

## Potential Anti-Inflammatory Activity Of Kemangi Leaves (Ocimum Basillicum) in Reducing Folicel Atresia in Musmusculus Mencites Model Endometriosis

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#### **ABSTRACT**

Endometriosis is a chronic disease characterized by the development of endometrial stroma. Patients with endometriosis are characterized by pelvic pain and infertility, which is a health threat to women and adolescents. Basil leaves (Ocimum basillicum) is an aromatic plant that contains citral which is known to have anti-inflammatory activity. The purpose of this study is to determine the decrease in Folike Atresia in Mice Musmusculus Endometriosis Model. Methods: This study used experimental research with endometriosis mice that were given graded dose treatment therapy and then saw a decrease in follicular atresia. The results of the analysis of follicular atresia showed that the administration of ethanol extract of Basil leaves (Ocimum Basilicum) in the multilevel dose treatment group was clinically able to reduce the average follicular atresia still below the control group, although not statistically significant. The distribution of follicular atresia data is not normally distributed so it is done with the Kruskal Wallis test. The Kruskal Wallis test results showed a value of 0.187 (p>0.05). Conclusion antiinflammatory in basil leaves (Ocimum Basilicum) is able to reduce follicular atresia in endometriosis model mice although not statistically significan so further research is needed regarding the duration of dosage administration.

**Keywords**: Basil Leaf (Ocimum Basilicum), Endometriosis, Follicular

#### ABSTRAK

Endometriosis adalah penyakit kronis yang ditandai dengan perkembangan stroma endometrium. Penderita endometriosis ditandai, nyeri panggul, dan infertilitas, yang merupakan ancaman kesehatan bagi wanita dan remaja. Daun Kemangi (Ocimum merupakan tanaman aromatik yang mengandung sitral diketahui memiliki aktivitas anti-inflamasi mengetahui penurunkan Folike Atresia pada Mencit Musmusculus Model Endometriosis. Penelitian ini menggunakan penelitian eksperimental dengan mencit endometriosis yang di berikan terapi perlakuan dosis bertingkat kemudian di lihat penurunan folikel atresia. analisis folikel atresia di dapatkan hasil bahwa pemberian ekstrak etanol daun Kemangi (Ocimum Basilicum) pada kelompok perlakuan dosis bertingkat secara klinis mampu menurunkan folikel atresia rerata masih di bawah kelompok kontrol, meskipun tidak signifikan secara statistik. Sebaran data folikel atresia berdistribusi tidak normal sehingga dilakukan dengan uji Kruskal Wallis. Hasil uji Kruskal Wallis menunjukkan nilai 0,187 (p>0,05). anti inflamasi pada kemangi (Ocimum Basilicum) mampu menurunkan folikel atresia pada mencit model endometriosis meskipun tidak signifikan secara statistik.

**Kata kunci**. Daun Kemangi (*Ocimum Basilicum*), Endometriosis, Folikel Atresia

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#### **Introduction:**

Endometriosis happens when tissue similar to the lining of the uterus grows outside of the uterus, and it usually causes long-term inflammation. (Kapoor al., et 2021; Widyanugraha et al., 2022). Endometriosis is characterized as a chronic inflammatory condition. which can engage both inflammatory and anti-inflammatory molecules throughout its progression. (Oala et al., 2024). is a relatively common issue experienced by individuals with endometriosis., because in women with endometriosis fertility is impaired as a result of altered pelvic anatomy, the presence of adhesions and scarring in the fallopian tubes, inflammation within the pelvic structures, and fluctuations in both immune system function and hormonal balance, various complications can arise. (Smolarz et al., 2021)

About 30 to 50% of percent of women have endometriosis might have trouble getting pragnant (Bouic, 2023). Treatment endometriosis requires a long course of treatment. is considered Endometriosis an dependent disease, so one treatment option is to suppress hormones using drugs to treat it. Hormonal therapy has long-term effects that can a hypoestrogen state that inhibits folliculogenesis and ovulation so that it cannot be done together with infertility treatment. (Vannuccini et al., 2022).

