# Genistein Affects Estrogen Receptor Alpha (ER-α)/Estrogen Receptor Beta (ER-β) Ratio, and Nuclear Factor-Kappa Beta (NF-κβ) in Mice Model of Endometriosis

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## ABSTRACT

**Background: Currently, treatment of endometriosis remains expensive. One of low-cost treatments for this disease is genistein. This study aimed to assess the effect of genistein on Estrogen Receptor Alpha (ER-α) and Estrogen Receptor Beta (ER-β) ratio and Nuclear Factor- Kappa Beta (NF-κβ) in mice model of endometriosis remain unclear.**

**Methods: This study is experimental using post-test research design. Twenty-four female mice (Mus musculus) model of endometriosis were fed genistein. The mice were divided into four groups with various doses of genistein, i.e., 1,30 mg/mice/day (group P1); 1,95 mg/mice/day (group P2); 2,60 mg/mice/day (P3), and; 3,25 mg/mouse/day (group P4). We fed the mouse using a sonde for 14 days. On the fifth day, the mice were sacrificed and dissected to take the peritoneal tissue. The tissue was coloured using Immunohistochemistry staining (IH) and microscopically assessed at 400x magnification calculated at 1000 cells in order to observe the expression of RE-α, RE-β, and NFkB.**

**Results: Genistein at various doses had a significant effect on the RE-α / RE-β ratio. The higher the genistein dose given, the lower the RE-α / RE-β ratio. In addition, genistein dose of 3.25 mg / day significantly reduced NFkB expression.**

**Conclusions: Genistein in various doses have been shown to significantly influence the RE-α / RE-β ratio. In other words, the higher the dose of genistein given will decrease the RE-α / RE-β ratio.**

**Keywords: Endometriosis, Genistein, Estrogen receptor-α, Estrogen receptor-β, Nuclear factor-κβ, Mice model of endometriosis**

## INTRODUCTION

Currently, endometriosis is one of the main issues in reproductive health as the incidence is quite high[1]. Endometriosis affected 6-10% of women of reproductive age from all ethnic and social groups[2]. Endometriosis and the etiopathogenesis of this disease have been extensively investigated and expressed in various theories ranging from clinical to biomolecular theories[2,3].

Estrogen is an inflammatory inductor and triggers a cascade of inflammatory reactions. Estrogen binds to the estrogen receptor α (RE-α) and estrogen receptor β (RE-β) and the dominant bond determines its final effect. RE-α is stimulatory and RE-β will inhibit RE-α. The RE-α / RE-β ratio determines the estrogen / SERM effect of being estrogenic or antiestrogenic. Estrogen triggers the activity of proinflammatory cytokines such as Tumor Necrosis Factor - α (TNF-α), Interleukin Iβ (IL1-β), Nappar Kappa Beta Nuclear Factor (NFκB) and triggers peritoneal macrophage reactivity to become hyperreactive and trigger a cascade of proinflammatory reactions and cause chain reactions with Beta (NFκB) and trigger reactivity of peritoneal macrophages to become hyperreactive and trigger a cascade of proinflammatory reactions and cause chain reactions. The end result of cell proliferation increases, apoptosis decreases, blood vessels increase, damage and progression of endometriosis increases[2,4].

An experimental study in a mice model of endometriosis discovered that genistein, which is the most potent phytoestrogen, is able to induce a decrease in the development of endometriosis cells that occur in a mice model of endometriosis[4,5]. Etiopathogenesis of endometriosis and its various treatment alternatives has long been known, but the end result of treatment always leaves many problems in the form of recurrence, side effects, high prices and wide-spread effectiveness. Therefore, genistein is a good agent as an alternative therapy for endometriosis in the future. However, the mechanism of genistein in reducing or inhibiting endometriosis cell proliferation and apoptosis remains unknown. Hence, the study aim to assess the influence of oral genistein administration on Estrogen Receptor Alpha (ER-α), Estrogen

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Receptor Beta (ER-β) and Nuclear Factor- Kappa Beta (NF-κβ) in a mice model of endometriosis.

## METHODS

**Objective:** In this proposed study we will prove the effect of various doses of genistein on the ratio of ERα and ERβ, and NFkB in a mice model of endometriosis.

**Design:** This experimental study used a post-test research design only with the control group.

**Preparation of Experimental Animal:** The study was conducted at the Laboratory of Reproductive Physiology Embryology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia between January and March 2017. The population of the study were healthy female mice (Mus musculus) with the following criteria: 1) weight between 20 and 30 grams; 2) aged between 2 and 3 months; 3) healthy (actively moved, had clean white hair and bright eyes). Furthermore, we randomly divided the mice that met the criteria into these six following groups: 1) negative control group (healthy mice that were not given with genistein); 2) positive control group (mice model of endometriosis that were given with genistein); 3) four experimental groups that were given genistein in four different doses.
The doses used in the study were based on our last study[6]. The doses were converted into doses for mice with conversion value was 0,0026[7].
**Table 1:** Calculation of genistein dose in each group from conversion of human dose to mice dose

| Experimental group | Human dose (mg/day) | Conversion factor | Mice dose (mg/day) |
|---|---|---|---|
| P1 | 500 | 0,0026 | 1,30 |
| P2 | 750 | 0,0026 | 1,95 |
| P3 | 1000 | 0,0026 | 2,60 |
| P4 | 1250 | 0,0026 | 3,25 |

To get the right number of sample, (t-1) (r-1) ≥ 15 was used[8]. T value is the number of groups, while r value is the number of replications in each experiment. The calculation result was that there should be four mice each group. However, we used six mice instead in order to prevent lack of sample as 20% of mice possibly die. Therefore, the total number of mice that we used in the study were 36 mice which consisted of thirty mice model of endometriosis (n=30) and six healthy mice (n=6).

