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# EFFECTS OF PHYTOESTROGENS RED BEAN EXTRACT *(Phaseolus vulgaris L.)* AGAINST ESTRADIOL LEVELS, VAGINAL MUCOSAL EPITHELIAL THICKNESS, ENDOMETRIAL THICKNESS AND FEMORAL BONE OSTEOCLASTS OF MENOPAUSAL RATS

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**Abstract**

**Background :** After menopause women will experience hypoestrogens due to loss of ovarian activity, causing changes in vaginal tissue, endometrial tissue and bone. The purpose of this study was to determine whether the effect of red bean extract with phytoestrogen content in it can be an alternative estrogen replacement therapy on estradiol levels, vaginal mucosal epithelial thickness, endometrial thickness and femoral bone osteoclasts in menopausal rats.

**Methods :** This study was conducted experimental laboratory using 6 groups, namely 1) control rat group (K), 2) ovariectomy rat group with no treatment (P-OVX), 3) ovariectomy rat group and given estradiol tablets 60µg/200gBW (P-OVXE), 4) ovariectomy rat group and given red bean extract dose I (35mg/200gBW) (P1), 5) ovariectomy rat group and given red bean extract dose II (70mg/200gBW) (P2), 6) ovariectomy rat group and given red bean extract dose III (140mg/200gBW) (P3). Estradiol levels in each rats were analyzed by *ELISA* method, vaginal and bone tissue was taken in each rat and then examinated using *Hematoxilin-Eosin*. The thickness of the vaginal mucosal epithelium and endometrial are calculated with a magnification of 400X as much as 10x the field of view and the number of osteoclasts is calculated with a magnification of 200X as much as 5 fields of view using *Leica Aperio ImageScope 12.4.6 Software* then calculated the average.

**Results :** There were significant differences between estradiol levels, vaginal mucosal epithelial thickness, endometrial thickness and the number of femoral bone osteoclasts in menopausal rats, as determined by the outcomes of One Way ANOVA and Post hoc Tukey HSD tests applied to these three variables. The last test is a linear regression of estradiol levels at 0.001, variable vaginal mucosal epithelial thickness at 0.000, variable endometrial thickness at 0.026 and variable osteoclasts at 0.040, showing that red bean extract at different doses affects estradiol, vaginal epithelial thickness, endometrial thickness and osteoclasts.

**Conclusions:** Red bean extract can increase estradiol levels, restore the vaginal mucosal epithelial thickness, increased endometrial thickness and prevent an increase in osteoclast cells in the femoral bone.

**Keywords:** Red beans, menopause, estradiol, vaginal mucosal epithelial, endometrial, osteoclasts

**Background**

Menopause is a physiological process experienced by every woman, usually occurs between the ages of 45-55 years due to loss of ovarian activity resulting in changes in hormone production, one of which estrogen (1). Clinically, natural menopause is defined as a period of amenorrhea for 1 year. A decrease in the hormone estrogen during menopause gives symptoms that can manifest in various organs. Possible symptoms are hot flushes, headaches, anxiety, irritability, depression, vaginal atrophy, urinary incontentesia, osteoporosis, coronary heart disease, and stroke (2). Based on data from the WHO *(Health Organization)* by 2030 it is estimated Women aged 50 years about 1.2 billion, of whom live in developing countries as much as 80% and every year women who experience menopause will increase by 3% (3). Indonesia has 30.3 million menopausal women in 2020. About 50% of women experience vaginal atrophy, also referred to as genitourinary syndrome, menopause during and after menopause (4). As women over 50 years of age in the world are at risk of osteoporosis with a ratio of 1 in 3 women (5)

One of the reproductive organs that experience the impact of a decrease in the hormone estrogen in menopause is the vagina. The clinical manifestation experienced is vaginal atrophy (5,6). Under normal estrogen levels, the vaginal mucosa is lined with epithelial cells that are thick enough, epithelial cells contain glycogen which is regularly released to the surface of the mucosa. When in hypoestrogen conditions there will be changes in the vaginal wall such as thinning of the mucosa, thinning of the vaginal wall and elasticity will also decrease so that it will have an impact on aches and pains during sexual intercourse (7,8)

Abnormal uterine bleeding (AUB) is a menstrual-related issue that might occur during perimenopause. This medical condition is most common among women aged 45 to 50, affecting 66% cases. The older the woman, the risk factors for AUB will increase as well (9). Endometrial atrophy due to hypoestrogen conditions will cause fluid in the intrauterine cavity to decrease, so friction will occur that causes micro-lesions on the epithelial surface. This will cause light bleeding or spotting (10). In addition, hyposetrogenic conditions can also have an impact on bone organs, which will experience osteoporosis due to the absence of a balance between the number of osteoblasts and osteoclasts. Osteoclasts are spesific cells involved for the resorption and degradation of injured bone tissue (11)

