RESEARCH ARTICLE

Effect of Phytoestrogens in Red Bean Extract (*Phaseolus vulgaris* L.) on Interleukin-6 (IL-6) Levels, Vaginal pH, Number of Endometrial Glands and Body Weight of Female Rats (*Rattus norvegicus*) with Ovariectomy as a Menopause Model

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ABSTRACT

Menopause, caused by decreased ovarian function and estrogen levels, can cause a variety of physical and emotional symptoms. A decrease in estrogen increases the risk of chronic conditions such as insulin resistance, type 2 diabetes, and osteoporosis. Atrophy of the endometrium decreased glandular numbers and thinning of the endometrial layer are characterized during menopause, which also increases the risk of postmenopausal bleeding. In addition, the decrease in estrogen also contributes to the thinning of the vaginal epithelium and changes in pH, increasing the risk of vaginal infections. Menopause is also associated with weight changes and the risk of other phenotypic changes. Although hormone replacement therapy (HRT) can help, it has risks, such as breast cancer. The use of phytoestrogens from plants such as red beans (*Phaseolus vulgaris* L) is a safe alternative treatment.

Objective: This study aimed to determine the effect of red bean extract (*P. vulgaris* L) on IL-6 levels, vaginal pH, number of endometrial glands and body weight in male rats model of menopause

Method: This study used a true experimental design with pre and post-test control group design. This study was conducted on female rats (*Rattus norvegicus*) Wistar strain menopausal model by dividing into control groups; ovariectomy group, standard estradiol treatment group, and three treatment groups of red bean extract (*P. vulgaris* L) at doses of 35 mg/200 gBW, 70 mg/200 gBW, and 140 mg/200 gBW. Interleukin-6 (IL-6) levels, vaginal pH, number of endometrial glands and rat body weight were observed in the study.

Result: Red bean extract (*P. vulgaris* L) can significantly improve IL-6 levels, vaginal ph, number of endometrial glands and body weight of rats with menopausal models ($p < 0.05$). In lowering IL-6 levels, the most optimal dose is 70 mg/200 gBW. While in improving the vaginal ph value, number of endometrial glands, and body weight of rats with an optimal menopausal model at a dose of 140 mg/200 gBW.

Conclusions: Red bean extract showed significant estrogenic activity as a phytoestrogen, showing that its use can be an alternative in overcoming symptoms due to menopause by being proven to reduce IL-6 levels, lower vaginal pH, increase the number of endometrial glands and reduce weight ovariectomy rats

Keywords: Menopause, Red bean *(Phaseolus vulgaris L)*, Phytoestrogens, IL-6, Vaginal pH, Number of endometrial glands, Body weight.

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INTRODUCTION

A woman is considered to have entered menopause when she has not had her period for an amount of a year or more continuously. It generally occurs between the ages of 45 and 55 and is a related outcome of physiological aging; it refers to the end of the reproductive years. There is an increasing global population of postmenopausal women. As of 2021, 26% of the global population of women and females were aged 50 or older; this represented a 22% increase from the previous decade. Loss of ovarian follicle function and a reduction in circulating estrogen levels result in menopause.[1,2]

Hormonal changes related to menopause can result in a variety of physical, emotional, mental, and social symptoms that vary between individuals. Although some experience only mild symptoms, others may experience severe symptoms, resulting in a disruption of everyday routines and a decrease in overall quality of life. Symptoms can last for several years and affect your personal as well as professional life, as well as overall

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health as you age.[2,3] During menopause, a decline in estrogen causes an elevation in IL-6 levels, a proinflammatory cytokine that is linked to chronic inflammation of the systemic nature. Estradiol is involved in the inhibition of IL-6 synthesis. Increased prevalence of metabolic diseases, including type 2 diabetes, insulin resistance, and cardiovascular disease, is associated with elevated levels of IL-6 in blood serum during menopause. In addition to being associated with osteoporosis, elevated levels of IL-6 can harm the endometrium.[4-7] During menopause, the reduction in estrogen levels causes the vaginal epithelium to become thinner.

This leads to a decrease in the shedding of vaginal epithelial cells, a decrease in the availability of glycogen, and a drop in the substances needed for acid production. Consequently, the vaginal pH increases. The reduction of glycogen in the vaginal epithelium of menopausal women results in an elevation of vaginal pH, making it more alkaline. As a result, this increases the vulnerability to vaginal infections or atrophic vaginitis. Vaginal pH is utilized as an additional diagnostic technique for menopause in certain research. It is established that the pH level in the vagina is equal to or less than 4.5 during the reproductive years and greater than 4.5 before the onset of menstruation and beyond menopause, provided there are no vaginal infections present.[8-10] The absence of estrogen

