

BMJ Open Effects of tanshinone on hyperandrogenism and the quality of life in women with polycystic ovary syndrome: protocol of a double-blind, placebo-controlled, randomised trial

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ABSTRACT

Introduction: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in reproductive-age women. Chinese herbal medicine has been used for the treatment of PCOS, but the evidence for its efficacy and safety is minimal. Tanshinones are a class of bioactive molecules isolated from *Salvia miltiorrhiza*, a commonly used herb in Traditional Chinese Medicine. This study aims to evaluate the efficacy of tanshinones on hyperandrogenism and quality of life in women with PCOS who do not attempt to conceive.

Methods and analysis: A total of 100 patients will be recruited and randomised into the tanshinone or placebo group. Tanshinone or placebo capsules will be taken orally for 12 weeks. The primary outcome parameter will be a change in plasma testosterone. Secondary end points will be changes in human chorionic gonadotropin-induced androgen response, insulin resistance, reproductive hormones, fasting lipid profiles, oral glucose tolerance test, quality of life and side effects.

Ethics and dissemination: Written informed consent will be obtained from each participant at the time of enrolling in the study. The trial has been approved by the Ethics Committee of First Affiliated Hospital of Heilongjiang University of Chinese Medicine. Results will be disseminated through a publicly accessible website.

Registration details: The study has been registered at the Chinese Clinical Trials Registry (ChiCTR-TRC-12002973) and at clinicaltrials.gov (NCT 01452477).

INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in reproductive-age women. Its prevalence rates depend on the diagnostic criteria used, but it can be up to 18% using the Rotterdam diagnostic criteria.¹ PCOS is characterised

by hyperandrogenism, oligo/amenorrhoea and polycystic ovary morphology and is often associated with insulin resistance. Hyperandrogenism is found in 60–80% of women with PCOS.² The major clinical and biochemical features of hyperandrogenism are hirsutism, acne, alopecia and seborrhoeic dermatitis; elevated androstenedione, testosterone and dehydroepiandrosterone sulfate (DHEAS) levels; and decreased sex hormone binding globulin (SHBG) levels. The syndrome presents not only with reproductive manifestations but also has metabolic implications including insulin resistance, obesity, dyslipidemia, systemic inflammation and type 2 diabetes.^{3–5}

PCOS is a clinical and public health issue because it adversely affects women's health and health-related quality of life and puts a significant strain on healthcare resources. PCOS and related complications are also a tremendous economic burden, and in 2006 the total annual cost to treat women with PCOS between the ages of 14 and 44 years was more than US\$430 million in the USA. Treatments for hirsutism and diabetes account for 14% and 40%, respectively, of the total healthcare costs related to PCOS.⁶

The long-term therapy for women of PCOS who do not desire to become pregnant depends on the specific clinical presentations and individual patient goals. Comprehensive treatment methods for hyperandrogenism and glucose and lipid metabolic dysfunction include lifestyle modifications, diuretic medicines, insulin-sensitising and anticholesteremic agents and oral contraceptives (OC).⁷ The first-line treatment in the management of overweight or obese women with PCOS is lifestyle modification, which consists of diet and

exercise, and this can often improve psychological outcomes, metabolic features and reproductive features.^{8–11} Lifestyle modifications can be combined with pharmacological interventions for optimal results.¹² OC can be used as the first-line medical agent in women with PCOS who have no desire to conceive, and OC can significantly reduce serum androgen concentrations and ameliorate the androgenic symptoms in the skin. In addition, antiandrogens for hyperandrogenism such as spironolactone, flutamide and cyproterone acetate can inhibit androgen-binding receptors and decrease androgen production.¹³

When choosing a medication, the side effects of the various medications must be taken into account, including weight gain, fatigue, nausea, oedema, diarrhoea, sinusitis, hypoglycaemia and kidney toxicity.^{13–14} Furthermore, some studies have shown that OC might decrease insulin sensitivity and aggravate glucose and lipid metabolism.^{15–16} Therefore, OC have not been approved by the US Food and Drug Administration for suppressing androgen production.