**Mice Model of Endometriosis**: The mouse model of endometriosis was made using the model making method with the best results as an endometriosis model mice from out previous study in endometriosis[9]. To ensure that the model that occurs has become endometriosis, one mouse each group was sacrificed.

**Preparing the Genistein:** The genistein used in this study was the Genistein that is a trade mark for genistein extract which is purified from soybeans, produced by Tokyo Chemical Industry, Japan with batch number OW86I-QF. It is a bottled packaging. The doses of genistein were weighed daily for 6 mice with the following details: 1) P1 = 1,30 mg/day x 6 mice = 7,80 mg/day; 2) P2 = 1,95 mg/day x 6 mice = 11,70 mg/day; 3) P3 = 2,60 mg/day x 6 mice = 15,60 mg/day; 4) P4 = 3,25 mg/day x 6 mice = 19,50 mg/day.
The weighed doses of genistein were stored in evendorf and labelled "P1, P2, P3, P4". Each genistein in evendorf P1-P4 was diluted using 1 ml of 100% sesame oil and subsequently was centrifuged. Hence, each mice was fed with 0.20 ml of genistein. Sesame oil was used as a solvent for genistein to simplify the oral administration process using sonde and prevent irritation of the esophagus. This method is easy and allows to control the dose given.

**Experimental Treatments:** Genistein dissolved in sesame oil was fed using sonde. The mouse of experimental group was fed once a day for 14 days (Yavuz et al., 2007). Genistein which has been dissolved with sesame oil was put into a 1 ml syringe that has been installed with a sonde at the edges. The nape of mice's neck was subsequently held slowly and carefully. A sonde was slowly inserted into the mouth of mice through the palatum to the pharynx and then to the esophagus. Subsequently, the genistein in the syringe was pushed into the esophagus so that it reached the stomach. Finally, after oral genistein administration, mice was put back into the cage

A mice was sacrificed using the cervical dislocation method. Thumb and index finger were placed on both sides of mice's neck at the base of the skull while another hand was placed at the base of the tail or hind leg and quickly pulled to separate of the neck and skull bones (the cervical dislocation method). The mice was placed on board with the stomach facing up. Subsequently, thumbtack was pinned on mice's feet. To open abdominal skin, scissors and tweezers was used carefully. The incision started from the midline and continued to the left and right side on the top and bottom to open the diaphragm. Finally, the peritoneal tissue was taken to observe the expression of RE-α, RE-β, and NFkB. The scarified mice were collected and buried into the ground that was safe and usually used for disposal of experimental animals.

**Histopathology Examination**: In microscopic examination, we used Immunohistochemistry staining (IH) from sagittal slices of the peritoneal tissue. The preparations were taken from the peritoneum in the most hyperemic areas of all mice model of endometriosis after being sacrificed with cervical dislocation.

**The expression of RE-α, RE-β, and NFκB:** To assess immunohistochemical of the level of mouse monoclonal antibody immunoreactivity against RE-α and RE-β, the intensity of staining and counting the number of positive cells containing RE-α and RE-β were observed using a microscope at 400x magnification in 1000 cells spread over 5 different fields. These were subsequently compared with positive control in the form of peritoneal tissue preparations which have also been carried out immunohistochemical examination of RE-α and RE-β receptors. Staining that occurs in RE-α and RE-β lies in the surface epithelium, glandular epithelium and stromal lesions of the peritoneal tissue of the peritoneum tissue of the mice. Positive result in microscopic examination is the presence of a brownish color in the nucleus while the cytoplasm is not colored. To observe, NFκB expression seen in endometriotic lesions peritoneum of mice model of endometriosis, we also used a light microscope at 400x magnification calculated at 1000 cells. We used a scoring for the expression of RE-α, RE-β, and NFkB.

**Table 2:** Score for the expression of RE-α, RE-β, and NFkB

| Score | Expression |
|---|---|
| 0 (-) | If no brown expression |
| 1 (±) or weak intensity | If the intensity of the brown color is very weak |
| 2 (+) or moderate intensity | If the intensity of the brown color is weaker than the positive control |
| 3 (+) or strong intensity | If the intensity of the brown color is the same or stronger than the positive control |

**Data Analysis:** The data was analyzed using normality test with Shapiro–Wilk test, comparative test using independent sample t-test (normally distributed data) or Mann– Whitney U-test (not normally distributed data) and ANOVA one-way test (F test) (normally distributed data) or Kruskal–Wallis test (not normally distributed data). After the calculations, path analysis was performed to know the relationship between the various variables studied. All calculations were carried out using the SPSS for Windows 19.0

## RESULTS

**ER-α Expression:** The ANOVA test results show that p-value was smaller than α = 0.05 (p <0.05) meaning there is a significant effect of oral genistein administration in different doses on RE-α expression. Based on the 5% LSD test results, a comparison between the positive control group (K +) with the treatment, it was shown that a significant increase in the expression of RE-α was shown in the P2 and P3 treatment groups. This is indicated by the mean ± SD treatment groups P2 and P3 which are higher and contain letters that are different from the positive control group.

