



## Effect of Extract Cocor Bebek (*Bryopyllum pinnatum*) to the Amount of Th17 Cells Spleen and Levels of IL-17A Serum in Mice Balb/C Pregnant Model of Systemic Lupus Erythematosus

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### Abstract

This study aims to determine a way to reduce the number of Th17 cells spleen and serum levels of IL 1A heart in mice Balb /C pregnant models with Systemic Lupus Erythematosus. This was studied, using mice Balb / C pregnant models with SLE. This study is divided into 5 groups: negative control, positive control, and treatment provision *Bryophyllum Pinnatum* dose of 10.5 mg/day, 21 mg/day and 42 mg/day. The Th cell number 17 was measured through flow cytometry. Levels of IL 17A were measured using the ELISA method. The data was analyzed using the Kruskal Wallis test. Giving *bryophyllum Pinnatum* dose 21mg / day ( $2.79 \pm 1.484$ ) has been proven to reduce the number of Th17 cells in mice Balb / C pregnant models with SLE when compared to the other dose treatment. Although, it did not significantly affect ( $p > 0.05$ ). There was no difference with the increased levels of IL *bryophyllum Pinnatum* 17A in Balb / C mice pregnant models with SLE ( $p > 0.05$ ). Conclusion: Giving *bryophyllum Pinnatum* has been proven to reduce the number of Th 17 cells in mice pregnant models with SLE.

**Keywords:** *Systemic Lupus Erythematosus (SLE), Bryopyllum Pinnatum, Th17, IL 17A.*

### Introduction

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune inflammatory disease, involving multiple organs. It has an unknown etiology, and breadth of clinical manifestations. The SLE disease affected nearly 90% of women in the age range of 15-40 years old. It had a ratio of women and men 5:1 [1].

SLE is most common in women of childbearing age and often causes health problems during pregnancy. These health problems can harm both the mother and the fetus.

The Unclear pathogenesis of SLE in pregnancy developed the study, using an animal model of lupus with induction methods Pristan intraperitoneally [2]. Giving Pristan in mice Balb / c, led to the occurrence of immune effects involving the antibody IgG, neutrophils, macrophages, and endothelial activation.

This led to the production of interferon (IFN), the production of interleukin 6 (IL-6), and it increases reverse a decline in the secretion of IL 2. Mechanism the underlying rise of autoimmunity due to Pristan injection in mice Balb/c [3].

Th 17 cells play an important role in the inflammatory process that leads to tissue damage. Interleukin (IL) -17 is a major cytokine of Th 17. Lymphocyte cytokine IL-17 (or IL-called 17A) is a potent inducer of inflammation, causing cellular infiltration and production of pro-inflammatory cytokines and chemokines. Whereas, the differentiation and regulation of Th17 influenced by IL-6 and TGF- $\beta$  also require transcription factor ROR $\gamma$ t, STAT3, and IRF-4. The presence of IL-6 will suppress the formation T-regulator (Treg), so that the formation of proinflammatory Th 17 cells will increase.

Increased levels of IL-17 produced by Th 17 cells have been detected in patients with autoimmune diseases such as SLE [4]. One natural ingredient that has the potential to be a treatment for SLE is *Bryophyllum pinnatum*. Several studies have shown that *pinnatum* *Bryophyllum* can be helpful as preventive medicine for the infection. It is an immunomodulatory, an anti-oxidant and an anti-inflammatory in both humans and animals.

Its content consisting of flavonoids, vitamin C, and fatty acids work as an antioxidant. It acts to eliminate free radicals and prevent cell damage [5-6]. Quercetin, and luteolin contained in flavonoids are believed to be responsible for the anti-inflammatory and immunomodulatory effects. However, there have been no previous studies investigating the effects of *Bryophyllum pinnatum* with relation to the disease SLE in pregnancy. Therefore, researchers are interested in studying the effects of *Bryophyllum pinnatum*, especially to decrease the number of Th 17 cells and the levels of IL 17 in mice Balb/c pregnant model of SLE.

## Materials and Methods

The samples used were Balb / C mice. The 30 pregnant SLE models were divided into 5 groups. There was 1 negative control group, 1 positive control group, and 3 groups treated with the extract *Bryophyllum pinnatum*. The *Bryophyllum pinnatum* was administered in doses of 10.5 mg / day, 21 mg / day and 42 mg / day. Tools used include: a set of sterile surgical instruments, a syringe, a sonde, an eppendorf, tubes, micro pipette 50  $\mu$ L, 100  $\mu$ L and 1000  $\mu$ L, vortex, centrifuges, incubators, antibody FITC CD4 anti-mouse antibodies,

PerCP IL-17A antimouse Biologend, Elisa kit Biologend, and Elisa Reader.

