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A randomized, double-blind, placebo-controlled trial of Chinese herbal medicine capsules for the treatment of premature ovarian insufficiency

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Abstract

Objective: This study was conducted to evaluate the treatment effectiveness of Chinese herbal medicine capsules containing the Yangyin Shugan formula (YYSG) in premature ovarian insufficiency (POI).

Methods: One-hundred forty-six women with POI participated in this stratified, randomized, double-blind, placebo-controlled clinical trial. Participants in two groups (n = 73 in each)—the YYSG group and control group—underwent treatment for 12 weeks. Outcome measures included the Chinese version Menopause-Specific Quality of Life questionnaire (CMS), serum levels of basal follicle-stimulating hormone (bFSH), basal estradiol, and anti-Mullerian hormone (AMH), the antral follicle count (AFC), and ovarian peak systolic velocity (PSV; cm/s).

Results: Treatment with YYSG significantly reduced the total scores of the CMS at the end of the 12th week with statistical significance ($P < 0.01$); the vasomotor, psychosocial, physical, and sexual domains significantly improvement after treatment ($P < 0.01$). Compared with the baseline hormone levels, YYSG markedly decreased the bFSH level with statistical significance ($P < 0.01$) and improved the AMH level ($P < 0.01$). Furthermore, YYSG greatly improved the participants' AFC and ovarian PSV, compared with placebo ($P < 0.01$). There were no serious adverse events, and the safety indices of whole blood counts, renal function, and liver function were within the normal range, both before and after treatment.

Conclusions: Treatment with YYSG was more effective than placebo for improving menopausal symptoms, basal hormone levels, and ovarian function in women with POI in Guangdong, China.

Key Words: Chinese herbal medicine – POI – YYSG decoction.

Premature ovarian insufficiency (POI) refers to a pathological condition characterized by amenorrhea, hypoestrogenic status, and elevated gonadotropin levels due to a decline in ovarian function before age 40.[1] The syndrome was first described by Albright and Fraser.[2] According to World Health Organization (WHO) statistics in 2007, it is estimated that approximately 1% of women become menopausal by the time they are 40, whereas the prevalence of POI in women younger than 30 is estimated to be 0.1%.[3] Qin et al[4] concluded that chromosomal abnormalities caused POI in approximately 10% to 13% of cases. Fenton[5] suggested that 19% of POI cases in New Zealand were caused by autoimmune ovarian damage, and, furthermore, some medical interventions such as chemotherapy, radiotherapy, and surgery may result in POI. In addition, an increasing number of premenopausal women who carry the breast cancer susceptibility genes mutation are undergoing risk-reducing surgery, including prophylactic oophorectomy. These factors have resulted in an increase in the numbers of women with iatrogenic POI.[6] Furthermore, studies have suggested an association between smoking and early menopause, and observational studies have suggested that HIV-positive women are at increased risk of early menopause.[7]

The treatment option used most often for POI is hormone therapy (HT), including estrogen therapy, progestogen therapy, and combined estrogen-progestogen therapy. Early intervention with a moderate dose of HT can alleviate some symptoms by compensating for decreased hormone levels. Although standard and rational application of HT was

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advocated on the basis of clinical trials, long-term use of HT remains controversial due to its side effects. A combination of estrogen and progestin could increase the risk of fatal and nonfatal malignancies.[8] The risks of HT differ, depending on type, dose, and duration of use, route of administration, timing of initiation, and whether a progestogen is used.[9]

Therefore, we undertook to find an effective alternative therapy for POI. Traditional Chinese medicine (TCM) has been used to treat POI through systematic regulation of physiological function, and also correction of pathophysiological conditions, and this has been carried out without obvious side effects.[10] According to the TCM theory, the main etiological factors of POI are ''yin deficiency and liver stagnation,'' and these disorders can lead to symptoms such as oligomenorrhea and amenorrhea. Moreover, women with yin deficiency may present with symptoms such as dizziness, insomnia, and constipation, whereas liver stasis may present with symptoms such as anxiety and irritability, and breast tenderness. Thus, TCM aims to supplement yin or the liver to create a balance.

In this study, we hoped to demonstrate clinical evidence for TCM treatment of POI. We carried out a randomized, double-blind, placebo-controlled clinical trial to evaluate the effectiveness of Chinese herbal medicine capsules for POI.

