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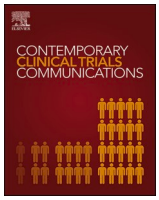

Efficacy, safety, and response predictors of Astragalus in patients with mild to moderate Alzheimer's disease: A study protocol of an assessor-blind, statistician-blind open-label randomized controlled trial

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ABSTRACT

Background: This pragmatic clinical trial aims to determine the efficacy and safety of add-on Astragalus membranaceus (AM) for cognition and non-cognition in patients with of mild to moderate Alzheimer's disease complicated with orthostatic hypotension in orthostatic hypotension, elucidate the underlying mechanisms, identify related response predictors, and explore effective drug components.

Methods: This is an add-on, assessor-blinded, parallel, pragmatic, randomized controlled trial. At least 66 adults with mild to moderate Alzheimer's disease (AD) and OH aged 50–85 years will be recruited. Participants will be randomized in a 1:1:1 ratio to receive 24 weeks of routine care or add-on low dose AM or add-on high dose AM group. The primary efficacy outcome will be measured by the Alzheimer's Disease Assessment Scale-Cognitive Subscale, Chinese version. Secondary efficacy outcome assessment will include neuropsychological tests, blood pressure, plasma biomarkers, multimodal electroencephalograms, and neuroimaging. Safety outcome measures will include physical examinations, vital signs, electrocardiography, laboratory tests (such as hematologic and blood chemical tests), and adverse event records.

Ethics and dissemination: This trial was approved and supervised by Fujian Medical University Union Hospital (2021KJCX040). Independent results, findings will be published in peer-reviewed journals and presented at national and international conferences.

Trial registration number: NCT05647473; ClinicalTrials.gov Identifier.

1. What is Already Known on this Topic

The clinical effects of add-on Astragalus in improving cognition and non-cognition in patients with of mild to moderate Alzheimer's disease complicated with orthostatic hypotension remain unclear.

2. What this study Adds

Add-on Astragalus may be another feasible treatment option for patients with of mild to moderate Alzheimer's disease complicated with orthostatic hypotension.

3. HOW this study Might Affect research, practice or Policy

The methodology and results of this trial may provide valuable insights into patient selection, outcome measurement, sample and effect size determination, and study duration for cognitive impairment with orthostatic hypotension drug trials.

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4. Introduction

Currently, more than 55 million people worldwide live with dementia, a number projected to reach 152 million by 2050 [1]. Alzheimer's disease (AD) is a chronic progressive neurodegenerative disorder, characterized by memory loss, executive function decline, language and motor impairments, personality changes, and behavioural and psychological disturbances [2]. AD is the most common form of dementia, accounting for approximately 60%–70 % of dementia cases [1]. The pathological features of AD include amyloid-beta (Aβ) deposition and hyperphosphorylation, tau neurofibrillary tangles, glial cell overactivation, and synaptic loss. In China, the population of AD patients is close to 9.83 million. AD and other dementias rank as the fifth leading cause of death, significantly impacting public health and sustainable development. Therefore, enhancing the prevention, diagnosis, and treatment of AD, as well as slowing its onset and progression, remains an urgent public health priority. Available medications for AD are very limited and act on similar mechanisms. The two most commonly used drugs are cholinesterase inhibitors (such as donepezil hydrochloride, rivastigmine, and galantamine) and excitatory amino acid receptor antagonists (memantine). However, due to the complex pathogenesis and diverse onset forms of AD, current drug treatments only delay disease progression and do not provide a cure, often causing adverse effects like nausea and vomiting [3]. Novel Aβ-targeted disease-modifying therapeutic drugs show promise and some have entered clinical use, yet their application inevitably raises immunological concerns, such as amyloid-related imaging abnormalities (ARIA) [4]. Hence, there is an urgent need to develop new therapeutic drugs for AD.

