

GENISTEIN TO DECREASE MATRIX METALLOPROTEINASE-2 (MMP-2) AND MATRIX METALLOPROTEINASE-9 (MMP-9) LEVELS IN PERITONEAL FLUID OF ENDOMETRIOSIS ON MICE MODEL

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ABSTRAK GENISTEIN MENURUNKAN KADAR MATRIKS METALLOPROTEINASE-2 (MMP-2) DAN MATRIKS METALLOPROTEINASE-9 (MMP-9) DALAM CAIRAN PERITONEAL MENCIT MODEL ENDOMETRIOSIS

Latar Belakang: Matrix metalloproteinases (MMPs) mempengaruhi progresivitas endometriosis, dalam invasi, perkembangan dan metastasis endometriosis. Penurunan kadar MMP-2 dan MMP-9 merupakan terapi yang tepat untuk kasus endometriosis menggunakan terapi hormonal alami yaitu genistein.

Tujuan: Penelitian ini bertujuan untuk mengetahui pemberian genistein dapat menurunkan kadar MMP-2 dan MMP-9 dalam cairan peritoneal mencit (*Mus musculus*) model endometriosis.

Metode: Penelitian ini merupakan penelitian eksperimental yang dilakukan di Laboratorium Fisiologi Reproduksi Embriologi Fakultas Kedokteran Hewan Universitas Airlangga Surabaya dan Laboratorium Fisiologi Fakultas Kedokteran Universitas Brawijaya Malang. Sampel menggunakan mencit (*Mus musculus*) model endometriosis. Penelitian dibagi menjadi 8 kelompok, yaitu : kontrol negatif, kontrol positif dan perlakuan pemberian genistein dosis 50 mg/hari, 100 mg/hari, 200 mg/hari, 300 mg/hari, 400 mg/hari, 500 mg/hari. Pengukuran kadar MMP-2 dan MMP-9 menggunakan ELISA kit. Data hasil pengamatan dianalisis dengan uji ANOVA, Tukey dan Regresi.

Hasil: Genistein mampu menurunkan kadar MMP-2 terendah pada dosis 50 mg/hari (5.61 ± 0.69 ng/mL), 100 mg/hari (4.91 ± 0.59 ng/mL) dan 400 mg/hari (4.95 ± 0.32 ng/mL) bila dibandingkan dengan perlakuan dosis yang lain. Genistein mampu menurunkan kadar MMP-9 terendah pada dosis 50 mg/hari (1.69 ± 0.45 ng/mL), 100 mg/hari (1.29 ± 0.32 ng/mL), 200 mg/hari (1.84 ± 0.25 ng/mL) dan 400 mg/hari (1.42 ± 0.11 ng/mL) bila dibandingkan dengan perlakuan dosis yang lain.

Kesimpulan: Genistein dapat menurunkan kadar MMP-2 dan MMP-9 dalam cairan peritoneal mencit (*Mus musculus*) model endometriosis.

Saran: Perlu studi lanjut untuk melihat pengaruh genistein pada hewan coba kelinci

Kata Kunci: Endometriosis, Genistein, MMP-2, MMP-9

ABSTRACT

Background: Matrix metalloproteinases (MMPs) affect the progression of endometriosis, in the invasion, development, and metastasis of endometriosis. Reducing levels of MMP-2 and MMP-9 is an appropriate therapy for endometriosis cases using natural hormonal therapy, namely genistein.

Purpose: To know the effect of genistein on the level of MMP-2 and MMP-9 in peritoneal fluid of endometriosis mice model.

Method: The design of this study was experimental, had been performed in Reproductive Physiology and Embryology Laboratory, Veterinary Medical Faculty of Airlangga University Surabaya and Physiology Laboratory, Medical Faculty of Brawijaya University Malang. The animal model was divided the 8 groups: negative group, positive group, and treated group with genistein doses of 50 mg/day, 100 mg/day, 200 mg/day, 300 mg/day, 400 mg/day, and 500 mg/day (human dose). The measurement of MMP-2 and MMP-9 levels using ELISA kits. The data were analyzed with ANOVA, Tukey, and Regression tests.