Traditional Chinese Medicine (TCM), which originated in China more than 3000 years ago, is an important part of complementary medicine (CM). CM includes many modalities such as Chinese herbal medicine (CHM), acupuncture, Tai Chi and other therapies. The theory of TCM is complex and includes Yin and Yang, Qi and Xue, Zang and Fu and the five elements. According to TCM theory, the aetiology and pathogenesis of PCOS are closely related to 'blood stasis' and 'kidney vacuity'.¹⁷ CHM is emerging as one of the most commonly practiced treatments for PCOS¹⁸ and it has been shown to aid in weight loss and improve the ovulation rate and insulin resistance as well as improve the patients' outlook.¹⁹ A clinical trial compared the efficacy of metformin and the Chinese herbal formula 'Tiangui Fang' in treating hyperandrogenism and hyperinsulinism in patients with PCOS. After treatment for 12 weeks, the Chinese herbal formula significantly lowered the serum testosterone and insulin levels compared to metformin alone.¹⁷ The mechanisms of some CHM formulations used to treat PCOS have been elucidated. For example, Gancao (*Radix glycyrrhizae*) can inhibit androgen synthesis and Baishao (*Radix paeoniae Alba*), Danggui (*Radix angelicae Sinensis*) and Danshen (*Salvia miltiorrhiza Bunge*) improve insulin sensitivity. Furthermore, Sanqi (*Radix Notoginseng*), Zelan (*Herba Lycophi*) and Zexie (*Rhizoma Alismatis*) can induce ovulation.¹⁹

Tanshinones are a class of bioactive constituents isolated from *S. miltiorrhiza* (Danshen), which is a commonly used herb in TCM. Cryptotanshinone is the major bioactive tanshinone in the plant and has several pharmacological effects including anti-inflammatory, antioxidative, anticholinesterase, antibacterial and antiplatelet aggregation and anticancer activities.^{20–22} CHM has been used for the treatment of PCOS, but the evidence for its efficacy and safety is minimal. Animal experiments showed that cryptotanshinone can induce favourable alterations in androgen excess and insulin

resistance as well as glucose metabolism,²³ but there is still a lack of scientific justification for the use of tanshinone in women with PCOS. In particular, no randomised controlled trials have been performed to evaluate the use of tanshinone on hyperandrogenism, metabolic profiles or the quality of life in women with PCOS who do not wish to conceive.

In the proposed study, we seek to evaluate the efficacy of tanshinone on hyperandrogenism, glucose and lipid metabolism as well as the quality of life in women with PCOS who do not attempt to conceive. Our hypothesis is that tanshinone is effective in the suppression of androgen production by directly inhibiting ovarian androgen production and by reducing insulin resistance and improving the lipid profile.

METHODS AND ANALYSIS

Study design

The study has been registered at the Chinese Clinical Trials Registry (ChiCTR-TRC-12002973) and at clinicaltrials.gov (NCT 01452477). This is a multicentre, randomised, double-blind and placebo-controlled clinical trial. Informed written consent will be obtained from eligible women prior to their participation in this study, and the recruited women will be randomised into either the tanshinone group or the placebo group. We will follow the CONSORT recommendations in reporting the results.²⁴

Setting and recruitment

This study will be conducted in the outpatient clinics of four hospitals in mainland China. The principal investigator at each clinic will recruit potentially eligible participants who will be informed of the study through internet, radio, newspaper or television advertisements. All of the potential participants can get full information about the study objectives, design and treatment as well as the benefits and risks of treatment from the investigators or research coordinators at each site.

Participants

A total of 100 eligible women will be recruited from four centres in China. They will be examined at the site centre and enrolled into the trial if they meet the selection criteria.

Inclusion criteria

1. Presence of PCOS diagnosed based on the Androgen Excess Society criteria. All participants must have hyperandrogenism (hirsutism and/or hyperandrogenaemia) and ovarian dysfunction (oligoanovulation and/or polycystic ovaries) and must not have other androgen excess-related disorders. Oligomenorrhoea is defined as an intermenstrual interval >35 days or <8 menstrual bleedings in the last year. Amenorrhoea is defined as an intermenstrual interval >90 days. Clinical hyperandrogenism is defined as a Ferriman-Gallwey (FG) score ≥ 5 .²⁵

2. Within the age range of 18–35 years.
3. No desire to become pregnant within 6 months and using condoms for contraception.

