

Original Article

Clinical therapy of Di-Huang-Yi-Zhi in treating patients with amnesic mild cognitive impairment: a prospective, open-label and randomized study

Chao Gu^{1*}, Ting Shen^{2*}, Hongmei An¹, Canxing Yuan¹, Tianli Zhang¹, Tingting Gu¹

¹Department of Neurology, Longhua Hospital of Traditional Chinese Medicine University, Shanghai, China;

²Department of Emergency, Chinese Medicine Hospital of Traditional Chinese Medicine University, Shanghai, China. *Co-first authors.

Received September 6, 2016; Accepted December 20, 2016; Epub February 15, 2017; Published February 28, 2017

Abstract: Amnesic mild cognitive impairment (aMCI) represents the early stage of Alzheimer disease (AD). Early diagnosis and treatment of aMCI is essential for preventing AD conversion. To investigate the effects of Di-Huang-Yi-Zhi (DHYZ) formula in aMCI. We perform a prospective, open-label and parallel-control trial, with aniracetam treatment as a positive control. After randomization, patients in DHYZ group were administered with orally with DHYZ decoction (75 ml each time, twice a day) in 12 months. Patients in aniracetam group received aniracetam tablet (400 mg each time, three times a day). The efficacy outcome included score changes in mini-mental state examination (MMSE), Montreal cognitive assessment (MoCA), Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog), the Barthel Index for activities of daily living (ADL) and Traditional Chinese medical (TCM) symptoms before and after treatment. Patients showed significant improvement in scores in MMSE, MoCA, ADAS-Cog, ADL and TCM ($P<0.05$) after treatment in both groups. Meanwhile, more obvious changes were obtained in patients with TCM treatment than those with aniracetam treatment ($P<0.05$). Considering the primary outcome in TCM syndrome score scale, there was a remarkable improvement on memory before and after treatment, while changes in score were more obvious after DHYZ treatment compared to that after aniracetam treatment ($P<0.05$). Additionally, both DHYZ and aniracetam treatment were safe and well-tolerated. The traditional medicine DHYZ formula is effective for treating patients with aMCI and preventing AD conversion from MCI.

Keywords: Amnesic mild cognitive impairment (aMCI), Di-Huang-Yi-Zhi (DHYZ), aniracetam, cognition, memory

Introduction

Mild cognitive impairment (MCI), which is characterized by deficit in memory or other cognitive domains under the condition of normal daily functioning, can be defined as the transitional state between the normal aging and dementia [1]. MCI can be roughly divided into amnesic MCI (aMCI), multi-domain slightly impaired MCI and single non-memory domain MCI [2]. Although the concept and operational definitions of MCI are still undefined and controversial, subjects with MCI are supposed to be at high risk for dementia, especially with aMCI [3]. Amnesic MCI with the main characteristic of reduced memory, especially as episodic memory is the most common type of MCI and often represents the early stage of Alzheimer's disease (AD) [4]. According to previ-

ous statistical report, the annual AD conversion rate from MCI subjects was 10-15%, whereas this conversion rate in normal elderly population was significantly less (1-2% annually) [5]. Thus early diagnosis and treatment for aMCI have been considered important and necessary.

Diagnosis of aMCI mainly depends on various neuropsychological scales and brain imaging techniques nowadays. The treatment for aMCI still focuses on delaying and preventing the progress from aMCI to AD. Therefore, several therapeutics for treating AD such as donepezil has been applied for aMCI subjects. Previous study suggested that improvements including ADAS-cog total scores, tests of attention and psychomotor speed, as well as PGA scores had been obtained in more donepezil-treated

DHYZ therapy treating for aMCI

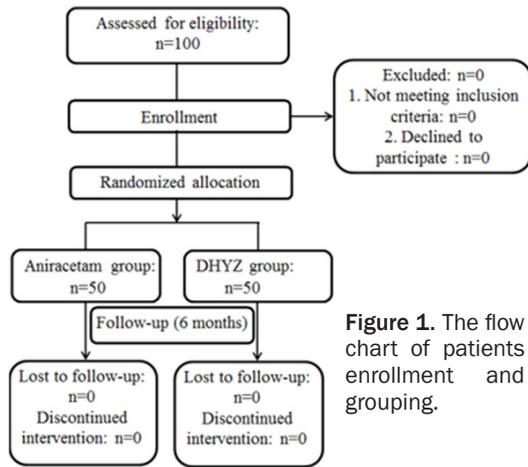


Figure 1. The flow chart of patients enrollment and grouping.

