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Effect of Red Bean Extract (*Phaseolus Vulgaris L. Sp*) on Il-6 Levels and AMH Levels as The Prevention of Premature Ovarian Failure in Mice Model of The Systemic Lupus Erythematosus

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Systemic Lupus Erythematous; POF; Isoflavone; AMH; IL-6

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ABSTRACT

Introduction: Continuous SLE treatment causes Premature Ovarian Failure (POF) and occurred in 27.3% SLE patients treated with cyclophosphamide. IL-6 plays a critical role in the B cell hyperactivity and immunopathology of human SLE. *Phaseolus Vulgaris L. Sp* have a high total phenolic content and have been shown to have anti-inflammatory and antioxidant effects. This study's objective is to prove the effect of red beans (*Phaseoulus vulgaris L. sp*.) in reducing inflammation (IL-6) and increasing AMH levels.

Methods: This is a true experimental design with a post-test-only controlled group design. The sample used in this study was 25 female Babic mice divided in two control groups and three treatment groups. Subjects were given intraperitoneal injection of Pristan (0.5 ml), busulfan (30 mg/kg BW), cyclophosphamide (120 mg/kg BW) to induce POF. ANA test was carried out to prone SLE induction was success. Red bean extract was given at 50 mg/kg BW (in treatment group 1), 75 mg/kg BW (in treatment group 2), and 100 mg/kg BW (in treatment group 3). Data of IL-6 and AMH levels in mice serum were obtained by measurement using ELISA.

Results: The study showed a significant difference in ANA levels with a p-value of 0.000 in every sample. The study has proven a significant decrease in IL-6 levels with a p-value of 0.000 and a significant increase in AMH levels at a dose of 100 mg/kg BW (P3) with a p-value of 0.000.

Conclusion: Phaseolus vulgaris extract had a significant effect on follicle changes in Balb/c mice in dose of 50 mg/kg and 75 mg/kg but not significantly reduce inflammation (IL-6).

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INTRODUCTION

Most women of reproductive age are affected by the autoimmune illness systemic lupus erythematosus (SLE). It is characterized by a body immune response deficit that results in the development of autoantibodies and immune complex clearance failure [1]. Continuous SLE treatment causes progressive and irreversible damage to oocytes in a dose dependent manner, thereby reducing the number of oocytes in ovaries [2]. With high doses and longer duration of cytotoxic medication, the oocyte number is reduced drastically resulting in POF. Premature ovarian failure (POF), also known as the loss of ovarian follicular activity before the age of 40, is characterized by the permanent end of menstruation [3]. Ovarian failure occurred in 27.3% patients of SLE patients treated with cyclophosphamide [4]. POF can be diagnosed by calculating Anti-Mullerian Hormone (AMH). The low AMH levels in SLE patients have been dichotomized for the presence of menstrual alterations [5].

IL-6 promotes systemic autoimmunity and pathologic inflammatory responses [6]. Data from several studies suggest that IL-6 plays a critical role in

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the B cell hyperactivity and immunopathology of human SLE, and may have a direct role in mediating tissue damage [7].

Red beans (*Phaseolus Vulgaris L. Sp*) have a high total phenolic content and have been shown to have anti-inflammatory and antioxidant effects. This study's objective is to prove the effect of red beans (*Phaseoulus vulgaris L. sp.)* in reducing inflammation and delaying premature ovarian failure in SLE.

MATERIAL AND METHODS

This study used a true experimental design with a post-test-only controlled group design. The population in this study were female Babic mice that would be treated.

Mice Model of The Systemic Lupus Erythematosus

The sample used in this study was a minimum of 25 female Baib/c mice. There are five groups in this study. Group one, the positive control group (K+), was female mice given an intraperitoneal injection of pristane with busulfan and cyclophosphamide. Group two, the treatment group, was female mice who were given an intraperitoneal injection of pristane, busulfan and cyclophosphamide and then red bean extract at 50 mg/kg BW (P1). In group three, the treatment group was female mice who were given an intraperitoneal injection of pristane, busulfan and cyclophosphamide and then red bean extract at a dose of 75 mg/kg BW (P2). Group four, the treatment group, was female mice who were given an intraperitoneal injection of pristane, busulfan and cyclophosphamide and then red bean extract at 100 mg/kg BW (P3). Group Five, the negative control group (K-) was female mice that were not given the injection and were not given red bean extract.

