The Effect of The Combination of *Phaleria macrocarpa* and *Phyllanthus niruri* Extracts on Peritoneal Macrophages Phagocytic Index of BALB/c Mice

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RESEARCH REPORT

Submitted to fulfill the assignment and fit-out requisite to obtain Bachelor of Medicine Degree

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APPROVAL PAGE

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a) This scientific research article is based on my genuine idea, formula and research, without any help from other parties, except supervisor and known parties.

b) This scientific research article is authentic and has never been published for any academic purpose in Diponegoro University or other university.

c) In this scientific research article there was no other people’s work or opinion, which have been used and published, unless there are clear acknowledgements of the original author names and the original paper titles are included in the references.

Semarang, August 21st 2013
The declarant,

Fadel Muhammad Garishah
Thank God I praise to Almighty God, for the blessings and His grace that I could finish these Academic Writing task. Writing the Academic Writing was done in order to fulfill one of the requirements to achieve the Bachelor of Medicine Degree from Faculty of Medicine Diponegoro University. I noticed it was very hard for me to finish this Academic Writing without help and guidance from various quarters since the restructuring proposal until the complete report of the results of this Scientific Writing. Together I deliver thanks and the highest appreciation to:

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8. And others we are not mentioned one by one on assistance directly or indirectly to this paper can be solved with good

Finally, I hope that God Almighty to recompense their kindness to everyone who had helped me. Hopefully this academic writing will be useful for all of us.

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Fadel Muhammad Garishah
ABSTRACT

Background Phaleria macrocarpa and Phyllanthus niruri have been known for their immunomodulatory effects to enhance and modulate the immune system. Nowadays, various herbal medicines combine herbs in purpose to obtain their benefits. There was not conducted research about the effects of combination both herbs in enhancing Macrophages Phagocytic Activity.

Aim To prove the effect of Phaleria macrocarpa and Phyllanthus niruri combination extracts to the differences of Peritoneal Macrophages Phagocytic Index compared to control group, single extract of Phaleria group and single extract of Phyllanthus group.

Methods This research was an experimental research with post-test only control group design. The samples were 20 healthy male BALB/c mice divided into 4 groups. The Control group given 0.5 cc water/day as placebo, T1 group given 0.14 mg Phaleria macrocarpa extract/day, T2 group given 0.4 mg Phyllanthus niruri/day and T3 group was given 0.4 mg and 0.14 mg combination extracts/day. The treatment was conducted in 7 days. The samples were terminated and peritoneal macrophages were isolated, cultured and measured for the phagocytic index at day 8.

Results Phagocytic Index Means were 0.038 for control, 0.114 for T1 group, 0.347 for T2 group and 0.068 for T3 group. The ANOVA test result showed at least a difference between two groups (p=0.001). From the post-hoc analysis, significant result was obtained from group T3 and T2 (p=0.020), while for the group T3 and Control (p=0.061) and group T3 and T1 (p=0.417) there was no difference.

Conclusions There is no difference of Peritoneal Macrophages Phagocytic Index of BALB/c mice that is treated with the combination extracts of Phaleria macrocarpa and Phyllanthus niruri compared to the control. The differences occur in the groups with the single-extract treatment.

Keywords Phaleria macrocarpa, Phyllanthus niruri, Peritoneal Macrophages, Macrophages Phagocytic Index
ABSTRAK

Latar Belakang Phaleria macrocarpa dan Phyllanthus niruri telah diketahui sebagai obat tradisional dengan kemampuan imunomodulasi yakni mampu meningkatkan maupun memodulasi sistem imunitas. Dewasa ini banyak obat herbal yang menggabungkan berbagai zat dengan tujuan memperoleh manfaatnya. Penelitian mengenai gabungan keduanya dalam meningkatkan imunitas seluler khususnya aktivitas fagositosis makrofag belum pernah dilakukan.

