Efficacy and Safety of Xiaoyao Pills for Mild to Moderate Depression With Syndrome of Liver Stagnation and Spleen Deficiency: Study Protocol for a Randomized Controlled Trial

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Study protocol

Keywords: Traditional Chinese Medicine, depression, randomized controlled trial

Posted Date: June 28th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-449004/v1

Abstract

Background: Depression is one of the most frequent and severe psychiatric conditions. Many chemical drugs to treat depression are associated with adverse reactions and have shortcomings. Traditional Chinese medicine is of great significance in the prevention and treatment of depression. Xiaoyao pills has achieved good results in clinical application, which has the advantages of quick effect and no obvious adverse reactions. The aim of our study is to evaluate the efficacy and safety of Xiaoyao pills on mild to moderate depression patients with syndrome of liver stagnation and spleen deficiency.

Methods: This study is a multi-center, double-blinded, randomized and placebo-controlled clinical trial. A total of 108 participants will be assigned to three groups: Xiaoyao pill group taking Xiaoyao pills twice daily for 4 weeks, placebo group taking placebos twice daily for 4 weeks and normal group without taking any drug. The primary and secondary outcome measures are the Hamilton Depression Scale (HAMD) and Traditional Chinese Medicine (TCM) Syndrome Scale. The assessment is at baseline(before treatment initiation), 1 week, 2 weeks 4 weeks after the first treatment. Exploratory outcome is also assessed to explore the mechanism of Xiaoyao pills at baseline and 4 weeks.

Discussion: The results from this study will provide clinical evidence on the efficacy and safety of Xiaoyao pills in patients with mild to moderate depression with syndrome of liver stagnation and spleen deficiency.

Trial registration: International Standard Randomized Controlled Trial Number Register: ISRCTN12746343. Registered on September 25, 2020.

Introduction

Background and rationale {6a}

Depression is one of the most frequent and severe psychiatric conditions, with an estimated prevalence reaching 15% in the general population[1]. Depression is characterized by sadness, loss of interest and pleasure, feelings of guilt, feeling of worthlessness, low appetite, fatigue, and poor concentration [2]. Depression is known to dramatically increase the risk of premature death by suicide or other general medical conditions, such as vascular diseases[1]. Considering the alarming impact of COVID-19 infection on mental health [3, 4], We should pay more attention to the treatment of depression. There are many treatments, including pharmacological, psychological and neurostimulatory options that are associated with remission of symptoms and full restoration of psychosocial function. Unfortunately, a significant proportion of patients do not achieve sustained remission, despite serial treatments [5]. Clinical practice has proven that many chemical drugs have beneficial therapeutic effects in depression, but they are also associated with adverse reactions, such as drowsiness and dystonia, and have shortcomings, such as a narrow antidepressant spectrum and a high rate of relapse[6].

Traditional Chinese medicine (TCM) is of great significance in the prevention and treatment of depression. Xiaoyao pills, which is one of Traditional Chinese medicine prescription, has been used for both the prevention and treatment of chronic dizziness for thousands of years. It has achieved good results in clinical application, which has the advantages of quick effect and no obvious adverse reactions[7]. Xiaoyao pills consists of Radix Bupleuri (root of Bupleurum Chinense DC.), Radix Paeoniae Alba (root of Paeonia lactiflora Pall.), Radix Angelicae Sinensis (root of Angelica sinensis (Oliv.) Diels), Rhizoma Atractylodis (root and rhizome of Atractylodes lancea (Thunb.) DC.), Poria (fungus nucleus of Poria cocos (Schw.) Wolf), Radix Glycyrrhizae (root and rhizome of Glycyrrhiza uralensis Fisch.), Herba Menthae (aboveground portions of Mentha haplocalyx Briq.), and Rhizoma Zingiberis Recens (fresh root and rhizome of Zingiber officinaleRosc.) in a ratio of 5:5:5:5:5:4:1:1[8]. The antidepressant mechanism of Xiaoyao pills was widely studied from the molecular biological level recent years [9, 10, 11]. At the same time, there are many clinical reports of Xiaoyao pills [12, 13, 14]. However, the quality of most clinical report methods needs to be improved.

Objectives {7}

This study intends to carry out a multi-center randomized controlled and double-blind trial to observe the efficacy of Xiaoyao pills in the treatment of mild to moderate depression.

Trial design {8}

This study is a multi-center, double-blinded, randomized and placebo-controlled clinical trial. The trial protocol has been approved by the ethical committee of Beijing University of Traditional Chinese Medicine (ref: 2020BZYLL0304) and is registered with ISRCTN at Current Controlled Trials (ISRCTN 12746343). The patients will provide written informed consent. The trial will be based on the principles of ICH-GCP [15]and appropriate legal regulations. For recommended items to address in a clinical trial protocol according to the SPIRIT 2013 Checklist [16] and the Consolidated Standards of Reporting Trials (CONSORT) statement [17].