In recent years, the adjuvant role of traditional Chinese medicine (TCM) in the treatment of cognitive disorders has gained increasing attention. Numerous studies have confirmed the efficacy of various Chinese medicines and formulas in treating AD [5,6]. Astragalus membranaceus (AM), a commonly used traditional Chinese medicine, is notable for its well-established cultivation techniques, cost-effectiveness, minimal adverse and toxic side effects, flexible clinical application, and high acceptability among elderly patients. According to TCM, AM enters the spleen and lung meridians. It can tonify qi, strengthen the spleen, elevate yang, raise the drooping, tonify Wei-defensive qi, secure the exterior, promote diuresis, reduce swelling, and promote pus discharge and tissue regeneration [7], and is extensively used to treat qi deficiency. TCM believes that dementia manifests as "spleen and kidney deficiency with insufficiency of qi and blood". Domestic studies have shown varying degrees of cognitive improvement using compound preparations containing AM for different types of senile dementia, along with observed improvements in microcirculation and increased cerebral blood flow [8]. Modern pharmacological research also indicates that AM possesses multiple neuronal anti-aging mechanisms, including antioxidative stress, stabilization of cell membrane ion channels, mitochondrial damage repair, inhibition of inflammatory factors, and telomerase activation [9]. Additionally, AM's active components exert effects through the above-mentioned pathways such as inhibiting microglial cell activation, reducing neuroinflammatory responses, enhancing intracellular protein aggregation transport, delaying neuronal apoptosis, and promoting myelin sheath repair and regeneration [10]. Various studies have demonstrated the positive therapeutic effects of AM extracts in AD model mice. Dietary supplementation with AM can reduce neuroinflammation and improve memory function [11]. Astragaloside IV has been shown to inhibit microglial cell activation and oxidative stress, thereby improving cognitive deficits [12] and reducing neuroinflammation [13] in AD mice. It also reduces tau hyperphosphorylation [14], Aβ toxicity [15], and synaptic damage [14] and apoptosis [12,14,16]. Astragalus polysaccharides have been found to alleviate cognitive dysfunction [17], Aβ accumulation [17], and neuroinflammation [18] in AD mice. Our previous studies also discovered that cycloastragenol promotes Aβ clearance, inhibits microglial cell inflammatory responses, and significantly reduces the deposition of aging-related substances, exhibiting anti-aging effects, as well as enhances glucose metabolism in the brain of AD mices.

Furthermore, the progression of AD pathology often relies on multiple abnormal physiological and biochemical changes resulting from aging stressors [19]. Telomeres (repetitive TTAGGG DNA sequences at the ends of linear chromosomes), critical indicators of aging, gradually shorten with age [20]. Increasing literature indicates the involvement of telomeres in AD neurodegeneration [21]. Telomere length can be regarded as a biomarker of cognitive aging [21,22]. The trend of telomere shortening is associated with the presence of dementia. Reduced telomerase activity and shortened telomeres increase the risk of AD [21,23,24] with greater neuronal apoptosis. Recently, the telomerase-activating effects of AM have captured interest [9,25,26]. Studies have shown that cycloastragenol and astragaloside IV activate telomerase, therefore protecting cells against aging and apoptosis [25–27].

Based on these characteristic effects of anti-aging, telomerase activation, and improving AD-related pathology, we hypothesize that AM may have therapeutic effects in delaying the progression of AD and improving cognitive dysfunction. Despite this, most current studies primarily investigate AM compound formulations, without a specific focus on AD and with key pathophysiological mechanisms still not well understood.

Currently, the etiology and pathogenesis of AD remain unclear. Cardiovascular health is considered a key factor in preventing AD [28], yet the mechanisms by which vascular damage leads to cognitive decline are yet to be fully clarified. Since cerebral hypoperfusion is widely associated with dementia [29–31], cerebral hemodynamics has been proposed as a potential link between vascular risk factors and AD. This underscores the importance of maintaining appropriate and continuous cerebral perfusion. Orthostatic hypotension (OH) is common among the elderly, characterized by a significant drop in blood pressure upon postural changes, with insufficient compensatory mechanisms from the sympathetic and parasympathetic nervous systems. This blood pressure drop can cause transient cerebral hypoperfusion [32]. Approximately 25 % of community-dwelling individuals over 60 years old live with OH, negatively impacting quality of life and increasing the risk of cardiovascular and cerebrovascular diseases. Studies indicate that OH is highly prevalent in dementia patients compared to healthy controls, with about 28 % of AD patients affected by OH [33–36], which significantly increases the risk of AD [37,38]. Additionally, even in non-OH subjects, great orthostatic blood pressure fluctuations significantly elevate the risk of dementia [38]. OH patients exhibit dysfunction in overall cognitive performance [39] and specific cognitive domains such as executive and memory functions [40], attention [41], and information processing speed [42]. Additionally, research has identified various abnormalities in white matter microstructure [43,44] and cortical electrical activity [45] of OH patients. Repeated episodes of cerebral hypoperfusion are thought to be a major cause of cognitive dysfunction in OH patients [32]. Moreover, previous studies have shown that medications that raise blood pressure can shorten task response times in patients with OH [46].Based on these findings, **we hypothesize that alleviating the recurrent cerebral hypoperfusion caused by OH may help improve cognitive and non-cognitive symptoms in patients with a combination of AD and OH.**

