ORIGINAL ARTICLE

Different expression of NF-κB and endometrial implant width in the administration of red fruit (*Pandanus conoideus* Lam) and leuprolide

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ABSTRACT

Objectives: This study aimed to prove the effect of different administration of red fruit (*Pandanus conoideus* Lam) extract and leuprolide on the expression of NF-κB and endometrial implant width in mouse models of endometriosis.
Materials and Methods: This study used completely randomized design with pre-post separated sample. The sample size was 36 female mice (*Mus muculus*), which were divided into 3 groups randomly. They were given with cyclosporine A, estrogen and endometrial tissue to form endometriosis model.
Results: The mean expression of NF-κBin in treatment group receiving leuprolide (4.13 ± 0.70) and red fruit (2.70 ± 1.08) were significantly lower than that in control group (6.94 ± 1.007). Whereas, in treatment group receiving red fruit (2.70 ± 1.08), it was significantly lower than in those receiving leuprolide (4.13 ± 0.70). Mean endometrial implant width in treatment group receiving leuprolide (13.62 ± 3.21) and red fruit (8.93 ± 2.74) were significantly lower than that in control group (28.89 ± 8.28). Whereas, in treatment group receiving leuprolide (13.62 ± 3.21), it was not significantly higher than in those receiving red fruit (8.93 ± 2.74).
Conclusion: The expression of NF-κB was significantly lower after the administration of red fruit than leuprolide. The width of endometrial implant was not significantly lower after the administration of red fruit than after the administration of leuprolide.

Keywords: Endometriosis; NF-κB; red fruit, leuprolide; lesion; maternal health

ABSTRAK

Tujuan: Membuktikan adanya perbedaan pemberian ekstrak buah merah (*Pandanus conoideus* Lam) dan leuprolide terhadap ekspresi NF-κB dan luas implan endometriosis pada mencit model endometriosis.
Bahan dan Metode: Desain penelitian eksperimental laboratorium rancangan acak lengkap dengan sampel sebelum dan sesudah perlakuan yang berbeda. Sampel terdiri atas 36 ekor mencit betina yang dibagi 3 kelompok secara acak. Model endometriosis dibuat dengan pemberian injeksi siklosporin A, injeksi estrogen dan jaringan endometrium.
Hasil: Rerata ekspresi NF-κB kelompok perlakuan leuprolide (4,13 ± 0,70) dan buah merah (2,70 ± 1,08) lebih rendah secara bermakna dibandingkan kelompok kontrol (6,94 ± 1,007). Sedangkan kelompok perlakuan buah merah (2,70 ± 1,08) lebih rendah secara bermakna dibandingkan kelompok perlakuan leuprolide (4,13 ± 0,70). Rerata luas implan endometriosis kelompok perlakuan leuprolide (13,62 ± 3,21) dan buah merah (8,93 ± 2,74) lebih kecil secara bermakna dibandingkan luas implan endometriosis kelompok kontrol (28,89 ± 8,28), sedangkan kelompok perlakuan leuprolide (13,62 ± 3,21) lebih tinggi tapi tidak bermakna dibandingkan kelompok perlakuan buah merah (8,93 ± 2,74).
Simpulan: Ekspresi NF-κB lebih rendah secara bermakna setelah mendapat ekstrak buah merah dibandingkan dengan yang mendapat leuprolide. Luas implan lebih rendah tapi tidak bermakna setelah mendapat ekstrak buah merah dibandingkan dengan yang mendapat leuprolide.