Result: Genistein was able to decrease the MMP-2 level in the lowest dose 50 mg/day (5.61 ± 0.69 ng/mL), 100 mg/day (4.91 ± 0.59 ng/mL) and 400 mg/day (4.95 ± 0.32 ng/mL) when compared with other treatment dose. Genistein was able to decrease the MMP-9 level in the lowest dose 50 mg/day (1.69 ± 0.45 ng/mL), 100 mg/day

(1.29±0.32 ng/mL), 200 mg/day (1.84±0.25 ng/mL) and 400 mg/day (1.42±0.11 ng/mL) when compared with other treatment doses.

Conclusion: Genistein may decrease MMP-2 and MMP-9 levels in peritoneal fluid of endometriosis mice model.

Suggestion: Further study is needed to see the effect of genistein on rabbit experimental animals.

Keywords: Endometriosis, Genistein, MMP-2, MMP-9

INTRODUCTION

Pathophysiologically Matrix metalloproteinases (MMPs) affect the progression of endometriosis, in the invasion, development and metastasis of endometriosis (Adamyani et al., 2020; Weigel et al., 2012). The presence of endometriosis is characterized by the presence of endometrial tissue outside the uterine cavity. The process of implantation of endometrial tissue requires the help of MMPs enzymes. MMPs are proteolytic enzymes that play a role in degrades basement membrane and extracellular matrix (MES) components (Shaco-Levy et al., 2008; Zhang et al., 2010). woman with Endometriosis tends to have high levels of MMPs in the peritoneal cavity, because in endometriosis there is an increase in estrogen in endometrial tissue through abnormal activation of the steroidogenesis process involving aromatase. The expression of these MMPs is stimulated by estrogen in endometrial tissue (Fassbender et al., 2014)

The pathogenesis of endometriosis begins with inflammation and is followed by degradation involving the enzyme MMPs, including the family of gelatinases (MMP-2/gelatinase A and MMP-9/gelatinase B). The degradative effects on MES, MMP-2 and MMP-9 have been believed to play an important role in the progression of endometriosis (Shaco-Levy et al., 2008). MMP-2 and MMP-9 are overproduced in patients with endometriosis. The increase in MMP-2 and MMP-9 levels is a result of the high concentration of estrogen secreted from endometrial tissue which causes endometrial cells to invade, differentiate, adhere, tissue remodeling and cells survive (Soares et al., 2012). Several studies have shown an increased expression of MMP-2 and MMP-9 in the peritoneal fluid in endometriosis patients (Collette et al., 2006).

Reducing levels of MMP-2 and MMP-9 is an appropriate therapy for cases of endometriosis. Anti-MMP is one of the future therapies for controlling tumor development and certain cancer therapies. MMPs are regulated at the level of gene expression and protein activation (Brinckerhoff, 2017). Currently, many anti-MMP drugs are being investigated in cancer as a therapeutic option for endometriosis (Mendis et al., 2009). Endometriosis

can be said to look like cancer, because endometriosis is a benign tumor disease, but has the characteristics of malignancy like cancer, namely invasiveness, uncontrolled cell growth, tendency to metastasize and recur (Leyland et al., 2010). MMP-2 and MMP-9 can be detected in malignant tissue and it is possible to use anti-MMP therapy on both enzymes. The rational chemical design of anti-MMP is possible to synthesize MMP subtype-specific compounds with inhibitory activities that are also more specific for certain diseases, such as cancer and arthritis (Mendis et al., 2009). There is a need for further research on anti-MMP as a treatment for endometriosis which is natural, inexpensive and easily available.

