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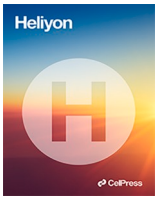

Research article

Effect of "Tiaoshen" acupuncture technique on mild depression and its underlying mechanism: A randomized controlled trial study protocol

Lu Bai [a], Wei Zou [b,c,*], Long Wang [d], Xueping Yu [b], Hongjun Lou [d], Xiaohong Dai [a], Wei Teng [a], Weiwei Yu [b], Mingyue Li [b], Hongtao Cao [b], Lei Zheng [b]

[a] *Heilongjiang University of Chinese Medicine, Harbin, 150040, China*
[b] *The third department of Acupuncture, the First Affiliated Hospital of Heilongjiang University of Chinese Medicine, Harbin, 150040, China*
[c] *Clinical Key Laboratory of Integrated Traditional Chinese and Western Medicine, Heilongjiang University of Chinese Medicine, Harbin, 150040, China*
[d] *The first Affiliated Hospital of Heilongjiang University of Chinese Medicine, Harbin, 150040, China*

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ABSTRACT

Background: Mild depression is not just a mental disease, but also a serious and long-term public health issue. It affects the quality of life of patients and can quickly develop into major depression. There are currently no effective drug treatments with high efficacy and few adverse reactions. Acupuncture may be an alternative treatment option. Preliminary experiments and practices have demonstrated that "Tiaoshen" acupuncture improves symptoms in patients who have depression, however the underlying data and method remain unclear at present.

Methods: This is a prospective, single-center, single-blind, randomized controlled trial. We plan to recruit 70 participants and randomly assign them to receive "Tiaoshen" acupuncture or traditional acupuncture at a ratio of 1:1. Then, all the participants will receive the appropriate acupuncture treatment for four weeks. The results of the Hamilton Depression Rating Scale (HDSR-24) will serve as the primary outcome, while the results of the Patient Health Questionnaire-9 (PHQ-9) and the World Health Organization Quality of Life Brief Version (WHOQOL-BREF) will serve as secondary outcomes. Evaluations will be conducted at baseline, 1, 2, and 4 weeks after treatment initiation, and 1 and 3 months after treatment completion. The safety of the intervention will be evaluated every week using the Columbia-Suicide Severity Rating Scale (C-SSRS) and the Treatment Emergent Symptoms Scale (TESS). Serum levels of oxidative stress markers 8-iso-prostaglandin F2α (8-iso-PGF2α), superoxide dismutase (SOD), uric acid (UA), and total bilirubin (TBIL) will be measured at baseline and the end of the treatment. We will conduct a statistical analysis of intention to treat (ITT) and conformance to protocol set (PPS) data.

Discussion: This research aims to provide high-quality evidence for the efficacy and safety of "Tiaoshen" acupuncture as a treatment for mild depression. In addition, the mechanism through which acupuncture heals mild depression will be investigated.

* Corresponding author. The third department of Acupuncture, the First Affiliated Hospital of Heilongjiang University of Chinese Medicine 26 Heping Road, Xiangfang District, Harbin, 150040, Heilongjiang, China.
E-mail address: zouweizzm@126.com (W. Zou).

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1. Introduction

Abbreviations

CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
C-SSRS	Columbia-Suicide Severity Rating Scale
FAS	Full Analysis Dataset
HDRS-24	Hamilton Depression Rating Scale-24
ICD-10	International Classification of Diseases 10th revision
ITT	Intention-to-treat
PHQ-9	Patient Health Questionnaire-9
PPS	Per-protocol Set
SOD	Superoxide Dismutase
SPIRIT	Standard Protocol Items: Recommendations for Interventional Trials
STRICTA	Standards for Reporting Interventions in Controlled Trials of Acupuncture
TBIL	Total Bilirubin
TCM	Tradition Chinese Medicine
TESS	Treatment Emergent Symptom Scale
UA	Uric Acid
WHOQOL-BREF	World Health Organization Quality of Life Brief Version

Depression is a common yet significant psychiatric disorder that affects roughly 280 million people worldwide and is one of the top 10 disabling diseases [1,2]. The condition is more prevalent in younger and older adults and affects more women than men [3]. The prevalence of novel coronavirus pneumonia (COVID-19) has imposed varying degrees of psychological burden on the general community. Studies have reported an increase in anxiety, depression, and stress in the general community, infected individuals, and medical personnel [4]. A stressful lifestyle has emerged as a primary cause of depressive episodes [5]. The characteristic of mild depression is that one of the core symptoms must be present and usually no more than four related symptoms. Although day to day functioning may feel a struggle it is rarely affected in any significant fashion. In this social setting, mild depression is more common than depression, but has received significantly less attention than depression. This neglect raises the probability that mild depression may progress to major depression [6]. Therefore, it is essential to conduct research into potential improvements in the treatment of mild depression.

