Research Paper

Yi-Chi-Tsung-Ming-Tang Reduced Aβ(1-40)-induced Neurotoxicity via of Acetylcholine and NMDA Receptors Expression, ROS Generation and Tau Phosphorylation

C. H. YEH[1,2], H. C. HUANG[3], K. S. CHANG[4], C. J. CHEN[3], M. L. YANG[5], S. L. TSAI, H. W. LIN[6,7] AND Y. H. KUAN[8,9]*

Department of Sport and Health Management, College of Nursing and Health, Da-Yeh University, [1]Department of Neurology, Yuan Rung Hospital, Changhua, [2]Department of Nursing, College of Medicine and Nursing, Hung-Kuang University, [3]Department of Chinese Pharmaceutical Sciences and Chinese Medicine Resources; College of Pharmacy, China Medical University, Taichung, [4]Department of Cardiology, Yuan Rung Hospital, Changhua, [5]Department of Anatomy, School of Medicine, Chung Shan Medical University, [6]Department of Optometry, Asia University, [7]Department of Medical Research, China Medical University Hospital, China Medical University, [8]Department of Pharmacology, School of Medicine, Chung Shan Medical University, [9]Department of Pharmacy, Chung Shan Medical University Hospital, Taichung, Taiwan

***Yi-Chi-Tsung-Ming-Tang* is a traditional Chinese medicine formula often prescribed for preventing or treating dizziness, tinnitus, mental fatigue and blurred vision. Recent study has demonstrated that Aβ(1-40)-induced neurotoxicity could be improved by this drug. The aim of present study was to determine the effects and protective mechanism of *Yi-Chi-Tsung-Ming-Tang* on Aβ(1-40)-induced death of primary cortical neurons. Primary cultures of Sprague-Dawley rat cortical neurons were exposed to Aβ(1-40), after the treatment with *Yi-Chi-Tsung-Ming-Tang* for 1 h. Methyl-thiazolyl-tetrazolium reduction assays were used to detect cell viability and the expression of acetylcholine receptors, N-methyl-D-aspartate receptors and phosphorylated and non-phosphorylated forms of tau were measured by western blot. Fluorometric assays were applied to detect the generation of reactive oxygen species. Pretreatment of primary cortical neurons with *Yi-Chi-Tsung-Ming-Tang* significantly inhibited Aβ(1-40)-induced cytotoxicity and reversed Aβ(1-40)-induced β-amyloid accumulation and acetylcholine receptor expression in a concentration-dependent manner. In addition, not only the Aβ(1-40)-reduced expression of N-methyl-D-aspartate receptors 1/2A was reversed but also the Aβ(1-40)-induced reactive oxygen species generation and tau phosphorylation expression were inhibited by *Yi-Chi-Tsung-Ming-Tang* in a concentration-dependent manner.**

Key words: *Yi-Chi-Tsung-Ming-Tang*, Aβ, reactive oxygen species, tau, NMDARs

Alzheimer's disease (AD) is an irreversible, progressive neurodegenerative disease characterized by the loss of memory, which results in dementia, cognitive defect, behavioural disturbance and neuropsychiatric symptoms[1]. A report published in 2006 had shown that 26.6 million people were affected by AD and approximately $156 billion were spent annually on caring for AD patients worldwide. By the year of 2050, the prevalence of AD is expected to quadruple and shall place a considerable burden about health cost on society[2]. Up to now, the drug treatment for AD mainly evolved around two mechanisms, which involved the inhibitors of acetyl cholinesterase and the stimulators of N-methyl-D-aspartate receptors (NMDAR). Unfortunately, these drugs do not much improve the symptoms caused by AD[3].

The neuropathological hallmarks of AD contain amyloid-beta (Aβ) aggregation, cerebral amyloid angiopathy, neurofibrillary tangles formation, neuron death, and synaptic loss[4]. Aβ is the peptide fragment derived from an integral membrane protein, the amyloid precursor protein (APP), by secreatases processing. Aβ(1-40) is one of the Aβ isoforms and the most abundant variant *in vivo*[5,6]. Aβ and its derived oligomeric species can bind to NMDAR thus facilitating the endocytosis and activation of NMDAR. In addition, Aβ and its derived oligomers induce

***Address for correspondence**
E-mail: kuanyh@csmu.edu.tw

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hyper phosphorylation of tau, which results in self-aggregation and microtubule destabilization. These molecule-associated pathogens, including up-regulated reactive oxygen species (ROS) generation and down-regulated acetylcholine receptor (AchR) expression, induce excitotoxicity in hippocampal and cortical neurons[7,8].

Yi-Chi-Tsung-Ming-Tang (YCTMT) is a traditional Chinese medicine formula and presented by the Chinese physician, Dong-Yuan Li, in Jin Dynasty (AD 1200s). In traditional medicine, YCTMT has been used to prevent symptoms such as dizziness, tinnitus, deafness, mental fatigue, and blurred vision[9-11]. In a previous study, we have demonstrated that YCTMT has a therapeutic effect on AD induced by Aβ(1-40) *in vivo*[12]. In the present, we aimed to study the molecular mechanism and protective effects of YCTMT on Aβ(1-40)-induced cytotoxicity in cultured primary cortical neurons.

