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Untargeted metabolomics reveals Shugan Jianpi Yangxin Prescription treatment for insomnia disorder: A randomized, double-blind, placebo-controlled, multi-center clinical trial

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Abstract

Background: Effective therapeutic approaches are needed to address insomnia disorder and ameliorate its associated symptoms. Traditional Chinese medicine (TCM), especially the Shugan Jianpi Yangxin Prescription (SJYP), evolved from TCM classic formula Xiaochaihu decoction, Guipi decoction and Xiaoyao San, demonstrated potential advantages in improving symptoms comorbid with insomnia disorder.

Purpose: To thoroughly assess the efficacy and safety of SJYP in the treatment of insomnia disorder, as well as to explore its potential impact of treatment on insomnia disorder patients' serum metabolomic profiles.

Methods: A total of 130 patients with liver depression and spleen deficiency type insomnia disorder who met the inclusion criteria were randomly assigned to control and SJYP groups in a 1:1 ratio (65 cases per group). Primary outcomes were changes in overall response rate. Secondary outcomes were assessed through various clinical parameters, including: Pittsburgh Sleep Quality Index (PSQI), Insomnia Severity Index (ISI), Chinese Medicine (CM) Syndrome Evaluation Scale, Hamilton Anxiety Scale (HAMA), Hamilton Depression Scale (HAMD). Safety was evaluated through vital signs, laboratory tests and electrocardiogram (ECG) results. Serum samples from 30 patients in the SJYP group before and after treatment were collected, as well as serum samples from 30 healthy individuals. Untargeted metabolomics was performed using ultra-performance liquid chromatography coupled with high-resolution mass spectrometry (LC-MS/MS). Multivariate statistical analyses, including orthogonal partial least squares-discriminant analysis (OPLS-DA), were employed to identify differential metabolites and metabolic pathways.

Results: 128 insomnia disorder patients were included in PPS (per protocol set). SJYP demonstrated a total effective rate of 93.85%, indicating favorable clinical efficacy. It significantly reduced PSQI scores in patients with insomnia disorder, improving overall sleep quality, and outperformed the control group in enhancing specific PSQI components such as sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbances, and daytime dysfunction

(p<0.05). Additionally, SJYP reduced ISI scores (p<0.05), alleviating the severity of insomnia. It also improved TCM syndrome evaluation scores, particularly in symptoms such as difficulty falling asleep, dream-disturbed and easy awakening, low or irritable mood, mental fatigue and lack of energy, poor appetite, forgetfulness, and dull complexion, with superior outcomes compared to the control group (p<0.05). Furthermore, it reduced scores on both HAMA and HAMD (p<0.05), ameliorating patients' anxiety and depressive symptoms. Follow-up assessments at 6 and 8 weeks of treatment showed continued reduction in ISI scores (p<0.05), suggesting that SJYP provided stable and sustained therapeutic effects for patients with insomnia disorder of liver depression and spleen deficiency type, leading to prolonged improvement in sleep quality. SJYP may influence metabolites such as Pectenotoxin 2, N-Lactoyl-Phenylalanine, and O-Acetylcarnitine in the treatment of insomnia disorder. Potential metabolic pathways involved include the FoxO signaling pathway, bile secretion, retrograde endocannabinoid signaling, glycine, serine and threonine metabolism, and the GABAergic synapse.

Conclusion: SJYP is both effective and safe in treating insomnia disorder and ameliorating its associated symptoms. Its potential therapeutic effects may be associated with the modulation of metabolites such as Pectenotoxin 2, N-Lactoyl-Phenylalanine, and O-Acetylcarnitine, and the regulation of metabolic pathways including the GABAergic synapse.

Keywords: Shugan Jianpi Yangxin Prescription; insomnia disorder; double-blind; randomized control trial; untargeted metabolomics; traditional Chinese medicine

Trial registration: ChiCTR2300067859. Registered 21 January 2023, https://www.chictr.org.cn.

Introduction

Insomnia disorder is recognized as one of the most prevalent types of sleep disorders. About 30% to 40% of people in the general population have trouble falling or staying asleep, while 10% to 15% suffer from chronic insomnia disorder[1]. Insomnia diminishes quality of life, and leads to substantial economic costs[2]. Benzodiazepine and nonbenzodiazepine hypnotics, including zolpidem, are the most commonly prescribed FDA-approved drugs for insomnia, but they come with limitations like daytime sleepiness, addiction (with withdrawal effects), tolerance, and a heightened risk of falls and cognitive issues, particularly in the elderly[3]. Traditional Chinese Medicine (TCM) can improve sleep effectively and alleviate the symptoms resulting from insomnia without causing addiction when used to treat chronic insomnia, and it is gaining more attention[4].

Shugan Jianpi Yangxin Prescription (SJYP), is derived from the modification and integration of three classic TCM formulas: Xiaochaihu Decoction, Guipi Decoction, and Xiaoyao Pills. It is used to treat insomnia disorder attributed to liver depression and spleen deficiency. Long-term clinical application has demonstrated its significant advantages in improving sleep symptoms, reducing comorbid conditions, and enhancing patients' quality of life. However, the lack of high-quality evidence-based evidence has limited its broader clinical adoption. Therefore, this study employs a multicenter, randomized, double-blind, placebo-controlled trial to investigate the clinical efficacy and safety of SJYP in treating insomnia disorder of this pattern. This study conducted untargeted metabolomic analysis on the serum of patients with insomnia disorders to explore the possible mechanism of SJYP in regulating sleep. The aim is to enhance clinical outcomes, expand treatment access for more patients with insomnia disorder, and provide new

insights along with high-quality evidence-based support for clinical practice.

Materials

Plant material

SJYP granules (Batch No. 21090158) were purchased from Sichuan Neo-Green Pharmaceutical. Each dose contains: *Bupleuri Radix* (Chaihu) 10 g, *Angelicae Sinensis Radix* (Danggui) 10 g, *Paeoniae Alba Radix* (Baishao) 15 g, *Scutellariae Radix* (Huangqin) 9 g, *Wolfiporia cocos* (Fuling) 15 g, *Codonopsis Radix* (Dangshen) 10 g, *Atractylodis Macrocephalae Rhizoma* (Baizhu) 15 g, *Ziziphi Spinosae Semen* (Suanzhaoren) 15 g, *Longan Arillus* (Longyanrou) 15 g, *Gardeniae Fructus* (Zhizi) 10 g, and *Glycyrrhizae Radix et Rhizoma Praeparata cum Melle* (Zhigancao) 10 g.

Reagents

Methanol, acetonitrile, ultrapure water were used and purchased from Fisher Chemica (China) in metabolomics work. Formic Acid was from Anpel Laboratory Technologies (China). 2-Propanol was purchased from Merck (Germany). 2-Chloro-L-Phenylalanine was from Merck (Germany).

Instrument

The LC-MS/MS analysis of sample was conducted on a UHPLC-Orbitrap Exploris 240 system (Thermo Fisher Scientifc (USA) Co.Ltd) equipped with an ACQUITY HSS T3 column (100 mm × 2.1 mm i.d., 1.8 μm (Waters, USA) at Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). JXDC-20 Nitrogen Blowing Instrument, Shanghai Jingxin Industrial Development Co., Ltd.; LNG-T88 Bench-top Rapid Centrifugal Concentrator Dryer, Taicang Huamei Biochemical Instrument Factory; Wonbio-96c High-throughput Tissue Grinder, Shanghai Wanbai Biotechnology Co., Ltd.; SBL-10DT Ultrasonic Cleaner (300W-10L), Ningbo Scientz Biotechnology Co., Ltd.; Centrifuge 5430R High-speed Refrigerated Centrifuge, Eppendorf, Germany; NewClassic MF MS105DU Electronic Balance, Mettler Toledo, Switzerland.

