Effecs of Tanshinone on hyperandrogenism and quality of life in women with polycystic ovary syndrome: protocol of a double-blind, placebo-controlled, randomized trial

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Effecs of Tanshinone on hyperandrogenism and quality of life in women with polycystic ovary syndrome: protocol of a double-blind, placebo-controlled, randomized trial

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ABSTRACT

Introduction: Polycystic ovary syndrome (PCOS) is one of the commonest endocrine disorder in reproductive age women. CHM has been used for the treatment of PCOS but the evidences for safety and efficacy are minimal. Tanshinones are a class of bioactive constituents isolated from Salvia miltiorrhiza, which is a common used herb in TCM. This study aims to evaluate the efficacy of tanshinone in women with PCOS who do not attempt to conceive on hyperandrogenism and quality of life.

Methods and analysis:

A total of 100 patients will be enrolled into this study and will be randomized into Tanshinones or placebo groups. Tanshinones or placebo capsules will be taken orally for 12 weeks. The primary endpoint is a change in plasma testosterone. Secondary end points are changes: in HCG induced response of androgens; insulin resistance; reproductive hormones; fasting gluco-lipid metabolic profiles; oral glucose tolerance test; quality of life; adverse events.

Ethics and dissemination:

Written informed consent will be obtained from each participant at study enrolment. The trial has been approved by the Ethis Committee of First Affiliated Hospital of Heilongjiang University of Chinese Medicine. Results will be disseminated via a publically accessible website.

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

1 INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the commonest endocrine disorder in reproductive age women. Its prevalence rates depend on different diagnostic criteria used and it can be up to 18% according to the Rotterdam diagnostic criteria [1]. PCOS is characterized by hyperandrogenism, oligo/amenorrhea and polycyctic ovary (PCO)

morphology and may be associated with insulin resistance. Hyperandrogenism can be found in 60%-80% in women with PCOS [2]. The major clinical and biochemical features of hyperandrogenism are hirsutism, acne, alopecia, seborrheic dermatitis, and abnormality sex steroid levels including elevated androstenedione, testosterone, dehydroepiandrosterone sulfate (DHEAS) levels, as well as decreased sex hormone binding globulin (SHBG) level. The syndrome presents not only with reproductive manifestations but also had metabolic implications including insulin resistance, obesity, dyslipidemia, systemic inflammation, and type 2 diabetes [3-5].

For the most part, PCOS is of both clinical and public health issues, as it adversely affects women's health, health-related quality of life and has significant implications on the health administration. The economic burden of PCOS and related complications is quite tremendous for the health care system. In 2006, the total annual cost among PCOS women aged 14-44yr was more than 430 million dollars in the United States, and moreover, the portion of health care-related economic burden for hirsutism and diabetes are 14% and 40% respectively [6].

The long-term therapy for women of PCOS who do not desire for pregnancy depends on the specific clinical presentations and individualized patient goals. Comprehensive treatment methods for hyperandrogenism, glucose and lipid metabolic dysfunction include lifestyle modifications, diuretic medicine, insulin-sensitizing and anticholesteremic agent, as well as oral contraceptives [7]. The first line treatment in the management of overweight or obese PCOS women is lifestyle modification, which consists of diet and exercise, and may have some benefits improving psychological outcomes, metabolic features and reproductive features [8-11]. Lifestyle modifications can be combined with pharmacologic interventions for optimal results[12]. Oral contraceptive pill (OCP) may be used as the first-line medical agent in women with PCOS who have no desire to conceive. The OCP can significantly reduce serum androgen concentration and ameliorate the skin androgenic symptoms. In addition, antiandrogens for hyperandrogenism such as

spironolactone, futamide or cyproterone acetate may inhibit androgen-binding receptors or decrease androgen production [13].

However, when choosing a treatment medication, the side effects of the various medications must be taken into account, including weight gain, fatigue, nausea, edema, diarrhea, sinusitis, hypoglycaemia and kidney toxicity[13-14]. Furthermore, some studies shown that the OCP may decrease insulin sensitivity and aggravate glucose and lipid metabolism [15-16]. Therefore, the OCP has not been approved by the U.S. Food and Drug Administration for suppressing androgen production.

Traditional Chinese Medicine (TCM) is an important part of complementary and alternative medicine (CAM), which includes many modalities, such as Chinese herbal medicine (CHM), acupuncture, Tai Chi and other therapies. TCM originated in china more than 3000 years ago. The theory of TCM is complex and includes Yin and Yang, Qi and Xue, Zang and Fu, as well as Five Elements. According to the TCM theory, the etiology and pathogenesis of PCOS are closely related with 'blood stasis' and ' kidney vacuity' [17]. CHM is emerging as one of the commonly practiced medicines for PCOS [18] and has been shown to aid in weight loss, improve ovulation rate and insulin resistance as well as improve patients' outlook [19]. A clinical trial observed the efficacy of Chinese herbal formula 'Tiangui Fang' and metformin in hyperandrogenism and hyperinsulinism patients of PCOS. After three months treatment, the Chinese herbal formula significantly lowered serum testosterone and insulin levels when compared with metformin [17]. The mechanisms of some CHM used in PCOS have been elucidated. For example, Gancao (Radix glycytthizae) can inhibit androgen synthesis and Baishao (Radix paeoniae Alba), Danggui (Radix angelicae Sinensis), and Danshen (Salvia miltiorrhiza Bunge) have the effect of improving insulin sensitivity. Furthermore, Sanqi (Radix Notoginseng), Zelan (Herba Lycopi) and Zexie (Rhizoma Alismatis) can induce ovulation [19].

Tanshinones are a class of bioactive constituents isolated from Salvia miltiorrhiza (Danshen), which is a common used herb in TCM. Cryptotanshinone (CT) is the major

bioactive tanshinone in the plant and has several pharmacological effects including anti-inflammatory, anti-oxidative, anti-cholinesterase, anti-bacterial, antiplatelet aggregation and anti-cancer [20-22]. CHM has been used for the treatment of PCOS but the evidences for safety and efficacy are minimal. The animal experiment showed that cryptotanshinone can induce favorable alterations in androgen excess and insulin resistance as well as glucose metabolism [23]. There is still a lack of scientific justification for the use of tanshinone in women with PCOS. Particularly, a randomized controlled trial (RCT) has not been performed to evaluate the use of tanshinones on hyperandrogenism, metabolic profiles and quality of life in women with PCOS who do not attempt to conceive.

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

2 METHODS AND ANALYSIS

2.1 Study design

The study was approved by the Ethis Committee of First Affiliated Hospital of Heilongjiang University of Chinese Medicine (2010HZYLL-016) and the other participating centre also have the ethics approvals. The study has been registered at ClinicalTrials.gov: NCT01452477. This is a multicentere, randomized, double blind and placebo-controlled clinical trial. Informed written consents will be obtained from eligible women prior to the participation of this study and recruited women will be randomized into either of the two groups (tanshinone capsules or placebo). We will follow the CONSORT recommendations in reporting the results [24].

