Research protocol: part 1

Project summary

Depression is a common mental health disorder affecting over 264 million people worldwide[1]. Current treatments often fail to achieve complete remission, highlighting the need for new therapeutic options. Xiaoyao Mixture (XYM), a Traditional Chinese Medicine formula, has shown promise in treating depressive disorders. However, its efficacy and safety have not been rigorously evaluated in randomized controlled trials for mild to moderate depression (MMD).

This multi-center, double-blinded, randomized controlled trial aims to assess the effectiveness and safety of XYM in treating MMD. 72 patients will be recruited from 4 hospitals in China and randomly assigned in a 1:1 ratio to either the XYM group or placebo group for 6 weeks. The primary outcome is change in the 17-item Hamilton Depression Rating Scale (HAMD-17) score. Secondary outcomes include measures of anxiety, anhedonia, somatic symptoms, sleep quality, quality of life, and Traditional Chinese Medicine syndromes. Safety will be monitored through adverse event reporting and laboratory tests.

If proven effective and safe, XYM could provide a new treatment strategy for MMD, meeting the urgent need for additional therapies. This trial follows rigorous methodological standards and systematically evaluates XYM's impact on various depression-related parameters. The results will provide high-quality evidence to guide clinical practice and benefit patients suffering from MMD.

General information

Protocol Title: Efficacy and Safety of Xiaoyao Mixture for the Treatment of Mild to Moderate Depression: A Randomized, Double-Blind, Placebo-Controlled Trial

Protocol Identifying Number: v 8.0
Date: June 8, 2023

Sponsor/Funder:
Lunan Pharmaceutical Group Co. Ltd., Linyi, Shandong Province, China
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Principal Investigator:
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Email: chenjiaxu@hotmail.com
Responsibilities: Prof. Chen will be responsible for the overall conduct of the study, including study design, participant recruitment, data collection, and analysis. He will also ensure that the study is conducted in accordance with the approved protocol, Good Clinical Practice (GCP) guidelines, and relevant regulations.

Research Sites:

1. The first affiliatted hospital of Jinan University
   613 Huangpu Avenue West, Tianhe District, Guangzhou Province, China 510632
   Tel: +86-20-3868-8077

2. Guangdong Provincial Hospital of Chinese Medicine
   111 Dade Road, Yuexiu District, Guangzhou, Guangdong Province, China 510120
   Tel: +86-20-8188-7233

3. Beijing Hospital of Traditional Chinese Medicine, Capital Medical University
   23 Meishuguan Back St, Dongcheng District, Beijing, China 100010
   Tel: +86-10-8471-2345

4. Ganzhou People's Hospital
   17 Hongqi Avenue, Zhanggong District, Ganzhou , Jiangxi Province, China 341099
   Tel: +86-0797-8083538

Clinical Laboratory:
Guangzhou Key Laboratory of Formula-Pattern of Traditional Chinese Medicine, School of Traditional Chinese Medicine, Jinan University
Tel: +86-20-8522-1321

Formula-Pattern Laboratory will be responsible for performing all laboratory tests required for the study, including blood chemistry, hematology, and urinalysis.

Rationale & background information

Rationale & Background Information

Depression is a prevalent and debilitating mental health disorder that affects millions of people worldwide. According to the World Health Organization, depression is a leading cause of disability and a major contributor to the global burden of disease [1,2]. Despite the availability of several treatment options, including pharmacotherapy and psychotherapy, many patients with depression do not achieve satisfactory outcomes or experience adverse effects that limit their adherence to treatment[3]. Therefore, there is a pressing need for novel and complementary approaches to managing depression.

