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Effect of Red Bean Extract *(Phaseolus vulgaris L.)* on IL-10 Levels in Systemic Lupus Erythematosus Model

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ABSTRACT

Introduction: Systemic Lupus Erythematosus (SLE) is a is a chronic autoimmune inflammatory disease of unknown etiologic. Multiorgan dysfunction in women with SLE causes menstrual irregularities, ovarian failure, follicular atresia, and premature ovarian failure (POF) leading to the cessation of menstruation. *Phaseolus vulgaris L.* contains isoflavones that can trigger oocyte maturation, stimulate the formation of antral follicles, increase FSH receptor expression and proliferate cell nuclear antigen (PCNA) expression. This study aims to prove the effect of red bean extract (isoflavones) in SLE by and increasing the secretion of anti-inflammatory IL-10 levels.

Material and Methods: This study used a true experimental design with a post-test-only controlled group design. The samples used in this study were at least 25 female Balb/c mice. Data on serum IL-10 levels in mice were obtained by measurement using ELISA. The data were statistically analysed using SPSS 16 for Windows software program. The data were tested for normality and homogeneity using parametric non-parametric analysis.

Results: The results showed that there was a significant difference in ANA levels with a p-value of 0.000 in each sample. From this test, it was proven that there was a significant increase in IL-10 levels at doses of 75 mg/KgBW (P2) and 100 mg/KgBW (P3) (p-value=0.000) due to the administration of various doses of red bean extract (isoflavones).

Conclusion: Red bean extract (isoflavones) had a significant effect in increasing IL-10 levels.

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune inflammatory disease of unknown etiologic and is often defined as a complex multisystem autoimmune disease that often causes irreversible damage and decreased quality of life [1]. The clinical manifestations, course of the disease, and prognosis of this disease vary based on the race or ethnicity and economic status of the patient [2]. SLE mainly affects women of childbearing age with a high mortality rate. Genetic, immunological, hormonal and environmental factors are thought to play a role in the pathophysiology of SLE [2,3].

The incidence of SLE in women is about six times higher than in men [1]. Based on data from the Hospital Information System (SIRS), in 2016 there were 2,166 inpatients diagnosed with lupus, of which 550 patients died. Lupus disease mostly attacks women at the age of 15-50 years (productive age) [4]. Multiorgan dysfunction in women with SLE causes menstrual irregularities, ovarian failure, follicular atresia, and premature ovarian failure (POF) leading to the cessation of menstruation.

POF is premature ovarian failure defined as the permanent cessation of menstruation due to loss of ovarian follicular activity before the age of 40 years [5,6]. The levels of various cytokines were found to be elevated in SLE patients, so cytokines are considered important

in the etiopathology of the disease. Cytokine functional polymorphisms can influence disease progression and severity. Interleukin 10 (IL-10) production is dominant in the systemic inflammatory response but antibodies that produced in SLE can reduce the anti-inflammatory cytokine IL-10 [7,8].

Red beans (*Phaseolus vulgaris L.)* has potential anti-inflammatory activity through its active components [9], namely flavanols such as catechins, epicatechins and proanthocyanidins. Phenolic components of *Phaseolus vulgaris L.* (isoflavones) are known to have antioxidant potential due to their ability to prevent the formation of free radicals [10,11]. Isoflavones that can trigger oocyte maturation, stimulate the formation of antral follicles, and stimulate IL-10 [12].

Several studies have shown a positive effect of red bean extract (isoflavones) on the development of ovarian follicles in experimental animals [13]. However, no research has been exploring the potential of red bean extract (isoflavones) on follicle growth of SLE mice with POF. This study aims to prove the effect of red bean extract (isoflavones) as an inhibitor of POF in SLE by increasing anti-inflammatory cytokine IL-10.

MATERIAL AND METHODS

This study used a true experimental design in a laboratory with a post-test-only controlled group design. This research was conducted in vivo with the aim of knowing the effect of red bean extract *Phaseolus vulgaris L. sp*. IL-10 levels, in serum of Balb/c mice modeled by POF-induced SLE. The population in this study were female Balb/c mice which were treated with an intra-peritoneal injection of pristane, busulfan, and cyclophosphamide and orally administered directly with red bean extract (isoflavones) [14].

