

Traditional Chinese medicine Lingguizhugan decoction treating non-alcoholic fatty liver disease with spleen-yang deficiency pattern: Study protocol for a multicenter randomized controlled trial

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Study protocol

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Abstract

Background Nonalcoholic fatty liver disease (NAFLD) is a metabolic stress liver injury induced by excessive fat accumulation in liver, which is closely related to insulin resistance (IR). Traditional Chinese medicine (TCM) has showed good curative effects and unique advantages in the prevention and treatment of NADLD, particularly in improving IR, liver function and clinical symptoms. This study will examine the efficacy and safety of Lingguizhugan decoction (LGZG) in the treatment of non-alcoholic fatty liver disease with spleen-yang deficiency pattern, and assess the optimal dosage.

Methods/design This study is a three-arm, dose-optimization, randomized, double-blinded, placebo-controlled clinical trial. A total of 243 NAFLD patients will be recruited and randomly assigned to standard dose Lingguizhugan decoction (SLGD) group, low dose Lingguizhugan decoction (LLGD) group, or the control group. The clinical indicator will be assessed during the 12-week intervention and follow-up 4 weeks. The primary outcome is the percentage of patients whose the decline of HOMA-IR reaches one standard unit. Secondary outcomes include body weight, body mass index, liver function, blood lipid metabolism, blood glucose metabolism, liver-kidney echo ratio, quantitative rating of clinical symptoms and signs, collecting sample(s) from participants, the medical outcomes study(MOS) item short from health survey(SF-36), Self-rating Depressive Scale(SAS), and Self-rating Anxiety Scale(SDS).

Discussion This study will provide initial evidence regarding the efficacy and safety of LGZG in the treatment of NAFLD with spleen-yang deficiency pattern, meanwhile establish the demonstration technique, and promote its application in community healthcare center. In addition, potential mechanism will be explored based on research of oral and gut microbiota.

Background

Non-alcoholic fatty liver disease (NAFLD) is one of the chronic liver diseases characterized by abnormal lipid accumulation in liver [1-3]. According to recent epidemiological studies, the prevalence of NAFLD is 25.24% worldwide and 27.37% in Asia-Pacific region [4], while the prevalence in China ranges from 24.47% to 29.70% [5]. Although NAFLD shows no obvious clinical manifestations in the early stage, it is an important risk factor for various cardiovascular diseases, type 2 diabetes mellitus and other metabolic diseases [6-8]. It has been reported that the all-cause mortality risk was also significantly elevated in NAFLD population [9, 10].

The pathogenesis mechanism of NAFLD is still vague except insulin resistance (IR) and genetic predisposition are relatively confirmed [11-13]. Generally based on disease progression, NAFLD could be divided into three stages, beginning from non-alcoholic fatty liver (NAFL), then to non-alcoholic steatohepatitis (NASH), and finally liver cirrhosis, which is a much greater risk factor for primary liver cancer [1, 14]. Therefore, how to prevent disease progression from potentially reversible NAFLD and NASH to irreversible cirrhosis is the key points for the treatment [15]. Currently, there is still no confirmed

interventions for NAFLD [16]. In view of this fact, many patients would seek medical help from Traditional Chinese Medicine (TCM) in China.

Based on TCM theory, NAFLD belongs to the category of "Gan Pi", and "phlegm dampness" and "phlegm fluid retention" are its pathological products. The basic pathogenesis of NAFLD is transportation dysfunction of spleen due to deficiency of spleen yang [17, 18]. The corresponding TCM pattern is spleen-yang deficiency. Main clinical manifestations contain laziness to speak, poor appetite, loose stool, cold limbs and so on. The basic treatment principle is warming yang to promote diuresis [19, 20].

Lingguizhugan decoction (LGZG), firstly documented in ancient classic "Jingui Yaolue", is a representative formula under the principle of warming yang to promote diuresis. It is composed of *Poria* (Fulin), *Ramulus Cinnamomi* (Guizhi), *Rhizoma Atractylodis Macrocephalae* (Baizhu), and *Radix Glycyrrhizae* (Gancao) [21]. Previous researches showed that LGZG could significantly decrease triglycerides (TG) and lipid contents of the liver tissue, and improve hepatic injury in high fat diet-induced NAFLD rats [20, 22]. LGZG was also reported to be capable of alleviating IR in diabetes patients [23]. However, there is still no high-level evidence to support LGZG application in treatment of NAFLD. In addition, the dose of LGZG is still following the classic. Dose optimization will also be clinically significant due to the variation of environment and lifestyle.