Traditional Chinese Medicine (TCM) has been used for centuries to treat various mental health conditions, including depression. Xiaoyao Mixture (XYM), a well-known TCM formula, has shown promising results in alleviating depressive symptoms and improving overall well-being[4]. XYM is composed of several herbs, including Radix Bupleuri (Chai Hu), Radix Paeoniae Alba (Bai Shao), Radix Angelicae Sinensis (Dang Gui), Rhizoma Atractylodis Macrocephalae (Bai Zhu), Poria (Fu Ling), Radix Glycyrrhizae (Gan Cao), Herba Menthae (Bo He), and Rhizoma Zingiberis Recens

(Sheng Jiang) [5]. These herbs work synergistically to regulate qi and blood, soothe the liver, and nourish the spleen, which are believed to be the underlying mechanisms of action for XYM in treating depression.

Several studies have investigated the efficacy and safety of XYM for depression. A meta-analysis [6] found that XYM, either alone or in combination with antidepressants, was more effective than antidepressants alone in reducing depressive symptoms and improving response and remission rates. Another systematic review[7] concluded that XYM was safe and effective for the treatment of depression, with fewer adverse effects compared to conventional antidepressants. However, these studies have limitations, such as small sample sizes, short treatment durations, and lack of placebo controls, which highlights the need for more rigorous, high-quality clinical trials.

The proposed study aims to evaluate the efficacy and safety of Xiaoyao Mixture (XYM) for the treatment of mild to moderate depression in a randomized, double-blind, placebo-controlled trial. By conducting this study, we hope to provide more robust evidence for the use of XYM as a complementary or alternative treatment for depression, which could potentially benefit a large number of patients who do not respond well to or tolerate conventional treatments.

The results of this study may have important implications for clinical practice and health policy. If XYM is found to be effective and safe for the treatment of depression, it could be integrated into existing mental health care services, providing patients with a wider range of treatment options.

In conclusion, the proposed study seeks to address the need for novel and complementary approaches to managing depression by evaluating the efficacy and safety of Xiaoyao Mixture, a promising TCM formula. The findings of this study may contribute to the growing body of evidence supporting the use of TCM in mental health care and ultimately improve outcomes for patients with depression.

References:

1. Malhi GS, Mann JJ. Depression. Lancet 2018;392(10161):2299-312. doi: 10.1016/S0140-6736(18)31948-2

2. Organization WH. The World Health Report 2001: Mental health: new understanding, new hope. 2001

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5. Zhou X, Ma Q, Yan Z, et al. Efficacy and safety of Chinese patent medicine Xiao Yao San in polycystic ovary syndrome: A systematic review and meta-analysis. J Ethnopharmacol 2023;313:116517. doi: 10.1016/j.jep.2023.116517 [published Online First: 20230425]

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in Treatment of Depression. J Tradit Chin Med 2010;51(6):500-05.
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Study goals and objectives

Depression is a prevalent mental health disorder that significantly impacts daily functioning and quality of life. Despite available treatments, many patients with mild to moderate depression (MMD) do not achieve full remission, underscoring the need for novel therapeutic approaches. Xiaoyao Mixture (XYM), a Traditional Chinese Medicine (TCM) formula, has demonstrated promising antidepressant effects in preliminary studies. However, its efficacy and safety have not been rigorously evaluated in randomized controlled trials (RCTs) for MMD.

The primary aim of this study is to assess the effectiveness and safety of XYM in treating MMD through a multi-center, double-blinded, placebo-controlled RCT. The secondary objectives are to evaluate XYM's impact on anxiety, anhedonia, somatic symptoms, sleep quality, quality of life, and TCM syndrome scores.

We hypothesize that XYM will be superior to placebo in reducing depressive symptoms, as measured by the 17-item Hamilton Depression Rating Scale (HAMD-17), and will demonstrate a favorable safety profile. We also expect improvements in secondary outcomes, reflecting XYM's potential to comprehensively alleviate depression-related symptoms and enhance overall well-being.

By generating high-quality evidence on XYM's efficacy and safety, this trial aims to provide a new treatment option for MMD and contribute to the advancement of mental health care. The findings may also shed light on the role of TCM in managing depression and guide future research in this area.

