., 1996), and Lipoteichoic acid (LTA) (Cleveland *et al*., 1996) or LTA-like molecule (Kusunoki *et al*., 1995) from Gram-positive bacteria.

TLRs are a critical first line of defense against bacterial, viral, and fungal invaders and play a vital role in microbial sensing (Tizard, 2013). TLR5 recognizes the flagellin protein of bacteria, a potent inflammatory stimulus present in the flagellar structure of many bacteria (Hayashi *et al*., 2001; Smith *et al*., 2003).

"*The Queen of Fruit*" that was the name given from traveler in the world Fairchild for *mangosteen*. It is well-recognized that consumption of fruits and vegetables can reduce the incidence of degenerative diseases including cancer, heart disease, inflammation, arthritis, immune system decline, brain dysfunction, and cataracts (Gordon, 1996; Feskanich *et al*., 2000)

The fruit pericarps, these are nature's most abundant sources of xanthones, which are natural chemical substances possessing numerous bio-active properties that help to maintain intestinal health, which neutralize free-radicals, which help and support joints and cartilage functions and promote immunomodulation systems (Suksamrarn *et al.*, 2006).

Materials And Methods

The research has been done in several places, which were Veterinary Feed and Nutrition Department, Veterinary Microbiology Department of Veterinary Medicine Faculty of Airlangga University; Dengue and Stem Cell Laboratory of Institute of Tropical Disease and Assessment Service Unit of Pharmacy Faculty of Airlangga University. The research has been done by April to June 2013. Animal used on the research are 36 female BALB/c mice (*Mus musculus*). All mice were obtained from Pusat Veterinaria Farma (Pusvetma), Surabaya, East Java, Indonesia. This research procedure was approved by Animal Case and Use Committee (ACUC) of Veterinary Medicine Faculty of Airlangga University (No. 249-KE).

On mangosteen pericarp preparation, it has used knife for chopping the pericarp, air stove for drying the percarp, grinding machine for grinding the pericarp, digital weight measurer, and plastic for storing the pericarp powder.

The extraction process undergo by rota evaporator, ultrasonic cator, separator tube, volumetric flask, air stove, Petri dish, filtration paper Whatman no. 1440 paper and incubator. Mangosteen pericap extract (MPE) has

stored in 6 bottles, and has administered orally using gavage needle to all mice.

Blood sample collection used needle 25' and syringe. All blood stored in centrifuge tubes before got centrifuged. Immunostaining in immunocytochemistry technique used microslides (Choke) and micropippete 10-100µL with tips according to (Javois., 2010) and (Rantam., 2003). Immunofluorescent microscope used for cell observation.

Data Analysis

The research results of cell counted for TLR5 and CD14 expression and analyzed using cell count and cell percentage comparison with normal cell

Results And Discussion

Immunocytochemistry technique was chosen to measured the expression of TLR5 and CD14 within the cell. Total cell counted for normal unlabelled cell culture was 510/10 µl.

The result shown in Table 1 is the number of cells that positively expressed TLR5 which noted as green color under immunofluorescent microscope observation at enlargment 40x.

Table 1. Data of Cells Counted for TLR5 Expression in Mice PBMCs

Group	Cell Count	Cell Percentage
T0	140/10 µl	27.45%
T1	400/10 µl	78.43%
T2	270/10 µl	52.94%
T3	280/10 µl	54.90%
T4	170/10 µl	33.33%
T5	300/10 µl	58.82%

T0 : PBS +ND Vaccine
T1 : MPE 40 mg/ml
T2 : MPE 20 mg/ml + ND Vaccine
T3 : MPE 40 mg/ml + ND Vaccine
T4 : MPE 60 mg/ml + ND Vaccine
T5 : Stimuno® 0.6 mg/25g/ml + ND Vaccine

According to Table 1 the negative control group, T0 group, yield the least result among treatment groups (140/10 µl). This group was administered with PBS/ml/day and ND vaccination. Cell percentage of T0 group and the other groups was compared to the normal cell culture which counted for 510/10 µl of normal cells. The least cell percentage yield by T0 group (27.45%) while the highest one was acquired by T1 group (78.43%). T1 group was administered only with MPE 40mg/ml/day without ND vaccination. Different with T2 group (52.94%) which administered with MPE 20mg/ml/day, and ND vaccination. This result similiar in T3 group (54.90%). This group was administered with the dose of MPE (40mg/ml/day) and ND vaccination. T4 group that was administered with highest dose of MPE (60mg/ml/day) showed the least effect for immunomodulation (33.33%). The positive control group, T5 group, was administered with Stimuno® 0.6 mg/25 g BW/ml. This group showed result (300/10 µl) below T1 (58.82%).