Methods: Participants, Interventions And Outcomes

Study setting and participants

A total number of 72 participants with mild-to-moderate depression (referred to symptoms of liver stagnation and spleen deficiency) and 36 healthy participants will be recruited at the five trial sites in china, including Peking university sixth hospital, the first affiliated hospital of Jinan university(Guangzhou overseas Chinese hospital), the affiliated brain hospital of Guangzhou medical university, Dongfang Hospital (Beijing University of Chinese Medicine second affiliated hospital) and Beijing Anding hospital Capital medical university. Participants will be informed of details about the study

which are purpose, duration, procedures, and key contacts, as well as risks and potential benefits. Participants may withdraw their consent for any reason without any consequences at any time. Eligible 72 participants with mild-to-moderate depression will be randomly allocated to Xiaoyao pill group (Group1) and placebo group (Group2), who will be required to take Xiaoyao pills and placebos respectively twice daily for four consecutive weeks. 36 healthy participants will be allocated to Normal group (Group 3) without taking any drug. The flow chart is listed in Figure 1.

Eligibility criteria {10}

Inclusion criteria

Participants meeting the following criteria will be included:

1. Meet the Diagnostic Statistics Manual of Mental Disorders (DSM-5) regarding the diagnosis of mild to moderate depression;

2. A score between 20 and 35 on HAMD;

3. Meet the TCM criteria of liver stagnation and spleen deficiency syndrome;

4. Aged between 16 - 18 years old, both genders;

5. Patients agree to participate in this trial and assign the informed consent;

6. Capable of reading and follow-up treatment, and permanently live locally.

Exclusion criteria

Participants meeting one or more of the following criteria will be excluded:

1. Bipolar depression, treatment-resistant depression and severe suicidal risk;

2. History of bipolar disorder, schizophrenia, obvious psychotic symptoms and depression disorder caused by non-addictive substances;

3. Combine with severe cardiovascular diseases, cerebrovascular diseases, hepatic diseases, renal diseases, hematological disease, cancer, or other severe primary diseases;

4. Pregnant or lactating women;

5. Inability to finish the compliance test, judge the efficacy and have complete data;

6. Involved with any other clinical trial at the time of consent.

Who will take informed consent? {26a}

All depressed subjects enrolled in the study as well as healthy controls will sign a paper version of informed consent prior to study initiation. Two copies of the informed consent were kept by the researchers and the subjects respectively.

Additional consent provisions for collection and use of participant data and biological specimens {26b}

Standard clinical laboratory findings (including complete blood count, liver and kidney function tests) as indicators of safety evaluation were routinely collected before and after the enrollment of patients to ensure the safety of subjects. Participants' urine samples and blood samples will be monitored by liquid chromatography-mass spectrometry (LC-MS) technology to explore the mechanism of action of the metyrapone at baseline and at 4 weeks.At the same time, participants' blood samples will be used to separate the total RNA. All the samples required for the study do not need to sign additional informed consent, because the Committee has already been approved by the ethics committee and can use the data after signing the informed consent.

Interventions

Explanation for the choice of comparators {6b}

Placebos were manufactured by Jiuzhitang Co., Ltd. Placebos are similar to the physical traits of Xiaoyao pills in size, flavor, scent, and color without the key ingredients. The placebos are composed of corn starch, Pregelatinized starch, maltose, Caramel color and water.

Intervention description {11a}

Participants randomized to treatment Group 1 will take Xiaoyao pills, which are composed of ChaiHu(Radix Bupleuri)-100g, DangGui(Radix Angelicae Sinensis)-100g, BaiShao(Radix Paeoniae Alba)-100g, BoHe(Mentha)-20g, ShengJiang(Ginger)-100g□BaiZhu (Atractylodes macrocephala Koidz) -100 g, FuLing (Poria cocos Wolf) -100g, ZhiGanCao (Glycyrrhiza uralensis Fisch)-80g.The action and batch number of each herb is summarized in Table 1. Xiaoyao pills are water honey pills (Z20013060) manufactured by Jiuzhitang Co., Ltd. Participants in Group 2 will take placebos which are manufactured by Jiuzhitang Co., Ltd. Placebos are similar to the physical traits of Xiaoyao pills in size, flavor, scent, and color without the key ingredients. The placebos are composed of corn starch, Pregelatinized starch, maltose, Caramel color and water. Participants will be required to take the medicine twice daily for four consecutive weeks and the dosage is 9g twice a day. The normal group will be not given any drug.