Several natural anti-MMP hormonal therapies have been developed from plants such as resveratrol, theaflavins, catechins, curcumin and genistein. Genistein is known for its anti-MMP properties. The most common source of genistein is soybeans

and processed products such as tofu, tempeh, tauco, soy sauce, are also found in fruits and green tea (Prakash & Gupta, 2011). One of these isoflavone groups has a chemical structure and mechanism pathway that is similar to estrogen in the body. Genistein works as Selective Estrogen Receptor Modulators (SERMs) which can act as anti-estrogen in high estrogen conditions (Anggraini, 2008). In in vivo studies with cancer cells, genistein was able to inhibit the invasion of these cancer cells by reducing the levels of MMP-2 and MMP-9 (Banerjee et al., 2008). Research conducted by Yavuz et al. (2006) in endometriosis mice with

administration of genistein at a dose of 500 mg/kg orally for 21 days can inhibit the implantation of endometriosis. This study has not yet explained the most ideal or appropriate dose for the treatment of endometriosis, so it is necessary to look at the appropriate administration of various doses of genistein starting from a dose of 50 to 500 mg.

Research on giving genistein to mice modeled with endometriosis has never been done in Indonesia. Based on the description above, researchers are interested in conducting research related to the administration of genistein to reduce

the levels of MMP-2 and MMP-9 in the peritoneal fluid of mice (*Mus musculus*) models of endometriosis. It is hoped that the administration of genistein can reduce the levels of MMP-2 and MMP-9 so that the development of endometriosis cells is inhibited.

RESEARCH METHODOLOGY

This study used an experimental design, Post test only with control group , carried out at the Laboratory of Embryology Reproductive Physiology, Faculty of Veterinary Medicine, Airlangga University, Surabaya and Physiology Laboratory, Faculty of Medicine, Brawijaya University, Malang.

The samples used were female mice (*Mus musculus*) model of endometriosis. Divided into 8 groups, namely 1 negative control group, 1 positive control group and 6 groups with genistein treatment, namely 50 mg/day, 100 mg/day, 200 mg/day, 300 mg/day, 400 mg/day and 500 mg/day (human dose).

The tools used include a set of sterile surgical instruments, analytical scales, sonde, stirrer, measuring cup, syringe, tube, digital camera , 1.5 ml Micro Centrifuge Tube , 50 L, 100 L and 1000 μ L micro pipettes, 15 ml falcon tip, centrifuge , and Mouse MMP-2 (Catalog# E-EL-M0780) Elisa kit Elabscience , Mouse MMP-9 (Catalog# E-ELM0627) Elisa kit Elabscience and ELISA Reader .

Procedure for Making Mice (*Mus musculus*) Endometriosis Model

The manufacture of endometriosis model mice refers to the research of Sutrisno et al. (2014). On the first day the mice (*Mus musculus*) in the K+ group and treatment were given 0.2 ml of Cyclosporin A injection IM, injection of endometrial and myometrial tissue from surgical material for benign tumors (adenomyosis) 0.1 ml IP. Ethynil Estradiol injection 0.1 ml IM on days 1 and 5. Then wait for 14 days, obtained mice as models of endometriosis.

Procedure for Administration of Various Doses of Genistein

The treatment group was exposed to various doses of genistein with doses of 0.13 mg/day, 0.26 mg/day, 0.52 mg/day, 0.78 mg/day and 1.04 mg/day and 1.30 mg/day for 14 days. The Genistein used is the trademark Genistein produced by the Tokyo Chemical Industry which is purified from soybeans. To facilitate the process of oral administration of genistein in powder form, it is necessary to dilute it using sesame oil until it reaches the desired volume.

Procedure for Measurement of MMP-2 and MMP-9 by ELISA method

Add 100 L of standard or sample into each well of the microplate and then cover the microplate with an adhesive strip. Incubate for 90 minutes at 37°C. Discard the liquid. Add 100 L of Biotinylated Detection Antibody . Incubate for 1 hour at 37°C. Aspirate and wash 3 times. Add 100 L of HRP Conjugate . Incubate for 30 minutes at 37°C. Aspirate and wash 5 times.

Add 90 L of Substrate Reagent to each well, then incubate for 15 minutes at 37°C. Add 50 L Stop Solution to each well and read at a wavelength of 450 nm. The levels of MMP-2 and MMP-9 were then calculated using a standard curve, which showed the Optical Density (OD) value and the levels of MMP-2 and MMP-9. Data on MMP-2 and MMP-9 levels were analyzed by ANOVA, Tukey and Regression tests. The statistical test is said to be meaningful if $p < 0.05$. The calculation process is carried out with the help of software (software) SPSS for windows 19.0.