2.2 Setting and recruitment

This study will be conducted in the outpatients of four hospitals in mainland China. The principal investigator in each site can recruit potentially eligible participants at outpatient clinic through internet, radio, paper or television advertisements. All the potentially

participants can get full information about the study objectives, design, treatment as well as benefits and risks of treatments from the investigators or research coordinators in each site.

2.3 Participants

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

2.3.1 Inclusion criteria

The inclusion criteria are as follows: (1) Presence of PCOS diagnosed based on the Androgen Excess Society criteria. All subjects must have hyperandrogenism (hirsutism and/or hyperandrogeniaemia), ovarian dysfunction (oligoanovulation and/or polycystic ovaries), and exclusion of other androgen excess related disorders. Oligomenorrhea is defined as an intermenstrual interval >35 days or <8 menstrual bleedings in the past year. Amenorrhea is defined as an intermenstrual interval >90 days. Clinical hyperandrogenism is defined as a Ferriman-Gallwey (FG) score ≥5 [25]. (2) Age of women from 18 to 35 years；(3) No desire of children within 6 month and use condoms for contraception.

2.3.2 Exclusion criteria

The exclusion criteria are as follows: (1) Use of hormonal drugs or other medications, which can affect the results of the study especially Chinese herbal prescriptions in the past 12 weeks；(2) Patients with other androgen excess endocrine disorders including 21-hydroxylase deficiency, hyperprolactinemia, Cushing syndrome, severe insulin resistance, thyroid dysfunction；(3) Patients with history of sever cardiac , pulmonary, hepatic, renal, neurologic disease or mental illness；(4) Pregnancy or lactation.

2.4 Interventions

Eligible participants will be randomized into one of the two arms: Tanshinone capsules (1g, three times/day) or placebo capsules. The tanshinone capsules (China State Food and Drug Administration (SFDA) approval no. Z13020110) and placebo both be provided by Hebei Xinglong Xili Pharmaceutical Co. LTD. They have the same outer packing, dosage，

shape,and predominant flavor. Tanshinone or placebo will be administrated orally for 12 weeks. The main pharmaceutical formulation of tanshinone capsules is cryptotanshinone, which comprises 90 % of total formulation in the experimental drug.

2.5 Study specific visits and procedures

The trial phase: treatment with either tanshinone or placebo for 12 weeks (Figure 1).

Participants will attend the clinic five times in total: a screening visit, a baseline visit, two monthly visits and the end of treatment visit.

At baseline and the end of treatment visits, participants will undergo the following tests : a HCG stimulation test, a 75 gm 2h oral glucose tolerance test, a hyperinsulinemic euglycemic clamp clamp test and blood sample for assays of reproductive hormones, plasma glucose and lipid. Adverse events and drug co-treatments will be recorded during visits. An overview of study visit is found in Table 1.

2.6 Study assessment (include questionnairs)

The primary outcome measure is a decrease of 10 ng/dl in basal testosterone. The secondary outcomes include:

(1) HCG induced response of androgens including 17-hydroxyprogesterone (17-OHP), androstenedione (A2), testosterone (T).

(2) Insulin resistance by the glucose disposal rate (GDR) with hyperinsulinemic euglycemic clamp test

(3) hyperinsulinemia by OGTT

(4) Reproductive hormones: testosterone (T), estradiol (E2), 17-α-hydroxyprogesterone (17-OHP), follicle stimulation hormone (FSH), leutinizing hormone (LH), sex hormone binding globulin (SHBG) and dehydoepiandrosterone sulphate (DHEAS).

(5) Fasting gluco-lipid metabolic profiles: fasting blood glucose, fasting insulin , C-peptide, glycosylated hemoglobin Alc(HbAlc), cholesterol, triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C).

(6) Oral glucose tolerance test (OGTT): serum for glucose, Insulin and c-peptide levels.

(7) Weight, blood pressure, waist/hip circumference, F-G score and acne.

(8) Adverse events.

2.6.1 Clinical examination and study tests

Height will be recorded to the nearest 1 cm without shoes, and weight will be recorded tothe nearest 0.01 kg. Patients will be weighed while dressed in light clothing, without shoes. The patient should sit quietly for a period of 5 minutes before the blood pressure is taken. The blood pressure may be taken in either arm using the appropriate cuff size. Acne lesions [26] will be assessed for this study. Trained study personnel will assess a patient's acne by evaluating 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 Ferriman and Gallwey 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 Hirsutism Score form.

HCG test: The ovarian androgen biosynthesis will be measured by human chorionic gonadotropin (hCG) test. On the morning of the experiment, between 8:00–0:900, a single injection of HCG 5,000 IU to the patient and blood samples will be drawn at 24, 48h therafter for assays of 17-OHP, A2 and testosterone .

OGTT test: All the 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 120min for glucose and insulin level determination.

Hyperinsulinemic euglycemic clamp: Insulin sensitivity will be assessed by the hyperinsulinemic euglycemic clamp before and after treatment. The hyperinsulinemic euglycemic clamp studies will be performed in Huaian Maternal and Child Health Hospital and 60 participants will receive clamp examination. The participants will be in a 10-12 h overnight fast. A small intravenous catheter will be placed in an antecubital vein for blood sampling. A second catheter will be inserted into the contralateral forearm for administration of insulin and glucose infusions. After a 30 min stabilization period, a 3-min priming insulin infusion will be administered, followed by a constant infusion of 120mU/m^2 surface area per min nearly 120 min. At the beginning 4 min after the insulin infusion, 20% dextrose will be infused at a variable rate to keep blood glucose concentrations at 4.5 ~ 5.0mmol/L. Blood samples will be collected every 5min. The glucose disposal rate (GDR;

milligrams per kilogram per minute), defined as the amount of glucose required to maintain stable blood glucose concentrations during the last 30 min of the clamping, will be used to calculate insulin resistance.

2.6.2 Questionnaires

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

2.7 Randomization and blinding

The randomization will be performed through a web-based randomization system operated by an independent data center (Institute of Basic Research of Clinical Medicine, China Academy of Chinese Medical Sciences, Beijing). Participants will be randomly assigned to tanshinone group (n=50) or placebo group (n=50) in a ratio of 1:1. 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 center. Participants, investigators and physicians taking care of subjects will be blind to the assignment.

2.8 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 version of CRF will be accomplished in a Web-based data management system at http://218.17.160.110:8081/login.aspx.

