Effectiveness of Yi-Zhi-An-Shen Granules on cognition and sleep quality in older adults with amnestic mild cognitive impairment: protocol for a randomized, double-blind, placebo-controlled trial

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Study protocol

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Abstract

Background Amnestic mild cognitive impairment (aMCI) is a syndrome characterized by significant forgetfulness that does not meet the criteria of dementia. Individuals with aMCI are at a bigger risk of progressing to dementia. Current studies suggest good sleep quality is linked with preserved cognition in the elderly, and sleep complaints are common among the elderly with amnesia. Therefore, improving their sleep may be helpful for maintaining and improving their cognitive capacity. According to the theory of traditional Chinese medicine, Yi-Zhi-An-Shen is an herbal compound which may ameliorate forgetfulness and sleep disorders. As growing evidence indicates that gut microbiome is associated with major mental symptoms, a hypothesis was proposed that Yi-Zhi-An-Shen Granules (YZASG) might work by alternating microbial abundance and diversity. In this study, the investigators intend to assess the efficacy of YZASG on global cognition in the elderly suffering from aMCI, and evaluate its safety as well as its potential mechanisms via sleep quality, fecal microbial 16SrDNA and metagenomics analyses, and serum markers. Methods/design This is a randomized, double-blind, placebo-controlled clinical trial. A total of 80 patients (aged 60~85 years) will be recruited and allocated randomly to a treatment group and a placebo group in a 1:1 ratio, then will be administered YZASG or isodose placebo three times a day. The intervention course is 16 weeks, with an 18-month follow-up. The primary outcome is Alzheimer Disease Assessment Scale-Cognitive subscale (ADAS-cog11). Secondary outcome measures are mini-mental state examination (MMSE), Montreal cognitive assessment (MoCA), Pittsburgh Sleep Quality Index (PSQI), serum concentrations of immunological factors and inflammatory cytokines, fecal microbiota. Fecal microbiota will only be collected at the baseline and endpoint of the intervention. Discussion The results of this trial will be conducive to assessing the safety and effectiveness on cognition of YZASG in intervening aMCI among the elderly and determining if it takes effect via the improvement of sleep quality, regulation of gut microbiota, concentration of certain serum markers.

Background

Alzheimer's disease (AD), one common type of dementia worldwide, is a neurodegenerative disease characterized by insidious onset and progressive cognitive decline [1]. It cannot only detrimentally affect physical condition and quality of life in patients, but also bring a huge burden on both family and society [2]. The prevalence of AD was 3.21% reportedly among the old in China [3]. It is proposed recently that research strategies of AD should focus on the preclinical stages – preclinical AD and mild cognitive impairment (MCI) due to AD, which are relatively ideal for intervention [4].

Patients with MCI always suffer cognitive decline severer than expected for an individual's age and education level, but which does not obviously affect daily function [5]. And the elderly with MCI are at a high risk of developing to dementia [6]. As the main subtype of MCI, nearly 90% of older adults with amnestic MCI (aMCI), MCI with memory complaints, reportedly progress to AD, and share similar pathophysiological characteristics with AD [7]. Nowadays, standard clinical management of MCI includes managing its risk factors, there're still limited pharmaceutical options for treating MCI from the NIA-AA
working group guidelines [8]. Thus effective drugs and other interventions are expected to be discovered to reduce the rate of progression from MCI to dementia.

Brain areas and systems of neurotransmitters involving regulation of sleep-wake cycle relate to memory and cognition mostly [9-11]. Individuals with various types of cognitive impairment generally have sleep disturbance [9, 12-14], and the study also suggested that the severity of these sleep disorders closely relates to that of cognitive decline [14]. Moreover, older adults with bad sleep quality always suffer poor cognitive performance [15-16], which might be related to higher level of AD-associated amyloid-β in brain [17]. Structural imaging data indicated that insomnia is associated with decreased volume of brain tissue, including hippocampus, as well [18]. The disorder of circadian rhythm often accompanies with high risk of cognitive decline and affects negatively cognition in multiple ways. Therefore, sleep problem is one risk factor of impaired cognitive function.