Based on the 5% LSD test results, a comparison between the positive control group (K +) with the treatment, it was shown that in the P2 and P3 groups a p-value of less than 0.05 (p <0.05) showed an increase in expression RE-α significantly (Figure 1). Whereas at P4, the p-value less than 0.05 (p <0.05) showed a significant decrease in the expression of RE-α. In the P1 group, the p-value was more than 0.05 (p> 0.05). Therefore, it was proven that the increase in RE-α expression significantly occurred in the group with oral genistein administration at a dose of 1.95 mg / day (P2) and 2.6 mg / day (P3). In the comparison between treatment groups, it was shown that all treatment groups had p-values less than 0.05 (p <0.05), which indicated differences in the expression of RE-α between treatment groups with oral genistein administration.

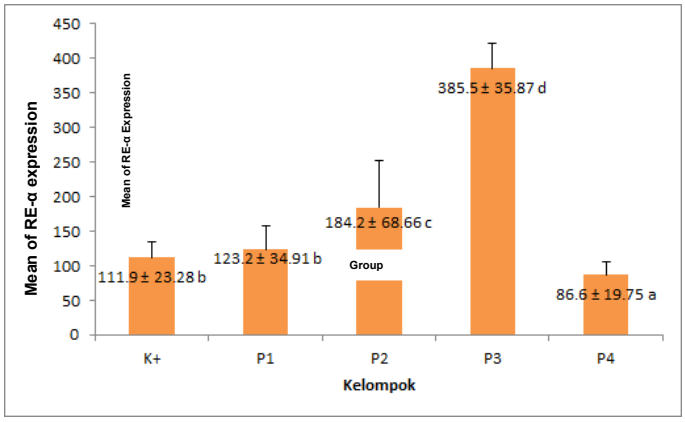


**Figure 1:** Mean of RE-α expression. Mean of RE-α expression of all control and treatment groups. The average expression of RE-α increased in the treatment group P1, P2 and P3 while the expression of RE-α decreased in group P4. Oral genistein administration statistically increase in RE-α expression significantly at a dose of 1.95 mg / day (P2) and 2.6 mg / day (P3).

