

The Effect of Soy Tempeh Flour Extract On Vagina Histological Structure of Swiss Webster Ovariectomized Mice (*Mus musculus*)

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Abstract—Estrogen deficiency can changes the reproductive system especially in menopausal women, such as a decreasing of smooth muscle contraction, a thinning of vaginal structure, epithelial atrophy and a collagen decreasing. The disorders can be overcome by phytoestrogens treatment. Tempeh is a fermented soy product contains isoflavones that has estrogen-like structure with 17 β -estradiol so that it can be able to bind estrogen receptors. Ovariectomized animal as animal test model has estrogen deficiency. Therefore, the purpose of this study to determine the effect of tempeh soy flour extract on vagina histological structure of Swiss Webster ovariectomized mice. The method used animals tested by treating 45 female Swiss Webster mice that were divided into five groups: negative control group (normal mice had not ovariectomized was not given tempeh flour extract), positive control (ovariectomized mice was not given tempeh flour extract), the treatment group (ovariectomized mice and was given tempeh flour extract D1 (0.21 g/ml / day), D2 (0.42 g/ml/day), and D3 (0.63 g/ml/day). Soy tempeh flour extract was given for 10, 20, and 30 days by gavage. The histological parameter were observed include tunica mucosa epithelium and tunica muscularis thickness of vagina. The result showed that tempeh flour extract increase epithelium thickness and tunica muscularis of the vagina ovariectomized mice. Treatment by taking longer time and giving higher dosage of tempeh flour extract could increase the epithelium thickness of tunica mucosa and tunica muscularis vagina. The treatment of 0.63 g/ml/day dosage during 30 days showed the highest thickness average values of epithelium and tunica muscularis. The epithelium thickness was 33.90 μ m and the tunica muscularis dense was 90.20 μ m.

Keywords—Extract tempeh flour, soybeans, vagina histology, ovariectomy, mice

INTRODUCTION

Reproduction is a physiological process that occurs in living organisms to maintain their offspring. One of determine factor in this process is the balance of reproductive hormones [21]. The important hormones in female reproduction are estrogen and progesterone that are produced by the ovary [14]. Estrogen stimulates cell proliferation in uterine endometrium and myometrium uterus [11] and progesterone function is maintenance the endometrium for embryo development. The endometrium lining the uterus are most responsive area to reproductive hormonal change so that endometrial lining has variable thickness [6] because of estrogen receptor that are found on and myometrial cells [3].

The decline in ovarian function lead to reduced levels of estrogen, causing physiological changes in the reproductive system as seen in menopausal women [5]. This condition will give impact on the uterus development [7]. Disturbances due to menopause can be overcome by administration of phytoestrogens which are naturally occurring compounds from plants [17]. Soybeans contain high concentration of phytoestrogens and isoflavones that have similarity structure with estrogen 17 β - estradiol in mammals [12]. Tempeh as consumer products of fermented soy isoflavone have higher content of isoflavones compared with unfermented soya [13] as much isoflavone compound 77.98mg / 100g dry weigh [1].

Research using natural materials containing isoflavones to increase the levels of estrogen and uterine function after ovariectomy has done by [19] using handeuleum leaf extract that containing isoflavones. The doses were used are 0.1 mg ; 0.5 mg ; and 1 mg, by gavage in mice for 8 weeks provide an increase in weight of the uterus . Other studies claim that mice fed a soy flour and tempeh flour dose 10 g dw/ 100 g bw / day showed an increase in the estrogen hormone [15]. Therefore, it is necessary to study the effects of tempeh flour extract on ovariectomized mice (*Mus musculus*) Swiss Webster strain.

MATERIALS AND METHODS

a. Time and Place

Research consisted of two activity which was conducted from April- June 2015 in Zoology Laboratory, Biology Department, Jember University.

b. Methods

The research was conducted from April to June 2015, in the Laboratory of Zoology, Department of Biology,

Faculty of Mathematics and Natural Science, University of Jember and Integrated Research and Testing Laboratory unit 4 (LPPT4), Gadjah Mada University, Yogyakarta.

Test animals used were ovariectomized female mice Swiss Webster strain 90 days old, \pm 35 gram weight. Mice were kept in plastic cages size of 30 x 20 x 15 cm³. Mice were fed by pellet CP511 as much as 1 per 10 gram body weight per day and drinking water ad libitum. In this study were used 35 female mice Swiss Webster strain that had been ovariectomized and maintained for 35 days for recovery. The mice were divided into two treatments that controls on ovariectomized mice fed a standard pellet and the other group were treated with tempeh flour extract orally (gavage) at a dose of 0.21 grams; 0.42 grams; 0.63 grams given daily for 10 days and 20 days after ovariectomy.

