© 2024 Journal of Pharmacy & Pharmacognosy Research, 12 (suppl. 1), S75-S79, 2024
ISSN 0719-4250
https://jppres.com

DOI: https://doi.org/10.56499/jppres23.1770_12.s1.75

Short Communication

Effect of kebar grass extract (*Biophytum petersianum* Klotszch) on luteinizing and follicle-stimulating hormone levels in rat model polycystic ovarium syndrome

Noviyati Rahardjo Putri[1*], Syarief Thaufik Hidayat[2], Melyana Nurul Widyawati[3], Ari Suwondo[4], Sudirman[5], Aida Amalia Nur Ramadhian[3]

[1]Midwifery Program, Faculty of Medicine, Sebelas Maret University, Jl Ir Sutami 36, Kentingan, Jebres, Surakarta, Indonesia.
[2]Medical Staff Group of Obstetric Gynecology Department, Dr. Kariadi Hospital, Jl. Dr. Sutomo 16 Randusari, Semarang, Indonesia.
[3]Postgraduate Program, Poltekkes Kemenkes Semarang, Jl Tirto Agung, Pedalangan, Banyumanik, Semarang, Indonesia.
[4]Health and Work Safety Department, Faculty of Public Health, Diponegoro University, Jl Prof Sudarto 13, Tembalang, Semarang, Indonesia.
[5]Nursing Program, Poltekkes Kemenkes Semarang, Jl Tirto Agung, Pedalangan, Banymanik, Semarang, Indonesia.
*E-mail address: novirahardjo@staff.uns.ac.id; ORCID: 0000-0002-4936-9066

ABSTRACT

Context: The major cause of infertility is polycystic ovary syndrome (PCOS). PCOS symptom is folliculogenesis disorder, increasing LH:FSH levels (3:1), and insulin resistence. As the fertilizer herb in Indonesia, kebar grass (*Biophytum petersianum* Klotzsch) has two types of protein with a molecular weight as same as pregnant mare serum gonadotrophin hormone (PMSG), which has follicle-stimulating hormone (FSH) and luteinizing hormone (LH) activity. It contains antioxidants, vitamin A, vitamin E, saponins, and amino acids. Unfortunately, it has not been conducted for PCOS.

Aims: To determine the effect of *B. petersianum* extract on LH and FSH levels in a PCOS rat model.

Methods: This study was a posttest only with a control group design. The sample consisted of five groups: C1 (healthy model), C2 (PCOS model), D1, D2, and D3 (dose 31.5, 63, and 94.5 mg/kg BW of kebab grass extract).

Results: *B. petersianum* extract reduced LH levels and increased FSH levels in blood serum measurement with p values of 0.007 and 0.013. The optimal dose was dose 2 (63 mg/kg bw). The glycoprotein content in *B. Petersianum*, which showed the same atomic weight as the PMSG, was thought to be a stimulator growth factor of follicles. The antioxidant content was also thought to inhibit prostaglandin precursors. It could restore the production of gonadotropin-releasing hormone to the normal hypothalamic-pituitary-ovarian (HPO).

Conclusion: *B. petersianum* extract can improve reproductive performance in the PCOS rat model. This research can be a reference for further research in reproductive disorders and the development of the clinical trial stage of traditional medical plants.

Keywords: follicle-stimulating hormone; kebar grass; luteinizing hormone; polycystic ovary syndrome.

INTRODUCTION

One of the causes of infertility is polycystic ovary syndrome (PCOS). It is a folliculogenesis disorder caused by disruption of the hypothalamic, pituitary, and ovarian cycles. In Europe, it is about 15-20%, and in Australia is 17.8% (Sirmans and Pate, 2013). In Indonesia, it is 4.5% and tends to increase up to 8-10% in women (Santoso, 2014).