Methods

Study design

This was a multicenter, randomized, double-blinded，placebo controlled clinical trial. Patient recruitment occurred between August 2023 and December 2024 from The First Affiliated Hospital of Henan University of Chinese Medicine, Henan Provincial People's Hospital, and Henan Province Hospital of Traditional Chinese Medicine. The trial was registered with Chinese Clinical Trial Registry (https://www.chictr.org.cn) under the identifier ChiCTR2300067859. Ethical approval was obtained from the Ethics Committee of The First Affiliated Hospital of Henan University of Chinese Medicine (2022HL-450-01), and the study was carried out following the Declaration of Helsinki and the guidelines for Good Clinical Practice. Participants provided

written informed consent for their participation and agreed to have their data published. The study was conducted in accordance with the CONSORT guidelines[5] to ensure methodological and reporting rigor.

Participants

The criteria for including patients were as follows: (1) Clinical Medicine Diagnostic Criteria: Based on the diagnostic criteria for insomnia disorder as specified in the International Classification of Diseases, 11th Revision (ICD-11) published in 2019[6].

(1) Chief complaints (at least one of the following must be present):

a. Difficulty falling asleep;

b. Difficulty maintaining sleep;

c. Early morning awakening;

d. Refusal to go to bed within an appropriate schedule;

e. Inability to fall asleep without assistance (requires the presence of another person to fall asleep).

(2) Daytime dysfunction (at least one of the following must be present):

a. Feelings of fatigue;

b. Difficulty concentrating;

c. Impaired communication or work performance;

d. Proneness to mood swings;

e. Daytime sleepiness;

f. Lack of physical energy;

g. Tension or headaches;

h. Increased errors or accidents;

i. Anxiety about sleep.

(3) The insomnia is not attributable to environmental or objective factors; (4) Sleep disturbances and associated daytime dysfunction occur at least three times per week;(5) Sleep disturbances and daytime dysfunction persist for at least three months; (6) Other sleep disorders have been ruled out. All six criteria must be met simultaneously for a diagnosis of insomnia disorder.

TCM Diagnostic Criteria: Based on the diagnostic guidelines outlined in the 2022 Expert Consensus on the Treatment of Adult Insomnia by Chinese Ethnic Medicine (issued by the Sleep Branch of the Chinese Ethnic Medicine Association)[7], and integrated with clinical expert experience, the diagnostic criteria for insomnia with liver depression and spleen deficiency syndrome were established as follows:

Symptoms: Insomnia with dream-disturbed sleep, depressive mood, hypochondriac and chest fullness, mental fatigue and reluctance to speak, physical weariness and lack of strength, poor appetite, belching and acid reflux. Pale tongue, slightly swollen tongue body or with teeth marks, white coating, wiry and weak pulse. Primary Symptoms: Difficulty falling asleep, easily awakened during sleep, difficulty returning to sleep after awakening, or in severe cases, sleeplessness throughout the night. Secondary Symptoms: Excessive dreaming, low mood or irritability, hypochondriac distension, epigastric fullness with poor appetite, palpitations, dizziness and blurred vision, mental exhaustion and fatigue, poor memory, dull or pale complexion. Tongue and Pulse Findings: Red tongue with white or greasy coating; wiry or thin pulse. Syndrome

Differentiation: Patients must present with corresponding tongue and pulse findings, along with at least one primary symptom and any three secondary symptoms.

The criteria for exclusion were: (1) Patients who regularly use sleep-inducing medications or health supplements; (2) History of other sleep-affecting disorders such as hypersomnia, circadian rhythm sleep disorder, sleepwalking, sleep-related breathing disorders, obstructive or central sleep apnea syndrome, restless legs syndrome, etc.; (3) Presence of severe psychiatric or emotional disorders within the past year (e.g., anxiety, depression, suicidal tendency or history), or long-term use of central nervous system depressants or stimulants; (4) A Hamilton Anxiety Scale (HAMA) score ≥ 14 or Hamilton Depression Scale (HAMD) score ≥ 17 at screening; (5) Other causes of insomnia within the past month, such as pain, fever, cough, surgery, external environmental disturbances, or stressful life events; (6) Comorbid severe systemic diseases affecting the cardiovascular, cerebrovascular, pulmonary, hepatic, renal, or hematopoietic systems, central nervous system disorders, or malignancy; (7) Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels exceeding 1.5 times the upper limit of normal, or serum creatinine (Scr) exceeding the upper limit of normal at screening; (8) Known allergy to any component of the investigational drug; (9) Pregnant or lactating women; (10) Individuals with alcohol abuse, substance abuse, or dependence on psychotropic drugs; (11) Participation in another drug clinical trial within the past month; (12) Any other condition deemed unsuitable for trial participation by the investigator.

Sample Size Calculation

Based on the results of a preliminary trial, the response rate was 95% in SJYP group and 83% in the control group. Using the formula for comparing two independent sample proportions, $n = 8pq / (p_1 - p_2)^2$, where $p_1 = 0.95$, $p_2 = 0.83$, $p = (p_1 + p_2)/2 = 0.89$, and $q = 0.11$, the calculated sample size per group was approximately $n \approx 55$. Accounting for an estimated dropout rate of 15%, the final sample size required was determined to be 65 participants per group, with a total of 130 participants across both groups.

Randomization and blinding

The grouping was performed using block randomization. The random allocation scheme and blinding codes were provided by the staff at the Center for Evidence-Based Medicine of the First Affiliated Hospital of Henan University of Chinese Medicine. Using R software and a predefined random seed number, the randomization details for this trial were generated, including random codes (i.e., drug numbers), group codes, and corresponding group information. After eligible patients provided signed informed consent, each center assigned participant numbers in ascending order based on the sequence of enrollment. Participants then received the study drugs corresponding to their assigned numbers.

The blinding methodology was implemented by personnel involved in drug preparation, statisticians, and individuals not otherwise associated with the trial. A two-level blinding system was adopted. First-level blinding referred to the blinding of the investigational drugs: medications in both groups were identical in appearance and uniformly packaged. Second-level blinding pertained to the coding of the drug packages: boxes for both groups were identical in size, color, label design, and other external features. The master blinding list was kept securely sealed and was

only accessible for emergency unblinding or at the conclusion of the trial.

Treatment Protocol

(1) Basic Treatment: Both groups received conventional sleep hygiene education to correct inappropriate sleep-related behaviors and beliefs[8]. Participants were instructed to take the medication regularly and cooperate actively with the treatment.

(2) Intervention Method for the SJYP Group: In addition to the conventional sleep hygiene education, participants in the SJYP group were administered the Chinese herbal compound SJYP in granule form (for composition is as shown in Table 1). The granules were dissolved in 200 ml of hot water and taken orally twice daily—once after lunch and once before bedtime - for a total duration of 4 weeks.

Table 1 Composition and proportion of SJYP

Chinese name	Botanical name	Latin name	English name	Medicinal part	Weight (g)	Category[a]
Chaihu	*Bupleurum chinense* DC.	*Bupleuri Radix*	Chinese Thorowax Root	root	10	Chief
Danggui	*Angelica sinensis* (Oliv.) Diels	*Angelicae Sinensis Radix*	Chinese Angelica	root	10	Deputy
Baishao	*Paeonia lactiflora* Pall.	*Paeoniae Alba Radix*	White Peony Root	root	15	Deputy
Huangqin	*Scutellaria baicalensis* Georgi	*Scutellariae Radix*	Baical Skullcap Root	root	9	Deputy
Fuling	*Poria cocos* (Schw.) Wolf	*Wolfiporia cocos*	Poria	sclerotium	15	Assistant
Dangshen	*Codonopsis pilosula* (Franch.) Nannf.	*Codonopsis Radix*	Codonopsis Root	root	10	Assistant
Baizhu	*Atractylodes macrocephala* Koidz.	*Atractylodis Macrocephalae Rhizoma*	White Atractylodes Rhizome	tuber	15	Assistant
Suanzhaoren	*Ziziphus jujuba var. spinosa* (Bunge) Hu ex H.F. Chow	*Ziziphi Spinosae Semen*	Spine Date Seed	seed	15	Assistant
Longyanrou	*Dimocarpus longan* Lour.	*Longan Arillus*	Longan Aril	arillus	15	Assistant
Zhizi	*Gardenia*	*Gardeniae*	Gardenia	fruit	10	Envoy

	jasminoides J. Ellis	*Fructus*	Fruit			
Zhigancao	*Glycyrrhiza uralensis* Fisch. ex DC.	*Glycyrrhizae Radix et Rhizoma Praeparata cum Melle*	Honey-fried Licorice Root	root	10	Envoy

[a] The herbs in Chinese formula are categoried into chief, Deputy, Asistant and Eivoy based on their finctions and efects. The Chief herbs) provides the mostpotentherapeutic action targeting main disease or symptoms, while the Deputy and Asistant strengthen the finction ofthe Chief or targetsthe concurent diseases orsymptoms. The Envoy works by guiding the treatment action and balancing the effect of other drugs.