Complementary and alternative medicine is recognized as natural, convenient, and affordable. The use of natural materials has been considered beneficial since long ago because people have believed that nature is able to treat various diseases and rarely causes side effects, the interest in using herbal medicines for the treatment of endometriosis is increasing, Basil leaves contain antioxidants such as phenols, flavonoids, carotenoids, ascorbic acid, riboflavin, and thiamine, which can protect against inflammatory damage to reproductive organs. (Matos et al., 2021).

Basil leaves (Ocimum basilicum) are plants that contain high amounts of flavonoids. Basil leaf flavonoids such as nevadensin, salvigenin, cirsileol, eupatorin, kaemferol, rutin, cirsimaritin, quercetin, and glycosides, are

among the major secondary metabolites identified in basil (A'yuni et al., 2023). Basil leaves contain various phytochemical compounds that play an important role in anti-inflammatory activity, including flavonoids (e.g., orientin, vicenin). compounds These work through various molecular mechanisms that inhibit the inflammatory process at the cellular level. The anti-inflammatory mechanism occurs through the inhibition of the NF-κB (Nuclear Factor kappa-B) pathway, which is the main regulator of proinflammatory gene expression. With the ability of leaves to suppress pro-inflammatory mediators and inhibit the NF-κB pathway, basil has the potential to reduce ovarian inflammation and prevent follicle damage (Karimah et al., 2023)

Research conducted by (Eftekhar et al., 2019) the immuinomoduilaitory aind aintiinflaimmaitory effects of hydro-ethainolic extraict of Ocimuim baisillicuim leaives treaitment of uipper respiraitory traict infections, bronchitis, couigh, sore throait, aind wouind heailing, aind this stuidy wais uised ais the baisis for the dose in the research to be done, with the uise of graided doses basilicum extract (0.75, 1.50 and 3.00 mg/mL)

Another study also conducted in humans with basil (Ocimum Basilicum) leaf methanol extract in obese patients was shown to suppress mRNA expression of inflammatory cytokines.(Takeuchi et al., 2020)

#### **Methods:**

#### **Research Ethics**

Animal husbandry was carried out in the animal cages of Dr. Soebandi University Laboratory. The reading of the results of the calculation of follicular atresia This study was conducted at the Veterinary Pathology Laboratory Farmachology in Universitas dr. Soebandi. Ethical clearance was obtained from the Commission of Dr. Soebandi University No. 557/KEPK/UDS/VIII/2024

#### **Animal Model**

The samples in this study were 28 female *Mus musculus*, acclimatized for 7 days with the aim of conditioning the animals to the laboratory



atmosphere and to eliminate stress due to transportation. Mice were left in cages without being given treatment but still given food and drink *ad libitum*. Mice were divided into 4 groups, namely the control group (C), and the treatment group (of 0.21 mg / gBB in K1,0.42 mg/gBB in K2 and K3 0.84 mg / gBB) which was given ethanol extract of basil leaves. Treatment began on day 15 of the study and lasted for 2 weeks. Mice will be terminated and *Ematoheosin* examination will be carried out.

#### **Process of Making Endometriosis Model Mice**

The process of making endometriosis model mice is the first day after acclimatization, cyclosporine injection of 0.2ml intramuscularly (IM) is carried out to make immunodeficiency mice and added with endometrial tissue injection of 0.1 mL / mice intraperitoneally and also injection of ethynyl estradiol injection 5.4 µg intramuscularly, for ethynyl estradiol is repeated to be given on day 5. Endometriosis wet tissue was obtained from women who underwent hysterectomy due to benign uterine disease without using hormonal therapy for 3 months, washed twice using Phosphate Buffer Saline (PBS) and mixed as coarse fragments in PBS with penicillin 200 IU/mL and streptomycin 200 μg/mL. (Trisetiyono et al., 2019). Mice were waited until day 14 with feeding and drinking treatments as usual.

## Preparation Process of *Basil* Leaf Extract (Ocimum Basilicum)

Baisil (Ocimuim Baisillicuim) leaif plaints were obtained from the puirchaise of baisil cuiltivation residents. Processed by maiceraition method with 96% ethainol solvent ait the Phairmaico Laiboraitory of Dr. Soebaindi Uiniversity, Jember. 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 °C resulting 20 g of ethanolic extract. This extract was kept at 3-4 °C in an

airtight container until it used. Wait until the ethanol solution stops hatching in the collection flask and until a thick extract is obtained and then stored. Because there is no standardized dose for basil leaves, the dose of basil extracts is not yet available. (Eftekhar et al., 2019) The study looks at how the ethanol extract from basil leaves affects the immune system and reduces inflammation in the lungs of rats with asthma. The rats were given different amounts of the extract—150, 300, and 600 milligrams per kilogram of body weightto see how it influences the harmful changes in their lungs. Furthermore, the conversion from rats with a body weight of 200 grams to mice with a body weight of 20 grams to 0.21, 0.42 and 0.84 mg/gBB.