Study design

This study is a prospective, multi-center, double-blinded, randomized controlled trial (RCT) with two parallel arms: XYM group and placebo group. The trial will recruit 72 patients with MMD from 4 hospitals in China, who will be randomly assigned in a 1:1 ratio to receive either XYM or placebo for 6 weeks.

Randomization will be performed using a central, concealed, web-based system, with stratification by site. An independent statistician will generate the allocation sequence. Participants, researchers, and outcome assessors will be blinded to the group assignments. Unblinding will occur only after the trial's conclusion or in case of emergency.

The study includes a screening period and a 6-week treatment period visit. Outcome assessments will be conducted at baseline and at weeks 1, 2, 4, and 6. The primary outcome is the change in

HAMD-17 score from baseline to week 6. Secondary outcomes encompass measures of anxiety, anhedonia, somatic symptoms, sleep quality, quality of life, and TCM syndromes. Safety will be monitored through adverse event reporting and laboratory tests.

Data will be collected using standardized case report forms and managed through an electronic data capture system. Statistical analyses will be performed on the intention-to-treat population, with additional per-protocol analyses.

This RCT follows the SPIRIT 2013 statement and SPIRIT-Outcomes 2022 extension, ensuring a rigorous and transparent design. The study has been approved by the ethics committees of participating institutions and registered in the Chinese Clinical Trial Registry.

Methodology

Study Population

Inclusion criteria:

- Meet the diagnostic criteria for depression in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) with a HAMD-17 score between 7 and 24 points.
- Meet the diagnosis criteria for liver depression and spleen deficiency syndrome according to Traditional Chinese Medicine (TCM).
- Aged 16-65 years, regardless of gender.
- Voluntarily provide informed consent and demonstrate good compliance.

Exclusion criteria:

- Bipolar depression, refractory depression, or suicidal tendency (item 3 score of HAMD scale > 2 points).
- Other mental disorders (e.g., organic mental disorders, schizophrenia, depression disorders caused by psychoactive or non-addictive substances).
- Severe organ lesions (heart, liver, kidney), hematological diseases, or tumors.
- Received systematic treatment within 2 weeks before enrollment.
- Pregnant, lactating, or planning to conceive during the trial.
- Participating in other clinical trials or have participated in other drug clinical trials within 3 months before screening.
- Known or suspected allergy to the investigational drugs and excipients, or hypersensitivity constitution.
- Used fluoxetine within 4 weeks before enrollment.
- Deemed unsuitable for the trial by the researchers.

Sample size calculation:

The sample size is determined based on the principles of feasibility and ethical considerations. With 30 participants per arm and considering a potential dropout rate of 15%, a total of 72 subjects (36 per group) will be required to detect potentially significant effects while minimizing unnecessary exposure.

Recruitment:

Participants will be recruited from outpatient clinics at the four trial sites in China. Recruitment strategies include displaying posters, publishing WeChat articles, and face-to-face screening by investigators. Eligible participants will provide informed consent before enrollment.

Study Intervention

The study intervention is Xiaoyao Mixture (XYM), a Traditional Chinese Medicine (TCM) formula registered with the National Medicine Permit No. Z37020339. XYM is manufactured by Lunan Hope Pharmaceutical Co., Ltd. and packaged in vials of 10 mL each (batch number: 3230501). The placebo, designed to match XYM in appearance, taste, and smell, is also provided by Lunan Hope Pharmaceutical Co., Ltd. (batch number: 3230501).

Participants in the XYM group will receive 30 mL of XYM twice daily for 6 weeks, while those in the placebo group will receive an identical dosage of the placebo. Medications will be individually packaged for each participant and managed by designated pharmacists at each center. Adherence will be monitored through medication returns at each visit.

Concomitant use of other psychiatric medications is prohibited during the trial, but non-pharmacological care and medications for physical ailments are allowed with proper documentation. Treatment duration is 6 weeks unless a participant experiences a serious adverse event or chooses to withdraw. Criteria for discontinuation include withdrawal of consent, loss to follow-up, poor compliance, and physician-determined unsuitability for continuation.