Kata kunci: Endometriosis; ekspresi; NF-κB; buah merah; leuprolide; lesi; kesehatan ibu

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● pISSN:0854-0381 ● eISSN: 2598-1013 ● doi: http://dx.doi.org/10.20473/mog.V30I12022.1-9
● Maj Obs Gin. 2022;30:1-9 ● Received 14 Jun 2021 ● Revised 11 Sep 2021 ● Accepted 25 Sep 2021 ● Published 1 Apr 2022

INTRODUCTION

Current management of endometriosis is still controversial. The European Society of Human Reproduction and Embryology (ESHRE) guidelines are still stuck in hormonal modulation and/or surgical removal of the lesion.[1] Both of these approaches have many drawbacks. Hormonal modulation creates a hypoestrogenic environment with hormonal contraceptives, progestogens, anti-progestogens, GnRH agonists and aromatase inhibitors.[2] Creation of a hormonal environment by inhibiting ovulation can temporarily suppress ectopic implants and reduce inflammation and associated painful symptoms. This approach is not appropriate for endometriosis patients with infertility who wish to conceive normally.[1] After the discontinuation of hormonal therapy, the recurrence rate is also high. Patients also experience intolerable side effects, and sometimes do not respond or are resistant to therapy. On the other hand, surgical intervention often leads to complications and the outcome is highly dependent on surgical ability. Recurrence due to incomplete resection or the presence of hidden endometriosis is common.[3] All of these pose difficult clinical challenges in the management of endometriosis, especially for symptomatic deep infiltrating endometriosis (DIE) lesions. Therefore, new therapeutic options are needed.

Altered ability of the microenvironment in endometriosis to support endometriotic cells is transmitted by kinase signaling pathways and has the potential to establish kinase-dependent lesional growth. Drugs targeting these kinases have proven successful in the treatment of other tumors and are increasingly being investigated as potential endometriosis treatments, particularly in the treatment of DIE lesions.[3] NF-κB may represent a potential therapeutic target because it has constitutive activation in peritoneal endometriosis lesions. There have been many studies on the design of pharmacological methods that contribute to the activity of NF-κB.[4] Several previous studies in mice showed that the expression of NF-κB decreased when red fruit extract was given.[5] This study aimed to compare the administration of red fruit extract and leuprolide, which have been widely used in the treatment of endometriosis, on the expression of NF-κB and the width of endometrial implants.

MATERIALS AND METHODS

This research was a laboratory experimental study with a completely randomized design with pre- and post-separated sample. This study used a sample of 36 mice which were divided into three groups randomly. Then the mouse model of endometriosis were made by the injection of cyclosporine A, the injection of 0.1 ml endometriosis isolate and the injection of ethinyl-estradiol at a dose of 30 gr/kgBW. Group A was a positive control group, sacrificed on day 14 (before treatment). Group B received a single dose of leuprolide injection of 100 mcg/kg BW, sacrificed on day 14, and group C received red fruit extract of 0.05 ml per day per sonde for 14 days. Groups B and C were sacrificed on day 28. All three groups were examined for the width of endometrial implant and NF-κB expression. During the study 7 mice died.

RESULTS

Table 1 shows that the mean expression of NF-κB in leuprolide (4.13 ± 0.70) and red fruit (2.70 ± 1.08) treatment groups is lower than NF-κB expression in control group (6.94 ± 1.007). Analysis with Anova test showed that the expression of NF-κB in the treatment group was significantly lower than in the control group ($p<0.05$). Meanwhile, the expression of NF-κB in the treatment group that received red fruit was significantly lower ($p<0.05$) than in the treatment group receiving leuprolide.

Table 1. Expression of NF-κB in mice groups receiving leuprolide, red fruit, and control

c	n	Groups	NFκB expressions				p
			$\bar{x}$	SD	Min	Max	
Control	10	Leuprolide	4.13	0.70	2.80	5.20	0.000
6.94 ± 1.007		Red fruit	2.70	1.08	1.40	4.00	0.000
Leuprolide	9	Control	6.94	1.007	5.6	8.20	0.000
4.13 ± 0.70		Red fruit	2.70	1.08	1.40	4.00	0.003
Red fruit	10	Control	6.94	1.007	5.6	8.20	0.000
2.70 ± 1.08		Leuprolide	4.13	0.70	2.80	5.20	0.003