Moreover, with the development of intestinal microbiota, many researches have verified the role of intestinal microbiota in the pathogenesis of NAFLD. Regulation of intestinal flora could increase appetite and energy intake in food, regulate gene expression regarding fat synthesis and beta oxidation, and improve insulin resistance [24-26]. These mechanisms may be involved in the occurrence of NAFLD. Other studies also reported that most gut microbiota origin from oral cavity, then speculated that oral microbiota are also correlated to NAFLD [27, 28]. TCM may play an important role in treating diseases through regulating microbiota. Therefore, it will also be valuable to explore the potential mechanisms of LGZG against NAFLD from the researches on oral and intestinal microbiota.

Hence, we designed this study to evaluate the efficacy and safety of LGZG for NAFLD with spleen-yang deficiency pattern, and to assess the optimal dosage. The results of this trial may help to establish the evidence base for LGZG application in community healthcare centers. In addition, the researches of oral and gut microbiota will benefit the exploration of potential mechanisms.

Methods/design

Study design

The study protocol was approved by the Medical ethics committee of Longhua Hospital Affiliated to Shanghai University of Traditional Chinese Medicine (2017LCSY069) and registered in Chinese Clinical Trial Registry (<http://www.chictr.org.cn/edit.aspx?pid=24555&htm=4>) with number ChiCTR1800014364 on 01 January 2018.

This study will be a 16-week, randomized, multi-center, double-blinded, three-arm, dose-optimization, placebo-controlled clinical trial. A total of 243 NAFLD patients will be recruited through recruitment advertisements and doctors' introduction from the following three community healthcare centers in Shanghai, namely Zhangjiang community healthcare center, Beicai community healthcare center, Sanlin community healthcare center. All three centers are based on Pudong New Area, Shanghai and no obvious difference will be supposed to exist in population and socioeconomic aspects. Eligible NAFLD patients who agree to participate in the study will be randomly assigned to standard dose Lingguizhugan decoction (SLGD) group, low dose Lingguizhugan decoction (LLGD) group, or the control group according to ratio of 1:1:1. On the basis of behavioral intervention therapy, patients in the SLGD and LLGD groups will be treated with different doses of LGZG, and the control group will receive placebo. LLGD group will be made of half of the standard dose, and the placebo will be one-tenth of the standard dose but with soluble starch, mixed colorant and bitter principle to achieve comparable appearance, smell and taste. The course of treatment will last 12 weeks. A 4-week follow-up will also be arranged to assess the continued effect. Participants will be assessed at week 0 (baseline), week 4, week 8, week 12 (end of treatment), week 16 (end of follow-up). All participants will be asked to provide written informed consent before entering the trial. The study flow chart is shown in Fig. 1, and participant timeline is presented in Table 1. The Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) Checklist is presented in Additional file 1.

Participants

Diagnostic criteria

All participants should meet the diagnostic criteria of NAFLD and TCM pattern differentiation criteria of spleen-yang deficiency. Diagnostic criteria of NAFLD will be based on the American Association for the Study of Liver Diseases (AASLD) in 2017 [12]. To be specific, four following criteria should be all met: (a) Imaging (ultrasound, computed tomography or magnetic resonance imaging) or histological evidence of hepatic steatosis; (b) No significant alcohol consumption, defined as >21 standard drinks per week in men and >14 standard drinks per week in women; (c) Exclusion of other reasons inducing hepatic steatosis, including but not limited to hepatitis C, specific drugs induced, parenteral nutrition and severe malnutrition; (d) No coexistence of other chronic liver disease, including but not limited to hemochromatosis, autoimmune liver disease, chronic viral hepatitis, alpha-1 antitrypsin deficiency, hepatolenticular degeneration and drug-induced liver disease.