MATERIALS AND METHODS

YCTMT is composed of *Astragalus membranaceus* (Fisch.) Bunge, *Ginseng quinquefolium* (L.) Alph. Wood, *Pueraria lobata* (Willd.) Ohwi, *Paeonia lactiflora* Pall, *Phellodendron chinense* C.K.Schneid, *Vitex rotundifolia* L.f., *Cimicifuga foetida* L. and *Glycyrrhiza uralensis* Fisch. in a ratio of 5:5:5:1:1:1.5:3:5 (dry weight). All components were purchased from a Chinese herbal shop in Taichung, Taiwan and were certified at the Department of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, China Medical University, Taichung, Taiwan. Voucher specimens (No. 2011YCTMT-A~H) have been deposited at the China Medical University. Aβ(1-40) was purchased from Tocris Bioscience (Ellisville, MO, USA) and dissolved in a vehicle containing 35% acetonitrile and 0.1% trifluoroacetic acid. Glycyrrhizin was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). Polyvinylidene fluoride (PVDF) membrane filter was obtained from Millipore Corp. (Bedford, MA, USA). Other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

High performance liquid chromatography (HPLC) analysis of YCTMT:

Hot water extract of YCTMT was prepared by dissolving it in methanol (5.0 mg/ml). Sample and standard solutions of glycyrrhizin were filtered through a filter cartridge (pore size of 0.22 μm) prior to analysis. The chemical content quantification was performed on a Shimadzu HPLC system equipped with Shimadzu LC-20AT pump, Shimadzu SIL-20 auto sampler, and Shimadzu SPD-M20A detector. The HPLC profile was performed on a RP-18 column (Cosmosil 5C18-AR-II, 4.6×250 mm, 5 μm) at a flow rate of 1.0 ml/min, detected at UV 254 nm. The injection volume was 10 μl. The mobile phase was composed of 0.1% TFA water solution (A) and methanol (B). The solvent gradient was as follows: 0-60 min from 5% B to 55% B. By diluting the stock solution, a series of standard solutions (glycyrrhizin) were prepared with concentrations of 2, 1, 0.5, 0.25, 0.1, 0.05, and 0.025 mg/ml used to calculate the concentration of examined compounds.

Liquid chromatography tandem-mass spectrometry (LC-MS/MS) analysis of YCTMT:

LC-MS/MS experiments were performed on a Dionex Ultimate 3000 HPLC system (Dionex, Germany) coupled with an ESI-ion trap MS (HCTultra PTM Discovery, Bruker Daltonics, Germany). A linear gradient on a C18 LC column (Atlantis T3 C18 5 μm 2.1×150 mm) was used to separate the water extract of YCTMT with a flow rate of 0.25 ml/min. The mobile phase A consisted of water containing 0.1% v/v formic acid and mobile phase B containing 99.9% v/v acetonitrile and 0.1% v/v formic acid. A gradient elution was applied from 5% v/v B to 60% B in the first 25 min, and to 90% B over 5 min. It was then held at 90% B for another 4 min at the flow rate of 0.25 ml/min, which was followed by a return to the starting conditions and re-equilibration of the column for 5 min with 5% B v/v prior to the next injection. The ESI source was operated in positive or negative ion mode. Nitrogen was used as a nebulizing (45 psi) and drying gas (10 l/min, 350°). For the MS/MS settings, the most eight intense ions from each MS full scan spectrum were automatically selected as the precursor ion peaks for the following auto MS/MS experiments. Helium was used as the collision gas.

Cortical neuron cultures:

Sprague-Dawley rats were used to obtain primary cultures of cortical neurons. The protocols for animal experiments were performed according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Animal Experimental Committee of China Medical University under permit number: 96-113-C. Cultures of primary cortical neurons were prepared from cerebral cortices of 18 d-old embryonic rats[13]. Briefly, pooled

dissected cerebral cortices were digested with papain solution and plated at a density of 8×10^5 cells/cm^2 on poly-D-lysine-coated dishes. One day after seeding, the culture medium was replaced with Neurobasal serum-free medium containing 1% B27 supplement, 0.5 mM l-glutamine, and 1% penicillin/streptomycin. Neurobasal serum-free medium was replaced every three days and 10 μM cytosine arabinoside was added to the medium on the third day. The purity of primary neurons was over 95% and was employed between 12 to 14 d in culture.

