Real-world effects of Liuwei Dihuang pill for menopausal syndrome□study protocol for a prospective,observational,multicenter cohort

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Abstract

Background: Liuwei Dihuang (LWDH) pill, an adaptation of Chinese herbal medicine, is commonly employed to alleviate menopausal symptoms. Experimental and randomized controlled trials indicated that LWDH pill was advantageous for patients with menopausal syndrome (MPS). However, the pertinent scientific evidence of LWDH in the real clinical practice remains insufficient.To systematically evaluate the effectiveness and safety of LWDH pill in the treatment of MPS under real-world clinical conditions, thereby providing high-quality evidence for rational clinical use.

Objective: To systematically evaluate the effectiveness and safety of LWDH pill in the treatment of MPS under real-world clinical conditions, thereby providing high-quality evidence for rational clinical use.

Methods: This prospective, multicenter, real-world, observational cohort study will be performed at six designated centers in China from March 9,2025 to Septemper 30,2025. A total of 1000 patients with menopausal syndrome will be allocated to either the exposure group (patients receiving LWDH treatment) or the non-exposed group (patients not receiving LWDH treatment), mostly based on patient desire in a genuine clinical setting. The main observation indicators will be changes in the modified kupperman scale and sex hormone levels.The secondary outcomes encompass alterations in the Menopausal quality of life scale, improvement in TCM syndrome scale, and variations in blood glucose and lipid levels.In addition, the multi-omics integrated analysis technique is employed to explore the potential mechanisms of action. Adverse events or clinically significant abnormalities in vital signs and laboratory parameters, irrespective of severity, will be documents during the trial to evaluated the safety of LWDH. Assessments will be performed at baseline, 2 weeks and 4 weeks after enrollment,with a subsequent 2-week follow-up. Data will be analyzed using a combination of artificial intelligence and traditional statistical methods.

Results: This study will, for the first time, systematically evaluate the clinical efficacy and safety of LWDH under real-world conditions, and quantitatively assess improvements in menopausal syndrome using multiple outcome measures.

Conclusions: Conclusion: This real-world cohort study is expected to provide important evidence regarding the clinical efficacy and safety of LWDH in treating menopausal syndrome, offering a scientific basis for its standardized use in clinical practice.

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Original Manuscript

Real-world effects of Liuwei Dihuang pill for menopausal syndrome：study protocol for a prospective, observational, multicenter cohort

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Background: Liuwei Dihuang (LWDH) pill, an adaptation of Chinese herbal medicine, is commonly employed to alleviate menopausal symptoms. Experimental and randomized controlled trials indicated that LWDH was advantageous for patients with menopausal syndrome (MPS). However, the pertinent scientific evidence of LWDH in the real clinical practice remains insufficient.

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Results: The clinical trial registration for this study was approved ,and the recruiting and enrolment of participants began in March 2025. We expect the future results of this study to be published in esteemed, peer-reviewed health research journals by around 2026.

Conclusion: This real-world cohort study is expected to provide important evidence regarding the clinical efficacy and safety of LWDH in treating menopausal syndrome, offering a scientific basis for its standardized use in clinical practice.

Study registration: This study was registered with the ClinicalTrials.gov (URL: https://clinicaltrials.gov/ ,Release Date: March 8, 2025 ,Unique identifier: NCT06874738).

Keywords: Liuwei Dihuang pill; real-world; menopasual syndrome; protocol; efficacy; safety

Introduction

Menopausal syndrome (MPS) is characterized by a range of physiological and psychological symptoms arising from the decline in ovarian function and fluctuations in oestrogen levels during the perimenopausal period[1]. The primary manifestations of this syndrome include vasomotor disturbances (hot flashes and night sweats), psychological symptoms (anxiety and depression), and metabolic disorders (dysglycemia and dyslipidemia)[2]. The World Health Organisation (WHO) projects that by 2030, more than 1.2 billion women globally would be approaching menopause or the post-menopausal period, with an annual increase of 47 million[3]. Recent studies have shown that the population of menopausal women in China has exceed 120 million, the most among all developing countries. It is estimated that by 2030, this number will rise to 210 million, accounting for approximately 1/7 of the global individuals affected by MPS[4]. Among these women, about 85% are expected to experience varying degrees of symptoms during their menopausal transition,which typically lasts around 3 to 5 years[5]. Menopausal syndrome may lead to a reduced quality of life and furthermore increase the risk of

cardiovascular diseases, osteoporosis, metabolic disorder, and Alzheimer's disease, imposing a certain burden on both families and society[6-8]. Therefore, lowering the prevalence of perimenopausal syndrome has become a significant public health issue that demands attention, receiving heightened focus from the medical community.

Western medicine mostly employs hormone therapy and non-hormonal pharmacotherapy as therapeutic modalities. Hormone therapy mitigates symptoms resulting from diminished oestrogen levels by providing oestrogen and progesterone; yet,its prolonged usage correlates with an elevated risk of breast cancer, endometrial cancer, vaginal cancer, and adverse cardiovascular events[9,10]. Antidepressants, gabapentin, and clonidine are non-hormonal medications that can ameliorate symptoms such as hot flashes and mood fluctuations,but may also cause side effects like nausea, insomnia, and sexual dysfunction[11,12]. Additionally, owing to individual variability among patients,symptoms may recur after the discontinuation of these treatments[13]. Therefore, finding safer and efficacy alternatives for treating MPS has become a current research focus. Traditional Chinese medicine (TCM) has a long history and rich experience in treating MPS, with its advantages lying in the emphasis on holistic management, which offers it unique benefits and broad prospects for application in this domain.In ancient China, MPS was viewed as a category of"depression syndrome, insomnia, fatigue,etc." based on its characteristic symptoms and signs[14]. The principle causes of this condition were thought to be deficiencies in the liver and kidneys[15]. Treatment methods included acupuncture, tuina (Chinese therapeutic massage), moxibustion, dietary therapy, and herbal medicine, all aimed at nourishing and tonifying these organs[16]. According to literature survey, herbal medicines can successfully alleviate symptoms and enhance quality of life, exhibit little side responses, and show high patient compliance, rendering it suitable for long-term use[17]. It was found that many formulas employed in the treatment of MPS are clinically based on the foundational recipe of

LWDH, which shows significant therapeutic effects[18,19]. The key element of the protocol is shown in Fig.1.