Several studies suggested that besides improving the cognitive performance and ability of daily living in individuals with AD, acetylcholinesterase inhibitors (ChEIs), like donepezil, galantamine and rivastigmine, also improved mental behavioral symptoms and sleep quality of these patients [19-21]. Furthermore, some trials indicated that melatonin could improve cognitive symptoms in patients with MCI and might delay their conversion to dementia [22-23]. As sleep disturbance appears to be linked with both aging and cognitive decline, strengthening sleep quality of the elderly might be an effective therapeutic target to slow the deterioration or improve cognitive impairment.

According to recent studies on MCI or AD, which involved donepezil [7], galantamine [24], memantine [25] and Solanezumab [26], pharmaceutical interventions showed poor amelioration of cognitive deficits. It might be the single pharmacological target of these drugs that did not adequately address the specific multiple pathophysiological characteristics. However, traditional Chinese medicine (TCM) is known for its multi-target, which could be used to address the complicated pathophysiological changes.

In the light of theory of TCM, kidney deficiency is the basis of amnesia and other cognitive deficits, while phlegm and blood stasis are significant pathological factors. For this reason, amounts of experiments and trials have been carried out and shown specific effects against cognitive decline, which involve Alpinae oxyphyllae fructus [27], Ligusticum wallichii [28-30], curcuma [31-32], Fructus gardenia [33-34], Radix notoginseng [35-36]. Meanwhile, an herbal formula named Yi-Zhi-An-Shen (YZAS) Granules has been composed of Lophatherum gracile and medical herbs mentioned above, which is designed for aiding the treatment of cognitive deficits. Table 1 details the pharmacological targets that the components in YZAS, which could be involved in the management of MCI. There have been preclinical researches contributing to the standardization of YZAS [37] and the mechanisms of its potential actions in animal experiments [38]. And some indications also manifested its safety and efficacy for improving cognitive function in clinical practice without causing daytime dysfunction.

Based on the theory of TCM, the actions of YZAS include soothing the nerves, that is to say, making someone calm down and helping sleep. Besides, as the emerging hypothesis of brain-gut axis, plenty of studies has shown changes of gut microbiome and the metabolite exert of gut microbiome exert an
influence on cognition impairment [39-41]. Because of being administrated orally, it should be noticed that the mechanism of action of YZAS might be via the gut microbiota. Consequently, a clinical trial with rigorous design is needed to confirm its safety in old individuals with MCI, efficacy on cognitive performance, and explore its potential mechanisms.

**Methods**

1. Objectives

The primary objective of this study is to evaluate the efficacy of Yi-Zhi-An-Shen Granules (YZASG) in optimizing cognitive performance over time in elderly individuals with aMCI. Secondly, the investigators intend to assess whether YZASG can improve sleep quality among aMCI patients, this herbal formula’s safety will also be assessed. Finally, participants’ serum samples and fecal genomic DNA will be extracted to analyze the differences of the indices of metabolism, cellular immune function, and gut microbiota between old individuals with aMCI and ones with normal cognition.

2. Design

This study is a randomized, double-blind, placebo-controlled trial with 16-week intervention and 18-month follow-up assessment. The current protocol (version v1.1) met the principles of the Declaration of Helsinki, and was in accordance of with Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) (see Additional file 1) and was approved by the Medical Ethics Committee of Teaching Hospital of Chengdu University of TCM. Then signed informed consents will be obtained from participants. And during the consent process, the caregiver or informant of the potential participants own should be present. The participants will be recruited from the communities, outpatient clinics of Teaching Hospital of Chengdu University of TCM and Welfare Institution of Emei Civil Administration in Sichuan Province, China. The Medical Ethics Committee has thoroughly reviewed this study, and then the ethical approval covers all these study sites.

The enrolled participants will be randomly assigned into the YZAS group or the placebo group with the allocation ratio of 1:1 using the statistical package. Allocation was concealed using batch numbers generated with SAS 9.2 software (Cary, NC, USA) by a statistician. The unique code will be assigned to each newly enrolled participant and preserved in the trial management board. The statistician expert who acts as the coder will be shielded from subject recruitment and statistical analysis, which will be performed by another statistician independent of the study group. Both participants and the research team are blinded to allocation. There will be six measurement sessions during this whole study, including the intervention period and the 18-month period (Figure 1). Any changes to the study protocol will be communicated with the study investigative team and the approving ethics committee.