The increasing number of cells expressed TLR5 started by negative control group (T0 group), dose of MPE (T1 group) explained that the MPE could altered the innate immune response and increase its activity. The T1 dose (40mg/ml) of MPE also showed better result compared with positive control group (T5 group) so that explain the optimal dose for MPE is 40mg/ml without any vaccination.

The relatively same result yielded by T2 and T3 group, which got the same dose of MPE with T2 and T3 group (20 mg/ml) and (40mg/ml). T4 that has the highest dose shown the low immune response compared with other dose of MPE. This result meant that the vaccination procedure did not exhibit a far different value of immune response on innate immunity compared with those on the adaptive immunity as signed as

antibody titer level. The phenomenon T1 have highest immune response might happen due of immune response in developing itself against foreign invaders (Tizzard, 2013). The innate immunity, which known to be only take several hours to react, also might explained how this result gained same regardless of what foreign invaders that challenged it, but more likely to what substance that stimulated its readily first before it's facing the antigen.

Nucleated cells, such as leukocytes, possess hundreds of different protein molecules on their surface. These proteins are good antigens and readily provoke an immune response when injected experimentally into a different species. These surface molecules are classified by the CD system. The result of CD14 in immunochemistry technique at Table 2

Table 2. Data of Cells Counted for CD14 Expression in Mice PBMCs

Group	Cell Count	Cell Percentage
T0	260/10 µl	50.98%
T1	330/10 µl	64.70%
T2	220/10 µl	43.13%
T3	250/10 µl	49.01%
T4	230/10 µl	45.09%
T5	450/10 µl	88.23%

T0 : PBS +ND Vaccine
T1 : MPE 40 mg/ml
T2 : MPE 20 mg/ml + ND Vaccine
T3 : MPE 40 mg/ml + ND Vaccine
T4 : MPE 60 mg/ml + ND Vaccine
T5 : Stimuno® 0.6 mg/25g/ml + ND Vaccine

According to Table 2 the negative control group, T0 group, yield the least result among treatment groups (110/10 µl). This group was administered with PBS/ml/day and ND vaccination. Cell percentage of T0 group and the other groups was compared to the normal cell culture which counted for 510/10 µl of normal cells. The least cell percentage yield by T0 group (21.56%) while the T1 group (64.70%). T1 group was administered only with MPE 40mg/ml/day without ND vaccination. Different with T2 group (43.13%) which administered with MPE 20mg/ml/day, and ND vaccination. This result similiar in T3 group (54.90%)%) was administered with the dose of MPE (40mg/ml/day) and ND vaccination and the T4 group (45.09%) was administered with highest dose of MPE(60mg/ml/day). The positive control group, T5 group, was administered with Stimuno® 0.6 mg/25 g BW/ml. This group showed the highest result (88.23%).

The result of CD14 has shown that T0 group that as the negative control showed the lowest immune response, that means MPE could explained that the MPE could altered the innate immune response and increase its activity. And the T0,T2, T3, and T4 showed the relatively same response immune. for T5 or control positive greatly increase in here. Unlike vaccine, most of immunomodulator agents are not real antigens but antigenomimetics or so called mitogens. Due to their actions as a non-specific and nonantigens properties, they do not stimulate the development of memory lymphocytes. Thus the effect of immunomodulator agents towards specific immune system will be reduced after a short of period of time (Wagner, 1999). Thus result could be mean that the MPE has been decreased and down regulate happened because the immune response has been at the peak.

Described that this MPE might be as increasing number of cells expressed CD14 started by negative control group (T0 group) to the dose of MPE (T1 group) explained that the MPE could altered the innate immune response and increase its activity and works optimal at 40mg/ml without any vaccination. As

activated CD14 acts not only as a surface receptor for LPS but might also function as a polyspecific receptor with broad recognition properties (Pugin *et al*., 1994).

Conclusion

Mangosteen Pericarp extract can induce an increasing value of TLR5 and CD14 expression by mice PBMCs, with optimal dose 40mg/ml.

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