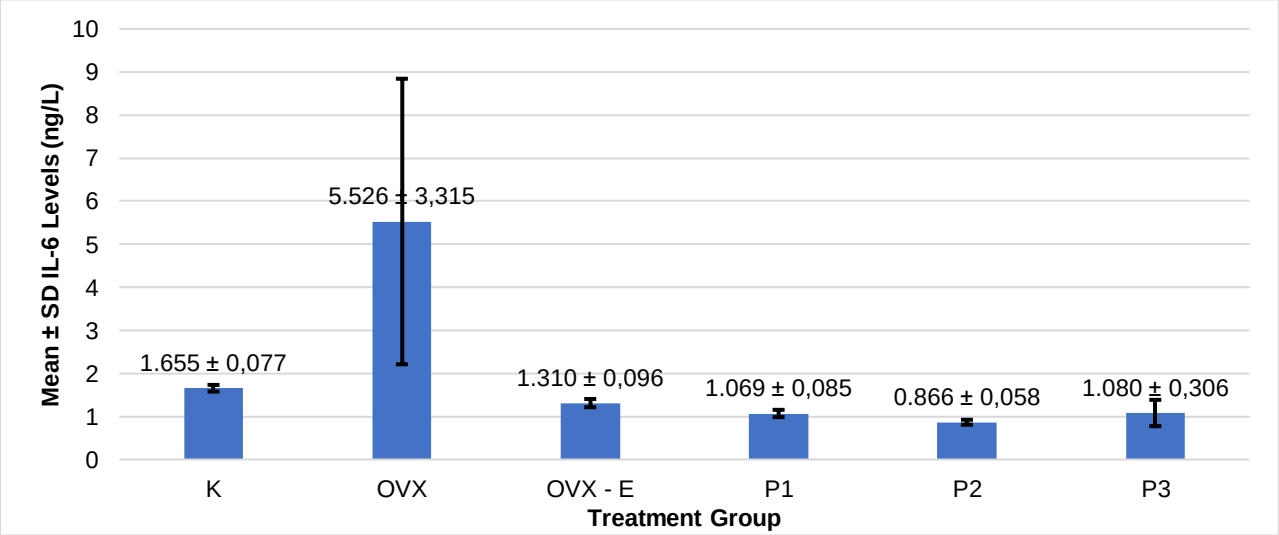

Figure 1: Histogram of average IL-6 levels (ng/L)