In order to keep the data qualities, we will adopt some valid measures to assure information authenticity, accuracy, integrality, seasonable sex. First, the site investigator or research coordinator should record or entry information accurately and timely. At the same time every site investigator must check the normative, accuracy and timeliness of recorded information at the CRFs monthly. Secondly, the data manager and programmer of the DCC will be in charge of data monitoring and validation regularly and supervise the findings found be resolved within the given time. Last but not least,

the unscheduled monitoring clinical site is very important for quality control and some procedure should be implemented to assure the collection and data of the study are normative, accurate and authentic. The CRFs will be compared against source documents. Make sure the errors have been resolved without delay. After each visit, monitor report must be distributed to the site principal investigator. The site visit is an effective action for data quality and patient protection.

2.9 Sample size calculation and statistical analysis

We hypothesis that serum testosterone level will be reduced by 10 ng/dl in the tanshinone group and will have no change in the placebo group. We hypothesis a standard deviation of 0.06 of the difference. To achieve 80% power to perceive a significant difference in T between the two treatment groups at the two-sided 5% level. The sample size will be estimated according to the parameters: α=0.05, β=0.1, 40 patients will be needed for each group. Considering a 25% dropout rate, 50 patients will be enrolled in each group and 100 patients will be enrolled in total.

All data will be analyzed by a specialized 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. its placebo capsules; within-participants effects before vs. after treatment) will be compared by ANOVA. The Pearson's chi-square test will be used to assess the different qualitative data between the two groups. Furthermore, the analysis for data statistical evaluation will be performed using the SPSS program, version 16.0 (SPSS Inc., Chicago, IL) and a P value < 0.05 will be considered statistically significant.

3. DISCUSSION

With the increase in the number of PCOS, more and more patients turn to complementary and alternative medicine for treatment. CHM can regulate and strengthen the hormonal systems of the whole body, which is a natural approach for treating PCOS. The significant advantages of CHM may be safe, effective, multi-targets and multiple methods for treating various aspects of PCOS including hyperandrogenism and quality of life [29-31]. In our previous studies, we have found that cryptotanshinone can decrease excessive androgens by inhibiting steroid hormones production of theca cells in ovary [32-33]. Moreover, cryptotanshinone can improve insulin resistance and glucose metabolism [33-34].

Tanshinone are extensively used for treating acne because of its antiandrogenic properties in China. The purpose of this study is to evaluate whether tanshinone has significant effect on hyperandrogenism in PCOS women and to explore new indications for tanshinone capsules whose major active ingredient is cryptotanshinone. In view of the good efficiency and fewer side effects of cryptotanshinone, we design this trial. To better evaluate the interventional therapeutic effects of tanshinone on hyperadrogenism, quality of life, insulin resistance, hyperinsulinemia, we applied HCG test, questionnaires(PCOS-QOL and ChQOL), hyperinsulinemic euglycemic clamp, OGTT test. Furthermore, there is no clinical trial design of tanshinone for PCOS. A well design of a double-blind, placebo-controlled, randomized trial not only attest the clinical efficacy but also could provide evidence-based therapy for PCOS.

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Contributors: WJS, YHZ and XKW conceptualised and designed the study. WJS, EHN and

XKW wrote the protocol. WL, JC and YZ reviewed the protocol for important intellectual content. All authors read and approved the final manuscript.

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

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Competing interests: None.

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Figure 1

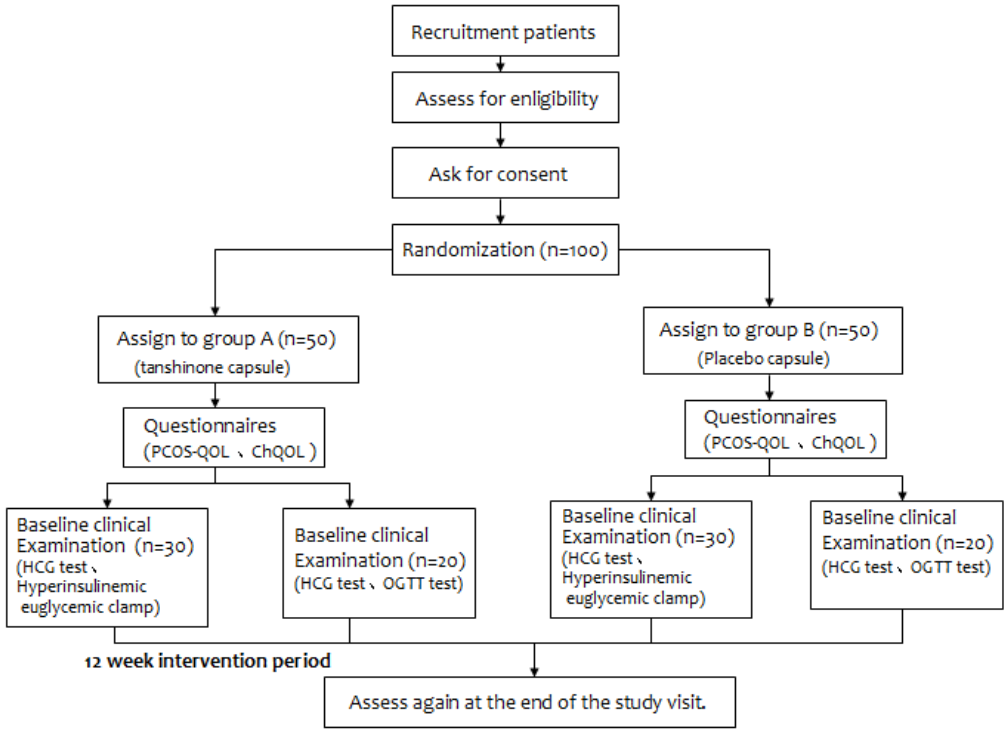

Table 1.Overview of study visits

	Screening visit	Baseline visit	Monthly visit 1	Monthly visit 2	Monthly visit 3 and End of treatment visit
Physical exam	X				X
Safety labs	X				X
Transvaginal ultrasound		X			X
Fasting phlebotomy for study parameters		X			X
Hyperinsulinemic euglycemic clamp		X			X
Query for adverse event and concomitant medications		X	X	X	X

Query for menstrual period	X	X	X	X	X
Medication dispensing and accounting		X	X	X	

Physical exam: Height, weight, hip and waist circumference, blood pressure, F-G score, 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.

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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 randomized 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 via a publically 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 characterized by hyperandrogenism, oligo/amenorrhea, 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 seborrheic 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 it also has metabolic implications including insulin resistance, obesity, dyslipidemia, systemic inflammation, and type 2 diabetes [3-5].