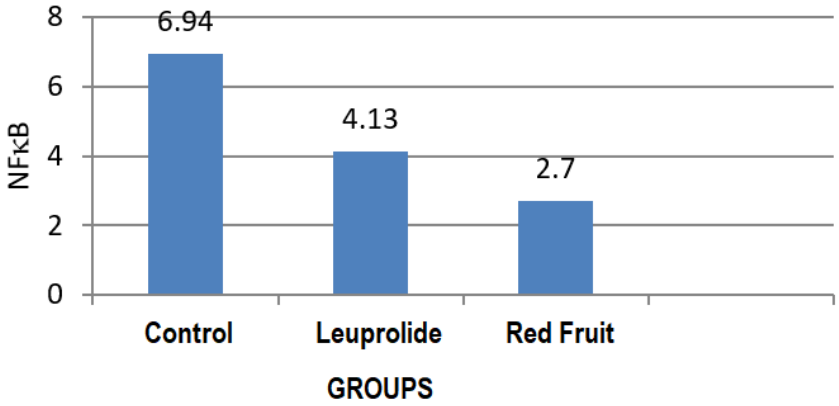

Figure 1. Expression of NF-κB in control, leuprolide and red fruit groups.

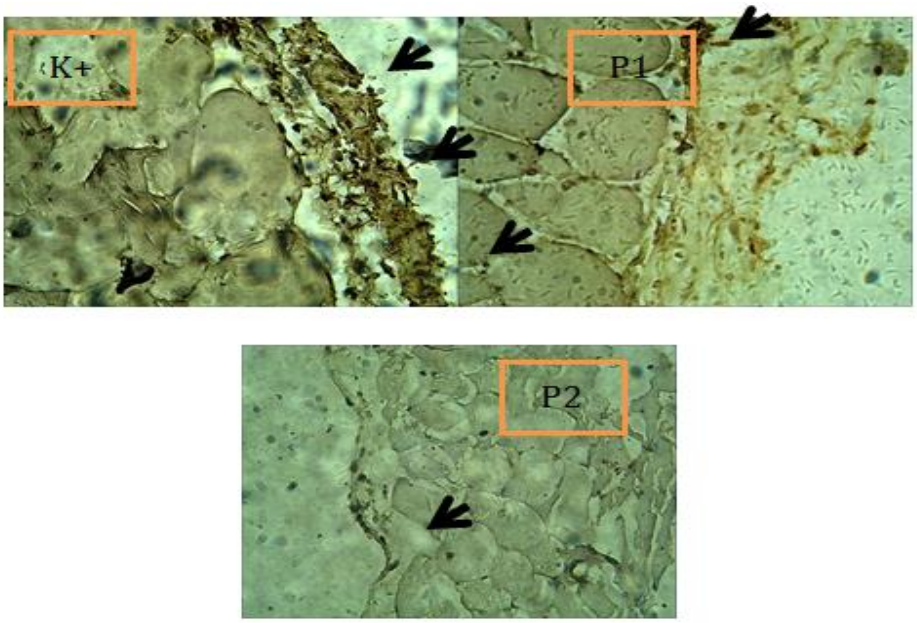

Figure 2. Differences in NF-κB expression (arrows) between K+, P1 (leuprolide) and P2 (red fruit) groups.

It was found that the expression of NF-κB in K+ group was stronger than in the other treatment groups, while the expression in P1 (leuprolide) group appeared to be stronger than in the other treatment groups. Its expression in P1 (leuprolide) group appeared to be stronger than in P2 (red fruit) group (Immunohisto-chemical staining, 400x magnification, Nikon Eclipse Ci, OptilabViewer 2.2, Image raster 3.0).

Table 2. The width of mice endometrial implant in leuprolide, red fruit and control groups.