### Histopathology Image of RE- α Expression:

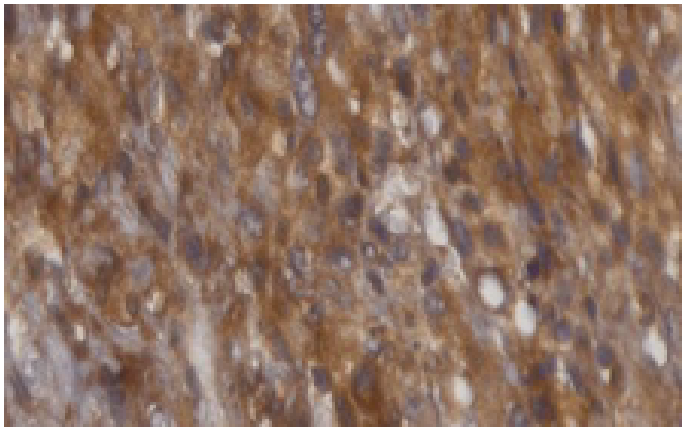

**Figure 2:** ER-α 1 121K+KK

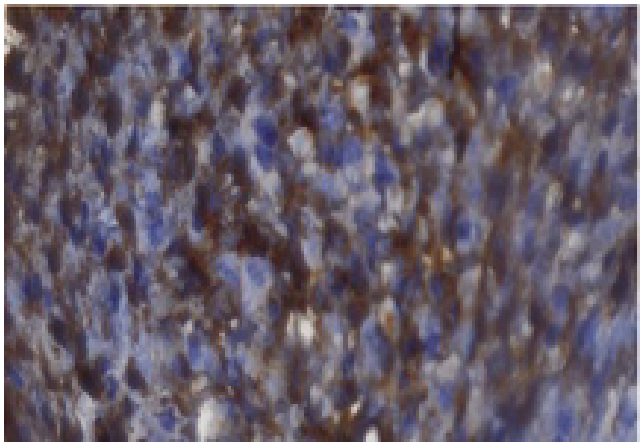

**Figure 3:** ER-α 2 121K+KK

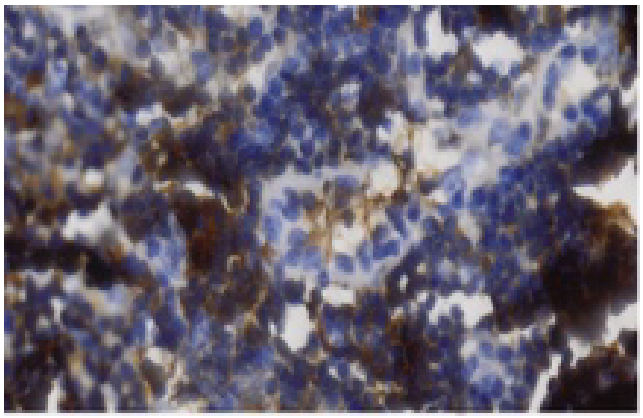

**Figure 4:** ER-α 3 121K+KK

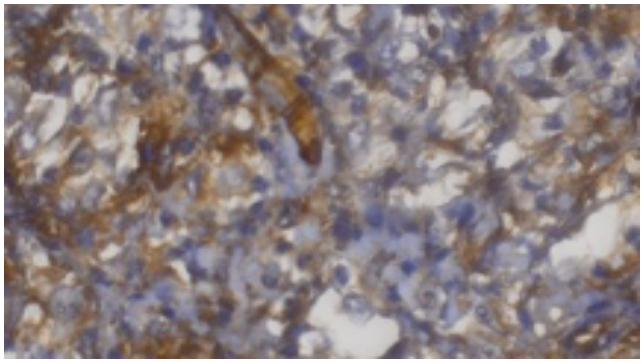

**Figure 5**: ER-α 4 121K+KK

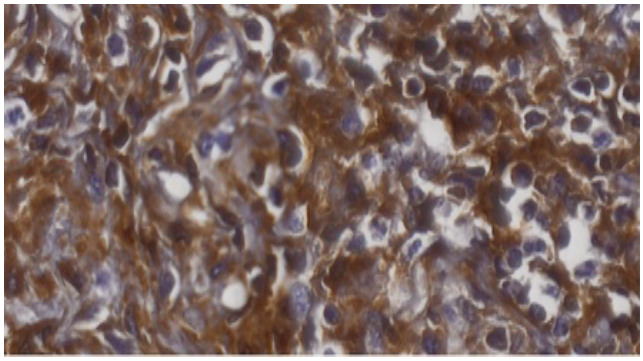

**Figure 6:** ER-α K(+) 121K+KK

Figure 2 to 6 shows immunohistochemical examination with HE staining shows that RE-α expression among all treatment groups were vary. ER-α expression in treatment group 3 had the strongest expressions, followed by treatment group 2 and treatment group 1. The hispathology image of ER-α expression used immunohistochemical staining, 400x magnification; Nikon H600L microscope; DS Fi2300 megapixel camera.

**ER-β Expression:** The ANOVA test results show that a p-value was 0,000 or smaller than α = 0.05 (p <0.05). Hence, there is a significant effect of oral genistein administration in different doses on RE-β expression.

The Dunnet T3 test 5% results reveal that the expression of RE-β significantly increased in the group of all treatment groups P1, P2, P3, and P4. In the comparison between treatment groups, it was shown that in the comparison between P1 with P2, P1 with P4, and P2 with P4 obtained p-values of more than 0.05 (p> 0.05). The average expression of RE-β in the treatment group P1 (1.30 mg / day dose) (P1), group P2 (1.95 mg / day dose) and group P3 (3.25 mg / day dose) did not differ significantly.

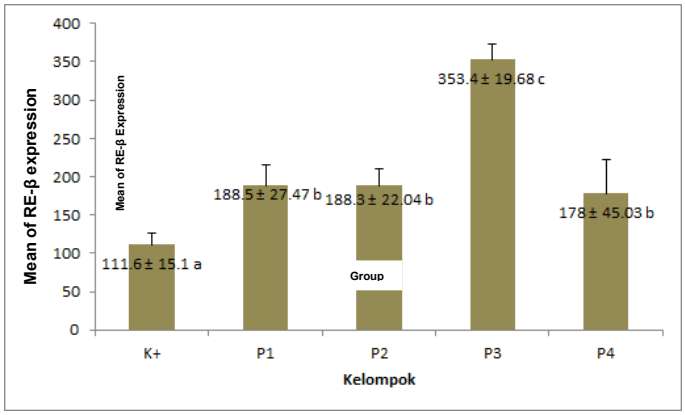


**Figure 7:** Mean of RE-β expression in all control and treatment groups Figure 8 illustrates that the mean of expression of RE-β increased in all treatment groups. Statistically, it was proven that there was a significant increase in the expression of RE-β in all treatment groups with various doses with the average expression of RE-β found in the group with oral genistein administration at a dose of 2.6 mg / day (P3).