The samples in this study were female Balb/c mice at least 25 Balb/c mice. Inclusion criteria in this study were female Balb/c mice, healthy condition (actively moving), normal behavior, no anatomical abnormalities, age 6-8 weeks, average body weight 18-25 grams, never used in research or intake any chemical. While the exclusion criteria were female Balb/c mice that experienced anatomical defects or abnormalities during the treatment process, white mice whose condition decreased or died during the study. The independent variable in this study was red bean extract (*Phaseolus vulgaris L*). The dependent variable in this study was the level of IL-10, while the control variables included the age of the experimental animal, the sex of the experimental animal, and the food intake of the experimental animal. Data on serum IL-10 levels in mice were obtained by measurement using ELISA.

The data were statistically analysed using SPSS 16 for Windows software program. Prior to data analysis, the data were tested for normality and homogeneity. If the data is normal and homogeneous, then the data is tested using parametric analysis. If the data is not normal and not homogeneous, then the data is tested using non-parametric analysis. A comparative test was conducted to compare serum IL-10 levels of positive control mice, negative control mice, and mice treated with red bean extract (isoflavones). The comparison test used was ANOVA followed by the LSD test if the data were normal and homogeneous. Kruskal-Wallis test followed by the Mann-Whitney test was used if the data were not normal. Data is presented in bar chart form in mean ± standard deviation (SD). Significant differences between each group are indicated by different notations. The p-value < 0.05 indicates a significant difference between the treatment groups.

RESULTS

Based on the test results of normality test obtained p value > 0.05 which indicates that the assumption of normality on these variables has been met. Based on Table 1, the result of the assumption of homogeneity of variance in the homogeneity test, the p-value> 0.05. Thus the assumption of homogeneity has been met and testing the effect of red bean extract (isoflavones) on IL-10 levels was carried out parametrically using the ANOVA test.

Table 1. Normality Test of The ANA Level and IL-10 Levels

Variable	Coefficient	p-value
ANA level	0.962	0.449
IL-10 . levels	0.962	0.459

Table 2. Homogeneity Test of The ANA Level and IL-10 Levels

Variable	Coefficient	p-value
ANA level	0.827	0.523
IL-10 . levels	2,693	0.061

Table 3. Comparison Test of ANA Levels with ANOVA and LSD

Treatment	Mean ± SD		p-value
K-	85.36 ± 46.71	a	
K+	273.93 ± 42.88	b	
P1	325.71 ± 90.18	b	0.000
P2	325.71 ± 108.84	b	
P3	256.07 ± 69.16	b	

Table 4. Comparison Test of IL-10 and LSD Level 5%

Treatment	Mean ± SD	p-value
K-	3.08±0.28 [c]	
K+	1.32±0.13 [a]	
P1	1.52±0.14 [a]	**0.000**
P2	2.53±0.22 [b]	
P3	2.96±0.15 [c]	

On the mean ± SD if it contains different letters it means there is a significant difference ($p < 0.05$) and if it contains the same letter it means there is no significant difference ($p>0.05$)

Based on Table 2, the results of the analysis using the ANOVA test, a p-value of 0.000 was obtained, which is smaller than $\alpha = 0.05$ ($p <0.05$). So from this test it can be concluded that there is a significant difference in the ANA levels of each group. Based on the results of the 5% LSD test in Table 4, the comparison between the K- and K+ groups showed that there was a significant difference between the K- and K+ groups. This is shown from the mean ± SD which contains different letters. From the results in this test, it was proven that there was a significant difference in ANA levels between the K- and K+ groups. Likewise, the comparison between K- and the treatment group given red bean extract (isoflavone), showed that there was a significant difference. The average ANA levels in the SLE model mice (Group K+ and treatment groups P1, P2, and P3) were higher than the K- group. From this test it is proven that the SLE model mice experienced an increase in ANA levels.

The results of the analysis using the ANOVA test obtained a p-value <0.05 which indicates a significant effect of red bean extract (isoflavones) on IL-10 levels. Or in other words, there are significant differences in IL-10 levels due to the administration of red bean extract (isoflavones) with different doses (Table 3). the test using Multiple Comparison LSD 5%, the comparison between K- and K+ obtained a p-value of less than 0.05 ($p <0.05$). This proves that there is a significant difference between the K- and K+ groups. As presented in Table 5, it was shown that the average IL-10 level in the K+ group was lower than that in the K- group. From this test proved a significant decrease in IL-10 levels in the K+ group.

