Contents

Efficacy and safety of three Ayurveda decoctions for Polycystic Ovary Syndrome: a study protocol for a randomized, single-blind, three-arm, clinical trial, Running title: Ayurveda decoctions for PCOS: a study protocol

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ABSTRACT

Polycystic Ovary Syndrome (PCOS) is one of the most common endocrine abnormalities in women of reproductive age. Due to fear of side effects and adverse effects of allopathic medicines, some patients prefer to take Ayurveda treatments for PCOS. Therefore, the aim of this research is to design a randomized, single-blind, three-arm, comparative clinical trial to detect the efficacy and safety of three Ayurveda decoctions (decoction of Nigellasativa, decoction of Sesamumindicumand decoction of Nigellasativa and Sesamumindicum). Rotterdam's (2003) diagnostic criteria will be used to diagnose PCOS. The selection of participants will be done according to the inclusion and exclusion criteria and allocated randomly into the three arms. Participants of Arm I, Arm II, and Arm III will receive an oral drug for twelve weeks and will be followed up for three months after the drug administration. Each patient will undergo hematological and biochemical investigations (FBS, FBC, ESR, AST/ALT, and serum creatinine/GFR) and a urine full report before and after the interventions, which are done primarily for safety assessment. Trans Abdominal Sonography or Trans-Vaginal Sonography will be carried out before, after the treatment, and during the follow-up period as primary outcome measures. Symptom severity and their impact on quality of life will be assessed at the beginning and end of the treatment by PCOS symptoms and the Health-Related Quality of life Questionnaire. Body constituent (Prakriti) identifying each at the screening with the help of a questionnaire which is already validated. The relation in-between constituent and PCOS will be evaluated at the beginning and the effect of therapy according to the body constituent will be evaluated at the end of the study. For primary and secondary

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outcome measures, the mean values at baseline and at the end of the study and the mean differences will be compared between the three arms using ANOVA (analysis of variance) or the non-parametric Kruskal–Wallis test, depending on the normality of the data. Intention-to-treat analysis will be performed for all efficacy outcomes and safety outcomes. At the end of this clinical research, it will be able to provide evidence-based scientific data on the classical Ayurveda treatment.

Keywords: *Ayurveda, Polycystic Ovary syndrome, Nigellasativa, Sesamumindicum*

INTRODUCTION

The ovarian factor is the second most common cause of infertility, and Polycystic Ovary Syndrome (PCOS) is one of the most common causes of anovulation (Dutta, 2007). The prevalence of PCOS depends entirely on ethnicity; however, most clinical research suggests that it is around 6–7%. PCOS affects women of South Asian origin earlier in life, with more severe symptoms and a higher prevalence (Wolf et al., 2018). The modern medical field has developed treatment modalities for PCOS and subfertility due to PCOS, such as hormonal therapy, in- vitro fertilization, embryo transfer and gamete intra-fallopian transfer, but some of them have not shown a considerable rate of success.

Though there are several treatment regimens for Nashtarajha (Oligo/anovulation) in Ayurveda, most are not proven scientifically. Thus, the study is setup to fill the gap to a certain extent. The purpose of this study is to establish basic clinical efficacy and safety data for three Ayurveda decoctions consisting of two herbs. Both herbs are used as single-ingredient drugs (Ayurveda Pharmacopoeia, 1994) and as a formula for Nashtarajha (Oligo/anovulation) in Ayurveda (Krishnedas, 1841 &Sastri, 1994). The main feature of PCOS is oligo/anovulation. Hence, these drugs can be given to PCOS patients. But those drugs are not clinically tested for efficacy and safety on PCOS so far. Therefore, the aim of this research was to design a randomized clinical trial to detect the efficacy and safety of three Ayurveda decoctions.

MATERIALS AND METHODS

Design of the study: A prospective randomized, single-blind, three-arm clinical study.

Study area: This study will be conducted in the Gynecology clinic of National Ayurveda Teaching Hospital, Borella from August 2022 to August 2023 and patient will be selected from those seeking treatments for PCOS and subfertility. **Participants:** Participation in this research is voluntary. Patient recruitment is done by screening for eligibility criteria (inclusion and exclusion).