The TCM pattern differentiation criteria of spleen-yang deficiency will refer to the expert consensus of spleen deficiency by China Association of Traditional Chinese Medicine in 2017, previous systematic review, and the clinical guidelines for the new Chinese medicine [29-31]. Ten symptoms and signs will be assessed by continuous 100-point scale, Higher score means severer degree. Every symptom or sign possesses with certain weight. The scoring will be calculated through multiplying the rating score by the weight. Spleen-yang deficiency pattern will be defined as total score of ten dimensions equal or greater than twenty. The detailed rating scale is shown in Table 2. *Inclusion criteria*

Participants who meet all of the following criteria will be included: (a) aged between 18 and 80 years, males or females; (b) confirmed diagnosis of NAFLD; (c) confirmed diagnosis of spleen-yang deficiency pattern; (d) voluntary informed content and agreement of participating in every visit, examinations and treatment according to the protocol.

Exclusion criteria

Participants who meet any of the following criteria will be excluded: (a) combination with other specific liver diseases which would induce fatty liver, including but not limited to alcoholic liver disease, chronic hepatitis C, autoimmune liver disease, and hepatocellular degeneration; (b) fatty liver induced by drugs (e.g. tamoxifen, ethylamine iodifurone, valproate, methotrexate, glucocorticoid), total parenteral nutrition, inflammatory bowel disease, hypothyroidism, Cushing syndrome, abetalipoproteinemia, and other syndromes related to insulin-resistance (e.g. lipid atrophic diabetes, Mauriac syndrome); (c) combination with serious primary diseases and mental diseases, including but not limited to cardiovascular and cerebrovascular diseases, hepatic diseases, renal diseases, hematologic diseases, cancers, and schizophrenia; (d) combination with diabetes or currently receiving anti-diabetic medicine treatment; (e) currently receiving treatments for NAFLD (including Chinese herbal decoction, Chinese patent medicine, and chemical agents); (f) antibiotics administration in recent one month; (g) allergy to compositions of experimental agents, or possessing with allergic constitution; (h) pregnancy and lactation women, and women who are likely to be pregnant but refuse to keep predefined contraception measures during the study; (i) participation in other clinical trials in recent three months or currently joining other trials; (j) mental or legal disability; (k) cannot obey medical advice for therapeutic lifestyle modifications; (l) suspicious of drug abuse or possessing other forbidden criteria.

Interventions

All clinical investigators responsible for diagnosis and treatment will be registered TCM practitioners. Before the start of the trial, a 3-day training session will be held for all clinical investigators involved in the study. All participants with NAFLD will receive treatment for 12 weeks according to the random assignment. LGZG has been utilized for a long history in clinical practice. Participation in this trial will not anticipate to bring any harms. Hence, no post-trial care will be arranged.

Behavioral intervention

Behavioral interventions will be given to all three groups and the prescription will be based on the Guidelines for Prevention and Control of Overweight and Obesity in Chinese Adults [32]. In brief, participants will be required to limit calorie intake (approximate 1660 kcal per day) and guarantee physical exercises (moderate aerobic exercise at least 150 minutes per week). Subjects will be informed to complete daily dietary and exercise records during treatment (additional file 2).

Drug intervention

LGZG is a traditional formula which is composed of *Poria* (Fulin), *Ramulus Cinnamomi* (Guizhi), *Rhizoma Atractylodis Macrocephalae* (Baizhu), and *Radix Glycyrrhizae* (Gancao). The standard dose for each herb is 12g, 9g, 6g, and 6g, respectively. LLGD group will be made of half of the standard dose, and the placebo will be one-tenth of the standard dose. The course of treatment last 12 weeks. Participants will be required to take one dosage (one pack) of granules 30 minutes after breakfast, once daily on weekdays. A total of 60 packs per patient will be administrated. The granules used in this study will be produced by Sichuan neo-green pharmaceutical technology development co., LTD (Sichuan, China). The crude herbs will be extracted and made into granules. The production process will be under the standard of Good Manufacturing Practice. No significant difference was found among granules samples from three groups in color, appearance, shape, smell and weight (Fig. 2).