METHODS

Participants and setting

This clinical trial was conducted in accordance with the principles of the Declaration of Helsinki (2008 Revision) and the guidelines of Good Clinical Practice of the International Conference on Harmonisation (ICH-GCP). The study was approved by the Medical Ethics Review Committee of the Guangdong Provincial Hospital of Chinese Medicine on July 10, 2015. The registration number of the Chinese Clinical Trial Registry (Chi CTR; ChiCTR-IOR-16007694) was generated on January 4, 2016, and the study design can be found online (http://www.chictr.org/cn/proj/show.aspx). All relevant information pertaining to this research can also be requested from the International Clinical Trials Registry Platform (ICTRP). All study participants were recruited for preliminary screening via advertisements in the outpatient and inpatient departments of the Guangdong Provincial Hospital of Chinese Medicine in Guangdong, China. All participants who enrolled in this clinical trial did so after comprehensively understanding the study procedures and providing written informed consent.

Diagnostic criteria

Criteria for diagnosing were specified as described in the 2016 European Society for Human Reproductive Embryology Guideline on POI.[1] The inclusion criteria were: Chinese women aged <40 years; oligomenorrhea/amenorrhea for >4 months; basal follicle-stimulating hormone (bFSH) level >25 IU/L, on two occasions >4 weeks apart; no HT or treatment with Chinese medicine for at least 3 months before study enrollment; and individuals meeting the above criteria who voluntarily consented to study participation. Exclusion criteria include: treatment with hormones or medications that induce reproductive toxicity, such as *Tripterygium wilfordii*, or participation in other clinical trials during the preceding 3 months that could have affected the menstrual cycle; oligomenorrhea/amenorrhea induced by surgical procedure or medical treatments (eg, chemotherapy, pelvic radiation therapy, ovarian cystectomy, ovarian drilling, ovarian wedge resection, attachment resection, tubal resection, tubal ligation, pelvic abscess surgery, uterine artery embolization); chronic illnesses, such as gastrointestinal disease, renal disease, liver disease, diabetes mellitus requiring treatment, and uncontrolled hypertension; serious diseases, such as cancer, autoimmune disease, thrombosis, and thrombophlebitis; hypersensitive constitution or allergies to the study medication; and nonprovision of written informed consent.

Study design

The study consisted of a 12-week treatment period. Eligibility screening and questionnaires on quality of life were undertaken, and information on demographic characteristics, reproductive history, habits such as smoking and alcohol consumption was also collected. In addition, responses to the CMS questionnaire were recorded. After the eligibility screening, participants underwent a series of medical tests to obtain baseline data. These comprised whole blood count, liver function test, renal function test; levels of bFSH, basal estradiol (bE_2), and anti-Mullerian hormone (AMH); and also breast color Doppler ultrasound and a transvaginal ultrasound. Blood samples were obtained both at baseline and at the study endpoint by our research nurse, and all samples were tested by the Medical Laboratory Department Guangdong Provincial Hospital of Chinese Medicine in China (see supplementary digital content 2-4, http://links.lww.com/MENO/A299, http://links.lww.com/MENO/A300, http://links.lww.com/MENO/A301). The ultrasound was undertaken by an experienced ultrasound expert. These data were used to ensure the minimal eligibility criteria for each woman and to screen out respondents with potentially poor adherence to study procedures. All study participants who fulfilled the inclusion and exclusion criteria were randomly assigned to either the Yangyin Shugan formula (YYSG) or placebo group. Consultations with a TCM practitioner were conducted every 4 weeks during the 12-week treatment period. During these consultations, weight, blood pressure, and lifestyle factors were monitored. All examiners who conducted blood assays and outcome measurements were blinded to the treatment group allocation. The randomization was done using sealed envelopes, and allocation was not revealed before the endpoints were recorded. Therefore, neither the participant nor the examiner knew who was in the study or the placebo group.

Interventions

The interventions used in this study were YYSG and placebo. The YYSG formula was modified from the Yiguan decoction description in the ''Supplement to the Classified

Case Records of Celebrated Physicians'' in the Qing Dynasty (first published in 1770 AD). In TCM theory, the composition of YYSG is used to nourish the kidney yin and soothe liver stasis. In this study, YYSG and placebo were administered to different groups, and their effectiveness was comparatively evaluated. All test medicines and placebos were manufactured by the Guangdong Provincial Hospital of Chinese Medicine. The test medication has had widespread application in our hospital for more than 30 years, with annual usages more than 30,000; and no adverse reactions have been reported, and it is produced in individualized separate wrapping, with controlled quality, and at an optimal dosage. This formula does not need to be boiled, unlike other traditional herbal medicines. The effectiveness of the capsules has been certified as being equal or superior to crude herbal medicine.[11,12]

Thus, these capsules were used to improve the quality-control element of this study and to reinforce participant compliance. The standard formula for YYSG consists of seven herbs. The dosages of each herb were standardized according to the latest Chinese pharmacopoeia (2015 edition). The composition, plant parts, main components, and function of each herb in the YYSG formulation are described in Table 1.

