

RINGKASAN

Pengaruh Imunomodulator Andrographolide Terhadap Kemampuan Daya Bunuh Intraseluler Makrofag pada *Mycobacterium Tuberculosis*

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Tuberkulosis adalah penyakit infeksi yang disebabkan oleh bakteri ganas *Mycobacterium tuberculosis* yang membunuh hampir 2-3 juta masyarakat di dunia per tahun. Pertambahan kasus baru mencapai jumlah sembilan juta kasus dan apabila tidak dikendalikan maka dalam 20 tahun mendatang tuberkulosis akan membunuh 35 juta orang.

Meskipun kemoterapi masih merupakan penatalaksanaan utama pada tuberkulosis namun karena pada 5 - 15 % kasus individu yang berkembang menjadi penderita tuberkulosis paru diduga sebagai konsekuensi adanya defek sel T dan fungsi makrofag atau kedua-duanya sehingga kemampuan fagositosis dan daya bunuh terhadap basil *Mycobacterium tuberculosis* menjadi lemah maka penggunaan imunoterapi tambahan mulai menarik perhatian untuk mengatasi tuberkulosis, terutama karena peningkatan persentase penderita yang resisten terhadap obat antituberkulosis.

Andrographolide adalah suatu diterpenoid lactone mempunyai rumus molekul $C_{20}H_{30}O_5$ dengan berat molekul 350,45. Gugus lakton akan berikatan dengan rantai karbohidrat dari protein transmembran pada permukaan sel T, akibatnya sel T helper menjadi aktif untuk selanjutnya mensekresikan sitokin yang dapat mengaktifasi sel lain seperti makrofag dan neutrofil.

Andrographolide dapat meningkatkan produksi IFN- α , IFN- γ pada PBMCs dan juga dapat meningkatkan kemampuan fagositosis peritoneal makrofag *guinea pig* untuk memfagositosis eritrosit ayam. Telah dilaporkan pula bahwa Andrographolide dapat berfungsi sebagai imunostimulan, hingga bisa dikatakan bahwa Andrographolide mempunyai aktivitas farmakologi yang meyakinkan sebagai kandidat dalam pengobatan imunomodulator.

Permasalahan dalam penelitian ini adalah apakah pemberian imunomodulator Andrographolide berpengaruh terhadap kemampuan daya bunuh intraseluler makrofag pada *Mycobacterium tuberculosis*, pada kultur *in vitro* ?

Tujuan dalam penelitian ini untuk membuktikan bahwa kemampuan daya bunuh intraseluler makrofag pada *Mycobacterium tuberculosis* yang diberi imunomodulator Andrographolide, lebih tinggi daripada daya bunuh makrofag tanpa pemberian Andrographolide, serta membuktikan bahwa waktu makrofag membunuh *Mycobacterium tuberculosis* yang diberi imunomodulator Andrographolide, lebih cepat daripada waktu makrofag membunuh *tuberculosis* tanpa pemberian Andrographolide, secara *in vitro*.

Dalam penelitian ini diharapkan dapat mengungkap informasi tentang pengaruh pemberian Andrographolide sebagai imunomodulator, khususnya terhadap daya bunuh intraseluler makrofag serta waktu makrofag membunuh *Mycobacterium tuberculosis*, pada kultur *in vitro*.

Rancangan penelitian ini adalah penelitian eksperimental laboratorium dengan rancangan penelitian *True Experimental Randomized Posttest Only Control Group Design*.

Sampel adalah makrofag manusia yang diperoleh dengan melakukan kultur *Peripheral Blood Mononuclear Cells* (PBMCs) dari darah relawan individu sehat.

Jumlah makrofag yang digunakan untuk tiap pengulangan atau replikasi adalah 10^5 sel/ml . Jumlah pengulangan ditentukan berdasarkan rumus Federer adalah 3 ulangan.

Penelitian dilakukan di Laboratorium *Tropical Disease Center (TDC)* Universitas Airlangga Surabaya yang dimulai pada bulan Oktober 2007 hingga bulan Januari 2008.

Data dianalisis menggunakan *Univariate Analysis of Variance* dengan uji F pada taraf nyata 5 % dan dilanjutkan dengan uji HSD menggunakan SPSS for Windows rel. 13.