Criteria for discontinuing or modifying allocated interventions {11b}

Shedding criteria:

Cases that have been enrolled but do not complete the clinical protocol should be considered drop outs in the following circumstances:

1. Patients withdrew from the trial on their own;

2. Lost to follow-up;

3. Poor adherence;

4. Because some diseases were considered by the study physician to be amenable to withdrawal from the trial.

The case should be explained. If the baseline pharmacodynamic data are available, the results of the last major outcome can be transferred to the final result for statistical analysis, and the research records should be kept for reference.

Discontinuing criteria:

The entire trial was completely stopped in multi-centers for the following reasons:

1. Serious safety concerns identified by the investigator;

2. There were major lapses in the program;

3. Reasons for funding or management by the sponsor;

4. Withdrawal of the trial from administration.

Total discontinuation of the trial can be temporary or permanent. When discontinuing a trial, full trial records should be retained.

Rejection criteria

Cases that have been enrolled but meet one of the following should be removed:

1. Cases were incorrectly diagnosed and incorrectly included;

2. Met the exclusion criteria;

3. One medication was not used;

4. Without any record of detection;

5. Due to the use of some prohibited medication, it was not possible to evaluate drug efficacy.

Rejected cases should state the reason, and their original medical records should be retained for statistical analysis of efficacy.

Strategies to improve adherence to interventions {11c}

To guarantee subject compliance, the study will set up a clinical research coordinator. Distribute drug use record cards to allow patients to record their medication status and follow-up time.

Relevant concomitant care permitted or prohibited during the trial {11d}

Concomitant treatments and forbidden drugs

1. Avoid cold and greasy foods that are difficult to digest.

2. During the period of taking the medicine, keep optimistic and avoid getting angry.

3. People with severe chronic diseases such as high blood pressure, heart disease, liver disease, diabetes, and kidney disease should take it under the guidance of a physician.

4. Normal menstruation, sudden excessive menstrual flow, prolonged menstrual period, or oligomenorrhea, wrong menstrual period, or irregular vaginal bleeding should go to the hospital for treatment.

5. People who are allergic to this product should not use it with caution.

6. It is forbidden to use this product when its properties change.

7. If you are using other drugs, please consult your physician or pharmacist before using this product.

Provisions for post-trial care {30}

There are no expected harms resulting from the trial. After the trial, patients were provided with guidance on specialized treatment protocols in psychiatry.

Outcomes {12}

Primary outcome measures

Hamilton Depression Scale (HAMD)

The HAMD is a tool developed for clinical evaluation of depression and is widely used in studies. It is composed of 17 questions. Each question is scored from 1 to 4 points, where a higher score indicates severe symptoms; 0 to 7 point is normal, 7 to17 point may have depression, 17 to 24 point definitely have depression, and more than 24 points means severe depression. Measurements are taken at the baseline, 1 week, 2 weeks, and 4 weeks after treatment.

Secondary outcome measures

Traditional Chinese Medicine (TCM) Syndrome Scale

TCM Syndrome Scale is used as a measure of depression. It consists of 1 primary symptom and 9 second diagnostic symptoms. The participants will be required with mental depression, and needs to have more than 4 other concurrent symptoms of the main symptoms at the same time, and the symptoms should last for a 4-week session. Moreover, participant will be provided with symptoms of

stagnation of liver qi and spleen deficiency as follows: (1) Suspicious; (2) Fullness of chest and thigh; (3) Chest tightness; (4) Easy to sigh;(5) Complexion is chlorosis; (6) Stomach fullness; (7) Abdominal pain;(8) Bloating; 9Nausea; 10Bowel; (11) Loose stools;(12) Foreign body sensation in the pharynx; (13) Light tongue, white tongue coating; (14) Pulse string is thin or slippery. Participant who is involved with more than 5 symptoms can be diagnosed. Measurements are taken at the baseline, 1 week, 2 weeks, and 4 weeks after treatment.

Exploratory outcome

Urine and blood of participants will be monitored to explore the mechanism of Xiaoyao pills at baseline and 4 weeks by liquid chromatograph-mass spectrometer (LC-MS) technology. Meanwhile, total RNA will be isolated from blood sample using TRIzol (Life Technologies). Differential expression will be analyzed by using TopHat method. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of differentially expressed genes will be implemented by Methylmion Specific PCR. And the exploratory outcomes will be expected by Network pharmacology to conduct correlation analysis with the differential genes.

Participant timeline {13}

Participant timeline is shown in Table 3.

Sample size {14}

The sample size calculation was performed using the central random distribution system and drug random system provided by BioZi. It is planned to recruit 60 depressive subjects with liver depression and spleen deficiency and 36 healthy subjects. Meanwhile, taking into account a dropout of 15%, we concluded that a total of 108 participants with 36 for each group would need to be recruited to ensure statistically significant results.