Concomitant treatment regulations

Except for research products, other drugs (including Chinese herbal decoction, Chinese patent drug and Chemical Drugs) and interventions (including acupuncture, cupping and massage) which may interfere effect will be forbidden during the study. For participants with other diseases combined who cannot stop relevant therapies, they should record concomitant interventions in details. If the disease progresses during the study period, patients could withdraw from the study and use other treatment methods. The case will be regarded as an invalid case, and the patient will be required to complete the relevant examinations and evaluations as much as possible.

Drug dispensation and compliance assessment

Drugs will be packed as required in double-blinded study. During the treatment period, a certain amount of packs will be distributed in week 0, week 4 and week 8 (22 packs). The additional two packs will be the reservation for unforeseen circumstances. After taking the medicine, the patients will be informed to return all the remaining products and packages at next visit. The clinical investigators will count the number of remaining products and calculate the compliance. The research team will establish a WeChat group for health guidance and periodically contact subjects by telephone or WeChat to check safety and improve compliance.

Outcome measures

Schedule for all outcome measures is shown in table 1. All demographic data will be determined before treatment, including the date of birth, gender, body weight, height, medication history, past medical history, smoking and drinking status and course of disease.

The primary outcome will be the proportion of participants with at least one-unit decrease of HOMA-IR from baseline to treatment endpoint (week 12) [33]. Secondary outcomes will include body measurements (body weight and body mass index), hepatic function (alanine aminotransferase (ALT), aspartate transaminase (AST), gamma-glutamyl transpeptidase (GGT), and alkaline phosphatase (ALP)), lipid metabolism profiles (total cholesterol (TC), TG, low density lipoprotein cholesterol (LDL-C), high

density lipoprotein cholesterol (HDL-C), apolipoprotein A (apoA), apolipoprotein B (apoB) and non-high density lipoprotein cholesterol (non-HDL-C)), glucose metabolism profiles (fasting blood glucose (FBG), fasting insulin and glycosylated hemoglobin (HbA1c)), inflammatory responses (blood cell count (WBC), C-reactive protein (CRP) and inflammatory cytokines), liver-kidney echo ratio by ultrasound, and scorings of various questionnaires (TCM spleen-yang pattern scale, the MOS item short form health survey (SF-36), self-rating depressive scale (SDS) and self-rating anxiety scale (SAS)). All secondary outcomes will also be evaluated from baseline to treatment endpoint.

Safety variables will contain vital signs (including blood pressure, respiratory, heart rate and body temperature) evaluated in every visit, electrocardiogram (ECG) and blood tests (including blood routine test and renal function) evaluated at baseline, week 4 and week 12. Adverse events (AEs) will be observed and recorded throughout the whole trial. LGZG is a classical herbal formula and possesses with satisfactory safety profiles. Potential AEs may be mild gastrointestinal reactions, which will be alleviated spontaneously without any additional medical interventions. Clinical investigators should document any AEs in case report form (CRF), containing following information: occurrence time, symptoms and signs, severity, duration, laboratory examinations, interventions, outcomes and follow-up time. If an AE occurs, clinical investigators should adopt appropriate measures to guarantee the safety of participants based on the actual situation. If serious AEs happen, clinical investigators should inform principal investigator in two hours. Then principal investigator should report to medical ethics committee and data monitoring committee within 24 hours. Whether termination of the trial will be decided after discussion.

Biological sample collection

In order to further explore the mechanism of LGZG on NAFLD and its effect on oral and gut microbiota, biological samples will be collected in this study. On the consent form, the collection of biological samples will be clearly stated. And the participants will have the right to refuse providing biological samples. All biological specimens will be used for auxiliary researches in the future. Blood samples will be planned for subsequent DNA methylation and metabolomics studies. Feces and coating on the tongue will be planned for gut and oral microbiota studies. Whole blood, morning urine, feces and coating on the tongue will be collected at baseline, 4 weeks, 12 weeks and 16 weeks. All samples will be transferred and stored in a 80°C refrigerator within 24 hours.

Sample size estimation

Sample size was estimated based on the clinical primary outcome. As far as we know, this is the first RCT evaluating the efficacy of LGZG versus placebo. No data is available for reference. So, based on our previous animal studies and consultation with TCM experts, we assumed that the effect size for SLGD group is 30%, LLGD group is 15%, and placebo group is 10%. If $\alpha = 0.05$, $\beta = 0.10$, the sample size can be calculated by the following formula:

[Please see the supplementary files section to access the equation.]

