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Original Article

The Ethanolic Extract of Basic Leaves (Ocimum basilicum) Effect on Expression of Follicle Stimulating Hormone Receptors (FSHr) and Folliculogenesis in Female Mice Model Endometriosis

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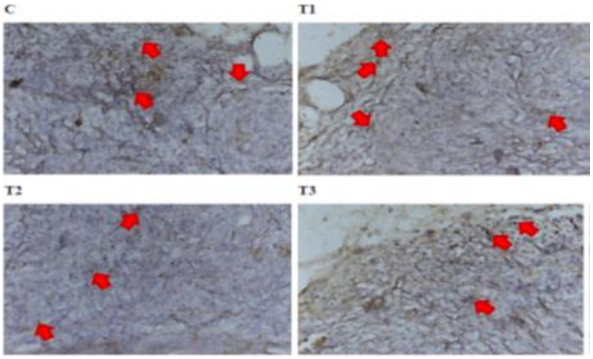
ABSTRACT

Background: Endometriosis is a tissue disease similar to the endometrial lining, either glands or stroma that grows outside the uterus which is one of the causes of infertility due to a decrease in the amount of folliculogenesis. Purpose/Aims. This study aims to analyze the effect of administration of ethanol extract of basil leaves (*Ocimum basilicum*) on the expression of follicle stimulating hormone receptor (FSHr) endometriosis tissue and folliculogenesis in female mice model endometriosis.
Method: Experimental research was conducted in the labomiceory with a simple experimental design (Posttest Only Control Group Design) involving two groups of subjects, one subject was treated (experimental group) with ethanol extract of basil leaves with a graded dose of 0.21mg/g-BW; 0.42 mg/g-BW; and 0.84 mg/g-BW and the other group was given a placebo (control group) by grouping 7 samples in simple random order per group. In the control group, 7 mices were terminated on day 15 to prove the endometriosis mice model. FSHr expression was evaluated from peritoneal tissue using immunohistochemistry and folliculogenesis was evaluated from ovarium using haematoxylin-eosin staining.
Results: This study showed a decrease in the percentage of FSHr expression between the control and treatment groups (p=0.259), an increase in the percentage of the number of antral follicles (p=0.203) and the number of corpus-luteum with significant values that were not significantly different (p=372) in endometriosis female mice model.
Conclusion: There was a decrease in FSHr expression especially at the dose of 0.42 mg/g-BW and an increase in the number of antral follicles and corpus luteum especially at the dose of 0.21 mg/g-BW, but not significantly.

GRAPHICAL ABSTRACT



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Introduction

Endometriosis is a tissue disease similar to the endometrial lining, either glandular or stroma that grows outside the uterus [1]. Endometriosis affects around 10% (190 million) of women and girls of reproductive age globally [2]. The disease is associated with severe pain and has a negative impact on life during menstruation, sexual intercourse, loose stools and/or urination, chronic pelvic pain, flatulence, nausea, fatigue, and sometimes depression, anxiety, and infertility. Until now, there is no medicine that can cure endometriosis, and treatment is still limited to controlling the symptoms [3].

Endometriosis is a disease that depends on the hormone estrogen. The hormone estrogen in endometriosis tissue is assumed from three sources, which are from the ovarium, external ovarium tissue (adipose tissue and skin) and the production of endometriosis tissue itself. The main source of estradiol is androstenedione (A) which is from the adrenals and ovarium, then is converted to estrone (E1) and then to estradiol (E2) [4]. Endometriosis is also able to produce local estrogen which comes from the activity of prostaglandin E2 (PGE2) which is induced by the enzyme cyclogeneses (COX)-2. Prostaglandin E2 stimulates the expression of all the genes encoding steroidogenic enzymes required for the synthesis of estradiol from cholesterol in stromal cells, especially STAR and CYP19A1 [5].

The use of herbal extracts has been proved to play a role in anti-inflammatory, anti-proliferative, anti-oxidant, as well as anti-angiogenic effects on endometrial and stromal cells/endometriosis lesions [6]. One of the plants from Southeast Asia, namely sweet basil (*Ocimum basilicum*) is a plant from the Lamiaceae family which is commonly used as an antipyretic, antifungal, analgesic, antiseptic, antibacterial, hepatoprotection, immune-modulator, anti-reppelent and anti-expectorant [6]. Basil leaves are plants that contain lots of essential oils, phenolic compounds and flavonoids. Flavonoids, including nevadensin, salvigenin, cirsileol, eupatorin, apigenin, acacetin, cirsimaritin, quercetin and ladanein, are among the major secondary metabolites identified in basil [7].