(3) Intervention Method for the control group: Along with conventional sleep hygiene education, the control group received a placebo granules matching SJYP. The placebo, prepared under the guidance of pharmaceutical experts, was identical to the SJYP medication in appearance, color, and odor. The administration method was the same as that in the SJYP group.

Outcomes

(1) Primary Efficacy Evaluation Indicator:

Efficacy was assessed based on the reduction rate in PSQI scores and improvement in clinical symptoms after treatment, categorized into four levels: cured, markedly effective, effective, and ineffective. The PSQI reduction rate (%) was calculated as follows: (Total score before treatment - Total score after treatment) / Total score before treatment × 100%. A reduction rate ≥ 75% was considered cured; 50% ≤ reduction rate < 75% as markedly effective; 25% ≤ reduction rate < 50% as effective; and reduction rate < 25% as ineffective. The total effective rate (%) = (Number of cured + markedly effective + effective cases) / Total cases × 100%.

(2) Secondary Efficacy Evaluation Indicators:

① PSQI Scores: Used to evaluate sleep quality[9]. This assessment tool covers seven dimensions: sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction. Evaluations were conducted at baseline (on the day of enrollment) and after 4 weeks of treatment.

② ISI Scores: A scale used to determine the severity of insomnia, reflecting the patient's sleep condition over the past two weeks. Symptoms are rated on a 5-point scale of severity: none, mild, moderate, severe, and very severe, corresponding to scores of 0, 1, 2, 3, and 4, respectively. Assessments were performed at enrollment, after 4 weeks of treatment, and at 6 and 8 weeks post-treatment.

③ TCM Syndrome Evaluation Scale Scores: Developed with reference to the Chinese Guidelines for Diagnosis and Treatment of Insomnia[10], this scale assesses symptoms of insomnia due to liver depression and spleen deficiency. The improvement of patients' primary and secondary symptoms was observed. Primary symptoms included difficulty falling asleep, dream-disturbed and easy awakening, difficulty in resuming sleep after awakening, and insomnia throughout the night. Secondary symptoms encompassed low or irritable mood, dizziness and blurred vision, fullness in chest, mental fatigue and lack of energy, epigastric stuffiness and poor appetite, belching and acid regurgitation, palpitations, forgetfulness, and dull complexion. Each symptom is rated as absent, mild, moderate, or severe, corresponding to scores of 0, 1, 2, and 3,

respectively. Evaluations were conducted at enrollment and after 4 weeks of treatment.

④ Patients' mental and psychological status were evaluated with the Hamilton Anxiety Rating Scale (HAMA) and the Hamilton Depression Rating Scale (HAMD)[11]. HAMA is primarily designed to assess anxiety symptoms in subjects, while HAMD is employed to evaluate the clinical depressive state of patients. Assessments were conducted at baseline and after 4 weeks of treatment.

(3) Safety Indicators:

Vital signs (body temperature, respiration, pulse, blood pressure), complete blood count, urinalysis, liver function, renal function, and electrocardiogram (ECG). These were assessed at enrollment and after 4 weeks of treatment.

Metabolomics

SJYP group patients serum samples were collected at 2 time points (pre-treatment baseline, 4 week) and 30 healthy individuals for Metabolomics testing and analysis. Blood samples from all participants, which were confirmed to have been collected after an overnight fasting period, were obtained in the morning and promptly centrifuged within 15 min at 3000 rpm for 10 min at 20 ℃. A 0.6 mL aliquot of the separated supernatant was transferred to cryovial tubes and immediately stored at -80℃ for subsequent serum metabolomic analysis.

The LC-MS/MS analysis of sample was conducted on a UHPLC-Orbitrap Exploris 240 system equipped with an ACQUITY HSS T3 column (100 mm × 2.1 mm i.d., 1.8 μm; Waters, USA) at Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The mobile phases consisted of 0.1% formic acid in water:acetonitrile (2:98, v/v) (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The flow rate was 0.40 mL/min and the column temperature was 40℃. The injection volume was 5 μL. MS conditions: The UPLC system was coupled to a UHPLC-Orbitrap Exploris 240 system Mass Spectrometer equipped with an electrospray ionization (ESI) source operating in positive mode and negative mode. The optimal conditions were set as followed: source temperature at 400℃; sheath gas flow rate at 40 arb; Aux gas flow rate at 10 arb; ion-spray voltage floating (ISVF) at -2800V in negative mode and 3500V in positive mode, respectively; Normalized collision energy , 20-40-60V rolling for MS/MS. Data acquisition was performed with the Data Dependent Acquisition (DDA) mode. The detection was carried out over a mass range of 70-1050 m/z.

Statistical Analysis

All data were analyzed using SPSS software (version 21.0). Categorical data were compared using the chi-square test and expressed as frequency and percentage (n, %). For continuous data, if normally distributed, results were presented as mean ± standard deviation ($\bar{x}\pm s$); if not normally distributed, median (IQR) were used. Ordinal data were analyzed using the rank-sum test. Comparisons of pre- and post-treatment scores were performed using t-tests, Mann-Whitney U tests, Wilcoxon signed-rank tests, or analysis of covariance (ANCOVA), as appropriate. A two-sided test was used, with $p<0.05$ considered statistically significant.

Results

Demographics and basic characteristics

The flowchart of this trial is presented in Fig.1. From August 2023 to December 2024, a total of 173 people with insomnia disorder. After being screened, 130 participants were randomly assigned toreceive SJYP (n=65) and placebo (n=65). 2 participants were excluded due to withdrawal or loss to follow-up in control group. Finally, 128 participants completed the study. Consequently, data from these 128 patients were utilized for subsequent analyses. Statistically significantdifferences were not observed between the two groups concerninggender, age, disease progression (Table 2).

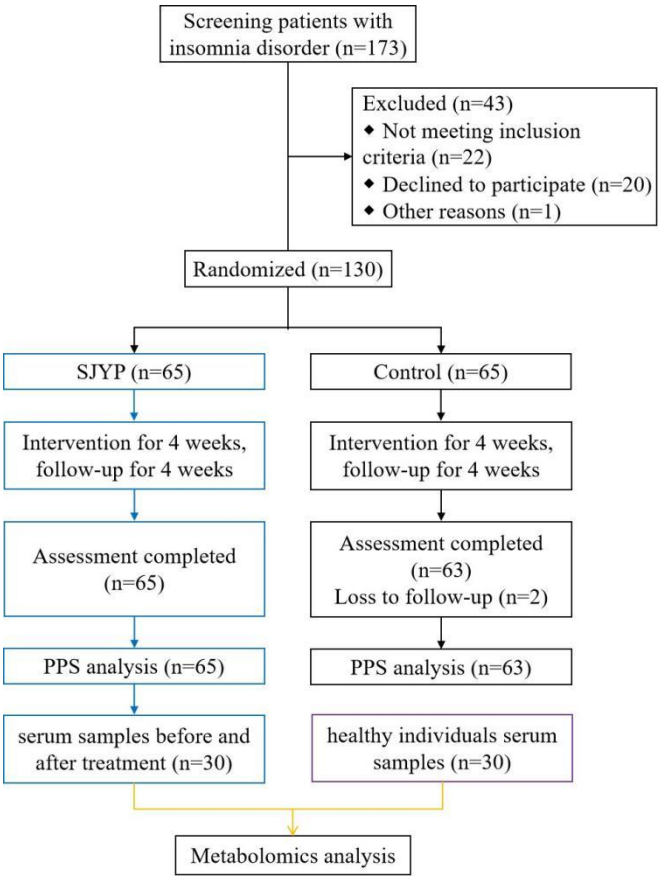


Fig.1 The flow chart of patients recruiting and following up. SJYP, Shugan Jianpi Yangxin Prescription. PPS, per protocol set.

