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Effect of Red Bean Extract (*Phaseolus vulgaris L. Sp*) on Interleukin-1, Inhibin B and PCNA Expression in Systemic Lupus Erythematosus Mice Model

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ABSTRACT

Introduction: Systemic Lupus Erythematosus (SLE) is a complex autoimmune disease characterized by multiple organ involvement. Chronic inflammation due to oxidative stress and autoimmune causes damage to related organs, one of which is the ovary which leads to Premature Ovarian Failure (POF). Red bean (*Phaseolus vulgaris L. Sp.*) is an anti-inflammatory and antioxidant agent. This study aims to examine the effect of red beans on IL-1, Inhibin B, and PCNA.

Methods: This is a true experimental study with a post-test-only controlled group design in vivo. The sample in this study were female Balb/c mice treated with red bean extract (*Phaseolus vulgaris L. sp.*) at doses of 50 mg/KgBW (P1), 75 mg/KgBW (P2), and 100 mg/KgBW (P3). IL-1, Inhibin B, and PCNA levels in each group were statistically analyzed using the SPSS 16 for Windows software program.

Results: Red bean extract (*Phaseolus vulgaris L. Sp.*) administration at various doses was statistically proven to significantly reduce IL-1 levels ($p<0,05$) and increase Inhibin levels in all groups ($p<0,05$). The highest average Inhibin B levels were found in the 100 mg/KgBW dose group (P3), significant increase in PCNA expression in all groups given various doses of red bean extract ($p<0,05$).

Conclusion: Administration of red bean extract (*Phaseoulus vulgaris L. Sp.*) can reduce levels of Interleukin-1, increase levels of Inhibin B and PCNA expression in SLE mice.

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INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a complex autoimmune disease characterized by multiple organ involvement. SLE can affect both men and women in all age groups, but the prevalence of SLE is highest at the age of 20 to 40 years and is rare at prepubertal age. Epidemiological data on SLE in Indonesia are uncertain, but in the United States, as much as 0.05 to 0.1% of the population suffers from SLE [1]. In women, hormonal influences are a significant risk factor for SLE. Estrogen and prolactin promote autoimmunity and increase the production of B-cell activating factors, and modulate lymphocyte and plasmacytoid dendritic cell activation [2].

Proliferating Cell Nuclear Antigen (PCNA) plays an important role in the induction of the lupus autoimmune response, which serologically plays a role in DNA replication and the cell cycle. PCNA plays a role in the ovarian cycle according to its biological function [3,4]. In addition, the protein Interleukin-1 (IL-1) is an important cytokine in the regulation of inflammation, innate immunity, angiogenesis, and hematopoiesis. Increased levels of IL-1 in follicular fluid in patients with primary ovarian insufficiency (POI) can cause inflammation and impair ovarian function by

accelerating the depletion of ovarian reserves [5]. Undetectable inhibin B and high FSH are markers of ovarian failure [6].

Chronic inflammation due to oxidative stress and autoimmune causes damage to related organs, one of which is the ovary which leads to POF [7]. Red bean (*Phaseolus vulgaris L. Sp.*) is an anti-inflammatory and antioxidant agent [8]. This study aimed to determine the effect of red bean extract (*Phaseoulus vulgaris L. Sp.*) in inhibiting the reduction of follicle reserves in Premature Ovarian Failure (POF) in SLE mice.

MATERIAL AND METHODS

This is a true experimental design with a post-test-only controlled group design in vivo. Inducing SLE in mices is done by giving a single dose intraperitoneal injection of 0.5 ml pristane in the middle or upper left part of the mice's abdomen with an inclination of 45o and a depth of 0.5 cm. The mice were observed for 8-16 weeks to get the SLE model mice. Mice were observed clinical and laboratory symptoms of SLE (ANA test) at the end of week 8-16. SLE model mice were injected with busulfan 30 mg/KgBW and cyclophosphamide 120 mg/KgBW in a single dose intraperitoneally in the middle or upper left part of the mice's abdomen with an inclination of 45° and a depth of 0.5 cm.

The sample in this study were female Balb/c mice treated with intraperitoneal injections of pristane, busulfan and cyclophosphamide as the positive control (K+), isoflavone of red bean extract administered orally at doses of 50 mg/KgBW (P1), 75mg/KgBW (P2), and 100 mg/KgBW (P3), and negative control without any treatment (K-). White mice with defects or anatomical abnormalities, decreased condition, and died during treatment were excluded.