RESEARCH RESULTS

On the 29th day, the mice were terminated for peritoneal fluid (*Mus musculus*) and then the levels of MMP-2 and MMP-9 were measured using the ELISA method.

MMP-2 Level Test Results Based on Genistein Doses

The difference in the mean levels of MMP-2 in the treatment groups is shown in the following histogram:



Figure 2
Histogram Mean MMP-2 . Levels

Note: In the Histogram if it contains different letters, it means that there is a significant difference (p -value < 0.05) and if it contains the same letters, it means that there is no significant difference (p -value > 0.05).

Figure 2 shows the histogram of the mean levels of MMP-2 in various treatment groups. The decrease in the mean levels of MMP-2 in the various treatment groups was not significantly different from

the negative control (1.34). This indicates that the administration of genistein in various doses ranging from 50 to 500 mg/day can reduce MMP-9 levels to close to MMP-2 levels in healthy mice.

However, as the genistein dose increased, the MMP-2 levels fluctuated, there was an increase in the P3 (6.71) and P4 (7.02) groups. Then it decreased in the P5 genistein dose of 400 mg/day (7.02), increased again at the P6 dose of 500 mg/day (6.96). Based on the ANOVA test, it was found that the average MMP-2 level based on the genistein dose can be seen in Table 1, indicating there was a significant difference ($P = 0.000 < \alpha$) in the decrease in the mean MMP-2 level between the control group and the treatment group at various doses. The highest mean was seen in the control group (8.73 ± 1.37) and then gradually decreased so that the lowest mean MMP-2 level was obtained at a dose of 100 mg/day (4.91 ± 0.59).

Table 1
MMP2 Level (ng/mL) Based on Dose

Observation Group	Means \pm SD	p
K(-)	5.61 \pm 0.23 ab	0.000
K(+)	8.73 \pm 1.37 c	
P1	5.61 \pm 0.69 ab	
P2	4.91 \pm 0.59 a	
P3	6.71 \pm 0.51 b	
P4	7.02 \pm 0.75 b	
P5	4.95 \pm 0.32 a	
P6	6.96 \pm 0.48 b	

Note: In the mean + SD, if it contains different letters, it means that there is a significant difference (p -value < 0.05) and if it contains the same letters, it means that there is no significant difference (p -value > 0.05).

Following are the results of the polynomial regression of the effect of genistein on MMP-2 levels:

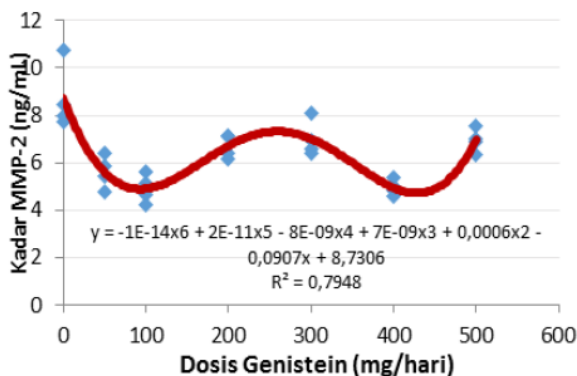


Figure 3
Scatter Plot Effect of Genistein on MMP-2 Levels with Polynomial Regression

Based on the results of the polynomial regression analysis, Figure 3 shows the R-square value of 0.7948 or 79.48%. Giving genistein was able to affect changes in MMP-2 levels by 79.48%. The remaining 20.52% is explained by other factors not involved in the study.