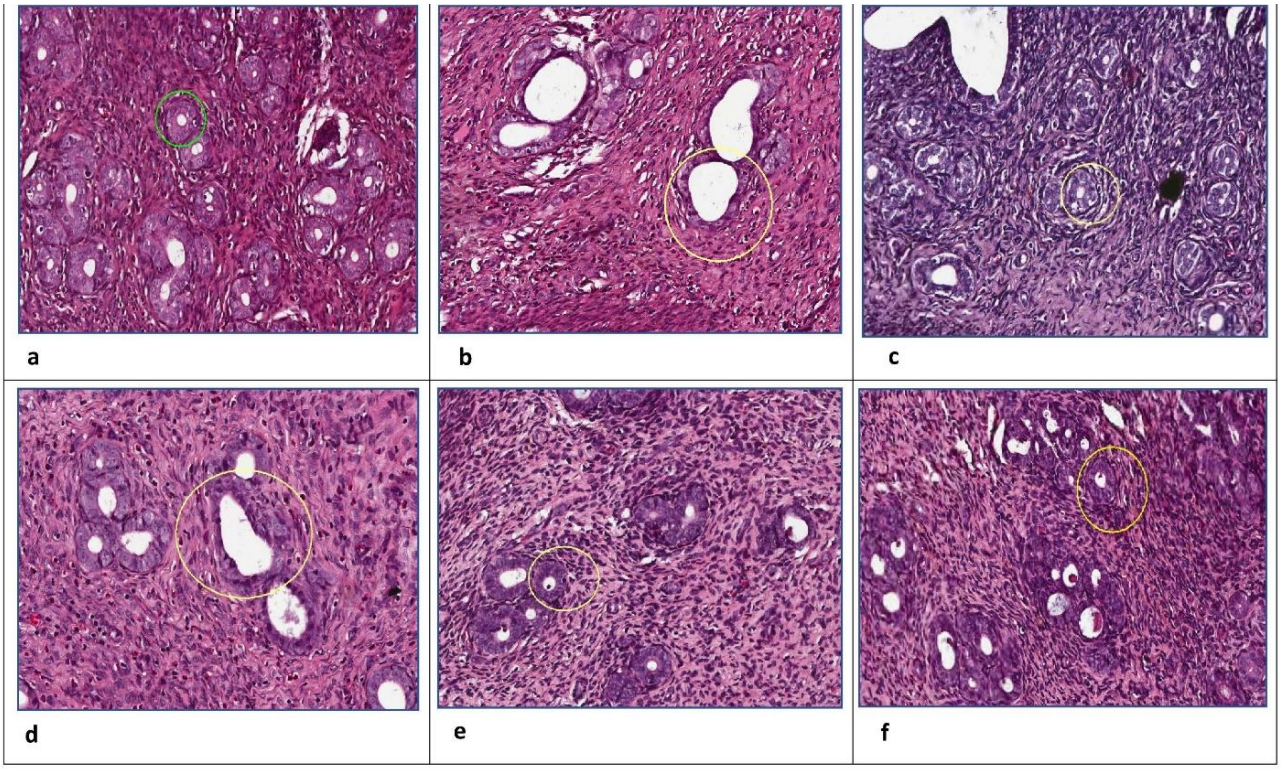

Figure 2: Histopathology of Number of Endometrial Glands in the treatment group

leads to atrophy of the endometrium. A decrease in estrogen will cause the binding with estrogen receptors (ERα, ERβ) to decrease so that estrogen receptors are inactive. Estrogen receptors, both ERα and ERβ, are key to endometrial epithelial cell proliferation. The absence of proliferation will lead to endometrial atrophy characterized by a thinning layer and consisting only of the basal stratum. A small and inactive number of glands lined by cuboidal to low columnar epithelium. Often, the gland undergoes cystic dilatation and is then lined by flattened epithelium. The stroma is less cellular and more collagen. There is no mitosis or secretory activity. The decrease in endometrial thickness is influenced by the absence of differentiation from the endometrial glands so the number of active endometrial glands decreases. The condition of the endometrium that atrophies during menopause causes the endometrial lining to become thinner so that it is susceptible to inflammation or inflammation that triggers postmenopausal bleeding.[11-14] Menopausal women experience phenotypic changes, such as weight gain accompanied by increased adipose tissue and a reduction in lean body mass resulting from diminished skeletal muscle mass and bone resorption. An increase in body mass is sufficient to contribute to a rise in the incidence of age-related overweight and obesity. Consequently, the likelihood of developing cardiometabolic disease, sarcopenia, and osteoporosis is heightened.[15,16] By administering estradiol from outside the body, also known as Hormone Replacement Therapy (HRT), this issue can be resolved. Although there is evidence supporting the effectiveness of HRT in reducing or preventing menopausal symptoms, further research suggests that prolonged use of HRT may be linked to an elevated risk of breast cancer, stroke, and thromboembolic events, among other negative consequences.[3,17]

Therefore, a potential and safe alternative treatment is needed, namely by using phytoestrogens from plants, one of which is red beans (*P. vulgaris* L). Red beans (*P. vulgaris* L) contain secondary metabolite compounds, namely isoflavones. Isoflavones exhibit a molecular structure that closely resembles that of 17-estradiol. As a result, they have the ability to attach themselves to both estrogen receptors ER α and ER β, leading to estrogen-like effects within the body. Experimental studies on animals have shown that they cause a variety of physiological effects.[18-22]

Objective

Currently, there is a lack of studies regarding the practical application of red beans (*Phaseolus vulgaris L.*) during menopause. Based on the relationship that has been described, the objective of this study is to demonstrate the efficacy of red bean extract. *P. vulgaris* L with isoflavone content and as phytoestrogens able to improve blood serum IL-6 levels, vaginal pH, number of endometrial glands and body weight in menopausal model rats.

MATERIALS AND METHOD

Experimental Animals and Research Design

This study used true experimental design, specifically utilizing a pre and post-test control group design. The

Table 1: Comparison Test of IL-6 levels with ANOVA and post hoc test

Group	Mean ± SD	*p-value*
K	1.65 ± 0.07[a]	
K OVX	5.52 ± 3.31[b]	
K OVX-E	1.31 ± 0.09[a]	0.001
P1	1.06 ± 0.08[a]	
P2	0.86 ± 0.05[a]	
P3	1.08 ± 0.3[a]	

Table 2: Correlation Test and linear regression of IL-6 levels

Variable	Regression	R-Square	Regression Equation	*p-value*
Red bean extract dosage	-0.544	29.6%	3.701–0.026	0.029

Tabel 3: Average vaginal pH value before treatment

Group	Mean±SD
K	7.07 ± 0.12
K OVX	8.05 ± 0.17
K OVX-E	7.7 ± 0.33
P1	7.8 ± 0.37
P2	7.8 ± 0.61
P3	8.2 ± 0.31

Table 4: Average vaginal pH after treatment

Group	Mean±SD
K	7.17 ± 0.12
K OVX	7.95 ± 0.35
K OVX-E	7.45 ± 0.31
P1	7.5 ± 0.48
P2	7.15 ± 0.42
P3	7 ± 0.14

Wistar strains of female white rats (*Rattus norvegicus*) used during this research were 9 to 12 weeks old, weighed 150 to 250 grams, and were in great condition. Acclimatized for seven days, rats were provided with *ad libitum* food and water.

In order to ascertain whether rats fulfill the inclusion criteria, their weight is recorded every two days during acclimatization. Following this, rats were divided into six different groups:

Control group (K): rats that were not treated with ovariectomy and given standard drinking feed by *ad libitum*

OVX group (K OVX): rats treated with ovariectomy and given standard feed and *ad libitum* drinking

OVX-E group (K OVX-E): rats treated with ovariectomy, given estradiol tablets 60 μg/200 gBW and given standard feed for 15 days

Treatment group 1 (P1): rats given ovariectomy treatment and given 35 mg/200 gBW red bean extract and standard feed for 15 days. [21]

Treatment group 2 (P2): rats given ovariectomy treatment and given 70 mg/200 gBW red bean extract and standard feed for 15 days. [21]

Treatment group 3 (P3): rats given ovariectomy treatment and given 140 mg/200 gBW of red bean extract and standard feed for 15 days.[21]

Table 5: Differences in vaginal pH values before and after administration of red bean extract *(P. vulgaris* L*)*

Group	Mean ± SD	Sig. (2-tailed)
P1 (35mg/200gBW) pre-post	0.3 ± 0.46	0.291
P2 (70mg/200gBW) pre-post	0.65 ± 0.58	0.111
P3 (140mg/200gBW) pre-post	1.2 ± 0.23	0.002