Table 2 Baseline demographic and clinical characteristics

Characteristics	**Control (n=63)**	**SJYP (n=65)**	***p* value**
Gender, n (%)			
Male	22 (17.19%)	19 (14.84%)	0.490
Female	41 (32.03%)	46 (35.94%)	
Age (years), median (IQR)	30 (25，42)	34 (25，49)	0.471
disease progression (months), median (IQR)	24 (6，72)	24 (6，48)	0.460

Primary outcomes

Based on the reduction rate of PSQI scores before and after treatment, the total effective rates were calculated. After 4 weeks of treatment, the effective rate was 69.84% (44/63) in the control group and 93.85% (61/65) in the treatment group. The treatment group showed a significantly higher total effective rate than the control group ($p < 0.05$) (Table 3).

Table 3 Comparison of clinical efficacy between two groups of patients

	Control (n=63)	**SJYP (n=65)**	***p* value**
Efficacy, n (%)			
Considered cured	5 (7.94%)	20 (30.77%)	
Markedly effective	21 (33.33%)	25 (38.46%)	
Effective	18 (28.57%)	16 (24.62%)	
Ineffective	19 (30.16%)	4 (6.15%)	
Total effective rate	44 (69.84%)	61 (93.85%)[a]	0.000

[a] compared with the control group after treatment, $p<0.05$

The change of PSQI scoring: In baseline, no significant difference was found between groups of PSQI scores ($p>0.05$). After a 4-week treatment, the difference in the PSQI total score between the treatment group and the control group was significantly lower ($p=0.000$)(Fig.2).The treatment group had significantly lower scores than the control group in the following factors: sleep quality ($p=0.010$), sleep latency ($p=0.016$), sleep duration ($p=0.002$), sleep efficiency ($p=0.027$), sleep disturbances ($p=0.009$), and daytime dysfunction ($p=0.015$). However, there was no significant difference in the use of sleep medication scores ($p=0.981$) between the treatment group and the control group (Table 4).

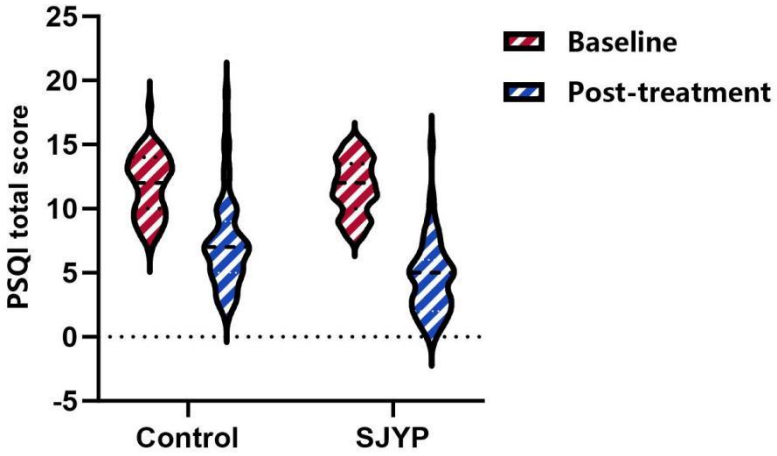


Fig.2 Violin plot of PSQI total score before and after treatment in both groups

Table 4 PSQI scores before and after treatment in both groups, median (IQR)

	Control (n=63)		**SJYP (n=65)**	
	Baseline	Post-treatment	Baseline	Post-treatment
sleep quality	2 (2，2)	1 (1，2)[a]	2 (2，3)	1 (1，1)[ab]

sleep latency	3 (2，3)	1 (1，3)[a]	3 (2，3)	1 (0，2)[ab]
sleep duration	2 (1，2)	1 (1，2)[a]	2 (1，2)	1 (0，1)[ab]
sleep efficiency	2 (1，3)	1 (0，2)[a]	2 (1，3)	0 (0，1)[ab]
sleep disturbances	1 (1，2)	1 (1，1)[a]	1 (1，2)	1 (0，1)[ab]
use of sleep medication	0 (0，1)	0 (0，0)[a]	0 (0，0.5)	0 (0，0)[a]
daytime dysfunction	1 (1，1)	1 (0，1)[a]	1 (1，2)	0 (0，1)[ab]
PSQI scores	12 (10，14)	7 (5，9)[a]	12 (10，13.5)	5 (2，6)[ab]

[a] defined as compared with the group before treatment, $p<0.05$

[b] comparison with the difference after treatment in the control group, $p<0.05$

Secondary Efficacy Evaluation Indicators

In baseline, no significant difference was found between groups of ISI scores ($p>0.05$). After a 4-week treatment, the treatment group demonstrated a significantly greater reduction in the ISI score compared to the control group ($p<0.05$). The reduction of ISI scoring in SJYP group persisted in the follow-up period (Table 5)(Fig.3).

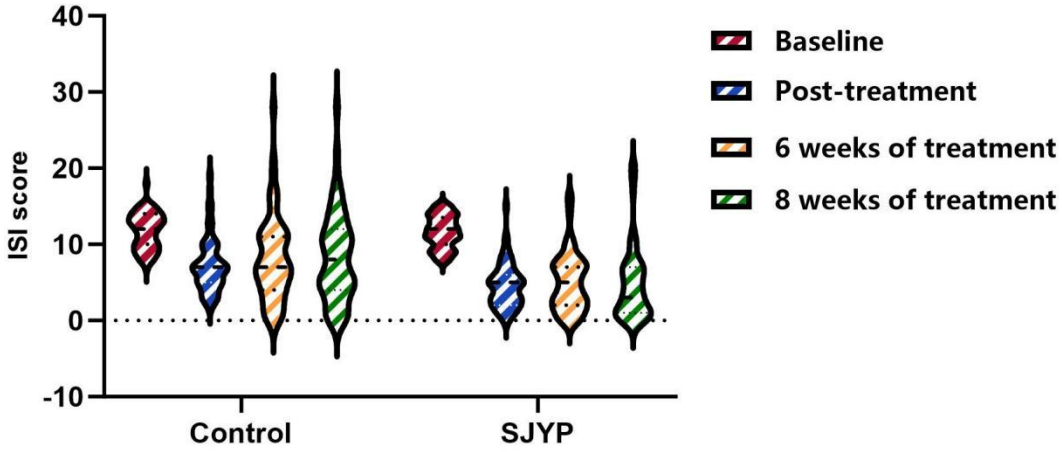


Fig.3 Violin plot of ISI scores before and after treatment in both groups

Table 5 ISI scores before ,after treatment and follow-up period in both groups

	Control (n=63)	**SJYP (n=65)**
Baseline, median (IQR)	15 (13，18)	14 (13，17)
Post-treatment, median (IQR)	7 (4，11)[a]	5 (2.5，7)[ab]
6 weeks of treatment, mean ± SD	8.05±5.48[a]	5.02±3.77[ab]
8 weeks of treatment, mean ± SD	8.25±5.82[a]	4.75±4.80[ab]

[a] defined as compared with the group before treatment, $p<0.05$

[b] comparison with the difference after treatment in the control group, $p<0.05$

SD: standard deviation

No significant difference in the total scores was observed between the two groups in baseline (p>0.05), both groups demonstrated reductions in the TCM syndrome scale scores after treatment, with the treatment group exhibiting a more pronounced effect.Comparison of the post-treatment reductions in the TCM syndrome scale factor scores between the two groups revealed that the SJYP group showed significantly greater reductions in scores for difficulty falling asleep, dream-disturbed and easy awakening, low or irritable mood, mental fatigue and lack of energy, poor appetite, forgetfulness, and dull complexion (p<0.05) (Table 6).

