

RESEARCH ARTICLE

The Effect of Administering Lemon Basil (*Ocimum sanctum*) Leaves on FSH and LH Estrogen Levels and Size of Ovarian Antral Follicle in Female Balb/C Mice

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ABSTRACT

Introduction: *Ocimum sanctum* have been proven effective in decreasing Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) levels, and sperm number, and also increase male testosterone levels. However, so far no evidence has been found on the effect of *Ocimum sanctum* leaves on the levels of estrogen, FSH, LH, and the size of ovarian antral follicles. **Objectives:** To evaluate the effect of *Ocimum sanctum* leaves on estrogen, FSH, LH and the size of antral follicle of female Balb/C mice.

Methods: In the post test only control group design, 12 female Balb/C mice were assigned into 2 groups. The treatment group (OS-G) was given 0.08 gram of *ocimum sanctum* leaves, three times a day for 30 days and the control group (C-G) received no *ocimum sanctum*. Estrogen, FSH and LH levels were measured on the 30th day, the mice were sacrificed and taken up by their ovaries. HE preparations were then made and HE was performed, and the size of the antral follicle of the ovary was measured.

Results: Mann Whitney analysis indicated that estrogen and FSH levels in OS-G group were significantly higher compared to that of C-G group, $p < 0.05$. Based on independent t-test, the level of LH in OS-G was significantly higher than that of C-G group, $p < 0.05$. On the contrary, the size of ovarian antral follicle in OS-G was smaller than that of C-G group, $p < 0.05$.

Conclusion: *Ocimum sanctum* increased the levels of estrogen, FSH, LH and decreased the size of ovarian antral follicle in female of Balb/C mice.

Keywords: *Ocimum sanctum*, estrogen, FSH, LH, ovary area

ABSTRAK

Pendahuluan: Daun kemangi (*Ocimum sanctum*) terbukti meningkatkan kadar testosteron, menurunkan kadar FSH (Follicle Stimulating Hormone), LH (Luteinizing Hormone), dan jumlah spermatozoa pada kelinci jantan. Namun, belum diketahui apakah daun kemangi berpengaruh terhadap kadar hormon estrogen, FSH, LH dan folikel ovarium pada mencit betina. **Tujuan:** Mengetahui pengaruh daun kemangi terhadap kadar hormon estrogen, FSH, LH dan ukuran antral follicle ovarium pada mencit betina Balb/C betina.

Metode: Penelitian eksperimental dengan *post test only control group design*, menggunakan sampel 12 ekor mencit betina galur Balb/c, dibagi 2 kelompok. Kelompok perlakuan (OS-G) mendapatkan bubuk daun kemangi 0.08 gram, dan kelompok kontrol (C-G) tidak mendapatkan bubuk daun kemangi. Pemberian daun kemangi dilakukan tiga kali sehari selama 30 hari. Hari ke-30 darah diambil untuk pemeriksaan kadar estrogen, FSH dan LH, Ovarium diambil untuk pengukuran antral folikel ovarium dengan pengecatan HE.

Hasil: Hasil uji *Mann Whitney* menunjukkan bahwa rerata kadar estrogen dan FSH pada kelompok OS-G lebih tinggi bermakna dibanding kelompok C-G, $p < 0.05$. Demikian pula dengan kadar hasil uji *Independent T-test* yang menunjukkan rerata kadar LH pada kelompok OS-G lebih tinggi bermakna dibanding kelompok C-G, $p < 0.05$. Sebaliknya ukuran antral follicle ovarium pada kelompok OS-G lebih kecil bermakna dibanding kelompok C-G, $p < 0.05$.

Kesimpulan: Pemberian bubuk daun kemangi (*Ocimum sanctum*) meningkatkan kadar hormon estrogen, FSH, LH dan memperkecil ukuran folikel antral ovarium pada mencit betina Balb/C.

