Effect of Herbal Medicinal Compounds on Alzheimer’s Disease Pathology in APP/PS1 Transgenic Mouse Model

Xuexia Li1,2, Zhijun He3, Chao Wang4, Yanjun Liu5, Zhifu Shan3, Lei Zhang3, Qingqing Shi3, Caiping Yue3, Yitong Lin3, Yun Liu3, Jiazuan Ni1,2,3, and Xiubo Du3

Abstract
The pathogenesis of Alzheimer’s disease (AD) is complex as various mechanisms interact with each other and, therefore, intervention from a single target is often ineffective. Many studies have shown that herbal medicines, such as curcumin, fisetin, icariin, and ginsenosides, have significant intervention effects on AD with different treatment mechanisms. Therefore, we have designed this study to know whether the combination of these herbal medicines can have an intervention effect on AD through multiple targets. Amyloid precursor protein/presenilin 1 (APP/PS1) double transgenic AD mice were used to study the protective effects of a combination of curcumin, piperine, icariin, and ginsenosides, as well as a combination of fisetin, piperine, icariin, and ginsenosides, which were separately mixed into the feed. These herbal medicinal compounds (HMCs) lowered the serum lipid levels, reduced the Aβ oligomers, decreased the pS404-tau protein, as well as neurofibrillary tangles, and restored the reduction of synaptic protein levels and neuronal death of AD mice without causing toxicity to liver and kidneys. In this study, we found that HMCs have significant intervention against AD through multiple targets, providing a novel therapeutic idea for the prevention of AD.

Keywords
Alzheimer’s disease, herbal medicines, β-amyloid pathology, tau pathology, synaptic deficit

Received: April 1st, 2020; Accepted: July 11th, 2020.

Alzheimer’s disease (AD), the most common form of dementia, currently affects approximately 50 million people, and is considered as one of the worst global health and social crises of the 21st century, becoming the world’s seventh leading cause of death.1 According to “World Alzheimer Report 2018,”4 there will be 1 new dementia patient every 3 seconds. It is predicted that by 2030, the number of patients will increase to 82 million, and 152 million by 2050. The global socioeconomic cost of dementia was around $1 trillion in 2018 and will rise to $2 trillion by 2030, bringing huge economic and human burden to society. However, there is still a lack of effective clinical drugs to either cure or control the progression of AD. Since 1998, more than 100 attempts have been made to develop effective drugs for the disease, but most of them have ended in failure. Therefore, in-depth study of the pathogenesis of AD, exploration of reliable early diagnostic indicators, and further development of AD drugs are still main research directions.

AD is an extremely complex pathogenic process mediated by many factors and mechanisms with multiple genes and targets.2 The amyloid cascade hypothesis, abnormal phosphorylation and aggregation of tau protein, and the hypothesis of neuronal loss currently occupy the main position in clinical and scientific research. Aβ, the core component of plaque, is generated from amyloid precursor protein (APP) progressively cleaved by β-secretase (BACE1) and γ-secretase. Many studies have shown that Aβ is a common pathway to induce AD, and a key factor in its occurrence and development.3 The neurotoxic mechanism of Aβ is complex, including triggering oxidative stress, inducing apoptosis, and causing inflammation.4,5 Tau is a microtubule-associated protein mainly in neurons. Its
primary function is to bind to tubules, facilitate the assembly of microtubules, and stabilize the microtubule structure.\textsuperscript{6} Tau plays an important role in the assembly of microtubules, and this process is regulated by its phosphorylation state. In AD patients, tau becomes abnormally phosphorylated and the hyperphosphorylated tau loses its ability to facilitate microtubule assembly, leading to axoplasmic transport impairment and the death of neurons.\textsuperscript{7-10} Hyperphosphorylated tau protein dissociates from the microtubule structure to form paired helical filaments, which further accumulate to form neurofibrillary tangles (NFT).\textsuperscript{11} The formation of NFT can also cause cytotoxicity, resulting in neuronal abnormalities. Both Aβ and tau pathology are highly correlated with synaptic loss, neuronal dysfunction, and memory decline in AD\textsuperscript{12-14}.