Although most guidelines recommend antidepressants as treatment for depression, antidepressants require weeks to months of treatment and there are withdrawal symptoms when discontinued [7]. Some experts deem it inappropriate to prescribe antidepressants to a large proportion of patients with mild depression [8]. Since the efficacy of antidepressants for mild and subthreshold depression has not been established, the various adverse effects of antidepressants may cause more harm than good for patients with mild depression [9]. Some depression practice guidelines therefore recommend that a period of active support and monitoring should be considered for patients with mild and subthreshold depression prior to initiating other evidence-based treatments and that psychotherapy be offered if depressive symptoms persist [10]. The vast majority of patients favor nonpharmaceutical therapies [11]. Certain psychotherapeutic approaches require individual treatment by trained clinicians, and even in countries with relatively high physician and psychotherapist densities, patients must wait several months to begin specific psychotherapy [12]. Low-income and middle-income countries also have fewer mental health intervention resources. Therefore, the search for a treatment that is safe, effective, has few side effects, is easily accessible, and is inexpensive is crucial.

Acupuncture, a form of complementary and alternative therapy, has been used in China for over 2500 years and has a long history of popularity. Acupuncture stimulation appears to improve depressive-like symptoms in rat models, according to research on animals [13]. Accumulating evidence from clinical studies suggests that acupuncture can play a vital role in the treatment of depressive symptoms, with significant reductions in depressive symptoms compared to normal care or psychotherapy alone [14,15], and it is safer and better tolerated than antidepressants alone, with better improvements in quality of life [16]. Our earlier research shows that "Tiaoshen" acupuncture treatment can considerably reduce the major symptoms of depression, with the added benefit of an early onset and short treatment duration [17,18].

The difference between traditional acupuncture and "Tiaoshen" is that traditional acupuncture is performed under the guidance of expert consensus, while in "Tiaoshen", another 5 points of Shenting, Sishencong, Taiyang, Shuigou and Danzhong is added compared with traditional acupuncture. These 5 points are mainly located in the head, of which "Before and after" Shencong, Shenting and Shuigou are located in the circulation of important Du meridians regulating the mind and brain. Taiyang is also an important point for regulating Qi and blood in the head. Danzhong has the effect of regulating qi in the chest. In addition to point selection, acupuncture manipulation is the key to effective treatment. Compared with the traditional acupuncture, “Tiaoshen” uses targeted special acupuncture techniques according to the patient’s physique, so that the effect of needle can be transmitted to the disease, so as to play the effect of regulating the Yuan spirit and achieve better curative effect. In recent years, several studies have demonstrated that the

activity of antioxidant enzymes and the level of oxidative products involve in the development of depression [19–22], and acupuncture had been reported having the potential to reduce oxidative stress [23–25].

Based on the findings of prior research, we speculate that "Tiaoshen" acupuncture may reduce the content of oxidation products in the serum of patients with mild depression, increase the content of antioxidants, and thus reduce the level of oxidative stress, and as a result, improve their quality of life, and have long-term efficacy and clinical application safety. To test this hypothesis, we aim to conduct a single-center, prospective, single-blind, randomized controlled trial with a four-week treatment duration and a three-month follow-up period. The purpose of this study is to observe the safety and efficacy of the "Tiaoshen" acupuncture protocol and the traditional acupuncture protocol in the treatment of mild depression and to compare the precise effects of various acupuncture techniques by measuring HDRS-24 scores, PHQ-9 scores and WHOQOL-BREF score. The measurement of peripheral levels of oxidative stress indicators can give a new scientific basis for clarifying the mechanism by which acupuncture treats depression.

This study aims to (1) evaluate the efficacy and safety of the treatment of mild depression using a comparison of the conventional acupuncture treatment technique and the "Tiaoshen" acupuncture treatment technique, (2) observe the changes in oxidative stress-related indices in patients with depression, and (3) explore the possible mechanisms of acupuncture as a treatment for depression.

2. Methods

2.1. Trial design

This study is designed as a prospective, single-center, single-blind, randomized, controlled trial. We will follow the Consolidated Standards of Reporting Trials (CONSORT 2010) guidelines [26], the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) [27], and the Standards for Reporting Interventions in Controlled Trials of Acupuncture (STRICTA) [28]. The flowchart of the trial design is depicted in Fig. 1.

The trial will be conducted in accordance with the Declaration of Helsinki and has been approved by the Ethics Committee of the First Affiliated Hospital of Heilongjiang University of Chinese Medicine (HZYLLKY202205001) and registered with the Chinese Clinical Trial Registry (ChiCTR2200062150).