Hasil penelitian menunjukkan bahwa rerata koloni *Mycobacterium tuberculosis* yang masih dapat tumbuh pada media Middlebrook 7H10 pasca ingesti, waktu inkubasi 24 jam dan 72 jam pada makrofag yang diberi Andrographolide 100 µg/ ml adalah 369,16 CFU/ml, 329,16 CFU/ml, 377, 50. Sedangkan untuk makrofag yang tidak diberi Andrographolide hasilnya adalah 608,33 CFU/ml, 611,66 CFU/ml, 640,27 CFU/ml. Hasil perhitungan rerata makrofag yang mengandung *Mycobacterium tuberculosis* hasil pengamatan dengan pengecatan Acridine Orange untuk makrofag yang diberi Andrographolide adalah 86,17, 89,50, 88,17. Sedangkan makrofag yang tidak diberi Andrographolide hasilnya adalah 69,50, 69,50, 69,50.

Dalam penelitian menggunakan nilai $p < 0,005$ hasilnya lebih kecil sehingga dapat disimpulkan bahwa kemampuan daya bunuh intraseluler makrofag pada *Mycobacterium tuberculosis* yang diberi imunomodulator Andrographolide, lebih tinggi daripada daya bunuh makrofag tanpa pemberian Andrographolide, secara *in vitro*. Sedangkan waktu makrofag membunuh *Mycobacterium tuberculosis* yang diberi imunomodulator Andrographolide, lebih cepat daripada waktu makrofag membunuh *tuberculosis* tanpa pemberian Andrographolide, secara *in vitro*, tidak terbukti sebab peningkatan kemampuan daya bunuh intraseluler hanya dapat dipertahankan hingga waktu inkubasi 24 jam. Dalam penelitian ini juga terungkap informasi bahwa makrofag yang diberi Andrographolide, daya fagositnya terhadap *Mycobacterium tuberculosis* lebih tinggi daripada makrofag yang tidak diberi Andrographolide.

Berdasarkan hasil penelitian, disarankan dalam menghadapi infeksi *Mycobacterium tuberculosis* sebaiknya para klinisi menambahkan imunomodulator Andrographolide baik untuk pencegahan atau sebagai pengobatan komplemen. Sedangkan untuk kepentingan penelitian yang terkait dengan penelitian yang dilakukan disarankan: perlu dilakukan penelitian lanjutan secara *in vitro*, pengaruh Andrographolide khususnya untuk mengetahui waktu makrofag membunuh *Mycobacterium tuberculosis* dalam kurun waktu stimulasi sampai 24 jam tetapi dengan konsentrasi *Mycobacterium tuberculosis* lebih kecil dari konsentrasi makrofag; perlu dilakukan penelitian untuk mengetahui pengaruh Andrographolide terhadap komponen sistem imun lain, baik pada sistem imun yang normal atau yang mengalami gangguan; perlu dilakukan penelitian secara *in vivo* untuk mengetahui pengaruh pemberian imunomodulator Andrographolide pada pemberian peroral.

SUMMARY

The influence of Immunomodulator Andrographolide for ability of energy killing Intraseluler Makrofag to *Mycobacterium Tuberculosis*

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Tuberculosis is infection disease which because of malignant bacterium *Mycobacterium tuberculosis* that kill approximated of 2-3 million public in world every year. Accretion of case newly reach amounts of nine million cases and if did not controlled hence in 20 year are coming of tuberculosis will kill 35 million people.

Though chemotherapy still is leading main at tuberculosis but because at 5 - 15 % individual case round into lung tuberculosis patient is anticipated as consequence of existence of defect T cell and Macrophage function or both of them until ability of phagocytosis and energy killing to bacillus *Mycobacterium tuberculosis* be weakened so usage of additional immunotherapy started to draw attention to overcome tuberculosis, especially because increasing of patient percentage which resistant to tuberculosis drug.

Andrographolide is a diterpenoid lactone has molecule formula $C_{20}H_{30}O_5$ with molecule weights of 350,45. Lacton bunch use with carbohydrate chain from protein transmembran at surface of T cell, as a result T cell helper be active henceforth secrete cytokine available for activated other cell like Macrophage and neutrofil.

Andrographolide can increase product $IFN-\alpha$?, $IFN-\gamma$? at PBMC and so increase ability of phagocytosis peritoneal guinea pig Macrophage for chicken erythrocyte phagocytosis. Have been reported that Andrographolide can use as immunostimulant, so can be said that Andrographolide has pharmacology activity tht assure as candidate in medication of immunomodulator.

This problem of research is giving of immunomodulator Andrographolide have an effect to ability of energy killing intraseluler Macrophage at *Mycobacterium tuberculosis*, at in vitro culture ?.

Purpose of in this research to prove that ability of energy killing intraseluler Macrophage at *Mycobacterium tuberculosis* which given by immunomodulator Andrographolide, higher than energy killing Macrophage without giving of Andrographolide, and also prove that Macrophage time killing *Mycobacterium tuberculosis* which given by immunomodulator Andrographolide, quicker than Macrophage time killed tuberculosis without giving of Andrographolide, in vitro.