Nevertheless, the majority of current research on LWDH is limited to small-scale clinical observations, as there is a paucity of large-scale real-world evidence regarding its therapeutic effects. To address this gap, this study is based on the real-world clinical data of 1,000 MPS patients from six medical institutions, analyzing the therapeutic effects of LWDH on MPS. The results are expected to provide evidence-based support for TCM to manage MPS in the future.

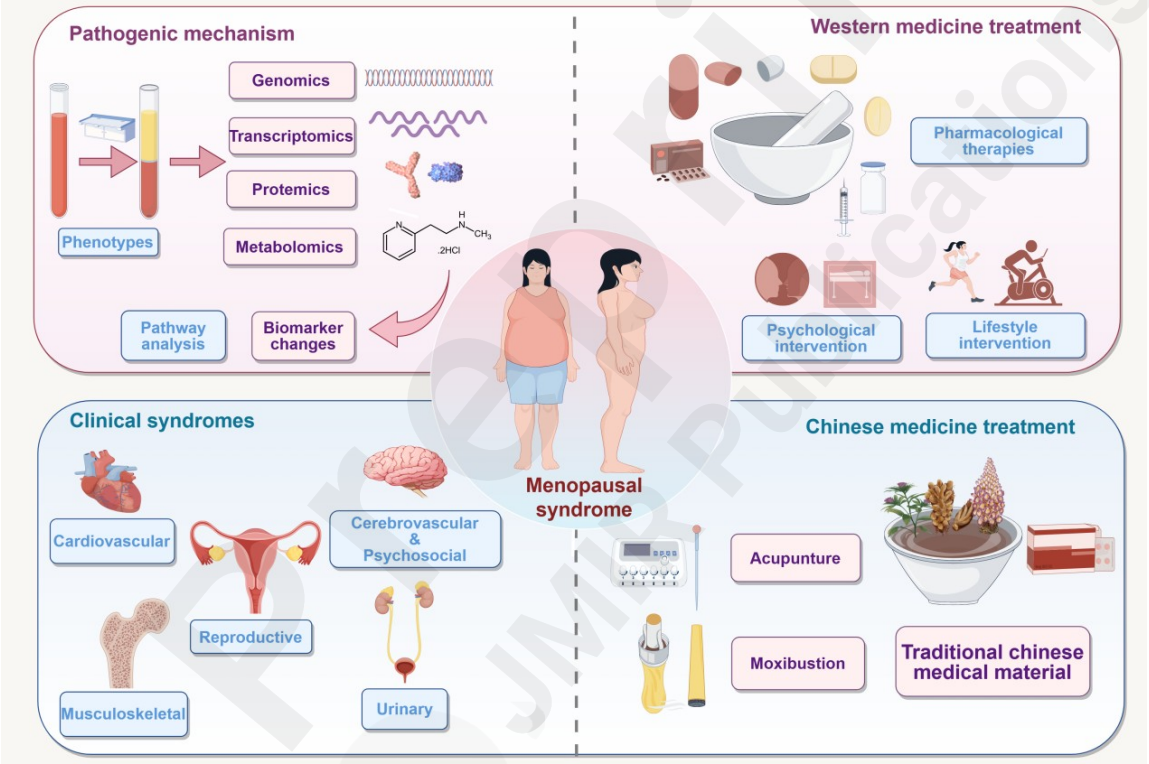

Fig.1 Summary of the Protocol

Methods

Research Design

It is a multicenter, prospective, observational cohort study designed to provide insight into the

administration of LWDH for patients with MPS in the real-world clinical practice setting. A total of 1000 patients with MPS from 6 centers will in different regions of China are divided into 2 cohorts: exposure group (patients with LWDH treatment) and non-exposed group (patients without LWDH treatment). Physicians decide whether to prescribe LWDH based on their assessment of the patient's overall condition, the drug's stated indications, clinical guidelines, and the patient's preferences. Assessments will be conducted at baseline, as well as at 2 weeks, 4 weeks, and 6 weeks after enrollment. The study protocol adheres to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) statement[20]. The completed SPIRIT checklist is supplied as Additional file 1. An overview of the study flowchart is presented in Fig 2.