Treatment is essential for alleviating the symptoms that manifest in postmenopausal women in order to prevent adverse effects on their health and overall well-being. Nowadays, hormone replacement therapy (HRT) is advised by physicians as an alternative to estrogen for the purpose of alleviating symptoms associated with menopause. Estrogen is an important hormone in the female reproductive system, there are 3 types of estrogen hormones namely estrone (E1), 17 β estradiol (E2) which is the main estrogen for women of childbearing age and estriol (E3) (12). Based on several studies, the use of HRT in the long term can cause the risk of cancer. Therefore, there is a need for safer alternatives to maintain health in menopausal women. The use of natural ingredients is needed as an alternative to HRT, one of which is phytoestrogens (13)

Phytoestrogens are chemical compounds that can bind to estrogen receptors inside naturally derived from plants whose chemical structure and function are similar to hormones 17 β estradiol (E2), have phenolic rings and hydroxyl groups (OH) with the distance between hydoxyl groups is 11.0-11.5 so that they can bind to estrogen receptors (14). Some active substances in plants that have potential as phytoestrogens are flavonoids, alkaloids, steroids. Food sources containing phytoestrogens include wheat, soybeans, tofu, sesame, nuts (13)

Red beans are one type of legume plant that contains phytoestrogen chemicals, namely flavonoids (15). Isoflavones are a class of flavonoids found in many legume, soybean and fruit plants (13,16). According to some studies, flavonoids can be used as an alternative to relieve menopausal symptoms such as vaginal and endometrial atrophy or osteoporosis in menopausal women (11). Rats undergoing bilateral ovariectomy procedures have similar characteristics to menopausal women, so female rats undergoing ovariectomy can be used

as a model in this study (17). On the basis of the aforementioned hypotheses, it is necessary to demonstrate whether red bean extract phytoestrogen can increase estradiol levels, vaginal mucosal epithelial thickness, and endometrial thickness while decreasing the number of osteoclasts in the femoral bone of menopausal model rats.

## Methods

### Research Design

Experimental design (pos-test control group) was adopted to conduct the research. Experimental animals, those who meet the inclusion and exclusion criteria are randomly divided into 6 treatments groups, each group consisting of 4 rats randomly (n=24). The first group (K) were control rat, the second group (P-OVX) be rats that were ovariectomy without any treatment, the third group (P-OVXE) was rat that were ovariectomy and given estradiol tablets 60 µg / 200gBW, the fourth group (P1) were rat that were ovariectomy and given red bean extract dose I (35 mg / 200gBW), the fifth group (P2) were rat that were ovariectomy and given red bean extract dose II (70 mg / 200gBW), sixth group (P3) were rats that were ovariectomy and given red bean extract dose III (140 mg / 200gBW).

### Red Bean Extract

The productin of red bean extracy is conducted at the laboratory of Herbal Materia Medika Batu. The red beans are rinsed with running water and then dried as part of the preparation procedure. Red beans that have been dried are blended into powder, the powder is weighed and put into an erlenmeyer glass and then soaked using 96% ethanol allowed to stand 1 night until settled. The next stage is the evaporation process, the top layer of the mixture of ethanol and active substance is taken and put into the evaporator flask, the ethanol solution is left to separate with the active substance left for approximately 2 hours. The extraction results are then put into plastic or glass bottles.

### Experimental Animal Treatment

For a period of one week, twenty-four white rats were acclimatized by being provided with food and water on an *ad-libitum* condition. After that, bilateral ovariectomy surgery was performed on 5 groups of rats (P-OVX, P-OVXE, P1, P2 and P3). After 14 days after bilateral ovariectomy, rats were given estradiol treatment orally as much as 60µg/200gBW, red bean extract was given orally at different doses in each group (35 mg/200gBW**,** 70 mg/200gBW and 140 mg/200gBW for 15 days.

### Estradiol Hormone Analysis

Blood sampling for analysis of the hormone estradiol is carried out on the day after treatment. Rats were anesthetized using 1% ketamine at a dose of 0.2 mL/100 grams BB, then blood was taken as much as 3 mL from the heart using a syringe. The blood obtained is accommodated into a vacuum tube that EDTA 1.8 mg/mL and centrifuged at a rate of 2000-3000 RPM for 20 minutes. Analysis of estrogen hormone levels is carried out by the ELISA method by following the instructions from the procedure manual listed in the Bioassay Technology Laboratory (BT LAB) Estradiol ELISA kit Cat.No: EA0011Ra.