Cell viability assays:

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide tetrazolium (MTT) reduction assay was used to monitor cell viability by measuring the mitochondrial succinic dehydrogenase activity as described previously[14]. Experimental neuron cultures were either treated with 0-20 μM of Aβ(1-40) alone for 24 h or first treated with various concentrations of YCTMT for 1 h followed by 10 μM of Aβ(1-40) for 24 h. As for the positive control group, 1% Triton X-100 was added to the well, followed by 0.5 mg/ml MTT solution. After 24 h incubation, the quantity of formazan was determined by eluting with dimethyl sulfoxide and measured at 550 nm using a microplate reader (MR4000; Dynatech, Chantilly, VA). Eqn. 1: percent cell viability (%) = OD_{550} value of treated groups/OD_{550} value of untreated control×100.

Western blot:

Cultures were washed twice with phosphate-buffered saline (PBS) and harvested in RIPA lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 50 mM sodium fluoride, 0.2 mM sodium orthovanadate, 1 μg/ml leupeptin, 1 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride). After centrifugation, the protein concentration in the supernatant was determined by Bradford assay. Protein extracts were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to PVDF membranes. After blocking with 5% non-fat milk in PBS for 1 h, the membranes were washed with PBS containing 0.1% Tween-20 (PBST), and incubated at room temperature for 2 h with antibodies including β-amyloid, AchR, NMDAR1, NMDAR2, β-actin, and phosphorylated and non-phosphorylated forms of tau, respectively. Then the membranes were washed again and the horseradish peroxidase-labelled IgG was added at room temperature for 1 h. The blots were developed using enhanced chemiluminescence reagents[15].

Measurement of intracellular ROS generation:

After YCTMT pretreatment, cultured neurons were incubated with 20 μM 2,7-dichlorfluorescein-diacetate (DCFH-DA) for 30 min in the dark, then washed, and the intracellular content of ROS was measured by using the semi-quantitative fluorescence technique[16] with a fluorescence microplate reader (Molecular Devices, CA, USA) at excitation/emission wavelengths of 400/505 nm. Results were expressed as fluorescence intensity.

Statistical analysis:

At least three independent experiments were performed as indicated in the figure legends. All data obtained were analysed using one-way analysis of variance (ANOVA) followed by post-hoc between-group analyses using Dunnett and Scheffé's test for multigroup comparisons and expressed as mean±standard deviation (SD). The criterion for statistical significance was $P<0.05$ for all evaluations.

RESULTS AND DISCUSSION

The reference compound of glycyrrhizin was identified in YCTMT by HPLC, which acted as an indicator compound for quality check of extraction procedure of each batch (fig. 1). The content of glycyrrhizin was calculated to be 0.78 mg/ml of YCTMT by HPLC quantification. The components of YCTMT extract were identified by LC-MS/MS. According to the molecular weights of precursor ion and their corresponded fragment ions in MS/MS spectra referring to published literature. The base peak chromatogram (BPC) of positive and negative ions of the YCTMT extract by LC-MS/MS analysis was shown in fig. 2. Albiflorin, paeoniflorin, palmatine, berberine, daidzein, formononetin, gallic acid, caffeic acid, puerarin, ferulic acid, and glycyrrhizin were identified in YCTMT extract by LC-MS/MS analysis according to MS and MS/MS ions (Table 1).

The viability of primary cortical neurons was measured by MTT assay. As shown in fig. 3A, Aβ(1-40) reduced cell viability in a concentration-dependent manner, significant effect started at 5 μM. Meanwhile, neurons incubated with various concentrations of YCTMT alone showed no significant effect on viability, even when the concentration of YCTMT was up to 320 μg/ml. But neurons pretreated with YCTMT attenuated Aβ(1-40)-impaired cell viability in a concentration-dependent manner starting from 12.8 μg/ml and up ($P<0.05$) (fig. 3B).