patients with MCI than placebo-treated patients [6], while other scholars indicate that oral AD drugs such as donepezil hydrochloride do not improve MCI [2]. Additionally, these cholinesterase inhibitors (ChEIs) always bring undesirable events like diarrhea, depression, and nausea [2]. So there is a need to explore other treatments with desired curative effects and fewer side effects preventing AD conversion from aMCI.

There is a long history for Traditional Chinese Medicine (TCM) preventing and treating cognitive decline [7]. Recent pharmacological and clinical studies confirmed that TCM could improve memory function and slow down aging with few side effects [8]. Di-Huang-Yi-Zhi (DHYZ), a compound herbal preparation of seven Chinese traditional herbs such as Shudi, Yizhi, Ziheche, has been applied for neurological disorders for approximately 900 years in China. The anti-senescence effects of DHYZ including regulating cell cycle-related protein expression in rats with early senile dementia and preventing oxidative damage in the brain tissue in a rat have been reported in several animal studies [9]. In addition, our previous clinical study concentrating on the effects of DHYZ in patients with PDD demonstrated that administration of DHYZ is safe and well-tolerated [10]. Therefore, we performed the present study to explore the efficacy of DHYZ on patients with clinical diagnosis of aMCI, with the aniracetam (a kind a nootropic agent widely applied in patients with cognitive disorders [11, 12]) treatment as a positive control. We hope our results would help to develop new strategies for treating aMCI as well as preventing AD.

Materials and methods

Study design

This prospective, open-label, randomized and parallel-control study was performed at the neurology department of LongHua Hospital affiliated to Shanghai University of Traditional Chinese Medicine from October 2012 to October 2013, aiming to evaluate the efficacy of Di-Huang-Yi-Zhi Formula for treating patients with aMCI. Patients treating with aniracetam were set as positive controls (Figure 1). The Medical Ethics Committee of Longhua Hospital affiliated to Shanghai University of Traditional Chinese Medicine approved this study and written informed consent was obtained from all participants after an adequate explanation for the study purpose, procedures, possible risks and discomforts.

Subjects

All subjects in our study were recruited from individuals with subjective memory complaints who wanted to visit a clinic at the neurology department in our hospital. Clinical diagnosis of aMCI was based on criteria establish by Petersen (1999). To enroll, patients should: 1) Age between 40 and 85 years; 2) Have subjective memory complaints confirmed by others; 3) Have mini-Mental State Examination (MMSE) score between 24 and 30, 17-item Hamilton depression scale score ≤ 12 , and Hach inskils chaemic Score (HIS) ≤ 4 ; 4) Have sufficient ability on vision, hearing and mental state to complete the neuropsychiatric assessment; 5) Have TCM syndromes with deficiency of kidney essence and phlegm-stasis consulting "Guiding principle of clinical research on new drugs of traditional Chinese medicine (2002 edition)" [13] and "Guiding principles of clinical research on mild cognitive impairment (protocol)" established by Tian *et al* [14]. If patients have one of the following exclusion criteria, they would be free from this study: 1) Presence of dementia; 2) Presence of cerebrovascular disease; 3) With depression or other psychiatric disorders within the last two years following the description in Diagnostic and Statistical Manual of Mental Disorders fourth edition (DSM-IV); 4) With a history of alcoholism, or drug abuse, or drug addiction within the preceding two years based on the DSM-IV criteria; 5) With a history of schizophrenia diagnosed by DSM-IV criteria; 6) Presence of other neuropsychological disor-

ders other than cMCI that may affect cognitive function; 7) Presence of hydrocephalus, cerebral tumor, progressive supranuclear palsy, epilepsy, subdural hematomas, disseminated sclerosis or other cerebral diseases.