Implant Width	n	Groups	Implant Width				p
			$\bar{x}$	SD	Min	Max	
Control	10	Leuprolide	13.62	3.21	9.34	17.54	0.000
28.89 ± 8.28		Red fruit	8.93	2.74	5.40	12.56	0.000
Leuprolide	9	Control	28.89	8.28	17.96	43.64	0.000
13.62 ± 3.21		Red fruit	8.93	2.74	5.40	12.56	0.074
Red fruit	10	Control	28.89	8.28	17.96	43.64	0.000
8.93 ± 2.74		Leuprolide	13.62	3.21	9.34	17.54	0.074

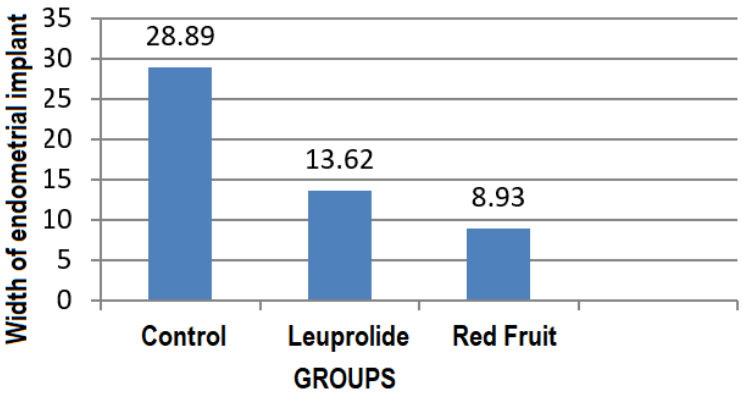

Figure 3. The size of endometrial lesions in control, leuprolide and red fruit groups.

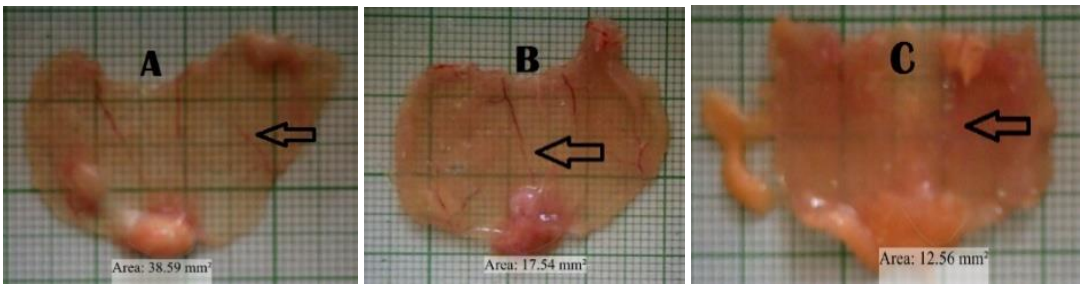

Figure 4. Profile of hypervascularization and implant width in the peritoneum of mouse model of endometriosis in control group (A), treatment group receiving leuprolide (B), and treatment group receiving red fruit (C).

Table 2 shows that the mean width of endometrial implants in leuprolide (13.62 ± 3.21) and red fruit (8.93 ± 2.74) treatment groups was smaller than the width in control group (28.89 ± 8.28). Anova test showed that the width of endometrial implants in treatment group was significantly smaller than the width in the control group ($p<0.05$), while the implant width in the leuprolide treatment group was higher but not significant ($p>0.05$) than in the treatment group receiving red fruit.

DISCUSSION

This study used a heterotransplantation technique, in which the tissue that was transplanted into the mice was derived from human endometrium. These implant fragments and endometriotic-like lesions resembled those found in the patients, both macroscopically and histologically, whereas the phase of the menstrual cycle at the time of tissue collection in humans did not appear to have an impact on the development of the ectopic lesions. Reservation of estrogen and progesterone receptors and estrogen responsiveness has been demonstrated in human ectopic tissue. Angiogenesis ensures maintenance of transplantation and systemic transport of administered drugs to human endometrial tissue. Vessel formation in mice occurs 4 days after the transplantation, independent of the ectopic lesion. When it is randomly inoculated into the peritoneal cavity, adhesion occurs within 2 days after implantation. The implantation site is mainly in the intestines of the abdominal wall muscles, liver and fat around the abdominal wall.[6] In a heterologous mouse model, human tissue is transplanted into the mice. Tissue maintenance in order to remain intact and well maintained requires a limited period of time. In most studies that have been performed, human endometrial cultures in mice have not exceeded 4 weeks. Three weeks after inoculation onwards, lymphocyte infiltration and other changes occur.[6] Therefore, in this study the process and intervention carried out on the mouse models did not exceed a period of 4 weeks.