Previously, OH was mainly treated with Western medicine, especially fludrocortisone [42] and midodrine [47]. However, these medications have many adverse effects such as headache, dizziness, nausea, urinary retention, and supine hypertension [48]. Traditional Chinese medicine (TCM) has seen **growing interest in the use of Chinese medicines to regulate OH**. In TCM theory, OH falls within the categories of "vertigo", "syncope", and "deficiency/consumption". According to TCM, OH occurs when spleen and stomach impairment as well as heart and lung deficiency cause yang qi depletion, and insufficient generation and transformation of qi and blood, resulting in the failure to raise qi and nourish the brain. The treatment strategy should be to tonify

qi, nourish blood, lift yang, raise the drooping, promote blood circulation, transform blood stasis, tonify the kidney, benefit the liver, and strengthen the spleen. AM is known for its roles in tonifying qi, generating fluids, astringing yin, stopping sweating, and nourishing the heart and lung. Modern pharmacological studies suggest that AM improves endothelial dysfunction, exhibits antioxidant properties, protects myocardium, maintains cardiac function, directly protects blood vessels, playing multiple roles in regulating blood pressure. It also improves material metabolism in the central nervous system, cardiovascular system, and endocrine system, reduces myocardial oxygen consumption and peripheral vascular resistance, promotes excitement in the adrenocortical system, enhances reticuloendothelial cell function under hypotension, increases cardiac output, improves microcirculation and immune function [49], exerting bidirectional regulation on blood pressure [50], ensuring cerebral perfusion, and correcting OH. Domestic studies have used various types of AM compound formulations to treat OH, resulting in varying degrees of symptom improvement [51]. Therefore, AM supports **the improvement of blood pressure abnormalities through its peripheral positive cardiotonic and vascular regulatory effects, illustrating its substantial therapeutic value in the treatment of OH.**

Current evidence supports the hypothesis that add-on AM can delay or even improve cognitive and non-cognitive symptoms in mild-to-moderate AD patients with OH (Fig. 1). Therefore, the current article presents the study design for this pragmatic clinical trial, aiming to determine whether AM improves cognitive and non-cognitive symptoms in these patients, elucidate the underlying mechanisms, identify relevant response predictors, and evaluate effective drug components.

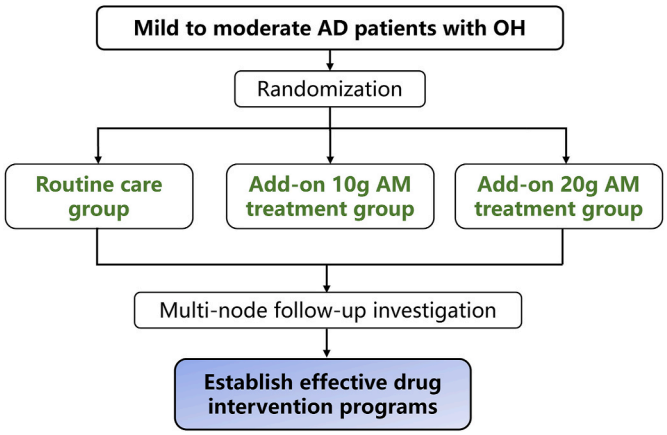

Fig. 2. Flowchart of the trial procedure.

5. Methods

5.1. Study design and population

An assessor-blinded, parallel, pragmatic, randomized controlled trial will be conducted at Union Hospital, affiliated with Fujian Medical University. This study will include eligible patients with mild-to-moderate AD with OH between February 2024 and May 2025 (Fig. 2, Fig. 3). The study used the SPIRIT reporting guidelines [52]. The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki. The inclusion and exclusion criteria are listed in Box 1.