Ethical Consideration: Ethics approval has been obtained from Ethics Review Committee, Institute of Indigenous Medicine (ERCIIM), University of Colombo, Sri Lanka (ERC no: 19/85) and this study protocol

was approved by the specialty board – Prasutitantrastreeroga of Postgraduate Institute of Indigenous Medicine, Colombo University, Sri Lanka.

Inclusion Criteria: PCOS women, diagnosed by Rotterdam (2003) Diagnostic criteria (Boyle &Teede, 2012) aged between 20-40 years are included. They need to have the ability to take medication, be willing to adhere to the medication regimen, willing and able to give informed consent to be included in the study.

Exclusion Criteria: To ensure correct clinical assessment, conditions other than PCOS, suffering from other diseases that cause the same similar signs and symptoms of PCOS. (Thyroid disorders, severe insulin resistance, androgen-secreting neoplasm, etc. will be excluded.) Patients having disorders of reproductive tract such as tuberculosis, carcinoma, and congenital deformities of reproductive tract and other chronic illnesses such as cardiac disease and hypertension, etc. and patients who are already on other treatment regime will be excluded.

Sample Size: In PCOS cases 3months conventional treatment response in about 30% according to clinical experience conventional group. In this clinical trial wishes to test if a new therapeutic regimen can increase the response in PCOS patients to 60% with a power (1- β) of 80%. The type 1 error risk (2α) should be 5%. With 10% dropouts into account, 43 patients per group will be included, resulting in a total of 129 patients.

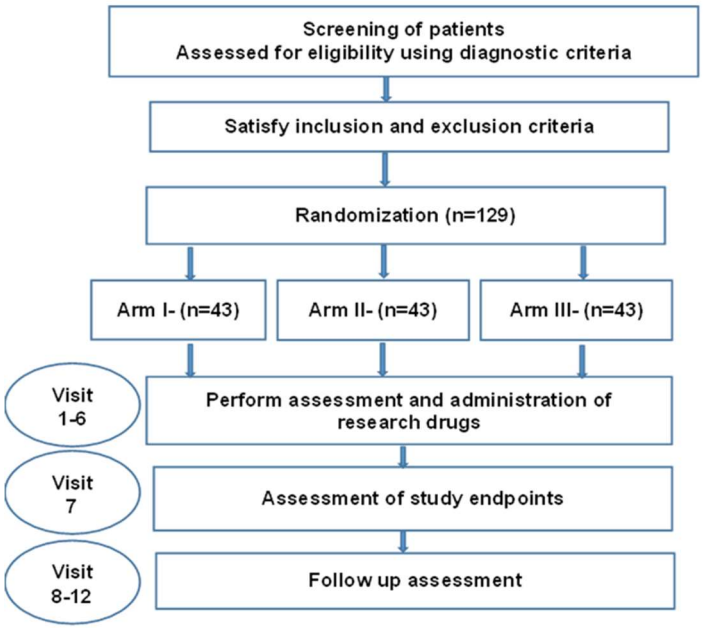

Figure 1: Study flow chart

Recruitment and Randomization: The participants will be provided with a detailed information sheet supplemented by a verbal explanation and obtain informed written consent. Consented participants

will be recruited randomly to the three arms Figure 1. Randomization sequence will be generated using an online randomization website. Each group will be enrolled with an allocation ratio of 1:1:1.

Treatment Protocol: Participant of Arm I, Arm II and Arm III will receive an oral drug for twelve weeks. Drug dosage and administration will be performed according to the following Table 1.

Table 1: Drug Dosage and Method of Drug Administration

Arm	Study Drugs	Dose	Mode of administration	Route	Period of intervention
I	*Nigella sativa* decoction	30 ml of Decoction	Morning and evening before meals	Oral	3 months
II	*Sesamum indicum* decoction	30 ml of Decoction	Morning and evening before meals	Oral	3 months
III	*Nigella sativa* + *Sesamum indicum* decoction	30 ml of Decoction	Morning and evening before meals	Oral	3 months

Follow Up:

Study participants will be followed up for three months after the drug administration period once a month. TVS/TAS, menstrual characters, associated features, and adverse effects will be performed in each visit. Hirsutism, Androgenic alopecia, Acne, and Acanthosis nigricans will be assessed in the last visit.

Drug Authentication and Standardization:

The various standardization parameters were studied as preliminary phytochemical analysis, heavy metal limits, Microbial limits, antioxidant activity, and developed the TLC fingerprint (Deepthika et al., 2022).