### Preparations og Histologycal Samples

*Vagina and Uterine organ*

During the organ collecting procedure, the rats were placed on a surgical board with their abdomen pointing upwards, fixed in position with pins inserted into their upper and lower extremities. Afterwards, the extracted organ is thoroughly cleansed to remove any attached fat. Then, the tissue sample is fixed with a 10% neutral buffer formalin solution and transported to Anatomical Pathology Laboratory for histology preparation, staining, and observation.

*Femoral bone organ*

After the removal of the vaginal organs, another organ extracted is the femur bone. To separate the posterior extremities of a rat from its body, the muscles, ligaments, and tendons that attach the femur to the proximal pelvis are dissected. Next, the femur bone of the posterior limb of rats was fixed using a 10% solution of neutral buffer formalin and transported to the Anatomical Pathology Laboratory for preparing histologycal samples.

### Statistical Analysis

The data was analyzed using the SPSS for Windows version 27 software. The analysus of the study's result starts with the Shapiro-Wilk test to determine if the data has a normal distribution. If the data is normally distributed, One-Way ANOVA test is applied. Post-Hoc test, Pearson's correlation test and linear regression test are applied if the result are significant.

## ETHICS

The procedure in this study is based on ethical approval by the Health Research Ethics Commission, Faculty of Medicine, Brawijaya University.

## RESULT

### Effect of Red Bean Extract on Estradiol Levels in Menopausal Rats

Table 1. Mean and Standard Deviation of Estradiol Levels

| Group Observation | Average ± Standard Deviation Estradiol Levels (ng/L) |
|---|---|
| K | 131±4.72[a] |
| P-OVX | 115±7.65[a] |
| P-OVXE | 555±1.51[b] |
| P1 | 172±8.71[a] |
| P2 | 210±1.33[a] |
| P3 | 453±3.63[ab] |

Description: (K) Control group of rats; (P-OVX) Group of ovariectomy rats; (P-OVXE) Group of ovariectomy rats + Estradiol tablet 60µg/200gBW; (P1) Ovariectomy group + red bean extract 35mg/200gBW; (P2) Ovariectomy group + red bean extract 70mg/200gBW; (P3) Ovariectomy group + red bean extract 140mg/200gBW. Different characters suggested significant differences ($p<0.05$)

The results of the One-way ANOVA test on estradiol levels found a significant difference with *p value* = 0.001 ($p<0.05$) so it was concluded that there was an effect of red bean extract on estradiol levels. In contrast to the K, P-OVX, P1, and P2 groups, significant difference was observed when assessing it to the P3 group. Observing the menopausal rats treated with red bean extract, it is noticeable that there is a rise in estradiol levels as the dosage of the extract increases. However, this increase is not statistically significant compared to P-OVX group.

Table 2. Correlation of red bean extract dose with estradiol levels

| Variable | N | R | $R^2$ | Line's Equation | P-value |
|---|---|---|---|---|---|
| Dosage of red bean extract | 12 | 0.817** | 0.668 | 509.73+27.89*Dose | 0.001 |

Description: (**) Correlation is significant at the 0.01 level

A statistically significant correlation ($p<0,001$) was observed between the dosage of red bean extract and estradiol levels in menopausal rats. A single increase in dose will result in an accompanying increase in estradiol levels, as the correlation between two varibles is very strong and in a positive value. The line's equation can adequately explain 66,8% of the variance in estradiol concentration.

### Effect of red bean extract on vaginal mucosal epithelial thickness of menopausal rats

The thickness of vaginal mucosal epithelial in menopausal rats was observed using Hematoxylin-Eosin staining. The measurements were conducted using Leica Aperio ImageScope 12.4.6 software at a magnification of 400X. A total of 10 fields of view were analyzed in all groups. The mean thickness of vaginal mucosal epithelium due to red bean extract administration is shown in Table 3.