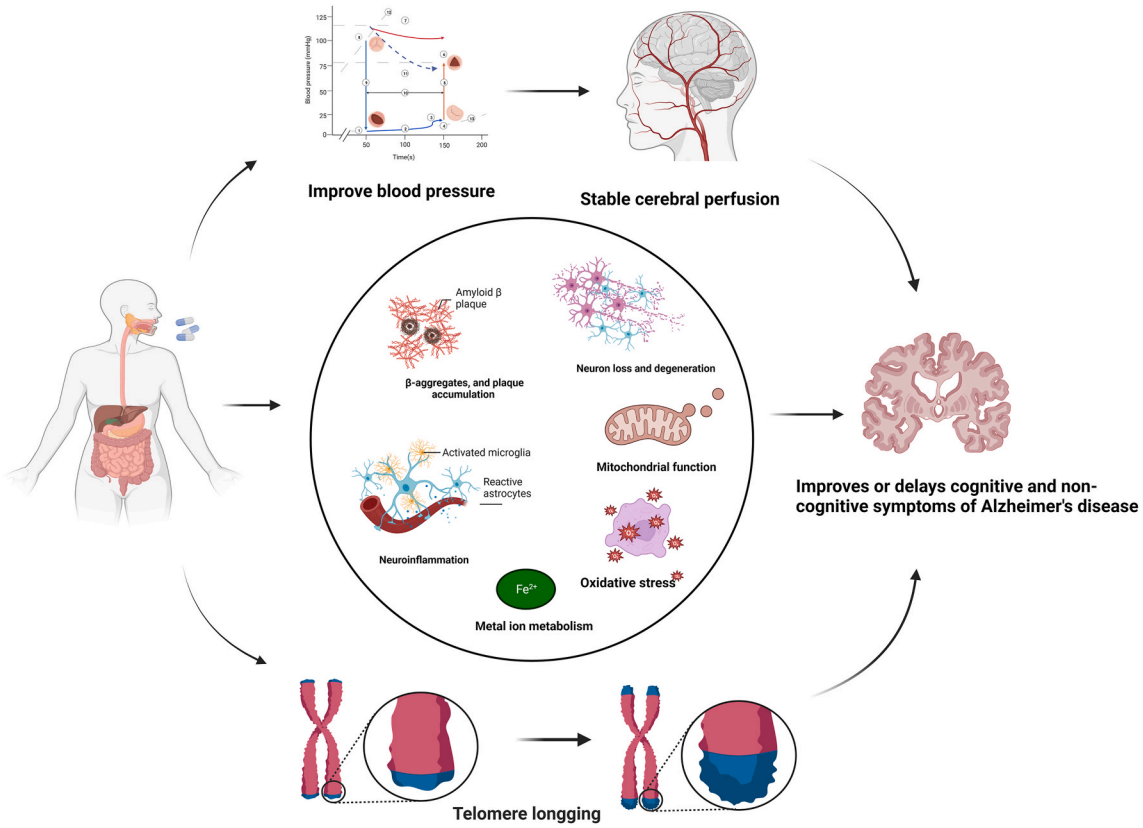

Fig. 1. Scientific hypothesis of the trial.

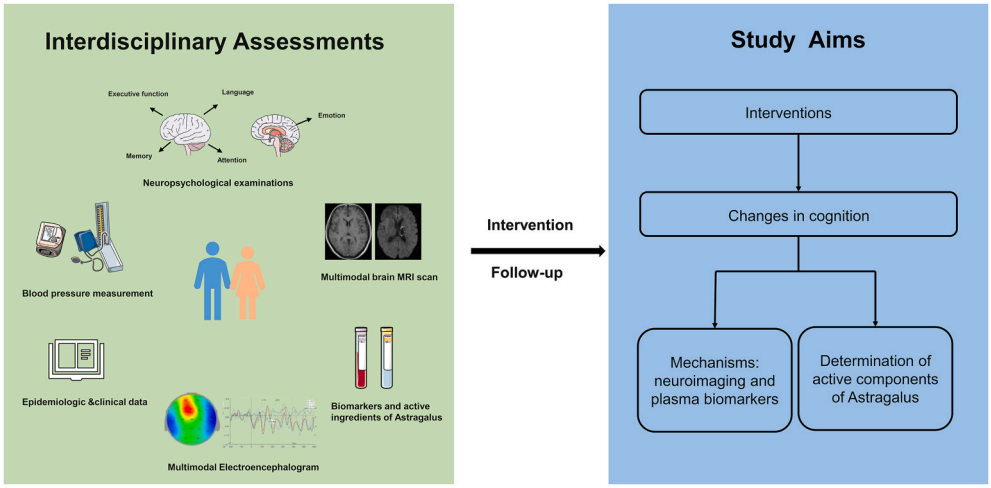

Fig. 3. This clinical trial examines whether AM improves cognitive and non-cognitive symptom in patients with of mild to moderate AD complicated with OH.
Abbreviation: AM, Astragalus membranaceus; AD, Alzheimer disease.
MRI, magnetic resonance imaging.