Preparation of The Drugs:

Decoctions will be prepared according to one of the basic formulations of Kwatha mentioned in Ayurveda Pharmacopeia Part I(Ayurveda Pharmacopoeia, 2006).

Storage, Packaging and Dispensing of Investigational Drugs:

All decoctions will be prepared separately under standard GMP conditions. Decoctions will be bottled in sterilized glass bottles with sealed plastic lids. Two bottles will be given for 14 days and labeled indicating the batch number, arm, dose, time of administration, mode of administration, etc. Drugs will dispense to the study participants at each visit with instructions. Treatment compliance will be assured by visual inspection of the drug containers, which will be carried by the patients at every clinic visit and questions by the investigator.

Data Collection, Record Keeping and Dissemination

Data will be collected by the principal investigator according to the given Table 2 and Consent, demographic characters, primary outcome measures, secondary outcome measures and safety measures will be taken as the table. Personal information will be kept confidentially until the end of the publication and will be discarded 2 years after the publication. Treatment and the scheduled interview will be held in a suitable environment where the privacy of the participants can be ensured. The results of the study will be published in peer-reviewed journals.

Table 2: Data Collection Schedule

Outcome Measures	**Screening of Patients**	**Treatment Period**			**Follow-up Period**		
		1st month	2nd month	3rd month	4th month	5th month	6th month
Informed consent	√						
Demographic characteristics	√						
Ultrasound Scan (TVS/TAS)	√	√	√	√	√	√	√
Assessment of acne using Global Acne grading System	√				√		√
Assessment of hirsutism using Ferriman-Gallwey Scale for Hirsutism	√				√		√
Assessment of Androgenic alopecia using Sinclar scale	√				√		√
Assessment of volume of menstrual blood using a Pictorial Blood Assessment chart	√	√	√	√	√	√	√
Assessment of dysmenorrhea using Visual Analogue Scale	√	√	√	√	√	√	√
Urine HCG		Will do if needed to exclude the pregnancy					
Safety assessment	√				√		
HQOL questionnaire	√				√		
Assessment of Prakriti	√						
Review of associated features	√	√	√	√	√	√	√
Screening for adverse effects		√	√	√	√	√	√

Primary Outcome Measurements:

Changes of PCOS Related Clinical findings:

- Findings of day 12 Trans Abdominal Sonography (TAS) or Trans-Vaginal Sonography (TVS) (ovarian volume, Largest Follicle size and endometrial thickness)
- Assessment of clinical features by Rotterdam (2003) Diagnostic criteria will be carried out before, after the treatment and during the follow-up period. Standard grading systems like the Global Acne grading System (Doshi, Zaheer and Stiller, 1997), Ferri- man-Gall-wey Scale (Ferriman& Gallwey 1996), Sinclair Scale (Sinclair et al., 2004), and the Burke's quantitative scale (Burke et al., 1999) will be used to assess those clinical features.

Secondary Outcome Measurements:

- Effect of intervention on Symptom severity and their impact on quality of life - will be assessed at the beginning and end of the treatment by PCOS symptoms and Health-Related Quality of life Questionnaire (Cronin et al., 1998).
- Relation in-between constituent and PCOS - Body constituent (Prakriti) is identifying of each at the screening with the help of a questionnaire which is already validated in India (Sanmugarajah, 2019). The relation in-between constituent and PCOS will be evaluated at the beginning, and the effect of therapy according to the body constituent will be evaluated at the end of the study.

Safety Assessment:

Each patient will undergo hematological and biochemical investigations (FBS, FBC, ESR, AST/ALT, and serum creatinine/GFR), urine full report before and after the interventions, which are done primary for safety assessment.

Safety Monitoring:

The patient will be advice to inform any adverse drug reaction immediately during the intervention and follow-up period. If found any adverse effects, it will be reported to the ERCIIM, and the participant will be directed to proper treatments after the termination of the research.

Termination:

The participants who do not wish to continue the treatment or participate in the study are free to inform the principal investigator at any time & do so. No loss of benefits/ medical care if withdrawn or discontinued the treatment.