## Procedure Making Mice Balb/C Model Systemic Lupus Erythematosus (SLE)

Making Balb / C mice model of SLE refers to research that have been done by Wang, et al (2005). On the first day, mice in group K (+) were given Pristan intraperitoneally injections at a dose of 0.5 ml. This took place once during the study, intending to create the immune effect. There was then a 12 week waiting period. After this, mice were examined for ANA, and signs of clinical manifestations of lupus. This was to ensure mice Balb / C into already mice model of SLE.

## Impregnate Mice Balb / C

After mice Balb / c became mice model of SLE, then mice Balb / C were impregnated in 3 stages. The stage Leebboth effect, the effect of pheromones and, then mated Whitten effect for 24 hours and examined vaginal plug in the morning [7].

## Procedure of Extracting *Bryophyllum Pinnatum*

Leaves of *Bryophyllum pinnatum* were cleaned, and then placed in the oven at a temperature of 40-60 ° C. They were then pureed in a blender. They weighed approximately 100 grams. They were then placed in Erlenmeyer flasks containing  $\pm$  1L, and soaked with ethanol to a volume of 1L. The vials were shaken, and left to settle. The top layer of ethanol with active substances that have been taken included in the evaporation flask. In the evaporator ethanol allowed the active substances already present in the evaporation flask. The flow of ethanol waited until dripping stopped at shelters  $\pm$  900ml flask. It was extracted, and placed into plastic bottles / glass, and then stored in a freezer [8].

## Giving Extract *Bryophyllum Pinnatum*

On the 9th day of pregnancy, the treatment group was given the extract *Bryophyllum pinnatum* in three doses. The dose of 10.5 mg/day/head, 21 mg/day/head and 42 mg/day/head for 10 days. On the 18th day, they performed the termination to take the spleen and heart serum of mice. The number of cells with Th 17 was then measured using flow cytometry methods. Levels of IL 17A were measured using the Elisa method.

### Measurement Procedure Number of Th 17 cells With Flow Cytometry

After isolating the spleen cells, the percentage of Th17 cells will be calculated using flow cytometry. To measure the percentage of Th17 cells labeled with FITC staining CD4 antibody anti-mouse (Biolegend, USA, catalog number 100 509) and PerCP IL-17A antibody anti-mouse (Biolegend, USA, catalog number 506 919). Overall, the Th17 staining procedures were performed by the procedure of the factory Biolegend.

### Measurement Procedures levels of IL-17A with Elisa

Levels of the cytokine IL-17 in the serum were examined using ELISA Kit mouse IL-17A (Biolegend, USA, catalog number 506 919).

### Data Analysis

All data is calculated, the mean ± SD and analyzed using SPSS 19.0 software. Data was tested for normality, dilanjutkan with a one-way ANOVA.

### Results

After doing research, obtained the following results:

**Table 1: Testing the effects of extracts Bryophyllum pinnatum to decrease the number of Th 17 cells in mice Balb / C pregnant model of SLE**

	Average ± stan. dev	p-value
K-	2,58 ± 0,938	
K+	5,028 ± 0,404	0,014
KP 1	7,56 ± 0,805	
KP 2	2,79 ± 1,484	
KP3	3,08 ± 0,150	

The above table shows that KP 1 has the highest number of Th 17 cells of all groups. KP 2 has a number of Th 17 cells approaching the number of Th 17 cells group K (-). This suggests a reduction in the number of Th cells 17 after being treated.

Based on the test Kruskal Walis has shown a p-value of 0.014 (p <0.05). So, it can be concluded that there is a correlation between the extract Bryophyllum pinnatum and a decrease in the number of Th 17 cells in mice Balb / c model of SLE was significantly (p <0,05).

**Table 2: Bonferroni test results to Total Th 17 cells in Mice Balb / C pregnant model of SLE**

	Average	Probability					
		KP 1	K+	KP 3	KP2	K-	Notasi
KP 1	7,56		0,023	0,000	0,000	0,000	a
K+	5,028	*		0,118	0,052	*	b
KP 3	3,08	0,000	NS		1,000	1,000	bc
KP 2	2,787	0,000	NS	1,000		1,000	bc
K-	2,580	0,000	*	1,000	1,000		c

Based on the above table, it can be seen that there is a significant difference in the mean number of cells Th 17 between K (+) compared with KP 1. It shows that the extract Bryophyllum pinnatum at a dose of

10.5 mg/head/day does not decrease the number of Th 17 cells. The results showed a decrease in the average number of cells Th 17 KP 2 has the approximate value K (-) but was not statistically significant.

