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Original Article

Effect and Safety of Huannao Yicong Formula (还脑益聪方) in Patients with Mild-to-Moderate Alzheimer's Disease: A Randomized, Double-Blinded, Donepezil-Controlled Trial*

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ABSTRACT **Objective**: To assess the effect and safety of Huannao Yicong Formula (还脑益聪方, HYF) in the treatment of patients with mild-to-moderate Alzheimer's disease (AD). **Methods**: Sixty patients with mild-to-moderate AD were evenly randomized into HYF group and donepezil group with the random number method. Patients in the HYF group took 5 g of HYF granules twice daily and 5 mg placebo of donepezil once daily. Patients in the donepezil group took 5 mg donepezil once daily and 5 g placebo of HYF granules twice daily. The intervention lasted for 6 months. Clinical researchers, participants and statisticians were blinded to the treatment assignment throughout the study. The primary outcomes were scores of Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog) and Chinese Medicine Symptom Scale (CM-SS). The secondary outcomes were scores of Montreal Cognitive Assessment (MoCA) test and Mini-Mental State Exam (MMSE). The serum levels of acetylcholinesterase (AchE) and amyloid-β protein 42 ($A\beta_{42}$) were detected with enzyme-linked immunosorbent assay kits. The scale assessments were conducted at baseline, the 3rd and 6th months of treatment, respectively. Biochemistry tests were conducted at baseline and the 6th month of treatment. **Results**: A total of 52 patients completed the trial, 28 in HYF group and 24 in donepezil group. Compared with the baseline, HYF and donepezil significantly decreased the total scores of ADAS-Cog and CM-SS, and significantly increased the scores of MoCA and MMSE after 6-month treatment (all $P<0.01$). Both treatments remarkably reduced the serum levels of AchE and $A\beta_{42}$ (both $P<0.05$). The CM-SS total effective rate of HYF was significantly higher than donepezil [75.00% (21/28) *vs*. 54.17% (13/24), $P<0.05$]. No severe adverse events were observed in both groups. **Conclusion**: HYF is effective and safe for improving the cognitive function in mild-to-moderate AD patients. [Trial registration: Chinese Clinical Trial Registry (Reg No. ChiCTR-IOR-17011746)]

KEYWORDS Alzheimer's disease, Huannao Yicong Formula, randomized controlled double-blinded trial, Chinese medicine

Alzheimer's disease (AD) is a neurodegenerative disease that manifests with memory loss, cognitive decline, or combined with emotion changes.[1] Forty-seven million people live with dementia worldwide. This number is reported increasing to 131.5 million by 2050 as population aging.[2] Patients with AD account for approximately two thirds of all cases of dementia. Due to the dramatic rise in the number of people with dementia, AD imposes an overwhelming burden on individual families and society.[3,4]

Neurofibrillary tangles and amyloid plaques in brain are the core molecular pathogeneses of AD.[5] Therefore, development of more effective, safe and affordable prevention and treatment for AD is very important. Recently a remarkable global effort has been made to

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discover new drugs solving this problem. However, many results were unsatisfactory in clinical studies. For example, verubecestat, a beta-secretase 1 (BACE1), was declared failure in reducing cognitive decline in the patients with mild-to-moderate AD in 2017,[6] and a similar result with a tau-aggregation inhibitor leuco-methylthioninium-bis.[7] The continuous failures in clinical trials trigger a conceptual shifting of discovery of the drugs with effect on single target to multiple targets. Chinese medicine (CM) may be more suitable in the treatment of age-related cognitive decline.[8] Besides, emerging evidences have been reported to prove that CM benefits cognitive function of patients with AD.[9-11] Huannao Yicong Formula (还脑益聪方, HYF) is prescribed based on the CM theory to treat AD and widely used in clinic. Our previous studies have shown that HYF could prevent neuronal apoptosis in hippocampal CA1 area,[12,13] inhibit the activity of γ secretase,[14] and reduce neurotoxicity caused by amyloid peptide (Aβ) in AD rat models.[15,16] This is a randomized, double-blinded, donepezil-controlled trial, aiming to provide clinical evidence on the possible cognitive benefits of HYF in the patients with mild-to-moderate AD.