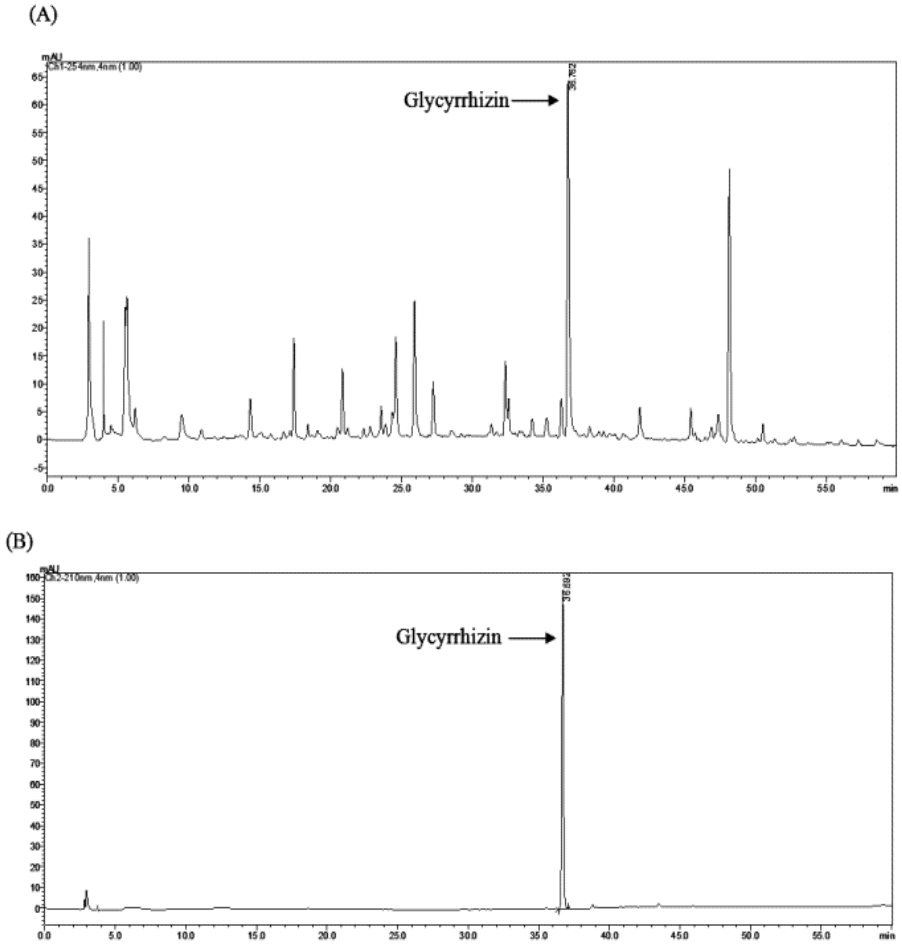

Fig. 1: HPLC profile of YCTMT
(A) The chemical profile of the YCTMT was performed using a RP-18 column and detected at UV 254 nm, (B) HPLC chromatograms of the reference compound (glycyrrhizin, t_R=36.692 min)

To explore the protective effect of YCTMT on cortical neurons, the expression of β-amyloid and AchR was measured using western blot. As shown in fig. 4, administration of Aβ(1-40) markedly increased the expression of β-amyloid as compared with the control. However, pre-incubation with YCTMT for 1 h inhibited the expression of β-amyloid in Aβ(1-40)-stimulated cortical neurons in a concentration-dependent manner starting at 12.8 μg/ml (P<0.05). Administration of Aβ(1-40) for 4 h markedly reduced the expression of AchR as compared with the control. β-actin plays as a stable and constitutive internal control and has the same expression. With 1 h pre-incubation of YCTMT, the reduced expression of AchR in Aβ(1-40)-stimulated cortical neurons was reversed in a concentration-dependent manner also starting at 12.8 μg/ml (P<0.05).

Administration of Aβ(1-40) markedly reduced the expression of NMDAR1 and NMDAR2A as compared with the control. However, pre-incubated with YCTMT for 1 h reversed the reduced expression of NMDAR1 and NMDAR2A in Aβ(1-40)-stimulated cortical neurons in a concentration-dependent manner starting from 12.8 μg/ml and up (P<0.05, fig. 5).

Administration of Aβ(1-40) markedly induced intracellular ROS generation as compared with the control. β-actin plays as a stable and constitutive internal control and has the same expression, as shown in fig. 5. However, pre-incubated with YCTMT for 1 h inhibited intracellular ROS generation in Aβ(1-40)-stimulated cortical neurons in a concentration-dependent manner, significant effect starting at 12.8 μg/ml (P<0.05) (fig. 6). Administration of Aβ(1-40) markedly induced phosphorylation of tau as compared with the control. However, pre-incubated with YCTMT for 1 h inhibited the phosphorylation of tau in Aβ(1-40)-stimulated cortical neurons in a concentration-dependent manner, significant effect starting at 12.8 μg/ml (P<0.05, fig. 7).