Table 6 TCM Syndrome Evaluation Scale Scores before and after treatment in both groups, median (IQR)

Factor	**Control (n=63)**		**SJYP (n=65)**	
	Baseline	Post-treatment	Baseline	Post-treatment
difficulty falling asleep	2 (2，3)	1 (1，2)[a]	3 (2，3)	1 (1，2)[ab]
dream-disturbed and easy awakening	2 (2，3)	1 (1，2)[a]	2 (1，3)	1 (0，1)[ab]
difficulty in resuming sleep after awakening	2 (1，3)	1 (0，2)[a]	2 (1，3)	1 (0，1)[a]
insomnia throughout the night	1 (1，1)	0 (0，1)[a]	1 (0，1)	0 (0，0)[a]
low or irritable mood	1 (1，1)	1 (1，2)	2 (1，2)	1 (0，1)[ab]
dizziness and blurred vision	1 (0，1)	0 (0，1)	1 (0，1)	0 (0，1)[a]
fullness in chest	1 (0，1)	0 (0，1)	1 (0，1)	0 (0，1)[a]
mental fatigue and lack of energy	1 (1，2)	1 (0，2)	2 (1，2)	1 (0，1)[ab]
epigastric stuffiness and poor appetite	1 (0，1)	0 (0，1)	1 (0，2)	0 (0，1)[ab]
belching and acid regurgitation	0 (0，1)	0 (0，1)	0 (0，1)	0 (0，0.5)
palpitations	1 (0，1)	0 (0，1)	1 (0，1)	0 (0，1)[a]
forgetfulness	1 (1，1)	1 (0，1)	1 (1，2)	1 (0，1)[ab]
dull complexion	1 (0，1)	1 (0，1)	1 (0，1)	0 (0，1)[ab]
Total scores, mean ± SD/ median (IQR)	14.57±3.25	9 (5，14)[a]	15.89±4.08	5 (3，8)[ab]

[a] defined as compared with the group before treatment, p<0.05

[b] comparison with the difference after treatment in the control group, p<0.05

SD: standard deviation

No significant differences in HAMA and HAMD scores were observed between the two groups at baseline (p>0.05). After four weeks of treatment, the treatment group demonstrated a significantly greater reduction in both HAMA and HAMD scores compared to the control group (p<0.05) (Table 7).

Table 7 HAMA and HAMD scores before and after treatment in both groups, median (IQR)

	Control (n=63)		**SJYP (n=65)**	
	Baseline	Post-treatment	Baseline	Post-treatment
HAMA	12（10，13）	6（4，9）[a]	11（8，13）	3（2，5）[ab]
HAMD	9（7，14）	6（4，8）[a]	10（6.5，12）	3（2，5.5）[ab]

[a] defined as compared with the group before treatment, p<0.05

[b] comparison with the difference after treatment in the control group, p<0.05

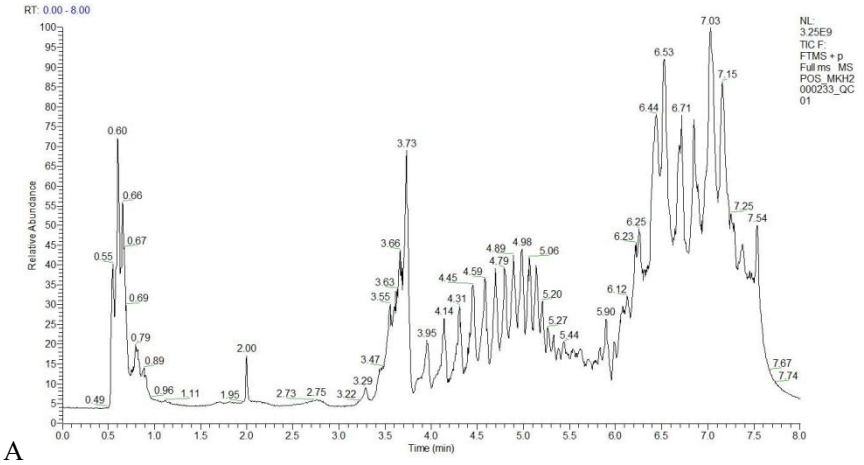
Safety profile

No adverse events were reported during the trial. No abnormal laboratory exami-nation was reported.

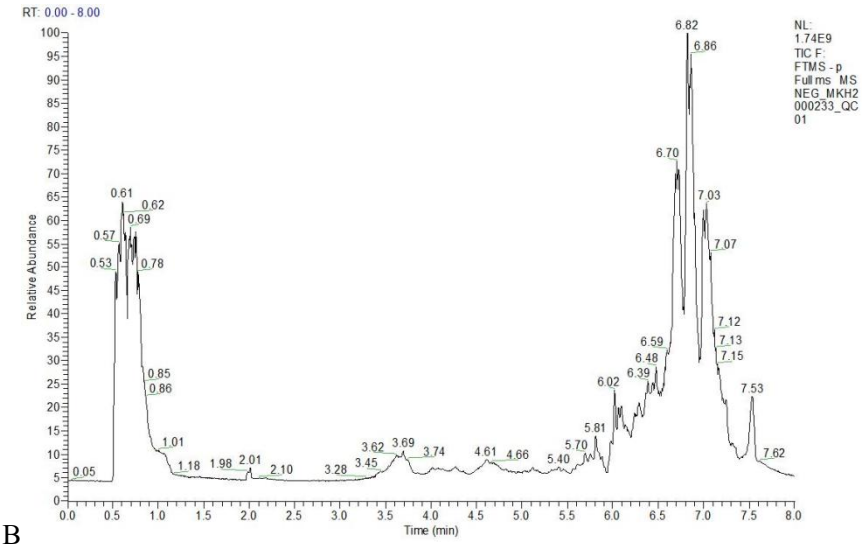
Metabolomics Results

Results of the total ion current chromatogram analysis for the QC sample

Serum samples were collected from 30 subjects each in the treatment group, the control group, and a cohort of healthy individuals for metabolomic analysis. The analysis was performed using LC-MS/MS technology, with quality control (QC) samples analyzed in both positive and negative ion modes to obtain total ion chromatograms (TIC), as shown in Fig.4. Overlay and visual analysis of the total ion chromatograms revealed a high degree of overlap at different time points, indicating strong instrumental signals, high peak intensity, and good reproducibility in retention time during repeated analysis of the same sample.



A



B

Fig.4 TIC diagram obtained by LC-MS/MS analysis in positive and negative ion modes

Results of pattern analysis of serum samples

The Normal group consisted of serum samples from 30 healthy individuals. The Insomnia group comprised the pre-treatment serum samples from the 30 patients in the SJYP group, while the Treatment group corresponded to their post-treatment serum samples. The processed data were imported into analytical software for multivariate statistical analysis. In this model, 200 random permutation tests were performed, and a PLS-DA permutation validation plot was generated. In both positive and negative ion modes, a separation trend was observed among the Normal, Insomnia, and Treatment groups, with model metrics of R2X = 0.639, R2Y = 0.974 (p<0.05), and Q2 = 0.816 (p<0.05), indicating high goodness-of-fit and predictive ability, as shown in Fig.5. To further characterize intergroup differences, an OPLS-DA model was employed to more accurately identify differential metabolites. Accordingly, OPLS-DA was performed pairwise among the Normal, Insomnia, and Treatment groups. Clear separation of metabolic profiles was achieved between all group comparisons. Results showed that in both positive and negative ion modes, the comparison between the Normal and Insomnia groups yielded R2Y (cum) and Q2 (cum) values of 0.986 and 0.895, respectively, while the comparison between the Treatment and Insomnia groups gave corresponding values of 0.993 and 0.588. These results confirm the absence of overfitting and demonstrate the reliability and validity of the OPLS-DA model, as depicted in Fig.6A and Fig.6B.

