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The Effect of Red Bean Extract (*Phaseoulus Vulgaris L. Sp.*) on T Helper 17, T Regulator Levels, and Atretic Follicle Count in Mice Models of Premature Ovarian Failure Induced by Systemic Lupus Erythematosus

Madina Paramitha Effendi*, Mukhamad Noryanto, I Wayan Arsana Wiyasa, Onni Dwi Arianto

Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Brawijaya/ dr. Saiful Anwar General Hospital, Malang, East Java, Indonesia

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CORRESPONDING AUTHOR*
Madina Paramitha Effendi
paramitha.madinna@gmail.com
Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Brawijaya/ dr. Saiful Anwar General Hospital, Malang, East Java, Indonesia

KEYWORDS
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ABSTRACT

Introduction: Systemic Lupus Erythematosus (SLE) is a long-term autoimmune disease that primarily impacts women of reproductive age. A major complication of SLE treatment with cyclophosphamide is premature ovarian failure (POF), leading to infertility. The imbalance between T-helper 17 (Th-17) and T-regulator (Treg) cells contributes to SLE progression. Red bean extract (*Phaseolus vulgaris* L. sp.), known for its antioxidant and anti-inflammatory effects, may help restore this balance and protect ovarian function.

Material and Methods: Female Balb/c mice were divided into five groups: control, untreated SLE, and three treatment groups receiving red bean extract at 50, 75, and 100 mg/kg. SLE was induced using pristane, followed by cyclophosphamide to trigger POF. The treatment groups received red bean extract for four weeks. Th-17 and Treg levels were measured using ELISA, and ovarian tissues were histologically analyzed to assess atretic follicle count. Data were statistically analyzed using ANOVA and Tukey's post hoc test.

Results: Red bean extract reduced Th-17 levels and increased Treg levels, indicating improved immune balance. Additionally, a decrease in atretic follicles suggested protective effects on ovarian function. The most significant results were observed at the 75 mg/kg dose. The extract's polyphenolic and phytoestrogenic content likely contributed to its anti-inflammatory and antioxidant activity.

Conclusion: Red bean extract shows promise as a natural therapeutic agent for preventing ovarian dysfunction in SLE-induced POF by modulating immune response and reducing follicular damage. Further research is needed to explore its molecular mechanisms and clinical potential in humans.

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INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease that affects multiple organ systems and is characterized by autoantibody production and immune complex deposition. It primarily affects women, occurring about eight times more frequently in females than in males, especially among individuals aged 20 to 40. SLE has a widespread global presence, with its prevalence differing across regions such as North America, Europe, and the Asia-Pacific, where annual incidence rates range from 4.3 to 4.53 cases per 100,000 individuals. The disease develops due to intricate interactions between genetic susceptibility, environmental influences, and immune system dysfunction, ultimately resulting in persistent inflammation and damage to multiple organs [1–3].

One major concern for women with SLE is its impact on reproductive health. Studies suggest that SLE can contribute to ovarian dysfunction, increasing the

likelihood of premature ovarian failure (POF). POF is characterized by the early loss of ovarian activity before the age of 40, leading to infertility and related health issues such as estrogen deficiency, osteoporosis, and cardiovascular complications. The development of POF in SLE patients is linked to autoimmune-related ovarian damage, persistent inflammation, and the negative impact of immunosuppressive treatments, including glucocorticoids and cyclophosphamide [4,5].

Given the limitations of current treatment options, there is growing interest in alternative therapies that can mitigate the progression of ovarian failure while minimizing adverse effects. One potential candidate is *Phaseolus vulgaris L.*, commonly known as red kidney bean. This plant contains bioactive compounds with anti-inflammatory, antioxidant, and phytoestrogenic properties. Phytoestrogens, particularly isoflavonoids and flavonoids found in red kidney beans, act as estrogen receptor agonists, which may help regulate hormonal balance and protect ovarian function [6].

