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Effect of Red Bean Extract (Phaseolus vulgaris, L) on the Development of Female Mice Eggs (Mus musculus) Balb/C strains

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Abstract: Research was conducted to study the effect of red bean extract (*Phaseolus vulgaris*, L) on the development of mouse egg follicles (*Mus musculus*) This study used a completely randomized design consisting of 4 treatment groups. Each treatment group used 5 female mice aged 2 months weighing 18-22 grams. The research parameters were the number of egg follicles at various stages of development which were observed from the histological incision of the ovarium. The data obtained were analyzed by Two Way Variance Analysis and DMRT Test. The results showed that the treatment of red bean extract had a significant effect on the development of egg follicles in mice with balb/c strains.

Keywords: *Phaseolus vulgaris,* L; Filokel; Mus musculus.

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Introduction

Egg maturation is regulated by hormones secreted by the anterior pituitary such as FSH and LH (Irianto, 2014). FSH functions to trigger the development of ovarian follicles. Estrogen formed by egg follicles can stimulate the growth of the uterine endometrial wall, inhibit FSH secretion, and stimulate LH (Luteinizing Hormone) secretion. LH surges cause ovulation (Ganong, 2014). If the egg is not fertilized, estrogen production will stop. This causes low blood estrogen levels, so LH production also falls.

Increased levels of FSH causes granulosa cells that wrap the egg cells to proliferate so that the number of layers of granulosa cells increases. FSH also stimulates granulosa cells to synthesize and secrete estrogen. Human estrogen stimulates the growth of the egg so that the egg is mature and ready for ovulation.

The process of egg maturation takes place in cycles during the reproductive phase. Towards menopause, the cycle begins to become irregular due to hormonal changes, such as reduced estrogen production which can cause a shortened follicular phase. This phenomenon is known as climacteric. Loss of ovarian function will gradually reduce its ability to respond to pituitary stimulation in synthesizing steroid hormones (Bellardin et al., 2014).

Some non-steroidal substances and compounds contained in plants have estrogenic activity. Phytoestrogens are compounds from plants whose structure is similar to the structure of estrogen so that it can show the agonist nature of Estrogen Receptors (ER). The ability of phytoestrogens to mimic the effects of estrogen is based on the presence of compounds with molecular weights equivalent to molecular weights of estrogens (272 g/mol), phenolic rings as binding sites and has a nucleus with two hydroxyl groups with a distance of 11.0-11.5Å (Mostrom and Evans, 2012). According to Biben (2012), phytoestrogens are compounds contained in a group of plants, both grains, nuts, vegetables, and fruits. Phytoestrogen compounds contained in plants include flavonoids, coumestants, lignans, and stilbene. One

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type of plant that contains phytoestrogens is kidney beans.

According to Ganong (2014), phytoestrogens can affect the ovarian cycle such as the growth and development of egg follicles. Nevertheless, the use of phytoestrogens in the world of health according to Biben (2012) and Irianto (2014) still raises many pros and cons. Opinions that still cause counter one of them is the benefits of phytoestrogens in the ovarian cycle. Hernawati (2014) said that 14 women given 52 mg of phytoestrogens per day for a month did not cause changes in levels of estradiol, estron, progesterone, FSH, or LH. This raises doubts about the benefits of phytoestrogens on the ovarian cycle. Therefore this study was conducted to determine the effect of giving red bean extract which is thought to contain phytoestrogens on the growth and development of egg follicles using mice as experimental animals.

Method

This research is an experimental laboratory using a completely randomized design (CRD) with factorial patterns. The first factor is the dose of red bean extract and the second factor is the duration of treatment. There were 4 doses of red bean extract that were tried namely 0 mg/kg bw, 37 mg/kg bw, 50 mg/kg bw, and 75mg/kg bw. The treatments are given for 15 or 30 consecutive days, respectively, so that 8 treatment combinations are obtained. Each treatment combination used 5 mice so that in this study 40 mice were used.

Results and Discussion

The results of research on the effect of giving red bean extract (Phaseolus vulgaris, L.) to the development of the egg follicles of test animals namely mice (Mus musculus) were taken based on data on the number of egg follicles obtained from observations and counting of egg follicles in histological preparations of ovarian mice (Mus musculus) microscopically at 100x magnification, using the Hematoxylin-Eosin (HE) staining technique.