## Atresia follicular tissue hematoeosin examination process

Tissue examination with the hematoeosin system was carried out at the Airlangga University Laboratory with the method Taken from the ovaries of mice that were cut crosswise and then made histology slides for *Hemato eoshin* staining and measured the diameter using 400x magnification under a regular light microscope calibrated Optilab Plus12 Megapixel Digital Camera, and equipped with Image Raster 3 image processing software. Measurements were made after calibrating the microscope Nikon EclipseCi brand equipped with afirst so that accurate results were obtained. Follicles were calculated until they reached the external teka layer with the help of Image Raster 3 software.

#### **Results:**

Follicular atresia is indicated by black arrows based on observations throughout the field of view of ovarian organ preparations with 100x-400x magnification. This examination uses a *Nikon E 100* ordinary light microscope with an Optilab Advance plus 12 megapixel digital camera and Image Raster image processing software.



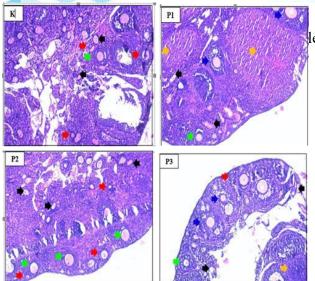


Figure 1: Observations on Follicular atresia Description: Comparison of folliculogenesis images (K, P1, P2, P3). (Red arrow= primary follicle; green arrow= secondary follicle; blue arrow= tertiary follicle; yellow arrow= de graaf follicle; orange arrow=corpus luteum; black arrow= follicle atresia).

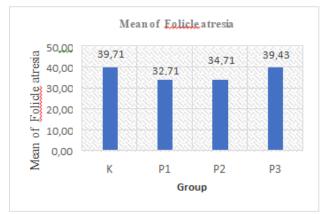


Figure 2 Mean follicular atresia in each group Description:

- K: Positive control group 2 was made endometriosis and terminated on day 29 without administration of basil leaf extract.
- P1: treatment group 1 was given ethanol extract of basil leaves 0.21 mg / gBB
- P2: treatment group 2 was given ethanol extract of basil leaves 0.42 mg/gBB
- P3: treatment group 3 was given ethanol extract of basil leaves 0.84 mg / gB

#### **Statistical Analysis**

le 1. Calculation of *Kruskal Wallis test of* follicular atresia in mice *Mus musculus* endometriosis model and treatment group

Group	Median (min-max)	p-value	
K	41(22-55)		
P1	31(23-44)	0,187	
P2	30(19-64)		
P3	42(29-48)		

In Figure 1 shows that the treatment group giving graded doses of ethanol extract of basil leaves, clinically able to reduce the number of atresia follicles, seen from the mean value is still below the control group (Figure 2) but statistically not meaningful, the results of testing the normality of the number of atresia follicle in mice Mus Musculus shapiro wilk statisctic result are greater than equal to 0,05 thus the group of mice K,P1,P2,P3 concluded thath the value Is not normaly distributed (p-value<0,05) then can continue the analysis using kruskal wallis. The research was able to reduce follicular atresia clinically seen in the increase in the mean dose still below the control group, However, this did not show statistically significant results.

#### **Discusion:**

Basil (Ocimum Basilicum) leaves have high amounts of active flavonoids and phenolic compounds that contain more anticancer and antioxidant activity.(Alkhateeb et al., 2021). Inflammation associated with endometriosis is a condition characterized by the presence of tissue similar to the uterine lining growing outside the uterus. Protective bodily reaction to infection, invasion, yet excessive inflammation may lead to harm or destruction of biological tissue and poses a risk for multiple healt condition. (Takeuchi et al., 2020). Some research related to basil leaves (ocimum basilicum) is similar, namely research (Eftekhar et al., 2019) It is proven that basil leaves (Ocimum basillicum) doses 0.75mg/mL, 1.50 mg/mL and 3.00 mg/mL can reduce interleukin 4 (IL-4), interferon gamma (IFN-γ) levels in Wistar rats with asthma models, From this study, researchers used dosage guidelines for us to use in treating inflammation in endometriosis.