Table 6: Comparative Test of Endometrial Glands with ANOVA and Post Hoc Test

Group	Mean±SD	*p-value*
K	15.75 ± 3.02^{ab}	
K OVX	10.92 ± 1.6^{a}	
K OVX-E	13.57 ± 1.98^{ab}	0.044
P1	12.6 ± 1.37^{ab}	
P2	13.4 ± 3.98^{ab}	
P3	17.7 ± 3.77^{b}	

Table 7: Correlation Test and linear regression of the number of endometrial glands

Variable	Regression	R-Square	Regression Equation	*p value*
Red bean extract dosage	0.693	48.1%	10.750 + 0.048	0.003

Table 8: Weight comparison test with ANOVA and post Hoc test

Group	Mean±SD	*p-value*
K	165.25 ± 8.05^{a}	
K OVX	235 ± 15.25^{b}	
K OVX-E	195 ± 14.07^{ab}	0.001
P1	190 ± 29.3^{a}	
P2	180.25 ± 23.72^{a}	
P3	174.25 ± 12.68^{a}	

Table 9: Correlation Test and linear regression of rat body weight

Variable	Regression	R-Square	Regression Equation	*p-value*
Red bean extract dosage	-0.658	43,3%	218.200 – 0.381	0.006

Menopause Rat Model

In making a menopausal model rat, bilateral ovariectomy was performed. Rats were given ketamine anesthesia mixed with xylasin (80 and 10 mg/kg) by intraperitoneal injection. Bilateral ovariectomy is performed under aseptic conditions.[17]

After 14 days, which is the healing period, follicle-stimulating hormone (FSH) levels are checked ELISA Test using Bioassay Technology Laboratory (BT LAB) FSH ELISA Kit Cat.No: EA0014Mo. It was proven that there was an increase in FSH in post-ovariectomy rats (23). The confirmation of menopause in humans is determined by an FSH level that surpasses 40 IU/L. Furthermore, specific studies indicate that vaginal pH can serve as an alternate diagnostic method for menopause (8). Therefore, in this study also carried out vaginal pH examination using vaginal fluid measured with a digital pH meter.

Treatment

Post-ovariectomy estradiol administration in the OVX-E group was given at a dose of 60 μg/200 gBW. The estradiol given is an estradiol valerate tablet (brand Progynova). Administration of red bean extract (*P. vulgaris* L) after ovariectomy and according to the therapeutic dose of each group. The treatment is given for 15 days using a sonde.

Extraction of red beans (*P. vulgaris* L)

Red bean extract (*P. vulgaris* L) used in this study was obtained through the maceration method extraction process with 96% ethanol solvent. The extraction was conducted at the Materia Medika Batu Laboratory, located in East Java, Indonesia.

Blood Serum IL-6 Levels

The rats were euthanized after a 15-day period of therapy. 3 mL of blood samples were extracted from the heart organ and subsequently analyzed for IL-6 levels using the ELISA technique. The ELISA test was conducted using the Mouse Interleukin 6, IL6 ELISA Kit Cat. No E0049Mo from Bioassay Technology Laboratory (BT LAB).

Vaginal pH

Vaginal pH examination is done before and after treatment. Vaginal pH measurement before treatment is performed after ovariectomy. The tool used to check the pH of the rat's vagina is a digital pH meter. The material used is vaginal mucosal secretions located in the vaginal introitus and rats vaginal canals.

Observation of the number of endometrial glands

The uterine organs are removed and stored in vials containing a 10% formalin fixative buffer solution. The number of endometrial glands was calculated from histological preparations of the uterus with haematoxylin-eosin staining by observing 10 fields of view with 400x magnification. The number of endometrial glands was calculated and averaged using e-slide Manager Software in each field of view previously scanned using Indomicrophoto Microscope.

Rat Body Weight

Rats' body weight was measured on all rats using digital scales. Weight measurements were taken while the rats were calm. Rats were measured after treatment (just before termination). Measurements were taken every 2 days to avoid rats experiencing stress.

Statistical Analysis

The data were expressed as mean ± standard deviation (SD) and analyzed using IBM SPSS Statistics 25. The One-Way ANOVA test was employed to assess the impact of red bean extract on vaginal pH, IL-6 levels, number of endometrial glands, and rat body weight. Subsequently,

Tukey's Post Hoc test was utilized to identify any statistically significant differences between the groups ($p < 0.05$). Additionally, to ascertain the causal relationship between variables, a linear regression analysis was conducted. To examine the association between the dosage of red bean extract and the measured variable, a Pearson correlation analysis was also performed. The T-Test is performed on the vaginal pH variable to compare the results of pre and post-treatment.

RESULT

IL-6 levels

IL-6 levels are checked after treatment. IL-6 levels in each group were shown to be normally distributed and homogeneous (p >0.05). The distinctions among each group were assessed utilizing ANOVA and Post Hoc analysis (Table 1). The findings indicated that the ovariectomy treatment (K OVX) resulted in a notable elevation in IL-6 levels in comparison to the control group (K) (p = 0.009, <0.05). The findings also demonstrated that the administration of red bean extract (*P. vulgaris* L) at different doses had a statistically significant effect in reducing IL-6 levels in all groups (p = 0.002, <0.05). The P2 group had the lowest average IL-6 levels, measuring 0.86 ± 0.05 (p = 0.002, <0.05), compared to the ovariectomy group (Fig. 1). It may be inferred that administering red bean extract (*P. vulgaris* L) has a similar impact to estradiol administration in lowering IL-6 levels in normal rats. However, there is no significant difference in the treatment outcomes when using different doses of red bean extract (P1, P2, P3 p >0.05).