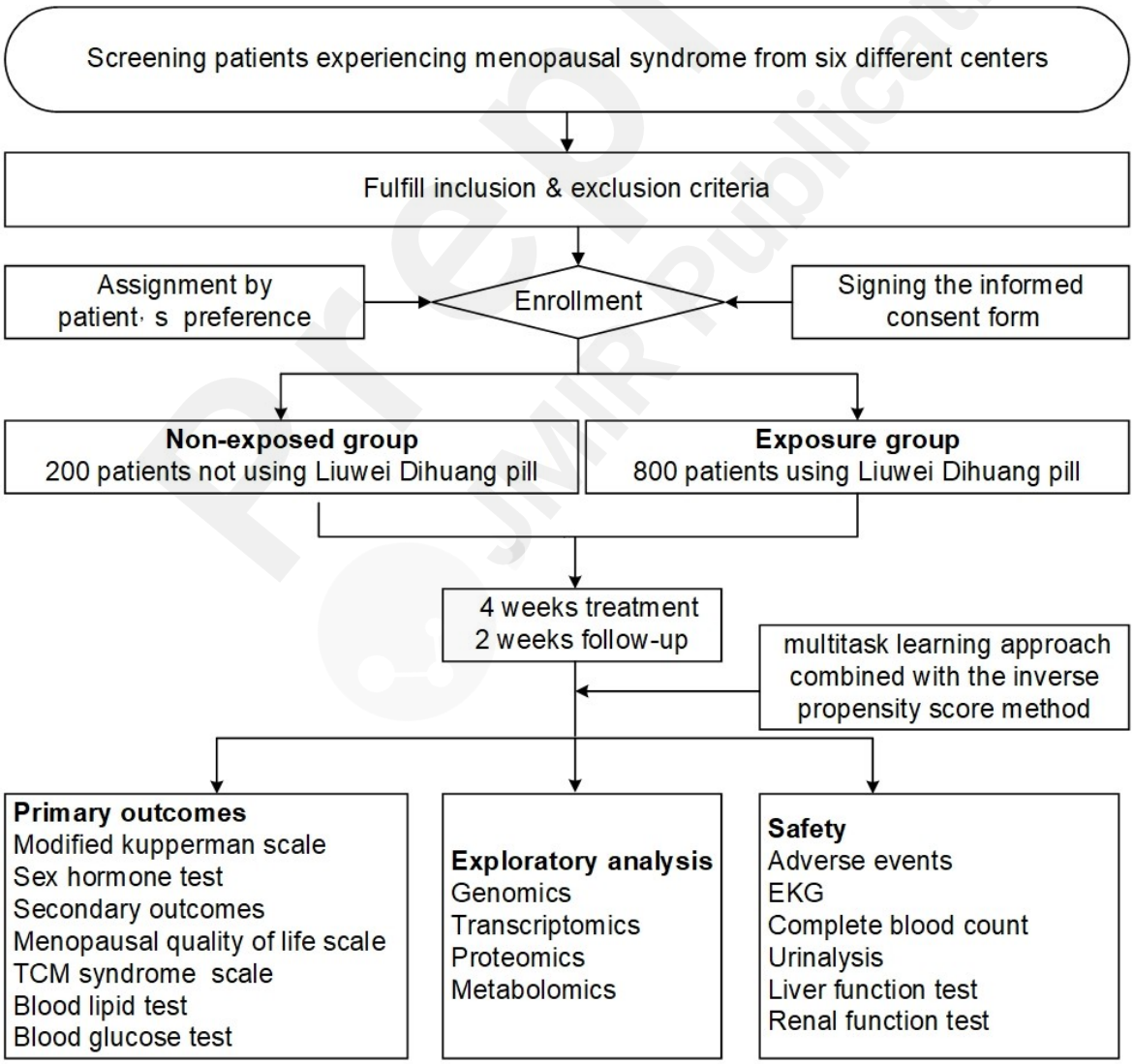

Fig.2 Flow diagram of study

Randomization and Blinding

Given that this study is a real - world observational study, patients are assigned to different groups based on their preferences and willingness for treatment options. Therefore, randomization and blinding are not applicable in this study. However, during the outcome evaluation, the statisticians and relevant personnel responsible for the final data analysis will remain blinded to the specific patient information and data, thereby eliminating potential biases in the data analysis results.

Sample Size Calculation

This study is a superiority trial. The sample size estimation is based on published randomized controlled trials of Liuwei Dihuang pill for the treatment of menopausal syndrome[21]. We estimate a response rate of 90% for the exposed group and 80% for the non-exposed group, using a one-sided test with a significance level of $\alpha = 0.025$ and a power of $1 - \beta = 0.9$. The exposed group and the non-exposed group will be included in a ratio of 1:4 based on the number of cases, with an anticipated dropout rate of 20%. This results in an estimated sample size of 171 for the non-exposed group and 684 for the exposed group. However, in real-world studies, a sufficiently large sample size is crucial to reduce bias and ensure the robustness of the study. Therefore, we have set the sample sizes to 200 for the non-exposed group and 800 for the exposed group, yielding a total sample size of 1000 participants.

Participants

Western medical diagnostic criteria

According to the relevant standards on menopause syndrome in "Obstetrics and Gynecology (9th Edition)"[1] and the "Guidelines for the Diagnosis and Treatment of MPS with Integrated Traditional Chinese and Western Medicine (2023 Edition)"[4], the diagnosis of the condition should be determined based on the patient's medical history, clinical symptoms, and laboratory test results.

Chinese medical diagnostic criteria

According to the clinical diagnostic criteria for menopause syndrome outlined in the "Guidelines for Clinical Research on New Chinese Medicines"[22], the diagnosis can be classified as a renal yin deficiency type.

Inclusion criteria

（1）Females aged 45 to 55 years old (inclusive);

（2）Meeting MPS Western medical diagnostic criteria;

（3）Meeting the criteria for syndrome differentiation of kidney yin deficiency according to TCM;

（4）Voluntarily signing the informed consent form.

Exclusion criteria

（1）Those with a known to be allergic to related ingredients of LWDH or contraindications to Chinese herbal preparations；

（2）Women who are pregnant, lactating, or intending to conceive；

（3）Patients with severe primary disorders of the liver, kidney, and haematopoietic system；

（4）Patients with comorbidities that significantly impact assessment, including severe cognitive impairment and aphasia.