Outcomes:

The primary outcome is the change in HAMD-17 score from baseline to week 6. Secondary outcomes include measures of anxiety (HAMA), anhedonia (DARS, TEPS), somatic symptoms (PHQ-15), sleep quality (PSQI), quality of life (Q-LES-Q-SF), overall clinical improvement (CGI), and TCM syndrome scores (LDSDSS). Safety outcomes include adverse events and laboratory tests.

Safety considerations

Adverse reactions throughout the study will be systematically checked by employing the Rating Scale for Side Effects (SERS) at every visit. Should any adverse events occur during the treatment phase, we will record all pertinent details in the case report forms (CRFs), including the time of the event, observed clinical symptoms and signs, the severity and duration of the event, relevant laboratory findings, any intervention applied, and the eventual outcome, as well as an assessment of the causal relationship with the treatment. Serious adverse events will be immediately reported to the research ethics committee, which will then review the situation and determine if further actions are warranted.

Follow-up

No follow-up.

Data management and statistical analysis

Data will be collected using standardized case report forms (CRFs) at baseline and at weeks 1, 2, 4, and 6. The CRFs will include demographic information, medical history, concomitant medications, adverse events, and outcome measures. All data will be entered into an electronic data capture (EDC) system by trained research staff, with regular monitoring to ensure data quality and

completeness.
Access to the EDC system will be restricted to authorized personnel, and data confidentiality will be maintained through secure storage and transmission. The trial manager and data coordinator will have access to the final dataset, and any requests for data sharing will be reviewed by the principal investigator.

Statistical Analysis
Statistical analyses will be performed on the intention-to-treat (ITT) population, which includes all randomized participants. Additional per-protocol (PP) analyses will be conducted on participants who complete the study without major protocol deviations. Continuous variables will be presented as means and standard deviations or medians and interquartile ranges, while categorical variables will be presented as frequencies and percentages.
There will be no examination of subgroups, nor will there be any interim analysis of efficacy.
All statistical tests will be two-sided, with a significance level of 0.05. Statistical analyses will be performed using R software.

Quality assurance

To ensure the integrity of the study and the quality of the data, a comprehensive quality assurance and quality control system will be implemented throughout the trial. The study will be conducted in accordance with the principles of Good Clinical Practice (GCP), as outlined in the International Conference on Harmonization (ICH) guidelines and relevant national regulations.

All study personnel will receive training on the study protocol, GCP, and standard operating procedures (SOPs) before the initiation of the trial. Regular study site monitoring visits will be conducted by qualified clinical research associates (CRAs) to ensure adherence to the protocol, GCP, and SOPs. The CRAs will review study documentation, participant consent forms, and source data verification to identify and resolve any discrepancies or issues.

An independent Data and Safety Monitoring Board (DSMB) will be established to oversee the trial's progress, review safety data, and make recommendations regarding study continuation or modification. The DSMB will consist of experts in the field of depression, TCM, and clinical trial methodology who are not directly involved in the study. The DSMB will review unblinded safety data at predefined intervals and ad hoc as needed, and will have the authority to recommend study termination or modification if significant safety concerns arise.

Data management will be conducted using a secure, validated electronic data capture (EDC) system. All data will be entered into the EDC system by trained research staff, with regular monitoring and validation to ensure data accuracy, completeness, and consistency. Data queries will be generated and resolved in a timely manner, and an audit trail will be maintained to track all data changes.

A central laboratory will be used for all study-related laboratory tests to ensure standardization and quality control. The laboratory will follow established SOPs and participate in external quality assurance programs to maintain high standards of performance.

Adverse event reporting will follow GCP guidelines and relevant national regulations. All adverse events will be recorded in the EDC system, and serious adverse events will be reported to the ethics committee, regulatory authorities, and the DSMB within the required timeframes.