Table 3. Mean and standard deviation on vaginal mucosal epithelial thickness

| Group Observation | Average ± Standard Deviation vaginal mucosal epithelial thickness (µm) |
|---|---|

| | |
|---|---|
| K | 78.83±8.53$^{bc}$ |
| P-OVX | 43.83±4.43$^{a}$ |
| P-OVXE | 88.56±3.86$^{c}$ |
| P1 | 66.4±16.31$^{b}$ |
| P2 | 81.22±7.58$^{bc}$ |
| P3 | 85.40±9.88$^{bc}$ |

Description: (K) Control group of rats; (P-OVX) Group of ovariectomy rats; (P-OVXE) Group of ovariectomy rats + Estradiol tablet 60µg/200gBW; (P1) Ovariectomy group + red bean extract 35mg/200gBW; (P2) Ovariectomy group + red bean extract 70mg/200gBW; (P3) Ovariectomy group + red bean extract 140mg/200gBW. Different characters suggested significant differences ($p<0.05$).

Statistically significant differences in the effects of administering red bean extract were identified using the One-way ANOVA test, resulting in a p-value of 0.000 ($p<0.05$). In contrast to groups K, P-OVXE, P1, P2, and P3, the average variability in vaginal mucosal ephitelial thickness was significantly different in the P-OVX group, which had the lowest group average. In comparison to the other groups, P-OVXE has the highest average thickness, which differs significantly. The group that received treatment with red bean extract showed different effects in the P1 group when compared to the P2 and P3 groups. However, no significant differeces were seen in the P2 and P3 groups. With a rise in mean, it is still possible to see the pattern of the epithelium of the vaginal mucosa getting thicker in red bean extract treatment group.

Table 4. Test results of correlation of red bean extract dose with vaginal mucosal epithelial thickness

| Variable Correlation | N | R | $R^2$ | Line's Equation | P-value |
|---|---|---|---|---|---|
| Dosage of red bean extract | 12 | 0.780** | 0.608 | 52.025+0.281*Dose | 0.000 |

Description: (**) Correlation is significant at the 0.01 level

Dosage of red bean extract demonstrated a strong and statistically significant correlation with the thickness of the vaginal mucosal epithelium (p=0.000; R=0.780). The association between the dose of the extract and thickness of the epithelium is positively correlated, suggesting that as the dose increases, the thickness of the epithelium simultaneously rises. The dosage administered can be represented by the line's equation, which accounts for 60,8% of the variability observed in the epithelium thickness of the vaginal mucosa.

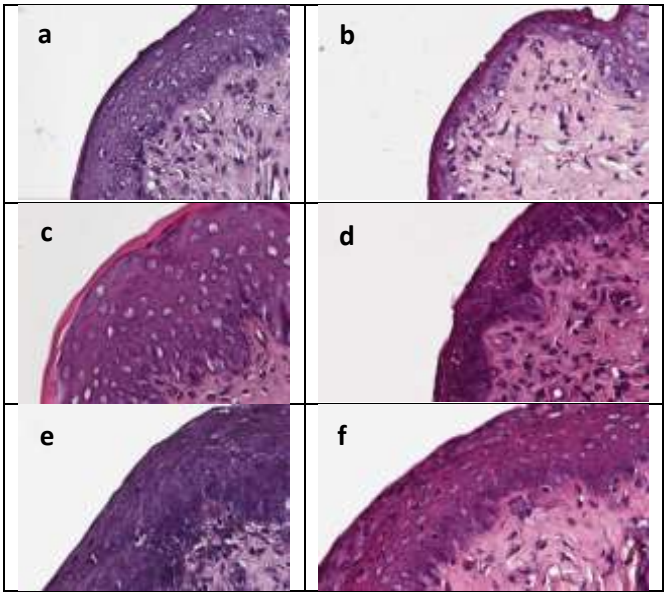


Figure 1. Histopathology of vaginal epithelium

Description: Epithelial tissue of vaginal mucosa performed Hematoxylin-Eosin staining, at 400X magnification. The picture above is a histopathological picture of epithelial cell tissue of the vaginal mucosa of each treatment group. (a) Control groups (78.83±8.53); (b) P-OVX Group (43.83±4.43); (c) P-OVXE Group (88.56±3.86); (d) P1 Group (66.4±16.31); (e) P2 Group (81.22±7.58); (f) P3 Group (85.40±9.88).

The figure above illustrates changes in the vaginal mucosal epthelial thickness of menopausal rats in each treatment group, as determined by the aforementioned figure, which is presented quantitatively. Normal vaginal mucosal epithelial thickness is shown as being greater in thickness compared to thatwhich occurs due to ovariectomy.

**Effect of red bean extract on Endometrial Thickness of menopausal rats**

The thickness of endometrial in menopausal rats was observed using Hematoxylin-Eosin staining. The measurements were conducted using Leica Aperio ImageScope 12.4.6 software at a magnification of 400X. A total of 10 fields of view were analyzed in all groups. The mean thickness of endometrial due to red bean extract administration is shown in Table 5.