METHODS

Diagnostic Criteria

Diagnoses of dementia and AD were according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-Ⅳ)[17] and Recommendations from the National Institute on Aging-Alzheimer's Association Workgroups on diagnostic guidelines for AD.[18]

Inclusion Criteria

The patient inclusion criteria were as follows: (1) aged between 50 and 80 years old, (2) meeting the above diagnostic criteria, (3) Mini-Mental State Examination (MMSE) score between 10 and 26,[19] (4) Clinical Dementia Rating (CDR) scores are 0.5, 1, or 2 (defined as mild-to-moderate cognitive impairments),[20] (5) Hamilton Depression Scale (HAMD) score<20, (6) Montreal Cognitive Assessment (MoCA) score<26,[21] (7) Hachinski Ischemic Scale (HIS) score<4, and (8) signed the written informed consent form.

Exclusion Criteria

The patient exclusion criteria included: (1) with a history of mental disease or alcohol addiction, (2) cognitive impairment caused by epilepsy, trauma, encephalitis, Parkinson's disease or other diseases, (3) with severe visual, hearing loss or other serious diseases that might interfere cognitive tests, (4) use of memantine within 30 days before the baseline or acetylcholinesterase inhibitors such as donepezil within 120 days before baseline, and (5) with severe heart, liver or renal system disease.

Participants

All eligible AD patients were recruited from Community Healthcare Centers of the Jingzhuang Town, Yanqing District, Beijing and Xiaoshi Town, Sanhe, Hebei Province, from October 2014 to September 2017. All subjects were free to withdraw from the study. A total of 60 subjects were recruited and randomized into the HYF and donepezil groups by 1:1 rate.

Estimation of Sample Size

This is a randomized, double-blinded, donepezil-controlled trial that was designed and reported according to the CONSORT 2010 Statement[22] and Standard Protocol Items: Recommendation for Interventional Trials 2013 (SPIRIT 2013).[23] The sample size of this trial was estimated based on a previous trial.[24] The result suggested that 60 participants as the sample size were sufficient to detect a meaningful change.

Ethical Approval and Registration

This trial was approved by the Ethics Committees of Xiyuan Hospital, Chinese Academy of Chinese Medical Science (No. 2015XL037-2) and was registered in the Chinese Clinical Trial Registry (Reg No. ChiCTR-IOR-17011746).

Randomization and Blinding

Participants were randomly and evenly assigned to 2 groups using block randomization. Random numbers were generated using SPSS software (version 19.0) and assigned by an independent statistician at the Good Clinical Practice Institute of Xiyuan Hospital. Drugs were numerically labeled and sequenced according to the random numbers. The drug randomization was conducted by Beijing CMages Pharmaceutical Co., Ltd. The patients were assigned to the intervention according to the sequence. The clinicians, patients and statistician were blind to the patients' grouping assignment and intervention. Database was locked after all data was inputted. The grouping information was revealed when statistical analyses were conducted. The researchers revealed the second blinding and wrote the study report finally. Unblinding was allowed only in emergency situation for the patients.

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Intervention

Eligible patients received corresponding intervention in the two groups. Patients in HYF group took 5 g of HYF granules twice daily and 5 mg placebo of donepezil once daily. Patients in donepezil group took 5 mg of donepezil once daily and 5 g placebo of HYF granules twice daily. The intervention lasted for 6 consecutive months. Placebo granules and tablets matched in size and shape with HYF granules or donepezil tablets. HYF granules, composed of *Radix Polygoni multiflori*, *Radix Ginseng*, *Rhizoma chuanxiong*, *Rhizoma Acori tatarinowii*, *Rhizoma Coptidis* with a weight ratio of 12:10:9:6:5. HYF granules placebo were made from dextrin (95%) and HYF granules (5%). HYF granules and placebo were manufactured by Beijing CMages Pharmaceutical Co., Ltd. Donepezil and placebo were manufactured by Eisai China Pharmaceutical Co., Ltd. Participants were advised not to take any additional drugs. The consumption of research medication was checked during each week of the studying period regularly.