Another study that was also conducted in humans with methanol extract of basil leaves was in obese patients who were shown to suppress the mRNA expression of inflammatory cytokines, including IL-6, IL1β, TNF-α, and CCL2 [7]. Furthermore, the anti-inflammatory activity of basil leaves can affect immunological changes by increasing levels of IFN-γ, IL4, IgE, PLA2 and TP as well as the IFN-γ/IL4 ratio as a Th1/Th2 index [8].

The ethanol extract of basil leaves expected to be able to inhibit the activation of NF-Kβ and COX therefore decrease in the binding of FSH hormone by the FSH receptor, thereby decreasing local estrogen (E2) production in endometriosis tissue as a result of a decrease in macrophages, cytokines and prostaglandins which can increase the expression of TGF-β in theca cells and increase the amount of folliculogenesis. Decreased expression of FSH receptors in endometriosis tissue will suppress local estrogen and CYP19A1 production thereby suppressing the development of endometriosis tissue. In addition, increasing the capture of FSH from the pituitary will help the process of steroidogenesis thereby increasing the number of primary, secondary, tertiary, and *de graff* follicles in the endometriosis mice model. From the result of this study, it is hoped that in the future, the ethanol extract of basil leaves (*Ocimum basilicum*) can be used as a complementary therapy for endometriosis.

Materials and Methods

This research is experimental research conducted in the Faculty of Veterinary Medicine laboratory, Universitas Airlangga, Surabaya. The ethic commission has approved this study with the following number: 2.KEH.169.12.2022. The experimental design used in this research is a simple experimental design (Posttest Only Control Group Design). This design involved two groups of subjects, one subject was treated (experimental group) ethanol extract of basil leaves with 0.21 mg/g-BW; 0.42 mg/g-BW; and 0.84 mg/g-BW and the other group was given nothing (control group) C which was treated with 10% Na-CMC placebo with simple random sampling. The samples used were female aged 2 months with a body weight of 25–30 grams as many as 7 samples each group. FSHr expression was examined using immunehistochemistry and folliculogenesis using hematoxylin eosin. Statistical

analysis using the Mann Whitney test.

The materials used in this study consisted of a variety of substances, including 0.9% physiological Sodium Chloride (NaCl), Distillated Water (DW), Phosphate Buffer Saline (PBS), MEM, endometrial tissue from cysts modeled at Dr. Soetomo General Hospital in Surabaya, penicillin, streptomycin, estradiol, ethanolic extract of *Ocimum basilicum* leaves, cyclosporin A, mineral oil, 70% alcohol, sterile paper tissue, and distilled water. The equipment utilized in this investigation included a Pasteur pipette, Eppendroff, disposable petri dish measuring 30×80 mm, glass petri dish, 1 ml and 5 ml disposable syringes, laminar flow, inverted microscopes, centrifuges, Bunsen burners, analytical balance, 5% CO_2 incubator set at a temperature of 38.5 degrees Celsius, and a digital camera.

Mice (*Mus musculus*) were injected with cyclosporine A intramuscularly in the thigh at a dose of 10 mg/kg/BW. Mice were then injected with endometrial tissue taken by excision from the uterus of benign tumor surgery patients 1×1×1 in 10 mL stored in PBS (phosphate buffered saline). The endometrial tissue was then washed using a centrifuge at 2500 rpm. The supernatant was discarded and then PBS, penicillin 200 IU/ml, and streptomycin 200 µg/ml were added, then the endometrial basal tissue was taken with a 3 mL syringe. Then the mice were given a dose of 0.1 ml by injecting intra-peritoneally slowly for 60 seconds at 45^{o} from the mice's abdomen using a 1 mL disposable syringe, with a 16-gauge needle so that the endometrial tissue could enter. Furthermore, Ethinyl estradiol was administered intramuscularly to the thighs of mice on days 8 and 12, then after endometriosis treatment on day 14, it is hoped that mice will become models of endometriosis.