PCOS is both a clinical and a public health issue because it adversely affects women's health and health-related quality of life and puts a significant strain on health care 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 430 million dollars in the United States. Treatments for hirsutism and diabetes account for 14% and 40%, respectively, of the total health care 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-sensitizing and anticholesteremic agents, and oral contraceptives [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 pharmacologic interventions for optimal results [12]. The oral contraceptive pill (OCP) can be used as the first-line medical agent in women with PCOS who have no desire to conceive, and the OCP can significantly reduce serum

androgen concentrations and ameliorate the androgenic symptoms in the skin. In addition, anti-androgens 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, edema, diarrhea, sinusitis, hypoglycemia, and kidney toxicity [13-14]. Furthermore, some studies have shown that the OCP might decrease insulin sensitivity and aggravate glucose and lipid metabolism [15-16]. Therefore, the OCP has not been approved by the U.S. 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 and alternative medicine (CAM). CAM 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 etiology 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 PCOS [18] and it has been shown to aid in weight loss and improve ovulation rate and insulin resistance as well as improve patients' outlook [19]. A clinical trial compared the efficacy of metformin and the Chinese herbal formula 'Tiangui Fang' in treating hyperandrogenism and hyperinsulinism in PCOS patients. After treatment for 12 weeks, the Chinese herbal formula significantly lowered serum testosterone and insulin levels compared to metformin [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 Lycopi*), and Zexie (*Rhizoma Alismatis*) can induce ovulation [19].

Tanshinones are a class of bioactive constituents isolated from *Salvia 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, anti-oxidative, anti-cholinesterase, anti-bacterial, anti-platelet aggregation, and anti-cancer activities [20-22]. CHM has been used for the treatment of PCOS but the evidence for its efficacy and safety are minimal. Animal experiments showed that cryptotanshinone can induce favorable 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 randomized controlled trials have been performed to evaluate the use of tanshinone on hyperandrogenism, metabolic profiles, or 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, and 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 was approved by the Ethics Committee of First Affiliated Hospital of Heilongjiang University of Chinese Medicine (2010HZYLL-016) and the other participating centers also have local ethics approvals. The study has been registered at the Chinese Clinical Trials Registry (ChiCTR-TRC-12002973) and at clinicaltrials.gov (NCT 01452477). This is a multicenter, randomized, 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 randomized 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 centers in China. They will be examined at the site center and will be 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 subjects must have hyperandrogenism (hirsutism and/or hyperandrogenemia) and ovarian dysfunction (oligoanovulation and/or polycystic ovaries) and must not have other androgen excess-related disorders. Oligomenorrhea is defined as an intermenstrual interval >35 days or <8 menstrual bleedings in the past year. Amenorrhea 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 to 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, hyperprolactinemia, Cushing syndrome, severe insulin resistance, and thyroid dysfunction.

(3) A history of severe cardiac, pulmonary, hepatic, renal, or neurologic disease or mental illness.

(4) Pregnancy or lactation.

Interventions

Eligible participants will be randomized into either the tanshinone group or the placebo group. The tanshinone capsules (1 g three times per day, China State Food and Drug Administration (SFDA) approval no. Z13020110) and placebo capsules will be provided by Hebei Xinglong Xili Pharmaceutical Co. Ltd. Both tanshinone and placebo capsules have the same outer packaging, color, shape, and flavor. Tanshinone or placebo will be administrated 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 a.m. and 12:00 noon after an overnight fast: a human chorionic gonadotrophin (HCG) stimulation test, a 75 g two-hour oral glucose tolerance test (OGTT), a hyperinsulinemic 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 hyperinsulinemic euglycemic clamp test.

(3) Hyperinsulinemia as determined by the OGTT.

(4) Plasma levels of reproductive hormones: estradiol, 17-OHP, follicle stimulation hormone, leutinizing hormone, SHBG, and DHEAS.

(5) Fasting glucose and lipid profiles: fasting blood glucose, fasting insulin, C-peptide, glycosylated hemoglobin 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 clothing without shoes. The patient will sit quietly for a period of 5 minutes before the blood pressure is taken. The blood pressure may be taken in either arm using the appropriate cuff size. Acne lesions [26] 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 a.m. and 9:00 a.m., a single injection of 5,000 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 glucose and insulin levels.

Hyperinsulinemic euglycemic clamp: Insulin sensitivity will be assessed by the hyperinsulinemic euglycemic clamp before and after treatment. The hyperinsulinemic euglycemic clamp studies will be performed at 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 stabilization period, a priming insulin infusion will be administered for 3 min followed by a constant infusion of 120 $mU \cdot m^{-2} \cdot min^{-1}$ 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 mmol/L and 5.0 mmol/L. Blood samples will be collected every 5 min during the insulin infusion. The GDR (measured as $mg \cdot kg^{-1} \cdot min^{-1}$) 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.

Randomization and blinding

The randomization will be performed through a web-based randomization system operated by an independent data center (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 center. Participants, investigators, and physicians taking care of subjects 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 with the data are resolved quickly and accurately. Third, unscheduled monitoring of the clinical sites will be important for quality control. These visits will assure that the collection method and study data are standardized, accurate, and authentic. The CRFs will be compared against 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 hypothesize that the basal serum testosterone level will be reduced by 10 ng/dL in the tanshinone group and will be unchanged in the placebo group. We assume a 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 analyzed by a specialized 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 ANOVA. Pearson's chi-square 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 program version 16.0 (SPSS Inc., Chicago, IL) and a P-value <0.05 will be considered statistically significant.

DISCUSSION

As the number of PCOS patients increases, it is anticipated that more and more patients will turn to complementary and alternative medicine for treatment. CHM can regulate and strengthen the hormonal systems of the whole body, and this is a natural approach for treating PCOS. The significant advantages of CHM are that it provides several options for the safe, effective, multi-targeted treatment of various aspects of PCOS including hyperandrogenism and poor quality of life [29-31]. 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 [32-33] and can improve insulin resistance and glucose metabolism [33-34].

Tanshinone is used extensively in China for treating acne because of its anti-androgenic 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 hyperinsulinemia, we will use the HCG test, questionnaires (PCOS-QOL and ChQOL), hyperinsulinemic 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, randomized trial will not only determine the clinical efficacy of such treatment but could also provide insights into new evidence-based therapies for PCOS.

Contributors: XKW conceptualized and designed the study. WJS, EHN, and XKW wrote the protocol. WL, JC, and YZ reviewed the protocol for important intellectual content. All authors read and approved the final manuscript.

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

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Competing interests: None.

Provenance and peer review: Not commissioned; externally peer reviewed.

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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 exam	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
Hyperinsulinemic 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 exam: Height, weight, hip and waist circumference, blood pressure, FG score, 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.