In order to do histological assesment, dissection was done in 11st and 21st days after the last treatment, to take the uterus. Furthermore, the method used for making preparations for paraffin histology method and the uterus is Haemotoxylin Eosin staining. Paraffin method is done by order of fixation, dehydration, clearing, infiltration, embedding, section, affixing and mounting.

Data were analyzed using one way ANOVA test at a level of 95 % or $\alpha = 0.05$ followed by Duncan's Multiple Range Test (DMRT) to know a significancy difference between the treatment group dose, as well as using T test with a level of 95 % or $\alpha = 0.05$, to determine the correlation between long day treatment with dose treatment.

RESULTS AND DISCUSSION

The results of mice uterus weight ovariectomized, after being treated by extracts of tempeh flour can be seen in Table 1. Based on the analysis of One Way Anova significance value on the 10th day $p = 0.00 < 0.05$, and the 20th day $p = 0.00 < 0.05$ showed that there are significancy differences between control and treatment. On day 10 and day 20, it appears the real difference between the negative and positive control.

On the 10th day, there is a significant difference between the positive control group to the treatment group tempe flour extract doses 0.21 g/mL, 0.42 g/mL and 0.63 g/mL, while on the 20th day of the positive control was not significant different from all treatment groups of tempe flour extract dose 0.21 g/mL, 0.42 g/mL and 0.63 g/mL but at a dose of 0.21 g/mL and 0.42 g/mL were not significantly different.

Increasing of uterus weight after tempeh flour extract treatment because of the ability of to increase cell

proliferation in the uterus . Isoflavones are given in ovariectomized rats is able to provide an increase in weight of the uterus [16]. On the negative control and a positive control looks a real difference suspected because of the effect of ovariectomy which causes a decrease in weight of the uterus. Removal of the ovaries causes a decrease in estrogen secretion , resulting in atrophy of the uterus due to inhibition of proliferation of epithelial cells in the uterus [9].

Table 1. The Average of Uterus Weight

Treatment	Uterus Weight (g)	
	Day 10 th ($\bar{x} \pm SD$)	Day 20 th ($\bar{x} \pm SD$)
Negative control	0.127 ± 0.0001 ^d	0.127 ± 0.0001 ^d
Positive control	0.019 ± 0.0001 ^a	0.022 ± 0.0001 ^a
0.21 g/ml	0.025 ± 0.0030 ^b	0.035 ± 0.0038 ^b
0.42 gr/ml	0.032 ± 0.0001 ^c	0.038 ± .0001 ^{bc}
0.63 gr/ml	0.037 ± 0.0001 ^d	0.043 ± 0.0033 ^c

Note : a number followed different letters indicate significant difference between dose at p < 0.05 . Negative controls: without ovariectomy and extract soybean flour; Positive control: ovariectomy without tempeh flour extract

Based on the test T did not seem significant difference in negative control which is normal mice without treatment ovariectomy and without extract powder tempe soybean so that the weight of the uterus does not change, whereas the positive control group and the treatment extract tempeh flour soy at all doses showed an increase in weight of the uterus when viewed from the old administration suspected that the long administration of isoflavones in soybean tempeh flour extract can increase cell proliferation in the uterus. The continuity consumption of isoflavones will result in high plasma isoflavones concentrations [5]. Based on the analysis of One Way Anova significance value day 10 p = 0.051 > 0.05, there is no significant difference between the control and treatment , whereas the 20th day p = 0.001 < 0.05. On the 10th day showed no dose effect of treatment when compared to controls . While on the 20th day is no difference between the positive control with dose 0.21 and 0.42 g/mL.

The observation of the endometrium thickness after tempeh flour extract treatment can be seen in Table 2 . These results suggested that the extract of tempeh flour at all doses for 10 and 20 days gavage have been able to stimulate the endometrium thickness, however there were not significant effect. These results are believed to dose 0.63 for 20 days gavage was high enough so that the isoflavones in soy tempeh flour extract has the potential to supply the back needs endogenous estrogen deficient due to ovariectomy . While the results are not real allegedly because tempeh flour extract doses range that have given were less optimum. Based on endometrial thickness T test did not show any significant difference , but the average is still increasing.

The results of the myometrium thickness in Swiss Webster mice strain after giving extract of tempeh flour can be seen in Table 3. Based on the analysis of One Way Anova significance value on the 10th day p = 0.028 < 0.05, and the 20th day p = 0.02 < 0.05. The results of the analysis of all doses for 10 days treatment showed no difference compare to positive control, but the thickness average of the myometrium tend to increase, whereas for the 20 days treatment, the positive control is a noticeable difference compare to the other doses.

Results One Way Anova analyst at day 10 on the measurement of the myometrium between control and treatment groups had an average value tends to increase although not significantly different.

