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Short Communication:
Effects of black soy phytoestrogens (*Glycine soja*) on elevated levels of estradiol in rat blood (*Rattus norvegicus*) ovariectomy

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Abstract. *Yuliawati D, Astuti WW, Yuniarti F. 2020. Effects of Black Soy phytoestrogens (*Glycine soja*) on elevated levels of estradiol in Rat Blood (*Rattus norvegicus*) ovariectomy. Nusantara Bioscience 12: 55-58.* Menopause is the transition from productive to non-productive times due to reduced estrogen and progesterone and has an impact on improving cardiovascular problems. The fulfillment of estrogen needs in menopause can use a type of phytoestrogens from black soybeans. The purpose of research is to prove the administration of black soy extract (*Glycine soja*) can increase the levels of estradiol in the rat blood (*Rattus norvegicus*) ovariectomy. Design research uses post-test only control group design. Twenty-five female rats were randomly divided into 5 groups (n = 5 tails per group), negative controls, positive and 3 treatments. Ovariectomy was performed in a group of positive controls and treatment. Thirty-day post ovariectomy rats were given appropriate group treatment (negative control, positive, and treatment with the administration of black soy extract dose of 50, 100, 150 mg/200 g/day for 30 days). The estradiol test used the Enzyme-linked immunosorbent assay (ELISA). Anova's test results with a significant degree of $\alpha = 0.05$ established that the p-value of 0.000 was less than $\alpha = 0.05$, meaning there was a significant effect of giving black soy extract on increased levels of estradiol in the rat blood ovariectomy. Post - Hoc Tukey testing shows increased the highest estradiol levels in the dose treatment group 100 mg/200 g/day. This research proves that the administration of black soy extract (*Glycine soja*) can increase the levels of estradiol in the rat blood (*Rattus norvegicus*) ovariectomy.

Keywords: Black soy extract, blood, estradiol, *Glycine soja,* ovariectomy, *Rattus norvegicus*

INTRODUCTION

Menopause which is the permanent cessation of menstruation with a minimum of 12 months is considered as the beginning of the aging process of women, characterized by some symptoms of menopause and associated with several diseases pathogenesis (Peacock and Ketvertis 2018). Some diseases experienced at the time of menopause are associated with decreased levels of estrogen. Epidemiological data suggest that female estrogens in the reproductive period provide significant protection in the cardiovascular and reproductive systems, bone organs, liver and brain, where the expression of estrogen receptors (RE-α) and β estrogen receptors (RE-β) are at varying levels (Cervellati and Bergamini 2016). In this time of menopause, there is a substantial increase in cardiovascular problems (CVD) that indicates reduced estrogen at the time of menopause which is likely the main cause of the increased risk of CVD (Ludgero-Correia et al. 2011). Epidemiological data shows a protective effect on cardiovascular disease and breast cancer in the East Asian population that makes soybeans their dominant diet (A Gil-Izquierdo et al. 2012; Chen et al. 2014).

Black soy (*Glycine soja*) which is a type of soybean plant is grouped into phytoestrogens because it is shown to contain isoflavones and anthocyanins. Isoflavones in black soybeans contain genistein which is similar to 17 β estradiol in its chemical structure, allowing genistein to bind to estrogen receptors and compete with endogenous estrogens. In a state of low endogenous estrogen (menopausal women, after ovariectomy, etc.), the estrogenic isoflavone estrogens that will occur as a weak substitute for estrogen so that the activity of estrogen isoflavones may increase (Pilsakova et al. 2010). Anthocyanins in black soybeans show its activity as phytoestrogens because it has a similar structure to estrogen which allows it to be bonded to estrogen receptors even if the density is very low compared to Endogenous estrogen (Chalopin et al. 2010).

The study of Yulia et al. (2015) suggests that genistein isoflavone was detected in the black soybean extract of var. Detam II with diethyl ether. While the research is Koh et al (2014) which identifies the anthocyanin pigment on black soy (*Glycine max* (L). Merr) Varieties of Tawonkong (TW) and Geomjeongkong-2 (G2) show the results that the most dominant anthocyanin is Cyanidin-3-O-β-D-glucoside (C3G) and G2 has a higher total rate of anthocyanins than TW.

