PROCEEDING

The International Conference on Pharmacy and Advanced Pharmaceutical Sciences

Faculty of Pharmacy UGM
Yogyakarta Indonesia
October 2009

UNIVERSITI SAINS MALAYSIA

NAIST

The International Conference on Pharmacy and Advanced Pharmaceutical Sciences

Faculty of Pharmacy UGM

PENGESAHAN

Salinan/ Foto kopi sesuai dengan aslinya

Surabaya, _____

Dekan,

Dr. Christina Avanti, M.Si.,Apt.

ISBN 978-979-95107-7-8

PROCEEDING

The International Conference on Pharmacy and Advanced Pharmaceutical Sciences Yogyakarta, Indonesia, 2009

Editors :

Pudjono

Hilda Ismail

Ronny Martien

Triana Hertiani

Ritmaleni

Published by:
Faculty of Pharmacy Universitas Gadjah Mada
Sekip Utara, Yogyakarta, 5281,
Indonesia

ISBN : 978-979-95107-7-8

First Edition, 2010

Printed in Yogyakarta, Indonesia

PROCEEDING

The International Conference on Pharmacy and Advanced Pharmaceutical Sciences Yogyakarta, Indonesia, 2009

Editors :

Pudjono

Hilda Ismail

Ronny Martien

Triana Hertiani

Ritmaleni

Published by:
Faculty of Pharmacy Universitas Gadjah Mada
Sekip Utara, Yogyakarta, 55281,
Indonesia

ISBN : 978-979-95107-7-8

First Edition, 2010

Printed in Yogyakarta, Indonesia

CONTENTS

Immunomodulatory activity of *Plantago major* L. on IgM titer of mice

Kartini*, A. Kirtishanti, Dessy, Fauziah, Isnaini
Faculty of Pharmacy, University of Surabaya
kartini@ubaya.ac.id

Abstract

Immunomodulatory activity of four fractions from *Plantago major* L. (n-hexane, ethyl acetate, n-butanol and water) on titer of IgM has been carried out on mice, ex vivo. Each test sample was taken orally during 7 days, single dose, at 3 different level dose. Titer of IgM has been determined using agglutination method, 5 days after immunization with sheep red blood cell (SRBC). All of the fractions has capability in increasing the titer of IgM, but the highest activity resulted from n-hexane fraction.

Key words : immunomodulator, *Plantago major* L., titer of IgM

Introduction

Immune system is a combination of cells, molecules and tissues that play a role in resistance to various diseases, especially infections. Drugs which can restore the imbalance in the immune system called as immunomodulator. The immune system is divided into two categories: specific and non-specific, each consisting of cellular and humoral immune system. Complements, interferon, C-reactive protein (CRP) are some examples of non-specific humoral defense, while the antibody including specific humoral immune components. The main function of antibodies is a defense against viral infections and extra cellular bacteria, also neutralize its toxin (1).

Antibodies are formed through the theory of "Two Signal Theory", meaning that antibodies will be formed if there are 2 signals: the first one is derived from the bond between the APC/Antigen Presenting Cell (B lymphocytes, monocytes, macrophages) and T-helper (especially TH_2); the second, is derived from cytokines released by activated T-helper (2).

One of the plants whose immunomodulatory activities is *Plantago major* L. ("daun sendok"). Their activity are : chemotactic activity in neutrophiles (3); increase NO & TNF-α production (4); enhance lymphocyte proliferation and secretion of IFN-γ (5); increase phagocytosis by monocytes and macrophages (6).

The activity of immune system components are interrelated, it is interesting to study thr role of *P. major* L. in enhancing phagocytosis by monocytes and macrophages, and the ability to increase the production of antibodies. Based on "Two Signal Theory", it is predicted that *P. major* L. be able to increase the production of antibody, especially IgM as the primary antibody response.

This study have been conducted to investigate the immunomodulatory activity of *P. major* L. leaves on the formation of IgM, which is determined by measuring IgM titer. In order to guide the discovery of bioactive compounds, tests carried out on chemical fractions of *P. major* L. leaves, i.e.: n-hexane, ethyl acetate, n-butanol and water.

Methodology

Materials

Materialsare the : leaves of *Plantago major* L. (obtained from the Village Tlekung, District Jungrejo, Batu; determination conducted by the Center for Information and Development of Traditional Medicine, UBAYA), methanol, grade (Mallinckrodt), n-hexane (Riedel-dehaen), ethyl acetate (Merck), n-butanol, Aqua bidestilata (Ikapharmindo Putramas), CMC Na, normal saline (B. Braun) and sheep red blood cells (SRBC).

Test animals are mices, BALB/c strain, male and female, aged 1-2 months (obtained from Laboratory of Pharmacology, Faculty of Pharmacy, UBAYA).