The study medication was tested for heavy metal and toxic elements, microbial contamination, and pesticide residues by the US Food and Drug Administration (FDA) Guangdong (Report no. Yue Z20080139). Water extracts of crude herbal materials were processed as described in the Chinese pharmacopoeia (2015 edition). The powder of both YYSG and placebo was formulated as warm water-soluble granules of similar shape, color, taste, and smell, as verified by senior Chinese herbalists. Each capsule contained 0.5 g crude herbal preparation and was prepackaged in two identical medicinal plastic bottles, each containing 36 capsules. Participants were instructed to take four capsules thrice daily.

Outcome measurements

The primary outcome measurement was the Chinese version of the Menopause-Specific Quality of Life (CMS) questionnaire.[13] The original English version, known as Menopause-Specific Quality of Life (MENQOL), was introduced in 1996, and has been widely used in many countries as a tool to assess the menopausal period health-related quality of life in clinical trials.[14] In 2002, our team introduced the MENQOL scale for the first time in China.[15] The Chinese version of the MENQOL questionnaire (see supplementary digital content 1, http://links.lww.com/MENO/A298) was approved, and has been permitted for use in China for nearly 15 years. To date, our team has carried out the largest psychometric study of the Chinese version of the MENQOL. A previous study confirmed that the CMS is a valid and reasonable tool for evaluating health-related quality-of-life factors for menopausal women in China that corresponds to the original English MENQOL.[13] There are 29 items in the CMS, which are divided into four domains including vasomotor symptoms, psychosocial symptoms, physical health, and sexual health-related questions. Lower scores in the CMS indicate a better quality of life of the participant. Each participant was asked to finish the questionnaire both before and after the intervention. This questionnaire was used to comprehensively evaluate important health issues that menopausal women may experience.

The secondary outcome measurement included serum hormone levels, AMH levels, antral follicle count (AFC), and ovarian blood flow. In this study, serum FSH and E_2 levels were measured to evaluate the effects of Chinese medicine on regulation of hormonal balance.

Circulating levels of AMH are derived from the total cohort of granulosa cells in the developing small ovarian follicles.[16] As it reflects the number of primordial follicles,[17] it is predictive of the reproductive lifespan.[18-20] The decrease in AMH levels that occurs with increased age may be noted before changes in other age-related variables are detected, suggesting that serum AMH levels may be the best marker of ovarian aging. In cross-sectional studies, serum AMH has been used as a sensitive and specific marker of ovarian function in women with POI.[21-23] Prior studies in infertile women have shown that the AFC is directly correlated with

TABLE 1. *Details of Yangyin Shugan decoction capsules*

Chinese name	Full scientific name	Plant parts used	%	Dose of dry plant	Constituent	Dose after extraction	Function in Chinese medicine
Chai hu	*Bupleuri Radix*	Dry root	19.4	12	Saikosaponin	2.90	Relieving qi stagnation, reinforcing yang,
Yu jin	*Curcumae Radix*	Dry root	19.4	12	Rhizoma curcumae Longae	2.90	Resolving qi stagnation
Bai shao	*Paeoniae Radix Alba*	Dry root	16.1	9	Peoniflorin	2.18	Regulating menstruation, nourishing yin, and arresting sweating
Shan yao	*Dioscoreae Rhizoma*	Dry root and stem	16.1	9	Diosgenin	2.18	Nourishing spleen and stomach, toning the kidney to arrest spontaneous emission
Di huang	*Rehmanniae Radix*	Dry root	14.5	10	Martynoside	2.42	Nourishing yin, strengthening marrow and essence
Fu ling	*Poria*	Dry sclerotia	14.5	10	Triterpenes	2.42	Invigorating spleen for eliminating,
Xiang fu	*Cyperi Rhizoma*	Dry root and stem	14.5	10	β-cyperone and cyperotundone		dispersing stagnated liver qi and stagnation, regulating menstruation and relieving pain
Total			100	62		15	

oocyte yield.[24-26] A transvaginal ultrasound is required to determine AFC by imaging and manually measuring the diameter of all small antral follicles and counting those with diameters between 2 and 10 mm. Beginning with the early antral follicular stage, folliculogenesis becomes cyclic, with maturation dependent on waves of FSH and other factors. Therefore, the AFC is typically measured at the beginning of a cycle. Certainly, in some cases, low AFC may be related to lower primordial follicle reserve in the ovary. We assessed ovarian reserve by measuring AFC and ovarian peak systolic velocity (PSV) at the initial consultation on reproductive function. The transvaginal ultrasound was done by an experienced ultrasonologist, and follicles with diameter between 2 and 10 mm (by clinician measurement, not automated calculation) were counted in both ovaries to account for the AFC.