Table.5 Mean and standard deviation on Endometrial Thickness

| Group Observation | Average ± Standard Deviation vaginal mucosal epithelial thickness (µm) |
|---|---|
| K | 508.31±31.35$^{b}$ |
| P-OVX | 276.57±75.14$^{a}$ |
| P-OVXE | 316.72±65.61$^{a}$ |
| P1 | 280.54±90.01$^{a}$ |
| P2 | 295.17±65.49$^{a}$ |
| P3 | 385.51±41.44$^{ab}$ |

Description: (K) Control group of rats; (P-OVX) Group of ovariectomy rats; (P-OVXE) Group of ovariectomy rats + Estradiol tablet 60µg/200gBW; (P1) Ovariectomy group + red bean extract 35mg/200gBW; (P2) Ovariectomy group + red bean extract 70mg/200gBW; (P3) Ovariectomy group + red bean extract 140mg/200gBW. Different characters suggested significant differences ($p<0.05$)

With increasing dose of red bean extract, the thickness of the uterine endometriumm in the experimental group demonstrated a tendency to increase. Notably, the P3 group demonstrated the greatest increase in thickness, measuring 385.51±41.44. The thickness is smaller than group K and greater than P-OVXE at 508.31±31.35 and 316.72±65.61. The thinnest endometrial layer is in the P-OVX group at 276.57±75.14. One-way ANOVA test results are p = 0.000, which means the effect of giving red bean extract on the endometrial thickness of menopausal rats. The thickness of the endometrium of group K differs significantly from groups P-OVX, P-OVXE, P1, P2, and P3. While the P-OVX, P-OVXE, P1, P2, and P3 groups did not have significant differences (Table 5).

Table 5. Test results of correlation of red bean extract dose with endometrial thickness

| Variable Correlation | N | R | $R^2$ | Line's Equation | P-value |
|---|---|---|---|---|---|
| Dosage of red bean extract | 12 | 0.553* | 0.306 | 260.091+0.806*Dose | 0.026 |

Description: (**) Correlation is significant at the 0.01 level

The dose of red bean extract and endometrial thickness had a significant relationship (p = 0.026). A moderate relationship was found with a value of R=0.553. An increase in the dose of red bean extract will be followed by an increase in endometrial thickness. The linear line equation represents 30.6% of the variation in endometrial thickness.

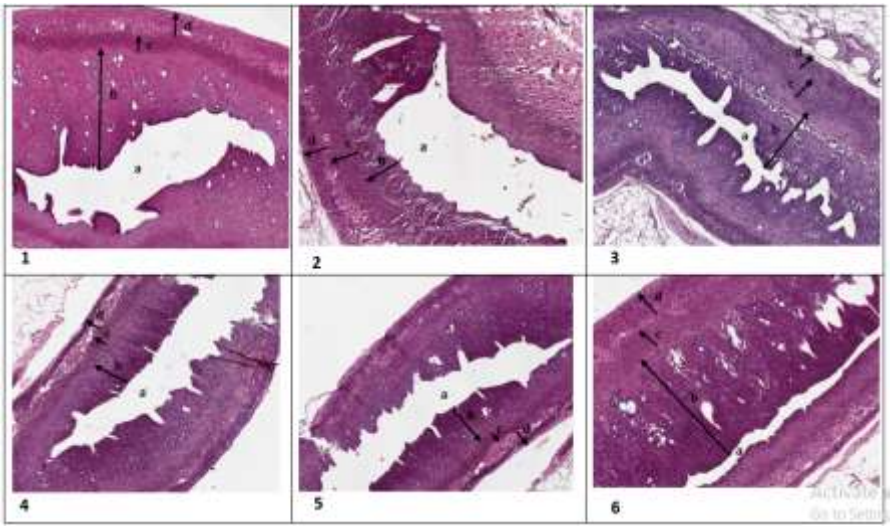

Figure 2. Histopathology of uterine

Description: Endometrial tissue stained with Hematoxylin-Eosin at 400X magnification. The figure above is a histopathological picture of endometrial thickness in each treatment group. (1) Control group (508.31±31.35); (2) P-OVX Group (276.57±75.14); (3) P-OVXE Group (316.72±65.61); (4) P1 Group (280.54±90.01); (5) P2 Group (295.17±65.49); (6) P3 Group (385.51±41.44); a. Lumen of the uterus; b. Endometrium; c. Myometrium; d. Perimetrium.