Drugs

DHYZ formula contains a mixture of 15 g Shudi (Radix RehmanniaePreparata), 3 g Ziheche (Placenta Hominis), 15 g Danshen (Salviae Miltiorrhizae Radix), 10 g Shichangpu (Rhizoma Acorigraminei), 3 g Guijiajiao (Tortoise-shell glue), 10 g Fushen (Indian Bread with Pine) and 10 g Yizhi (Alpiniaoxyphylla). This mixture was processed into a decoction by the Pharmacy of Longhua Hospital affiliated to Shanghai University of Traditional Chinese Medicine according to the Pharmacopoeia of the People's Republic of China, 2000 edition [15]. Aniracetam tablets were provided by Jin Cheng Hai Si Pharmaceutical Co. Ltd (H20066667, Jincheng, Shanxi, China).

Grouping and treatment

Eligible patients were randomly assigned to either the Di-Huang-Yi-Zhi (DHYZ) group or aniracetam group at a ratio of 1:1. Randomization was performed using a computer-generated procedure. Patients in the DHYZ group were administered orally with DHYZ decoction (75 ml each time, twice a day). Patients in the aniracetam group received aniracetam tablet (two tablets each time, 200 mg/tablet, three times a day). Patients were treated with DHYZ decoction or aniracetam for 12 months.

Outcomes measures

Patients from each group underwent neuropsychological assessments, syndrome differentiation and physical examination before and after drug treatment.

No formal primary endpoint was predefined in our study. The main efficacy outcomes were evaluated through measurement of cognition, behavior and global function by MMSE (Chinese) [16] and Montreal cognitive assessment (MoCA) [17], Assessment Scale-Cognitive Sub-scale (ADAS-Cog) [18] and the activities of daily living (ADL) scales [19].

Additionally, efficacy outcomes were also assessed by TCM syndrome score scale, which was established by the Guideline Principle of Clinical Study on TCM new drug for dementia

and the Guiding principles of clinical research on mild cognitive impairment (protocol), for systemic manifestations, tongues and pulses before and after treatment. Based on the depiction of TCM syndrome score scale in our previous paper [10], two items including "Dry eye and throat" and "Paraphronia" belonging to secondary outcomes were deleted, since they were unrelated with the TCM syndrome of "deficiency of kidney essence and phlegm-stasis".

Drug safety was assessed on the basis of regular physical examination for body temperature, respiratory rate, pulse and blood pressure; routine tests for blood, urine and stool; Hepatic and renal functions detection for blood urea nitrogen and creatinine as well as electrocardiogram before and after treatment in both groups.

Statistical analysis

In our study, data were presented as mean \pm standard deviation (SD) or number (n). The TCM syndrome scores for patients with aMCI were determined by two independent expert TCM physicians in our hospital and both of them were blind to our study design and grouping. If they had any discrepancies, scores were determined by the third TCM physician in our hospital. Demographic data between two groups were compared using independent sample *t*-tests (continuous variables) or chi-square test (categorical variables). For efficacy, paired *t*-tests and independent sample *t*-tests were used for intra-group and inter-group comparisons, respectively. All statistical analyses were performed using the SPSS 19.0 software (SPSS, Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

Results

Subjects characteristics

Total of 100 patients with clinical diagnosis of aMCI were included in this trial: 50 patients in the aniracetam group and 50 patients in the DHYZ group. The aniracetam group consisted of 26 males and 24 females, with the average age of 67.81 ± 5.63 years (age range 56-80 years). The DHYZ group included 23 males and 27 females, with the average age of 69.50 ± 4.96 years (age range 59-78 years). As shown in **Table 1**, no significant differences were observed between these two groups in terms of

DHYZ therapy treating for aMCI

Table 1. Comparison of basic information and clinical features between aniracetam and DHYZ group

Basic information and clinical features		Aniracetam (n=50)	DHYZ (n=50)	P
Sex	Males	26 (52%)	23 (46%)	0.548
	Females	24 (48%)	27 (54%)	
Age (years)		67.81±5.63	69.50±4.96	0.724
BMI (kg/m ²)		25.62±3.21	27.53±5.26	0.676
Smoking	Presence	16 (32%)	19 (38%)	0.529
	Absence	34 (68%)	31 (62%)	
Diabetes	Presence	11 (22%)	8 (16%)	0.444
	Absence	39 (78%)	42 (84%)	

Notes: Data were presented as mean ± standard deviation (S.D.) or n (%). DYH: Di-Huang-Yi-Zhi. BMI: Body mass index.