Data on demographic and clinical characteristics such as age, height, weight, education level, age at menarche, regularity of the menstrual cycle, duration of each cycle, medical history, medication history, and allergies were collected as baseline information. To scrutinize the impact of the tested medication on the breast and uterus,[8,9] a color Doppler was used to examine the breast and uterus of each participant both before and after the administration of TCM/placebo, as well as routine examination of blood and urine, and also an electrocardiogram (ECG). In addition, participants were asked to report any adverse events noticed after receiving treatment. For participants who dropped out, reasons for withdrawal were recorded.

Randomization and masking

In this study, participants were randomized into two groups on the basis of their age and bFSH level. Both the participants and the investigators were blinded to treatment allocation. The investigational drugs were coded and uniformly packaged. Medication to last for 4 weeks was packed in a separate box, labeled with the drug name, observation period, codes, manufacturer, and also usage, dosage, and storage conditions. The portion of drug dispensed to a participant for each treatment session contained the same number of bottles of capsules, either herbal medicine or placebo. Meanwhile, emergency envelopes for code breaking were prepared in case of serious adverse events or for emergency unblinding, if necessary. Finally, the computer-generated random digits and program were stored confidentially for safekeeping. The random allocation sequence was sealed in opaque envelopes.

Unblinding provisions

The method of unblinding twice was used in this double-blind clinical trial. The database was locked after the blinded review, and the grouping information was revealed to enable statistical analysis. On completion of the statistical analysis and preparation of the report, treatment allocation was unblinded at the final review conference.

Sample size calculation

Sample size in this study was calculated on the basis of changes in the number of antral follicles as described in previous research.[27] We used the following formula:

$$n = \frac{(u_\alpha + u_\beta)^2(1 + 1/k)\sigma^2}{(\Delta - \delta)^2}$$

In previous research,[27] the change of numbers of antral follicles between weeks 0 and 12 ranged from 4.30 ± 3.19 to 5.65 ± 3.03 in the treatment group and 3.29 ± 3.17 to 3.45 ± 3.30 in the control group. The 12-week difference between the two groups was 2.2, with a standard deviation (SD) of 3.17. Using the difference significance test to estimate the sample content, and taking $\alpha = 0.05$ and $\beta = 0.10$, power was calculated as $(1 - \beta) = 90\%$, when two-tailed α and power were set at 0.05 and 90%, respectively. We calculated that 51 participants were needed in each group. With an estimated 30% dropout rate during 12 weeks of treatment, each group needed to include at least 73 participants; therefore, 146 participants were needed to adequately power this study.

Statistical analysis

All efficacy and safety analyses were conducted according to the intention-to-treat principle. Missing values were imputed by the last-observation-carried-forward method. Statistical analysis was conducted using the Statistical Package of Social Sciences 19.0 for Windows. Statistical significance was defined as being determined by two-sided $P < 0.05$. Baseline characteristics were reported as mean (SD) and frequency (%). Baseline differences between groups were assessed with the Student's t test for normally distributed continuous variables and with a nonparametric Mann-Whitney U test for non-normally distributed continuous variables.

Measurement data were designated as mean (SD) in the statistical description. These data were usually analyzed by analysis of paired t test (where data satisfied normal distribution and homogeneity of variance) and paired nonparametric test (where data satisfied neither normal distribution nor homogeneity of variance). The chi-square test is optimal for categorical variables. If there was a statistically significant difference within any group, pair-wise comparison was conducted.

Demographic data and clinical characteristics of participants such as age, body mass index, employment, marital status, history of smoking and alcoholism, age at menarche, dysmenorrhea, history of ovulation induction, gravidity, and parity were noted as constituent ratios in a descriptive analysis.

RESULTS

Flow diagram of participant enrollment

A participant enrollment flow diagram is presented in Fig. 1. In all, 445 Chinese women were recruited for preliminary screening via advertisements from the outpatient and inpatient departments of the Guangdong Provincial Hospital of Chinese Medicine in China from August 1, 2015 to January 31, 2017. In all, 174 women participated in the baseline