The figure above shows the thickness of the endometrium in each treatment group. The thickness of the endometrium is calculated from the upper edge of the endometrial epithelium to the myometrial limit. In the ovariectomy group, the thickness of the endometrium was thinner than the control group. In the red bean extract treatment group, endometrial thickness increased compared to the ovariectomy group.

**Effect of red bean extract on the number of femoral bone osteoclast cells in menopausal model rats**

Table 6. Average Data on the Number of Femoral Bone Osteoclasts in Experimental Animals

| Group Observation | Average ± Standard Deviation Number of osteoclast cells (n) |
|---|---|
| K | 3.15±0.70$^{a}$ |
| P-OVX | 5.57±1.52$^{b}$ |
| P-OVXE | 2.75±0.5$^{a}$ |
| P1 | 5.05±1.21$^{ab}$ |
| P2 | 3.1±1.27$^{a}$ |
| P3 | 2.8±0.76$^{a}$ |

Description: (K) Control group of rats; (P-OVX) Group of ovariectomy rats; (P-OVXE) Group of ovariectomy rats + Estradiol tablet 60µg/200gBW; (P1) Ovariectomy group + red bean extract 35mg/200gBW; (P2) Ovariectomy group + red bean extract 70mg/200gBW; (P3) Ovariectomy group + red bean extract 140mg/200gBW. Different characters suggested significant differences ($p<0.05$).

A p-value of 0.03 ($p<0.05$) indicated that red bean extract had a statistically significant effect on the treatment group. Table 5 shows that the P-OVX group had a significantly different number of osteoclast compared to K, P-OVXE, P2, and P3 groups. However, there was no significant difference between the P-OVX and P1 group. P-OVX group showed the highest mean number of osteoclast when compared with another groups. The administration of red bean extract resulted in a reduction in number of osteoclasts in menopausal rats, however the difference was not statistically significant.

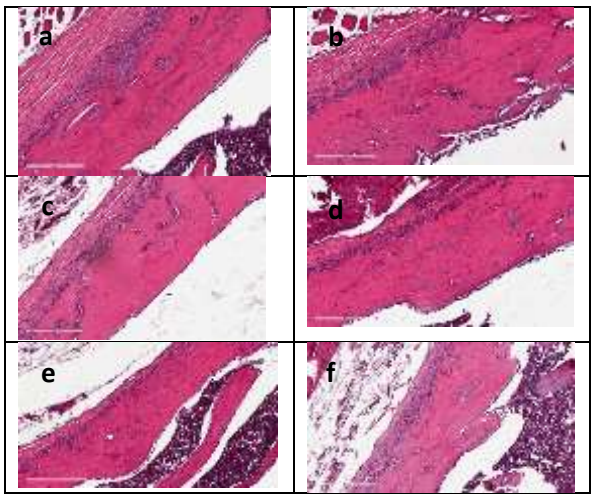


Figure 3. Histopathology of femoral bone tissue

Description: Femoral bone osteoclast cell tissue performed Hematoxylin-Eosin staining at 200X magnification. The picture above is a histopathological picture of femoral bone tissue in each treatment group. (a) Control groups (3.15±0.70); (b) P-OVX Group (5.57±1.52); (c) P-OVXE Group (2.75±0.5); (d) P1 Group (5.05±1.21); (e) P2 Group (3.1±1.27); (f) P3 Group (2.8±0.76).

Table 7. Test results correlation of red bean extract dose with the number of osteoclasts

| Variable Correlation | N | R | $R^2$ | Line's Equation | P-value |
|---|---|---|---|---|---|
| Dosage of red bean extract | 12 | -0.599* | 0.359 | 5.200-0.019*Dose | 0.040 |

Description: (*) Correlation is significant at the 0.05 level

The number of osteoclasts in menopausal rats showed a strong and statistically significant correlatoin with the dosage of red bean extract administered (p=0.040; R=-0.559). The correlation between the two variables is negative, indicating that an escalation in the dosage of red bean extact will lead to a reduction number of osteoclasts in menopausal rats. The equation of the lines shown in table 6, accounts for 35,9%o of the variation in the number of osteoclasts.

## DISCUSSION

### Effect of Red Bean Extract on Estradiol Levels in Menopausal Rats

The results of estradiol levels showed that the highest average estradiol levels were in the P3 group given 140mg/200gBW red bean extract, while the lowest average estradiol levels were in the P-OVX group that was not given any treatment. The results of the One-Way ANOVA test determined that the p-value= 0.001(p>0.05) so that there significant differences in average estradiol levels were concluded, especially in the P-OVX group with groups P1, P2 and P3.