MMP-9 Level Test Results Based on Genistein Doses

The difference in the mean levels of MMP-2 in the complete treatment group is shown in the following histogram:

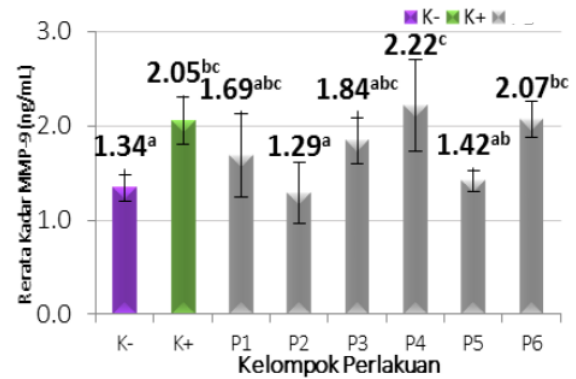


Figure 4
Histogram Mean MMP-9 . Levels

Note: In the Histogram, if it contains different letters, it means that there is a significant difference (p -value < 0.05) and if it contains the same letters, it means that there is no significant difference (p -value > 0.05).

In Figure 4 the mean decrease in MMP-9 levels at P1 at a dose of 50 mg/day of genistein (1.69), P2 at a dose of 100 mg/day (1.29), P3 at a dose of 200 mg/day (1.84) and P5 at a dose of 400 mg/day (1.42) was not significantly different from the negative control (1.34). This shows that the administration of genistein doses of 50 mg/day (1.69), 100 mg/day (1.29), 200 mg/day (1.84), 400 mg/day (1.28) can reduce MMP levels. -9 to close to MMP-9 levels in healthy mice.

However, as the genistein dose increased, MMP-9 levels fluctuated, there was an increase in P3 (1.84) and P4 (2.22). Then decreased at P5 genistein dose of 400 mg/day (1.42), increased again at P6 dose of 500 mg/day (2.07).

Based on the ANOVA test, the mean MMP-9 levels by dose of genistein (Table 2)

Table 2
MMP-9 Level (ng/mL) Based on Dose

Observation Group	Means ± SD	p
K(-)	1.34 ± 0.14 ab	0.001
K(+)	2.05 ± 0.26 bc	
P1	1.69 ± 0.45 abc	
P2	1.29 ± 0.32 a	
P3	1.84 ± 0.25 abc	
P4	2.22 ± 0.48 c	
P5	1.42 ± 0.11 ab	
P6	2.07 ± 0.19 c	

Note: In the mean + SD, if it contains different letters, it means that there is a significant difference (p-value <0.05) and if it contains the same letters, it means that there is no significant difference (p-value >0.05). K(-): healthy mice without genistein; K(+): endometriosis model mice without being given genistein; P1: endometriosis model mice were given 50 mg/day of genistein (~0.13 mg/day/mouse); P2: endometriosis model mice were given genistein at a dose of 100 mg/day (~0.26 mg/day/mouse); P3: endometriosis model mice were given genistein at a dose of 200 mg/day (~0.52 mg/day/mouse); P4: endometriosis model mice were given genistein at a dose of 300 mg/day (~0.78 mg/day/mouse); P5: endometriosis model mice were given genistein at a dose of 400 mg/day (~1.04 mg/day/mouse); P6: endometriosis model mice were given genistein at a dose of 500 mg/day (~1.30 mg/day/mouse)

Table 2 shows that there was a significant difference (P=0.001<α) in the mean decrease in MMP-9 levels between the control group and the treatment group at various doses. The highest mean was seen in P3 giving genistein at a dose of 300 mg/day (2.22 ± 0.48). The lowest mean MMP-9 level was at a dose of 100 mg/day (1.29±0.32) . The following are the results of the polynomial regression of the effect of genistein on MMP- 9 levels:

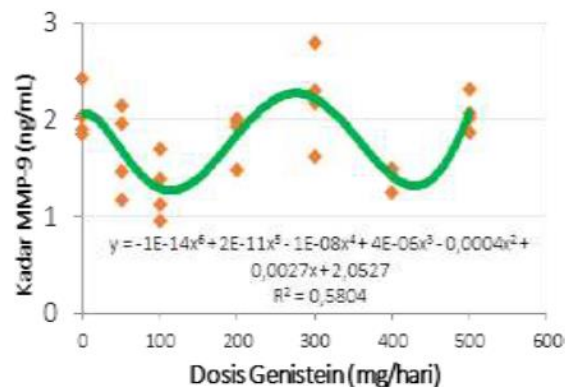


Figure 5
Scatter Plot Effect of Genistein on MMP-9 Levels with Polynomial Regression

Based on the results of the polynomial regression analysis, Figure 5 shows the R-square value of 0.5804 or 58.04%. Giving genistein was able to affect changes in MMP-9 levels by 58.04 %. The remaining 41,96 % is explained by other factors not involved in the study.