The same variety of Basil leaves (*Ocimum basilicum*) was harvested from the same agriculture in Medan (North Sumatra, Indonesia) in May 2022. The authentication of the basil leaves, flowers, and seeds was done in “Eka Karya” Bothanical Garden in Bali and the ethanolic extract of basil leaves was obtained in Pharmacology Laboratory of Veterinary Medicine, Universitas Airlangga. Dried basil leaves were chopped and blended into 100 grams powder and macerated in 900 ml of ethanol 96% for 48 hours at room temperature. The resulting solution was filtered using Wattman paper no. 40 and then shake using digital shaker with 50 rpm. At the end, the mixture was evaporated using rotary evaporator at 90 ^{o}C resulting 20 g of ethanolic extract. This extract was kept at 3-4 ^{o}C in an airtight container until it used.

The dose of ethanol extract of basil leaves which was given for 14 days orally and suspended by 1% Na-CMC solution refers to the research that regarding the effect of the ethanol extract of *Ocimum basilicum* leaves as an immodulator and anti-inflammatory in the rat (*Rattus norvegicus*) model of asthma [9]. In that study it was stated that the ethanol extract of basil leaves had an anti-inflammatory effect at doses of 150, 300, and 600 mg/kg-BW in rats. The dose conversion was carried out from rats weighing 200 g to mice weighing 20 g.

Follicle Stimulating Hormone receptor (FSHr) expression was examined using immunohistochemistry and folliculogenesis count using haematoxylin-eosin. The information was documented in Microsoft Excel 2016 and transferred to SPSS Version 25 for statistical assessment. Numerical measures were expressed as percentages. The data was presented as mean ± standard deviation (SD). The outcomes were evaluated with Kruskal-Wallis's test followed by the Mann-Whitney test. The statistical significance level used was 95%, with a significance value of 5%. Any p values that demonstrated $p < 0.05$ were deemed statistically significant.

Results and Discussion

The results of this study indicated that administration of ethanol extract of basil leaves (*Ocimum basilicum)* was able to reduce FSHr expression in endometriosis tissue of female endometriosis mice model but this was not statistically proven. The variation in the doses given also did not show a significant difference between all treatment groups. The treatment group that was given *Ocimum basilicum* ethanol extract 0.42 mg/g-BW (treatment 2 or T2) showed the lowest average value of FSHr expression (16.285 ± 6.89), followed by P3 (23.28 ± 15.649), and P1 (25.14 ± 7.998). The highest mean FSHr expression was in control group (C) which was only given placebo (28.6 ± 12.998). The highest number of antral follicles was in the 0.21mg/g-BW group (treatment 1 or T1) with a

mean (2(0-5)), then T3 (1(0-4)) followed by C (1(0-2)). The lowest number of antral follicles is at T2 (0(0-1)). The highest number of corpus luteum was in the 0.42 mg/g-BW group (T2) which had the same value as C(2(0-3)), then T1 (1(1-3)) and lowest was T3 (0(0-3)). The distribution of FSHr expression data is normal and homogeneous so that it can be continued with the *One-Way ANOVA* test. The results of the different test with *One-Way ANOVA* showed a value of p> 0.05 while folliculogenesis was not normally distributed therefore proceed with the Kruskal-Wallis test. The results of the Kruskal-Wallis test showed a value of p > 0.05.

The result of the statistical data are shown in Table 1 with the average expression of FSHr in the treatment group (T1, T2, T3) decreased in the average percentage of FSHr expression along with increasing doses of basil leaves ethanol extract in T1, T2, T3 endometriosis female mice model compared to the control group (C). The results of the *One-Way Anova* statistical test with a confidence level of 0.05 obtained a result of 0.259 (p-value ≥0.05), so it was concluded that there was no significant difference between the expression value of FSHr in the group that is not given basil leaves ethanol extract (C) and those given basil leaves ethanol extract bacilli (T1, T2, and T3). Meanwhile, the mean percentage number of antral follicles T1 (2 ± 1.91) increased the highest followed by T3 (1.28 ± 1.60) compared to C (0.85 ± 0.9). At T2 (0.28 ± 0.49) it decreased compared to C. The test results showed that the ethanol extract of basil leaves at the lowest dose of 0.21g/g-BW were able to increase antral follicles compared to controls. This can be seen from the average percentage that is higher than placebo as a control, as well as a decrease in the number of follicles with increasing doses in the endometriosis mice model. When the Kruskal-Wallis test was carried out, it was said to be significant if the results were less than or equal to 0.05. In the number of antral follicles, a sig value of 0.203 (p-value ≥0.05) was obtained, thus there was no statistically significant difference (Table 2).