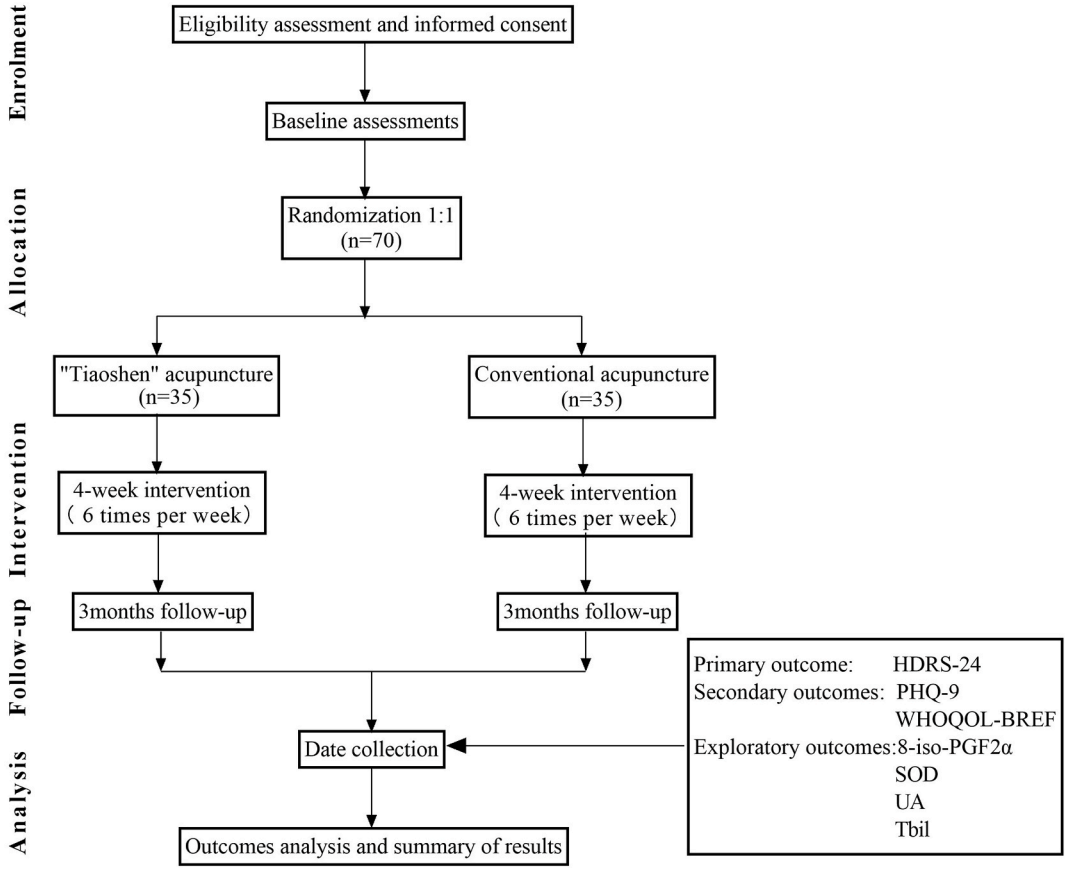

Fig. 1. CONSORT 2010 flow diagram.

2.2. Setting

The trial will be conducted in the outpatient department of the First Affiliated Hospital of Heilongjiang University of Chinese Medicine.

2.3. Participants

Participants will be recruited for the trial from the patients at the Acupuncture Department of the First Affiliated Hospital of Heilongjiang University of Chinese Medicine. Additionally, recruitment will be conducted via advertisements posted on the WeChat platform, WeChat, or QQ chat group (Tencent Corp, WeChat version: 8.0.1, QQ version: 8.8.5, Shenzhen, China). Only those participants who meet all inclusion criteria will be recruited for the study after they sign the informed consent form.

2.4. Inclusion criteria

Participants who meet the following criteria will be included.

1. Patients who fit the diagnostic criteria for mild depression according to the International Classification of Diseases, tenth revision (ICD-10) [29].
2. Patients between the ages of 18 and 70 years.
3. Patients with an HDRS-24 score between 20 and 26 (inclusive).
4. Patients who have not participated in any other clinical studies in the preceding month.
5. Patients who have not received any treatment for depression such as acupuncture and medication, within the past week.
6. Patients who have not been diagnosed with aphasia or mental retardation, who can comprehend the content of the scale, who are willing to undergo four weeks of acupuncture therapy, and who sign an informed consent form are eligible for the study.

2.5. Exclusion criteria

Participants with the following conditions will be excluded.

1. Patients having suicidal tendencies who fit the diagnostic criteria for schizophrenia, bipolar disorder, or other psychotic diseases.
2. Patients with severe heart, liver, kidney, and other systemic diseases.
3. Pregnant or lactating women.
4. Patients who cannot tolerate needling due to a skin infection or breakdown at the site of needling, coagulation disorders, dizziness, or extreme weakness.
5. Patients who have participated in other clinical trials within one month prior to the start of this trial.
6. Patients who have taken antidepressants or received acupuncture for depression within one week prior to the start of this trial will be excluded.
7. Patients with severe cognitive and consciousness problems that limit communication, patients who are unwilling to cooperate, and patients who have not signed the informed consent form.

2.6. Withdrawal and termination criteria and management

2.6.1. Withdrawn criteria

1. Patients cannot continue to receive treatment due to personal reasons, or poor compliance is difficult to cooperate with treatment and later lost to follow-up;
2. Patients had adverse reactions related to acupuncture treatment;
3. Patients received other related drugs or treatment measures other than the prescribed therapy during the study by themselves.

2.6.2. Termination criteria

Patients who had a mental health crisis or apparent suicide attempt during the study were withdrawn by their physicians.