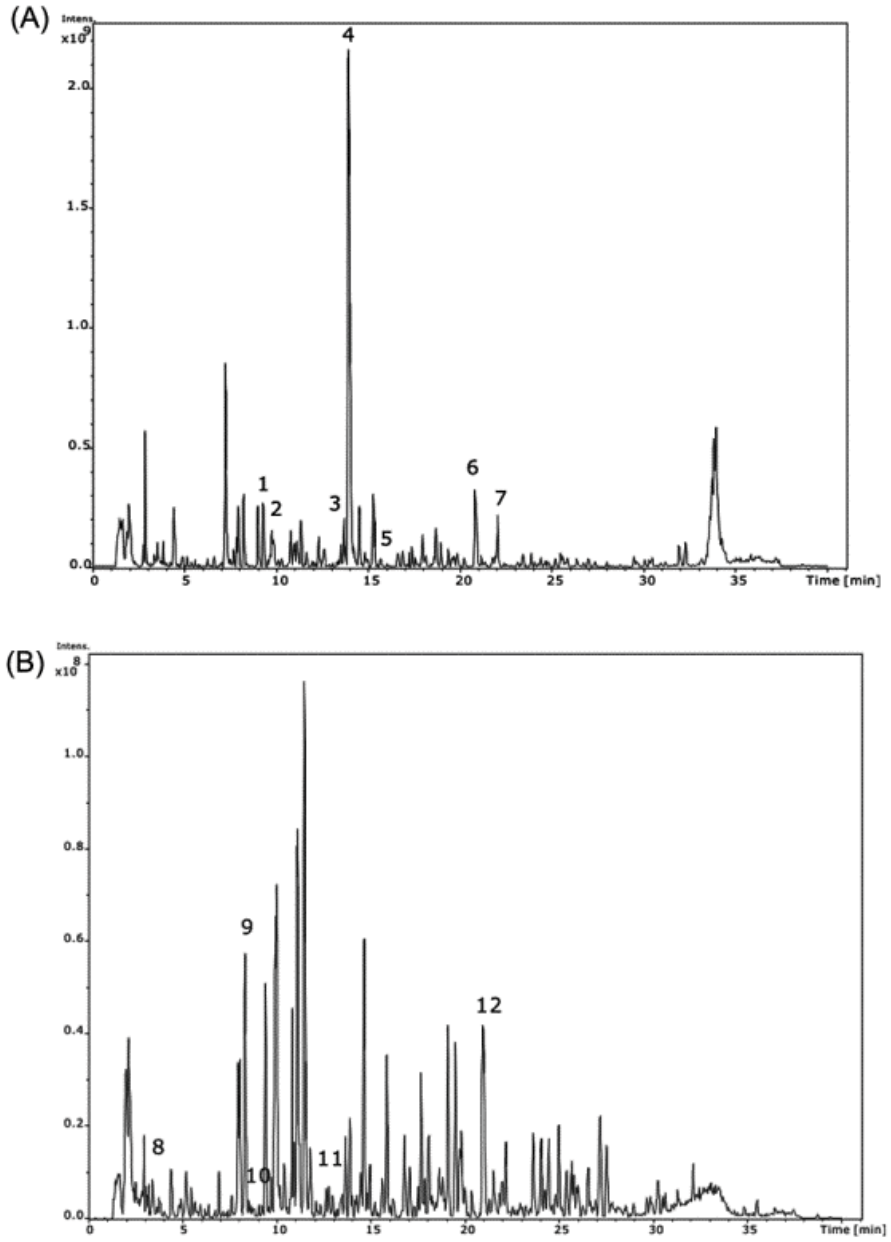

Fig. 2: The BPC of YCTMT extract by LC-MS/MS analysis
(A) Positive and (B) negative ions

TABLE 1: HPLC-MS-MS IDENTIFICATION OF THE CONSTITUENTS IN YCTMT EXTRACT

Peak no.	Selected ion	MS (ESI)	MS/MS (ESI)	Molecular formula	Proposed compound
1	$[M+H]^+$	481.2	319.2, 196.9	$C_{23}H_{28}O_{11}$	Albiflorin
2	$[M+NH_4]^+$	498.3	301.1, 178.9	$C_{23}H_{28}O_{11}$	Paeoniflorin
3	$[M]^+$	352.5	337.1, 308.1	$C_{21}H_{22}NO_4$	Palmatine
4	$[M]^+$	336.1	321.1, 292.1	$C_{20}H_{18}NO_4$	Berberine
5	$[M]^+$	254.1	198.9, 137.0	$C_{15}H_{10}O_4$	Daidzein
6	$[M+H]^+$	823.5	647.5, 453.4	$C_{42}H_{62}O_{16}$	Glycyrrhizin
7	$[M]^+$	268.3	254.0, 237.0, 213.0	$C_{16}H_{12}O_4$	Formononetin
8	$[M-H]^-$	169	125.0	$C_7H_6O_5$	Gallic acid
9	$[M-H]^-$	415.2	295.1, 267.0	$C_{21}H_{20}O_9$	Puerarin
10	$[M-H]^-$	179.1	135.0	$C_9H_8O_4$	Caffeic acid
11	$[M-H]^-$	193.0	177.9, 149.0, 134.0	$C_{10}H_{10}O_4$	Ferulic acid
12	$[M-H]^-$	821.5	627.1, 361.1	$C_{42}H_{62}O_{16}$	Glycyrrhizin