3. Methods
3.1 Participants

The investigators anticipate recruiting 80 participants from Teaching hospital of Chengdu University of TCM, communities, and Welfare Institution of Emei Civil Administration. All patients will undergo standard medical examination and neuropsychological testing to ensure correct diagnosis of aMCI.

The inclusion criteria are:

(1) subject has assigned informed consent to participate in the study and continues to give willing consent for participation with

(2) age from 60 to 85 years with a diagnosis of aMCI

(3) educational level of at least 6 years

(4) availability of a caregiver or informant who can assist in completing rating scales for the duration of the study

(5) cognitive complaints reported by the subject and confirmed by the caregiver or informant

(6) Clinical Dementia Rating (CDR) global score of 0.5, and memory item score of 0.5

(7) Mini-mental state examination (MMSE) score of 24-30 (for the participant with educational level of 6 years, MMSE score of 20-30)

(8) Diagnostic and Statistical Manual of Mental Disorders, Version 5 (DSM-V) criteria of dementia not fulfilled

A patient will be excluded when he or she:

(1) has been previously enrolled in this study and received the investigational product

(2) has received an investigational product within 30 days prior to screening

(3) has received disease-modifying therapy in the past 6 months (e.g., donepezil, rivastigmine, galantamine, memantine, and any other existing drugs that are declared to have the function of improving cognition)

(4) has a known allergy to the study drug or any of its constituents;

(5) has a history of alcohol abuse or alcohol dependency in the 3 years prior to study entry, or is an alcoholic or drug addict, as determined by the investigator

(6) has ongoing clinically significant (as judged by the investigator), metabolic or any other disease that could currently cause impaired memory (e.g., untreated thyroid disease, vitamin or other nutritional
deficiencies, chronic kidney, or liver disease)

(7) has memory impairments that can be attributed to a disease or condition other than an early phase neurodegenerative syndrome

(8) has a parkinsonian movement disorder

(9) uses psychoactive medications that would affect the subject's ability to reliably perform neurocognitive testing or create uncertainty in distinguishing between the effects of the psychoactive medication and the subject's underlying cognitive impairment (e.g., benzodiazepines, sedatives, antipsychotics)

(10) has a history of major recurrent depressive disorder (DSM-V) within the last 5 years prior to screening

(11) has a brain tumor or other intracranial lesion, a disturbance of cerebral spinal fluid circulation (e.g., normal pressure hydrocephalus), and/or a significant history of head trauma or brain surgery

(12) has signs of major cerebrovascular disease, with score of modified Hachinski Ischemia Score (mHIS) at more than 4, or as verified by medical history and/or brain MRI or CT

(13) has severe visual or hearing impairments that cannot cooperate with examinations

(14) has severe digestive system diseases

(15) has received antibiotics within 60 days prior to screening.

Handling of withdrawal and dropout: (1) Voluntarily withdrawal, (2) loss of follow-up, (3) poor compliance and presence of severe adverse effects, (4) revealing and uncovering blind in urgency, (5) misdiagnosis, (6) using forbidden drugs or treatments in the course of the trial, (7) taking no medication during the trial, (8) no evaluable records after medication. Reasons for withdrawing participants will be recorded in case report forms (CRFs), and the last data will be included in data analysis. All these criteria will be ascertained by the supervisor of this study.

### 3.2 Interventions

All the participants will receive the same basic treatment, including health education, moderate aerobic exercise (30-60 minutes per day) and general nutritional support. The participants assigned to the YZAS group will take Yi-Zhi-An-Shen granule, which is composed of YiZhiRen (*Alpinia oxyphylla Miq.*) - 5g, SanQi (*Panax notoginseng*) - 3g, ChuanXiong (*Ligusticum chuanxiong hort*) - 10g, ZhiZi (*Gardenia jasminoides Ellis*) - 10g, YuJin (*Curcuma longa L.*) - 10g, DanZhuYe (*Lophatherum gracile*) - 10g, while ones in the placebo group will take placebo made from starch which appears the same shape, color, smell, taste, texture package and Lot Number. Participants will be instructed to dissolve granules into 100ml of boiled water and to take the solution orally between 30°C to 37 °C three times daily for 16
weeks. Each granule is prepared by Sichuan Neo-green Pharmaceutical Technology Development Co., Ltd, Sichuan China according to the standards of Good Manufactory Practice (GMP). During the whole study period, relevant health care and treatment for medical needs will be permitted without adverse reactions with YZASG or hindering the study process.