Box 1
Inclusion and exclusion criteria

Inclusion criteria

(1) Patients aged 50 to 85.
(2) A decrease in systolic blood pressure of 20 mm Hg or a decrease in diastolic blood pressure of 10 mm Hg within 3 min after standing [57].
(3) Memory loss for at least 6 months, with a progressive worsening trend.
(4) Patients with mild or moderate disease degree, that is, the total score of MMSE: 14 points < total score of MMSE <24 points, 0.5 ≤ CDR≤2 points [58], and the total score of HAMD (24-item version) ≤20 points [59].
(5) Brain magnetic resonance imaging shows the degree of hippocampal atrophy is greater than or equal to grade 1.
(6) The modified Hachinski Ischemia Scale (m-HIS) score was <4 points [60].
(7) The criteria described by the diagnostic and statistical manual of mental disorder-V for the diagnosis of dementia comply with the National Institute on Aging - Alzheimer's Association "Very likely AD" (National Institute of Aging-Alzheimer's Association, 2011) [61].
(8) There are no obvious positive signs in nervous system examination;
(9) The subjects have the ability of reading, writing and communication, have a stable caregiver, accompany to attend the visit.
(10) The basic treatment of AD before enrollment remained unchanged, and if long-term users needed to use it steadily for more than 4 weeks before randomization, the dose was kept as stable as possible during the study. Such drugs include: cholinesterase inhibitors and diamantine.

Exclusion criteria

(1) MRI showed significant focal lesions, including one of the following: a. There were more than 2 infarcts with a diameter greater than 2 cm; b. Infarcts in key areas such as the thalamus, hippocampus, entorhinal cortex, parorhinal cortex, angular gyrus, cortex, and other subcortical gray matter nuclei; c. White matter lesion Fazekas Scale ≥3.
(2) Patients who have taken other Chinese medicine preparations in the past three months
(3) Allergy or contraindication of astragalus
(4) There are other neurological diseases that can cause brain dysfunction or cognitive impairment; Mental and neurological retardation is present; Presence of malignant tumor
(5) The modified Hachinski Ischemia Scale (m-HIS) score was ≥4 points.
(6) Patients who refuse or have MRI or EEG contraindications (pacemakers, coronary and peripheral arterial stents, Metal implants, claustrophobia, or severe visual or hearing impairment), refusing to draw blood
(7) Pregnant or lactating patients;
(8) Patients who have participated in other clinical studies within the past 3 months
(9) Patients who refuse or have contraindications to magnetic resonance imaging (MRI) or electroencephalography (EEG) (pacemakers, coronary and peripheral artery stents, metal implants, claustrophobia, severe visual and/or hearing impairment);
(10) Patients using a cholinesterase inhibitor, psychoactive drugs, medications that affect the cardiovascular system, or any medication known to interact with AM;
(11) Patients who are pregnant or lactating;
(12) Patients who have participated in other clinical studies within the past 3 months.

5.2. Sample size calculation

The sample size will be calculated using PASS version 11. α and the loss to follow-up rate will be set to 0.05 and 10 %, respectively, to achieve a statistical power (1-β) of 90 %. Information from our pilot study suggests that 20 patients will be required in each group. Therefore, we will recruit 22 patients per group for potential dropouts.

5.3. Randomization and blinding

We will recruit a minimum of 66 eligible adults. A non-investigator will generate a randomization sequence using a centralized computer system. Enrolled patients will be randomized in a 1:1:1 ratio to receive 24 weeks of routine care, add-on 10g AM or add-on 20g AM. The study participants will not be masked because of the nature of the treatment. But statisticians and outcome assessors will be blind to the allocation of treatment. Fig. 2 presents the flowchart of this study.

5.4. Intervention and control

5.4.1. Routine care group

Participants will be educated on ways to avoid induced hypotensive states, such as avoiding prolonged standing, standing after exercise, being nervous, eating several carbohydrate-rich foods, drinking alcohol, and being in a warm environment (such as a sauna). Participants will be encouraged in a comfortable home environment, such as a sit-down bath [53]. If there are no contraindications, they are advised to increase their salt intake to approximately 10 g per day and adjust their fluid intake to 2–3 L per day [53]. They will also be encouraged to perform lower-body strength training and moderate, non-strenuous activities [53]. Seriously ill patients will be proposed to raise the head of their bed during sleep, wear tight clothing, eat small meals, and reduce alcohol intake [53]. Concurrent use of cholinesterase inhibitors, memantine, or both will be permitted.

5.4.2. Add-on 10g AM treatment group

Patients will receive daily 10g AM treatment and routine care for 24 weeks.

5.4.3. Add-on 20g AM treatment group

Patients will receive daily 10g AM treatment and routine care for 24 weeks.