Based on Table 5, in a comparison between K+ and all treatment groups given red bean extract (isoflavone), the p-value was less than 0.05 ($p<0.05$) in groups P2 and T3. From this test it was proven that there was a significant difference in the average IL-10 level in the K+ group and the treatment group given red bean extract (isoflavone) 75 mg/KgBW (P2) and 100 mg/KgBW (P3). As explained in Table 3, the average IL-10 level in all treatment groups was higher than in the K+ group. This proves that there was a significant increase in IL-10 levels in the treatment group given red bean extract (isoflavone) 75 mg/KgBW (P2) and 100 mg/KgBW (P3).

In a comparison between K- and all treatment groups given red bean extract (isoflavone), a p-value of more than 0.05 ($p>0.05$) was obtained in the P3 group. From this test it was proven that there was no significant difference in the average IL-10 levels in the K- group and the P3 treatment group. Or in other words, administration of red bean extract (isoflavone) at a dose of 100 mg/KgBW was proven to be able to increase IL-10 levels so that they were relatively the same as the K- group (Fig.1).

Fig. 2 shows the average histogram of IL-10 levels in all control and treatment groups. Starting from the K- group, it can be seen that the average IL-10 level decreased significantly in the K+ group. Then, when given various doses of red bean extract (isoflavone) (P1, P2, P3), it was statistically proven that there was a

Table 5. Multiple Comparison Test of IL-10 Levels with LSD Level 5%

Ratio		Average Difference	p-value
K-	K+	1.76173	0.000
	P1	1.56042	0.000
	P2	0.55255	0.000
	P3	0.12598	0.311
K+	P1	-0.20130	0.113
	P2	-1.20917	0.000
	P3	-1.63574	0.000
P1	P2	-1.00787	0.000
	P3	-1.43444	0.000
P2	P3	-0.42657	0.002

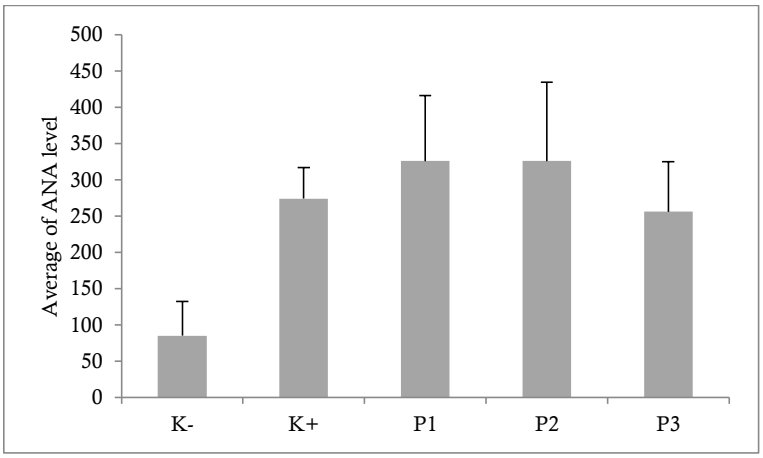


Fig.1. Histogram of Average ANA Levels

(K- : The negative control group showed pristane injection; K+ : The positive control group was injected with pristine, busulphan, and cyclophosphamide; P1: The positive control group was injected with pristine, busulphan, cyclophosphamide, and isoflavone 50 mg/KgBB; P2: The positive control group was injected with pristine, busulphan, cyclophosphamide, and isoflavone 75 mg/KgBB; P3: The positive control group was injected with pristine, busulphan, cyclophosphamide, and isoflavone 100 mg/KgBB)