Safety Monitoring

To evaluate the safety of HYF, the biochemical assessments of the kidney and liver functions were conducted before test, at the 3rd and 6th months of treatment. Any adverse event occurring in the trial was recorded in details, including symptoms, degree, time, duration and treatments, and documented in case report form.

Outcome Assessment

The scale assessments were conducted by trained testers at baseline, the 3rd and 6th months of treatment, respectively. Biochemistry tests were conducted at baseline and endpoint of the trial.

Primary Outcomes

The Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog) is designed to measure cognitive ability and evaluate the efficacy of the anti-AD treatments.[25-27] It consists of 12 items. The total score ranges from 0 to 70, with the higher score indicating severer cognitive dysfunction.

The CM Symptom Scale (CM-SS) is a tool, according to the Guiding Principles of Clinical Research on New Drugs of Traditional Chinese Medicine issued in 2002, to evaluate the effects of treatments on CM syndromes,[28] including 4 main symptoms, 6 secondary symptoms, tongue coating and pulse. The efficacy rates of HYF and donepezil were categorized in 3 levels: (1) markedly effective: improved in the clinical symptoms and signs of CM, and a reduction rate of symptom score≥70%; (2) effective: improved in the clinical symptoms and signs of CM, and a 30%–69% reduction rate of symptom score; (3) inefficacious: no significant improvements in the clinical symptoms and signs of CM, or even worsened, and a reduction rate of symptom score<30%. Efficacy rate (%)=(score before treatment–score after treatment)/score before treatment × 100%.

Secondary Outcomes

MMSE and MoCA were used to evaluate the degree of cognitive dysfunction.[21,29] MMSE contains items assessing orientation, memory, attention and calculation, naming and visuospatial skills of patients. MoCA evaluates orientation, executive function, language ability, short-term memory, attention and visuospatial ability.

Serum Levels of Acetylcholinesterase (AchE) and Amyloid-β Protein 42

The level of acetylcholinesterase (AchE) was determined with the AchE activity assay kit (Nanjing Jiancheng Bioengineering Institute, China, lot No. 20161226-A). The level of amyloid-β protein 42 ($A\beta_{42}$) was determined with the human $A\beta_{42}$ enzyme-linked immunosorbent assay (ELISA) kit (Beijing Sinouk Institute of Biological Technology, China, lot No. 20170220).

Statistical Analysis

The SPSS 19.0 software was used for statistical analyses conducted by an independent statistician in a blinding manner. All analyses were in accordance with the intention-to-treat principle. The data were expressed as mean ± standard deviation ($\bar{x} \pm s$) or mean ± standard error of mean ($\bar{x} \pm SEM$). All baseline characteristics of both groups were compared using χ^2 test or Student's *t*-tests without any significant difference. The primary outcomes and secondary outcomes were analyzed with paired *t*-tests, independent *t*-tests or Wilcoxon signed-rank tests. Significance was defined as two-sided $P<0.05$.

RESULTS

Characteristics of Demography and Baseline

Sixty patients were recruited and evenly randomized into HYF group and donepezil group.

A total of 52 patients completed the trial, 28 in HYF group and 24 in donepezil group. The flow diagram of process in this trial is presented in Figure 1. No significant difference was identified between the two groups at the baseline (P>0.05, Table 1).