Description: The values represent the mean ± SD. K: control; K OVX: ovariectomy rats; K OVX-E: ovariectomy rats treated with estradiol; P1: ovariectomy rats treated with 35 mg/200 gBW red bean extract (*P. vulgaris* L); P2: ovariectomy rats treated with 70 mg/200 gBW red bean extract (*P. vulgaris* L); P3: ovariectomy rats treated with 140 mg/200 gBW red bean extract (*P. vulgaris* L). If there are different letters in a mean ± SD, it indicates a significant difference ($p < 0.05$), and if there are the same letters, it indicates no significant difference ($p > 0.05$).

Relationship of Red Bean Extract (*P. vulgaris* L) to IL-6 levels

Pearson's correlation between red bean dose and IL-6 R = -0.544 with significance p = 0.029 (Table 2). This shows a strong negative correlation between red bean doses and IL-6 levels. Variability in IL-6 levels is 29.6%; as the dose of red beans ingested increases, IL-6 levels decrease.

Vaginal pH

The vaginal pH values before each treatment group had different averages (Table 3). The average results indicated that the group that underwent ovariectomy (K OVX; K OVX-E; P1; P2; P3) had a higher mean vaginal pH value compared to the control group.

Description: The values represent the mean ± SD. K: control; K OVX: ovariectomy rats; K OVX-E: ovariectomy rats treated with estradiol; P1: ovariectomy rats treated with 35 mg/200 gBW red bean extract (*P. vulgaris* L); P2: ovariectomy rats treated with 70 mg/200 gBW red bean extract (*P. vulgaris* L); P3: ovariectomy rats treated with 140 mg/200 gBW red bean extract (*P. vulgaris* L).

The vaginal pH values after each group's treatment had different averages (Table 4). Based on the average results, it showed that the vaginal pH value was lowest in the P3 treatment group and the highest vaginal pH value in the K OVX group.

Description: The values represent the mean ± SD. K: control; K OVX: ovariectomy rats; K OVX-E: ovariectomy rats treated with estradiol; P1: ovariectomy rats treated with 35 mg/200 gBW red bean extract (*P. vulgaris* L); P2: ovariectomy rats treated with 70 mg/200 gBW red bean extract (*P. vulgaris* L); P3: ovariectomy rats treated with 140 mg/200 gBW red bean extract (*P. vulgaris* L).

The paired T-test was utilized to examine the variations in vaginal pH values before and after treatment in the group that received varying amounts of red bean extract (*P. vulgaris* L) (Table 5). Previous research has established that the data follows a normal distribution (p >0.05). The data indicate that the most significant result group was the

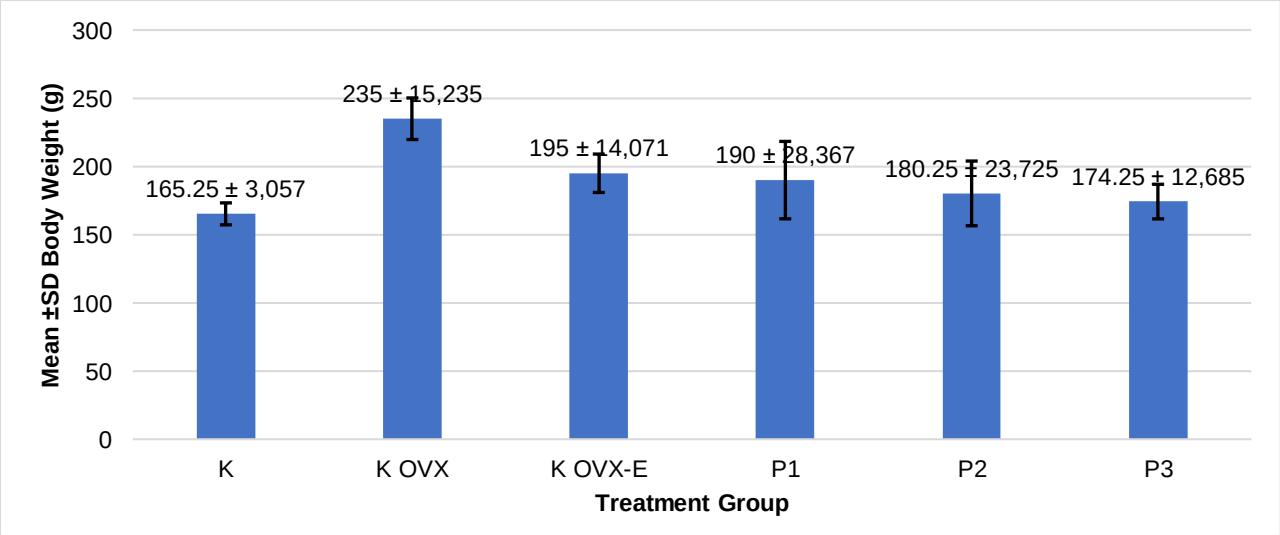

Figure 3: Histogram of average rat body weight (g)

P3 group, where the significance level (Sig.) produced was 0.002, sig < 0.05. Therefore, it can be interpreted that the red bean extract treatment dose of 140 mg/200 gBW has a significant effect on vaginal pH, with an average decrease of 1.2 ± 0.23.