PCNA was analyzed by immunohistochemistry using an anti-PCNA antibody (Santa Cruz Biotechnology). Interleukin-1 (IL-1) was analyzed by ELISA using Mouse Interleukin-1 Elisa kit 48T. Inhibin B was analyzed by ELISA using Mouse inhibin B Elisa kit 48T. Data were statistically analyzed using the SPSS 16 for Windows software program. If the data are normally distributed and homogeneous, the comparison test is ANOVA, followed by a Tukey post hoc test. If the data are not normally distributed, the Kruskal-Wallis test is used, then the Mann-Whitney (Dunn) test.

RESULTS

The IL-1 and Inhibin B levels in each group were normally distributed and homogenous (p>0,05). ANOVA and LSD 5% test were carried out to determine differences (Table 1). The results showed red bean extract *(Phaseolus vulgaris L. Sp.)* administration at various doses was statistically proven to significantly reduce IL-1 levels in all groups and increase Inhibin B levels in all groups. The highest averages of Inhibin B levels were found in the 100 mg/KgBB dose group (P3).

The results also showed that the PCNA expression were not normally distributed and homogeneous (p<0,05). Kruskal-Wallis and Dunn Test 5% was carried out to determine differences in PCNA expression (Table 3). The administration of red bean extract at various doses (P1, P2, P3) was statistically proven to significantly increase PCNA expression in all treatment groups.

Regression analysis can be used to investigate the effect of red bean extract *(Phaseolus vulgaris L. Sp.)* on IL-1 levels, Inhibin B levels, and PCNA expression. (Table 4). Red bean extract can affect the decrease in IL-1 levels by 33.68%. Other factors not included in the study account for the remaining 66.32%. The regression coefficient of -0.032 indicates that an increase in the red bean extract *(Phaseolus vulgaris L. Sp.)*

Table 1. Comparison Test of IL-1 Levels with ANOVA Test and LSD 5%

Group	Mean±SD	*p-value*
K-	7.11 ± 0.32^{a}	
K+	18.77 ± 0.84^{c}	
P1	14.43 ± 1.31^{b}	0.000
P2	14.92 ± 1.11^{b}	
P3	15.75 ± 1.84^{b}	

On the average ± 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).

Table 2. Comparison Test of Inhibin B Levels with ANOVA Test and LSD 5%

Group	Mean±SD	*p-value*
K-	270.04 ± 14.4^{d}	
K+	125.93 ± 31.17^{a}	
P1	159.81 ± 12.31^{b}	0.000
P2	219.07 ± 24.51^{c}	
P3	$243.89 \pm 25.39^{c,d}$	

On the average ± 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).

Table 3. Comparison Test of PCNA Expression with Kruskal-Wallis Test and Dunn 5%

Group	Mean±SD	*p-value*
K-	60.58 ± 20.03^{c}	
K+	13.53 ± 2.07^{a}	
P1	47.73 ± 16.76^{bc}	0.000
P2	27.23 ± 7.42^{b}	
P3	36.01 ± 35.55^{ab}	

On the average ± 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)

Table 1. Regression Analysis Results of IL-1 Levels, Inhibin Levels, and PCNA Expression

Dependent Variable	Regression Equation	*p-value*	Correlation and *p-value*	R-Square
IL-1 Level	Y1 = 17.785 - 0.032 X	0.007	r = -0.58 p = 0.007	33.68%
Inhibin B Level	Y2 = 118.307 + 1.224 X	0.000	r = 0.878 p = 0,000	77.12%
PCNA Expression	Y3 = 21.966 + 0.17 X	0.254	r = 0.275 p = 0.254	7.56%

Y1 : IL-1 level; Y2 : Inhibin B Levels; Y3 : PCNA expression; X: Red Bean Extract Dosage

dose of 1 mg/kgBW is predicted to reduce IL-1 levels by 0.032 pg/ml. Administration of Red Bean Extract were affect affect an increase in Inhibin B Levels of 77.12%. The remaining 22.88% is explained by other factors not involved in the study. The regression coefficient of 1,224 indicates that an increase in the red bean extract (*Phaseolus vulgaris L. Sp.*) dose of 1 mg/kg BW is predicted to increase Inhibin B levels by 1,224 ng/ml. the effect of red bean extract on PCNA expression was 17.26%. the remaining 82.74% is explained by other factors not involved in the study.

