Potential Honey Propolis Trigona to Prevent Arthritis Rheumatoid by Increase Foxp3 Level; A Preliminary Study

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ABSTRACT

Background: Rheumatoid arthritis is an autoimmune disease and need the balancing of immune system. Foxp3 Treg level is one of critical regulator to balance system immune. This study aim to analyze potential of propolis and honey propolis for prevention rheumatoid arthritis based on Foxp3 Treg as a target. **Methods:** It was a quasi-experimental study with pretest-posttest control design, Subject divided into 3 groups, group 1 control did not give anything, group2 given propolis 60 mg/capsule and group 3 given honey propolis 35 mg/pack. Propolis and Honey Propolis Trigona was giving through 14 days and blood sample for ELISA examination taken three times, Before intervention (baseline), 7 day after intervention and 14 days after intervention. **Results:** Analysis mean difference between baseline data with 14 days data show giving honey propolis has the highest increase of Foxp3 Treg and giving propolis has the lowest increase of Foxp3 Treg. Among the three groups, only the group given honey propolis has a significant effect. **Conclusion:** Honey propolis has potential effect as therapy for prevention arthritis rheumatoid by increase Foxp3 Treg level. Adherence to consume honey propolis is necessary to provide the maximum effect.

Keywords: *Honey Propolis, Trigona, Arthritis Rheumatoid, Autoimmune*

INTRODUCTION

Rheumatoid arthritis (RA) is a severe inflammatory disease and categorized as one of autoimmune disease, unproper treatment will make it improve to joint damage and disability. The most disturbing is pain and swelling due to inflammation. In advanced conditions, the disease may increase the risk of cardiovascular disease and changes in bone metabolism. [1 2]

T regulatory (Treg) cell is critical regulator in autoimmune disease, impaired self-tolerance in autoimmune diseases such as rheumatoid arthritis occurs due to Treg cell failure to regulate its suppressive function. Reduction of Treg frequency correlate with increase of RA.

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Manipulating Tregulator cells with Foxp3 as transcription factor or marker of Treg as a target of prevention and therapeutic is a very important factor in immune homeostasis. [1 3-5]

Several studies has tried Treg injection to the the patient and successfully affect the duration of remission. [6 7] Several studies also use Salmonella bacteria that has been attenuated as a vaccine for cases autoimmune diseases, it has ability to induce Foxp3 Treg. Although successful in inducing the expression of Foxp3 Treg, using pathogenic bacteria is still being debated. [8 9] Tregulator cell involvement is the latest pathologic mechanism developed in the therapeutic target of rheumatoid arthritis, but it requires intervention to increase of Foxp3 Treg without debate and doubts about intervention safety.

South Sulawesi is one of honey and propolis producing regions and honey bee products include propolis and honey has ability to promote Foxp3 Treg both in healthy and unhealthy subject. This ability

associated with polyphenol, quercetin and vitamin content of its. [10-12] Studies of honey and propolis and its correlations with immune systems have been numerous, but studies that correlate honey and propolis with Foxp3 Treg are rare. The combination of honey and propolis is also rarely studied and usually researched distinctly.

This study aim to analyze potential of propolis and honey propolis for prevention rheumatoid arthritis based on Foxp3 Treg as a target.

MATERIAL AND METHOD

Material

Trigona honey and propolis used was local honey bee products, it was taken from Masamba, a district in South Sulawesi. Honey bee farmer has been produce in a large scale and become the raw material for some industries of honey and propolis.

The water content of honey is lowered dehumidifier up to 20% then stored for 3 days in a stainless container to precipitate the dirt. Propolis extracted using water and dried using fresh dryer as long as 24 hours. Honey propolis then mixed with 85% honey and 15% honey in 35 mg/pack. Propolis packed in capsule as much as 60 mg/capsule.

Methods

Protocol Recruitment of Subject

Ethical clearance has been accepted by Hasanuddin University Ethical Committee (1046/H4.8.4.5.31/ PP36-KOMETIK/2017). To recruit subject of study, we offering free rheumatic screening to those who have a rheumatic-like symptom. Thirty people were willing to take part and twenty of them were positive for rheumatic suspect (examination of ASTO, CRP and Rheumatoid Factor). They were giving information about protocol of study and asked to join the study, informed consent were giving both to them who had suspect of rheumatoid and normal subject.

Design and protocol of Study

It was a quasi experimental study with pretest post control design, Ethical Clearance from Ethical Committee of Medical faculty, Hasanuddin University). Subject divided into 3 groups, group 1 was control did not given anything, group2 given propolis 60 mg/ capsule and group 3 given honey propolis 35 mg/pack. Group 1 and 3 consist of 10 subject and group 2 consist of 7 subject, because 2 subject exclude after informed consent given because they had allergic with honey.

It was a quasi-experimental study with pretest-posttest control design, Subject divided into 3 groups, group 1 control did not give anything, group2 given propolis 60 mg/capsule and group 3 given honey propolis 35 mg/pack. Group 1 and 3 consist of 10 subject and group 2 consist of 7 subjects, because 2 subjects exclude after informed consent given because they had allergic to honey.

Propolis and Honey Propolis Trigona was giving through 14 days and blood sample for ELISA examination taken three times, Before intervention (baseline), 7 day after intervention and 14 days after intervention. Protocol of ELISA was use standard od human ELISA Kit produced by mybiosource.