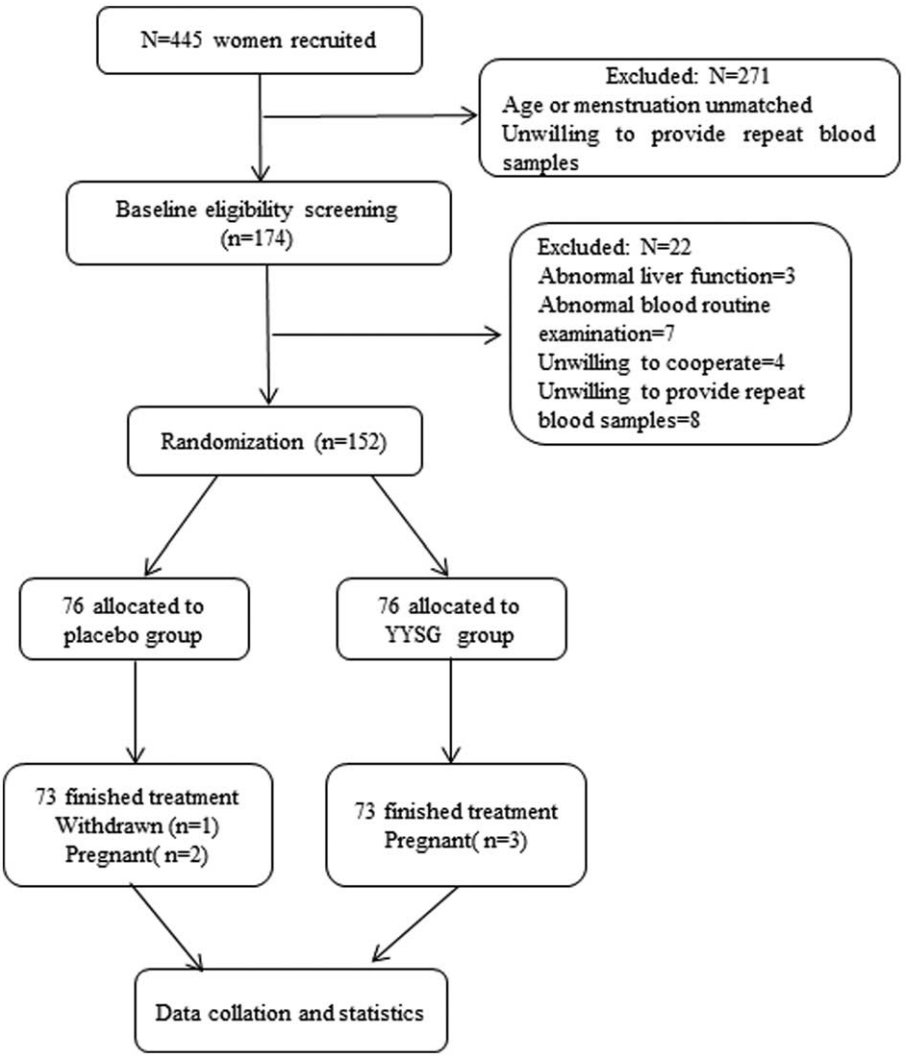

FIG. 1. Flow diagram of participant enrollment. YYSG, Yangyin Shugan formula.

eligibility screening for randomization. Three women were excluded due to abnormal results of liver function; 7 women were excluded due to abnormal results of routine blood examination (including white blood cell, red blood cell, and platelet counts). In addition, four women were excluded for refusal to cooperate with study procedures, and eight women were excluded as they declined to provide repeat blood samples; apart from these, six participants (three each from the YYSG and placebo groups) withdrew during the study period (for reasons listed in the flow diagram).

Finally, 146 participants completed the assigned treatment. Both study groups were comparable with regard to baseline demographic and clinical characteristics of participants (Table 2).

Baseline data

The baseline characteristics of study participants are summarized in Table 2. The mean (SD) age was 38.3 (2.8) years for the YYSG group and 38.0 (3.6) years for the placebo group. The mean (SD) body mass index was 21.1 (2.9) kg/m^2 for the YYSG group and 20.8 (2.7) kg/m^2 for the placebo group. No significant differences were observed in the age, body mass index, job, marital status, history of smoking and alcoholism, age at menarche, dysmenorrhea, history of ovulation induction, gravidity, and parity ($P > 0.05$) between the study groups.

Primary outcome assessment

Total CMS scores and different domains of CMS scores

The CMS scores for POI in all groups are summarized in Table 3. A significant reduction in total CMS scores was noted within the YYSG group and on comparison between this group and the placebo group. After the 12-week treatment period, the mean (SD) of the total CMS score had significantly decreased in both groups—from 67.64 (7.42) to 38.64 (5.69) in the YYSG group, and from 68.29 (4.02) to 65.04 (4.40) in the placebo group ($P < 0.01$). Scores were reduced in the vasomotor domain from 7.08 (1.09) to 4.21 (0.96) in the