At present, the treatment of AD using modern medicines is mainly to improve clinical symptoms, which cannot stop the Table 1. Composition and Dose of Experimental Standard Chow Diet.

|                         | Curcumin | Fisetin | Piperine | Icarin | Ginsenosides |
|-------------------------|----------|---------|----------|--------|--------------|
| CPIG-H\textsuperscript{1} | 136      | -       | 0.8      | 90     | 52           |
| CPIG-L\textsuperscript{2} | 68       | -       | 0.4      | 45     | 26           |
| FPIG-H                   | -        | 360     | 0.8      | 90     | 52           |
| FPIG-L                   | -        | 180     | 0.4      | 45     | 26           |

\textsuperscript{H} stands for high dose. L\textsuperscript{2} stands for the low dose.
disease process. Herbal medicines have the advantages of overall regulation of the occurrence and development of diseases. At the same time, it can maintain the homeostasis of important organelles, such as mitochondria and endoplasmic reticulum. Therefore, the development of drugs from plants to treat and delay the pathological process of AD has attracted much attention in recent years. Curcumin is a component of turmeric with anticancer, anti- Alzheimer’s disease activity, and antioxidative effects. However, the efficacy of curcumin is limited by its quite low bioavailability. However, studies have shown that piperine can improve the bioavailability of curcumin. Fisetin is one of the most antioxidant potent flavonoids, a family of antioxidants found in vegetables, fruits, red wine, and tea, which has a range of pharmacological activities, such as antioxidant, anti-inflammatory, and anticancer effects. Icariin, the main pharmacologically active component of herba Epimedum, has been traditionally used as an antirheumatic remedy and aphrodisiac. Our previous studies have shown that icariin significantly improved the spatial learning and memory of AD model mice. Ginsenosides, with a wide range of pharmacological activities, including anticancer, antioxidation, anti-inflammation, antifatigue, antiaging, and other biological activities, are the major constituents of Panax species (Araliaceae), which are traditional, valuable, medicinal herbs. Many studies have shown that curcumin, fisetin, icariin, and ginsenosides have significant intervention effects on AD, with different treatment mechanisms.

AD is a complex disease caused by multiple pathogenic factors. Thus, the current “One molecule-one-target” (OMOT) strategy cannot solve the multiple pathological stages of AD. In contrast, the “Combination-drugs-multi-targets” strategy, which is an inevitable trend in future AD treatment, is more effective in addressing multiple pathological processes in AD. This may overcome the shortcomings of poor efficacy of OMOT. Thus, we have designed this study to explore tentatively the effects of the combination of curcumin, piperine, icariin, and ginsenosides (CPIG), and the combination of fisetin, piperine, icariin, and ginsenosides (FPIG), on AD pathology.

Materials and Methods

Materials and Reagents

Curcumin, piperine, fisetin, icariin, and ginsenosides were all purchased from Shanxi Senlang Biochemical Co., Ltd. Ginsenosides (80.6%) used in the experiment were a mixture of a variety of ginsenoside analogs, consisting of Rg1 (9%), Re (22.6%), Rb1 (4.7%), Rc (3.1%), Rb2 (5.1%), Rd (9.8%), and other types of ginsenoside analogs (26.3%). Biochemical reagents were obtained from Shenzhen iCubio Biomedical Technology Co., Ltd. The primary antibody of 6E10 was obtained from Biolegend. Antibodies of pS404-tau, postsynaptic density protein 95 (PSD95), Synapsin I (Syn1), and Synaptophysin (Syna) were acquired from Abcam. Secondary antibodies were purchased from Abmart. Glycine silver staining kit and Nissl staining kit were obtained from Wuhan Servicebio Technology Co., Ltd and Solarbio Life Sciences, respectively. All the other reagents were of analytical grade.