Animal studies have shown promising results in using red kidney bean extract to modulate immune responses and reduce inflammatory markers such as T-helper 17 (Th-17) cells while increasing T-regulatory (Treg) cells. Th-17 cells are pivotal in driving the inflammatory response associated with SLE, whereas Treg cells function to regulate and suppress an overactive immune system. Furthermore, experimental data suggest that supplementation with red kidney bean extract can reduce ovarian follicle atresia, thereby preserving ovarian function in SLE models [7,8].

This study aims to explore the therapeutic potential of red kidney bean extract in modulating immune balance and preventing ovarian damage in a murine model of POF induced by SLE. By examining its effects on Th-17, Treg levels, and ovarian follicle atresia, this research seeks to provide a scientific basis for its application in clinical settings. Understanding the impact of natural compounds on autoimmune-induced ovarian dysfunction may pave the way for novel, less toxic treatment strategies for women with SLE.

MATERIAL AND METHODS

This study implemented a true experimental design with a post-test-only controlled group approach to evaluate the effects of *Phaseolus vulgaris L.* (red kidney bean) extract on Th-17 levels, Treg levels, and atretic follicle count in a murine model of Systemic Lupus Erythematosus (SLE)-induced Premature Ovarian Failure (POF). Female Balb/c mice were utilized as test subjects and underwent a series of experimental treatments, including intraperitoneal injections of pristane, busulfan, and cyclophosphamide, followed by oral administration of red kidney bean extract.

The study involved 25 mice, randomly assigned to five groups: a negative control (K-), which received no injections or treatment; a positive control (K+), which was given pristane, busulfan, and cyclophosphamide without any red kidney bean extract; and three treatment groups (P1, P2, and P3), which received the same injections as the positive control but were administered red kidney bean extract at doses of 50 m per kgBW, 75 mg per kgBW, and 100 mg per kgBW, respectively. The sample size was determined using the Federer formula, ensuring that each group consisted of at least five mice.

In this study, the independent variable was the administration of Phaseolus vulgaris L. extract, while the dependent variables included Th-17 levels, Treg levels, and the number of atretic follicles. Several factors were controlled, such as the age, sex, diet, and overall health of the mice. The experiment began with the induction of SLE, where mice received an intraperitoneal injection of pristane (0.5 ml) and were monitored for 18–20 weeks for lupus-related symptoms, including hair loss, arthritis, glomerulonephritis, anemia, and the presence of anti-dsDNA antibodies. To induce POF, mice were given intraperitoneal injections of busulfan (30 mg/kgBW) and cyclophosphamide (120 mg/kgBW), resulting in progressive follicle depletion and estrogen deficiency, simulating premature ovarian failure in humans.

Table 1. Levels of Anti-dsDNA, Th-17, Treg, and Atretic Follicles in SLE-Induced POF Mice

Group	Anti-dsDNA (IU/mL)	Th-17 (pg/mL)	Treg (pg/mL)	Atretic Follicles
K-	30.72 ± 2.1	40.24 ± 2.3	18.12 ± 2.4	1.00 ± 1.00
K+	41.39 ± 3.5	59.73 ± 1.1	12.68 ± 2.6	16.00 ± 3.2
P1	38.48 ± 2.8	45.94 ± 3.7	15.42 ± 2.5	9.00 ± 2.9
P2	33.50 ± 3.2	42.16 ± 2.6	17.35 ± 2.8	4.00 ± 1.7
P3	30.25 ± 2.5	30.25 ± 1.8	19.47 ± 2.5	0.80 ± 0.84