Equipments

Instrument use are : rotary evaporator (Heidolph), centrifuge (Hettich), haemositometer, electrical mixer (Ika Labortechnik), water bath (Memmert), Oven (Binder), ultrasonic bath (Branson), injection syringes (Terumo), sonde, incubator shaker (GFL), micro titer plate, micropipette (Socorex), Pasteur pipette, magnifying glass, surgical instruments and glass instruments.

Preparation of *P. major* L. fractions:

All of the fractions (n-hexane, ethyl acetate, n-butanol and water) are prepared according to the Markham (7) with slight modifications. Dried powder of samples (±800 g) was extracted with methanol trought kinetic maceration (3 hours), then soaked (24 hours) and filtered. Residue is extracted again in, similar using methanol-water (9:1) and methanol-water (1:1), respectively. Three extracts are combined, concentrated under vacuum and water bath (± 60^0C). The viscous extract are diluted in hot water (10 x weight of viscous extract), then shaked in a separation funnel with n-hexane, ethyl acetate and n-butanol, respectively.

Preparation of sample for oral

In this study, a negative control (0.5% CMC-Na) and three different doses of each fraction have been used (Table 1). Each fraction was in suspension in 0.5% CMC-Na, given in 0.5 ml, orally once daily for 7 consecutive days.

Table 1. Dose of *P. major* L. fractions in immunomodulatory activity test

Dose	Fraction (equivalent to mg methanol extract/kg BW of mice)	
	n-hexane, n-butanol, water	Ethyl acetate
I	400	207,1
II	1200	400
III	2000	800

Determination of the primary antibody response (IgM)

Antibody titer is the highest of the serum dilution that still gives agglutination reaction. Agglutination is positive when there is a visible layers of erythrocytes with evenly or diffuse edge (not flat) on the basis of sinks (micro titer plate) and transparent liquids without the occurrence of the point-shaped erythrocyte sedimentation in the middle of sinks. IgM titer is determined as follows (8): serum of mice taken from the heart, 5 days after immunization with SRBC (10^8 cells/ml, intra-peritoneally). Serial dilutions of serum using double dilution-method was carried out on sinks (100 µl). 100 µl phosphate buffer solution was used as control solvent. 100µl SRBC (10^8 cells/ml) is added into each sinks, shaken for 5 minutes and then incubated for 30 minutes in 37^0C. Finally, the mixture left overnight at room temperature. Agglutination results read under a magnifying mirror, titer expressed as the denominator of serial dilutions at the numerator=1 (reciprocal).

Analysis

To determine the influence of each *P. major* L. leaves fractions on the formation of IgM titer of mice, the average IgM titer of the treatment group are compared with the control group using non-parametric statistical analysis (Kruskal-Wallis at α = 0.05).

Results and Discussions

The extraction carried out on 876.4968 g of dry leaf powder, yielded 166.7127 g (19.02%) methanol extract. N-hexane, ethyl acetate, n-butanol and water factions produced from this extraction is: 14.72; 4.36; 18.56 and 48.07% methanol extracts, respectively. Influence of each fraction on the titer of IgM is shown in table 2 and figure 1.

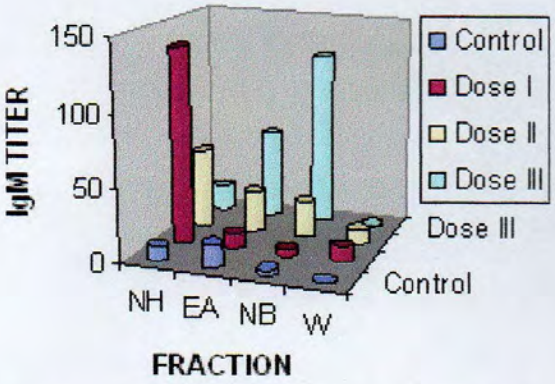


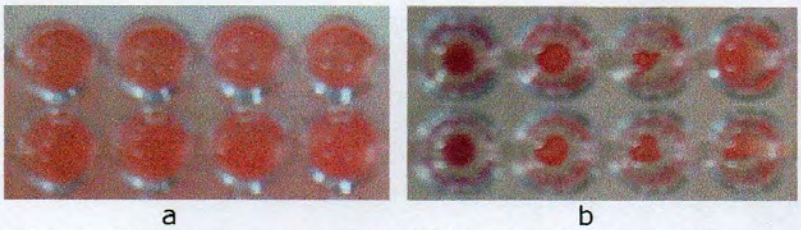
Figure 1. Graph of the Influence of n-hexane (NH), ethyl acetate (EA), n-butanol (NB) and water (W) fraction of *P. major* L. leaves on IgM titer of mice.