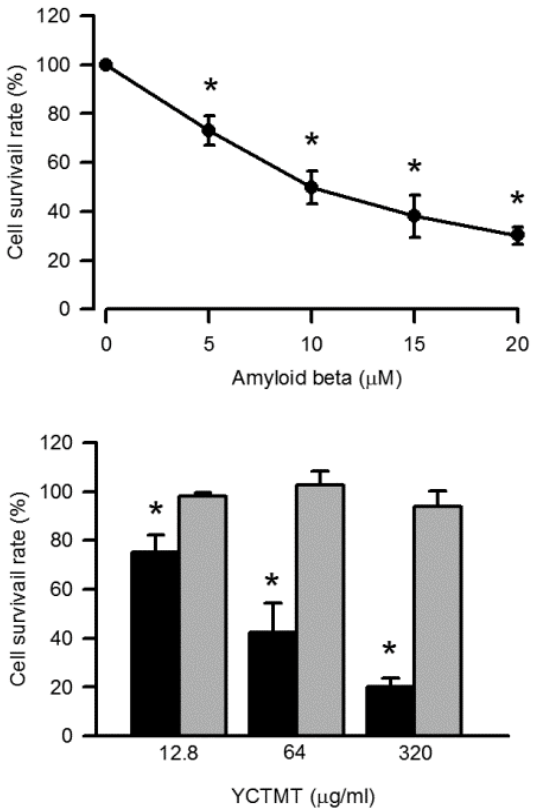

Fig. 3: Effects of YCTMT on Aβ(1-40)-reduced cell viability
Cell viability was measured by MTT assay. Data are expressed as mean±SD (n=4). *P<0.05 was considered significant as compared with the vehicle values. ■ With amyloid beta; □ without amyloid-β

YCTMT is one of the most important traditional Chinese medicines and widely used in Asian countries such as China, Japan, and Korea. However, there are several pieces of evidence, which demonstrate that some of the YCTMT composites, such as *A. membranaceus* (Fisch.) Bunge, *G. quinquefolium* (L.) Alph. Wood, *P. lobata* (Willd.) Ohwi, and *G. uralensis*, have the ability to protect against AD[17-20]. Moreover, YCTMT improves Aβ(1-40)-induced AD-like phenotype, including learning and memory loss[12]. The death of neuron in cerebral cortex is an important feature of AD[3]. In the present study, we also found Aβ(1-40), the most abundant of Aβ isoform-induced cytotoxicity in primary cortical neurons. Though YCTMT alone has no effect on cell viability of cortical neurons, Aβ(1-40)-induced neuron death is inhibited by pretreatment of YCTMT in a concentration-dependent manner. These results suggested that AD could be improved by YCTMT via reduced death of cortical neuron.

The imbalance between clearance and production of Aβ causes its intracellular accumulation, which is the initiating factor of AD. These accumulated intracellular Aβ will further deposit and assemble into fibrils, which arrange themselves into parallel β-pleated sheets to form insoluble plaques[8]. These Aβ plaques are the conspicuous pathological feature in brains of AD patients. In addition, there are evidences proposed that among the Aβ isoforms, Aβ(1-40) and Aβ(1-42) are the most toxic species. In AD caused by APP gene duplication, the intracellular Aβ(1-40) has shown prominent existence in patients' neurons[21,22]. Murine model of AD showed that intracellular Aβ accumulation results in neuron death[23]. At present, we had demonstrated the increased expression of Aβ in Aβ(1-40)-treated primary cortical neurons, and which was attenuated by YCTMT in a dose-dependent manner. These results were in accordance with pervious study, which demonstrated that Aβ plague accumulation was reduced by YCTMT in brain of Aβ(1-40)-induced AD rats[12]. In addition, the Aβ-induced toxic effect appeared more rapidly on cholinergic axon terminals than on cell bodies[24]. The activation of choline acetyltransferase and acetylcholinesterase are significantly reduced in AD patients[25]. In Aβ(1-40)-induced AD rats, the

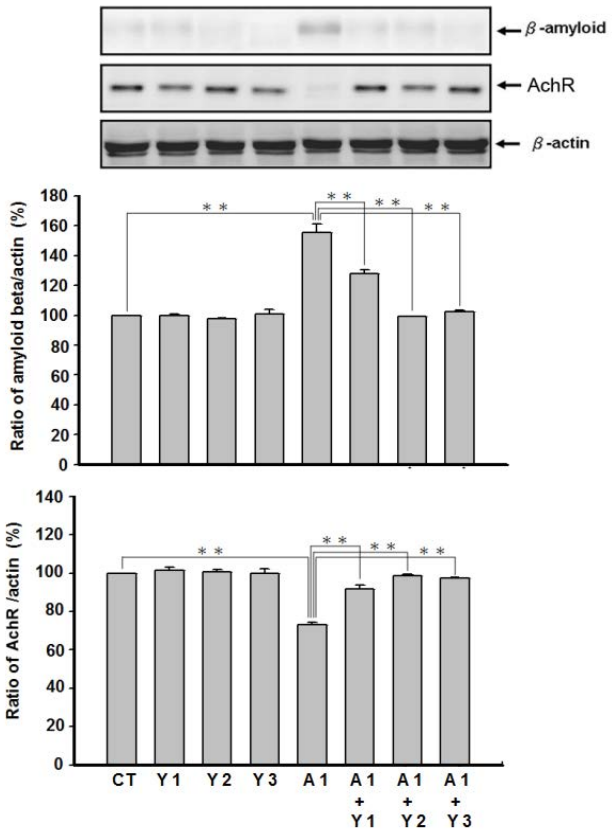

Fig. 4: Effect of YCTMT on Aβ (1-40)-induced accumulation of β-amyloid and down-regulation of AchR
Values were expressed as mean±SD (n=3 in each group). *P<0.05 was considered significant as compared with the Aβ (1-40) values. C T: control; Y 1: YCMT (12.8 μg/ml); Y 2: YCMT (64 μg/ml); Y 3: YCMT (320 μg/ml); A 1: Amyloid-β (10 μM)