5.5. Herbal safety

Soluble herbal granules that comply with Good Manufacturing Practice standards will be used, prepared by ShengGuoRong Institute of Traditional Chinese Medicine (Fujian Province, China). Senior TCM doctors from the Union Hospital Affiliated to Fujian Medical University will be responsible for the prescription. Four to 6 weeks after randomization, liver and kidney functions will be examined in all participants to monitor acute changes in these functions.

5.6. Outcomes

Efficacy and safety will be evaluated at baseline and 12, 24 and 48 weeks (Table 1).

5.7. Primary outcome

The primary outcome measure was cognition as measured by the ADAS-Cog-C score between baseline and week 48 or at the end of individual treatment in cases of premature withdrawal.

5.8. Secondary outcomes

Secondary outcomes will include neuropsychological, BP, biomarker, EEG, and neuroimaging (NODDI) changes from baseline to week 48 or at the end of individual treatment in cases of early discontinuation of the intervention.

5.8.1. Neuropsychological assessments

All participants will undergo various neuropsychological tests to assess cognition. Neuropsychological measures consist of seven cognitive areas (tests to assess each area are in parentheses), including the following: (1) general mental status (MoCA—Chinese version), (2) memory (Rey–Osterrieth Complex Figure Test [ROCF] recall, Rey Auditory-Verbal Learning Test [RAVLT] learning/immediate free recall, and RAVLT long-delayed recall); (3) spatial processing (ROCF-copy and

Table 1
Trail design time table.

Timepoints (weeks)	Study process									
	Enrollment	Allocation	Post allocation—intervention period							
	−1	0	1	4	8	12	16	20	24	48
Enrolment										
Qualification examination	X									
Baseline	X									
Informed consent	X									
Randomization	X									
Medical history	X									
Treatment history	X									
Other diseases	X									
Allocation		X								
Interventions										
Routine care			X	X	X	X	X	X	X	X
Add-on 10g AM			X	X	X	X	X	X	X	X
Add-on 20g AM			X	X	X	X	X	X	X	X
MRI scan		X				X			X	X
EEG examination		X				X			X	X
Neuropsychological		X				X			X	X
BP		X				X			X	X
Safety		X				X			X	X

Note: Adverse events will be recorded during the intervention.
Abbreviations: MRI, magnetic resonance imaging; EEG, electroencephalogram; Aβ, β-amyloid; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; P-tau, hyper-phosphorylated tau; BP, blood pressure; ECG, electrocardiography; AEs, adverse events.

Clock-Drawing Test); (4) attention (Trail Making Test-A and Digit Span Forward); (5) executive function (Trail Making Test-B and Digit Span Backward); (6) language (Verbal Fluency Test); and (7) emotion (Hamilton Anxiety Scale, Hamilton Depression Scale and Neuropsychiatric Inventory).

5.8.2. Blood pressure measurements

The test will be scheduled at noon to minimize the diurnal effects of hemodynamics. All participants will be instructed to lie on their backs for 20 min to establish psychological and physiological equilibrium before the testing. The same examiner will perform all assessments in a quiet room. All participants will be in the supine position after resting for 5 min and will have their right upper arm BP measured at 1-min intervals (Omron HBP-1300; Omron Healthcare Co., Ltd., Dalian, China). A total of three measurements will be taken, and the average of these measurements will be taken as supine BP. Subsequently, participants will remain in a standing position and have their right upper arm BP measured at 1-min intervals for 3 min. The maximum change in BP relative to supine position while standing will be used to define OH.

5.8.3. Measurement of biomarkers

In recent years, plasma biomarkers have been used as a noninvasive, low-cost, and easily accessible tool to assess pathology during cognitive impairment. Venous blood samples will be collected from all participants using 9-mL K3-ethylenediamine tetraacetic acid tubes, and plasma will be obtained within 1 h after collection by centrifugation at 25 °C at 2000 g for 20 min. The plasma samples will then be reserved at −80 °C until testing. Plasma samples with hemolysis will be excluded. Plasma β-amyloid (Aβ) 40, Aβ42, glial fibrillary acidic protein, neurofilament light chain, and hyper-phosphorylated tau-181 will be measured using the SIMOA. Telomerase activity will be evaluated using a TeloTAGGG Telomerase PCR ELISA Kit (Roche Diagnostics, Mannheim, Germany).