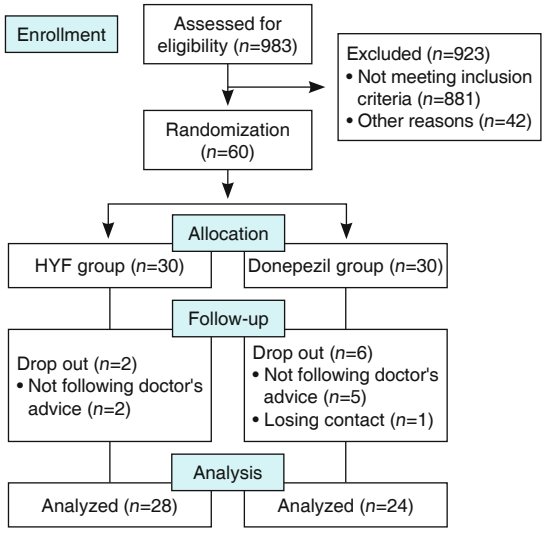

Figure 1. Flow Diagram of Process in HYF Trial

Table 1. Baseline Demographics and Clinical Characteristics of Patients ($\bar{x} \pm s$)

Characteristic	Donepezil group (24 Cases)	HYF group (28 Cases)	t	P value
Gender [Case (%)]				
Male	4 (16.67)	9 (32.14)	1.65	0.199
Female	20 (83.33)	19 (67.86)		
Age (Year)	60.88 ± 8.93	63.44 ± 6.42	–1.19	0.240
Education level (Year)	5.83 ± 3.59	5.25 ± 3.79	0.57	0.402
BMI (kg/m^2)	25.41 ± 4.29	24.08 ± 2.71	1.36	0.090
CDR (Score)	0.75 ± 0.44	0.68 ± 0.41	0.60	0.550
ADAS-Cog (Score)	20.20 ± 7.29	19.50 ± 6.05	0.38	0.709
CM-SS (Score)	12.67 ± 5.27	12.32 ± 5.31	–0.23	0.818
MMSE (Score)	21.96 ± 2.65	21.64 ± 2.47	0.45	0.659
MoCA (Score)	18.00 ± 4.25	17.00 ± 4.50	0.82	0.416
AchE (IU/L)	3801.22 ± 739.22	3990.96 ± 473.80	–1.94	0.053
Aβ_{42} (ng/mL)	1.19 ± 0.48	1.33 ± 0.51	–0.66	0.509

Notes: BMI: body mass index, CDR: Clinical Dementia Rating, ADAS-Cog: Alzheimer's Disease Assessment Scale-Cognitive Subscale, CM-SS: Chinese Medicine Symptom Scale, MMSE: Mini-Mental State Exam, MoCA: Montreal Cognitive Assessment, AchE: acetylcholinesterase, Aβ_{42}: amyloid-β protein 42

Primary Outcomes

ADAS-Cog

Compared with the baseline, the total scores of ADAS-Cog significantly decreased in HYF and donepezil groups at the endpoint (P<0.01), but no significant difference was observed between the two groups (P>0.05, Figure 2).

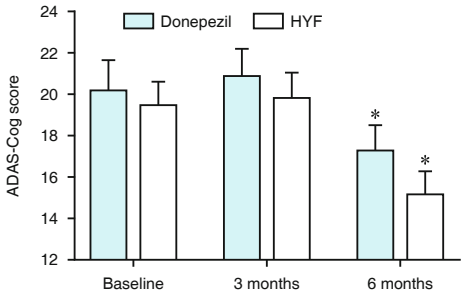

Figure 2. Comparison of ADAS-Cog Score between Two Groups ($\bar{x} \pm$ SEM)

Notes: *P<0.01 *vs*. baseline within group. ADAS-Cog: Alzheimer's Disease Assessment Scale-Cognitive Subscale