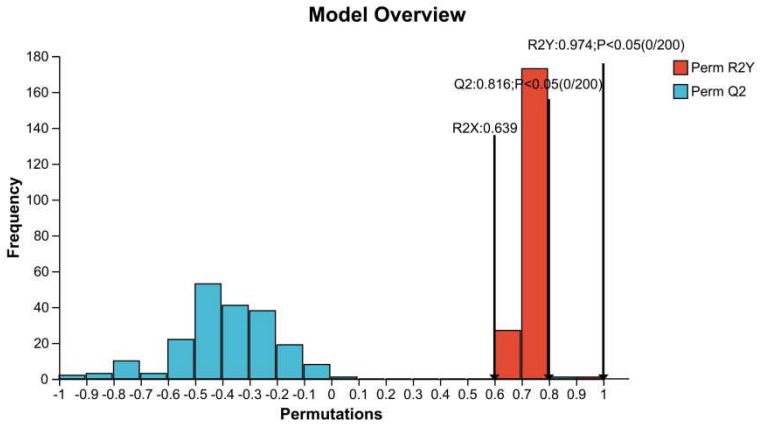
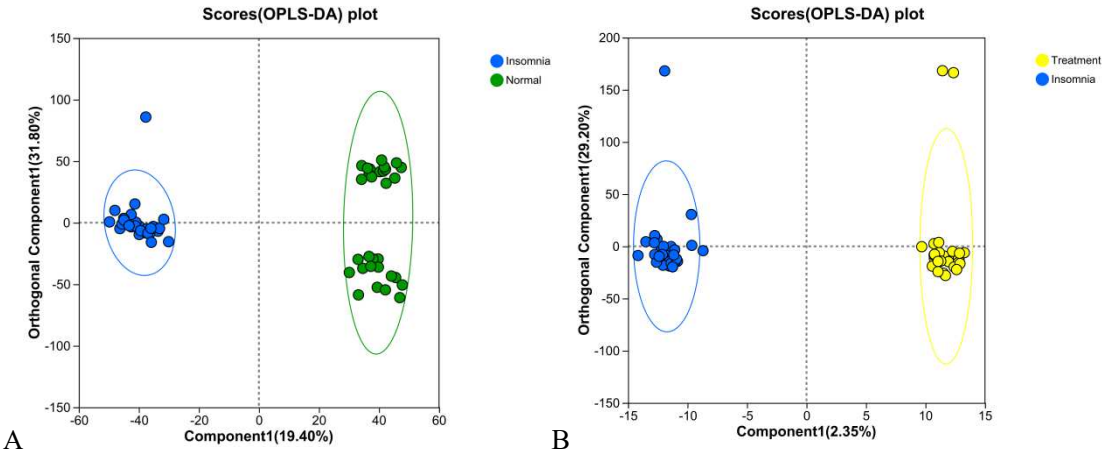


Fig.5 PLS-DA permutation test chart



A B

Fig.6 A：Normal group and Insomnia group OPLS-DA shot chart; B：Treatment group and Insomnia group OPLS-DA shot chart

Identification of differential metabolites

To further elucidate the potential mechanism of SJYP in treating insomnia disorder, differential metabolites were screened. Under the OPLS-DA model, variables between the Normal and Insomnia groups, as well as between the Treatment and Insomnia groups, in both positive and negative ion modes were subjected to univariate analysis. Metabolites with VIP > 1, $p < 0.05$, and FC > 1 were selected as significantly altered variables between groups[12]. Statistical analysis, including OPLS-DA, was then performed on the abundance values of differential metabolites using the ropls package (Version1.6.2) in R. By leveraging the HMDB and KEGG database, common differential metabolites between the Normal and Insomnia groups and the Treatment and Insomnia groups were identified. Pathway enrichment analysis was carried out with the scipy.stats package in Python. After excluding non-endogenous metabolites and duplicates, 370 metabolites with significant alterations between the Insomnia and Normal groups were predicted as potential biomarkers for insomnia disorder. Among these, 260 were up-regulated and 110 were down-regulated. Similarly, 202 metabolites showing significant differences between the Treatment and Insomnia groups were predicted as potential biomarkers for the therapeutic model of insomnia. Of these, 66 were up-regulated and 136 were down-regulated.Finally, 28 common differential

metabolites were identified, including Pectenotoxin 2, Val-Arg, Thr-Leu, Ala-Leu, Ile-Leu, Val-Ile, N-Lactoyl-Phenylalanine, and O-Acetylcarnitine. Compared to the Insomnia group, the Treatment group exhibited up-regulation of 8 differential metabolites and down-regulation of 20 differential metabolites, as listed in Table 8. Among these, 3 metabolites were lipids, 4 were amino acids, 3 were terpenoids and their glycosides, 9 were peptides, and 9 were other organic compounds. This regulatory pattern suggests that SJYP may exert its therapeutic effects on insomnia disorder by modulating the expression of these characteristic metabolites.

Table 8 differential metabolites

metabolite	M/Z	VIP	Relative content of differential metabolites	
			Insomnia group-Normal group	Treatment group-Insomnia group
Ethyl 2-Cyano-3-[(2-Oxoazepan-3-Yl)Amino]Acrylate	315.141	4.7927	↑[1)]	↓[2)]
1-(2,8-Dioxo-1,7-Diazatricyclo[7.3.0.03,7]Dodecan-3-Yl)Propan-2-Yl Carbamate	359.167	4.261	↑[1)]	↓[2)]
Benazeprilat	837.3394	3.1226	↑[1)]	↓[2)]
Pectenotoxin 2	903.4711	2.9847	↑[1)]	↓[2)]
Protoporphyrinogen Ix	569.3132	2.9385	↑[1)]	↓[2)]
Phe Leu Ala	350.2067	2.8645	↑[1)]	↓[2)]
3-[(2-Fluorophenyl)Methyl]-6-(Methoxymethyl)-2-Pyrrolidin-1-Ylpyrimidin-4-One	318.1642	2.4303	↑[1)]	↓[2)]
Val-Met-His	769.3518	2.3744	↑[1)]	↓[2)]
Rubiscolin-6	747.3697	2.1254	↑[1)]	↓[3)]
(3B,16A,20R)-25-Acetoxy-3,16,20,22-Tetrahydroxy-5-Cucurbiten-11-One 3-Glucoside	749.381	2.1057	↑[1)]	↓[2)]
Alpha-Ionol O-[Arabinosyl-(1->6)-Glucoside]	453.2447	2.087	↑[1)]	↓[2)]
N,N'-Diethylthiourea	133.079	2.0028	↑[1)]	↓[2)]
5-(4-Phenoxybutoxy)Psoralen	351.123	1.8756	↑[1)]	↓[2)]
[(2R,3S,4R,5R)-3,4,5,6-Tetrahydroxy-1-Oxohexan-2-Yl] (2S)-2-Aminopropanoate	290.0657	1.7931	↑[1)]	↓[2)]
Thr-Leu	233.1491	1.6808	↑[1)]	↓[2)]
Val Arg	274.1864	1.6169	↑[1)]	↓[2)]
Ala-Leu	203.1385	1.4331	↑[1)]	↓[2)]
2-Amino-2-(5-Methyl-3-Oxo-1,2-Oxazol-4-Yl)Propanoic Acid	209.0529	1.2887	↑[1)]	↓[2)]
Ile-Leu	245.1854	1.2034	↑[2)]	↓[4)]
Val-Ile	231.1698	1.1737	↑[1)]	↓[2)]

N-Lactoyl-Phenylalanine	238.1069	2.9681	↓[1)]	↑[2)]
Citronellyl Beta-Sophoroside	481.2611	1.7079	↓[1)]	↑[2)]
O-Acetylcarnitine	204.1225	1.4915	↓[1)]	↑[2)]
Pe(5-Iso Pgf2Vi/14:1(9Z))	754.4255	2.6763	↓[1)]	↑[2)]
N-Lactoylleucine	204.1227	2.0215	↓[1)]	↑[2)]
Nodularin	842.4767	3.178	↓[1)]	↑[2)]
Moxidectin	644.3559	1.0609	↓[1)]	↑[2)]
N,N-Dibutylformamide	158.1537	1.6022	↓[1)]	↑[2)]

[1)] compared with the Normal group, $p<0.05$; [2)] compared with the Insomnia group, $p<0.05$

Analysis of metabolic pathways of metabolites with differential expression in samples

Metabolic pathway analysis of the differential metabolites between the Treatment and Insomnia groups was performed. This study identified the metabolic pathways influenced by SJYP in insomnia disorder, which included pathways such as FoxO signaling pathway, Bile secretion, Retrograde endocannabinoid signaling, Glycine, serine and threonine metabolism, Protein digestion and absorption, Synaptic vesicle cycle, GABAergic synapse, Arginine and proline metabolism, etc(Fig.7). The results of the pathway analysis suggest that SJYP may exert its therapeutic effects on insomnia disorder by modulating these specific metabolic pathways.