2.6.3. Withdrawal/termination management

1. After the patient dropped out, the investigator should contact the patient who withdrew to inquire about the potential cause, record the last treatment time, complete the evaluation of the scale that can be completed, and fill in the case report form (CRF) together with the details and date of the patient who withdrew for further analysis;
2. For the patients who withdrew due to adverse reactions, the relevant situation of adverse reactions should be recorded in detail in the CRF, and the corresponding treatment measures should be given;

3. For patients whose cases have been identified as Withdrawal/termination of the study and require further treatment, the treatment should be provided by the investigator, but the relevant data of the subject will be considered missing data and will not be recorded or analyzed.

2.7. Sample size estimation

Non-inferiority test will be adopted in this study. The sample size was determined by utilizing the HDRS-24 scale score as the evaluative index. We anticipated that the HDRS-24 scale score for patients with mild depression treated with "Tiaoshen" acupuncture would be 8 ± 4.02, which is 3 points lower than the change in HDRS-24 scale score for patients with mild depression treated with conventional acupuncture, and the standard deviation was comparable between the two groups. A two-sided test was used to determine the significance level α as 0.05 and the test efficacy Power = 1-β = 0.8. The following formula was utilized to estimate the sample size required:

$$n1 = n2 = \frac{(Z_\alpha + Z_\beta)^2 2\sigma^2}{\delta^2}$$

$$= \frac{(1.960 + 0.842)^2 \times 2 \times 4.02 \times 4.02}{3^2} = 28.19$$

According to our calculations, a minimum of 29 participants will be included in each group, and based on a 15% withdrawal rate, the total sample size would be 70 participants, with 35 participants in each group.

	STURY PERIOD								
	Enrolment	Allocation	Post-Allocation						Close-out
			Intervention				Follow-up		
TIMEPOINT	Week-1	Week0	Week1	Week2	Week3	Week4	Month2	Month4	>Month4
ENROLMENT									
Eligibility screen	×								
Informed consent	×								
Allocation		×							
INTERVENTIONS									
Conventional acupuncture			♦	—	—	♦			
"Tiaoshen" acupuncture			♦	—	—	♦			
ASSESSMENTS									
Sociodemographic characteristics	×								
HDRS-24		×	×	×	×	×	×	×	
PHQ-9		×	×	×	×	×	×	×	
WHOQOL-BREF		×	×	×	×	×	×	×	
TESS		×	×	×	×	×			
C-SSRS		×	×	×	×	×			
Blood sample (8-iso-PGF2α, SOD, UA, Tbil)		×				×			
Analysis of trial outcomes									×

Fig. 2. SPIRIT figure.

2.8. Randomization and allocation concealment

Researchers who are unaware of the trial will utilize SAS statistical software (SAS Inc., NCSU, version: 9.4, USA) to generate random numbers using a randomized block design in order to balance the effect of time of entrance on participants characteristics and to ensure a balance between groups. A strategy of not fixing the block size was used to reduce group predictability, stratifying participants by the center with a 1:1 allocation of random block sizes of 4 and 6. Only the randomization researcher is aware of the block size, which shall not be revealed until the end of the trial.

The corresponding grouping codes will be placed in opaque, sealed envelopes. Each participant who meets the inclusion criteria will receive a sealed envelope with the assigned number. Different treatment will be delivered based on the subgroup code included within the envelope. Assessors and practitioners participating in the study will not be a part of the randomization procedure. After the patients were enrolled, the researchers would give the patients a number, then open the corresponding numbered envelope, and intervene according to the grouping plan within the envelope. The treatment regimen received by each subject was determined by the generated random sequence. The researchers in charge of grouping are not related to this experiment.

2.9. Blinding

Participants will be informed that the study is a comparison of the advantages and disadvantages of two effective acupuncture treatments, and that the two groups of participants will avoid getting acquainted and communicating with each other after randomization, and will receive treatment in separate treatment rooms to minimize subjective effects. Acupuncturists will be advised not to share relevant information with participants, and participants will be unable to discern which therapy they are receiving. Due to the unique nature of acupuncture, acupuncturists cannot be blinded to treatment allocation, and double blinding will be challenging to accomplish in this trial. In addition, we will ensure that practitioners doing outcome evaluations and data analysis are unaware of the groups. When participants withdraw or the trial is terminated, acupuncturists would be able to unblind the withdrawn patients.

2.10. Intervention and participant timelines

The participants will be randomly assigned either to the "Tiaoshen" or the conventional acupuncture group. All acupuncture sessions will be performed by two acupuncturists from China who are licensed as traditional Chinese medicine (TCM) practitioners by the Ministry of Health of the People's Republic of China and registered with the Chinese Medical Association, have a master's degree in acupuncture, and at least five years of clinical experience in acupuncture. Prior to the beginning of the trial, acupuncturists will participate in training sessions to ensure that the skills of the acupuncturists are consistent. Patients will undergo 24 acupuncture sessions over the course of 4 weeks (one treatment per day for 6 days, 50 min per session, followed by 1 day off). The trial will utilize a disposable sterile needle (Hwato disposable acupuncture needle, size 0.35 × 40 mm, Suzhou Medical Appliance Factory, China). For the location of acupuncture points, we shall refer to the national standard "Nomenclature and location of meridian points" (GB/T12346-2021) and "Nomenclature and location of extra points in common use" (GB/T40997-2021) [30,31]. As the acupuncture site must be completely exposed, we will ensure that the room temperature is around 26 °C. The schedule of the trial is described in detail in Fig. 2.