Some studies have shown an influence on the introduction of phytoestrogens on estradiol levels in the blood. One of the research of Abbasabadi and Mina (2016) suggests that soy milk that is a processed product from soybeans is able to increase the serum levels of 17-β estradiol significantly in the treatment group of rats ovariectomy given milk soy compared to control groups of rat ovariectomy are not given soy milk. Based on this, researchers want to prove the administration of black soy extract (*Glycine soja*) can increase the levels of estradiol in the blood of rats (*Rattus norvegicus*) ovariectomy.

MATERIALS AND METHODS

Animals

The study used 25 healthy female rats (*Rattus norvegicus*), aged 2-3 months, had a bodyweight of 200-400 grams in the laboratory and analysis of Bioscience of Brawijaya University, Malang. The rats were left in a normal state without treatment (adaptation period) for 7 days, and then rat randomization was performed. The rats were divided into 5 groups (n = 5 tails per group) namely the negative control group (without any treatment), positive control (OVX), 3 treatment groups (OVX + black soy extract at a dose of 50, 100.150 mg/200 g/day). Furthermore, Ovariectomy was performed on a group of positive controls and treatment groups. Thirty-day post ovariectomy rats were given appropriate group treatment (negative control, positive control, and group treatment with black soy extract for 30 days).

Extraction of black soy (Glycine soja)

Black soy (*Glycine soja*) is obtained from the research Hall of various nuts and bulbs (Balitkabi), Malang, East Java, Indonesia. The black soybeans are dried first in the oven at 50-70ºC. The dried material is crushed with a blender to obtain black soy powder. In the initial extraction process, the black soy powder is soaked with ethanol 96% as much as 900 ml and shaken until thoroughly mixed. The Immersion process is done for 3 days. The bath result is inserted in the evaporation flask 1 L. The ethanol solution is left to evaporate in the evaporated flask and left to stop dripping on the passenger flask (± 3 hours for 1 pumpkin). The extraction result is inserted into the glass bottle and stored in the freezer.

Ovariectomy

Anesthesia rats before ovariectomy uses Ketamine 120mg/kg, and Xylazine 20mg/kg. The transabdominal incision is performed approximately 1.5-2 cm above the uterus. The uterus sought and after it is found fastened cornu utero until the left ovarian oviduct. The oviduct and left ovarian are found free from the fatty tissues and connective tissues surrounding it. Furthermore, the oviduct of distal and ovarian parts are ligated and removed. Betadine solution is given to scar cuts. The procedure ovariectomy for the right ovary is the same as the left ovarian. Incision wound is closed and stitched layer after layer, then wound smeared Betadine. Post ovariectomy recovery time is 30 days.

Analysis of estradiol levels

Blood sampling in all groups (negative, positive control and treatment) is performed 30 days after administration of black soy extract. The blood samples are obtained and then centrifuged to obtain a clear filtrate (blood plasma). The estradiol test on the blood plasma of rats is performed through the method of ELISA (Enzyme-Linked Immunosorbent Assay) with pg/ml units.

Statistical Analysis

Data is displayed in average form ± SD. A Comparative test with One Way Anova is followed by Post-Hoc Tukey (LSD 5%) if the result is significant (p-value of < 0.05). Data analysis is assisted by program SPSS 23 for Windows.

Ethics

All research procedures are expressed by the Research Ethics Commission (Animal Care and Use Committee) of Universitas Brawijaya, Malang.

RESULTS AND DISCUSSION

Overview of estradiol levels in rat blood ovariectomy

The results of estradiol test on the blood plasma of rats through ELISA method are shown in Table 1. Based on this table, the highest increased average levels of estradiol in the treatment group after administration of black soy extract namely in the treatment group 2 (P2) is a dose of 100 mg/200 g/day

Effect of administering black soy extract to elevated levels of estradiol in rat blood ovariectomy

The results of the analysis with Anova show a P-value of 0.000, smaller than α = 0.05 which means there is a significant effect of administering the black soy extract (*Glycine soja*) to increased expression of estradiol levels in the blood of rat ovariectomy Or in other words, there is a significant difference in the concentration of estradiol in the blood of rats ovariectomy due to the administration of black soy extract (*Glycine soja*) with different doses. Subsequently, Post-Hoc Tukey testing is conducted to determine which group had a significant difference in estradiol levels. The Post-Hoc Tukey test results can be seen in Table 2.