DISCUSSIONS

Endometriosis is a chronic inflammatory disease characterized by implantation of endometrial tissue outside the uterine cavity involving proteolytic enzymes, namely MMPs. With a degradative effect on basement membrane and extracellular matrix (MES) components, MMPs are believed to play an important role in the progression of endometriosis.

This study was conducted in vivo using mice (Mus musculus) model of endometriosis, referring to the study of Sutrisno et al. (2014). Based on the results of this study, the mean value of MMP-2 and MMP-9 levels in the group of mice modeled with endometriosis was higher than the group of healthy mice. This is in accordance with previous studies that in women with endometriosis tend to increase MMP levels in the peritoneal cavity as a result of the high estrogen hormone influenced by abnormal activation of the steroidogenesis process involving aromatase. The high concentration of estrogen secreted from the endometrial tissue causes endometriosis cells to invade, differentiate, adhere to, tissue remodeling and cells survive.

Based on the results of the research, genistein began to show a decrease in MMP-2 levels at doses of 50, 100 and 400 mg/day. Furthermore, decreased levels of MMP-9 at doses of 50, 100, 200 and 400 mg/day. Changes in the mean levels of MMP-2 and MMP-9 at a dose of 100 mg/day genistein showed a very significant change statistically (p-value = 0.005 <) . These results are consistent with the study that a dose of 100 mg/kg

genistein was able to inhibit the metastasis of prostate cancer cells implanted in mice in vivo (Lakshman et al., 2008). This opinion is supported by research, genistein can significantly reduce the expression of MMP which is involved in controlling invasion and metastasis in several types of cancer (Noori-Daloi et al., 2012). Genistein has an antiestrogenic effect on several reproductive tissues including the endometrium (Kayisli et al., 2002). Genistein is also an antagonist and reduces estrogenic effects on endometrial glands with high levels of estrogen (Sha & Lin, 2008).

In this study, it was shown that genistein could decrease the levels of MMP-2 and MMP-9 as the dose of genistein increased. It is suspected that the chemical structure of genistein has a similarity with 17β -estradiol so that it is able to bind to the ER (Pavese et al., 2010). The similarity between estrogen and genistein lies in the two hydroxide (-OH) or hydroxyl (C4 and C7) groups, spaced 11.0 - 11.5 Å which essentially resembles the nucleus of estrogen. The C7 hydroxyl group is required for genistein to bind to the estrogen receptor. The distance between C4 and C7 is agreed upon as the main structure of a substrate in order to have an estrogenic effect that has a certain affinity for the ER (Pavese et al., 2010). Sha and Lin (2008) explained that the structural similarity of genistein with 17β -estradiol causes genistein to compete with endogenous estrogens produced in terms of binding to RE- β and inhibiting RE- α . Genistein has a higher ability to bind to RE- β which is 20-30 times than RE- α and is comparable to the affinity of 17β -estradiol, but has lower activity than 17β -estradiol (Pilsakova et al., 2010). Administration of low concentrations of genistein showed little estrogenic activity but at higher concentrations it would give an ER antagonist effect. Genistein binding and receptors

Estrogen forms an estrogen-estrogen receptor complex and activates the ERE so that it affects the transcription process (Gruber et al., 2002). Genistein modulates several cellular signal transduction pathways related to cell proliferation, apoptosis, angiogenesis, invasion, metastasis, oxidative stress through RE- β binding (Lee et al., 2012).