~~Effecs~~Effects of ~~Tanshinone~~tanshinone on hyperandrogenism and quality of life in women with polycystic ovary syndrome: protocol of a double-blind, placebo-controlled, randomized trial

Wenjuan Shen [1], Yuehui ~~Zhang~~Zhang [1], Wei ~~Li~~Li [1], Jing ~~Cong~~Cong [1], Ying ~~Zhou~~Zhou [2], Ernest HY ~~Ng~~Ng [3], Xiaoke Wu [1*]

ABSTRACT

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

Methods and analysis:

A total of 100 patients will be ~~enrolled into this study~~recruited and ~~will be~~ randomized into ~~Tanshinones~~ the tanshinone or placebo ~~groups. Tanshinones~~group. Tanshinone or placebo capsules will be taken orally for 12 weeks. The primary ~~endpoint is~~ outcome parameter will be a change in plasma testosterone. Secondary end points ~~are~~will be changes~~:~~ in ~~HCG~~ human chorionic gonadotropin-induced androgen response ~~of androgens;~~, insulin resistance~~;~~, reproductive hormones~~;~~, fasting ~~gluco~~lipid ~~metabolic~~ profiles~~;~~, oral glucose tolerance test~~;~~, quality of life~~; adverse events,~~ and side effects.

Ethics and dissemination:

Written informed consent will be obtained from each participant at the time of enrolling in the study ~~enrolment~~. The trial has been approved by the ~~Ethis~~Ethics Committee of First Affiliated Hospital of Heilongjiang University of Chinese Medicine. Results will be disseminated via a publically accessible website.

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

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the ~~commonest~~most common endocrine ~~disorder~~disorders in reproductive ~~-~~age women. Its prevalence rates depend on ~~different~~the diagnostic criteria used ~~and~~, but it can be up to 18% ~~according to~~using the Rotterdam diagnostic criteria [1]. PCOS is characterized by hyperandrogenism, oligo/amenorrhea, and ~~polycystic~~polycystic ovary ~~(PCO)~~ morphology and ~~may be~~is often associated with insulin resistance. Hyperandrogenism ~~can be~~is found in 60~~%~~%–80% ~~in~~of women with PCOS [2]. The major clinical and biochemical features of hyperandrogenism are hirsutism, acne, alopecia, and seborrheic dermatitis~~, and abnormality sex steroid levels including;~~ elevated androstenedione, testosterone, and dehydroepiandrosterone sulfate (DHEAS) levels~~, as well as;~~ and decreased sex hormone binding globulin (SHBG) ~~level~~levels. The syndrome presents not only with reproductive manifestations but it also ~~had~~has metabolic implications including insulin resistance, obesity, dyslipidemia, systemic inflammation, and type 2 diabetes [3-5].

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exercise, and ~~may have some benefits improving~~this can often improve psychological outcomes, metabolic features, and reproductive features [8-11]. Lifestyle modifications can be combined with pharmacologic interventions for optimal results [12]. ~~Oral~~The oral contraceptive pill (OCP) ~~may~~can be used as the first-line medical agent in women with PCOS who have no desire to conceive~~. The,~~ and the OCP can significantly reduce serum androgen ~~concentration~~concentrations and ameliorate the ~~skin~~ androgenic symptoms in the skin. In addition, ~~antiandrogens~~anti-androgens for hyperandrogenism such as spironolactone, ~~flutamide or~~flutamide, and cyproterone acetate ~~may~~can inhibit androgen-binding receptors ~~or~~and decrease androgen production [13].

~~However, when~~When choosing a ~~treatment~~ medication, the side effects of the various medications must be taken into account, including weight gain, fatigue, nausea, edema, diarrhea, sinusitis, ~~hypoglycaemia~~hypoglycemia, and kidney toxicity [13-14]. Furthermore, some studies have shown that the OCP ~~may~~might decrease insulin sensitivity and aggravate glucose and lipid metabolism [15-16]. Therefore, the OCP has not been approved by the U.S. 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 and alternative medicine (CAM~~), which~~). CAM includes many modalities~~,~~ such as Chinese herbal medicine (CHM), acupuncture, Tai Chi, and other therapies. ~~TCM originated in china more than 3000 years ago.~~ The theory of TCM is complex and includes Yin and Yang, Qi and Xue, Zang and Fu, ~~as well as~~and the Five Elements. According to ~~the~~ TCM theory, the etiology and pathogenesis of PCOS are closely related ~~with~~to 'blood stasis' and '~~-~~kidney vacuity' [17]. CHM is emerging as one of the most commonly practiced ~~medicines~~treatments for PCOS [18] and it has been shown to aid in weight loss~~,~~ and improve ovulation rate and insulin resistance as well as improve patients' outlook [19]. A clinical trial ~~observed~~ compared the efficacy of metformin and the Chinese herbal formula 'Tiangui~~-~~Fang' ~~and metformin~~ in treating hyperandrogenism and hyperinsulinism in PCOS patients ~~of PCOS~~. After ~~three months~~ treatment for 12 weeks, the Chinese herbal formula significantly lowered serum testosterone and insulin levels ~~when~~ compared ~~with~~to metformin [17]. The mechanisms

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of some CHM formulations used ~~in~~to treat PCOS have been elucidated. For example, Gancao (*Radix* ~~glycytthizae~~*glycyrrhizae*) can inhibit androgen synthesis and Baishao (*Radix paeoniae Alba*), Danggui (*Radix angelicae Sinensis*), and Danshen (*Salvia miltiorrhiza Bunge*) ~~have the effect of improving~~improve insulin sensitivity. Furthermore, Sanqi (*Radix Notoginseng*), Zelan (*Herba Lycopi*~~)~~), and Zexie (*Rhizoma Alismatis*) can induce ovulation [19].

Tanshinones are a class of bioactive constituents isolated from *Salvia miltiorrhiza* (Danshen), which is a ~~common~~commonly used herb in TCM. Cryptotanshinone ~~(CT)~~ is the major bioactive tanshinone in the plant and has several pharmacological effects including anti-inflammatory, anti-oxidative, anti-cholinesterase, anti-bacterial, ~~antiplatelet~~anti-platelet aggregation, and anti-cancer activities [20-22]. CHM has been used for the treatment of PCOS but the ~~evidences~~evidence for its efficacy and safety ~~and efficacy~~ are minimal. ~~The animal experiment~~Animal experiments showed that cryptotanshinone can induce favorable alterations in androgen excess and insulin resistance as well as glucose metabolism [23~~]. There~~], but there is still a lack of scientific justification for the use of tanshinone in women with PCOS. ~~Particularly, a~~In particular, no randomized controlled ~~trial (RCT) has not~~trials have been performed to evaluate the use of ~~tanshinones~~tanshinone on hyperandrogenism, metabolic profiles ~~and~~, or quality of life in women with PCOS who do not ~~attempt~~wish to conceive.

~~With this~~In the proposed study, we ~~sought~~seek to evaluate the efficacy of tanshinone ~~in women with PCOS who do not attempt to conceive~~ on hyperandrogenism, glucose and lipid metabolism, and 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 ~~directly~~ and ~~the reduction of~~by reducing insulin resistance ~~as well as on~~and improving the ~~improvement of~~ lipid profile.