Regular study progress reports will be provided to the ethics committee and regulatory authorities, including information on participant recruitment, retention, safety, and data quality. Any protocol deviations or violations will be documented and reported as required.

The study will undergo regular audits by an independent quality assurance team to ensure compliance with GCP, the study protocol, and applicable regulations. Audit findings will be reported to the study sponsor and principal investigator, and corrective actions will be implemented as necessary.

By implementing a robust quality assurance and quality control system, this study aims to generate high-quality, reliable data that can inform clinical practice and advance the understanding of Xiaoyao Mixture in the treatment of mild to moderate depression.

Expected outcomes of the study

This study aims to generate high-quality evidence on the efficacy and safety of Xiaoyao Mixture (XYM) in the treatment of mild to moderate depression (MMD). The results of this trial are expected to contribute to the advancement of knowledge in several ways:

Expanding treatment options for MMD: If XYM demonstrates superior efficacy compared to placebo, it could provide a novel, effective, and safe treatment option for patients with MMD, particularly those who do not respond adequately to conventional therapies or prefer a more natural approach. This could expand the therapeutic arsenal for MMD and improve patient outcomes.

Advancing understanding of TCM in mental health: The systematic evaluation of XYM's efficacy and safety could contribute to the growing body of evidence supporting the role of Traditional Chinese Medicine (TCM) in the management of mental health disorders. The findings may encourage further research into the mechanisms of action and potential synergistic effects of TCM formulas in the treatment of depression.

Informing personalized treatment strategies: The inclusion of TCM syndrome scores as a secondary outcome may help identify subgroups of patients who are more likely to benefit from XYM. This could inform the development of personalized treatment strategies that take into account individual patient characteristics, aligning with the principles of precision medicine.

The results of this study will be disseminated through various channels to maximize their impact on health care, health systems, and health policies:

Publication in peer-reviewed journals: The findings will be submitted for publication in

high-impact, peer-reviewed scientific journals in the fields of psychiatry, TCM, and integrative medicine. This will ensure that the results reach a broad audience of researchers, clinicians, and policymakers.

Presentation at conferences: The results will be presented at national and international conferences focused on mental health, TCM, and integrative medicine. This will provide opportunities for direct engagement with the scientific community, fostering discussion and collaboration.

Integration into clinical practice guidelines: If the results demonstrate a significant benefit of XYM in the treatment of MMD, they may be incorporated into clinical practice guidelines for the management of depression. This could influence the standard of care and encourage the adoption of XYM as a treatment option in clinical settings.

Health policy implications: The findings of this study may have implications for health policies related to the integration of TCM into mental health care. Demonstrating the efficacy and safety of XYM could support the inclusion of TCM in national health insurance programs, increasing access to these treatments for patients with MMD.

Public dissemination: The results will be communicated to the general public through media releases, patient advocacy groups, and online platforms. This will raise awareness about the potential benefits of XYM and TCM in the management of depression, empowering patients to make informed decisions about their treatment options.

Dissemination of results and publication policy

The results of this study will be disseminated through multiple channels to ensure that the findings reach a wide audience, including the scientific community, healthcare professionals, policymakers, and the general public. The dissemination plan will adhere to the principles of transparency, accuracy, and accessibility.

Scientific Media:

Peer-reviewed journals: The main study results will be submitted for publication in high-impact, peer-reviewed journals in psychiatry, Traditional Chinese Medicine (TCM), and integrative medicine.

Conference presentations: The findings will be presented at national and international conferences on mental health, TCM, and integrative medicine.

Community and Participant Dissemination:

Participant feedback: All participants will receive a lay summary of the study results within 6 months of the trial's completion.

Patient advocacy groups: The results will be shared with relevant patient advocacy groups and mental health organizations.

Media releases: Press releases and media kits will be prepared to communicate the study's key findings to the general public.