80% to 120% of drug usages are eligible for protocol plan. The package, drug name, function and indication, usage and dosage, storage condition, valid period and name of the manufacturer will be marked and a tag indicating ‘trial use’ will be attached. Drugs must be kept in the appropriate temperature in a dry, cool and shady place. Drug administrators should take back unused drugs to estimate participant compliance and record these in the CRFs.

### 3.3 Outcomes

#### 3.3.1 Primary outcome

Cognitive decline is measured using the Chinese version of the Alzheimer Disease Assessment Scale-Cognitive subscale (ADAS-cog11). It was also chosen to calculate the sample size, for a 4-point change in it as the measure of clinical significance [42]. The total score is 70. A score increase indicates greater severity of impairment. The specific hypothesis is that the increase from baseline to endpoint will be significantly less at least 2.5 points than that for placebo, which will be considered effective in this study.

The ADAS-cog will be assessed at baseline (before intervention), at 16 weeks (the end of intervention) and 6, 12, and 18 months after intervention.

#### 3.3.2 Secondary outcome

Secondary measures include the MMSE, Montreal Cognitive Assessment (MoCA, Changsha Version), CDR, Pittsburgh sleep quality index (PSQI), activity of daily living (ADL), gut microbiome, and serum markers.

The MMSE is an 11-question measure that tests five areas of cognitive function (orientation, registration, attention and calculation, recall, and language). The maximum score is 30 and a score below 24 is considered abnormal for dementia screening.

The MoCA (Changsha Version) will also be used to evaluate general cognitive function, as it contains visuospatial processing and organizational capability which can make up for the shortcoming of the MMSE. The total score for it is 30, with a higher number indicating a more intact cognitive function. Meanwhile, the MoCA has been shown to be a promising tool to detect MCI and early AD.
The CDR will be used as an assistant evaluation for patients’ dementia severity, which scored 0-3, with higher scores indicating more severity. It is a semi-structured interview performed with the patient and caregiver (informant), characterizing six domains of cognitive and functional performance. The CDR sum of boxes (CDR-SB) scored 0-18, will also be applied to assess patients’ cognitive status, with higher scores indicating worse functioning.

The PSQI will be used to assess participants' comprehensive quality of sleep, which involves sleep quality, sleep duration, sleep efficiency, sleep disorders, daytime dysfunction, sleeping aids, etc. The total score for it is 21, with a higher score indicating a worse sleep quality.

ADL will be assessed including basic activities of daily living (BADL) and instrumental activities of daily living (IADL). An individual's BADL will be evaluated mainly by the subjects' performance from the perspectives of bathing, dressing, grooming, initiation, toileting and feeding, with six items and a sum of scores ranging from 0 (normal) to 24 (complete dependence on others). And modified Lawton Instrumental Activities of Daily Living Scale will be used to measure the IADL of a subject, with eight items and a sum of scores ranging from 0 (normal) to 32 (complete dependence on others).

These clinical tests will be administrated by a trained, certified clinician or rater experienced in the assessment of patients with cognitive deficits. And the rater who will conduct the CDR for a patient cannot complete any other rating scales for the same patient, and will be blinded to the results of all other neuropsychological scales. Scales mentioned above will be assessed at baseline (before intervention), at 8 and 16 weeks (during intervention) and 6, 12, and 18 months after intervention.

Blood samples will be collected from all participants for further assessing the mechanisms of YZASG via changes in serum metabolic, inflammatory, and immunologic markers. All these tests will be entrusted to the laboratory medicine of Sichuan Academy of Medical Science & Sichuan Provincial People’s Hospital for conduction.