5.8.4. Electrophysiological data acquisition and processing

5.8.4.1. Electroencephalography data acquisition and processing. The NVX52 fast automatic event-related potential module (NeuroMed, China), associated with the EEG acquisition equipment amplifier, can detect three event-related potential components (N100, MMN, and P300) in 3 min. The superimposed waveforms of the multi-brain regions of the standard and target stimuli will be labeled in the multitask state, and digital correction will be performed using the component characteristics. EEG signal processing will be performed using the public EEGLAB_toolbox (https://sccn.ucsd.edu/eeglab/index.php) in MATLAB (MathWorks, Natick, MA, USA).

5.8.4.2. Magnetic resonance imaging data acquisition and processing. All MR data will be obtained using a 3T MAGNETOM Trio A Tim System (Siemens Healthcare, Erlangen, Germany). All participants will undergo MRI scans and neurite-oriented diffusion and density imaging (NODDI) using 3T MR tomography. FSL will be used to unless the brain tissue, and the MATLAB NODDI_toolbox NODDI (www.nitrc.org/projects/noddi_toolbox) calculation parameter (including neurite density index, orientation dispersion index, and isotropic volume fraction) will then be used [54].

5.9. Safety outcomes

Safety outcomes will include basic vital signs, electrocardiograph, laboratory tests (e.g., vital signs, Electrocardiograms, liver and kidney function tests), and adverse event (AE) records.

5.10. Termination criteria

The intervention will be terminated if the participant has a severe AE (SAE). This study will be terminated under two conditions:(1) the occurrence of a cluster of SAE associated with AM and (2) completion of all subsequent assessments. For participants with SAE, the study sponsor will be responsible for the cost of all medical examinations and provide assistance and care to the extent possible.

5.11. Data collection and management

The demographic data of the participants, including age, sex, education, body mass index, BP, history of hypertension and use of hypertension drugs, other medical history, and co-taking drugs, will be acquired from the hospital's electronic medical records system and outpatient system.

Participants will be provided with a self-filled questionnaire to record and report adverse reactions, and all AEs will be coded according to Standard 5.0 Common Terminology for Adverse Events and follow the recommendations of the Comprehensive Standard for Extended Reporting of Trials of Chinese Herbal Medicine Formulations.

All participants will be scheduled for weekly follow-up during the first month and monthly follow-up after the first month until the end of intervention. Fine-tuning will be performed according to clinical needs. Outcomes will be assessed at baseline, week 12, and week 24. Table 1 displays the experimental procedure.

5.12. Pharmacokinetics of AM active ingredients

Blood (500 μL) will be drawn from the elbow vein baseline and 12, 24 and 48 weeks after administration and transferred into 1.5 mL heparin-containing polyethylene tubes. The plasma samples will be immediately centrifuged at 25 °C at 2000×*g* for 20 min, and all samples will be reserved at −80 °C pending further analysis. After the sample preparation described in the "Sample preparation" section, the developed bioassays based on ultra performance tandem mass spectrometry technology will be used to assess AM plasma concentration levels. Nonventricular analyses were performed to examine and calculate important pharmacokinetic parameters using the Drug and Statistics 3.0 software (Chinese Committee of Mathematical Pharmacology, Shanghai, China).

5.13. Termination criteria

The intervention is terminated if the participant has a severe AE (SAE). This study will be terminated if (1) there is a cluster SAE associated with AM and (2) all subsequent assessments are completed. For participants with SAE, the study sponsor will be responsible for the cost of all medical examinations and provide assistance and care to the extent possible.

5.14. Quality control procedures

The research program was developed after the brainstorming of Chinese scientists and renowned senior neurologists. All data will be obtained through face-to-face interviews, clinical investigation, neuropsychological assessments, and laboratory inspection. All researchers (senior neurologists) will be trained based on the investigator's manual prior to assessment. We initiated a pilot study to monitor the applicability of the evaluated processes and questionnaires. In order to monitor compliance with the study medication, irregular visits will be scheduled. To ensure data privacy, security and accuracy, only statisticians will have access to the final data set, and investigators will not be allowed access. No one may make changes to the final data set.

5.15. Patient and public involvement

Participants will be continuously recruited through standardized public advertising and referrals by general practitioners, memory clinics, and informants. Patients and general public will not join in

developing research questions or study designs. Participants will be informed of the results by phone and email. Participants' personal information will not be disclosed.

5.16. Statistical analyses

All the data of this study will be assessed by two professional data entry personnel and uniformly filled in the research report form. It will then be reviewed by a professional auditor and entered by two data entry personnel to assess each other and prevent errors.