Number of Endometrial Glands

The measurement of the number of endometrial glands was calculated from histological preparations with hematoxylin-eosin staining by observing 10 fields of view of 400X magnification. The number of endometrial glands in each group was shown to be normally distributed and homogeneous (p >0.05). ANOVA and Post Hoc tests were used to assess the differences among each group, as shown in Table 6. The findings indicated a reduction in the quantity of endometrial glands in the ovariectomy group (K OVX). However, this decrease was not statistically significant (p = 0.2, >0.05). The treatment group that received red bean extract showed a noteworthy rise in the number of endometrial glands. The P3 group had the highest average of 17.7 ± 3.77 (0.032, <0.05). Administration of red bean extract with the highest dose of 140 mg/200 gBW produced a better number of glands than in the estradiol treatment group which had an average of 13.57 ± 1.98, although not significant (p = 0.344, <0.05).

Description: The values represent the mean ± SD. K: control; K OVX: ovariectomy rats; K OVX-E: ovariectomy rats treated with estradiol; P1: ovariectomy rats treated with 35 mg/200 gBW red bean extract (*P. vulgaris* L); P2: ovariectomy rats treated with 70 mg/200 gBW red bean extract (*P. vulgaris* L); P3: ovariectomy rats treated with 140 mg/200 gBW red bean extract (*P. vulgaris* L). If there are different letters in a mean ± SD, it indicates a significant difference (p < 0.05), and if there are the same letters, it indicates no significant difference (p > 0.05).

Relationship of Red Bean Extract (*P. vulgaris* L) to Number of Endometrial Glands

The relationship between the dosage of red bean extract and the number of endometrial glands exhibited a Pearson correlation coefficient of 0.693 as shown in Table 7, indicating a strong positive association. This correlation was statistically significant, with a *p-value* of 0.003. There was a clear and meaningful correlation seen between the dosage of red bean extract and the quantity of endometrial glands. There is a direct correlation between the dosage of red beans given and the quantity of endometrial glands produced. The range in the number of endometrial glands is 48.1%.

Description: Endometrial tissue stained with haematoxylin-eosin at 400X magnification. The figure above is a histopathological picture of the number of endometrial glands in each treatment group. (1) Control group (K) =15.75 ± 3.02; (2) Group OVX (K OVX) =10.92 ± 1.6; (3) Group OVX-E (K OVX-E) =13.57 ± 1.98; (4) Group P1=12.6 ± 1.37; (5) Group P2 =13.4 ± 3.98; (6) Group P3 =17.7 ± 3.77.

Rat Body Weight

Weight measurement is carried out after treatment. The body weight in each group was found to follow a normal distribution and exhibit homogeneity (p >0.05). The differences between each group were assessed using analysis of variance (ANOVA) and post hoc analysis (Table 8). The findings indicated that the group that underwent ovariectomy exhibited a substantial rise in body weight, with an average of 235 ± 15.25, in comparison to the control group (p = 0.001, <0.05). In the P3 group, the average body weight was 174.25 ± 12.68 (Fig. 2), which was significantly lower than the ovariectomy group (p = 0.002, <0.05). Therefore, it can be inferred that administering red bean extract (*P. vulgaris* L) has a comparable impact to estradiol administration in promoting weight loss in normal rats. However, there is no statistically significant difference observed when comparing the effects of different doses of red bean extract (P1, P2, P3 p >0.05) (Fig. 3).

Description: The values represent the mean ± SD. K: control; K OVX: ovariectomy rats; K OVX-E: ovariectomy rats treated with estradiol; P1: ovariectomy rats treated with 35 mg/200 gBW red bean extract (*P. vulgaris* L); P2: ovariectomy rats treated with 70 mg/200 gBW red bean extract (*P. vulgaris* L); P3: ovariectomy rats treated with 140 mg/200 gBW red bean extract (*P. vulgaris* L). If there are different letters in a mean ± SD, it indicates a significant difference (p < 0.05), and if there are the same letters, it indicates no significant difference (p > 0.05).

Relationship of Red Bean Extract (*P. vulgaris* L) to Rats' Body Weight

The correlation test between red bean dose and rat body weight resulted in a Pearson correlation of -0.658 with a p-value significance of 0.006. This showed a strong and significant negative correlation between red bean doses and rat body weight. An increase in the dosage of red beans led to a corresponding decrease of 43.3% in the body weight of the rats.