One of the clinical signs of PCOS is increased luteinizing hormone (LH) and gonadotropin-releasing hormone (GnRH) levels. At the beginning of the fertilization phase, some PCOS women have an increasing ratio between LH and follicle-stimulating hormone **(**FSH**)** (3:1), which the normal levels are balanced (Mitrašinović-Brulić et al., 2021; Morshed et al., 2021). Higher IR, especially in the ovary, can suppress sex hormone-binding globulin (SHGB) production and stimulate hyperandrogenism. Low SHGB levels and hyperandrogenism can suppress FSH production. High LH levels and a hyperandrogenism environment cause the concentration of cyclic adenosine monophosphate (cAMP) in the granulosa cells to increase. These conditions result in premature terminal differentiation of granulosa cells that can cause anovulation or oligoovulation (Giovanni, 2016).

Kebar grass (*Biophytum petersianum* Klotzsch) is one of the herbal therapies that is empirically used as a fertilizer in Indonesia, especially in Kebar District, Manokwari, West Papua Province (Sembiring and Darwati, 2016). *B. petersianum* has two types of protein with a molecular weight, the same as pregnant mare serum gonadotrophin hormone (PMSG), which has FSH and LH activity. It contains antioxidants, vitamin A, vitamin E, saponins, and amino acids. Pre-clinical research suggests that *B. petersianum* could shorten the estrus cycle, prolong the estrus phase, increase ovarian and uterine hypervascularization, and increase the number of children conceived (Effendi et al., 2018; Lefaan, 2014). To the best of our knowledge, no studies

Paper presented at the 4th International Conference on Medicine and Health Sciences (ICO-Helics), 2023.

evaluate *B. petersianum* for the PCOS model specifically. In the present study, a novel *B. petersianum* extract that could be a potential alternative therapy for PCOS is provided.

MATERIAL AND METHODS

Ethical statement

This research obtained ethical clearance from the Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine, Public Health and Nursing Gadjah Mada University – Dr. Sardjito General Hospital with No. KE/FK/0181/EC/ 2020.

Study design

This research was a laboratory study with a post-test control group design.

Housing and husbandry

The research was conducted at the animal house of the Department of Pharmacology and Therapy, Faculty of Medicine, Public Health and Nursing, Gadjah Mada University, Indonesia. The feeding using pellets with a dose of 15-20 g for each rat.

Sample size and allocating animals to experimental groups

Rattus norvegicus Wistar strain is one of the animals developed as a polycystic ovary syndrome model. The rats in this study weighed 200 g. The sample size was 25 female Wistar rats and a dropout number (10%), for a total of 30. The animals were divided into 5 groups, with 6 animals in each group. Sampling was carried out by simple random sampling.

The research group was divided into two major proportions (the control and the treatment group). The control group consisted of the negative control group (C1), which was healthy rats, and the positive control group (C2), which was the PCOS model. The treatment group was given *B. petersianum* extract in three doses (D_{1-3}). These doses were in accordance with a previous report, which were 0.045 mg/g bw (1st dose), 0.09 mg/g bw (2nd dose) and 0.135 mg/g bw (3rd dose) in mice model (Sadsoeitoeboen, 2005). The conversion in the rat model was 31.5 mg/kg bw (1st dose), the 2nd dose was 63 mg/kg bw, and the 3rd dose was 94.5 mg/kg bw. The weight of the animals was approximately 200 g.

Plant material

Biophytum petersianum Klotszch was registered in the International Plant Names Index (IPNI) under the Life Sciences Identifier (LSID) number: 51869-3 (International Plant Names Index, 2024). In Indonesia, *B. petersianum* was recognized as part of the Intangible Cultural Heritage of Indonesia with the registration number 2011001583, originating from Manokwari Regency, West Papua Province, located at North -0° 54' 0" N and East 133° 45' 0" E (Warisan Budaya Tak Benda Indonesia, 2011). The extraction of Kebar grass was conducted at the Center for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta, Indonesia, using all parts of the plant.

The first step of the extraction process was boiling the grass at 60°C for 6 h in 3 days, then filtering. The second step was evaporation until a thick liquid formed. The final form was a freeze-drying process for 3 × 24 hours. About 300 g of dry *B. petersianum* extract yielded 22 g (7.3%).

Animal model

The PCOS model was inducted by injecting testosterone propionate at a dose of 1 mg/100 g bw. This hormone was injected subcutaneously for 28 days in PCOS rat model groups. Synchronization of the estrus phase was carried out using the continuous white effect method (Osuka et al., 2019).