Standard treatments

Eligible individuals who meet the inclusion criteria and provide informed consent will be assigned to either the exposed group or the non-exposed group based on their preferences and whether they are taking LWDH in real clinical practice. To facilitate the trial process and minimize drop-out rates, enrolled patients will receive guidance on medication, cognitive assessments, and other relevant evaluations, as well as essential health education before the trial begins. They will also be thoroughly informed about the potential risks and benefits of participating in this study, their rights and responsibilities during the research process, and the procedures for handling emergency situations. Patients in the non-exposed group will receive standardized western medication according to patient's clinical conditions, including Hormonal drugs,antidepressants, and anti-anxiety drugs. Patients in the exposure group will be recommended to to take 8 pill (3g) of LWDH orally three times daily for four weeks, in addition to standardised Western medicine. The actual dosage and treatment course of LWDH will be determined at the discretion of the attending physician, as well as the discussion between the patients and the physician.During the entire monitoring phase, the use of combination therapy and other treatments is permitted. All pharmaceuticals will be recorded in the case report form.

Outcome measures

Primary outcome

（1）The severity of MPS will be assessed using the Modified Kupperman Scale[23]. This scale comprises various items, including hot flashes, sweating, insomnia, dizziness, fatigue, joint pain, depression, and anxiety, with each item rated from 0 to 3. Individual scores for each

assessment item will be calculated by multiplying the score by its corresponding weighting factor. The total score ranges from 0 to 63, where a score above 30 indicates severe symptoms, a score between 16 and 30 reflects moderate symptoms, a score between 6 and 15 denotes mild symptoms, and a score below 6 signifies normal symptomatology.

（2）The hormone levels in patients with MPS primarily focus on six sex hormone indicators, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), oestradiol (E2), progesterone (P), testosterone (T), and prolactin (PRL).

Secondary outcome

（1）The quality of life of perimenopausal women is assessed using the Perimenopausal Quality of Life Scale[24]. This scale encompasses four dimensions, comprising a total of 29 items: vasomotor symptoms (3 items), psychological status (7 items), sexual life (3 items), and physical status (16 items). Each item is rated on a scale from 0 to 6, with lower scores reflecting a higher quality of life for the patient.

（2）The clinical symptoms and signs associated with kidney-yin deficiency in MPS will be evaluated using a TCM Syndrome Scale[25]. This scale evaluates three dimensions: physical sensations, psychological feelings, and urogenital symptoms.Each item is rated on a scale from 0 to 3,with higher score indicating more severe symptoms.

（3）The blood glucose levels will be assessed using the following indicators: fasting blood glucose (FPG), 2-hour postprandial blood glucose (2hPG), and glycated albumin (GA). These indicators provide insights into the status of glucose metabolism.

（4）The blood lipid levels will be assessed using the following indicators: total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). These indicators will help evaluate the status of lipid metabolism.

Exploratory analysis

(1) Genomic analysis: Susceptibility genes associated with the disease and their mutation status are identified using gene sequencing or whole exome sequencing techniques, clarifying the relationship between gene mutations and changes in signaling pathways.

(2) Transcriptomic analysis: Differentially expressed genes are screened for by utilizing mRNA high-throughput sequencing technology, and the mechanisms of gene regulation are explored.

(3) Proteomic analysis: Differentially expressed proteins are identified using LC-MS technology, and key protein targets and signaling pathways are clarified.

(4) Metabolomics analysis: The changes in metabolites are revealed through the analysis using GC-MS technology, highlighting the regulatory roles of metabolic pathways and their potential mechanisms within the body.

The above indicators will be evaluated and compared both before the medication and 4 weeks after the treatment. All blood tests will be conducted after an 8-hour fasting period and a minimum of 30 minutes of rest before collecting venous blood. For menopausal participants, blood samples will be taken prior to treatment and on the last day of treatment, while for menstruating participants, blood samples will be collected on the second day of their menstrual period.

To enable exploratory multi-omics analyses, 6.5 mL of peripheral blood will be collected at each time point. Of this, 4 mL of whole blood will be maintained at 4 °C for 1 h, centrifuged at 4,000 rpm for 10 min at 4 °C, and the resulting serum aliquoted into four 0.5 mL cryovials. The remaining 2.5 mL will be drawn into a BD PAXgene Blood RNA tube (Cat. No. 762165), gently inverted to ensure thorough mixing. All vials will be labeled with the participant ID, placed into

pre-labeled freezer boxes, and promptly stored at −80 °C (with a maximum holding time of 30 min at 4 °C if immediate transfer is not possible). Identical procedures will be applied for both pre- and post-treatment samples.

Safety outcomes

Adverse events, vital signs, and some laboratory tests are considered as safety outcomes. Vital sign (blood pressure and heart rate) will be measured before and after medication administration. Routine laboratory tests (hemanalysis,urinalysis, hepatic and renal function) and electrocardiograms will be measuredbefore and after medication administration. The adverse events will be documented after medication. The prognosis of adverse events will be observed until the adverse reactions disappear or relieve.

Data collection

The data collection and follow-up schedule is presented in Table 1.

（1）Screening and enrollment phase

Participants will be screened to determine their eligibility based on inclusion and exclusion criteria.Before administering the medication, data will be collected on general demographic information (age, gender, ethnicity, occupation, height, weight, etc.), vital signs (temperature, blood pressure, heart rate, respiration), medical history, treatment history, and medication history. Patients enrolled in the study will undergo hematological tests(Sex hormon e test,Complete blood count,Urinalysis,Blood lipid test,Blood glucose test,Liver function test,and Renal function test),Electrocardiogram(EKG),and questionnaire surveys (Modified Kupperman Scale, Menopause Quality of Life Scale, TCM Syndrome Scale). The collected

information will be recorded in the screening and enrollment section of the Case Report Form (CRF).