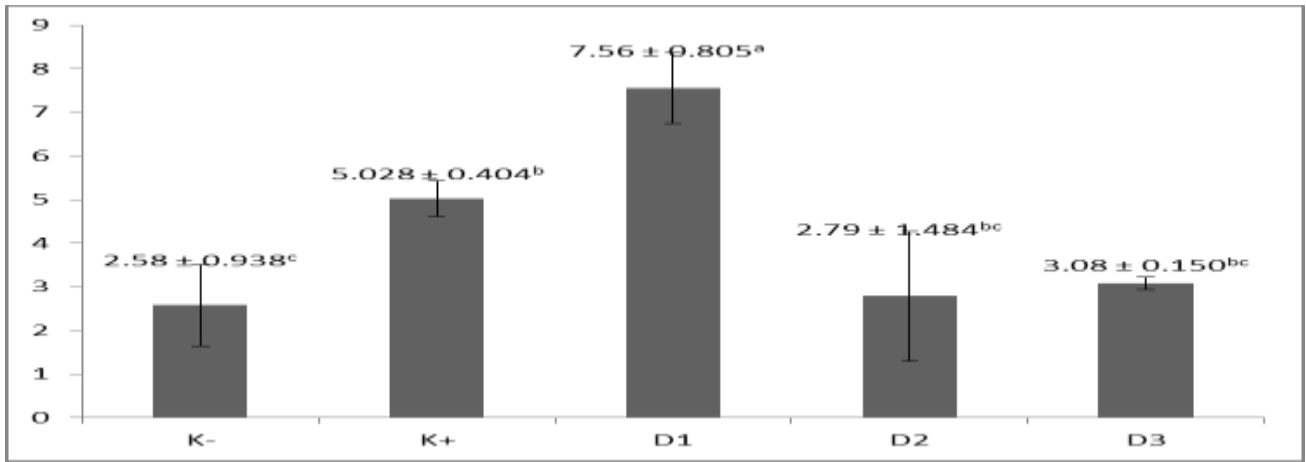


Figure 1: The administration of the extract *Bryophyllum pinnatum* to the number of Th 17 cells in mice Balb / c model of SLE,

From the histogram above it can be seen that there are significant differences between the number of Th17 cells K (-) and K (+), while the value of the highest rates found in the treatment group dose of 10.5 mg / day (D1). There was no significant difference between the treatment group K (-) (D2 and D3) or between K (+) to the treatment group (D2 and D3). So it can be concluded that the administration of the extract *Bryophyllum*

*pinnatum* was quantitatively able to reduce the number of Th 17 cells in mice Balb / c model of SLE, although not statistically significant.

### Testing the Effects of Extracts *Bryophyllum Pinnatum* to Decreased Levels of IL-17A in Mice Balb / C Pregnant Model of SLE

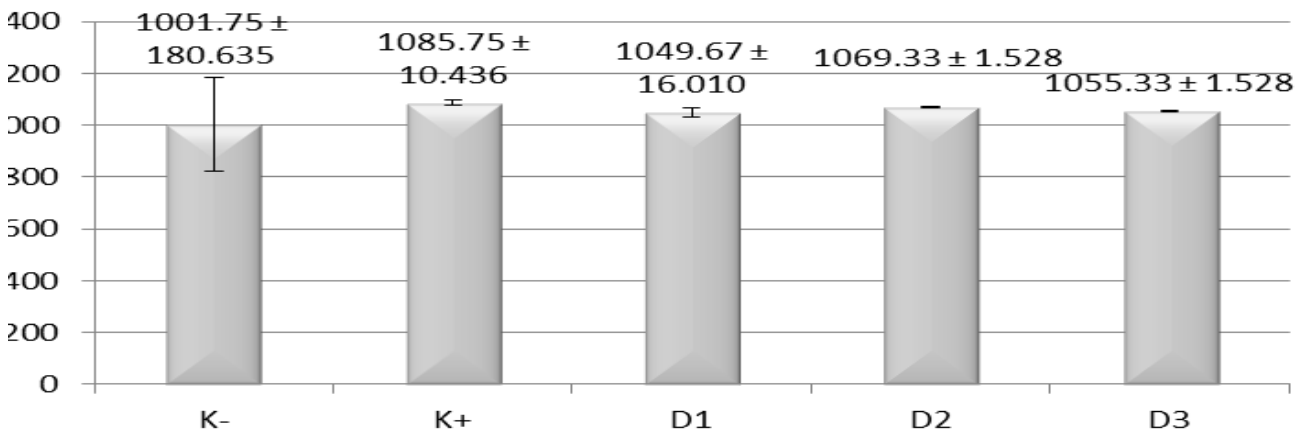


Figure 2: The effects of extracts *Bryophyllum pinnatum* to decreased levels of IL-17A in mice Balb / C pregnant model of SLE

The above picture shows that the group K (+) had a mean level of IL 17A higher than the treatment group. Kruskal Walis' tests showed a p-value of 0.071 ( $p > 0.05$ ), so it can be concluded that there is no significant correlation between extracts of *Bryophyllum pinnatum* and decreased levels of IL 17A in mice Balb / c model of SLE.