Fecal genomic DNA will be extracted from frozen stools using QIAamp DNA mini stool kit (Qiagen, Hiden, Germany), obtained from the patients with aMCI and 15-20 participants with normal cognition at baseline and the end of the intervention. After PCR amplification, DNA fragments will be sequenced on an Illumina HiSeq 2500 instrument and an Illumina HiSeq X instrument for 16SrDNA and metagenomics analyses (which will be chosen from some representative samples in the results of 16SrDNA analysis) respectively at Biomarker Technologies Co, Ltd (Beijing, China) to analyze the differences in gut microbiome between patients with aMCI and individuals with normal cognition. When it is available, the investigators will also assess the changes in gut microbiome between the treatment group and the placebo group after the intervention of YZASG in the same way.

3.3.3 Safety outcomes
To assess the safety of YZASG compared to placebo in subjects with aMCI, the investigators will record the incidence and severity of treatment-emergent adverse events (TEAEs), and clinically important changes in safety assessment results. These safety indicators, including vital signs, weight, clinical laboratory tests, physical and neurological exams, ECGs, and CTs/MRIs, will gather at the baseline and the end of 16th week.

4. Statistical considerations and data management

4.1 Sample size

Using pre-intervention and post-intervention scores obtained from Miao et al. [43], on the basis of non-inferiority trial principle, one-sided test, at $\alpha = 0.05$, then at least 33 patients are needed for inclusion in the treatment group who will be administrated with YZASG to achieve a prospective power of 90% (ie. $\beta = 0.1$) and detect a minimum clinical between-group difference of 1.30 on ADAS-cog [43] at 16 weeks. Allowing for a maximum dropout rate of 20%, the number of subjects in the treatment group has been set to 40 patients with aMCI. With the allocation ratio of 1:1, 80 subjects are required.

4.2 Statistic analysis

Analysis will be conducted by another statistician of the National Clinical Trial Center of Chinese Medicine (Chengdu, China), who will be also blinded to the whole trial, using SAS 9.2 software (Cary, NC, USA) and SPSS 21.0 software (IBM, NY, USA).

The analysis data set will consist of a modified intention-to-treat data set, a per protocol (PP) set, and a safety data set. All mechanism and efficacy analyses will be conducted according to the modified intention-to-treat (mITT) principle. The mITT data set will include the participants who have completed at least one observation since the intervention begins. The PP population will only include participants who adhered to the trial protocol and completed the clinical trial. And the minimum compliance rate for participants taking the investigational drugs in the PP data set is 80%. Besides, the safety analysis will be conducted according to the safety data set, which will include any participants who were assigned to randomly and took at least one dose of the investigational drug. Missing values will be replaced by the last observation carried forward (LOCF) method. ADAS-cog (including its monomial item) changes from baseline and the secondary outcomes will be assessed using an analysis of covariance with treatment groups as factors and baseline values as covariates. Mean differences will be used to express effect sizes. The baseline homogeneity of the baseline characteristics and differences between the two groups will be analyzed with Fisher’s exact test, or $^2$ test for categorical measures and with the $t$ test or Wilcoxon rank-sum test for continuous measures. The statistical significance is defined as a one-sided P-value of $<0.05$ and 90% confidence interval.
For the gut microbial 16SrDNA analysis, sequenced data will be interpreted using the bioinformatics tools programmed in the Ion Reporter software. Based on the specified similarity, QIIME algorithms will be used to classify operational taxonomic units (OTU) and statistically analyze biological information, and then to understand the diversity and abundance of the flora community, and further to determine the bacterial diversity within a sample (α diversity) and among all the samples (β diversity). Alpha diversity includes four indicators that represent total number, richness, phylogenetic diversity, and dispersible uniformity of species and community abundance. These four algorithms, including binary jaccard, bray curtis, weighted unifrac, and unweighted unifrac, will be performed to analyze β diversity to compare the similarity of different samples in species diversity. According to the above data, principal component analysis will be conducted to observe the differences between floras. Additionally, multivariate data analysis with principal component analysis on the diversity indexes and comparisons of genus and species level data will be performed to reveal differences in the microbial composition between individuals with normal cognition and ones with aMCI. Metastats software will be used to perform t test on the species abundance data between two groups, and p value will be obtained, then by correcting p value q value will also be obtained. Subsequently, according to p or q value, species that cause the differences in the microbial composition of the two sets will be screened out. Significant analyses between two groups will be performed at the level of classification of the gate, class, subject, family, genera and species, respectively. Sparcc algorithm will be used to conduct correlation analysis (including positive and negative correlation) and statistical tests. Next, a co-expression analysis network map will be drawn using python. The subsequent statistical analyses will be performed with the R Programming Language 3.0.1 (NZL).