Animals and Treatment

The APP/PS1 transgenic mice (B6C3-Tg [APPswe, PSEN1dE9] 85Dbo/Mmjax) were purchased from Guangdong Medical Lab Animal Center. All mice were kept in a 12-hour light/12-hour dark cycle and given access to food and water ad libitum. Fifty male mice (4 months old) were randomly divided into 5 groups: high dose of CPIG (CH), low dose of CPIG (CL), high dose of FPIG (FH), low dose of FPIG (FL), and AD control mice. Ten wild-type (WT) mice (C57/BL6) were used as the normal control group. Detail of the composition and dose of the experimental diet is shown in Table 1.

Figure 1. HMCs administration improved locomotor and exploring ability of APP/PS1 transgenic mice by open-field test. (A) The numbers of crossed grids; (B) frequencies of rearing (***P < .001, *P < .05; n = 10). AD, Alzheimer’s disease; CH, high dose of CPIG; CL, low dose of CPIG; CPIG, combination of curcumin, piperine, icariin, and ginsenosides; FH, high dose of FPIG; FL; low dose of FPIG; FPIG, combination of fisetin, piperine, icariin, and ginsenosides; HMC, herbal medicinal compound.
After 4 months of drug treatment, all the mice were tested by the open-field test to assess their cognitive functions. After the behavioral test, the mice were euthanized, and eyeballs blood and brain tissues rapidly collected. The left hemisphere was fixed for frozen sections, which were used for immunofluorescence analysis, glycine silver staining, and Nissl staining. The right hemisphere was immediately frozen at −80 °C for western blot analysis.

All the procedures and experiments were approved by the Animal Ethical and Welfare Committee of Shenzhen University with permit number AEWC-20140615-002. Every effort was made to reduce mice suffering.

**Open-Field Test**

The apparatus is an open field made of gray plastic, measuring 40 × 40 × 40 cm. The floor is made up of hard plastic and divided into 25 equal-sized grids. Mice were put in the corner of the field and moved freely for 3 minutes to monitor the spontaneous locomotor activity of the mice in the CH, CL, FH, FL, AD, and WT groups. Between trials, the arena was cleaned with 75% ethanol to reduce olfactory cues. Each mouse was introduced into the field and the number of grids entered with all paws and rearing, i.e., the animal stood on its hind legs, were recorded.

**Biochemical Analysis**

Eyeballs blood was collected and serum was obtained after centrifugation at 12,000 rpm for 30 minutes. Serum biochemical indexes were measured using a biochemical analyzer (iMag-ic-M7, China) according to the manufacturer’s protocol.

**Western Blot Assay**

The hippocampal tissue of mice was fully lysed in lysis buffer with the addition of phosphatase inhibitors, protease inhibitors, and phenylmethanesulfonyl fluoride. The protein supernatant was obtained after centrifugation at 12,000 rpm for 30 minutes and the protein concentration was determined using the bicinchoninic acid method. After heating at 100 °C for 3-5 minutes with the addition of loading buffer, 12 µg of each protein extract was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes. The membranes were blocked with 5% fat-free milk and 6E10, pS-404 tau, PSD95, Syn1, and Syna were detected by corresponding primary antibodies, followed by a combination of horseradish peroxidase-conjugated secondary antibody. The protein blots were detected by enhanced chemiluminescence reagents and imaged via Image Station (Tanon-5200, China). Relative band intensities were quantified by Quantity One software.