Statistical Analysis:

Statistical analysis will be performed using the SPSS statistical package program (ver. 22.0), and the level of significance was established at $\alpha = 0.05$. Descriptive analysis for categorical variables will be expressed as numbers and proportions. The continuous data will be assessed for normalcy using QQ plots and the Kolmogorov–Smirnov test. Continuous data will be described using mean and standard deviation for normally distributed variables, while skewed data were described using IQR. Between-group comparisons will be carried out by analysis of variance (ANOVA) for normal data, while for non-normal data, the Kruskal–Walli's test will be used. For before and after data comparisons, the paired t-test and the related samples Wilcoxon sign rank test will be used for normally distributed and non-normal distrusted data, respectively. Similarly, for categorical variables, the chi-square test and Mac Nemar chi-square test will be used, respectively. Intention to treat analysis was performed for all efficacy outcomes and safety outcomes.

DISCUSSION

According to Yavari and others, Sesamum indicum L. may be an excellent choice for initiating bleeding in women with oligomenorrhea with no adverse effects when compared to existing hormonal therapy (Yavari et al., 2014). The sesame seeds therapy increased FSH activity, according to Rahman, Majeed, and Alkatan (Rahman, Majeed &Alkatan, 2009). Siriwardena et al. conducted clinical research for PCOS using a formula that included Nigella sativa, and only 25% of the patients had irregularities at the end of the treatment. At the completion of the treatment, 57.5 percent of patients had normal monthly bleeding length, 75 percent were free of dysmenorrhea, and most patients (70 percent) had normal menstrual blood quantity. When it came to skin discoloration, 87.5 percent of patients reported feeling better (Siriwardene et al., 2010). According to a comparative study conducted in Alta'ee, Ewadh, and Zaidan, Nigella sativa contains a significant amount of the sex hormones estrogen, progesterone, prolactin, testosterone, FSH, and LH (Alta'ee, Ewadh&Zaidan, 2006). Modaresi and Poor-Naji demonstrated that a hydro-alcoholic extract of Nigella sativa could increase the number of follicles and corpus luteum in female mice, resulting in improved fertility (Modaresi & Poor-Naji, 2012).

The use of Ayurveda medicines for PCOS may not be acceptable in scientific foru ms unless firm evidence of their efficacy and safety has proved. Therefore, this kind of trial protocol will be helpful to prove evidence-based scientific data on this subject area. The symptoms such as hirsutism, irregular bleeding, acne, obesity, and subfertility of PCOS may affect a patient's quality of life. In this study, it is expected to discover that how PCOS affect patient's quality of life and it is further expected to prove that Ayurveda treatments can be more helpful in improving quality of life. Prakriti (Constitution) concept of Ayurveda is claimed to be useful in predicting an individual's susceptibility to a disease, prognosis of disease and selection of treatment. This study is going to find the relationship in-between PCOS and constitution, and the effect of therapy will be analyzed by a participant's constitution.

CONCLUSIONS

At the end of this clinical research, it will be developed effective Ayurveda treatment for PCOS. Also, will be able to provide evidence-based scientific data on the classical Ayurveda treatment. Recruitment of participants commenced in March 2022, and results are expected to be available in March 2023.

Conflicts of Interest: The authors declare no conflict of interest.

Significance Statement: The use of herbal medicine goes back thousands of years. Herbal medicines are now recognized as dietary supplements for preventive care and complementary/alternative medicine. Polycystic Ovary Syndrome is the most common hormonal abnormality. There are several treatment regimens for PCOS in Ayurveda. However, most are not proven scientifically. This study aims to conduct a clinical trial to compare the efficacy and safety of three Ayurveda decoctions used in Ayurveda. Participants will be randomly divided into three groups, Arm I, Arm II, and Arm III and will receive an oral drug for twelve weeks and will be followed up for three months after the drug administration. Decoctions will be prepared according to one of the basic formulations mentioned in Ayurveda. The effect of the intervention on Symptom severity and their impact on quality of life - will be assessed at the beginning and end of the treatment. The use of Ayurveda medicines for PCOS may not be acceptable scientifically unless firm evidence of their efficacy and safety has been proven. This trial will help prove evidence-based scientific practice in this subject area.

REFERENCES

1. Alta'ee, A.H., Ewadh, M.J., Zaidan, H.K. (2006) *Deepthika et al., JSLAAS, Vol 4 Issue 1 (2022) 2-11* (Nigella sativa) Oil: Comparative Study. *Medical Journal of Babylon,3*(1):17-21.
2. Anonymous. (1994). *Ayurveda Pharmacopoeia* Vol.1, Part II, Department of Ayurveda, Sri Lanka.53.