The λ is the degree of freedom, taking 12.65 in this case. The p_{\max} is 30% and the p_{\min} is 10%. Assuming the maximum dropout rate is 20%, 81 patients will be required for each group, with a total of 243 patients.

Randomization and blinding

All participants will be randomly allocated to SLGD group, LLGD group, or the control group based on the ratio of 1:1:1. The random number table will be generated by SPSS 19.0 for Windows software. A research assistant who will not participate in this clinical trial will send the random number table to the pharmaceutical company for packaging to maintain the blinding of participants and clinical investigators. According to the order of enrollment, the clinical investigators will assign the number from small to large, and the pharmacists will distribute the corresponding drug. Participants, clinical investigators and statisticians will all be blinded until the completion of whole trial. The randomization list and blinding codes will be kept strictly confidential.

Based on the requirements of medical ethics, a double-blind study should set up an emergency letter for each blind number. Emergency letters will be sealed in opaque envelopes. The contents of the letter will contain group details and emergency situations. Emergency letters should be sealed and could only be opened when necessary. The emergency letter will be read by clinical investigators in cases of severe AEs, in which knowing the experimental treatment is compulsory for further medical interventions. The case will be treated as withdrawal and the clinical investigator will record the corresponding reason in the CRF. All emergency letters will be retrieved together with CRF after the study. Twice unblinding will be used in this trial. Briefly speaking, first unblinding will uncover the codes of treatments, namely A, B and C. After the completion of statistical analysis, the second unblinding will uncover the specific drugs corresponding to the cords.

Data collection and management

The trial steering committee will be composed of the principal investigator (GJ) and coordinators from three centers. The committee will be responsible for revising study design, reviewing overall progress and communicating with medical ethics committee per six months. An independent data monitoring committee (DMC) will also be established by the trial steering committee. The DMC will include clinical epidemiologists, data monitors and statisticians. No competing interest will exist. The DMC will review the efficacy and safety data according to the participants' schedule, and provide suggestions to the trial steering committee on revising study design or terminating the trial.

Clinical investigators will be responsible for the data collection during this study according to the schedule. CRFs should be filled in after every visit to guarantee the accuracy and timeliness. Data monitors from DMC will review the CRFs per month. When a participant finishes all treatments and follow-up, the completed CRF will be verified by the coordinator. During the research process, the trial steering committee will also assign research assistants every month from Shanghai University of Traditional Chinese Medicine to solve potential problems encountered during the trial and guarantee the quality of the study execution.

In order to improve the compliance of patients to the intervention, free tests and free medical education will be provided. On the consent form, participants will be informed that they could withdraw the trial at any time, and the withdrawal decision will not interfere the regular medical care. Participants will also be noticed that their personal information will be de-identified throughout the trial to guarantee the confidentiality. For participants who discontinue or deviate from intervention protocols, the researcher should contact the subjects, ask the reasons, record the last time of taking medicine, and complete the evaluation items as much as possible.

Statistical analysis

The analysis of efficacy and safety will be based on the principle of intention-to-treat (ITT) principle, namely all randomized participants will enter the final analysis. The missing value will be filled by the method of the last observation carried forward. Data will be presented as mean with standard deviation, median with range or number with percentage. Data analysis will use the SPSS 19.0 for Window software. All statistical inferences will be performed using a two-sided test with a statistically significant test level of $P < 0.05$, and the confidence interval of parameters will be estimated by 95% confidence interval. Difference with groups will be assessed by paired t-test, chi-square test or Wilcoxon signed rank test according to specific variables. Difference among groups will be assessed by one-way analysis of variance or Kruskal-Wallis test based on characteristics of data. Post-hoc pairwise multiple comparisons will be performed if the statistical significance is found.

No interim analysis was arranged in this trial due to short intervention period and safety profiles of LGZG. Potential sub-group analyses will be determined based on characteristics of included participants, for instance, different categories of body mass index and different combined diseases.