A single intraperitoneal injection of Pristan (0.5 ml), busulfan (30 mg/kg BW), and cyclophosphamide (120 mg/kg BW) were administered to mice. The injection site was angled at a 45-degree angle and was 0.5 cm deep. The animals were examined for four weeks to make POF. Every week, the mice's weight was recorded. ANA test was carried out on the mice after an 8-week observation following Pristane injection to prone SLE induction was success.

Il-6 Levels

IL-6 levels in mouse blood serum were assessed using an ELISA kit (enzyme-linked immunosorbent assay) (SIGMA-Aldrich Mice IL-6 ELISA Kit catalogue number RAB0245). IL-6 concentrations are measured in units per millilitre. Scale: Interval.

AMH Levels

Santa Cruz Biotechnology's primary anti-AMH antibody and secondary antibody were used in an ELISA to examine the samples (goat anti-mouse).

Red Bean Extract (Phaseolus Vulgaris L. Sp)

Balai Material Medica Batu-obtained red bean extract. Using 96 per cent ethanol and 800 grams of red bean Simplicia was macerated for two days. The mixture was then filtered through Whatman No. 42 (125 mm) paper, and the extract was then dried in a rotary evaporator at 45 °C before being placed in a desiccator. The extract is thought to yield roughly 80 grams.

Data Analyse

Data were statistically analysed using SPSS 16 for Windows software program for normality and homogeneity tests. Hypothesis analysis for IL-6 was done parametrically with ANOVA and followed by Dunnet T3. Meanwhile, Hypothesis analysis for AMH was done parametrically with ANOVA and followed by LSD.

Ethics

This study was conducted based on the protocol, which has been granted ethical approval from the RSUD Dr. Saiful Anwar Malang Review Board.

RESULTS

The homogeneity test on the IL-6 level variable obtained a p-value of 0.002 ($p<0.05$), while for the AMH variable, the p-value was 0.090 ($p>0.05$). Therefore, the process of testing the hypothesis on the variable IL-6 levels was carried out parametrically using ANOVA and then followed by a post hoc test using the Dunnet T3 test. While on the variable AMH levels, the testing process was carried out using ANOVA and continued with the LSD test.

Fig. 1 shows the histogram of the average IL-6 levels of all control and treatment groups. Starting from the K- group, we saw that the mean IL-6 levels increased significantly in the K+ group. Then, when given various doses of red bean extract (P1, P2, P3), it was not statistically proven that there was a significant decrease in IL-6 levels in all groups given various doses of red beans extract.

Judging from the test using Multiple Comparison Dunnet T3 5%, the comparison between K- and K+ obtained a p-value less than 0.05 ($p<0.05$). Proves a significant difference between the K- and K+ groups. As described in Table 1, it is shown that the average level of IL-6 in the K+ group is higher than in the K- group. From this test, it was proven that there was a significant increase in IL-6 levels in the K+ group.

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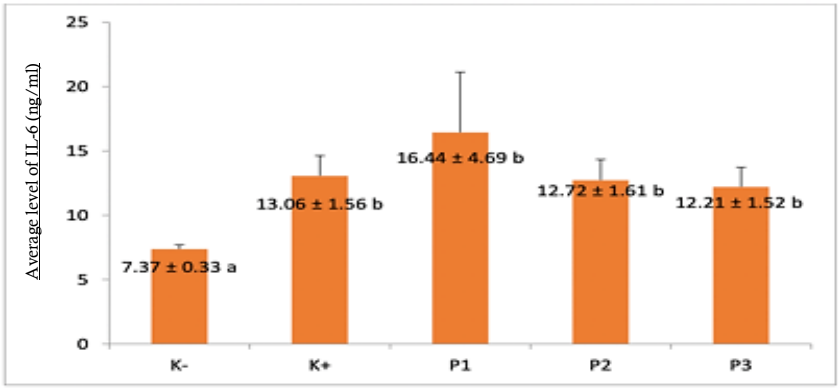


Fig. 1. Histogram of Average Il-6 Levels

Fig. 2 shows the histogram of the average AMH levels of all control and treatment groups. Starting from the K- group, we saw that the average AMH levels increased significantly in the K+ group. Then, at various doses of red bean extract (P1, P2, P3), it was statistically proven that there was a significant increase in AMH levels in the 75 mg/kg BW (P2) and 100 mg/kg BW (P3) red bean extract groups.