Recruitment {15}

A total number of 72 participants with mild-to-moderate depression (referred to symptoms of liver stagnation and spleen deficiency) and 36 healthy participants will be recruited at the five trial sites in china. The number of registrations at each facility is monitored by the monitoring committee via Electronic data capture (EDC).

Assignment of interventions: allocation

Sequence generation {16a}

The study adopts the method of central stratified block randomization. According to the given seed number and segment length, the random number table of 72 subjects was generated by an independent statistician using SAS 9.4 statistical software according to the ratio of 1:1 of the Xiaoyao pills group and the placebo group.

Concealment mechanism {16b}

Not applicable

Implementation {16c}

Not applicable

Assignment of interventions: Blinding

Who will be blinded {17a}

The study used a double-blind design and drugs were coded and packaged blind according to a generated random number table by personnel unrelated to this trial. The drugs in each group were uniformly packaged, while guaranteeing that the Xiaoyao pills and placebos were not different in appearance. The centers dispensed the drugs sequentially with the assigned drug number and in the order of subject enrollment. The blind bottom was kept in duplicate by the principal investigator and the sponsor after sealing, and the blind bottom could not be removed during the trial.

Procedure for unblinding if needed {17b}

Not applicable.

Data collection and management

Plans for assessment and collection of outcomes {18a}

The results and baseline of all participants will be collected and evaluated on EDC.

Plans to promote participant retention and complete follow-up {18b}

A subject follow-up record form was set up for this study, and the follow-up of subjects was managed by the clinical research coordinator.

Data management {19}

The sponsor will collect paper version CRFs of patient data for proper storage as study raw materials. All data will be entered into the EDC, any traces of entries, modifications, deletions etc. in the EDC will be retained in the log showing who and when they were changed.

Confidentiality {27}

All patients' data are kept confidential and not disclosed. Only study physicians granted authority by the sponsor have access to the EDC by account number and password, and can only enter and review patient data at their site. The statistician and sponsor have access to the data for all participants.

Plans for collection, laboratory evaluation and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}

After signing an informed consent form, patients will be given a baseline blood sample and retention urine sample, in which blood routine, liver and kidney function and urine routine are safety evaluation indicators. Meanwhile, a portion of blood samples will be used for DNA methylation detection and a portion of urine samples will be used for metabolomics detection. These blood and urine tests will be performed again when the patient is out of the group.

Blood and urine samples will be stored properly and will not be used by any other route than the study. Meanwhile, existing as well as further sample specific studies will be conducted with ethics committee approval.

Statistical methods

Statistical methods for primary and secondary outcomes {20a}

Investigators will establish the electronic case into the EXCEL form when making follow-up observations by interviewing the participants. Paper will be entered into a database by those investigators in order to ensure data validity. All analyses will be performed using SPSS software version 21.0. Differences are considered to be statistically significant for two-sided $P<0.05$.Comparisons between groups will be conducted by using an analysis of covariance (ANCOVA). The significance of the cure rate, improvement rate and unhealed rate differences between the groups will be compared using ridit analysis and chi-square test.

Interim analyses {21b}

Security monitoring will follow through the study at all times. Adverse events are reported immediately to the principal investigator, who is required to report them to the clinical trial monitoring committee within the prescribed period of time, depending on the severity. The clinical trial monitoring committee decided on a case by case basis whether to terminate the study.

No efficacy interim analysis was set up in this study.

Methods for additional analyses (e.g. subgroup analyses) {20b}

Not applicable.

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}

Participants with missing primary or secondary outcome data will be excluded.

Plans to give access to the full protocol, participant level-data and statistical code {31c}

This study protocol is registered with the registration number ISRCTN12746343 on the International Standard Randomized Controlled Trial (http://www.isrctn.com/ISRCTN12746343).

Oversight and monitoring

Composition of the coordinating centre and trial steering committee {5d}

There are several clinical study coordinators at each study center. And a clinical research monitoring board is set up to monitor the safety of the study, the authenticity and integrity of the data. The clinical study monitor is held by the sponsor and a third party other than the investigator.

Composition of the data monitoring committee, its role and reporting structure {21a}

Not applicable.

Adverse event reporting and harms {22}

1. Once an adverse event occurs, the investigator shall state to the subject that the subject is required to report truthfully the changes in his condition following the medication. Physicians are to avoid inducing questions.

2. While observing efficacy, pay close attention to observing adverse events or unanticipated toxic side effects (including symptoms, signs, laboratory tests), analyze the causes, make judgment, and follow-up observations and records.

3. For adverse events occurring during the study period, their symptoms, extent, time of appearance, duration, handling measures, experience, etc. should be recorded on a case report form, evaluated their relevance to the study drug, and recorded in detail by the investigator, signed and dated.