Exclusion criteria

1. Use of hormonal drugs or other medications in the past 12 weeks that can affect the results from the Chinese herbal prescriptions.
2. Other androgen excess endocrine disorders including 21-hydroxylase deficiency, hyperprolactinaemia, Cushing syndrome, severe insulin resistance and thyroid dysfunction.
3. A history of severe cardiac, pulmonary, hepatic, renal or neurological disease or mental illness.
4. Pregnancy or lactation.

Interventions

Eligible participants will be randomised into either the tanshinone group or the placebo group. The tanshinone capsules (1 g 3 times/day, China State Food and Drug Administration (SFDA) approval number Z13020110) and placebo capsules will be provided by Hebei Xinglong Xili Pharmaceutical Co Ltd. The tanshinone and placebo capsules have the same outer packaging, colour, shape and flavour. Tanshinone or placebo will be administered orally for 12 weeks. The main pharmaceutical formulation of the tanshinone capsules is cryptotanshinone, which comprises 90% of the total formulation in the experimental drug.

Study-specific visits and procedures

The trial phase will involve treatment with either tanshinone or placebo for 12 weeks (figure 1). Participants will attend the clinic five times in total for a screening visit, a baseline visit, two monthly visits and the end-of-treatment visit.

At the baseline and the end-of-treatment visits, participants will undergo the following tests between 8:00 and

12:00 after an overnight fast: a human chorionic gonadotrophin (HCG) stimulation test, a 75 g 2 h oral glucose tolerance test (OGTT), a hyperinsulinaemic euglycemic clamp test and measurement of fasting lipid profiles and levels of reproductive hormones. Side effects, adverse events and other current drug treatments will be recorded during the visits. An overview of the study visits is found in table 1.

Study assessment (including questionnaires)

The primary outcome measure is a decrease in basal serum testosterone concentration. The secondary outcomes include

1. HCG-induced response of androgens including 17- α -hydroxyprogesterone (17-OHP), androstenedione (A2) and testosterone;
2. Insulin resistance as determined by measuring the glucose disposal rate (GDR) with the hyperinsulinaemic euglycemic clamp test;
3. Hyperinsulinaemia as determined by OGTT.
4. Plasma levels of reproductive hormones: oestradiol, 17-OHP, follicle stimulation hormone, luteinising hormone, SHBG and DHEAS;
5. Fasting glucose and lipid profiles: fasting blood glucose, fasting insulin, C peptide, glycosylated haemoglobin A1c, cholesterol, triglycerides, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol;
6. Weight, blood pressure, waist/hip circumference, FG score and acne;
7. Side effects and adverse events.

Clinical examination and study tests

Height will be recorded to the nearest 1 cm without shoes, and weight will be recorded to the nearest 0.01 kg. Patients will be weighed while dressed in light

Figure 1 Flow chart of the study.