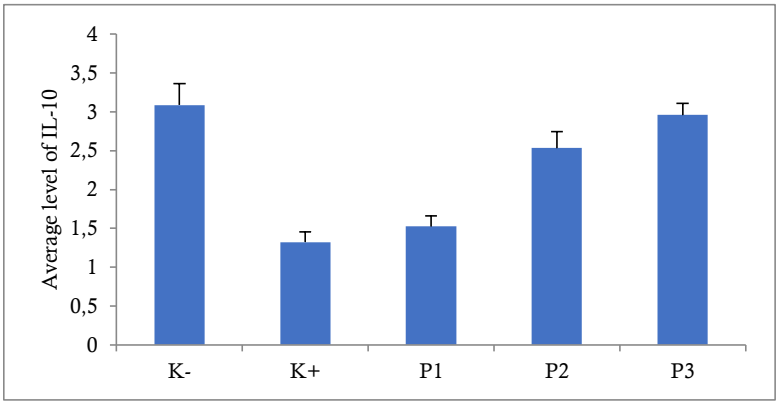


Fig.2. Histogram of Average IL-10

(K- : The negative control group showed pristane injection; K+ : The positive control group was injected with pristine, busulphan, and cyclophosphamide; P1: The positive control group was injected with pristine, busulphan, cyclophosphamide, and isoflavone 50 mg/KgBB; P2: The positive control group was injected with pristine, busulphan, cyclophosphamide, and isoflavone 75 mg/KgBB; P3: The positive control group was injected with pristine, busulphan, cyclophosphamide, and isoflavone 100 mg/KgBB)

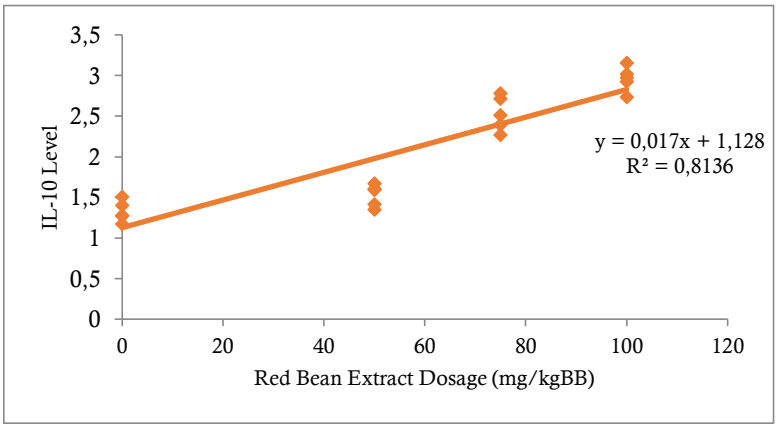


Fig.3. Scatter Plot: The Effect of Red Bean Extract (Isoflavone) on IL-10 Levels

mg/KgBW (P2) and 100 mg/kg red bean extract (isoflavone). kgBB (P3).

Based on Fig.3, the results of the IL-10 level regression analysis, a regression coefficient of 0.017 was obtained with a p-value of 0.000. The coefficient of determination (R-square) of 81.36% indicates that the diversity of the data is explained by the effect of red bean extract (isoflavone) on IL-10 levels of 81.36%. The high R-square value indicates that the linear model accurately describes the effect of red bean extract (isoflavone) on IL-10 levels. Giving red bean extract (isoflavone) can affect the increase in IL-10 levels by 81.36%. The remaining 18.64% is explained by other factors not involved in the study. The regression coefficient of 0.017 indicates that an increase in the dose of red bean extract (isoflavone) of 1 mg/kgBW is predicted to increase IL-10 levels by 0.017

DISCUSSION

Regulatory B cells (B Reg) are known to have an immunosuppressive effect on SLE homeostasis. B reg can suppress the inflammatory process through the production of anti-inflammatory cytokines such as IL-10, IL-35 and TGF-β. IL-10 is synthesized by Breg when the bond between IFN-α and CD40 occurs. An increase in the severity of SLE which is marked by an increase in the SLEDAI score will be followed by a decrease in IL-10 and TGF-β levels [15]. IL-10 is a cytokine that has a complex heterodimer receptor. IL-10 inhibits the activation of monocytes, dendritic cells and macrophages thereby reducing pro-inflammatory mediators. Administration of anti-IL-10 in new born SLE NZB/W F1 mice can improve the survival rate by around 10-80% which is characterized by decreased anti-dsDNA, proteinuria and glomerular lesions [16]. Meanwhile, administration of anti-Il-10 in SLE NZB/W F1 mice aged 4 weeks showed an acceleration of the autoimmunity process. IL-10-mediated protection from therapy appears to be related to the effect of TNF-α. IL-10 can inhibit TNF-α production by macrophages and T lymphocyte cells [17].

Nonetheless, some other literature shows that an increase in IL-10 occurs significantly in patients with SLE when compared to healthy individuals. This is because B reg hyperactivity occurs in the balance of inflammatory and anti-inflammatory responses. The process of increasing IL-10 in SLE occurs secondary to follicular Th expansion against B lymphocyte effector cells in conditions of autoimmunity [18,19].