Usually the levels of estradiol produced are highly dependent on the follicles in the ovaries needed for ovulation, if follicular development takes place normally then hormone levels will be produced as well. Conversely, if during the process of follicular development is disrupted, then the follicle becomes aresia which will affect the levels of the hormone estrogen formed (18).

In menopausal women or ovariectomy will result in a decrease in estrogen levels due to loss of ovarian follicle activity. Estrogen hormone consists of 3 types, namely estrone (E1), estradiol (E2) and estriol (E3). Estradiol (E2) is the most dominant estrogen in the perimenopausal period and as the main product of the entire process of estrogen biosynthesis in a woman's life (19). This is in accordance with previous studies showing that there is a significant effect of ovariectomy on reducing estradiol levels in the blood of rats (20)

Red bean extract is one type of legume plant that contains phytoestrogen chemicals (13,16). Phytoestrogens are natural, plant-derived nonsteroidal polyphenolic compounds whose structure and chemical function are similar to the 17 β estradiol (E2) hormones that can interact with ER-α, ER-ß or interact through both (13,19). According to previous research, phytoestrogens such as steroid compounds can turn into estrogen through the aromatase process. Phytoestrogens that enter the body, are metabolized first before entering the tissues. Phytoestrogens of isoflavone compounds are metabolized with the help of glucosidase enzymes into active genisten and daidzein. In the intestine through a complex enzyme system of metabolic processes, the element daidzein is aromatized to equol and O desmethylangiolensin (O-DMA) and genistein to heterocyclic phenol. Equol, O desmethylangiolensin (O-DMA) and genistein become phenol heterocyclics whose structure has similarities with the hormone estrogen so that it is able to bind to estrogen receptors (21,22). Research on the effects of other phytoestrogens was also conducted previously which showed the results of giving red clover extract significantly increased levels of estrogen and progesterone hormones through binding to ER-α and ER-β (23).

**Effect of red bean extract on vaginal mucosal epithelial thickness of menopausal rats**

The results of data analysis of the effect of red bean extract on the vaginal mucosal epithelial thickness are significant in vaginal mucosal epithelial thickness in rats group that given red bean extract at various doses. In the control group, ovariectomy but without red bean extract had the lowest vaginal mucosal epithelial thickness compared to the treatment group given red bean extract. The vagina is one of the female reproductive organs that undergoes changes due to postmenopausal female hypoestrogen. At usual estrogen levels, the vaginal mucosa is lined with fairly thick epithelial, epithelial containing glycogen that are regularly released into the mucosal surface (7,24).

Menopause is characterized by a decline in estrogen levels in women, which can potentially increase the probability of vaginal atrophy. Woman may experience pain during sexual intercourse due to the thinning and decreased flexibility of the vaginal wall, as well as the thinning of the vaginal mucosa (7,8). Ovariectomy in rats leads to decreasing of the vaginal epithelial wall, which is also observed in postmenopausal women. Previous research showed that the thickness of the vaginal mucosal epithelium in ovariectomy rat increased upon estrogen administration compared to ovariectomy rat without any treatment (20). This is in line with this study where the P-OVX group has a lower vaginal mucosal epithelial thickness than the P-OVXE group.

Decreased estrogen binding to ER-α vaginal epithelial tissue occurs due to hypoestrogen conditions during menopause. The absence of mTORC1 induction, which is involved in the regulation of estrogen via the PI3K/Akt pathway, will result in the inhibition of growth factor expression, specifically EGF-like factor, which in turn will prevent the proliferation and differentiation of vaginal cells (25). EGF stimulates cell proliferation to regenerate the mucous epithelial lining and increase the thickness of the vaginal wall. Red bean extract includes flavonoids, especially genistein. In order to stimulate the proliferation of vaginal epithelial cells, genistein is capable of increasing EGF expression. Additionally, this will stimulate a rise in the quantity of cells and the epithelial lining of the vaginal mucosa (26)

Another mechanism that can occur, during menopause, a decrease in estrogen will cause the ER in the tissues to decrease resulting in increased expression of heat shock protein 70 (Hsp70). Hsp70 will allow the ER ligand to be in an inactive state. Flavonoids are able to increase ER expression in tissues so as to reduce cellular stress and decrease Hsp70. This will cause the vaginal mucosal epithelial thickness to re-proliferate (21,23,27).