Policy Makers:
Policy briefs: Concise policy briefs will be prepared, summarizing the study's findings and potential implications for health policies.
Meetings with policymakers: The study team will present the findings to policymakers through meetings, workshops, and other forums.
Publication Policy:

Authorship: Authorship will be determined based on the ICMJE criteria.
Acknowledgments: All study personnel, funding sources, and contributors will be acknowledged in the publications.
Conflicts of interest: All authors will disclose any potential conflicts of interest.
Data sharing: Study data will be made available for secondary analyses upon request, subject to approval and adherence to data protection and confidentiality principles.

Duration of the project

This study is expected to be completed within a period of 24 months. The timeline for each phase of the project is outlined below:

Phase 1: Study Preparation (Months 1-3)
Month 1: Study protocol finalization, ethics committee submission, and clinical trial registration
Month 2: Recruitment and training of study personnel, preparation of study materials, and establishment of the Data and Safety Monitoring Board (DSMB)
Month 3: Initiation of study site setup, including equipment and supplies procurement, and development of the electronic data capture (EDC) system

Phase 2: Participant Recruitment and Intervention (Months 4-15)
Months 4-6: Participant screening and enrollment at all study sites
Months 7-15: Intervention period (6 weeks per participant), with ongoing data collection and monitoring

Detailed timeline for Phase 2:
Month 4: Screening and enrollment of the first 20 participants
Month 5: Screening and enrollment of an additional 30 participants
Month 6: Screening and enrollment of the remaining 22 participants
Months 7-8: Intervention and data collection for the first 20 participants
Months 8-9: Intervention and data collection for the next 30 participants
Months 9-10: Intervention and data collection for the remaining 22 participants
Months 11-15: Continued intervention and data collection, with ongoing data monitoring and quality control

Phase 3: Data Analysis and Reporting (Months 16-24)
Months 16-18: Data cleaning, validation, and database lock
Months 19-21: Statistical analysis and preparation of study reports
Months 22-23: Manuscript drafting and submission to peer-reviewed journals

Month 24: Dissemination of results to participants, community, and policymakers

Detailed timeline for Phase 3:
Month 16: Data cleaning and validation for the first 20 participants
Month 17: Data cleaning and validation for the next 30 participants
Month 18: Data cleaning and validation for the remaining 22 participants, and database lock
Month 19: Primary and secondary outcome analyses
Month 20: Subgroup and sensitivity analyses
Month 21: Preparation of study reports and initial manuscript drafts
Month 22: Finalization of manuscripts and submission to peer-reviewed journals
Month 23: Preparation of lay summaries and policy briefs
Month 24: Dissemination of results to participants, community, and policymakers, and project closure
Throughout the study, regular meetings will be held among the research team to monitor progress, address any issues, and ensure adherence to the project timeline. The DSMB will convene at predefined intervals (e.g., every 6 months) to review safety data and provide recommendations regarding study continuation or modification.

Problems anticipated
While we have taken steps to ensure the smooth execution of this study, we anticipate potential difficulties that may arise during the course of the project. These challenges, along with their possible solutions, are discussed below:

Participant retention:
Challenge: Ensuring participant retention throughout the 6-week intervention period could pose a challenge.
Solution: To promote participant retention, we will maintain regular communication with participants, provide clear instructions and support, and offer incentives for completing the study, such as travel reimbursements or gift cards.

Adherence to the intervention:
Challenge: Participants may not adhere to the prescribed dosage and frequency of the Xiaoyao Mixture (XYM) or placebo, which could impact the study results.
Solution: To encourage adherence, we will provide participants with clear instructions on how to use the study medication, along with tips for incorporating it into their daily routine. We will also use medication diaries and conduct pill counts at each study visit to monitor adherence. Participants will receive reminders via phone calls or text messages to take their medication as prescribed.