### Histopathology Image of RE- β Expression:

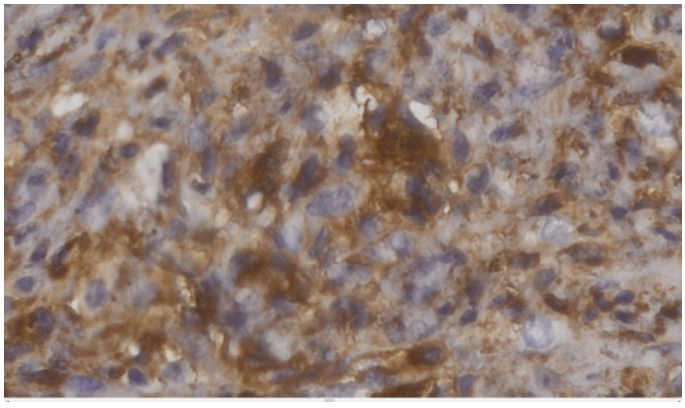

**Figure 8:** ERB 1

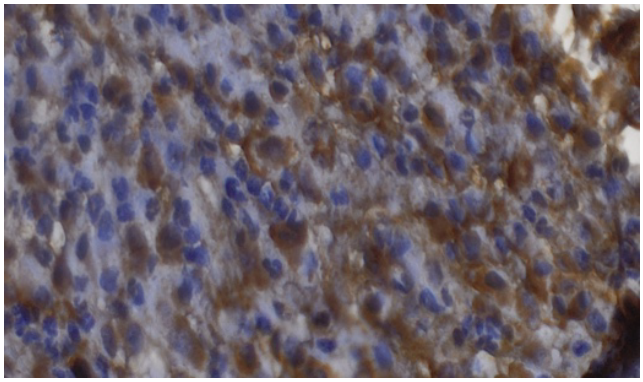

**Figure 9:** ERB 2

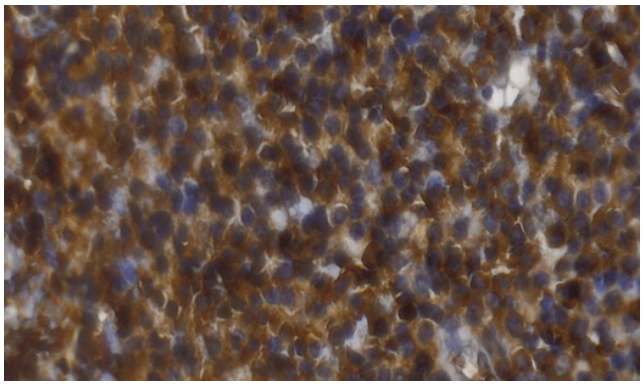

**Figure 10:** ERB 3

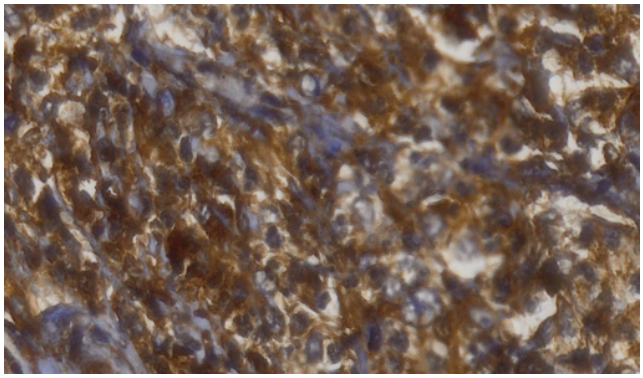

**Figure 11:** ERB 4

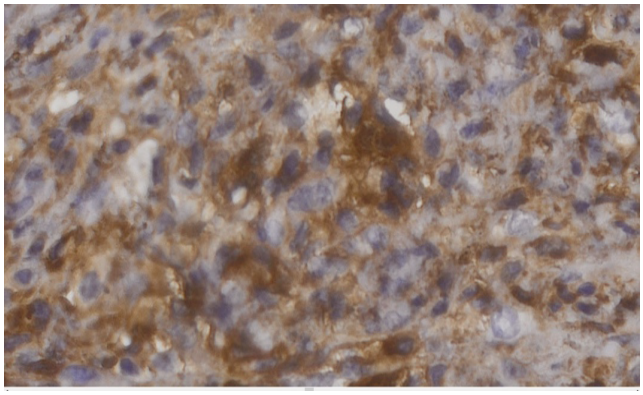

**Figure 12. ERB K(+)**

Figure 8 to 12 illustrates that in the endometrial lesion staining, the strongest expression of RE-β expression was shown in treatment group 3, followed by treatment group 2 and treatment group 1. Figure 4 shows that the expression of RE-β produced are different among groups. The Hispathology image of ER-β expression used immunohistochemical staining, 400x magnification; Nikon H600L microscope; DS Fi2300 megapixel camera.

**ER-α/ER-β Ratio:** The ANOVA test results found that a p-value was 0,000 or smaller than α = 0.05 (p <0.05. Therefore, there is a significant effect of oral genistein administration in various doses on the RE-α / RE-β ratio.

The Dunnet T3 5% test results showed that there was a significant decrease in the RE-α / RE-β ratio in the treatment group P1 and treatment group P4. There was no statistically significant difference in the RE-α / RE-β ratio among all treatment groups (p> 0.05).

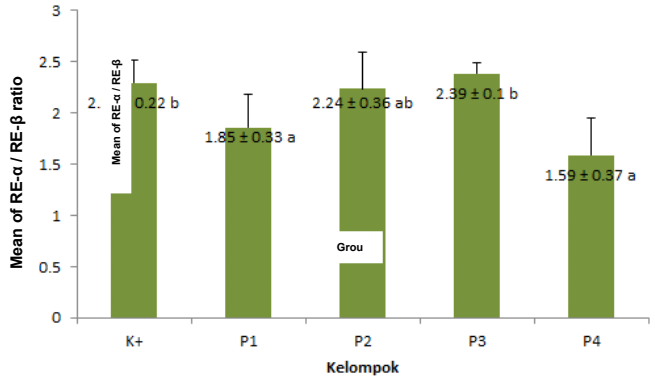


**Figure 13:** Mean of RE-α/RE-β ratio of all control and treatment groups. It shows that the average RE-α / RE-β ratio decreased in the treatment group P1, P2, and P4 while the average RE-α / RE-β ratio increased the treatment group P3. Statistically, there was significant decrease in the RE-α / RE-β ratio in treatment group P1 (1.3 mg / day dose) and treatment group P4 (3.25 mg / day dose)

**NF-κβ:** The ANOVA test results shows that a p-value was 0,000 or smaller than α = 0.05 (p <0.05). Hence, there was a significant effect of oral genistein administration in different doses on NFkB expression.