CM-SS

Compared with the baseline, the total CM-SS scores of the patients in both groups significantly decreased at the 3rd and 6th months (P<0.05 or P<0.01), however, the score of HYF group showed a greater decrease than that in donepezil group (P<0.05). HYF significantly improved the symptom domains of intelligent impairment, dull look and cold limbs at the 3rd month (P<0.05 or P<0.01). After 6 months of treatment, significantly decreases of scores were observed in domains of intelligent impairment, fatigue, dull look, fatigued cumbersome limbs, heavy-headedness and cold limbs in HYF group (P<0.01 or P<0.05), while only in the symptom domains of intelligent impairment, fatigue and dull look, the scores significantly decreased in donepezil group (P<0.01 or P<0.05). Compared with the donepezil group, the symptoms of dull look, fatigued cumbersome limbs, palpitation, heavy-headedness and cold limbs were significantly improved in HYF group (P<0.05, Figure 3).

The CM-SS total effective rate of HYF was significantly higher than donepezil [75.00% (21/28) *vs*. 54.17% (13/24), P<0.05].

Secondary Outcomes

MMSE Score

At the endpoint, MMSE scores significantly increased in both donepezil and HYF groups (P<0.05 or P<0.01), and no difference was found between the two groups (P>0.05). After 3 months of treatment, significant improvements in delayed recall, attention and calculation in HYF group, and in delayed recall and language ability in donepezil group were observed (P<0.05). After

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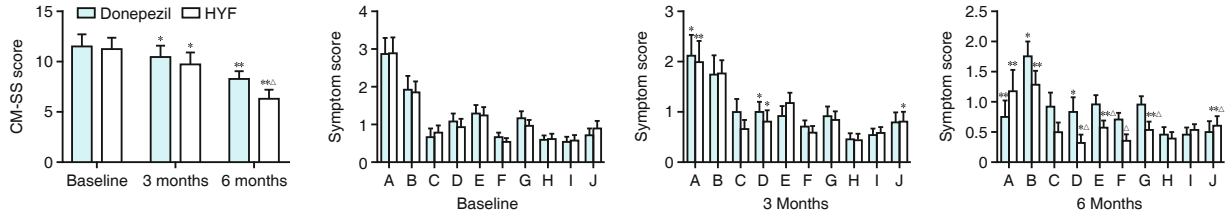

Figure 3. Comparison of CM-SS Score between Two Groups ($\bar{x} \pm$ SEM)

Notes: $^{*}P<0.05$, $^{**}P<0.01$ *vs*. baseline within group; $^{\triangle}P<0.05$ *vs*. donepezil group at the same time. CM-SS: Chinese Medicine Symptom Scale. A: intelligent impairment, B: fatigue, C: chest tightness not hunger, D: dull look, E: fatigued cumbersome limbs, F: palpitation, G: heavy-headedness, H: blows hot and cold, I: blausucht, J: cold limbs

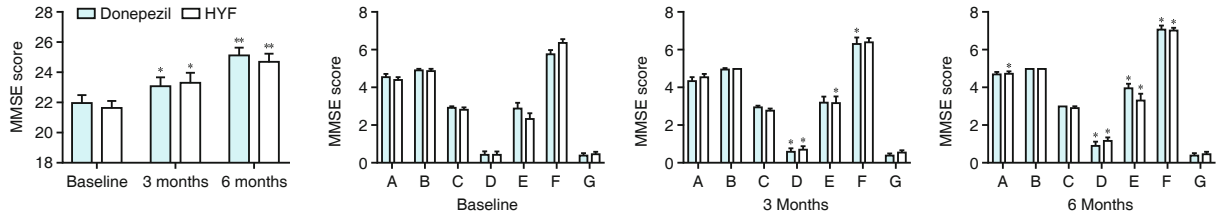

Figure 4. Comparison of MMSE Score between Two Groups ($\bar{x} \pm$ SEM)

Notes: $^{*}P<0.05$, $^{**}P<0.01$ *vs*. baseline within group. MMSE: Mini-Mental State Exam Scale. A: time orientation, B: spatial orientation, C: instant memory, D: delay recall, E: attention and calculation, F: language ability, G: visuospatial ability