Differences in NF-κB expression in mouse models of endometriosis in red fruit, leuprolide, and control groups

In endometriosis there is a chronic inflammatory process. Cytokines, as proinflammatory mediators such

as TNF-α, and IL-1β, will activate NF-κB through a canonical pathway. Stimulation of the canonical NF-κB pathway leads to IKKβ phosphorylation. IKKβ is part of the IKK complex along with IKKα and IKKγ and activated IKKβ to phosphorylate the inhibitory protein IκB at p50-p65, removing it from the complex and targeting it for proteasomal degradation. The unbound p50-p65 complex translocate into the nucleus and stimulates gene transcription.[3]

Differences in NF-κB expression in red fruit and control groups

This study showed that the mean expression of NF-κB in the treatment group that received red fruit (2.70 ± 1.08) was significantly lower than the expression of NF-κB in control group (6.94 ± 1.007). Many laboratory studies proved that various antioxidants had the ability to inhibit NF-κB activation with various mechanisms from phorbol ester to TNF-α, to hydrogen peroxide. These evidences prove the involvement of ROS in the NF-κB activation pathway.[7] Vitamin E derivatives inhibit TNF-α which induces NF-κB activation in human Jurkat T cells. Incubation of cells with different concentrations of α-tocopherol acetate or α-tocopherol succinate can inhibit NF-κB activation.[7] Only with 10 μM pentamethyl hydroxy chromane, a vitamin E derivative without a phytyl tail is able to completely inhibit NF-κB activation. A-tocopherol succinate not only inhibits the activation and translocation of NF-κB to the nucleus, but also inhibits in vitro binding of the active protein to κB DNA.[8]

The components of vitamin E that have this activity are tocopherols and tocotrienols. The tocopherol group has a saturated isoprene side chain and is divided into alpha, beta, gamma and sigma tocopherols. The tocotrienol group has an unsaturated isoprene side chain and is divided into alpha, beta, gamma, and sigma tocotrienols.[9] The biological activity of tocopherols is alpha>beta>gamma> sigma. The biological activity of vitamin E is related to its function in the body. Broadly speaking, the main function of tocopherols in vivo is antioxidant, that is by protecting the unsaturated fatty acids in cell membranes from peroxidative degradation. The action of vitamin E as an antioxidant can be demonstrated by two different mechanisms, ie. 1) vitamin E reacts directly with singlet oxygen and 2) vitamin E acts by capturing radicals derived from unsaturated fatty acids and stop autoxidation.[9]

The results of Selly's research[10] showed that the tocopherol content in red fruit was very high of 22940.35 ppm, and α tocopherol was 481.48 ppm. Research by Irawan[11] showed that the total tocopherol content of red fruit extract was 11000 ppm. Meanwhile, Susanti's research[12] showed that the total tocopherol and α-tocopherol in red fruit extract could reach 10832 ppm and 1368.26 ppm, respectively. In addition to containing high total tocopherols and α-tocopherols, red fruit extract also contains oleic acid, linoleic acid, linolenic acid and decanoic acid, all of which are active compounds to inhibit TNF-α which induces NF-κB activation in Jurkat T cells, inhibiting activation and translocation of NF-κB to the nucleus, and inhibited in vitro binding of the active protein to κB DNA. All of these activities cause the expression of NF-κB to decrease.