When comparing K+ and all treatment groups that were given red bean extract, the p-value was less than 0.05 ($p<0.05$). In groups P2 and P3. This test proved a significant difference in the average AMH levels in the K+ group, with the treatment group giving red bean extract 75 mg/kg BW (P2) and 100 mg/kg BW (P3). As described in Table 2, the average AMH levels in all treatment groups were higher than in the K+ group. Thus, the treatment group showed a significant increase in AMH levels with 75 mg/kg BW (P2) and 100 mg/kg BW (P3) red bean extract.

The quadratic regression model of the effect of Red Bean Extract on IL-6 levels has an R2 value of 0.2103 (21.03%). Giving Red Bean Extract affected the decrease in IL-6 levels only by 21.03%. The remaining 78.97% is explained by other factors not involved in the study (Fig. 3).

Based on the regression analysis of AMH levels, a regression coefficient of 8.188 was obtained with a p-value of 0.000. The coefficient of determination (R-square) of 54.9% indicates that the diversity of the data explained by the effect of giving Red Bean Extract to AMH levels is 54.9%. The reasonably high R-square value indicates that the linear model explaining the effect of Red Bean Extract on AMH levels is entirely accurate. Giving Red Bean Extract affected the increase in AMH levels by 54.9%. The remaining 45.1% is explained by other factors not involved in the study. The regression coefficient of 8.188 indicates that an increase in the dose of red bean extract by 1 mg/kg BW is predicted to increase AMH levels by 8.188 pg/mL (Fig. 4).

AMH has been proven in various studies as a marker of ovarian reserve. Serum AMH levels have also been shown to reflect ovarian reserve, and are more sensitive than FSH, and relatively stable in various menstrual cycles, so that it is a good serum parameter to determine ovarian reserve [2,8].

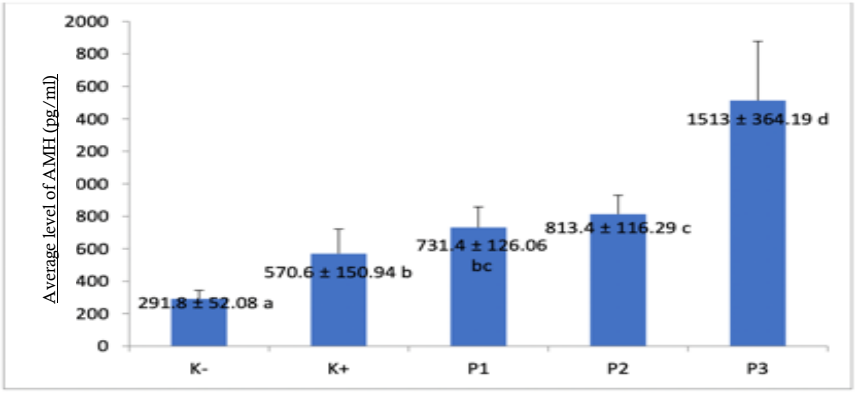


Fig. 2. Histogram of Average AMH Levels

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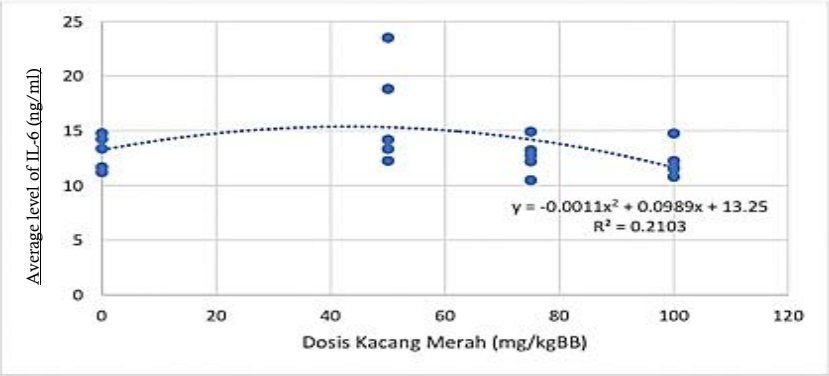


Fig. 3. Scatter Plot Polynomial Regression Effect of Red Bean Extract on IL-6 Levels

Red beans (*Phaseolus Vulgaris L. Sp*) showed an inhibitory effect on gene expression of IL-1β, IL-6 and TNF-α which were higher than white beans. The anti-inflammatory effect of red bean is related to the antioxidant potential of its phenolic components. Kidney bean extract inhibits the expression of iNOS, IL-1β, IL-6 and TNF-α through inactivation of the NF-κB pathway [9]. Transcriptional regulation of genes involved in inflammatory and immune responses is strongly influenced by NF-B. NFκB protein is normally intracytoplasmic and is in an inactive state. When stimulated, NF-B is activated and translocated into the nucleus to induce transcription of various inflammatory genes [10].