2.10.1. Conventional acupuncture group

The conventional acupuncture treatment plan refers to clinical practice guidelines for treating depression using acupuncture. Acupuncture treatment will target Baihui (GV20), Yintang (GV24+), bilateral Fengchi (GB20), Neiguan (PC6), Shenmen (HT7), Hegu (LI4), and Taichong (LR3) acupoints [32]. Before acupuncture treatment, the skin around the acupuncture points will be disinfected with 75% alcohol. The acupuncturist will then insert the needles into the acupuncture points and manipulate the needles using a reinforcing-reducing technique at each acupuncture point until the typical deqi sensations are achieved, including pain, distention, numbness, or heaviness felt by the patient and heaviness, stagnation, or tightness felt by the acupuncturist.

2.11. "Tiaoshen" acupuncture group

The "Tiaoshen" acupuncture treatment strategy depends on the clinical experience of the acupuncturist. Baihui (GV20), Sishencong (EX-HN1), Shenting (GV24), Yintang (GV24+), Shuigou (GV26), Danzhong (CV17) and bilateral Taiyang (EX-HN5), Fengchi (GB20), Neiguan (PC6), Shenmen (HT7), and Taichong (LR3) acupoints will be used in the acupuncture treatment. Compared to the standard acupuncture plan, more head acupuncture points will be selected, and the role of acupuncture manipulation will be emphasized more. After the patient is prepared and their breathing has been stabilized, the area around the acupuncture point shall be routinely disinfected. As soon as the needle points positioned at Baihui (GV20), Sishencong (EX-HN1), and Shenting (GV24) reach the galea aponeurotica, a rapid twisting technique will be conducted with an amplitude of 180°–200°, 200 times per minute, for 3–5 min. At Yintang (GV24+) and Taiyang (EX-HN5), a small one-way twisting manipulation with small amplitude lifting and thrusting will be performed. A modest twisting method at Fengchi (GB20) will enable transmission of the needle sensation to the inner brain. Shuigou (GV26) with sparrow-pecking twisting will be implemented to get the patient to experience the moistness of the eye. Using Danzhong (LR3) with rapid, small-scale lifting and twisting, the sensation of the needle will be transferred from the local point to the chest. The needles at Neiguan (PC6), Shenmen (HT7), and Taichong (LR3) will be handled with small, one-way twists with lifting and inserting. The previously described points will be stimulated at a rate of approximately 60 times per minute until the corresponding sensation of

Table 1
Location and direction depth of the acupoints.

Acupoints	Location	Direction	Depth (cun)
GV20 (Baihui)	On the head, 5 cun straight up from the middle of the front hairline.	Anterior to posterior along the scalp. Horizontal insertion: 15° to the body surface	0.5-0.8 cun
GV24 (Shenting)	On the head, 0.5 cun straight up from the middle of the front hairline.	Anterior to posterior along the scalp. Horizontal insertion: 15° to the body surface	0.5-0.8 cun
GV24^{+} (Yintang)	On the head, in the depression between the medial ends of the two eyebrows.	From the upper end downward. Horizontal insertion: 15° to the body surface.	0.3-0.5 cun
GV26 (Shuigou)	On the face, at the intersection of the upper 1/3 and middle 1/3 of the human middle sulcus.	Needle tip diagonally upward. Oblique insertion: 45° to the body surface.	0.3-0.5 cun
EX-HN1 (Sishencong)	On the head, 1 cun in front, behind, left and right of GV20 (Baihui), a total of 4 points.	Anterior to posterior along the scalp. Horizontal insertion: 15° to the body surface.	0.5-0.8 cun
EX-HN5 (Taiyang)	On the head, between the tip of the eyebrow and the outer canthus of the eye, in the depression about one finger back (middle finger).	Oblique insertion: 45° to the body surface	0.3-0.5 cun
GB20 (Fengchi)	On the nape, under the occipital bone, in the depression between the upper end of the sternocleidomastoid muscle and the upper end of the trapezius muscle.	Towards the tip of the nose. Oblique insertion: 45° to the body surface	0.8-1.2 cun
CV17 (Danzhong)	On the front median line of the chest, the same level as the fourth intercostal space.	Immediately adjacent to the sternal stalk from above downward. Horizontal insertion: 15° to the body surface.	0.5-0.8 cun
PC6 (Neiguan)	On the anterior region of the forearm, 2 cun above the transverse stripe of the distal palmar aspect of the wrist, between the palmaris longus tendon and the radial carpal flexor tendon.	Perpendicular insertion: 90° to the body surface.	0.5-1.0 cun
HT7 (Shenmen)	On the medial wrist area, at the ulnar end of the distal palmar transverse wrist stripe. The radial margin of the ulnar carpal flexor tendon.	Perpendicular insertion: 90° to the body surface.	0.3-0.5 cun
LI4 (Hegu)	On the back of the hand, between the first and second metacarpal bones, about the midpoint of the radial aspect of the 2nd metacarpal.	Perpendicular insertion: 90° to the body surface.	0.5-1.0 cun
LR3 (Taichong)	On the dorsum of the foot, between the 1st and 2nd metatarsal bones, in the four sunken areas in front of the metatarsal plantar joint or touching the arterial pulsation.	Perpendicular insertion: 90° to the body surface.	0.5-1.0 cun