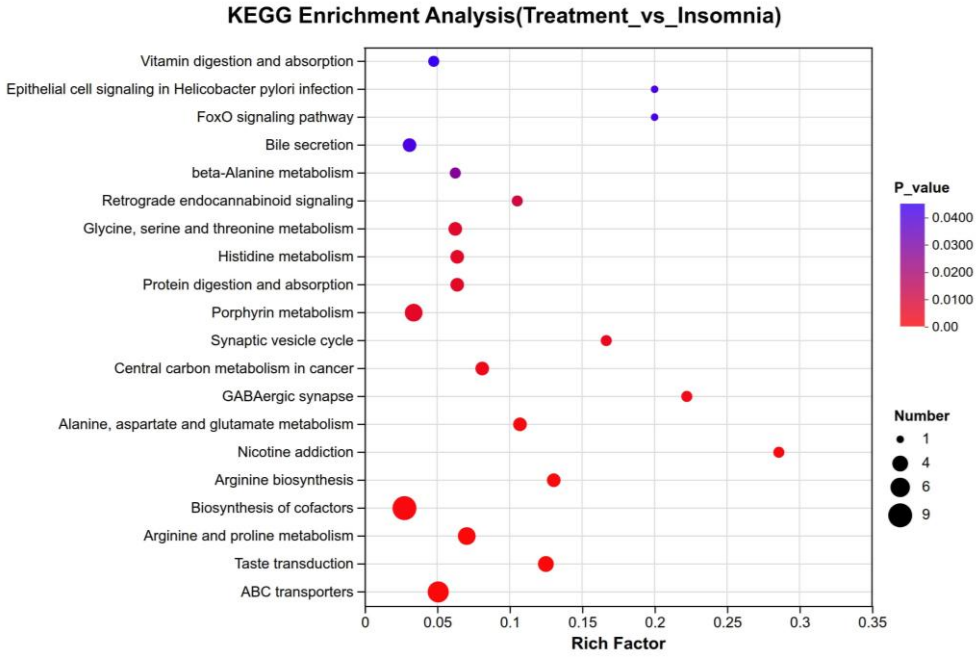


Fig.7 SJYP improves insomnia disorder related metabolic pathway

Discussion

This multicenter, randomized, double-blind, placebo-controlled trial demonstrated that the SJYP significantly improved sleep quality, reduced insomnia severity, alleviated TCM syndrome manifestations, and ameliorated comorbid anxiety and depressive symptoms in patients with insomnia disorder of liver depression and spleen deficiency type. These clinical benefits were sustained during follow-up, underscoring the prescription's stable and prolonged therapeutic effect. Importantly, the integrated metabolomic analysis revealed that SJYP's mechanism may involve the modulation of specific metabolic pathways, including GABAergic synapse, FoxO signaling, and retrograde endocannabinoid signaling, providing a novel biochemical perspective on its efficacy.

Insomnia disorder has a high disease and economic burden, it is a risk factor for mental and physical disorders[13]. Chronic use of sedative-hypnotics is detrimental to sleep regulation and cognition at the macro and micro-level[14]. In recent years, TCM holds great potential for advancing personalized and effective insomnia disorder treatments globally[15]. A study investigating the distribution of liver depression-related syndromes in primary insomnia found that liver depression-spleen deficiency syndrome accounted for the highest proportion[16]. To our knowledge, there is currently a relative scarcity of randomized controlled trials on TCM for the treatment of insomnia disorder, particularly regarding insomnia disorder with the type of liver depression and spleen deficiency. Therefore, we studied the clinical efficacy of SJYP for insomnia disorder with liver depression and spleen deficiency type and employed untargeted metabolomics to explore its potential mechanism of action.

Insomnia disorder is primarily characterized by clinical manifestations such as difficulty initiating sleep, difficulty maintaining sleep, early morning awakening, easy awakening with difficulty returning to sleep, or even sleeplessness throughout the night, accompanied by daytime functional impairment (e.g., fatigue, depressed mood, cognitive deficits)[17]. Our findings align with and extend previous reports on TCM interventions for insomnia[18,19]. While prior studies have highlighted the role of herbal compounds in improving sleep, high-quality evidence from rigorous randomized controlled trials remains limited, particularly for syndrome-specific formulations such as SJYP. The observed reduction in PSQI and ISI scores corroborates the prescription's core hypnotic effect, but more notably, its positive impact on daytime dysfunction and emotional symptoms addresses broader aspects of insomnia-related impairment. This multifactorial improvement supports the TCM holistic approach, which targets both the symptoms and root causes of the disorder[20].

TCM theory considers sleep to be a process of harmony between Yin and Yang works as Yang enters Yin leads to sleep and Yang comes out of Yin leads to wakefulness. Insomnia disorder is considered a result of disharmony between Yin and Yang[21]. The clinical manifestations of TCM symptoms in patients with insomnia disorder also deserve attention. Most patients with chronic insomnia disorder typically experience persistent negative emotional states and symptoms, such as low mood or irritability[22]. Chronic insomnia can lead to loss of appetite by affecting the nervous and digestive systems[23]. According to TCM theory , SJYP evolved from TCM classic formula Xiaochaihu decoction, Guipi decoction and Xiaoyao San. SJYP targets the TCM symptoms caused by insomnia disorder for therapeutic intervention. In our study, SYJP effectively improved patients' symptoms of difficulty falling asleep, dream-disturbed and easy awakening,

difficulty in resuming sleep after awakening, and insomnia throughout the night. Furthermore, TCM syndromes such as low or irritable mood, mental fatigue and lack of energy, epigastric stuffiness and poor appetite, forgetfulness, and dull complexion were significantly alleviated after SYJP intervention, demonstrating significantly greater improvement compared to the control group.

There is a well-established link between sleep disorders and psychological problems such as anxiety and depression[24]. Insomnia may contribute to the development of psychiatric disorders and increase the risk of depression[25]. Therefore, insomnia disorder patients' mental and psychological well-being also deserves attention. Our study found that SJYP can reduce both HAMA and HAMD scale scores in patients with insomnia disorder and alleviate their anxiety and depressive symptoms.

Prior studies have confirmed that the SJYP exhibits sedative and hypnotic effects, demonstrating certain therapeutic efficacy against insomnia disorder. The underlying mechanism is associated with increased 5-HT levels in the brain and suppressed expression of orexin-A (OXA) and its receptors in the hypothalamus[26]. In studies on sleep-related psychiatric disorders, serum or plasma is typically utilized for metabolomic analysis to assess aberrant sleep regulation mechanisms or pharmacological actions[27]. The metabolomic results offer a compelling explanatory layer for SJYP’s clinical effects. The identification of 28 common differential metabolites—including peptides such as Val-Arg, Thr-Leu, and lipids like O-Acetylcarnitine - suggests that SJYP may rectify metabolic disturbances characteristic of insomnia disorder. These differential metabolites primarily involve peptides, amino acid derivatives, lipids, terpenoids, phenylpropanoids, polyketides, pharmaceuticals and their metabolites, as well as other organic compounds. Among them, peptides and amino acid derivatives may be associated with neuromodulation and sleep-related pathways[28]; lipids and terpenoids may reflect energy metabolism and the influence of plant secondary metabolites[29,30]; phenylpropanoids and polyketides, likely derived from bioactive constituents of Chinese herbal medicine, have been reported to exhibit sedative-hypnotic effects[31]. These metabolites offer valuable clues for further exploration of the metabolic mechanisms underlying the treatment of insomnia with Chinese herbal formulas. Notably, several of these metabolites are linked to neurotransmitter synthesis and energy metabolism, processes known to be disrupted in sleep disorders[32]. Previous study have indicated that N-Lactoyl-phenylalanine is associated with sleep phenotypes[33]. However, their specific regulatory roles and underlying mechanisms in the treatment of insomnia disorder warrant further investigation.