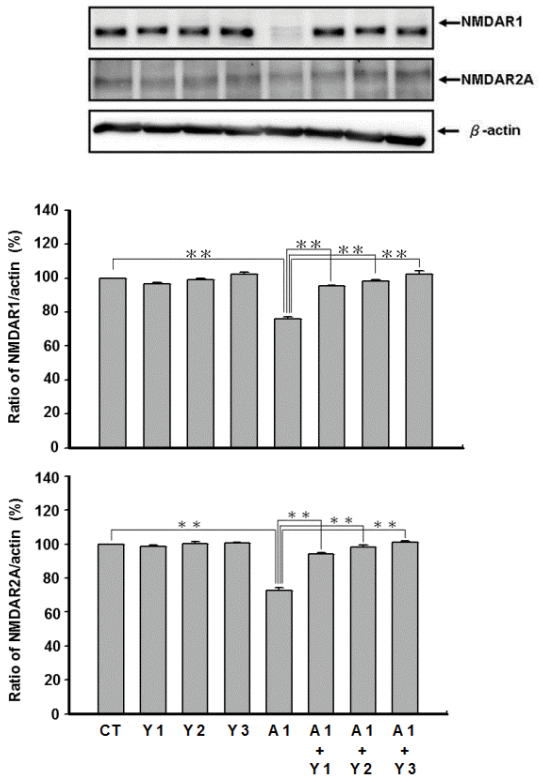

Fig. 5: Effect of YCTMT on Aβ (1-40)-induced down-regulation of NMDAR1 and NMDAR2A

Values were expressed as mean±SD (n=3 in each group). *P<0.05 was considered significant as compared with the Aβ (1-40) values. C T: control; Y 1: YCMT (12.8 µg/ml); Y 2: YCMT (64 µg/ml); Y 3: YCMT (320 µg/ml); A 1: Amyloid-β (10 µM)

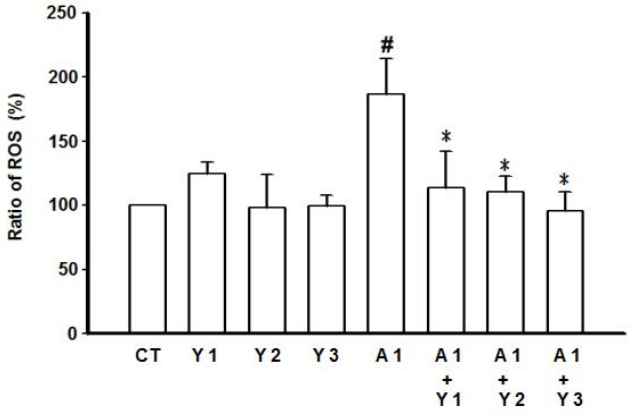

Fig. 6: Effect of YCTMT on Aβ (1-40)-induced intracellular ROS generation

Values were expressed as mean±SD (n=5). #P<0.05 was considered significant as compared with the vehicle values (CT). *P<0.05 was considered significant as compared with the Aβ (1-40) values. CT: control; Y 1: YCMT (12.8 µg/ml); Y 2: YCMT (64 µg/ml); Y 3: YCMT (320 µg/ml); A 1: Amyloid-β (10 µM)

expression of acetylcholine in brain was recovered by YCTMT[12]. The results of present study indicated that the Aβ(1-40)-induced AchR expression was inhibited by YCTMT in primary cortical neurons. All these data gathered had made it clear that YCTMT could improve Aβ-induced neuronal death via reduction of Aβ accumulation and increased AchR expression.

NMDARs are the glutamate-gated cation channels with high calcium permeability and widely expressed in the central nervous system and play an important role in both excitatory synaptic transmission under physiological conditions and excitotoxic neuronal death under pathological conditions[26]. NMDARs are tetrameric complexes form by several heteromeric complexes from three NMDAR classes and their various subunits, which are NMDAR1, NMDAR2 with subunits A, B, C, and D, and NMDAR3 with subunits A and B. NMDAR1/2A, the di-heteromeric component of NMDARs, is widely and abundantly expressed in CNS in the mammalian[27]. It has been demonstrated that during the progression process of AD, mRNA and DNA expression of NMDAR1 subunit was down regulated in human[28]. In AD, NMDAR1 containing an N-terminal splice cassette appears to be reduced significantly, which may result in susceptible cells accession[29]. In the hippocampus and cortex of post-mortem br ain from AD patients, levels of NMDAR2A mRNA and protein similar to NMDAR1 are also decreased[30]. It was found that while the expression of NMDAR1/2A was reduced by Aβ(1-40) in primary cortical neurons, pretreatment with YCTMT reversed the effect in a dose-dependent manner. These