Publication and dissemination

Regardless of the efficacy of drugs, the results will be disseminated through publications. The future report of this clinical trial will follow the requirements of CONSORT statement [34] and Chinese herbal medicine Formulas extension [35]. Authorship for the final report will be determined based on the contributions to the trial. We will not intend to use professional writers.

Discussion

NAFLD is a widely health problem, and more than 80% of obese people suffer from NAFLD globally [36]. A large population-based study has demonstrated that NAFLD is one of the important causes of hepatocellular carcinoma (HCC), and the incidence of NAFLD related HCC is increasing by approximate 10% per year [37]. However, the pathogenesis mechanism of NAFLD is vague [38]. There is still no drug currently approved for the treatment of NAFLD by the US Food and Drug Administration (FDA) or the European Medicines Evaluation Agency (EMA) [10, 39]. Hence, many NAFLD patients will seek help from TCM interventions, especially Chinese herbal medicine.

Treatment based on pattern differentiation is the character of understanding and treating disease by TCM. Therefore, we introduce pattern differentiation as one of inclusion criteria for NAFLD participants, namely spleen-yang deficiency. LGZG is the corresponding formula for this pattern. Previous animal researches also indicated the potential efficacy of LGZG against NAFLD [20, 22]. Then, a clinical trial will be necessary to demonstrate the application. To our knowledge, our trial is the first multi-center, randomized, double-blinded, placebo-controlled clinical trial to assess the efficacy and safety of LGZG for NAFLD.

To ensure the quality of this research, a strict system of training and supervision will be established. Training sessions will be held for each center to explain the study protocol and potential upcoming matters. How to confirm the TCM pattern of spleen-yang deficiency and how to collect biological samples will also be explained in training sessions. The biochemical measurements of each participating center will be tested uniformly by KingMed Diagnostics (Shanghai) to guarantee the consistency. The ECG and liver-kidney echo ratio by ultrasound will be examined in the corresponding experimental center with uniform standard.

The results of this trial will provide initial evidence regarding the efficacy and safety of LGZG in treatment of NAFLD with spleen-yang deficiency pattern. Biological samples collected in this trial may explain the possible action mechanisms of LGZG associated with oral and gut microbiota.

Trial status

We recruited participants from July 2018 and completed in February 2019. All clinical data have been locked in June 2019. This protocol was submitted before completion of recruitment.

Abbreviations

AASLD: American Association for the Study of Liver Diseases; AEs: Adverse events; ALP: alkaline phosphatase; ALT: Alanine aminotransferase; apoA: apolipoprotein A; apoB: apolipoprotein B; AST: Glutamic pyruvic transaminase; CRF: Case report form; CRP: C- reactive protein; ECG: Electrocardiogram; EMEA: European Medicines uation Agency; FDA: Food and Drug Administration; FPG: fasting blood glucose; GGT: gamma-glutamyl transpeptidase; HbA1c: fasting insulin and glycosylated hemoglobin; HCC: hepatocellular carcinoma; HDL-C: high density lipoprotein cholesterol; IR: insulin resistance; ITT: intention-to-treat; LDL-C: low density lipoprotein cholesterol; LGZG: Lingguizhugan decoction; LLGD: low dose Lingguizhugan decoction; MOS: the medical outcomes study; NAFL: non-alcoholic fatty liver; NAFLD: Nonalcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; non-HDL-C: non-high density lipoprotein cholesterol; SAS: Self-rating Depressive Scale; SDS: Self-rating Anxiety Scale; SF-36: MOS item short form health survey; SLGD: standard dose Lingguizhugan decoction; SPIRIT: Recommendations for Interventional Trials; TC: total cholesterol; TG: triglycerides; TCM: Traditional Chinese medicine; WBC: white blood cell count.

Declarations

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Availability of data and materials

Because of no data sets were analyzed or generated during the present study, data sharing does not apply to this article. The data generated in this clinical trial will be available for non-commercial scientific researches after completing all data analysis and de-identification. Application should contain the relevant research aim and methods, and be sent to the trial steering committee. The data sharing decision will be made after discussion.