4. When an adverse event is identified, the observing physician may decide whether to discontinue observation based on the condition, and cases who discontinue because of adverse effects should be followed up with a detailed record of the handling and outcome.

5. In the event of a serious adverse event in a study, the unit that assumes the responsibility for the clinical study must take immediate measures to protect the safety of subjects, must report to the project leader and the Research Center for clinical trials of drugs, should report within 24 hours to the drug administration, the subject responsible unit and the ethics committee. Investigator to sign and date on report. The subject responsible unit will guarantee reporting procedures that meet all legal and regulatory requirements.

6. When urgent breaking of blinding is required for a serious adverse event to occur in a clinical study, the blinding should be broken jointly by the project leader, investigator, clinical monitor.

Frequency and plans for auditing trial conduct {23}

The sponsor and the study center will meet regularly to ensure that the study is being conducted in accordance with the study protocol.

Plans for communicating important protocol amendments to relevant parties (e.g. trial participants, ethical committees) {25}

If the study protocol is amended, it must be approved by the ethics committee of Beijing University of Chinese medicine, and patients will also be provided written informed consent to inform the modification.

Dissemination plans {31a}

The results of this study will be published in a scientific journal.

Discussion

Depression is a common affective mental disorder, which is associated with a high burden of disease. Research suggests that chronic diseases, genetic factors, social and environmental factors can induce depression [18]. The common therapy for depression is antidepressants. Due to the side effects of drugs for depression, the compliance of patients is rather poor, and then affect the drug efficacy [19]. Therefore, it is urgent to find safe and no side-effect treatment. A number of patients with depression would prefer Traditional Chinese medicine over pharmacotherapy when our study demonstrates the effectiveness and safety of Traditional Chinese medicine to treat depression. This will be especially relevant for patients who do not respond to medical therapy or who experience adverse side effects to drug therapy.

According to Traditional Chinese Medicine theory, liver stagnation and spleen deficiency syndrome is one of the main syndromes of depression. Especially due to people's unhealthy eating habits and long-term emotional stress from life and work in modern society, the symptoms of liver stagnation and spleen deficiency are very common. In clinical practice, the syndrome of liver stagnation and spleen deficiency has become a high incidence and common syndrome. Xiaoyao pills has good effect of soothing the stagnated liver and strengthening the spleen. In our study, TCM syndrome efficacy evaluation is added in order to determine more suitable patient populations to take Xiaoyao pills and optimize indications.

The potential mechanism of Xiaoyao pills to treat depression mainly focused on animal experiments [20,21,22,23,24,25]. There is little evidence about the potential mechanism about Xiaoyao pills to treat depression in clinical studies. Therefore, in addition to the efficacy evaluation of Xiaoyao pills, the action mechanism of Xiaoyao pills from the perspective of transcriptomics and DNA methylation sequencing will also be studied in our trial.

However, our study has limitation. The limitation concerns the fact that we will only recruit mild to moderate depression patients with syndrome of liver stagnation and spleen deficiency in order to ensure the curative effect. Effectiveness of TCM for different types of depression is not evaluated.

Therefore, a clinical trial about Traditional Chinese Medicine to treat different types of depression are still needed.

Trial status

The trial is currently in the recruitment phase. *This study protocol is version 3 made on June 1, 2020. Recruitment commenced in March 2020 at Peking university sixth hospital and is expected to be completed in December 2021.*

Abbreviations

DSM-5： Diagnostic Statistics Manual of Mental Disorders; HAMD：Hamilton Depression Scale; TCM： Traditional Chinese Medicine; LC-MS：liquid chromatograph-mass spectrometer; GO：Gene Ontology; KEGG：Kyoto Encyclopedia of Genes and Genomes; AEs：Adverse events; CRF：Case Report Forms; ANCOVA: analysis of covariance; RCT: Randomized controlled trial; EDC: Electronic data capture.

Declarations

Acknowledgements

We would like to thank National Natural Science Foundation of China for funding.

Authors' contributions {31b}

JXC conceived the study and prepared the initial protocol. ZX drafted the manuscript and participated in the study design. ZH participated in the formulation of research protocols in each clinical sub center and polished the language of the manuscript. SHL and XHW were in charge of coordination and directed implementation. XZ helped to develop the study analysis. QYM made amendments and participated in designing the trial protocol. All authors read and approved the final manuscript.

Funding {4}

The study were supported by Key projects of National Natural Science Foundation of China (NO.81630104) and Youth Fund Project of National Natural Science Foundation of China (NO.81803972).

Availability of data and materials {29}

The sponsor, Beijing University of Chinese medicine, has access to the final dataset. The researchers need the consent of the sponsor to use the data.

Ethics approval and consent to participate {24}

Ethical Review Board of Beijing University of Chinese Medicine approved the study (2020BZYLL0304). Written informed consent to participate will be obtained from all participants.