（2）Treatment phase

Patients will be followed up at the 2 weeks(±7 days) and 4 weeks(±7 days) after medication administration to check vital signs. Additionally, assessments will include hematological tests, questionnaire surveys, adverse events, medical history, medication history, and treatment history since enrollment. Furthermore, information regarding the prescription and distribution of LWDH will also be collected at V0 and V 1. When LWDH is newly prescribed, it will be checked at V2 and V3. Relevant information will be written in the treatment section of CRF.

（3）Follow-up visit

A follow-up will be conducted by phone 6 weeks (±7 days) after study enrollment to identify adverse events and changes in medical history, medication history, and treatment history. Relevant information will be documented in the follow-up section of CRF. Finally, a summary of the completion status of the cases will be provided.

Table 1 Time schedule of participant enrollment and follow-up

Study protocol				
	Screening and enrolment	**Treatment phase**		**Follow-up**
Timepoint	Baseline V0	2weeks±7 days V1	4weeks±7 days V2	6weeks±7 days V3
Enrolment				
Confirm eligility	×			
Informed consent	×			
Demographics	×			
Medical history-treatment history-medication history	×			
Interventions				
LWDH pill		×	×	
Assessments				
Vital signs	×	×	×	
Hematological examination	×	×	×	
Questionnaire surveys	×	×	×	
EKG	×	×	×	
Prescription and distribution of LWDH	×	×		
Evaluation of medication compliance		×	×	
Combined medication	×	×	×	
Adverse events		×	×	×
Changes in medical history, treatment, or medication		×	×	×
Conclusion of completion of the case				×

Quality Control

Quality Control Measures

（1）In order to guarantee the successful implementation of quality control in clinical research, it is imperative that investigators comply with standard operating procedures (SOPs).

(2) In order to guarantee that all conclusions are derived from original data, it is essential that all observations and findings in clinical research be verified.

(3) In order to guarantee the reliability and proper administration of data, quality assurance must be performed at each stage of the data processing process.

Investigator Training

(1) Prior to the commencement of clinical research, the principal investigator at each research centre must offer training on the study protocol to ensure that investigators comprehend and are acquainted with the properties, effects, efficacy, and safety of the study drug, including pertinent preclinical data.

(2) Investigators must remain informed about all new information pertaining to the medicine that arises during the research.

(3) This information for the study should be documented on the case report form.

Enhancing Participant Compliance

(1) The investigators articulates the mechanism of action, therapeutic effects, and potential side effects of LWDH using accessible language for the patients. This strategy enables patients to understand the necessity and significance of the treatment, thus fostering their cooperation with the study.

(2) Encouraging patients to actively engage in self-management is essential. This includes keeping track of their medication usage and noting any changes in their symptoms. Such practices not only enhance their understanding and skills in self-management but also boost their confidence in the treatment process.

(3) Send reminders through email, phone calls, or text messages to help promptly identify and address any issues patients may face during the treatment process. This approach ensures

that patients attend their follow-up appointments and adhere to their medication schedule.

Statistical analysis

Situations and treatment of missing values

Data missingness is described as variables explicitly stated as missing or unobtainable, unreported variables (i.e., blank observations), reported data that are ambiguous, or data that are inconsistent or beyond acceptable ranges. This study will only address missing data for the primary outcome measures. The approach may include "carrying forward the last observation to replace missing experimental data, ensuring that the number of subjects evaluating efficacy at the endpoint remains consistent with that at the start of the trial," as well as using multiple imputation methods for handling missing data.

Data entry and processing

The information of MPS patients who meet the criteria will be collected by researchers and entered into the Traditional Chinese Medicine Research Case Collection System (https://www.yiankb.com/edc/) to establish a research database. Statistical analysis will be performed using SPSS 26.0, and the significance level for all statistical tests will be set at $\alpha = 0.05$.

Descriptive statistics will be conducted for the demographic and baseline information of participants. Continuous variables will be presented as mean±SD, whereas categorical variables will be described using frequency and percentage.

The results of each scale, the values of the six sex hormones, and the data on blood glucose and lipids will be assessed for normality using the Shapiro-Wilk test. If the data follows a

normal distribution, group comparisons will be conducted using independent samples t-tests, while within-group comparisons will be performed using paired samples t-tests. For variables that do not follow a normal distribution, the Mann-Whitney U test will be used.

A multi-level integrative analysis strategy is adopted to systematically elucidate the multi-omics mechanisms of LWDH in its intervention for MPS.Differential features in single-omics will be selected using orthogonal partial least squares discriminant analysis (OPLS-DA) along with the Benjamini-Hochberg correction. Multi-omics factor analysis (MOFA) and the DIABLO algorithm will be employed for the integration of cross-omics data, facilitating the construction of a gene-protein-metabolite regulatory network.Weighted Gene Co-expression Network Analysis (WGCNA) will be utilized to identify core modules that are significantly associated with clinical phenotypes. This analysis will be complemented by KEGG pathway enrichment and the Reactome database to facilitate cross-omics pathway integration. The random forest algorithm will be applied to identify key biomarker combinations, while Spearman correlation analysis will be used to establish the regulatory relationships between genomic variations and downstream molecular phenotypes. The MetaboAnalyst platform will be utilized for metabolic pathway topology analysis, and Cytoscape will be employed to visualize the multi-omics interaction network.

To control for potential confounding variables, we will use a multitask learning approach combined with the inverse propensity score method. The following covariates will be considered: sex, age, BMI, duration of menopausal syndrome, comorbidities, and whether hormonal medication is being taken, as well as the type of hormonal medication if applicable.