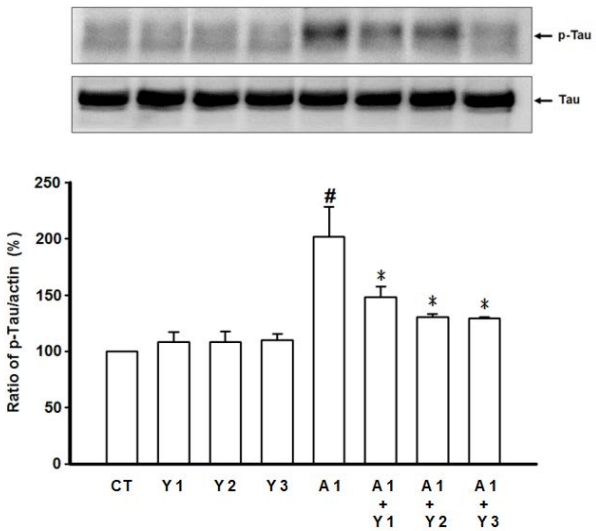

Fig. 7: Effect of YCTMT on Aβ(1-40)-induced phosphorylation of tau

Values were expressed as mean±SD (n=3 in each group). #P<0.05 was considered significant as compared with the vehicle values (CT). *P<0.05 was considered significant as compared with the Aβ (1-40) values. CT: control; Y 1: YCMT (12.8 µg/ml); Y 2: YCMT (64 µg/ml); Y 3: YCMT (320 µg/ml); A 1: Amyloid-β (10 µM)

results indicated that YCTMT could improve Aβ-induced neuronal death via destructing the diminishing expression of NMDAR1/2A.

Excessive activation of NMDARs is stimulated by Aβ, which in turn leads to calcium influx. The abnormally increased concentration of calcium in cytosol and mitochondria leads to mitochondria disruption. In addition, intracellular Aβ causes damage to cytochrome *c* oxidase, α-ketoglutarate and pyruvate dehydrogenase, and DNA in mitochondria. Therefore, Aβ directly or indirectly harms the mitochondrial functions and results in overproduction of ROS[8,31]. Oxidative stress generated from these ROS plays an important role in Aβ-induced excitotoxicity[32]. Aβ-induced generation of oxidative stress is inhibited by NMDAR inhibitors[33]. It was found that the generation of ROS was stimulated by Aβ(1-40) in primary cortical neurons, while pretreatment with YCTMT reversed the effect in a dose-dependent manner. These results indicated that YCTMT could improve Aβ-induced excitotoxicity via reducing the generation of oxidative stress.

Tau, the highly soluble microtubule-associated protein, promotes tubulin assembly into microtubules and stabilizes formed microtubules[34]. There are several critical phosphorylation sites on serine, threonine, and tyrosine residues for regulating the affinity of microtubules[34,35]. Normally, tau stabilizes microtubule structure, but such stabilization is converted into disruption when hyper phosphorylation of tau is induced by several kinases, including glycogen synthase kinase-3β, cyclin-dependent kinase-5, mitogen-activated protein kinase, Akt, Fyn, protein kinase A, calcium-calmodulin protein kinase 2, and microtubule affinity-regulating kinase[36]. The sites of hyperphosphorylation on tau form paired helical filaments, which result in neurofibrillary lesions in brains of AD patients[8]. It has been proposed that phosphorylation of tau is induced by Aβ in cortical neurons[37]. It was demonstrated that Aβ(1-40)-induced tau phosphorylation in primary cortical neurons, of which neurons treated with YCTMT alone has no effect. On the other hand, pretreatment with YCTMT reversed the Aβ(1-40)-induced tau phosphorylation in a dose-dependent manner. The evidence suggested that Aβ-induced hyper phosphorylation of tau via NMDAR activation through glycogen synthase kinase-3β, and the positive feedback from hyper phosphorylation of tau activated the NMDAR via tyrosine kinase, Fyn[38]. All these results indicated that YCTMT could improve Aβ-induced excitotoxicity via the reduction of tau phosphorylation.

In present study, we have demonstrated that YCTMT effectively attenuates Aβ-induced neuronal death. The mechanisms underlying this protective effect include improvement of β-amyloid accumulation and AchR reduction; reversal of NMDAR1 and NMDAR2A expression; reduction of intracellular ROS generation; and inhibition of tau phosphorylation. Experimental findings support the potential use of YCTMT as a therapeutic agent for AD prevention. However, Aβ(1-42) is the other toxic species in AD. The further study about Aβ(1–42)-induced neurocytotoxicity reduced by YCTMT will be more confirm the hypothesis.

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Conflict of interest:

All authors declare no conflict of interests.

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Nil.

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