Statistical analysis

Univariate analysis determined the levels of LH and FSH. Prior to the bivariate test, the normality test was first performed using Saphiro-Wilk. The data was normally distributed when the p-value was >0.05. In this study, the Kruskal Walls test was used with non-parametric data. After a significant result, the Mann-Whitney test was carried out to find out which group had a significant difference ($p<0.05$) or optimum dose.

RESULTS

Evaluation animal model

To measure the PCOS model, one female rat was taken from each group and executed after synchronization. Based on the evaluation model, in the proestrus phase of the rat, the healthy rat model has the lowest LH level of 4.8 ng/mL and the highest FSH level of 4.9 ng/dL compared to the four groups of the PCOS model (C_2, D_{1-3}). PCOS model had a higher LH and lower FSH level in the proestrus phase due to exogenous

androgen exposure. An exogenous androgen can stimulate the occurrence of hyperandrogenism, thereby suppressing the stimulation of SHGB in the liver. Low SHGB production causes suppressed levels of FSH, resulting in anovulation or follicular maturation arrest and an increase in LH levels (Osuka et al., 2019).

Descriptive data analysis

Table 1 presents the levels of FSH and LH in the proestrus phase. Table 1 interprets that the average proestrus phase LH levels in the positive control group/C_2 (baseline of PCOS model) were 6.7 ± 2.4 ng/mL. Meanwhile, the negative control group/C_1 (baseline of the healthy model) had a lower average LH level, which was 3.6 ± 0.5 ng/mL.

Table 1. Descriptive data analysis of LH and FSH.

No.	Group	n	LH (ng/mL)	FSH (ng/mL)	FSH:LH
			Mean ± SD		
1.	Negative Control (C_1)*	5	3.6 ± 0.5	3.9 ± 0.5	1:0.9
2.	Positive Control (C_2)**	5	6.7 ± 2.4	2.4 ± 0.4	1:2.7
3.	Dose 1 (D_1)	5	4.5 ± 0.9	3.5 ± 0.2	1:1.2
4.	Dose 2 (D_2)	5	3.5 ± 0.7	3.8 ± 0.4	1:0.9
5.	Dose 3 (D_3)	5	4.6 ± 0.7	3.8 ± 0.6	1:1.2

*Healthy rat model. **PCOS rat model.

Table 2. Inferential data analysis.

No.	Group	p-value* FSH	p-value* LH	p-value** FSH	p-value** LH
1.	Negative Control (C_1)	0.013	0.007	C_2 against C_1 (0.008)	
2.	Positive Control (C_2)			C_2 against D_1 (0.008)	C_2 against C_1 (0.009)
3.	Dose 1 (D_1)			C_3 against D_2 (0.008)	C_2 against D_2 (0.012)
4.	Dose 2 (D_2)			C_4 against D_3 (0.008)	
5.	Dose 3 (D_3)				

*Kruskal Wallis test. **Mann Whitney test.

The average in proestrus phase of FSH levels in the positive control group/C_2 (baseline model of PCOS) was the lowest at 2.4 ± 0.4 ng/ml, while in the negative control group/C1 (baseline of the healthy model) was 3.9 ± 0.5 ng/mL. The ratio of FSH:LH in the proestrus phase in the PCOS baseline group was 1:2.7, and the healthy group was 1:0.9.

The results illustrated higher LH levels and lower FSH levels in the PCOS model versus the healthy model and the dose$_{1\text{-}3}$ model. Exposure to exogenous androgens (testosterone) stimulated a lower expression of SHBG production. SHGB is a reversible sex hormone binding protein and has a high affinity to tie up androgens. Amount free androgens, especially testosterone, which are produced by this condition, cause a disruption of the hormonal balance, especially reproductive hormones. The manifestation of reproductive hormone disorder in women is excess LH by the anterior pituitary gland and suppression of FSH production (Guyansyah and Parwanto, 2019). Additionally, increased LH causes theca cells to produce large amounts of androgens, thereby inhibiting the development of ovarian follicles and producing clinical signs and symptoms of PCOS. At the beginning of fertilization, the ratio between FSH and LH is balanced, but some women with PCOS experience a 3-time increase in LH compared to normal women at the beginning of folliculogenesis (Sterling, 2011).