TABLE 2. *Baseline characteristics data*

Characteristics	YYSG group (n = 73)	Placebo (n = 73)	Between-group *P*
Age, mean (SD), y	38.3 (2.8)	38.0 (3.6)	0.81
BMI, kg/m^2, mean (SD)	21.1 (2.9)	20.7 (2.7)	0.46
Employment, n (%)			
Administrative worker	21 (28.8%)	19 (26%)	0.83
Waitress	5 (6.8%)	5 (6.8%)	
Professionals	15 (20.5%)	12 (16.4%)	
Self-employed	15 (20.5%)	21 (28.8%)	
Others	17 (23.3%)	16 (21.9%)	
Marital status			
Single	1 (1.4%)	4 (5.5%)	0.37
Married	72 (98.6%)	69 (94.5%)	
Other	0 (0%)	0 (0%)	
History of smoking	2 (2.7%)	4 (5.5%)	0.68
History of alcoholism	0 (0%)	0 (0%)	1.0
Age at menarche, mean (SD), y	13.1 (1.2)	13.4 (1.3)	0.15
Dysmenorrhea, n (%)	10 (13.7%)	18 (24.7%)	0.09
History of ovulation induction, n (%)	12 (16.4%)	8 (11.0%)	0.34
Gravidity, n (%)			
0	9 (12.3%)	6 (8.2%)	0.59
1-3	48 (65.8%)	47 (64.4%)	
≥4	16 (21.9%)	20 (27.4%)	
Parity			
≤1	65 (89.0%)	58 (79.5%)	0.11
≥2	8 (11.0%)	15 (20.5%)	

Results from Student's *t* test for normally distributed continuous variables and from nonparametric Mann-Whitney *U* test for non-normally distributed continuous variables.
BMI, body mass index; YYSG, Yangyin Shugan decoction.

YYSG group, and from 7.12 (1.08) to 6.93 (0.90) in the placebo group ($P < 0.01$); in the psychosocial domain from 19.12 (1.98) to 14.37 (2.78) in the YYSG group, and from 19.66 (1.42) to 17.05 (1.66) in the placebo group ($P < 0.01$); in the physical domain from 34.32 (5.53) to 16.88 (3.92) in the YYSG group, and from 34.07 (2.57) to 33.60 (2.78) in the placebo group ($P < 0.01$); and in the sexual domain from 7.12 (1.17) to 3.19 (1.59) in the YYSG group, and from 7.44 (1.22) to 7.45 (1.24) in the placebo group ($P < 0.01$). There were significant differences within the YYSG group and between this group and the placebo group after the 12-week treatment period.

Secondary outcome assessment

Levels of serum bFSH, bE$_2$, and AMH, AFC, and ovarian PSV

Levels of serum bFSH, bE$_2$, and AMH, the AFC, and ovarian PSV were tested at baseline and at the culmination of the 12-week treatment period, with significant differences between the YYSG and placebo groups (Table 4).

Safety assessment and compliance

Routine examinations found no abnormalities in blood, urine, and ECG after the 12-week treatment. There were no abnormal findings in the breast or uterus.

Participant adherence to the protocol after the 12-week treatment was fair, with only one participant withdrawing from the placebo group (Fig. 1). The overall treatment compliance among the remaining participants was good. Participants were found to have taken at least 95% of scheduled doses, as calculated by counting the remaining medication capsules.

Safety and adverse events

Whole blood count, renal function, and liver function were evaluated at baseline and after treatments. There were no differences in these safety indices within the YYSG group or between the study groups. Both YYSG and placebo were well-tolerated. There were no serious adverse events (eg, conditions requiring hospital admission). In the YYSG group, no adverse events occurred. In the placebo group, a single adverse event of stomach ache was reported (n = 1). No serious adverse events occurred.

TABLE 3. *Comparison of main outcome measures within and between the YYSG group and the placebo group*

Outcome measures	YYSG (n = 73)		Placebo (n = 73)		*P*[a]
	Mean ± SD	M (P75-P25)	Mean ± SD	M (P75-P25)	
CMS total score					
Baseline	67.64 ± 7.42	67.0 (7.0)	68.29 ± 4.02	69.0 (4.5)	0.11
12 wks	38.64 ± 5.69[b]	38.0 (7.5)	65.04 ± 4.40[b]	65.0 (5.0)	**<0.01**
Vasomotor domain					
Baseline	7.08 ± 1.09	7.0 (1.0)	7.12 ± 1.08	7.0 (2.0)	0.86
12 wks	4.21 ± 0.96[b]	4.0 (2.0)	6.93 ± 0.90[b]	7.0 (1.0)	**<0.01**
Psychosocial domain					
Baseline	19.12 ± 1.98	19.0 (2.0)	19.66 ± 1.42	20.0 (1.5)	0.55
12 wks	14.37 ± 2.78[b]	15.0 (3.0)	17.05 ± 1.66[b]	17.0 (2.0)	**<0.01**
Physical domain					
Baseline	34.32 ± 5.53	34.0 (5.5)	34.07 ± 2.57	34.0 (2.0)	0.88
12 wks	16.88 ± 3.92[b]	17.0 (4.0)	33.60 ± 2.78[b]	33.0 (3.0)	**<0.01**
Sexual domain					
Baseline	7.12 ± 1.17	7.0 (2.0)	7.44 ± 1.22	7.0 (3.0)	0.12
12 wks	3.19 ± 1.59[b]	3.0 (2.5)	7.45 ± 1.24[b]	7.0 (1.5)	**<0.01**

CMS, Chinese version of Menopause-Specific Quality of Life; M, median; YYSG, Yangyin Shugan decoction.
[a] *P* values represent baseline and 12-week comparisons between groups. The results are derived from the Wilcoxon tests.
[b] $P < 0.05$, compared with the baseline within the group. The result is derived from the Mann-Whitney *U* tests.
Bold values indicate statistical significance.