### Discussion

The results of this study indicate that there are significant differences between the number of Th 17 cells K (-) and K (+). It is similar to past research, which shows that Pristan in mice Balb/c led to immune effects involving antibody IgG, neutrophils,

macrophages and endothelial activation. The activation of IgG will provide signaling in neutrophils and macrophages via FCyR, for their secretion of neutrophils. The percentage of IL-6 increased, while the percentage of IL 2 decreased.

The activated endothelial due to inflammatory mechanisms also secrete IL-6, so that the mice Balb/c induced Pristan, will produce IL 6 more. Pristan also increases the production of IFN. The increase in IFN and IL-6 plays an important role in the formation of autoantibodies in mice Balb/c. IL-6 is high, and a low TGF-β cause's cell T CD4 to differentiate into Th 17 cells. Other studies have reported that in autoimmune diseases

such as SLE occurs an imbalance between T helper 17 (Th17) and regulatory T (Treg) that there is increasing activity of Th 17 cells and a decrease in the activity of Treg cells [9-10]. This study also showed that the average value is highest in the treatment group dose of 10.5 mg/day (D1). There was no significant difference between K (-) in the treatment group (D2 and D3) or between K (+) to the treatment group (D2 and D3). So it can be concluded that the extract *Bryophyllum pinnatum* can reduce the number of Th 17 cells in mice Balb/c model of SLE.

Although this is not statistically significant. Several studies have reported that the flavonoid compounds in the plant *Bryophyllum pinnatum* are mainly quercetin, kaempferol, rutin and luteolin. These have been shown to have immunomodulatory effects [11-14]. Other studies have reported that polyphenols and flavonoids have a positive correlation with immunomodulating capabilities.

This occurs through the stimulation and the proliferation of splenocytes. Also, it was reported that the flavonoid quercetin can stimulate the activity of peritoneal macrophages and increase NK cell activity [16]. Some studies have also proved their immunomodulatory effects on plants *Bryophyllum pinnatum*.

An immunomodulator is a compound that can affect the humoral and cellular immune system. There are two types of immunomodulatory, ie immunostimulatory, boosts the immune system. Immunosupresor suppresses the immune system. The mechanism of action of immunomodulators includes increased immunity against infection through cells of the immune system.

These cells include lymphocytes, macrophages, dendritic cells, and NK cells. Advanced mechanisms could involve the induction or improvement of immune system effector functions and cytokine balance settings. The results of this study are supported by studies with similar findings.

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These studies show that luteolin contained in flavonoids can inhibit the stimulation of TNF  $\alpha$  and IL-6 in macrophages. the production of IL-6 will be reduced, thereby inhibiting the differentiation of the Th 17 cells. Other studies also state that ethanol extracted from leaves of *Bryophyllum pinnatum* has antinociception, anti-inflammatory, and immunomodulatory effects. This is due to flavonoids, polyphenols, and triterpenoids [17-18]. The results showed that there was no correlation between the extract *bryophyllum Pinnatum*, and decreased levels of IL 17A in mice Balb/c model of SLE.

Some past research regarding the mechanisms *bryophyllum Pinnatum* showed that it was an anti-inflammatory, and useful in inhibiting the activation of IL 17, but the results of this study indicate that the extract *bryophyllum Pinnatum* not been able to reduce the levels of IL-17A in pregnant mice models of SLE. This is likely due to the way the endocrine and immune systems are closely linked and mutually influence each other. In pregnancy with SLE increased levels of cytokines IL-6, IL-10, IL-17 and TNF during pregnancy [19], whereas in normal pregnancy serum levels of IL 17 increased in the third trimester, it shows that increased levels of IL-17 may be involved in labor and inflammation [20].

## Conclusion

In this study, it can be concluded that administering the extract *bryophyllum Pinnatum* has been proven to reduce the number of Th 17 cells in mice Balb / c pregnant model of Systemic Lupus Eritematosus (SLE). Giving the extract *bryophyllum Pinnatum* has not been shown to reduce levels of IL-17A in mice Balb / c pregnant model of Systemic Lupus Eritematosus (SLE).

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