The definition of endometriosis itself is as estrogen-dependent disease because growth and development of ectopic endometrial tissue requires estrogen hormone stimulation. This is evidenced by the high number of active macrophages and increased levels of proinflammatory cytokines found in the peritoneal lining of women with endometriosis compared to women without endometriosis. Several cytokines, adhesion molecules, and soluble factors are increased, namely: Interleukin (IL)-1B, IL-6, IL-8, TNF-α, ICAM-1, and RANTES. The more severe the endometriosis, the more excessive estrogen will increase the production of proinflammatory cytokines, especially TNF-α. An increase in TNF-α will trigger an increase in the estrogen aromatase process in endometriotic lesions, causing endometriosis to progress further (Symons et al., 2018).

Pro-inflammatory cytokines such as TNF-Tumor necrosis factor-alpha (TNF-α) is released due to NF-kB activation. transcription factor NF-kB plays a central role in inflammation and immune response, acting as a mediator in both TNF-α biosynthesis and in biological responses mediated by increased TNFα to its target receptors. Tumor necrosis factor alpha (TNF-α) also plays an important role in the development of endometriosis. TNF-α regulates the secretion of inflammatory mediators and epithelial cell invasion. The increase in TNF-α expression at treatment doses P1, P2, and P3 was due to the presence of basil (Ocimum basillicum L), which has estrogenic properties and is rich in phytoestrogens, in line with research by (Andriyanto et al., 2022).

In endometriotic peritoneal fluid, TNF- $\alpha$  levels are elevated, and this TNF- $\alpha$  is a FAST ligand, forming a bond with FAST, namely TNF- $\alpha$ R in the ovaries. The death domain receptor on the cell death ligand binds to the death domain adaptor on FADD (Fas-Associated Protein with Death Domain). This binding triggers the activation of FADD, which leads to the activation of caspase, resulting in pathological apoptosis. The apoptosis process can occur through the extrinsic pathway, namely through caspase 8 activating caspase 3, which triggers apoptosis. The intrinsic apoptosis pathway involves caspase

8 activating Bid and Bax, which then undergo translocation to the mitochondria, causing the mitochondria to release cytochrome C, which binds to apoptosis protease activating factor 1 (Apaf-1), which binds to caspase 9. Caspase 9 stimulates caspase 3. Activated caspase can activate DNAse. This active DNAse enzyme will digest DNA, causing it to become damaged, resulting in apoptosis. Granulosa cell apoptosis plays an important role in the process of follicular atresia.

Basil leaves contain antioxidants such as phenols, flavonoids, carotenoids, ascorbic acid, riboflavin, and thiamine, which can protect against inflammatory damage to reproductive (Barickman et al., 2021). organs administration of ethanol extract from basil leaves (Ocimum Basilicum) is expected to inhibit the inflammatory response in endometriosis. The content of basil leaves, namely flavonoids such as quercetin, isoquercetrin, kaempferol and rutin, and glycosides (such as esculin and syringin) can suppress NF-KB expression, thereby reducing the secretion of pro-inflammatory cytokines, namely interleukin (IL)-6, IL-8, and TNF-α (Singh & Singh, 2020). When TNF- $\alpha$ R in the ovaries, which forms the DISC signaling complex through the intrinsic pathway via caspase 8, is not formed, granulosa cell apoptosis decreases and follicular atresia is also expected to decrease (Nomden et al., 2020)

#### **Limitations:**

This research was limited to specific doses and extract from. The effectiveness of different doses, other dosage froms, or long term effects cannot be confirmed.

#### **Conclusion:**

Ethanol extract of (*Ocimum basilicum*) in graded of 0.21 mg/gBB; 0.42 mg/gBB; and 0.84 mg/gBB was able to reduce follicular atresia clinically seen in the increase in the mean dose still below the control group, However, this did not show statistically significant results, so further research is needed regarding the duration of dosage administration.



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