~~2.~~ METHODS AND ANALYSIS

~~2.1~~ Study design

The study was approved by the ~~Ethis~~Ethics Committee of First Affiliated Hospital of Heilongjiang University of Chinese Medicine (2010HZYLL-016) and the other participating

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centrecenters also have thelocal ethics approvals. The study has been registered at ClinicalTrialsthe Chinese Clinical Trials Registry (ChiCTR-TRC-12002973) and at clinicaltrials.gov: NCT01452477. (NCT 01452477). This is a multicentermulticenter, randomized, double—blind, and placebo-controlled clinical trial. Informed written consentsconsent will be obtained from eligible women prior to thetheir participation ofin this study, and the recruited women will be randomized into either of the two groups (tanshinone capsulesgroup or the placebo). group. We will follow the CONSORT recommendations in reporting the results [24].–

2.2 Setting and recruitment

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

2.3 Participants

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

2.3.1 Inclusion criteria

(1) The inclusion criteria are as follows: (1) Presence of PCOS diagnosed based on the Androgen Excess Society criteria. All subjects must have hyperandrogenism (hirsutism and/or hyperandrogeniaemia),hyperandrogenemia) and ovarian dysfunction (oligoanovulation and/or polycystic ovaries),) and exclusion ofmust not have other androgen excess–related disorders. Oligomenorrhea is defined as an intermenstrual interval >35 days or <8 menstrual bleedings in the past year. Amenorrhea is defined as an intermenstrual interval >90 days. Clinical

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hyperandrogenism is defined as a Ferriman-Gallwey (FG) score ≥5 [25]. ~~(2) Age of women from 18 to 35 years; (3) No desire of children within 6 month and use condoms for contraception.~~

(2) ~~2.3.2~~ Within the age range of 18 to 35 years.

(3) No desire to become pregnant within 6 months and using condoms for contraception.

Exclusion criteria

(1) ~~The exclusion criteria are as follows: (1)~~ Use of hormonal drugs or other medications~~, which~~ in the past 12 weeks that can affect the results ~~of~~from the ~~study especially~~ Chinese herbal prescriptions ~~in the past 12 weeks; (2) Patients with other.~~

(2) Other androgen excess endocrine disorders including 21-hydroxylase deficiency, hyperprolactinemia, Cushing syndrome, severe insulin resistance, ~~thyroid dysfunction; (3) Patients with history of sever cardiac , pulmonary, hepatic, renal, neurologic disease or mental illness; (4) Pregnancy or lactation~~and thyroid dysfunction.

(3) ~~2.4~~ A history of severe cardiac, pulmonary, hepatic, renal, or neurologic disease or mental illness.

(4) Pregnancy or lactation.

Interventions

Eligible participants will be randomized into ~~one of~~either the ~~two arms: Tanshinone capsules (1g, three times/day) or~~ tanshinone group or the placebo ~~capsules~~group. The tanshinone capsules (1 g three times per day, China State Food and Drug Administration (SFDA) approval no. Z13020110) and placebo ~~both~~capsules will be provided by Hebei Xinglong Xili Pharmaceutical Co. ~~LTD. They~~ Ltd. Both tanshinone and placebo capsules have the same outer ~~packing, dosage,~~ packaging, color, shape, and ~~predominant~~ flavor. Tanshinone or placebo will be administrated orally for 12 weeks. The main pharmaceutical

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formulation of the tanshinone capsules is cryptotanshinone, which comprises 90% of the total formulation in the experimental drug.

2.5 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 a.m. and 12:00 noon after an overnight fast: a human chorionic gonadotrophin (HCG) stimulation test, a 75 gm 2hg two-hour oral glucose tolerance test, (OGTT), a hyperinsulinemic euglycemic clamp clamp test and blood sample for assays, and measurement of fasting lipid profiles and levels of reproductive hormones, plasma glucose and lipid. Adverse. Side effects, adverse events, and other current drug co-treatments will be recorded during the visits. An overview of the study visitvisits is found in Table 1.

2.6 Study assessment (include questionnairsincluding questionnaires)

The primary outcome measure is a decrease of 10 ng/dl 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 (T).

(2) Insulin resistance byas determined by measuring the glucose disposal rate (GDR) with the hyperinsulinemic euglycemic clamp test.

(3) hyperinsulinemiaHyperinsulinemia as determined by the OGTT.

(4) ReproductivePlasma levels of reproductive hormones: testosterone (T), estradiol (E2),, 17-α hydroxyprogesterone (17-OHP),, follicle stimulation hormone (FSH),, leutinizing hormone (LH), sex hormone binding globulin (, SHBG), and dehydroepiandrosterone sulphate (DHEAS). .

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(5) Fasting ~~gluco~~ glucose and lipid ~~metabolic~~ profiles: fasting blood glucose, fasting insulin, C-peptide, glycosylated hemoglobin ~~Alc(HbAlc),~~ A1c, cholesterol, triglycerides ~~(TG),~~ high-density lipoprotein cholesterol ~~(HDL-C),~~ and low-density lipoprotein cholesterol ~~(LDL-C).~~

~~(6) Oral glucose tolerance test (OGTT): serum for glucose, Insulin and c-peptide levels.~~

~~(7~~(6) Weight, blood pressure, waist/hip circumference, ~~F-G~~FG score, and acne.

~~(8) Adverse~~(7) Side effects and adverse events.

~~2.6.1~~

Clinical examination and study tests

Height will be recorded to the nearest 1 cm without shoes, and weight will be recorded ~~tothe~~to the nearest 0.01 kg. Patients will be weighed while dressed in light clothing~~,~~ without shoes. The patient ~~should~~will sit quietly for a period of 5 minutes before the blood pressure is taken. The blood pressure may be taken in either arm using the appropriate cuff size. Acne lesions [26] will be assessed ~~for this study. Trained~~by trained study personnel who will ~~assess a patient's acne by evaluating~~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 ~~Ferriman and Gallwey~~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 ~~Hirsutism Score~~ form.

HCG test: ~~The ovarian~~Ovarian androgen biosynthesis will be measured ~~by human chorionic gonadotropin (hCG)~~ with the HCG test. On the morning of the experiment, between 8:00 ~~o:900,~~ a.m. and 9:00 a.m., a single injection of ~~HCG~~ 5,000 IU HCG will be given to the patient and blood samples will be drawn ~~at 24, 48h therafter~~24 h and 48 h later for assays of 17-OHP, A2, and testosterone.

OGTT test: All ~~the~~ 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 ~~120min~~120 min for determining glucose and insulin ~~level determination~~levels.