Figure 1. Histological Preparations of 40 x 60 Mice Enlarged Ovaries.

Figure 1 shows the various growth phases in test animal egg follicles. These follicles include primordial follicles, primary follicles, secondary follicles, tertiary follicles, de Graff follicles, and corpus luteum. Primary follicles are characterized by a single layer of granulosa cells. The size of the primary follicle is usually the smallest of the other follicular types. Primary follicles are almost the same as secondary follicles, the thing that distinguishes is characterized by the presence of two or more layers of granulosa cells.

Tertiary follicles are a stage of further development of secondary follicles, which distinguishes between tertiary and secondary follicles which are characterized by the presence of a gap that has been filled with follicular fluid on both sides of the oocyte, this part is called the antrum.

The de Graff follicle is characterized by a gap that is already filled with follicular fluid which is much larger than the tertiary follicles and oocytes located on the edge of the follicle which is connected by several granulose cells called corona radiata. In addition, the number of granulosa cells surrounding the ovum is getting smaller. The size of the de Graff follicle is usually large, so it can be more easily observed.

The corpus luteum, a follicular space will be filled with blood and splenic fluid after ovulation. Usually large and red.

Data from the calculation of the average number of egg follicles of test animals, observed based on each type of follicle, by counting the total number of follicles contained in the ovaries of test animals. The data in figure 2 shows the average number of egg follicles.

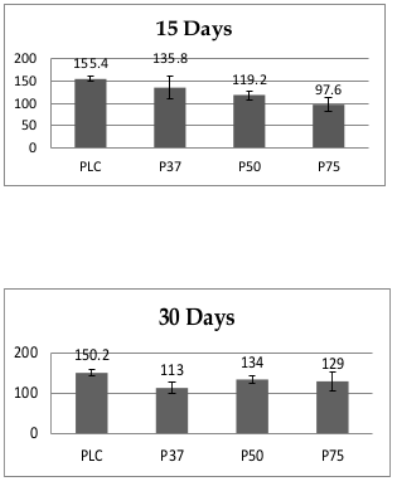
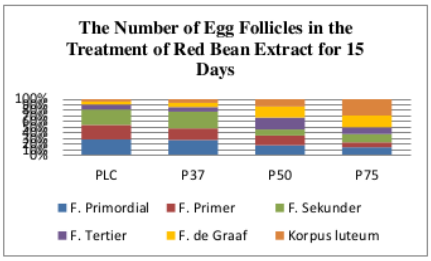


Figure 2. The average number of egg follicles of test animals treated for 15 days and 30 days.

The results showed a difference in the average number of egg follicles of test animals treated with red bean extract for 15 days. The PLC group had more egg follicles on average compared to the group treated with red bean extract (Figure 2). The average egg follicles of test animals also decreased consecutively as the higher dose of red bean extract was given. This shows that the treatment of red bean extract decreased the average number of egg follicles of test animals.

The same phenomenon also occurs in the treatment of red bean extract which was given for 30 days. PLC has the most average number of follicles compared to P37, P50, and P75 treatments (Figure 3). The average number of egg follicles is at least in P37, while for P50 and P75 have an average egg follicle that is not much different.



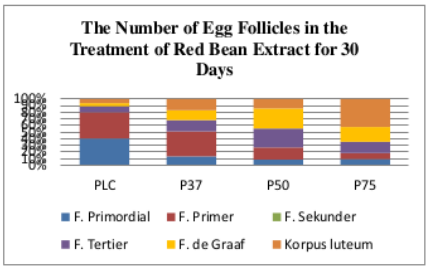


Figure 3. Composition of mouse ovary follicles (Mus musculus) per visual field unit (1.83 x 106µm2).

The results number of egg follicles showed that the test of animals treatment with red bean extract had a higher percentage of follicular stages (de Graff and corpus luteum) than PLC on day 15 (Figure 2). The PLC group had a final follicular percentage of only 10%, whereas in P37, P50, and P75 the percentage of end-stage follicles was respectively 15, 28, and 50%. The higher concentration of red bean exract that given to test animals are the higher percentage of follicles in this final stages.