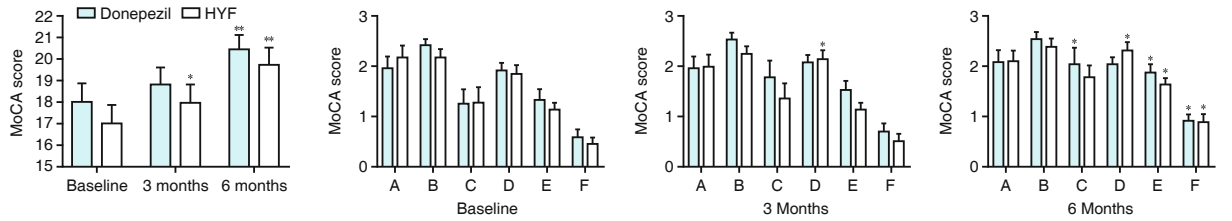

Figure 5. Comparison of MoCA Score between Two Groups ($\bar{x} \pm$ SEM)

Notes: $^{*}P<0.05$, $^{**}P<0.01$ *vs*. baseline within group. MoCA: Montreal Cognitive Assessment Scale. A: visuospatial ability and executive function; B: naming; C: short-term memory; D: attention; E: language ability; F: abstraction

6 months of treatment, the scores of delayed recall, attention and calculation, and language ability in both groups remarkably increased ($P<0.05$). Additionally, the patients in HYF group showed a significant improvement in time orientation ($P<0.05$, Figure 4).

MoCA Score

After 3 months of HYF treatment, MoCA score increased significantly ($P<0.05$), while no difference in donepezil group. At the endpoint, MoCA score significantly increased in donepezil and HYF groups ($P<0.01$). Attention was significantly improved in HYF group at the 3rd month ($P<0.05$). Attention, language ability and abstraction were significantly improved in HYF group after 6 months of treatment ($P<0.05$). Short-term memory, language ability and abstraction were significantly improved in donepezil group ($P<0.05$, Figure 5).

Serum Level of AchE and Aβ_{42}

Compared with the baseline, the levels of both AchE and Aβ_{42} significantly decreased in the two groups after 6-month treatment ($P<0.05$). Donepezil showed better effect than HYF in decreasing the serum level of AchE ($P<0.05$). The effects of donepezil and HYF in decreasing the serum level of Aβ_{42} were equivalent ($P>0.05$, Figure 6).

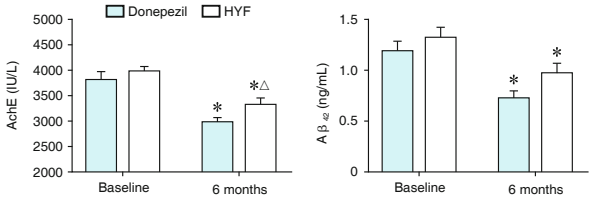

Figure 6. Comparison of Serum Levels of AchE and Aβ_{42} between Two Groups ($\bar{x} \pm$ SEM)

Notes: $^{*}P<0.05$ *vs*. baseline within group; $^{\triangle}P<0.05$ *vs*. donepezil group at the same time. AchE: acetylcholinesterase, Aβ_{42}: amyloid-β protein 42

Safety

In the trial there was no death event observed. However, there were adverse events with 1 patient in HYF group and 2 in donepezil group. In HYF group, 1 patient

had a headache due to hypertension. The headache gradually reduced after regularly taking anti-hypertension drugs. In donepezil group, 1 patient had dizziness and nausea, which disappeared after reducing donepezil to half dosage, twice daily. Another patient had dreaminess and facial fever, which disappeared after a few days. In addition, no clinically significant changes were found in laboratory assessments or physical examinations for the safety evaluation.