The Dunnet T3 5% test results shows that there was a significant increase in NFkB expression in the treatment group P2 and treatment group P3. In the comparison between treatment groups, it was shown that the comparison between P1 and P2 and P1 with P4 obtained p-values of more than 0.05 (p> 0.05). The average of NFkB expression in the treatment group P1 (1.30 mg / day dose), P2 (1.95 mg / day dose) and P4 (3.25 mg / day dose) did not differ significantly.

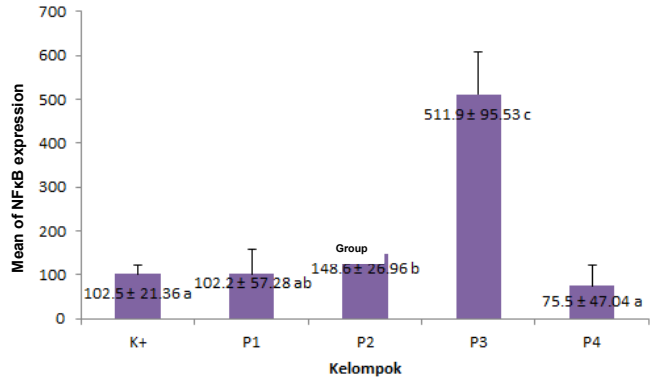


**Figure 14:** The average histogram of NFkB expression of all control and treatment groups. The man of NFκB expression increased in the treatment group P1, P2 and P3. Statistically, there was a significant increase in NFκB expression treatment group P2(1.95 mg / day dose) and P3 (2.6 mg / day dose)