When it comes to metagenomics analysis, after getting Clean Reads, taxonomic analysis will be conducted to measure species composition and abundance information of samples. After the significance test of difference has been performed, p value will be obtained. Then by correcting the p value, false discovery rate (FDR) will be obtained. The Benjamini Hochberg false discovery rate adjustment will be used to account for the number of taxa tested in each comparison.

### 4.3 Data management

All data will be stored on a secure server with two back-up copies on external hard drives. Paper-based forms will be digitized and the original copies stored in locked filing cabinets in the archives room of Good Clinical Practice (GCP) in Department of Geriatrics, and managed by a department staff member who is external to the research team. All participants are de-identified upon randomization and referred to on all forms with a participant ID. A password-protected spreadsheet stored on the secure server links participant names to ID codes to all for re-identification to occur if required. As this is a relatively small investigator-initiated trial, a data monitoring committee and auditing process are not required.

### 5. Quality control and monitoring
Each trial center has a project manager who takes charge of the quality of research. All investigators were qualified and trained before. After the baseline measurement, the 16 weeks of intervention will be dispensed. Participants will be required to return any unused medication at every 2 weeks, which will be used for determining compliance. The number of returned granules will be counted by a department member who is external to the study team. During the whole course, attentive follow-up will also be conducted every 2 weeks. Participants who exit the study early will be contacted via telephone and requested to complete the exit interview.

To monitor safety, participants will be referred to the Teaching Hospital of Chengdu University of TCM and the local cooperating hospital of Welfare Institution of Emei Civil Administration, which is next to the institution. Standard blood safety tests (full blood count, blood coagulation function, liver and renal function tests) and ECGs will be carried out at the baseline and the end of the 1st and 16th weeks of intervention, while brain CTs/MRIs will be done at the baseline and the end of 16th week. And the reports of annual physical checkup of participants are also encouraged to share with the investigators in this study.

Adverse events will be closely monitored and recorded throughout the whole course of the study. Once a serious adverse event happens, if the study drug is suspected to be a potential cause and receiving appropriate medical care is identified to be essential for the participant, then unblinding of that participant will occur and at least two persons should present. After treated, that participant will be stopped from the subsequent investigation and regarded as the dropout case. Anyway, the investigators will also visit that participant regularly and record his/her reactions and treatments of the adverse events until the endpoint of this study. And this will be performed by the department member external to this study team. A development update safety report must be submitted annually to the Medical Ethics Committee of Teaching Hospital of Chengdu University of TCM.

The random code and allocation information will be kept concealed from the study team and participants by the end of this study when all statistical analyses have been finished.

**Discussion**

YZAS has shown effectiveness in improving cognitive performance and neuroinflammation in animal experiments. Provided that it can also be proved to address cognitive deficits in this clinical trial, it may act via multiple mechanisms involved in the pathology of aMCI, which will be determined in the study.

Supposing that old individuals with aMCI almost experience poor sleep, this study may provide an evidence-based medical approach to improving sleep quality and then maintaining cognition among these patients. However, in terms of testing specifically the differences of fecal microbiome between the old with normal cognition and those with aMCI, this project has a relatively small sample size, which is calculated according to the primary outcome. Owing to the number and the length of the tasks, participants’ burden is a bit high, breaks will be scheduled during each period of follow-up assessments.
The results of this randomized controlled trial will inform the development of future interventional studies to prevent or delay the cognitive decline among patients with MCI or dementia.