the needle is produced. The location of acupuncture points and the direction of needle depth are provided in Table 1.

2.12. Outcomes

All assessments will be conducted by researchers who are unaware of the assigned treatment. In addition to measures, socio-demographic information such as age, gender, educational background, and occupation will be included. Fig. 2 depicts the time points for data collection during the trial.

2.13. Primary outcome

The primary outcome will be the change in HDRS-24 scores between baseline, post-treatment and follow-up. The Hamilton Depression Rating Scale is the most commonly employed classical clinical depression symptom rating scale [33]. A total score of $\geq$35 points is regarded as severe depression, a total score between 26 and 34 points is considered moderate depression, between 20 and 26 points is considered moderate depression, and $\leq$8 points shows the absence of depressive symptoms.

2.14. Secondary outcomes

Secondary outcomes included scores on the Patient Health Questionnaire-9 (PHQ-9) [34] and World Health Organization Quality of Life Brief Version (WHOQOL-BREF) [35].

(1) PHQ-9: The Patient Health Questionnaire-9 is a depressive symptom self-rating scale for the rapid screening and assessment of depressive symptoms. Mild depression is indicated by a score between 5 and 9 points, moderate depression between 10 and 14 points, moderate to severe depression between 15 and 19 points, and severe depression between 20 and 27 points.
(2) WHOQOL-BREF: The World Health Organization Quality of Life Bref is a short version of the WHOQOL-100 scale that retains the scale's comprehensiveness and incorporates all variables linked to survival quality. Twenty-six items will be graded to determine the psychological status, physical changes, social relationships, and environment of the participants, and two items will measure the general quality of life condition of participants during the past two weeks.

2.15. Exploratory outcomes

In addition to evaluating the efficacy and safety of acupuncture, we will assess the oxidative stress response with acupuncture treatment by measuring serum levels of the oxidative product 8-iso-prostaglandin F2α (8-iso-PGF2α), the enzymatic antioxidant superoxide dismutase (SOD), and the nonenzymatic antioxidants uric acid (UA) and total bilirubin (TBIL).

2.16. Adverse events and safety monitoring

Throughout the treatment, the TESS and the Columbia Suicide Severity Rating Scale (C-SSRS) will be administered weekly to ensure safety [36,37]. When adverse events occur, corrective measures and prognostic treatment will be offered based on the severity of adverse events, adverse events will be recorded and treated within 24 h, and adverse reaction data will be used for an objective and realistic evaluation. In addition, research will be conducted to discover whether adverse events are related to therapy, the treatment with which they are associated, and whether treatment changes within the scope of the trial are beneficial. In case of adverse events that may occur during acupuncture treatment, such as dizziness, needle lag, nausea, hematoma formation, local infection, and other minor events, the patient may be allowed to continue with the early intervention trial only after consent has been obtained and influencing factors have been excluded. After excluding factors unrelated to treatment, researchers will assess if the early intervention trial can continue; if adverse events are serious and trial-related, patients will be withdrawn from the study and provided with appropriate medical care.

2.17. Data collection

In the follow-up phase, the HDRS-24, PHQ-9, and WHOQOL-BREF data required to assess treatment efficacy will be collected at baseline, weeks 1, 2, and 4 after treatment initiation, and months 1 and 3 at the end of treatment in the follow-up phase. The First Affiliated Hospital of Heilongjiang University of Chinese Medicine will collect venous blood to measure the levels of biological indicators of oxidative stress. After collecting venous blood, the upper serum layer will be collected and stored at −80 °C for testing after centrifugation at 3500 r/min. The 8-iso-PGF2α concentration will be measured using an enzyme-linked immunosorbent assay (ELISA), whereas the SOD concentration will be measured using the water-soluble tetrazol-1 (WST-1) assay. The levels in each sample will be measured using assay kits from Nanjing Jiancheng Bioengineering Institute and a BioTek Instruments ELX808 microplate reader. The levels of UA and TBIL will be measured using a Hitachi 7600 automatic biochemical analyzer. The detection of biomarkers will be conducted with the aid of the Clinical Laboratory of the First Affiliated Hospital of Heilongjiang University of Chinese Medicine, and the procedure will be carried out in accordance with the instructions. All blood samples will be discarded after use. To guarantee confidentiality, all data will be entered on a paper case report form (CRF), and participant information will be recorded anonymously. Corrections to the CRF shall be signed and dated by the person in charge. Two independent investigators who are unaware of the group

assignment will enter all the data into a password-protected, predesigned computerized data set. The second investigator will verify the accuracy of the entered data. Access to the final trial dataset will be confined to the research team and no third parties will be granted access.