Data quality and completeness:
Challenge: Ensuring high-quality, complete data collection across all study sites may be challenging, particularly given the subjective nature of some outcome measures.
Solution: We will provide comprehensive training to all study personnel on data collection procedures, including the proper administration of outcome measures. Regular monitoring visits

by the clinical research associates (CRAs) will be conducted to ensure data quality and completeness. The electronic data capture (EDC) system will have built-in data validation and quality checks to minimize errors and inconsistencies.

Timeframe and budget constraints:
Challenge: Unforeseen circumstances, such as delays in participant recruitment or unexpected costs, may impact the study's ability to be completed within the stipulated timeframe and funding.
Solution: We have built a contingency plan into the study timeline and budget to account for potential delays or additional expenses. Regular monitoring of study progress and expenses will help identify any issues early on, allowing for timely adjustments to be made. If significant deviations from the planned timeline or budget occur, we will promptly communicate with the funding agency and ethics committee to discuss appropriate solutions.

Project management

The success of this study relies on the effective collaboration and coordination of a multidisciplinary team. Each team member has a specific role and set of responsibilities to ensure the project's smooth execution. The key roles and responsibilities are as follows:

1. Principal Investigator (PI):
Oversees the entire study, ensuring its scientific integrity and adherence to ethical standards
Develops and finalizes the study protocol, and obtains ethical and regulatory approvals
Coordinates with co-investigators, study sites, and other collaborators
Monitors study progress, data quality, and safety, and takes corrective actions as needed
Leads data analysis, interpretation, and publication of study results

2. Co-Investigators:
Contribute to the development and refinement of the study protocol
Assist in obtaining ethical and regulatory approvals at their respective study sites
Oversee participant recruitment, enrollment, and data collection at their sites
Ensure adherence to the study protocol and Good Clinical Practice (GCP) guidelines
Collaborate with the PI in data analysis, interpretation, and publication

3. Study Coordinator:
Manages day-to-day study operations and serves as a liaison between the PI, co-investigators, and study sites
Assists in the development and distribution of study materials, such as case report forms (CRFs) and study manuals
Coordinates study site initiation, training, and monitoring visits
Monitors participant recruitment, retention, and data quality across all sites
Assists in data management, analysis, and report preparation

4. Clinical Research Associates (CRAs):
Conduct regular monitoring visits at study sites to ensure compliance with the protocol, GCP, and

regulatory requirements
Review study documentation, participant consent forms, and source data verification
Identify and resolve data discrepancies or issues, and provide feedback to study sites
Assist in the preparation of monitoring reports and communication with the PI and study coordinator

5. Data Manager:
Develops and maintains the electronic data capture (EDC) system
Ensures data security, backup, and access control
Performs data validation, cleaning, and query resolution
Generates data reports for the PI, DSMB, and other stakeholders
Assists in data analysis and preparation of study reports

6. Statistician:
Contributes to the development of the statistical analysis plan
Provides input on sample size calculation and randomization procedures
Conducts statistical analyses of primary, secondary, and exploratory outcomes
Assists in the interpretation of study results and preparation of manuscripts

7. Study Physicians:
Assess participant eligibility and conduct medical evaluations
Monitor participant safety and report adverse events
Provide medical guidance to participants and study staff
Collaborate with the PI and co-investigators in data interpretation and publication

8. Study Nurses:
Assist in participant screening, enrollment, and informed consent processes
Administer study interventions and conduct study assessments
Monitor participant compliance and provide education and support
Maintain accurate and complete study documentation

9. Laboratory Personnel:
Process, analyze, and store biological samples according to the study protocol
Ensure quality control and adherence to laboratory standards
Provide timely and accurate laboratory results to the study team

10. Administrative Staff:
Provide administrative support to the study team, including scheduling, correspondence, and document management
Assist in the preparation and submission of ethics committee and regulatory documents
Manage study finances, including budgeting, expense tracking, and reimbursements

Regular team meetings will be held to discuss study progress, address any issues, and ensure effective communication among all team members. The PI will have ultimate responsibility for

the study's conduct and will work closely with the co-investigators, study coordinator, and other team members to ensure the project's success.