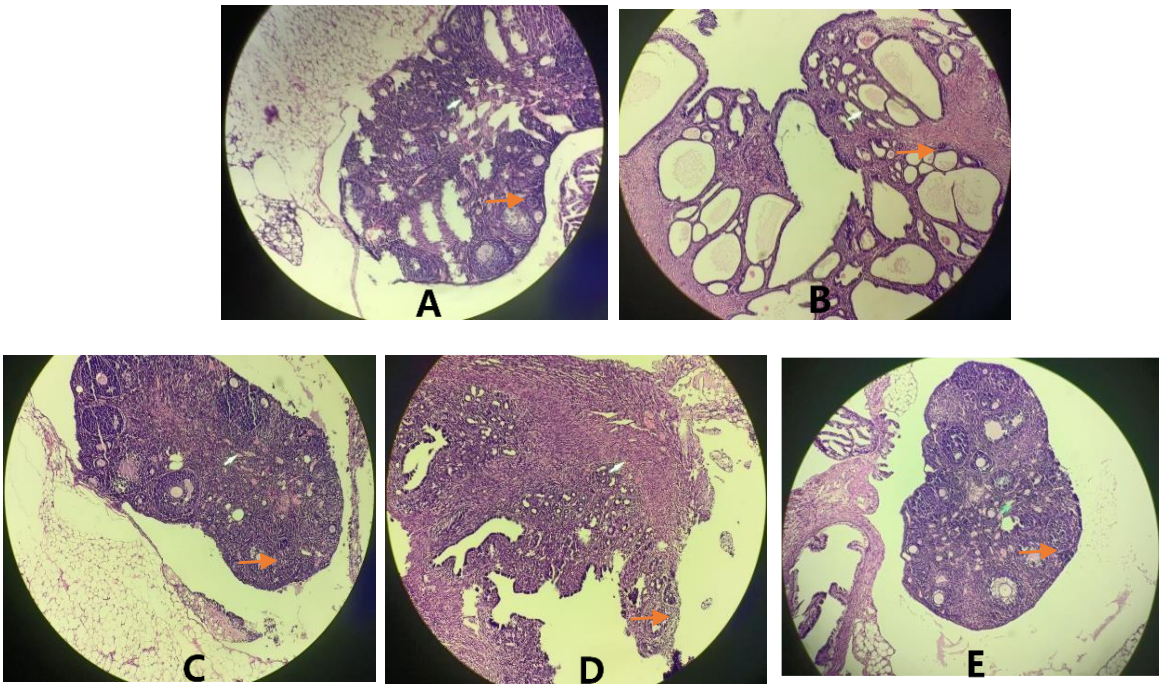


Fig. 1. Histological Images of Atretic Follicles in Different Groups; (A). Ovarian tissue from the K+ group, showing severe follicular atresia; (B). Ovarian tissue from the K- group, showing healthy follicular structure; (C, D, E). Ovarian tissues from P1, P2, and P3, demonstrating a gradual decrease in atretic follicles in response to increasing doses of red kidney bean extract.

Once the disease model was successfully established, red kidney bean extract was administered orally at varying doses (50 mg/kgBW, 75 mg/kgBW, and 100 mg/kgBW) daily for four weeks. To analyze the treatment effects, Th-17 and Treg levels were measured using enzyme-linked immunosorbent assay (ELISA), while ovarian tissue was examined histologically to determine the count of atretic follicles. Statistical analysis commenced with normality and homogeneity tests to assess data distribution. If the data followed a normal distribution, a one-way ANOVA was conducted, followed by Tukey's post hoc test to identify significant differences between groups. For non-normally distributed data, the Kruskal-Wallis test and Mann-Whitney U test were applied instead. A p-value of less than 0.05 was considered statistically significant.

Ethics Approval

The study was approved by the Ethical Committee of the Medical Faculty at Brawijaya University.

RESULTS

This research explored the impact of *Phaseolus vulgaris L.* (red kidney bean) extract on Th-17 and Treg levels, as well as the atretic follicle count, in a mouse model of SLE-induced premature ovarian failure (POF). The study utilized female Balb/c mice, which were assigned to five distinct groups: three treatment groups (P1, P2, and P3), a negative control (K-) and a positive control (K+), The K- group received no disease induction or treatment, while the K+ group received pristane, busulfan, and cyclophosphamide injections to induce SLE and POF but no treatment. The treatment groups were administered red kidney bean extract at doses of 50 mg per kgBW, 75 mg per kgBW, and 100 mg per kgBW, respectively. After four weeks of treatment, various parameters, including Th-17 and Treg levels (measured using ELISA) and atretic follicle counts (evaluated histologically), were analyzed to determine the efficacy of red kidney bean extract.