2.18. Statistical analyses

Intention-to-treat (ITT) will be used for the complete follow-up analysis. All participants who received at least one intervention and for whom at least one outcome evaluation that matched the inclusion criteria was available will be included for the full analysis in the full analytic dataset (FAS). In the statistical analysis, missing variables will be substituted with missing data according to the ITT principle using the latest observation carried forward (LOCF) approach. Participants who comply with the trial protocol and finish the trial treatment will also be included in the complying per-protocol set (PPS) and their data will also be analyzed.

SPSS (IBM Corp, Version 25.0, New York, USA) will be utilized to analyze the data. The means and standard deviations of data with a normal distribution will be reported, whereas data with a non-normal distribution will be characterized using medians and inter-quartile ranges (25%–75%), along with confidence intervals of 95%. Qualitative data will be provided as percentages or proportions and compared using the Pearson's chi-squared test; cell theoretical frequencies that do not fulfill the Pearson's chi-squared test will be analyzed using the Fisher's exact test. Quantitative data will be compared within groups and those with a normal distribution will be analyzed using a paired *t*-test. The Wilcoxon signed-rank test will be utilized for non-normally distributed data. For between-group comparisons, normally distributed data with homogeneous variances will be analyzed using the two independent samples *t*-test; otherwise, the Mann-Whitney test will be utilized. Data from repeated measurements at multiple time points will be examined using repeated-measures analysis of variance. The Bonferroni test will be used for post hoc pairwise comparisons. Statistical tests will be performed using two-sided tests; 5% will be utilized as the significance level for two-tailed testing in statistical analysis. Independent data analysis practitioners who are blinded to the treatment assignment will conduct statistical analyses.

2.19. Quality control

To ensure the quality of the study, all investigators will get standardized training prior to the start of the trial. The training course will introduce investigators to the trial's specifics. To minimize patient dropouts, we will communicate with patients during the trial, give health information, and encourage patients to stick to therapy for as long as possible. We will enhance patient adherence and reduce dropout rates. All participants who withdraw from the trial will be required to provide an explanation, which will be recorded and analyzed.

Safety monitoring is the responsibility of the ethics committee of the First Affiliated Hospital of Heilongjiang University of Chinese Medicine. The ethics committee can inspect original documents, evaluate clinical trial records, and conduct interviews with assessors to ensure the study adheres to the protocol's guiding principles. During treatment, data and progress monitoring will be conducted every two weeks, and periodic reviews will be conducted every two months. The ethics committee is independent of the trial researchers and has no competing interests.

3. Discussion

Patients with mild depression exhibit fewer and less depressive symptoms. Typically, there are no outward manifestations of low mood or slow movement. Mild depression, however, can easily progress to major depression. Major depression has a significant impact on the patients' physical health, social functioning, and daily life, and is a condition with a high disease burden [38]. Clinicians and researchers must discover remedies to alleviate mild depression and prevent disease progression.

Acupuncture is frequently utilized in clinical practices in China. Acupuncture's effectiveness is largely determined by the selection and combination of acupuncture points and acupuncture procedures. Our study's acupuncture technique was meticulously designed based on the TCM theory and the opinion of experienced acupuncturists. Mild depression is directly associated with the dysfunction of the brain spirit and heart spirit based on acupuncture and TCM theories. The governor channel is an important channel that regulates the brain spirit and heart spirit. Therefore, the majority of our acupuncture point prescription consisted of the head points in the governor channel, including GV20, GV24, GV24^{+} and GV26. Although EX-HN1 is an extra meridian point, both the front and back EX-HN1 are located on the governor's channel's running course. EX-HN5 and GB20 are vital locations for regulating the head's qi and blood. PC6 and HT7 are associated with the hand reverting yin pericardium channel and the hand yin heart channel, respectively. Activating the brain spirit and regulating the heart spirit, the two points complement one another. The CV17 not only relaxes the chest and controls qi, but it also regulates and calms the tranquilizing spirit. The therapeutic benefits of LR3 include dispersing liver and regulating qi.

Moreover, acupuncture manipulation is essential for obtaining qi. To obtain qi in the "Tiaoshen" acupuncture group, we shall apply a specific and unique acupuncture technique. For instance, when acupuncture points on the head are stimulated with high-frequency twirling techniques to transmit the needling sensation to the diseased area, the primal spirit is regulated and acupuncture's unique benefits can be observed. After rapid skin-piercing needle insertion, the acupuncture sites on the limbs will be manipulated with a small unidirectional twirling motion, allowing the patient to obtain qi for a longer duration, hence enhancing the therapeutic effect. We will set the acupuncture intervention time to 50 min, which allows for many manipulations of the needle to activate the acupuncture points and provide the desired result. Our earlier experiments have verified that lengthy needle retention time improves physical symptoms and sleep disorder factors, which are readily apparent in individuals with mild depression [17,39].