Hyperinsulinemic euglycemic clamp: Insulin sensitivity will be assessed by the hyperinsulinemic euglycemic clamp before and after treatment. The hyperinsulinemic euglycemic clamp studies will be performed ~~in~~at Huaian Maternal and Child Health

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Hospital and 60 participants will receive clamp ~~examination~~examinations. The participants will ~~be in~~undertake a 10~~-~~–12 h overnight fast. A small intravenous catheter will be placed in an antecubital vein for blood sampling~~. A,~~ and a second catheter will be inserted into the contralateral forearm for administration of insulin and glucose infusions. After a 30 min stabilization period, a ~~3-min~~ priming insulin infusion will be administered~~,~~ for 3 min followed by a constant infusion of ~~120mU/m²surface area per~~ 120 $mU \cdot m^{-2} \cdot min$ ~~nearly~~$^{-1}$ for 120 min. ~~At the beginning 4 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 ~~at~~between 4.5 ~~–~~mmol/L and 5.~~0mmol~~0 mmol/L. Blood samples will be collected every ~~5min.~~5 min during the insulin infusion. The ~~glucose disposal rate (~~GDR~~; milligrams per kilogram per minute),~~ (measured as $mg \cdot kg^{-1} \cdot min^{-1}$) 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 ~~to calculate~~as the measure of insulin resistance.

~~2.6.2~~ Questionnaires

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

~~2.7~~ Randomization and blinding

The randomization will be performed through a web-based randomization system operated by an independent data center (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~~) in a ratio of 1:1.~~). 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 center. Participants, investigators~~,~~ and physicians taking care of subjects will be blind to the assignment.

~~2.8~~ 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 ~~version~~versions of ~~CRF~~the CRFs will be ~~accomplished in~~deposited into a ~~Web~~web-based data management system at http://218.17.160.110:8081/login.aspx.

In order to ~~keep~~maintain the quality of the data ~~qualities~~, we will adopt ~~some~~ valid measures to assure information authenticity, ~~-~~ accuracy, ~~integrality, seasonable sex.~~ and integrity. First, the site investigator or research coordinator should record or ~~entry~~enter information accurately and in a timely~~. At the same time~~ manner, and every site investigator must make a monthly check of the ~~normative,~~ accuracy and timeliness of the recorded information ~~at~~on the CRFs ~~monthly. Secondly.~~ Second, the data manager and programmer of the Data coordination center (DCC) will be in charge of data monitoring and validation ~~regularly~~ and ~~supervise~~will ensure that issues arising with the ~~findings found be~~data are resolved ~~within the given time. Last but not least, the~~ quickly and accurately. Third, unscheduled ~~-~~ monitoring of the clinical ~~site is very~~ sites will be important for quality control ~~and some procedure should be implemented to~~ . These visits will assure that the collection method and ~~data of the~~ study data are ~~normative~~standardized, accurate, and authentic. The CRFs will be compared against source documents~~. Make~~ to make sure ~~the~~that errors have been resolved without delay. After each visit, ~~monitor~~the monitoring report ~~must~~will be distributed to the site principal investigator. The site visit is an effective action for maintaining data quality and patient protection.

~~2.9~~ Sample size calculation and statistical analysis

We ~~hypothesis~~hypothesize that the basal serum testosterone level will be reduced by 10 ng/~~dl~~dL in the tanshinone group and will ~~have no change~~be unchanged in the placebo group. We ~~hypothesis~~assume a standard deviation of 0.06 of the difference~~. To~~ of two groups. The sample size needed to achieve an 80% power to perceive a significant difference in ~~T~~serum testosterone concentration between the two treatment groups at the two-sided 5% level~~. The sample size will~~ can be estimated ~~according to~~with the

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parameters~~;~~ α~~= =~~ 0.05~~,~~ and β~~= =~~ 0.1~~,~~. This power analysis suggests that 40 patients will be needed for each group. ~~Considering~~Assuming a ~~25~~20% dropout rate based on our past experience, 50 patients will be enrolled in ~~each group~~both groups and 100 patients will be enrolled in total.

All data will be analyzed by a specialized 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~~tanshinone vs. ~~its~~ placebo capsules~~;~~ and within-~~participants~~participant effects before vs. after treatment) will be compared by ANOVA. ~~The~~ Pearson's chi-square test will be used to assess the different qualitative data between the two groups. ~~Furthermore, the analysis for data statistical~~Statistical evaluation of the data will be performed using the SPSS program~~,~~ version 16.0 (SPSS Inc., Chicago, IL) and a P~~-~~value <~~-~~0.05 will be considered statistically significant.

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3.

DISCUSSION

~~With the increase in~~As the number of PCOS~~,~~ patients increases, it is anticipated that more and more patients will turn to~~—~~ complementary and alternative medicine for treatment. CHM can regulate and strengthen the hormonal systems of the whole body, ~~which~~and this is a natural approach for treating PCOS. The significant advantages of CHM ~~may be~~are that it provides several options for the safe, effective, multi-~~targets and multiple methods for treating~~targeted treatment of various aspects of PCOS including hyperandrogenism and poor quality of life [29-31]. In our previous studies, we have found that cryptotanshinone can decrease excessive androgens by inhibiting steroid ~~hormones.~~ hormone production ~~of~~in the theca cells in the ovary [32-33~~].~~ ~~Moreover, cryptotanshinone~~] and can improve insulin resistance and glucose metabolism [33-34].

Tanshinone ~~are~~is used extensively ~~used~~in China for treating acne because of its ~~antiandrogenic~~anti-androgenic properties ~~in China~~. The purpose of this study is to evaluate whether tanshinone has a significant effect on hyperandrogenism in ~~PCOS~~ women with PCOS and to explore new ~~indications~~uses for ~~tanshinone capsules whose major active ingredient is~~ cryptotanshinone~~. In view~~, the primary biologically active form of ~~the good efficiency and fewer~~tanshinone. This trial was designed based on the high efficacy and few side effects of cryptotanshinone~~, we design this trial.~~ To better evaluate the ~~interventional~~ therapeutic effects of tanshinone on ~~hyperadrogenism~~hyperandrogenism, quality of life, insulin resistance, and hyperinsulinemia, we ~~applied~~will use the HCG test, questionnaires (PCOS-QOL and ChQOL), hyperinsulinemic euglycemic clamp, and OGTT test. ~~Furthermore, there is~~There have been no clinical ~~trial design~~trials performed to determine the efficacy of tanshinone for PCOS~~. A~~, and a well ~~design of a~~designed double-blind, placebo-controlled, randomized trial will not only ~~attest~~determine the clinical efficacy of such treatment but ~~also~~ could also provide insights into new evidence-based ~~therapy~~therapies for PCOS.

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Contributors: ~~WJS, YHZ and~~ XKW ~~conceptualised~~conceptualized and designed the study. WJS, EHN, and XKW wrote the protocol. WL, JC, and YZ reviewed the protocol for important intellectual content. All authors read and approved the final manuscript.

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

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Competing interests: None.