Consent for publication {32}

Not applicable.

Competing interests {28}

The authors declare that they have no competing interests.

References

1. Troubat R, Barone P, Leman S, Desmidt T, Cressant A, Atanasova B, Brizard B, El Hage W, Surget A, Belzung C, Camus V. Neuroinflammation and depression: A review. Eur J Neurosci. 2021 Jan;53(1):151-171.
2. Vasile C. CBT and medication in depression (Review). Exp Ther Med. 2020 Oct;20(4):3513-3516.
3. Shader RI. COVID-19 and Depression. Clin Ther. 2020 Jun;42(6):962-963.
4. Mazza MG, De Lorenzo R, Conte C, Poletti S, Vai B, Bollettini I, Melloni EMT, Furlan R, Ciceri F, Rovere-Querini P; COVID-19 BioB Outpatient Clinic Study group, Benedetti F. Anxiety and depression in COVID-19 survivors: Role of inflammatory and clinical predictors. Brain Behav Immun. 2020 Oct;89:594-600.
5. McAllister-Williams RH, Arango C, Blier P, Demyttenaere K, Falkai P, Gorwood P, Hopwood M, Javed A, Kasper S, Malhi GS, Soares JC, Vieta E, Young AH, Papadopoulos A, Rush AJ. The identification, assessment and management of difficult-to-treat depression: An international consensus statement. J Affect Disord. 2020 Apr 15;267:264-282.
6. Boer J, Ederveen E, Grundmark B. Desloratadine and depression, a drug safety signal based on worldwide spontaneous reporting of side effects. Ups J Med Sci. 2018 Sep;123(3):174-178.
7. Cipriani A, Furukawa TA, Salanti G, Geddes JR, Higgins JP, Churchill R, Watanabe N, Nakagawa A, Omori IM, McGuire H, Tansella M, Barbui C. Comparative efficacy and acceptability of 12 new-generation antidepressants: a multiple-treatments meta-analysis. Lancet. 2009 Feb 28;373(9665):746-758.
8. Chinese Pharmacopoeia Commission Pharmacopoeia of the People's Republic of China; Chinese Medical Science and Technology Press: Beijing. China 2015;1:11354.
9. Xi S, Yue G, Liu Y, Wang Y, Qiu Y, Li Z, Ma P, Liu T, Jiang Y, Liang Y, Liu Q, Shi J, Chen J, Yue L. Free Wanderer Powder regulates AMPA receptor homeostasis in chronic restraint stress-induced rat model of depression with liver-depression and spleen-deficiency syndrome. Aging (Albany NY). 2020 Oct 14;12(19):19563-19584.
10. Ding F, Wu J, Liu C, Bian Q, Qiu W, Ma Q, Li X, Long M, Zou X, Chen J. Effect of Xiaoyaosan on Colon Morphology and Intestinal Permeability in Rats With Chronic Unpredictable Mild Stress. Front Pharmacol. 2020 Jul 16;11:1069.

11. Zhu HZ, Liang YD, Ma QY, Hao WZ, Li XJ, Wu MS, Deng LJ, Li YM, Chen JX. Xiaoyaosan improves depressive-like behavior in rats with chronic immobilization stress through modulation of the gut microbiota. Biomed Pharmacother. 2019 Apr;112:108621.
12. Liu CC, Wu YF, Feng GM, Gao XX, Zhou YZ, Hou WJ, Qin XM, Du GH, Tian JS. Plasma-metabolite-biomarkers for the therapeutic response in depressed patients by the traditional Chinese medicine formula Xiaoyaosan: A (1)H NMR-based metabolomics approach. J Affect Disord. 2015 Oct 1;185:156-63.
13. Liu X, Liu C, Tian J, Gao X, Li K, Du G, Qin X. Plasma metabolomics of depressed patients and treatment with Xiaoyaosan based on mass spectrometry technique. J Ethnopharmacol. 2020 Jan 10;246:112219.
14. Zhang Y, Han M, Liu Z, Wang J, He Q, Liu J. Chinese herbal formula xiao yao san for treatment of depression: a systematic review of randomized controlled trials. Evid Based Complement Alternat Med. 2012;2012:931636.
15. Englev E, Petersen KP. ICH-GCP Guideline: kvalitetssikring af kliniske laegemiddelforsøg. Status og perspektiver [ICH-GCP Guideline: quality assurance of clinical trials. Status and perspectives]. Ugeskr Laeger. 2003 Apr 14;165(16):1659-62.
16. Chan AW, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin JA, Doré CJ, Parulekar WR, Summerskill WS, Groves T, Schulz KF, Sox HC, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 statement: defining standard protocol items for clinical trials. Ann Intern Med. 2013 Feb 5;158(3):200-7.
17. Eldridge SM, Chan CL, Campbell MJ, Bond CM, Hopewell S, Thabane L, Lancaster GA; PAFS consensus group. CONSORT 2010 statement: extension to randomised pilot and feasibility trials. BMJ. 2016 Oct 24;355:i5239.
18. Raic M. Depression and heart diseases: leading health problems. Psychiatr Danub. 2017;29 Suppl 4(Suppl 4):770–777.
19. Hu XH, Bull SA, Hunkeler EM, et al. Incidence and duration of side effects and those rated as bothersome with selective serotonin reuptake inhibitor treatment for depression: patient report versus physician estimate. J Clin Psychiatry 2004;65:959–65.
20. Song M, Zhang J, Li X, Liu Y, Wang T, Yan Z, Chen J. Effects of Xiaoyaosan on Depressive-Like Behaviors in Rats With Chronic Unpredictable Mild Stress Through HPA Axis Induced Astrocytic Activities. Front Psychiatry. 2020 Oct 20;11:545823.
21. Li X, Qiu W, Li N, Da X, Ma Q, Hou Y, Wang T, Song M, Chen J. Susceptibility to Hyperglycemia in Rats With Stress-Induced Depressive-Like Behavior: Involvement of IL-6 Mediated Glucose Homeostasis Signaling. Front Psychiatry. 2020 Jun 23;11:557.
22. Pan Q, Wu J, Liu Y, Li X, Chen J. Involvement of Hepatic SHIP2 and PI3K/Akt Signalling in the Regulation of Plasma Insulin by Xiaoyaosan in Chronic Immobilization-Stressed Rats. Molecules. 2019 Jan 29;24(3):480.