Table 2. Changes in MMSE, MoCA, ADAS-Cog and ADL scores before and after treatment

Neuropsychological scales	Aniracetam (n=50)		DHYZ (n=50)	
	Before	After	Before	After
MMSE	24.82±1.69	25.01±1.53*	24.70±1.78	25.72±1.68*,#
MoCA	22.84±1.47	23.78±1.76*	23.18±1.66	24.66±1.93*,#
ADAS-Cog	20.14±3.36	17.06±2.08*	20.06±3.18	17.18±2.33*,#
ADL	24.06±3.11	22.88±2.01*	23.88±3.09	21.74±2.17*,#

Notes: Data were presented as mean±standard deviation (S.D.). *P<0.05, compare with before treatment; #P<0.05, compared with aniracetam group. MMSE: Mini-mental state examination; MoCA: Montreal cognitive assessment; ADAS-Cog: Alzheimer's Disease Assessment Scale-Cognitive Subscale; ADL: activity of daily living; DHYZ: Di-Huang-Yi-Zhi.

Table 3. Changes in TCM symptom scores before and after treatment

TCM syndrome scores	Aniracetam (n=50)		DHYZ (n=50)	
	Before	After	Before	After
Total	11.93±2.18	9.90±2.87*	12.32±2.38	7.39±2.16*,#
Primary syndrome				
Memory decline	3.08±0.86	2.32±0.63*	3.12±1.02	1.72±0.87*,#
Secondary syndrome				
Slow response	1.72±1.11	1.04±0.86*	1.68±1.03	1.06±0.98*
Soreness and weakness of waist and knees	2.24±0.97	2.04±0.53	2.30±1.03	1.38±0.66*,#
Dizziness and tinnitus	1.72±1.08	1.48±1.03	1.78±1.02	0.80±0.93*,#
Anorexia and dull stomach	1.56±1.03	1.44±1.05	1.54±1.01	1.48±1.03
Less sleep and dreaminess	1.80±1.03	1.62±1.05	1.88±1.01	0.96±0.73*,#

Notes: Data were presented as mean±standard deviation (S.D.). *P<0.05, compare with before treatment; #P<0.05, compared with aniracetam group. DHYZ: Di-Huang-Yi-Zhi.

sex, age, BMI, presence of smoking and diabetes, as well as the scores of MMSE, MoCA, ADAS-Cog, ADL or TCM before treatment ($P>0.05$).

Efficacy outcomes

Scores of several neuropsychological scales including MMSE, MoCA, ADAS-Cog and ADL before and after treatment were presented in **Table 2**. Patients with aMCI showed significant improvement in terms of MMSE, MoCA, ADAS-

Cog and ADL scores after both aniracetam and DHYZ treatment when compared to that before ($P<0.05$). Moreover, there were no statistical differences for these scale scores before treatment between aniracetam and DHYZ group, while more obvious changes in scores were observed in patients after DHYZ treatment (aniracetam vs DHYZ: 25.01±1.53 vs 25.72±1.68 in MMSE score; 23.78±1.76 vs 24.66±1.93 in MoCA score; 17.06±2.08 vs 17.18±2.33 in ADAS-Cog score; 22.88±2.01 vs 21.74±2.17 in ADL score; $P<0.05$). These results indicated

DHYZ therapy treating for aMCI

Table 4. Comparison of vital signs between aniracetam and DHYZ group

Parameters	Aniracetam (n=50)		DHYZ (n=50)	
	Before	After	Before	After
Body temperature (°C)	36.55±0.24	36.68±0.35	35.58±0.19	35.78±0.22
Respiratory rate (breaths/min)	18.12±0.56	17.38±0.89	19.11±0.32	19.03±0.28
Pulse (beats/min)	80.37±2.68	78.24±3.44	77.35±4.21	78.21±5.05
Blood pressure (mmHg)	128.23±10.33/82.03±7.27	122.45±12.12/78.04±6.26	135.16±13.48/78.02±8.34	130.08±9.25/76.22±6.35
Blood urea nitrogen level (mmol/L)	5.26±0.15	4.83±0.26	6.02±0.27	8.89±0.33
Blood creatinine level (μmol/L)	77.43±5.24	72.26±4.14	80.16±6.28	82.24±7.34

Notes: Data were presented as mean±standard deviation (S.D.). DHYZ: Di-Huang-Yi-Zhi.

that DHYZ seemed more effective for cognitive improvement in patients with aMCI than aniracetam.