**Effect of red bean extract on endometrial thickness of menopausal rats**

Based on the results showed that the ovariectomy (OVX) group had the least average endometrial thickness. This explains that the decrease in endometrial thickness during menopause is caused by a decrease in estrogen levels. Estrogen plays an important role in maintaining the growth and proliferation of endometrial cells. As estrogen levels decrease,

estrogen receptor activity (ERα and ERβ) also decreases, resulting in decreased proliferation of endometrial epithelial cells (28)

The P3 group, which received a dosage of 140mg/200gBB of red bean extract (Phaseolus vulgaris L), exhibited the greatest endometrial thickness. This demonstrates that the phytoestrogen content has an impact on the thickness of the endometrium. Giving red bean extract to menopausal rats has a significant effect on increasing endometrial thickness. Previous studies related to the consumption of isoflavones from soybean protein showed no effect on endometrial thickness (29). These results were also supported by a meta-analysis related to phytoestrogen consumption in menopause there was no change in endometrial thickness (30). Menopause will cause a decrease in estrogen receptors and growth factors resulting in changes in the biological environment of the endometrium. Growth factors that play a physiological role in the endometrium are TGF-β1 and IGF-1. An increase in TGF-β1 mRNA and a decrease in IGF-1 occur at menopause, in which the proteins IGF-1 and TGF-β1 have opposite roles. IGF-1 will usually be found in the proliferative phase, while TGF-β1 plays a role in suppressing immunity and inhibiting epithelial cell growth so that high levels of protein will cause endometrial atrophy (31). Estrogen induces IGF-1 secretion through its binding to the ER (32). Previous research on soy protein suggests flavonoids, a type of isoflavone, can increase IGF-1 (33).The increase in endometrial thickness could be caused by flavonoids in red bean extract that have properties able to binding ER and stimulating IGF-1 secretion, which is reuwired for encometrial cell proliferation, according to this study.

An alternative mechanism has been proposed by prior research to elucidate how phytoestrogens can augment the mRNA expression of ERα and ERβ, thereby mediating the multifaceted impacts of estrogen on distinct target tissues. Additionally, phytoestrogens can affect the thickness of the uterine epithelium and the expression of epithelial proliferating cell nuclear antigen (PCNA) (34,35).

**Effect of red bean extract on the number of femoral bone osteoclast cells in menopausal model rats**

Compared to the ovariectomy and untreated (P-OVX) group, the average number of osteoclasts in the control group (K) tended to increase. This phenomenon is hypothesized to occur due to the fact that ovariectomy induces estrogen deficiency, which in turn activates osteoclast activity. Based on the results of previous studies, the bilateral ovariectomy rat group showed a greater number of osteoclasts compared to the control rats group. It is explained that the lack of estrogen in the body can be an inhibitor of the interaction of estrogen with ER-ß in osteoblasts, so that it can inhibit osteoblast progenitors in the secretion of osteoprotogerin (OPG) which is an inhibitory factor of osteoclastogenesis (22,36). Estrogen deficiency conditions can be overcome by giving phytoestrogens. Research shows that a diet rich in phytoestrogens can relieve menopausal symptoms and protect against estrogen-related diseases including osteoporosis (37).

Red bean extract contains phytoestrogens, namely isoflavones which belong to the flavonoid group. Phytoestrogens affect health through *genomic* and *non-genomic mechanisms*. Because of their low molecular weight, phytoestrogens can pass through cell membranes and interact with receptors and enzymes. Genomic mechanisms include extrogenic and antiestrogenic effects on *Estrogen Receptors* (ER). *Nongenomic* mechanisms include angiogenesis inhibition, SHBG excitability, 5α reductase inhibition, 17 β-OH-steroid-dehydrogenase and aromatase enzymes. The estrogenic properties of isoflavones are due to the A-C ring resembling the hydroxyl group in position 5, which is seen to play an important role in increasing estrogen activity (38). A decrease in the quantity of osteoclasts was observed in all treatment groups of this study, indicating that red bean extract demonstrated a preventative effect against osteoporosis. In numerous animal studies that have had ovariectomy, phytoestrogens have been shown to preserve bone, reduce loss of bone cells and increase bone mass (39,40).

## CONCLUSION

In this study, red bean extract can increase estradiol levels, vaginal mucosal epithelial cell thickness, endometrial thickness and reduce the number of osteoclast cells. Reduced estradiol levels, vaginal atrophy, endometrial atrophy and osteoporosis are just a few of the symptoms and health potential risks linkes to menopause that this research suggests red bean extract may be able to hel with safely. However, further research is necessary to support these findings and understand the underlying mechanism of red bean extract's effects.

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