TABLE 4. *Comparison of secondary outcome measures within and between the YYSG group and the placebo group*

Outcome measures	YYSG (n = 73)		Placebo (n = 73)		P^a
	Mean ± SD	M (P75-P25)	Mean ± SD	M (P75-P25)	
bFSH, IU/L					
Baseline	34.04 ± 4.19	34.8 (6.1)	32.94 ± 4.20	32.2 (6.5)	0.09
12 wks	10.11 ± 4.63[b]	8.6 (9.0)	32.66 ± 13.81[c]	31.3 (10.4)	**<0.01**
Difference value	−23.92 ± 5.68	−23.9 (10.5)	−0.29 ± 13.98	−2.7 (11.0)	**<0.01**
bE_2, pmol/L					
Baseline	183.64 ± 133.14	155.8 (108.6)	201.01 ± 95.67	183.9 (161.6)	0.07
12 wks	201.08 ± 126.05[c]	164.4 (173.8)	205.92 ± 121.09[c]	179.2 (162.9)	0.79
Difference value	148.26 ± 497.33	34.4 (162.6)	19.30 ± 220.07	−9.6 (174.7)	**<0.05**
AMH, ng/mL					
Baseline	1.14 ± 1.58	0.5 (1.7)	1.55 ± 2.35	0.6 (1.7)	0.64
12 wks	1.76 ± 2.11[b]	0.9 (3.0)	0.73 ± 1.61[b]	0.2 (0.9)	**<0.01**
Difference value	0.62 ± 0.91	0.3 (1.2)	−0.82 ± 1.66	-0.1 (0.9)	**<0.01**
AFC					
Baseline	4.90 ± 3.17	5.0 (5.0)	4.56 ± 3.42	4.0 (5.5)	0.46
12 wks	6.97 ± 3.35[b]	6.0 (4.5)	4.43 ± 3.06[c]	4.8 (4.0)	**<0.01**
Difference value	2.07 ± 2.51	2.0 (3.8)	−0.13 ± 2.96	0.0 (3.4)	**<0.01**
LEFPSV, cm/s					
Baseline	11.20 ± 7.67	10.5 (6.2)	10.03 ± 8.11	9.3 (7.8)	0.28
12 wks	15.79 ± 7.40[b]	14.7 (7.6)	8.56 ± 4.68[c]	9.9 (5.4)	**<0.01**
Difference value	4.59 ± 5.00	4.8 (6.9)	−1.47 ± 8.48	−1.1 (10.2)	**<0.01**
RIGPSV, cm/s					
Baseline	9.45 ± 6.82	9.7 (6.7)	12.46 ± 9.03	10.9 (9.2)	0.06
12 wks	11.79 ± 7.71[b]	10.7 (7.7)	9.69 ± 7.48[b]	9.7 (6.3)	**<0.05**
Difference value	2.35 ± 9.26	0.7 (13.0)	−2.77 ± 10.13	−1.6 (10.4)	**<0.01**

AFC, antral follicle count; AMH, anti-Mullerian hormone; bE_2, basal estradiol; bFSH, basal follicle-stimulating hormone; CMS, Chinese version of Menopause-Specific Quality of Life; LEFPSV, left ovarian peak systolic velocity; RIGPSV, right ovarian peak systolic velocity; YYSG, Yangyin Shugan decoction.

[a] *P* values represent baseline and 12-week comparisons between groups. The results are derived from the Wilcoxon tests.

[b] $P < 0.05$, compared with the baseline within the group. The result is derived from the Mann-Whitney *U* tests.

[c] $P > 0.05$, compared with the baseline within the group. The result is derived from the Mann-Whitney *U* tests.

Bold values indicate statistical significance.

DISCUSSION

The report of this randomized controlled trial was prepared according to Consolidated Standards of Reporting Trials for TCM statement.[28] Based on data analysis, we found that it was a meaningful and rewarding method to evaluate the effects of a Chinese herbal medicine through its effect on the particular symptoms of a disorder; additionally, the method allowed us to assess the efficacy of a drug, thus facilitating the development of precision medicine.