Based on the results of the analysis using the ANOVA test, a p-value of 0.000 was obtained, which is smaller than $\alpha = 0.05$ ($p < 0.05$). So from this test it can be concluded that there is a significant influence. Isoflavone significant effect on IL-10 levels in SLE and POF mice. Based on the multiple comparison test with 5% LSD, IL-10 levels were found to be significantly different in the K- and K+ groups. IL-10 levels in the treatment group were also found to have significant differences between P1, P2 and P3. The highest IL-10 level was found in the K- group, which was 3.08 ± 0.28 pg/mL. The IL-10 level in the K- group was not significantly different from the P3 group (2.96 ± 0.15). This study shows that administration of isoflavones can suppress the inflammatory process in SLE and POF model mice through the production of the anti-inflammatory cytokine IL-10.

The results of this study indicated that the lowest IL-10 level was in the K+ group (1.32 ± 0.13 pg/mL) and significantly increased in the P3 treatment (2.96 ± 0.15 pg/mL) (p-value = 0.000). Giving various doses of red bean extract (isoflavones) (P1, P2, P3) has been proven to cause a significant increase in IL-10 levels. This suggests that the anti-inflammatory effect of isoflavones is affected by the IL-10 response in SLE and POF mice.

This study is in line with that of Abbassifard and colleagues, the aim of their study was to investigate the expression of cytokines produced by B cells in newly diagnosed SLE patients. The results showed that serum levels of IL-10 and TGF-β decreased significantly with increasing SLEDAI scores in the patients studied ($P < 0.05$). It was concluded that the release of anti-inflammatory cytokines, especially IL-10 and TGF-β, can inhibit the immune response and autoreactive immune cells in SLE patients with mild to moderate disease activity [15].

Luo, Q and colleagues conducted a study to determine the effect of soy isoflavones in controlling obesity and reducing blood lipid levels associated with intestinal barrier function. Research methods that performed by Luo, Q is a rat model of obesity prepared on a high-fat diet. Then the obese rats were given soy isoflavone supplements with different doses for 4 weeks. Our results show that obesity induces pro-inflammatory cytokine expression, decreases anti-inflammatory cytokine (IL-10) expression, increases gut permeability, alters gut microbiota and exacerbates oxidative damage in the colon. Administration of soy isoflavone reversed these changes in obese rats, showing increased IL-10 as an anti-inflammatory, improving intestinal immune function and permeability, attenuation of oxidative damage, increasing the fraction of beneficial bacteria that produce short chain fatty acids and short chain fatty acid production, and reducing harmful bacteria. Furthermore, soy isoflavones blocked TLR4 and NF-κB expression in the colon of obese mice. The conclusion obtained from Luo's research is that soy isoflavones can improve obesity by increasing anti-inflammatory (IL-10), weakening intestinal oxidative stress, restoring immune and mucosal barriers [20].

Chen *et al.* (2019) said that in the current study there were no adverse clinical responses, including immune stress, and the development of inhibitory reactions to isoflavones. In addition, Chen e*t al.* suggested that isoflavones could act as additional compounds to enhance the cellular immune response in OVA mice. Compared to the control group, IgG antibody levels and IgG subclasses increased significantly, while splenocyte and cytokine expression were also significantly increased in the OVA group by isoflavone administration. Based on the research by Chen *et al.,* it can be concluded that the use of isoflavones as additional compounds can increase specific antibodies and cellular responses to OVA in mice by regulating gene expression of Th1/Th2 cytokines and transcription factors. It is known that the cytokines in Th1/Th2 are IL-2, IFN-γ, IL-4 and IL-10, all of these cytokines are found increased by isoflavone administration. This is in accordance with this study where the administration of isoflavones will increase anti-inflammatory in the form of IL-10.

In this study, Il-10 levels were measured using the ANOVA and 5% LSD test methods. Administration of various doses of isoflavone to Balb/c mice models of SLE and POF increased IL-10 levels. Therefore, in this study giving red bean extract (isoflavone) can significantly increase IL-10 levels. The limitation of this study is need to be done a test of toxic dose and lethal dose of red bean extract (Isoflavone) to define the therapeutic dose for further research, and also it is necessary to conduct research on the types of flavonoids and the potential of isoflavones in red bead extract (isoflavone) which play a role in preventing POF in SLE.

CONCLUSION

Red bean extract (isoflavones) with a dose of 100 mg/KgBW significantly affected the increase in the highest IL-10 levels and was not significantly different from the K(-) group in POF and SLE mice.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest regarding the publication of this article.

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