DISCUSSION

This study aims to utilize soybean as a raw material to evaluate its anti-inflammatory activity. Administration of various doses of red bean extract (P1, P2, and P3) statistically proven that there was a significant decrease in IL-1 levels in all groups. This finding is in line with research conducted by Kao et al., showed that all isoflavones and genistein powders from soybeans were effective in inhibiting inflammation induced by intraperitoneal lipopolysaccharide, reducing the number of leukocytes in rat blood, and reducing the production of IL-1, IL-6, NO, and PGE2 in peritoneal exudate cells and peritoneal exudate fluid [9]. The anti-inflammatory effect of red bean *(Phaseolus vulgaris L. Sp.)* is related to the antioxidant potential of its phenolic components. Kidney bean extract inhibits the expression of iNOS, IL1β, IL-6 and TNF-α through inactivation of the NF-κB pathway [10].

Transcriptional regulation of genes involved in inflammatory and immune responses is strongly influenced by NF-B. The NFκB protein is normally cytoplasmic and in an inactive state. Upon stimulation, NF-B is activated and translocates to the nucleus to induce the transcription of various proinflammatory genes [11].

A study examined the content of *Phaseolus vulgaris L. Sp.* from various Canadian manufacturers (Black Violet, Othelo, Polaris, Viva). This study shows levels of phenols, flavonols, and anthocyanins in *Phaseolus vulgaris L. Sp.*, despite different sources. In addition, an ORAC (Oxygen Radical Absorption Capacity) assessment was also performed, demonstrating the antioxidant properties of this red bean [12]. This research is also in line with the anti-inflammatory properties affecting granulocytes, monocytes, and lymphocytes. A diet containing isoflavones prevented inflammation associated with the induction of metallothionein in the gut and the induction of manganese superoxide dismutase (Mn-SOD) in the liver of mice injected with endotoxin lipopolysaccharide (LPS), and suppressed the gut response to inflammation by modulating the action of the pro-inflammatory cytokine interleukin (IL-6). Other in vivo findings also show that various isoflavones consistently exhibit anti-inflammatory effects in several animal models by lowering interleukin levels [13].

Higher inhibin A serum levels were found in patients with highly differentiated ovarian carcinoma compared to the group of patients with a poorly differentiated cancer, which may confirm the influence of inhibin A on cell proliferation processes [14]. Inhibin A is a gonadal hormone that acts in conjunction with estradiol to exert specific negative feedback to control FSH secretion by the pituitary gland [15]. Therefore, inhibin A may promote cell proliferation under estradiol secretion [16]. There is a significant difference in Inhibin levels as a result of giving red bean extract at different doses. This is in line with research conducted by Britt et al. [17].

Most of the soybean diets containing isoflavones improve ovarian morphology. The genistein diet reduced FSH levels in ArKO mice and correlated with increased ovarian inhibin subunit expression. This is consistent with the increase in the expression of the Inhibin B subunit by 1.7 times (P = 0.005) in ArKO mice on a soy diet compared to controls. The higher the dose of red bean extract given, the higher the isoflavone levels in the rats' bodies. Red beans contain other substances called phytoestrogens with properties similar to the hormone estrogen. The secretion of the hormone estrogen will affect the reproductive organs in female animals, including the ovaries, which are related to the development of the endometrium and its follicles. Phytoestrogens are agonists against estrogen by helping estrogen bind to its receptors, so that high doses of red bean extract will accelerate the maturation of egg follicles [18].

Phytoestrogens are thought to cause faster follicular growth by affecting FSH secretion and estrogen

secretion from the ovaries. High concentrations of the two hormones cause an increase in the speed of development of tertiary follicles. The higher the dose is given, the higher the isoflavone levels in the body of the test animal. Isoflavones are estrogen agonists by helping estrogen to bind to its receptors, thus a high dose of red bean extract will accelerate the development of egg follicles [19].

There was a significant difference in PCNA expression as a result of giving red bean extract at different doses. The results of this study are in line with a study by Sirotkin, et al., 2015. that these soy isoflavones affect the basic functions of ovarian cells (proliferation, apoptosis, and hormone release) and modify the effects of FSH. Doses of isoflavone daidzein (10 and 100 μM) increased PCNA accumulation showed a significant daidzein effect ($p < 0.05$) on porcine ovarian granulosa cells, but did not increase at 1 μM dose [20].

CONCLUSION

Red bean extract (*Phaseoulus vulgaris L. Sp.*) adminisitration can reduce levels of Interleukin-1, increase levels of Inhibin B and increase PCNA expression in SLE mice.

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CONFLICT OF INTEREST

The authors declare there is no potential conflict of interest.

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