Table 1. Mean and standard deviati00on value of FSHr expression

Group	n	Mean ± Std. FSHr	p-value
C	7	28.6 ± 12.998	0.259
T1	7	25.14 ± 7.998	
T2	7	16.285 ± 6.89	
T3	7	23.28 ± 15.649	

Table 2. Kruskal-Wallis test result for antral follicles in endometriosis mice model which given *Ocimum basilicum* extract as a complementary therapy for endometriosis

Group	n	Median (Min-Max) Antral Follicle	p-value
C	7	1 (0-2)	0.203
T1	7	2 (0-5)	
T2	7	0 (0-1)	
T3	7	1 (0-4)	

Table 3. Kruskal-Wallis test result for corpus luteum in endometriosis mice model which given *Ocimum basilicum* extract as a complementary therapy for endometriosis

Group	n	Median (Min-Max) *Corpus luteum*	p-value
C	7	2 (0-3)	0.372
T1	7	1 (1-3)	
T2	7	2 (0-3)	
T3	7	0 (0-3)	

Whereas in the corpus luteum, the test results showed that the ethanol extract of basil leaves at the lowest dose of 0.21 g/g-BW were able to increase the corpus luteum compared to the control. This can be seen from the higher average percentage of placebo as a control, the second dose of 0.42 g/g-BW and the highest dose of 0.84 g/g-BW for increased folliculogenesis in the endometriosis mice model. However, the results of the Kruskal-Wallis test get significant results if it is less than equal to 0.05. In the corpus luteum, a sig value of 0.372 (p-value ≥0.05) was obtained, thus the results showed that there was no significant difference (Table 3).

Besides being present in the gonads, the FSH receptor (FSHr) is also present in certain extragonadal tissues, albeit at a low level. The mRNA and protein levels of FSHR are observed in endometriosis lesions and normal secretory phase endometrium. Prior research discovered that endometriotic tissue generates significantly greater quantities of estrone (E1) and E2 compared to normal secretory endometrium [10]. FSH also plays a role in angiogenesis and inflammation in the pathophysiology of disseminated endometriosis lesions [11].

Endometrial estrogen production contributes only a few to serum estrogen compared to ovarian estrogen production. It is estimated that the relationship between poor estrogen production between the endometrium and endometriosis is caused by different CYP19A1 expression levels, substrate availability and metabolism. According to reference [9], the levels of E1 and E2 in endometriosis are not consistent with the levels found in serum estrogen or endometrial tissue, indicating that there is a tissue-specific mechanism regulating the expression of CYP19A1 and estrogen production and metabolism. Reference [9, 10] states that in endometriotic lesions, E2 is the main estrogen and is present at higher concentrations than E1 throughout the menstrual cycle. The elevated steroid hormone levels in endometriosis tissue are directly linked to increased expression of StAR, HSD3B2, CYP17A1, CYP19A1, and SF1, and decreased expression of CYP11A1 [9, 12]. In the human endometrium during the secretory phase of the menstrual cycle, local estrogen production has also been observed. Although local estrogen production and overexpression of CYP19A1 have been linked to ovarian endometriosis and endometrial cancer, this does not necessarily exclude the possibility of it occurring in the normal endometrium [13, 14], as per the reference material.

In this study, complementary therapy for endometriosis used ethanol extract of basil leaves at a dose of 0.21 mg/g-BW; 0.42 mg/g-BW; 0.84 mg/g-BW to suppress FSHr expression in endometriosis were proven to be able to suppress the average percentage as shown in table 5.1, but did not have a significant difference (p>0.05). This is in line with research conducted by [15] that treatment with an emphasis on FSHr can be an alternative to endometriosis [15]. The decrease in FSHr expression in this treatment group was due to basil leaves extract containing antioxidants such as phenols, flavonoids, carotenoids, ascorbic acid, riboflavin, and thiamine which are able to protect reproductive organs from oxidative stress due to increased ROS in the body [16]. In patients with endometriosis, increased production of ROS by mononuclear cells in the peritoneal cavity. ROS release is induced by stimulation of macrophages in the peritoneal cavity [17]. In the treatment group 1, the ethanol extract of basil leaves at a dose of 0.21 mg/gBW could not reduce FSHr expression in endometriosis tissue, presumably because it could not suppress oxidative stress caused by endometriosis. The levels of exogenous antioxidants obtained are not sufficient to balance the levels of oxidants in the body so that the levels of oxidants or ROS as a cause of oxidative stress are still high compared to antioxidants.