All results are presented with p-values. At $P < 0.05$, the differences were considered statistically significant, while at $P > 0.05$, they were deemed non-significant.

Unlike RCTs, this study did not interfere with clinical diagnosis and treatment; it merely recorded real-world data. When the volume of data reaches a certain threshold, fundamental

statistics and stratified analyses can be conducted using patient information and actual treatment data.

Ethics issues

During participation in this study, while the blood sample collection is generally safe, participants may experience brief discomfort and localized bruising at the collection site. Additionally, a small number of individuals may encounter mild dizziness, which represents some of the potential risks associated with this study.

Participants in this study will receive free laboratory tests, medical consultation services, and feedback on omics test results. Additionally, this study will not provide participants with any medications or other forms of compensation. In the event of any injuries related to this study, the research team will assume corresponding responsibilities in accordance with national laws and regulations and will provide appropriate compensation for any damages related to the study. Before enrollment, the researchers should provide the participants or their legally authorised representatives with ample time and opportunity to understand the details of the clinical trial, and thoroughly answer any questions they may have related to the clinical trial. Participants or their guardians should receive a signed copy of the informed consent form, which includes their names and the date. During the trial, if participants have any questions, they can ask the research physician at any time. Additionally, they have the right to withdraw from the study at any time without any external pressure, and the research physician will appropriately manage their subsequent treatment. To safeguard data security, an ethics-approved, comprehensive data-storage and management plan has been implemented to minimize any risk of breach.

Result

Protocol version 1.0, February 2025. The clinical trial registration for this study was approved , and the recruiting and enrolment of participants began in March 2025. We expect the future results of this study to be published in esteemed, peer-reviewed health research journals by around 2026.

Discussion

Currently, LWDH combined with western medicine is widely used for patients with MPS, especially for those who are dissatisfied with the efficacy or want to relieve the side effects from western medication[26]. In this context, the rationale and methodology of a prospective, multicenter observational study are presented, aiming to evaluate the clinical efficacy and safety of LWDH alone in the therapy of MPS in real-world settings. This study will provide valuable insights into the potential benefits and risks of using LWDH as a standalone therapy, contributing to a better understanding of its role in managing menopausal symptoms.

LWDH originates from the medical text "Xiao Er Yao Zheng Zhi Jue," written by the renowned physician Qian Yi during the Song Dynasty.This formula is composed of six traditional Chinese herbs: Rehmannia glutinosa (Shudihuang), Cornus officinalis (Shanzhuyu), Dioscorea opposita (Shanyao), Poria (Fuling), Alisma orientale (Zexie), and Moutan Cortex (Mudanpi). Each ingredient contributes to its therapeutic effects, targeting nourishing yin and tonifying the kidneys[27]. Modern pharmacological studies indicate that LWDH exhibits a range of effects, including antioxidant, anti-aging, immune regulation, anti-inflammatory, anti-mutagenic, and anti-tumor properties, along with antihypertensive and lipid-lowering effects[28].

Through systematic pharmacology research, it has been reported that LWDH may exert therapeutic effects through its active components such as kaempferol, β-sitosterol,

stigmastanol, and tetrahydroharmine. These constituents are thought to interact with targets like NCOA2, PTGS2, PRKACA, and PGR, thereby intervening in pathways related to estrogen signaling, endocrine resistance, cholinergic synapses, and serotonergic synapses. This multifaceted mechanism of action underscores the potential of LWDH as a therapeutic option for managing perimenopausal syndrome[29]. In rats with premature ovarian insufficiency, a study showed that LWDH effectivenessly inhibits the apoptosis of ovarian granulosa cells, improves ovarian endocrine function, enhances antioxidant capacity, and mitigates oxidative stress damage[30]. The downregulation of 12 differential proteins related to Diminished Ovarian Reserve (DOR) by LWDH is significant at the proteomics level. These proteins play crucial roles in lipid metabolism, inflammation, and immune regulation, with notable enrichment in pathways such as sphingolipid metabolism and arachidonic acid metabolism[31]. A systematic review revealed that LWDH is effectiveness in improving symptoms in MPS, such as vasomotor symptoms, mood disorders, sleep disturbances, glucose and lipid metabolism disorders, and bone loss[32,33]. However, there is currently no prospective research evidence to support these findings.

Randomized controlled trials (RCTs) hold significant value in scientific research; however, their design often focuses on single symptoms and frequently excludes patients with comorbidities. The limitation of this research method is that it cannot fully reflect the clinical practice of TCM in the real world. In contrast, the prospective observational cohort study does not interfere with the clinical treatment process of patients but instead accurately records treatment data, making it more aligned with the clinical environment of the real world. This study allows for the evaluation of the efficacy of LWDH in a diverse patient population, capturing its effects on various heterogeneous symptoms, such as metabolic disorders and psychological symptoms, and exploring its potential synergistic effects with other therapies.

This study integrates genomic, transcriptomic, proteomic, and metabolomic data to uncover the potential mechanisms of action of LWDH in treating MPS from multiple perspectives, thereby addressing the limitations inherent in individual omics. The combined multi-omics analysis identifies a series of differentially expressed biomarkers associated with the disease, revealing the key mechanisms by which LWDH exerts its effects on MPS.

The results of this study will provide crucial evidence for incorporating TCM into clinical guidelines for menopause management. Multimodal data are integrated in this study, aiming to promote the precision medicine and tailored treatment strategies of MPS treatment. By further validating the effectiveness, safety and mechanism of LWDH, it can offer a safe and effectiveness alternative therapy for menopausal women. Moreover, the findings of this study will also offer new directions for subsequent mechanistic research, promoting the development of integrated approaches that combine TCM and Western medicine in the treatment of MPS.