Ethics

The study protocol has been approved by the Ethics Committee of Jinan University (JNUKY-2022-100, version 8.0, June 2023) and registered in the Chinese Clinical Trial Registry (ChiCTR2300074953, registered on August 28, 2023). The trial will be conducted in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines, and relevant national regulations.

Informed consent forms

All participants will provide written informed consent before enrollment, and their privacy and confidentiality will be protected throughout the study. Participants will be informed of their right to withdraw from the study at any time without consequences.

Research protocol: part 2

Budget

N.A

Other support for the project

NO.

Collaboration with other scientists or research institutions

NO.

Curriculum Vitae of investigators

Principal Investigator: Dr. Jiaxu Chen, MD, PhD

Current Position: Professor of Traditional Chinese Medicine, Beijing University of Traditional Chinese Medicine

Education: MD (Beijing University of Chinese Medicine)

Research Interests: Traditional Chinese Medicine formulas for mental health, herbal pharmacology, clinical applications of Chinese herbal medicine

Selected Publications:

1. Zhao Xin, Wang Limin, Liu Yue, Jiang Youming, Chen Jiaxu, Xue Feifei. Epidemiological Investigation on Sub-health State of Liver Depression and Spleen Deficiency Syndrome. Modernization of Traditional Chinese Medicine and Materia Medica--World Science and Technology 2018, 20(11): 1979-1983.

2. Zhao Xin, Liu Yueyun, Chen Jiaxu, Xue Feifei. Discussion on Diagnostic Criteria of Liver Depression and Spleen Deficiency Syndrome. Modernization of Traditional Chinese Medicine and Materia Medica--World Science and Technology 2018, 20(11): 1974-1978.

3. Teng Xuejiao, Chu Yanhong, Zhai Chengcheng, Yu Yingfang, Cai Yuchun, Chen Shaohong, et al. The epidemiological characteristics and influencing factors for Blastocystis hominis infection

among human immunodeficiency virus seropositive individuals in Tengchong of Yunnan Province. Chinese Journal of Parasitology & Parasitic Diseases 2018, 36(2): 129-134.
Grants and Awards:
He is a recipient of the National Science Fund for Distinguished Young Scholars, a Distinguished Professor of the Yangtze River Scholar Award Program by the Ministry of Education, and a national-level candidate of the "Hundred, Thousand, and Ten Thousand Talents Project."

Other research activities of the investigators

NO

Financing and insurance

Financing:

This study will be financed by a grant from the Lunan Pharmaceutical Group Co. Ltd. The grant will cover all expenses related to the study, including:

- Study medication (Xiaoyao Mixture and placebo)
- Laboratory tests and medical examinations
- Participant recruitment and compensation
- Data management and statistical analysis
- Publication and dissemination of results

The Principal Investigator, Prof. Jiaxu Chen, will be responsible for managing the grant funds and ensuring that they are used in accordance with the approved budget.

Insurance:

All participants in this study will be covered by clinical trial insurance, which will be provided by a reputable insurance company in China. The insurance policy will cover any potential harm or injury that may result from participation in the study, including:

- Medical expenses related to the treatment of study-related adverse events
- Compensation for any temporary or permanent disability resulting from the study
- Compensation for death resulting from the study

The insurance policy will be in accordance with the regulations and guidelines set forth by the National Medical Products Administration (NMPA) and the Ministry of Finance of the People's Republic of China. The Principal Investigator will ensure that the insurance policy is in place before the start of the study and that all participants are fully informed about the coverage and the process for making a claim, if necessary.

In addition to the clinical trial insurance, the study sites will maintain their own liability insurance to cover any potential claims arising from the conduct of the study.

The study team will adhere to all relevant regulations and guidelines regarding clinical trial insurance and will promptly report any adverse events or potential claims to the insurance company and the appropriate regulatory authorities.