The same phenomenon also occurs in the treatment of red bean extract given to test animals for 30 days. The percentage of end-stage follicles in the PLC is 11%. The percentage is much smaller compared to P37 by 32%, P50 by 45%, and P75 by 65%. Large variations in the percentage of end-stage follicles in each treatment also showed that the higher the dose of red bean extract given, the higher the percentage of the number of follicles in the final stage of the test animals. It also shows the difference in the percentage of end-stage follicles in the duration of administration of red bean extract. Test animals that were given red bean extract treatment for 15 days had a smaller percentage of the final follicle compared to red bean extract treatment for 30 days. Red bean extract treatment for 15 days 5 times accelerates follicular development compared to PLC. Red bean extract treatment for 30 days 6 times accelerates follicular development. This shows that the higher the concentration of red beans, the faster the development of follicles in test animals. Regression test results show red bean extract influences the development of the final stage follicles. This can be seen in Figures 4 and 5.

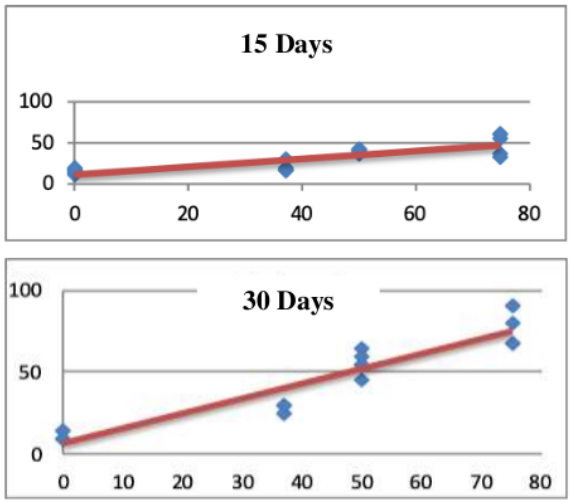


Figure 4. Graphic Effect of red bean extract on final stage follicles.

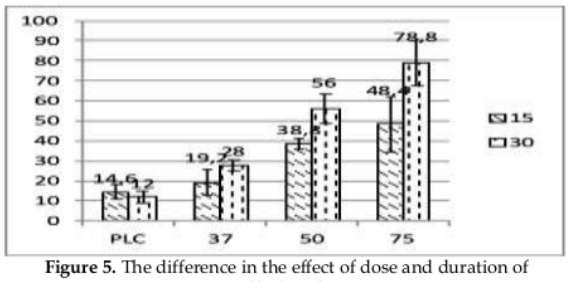


Figure 5. The difference in the effect of dose and duration of treatment of kidney bean extract.

The results of the analysis of variance showed that red bean extract treatment significantly affected the total number of egg follicles in the number of primordial follicles, the number of tertiary follicles, the number of deGraff follicles, and the number of corpus luteum.

After the DMRT test results were obtained that the largest number of follicles were found in the P3 treatment (30). Although this number is not significantly different from P2 (30), it is significantly different from the total number of egg follicles in other treatments.

Statistical analysis showed that all treatment combinations except P1 (15) treatment were significantly different from PLC (30) (control). This means that red bean extract treatment generally causes a decrease in the number of primordial follicles, the number of primary follicles, and the number of secondary follicles. The decline is probably caused by the phytoestrogen effect contained in the red bean extract used as a treatment in this study.

According to Mastuti (2012), red bean extract contains isoflavone-type phytoestrogen compounds. According to the USDA (2015), the isoflavone content in red beans consists of genistein, daidzein, and coumestan. While in the digestive tract, isoflavones are converted enzymatically to heterocyclic phenols. The structure of heterocyclic phenols is similar to estrogen (Mostrom and Evans, 2012). Genistein also undergoes metabolism in the gastrointestinal tract into a component of pethylphenol, while daidzein is also converted enzymatically to equol, dihydrodaidzein, and O desmethylangiolensin (O-DMA) (Narozna et al, 2017). According to Mostrom and Evans (2012), genistein and daidzein are isoflavone compounds whose estrogenic activity is higher than other isoflavone compounds. Estrogenic activity is related to the structure of isoflavones which can be transformed into equol, wherein equol has a phenolic structure similar to the hormone estrogen. So that it can increase the development of mouse egg follicles.

Tertiary follicles are a stage of further development of secondary follicles. The difference between tertiary and secondary follicles, that is, tertiary follicles is characterized by the presence of a gap that is already filled with follicular fluid which makes it larger. Usually, the gap that contains the follicular fluid is divided into two parts separated by the ovum. This is what distinguishes tertiary follicles from de Graff follicles.