Table 2. The Average of Endometrium Thickness

Treatment	Endometrium thickness (µm)	
	Day 10 th ($\bar{x} \pm SD$)	Day 20 th ($\bar{x} \pm SD$)
Negative control	478.37 ± 86.55	642.23 ± 142.93 ^c
Positive control	256.07 ± 30.87	232.53 ± 21.98 ^a

0.21 g/mL	282.16 ± 111	324.53 ± 51.06 ^{ab}
0.42 g/mL	288.77 ± 55.09	377.17 ± 34.2 ^{ab}
0.63 g/mL	350.30 ± 104.26	389.46 ± 73.59 ^b

Note: difference between treatment dose at p < 0.05. Negative controls: without ovariectomy and extract tempeh flour; Positive control: ovariectomy without tempeh flour extract

These results are expected because the dose tempeh flour extract containing isoflavones which are used not optimum. Isoflavones also have a structure similar to endogenous estrogen and can cause isoflavones have estrogenic effects but lower affinity to the estrogen receptor than the endogenous estrogen in the body [10] [18]. While the analysis of the 20th day inter-dose treatment showed no significant differences, although the average value increases. It is suspected because of the effect of tempe soybean flour extract that can increase estrogen levels in the body, but the dose used has not shown that the optimum range to increase significantly the thick myometrium. Estrogen causes increased mitotic activity in cells that lead to an increase in the number of smooth muscle layer of the myometrium [14]. Based on the test T of extract of soybean tempeh flour for 10 days and 20 days in the negative control group did not seem difference as a negative control is normal mice without treatment ovariectomy and without giving soybean tempeh flour extract so that the weight of the uterus does not change. Positive controls and soybean tempeh flour extract treatment at all doses showed significant differences allegedly because the old administration of isoflavones in soybean tempeh flour extract can increase muscle cell proliferation in uterine myometrium.

Table 3. The Average of Myometrium Thickness

Treatment	Myometrium Thickness (µm)	
	Day 10 th ($\bar{x} \pm SD$)	Day 20 th ($\bar{x} \pm SD$)
Negative control	83.60 ± 23.68 ^b	82.23 ± 15.02 ^c
Positive control	34.73 ± 1.74 ^a	43.67 ± 1.98 ^c
0.21 g/mL	44.26 ± 2.64 ^a	59.73 ± 6.06 ^b
0.42 g/mL	54.13 ± 15.48 ^a	61.50 ± 2.65 ^b
0.63 g/mL	56.30 ± 19.05 ^{ab}	71.30 ± 16.04 ^b

Note: difference between treatment dose at p < 0.05. Negative controls: without ovariectomy and extract tempeh flour; Positive control: ovariectomy without tempeh flour extract

Outcome of this study indicate that the weight of the uterus , the endometrium and myometrium thickness is affected because of the presence of estrogen. Estrogen works in the uterus to increase the mass of the endometrium and myometrium through the proliferation and growth of the epithelial cells of the endometrium and myometrium muscle cells [8].

Ovariectomized causes a deficiency of estrogen in the body because of the decline of ovarium function. Furthermore the epithelial cell proliferation and growth of the endometrium and myometrium muscle cells is inhibited. To restore the availability of estrogen, do tempe soybean flour extract containing isoflavones, plant compounds that are structurally similar to estrogen 17β-estradiol in mammals and can bind to estrogen receptors [12]. Effect of estrogen on the cells began after the binding of estrogen to the receptor in the cytosol. Estrogen receptor complex and subsequently diffuses into the cell nucleus and attach to DNA. Complex bond with estrogen-receptor induces DNA synthesis and mRNA expression in the form of protein synthesis thereby increasing the activity of target cells, as indicated by cell proliferation [4]. Increased thickness of the myometrium probably derived from estrogen binding to estrogen receptors (ERα) in the DNA binding site in the stromal cells of the myometrium, the bond resulting in transcriptional activity resulting in the synthesis of proteins that trigger mitotic activity in cells [2]. Estrogen increases the number and smooth muscle contractile protein levels [7].

The extract soybean tempeh flour at all doses and duration of administration can increase the average

weight of the uterus, endometrial thickness and thick myometrium. Dose 0.63 g/mL of the extract for 20 days showed the highest result compared with the other treatment groups, but these dose has not been able to recover the uterus histology. This was allegedly because of their low isoflavones bind to the receptor. Isoflavones have a low affinity to the estrogen receptor than the endogenous estrogen so that the required amount of phytoestrogen more to obtain endogenous estrogen-like effects [20].

CONCLUSION

Soybean flour extract at dose 0.63 g/ml/day for 20 days treatment was the most influential to the increase in weight of the uterus, endometrial thickness and thick myometrium compared with the treatment for 10 days. There were an average increasing in weight of the uterus, endometrial and myometrium thickness.

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