Differences in the expression of NF-κB in leuprolide and control groups

This study showed that the mean expression of NF-κB in leuprolide treatment group (4.13 ± 0.70) was significantly lower than in control group (6.94 ± 1.007). The positive interaction between ER and the NF-κB signaling pathway is explained by the finding that estradiol induces cell proliferation by an NF-κB-dependent mechanism, which is enhanced by the presence of TNF-α and the formation of a complex containing ER, P65 and the ras-related C3 botulinum substrate 3 (RAC 3) coactivator. In endometrial stromal cells, estradiol increases TNF-α-induced IL8 production through NF-κB activation.[4] A laboratory experiment conducted by Stice et al.[13] stated that E2 causes activation of p50 NF-κB. IκB degradation was also detected with E2. The p50 and p65 binding and nuclear translocations increased. The activation of NF-κB by E2 is through ERK 1/2. ERK 1/2 knockdown inhibits NF-κB activation by E2, which supports the notion that ERK 1/2 phosphorylation is the point of convergence for the upstream signaling pathway for NF-κB activation by E2. Leuprolide induces medical menopause by decreasing the hypothalamic-pituitary GnRH receptor, resulting in decreased gonadotropin secretion, suppression of ovulation and a significant decrease in serum estrogen levels.[14] Under hypoestrogen conditions, the expression of NF-κB will decrease.

Differences in NF-κB expression in red fruit and leuprolide groups

This study showed that the mean expression of NF-κB in red fruit treatment group (2.70 ± 1.08) was significantly lower than in leuprolide group (4.13 ± 0.70). Red fruit extract contains high total tocopherol and α-tocopherol and in addition it also contains oleic acid, linoleic acid, linolenic acid and decanoic acid, all of which are active compounds that will inhibit TNF-α to induce NF-κB activation in Jurkat T cells, inhibiting

activation and translocation of NF-κB to the nucleus, and inhibiting in vitro active protein binding to κB DNA. This activity is able to suppress the expression of NF-κB more strongly than leuprolide.

Differences in the width of endometrial implants in mouse model of endometriosis in red fruit, leprolide and control groups

The extent of endometriosis lesions was obtained by measuring the area of hyperemia in the form of red lesions on the peritoneal wall of the mice. This hyperemia area indicates the angiogenesis process to support the life and development of endometrial tissue in the peritoneum. There are some data to suggest that suppressed cytotoxic functions in the peritoneum may allow ectopic endometrial cells to survive and implant on the peritoneal surface. NK cells are cytotoxic lymphocytes with the ability to lyse target cells. Peritoneal NK cells are involved in retrograde menstrual clearance and endometriosis patients show decreased NK cell cytotoxicity. A study by Aoki et al.[15] using subcutaneously implanted human endometrial tissue in a mouse model of endometriosis, demonstrated the importance of NK cells in the formation of endometrial lesions. Lesions were found in only 40% of untreated mice, but were found in 100% of mice treated with NK inhibitors. In endometriosis there is a change in the immune system in the form of an immune system deficiency. Research on patients with endometriosis has shown changes in several immunological components in the peritoneal fluid, including macrophages, NK monocytes, T lymphocytes, B cells, inflammatory mediators such as complement and cytokines, and endometrial destroying cells that allow attachment, migration and angiogenesis.[16] Women with endometriosis have higher macrophage activity. This can be seen through an increase in size, an increase in the number of complement C3 and C4 and an increase in the secretion of lysosomal phospholipase. This phospholipase acts on the phospholipid membrane, releasing arachidonic acid which is then used for the synthesis of prostaglandins. This causes an increase in peritoneal prostaglandins in women with endometriosis and causes pain.

Macrophages play a role in the initiation, development and growth of endometriosis. Macrophages are also involved in the process of adhesion of endometrial cells to the peritoneum by secreting fibronectin which plays a role in changing cell stages in the cell cycle, so that they become cells that are sensitive to growth factors, and estrogen is one of the growth factors. Increased activation of macrophages accompanied by hyperestrogenism is a factor in the proliferation of endometrial implant cells.[17] Red endometriotic lesions and surrounding peritoneum were shown to have the greatest number of macrophages compared to black and white lesions, a finding consistent with the observation that red lesions are more inflamed and this may explain the difference in macrophage numbers seen in different studies. An increase in the number of macrophages can lead to a more inflammatory environment and increased production of inflammatory cytokines that will not only affect the endometriotic lesion, but also the surrounding area. The inflammatory response can lead to disease progression by stimulating cell proliferation, increasing cell adhesion protein expression and promoting neovascularization. Chronic inflammation within the peritoneum can also cause a fibrotic response and may be associated with progression to white fibrotic endometrial lesions. Retrograde menstrual blood can initiate an inflammatory response and macrophage recruitment but it is also possible that the inflammatory response precedes endometriosis and contributes to disease progression and sustaining its development.[18]