Conclusion

This prospective, multicenter, real-world cohort study will provide the first large-scale clinical evidence regarding the effectiveness and safety of Liuwei Dihuang pill in women with menopausal syndrome. By integrating multi-omics approaches, the study is expected to elucidate the underlying mechanisms of action and broaden the scientific understanding of traditional Chinese medicine in menopause management. The findings will offer robust evidence to support clinical practice, promote precision medicine, and facilitate the development of integrative strategies for improving women's health during the menopausal transition.

Abbreviation

LWDH, Liuwei Dihuang; MPS, Menopausal syndrome; TCM, Traditional Chinese Medicine; WHO, World Health Organisation; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, oestradiol; P, progesterone; T, testosterone; PRL, prolactin; FPG, fasting blood glucose; 2hPG, 2-hour postprandial blood glucose; GA, glycated albumin; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; EKG, Electrocardiogram; CRF, Case Report Form; SOPs, standard operating procedures; OPLS-DA, orthogonal partial least squares discriminant analysis; MOFA, Multi-omics factor analysis; WGCNA, Weighted Gene Co-expression Network Analysis; Shudihuang, Rehmannia glutinosa; Shanzhuyu, Cornus officinalis; Shanyao, Dioscorea opposita; Fuling,Poria; Zexie, Alisma orientale; Mudanpi, Moutan Cortex; DOR, Diminished Ovarian Rese.

Data avaliabilty

The present study did not generate or analyse any datasets. The dataset generated during this study can be obtained from the corresponding author upon reasonable request.

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Authors' Contributions

LW, SW, QS, and SZ set the research topic. LW, SW, and HZ conducted the design of the study. SW drafted the manuscript.MW and LW critically revised the overall manuscript. YS, TS, WZ, YY, LM, YY, WT, and XD served as local primary investigators at sub-centers, responsible for patient recruitment and data collection. All co-authors endorsed the final paper and took accountability for the decision to submit it for publication.

Declaration of competing interest

The authors declare that they have no conflicts of interest. No funding was received from the relevant pharmaceutical companies in this study.

References

1.Ge JB, Xu YJ, Wang C. Internal Medicine[M]. 9th Edition. Beijing: People's Medical Publishing House, 2018: 188.

2.Fang Y, Liu F, Zhang X, et al. Mapping global prevalence of menopausal symptoms among middle-aged women: a systematic review and meta-analysis. BMC Public Health. 2024; 24(1): 1767. https://doi.org/10.1186/s12889-024-19280-5.PMID:38956480.

3.Hill K. The demography of menopause. Maturitas. 1996; 23(2): 113-127. https://doi.org/10.1016/0378-5122(95)00968-x. PMID:8735350.

4.Chinese Society of Integrated Traditional and Western Medicine Obstetrics and Gynecology Professional Committee.Guidelines for the Diagnosis and Treatment of Menopausal Syndrome with Integrated Traditional and Western Medicine (2023 Edition) [J].Chinese Journal of Practical Gynecology and Obstetrics, 2023, 29 (8): 799-808.

5.Zhu D, Chung HF, Dobson AJ,et al.Age at natural menopause and risk of incident cardiovascular disease:a pooled analysis of individual patient data.Lancet Public Health. 2019;

4(11): e553-e564. https://doi.org/10.1016/S2468-2667(19)30155-0.PMID:31588031.

6.Mou H, Zhang J, Guo Y, et al. Publisher Correction:Effects of key physiological parameters on cardiovascular disease and osteoporosis risk in perimenopausal and postmenopausal women.Sci Rep. 2025; 15(1): 10251. https://doi.org/10.3389/fendo.2024.1514913. PMID: 39574957.

7.Klisic A, Ahmad R, Daka B, et al. Editorial: Cardiometabolic diseases in postmenopausal women. Front Endocrinol (Lausanne). 2024; 15:1514913. https://doi.org/10.3389/fendo. 2024. 1514913. PMID: 39574957.

8.Lan Y, Huang Y, Song Y, et al. Prevalence, severity,and associated factors of menopausal symptoms in middle-aged Chinese women: a community-based cross-sectional study in southeast China. Menopause. 2017; 24(10): 1200-1207. https://doi.org/10.1097/GME. 0000000000000906. PMID: 28609386.

9.Mehta J, Kling JM, Manson JE. Risks, Benefits, and Treatment Modalities of Menopausal Hormone Therapy: Current Concepts. Front Endocrinol (Lausanne). 2021;12:564781. https://doi.org/10.3389/fendo.2021.564781. PMID:33841322.

10.Gambrell RD Jr. The menopause: benefits and risks of estrogen-progestogen replacement therapy. Fertil Steril. 1982; 37(4): 457-474. https://doi.org/10.1016/s0015-0282(16)46149-2. PMID: 7040116.

11.Sahni S, Lobo-Romero A, Smith T. Contemporary Non-hormonal Therapies for the Management of Vasomotor Symptoms Associated with Menopause:A Literature Review. touch REV Endocrinol. 2021; 17(2): 133-137. https://doi.org/10.17925/EE.2021.17.2.133. PMID: 35118459.

12.Witten T, Staszkiewicz J, Gold L, et al. Nonhormonal Pharmacotherapies for the Treatment of Postmenopausal Vasomotor Symptoms. Cureus. 2024; 16(1): e52467. https://doi.org/10.7759/cureus.52467. PMID: 38371081.