The findings revealed a significant decrease in Th-17 levels in the treatment groups compared to the K+ group ($p < 0.05$). The K+ group exhibited the highest Th-17 levels, confirming the inflammatory nature of SLE-induced ovarian damage. In contrast, Th-17 levels progressively decreased with increasing doses of red kidney bean extract, with the P3 group (100 mg per kgBW) showing the lowest Th-17 levels, approaching those of the K- group. These results indicate that red kidney bean extract possesses potent anti-inflammatory properties, which may help mitigate immune-driven ovarian damage. Conversely, Treg levels were significantly increased following red kidney bean extract administration ($p < 0.05$). The K+ group had the lowest Treg levels, indicating an impaired immune regulatory

function in SLE-induced POF. Nevertheless, all treatment groups displayed a dose-dependent rise in Treg levels, with the P3 group (100 mg per kgBW) showing the most substantial increase, closely resembling the levels observed in the K- group. This suggests that red kidney bean extract may help restore immune balance by enhancing Treg-mediated immune suppression, which is essential for controlling autoimmunity.

Histological analysis of ovarian tissue revealed a significant reduction in atretic follicles in the treatment groups compared to the K+ group ($p < 0.05$). The K+ group exhibited severe follicular atresia, confirming that SLE and POF induction resulted in accelerated follicular depletion. However, treatment with red kidney bean extract led to a dose-dependent decrease in follicular atresia, with the P3 group (100 mg/kgBW) showing the lowest number of atretic follicles. This suggests that red kidney bean extract plays a protective role in preserving ovarian follicular integrity, potentially delaying the onset of ovarian failure.

Table 1 summarizes the levels of anti-dsDNA, Th-17, Treg, and atretic follicles in each group. The K+ group exhibited the highest anti-dsDNA and Th-17 levels, confirming immune dysregulation, whereas the P3 group demonstrated a notable improvement in all parameters, with values approaching those of the K- group. Histological analysis further confirmed these findings, as shown in Fig.1. In the K+ group, ovarian tissue exhibited extensive follicular atresia with numerous degenerated follicles. In contrast, treatment groups (P1, P2, and P3) showed a gradual reduction in atretic follicles, with P3 exhibiting the most preserved follicular structures.

Overall, these findings suggest that red kidney bean extract exerts immunomodulatory and ovarian-protective effects in SLE-induced POF mice. The extract significantly reduces inflammation (Th-17 suppression), enhances immune regulation (Treg upregulation), and protects ovarian follicles from atresia. The most pronounced effects were observed in the P3 group (100 mg/kgBW), indicating that higher doses of red kidney bean extract may offer greater therapeutic benefits. These results support the hypothesis that red kidney bean extract could be a potential natural intervention for preventing premature ovarian failure in SLE patients.

DISCUSSION

The research findings indicate that administering red bean extract had a notable impact on Th-17 levels in mice with systemic lupus erythematosus (SLE)-induced premature ovarian failure (POF). Statistical analysis using a one-way ANOVA test revealed a p-value of less than 0.05, signifying a significant difference in Th-17 levels among the treatment groups. Further analysis with Tukey's post hoc test confirmed that mice receiving the highest dose of red bean extract (100 mg/kg BW) experienced a significant reduction in Th-17 levels compared to the positive control group, which did not receive treatment. These results suggest that red bean extract actively contributes to immune regulation in POF and SLE.

