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Effect of Purple Miana Leaf (Coleus Scutellorioide (L) Benth) from Tana Toraja District on IL-10 mRNA Expression in Mice Induced Mycobacterium Tuberculosis

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Abstract

Tuberculosis therapy derived from local natural resouces continues study and develop, one of natural resources from Tana Toraja district in South Sulawesi, Indonesia is Miana leaves. This tudy was aimed to investigate effect of purple miana leaf (Coleus Scutellorioide (L) Benth) from Tana Toraja district On IL-10 mRNA Expression In Mice Induced Mycobacterium Tuberculosis. It was a laboratory research using Balb/c mice divided into three groups (each group consist 5 mices); negative control (Standard diet), positive control (Rifampicin) and intervention group (Miana leaf). All mices injected *mycobacterium tuberculosis* 0.2 ml x 10^5 ml/CFU then given control positive was given Rifampicin as much as 5,4 mg/bw and intervention group was given miana leaf extract as much as 0.3 ml/30 grbw for 14 days respectively. Examination of IL-10 mRNA expression by Realtime PCR was done before and after intervention. Data analyzed used Paired sample T Test and Wilcoxon. Result showed that purple Miana extract leaf decrease mRNA expression sigficantly and this decline was lower than negative and positive control. Comparison between groups showed that there was no significance difference between positive control and intervention control. Conclusion of this study is purple Miana leaf extract was able to decrease the expression of IL-10 mRNA and this ability was not significantly different with rifampicin ability.

Keywords: Miana Leaves; IL-10; Mycobacterium Tuberculosis.

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1. Introduction

Incidence of tuberculosis continues to increase despite continued handling of efforts especially among at-risk populations, that is poor and socially marginalized people [1]. Some of areas as Papua in Indonesia have high tuberculosis cases and even those with drug resistance are also very [2]. This condition makes researchers should keep trying to develop therapies derived from local materials available in community, one of which is purple miana leaves.

Miana leaves have been shown immunomodulatory effects through its ability to prevent tuberculosis infection by increasing T-lymphocytes, CD4 T-Cells and levels of IFN-γ [3]. Several studies have shown that miana leaves contain flavonoids (Lumbessy 2013; Ridwan, 2007). Flavonoids are considered as inhibitors for mycobacteria including tuberculosis and become an anti-inflammatory that is useful in excessive inflammation in lungs [4-6].

Inflammatory reactions in lungs with granulomas somewhile will be develop as a systemic disease such as sarcoidosis. If there is inflammation due to excessive granulomas, this may lead to tissue remodeling and becomes to fibrosis and/or cavitation [7]. Reduction of inflammation in chronic condition may help CD14 which is one of extracellular adaptor proteins needed for introduction of gram-negative bacteria such as mycobacterium tuberculosis [8].

Some ingredients from nature such as honey and propolis containing flavonoids that can provide balance to the immune system to prevent excessive immune responses [9]. Purple Miana leaf as natural resources containg flavonoid will be investigate in this study whether it has ability to provide anti-inflammatory effects in mice induced by mycobacterium tuberculosis.

2. Material and Methods

This study was a laboratory study that intervened purple miana leaves from Tana Toraja district in Balb / c strain mice infected with mycobacterium tuberculosis. Leaves of miana extracted at Phytochemical Laboratory, Hasanuddin University Faculty of Pharmacy and IL-10 expression examination were conducted at Hasanuddin University's Immunology and Biomolecular Laboratory, faculty of medicine. Ethical permission has been obtained from the Ethics Commission of Faculty of Medicine, University of Hasanuddin.

Balc/c mice were adapted for seven days then infected with mycobacterium tuberculosis 0.2 ml x 105 ml / CFU. After infected with mycobacterium, it was divided into 3 groups (each group consists of 5 mices); negative control group was given a standard diet, positive control group given Rifampicin 5.4 mg/20 grbw and intervention group were given 0.3 ml/20 grbw purple leaf Miana extract as long as 14 days.

Examination of IL-10 mRNA expression was performed on the 8th day before bacterial injections and after intervention or on day 15. Expressions of IL-10 mRNAs were performed by taking 0.1 mL blood sample which was then extracted using the Boom protocol and analyzed using the Real-Time Pulymerase Chain Reaction (PCR). Primer used was 5'-TGGCCCAGAAATCAAGGAGC-3'.

Data analysis using statistical package for Statistical Science (SPSS), data are presented in graphical form and tables that are equipped with different mean and probability values. Statistical test using paired sample T-test for normal distributed data and Wilcoxon test for non-distributed data.

3. Result

The results showed that IL-10 mRNA expression in the group treated with purple Miana leaf extract was higher with the group given rifampicin but lower if compared with the group given standard diet (Figure 1).