Authors contributions

JJX initiated the study protocol. RRW contributed to the development and refinement of the study protocol. SFY and JJX prepared the data management and statistical analysis plan. GJ, PYZ, LZ and BCL provided critical advice and made substantial contributions to the conception and design of the study and subsequent protocol revisions. JJX drafted the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The study protocol was approved by the Medical ethics committee of Longhua Hospital Affiliated to Shanghai University of Traditional Chinese Medicine (ref approval no. 2017LCSY069) and we will not begin recruiting until obtaining local ethical approval. Before the implementation of the study, the study protocol was modified three times, and approved by the ethics committee respectively. The final protocol version was V3.0. We will guarantee that this study is conducted in the principles of the Declaration of Helsinki and the principles of the Quality Control of Clinical Trial of Drugs in China. All participants need to sign the informed consent before enrollment, and will be informed about the purpose, probable risks and benefits, arrangement, the rights and obligations, and can quit the study at any time. Signed informed consent from patient willing to participate in the trial will be collected by clinical investigators. The Informed consent form will be available from the corresponding author on request.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Tables

Table 1. Participant timeline

	Study phase					
	Screening	Allocation	Intervention			Follow-up
Time point (weeks)	-2	0	4	8	12	16
Enrollment						
Eligibility	X					
Informed consent	X					
Allocation		X				
Intervention						
SLGD			X	X	X	
LLGG			X	X	X	
Placebo			X	X	X	
Assessment						
Demographic indexes	X	X				
HOMA-IR		X	X		X	X
Hepatic function		X	X		X	X
Lipid metabolism		X	X		X	X
Glucose metabolism		X	X		X	X
Inflammation response		X	X		X	X
Liver-kidney echo ratio		X			X	
Body measurements		X	X	X	X	X
TCM pattern scale		X	X	X	X	X
SF-36		X			X	X
SAS		X			X	X
SDS		X			X	X
Vital signs		X	X	X	X	X
Blood routine test		X	X		X	X
Electrocardiograph		X	X		X	
AEs		X	X	X	X	X

Biological sample collection		X	X		X	X
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Table 2: Spleen-yang deficiency pattern scale

Symptoms	Score (0-100)
Do you feel lazy to speak?	20%
Do you sweat often or have night sweats even though you are not active and it is not hot?	5%
Do you have bland and tasteless feeling in your mouth?	10%
Does your stool thin texture and loose?	20%
Do you feel more saliva?	10%
Do you have bleeding gums?	5%
Do you feel lack of warmth in the extremities	10%
Do you have insomnia, have trouble sleeping at night	5%
Are you likely to catch cold?	10%
Do these symptoms affect your eating habits (such as hate greasy good)?	5%
Total	