In endometriosis with high estrogen levels, genistein is antagonistic and reduces estrogenic effects (Kayisli et al., 2002; Sha and Lin, 2008). Inhibition of MMPs activity may provide benefits in the treatment of endometriosis (Amalinei et al., 2010; Soares et al., 2012). Genistein inhibition of MMP-2 and MMP-9 was carried out in the genomic pathway, namely directly on the ER. According to Sutrisno et al. (2010) the genomic mechanism is divided into

two, namely: 1) Isoflavones directly bind to the ER (direct genomic), causing effects such as the effect of endogenous estrogen in the form of gene transcription and 2) Isoflavones indirectly (indirect genomic), namely by influencing endogenous estrogen levels in the body. circulation (competitive inhibitor mechanism) so that it can increase the synthesis of Sex Hormone Binding Globulin (SHBG) which functions in binding endogenous estrogen with globulin so that less estrogen is free. Genistein modulates several cellular signal transduction pathways related to cell proliferation, apoptosis, angiogenesis, invasion, metastasis, oxidative stress through RE- β binding (Lee et al., 2012).

In accordance with the study of Lakshman et al. (2008) where genistein at a dose of 100 mg/kg was able to inhibit the metastasis of prostate cancer cells implanted in mice in vivo. Lee et al. (2012) said genistein can inhibit MMPs, proteolytic enzymes involved in the invasion of breast cancer cells. This opinion is supported by research by Noori-Daloi et al. (2012) that genistein can significantly reduce MMP expression which is involved in controlling invasion and metastasis in several types of cancer including prostate cancer, lung damage and breast cancer. In in vivo studies with cancer cells, genistein was able to inhibit cancer cell invasion by reducing MMP-2 & MMP-9 levels (Banerjee et al., 2008; Pavese et al., 2010; Lee et al., 2011). This is in accordance with the research conducted by Sutrisno et al. (2014) stated that genistein can reduce MMP-2 levels by acting as anti-inflammatory and anti-angiogenesis in endometriosis cell cultures.

One of the ways genistein works is SERM (Selective Estrogen Receptor Modulator) which works depending on the dose, duration of administration and organ. Genistein as a SERM is a pure antagonist when acting through RE- β provides anti-estrogenic effects and activates corepressor proteins to inhibit the gene transcription process so that the synthesis of mRNA into DNA, protein synthesis is inhibited resulting in a decrease in MMP-2 and MMP-9 levels. In vitro studies, genistein works as a SERM capable of providing antiestrogenic effects on endometriosis conditions and inhibiting the proliferation of endometrial cells. In this study, it was shown that as the genistein dose increased, the mean MMP-2 and MMP-9 levels fluctuated. At different doses give different responses. Genistein can reduce MMP-2 and MMP-9 levels by acting as anti-inflammatory and anti-angiogenesis in endometriosis cell cultures (Sutrisno et al., 2014).

This study used mice (*Mus musculus*) endometriosis model, where the level of estrogen levels in endometriosis tissue is very high.

Endometriosis is closely related to the presence of RE- α and RE- β . RE- α is associated with the effect of estrogen on cell proliferation, while RE- β is antiproliferative. Genistein has a 20-30 times higher affinity for RE- β than RE- α . Affinity will be further increased when genistein is given in high doses. Genistein which interacts with RE- β will be antagonistic (antiestrogenic) reducing estrogenic effects on endometrial tissue and perform its function in inhibiting RE- α from binding to endogenous estrogens by forming heterodimers with RE- α , which causes the corepressor protein to become active so that the process of gene transcription and protein synthesis is inhibited, resulting in a decrease in MMP-2 and MMP-9 levels. This provides an explanation that genistein therapy for endometriosis cases through an antagonistic effect that reduces the action of estradiol and causes a decrease in MMP-2 and MMP-9 levels .

CONCLUSION

Genistein has been shown to have an effect on decreasing levels of MMP-2 and MMP-9 in the peritoneal fluid of mice (*Mus musculus*) models of endometriosis.

SUGGESTION

Further studies are needed to see the effect of genistein on rabbits.

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