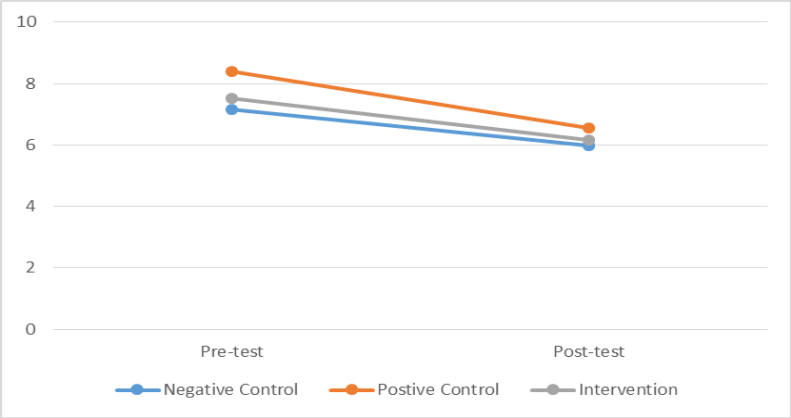

Figure 1: IL-10 mRNA Expression Before and After Intervention

The data in Table 1 showed that the group given purple miana leaf extract was able to decrease the expression of the IL-10 mRNA but this decrease in expression was the lowest compared to the negative control group and the positive control group. Although it only decreased an average of 0.951, this decline was considered statistically significant (P <0.005).

Table 1: Analysis Mean Difference of IL-10 mRNA expression before and after Intervention

Group	Test	Expression of mRNA IL10		Mean Difference		p*
		Mean	SD	Mean	SD	
Negatif Control	Pre	7,159	0,638	2,393	0,450	**0,000**[a]
	Post	5,995	0,499			
Negative Control	Pre	8,388	0,436	1,012	0,378	**0,004**[b]
	Post	6,560	0,507			
Miana Leaves Extract	Pre	7,511	0,542	0,951	0,236	**0,001**[b]
	Post	6,147	0,639			

[a]Pair Sampel T Test

[b]*Wilcoxon* Test

After giving intervention for 14 days, there was no significant difference between the intervention group given Miana leaf extraxt and the positive control group given Rifampicin. While intervention group with negative control group differed significantly, negative control group was also different significantly from rifampicin group.

Table 2: Comparison Expression of IL-10 mRNA Expression Between Groups

Group	Mean	Difference Mean	P**	P
Intervention	7,511	0.877	**0.056**	
Negative Control	8,388			
Intervention	7,511	0.352		
Positive Control	7,159		**0.548**	**0.030**
Negative Control	8,388			
Positive Control	7,159	1.229	**0.016**	

4. Discussion

The presence of IL-10 is necessary to prevent excessive granulomas in inflamed lungs but an excessive increasing in infectious conditions may prevent bacterial elimination. Based on this study, administration of Miana leaf extract has potential beneficial effect on tuberculosis therapy because it can decrease IL-10 but this decline was smaller than group given only standard diet or negative control group but did not differ significantly with positive control groups given Rifampicin. Animal experiments show that lowering IL-10 activity during chronic tuberculosis infection provides a stabilizing effect on pumonary bacterial load and improves survival. Overly high activity of IL-10 can suppress immunity against bacteria and also increase bacterial survival [10, 11]. Blockade to IL-10 is considered to be able to optimize resistance to tuberculosis and even substances that have antagonistic effects on IL-10 have the advantage of being adjuvants for prevention [12]. Tumbuhan seperti daun miana yang mengandung flavonoid natural patut dipertimbangan untuk menjadi antiinflamasi pada penyakit infeksi karena kemampuan flavonoid dalam tumbuhan sudah terbukti mampu untuk mempengaruhi IL-10 [13]. Meskipun tumbuhan yang mengandung flavonoid bisa berfungsi sebagai anti infamasi namun di sisi yang lain kemampuannya untuk menjadi anti mikroba juga tetap ada, hal ini memberikan efek yang sangat menguntungkan dalam terapi [14]. Plants such as Miana leaves containing natural flavonoids should be considered to be anti-inflammatory in infectious diseases because flavonoid in plants has ability to decline IL-10 [13]. Although flavonoid-containing plants may act as anti-inflammatory but on the other hand their ability to be anti-microbial also persists, this provides a very beneficial effect in therapy [14]. Further explorations is needed related to the ability of miana leaf extracts to influence pro-inflammatory activity and also appropriate dosage because this study did not explore dose response. It is also important to explore difference effect between types of Minana leaves by color and geographic location.

5. Conclusion

Purple Miana leaf extract has potential beneficial effects on tuberculosis therapy because it is able to influence anti-inflammatory activity, which decreases expression of IL-10 mRNA and this ability is not significantly different from Rifampicin's ability.

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