Treatment group 2 which was given basil leaves ethanol extract at a dose of 0.42 mg/gBW could not significantly reduce FSHr expression, but showed a decrease in the highest average percentage of FSHr expression in the endometriosis mice model. The levels of exogenous antioxidants obtained from the ethanol extract of basil leaves is not sufficient to balance the levels of oxidants in the body so that the levels of oxidants or ROS as the cause of oxidative stress are still high compared to antioxidants.

Numerous studies have demonstrated that the use of leaf powder from the Ocimum genus can effectively reduce FSH and LH levels while also increasing testosterone levels and decreasing sperm count in male rabbits [18, 19]. The existence of certain substances, including eugenol, euginal, ursolic acid,

sitosterol, and stigmasterol, in basil leaves suggests that there are phytochemicals present that act as either Phyto androgens or phytoestrogens. These compounds have the ability to negatively regulate the secretion of FSH, LH, and sperm count through negative feedback in the pituitary gland [20].

Treatment group 3 could not significantly reduce FSHr expression, presumably due to administration of ethanol extract of basil leaves at a dose of 0.84mg/g-BW, which is included in a dose that can cause toxicity in , causing a condition called "antioxidant stress" [21]. This statement is in line with [22] who gave doses of basil leaves extract with graded doses of 140, 280, and 560 mg/kg-BW in exposed to lead acetate resulting in experiencing an increase in Serum Glutamic Oxaloacetic Transaminase (SGOT) values at doses 560 mg/kgBW compared to a dose of 280 mg/kg-BW. Increased levels of SGOT which is an important indicator of liver function related to toxicity that occurs in the body. It will disrupt the balance of oxidant-antioxidants when antioxidant levels exceed oxidant levels.

Free radicals or low ROS that function in chemical signaling that regulates glucose metabolism, cell growth, to proliferation. Excess antioxidants result in the body being unable to distinguish between radicals that play a physiological role and radicals that cause oxidative damage to biomolecules. Elimination of all free radicals will not prolong the body's normal functions, but on the contrary, will damage the body's overall performance [23, 24].

The increase in FSHr which coincided with the increase in the dose of ethanol extract also did not rule out the possibility of a sign of a polymorphism gene in FSHr due to the appearance of an anomaly at the third dose. This cannot be confirmed because there was no examination of the polymorphism gene in FSHr [25].

At all stages of folliculogenesis, most of the follicles will experience atresia and only a few qualified follicles will become graff follicles or mature follicles ready to be fertilized. In primordial follicles and primary follicles, atresia in the follicle begins with the process of oocyte apoptosis which is then followed by granulosa cell apoptosis. Whereas atresia of secondary, tertiary, and de graff follicles begins directly with apoptosis of granulosa cells [26].

Research on giving complementary therapy as a treatment for infertility from herbal medicines has been carried out to date. One of them uses ethanol extract of basil leaves. According to a study conducted on healthy adult albino mice, maceration of basil leaves extract can enhance the growth and development of follicles, which in turn increases the synthesis and secretion of estradiol, thereby increasing the preparation of the uterus for implantation [27]. In other Lamiacea family plants, ethanol extract of basil leaves (*Ocimum sanctum*) is very helpful in treating clinical and pathological disorders of polycystic ovarian syndrome, preventing ovarian cell dysfunction, increasing fertility, and significantly increasing uterine weight [28].

The result of this study indicates that the ethanol extract of basil leaves can increase the number of antral follicles and the corpus luteum. Of the three doses used (0.21mg/gBW; 0.42mg/gBW; and 0.84mg/gBW) it showed that only the doses of 0.21mg/gBW and 0.84mg/gb could increase the number of antral follicles and corpus luteum compared to the control group, however there was no significant difference between the graded doses used. This shows that giving the lowest dose of 0.21 mg/gBW of ethanol extract of basil leaves in this study has the potential to be quite effective for increasing the number of antral follicles and corpus luteum. This study is in line with the administration of curcumin extract as a complementary therapy to endometriosis model, in which increasing the dose decreases the amount of folliculogenesis [24].

Granulosa cell apoptosis leads to a prolonged follicular period which hinders follicular growth and results in impaired oocyte follicle growth, oocyte proliferation, and differentiation. This leads to a decline in FSH and GDF9 levels. The expression mechanism of FSH receptors, activin, and GDF-9 produced by granulosa cells is disrupted, and they are unable to stimulate FSH receptor expression through an autocrine/paracrine mechanism.