Th-17 cells, a subset of CD4+ T-helper cells, play a key role in autoimmune and inflammatory responses. Elevated Th-17 levels are commonly linked to chronic inflammatory diseases like SLE, as these cells produce pro-inflammatory cytokines, particularly IL-17, which drives inflammation and tissue damage. Red bean extract contains bioactive compounds such as isoflavonoids and flavonoids, which have been shown to inhibit inflammatory pathways by reducing the expression of cytokines like IL-6 and IL-17. By suppressing these inflammatory markers, red bean extract demonstrates potential as an immunomodulatory agent that may help alleviate inflammation in SLE-associated POF [3, 9–11].

Besides lowering Th-17 levels, red bean extract administration was also shown to enhance T-regulatory (Treg) cell levels. Statistical analysis revealed a significant rise in Treg levels in the groups receiving higher doses of the extract compared to the positive control group (K+). Treg cells play a vital role in maintaining immune balance by preventing excessive immune activation and reducing autoimmunity [12].

The increase in Treg levels is believed to be primarily driven by the phytoestrogens present in red bean extract, which can interact with estrogen receptors and regulate Foxp3 expression, a crucial transcription factor involved in Treg cell differentiation. Higher Foxp3 expression enhances the suppressive function of Treg cells, helping to restore immune balance. This is particularly important in SLE and POF, where immune system dysregulation significantly contributes to disease development. Therefore, red bean extract may aid in stabilizing the Th-17/Treg ratio, which is often disrupted in individuals with SLE and POF [4,13,14].

Additionally, the study demonstrated that red bean extract could decrease the number of atretic follicles in POF model mice with SLE. Statistical analysis revealed that the group receiving the highest dose (100 mg/kg BW) had fewer atretic follicles compared to the positive control group (K+), with numbers approaching those found in the negative control group (K−). This suggests that red bean extract may play a protective role in ovarian function, helping to maintain follicular integrity even in an inflammatory environment.

Atretic follicles are follicles that undergo degeneration and fail to mature. In POF conditions induced by SLE, increased inflammatory cytokines such as IL-6 and TNF-α can accelerate the follicular atresia

process, ultimately contributing to ovarian dysfunction and infertility. The phytoestrogen content in red bean extract is believed to play a role in inhibiting this process through protective mechanisms on granulosa cells and oocytes. Additionally, the antioxidant properties of red bean extract can reduce oxidative stress, which contributes to ovarian cell apoptosis. By counteracting oxidative stress and inflammation, red bean extract may promote better ovarian health and improve reproductive outcomes [15–18].

This study highlights the potential of red bean extract as a therapeutic agent for reducing inflammation and enhancing ovarian function in a mouse model of SLE-induced POF. However, several limitations remain. The long-term toxicity effects of red bean extract and the precise molecular mechanisms underlying Th-17/Treg regulation were not examined. Additionally, the optimal dosage and treatment duration for achieving the greatest therapeutic benefit require further study. Therefore, additional research is necessary to assess the long-term effects of red bean extract in animal models and to conduct clinical trials in humans [19].

Overall, the findings of this study suggest that red bean extract may be a potential alternative therapy for managing SLE-induced POF by lowering Th-17 levels, enhancing Treg levels, and reducing atretic follicles. With its immunomodulatory, antioxidant, and estrogenic properties, red bean extract shows promise as a natural supplement that could help regulate immune balance and support reproductive health in individuals with autoimmune conditions [20,21].

CONCLUSION

This study provides evidence that *Phaseolus vulgaris L.* (red kidney bean) extract effectively modulates immune responses and protects ovarian function in SLE-induced POF. By significantly reducing Th-17 levels, increasing Treg levels, and preventing follicular atresia, the extract demonstrates strong immunomodulatory and ovarian-protective properties. The 100 mg/kgBW dose showed the most significant effects, suggesting a dose-dependent therapeutic potential. These findings highlight the possibility of red kidney bean extract as a natural intervention for managing autoimmune-related ovarian dysfunction, although further research is needed to explore its clinical applications in human SLE patients at risk of premature ovarian failure.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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