**Immunofluorescence Analysis**

For immunofluorescence, frozen sections were washed with phosphate-buffered saline (PBS) for 5 minutes, repaired by antigen repair solution for 5 minutes, permeabilized with 2% Triton X-100 in PBS (PBST) for 10 minutes, blocked with 3% bovine serum in PBST for 1.5 hours, and incubated with primary anti-6E10 antibody (1:1000, mouse monoclonal, Biolegend, CA, USA) diluted in blocking solution overnight at 4 °C. These were followed by 3 washes with PBS. Secondary antibody conjugated to Dylight-488 (1:1000, Abcam, Cambridgeshire, England) was then added and incubated at room temperature for 2 hours. After 3 times of washing with PBS, frozen sections were incubated with DAPI (1:500, Beyotime, Shanghai, China) for 5 minutes, washed 3 times with PBS again, and then visualized using a confocal microscope (ZEISS, Jena, Germany).

**Glycine Silver Staining**

Glycine silver staining was performed according to the instructions of the manufacturer. Briefly, the frozen sections were stained with acid formaldehyde for 5 minutes and washed 3 times with double deionized water. The sections were then treated with silver glycine solution preheated at
Li et al.

37 °C for 4 minutes, and subsequently put into reduction solution preheated at 45 °C for several seconds followed again by 3 times washing with double deionized water, and finally visualized using an upright fluorescence microscope (OLYMPUS BX51).

Nissl Staining

Nissl staining was performed according to the instructions of the kit manufacturer. Briefly, the frozen sections were stained with methyl violet stain solution for 15 minutes and washed with double deionized water. The sections were then washed with Nissl differentiation for seconds until most of the stain was eliminated, and subsequently dehydrated with anhydrous ethanol and xylene, and visualized using an upright fluorescence microscope (OLYMPUS BX51).

Statistical Analysis

The data were analyzed with GraphPad Prism 7.0 software and expressed as mean ± standard error of the mean. The degree of statistical significance is shown as follows: **** P < .0001; *** P < .001; ** P < .01; * P < .05.

Results

HMCs Significantly Improved Locomotor and Exploring Ability of APP/PS1 Transgenic Mice

The open-field test was used to assess locomotor and exploring activity behavior indicated by the numbers of crossed grids and rearing of the mice in C57, AD, CH, CL, FH, and FL groups. As shown in Figure 1, compared with the C57 group, the mice in AD...
group showed a significant decrease in the number of crossed grids and rearing within 180 seconds (Figure 1(A) and (B)), indicating the decreased exploratory and locomotor activity of AD mice. Furthermore, the mice in FH group demonstrated a significant increase in the number of crossed grids (Figure 1); CH as well as CL mice reared more frequently in comparison with the AD mice (Figure 1(B)), indicating a notable increase in locomotor and exploring ability, respectively. However, there was no significant difference in either the crossed grids or rearing between FL and AD mice.

**Effect of HMCs on Serum Lipids and Toxicity to Liver and Kidneys**

To explore the toxicity of herbal medicinal compounds (HMCs) to liver and kidneys of mice, several biochemical indicators in the serum of mice were analyzed. As shown in Figure 2, there was no difference in the protein levels of either aspartate transferase or glutamine transferase, the 2 biochemical indexes of liver cell injury, among the mice from all groups (Figure 2(A)). Similarly, the levels of uric acid and creatinine that are the main biochemical indicators reflecting the metabolic capacity of the kidney in HMCs-treated groups and C57 mice did not differ either (Figure 2(B)), suggesting that HMCs had no toxicity to liver and kidneys of mice. Interestingly, triglycerides of all the CH, CL, FH, and FL groups were remarkably lower than that of AD mice, indicating the serum lipid reduction ability of HMCs (Figure 2(C)).