Inferential data analysis

The results of the study regarding the effect of *B. petersianum* extract on LH and FSH levels between groups are described in Table 2. Table 2 shows that there was a difference in LH and FSH levels between groups after being given *B. petersianum* extract for 16 days with p = 0.007 and 0.013. It could be concluded that administration of *B. petersianum* extract can reduce LH and increase FSH levels in the PCOS model based on average hormone level. Mann Whitney test in LH level concluded the smallest p-value was D_2 (63 mg/kg bw) or 0.0012. Therefore, it was the optimum dose for reducing LH levels in the PCOS rat model during the proestrus phase. The mean value in D_2 was close to the normal baseline in the negative control group (healthy model) (Table 2).

DISCUSSION

The results showed that administration of *B. petersianum* extracts for 16 days could reduce the levels of LH and FSH in the proestrus phase in the PCOS rat model. The LH and FSH levels in the intervention group at all doses were lower than those in the PCOS model group and closely matched those in the healthy control group.

The LH level in the proestrus phase could be suppressed due to *B. petersianum* extract because of PMSG. PMSG is a complex glycoprotein that has a high function of FSH and low LH activity. Several pre-clinical studies have proven that PMSG can improve the reproductive system, including restoring the regularity of the estrus cycle, increasing uterine weight, and increasing follicle growth stimulating hormone when compared to the control group. Restoring the regularity of the estrous cycle improves the balance of the hypothalamic–pituitary–ovarium (HPO) axis in reproductive hormones (Nur et al., 2016; Wei et al., 2015). It can be used to increase ovulation by stimulating the growth of follicles (Nur et al., 2016; Wei et al., 2015).

A decrease in LH levels was also found in a study with the PCOS rat model due to the administration of natural plant components, which are antioxidants. Antioxidants are thought to inhibit unsaturated fatty acids, which are prostaglandin precursors. Prostaglandins are substances that resemble hormones but are derived from enzymatic reactions of fat. The decrease in prostaglandins will eventually restore the production of gonadotropin-releasing hormone to the normal HPO axis. So that in the proestrus stage, FSH production can return to normal (Atashpour et al., 2017).

The limitation of this research is that diagnosis of PCOS cannot be carried out using the analysis of the development of the Graff follicle. In the future, the evaluation of follicular development can be used as supporting data for the hypothesis.

CONCLUSION

The results showed that administration of *B. petersianum* extract could reduce LH levels, escalate FSH levels, and balance the LH:FSH ratio in the proestrus phase in a PCOS rat model in a preclinical study. *B. petersianum* contains PMSG, a complex glycoprotein that has a high FSH function and low LH activity. However, this research can be a reference for further research, especially in PCOS and other reproductive disorders.

CONFLICT OF INTEREST

The authors declare no conflicts of interest with the data contained in the document.

ACKNOWLEDGMENT

The authors acknowledged the LPPM Sebelas Maret University.

REFERENCES

Atashpour S, Kargar Jahromi H, Kargar Jahromi Z, Maleknasab M (2017) Comparison of the effects of ginger extract with clomiphene citrate on sex hormones in rats with polycystic ovarian syndrome. Int J Reprod Biomed 15(9): 561–568. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5894472/

Effendi M, Prasetyorini, Azzahra S (2018) The estrogenic activities of kebar grass extract (*Biophytum petersianum*) on white female mice (*Mus musculus*). Int J Recent Technol Eng 8(2S7): 153–157. https://doi.org/10.35940/ijrte.B1038.0782S719

Giovanni M (2016) Induksi Ovulasi pada Pasien Sindroma Ovarium Polikistik (SOPK) (1st ed.). Universitas Udaya.