FSH secreted by the anterior pituitary is transported to the ovaries and binds to receptors on the plasma membrane. The binding between the FSH and the receptor will allow the receptor to be attached to the protein G stimulator which stimulates the enzyme adenylyl cyclase. Adenylyl cyclase is an enzyme that is bound to the membrane, by protein G will catalyze the conversion of small amounts of cytoplasmic adenosine triphosphate (ATP) into cAMP in cells (Ganong, 2014). Protein kinases that bind to cAMP are then activated which phosphorylate specific proteins in the follicle.

Increased levels of cAMP trigger estrogen secretion in egg follicles by stimulating the activity of the aromatase enzyme in cells that catalyzes estrogen synthesis. Primordial follicular granulosa cells begin to form estrogen receptors that bind to estrogen to form Estrogen Receptor Element (ERE). The ERE and estrogen complex diffuses into the nucleus and attaches to the promoter portion of specific DNA strands, thereby affecting the process of protein synthesis that occurs in secondary follicles.

Phytoestrogens are thought to cause faster follicular growth by affecting FSH secretion and estrogen secretion from the ovaries. High concentrations of the two hormones cause an increase in the speed of development of tertiary follicles. The higher the dose is given the higher isoflavone levels in the body of the test animal. Isoflavones are estrogen agonists by helping estrogen to bind to their receptors so that a high dose of red bean extract will accelerate the development of egg follicles (Mastuti, 2012).

The de Graff follicle is characterized by a gap that is already filled with follicular fluid which is much larger than a tertiary follicle, while the ovum is pushed to the edge of the follicle which is connected with several granulosa cells. In addition, the number of granulosa cells surrounding the ovum is getting smaller. These cells are called corona radiata. The size of the de Graff follicle is usually very large.

Increased levels of estrogen in the follicles and increased levels of LH cause the proliferation of follicular theca cells and their secretions. This causes the diameter of the follicle to increase so that there is an increase in mass up to a thousandfold compared to the diameter of the primordial follicle. When the follicle enlarges, the ovum itself remains embedded in the mass of granulosa cells located in the follicular valve.

Phytoestrogens cause follicular maturation to accelerate by affecting the speed of estrogen secretion. High concentrations of these hormones provide positive feedback on LH secretion so that the maturation of the egg follicles becomes rapid. The higher the dose given, the higher isoflavone levels in the body of mice. Phytoestrogens are agonists against estrogens by helping estrogens to bind to their receptors, so high doses of red bean extract will accelerate the maturation of egg follicles.

According to Sirotkin (2014) isoflavones whose action resembles endogenous estrogen is likely to provide a positive feedback effect on LH secretion, so

that LH levels increase. According to Cederroth, et al (2012) this spurred the synthesis and secretion of progesterone. The external sensitivity begins to release proteolytic enzymes from lysosomes. This enzyme results in the dissolution of the follicular capsule wall so that the wall weakens and causes more swelling of the entire follicle and stigma degeneration will simultaneously occur the growth of new blood vessels that take place quickly into the follicular wall, and at the same time prostaglandins (hormones that cause vasodilation) will be secreted into follicular tissue. Both of these effects will result in plasma transudation into the follicle which plays a role in follicular swelling. Finally, the combination of follicular swelling and stigma degeneration results in follicular rupture accompanied by ova removal.

Ovulation follicles will leave granulosa and internal theca cells which quickly turn into lutein cells. This cell diameter enlarges twice or more and is filled with lipid inclusions which give a yellowish appearance. This process is called luteinization, and all the mass of cells together is called the corpus luteum (Cederroth et al, 2012).

Based on the above discussion, phytoestrogens affect ovulation and the development of the corpus luteum, so high levels of phytoestrogens in the ovary will have a significant effect on the ovulation process. Egg follicles that have matured will experience an increase in the speed of ovulation so that it has an impact on increasing the number of corpus luteum as well. This is consistent with the data in this study.

Conclusions

The treatment of red bean extracts significantly affected the speed of the development of mouse egg follicles. The higher the treatment dose the greater the effect on the speed of egg follicle development. The duration of red bean extract gave a significant effect on the development of mouse egg follicles. The longer the treatment is given a greater effect on the development of egg follicles. There is an interaction between dose and duration of treatment of red bean extract on the speed of egg development.

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