DISCUSSION

The primary analyses in this trial proved the effect of HYF in benefitting the cognitive function. Besides, it also showed that HYF was safe to be used. ADAS-Cog test is commonly used to assess the cognitive function, and is regarded as the gold standard for evaluating the efficacy of anti-dementia drugs.[30] After treatment, ADAS-Cog scores significantly decreased in both groups, without significant difference between groups. HYF significantly improved more symptoms of CM-SS than donepezil.

MMSE and MoCA are the two most widely used tests for cognitive screening.[31] Compared with MMSE, MoCA contains measurement of executive function.[31] But application of MoCA needs more time and it also requires higher level of education of the patients. The MMSE and MoCA scales were used together to improve the detection rate of cognitive impairment and increase the sensitivity and specificity. There are statistically improvements of MoCA and MMSE scores at the endpoint compared with baseline in both groups, with no significant difference between groups.

Now, the treatment of AD is still a problem of global concern. So far only acetylcholinesterase inhibitors and N-methyl-D-aspartate receptor antagonist were approved for the medical treatment of AD. Unfortunately, they cannot retard the pathological progress of AD, but only provide symptomatic benefit. Therefore, we need to broaden our horizons seeking more drugs. CM has gained considerable wisdom in the treatment of dementia during thousands of years of practice. Modern pharmacological studies also conform the therapeutic effects of Chinese herbal medicine (CHM), including numerous active components derived from CHM.

Modern pharmacological research provides clinical and experimental evidence for HYF and the ingredients contained in treating AD effectively. Some bioactive compounds identified in HYF,[32] such as ferulic acid,[33,34] berberine,[35,36] ginsenoside Rg1, Rb1 and Re,[37,38] and emodin,[39,40] have been shown to inhibit Aβ toxicity and the activity of AchE, thus exerting neuro-protective potentials. Moreover, oral consumption of ginseng slices or its extracts have been reported to be beneficial for the cognitive performance both in patients and different animal models, which were comprehensively reviewed elsewhere.[41,42] Besides, our previous clinical study showed that HYF could improve cerebral blood flow and alleviate circulatory inflammatory responses in senile patients with mild cognitive impairment.[24]

In the current study, the results showed that HYF improved the cognitive function of patients with mild-to-moderate AD. This may be explained by the decreased serum levels of $A\beta_{42}$ and AchE. Mounting evidences demonstrate that Aβ plays a crucial role in initiating AD.[43] Aβ derives from amyloid-β precursor protein,[44] and accumulates in the brain, resulting in damage to neuronal cells. Experimental evidences show that soluble Aβ oligomers, especially $A\beta_{42}$ oligomers, are the key neurotoxic substances.[45,46] Due to the high cost of amyloid positron emission tomography imaging and the invasive invention of cerebrospinal fluid collection, serum $A\beta_{42}$ was evaluated in this study.

AchE is considered to be another promising target for the treatment of AD. Considerable evidence show that AchE interacts with Aβ, promotes amyloid fibril formation[47] and triggers Aβ aggregation and deposition.[48] Previous animal experiments showed that HYF could reduce the deposition of Aβ in aging rats with cognitive impairment,[49] and decrease the content of AchE in β-amyloid precursor protein transgenic mice.[12] Our current study further proved its clinic effect in decreasing the serum levels of AchE in mild-to-moderate AD patients.

In summary, the findings indicated that HYF was effective in improving cognitive function and alleviating CM symptoms in patients with mild-to-moderate AD. No serious side-effect was observed during the trial. Our study indicated that HYF might be a potential new drug for the treatment of mild-to-moderate AD. However, larger sample size, multicenter studies are needed to confirm these findings, which is within our future research plan.

Conflict of Interest

We declare that there was no conflict of interest.

Author Contributions

Li H, Liu JP, Liu JG and Liu LT contributed to trial design and supervising the whole process. Yang Y, Fang JY, Wei Y and Cao Y were involved in the experimental conducting of the trial. Yang Y, Fang JY, and Wang HC contributed to the data analysis and manuscript preparation. All the authors read, revised and approved the final manuscript.

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