Due to excessive granulosa cell apoptosis, FSH and GDF-9 levels in granulosa cells decrease, which hampers enzymatic activity necessary for catalyzing androgen aromatization to produce estrogen. As a consequence of this, the steroidogenesis process is suppressed, and there is a reduction in estrogen sensitivity which results in feedback to the anterior

pituitary gland to lower LH production by GnRH. This, in turn, impacts the process of oocyte maturation, leading to a prolonged follicular period and impaired folliculogenesis [28].

In the treatment group 1 with a dose of 0.21 mg/gBW, the average number of antral follicles and corpus luteum was high. This may be due to the less effective doses of ethanol extract of basil leaves in this group, so the process of inhibiting several cytokines such as TNF-alpha, COX-2 and NF-kB is not optimal in improving endometriosis conditions [28].

The prolongation of the estrus cycle is in line with studies of giving basil leaves extract to healthy mice which resulted in an extension of the estrus cycle [27]. The results of this study indicated that there was an extension of the length of the estrus cycle, especially during the estrous and met-estrous phases due to the administration of basil leaves extract. Giving basil leaves extract at the end of the estrus phase causes an increase in the length of the estrus and metestrus phases. This is because the content of basil leaves in the form of steroids (sitosterol) can turn into estrogen through the aromatization process so that at the end of the estrous phase where the concentration of estrogen begins to decrease will increase again and cause the signs of estrus to be maintained. Ahmed et al. (2002) stated that giving basil leaves for a long time can cause changes in the estrus cycle, which is the prolongation of the estrus cycle.

The flavonoids present in basil leaves such as orientin, vicenin, and isoflavones exhibit estrogenic properties by binding to the estrogen receptors in the body, as per the research conducted by Mousavi et al. (2018). These isoflavones can mimic the effects of estrogen in various tissues, and can induce negative feedback to the pituitary gland, leading to the reduction of endogenous estrogen levels [29].

Various studies have produced different findings. While the expression of FSHr in endometriosis tissue reduced by an average percentage at doses of 0.42mg/gBW and 0.84mg/gBW, the number of antral follicles and corpus luteum decreased simultaneously. Basil leaves, which contain isoflavones and eugenol, also include sitosterol and stigmasterol, both of which are androgenic, according to reference [30]. Therefore, the decrease in the number of antral follicles and corpus luteum may be caused by an increase in sitosterol and stigmasterol. Furthermore, sitosterol and stigmasterol are androgen precursors that can convert to testosterone, resulting in increased testosterone levels and inhibition of ovarian follicular development [30].

The main function of endogenous estrogen is to enhance the growth and proliferation of tissues and organs that possess estrogen receptors [29]. This steroid hormone is produced from cholesterol by the theca interna cells and granulosa cells located in ovarian follicles, and to a lesser extent by the corpus luteum, placenta, and adrenal cortex. In response to follicular development driven by FSH, estrogen levels increase during the menstrual cycle

In the final stage of the follicular phase, estrogen gives rise to positive feedback on GnRH levels, prompting the secretion of LH and causing ovulation to occur [29]. Consequently, it is proposed that the use of basil leaves, which possess estrogenic properties, may be an effective method for introducing negative feedback to the hypothalamus and pituitary gland, thereby suppressing the production of FSH and LH. This in turn leads to an arrested follicle development, preventing ovulation from taking place [31].

Conclusions

After analyzing the data, it can be inferred that the average percentage of FSHr expression decreased in the treatment group compared to the control group. The increase in the mean percentage of antral follicles and corpus luteum was not significantly different between the two groups ($p>0.05$) in the female mice model of endometriosis. Future research should focus on evaluating the FSHr polymorphism gene and the extent of endometriosis lesions. Additionally, the use of ethanol extract of basil leaves as a complementary therapy should be reconsidered as it is believed that the suppression of FSH receptors in endometriosis tissue may affect FSHr expression in granulosa cells.

Study Limitations

This study was limited to examining the expression of follicle stimulating hormone receptor (FSHr) and the amount of folliculogenesis, did not measure the extent of the lesion to prove that decreased and increased FSHr expression was associated with the

severity of endometriosis and increased doses of basil leaf ethanol extract, did not examine the possibility of the FSHr polymorphism gene, did not examined the quality of folliculogenesis, did not examine estrogen levels as a consequence of giving phytoestrogens to endometriosis model mice and did not perform toxicity tests on ethanol extract of basil leaves.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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