DISCUSSION

Ovarian dysfunction occurs as a result of menopause, causing a decrease in the number of ovarian follicles. The decline in the quantity of preantral and antral follicles as one ages is believed to be linked to a drop in anti-Mullerian hormones and inhibin B. The lack of inhibin B's regulatory impact on the synthesis of gonadotropins in the pituitary gland results in an increase in the levels of FSH throughout the body. The decrease in estrogen becomes one of the last changes that occur.[24] This study aimed to ascertain the impact of red bean extract (*P. vulgaris* L) as an alternative to HRT in reducing and overcoming symptoms and complications during menopause. Red beans also contain chemicals in the form of phytoestrogens in the form of flavonoid compounds with secondary metabolite compounds, namely isoflavones. Based on their chemical structure, isoflavones resemble the chemical structure of 17-estradiol and can bind to both ER α and ER β estrogen receptors, resulting in endogenous estrogen-like effects. The USDA states that 100g of raw red beans (*P. vulgaris* L.) contain isoflavone-type phytoestrogens, namely 0.29 mg genistein and 0.30 mg

daidzein, so the total amount of isoflavones in 100 g of red beans is 0.59 mg (18,21). Red beans (*P. vulgaris* L.) possess a significant amount of total phenolic compounds. Research has demonstrated their ability to exhibit anti-inflammatory and antioxidant properties.[25]

Estrogen decline during menopause contributes to the elevation of IL-6 levels. Estradiol functions by suppressing the activation of genes responsible for proinflammatory cytokines and the synthesis of the proinflammatory cytokine interleukin-6 (IL-6). Estrogen, through its activated receptor (ER) directly inhibits IL-6 gene expression through NF-kB-dependent mechanisms. Low estrogen in the body during menopause increases the production of free radicals (ROS) which are another source in the production of cytokine IL-6 (4,26). High IL-6 in menopause is also proven in previous human studies, where postmenopausal women aged 52-63 years have higher serum IL-6 levels than younger ones (6). Based on this study, the group of rats who underwent ovariectomy showed noticeably higher levels of IL-6 in comparison to the control group. IL-6 levels were found to be substantially reduced when red bean extract (*P. vulgaris* L) was administered in this study (Table 1). These results are also consistent with previous studies conducted on mouse models of systemic lupus erythematosus (SLE), although the data showed no significance.[25] In this study, there was an increase in the P3 group with a dose of 140 mg/200 gBW, this showed a slight increase in IL-6 levels. This indicates the possibility of toxicity if the dose is further increased. In previous studies, the maximum dose used was 100mg (25).

Red bean extract (*P. vulgaris* L) contains flavonoids-isoflavones which are natural phenolic compounds with various biological activities, especially antioxidant, antitumor, and anti-inflammatory effects. Red bean extract can lower IL-6 levels through inhibition of gene expression by disabling the NF-κB pathway (27). NF-κB is crucial in controlling the transcription of several genes related to inflammatory and immunological responses. NF-κB proteins typically exist in the cytoplasm of dormant cells. However, when they are stimulated, they become active and move to the cell nucleus. This movement leads to the activation of several inflammatory genes through the process of transcription. Prior research has demonstrated that extracts containing high levels of phenolic compounds derived from peanuts have the ability to decrease the production of NF-κB p65 within the cell nucleus.[28,29] Vaginal pH was also examined in this experiment. Before ovariectomy, the pH value of the vagina in each group was different. The situation showed that in experimental animals (rat), the pH level in the proestrus phase was 5.57 and decreased in the estrus phase to 4.53 (30). After ovariectomy, the pH value of the vagina increased higher than in the control group. Menopause has two main influences on the pH of the vagina: the menopausal status itself and the existence of potentially harmful organisms. Lactobacilli in fertile women help maintain a naturally acidic vaginal pH, which prevents the growth of undesirable bacteria in the vagina. Certain strains of lactobacilli also synthesize hydrogen peroxide, which serves to inhibit the proliferation of harmful bacteria inside the vaginal environment. However, the onset of menopause causes a reduction in estrogen levels, which in turn results in the weakening of the vaginal epithelium. This leads to a decrease in the shedding of epithelial cells into the vagina resulting in an elevation in the pH level. The alteration in pH results in a reduction in the population of lactobacilli and a proliferation of other bacteria, hence elevating the susceptibility to urogenital infections. Hence, it is crucial to eliminate the occurrence of vaginitis in order to ensure that the pH level of the vagina accurately reflects the true condition after menopause. The typical pH level of the vagina is below 4.5, although it might rise if there are vaginal infections. If there are no vaginal infections present, a pH level higher than 4.5 is a clear indication of reduced estrogen levels. Consequently, pH monitoring can be utilized as a diagnostic tool for menopause when there are no vaginal infections present.[8,9,31] After administration of red bean extract (*P. vulgaris* L), vaginal pH decreased significantly in the P3 group at a dose of 140 mg/200 gBW (Table 5). Consistent with prior studies, it has been found that phytoestrogens stimulate the synthesis of glycogen. Lactobacillus Döderlein bacteria, present in the natural vaginal flora, metabolize glycogen into lactic acid, maintaining an acidic pH in the vagina and preventing the proliferation of harmful microbes. Estrogen increases the capacity of basal cells in the vaginal epithelium to store glycogen. Glycogen is synthesized and accumulated by epithelial cells. Upon cell discharge, Lactobacillus bacteria will enzymatically break down glycogen into lactic acid, leading to a vaginal pH of approximately 4.5. Vaginal acidity serves as a defense mechanism against infections and certain anaerobic microbes and pathogens.[32] The data indicate that the ovariectomy group (K OVX) had the lowest average number of endometrial glands. However, the difference was not statistically significant. The decline in the quantity of endometrial glands following menopause is attributed to a reduction in estrogen levels. Estrogen is crucial for the growth and multiplication of endometrial cells. When estrogen levels decline, the activity of estrogen receptors (ERα and ERβ) similarly declines, leading to a reduction in the growth of endometrial epithelial cells. Estrogen has a notable impact on female reproduction by interacting with two main estrogen receptors, ERα and ERβ, and potentially through the G-protein-coupled estrogen receptor (GPER; GPR30).[14] In this case, there is still not much research on the exact path of menopause to the number of endometrial glands. The highest number of endometrial glands was found in the P3 group with the administration of red bean extract (*P. vulgaris* L) at a dose of 140 mg/200 gBW. This demonstrates the correlation between the phytoestrogen level and the quantity of endometrial glands. Previous studies have also confirmed these findings, demonstrating that the use of genistein phytoestrogens led to an increase in cell growth. However, the effectiveness of genistein phytoestrogens was not as high as that of endogenous estrogens or synthetic estrogens like 17ß-estradiol (E2) or ethinylestradiol (EE).[33] Phytoestrogens have the ability to