### Histopathology Image of NFkB Expression:

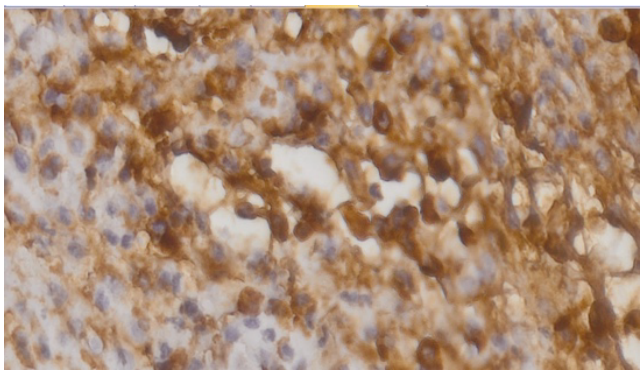

**Figure 15:** NFKB 1

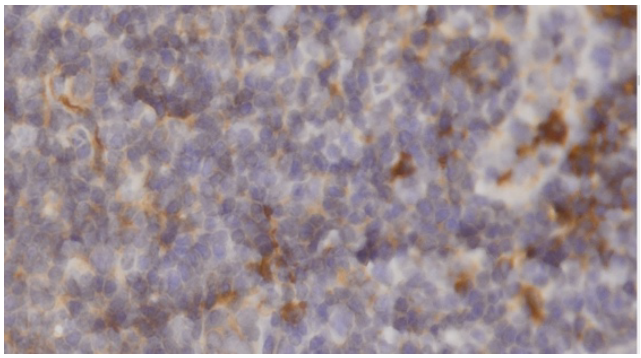

**Figure 16:** NFKB 2

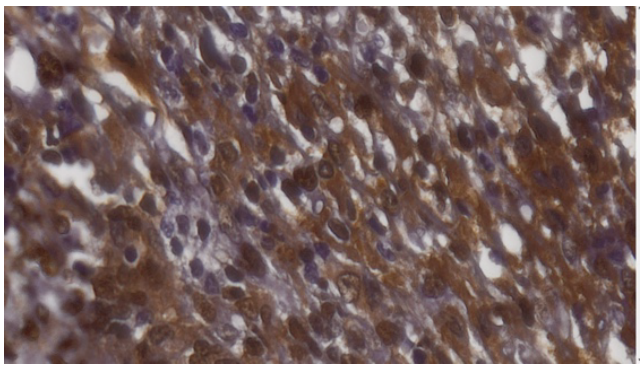

**Figure 17:** NFKB 3

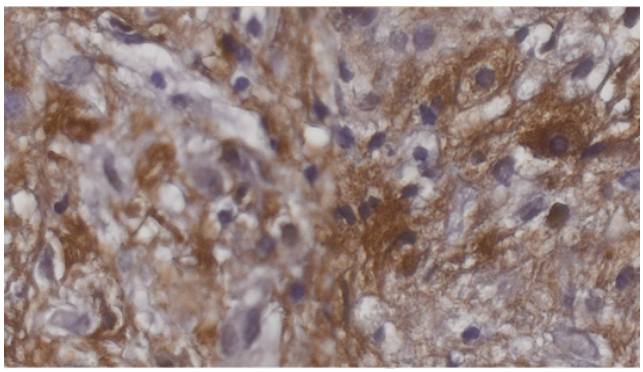

**Figure 18:** NFKB 4

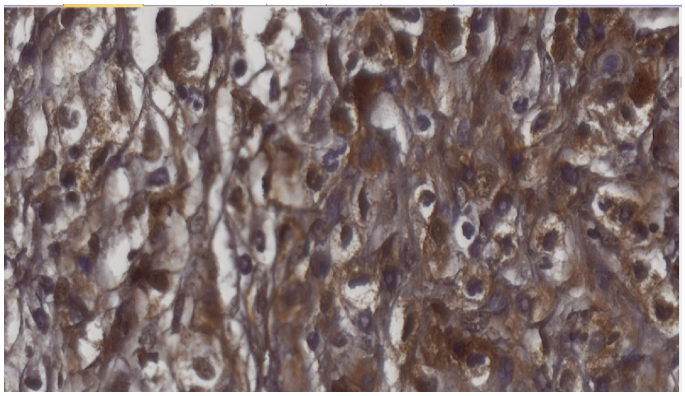

**Figure 19:** NFKB K(+)

Figure 15 to 19 illustrates the Immunohistochemical examination which carried out on endometriosis spots obtained in each mouse. It shows that the treatment group P3 had the strongest NFκB expression, followed by treatment group P2 and P1. The hispathology image of NFkB expression used immunohistochemical staining, 400x magnification; Nikon H600L microscope; DS Fi2300 megapixel camera.

## DISCUSSION

**Alpha:** In our study, the effect of high dose of oral genistein administration on alpha estrogen receptor expression was explored because the basic characteristic of alpha estrogen (RE-α) is the stimulating effect. RE-α expression significantly increased in the group with genistein dose of 1.95 mg / day (P2) and 2.6 mg / day (P3). In the comparison to treatment groups, the group with the highest genistein dose of 3.25 mg / day (P4) had lower decrease than positive control groups, meaning that it consistently decreased alpha estrogen receptor expression.

An endometriosis cell culture research found that genistein was able to reduce the proliferation of endometriosis cell cultures by decreasing the expression of alpha (RE-α) and beta (RE-β) receptors. The decrease is consistent for each dose, hence, genistein suppresses the expression of RE-α in in vitro studies. Moreover, this study used a mice model of endometriosis (in-vivo) with a dose of 2.6 mg / day and still showed an increase in the expression of RE-α. In conclusion, it still showed a stimulating effect[6]. A research on endometriosis cell cultures exposed to genistein, also obtained similar results. In the study, when the dose was given at 3.25 mg / day (P4), it was seen that consistently reduced RE-α expression, and decreased RE-α expression would be followed by decreasing endometriosis cell proliferation. It can be concluded that with a dose of 3.25 mg / day genistein can reduce the expression of RE-α in mouse model of endometriosis[10].

However, another study obtained different results between administration of genistein through injection and oral[11]. By using a low dose given to mice that have been implanted with endometriosis tissue, administration by injection resulted an endometriosis implant growth while oral administration resulted no growth or regression of endometriotic implants. Oral administration is a natural route for medicine and nutrition so that foods containing high genistein (nuts) will be very beneficial if it is given via the oral route.

High-dose genistein causes endometriosis cell regression[5]. Genistein is a natural SERM which the character of its effect on target organs (endometriosis) depends on the dose, duration of exposure and type of tissue or organ. The group P4 with a dose of 3.25 mg / day, which is equivalent to 1,250 mg of genistein, had anti-estrogen character in human so that it suppresses endometriosis cell proliferation. This SERM character proved that high doses actually suppress RE-α expression.

**Beta:** Oral genistein administration in different doses also had significant effect on the expression of RE-β. When the genistein was administered up to 2.6 mg / day (P3), the expression of RE-β increased, however, when the dose was increased up to 3.25 mg / day (P4), the expression of RE-β decreased significantly. This decrease also consistently occurred in other variables, hence at a dose of 3.25 mg / day, genistein was able to suppress the expression of RE-β and result in decreased proliferation and increased apoptosis of endometriosis cells. Normally, the estrogen hormone activates alpha and beta estrogen receptors in the cell nucleus and binds to estrogen responsive elements (EREs) and initiates the next response. Estrogen not only works genomically, but also it works non-genomically so that the response can be very fast or long term. Endometriosis and endometrial cancer are disorders that depend on the action of estrogen, so the regulation of the hormone estrogen will affect both diseases[12]. A similar study in endometriosis cell culture also found that the administration of genistein to endometriosis cell culture caused a decrease in the expression of RE-β[10].

A study in endometriosis cell culture, genistein administration caused a decrease in the expression of RE-α and RE-β but an increase in apoptosis. It was concluded that the change in expression of both estrogen receptors is closely related to increased apoptosis. In this study, a different pattern was found wherein there was an increase in the expression of estrogen receptors, and when the dose was high the opposite effect occurred. Genistein belongs to the SERM group and has a higher affinity for RE-β. RE-β at the molecular level has the function of regulating RE-α and results in suppressing the activation function of RE-α. The results of the study were consistent i.e. genistein administration was able to regulate apoptosis and proliferation of endometriosis progression in a mice model of endometriosis[6].