Figures

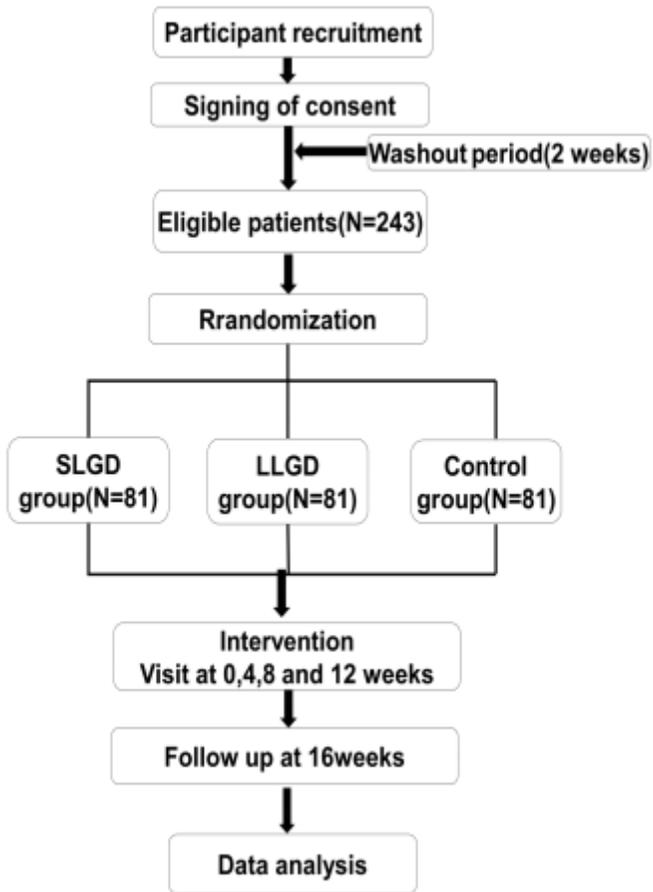


Figure 1

Flow chart of the clinical study

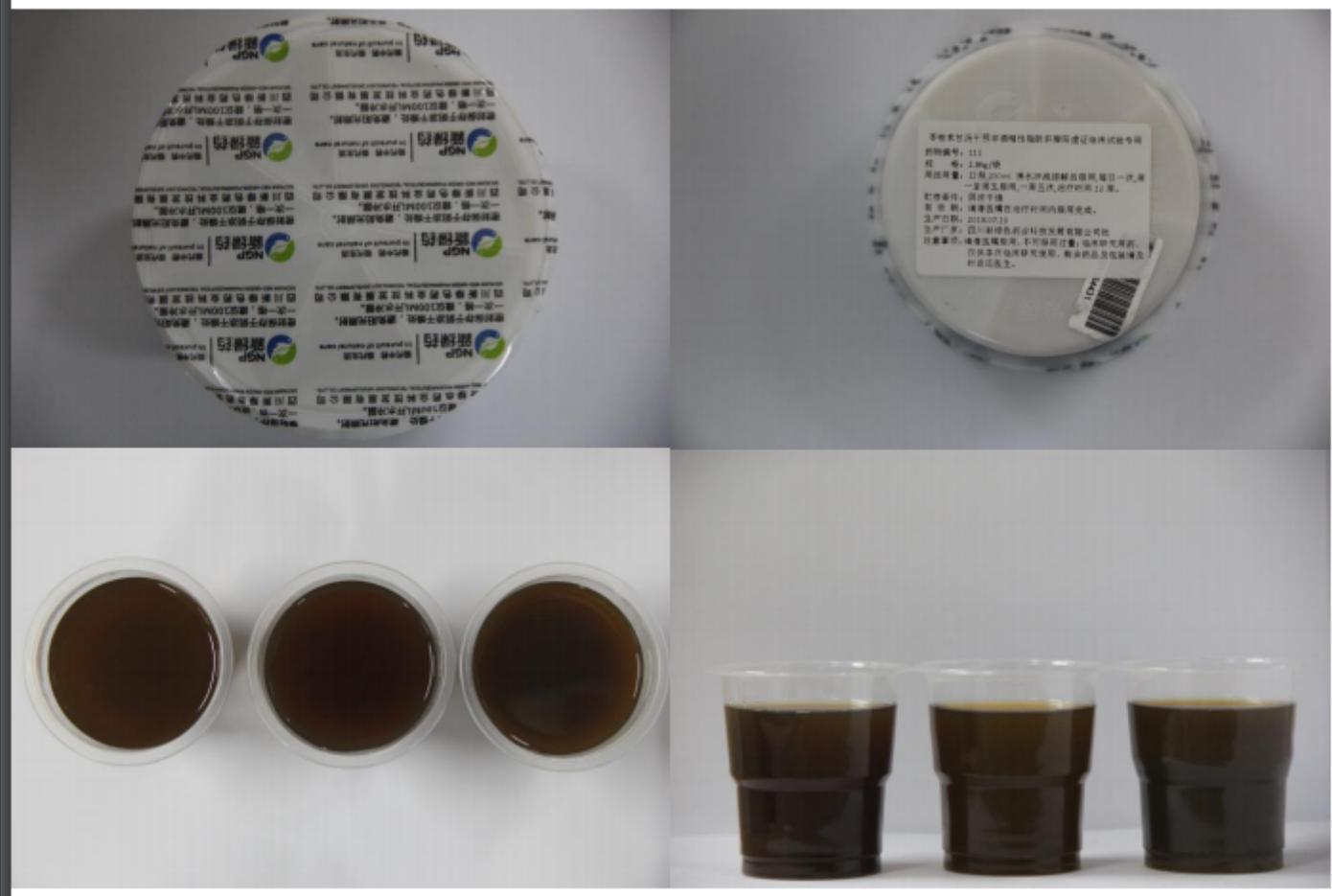


Figure 2

Appearance of drug samples from three groups

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