Table 2. The Influence of *P. major* L. fractions on the titer of IgM

Group of mice	Titer of IgM (fraction)			
	n-hexane	Ethyl acetate	n-butanol	Water
Control	10,00±15,95	16,00±9,24	3,33±6,41	0,66±1,63
Dose I	**138,00±141,53**	11,20±4,13	5,33±9,69	**12,00±29,39**
Dose II	56,00±40,48	29,60±15,11	26,00±27,45	10,33±8,61
Dose III	19,17±15,05	**32,00±0,00**	**122,67±74,13**	1,33±2,06

Table 2 and figure 1 showed that IgM titer at all doses treatment groups (except the EA fraction) is relatively higher compared to the control group. Therefore, it is proved that all of fractions of *P. major* L. leaves increase the titer of IgM, but at different dose, also in different enhancement level. Statistical analysis (Kruskal-Wallis, α=0.05) shown a significantly differences (significance of four fractions: 0.048; 0.000; 0.001 and 0.018, respectively). All are smaller than 0.05, so it can be concluded that all of fractions can increase the IgM titer of mice.

Figure 1 shown the differences between doses and IgM titer of each fractions. In n-hexane and water fractions, higher dose of the test material, give the lower of the titer IgM. However, the opposite occurs in ethyl acetate and n-butanol. By considering the doses, it can be concluded that the n-hexane fraction at dose equivalent to 400 mg of methanol extract/kg BW of mice can increase the IgM titer at highest level.



a b

Figure 2. Result of Hemagglutination test using SRBC as antigen: before (a) and after (b) incubation overnight at room temperature.

In this study the measurement of antibody titer was done by hemagglutination method (Figure 2), where particulate antigen bounded by IgM antibodies in the form of pentavalent/pentamer, so lattice (agglutination results) can be seen easier. SRBC is the best antigen for antibody production test on mice. More over, it is belong to T-dependent, so in stimulating the formation of antibodies requires T lymphocytes facility. Another antigen that can be used is: fowl gamma globulins/immunoglobulin from poultry) (9).

Based on the "Two Signal Theory", the role of each *P. major* L. leaves fractions in improving the IgM titer was estimated as adjuvant to stimulate phagocytosis process by

monocytes/macrophages. So many more antigens presented by monocytes/macrophages with MHC II molecules to be recognized by TH_2. It will stimulate TH_2 to secretes IL-4 and IL-5. When the secretion of IL-4 and IL-5 increase, the stimulation of B cells increased, IgM secretion was also increased. This is supported by previous research which proved that *P. major* L. can enhance phagocytosis by monocytes and macrophages (6). The other mechanisms, fractions of *P. major* L. leaves stimulate effectors cells (B lymphocytes) directly to synthesize antibody.

Since P. major L. leaves enhance cellular immune responses as well as the humoral, this plant can be predicted as a potential candidate for an immunomodulator. One to consider is as adjuvant or supportive therapy to overcome the unwanted side effect of chemotherapy (most of them are immunosuppressant).

Conclusion

N-hexane, ethyl acetate, n-butanol and water fraction of *Plantago major* L. leaves given orally to mice can increase the titer of their IgM. N-hexane fraction of *Plantago major* L. leaves has the highest activity in increasing IgM titer.

References

Baratawidjaja, K.G., 2004, Imunologi Dasar, Edisi ke-6, Jakarta: Balai Penerbit FKUI

Chiang, L.C., Chiang, W., Chang, M.Y., Lin, C.C., 2003, In vitro Cytotoxic, Antiviral and Immunomodulatory Effects of *Plantago major* and *Plantago asiatica*, Am J Chin Med., 31(2), 225-34

Clancy, J., 2000, Basic Concepts in Immunology, A Student's Survival Guide, New York: The McGraw-Hill Companies

Dorhoi, A., Dobrean, V., Zahan, M., Virag, P., 2006, Modulatory Effects of Several Herbal Extracts on Avian Peripheral Blood Cell Immune Responses, Phytother Res., 20(5), 352-8

Gomez-Flores, R., Calderon, C.L., Scheibel, L.W., Tamez-Guerra, P., Rodriguez-Padilla, C., Tamez-Guerra, R., Weber, R.J., 2000, Immunoenhancing Properties of *Plantago major* Leaf Extracts, Phytother Res.,14(8), 617-22

Hudson, L., Hay, F.C., 1989, Practical Imunology, 3rd ed. Oxford: Blackwell Scientific Publications, 207-63.

Markham, K.R., 1988, Cara mengidentifikasi Flavonoid, Bandung, Penerbit ITB

Samuelsen, A.B., 2000, The Traditional Uses, Chemical Constituents and Biological Activities of *Platago major* L. A Review, J. Ethnopharmacol.,71, 1-21

Sigal, L.H., Ron, Y., 1994, Immunology and Inflamation, Basic Mechanisms and Clinical Consequences, Mc Graw Hill, Inc., Health Profession Division