High-dose genistein causes endometriosis cell regression[5]. Genistein is a natural SERM which the character of its effect on target organs (endometriosis) depends on the dose, duration of exposure and type of tissue or organ. The group P4 with a dose of 3.25 mg / day, which is equivalent to 1,250 mg of genistein, had anti-estrogen character in human so that it suppresses endometriosis cell proliferation. This SERM character proved that high doses actually also suppress RE-β expression.

**Alpha and beta:** Our study showed that there is a significant decrease in the RE-α / RE-β ratio. i.e., the genistein administration group at a dose of 1.3 mg / day (P1) and 3.25 mg / day (P4). In the average histogram of the RE-α / RE-β ratio, the average RE-α / RE-β ratio decreases in the treatment group with oral genistein administration P1, P2, and P4. A decrease in the RE-α / RE-β ratio means a higher increase in expression of RE-β expression than RE-α. In other words, the effect of RE-β is more dominant in regulating the effect of RE-α that results the inhibition of the effect of RE-α. RE-α inhibition plays important role in reducing the rate of endometriosis cell proliferation. The increase of expression of RE-β is associated with the decrease of inflammatory conditions (TNF-α) of endometriosis cells with the administration of genistein. This decrease in inflammatory is also accompanied by the decrease in endometriosis cell proliferation and the increase in apoptosis[13]. RE-β is a prospective target of therapy in the future considering that more diseases will initiate RE-α. Manipulation of RE-β will be an alternative therapy for endometriosis, uterine cancer and others[14].

Another study found that the expression of RE-α / RE-β decreased as the result of the increase of genistein administration[10]. However, in

this study there was a consistent increase in the RE-α / RE-β ratio and showed that the expression of RE-β remained consistently high, so that the ratio analysis, it was more consistent at a high level. It would be different if RE- α or RE-β was analyzed one by one. Helguero et al. (2005) explained that RE-β becomes the regulator of RE-α so as long as there is RE-β, the effects of RE-α proliferation can be balanced and controlled. Therefore, the RE-α / RE-β ratio becomes more sensitive to be a predictor of apoptosis in breast malignancies (cell line). The ratio of RE-α / RE-β is related to the rate of apoptosis of heart cells[15]. In addition, the high expression of the RE-α / RE-β ratio is correlated with high apoptosis in cells heart muscle cells. Another study obtained data that the balance of RE-α and RE-β is the key to apoptosis in endometriosis. The RE-α / RE-β ratio will affect BAX expression and high BAX expression will shift toward apoptosis and have a prognostic value in improving endometriosis progression[16].

In this study, when the expressions of RE-α and RE-β were compared one by one, irregular patterns showed up. However, when it was viewed from the RE-α / RE-β ratio as a single unit, the results were consistent for each group and group 4 (P4) seen a statistically significant decrease. A stable ratio makes the RE-α / RE-β ratio a good parameter for seeing the progression of endometriosis. A low ratio indicates that RE-β expression is more dominant and the effect of cell proliferation suppression and endometriosis progression will decrease. The RE-α / RE-β ratio can be a good parameter to see the effect of drugs / genistein on endometriosis.

**NFkB Kappa beta:** The increase in NFkB expression significantly occurred in the group with oral genistein administration at a dose of 1.95 mg / day (P2) and 2.6 mg / day (P3). There was an ATTG insertion / deletion mutation in the NFkB1 grn promoter and was closely related to the severity of endometriosis and infertility[17]. Furthermore, a research on hyperplasia endometrial cell culture (a condition that resembles endometriosis / endometrial cancer) found that genistein was able to inhibit the growth of endometrial cells through inhibition of EGFR and decreased expression of PI3K / Akt and NFkB, genistein increases apoptosis in endometrial cells through intrinsic pathways. Genistein also decreases NFkB expression and influences the work of p53 protein which in turn affects the increase in endometrial cell apoptosis. In this study it appears that in treatment group 4 (P4) there was a consistent decrease in NFkB expression. Genistein dose of 3.25 mg / day consistently decreases NFkB expression[18]. Another research using mouse model of endometriosis by administering various doses of genistein with doses below 1.3 mg / day, shows that genistein influences the expression of NFkB, but the effect is still inconsistent and does not provide predictions for doses higher dose[19]. In this study with the dose increased, it turned out that the new dose of 3.25 mg / day consistently reduced the expression of NFkB in mouse model of endometriosis.

To the best of our knowledge this is the first study using the model of adenomyosis. In addition, to date there is no study assessing the role of genistein in endometriosis treatment. However, future study is needed to assess the effect of genistein administration on various factors that have an influence on endometriosis lesions, especially through various signalling pathways that affect cell proliferation and apoptosis such as thyroxine kinase, topoisomerase, various stem cell markers, and etc. In addition, a study carried out to identify the role of genistein in apoptosis through different pathways and using a pure or natural genistein trial which is performed on humans with endometriosis are needed.

## CONCLUSION

**Genistein various doses significantly influenced the RE-α / RE-β ratio. The higher the dose of genistein given will decrease the RE-α / RE-β ratio. In addition, genistein dose of 3.25 mg/ day significantly reduced NFkB expression. Genistein should be taken into account in the treatment of endometriosis as it is effective yet affordable.**

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**Competing Interest**: None.

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