13. Academic Committee of the Korean Society of Menopause, Lee SR, Cho MK, et al. The 2020 Menopausal Hormone Therapy Guidelines. J Menopausal Med. 2020; 26(2): 69-98. https://doi.org/10.6118/jmm.20000. PMID: 32893509.

14. Hu SW, Liang HY, Ma K, et al. Discussion on the Clinical Advantages of Traditional Chinese Medicine for Specific Diseases-Perimenopausal Syndrome[J]. Chinese Journal of Experimental Formulas, 2024, 30(21): 234-242.

15. Gong MF, Hu Y. Analysis of the Distribution of Traditional Chinese Medicine Syndromes and Influencing Factors in Patients with Perimenopausal Syndrome[J]. Chinese Journal of Maternal and Child Health Care,2021,36(11):2645-2648.

16. Zhang M, Sun Y, Shen MH, et al. Current Status of Non-Pharmacological Therapies for Perimenopausal Syndrome[J].Chinese Journal of Geriatrics, 2014, 34(24): 7132-7134.

17. Zhang XQ. Analysis of the Current Status of Hormone Replacement Therapy and Traditional Chinese Medicine Treatment for Menopause in the Last Five Years in China [J]. Journal of Guangdong Medical University, 2019, 37(6): 666-671.

18. Han YY, Yuan YH. Observation on the Efficacy of Self-Formulated Liuwei Gengnianan in Treating 80 Cases of Perimenopausal Syndrome of Liver and Kidney Yin Deficiency Type[J]. Journal of Traditional Chinese Medicine, 2024, 52(10): 88-92.

19. Huang JX. Clinical Effect of Zishen Ningxin Decoction in Treating Female Perimenopausal Syndrome[J]. Heilongjiang Journal of Traditional Chinese Medicine, 2024, 53(04): 14-15.

20. Chan A, Tetzlaff JM, Gøtzsche PC, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. BMJ. Jan 08, 2013;346(jan08 15):e7586-e7586. https://doi.org/10.1136/bmj.e7586. PMID: 23303884.

21. Chen J. Clinical Effect of Modified Liuwei Dihuang Wan in Treating Kidney Yin Deficiency Type Perimenopausal Syndrome. Journal of Clinical Medicine Research and Practice, 2020, 5(33): 152-153.

22. Zheng XY. Guidelines for Clinical Research on New Chinese Medicinal Drugs (Trial) [M]. Beijing: China Medical Science and Technology Press, 2002: 254-259.

23. Liu Y, Zhang J. The Effect of Estradiol Valerate Tablets on Menopausal Syndrome Scores, Estrogen Levels, and Adverse Reactions in Patients with Perimenopausal Syndrome[J]. Journal of Practical Clinical Medicine, 2020,24(1): 109-111.

24. Hilditch JR, Lewis J, Peter A, et al. A menopause-specific quality of life questionnaire: development and psychometric properties [published correction appears in Maturitas 1996 Nov; 25(3): 231]. Maturitas. 1996; 24(3): 161-175. https: //doi.org/10.1016/s0378-5122 (96) 82006-8. PMID: 8844630.

25. Zhang X, Gao S, Wang YX, et al. The Effects of Modified Chaihu Guizhi Decoction on Vascular Dysfunction, Serum FSH, LH, E2 Levels, and Endometrial Thickness in Patients with Perimenopausal Syndrome[J].Chinese Journal of Traditional Chinese Medicine, 2019, 37 (12): 3010-3012.

26. Chen Y, Yin L, Wu L. Analysis of the Effects of Hormone Replacement Therapy Combined with Liuwei Dihuang Decoction in the Treatment of Perimenopausal Syndrome[J]. Journal of Practical Gynecological Endocrinology, 2022, 9(12): 58-61.

27. Hou WX, Si RH, Liu YQ, et al. The Molecular Mechanism of Liuwei Dihuang Decoction Intervening Autophagy through the FcγRⅡB/c-Src Pathway in the Prevention and Treatment of Alzheimer's Disease [J/OL]. World Science and Technology-Modernization of Traditional Chinese Medicine, 1-15 [2025-04-07].
http://kns.cnki.net/kcms/detail/11.5699.R.20250312.1444.004.html.

28. Qin L, Gao T, Zhong JW, et al. Advances in Modern Pharmacological Research of Liuwei Dihuang Decoction and Its Applications in Reproductive Medicine[J].China Modern Distance Education of Traditional Chinese Medicine, 2024, 22(20): 185-188.

29. Zhang R. Network Pharmacology Study of Liuwei Dihuang Decoction in the Treatment of

Perimenopausal Syndrome [J]. Medical Information, 2022, 35(09):2 8-33.

30. Li XR, Zhong JW, Luo YX, et al. Molecular Mechanism of Liuwei Dihuang Decoction in Treating Model Mice with Premature Ovarian Insufficiency [J]. China Tissue Engineering Research, 2024, 28(26): 4151-4157.

31. Gao T, Zhong JW, Qin L, et al. Exploring the Mechanism of Liuwei Dihuang Decoction in Treating Mice with Reduced Ovarian Reserve Based on Proteomics Technology [J]. China Journal of Chinese Medicine, 2023, 48(12): 3224-3234.

32. Yue C, Xiao XF, Qin WJ. Clinical Research Progress of Liuwei Dihuang Decoction in Treating Perimenopausal Syndrome [J]. Drug Evaluation Research,2022,45(04): 804-809.

33. Xie HY. Clinical Progress of Liuwei Dihuang Wan in the Treatment of Diabetes[J]. Chinese and Foreign Medical Research, 2023, 21(28):181-184.

Supplementary Files

CONSORT (or other) checklists

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