increase the mRNA expression of ERα and ERβ, which are involved in facilitating the diverse actions of estrogen on various target organs. These receptors can be stimulated by either attaching to estrogens and estrogen-like ligands pr by engaging with various signal transduction pathways. ERα and ERβ play a vital role in regulating cellular responses to estrogen and similar chemicals. They accomplish this by directly engaging with estrogen and by employing alternative signaling pathways. Phytoestrogens can influence the thickness of the uterine epithelium and the expression of proliferating cell nuclear antigen (PCNA) in the epithelial cells. PCNA is a very stable central protein with a molecular weight of around 36 kDa and exhibits an acidic character. These proteins are produced during the processes of DNA replication and DNA repair. PCNA establishes connections with DNA polymerase δ and RF-C proteins to bind at the crossover site where DNA primers and templates converge. The expression of PCNA protein and mRNA in the cell nucleus during the S stage is positively associated with DNA synthesis. Thus, the presence and expression of PCNA can be used as an important indicator for DNA replication and synthesis activity in a cell (33,34). In addition to affecting mRNA from ERα, ERβ and PCNA, the phytoestrogen genistein is also capable of influencing mRNA from C3. Where C3 expression occurs in new glandular epithelial cells and lumen synthesized from the endometrium.[33,35] Weight gain during menopause has been shown to increase body weight. In this study, the ovariectomy group (K OVX) showed significantly higher body weight compared to the other group (Table 5), this is also in accordance with previous research (36). After menopause, there is usually an increase in body weight in women. This increase is often due to general aging and hormonal changes. Estradiol levels affect mitochondrial function. Mitochondria are considered important centers in the cellular aging process. Estradiol receptors are present in various parts of the cell, including in the mitochondrial membrane and plasma membrane of the cell, as well as in the cell nucleus and cytoplasm. A decrease in estradiol levels during menopause can potentially affect ATP synthesis and alter cellular metabolic pathways. Imperfections in mitochondrial function can lead to cellular aging, particularly in brain tissue, fat, and muscle, ultimately affecting the regulation of cellular metabolism, fat distribution, and weight gain.[37-39] Administration of red bean extract (*P. vulgaris* L) was able to significantly reduce body weight in the treatment group, with P3 (dose 140 mg/200 gBW) having the lowest body weight (Table 8). Adipose tissue exhibits a high degree of sensitivity to estrogen, with both human and mouse fat tissue including estrogen receptors (ER) α and ERβ. Phytoestrogens can impact the composition of the body by directly attaching to estrogen receptors, particularly the ER α. They can also influence the regulation of body composition through hormones such as adiponectin, ghrelin, insulin, and leptin. Additionally, phytoestrogens can modify the metabolic activity of adipose tissue.[40–42] An inherent limitation of this study is the absence of a low-dose range, which precludes the determination of dangerous dosage levels. Researchers also did not examine the effects of red bean extract on other proinflammatory cytokines affected by the hypoestrogen state in menopause. The specific content of red bean extract is still unknown in this study. The effect of red bean extract phytoestrogens on estrogen receptors (ER), specifically on reproductive organs, has also not been studied. Therefore, further research is needed on phytoestrogens from red beans (*P. vulgaris* L).

CONCLUSION

Various results showed that red bean extract had a strong association with decreased IL-6 levels, decreased vaginal pH, increased number of endometrial glands, and weight loss in rats who performed ovariectomy (OVX) as a menopausal model. These findings indicate that red beans (*P. vulgaris* L) have the potential to treat or reduce the effects and symptoms of hypoestrogen in menopause. Red bean extract also shows significant estrogenic activity as a phytoestrogen, which suggests that its use may be an alternative to hormone replacement therapy (HRT). However, more research is needed to evaluate the dose and its effect on proinflammatory cytokines as well as other organs affected by low estrogen in menopause.

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Ethics

This research has received ethical approval for all research procedures approved by the Ethics Commission of the Faculty of Medicine, Universitas Brawijaya, with ethical approval certificate numbers 429/EC/KEPK-S2/12/2023, 430/EC/KEPK-S2/12/2023, 431/EC/KEPK-s2/12/2023.

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