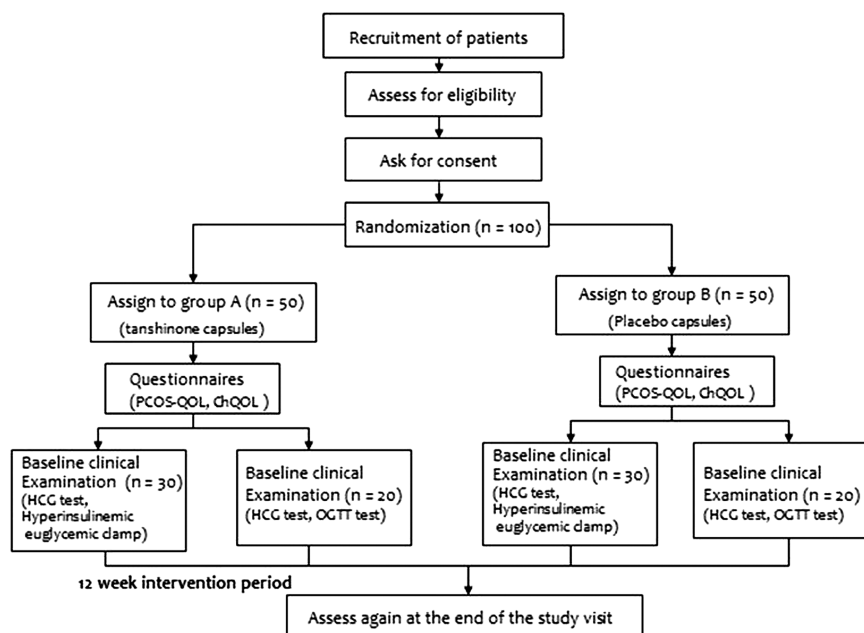


Table 1 Overview of study visits

	Screening visit	Baseline visit	Monthly visit 1	Monthly visit 2	Monthly visit 3 and end-of-treatment visit
Physical examination	X				X
Safety labs (liver profile: ALT/AST, total bilirubin Renal profile: BUN or creatinine)	X				X
Transvaginal ultrasound		X			X
Fasting phlebotomy for study parameters		X			X
Hyperinsulinaemic euglycemic clamp		X			X
Ask about adverse events and concurrent medications		X	X	X	X
Ask about menstrual period	X	X	X	X	X
Medication dispensing and accounting		X	X	X	

Physical examination: Height, weight, hip and waist circumference, blood pressure, FG score and acne.

Fasting phlebotomy: Serum for the Central Core Laboratory.

Transvaginal ultrasound: Endometrial thickness, ovarian volume, antral follicle count and size of ovarian cysts or developing follicles.
ALT, alanine transaminase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; FG score, Ferriman-Gallwey score.

clothing without shoes. The patient will sit quietly for a period of 5 min before the blood pressure is taken. The blood pressure may be taken in either arm using the appropriate cuff size. Acne lesions²⁶ will be assessed by trained study personnel who will evaluate the patient's face. The areas of the face to be evaluated are the right forehead, left forehead, right cheek, left cheek and chin. Hirsutism will be graded according to the modified FG method by trained study personnel. Patients will be given the Hirsutism Score form and the study personnel will tell the patients to assess the areas outlined on the form.

HCG test: Ovarian androgen biosynthesis will be measured with the HCG test. On the morning of the experiment, between 8:00 and 9:00, a single injection of 5000 IU HCG will be given to the patient and blood samples will be drawn 24 h and 48 h later for assays of 17-OHP, A2 and testosterone.

OGTT test: All participants will undergo an overnight fast. After ingestion of a 75 g glucose load, blood samples will be obtained at 0, 30, 60, 90 and 120 min for determining the glucose and insulin levels.

Hyperinsulinaemic euglycemic clamp: Insulin sensitivity will be assessed by the hyperinsulinaemic euglycemic clamp before and after treatment. The hyperinsulinaemic euglycemic clamp studies will be performed at the Huaian Maternal and Child Health Hospital and 60 participants will receive clamp examinations. The participants will undertake a 10–12 h overnight fast. A small intravenous catheter will be placed in an antecubital vein for blood sampling and a second catheter will be inserted into the contralateral forearm for administration of insulin and glucose infusions. After a 30 min stabilisation period, a priming insulin infusion will be administered for 3 min followed by a constant infusion of 120 mU/m²/min for 120 min. Three minutes after the start of the priming insulin infusion, 20% dextrose will be infused at a variable rate to keep blood glucose concentrations between 4.5 and 5.0 mmol/L. Blood samples

will be collected every 5 min during the insulin infusion. GDR (measured as mg/kg/min) is defined as the amount of glucose required to maintain stable blood glucose concentrations during the last 30 min of the clamping. This value will be used as the measure of insulin resistance.

Questionnaires

All of the participants will be requested to complete the Polycystic Ovary Syndrome Quality of Life (PCOS-QOL)²⁷ and the Chinese Quality of Life (ChQOL)²⁸ questionnaires before and after treatment.

Randomisation and blinding

Randomisation will be performed through a web-based randomisation system operated by an independent data centre (Institute of Basic Research of Clinical Medicine, China Academy of Chinese Medical Sciences, Beijing). Participants will be randomly assigned to the tanshinone group (n=50) or the placebo group (n=50). The identification code and random number, which are unique for each participant, will be given by a web-based system (<http://210.76.97.192:8080/dst/>) produced by the independent data centre. Participants, investigators and physicians taking care of participants will be blind to the assignment.