This study will be analyzed using data from the intention-to-treat (ITT) and per-protocol populations. The ITT population will include all participants who receive the intervention and receive a complete baseline assessment and at least one assessment of the primary outcome variable. Missing data will be processed using multiple interpolation. Covariance analysis will be used to evaluate efficacy and safety outcome measures, with intervention groupings as factors and baseline data as covariates. The role of neuroimaging, multimodal EEG, and plasma biomarkers in the transition from treatment (A vs. R) to outcome (i.e., neuropsychological manifestations) will be assessed. Changes in neuroimaging, multimodal EEG, and plasma biomarkers will be considered potential mediator variables. Both sets of baseline features will be categorically measured using the Fisher exact, chi-squares, or Cochran–Mantel–Haenszel test, and continuous measurements will be performed using either the *t*-test or Wilcoxon rank-sum test. The chi-squared or Fisher's exact test will be used to analyze AE incidence. All analyses will be performed using IBM SPSS Statistics version 25 (IBM Corp, Armonk, NY, USA). An independent statistician who is blinded to the assignment of the participants will perform statistical analyses. All hypothesis tests will be significant with a two-tailed p value < 0.05.

6. Discussion

The relationship between PH and cognition has received widespread attention over the past few decades. However, OH has recently been recognized as an important risk factor for cognitive impairment and AD. The vascular hypothesis considers that recurrent hypotension leads to cerebral hypoperfusion, leading to hypoxia damage and impaired cognition in vulnerable areas of the brain. AM can relieve cognitive dysfunction caused by inadequate brain perfusion through anti-inflammatory mechanisms. Furthermore, AM regulates neurotransmitters and receptors, inhibits amyloid aggregation, induces myelin repair and neurogenesis, and activates cognition-related signaling pathways [10]. Hence, we hypothesize that AM treatment can improve cognition in patients with of mild to moderate AD complicated with OH. Here, we will test this hypothesis and analyze which active ingredients in AM aid in improving cognitive impairment.

This trial uses an innovative and meticulous design. Previous drug trials forAD have included only changes in neuropsychological assessments as outcome measures; however, our study will combine plasma biomarkers, multimodal EEG, neuroimaging indicators, and network pharmacology. This approach will increase the credibility of the results and explore the mechanisms underlying cognition improvement. We plan to apply advanced diffusion imaging techniques with 3T MR tomography for NODDI. NODDI is a state-of-the-art microstructural imaging technique based on diffuse MRI that utilizes diffusion gradients of varying intensities to provide finer microstructural indicators of tissue than diffusion-weighted imaging [55]. This trial may provide a basis for subsequent clinical implementation of individualized drug administration, improve efficacy, avoid or reduce side effects, and achieve optimal treatment outcomes. Compared with double-blind trials, open-label trials are more closely aligned with clinical practice, exhibit higher participant compliance, and are more economical [56]. However, it may influence post-randomization management decisions, outcome reporting, and evaluation. External evaluators and statisticians will be blinded to the allocation of interventions.

This paper presents a rigorously designed, randomized controlled two-arm trial investigating the efficacy of AM in treating cognitive impairment in patients with OH. The methodology and results of this trial provide valuable insights into patient selection, outcome measurement, sample and effect size determination, and study duration for cognitive impairment with OH drug trials. These results may provide an effective treatment for individuals with the disease.

Ethics and dissemination

This trial was approved and supervised by Fujian Medical University Union Hospital (2021KJCX040). All participants give written informed consent and can withdraw at any time without giving a reason without prejudice to further treatment. Any adverse events related or unrelated to this trial will be addressed and records maintained for all participants. The results of this study will be published in high-impact interdisciplinary journals and at international and national conferences. In addition, all results of clinical trials will be posted on ClinicalTrials.gov.

Patient consent for publication

Not applicable.

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CRediT authorship contribution statement

Yingzhe Cheng: Writing – review & editing, Writing – original draft. **Lin Lin:** Writing – review & editing, Software. **Peilin Huang:** Writing – review & editing, Project administration, Formal analysis. **Jiejun Zhang:** Writing – review & editing, Methodology, Investigation. **Xiaodong Pan:** Writing – review & editing, Writing – original draft, Funding acquisition.

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.

Data availability

No data was used for the research described in the article.

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Abbreviation

OH	Orthostatic hypotension
AM	Astragalus membranaceus
AD	Alzheimer disease
BP	blood pressure
TCM	traditional Chinese medicine;
MRI	magnetic resonance imaging

EEG	electroencephalography
AE	adverse event
Simoa	Single-molecule Array
NODDI	neurite-oriented diffusion and density imaging
Aβ	β-amyloid
GFAP	glial fibrillary acidic protein
NfL	neurofilament light chain
P-tau	hyper-phosphorylated tau
ITT	intention-to-treat

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