23. Yan Z, Jiao H, Ding X, Ma Q, Li X, Pan Q, Wang T, Hou Y, Jiang Y, Liu Y, Chen J. Xiaoyaosan Improves Depressive-Like Behaviors in Mice through Regulating Apelin-APJ System in Hypothalamus. Molecules. 2018 May 3;23(5):1073.
24. Li XJ, Ma QY, Jiang YM, Bai XH, Yan ZY, Liu Q, Pan QX, Liu YY, Chen JX. Xiaoyaosan exerts anxiolytic-like effects by down-regulating the TNF-α/JAK2-STAT3 pathway in the rat hippocampus. Sci Rep. 2017 Mar 23;7(1):353.

Tables

Table 1 The action and batch number of each herb

Ingredients	Lot number	Action
BaiZhu ***(Atractylodes*** ***Macrocephala Koidz)***	018AJ-2005005-725	TCM: Tonifying *Qi* of spleen, eliminating dampness and diuresis.
Chaihu ***(Radix Bupleuri)***	FM201538	TCM: Soothing the liver and tonifying qi, relieving depression, and reducing fever. Pharmaceutical study: 1.Sedative effect. 2.Analgesic effect. 3.Anti-pathogen effect.
Danggui ***(Radix Angelicae Sinensis)***	FM201541	TCM: Moisturizing the intestines promoting blood circulation and removing blood stasis，regulating menstruation and relieving pain.
Baishao ***(Radix Paeoniae alba)***	FM201030 FM201037 FM201041	TCM: Restraining yin and stopping sweating, nourishing blood and regulating menstruation，calming liver yang，softening liver and relieving pain.
Fuling ***(Poria cocos Wolf)***	FM201540	TCM：Diuresis, detumescence, tonifying *Qi* of spleen and tranquilization. Pharmaceutical study: 1.Diuresis effect. 2.Calming effect. 3.Anti-tumor effect. 4.Anti-diabetic effect. 5.Enhance myocardial contractility.
Zhigancao ***(Glycyrrhiza uralensis Fisch)***	FM200983	TCM: Tonifying *Qi* of spleen, eliminating phlegm, anti-cough, heat-clearing and detoxifying and reconciling. Pharmaceutical study: 1.Anti-arrhythmia effect. 2.Anti-ulcer effect. 3.Diuresis effect. 4.Analgesia effect. 5.Anti-tussive effect. 6.Eliminating phlegm effect.
Bohe ***(Mentha)***	FM201542	TCM: Evacuating wind-heat, clearing the boss, relieving the throat, clearing the rash, soothing the liver and promoting qi. Pharmaceutical study: 1.Anti-inflammatory effect. 2.Sedative effect. 3.Choleretic effect. 4.Penetration promoting effect.
Shengjiang ***(Ginger)***	FM201539	TCM: Relieving the appearance and dispelling cold, warming the middle to stop vomiting, warming the lungs and detoxifying. Pharmaceutical study: 1.Antibacterial effect. 2.Anti-cancer effect. 3.Anti-oxidation effect. 4.Anti-aging effects.