Data entry and quality control of data

The data will be recorded in the Case Report Forms (CRFs). The CRFs will be filled out truly and accurately, and the electronic versions of the CRFs will be deposited into a web-based data management system at <http://218.17.160.110:8081/login.aspx>.

In order to maintain the quality of the data, we will adopt valid measures to assure information authenticity, accuracy and integrity. First, the site investigator or research coordinator should record or enter information accurately and in a timely manner, and every site investigator must make a monthly check of the accuracy

and timeliness of the recorded information on the CRFs. Second, the data manager and programmer of the Data Coordination Center (DCC) will be in charge of data monitoring and validation and will ensure that issues arising from the data are resolved quickly and accurately. Third, unscheduled monitoring of the clinical sites will be important for quality control. These visits will ensure that the collection method and study data are standardised, accurate and authentic. The CRFs will be compared with source documents to make sure that errors have been resolved without delay. After each visit, the monitoring report will be distributed to the site principal investigator. The site visit is an effective action for maintaining data quality and patient protection.

Sample size calculation and statistical analysis

We hypothesise that the basal serum testosterone level will be reduced by 10 ng/dL in the tanshinone group and remain unchanged in the placebo group. We assume standard deviation of 0.06 of the difference of two groups. The sample size needed to achieve an 80% power to perceive a significant difference in serum testosterone concentration between the two treatment groups at the two-sided 5% level can be estimated with the parameters $\alpha=0.05$ and $\beta=0.1$. This power analysis suggests that 40 patients will be needed for each group. Assuming a 20% dropout rate based on our past experience, 50 patients will be enrolled in both groups and 100 patients will be enrolled in total.

All data will be analysed by a specialised statistician using the intent-to-treat approach for the evaluation of drug efficacy, the per-protocol analysis for adherence and safety analysis for adverse events. The efficacy of two treatments (tanshinone vs placebo capsules and within-participant effects before vs after treatment) will be compared by analysis of variance (ANOVA). Pearson's χ^2 test will be used to assess the different qualitative data between the two groups. Statistical evaluation of the data will be performed using the SPSS programme V.16.0 (SPSS Inc, Chicago, Illinois, USA) and a p value <0.05 will be considered statistically significant.

DISCUSSION

As the number of patients with PCOS increases, it is anticipated that more and more patients will turn to CM for treatment. CHM can regulate and strengthen the hormonal systems of the whole body and is a natural approach for treating PCOS. The significant advantages of CHM are that it provides several options for the safe, effective, multitargeted treatment of various aspects of PCOS including hyperandrogenism and poor quality of life.^{14 29 30} In our previous studies, we have found that cryptotanshinone can decrease excessive androgens by inhibiting steroid hormone production in the theca cells in the ovary^{31 32} and also improve insulin resistance and glucose metabolism.^{32 33}

Tanshinone is used extensively in China for treating acne because of its antiandrogenic properties. The purpose of this study is to evaluate whether tanshinone has a significant effect on hyperandrogenism in women with PCOS and to explore new uses for cryptotanshinone, the primary biologically active form of tanshinone. This trial was designed based on the high efficacy and few side effects of cryptotanshinone. To better evaluate the therapeutic effects of tanshinone on hyperandrogenism, quality of life, insulin resistance and hyperinsulinaemia, we will use the HCG test, questionnaires (PCOS-QOL and ChQOL), hyperinsulinaemic euglycemic clamp and OGTT test. There have been no clinical trials performed to determine the efficacy of tanshinone for PCOS, and a well-designed, double-blind, placebo-controlled randomised trial will not only determine the clinical efficacy of such treatment but also could provide insights into new evidence-based therapies for PCOS.

Contributors XW conceptualised and designed the study. WS, EHYN and XW wrote the protocol. WL, JC and YZ reviewed the protocol for important intellectual content. All authors read and approved the final manuscript.

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Competing interests None.

Patient consent Obtained.

Ethics approval The study was approved by the Ethics Committee of the First Affiliated Hospital of Heilongjiang University of Chinese Medicine (2010HZYLL-016).

Provenance and peer review Not commissioned; internally peer reviewed.

Data sharing statement The trial results will be published in a peer-reviewed scientific journal.

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