Table 1. Overview of estradiol levels in rat blood

Group	Mean	SD	Min	Max
Negative control (K-)	12.300	2.865	10.000	17.250
Positive control (K+)	7.284	2.243	5.208	10.590
P1 (50mg/200 g/day)	9.175	2.282	6.500	11.875
P2 (100mg/200 g/day)	12.346	1.643	10.610	14.250
P3 (150mg/200 g/day)	12.081	1.037	10.559	13.227

Table 2. The Post Hoc Tukey Test Results

Group	N	Subset *for alpha*=0,05	
		1	2
Positive control (K+)	5	262.68420	
P1 (50mg/200 g/day)	5	322.44620	
Negative control (K-)	5	337.91040	
P3 (150mg/200 g/day)	5		442.24400
P2 (100mg/200 g/day)	5		485.39480

Based on the results of the test analysis Tukey 5% shown in table 2 above, the comparison of estradiol levels in the positive control group (K +) with the treatment group, that increased levels of estradiol are significantly shown in the treatment group, P2 dose of 100 mg/200 g/day and P3 dose of 150 mg/200 g/day. It is shown from the average value of ± SD group treatment P2 and P3 higher and is on a different subset with a positive control group (K +).

Discussion

Results of the test analysis Tukey 5% show that the comparison of estradiol levels in the positive control group (K +) with the treatment group, that increased levels of estradiol are significantly shown in the treatment group, P2 dose of 100 mg/200 g/day and P3 dose of 150 mg/200 g/day.

Menopause which is a critical period in the life of women is characterized by the decline of ovarian hormone production due to age (Ludgero-Correia et al. 2011). In post-menopausal women who experience decreased ovarian function to produce estrogen, peripheral aromatase activity becomes the main source of estradiol synthesis. Some studies have also shown that aromatase activity is increasing as it grows older (Purohit and Reed 2002). This aromatase enzyme is widely distributed both in the tissue of the gonads and out of the gonads, as is the case: bones, brain, adipose tissue and blood vessels (Bulun et al. 2003). The existence of aromatase in coronary endothelial means that the conversion of androgens into estradiol at the local heart may affect the function and structure of coronary endothelial modeling (Jazbutyte et al. 2012).

The fulfillment of estrogen needs in menopausal women (hypoestrogens) can use black soy (*Glycine soja*) caused by black soy that contains isoflavones and anthocyanins which show its activity as phytoestrogens. Isoflavones in black soybeans contain genistein which is similar to 17 β estradiol in its chemical structure allowing genistein to bind to estrogen receptors and compete with endogenous estrogens. Genistein affinity from RE-β 87% or approximately 20-30 times higher than the RE-α which is 4% in proportion and comparable to the affinity of 17 β-estradiol. Interactions between genistein with estrogen receptors lead to an activation called estrogen receptor element (ERE), which is a genomic mechanism line, particularly the transcription process (Pilsakova 2010; Kim and Park 2012). Anthocyanins show its activity as phytoestrogens because it has a structure similar to estrogen, making it possible to bind to estrogen receptors even if the severity is very low compared to endogenous estrogens (Chalopin et al. 2010).

With regard to the above, interactions between genistein and anthocyanins in black soy (*Glycine soja*) with estrogen receptors can lead to the activation of estrogen receptor element (ERE) for gene transcription process, one of which is the aromatase enzyme in The aortic remembers in the tissues of the blood vessels both men and women express the re-α and re-β (Mendelsohn and Karas 2005). It is known that the aromatase enzyme plays a role in androgen conversion into important estradiol in cardiovascular protection. The Villablanca et al. study (2013) indicates that the aortic endothelial cell of the male is generating a lot of estradiol through the conversion of testosterone by aromatase and the regulation of this process is mediated by the primary estrogen receptor RE-α. It supports the results of the study that administering black soy extract (*Glycine soja*) may increase the levels of estradiol in rat blood which is likely that estradiol is produced from the aromatase activity due to the primary source of estradiol i.e. the ovaries already Is missing from the results of ovariectomy and the regulation of the process is mediated by estrogen receptors.

In conclusion, the administration of black soy extract (*Glycine soja*) may increase levels of estradiol in the Rat blood (*Rattus norvegicus*) ovariectomy, wherein increased levels of estradiol are significantly indicated in the treatment group, P2 dose of 100 mg/200 g/day and P3 dose of 150 mg/200 g/day

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