Kata Kunci: *Ocimum sanctum*, estrogen, FSH, LH, ukuran Follicle ovarium

INTRODUCTION

One of serious issues the Indonesian government is currently facing is the rapid population growth. They endeavour to suppress this population growth rate using family planning program. Contraception is one of family planning methods to prevent sperm and egg

cells from meeting (Wilopo, 2009). Several literatures indicate that 74 plant species are empirically used by people in some regions for contraception, and one of them is Basil (*Ocimum sanctum* Linn; OS) leaves. Some studies have shown that OS leaves are effective as an anti-fertility in male rabbits since it has been

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found capable of lowering the number and motility of spermatozoa (Ahmed et al., 2002; P. Prakash and Gupta, 2005). Another research conducted by Sethi J, et al also reports that OS leaves is proven effective in increasing the testosterone level and decreasing the Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) levels and the number of spermatozoa (Sethi et al., 2010). All these study results indicate the effect of OS leaves as an anti-fertility in male rabbits through negative feedback as mediated by increased exogenous testosterone level. Considering that the arrangement of reproductive hormones in men and women have something in common, i.e. through hypothalamus-hypophysis-target organ axis (Plant, 2015). Thus, it is important to conduct a study to evaluate the effect of OS leaves on reproductive hormones in women.

Basil is a plant which many Indonesians are quite familiar since its ability to grow in almost all places, its leaves are commonly consumed raw. This lemon basil has fragrant leaves and it can serve many purposes for human. Basil leaves contain flavonoid which constitutes one of the largest natural phenols (Widyawati, 2017). So far, this basil use is directed toward medical research purposes, such as to cure pityriasis versicolour, stomatitis, common cold, and anticancer (Akbar, 2010). Several evidences showed that OS leaf powder has been proven effective in lowering FSH and LH levels and number of sperms and increasing testosterone levels in male rabbits (Pattanayak et al., 2010; Sethi et al., 2010). This indicates that the compounds contained in basil leaves (euginol, euginal, ursolic acid, β sitosterol, and stigmasterol) are phytochemical, which act as either phytoandrogen or phytoestrogen which can suppress the secretion of FSH, LH, and number of sperms through negative feedback in hypophysis. (Baliga et al., 2016; Sethi et al., 2010) Additionally, basil leaves also contain orientin, vicenin, and isoflavone flavonoids which are estrogenic and thus, bind estrogen receptors within the body (Mousavi et al., 2018). Isoflavone can cause estrogen-like effect in numerous tissues, including the negative feedback to hypophysis and, in turn, it can reduce the endogenous estrogen level (Cui et al., 2013).

The main function of endogenous estrogen is to cause cell proliferation and tissue growth, female genital organ, and other tissues which have estrogen receptors (Cui et al., 2013). Endogenous estrogen is a steroid hormone synthesized from cholesterol by theca interna cells and granulosa cells in ovarian follicles. In addition, in a few number estrogen is also synthesized by corpus luteum, placenta and adrenal cortex. During the menstruation cycle, the estrogen level increases with the development of ovarian follicle stimulated by FSH.

At the end of the follicular phase, estrogen gives positive feedback to GnRH level to secrete LH, that it creates LH surge and triggers ovulation (Cui et al., 2013). Therefore, administering basil leaves, which is estrogenic in nature, is hypothesized to be effective in providing negative feedback to hypothalamus and hypophysis, hence it no longer produces FSH and LH. As a result, follicles do not develop and ovulation never takes place. For this reason, this research aims to evaluate the effect of administering basil (*Ocimum sanctum*) leaves on the levels of reproductive hormones which consist of FSH, LH, estrogen, and size of ovarian antral follicles in female Balb/C mice.

METHODS

This was a laboratoric experimental study with Posttest only Control Group Design. Twelve female Balb/c mice aged 10-12 weeks with 20-30 grams weight were divided into 2 groups randomly. One group was the control group (C-G) and the other was treatment group (Os-G). The control group received no basil leaves, and the treatment group was given basil leaf powder at 0.08 gram dose. All treatments were given for 30 days. The estrogen hormone, FSH and LH levels were measured on the 30th day, by sampling the mice's blood from medial canthus of orbital sinus of the right eye for measurement using ELISA method. This research had been approved by the Ethical Commission of Medicine Faculty at UNISSULA with its approval No. 46/II/2017/Komisi Bioetik

Basil Leaf Powder Preparation

The freshly picked basil leaves were washed using tap water, drained well and aerated overnight. The leaves used were the brightest green to the last third leaves from the stem base. After being aerated overnight, the leaves then dried using oven at 40° C until dried and brittle. The dried leaves were blended to pieces and then sifted using calico fabric until became powder. The lemon basil leaf powder was saved in a transparent plastic bag to avoid contaminant and stored in a closed container. The basil leaf powder was then mixed with the feed materials before being turned into pellets. The pellets which had been mixed with lemon basil leaf powder were then dissolved using distilled water and saved in vial bottles in a refrigerator.