**Trial Status**

This trial (protocol version: v1.1, Dec. 18th, 2017) began recruitment on 21 April 2018 and is currently ongoing. The recruitment will be approximately completed by the end of April in 2019.

**Declarations**

**Additional file:**

SPIRIT 2013 checklist: recommended items to address in a clinical trial protocol and related documents.

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**Availability of data and materials**

The datasets used and/or analyzed during this study are available from the corresponding author on reasonable requests.
Consent for publication

Not Applicable.

Authors' contributions

WBW, the lead principal investigator, contributed to the study design and the development of this trial with SNY. SNY drafted the initial protocol, and WBW provided review. TH and BYL participated in the coordination of the trial, and recruited patients with YQQ and JXC. HMP assisted with the collection of data. ML and CLC advised on medical assessment and clinical issues. CLC also worked as the clinical supervisor. All authors reviewed the content and approved the final version.

Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

Ethical approval for this study was provided by the Teaching Hospital of Chengdu University of Traditional Chinese Medicine Medical Ethics Committee (2017KL-035, 2017KL-036). All participants for this study will provide written informed consent.

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Tables

Due to technical limitations, Table 1 has been placed in the supplementary files section.

Figures
| Time point (weeks) | Study Period |
|-------------------|--------------|
|                   | Enrollment       | Baseline | Intervention Period | Follow-up Period |
|                   | -4              | 0        | 1 | 8  | 16 | 42 | 68 | 94 |
| **Enrollment:**   |                 |          |   |    |    |    |    |    |
| Eligibility       | ×               |          |   |    |    |    |    |    |
| Informed Consent  | ×               |          |   |    |    |    |    |    |
| Allocation        | ×               |          |   |    |    |    |    |    |
| **Interventions:**|                 |          |   |    |    |    |    |    |
| YZASG or placebo  | ×               |          |   |    |    |    |    |    |
| **Assessments:**  |                 |          |   |    |    |    |    |    |
| **Primary Outcome** |               |          |   |    |    |    |    |    |
| ADAS-cog          | ×               |          |   |    |    |    |    |    |
| **Secondary Outcomes** |           |          |   |    |    |    |    |    |
| MMSE              | ×               |          |   |    |    |    |    |    |
| MoCA              | ×               |          |   |    |    |    |    |    |
| CDR               | ×               |          |   |    |    |    |    |    |
| PSQI              | ×               |          |   |    |    |    |    |    |
| ADL               | ×               |          |   |    |    |    |    |    |
| Blood Sample      | ×               |          |   |    |    |    |    |    |
| Metabolic markers |                |          |   |    |    |    |    |    |
| Inflammatory markers |            |          |   |    |    |    |    |    |
| Immunologic markers |              |          |   |    |    |    |    |    |
| Fecal Sample      | ×               |          |   |    |    |    |    |    |
| 16SrDNA           |                |          |   |    |    |    |    |    |
| Metagenomics      |                |          |   |    |    |    |    |    |
| Safety Outcomes   |                 |          |   |    |    |    |    |    |
| Vital signs       | ×               |          |   |    |    |    |    |    |
| Weight            | ×               |          |   |    |    |    |    |    |
| Full blood count  | ×               |          |   |    |    |    |    |    |
| Coagulation function |              |          |   |    |    |    |    |    |
| Liver function test|              |          |   |    |    |    |    |    |
| Renal function test|              |          |   |    |    |    |    |    |
| ECG               | ×               |          |   |    |    |    |    |    |
| Brain CT/MRI      | ×               |          |   |    |    |    |    |    |

**Figure 1**

Schedule of interventions and assessments. Abbreviation: ADAS-cog, Alzheimer disease assessment scale-cognitive subscale; MMSE, mini-mental state examination; MoCA, Montreal Cognitive Assessment; CDR, clinical dementia rating; PSQI, Pittsburgh sleep quality index; ADL, activity of daily living; 16SrDNA: 16S ribosomal deoxyribonucleic acid; ECG, electrocardiogram; CT, computed tomography; MRI, magnetic...
resonance imaging. And vital signs include individual's temperature, breath and pulse per minute, and blood pressure.

Supplementary Files

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