Guyansyah A, Parwanto ME (2019) Protein pengikat hormon seks: Sex hormone binding globulin (SHBG) dan aksi steroid seks. J Biomed Kesehatan 2(1): 45–50. https://doi.org/10.18051/JBiomedKes.2019.v2.45-50

International Plant Names Index (2024) *Biophytum petersianum* Klotzsch. https://www.ipni.org/n/51869-3 [Consulted 12 August, 2024].

Lefaan PN (2014) The influence of kebar grass infuse to mice (*Mus musculus*) spermatogenesis. J Sain Vet 32(1): 55–67.

Mitrašinović-Brulić M, Buljan M, Suljević D (2021) Association of LH/FSH ratio with menstrual cycle regularity and clinical features of patients with polycystic ovary syndrome. Middle East Fert Soc J 26: 40. https://doi.org/10.1186/s43043-021-00085-0

Morshed MS, Banu H, Akhtar N, Sultana T, Begum A, Zamilla M, Tuqan S, Shah S, Hossain A, Afrine S, Rashid EU, Jahan IA, Hasanat MA (2021) Luteinizing hormone to follicle-stimulating hormone ratio significantly correlates with androgen level and manifestations are more frequent with hyperandrogenemia in women with polycystic ovary syndrome. J Endocrinol Metab 11(1): 14–21. https://doi.org/10.14740/jem716

Nur NA, Siregar TN, Hamdan, Nasution I, Thasmi CN, Dasrul (2016) Effect of PMSG and hCG to increase birth number in Campbell's hamsters (*Phodopus campbelli*). J Med Vet 10(1): 59–61. http://dx.doi.org/10.21157/j.med.vet..v10i1.4041

Osuka S, Nakanishi N, Murase T, Nakamura T, Goto M, Iwase A, Kikkawa F (2019) Animal models of polycystic ovary syndrome: A review of hormone-induced rodent models focused on hypothalamus-pituitary-ovary axis and neuropeptides. Reprod Med Biol 18(2): 151–160. https://doi.org/10.1002/rmb2.12262

Sadsoeitoeboen PD (2005) Manfaat Ekstrak Rumput Kebar (*Biophytum petersianum* Klotzsch) Terhadap Penampilan Reproduksi Mencit Putih Betina. Institut Pertanian Bogor.

Santoso B (2014) Sindroma Ovarium Polikistik: Masalah Reproduksi Dan Tantangannya Terkait Dengan Gaya Hidup. In Airlangga University Press (Issue September). Airlangga University Press.

Sembiring B, Darwati I (2016) Identification of chemical component of kebar grass accession (*Biophytum petersianum*) from Papua and Java. Bul Littro 25(1): 37–44. https://doi.org/10.21082/bullittro.v25n1.2014.37-44

Sirmans S, Pate K (2013) Epidemiology, diagnosis, and management of polycystic ovary syndrome. Clin Epidemiol 2014(6): 1–13. https://doi.org/10.2147/CLEP.S37559

Sterling E (2011) Hormone levels and PCOS. Obsgyn.Net. https://www.contemporaryobgyn.net/view/hormone-levels-and-pcos [Consulted 1 May, 2020].

Warisan Budaya Tak Benda Indonesia (2011) Rumput Kebar. https://warisanbudaya.kemdikbud.go.id/?Newdetail&detailCatat=1583 [Consulted 12 August, 2024].

Wei S, Gong Z, An L, Zhang T, Dai H, Chen S (2015) Cloprostenol and pregnant mare serum gonadotropin promote estrus synchronization, uterine development, and follicle-stimulating hormone receptor expression in mice. Genet Mol Res 14(2): 7184–7195. https://doi.org/10.4238/2015.June.29.12

Citation Format: Putri NR, Hidayat ST, Widyawati MN, Suwondo A, Sudirman, Ramadhian AAN (2024) Effect of kebar grass extract (*Biophytum petersianum* Klotszch) on luteinizing and follicle-stimulating hormone levels in rat model polycystic ovarium syndrome. J Pharm Pharmacogn Res 12(suppl. 1): S75–S79. https://doi.org/10.56499/jppres23.1770_12.s1.75

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Open Access: This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/ licenses/by/4.0/), which permits use, duplication, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.