There was significant improvement of TCM syndrome scores in both DHYZ and aniracetam group after treatment (**Table 3**). As displayed in **Table 3**, no obvious difference of TCM syndrome scores including total score, primary and secondary syndrome scores were found between two groups before treatment, while patients treating with DHYZ had more remarkable improvement on total TCM syndrome score than those with aniracetam (7.39±2.16 vs 9.90±2.87, $P<0.05$). In addition, for the primary syndrome of memory decline, both treatments could decrease the score, while score after treatment was dramatically lower in DHYZ group than that in aniracetam group (1.72±0.87 vs 2.32±0.63, $P<0.05$). Furthermore, for several secondary syndromes including soreness and weakness of waist and knees, dizziness and tinnitus and less sleep and dreaminess, more obvious changes of scores were observed in DHYZ group ($P<0.05$).

Safety outcomes

There were no significant changes in body temperature, respiratory rate, pulse and blood pressure, blood, urine and stool laboratory test, blood urea nitrogen and creatinine level as well as electrocardiogram in patients with aMCI before and after treatment in both groups (**Table 4**). These treatments were safe and well-tolerated for patients.

Discussion

Early diagnosis and treatment of aMCI are helpful to prevent AD conversion from MCI. Effective therapeutics for treating patients with aMCI has been investigated in a number of studies. We preformed the present study to explore the effects of DHYZ, a compound herbal prepara-

tion of Chinese traditional herbs, on patients with aMCI. Following the results in our study, patients with both treatments achieved significant improvement in MMSE, MoCA, ADAS-Cog, ADL as well as TCM syndrome scores compared to that before treatment. Additionally, DHYZ treatment seemed more effective in patients with aMCI. The beneficial effects obtained from TCM treatment included improvements on memory, global function as well as several clinical manifestations. Additionally, DHYZ therapeutics for treating patients with aMCI is safe and well-tolerated, based on our results and our previous description.

The main feature of aMCI is reduced memory. Considering the changes in primary syndrome of "Memory decline" in TCM syndrome score, patients with DHYZ treatment had an obvious improvement in memory, and changes in score were more significant than those with aniracetam treatment. The main bioactive substances from DHYZ formula for restoring memory and improving cognitive function are Shichangpu, Danshen and Yizhi and their beneficial effects on patients with aMCI are supposed to be related to the improvement of cholinergic system in the central nervous system. Previous studies indicated that Shichangpu had a neuroprotective effects on the central acetylcholine system, which can improve the ability of learning and memory in several model rats with neuronal and cognitive impairment [20, 21]. Meanwhile, Yizhi and its extracts such as sesquiterpenoids can suppress the activity of acetylcholinesterase [22, 23]. Additionally, Danshen a bioactive constituent in many other Chinese herbal remedies can improve the cognitive function via inhibiting the acetylcholinesterase activity [24, 25]. However, whether the beneficial effects of DHYZ on patients with aMCI are attributed to the regulatory effects on cholinergic system should be explored in further study.

Previous studies based on the TCM diagnostic criteria for MCI have found that the TCM pathological features of aMCI are described as kidney essence deficiency and phlegm and blood stasis. In TCM, kidney is supposed to play a vital role for the brain running normally and the energy from the kidney named as kidney essence can produce marrows such as cerebral marrow and bone marrow. A classical work of Chinese Medicine "Huang Di Nei Jing" said, "The brain is the sea of marrow" and "kidney stores essence to generate marrow". So the cerebral marrow generated from bone marrow is very important to maintain the normal physiological function of brain. The insufficient kidney essence directly reduces the generation of cerebral marrow, leading to various pathological conditions including diminished responsiveness. Meanwhile, phlegm is considered another cause for all prolonged diseases in TCM. Following the doctrines in TCM, phlegm-dampness and turbidity can obstruct the apertures, and then lead to a turbidity of the sea of marrow. Then the nourishment for the spirit of brain loses, finally resulting in a damaged intelligence as well as a poor memory. DHYZ formula consisting of seven herbs was created in the early 1990s based on the abundant academic experience of the old Chinese medicine. This formula is established using tonifying kidney and generating cerebral marrow as a principle, which is supposed to regulate the spirit and improve memory. As show in present study,