Phenolic compounds, besides from contributing to the smell, taste, and color of food, have a long-term intake that could play a bioactive role due to their antioxidant activity which has been related to the prevention of obesity, cardiovascular and neurodegenerative diseases, cancer, and diabetes, as well as exhibiting anti-inflammatory, antimutagenic, and antibacterial properties [11]. Madhujith et al. demonstrated that beans, especially those with colored skins, possess strong antioxidant activity as measured by different model systems [12]. Epidemiological studies correlated the consumption of procyanidin-rich foods with a lower incidence of inflammatory disease and diseases of multifactorial pathogenesis [13]. Similarly, the transcription and secretion of proinflammatory cytokines, including IL-1β, IL-2, IL-6, TNF-α, and interferon-γ, could be down-regulated by procyanidins, as reported in some in vitro and in vivo studies [14].

A study examined the content of *Phaseolus vulgaris* from various manufacturers in Canada (Black Violet, Othelo, Polaris, Viva). This study shows the content of phenols, flavonols, and anthocyanins in red beans, although from different manufacturers. In addition, an ORAC (Oxygen Radical Absorbance Capacity) assessment was also carried out which showed the antioxidant properties of this red bean [15]. The high total phenolic content and ORAC activity indicate the anti-inflammatory and antioxidant effects of kidney beans. Studies prove the anti-inflammatory effect of red bean extract, in addition to the antioxidant effects that have been proven by ORAC earlier. The anti-inflammatory effect is assessed from the inhibition of the Cyclooxygenase (COX) and Lipoxygenase (LOX) pathways that play a role in the formation of various

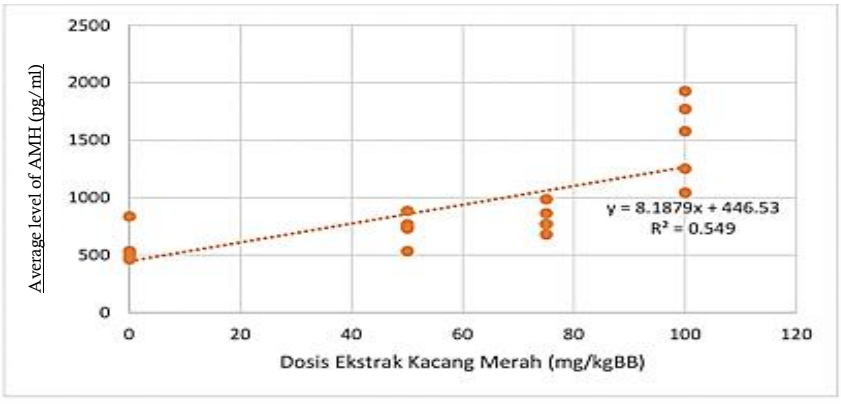


Fig. 4. Scatter Plot Effect of Red Bean Extract on AMH Levels

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inflammatory mediators such as leukotrienes and prostaglandins [16]. Another study also investigated the specificity of COX inhibition specifically, either COX-1 or COX-2. Specific inhibition of COX-2 is preferred because COX-2 plays a more important role in the formation of inflammatory mediators, while COX-1 acts more as a gastroprotectant [17]. From this study it can also be concluded that red bean extract has anti-inflammatory and antioxidant effects. Consideration of dosage of *Phaseolus vulgaris* extract based on a study conducted by Hasanah et al. In this study, the dose of Phaseolus vulgaris extract which had a significant effect on follicle changes in Balb/c mice was 50 mg/kg and 75 mg/kg. In addition, this study also added a dose of 100 mg/kg to determine the significance of a higher dose [18].

DISCUSSION

AMH has been proven in various studies as a marker of ovarian reserve. Serum AMH levels have also been shown to reflect ovarian reserve, and are more sensitive than FSH, and relatively stable in various menstrual cycles, so that it is a good serum parameter to determine ovarian reserve [2,8].

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CONCLUSION

Red bean extract containing isoflavone not significantly reduces Il- 6 levels due to pro-inflammatory cytokine and increases AMH levels due to anti-inflammatory cytokine.

ACKNOWLEDGMENT

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this study.

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