Measurement of FSH, LH, dan Estrogen

The levels of FSH, LH, and estrogen were examined using ELISA method. The principle of ELISA was to detect antigen or antibody (hormones) in a serum using specific antibody or antigen and

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to quantify them using an enzymatic reaction. The quantity of hormones was determined by the change in color as measured using spectrophotometer at 450 nm wavelength.

in the left and right ovaries using Hematoxylin-Eosin staining. The antral follicles were then observed in five fields of view using BX51-type Olympus microscope at 400 time magnification.

Measurement of the Antral Follicle Ovarian Size

The width of antral follicles in the mice's ovary was measured by observing the histologic preparation

Statistical Analysis

The data of estrogen and LH levels were not normally distributed nor homogenous, so the statistical

Table 1. Mean FSH, LH and estrogen levels and antral follicle diameter of female Balb/C mice

VARIABLES	GROUPS	
	C-G N=5 (Mean ± SD)	OS-G N=5 (Mean ± SD)
Estrogen Level (mIU/mL)	111.54 ± 38.69	1595.70 ± 12.41
Shapiro wilk	0.049	0.754
Levene test		0.174
Mann Whitney		0.004
FSH level (mIU/mL)	22.28 ± 5.87	99.15 ± 0.83
Shapiro wilk	0.254	0.297
Levene test		0.000
Mann Whitney		0.004
LH level (mIU/mL)	2.10 ± 0.25	19.82 ± 0.24
Shapiro wilk	0.762	0.230
Levene test		0.859
Independent t-test		0.000
Antral Follicle Width (µm)	864,642.50 ± 317,300.17	381,208.33 ± 226,272.07
Shapiro wilk	0.994	0.564
Levene test		0.329
Independent t-test		0.012

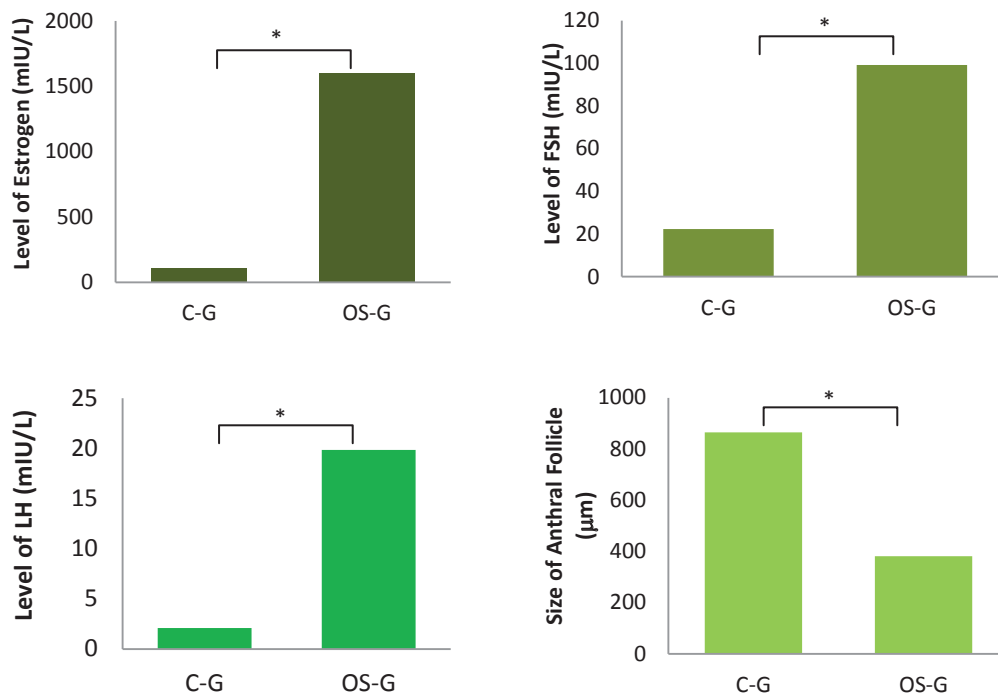


Figure 1. Mann Withney Analysis on Estrogen and FSH concentration and Independent t test on LH concentration and the size of Ovary's Antral Follicle between two Groups. * P < 0.05