Table 3

	STUDY PERIOD					
	Enrolment	Allocation	Post-allocation			Close-out
TIMEPOINT**	0	0	t_1	interim	t_2	t16
Trial days	-1	0	1	14	28	112
ENROLMENT:						
Informed consent	X					
Eligibility screen	X					
Demographic characteristics	X					
Randomization		X				
INTERVENTIONS:						
Placebo Group			←	—	→	
Xiaoyaopills Group			←	—	→	
ASSESSMENTS:						
HAMD	X			X	X	X
TCM Syndrome Scale	X			X	X	X
SERS	X			X	X	X
C-SSRS	X			X	X	X
Blood routine examination	X				X	
Liver function (ALT、AST)	X				X	
Renal function（Cr、BUN）	X				X	
Electrocardiogram	X				X	

Figures

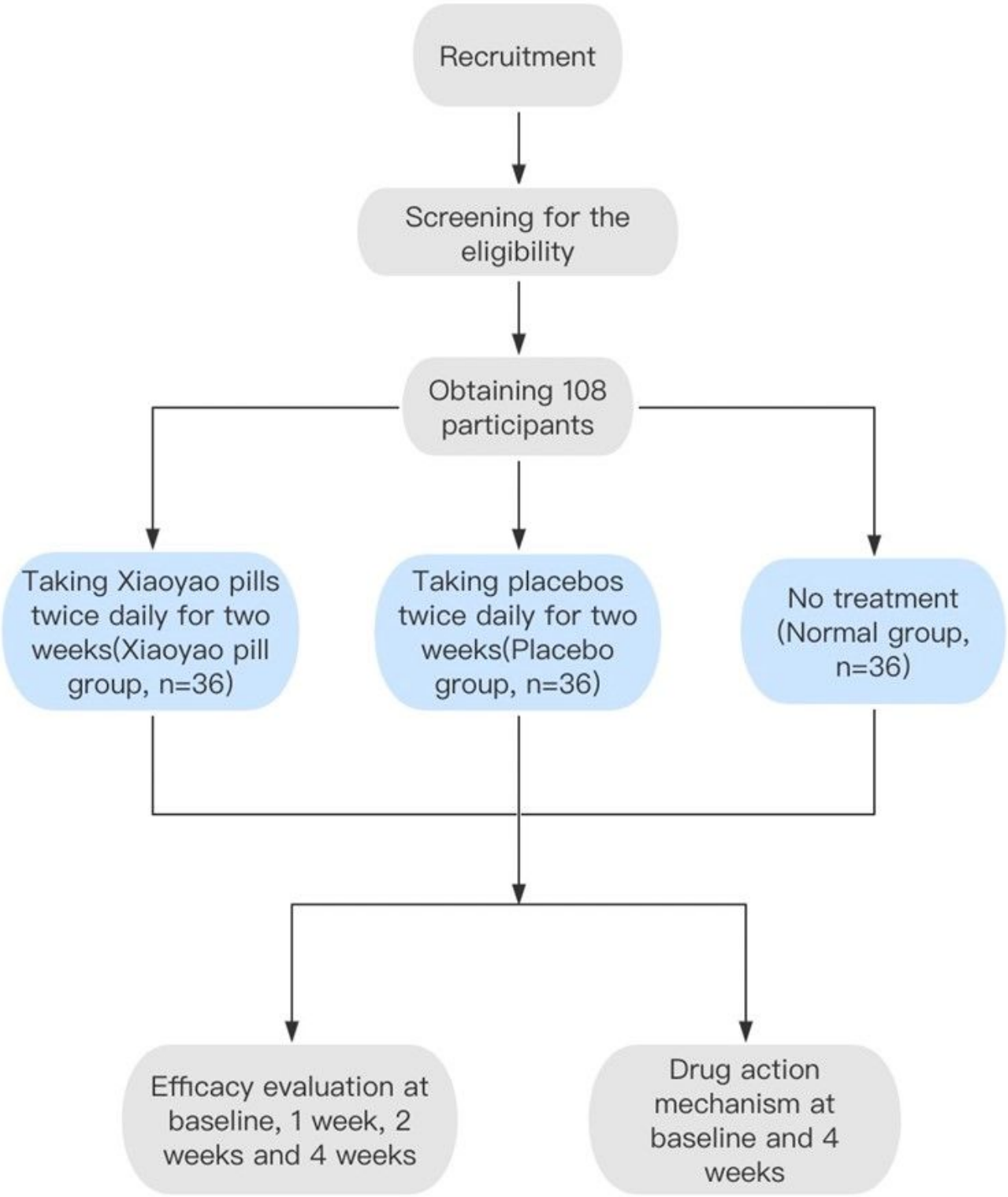

Figure 1

Flow chart

Supplementary Files

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