Statistical Analysis

Data analyse using Statistical Package for Social Sciences (SPSS), statistical test were Wilcoxon and Paired-Sample T Test to compare of mean. Mean difference considered significant if probability value (p-value) less than 0.05.

RESULT

Analysis result after seven days giving honey propolis, it has the highest effect to increase Foxp3 Treg, and it is significant statistically while propolis and control also increase Foxp3 Treg but it is not significant (Table 1). After 14 days, data indicated a decline of foxp3 both group giving honey and honey propolis while control group shows an increase of Foxp3 Treg (Table 2).

Table 1. Comparison of Mean Difference Between Baseline with 7 days After Intervention

Groups	Level of Foxp3 (pg/ml)			P
	Mean±SD			
	Baseline	7 days	Mean Difference	
Control (n=10)	0.898±0.343	1.071±0.612	0.173	0.416*
Honey Propolis (n=10)	0.538±0.539	0.901±0.581	0.363	0.005**
Propolis (n=7)	0.417±0.581	0.425±0.110	0.008	0.917**

**Paired Sample T-Test*

***Wilcoxon*

Analysis mean difference between baseline data with 14 days data show giving honey propolis has the highest increase of Foxp3 Treg and giving propolis has the lowest increase of Foxp3 Treg. Among the three groups, only the group given honey propolis has a significant effect (table 3 and 4).

Table 2. Comparison of Mean Difference Between 7 days with 14 days After Intervention

Groups	Level of Foxp3 (pg/ml)			P
	Mean±SD			
	7 days	14 days	Mean Difference	
Control (n=10)	1.071±0.612	1.194±0.916	0.123	0.441**
Honey Propolis (n=10)	0.901±0.581	0.888±0.529	-0.013	0.897*
Propolis (n=7)	0.425±0.110	0.616±0.262	-0.191	0.172**

**Paired Sample T-Test*

***Wilcoxon*

Table 3. Comparison of Mean Difference Between Baseline with 14 days Afte Intervention

Groups	Level of Foxp3 (pg/ml)			P
	Mean±SD			
	Baseline	14 days	Mean Difference	
Control (n=10)	0.898±0.343	1.194±0.916	0.296	0.646*
Honey Propolis (n=10)	0.538±0.539	0.888±0.529	0.350	0.037*
Propolis (n=7)	0.417±0.581	0.616±0.262	0.199	0.173*

**Uji Wilcoxon*

Table 4. Comparison of Mean Difference Between Baseline with 7 days After Intervention

Groups	Mean Difference of Foxp3 (pg/ml)		
	Mean±SD		
	Baseline-7 days	7 days-14 days	Baseline-14 days
Control (n=10)	0.173	0.123	0.296
Honey Propolis (n=10)	0.363	-0.013	0.350
Propolis (n=7)	0.008	-0.191	0.199

**Paired Sample T-Test*

***Wilcoxon*

DISCUSSION

Intervention use honey propolis showed the significant increase of Foxp3 Treg compare to another group, both control and propolis intervention. Analysis in detail shows that increase of Foxp3 Treg happens in the first week and in the second week Foxp3 decline, we have an assumption that adherence of subject is determinant why the data is not consistent. In the first week, all subjects reported that they did not take their honey propolis and propolis regularly. Majority of subjects give a reason, they forget ones or too busy even we have remembered them. It shows that adherence is just consistent in the first week.

Propolis has the anti-inflammatory and immunosuppressant effect, the component of it that maybe has a role is Artepillin. Mechanism how propolis and honey bee products affect immunosuppressant still not clear and this study may be one explanation that Foxp3 Treg is the keyword of it. [13-15]

Prevention from arthritis was associated with increased of Foxp3 Treg ratio and decrease of inflammation such as IL-17. [16] Rheumatoid arthritis is a chronic systemic autoimmune disease characterized by synovitis and joint erosion and involves activation of T cells in its pathogenesis. [17] The abnormality that is encountered in rheumatoid arthritis due to the growth of synovial cells that damage bone and cartilage. The pathogenesis is the activation of autoreactive T cells that migrate into the synovial cavity and B cells, through a cytokine that is folded by T cells. The synovial membrane becomes hyperselular due to the accumulation large amounts of lymphocytes in various stages of activation, plasma cells and macrophages. All cells exhibit high activity and interactions between the cells that form the formation of immunoglobulin and rheumatoid factor. [18-20] Activation of excessive immune cells and the inability to recognize self-antigen is a determinant for arthritis rheumatoid an another autoimmune diseases, for this problem requires therapy that can lead to homeostasis of the immune system.

One of the main targets of therapy in rheumatoid arthritis patients is restore the immune balance system, regulatory T cells are the ideal targets in this case because their function can suppress inflammation. The better thing is that the increased amount of Treg to prevent inflammation will not inhibit the ability of the immune response to fight infection. [6 21 22]

Honey propolis has a potential effect to promote Foxp3 Treg in human suspect artritis rheumatoid. This study just an eliminary study and need more studies that controls more variables so it is clear that the effect of immune balance is derived from the intervention that is done.

CONCLUSION

Honey propolis has potential effect as therapy for prevention arthritis rheumatoid by increase Foxp3 Treg level. Adherence to consume honey propolis is necessary to provide the maximum effect.

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Conflict of Interest: Authors declare that there is no conflict interest related this study.

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