Tujuan Membuktikan pengaruh pemberian gabungan Phaleria macrocarpa dan Phyllanthus niruri terhadap perbedaan indeks fagositosis makrofag peritoneal mencit BALB/c

Metode Penelitian eksperimental post-test only design. Sampel 20 mencit BALB/c dibagi menjadi 4 kelompok. Kelompok K diberikan 0.5 cc air/hari sebagai placebo, Kelompok T1 diberikan 0,14 mg ekstrak Phaleria macrocarpa dalam 0,5 cc air/hari, Kelompok T2 diberikan 0,4 mg ekstrak Phyllanthus niruri dalam 0,5 cc air/hari dan Kelompok T3 diberikan 0,4 mg dan 0,14 mg ekstrak gabungan dalam 0,5 cc air/hari. Perlakuan diberikan selama 7 hari. Pada hari ke-8 dilakukan terminasi. Makrofag peritoneal diisolasi, dikultur dan diperiksa aktivitas fagositosisnya.

Hasil Rata-rata Indeks Fagositosis Kontrol 0,038, Kelompok T1 0,114, Kelompok T2 0,347 dan Kelompok T3 0,068. Pada uji ANOVA diperoleh ada perbedaan yang signifikan pada satu kelompok (p=0,001). Dari hasil uji post-hoc perbedaan signifikan didapatkan pada Kelompok T3 dan T2 (p=0,020), sedangkan pada Kelompok T3 dan Kontrol (p=0,061) dan Kelompok T3 dan T1 (p=0,417) tidak diperoleh adanya perbedaan.

Kesimpulan Tidak didapatkan perbedaan Indeks Fagositosis Makrofag Peritoneal mencit BALB/c yang diberikan ekstrak gabungan Phaleria macrocarpa dan Phyllanthus niruri dibandingkan kelompok kontrol. Perbedaan didapatkan pada kelompok yang diberikan ekstrak tunggal.

Kata kunci Phaleria macrocarpa, Phyllanthus niruri, Makrofag Peritoneal, Indeks Fagositosis Makrofag
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**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Explanation</th>
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<tr>
<td>TLRs</td>
<td>Toll-like receptors</td>
</tr>
<tr>
<td>NLRs</td>
<td>NOD-like receptors</td>
</tr>
<tr>
<td>RLRs</td>
<td>RIG-like receptors</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>NOs</td>
<td>Nitrite Oxides</td>
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<tr>
<td>T_{H1} cells</td>
<td>CD4$^+$ T Lymphocytes Helper Subset 1</td>
</tr>
<tr>
<td>T_{H2} cells</td>
<td>CD4$^+$ T Lymphocytes Helper Subset 2</td>
</tr>
<tr>
<td>T_{H17} cells</td>
<td>CD4$^+$ T Lymphocytes Helper Subset 17</td>
</tr>
<tr>
<td>NK cells</td>
<td>Natural Killer cells</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Interferon Gamma</td>
</tr>
<tr>
<td>f-MLP</td>
<td>f-Methionine-Leucine-Phenylalanine</td>
</tr>
<tr>
<td>PRRs</td>
<td>Pattern Recognition Receptors</td>
</tr>
<tr>
<td>APCs</td>
<td>Antigen-Presenting Cells</td>
</tr>
<tr>
<td>PAMPS</td>
<td>Pathogen Associated Molecular Patterns</td>
</tr>
<tr>
<td>CTLs</td>
<td>CD8$^+$ T Lymphocytes (cytotoxic lymphocytes)</td>
</tr>
<tr>
<td>MPS</td>
<td>Mononuclear Phagocytic System</td>
</tr>
<tr>
<td>HPSCs</td>
<td>Hematopoietic Stem Cells</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Granulocyte Monocyte Colony Stimulating Factor</td>
</tr>
<tr>
<td>M-CSF</td>
<td>Monocyte Colony Stimulating Factor</td>
</tr>
<tr>
<td>IILs (2, 4, 10, 12, 18 etc.)</td>
<td>Interleukins</td>
</tr>
<tr>
<td>MHC</td>
<td>Major Histocompatibility Complex</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor Necrosis Factor</td>
</tr>
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<td>NF-κB</td>
<td>Nuclear Factor Kappa B</td>
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<tr>
<td>IκB</td>
<td>I Kappa B</td>
</tr>
<tr>
<td>BM DCs</td>
<td>Bone-Marrow-derived Dendritic Cells</td>
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<tr>
<td>T_{Reg} Cells/ Reg T cells</td>
<td>Regulatory T Cells</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Transforming Growth Factor Beta</td>
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