In this study, the mean width of the endometrial implant was found to be quite large in the leuprolide group, red fruit and control group. This shows that the statistical distribution of the width of endometrial implant in the leuprolide, red fruit and control groups each had a deviation that was far from the mean value. This condition could be caused by the process and the degree of occurrence of endometriosis lesions in each different mice, so that each sample started at a different level of endometriosis lesions. The level of immunity of each mouse was different, causing the level of endometriosis lesions formed was also different. In this study, randomization and strict control were carried out.

Difference in the width of endometrial implants in red fruit and control groups

In this study, the mean width of endometrial implant in the red fruit group (8.93 $\pm$ 2.74) was significantly smaller than the control group (28.89 $\pm$ 8.28). This research used red fruit that had passed a standardized extraction process. Red fruit, in addition to containing tocopherol, also has a high content of carotene. According to Budi,[19] red fruit contains 59.7 ppm β-carotene. The results of Susanti's research[12] showed that the total carotene content of red fruit extract extracted by modification method 2 could reach 21,430 ppm with β-carotene of 4,583 ppm. The high carotene content in red fruit can be seen from the color of the red fruit extract (in the form of oil) which is dark red. Red fruit, in addition to containing α-carotene and β-carotene, also contains oleic acid, linoleic acid, linolenic acid and decanoic acid, all of which are active compounds that have the potential as antioxidants and boost immunity. Natural substances that act as antioxidants can function

in preventing the development of cancer cells as well as regulating the balance of hormones that play a role in causing cancer.

In addition, the interaction of β carotene with protein is known to increase the production of antibodies in the body's immune system.[20] B carotene is able to increase the number of natural killer cells and increase the activity of T cells and lymphocytes. This will suppress free radicals, carcinogenic compounds, and the presence of cancer cells. Tocopherol plays a role in improving the immune system and reducing tissue cell mortality, while unsaturated fatty acids are easily digested and absorbed by the body so as to facilitate metabolic processes.[19] As we know that the development of endometrial implants outside the uterine cavity endometrium is associated with a chronic inflammatory process. In endometriosis, there is a decrease in the phagocytosis of macrophages, the number of NK cells and lymphocytes. The administration of red fruit extract which has a very high carotene content has been shown to increase macrophage phagocytosis, increase the number of NK cells and lymphocyte proliferation. In the final result, the width of endometrial implants in mouse model of endometriosis receiving red fruit extract was smaller than that in control.

Differences in the width of endometrial implants in leuprolide and control groups

In this study, the mean width of endometrial implants in the leuprolide group (13.62 $\pm$ 3.21) was significantly smaller than the width of endometrial implant area in control group (28.89 $\pm$ 8.28). Estradiol is a biologically active estrogen that is produced mainly at three sites in the body of women with endometriosis. In all these sites, the expression of aromatase enzyme is critical for the production of estradiol. In addition, several other steroidogenic proteins are expressed and complement aromatase activity for estradiol production. The classic site for estrogen production is the ovary. Theca and granulosa cells of the preovulatory follicle convert cholesterol to estradiol which is actively released into the circulation in a cyclic manner. The second group of the body collectively referred to as the peripheral tissues, includes large tissues such as fat, skin, and skeletal muscle, all of which express aromatase. In these peripheral tissues, circulating androstenedione is converted to estrone, which is further converted to estradiol. Peripheral tissues do not secrete estradiol in the classical sense, but, because of their large quantity, can produce sufficient levels of estradiol to increase blood levels, especially in obese women. The third site for estradiol production is the endometriotic tissue itself. Endometriotic stromal cells uniquely express a full complement of genes in the steroidogenic cascade, which is sufficient to convert cholesterol to estradiol.[21] A constant supply of estrogen from multiple sources is essential for the growth and persistence of endometriotic implants. As mentioned above, endometrial implants have intrinsic aromatase activity, which leads to the conversion of cholesterol to estradiol. The endometrium is rich in PG-E2 receptors and activation of the PG receptor subtype EP-2 leads to the activation of cyclic AMP, which increases steroidogenic gene expression, and aromatase activity ultimately leads to increased production of estradiol. Simultaneously, the intrinsic aromatase activity of estradiol produced from ovarian and peripheral fat also reaches the site of endometriosis. This continuous supply of estrogen is essential for the continued growth and survival of the endometrial implants.[22]