3. Anonymous. (2006). *Ayurveda Pharmacopoeia* Vol.1, Part I, Department of Ayurveda, Sri Lanka, 20.31.
4. Boyle, J., Teede, H.J. (2012). Polycystic ovary syndrome - an update. *Aust Fam Physician*. 41(10):752-6. PMID: 23210095.
5. Burke, J.P., Hale, D.E., Hazuda, H.P., Stern, M.P. (1999). A Quantitative Scale of Acanthosis Nigricans. *Diabetes Care, 22*:1655-1559.
6. Cronin, L., Guyatt, G., Griffith, L., Wong, E., Azziz, R., Futterweit, W., Cook, D., Dunalf, A. (1998). Development of a health-related quality of life questionnaire (PCOSQ) for women with Polycystic Ovary Syndrome. *The Journal of Clinical Endocrinology and Metabolism,83*(6):1976-1987.
7. Deepthika, S.H.K., Karunagoda, K., Perera, P.K., Arawwawala, L.D.A.M. (2022). Quality assessment of a decocotion of Sesamum indicum and Nigella sativa L.;Polycystic Ovary Syndrome, *Asian Journal of Pharmacognosy, 6*(1): 32-41.
8. Doshi, A., Zaheer, A., Stiller, M.J. (1997). A comparison of current acne grading systems and proposal of a novel system. *Int J Dermatol, 36*(6):416–418.
9. Dutta, D.C. (2007). *Textbook of Gynecology Including Contraception*. 4th edition, Calcutta: New Central Book Agency, India, 216.
10. Ferriman, D.M., Gallwey, J.D. (1996). Clinical Assessment of Body Hair Growth in Women. *The Journal of Clinical Endocrinology & Metabolism,21*(11):1440-1447.
11. Krishnedas, G.V. (1841). *Vrihatniganturatnakaraya*, Chaukhambhaorientalia, India. 432.
12. Modaresi, M., Poor-Naji, N. (2012). The effect of black seed (Nigella sativa) hydro-alcoholic extract on breeding factors in female mice. *Journal of Shahrekord University of Medical Sciences,13*(6):63-70.
13. Rahman, S.Y.A., Majeed, A.F.A., Alkatan, M.M. (2009). Effect of sesame seeds on blood physiological and biochemmical parameters in broiler breeder hens. *Iraqi Journal of Veterinary Sciences,23*(1):25-28.
14. Sanmugarajah, V. (2019). A Study of the standard tool for prakriti assessment in indigenous medicine. *World Journal of Pharmaceutical Research,8*(5):574-582. Retrieved from DOI:10.20959/wjpr20195-14705
15. Sastri, L. (1994). *Yogaratnakara*, Chaukhambha Sanskrit sansthan, Varanasi. 406.
16. Sinclair, R., Jolley, D., Mallari, R., Magee, J. (2004). The reliability of horizontally sectioned scalp biopsies in the diagnosis of chronic diffuse telogen hair loss in women. *J Am Acad Dermatol, 51*:189-199.

17. Siriwardene, S.A.D., Karunathilaka, L.P.A., Kodituwakku, N.D., Karunarathne, Y.A.U.D. (2010). Clinical efficacy of Ayurveda treatment regimen on subfertility with poly cystic ovarian syndrome (PCOS). *AYU,31*(1): 24 – 27. Retrieved from DOI:10.4103/0974-8520.68203
18. Wolf, W.M., Wattick, R.A., Kinkade, O.N.and Olfert, M, D. (2018). Geographical Prevalence of Polycystic Ovary Syndrome as Determined by Region and Race/Ethnicity. *Int. J. Environ. Res. Public Health, 15*, 2589. Retrieved from DOI:10.3390/ijerph15112589
19. Yavari, M., Rouholamin, S., Tansaz, M., Bioos, S., Esmaeili, S. (2014). Sesame a Treatment of Menstrual Bleeding Cessation in Iranian Traditional Medicine: Results from a Pilot Study. *Shiraz E Medical Journal,15* (3): e21893. Retrieved from DOI:10.17795/semj21893.