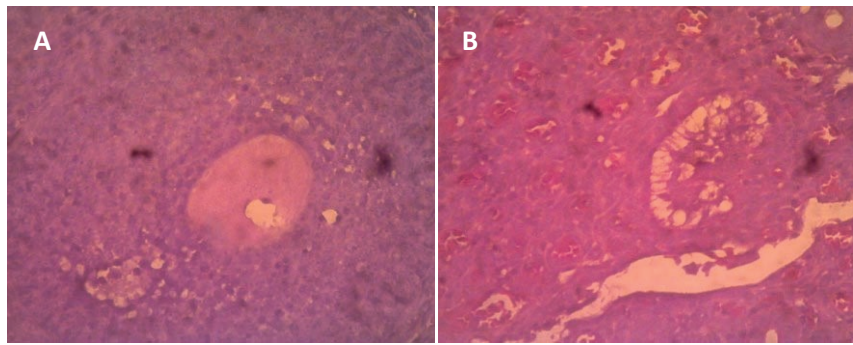


Figure 2. The Size of Ovary's Antral Follicle in C-G (A) and OS-G (B)

test used was Mann-Whitney. Meanwhile, the data for FSH level and antral follicle width were normally distributed and homogenous, thus the statistical test used was Independent T-test. The analysis result was considered as significant if the value of $p < 0.05$.

RESULTS

The study was conducted for 30 days, the mean FSH, LH and estrogen levels and antral follicle width of female Balb/C mice in each group were found as shown in Table 1.

The research results as shown in table 1 indicated that the FSH, LH and Estrogen levels in OS-G group was higher than C-G group.

Mann Whitney analysis results showed the mean estrogen and FSH levels in OS-G group were significantly higher than C-G group, $p < 0.05$ (figure 1). The same applied to the Independent T-test result which showed the mean LH level in OS-G group was significantly higher than C-G group, $p < 0.05$ (figure 1). On the other hand, the mean size of ovarian antral follicle in OS-G group was significantly smaller than C-G group, $p < 0.05$ (figure 1,2).

DISCUSSION

This research's results indicated that administering basil (*Ocimum sanctum*) leaves at 0.08 gram dose three times a day for 30 days had been found to have an effect on increasing FSH, LH, and Estrogen levels and, on the other hand, decreasing the size of ovarian antral follicle in female BALB/C mice. This finding was different from Sethi who found that administering OS 2 grams per day for 30 days in albino male rabbits showed an increased testosterone level, decreased LH and FSH levels and number of sperm (Sethi et al., 2010). The hypothalamus hypophysis, and target organ axis between men and women was similar, it was actually expected that this research would find the estrogen level would increase after OS leaves were administered (Plant, 2015).

The increase in estrogen as a result of

administering OS constituted exogenous estrogen, hence this estrogen increase should have been followed by decreased in LH and FSH levels and atresia antral follicle through negative feedback (Plant, 2015). This made sense since, in addition to containing isoflavone, OS also contained euginol which was estrogenic (Baliga et al., 2016; Poli and Challa, 2019). However, this research showed different result, the FSH, LH and estrogen levels increased and the size of ovarian antral follicle decreased simultaneously. On the other hand, in addition to containing isoflavone and euginol, it also contained β sitosterol and stigmasterol which were androgenic (Nasihun, 2012). Therefore, it was postulated that the decrease in size of ovarian antral follicle was due to increased sitosterol and stigmasterol. Furthermore, sitosterol and stigmasterol were androgen precursor which could be converted into testosterone, thus the testosterone level was higher and suppress the development of ovarian follicle (Nasihun, 2012). Such a circumstance was similar to hyperandrogenic condition in infertile women as a result of polycystic ovary syndrome (Abasian et al., 2018). Refer to this research result, it could be inferred that the various compounds in OS leaves can cause a decrease in the size of antral follicles and even ovarian follicle atresia, yet it remained unknown whether or not it is through an increase in testosterone level, thus it is important to conduct further research on this matter.

CONCLUSION

Administering basil (*Ocimum sanctum*) leaf powder at 0.08 gram/BW dose 3 times a day for 30 days could increase the estrogen hormone, FSH and LH levels in female balb/C mice, and decrease the size of ovarian antral follicle in female Balb/C mice.

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