In this research expected can express information concerning influence of giving of Andrographolide as immunomodulator, specially to energy killing intraseluler Macrophage and also Macrophage time kill *Mycobacterium tuberculosis*, at in vitro culture.

This research device is research of eksperimental laboratory with device research of True Experimental Randomized Postest Only Control Group Design.

Sample is man Macrophage which obtained by doing culture Peripheral Blood Mononuclear Cells (PBMCs) from healthy individual volunteer blood.

Amount of Macrophages which applied for every replications or repetitions are 10^5 cel/ml . Amount of repetitions are determined based on formulas Federer are 3 restarting.

Research is done in Laboratory Tropical Disease Center (TDC) University Airlangga Surabaya which started in October 2007 until month of January 2008.

Data is analysed to apply Univariate Analysis of Variance with F test at significance level of 5 % and continued with test HSD apply SPSS for Windows rail. 13.

Research result indicate that colony average *Mycobacterium tuberculosis* admiting of emergence at media Middlebrook 7H10 post ingesti, incubation is of 24 hour and 72 hour at given by Macrophage Andrographolide 100 μ g/ ml is 369,16 CFU/ml, 329,16 CFU/ml, 377, 50. While for Macrophage which be not given by Andrographolide the result is 608,33 CFU/ml, 611,66 CFU/ml, 640,27 CFU/ml. Result calculation Macrophage average containing *Mycobacterium tuberculosis* observation result with painting of Acridine Orange for Macrophage given by Andrographolide is 86,17; 89,50; 88,17. While Macrophage which be not given by Andrographolide the result is 69,50; 69,50; 69,50.

In research apply value $p < 0,005$ the result is smaller so that inferential that ability of energy killing intraseluler Macrophage at *Mycobacterium tuberculosis* which given immunomodulator Andrographolide, higher than energy kill Macrophage without giving of Andrographolide, in vitro. While Macrophage time killed *Mycobacterium tuberculosis* which given by was immunomodulator Andrographolide, quicker than Macrophage time killed tuberculosis without giving] of Andrographolide, in vitro, unprovable because increasing of ability of energy kill intraseluler can only defended till incubation time of 24 hours. This research also expressed by information that given by Macrophage Andrographolide, the phagocyte energy to *Mycobacterium tuberculosis* is higher than Macrophage which be not given by Andrographolide.

Based on research result, suggested in face of infection *Mycobacterium tuberculosis* better of klinisi add immunomodulator Andrographolide good to preventions or as medication of complement. While for the sake of research related to research which suggested done: require to be done research of continuation in vitro, influence Andrographolide specially to know Macrophage time kill *Mycobacterium tuberculosis* in stimulation range of time until 24 hour but with concentration of *Mycobacterium tuberculosis* smaller than concentration of Macrophage; require to be done by research for knowing influence Andrographolide to other immune system component, either at normal immune system or experiencing trouble; require to be done by research in vitro for knowing influence of giving of immunomodulator Andrographolide at giving of peroral.

ABSTRACT**The influence of Immunomodulator Andrographolide for ability of energy killing Intraseluler Makrofag to *Mycobacterium Tuberculosis*****Suhariyadi**

This research aim to prove that given by Macrophage is given immunomodulator Andrographolide, energy killing the intracelluler to *Mycobacterium tuberculosis* is higher, and also time killed it quicker than Macrophage without giving of Andrographolide, in vitro.

This research device is research of eksperimental laboratory with device of research of True Experimental Randomized Posttest Only Control Group Design by 3 restating .

Macrophage culture from PBMC come from healthy volunteer blood, stimulated by 100 µg/ml during 24 hour here in after infection with *Mycobacterium tuberculosis* during 1 hour. Macrophage culture post ingesti, incubation of 24 hour(clock and 72 hour, painted with Acridine Orange and calculated percentage of Macrophage containing *Mycobacterium tuberculosis*. Macrophage which culture and lysis at media Middlebrook 7H10 observed is energy killing the intracelluler apply method CFU/ml. Data is analysed use Univariate Analysis of Variance with F test at significance level of 5 % and continued with test HSD.

Result in this research concluded that at culture invitro, given by Macrophage is given by Andrographolide ability of energy killing the intraseluler is higher than Macrophage without giving of Andrographolide. While time killed given by Macrophage Andrographoliede to *Mycobacterium tuberculosis* was quicker than which without Andrographolide, unprovable, because energy killing the intraseluler increased only until incubation time of 24 hour. So is concluded also that Macrophage is given by Andrographolide, the phagocyte energy to *Mycobacterium tuberculosis* is higher than which without Andrographolide.

Keyword: Andrographolide, Macrophage, killing intracelluler, *Mycobacterium tuberculosis*.