This study identified the metabolic pathways influenced by SJYP in insomnia disorder, which included pathways such as FoxO signaling pathway, Bile secretion, Retrograde endocannabinoid signaling, Glycine, serine and threonine metabolism, Protein digestion and absorption, Synaptic vesicle cycle, GABAergic synapse, Arginine and proline metabolism. The enrichment of pathways such as GABAergic synapse and retrograde endocannabinoid signaling is particularly relevant, as both are critically involved in sleep-wake regulation and synaptic homeostasis. Research has shown that GABAergic synaptic transmission and plasticity were closely connected with sleep and wake[34]. Pathway enrichment highlighted GABAergic synapse signaling as a key mechanism underlying insomnia[35]. Recent studies have demonstrated that Chinese herbal formulas can regulate the GABAergic system through multiple pathways, including increasing GABA levels, activating GABA receptors, and inhibiting GABA transaminase activity, thereby exerting

synergistic sedative and sleep-promoting effects[36]. Our study found that following SJYP treatment, patients with insomnia disorder exhibited significant alterations in serum metabolomic profiles. The marked enrichment of the GABAergic synapse pathway suggests that SJYP may restore sleep-wake homeostasis by improving GABAergic signaling. The activation of retrograde endocannabinoid signaling facilitates the recovery of synaptic impairments caused by sleep deprivation[37]. Protein digestion and absorption provides essential amino acids for the synthesis of sleep related neurotransmitters. Older adults with Insomnia with short sleep duration consumed lower levels of amino acids such as phenylalanine, tyrosine, and valine, which are known to play a role in sleep regulation[38]. Sleep disruption alters bile acid homeostasis[39]. Research has shown that insomnia is associated with elevated levels of secondary bile acids, such as deoxycholic acid (DCA), DCA could be considered a potential biomarker for this disease[38]. One of the key metabolic pathways which alleviates sleep deprivation was arginine and proline metabolism[18]. These data imply that SJYP does not merely induce sedation but may facilitate the restoration of neurochemical balance, thereby promoting natural sleep architecture. However, their specific regulatory roles and mechanisms in the treatment of insomnia disorder remain to be further explored.

A key innovative aspect of this study is the integration of rigorous clinical trial design with untargeted metabolomics—a approach seldom applied in TCM insomnia disorder research. This methodology not only validates SJYP's efficacy but also illuminates potential mechanistic targets, bridging TCM theory with contemporary pathophysiology. For instance, the modulation of FoxO signaling and glycine-serine-threonine metabolism aligns with emerging evidence on circadian regulation and sleep-related metabolic adaptation. FOXO, which has been reported to regulate synapse plasticity of neurons,could alleviate the influence of sleep loss[40]. A study explored potential connections between FoxO signaling pathway and circadian dysregulation[41]. Chronic sleep disorder significantly suppressed glycine, serine and threonine metabolism[42]. Further studies confirmed that increasing the levels of glycine, serine, and threonine can significantly prolong sleep duration and improve sleep quality[43]. The enrichment of the FoxO signaling pathway, as well as glycine, serine, and threonine metabolism in this study suggests that SJYP may synergistically ameliorate energy metabolism disturbances and sleep-wake rhythm dysregulation in patients with insomnia disorder by restoring the normal function of FoxO signaling and improving amino acid metabolic processes. Such findings position SJYP as a modulator of multiple intersecting pathways, offering a plausible explanation for its broad-spectrum efficacy on sleep, mood, and somatic symptoms.

The enrichment of the bile secretion pathway provides a new perspective on gut-brain axis regulation in SJYP's amelioration of insomnia disorder. Circadian rhythm disruption can disturb bile acid homeostasis, leading to liver and intestinal inflammation as well as microecological dysbiosis. Studies have shown that a prebiotic diet can promote the recovery and recalibration of sleep and core body temperature rhythms following circadian disruption by increasing the abundance of beneficial gut bacteria and altering the fecal bile acid profile. Notably, specific gut microbiota such as Ruminiclostridium 5 are significantly associated with changes in bile acids and the recovery of sleep[44]. This suggests that bile acid metabolism may participate in the maintenance of sleep-wake cycle homeostasis via the microbiota-gut-brain axis, and that abnormalities in this pathway may constitute a key component of the metabolic disturbances underlying insomnia disorder. Therefore, we hypothesize that SJYP may synergistically improve

sleep by modulating bile acid metabolism and indirectly influencing the function of the microbiota-gut-brain axis, thereby providing further mechanistic evidence for the holistic regulatory effects of this formula.

This study represents the first randomized controlled trial to investigate Traditional Chinese Medicine for insomnia disorder combined with metabolomic analysis. However, our trial had some limitations. First, the study enrolled only Chinese participants, who are mostly familiar with CHM interventions. This may potentially impact the generalizability of our findings, as patients from other countries may not exhibit the same level of acceptance. Second, while the metabolomic analysis identified promising pathways, further functional validation in experimental models is warranted to establish causality. Third, the 8-week follow-up period, though suggestive of sustained benefit, calls for longer-term studies to assess durability and relapse prevention. Future research could focus on the following directions: expanding the ethnic and cultural diversity of the sample to verify generalizability; conducting longer-term follow-up to evaluate the sustainability of therapeutic effects; using animal or cell models to validate the key metabolic pathways and biomarkers identified; and performing head-to-head comparative studies with conventional Western medications to clarify relative advantages and positioning.

In summary, by integrating clinical evaluation with systems biology approaches, this study confirms the comprehensive benefits of SJYP in treating insomnia disorder with liver depression and spleen deficiency and preliminarily elucidates its potential functional network. It provides valuable evidence and insights for advancing the modernization and internationalization of TCM in the treatment of insomnia disorder.

Conclusion

In summary, Shugan Jianpi Yangxin Prescription (SJYP) demonstrated definite clinical efficacy and a favorable safety profile in treating insomnia disorder of the liver depression and spleen deficiency type. SJYP not only significantly reduced the PSQI and ISI scores and improved the overall response rate, but also effectively ameliorated TCM syndromes such as difficulty falling asleep, low mood, and mental fatigue, while relieving anxiety and depressive symptoms. These therapeutic benefits remained stable at the 8-week follow-up. Serum untargeted metabolomics analysis revealed that SJYP may exert its therapeutic effects by bidirectionally regulating 28 differential metabolites, including Pectenotoxin 2, N-Lactoyl-Phenylalanine, and O-Acetylcarnitine, and by coordinately modulating multiple metabolic pathways—such as GABAergic synapse, FoxO signaling pathway, retrograde endocannabinoid signaling, glycine/serine/threonine metabolism, bile secretion, and protein digestion and absorption. These multidimensional actions collectively contribute to restoring central inhibitory neurotransmission, improving synaptic plasticity, and re-establishing circadian rhythm and energy metabolism homeostasis. By rigorously integrating a randomized controlled clinical trial with metabolomics methodology, this study provides high-quality evidence-based medical evidence and a mechanistic elucidation for SJYP in the treatment of insomnia disorder, and provides a valuable reference for systems biology-based research on Chinese herbal compound formulas.

CRediT authorship contribution statement

Xu Wang: Data curation, Conceptualization, Investigation, Visualization, Writing – original draft. Jie Zhang: review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. Min Zhao: quality control. Jianping Duan: Investigation, Data curation. Sen Ma: Investigation, Data curation. Yang Guo: Investigation, Data curation. Xiaochun Wang: Investigation, Data curation. Hongju Zhang: Investigation. Feng Wang: 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.

Ethics statement and registration

The Ethical Committee of The First Affiliated Hospital of Henan University of Chinese Medicine has approved the study protocol. The trial was registered with Chinese Clinical Trial Registry under the identifier ChiCTR2300067859. Registered 21 January 2023, https://www.chictr.org.cn. And the study was carried out following the Declaration of Helsinki and the guidelines for Good Clinical Practice. Signed informed consent was obtained from all participants in the study.

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