However, there were several limitations in our study. Firstly, since the pathological mechanism of aMCI is complicated and the TCM syndromes are also various, analyses for TCM syndromes in our study were insufficient. Secondly, there were no confirmed operational definitions of MCI. The improvements in memory, cognition, global function as well as clinical manifestation were only assessed by several neuropsychological scales and TCM syndrome score scales, further study with these scales combined with brain imaging is needed. Although our study should be faced with these limitations, it still provided a novel insight and a safe therapy in treating aMCI and preventing AD conversion from MCI.

In summary, our results showed that DHYZ therapy could significantly improve the memory as well as other cognitive function in patients with aMCI meanwhile the underlying mechanism may be associated with the improvement

of cholinergic system in the central nervous system in patients with aMCI. However, considering the limitations existed in our study, further study with more comprehensive analysis in TCM syndrome and more reasonable assessment methods is still needed.

Acknowledgements

This study was supported by Project within budget of Shanghai Pudong new district (No. PDYNZJ2015-19), The general projects of Shanghai municipal commission of health and family planning (No. 201540184), Project within budget of Shanghai committee of science and technology (No. 2012JW34) and The medical guide project of Shanghai committee of science and technology (No. 16401931400).

Disclosure of conflict of interest

None.

Address correspondence to: Hongmei An and Canxing Yuan, Department of Neurology, Longhua Hospital of Traditional Chinese Medicine University, 725 South Wanping Road, Shanghai 200032, China. Tel: +86-21-64385700; Fax: +86-21-64398310; E-mail: shdoctorchao@163.com (HMA); ycanxing@hotmail.com (CXY)

References

- [1] Arrieu S, Letenneur L, Orgogozo JM, Fabrigoule C, Amieva H, Le Carret N, Barberger-Gateau P and Dartigues JF. Incidence and outcome of mild cognitive impairment in a population-based prospective cohort. *Neurology* 2002; 59: 1594-1599.
- [2] Park SK, Jung IC, Lee WK, Lee YS, Park HK, Go HJ, Kim K, Lim NK, Hong JT, Ly SY, Rho SS. A combination of green tea extract and l-theanine improves memory and attention in subjects with mild cognitive impairment: a double-blind placebo-controlled study. *J Med Food* 2011; 14: 334-343.
- [3] Fisk JD and Rockwood K. Outcomes of incident mild cognitive impairment in relation to case definition. *J Neurol Neurosurg Psychiatry* 2005; 76: 1175-1177.
- [4] Dubois B and Albert ML. Amnesic MCI or prodromal Alzheimer's disease? *Lancet Neurol* 2004; 3: 246-248.
- [5] Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG and Kokmen E. Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol* 1999; 56: 303-308.
- [6] Salloway S, Ferris S, Kluger A, Goldman R, Griesing T, Kumar D and Richardson S. Efficacy