In another study, in endometriosis tissue there was an increase in the expression of the aromatase enzyme and a decrease in the expression of the 17β-hydroxysteroid dehydrogenase (17β-HSD) type 2 enzyme. This indicated an increase in the bioavailability of the estradiol concentration. Estradiol stimulates the production of prostaglandin E2 (PGE2) which then stimulates aromatase activity,[23] while leuprolide is the drug most widely used for medical treatment of endometriosis. Prolonged administration of leuprolide suppresses gonadotropin secretion and suppresses steroidogenesis. Leuprolide binds to the receptor for a long time and induces a prolonged period of down-regulation. Leuprolide is 200 times more potent than natural GnRH. Long-term use of leuprolide produces many endocrine effects. Serum estrone, estradiol, testosterone, and androstenedione levels decreased significantly. Total serum estrone and estradiol and serum estradiol concentrations are two to three times lower than those induced by long-term administration of danazol. Leuprolide therapy relieves symptoms in up to 90% of patients with endometriosis. Maximum therapeutic effect is seen with endometriotic spots smaller than 1 cm.[24] GnRHa can reduce inflammatory reactions and angiogenesis, induce apoptosis in tissues, and reduce proliferation in endometrial tissue.[25]

Differences in the extent of endometriosis lesions in red fruit and leuprolide groups

This study showed that the mean area of endometriosis implants in leuprolide treatment group (13.62 $\pm$ 3.21) was higher but not significantly different than that in red fruit group (8.93 $\pm$ 2.74). Anova test showed the results of $p > 0.05$. An in vitro study by Andini showed that red fruit has anti-proliferative activity against HeLa and K-562 cancer cells. Its anti-proliferative activity can approach, or even exceed, the activity of the positive control (doxorubicin) at higher concentrations.[26]

Endometriosis itself, although actually a benign disease, has features that resemble cancer cells, such as a tendency to invasion, uncontrolled growth, neoangiogenesis, and distant spread.[27]

In this study, red fruit extract was shown to suppress endometrial implants more strongly (but not statistically significant) compared to the effect of leuprolide. When compared to leuprolide, red fruit is cheaper and abundantly available in Indonesia, especially in Papua Island. The way leuprolide acts is through the hormonal pathway, so long-term use will cause many endocrine effects. Serum estrone, estradiol, testosterone, and androstenedione levels decreased significantly. The most common side effects of leuprolide are hot flushes, vaginal dryness, insomnia, etc. Decreased bone mineral density has been shown in the lumbar spine, but until now there have been no reports of endocrine effects in people who consume red fruit which until now is consumed freely by the Papuan people and is safe for long-term use. Research conducted by Widowati et al.[28] did not find any toxic effects as abnormal behavior or mortality in rats, which were given red fruit even at high doses, 5 ml/200 g body weight, or about 9.4 times the human dose. The expression of NF-κB in endometriosis model mice that received red fruit was significantly lower than in those receiving leuprolide, while the width of endometrial implant in the mouse models that received red fruit was found to be lower but not statistically significant than those receiving leuprolide. This is because the expression of NF-κB is a molecular change, while the implant area is a cellular change. Molecular changes are more sensitive and occur before cellular changes. Not all molecular changes are immediately followed by cellular changes. A certain amount of time is required for the cellular changes to follow.

CONCLUSION

The expression of NF-κB was significantly lower in the group of mouse models of endometriosis receiving red fruit extract than in those receiving leuprolide. The width of endometrial implant was lower but not significant in the group of mouse models of endometriosis receiving red fruit extract compared to those receiving leuprolide.

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