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Figure 1

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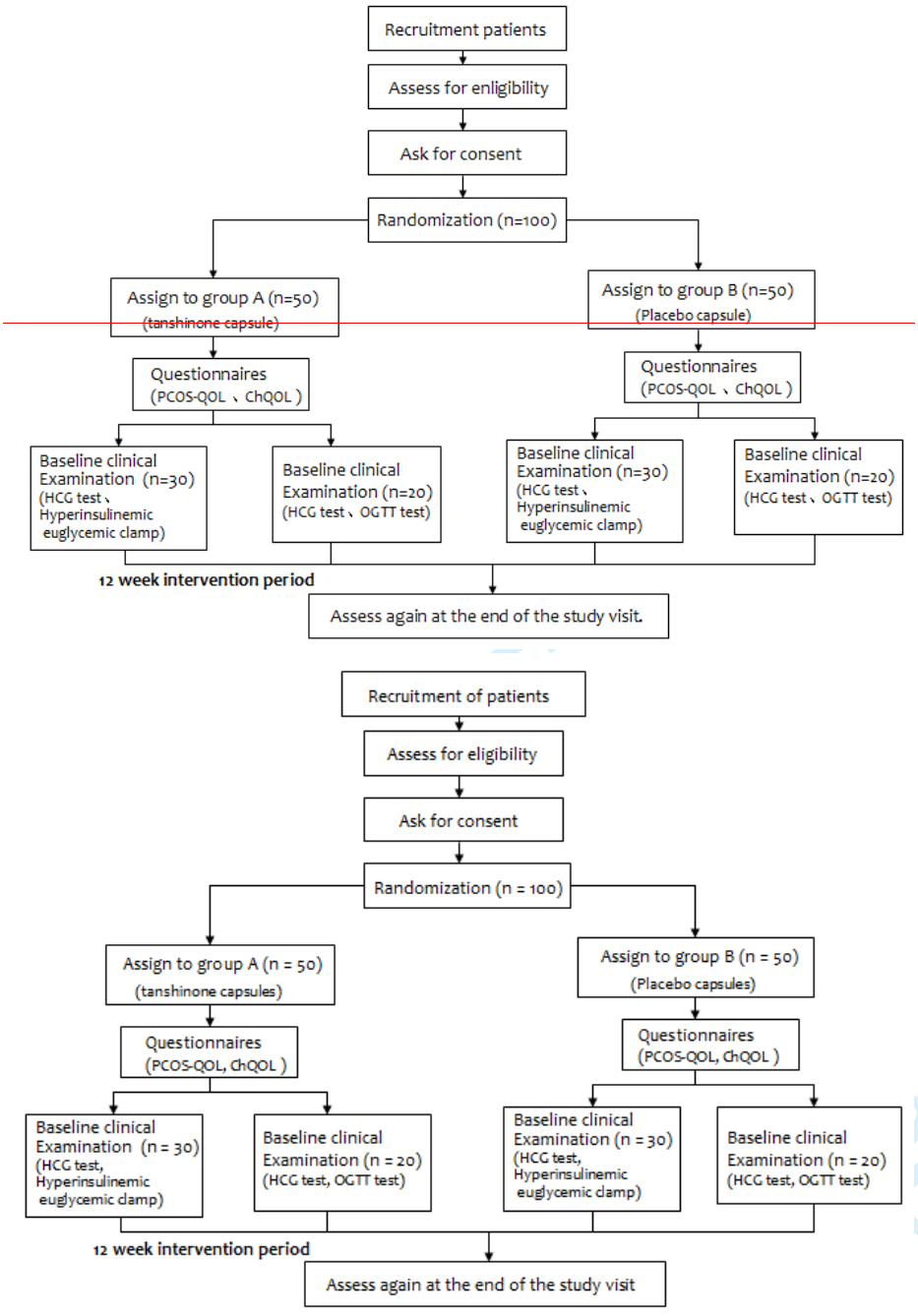

Table 1. Overview of study visits

	Screening visit	Baseline visit	Monthly visit 1	Monthly visit 2	Monthly visit 3 and End of treatment visit
Physical exam	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
Hyperinsulinemic euglycemic clamp		X			X
~~Query for~~Ask about adverse ~~event~~events and ~~concomitant~~concurrent medications		X	X	X	X
~~Query for~~Ask about menstrual period	X	X	X	X	X
Medication dispensing and accounting		X	X	X	

Physical exam: Height, weight, hip and waist circumference, blood pressure, ~~F-G~~FG score, acne.

Fasting phlebotomy: Serum for the Central Core Laboratory.

Transvaginal ultrasound: ~~endometrial~~Endometrial thickness, ovarian volume, antral follicle count, and size of ovarian cysts or developing follicles.

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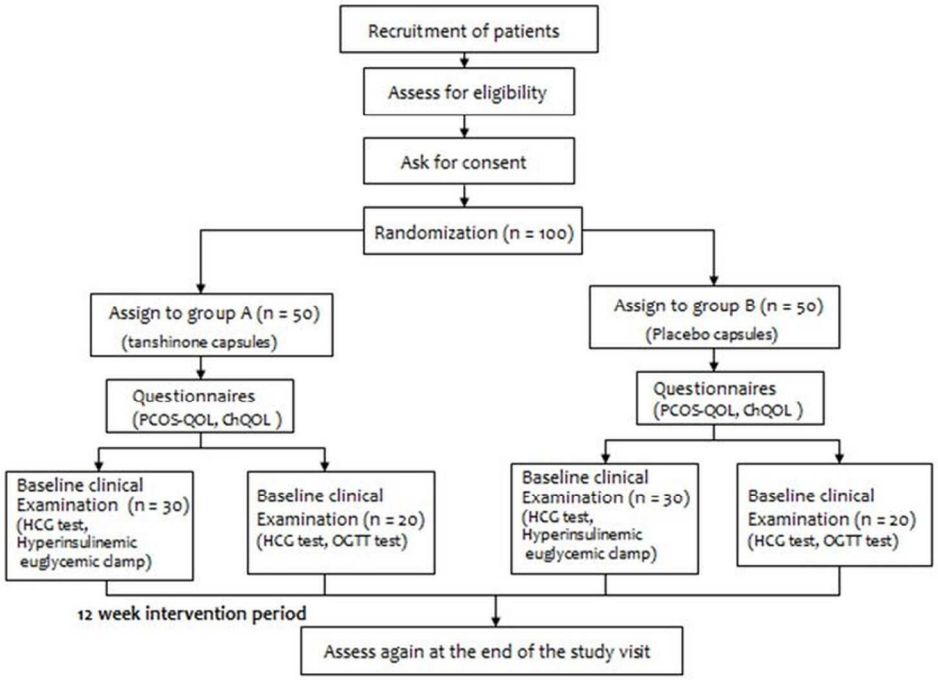
Figure 1



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