As antidepressants are inappropriate for patients with mild depression, we did not select antidepressants as positive controls in this trial's control group. Our study did not include a sham acupuncture control group for two other reasons. Due to China's national conditions, most patients have acupuncture treatment experience, making it impossible to adopt a blinding method. On the other hand, there is no unified standard for sham acupuncture, and it is difficult to achieve complete obliviousness. However, it is difficult to control the positive effects of patients' expectations of acupuncture and moxibustion on the research results because the trials are conducted in TCM hospitals, and it is difficult to separate the nonspecific effects of acupuncture and moxibustion. In the clinical practice guidelines for acupuncture for depression, we chose conventional acupuncture as the control intervention due to the aforementioned factors. In China, conventional acupuncture methods are also extensively used to treat patients with depression. Several studies have demonstrated that it can effectively improve the symptoms of patients, which lends credence and clinical value [40]. Therefore, conventional acupuncture is a reasonable control intervention that is consistent with ethical standards and current clinical practice, and it is more conducive to protecting patients' rights, avoiding a treatment delay as much as possible due to study design bias, and maximizing the therapeutic effect of acupuncture on mild depression in clinical practice.

Depression is related to multiple factors in its pathogenesis, and acupuncture treatment for depression is also the result of the combined action of multiple targets. In recent years, research has found that the pathophysiology of depression may involve multiple interrelated biochemical pathways, including oxidative stress [41]. Based on the analysis of clinical scale scores, peripheral blood markers related to oxidative stress such as 8-isoprostaglandin F2α (8-iso-PGF2α), superoxide dismutase (SOD), uric acid (UA), and total bilirubin (Tbil) were detected to observe the occurrence of oxidative stress. Furthermore, changes in oxidative stress reaction markers in the serum of patients with mild depression under acupuncture intervention were observed to analyze the potential mechanism of acupuncture treatment for depression.

Most of the previous trials did not report adverse events, and there is a lack of reliable evidence about the safety of acupuncture. More rigorous, high-quality randomized controlled trials are still required to confirm the efficacy and safety of acupuncture [42]. The results of a meta-analysis recommend that, when applicable, studies should employ suitable blinding procedures and include quality of life assessments, treatment acceptability evaluations, and medium- and long-term follow-up [43]. Therefore, in this trial, we will strictly adhere to the CONSORT statement and STRICTA recommendations, standardize the study protocol, evaluate adverse events using the TSEE scale with reference to a meta-analysis, and measure the quality of life of participants using WHOQOL-BREF. To assess the treatment's long-term efficacy, a three-month-long follow-up survey will be undertaken after its completion. In addition, we want to measure the levels of oxidative stress indicators to examine the potential mechanism of acupuncture in the treatment of mild depression.

Our experiment minimizes any potential biases that could influence the results of the study, and it addresses some of the issues with the previous experiments. However, certain restrictions should be noted. First, it is a single-center study that does not differentiate between regional differences. Second, the diagnosis and evaluation of depression remains subjective, and there are inadequate objective measurement indicators, which may lead to bias in participant inclusion and outcome evaluations. Due to the unique nature of the treatment, the acupuncturist cannot be blinded to the groups, which could influence the study's findings. We are currently working to standardize this trial's procedure.

In conclusion, it is expected that the results of this study would indicate that tone-and-spirit acupuncture efficiently improves the symptoms of mild depression, enhancing quality of life, and is safe. The evaluation of relevant plasma indicators to determine whether the mechanism underlying the effect of acupuncture is to reduce the oxidative stress response will have a positive effect on the treatment of mild depression. This is the first randomized controlled trial to assess the effects of acupuncture on oxidative stress in patients with mild depression, to our knowledge. Despite its limitations, this study aims to investigate the efficacy and safety of acupuncture for mild depression and provide high-quality, intuitive medical evidence for the design of larger randomized controlled trials in the future.

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Ethical statement

The trial will be conducted in accordance with the Declaration of Helsinki and has been approved by the Ethics Committee of the First Affiliated Hospital of Heilongjiang University of Chinese Medicine (HZYLLKY202205001). Informed consent will be obtained from all participants prior to starting the trial.

4. Clinical registration details

URL: https://www.chictr.org.cn/showproj.html?proj=174216.

CRediT authorship contribution statement

Lu Bai: Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Wei Zou:** Writing – original draft, Funding acquisition, Formal analysis, Data curation, Conceptualization, Writing – review & editing. **Long Wang:** Methodology, Data curation. **Xueping Yu:** Resources, Methodology, Investigation. **Hongjun Lou:** Supervision, Software, Formal analysis. **Xiaohong Dai:** Supervision, Investigation, Data curation. **Wei Teng:** Validation, Formal analysis. **Weiwei Yu:** Validation, Investigation. **Mingyue Li:** Visualization, Methodology. **Hongtao Cao:** Methodology, Formal analysis. **Lei Zheng:** Software, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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