It is shown in our research report that the YYSG formula contained seven compounds: *Paeoniae Radix Alba*, *Rehmanniae Radix Praeparata*, *Curcumae Radix*, *Poria*, *Bupleuri Radix*, *Cyperi Rhizom*, and *Dioscoreae Rhizoma*[29]—all of which are traditional Chinese herbal prescriptions used for menopausal symptoms. Extensive research has been conducted in the past 30 years, focusing on the pharmacodynamics and pharmacokinetics of YYSG. Our previous studies indicated that YYSG alleviated the POI menopausal syndrome, with all compounds displaying strong estrogen-like effects and increasing endocrine and antioxidant functions through activation of the aromatase and catalase detoxifying pathways.[11,12] Furthermore, YYSG is involved in prolonging antiosteoporotic effects and delaying the aging of the hypothalamus-pituitary-target gland axis.[27,30] These studies support the clinical application of YYSG for treating POI symptoms. To our knowledge, this trial is the first placebo-controlled, double-blind clinical trial of YYSG for the treatment of POI. Our method provides a standardized preparation that includes capsules, documented screening, a randomization process, validated biomarkers, and objective measurement.

This study showed that the YYSG can effectively decrease the total CMS scores and scores of its four domains in women with POI. Especially in the vasomotor domain, the score noticeably decreased from 7.08 (1.09) to 4.21 (0.96) in the YYSG group—a nearly 40% change. In addition, the physical domain score decreased by more than 50%—from 34.32 (5.53) to 16.88 (3.92)—in the YYSG group; this domain includes the items of flatulence or gas pains, aching of muscle and joints, feelings of tiredness or exhaustion, difficulty sleeping, aches at the back of the neck or head, and so on. According to TCM theory, menopausal symptoms, such as hot flashes and night sweats, are mainly related to renal deficiency, whereas physical and mental exhaustion, sexual problems, and joint disorders are mainly related to liver stasis. Simple treatment strategies that nourish the kidney or soothe the liver cannot solve the problems of POI. The composition of YYSG includes herbs that act as a kidney tonic and soothe the liver to effectively treat POI symptoms arising from a pattern of renal deficiency and hepatic stasis. These results are consistent with previous clinical studies on YYSG.[11,27,30]

As we know, ovarian reserve is a measurement of the reproductive potential of the ovaries. The parameters of ovarian reserve include a woman's age, serum levels of sex hormones (FSH and E_2), AMH, AFCs, and the PSV (the stromal blood flow determined by pelvic ultrasound on days 4-8 of the menstrual cycle), which are among factors that

have been measured to evaluate ovarian reserve.[31-40] In this trial, the serum hormones (FSH and E_2), AMH, AFC, and ovarian PSV were checked at baseline and after 12 weeks of treatment. Compared with placebo, the YYSG formula exhibited the effect of down-regulating the FSH level and up-regulating AMH and E_2 levels, and also the AFC and PSV effectively (Table 4), all of which are consistent with findings from our previous studies.[11,12,27,41] The safety indices of whole blood counts, renal function, liver function, breast, and ECG remained at normal levels after treatment. Apart from gastric discomfort, no obvious side effects were found in this study. Participants lost to the study were low in both the treatment and placebo groups. These findings suggest that YYSG is a potential well-tolerated intervention for treating POI.[11,12,27,30,41,42]

Although our findings indicate YYSG was superior to placebo in improving symptoms and regulating hormone levels, this study has three major limitations. First, a self-selection bias is probably present, as study participants were recruited through newspaper advertisements. Most of them expressed negative views about HT during intake interviews, and many even had high prior expectations regarding the effectiveness of Chinese medicine. Such expectations may lead to the inaccuracy of subjective reporting, which inputs variations into our CMS scores. Second, the research cycle was relatively short, as compared with the traditional treatment for POI which could continue for years. Because of the long treatment cycle and high expense, the compliance of participants in our trial gradually decreased; therefore, we stopped our study at the end of 3 months. Moreover, many studies adopt a 3 to 6-month medication observation period to observe the effectiveness and safety of the therapy.[43-46] With better methods to ensure adherence, we would like to extend the treatment and follow-up periods, because the effects of our intervention are still evident at the end of the treatment period. Third, this study only included participants with POI with symptoms of renal deficiency and hepatic stasis. Due to financial constraints, our study was a small single-center trial in Guangzhou. Given the wide prevalence of POI in TCM, future multicenter studies will be needed to differentiate and treat POI according to the different syndrome types. In the near future, large-scale, multicenter studies will be carried out to study the use of Chinese herbal medicine to treat POI at different stages.

The measurements selected in this study were mainly specified to explore diagnostic or therapeutic factors rather than pathogenesis. In future studies, we would like to test etiological factors of POI, such as granule cell apoptosis and autophagic mechanisms, to better understand POI.

CONCLUSIONS

Our study shows that YYSG is effective in regulating hormonal levels and improving ovarian function among Guangdong women with POI. It is well-tolerated, with no serious adverse events noted during the study period. Larger-scale multicenter clinical studies of YYSG among more diverse populations are needed to further confirm these findings.

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