DHYZ therapy treating for aMCI

- of donepezil in mild cognitive impairment: a randomized placebo-controlled trial. *Neurology* 2004; 63: 651-657.
- [7] Wu TY, Chen CP and Jinn TR. Traditional Chinese medicines and Alzheimer's disease. *Taiwan J Obstet Gynecol* 2011; 50: 131-135.
- [8] Liu P, Kong M, Liu S, Chen G and Wang P. Effect of reinforcing kidney-essence, removing phlegm, and promoting mental therapy on treating Alzheimer's disease. *J Tradit Chin Med* 2013; 33: 449-454.
- [9] Tang B, Shi K, Li X, Wang H, Fang H, Xiong B and Wu Y. Effects of "Yang-warming and kidney essence-replenishing" herbal paste on cold-related asthma exacerbation. *J Tradit Chin Med* 2013; 33: 468-472.
- [10] Gu C, Shen T, An H, Yuan C, Zhou J, Ye Q, Liu T, Wang X and Zhang T. Combined therapy of Di-Huang-Yi-Zhi with Donepezil in patients with Parkinson's disease dementia. *Neurosci Lett* 2015; 606: 13-17.
- [11] Endo H, Tajima T, Yamada H, Igata A, Yamamoto Y, Tsuchida H, Nakashima Y, Suzuki Y, Ikari H and Iguchi A. Pharmacokinetic study of aniracetam in elderly patients with cerebrovascular disease. *Behav Brain Res* 1997; 83: 243-244.
- [12] Koliaki CC, Messini C and Tsolaki M. Clinical efficacy of aniracetam, either as monotherapy or combined with cholinesterase inhibitors, in patients with cognitive impairment: a comparative open study. *CNS Neurosci Ther* 2012; 18: 302-312.
- [13] Zheng X. Guiding principle of clinical research on new drugs of traditional Chinese medicine. Beijing: China medic-pharmaceutical sciences and technology publishing house; 2002. pp. 143.
- [14] Tian JZ, Shi J, Zhang XQ, Bi Q, Ma X, Wang ZL, Li XB, Sheng SL, Li L, Wu ZY, Fang LY, Zhao XD, Miao YC, Wang PW, Ren Y, Yin JX, Wang YY; Beijing United Study Group On MCI Of The Capital Foundation Of Medical Developments. Guiding principles of clinical research on mild cognitive impairment (protocol). *Zhong Xi Yi Jie He Xue Bao* 2008; 6: 15-21.
- [15] Weyand CM and Goronzy JJ. Association of MHC and rheumatoid arthritis. HLA polymorphisms in phenotypic variants of rheumatoid arthritis. *Arthritis Res* 2000; 2: 212-216.
- [16] Folstein MF, Folstein SE and McHugh PR. "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; 12: 189-198.
- [17] Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, Cummings JL and Chertkow H. The montreal cognitive assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc* 2005; 53: 695-699.
- [18] Rosen WG, Mohs RC and Davis KL. A new rating scale for Alzheimer's disease. *Am J Psychiatry* 1984; 141: 1356-64.
- [19] Collin C, Wade D, Davies S and Horne V. The barthel ADL index: a reliability study. *Int Disabil Stud* 1988; 10: 61-63.
- [20] Kim JH, Hahm DH, Lee HJ, Pyun KH and Shim I. *Acori graminei rhizoma* ameliorated ibotenic acid-induced amnesia in rats. *Evid Based Complement Alternat Med* 2009; 6: 457-464.
- [21] Lee B, Choi EJ, Lee EJ, Han SM, Hahm DH, Lee HJ and Shim I. The neuroprotective effect of methanol extract of *gagamjungjihwan* and *fructus euodiae* on ischemia-induced neuronal and cognitive impairment in the rat. *Evid Based Complement Alternat Med* 2010; 2011: 685254-685254.
- [22] Chen P, Wang PP, Jiao ZZ and Xiang L. Sesquiterpenoids from the fruits of *alpinia oxyphylla* and their anti-acetylcholinesterase activity. *Helvetica Chimica Acta* 2014; 97: 388-397.
- [23] Shi SH, Zhao X, Liu B, Li H, Liu AJ, Wu B, Bi KS and Jia Y. The effects of sesquiterpenes-rich extract of *alpinia oxyphylla* miq. on amyloid- β -induced cognitive impairment and neuronal abnormalities in the cortex and hippocampus of mice. *Oxid Med Cell Longev* 2014; 2014: 451802.
- [24] Lin HQ, Ho MT, Lau LS, Wong KK, Shaw PC, Wan DC. Anti-acetylcholinesterase activities of traditional Chinese medicine for treating Alzheimer's disease. *Chem Biol Interact* 2008; 175: 352-354.
- [25] Zhao Q, Matsumoto K, Tsuneyama K, Tanaka K, Li F, Shibahara N, Miyata T and Yokozawa T. Diabetes-induced central cholinergic neuronal loss and cognitive deficit are attenuated by tacrine and a Chinese herbal prescription, *kangen-karyu*: elucidation in type 2 diabetes db/db mice. *J Pharmacol Sci* 2011; 117: 230-242.