**HMCs Inhibited Aβ Generation and Aggregation in the Brains of APP/PS1 TranCH, high dose of CPIG; CL, low dose of CPIG; CPIG, combination of curcumin, piperine, icariin, and ginsenosides; sgenic Mice**

Amyloid plaques formed by Aβ aggregation are one of the hallmarks of AD. Thus, we examined the effects of HMCs on Aβ generation and deposition in the brains of AD mice. As shown in Figure 3, the levels of Aβ oligomers (14× and 6×) in the hippocampus of AD mice were much higher than those of C57 mice by western blot analysis (Figure 3(A) and (B)), while HMC treatments significantly reduced the levels of Aβ oligomers (Figure 3(A) and (B)). In agreement with this result, immunofluorescence analysis with anti-Aβ antibody (6E10) has shown that Aβ immunoreactivity in CA1, CA3, DG, and cortex of AD mice were much higher than that in the C57 group, and HMCs inhibited the deposition of Aβ in these regions of AD mice (Figure 3(C)-(F)).

**HMCs Attenuated Tau Hyperphosphorylation and NFT Formation in APP/PS1 Transgenic Mice**

NFT formed by hyperphosphorylated tau protein are another important feature of AD, which induce toxicity in the nervous system. Herein, we determined the level of tau phosphorylated at serine 404 (pS404-tau) in hippocampus of AD mice by western blots. As shown in Figure 4, the expression level of pS404-tau protein in hippocampus of AD mice is higher than that of C57 mice (Figure 4(A) and (B)), while CH and CL decreased the pS404-tau protein level. Moreover, FH and FL significantly reduced the pS404-tau protein level in hippocampus of AD mice (Figure 4(A) and (B)). Meanwhile, we performed glycine silver staining of brain frozen slices using a glycine silver staining kit to directly observe the levels and distributions of NFT. The results showed that the contents of NFT in CA1, CA3, DG, and cortex of HMCs-treated AD mice were significantly decreased as compared with untreated AD mice (Figure 4(C) and (D)).

**HMCs Restored the Reduction of Synaptic Protein Levels and Neuronal Death in APP/PS1 Transgenic Mice**

Synapses are the sites of functional communication between neurons and the key sites for information transmission.
Numerous studies have demonstrated that synaptic damage occurred in the brain of APP/PS1 transgenic mice. We, therefore, have investigated the expression of synaptic proteins including PSD95, Syn1, and Syna of hippocampal tissues, which were all decreased in AD mice and reversed in HMCs-treated mice (Figure 5(A) and (B)). This reveals the recovery of synaptic deficits in AD by HMCs. The loss and death of neurons is an important cause of synaptic dysfunction, which is closely related to the development of AD. Here, Nissl staining was conducted to detect the density of neurons...
in AD mice treated with or without HMCs. As shown in Figure 5, the number and density of Nissl bodies in CA1, CA3, DG, and cortex regions of AD mice were significantly decreased in comparison with C57 mice (Figure 5(C) and (D)). This reflects that the neurons of AD mice were damaged to some extent. As expected, the amount and density of Nissl bodies in these regions of HMCs-treated AD mice were restored (Figure 5(C) and (D)). Our results illustrated that HMCs administration not only protected the synaptic proteins but also alleviated neuronal death and impairment in AD mice.

Discussion

The pathogenesis of AD is very complex and involves many factors and organs. At present, the main drugs used to treat the disease are single-target drugs, which can only improve or alleviate the symptoms to a certain extent and cannot reverse or even delay the development of AD. In addition, the patients may have adverse reactions after drug administration. Herbal medicines have long been used to prevent and treat cognitive impairment and have the advantages of little side effect, multiple targets, and overall regulatory effect, focusing not only on the remission of symptoms, but also on restoring and maintaining homeostasis. This is like the principle of modern multitarget therapy for complex diseases such as AD and is more appropriate for long-term use.

Curcumin, fisetin, icariin, and ginsenosides were reported having significant intervention effects on AD. Curcumin showed significant protection, especially in inhibiting the formation of amyloid beta plaques and promoting their disaggregation. It has been reported that curcumin stimulated phagocytosis, altered expression of inflammatory cytokines by decreasing CD33, and increased TREM2 expression to emulate Aβ immunotherapy and control signaling and phagocytic clearance of amyloid. In addition, curcumin can attenuate the hyperphosphorylation of tau and enhance its clearance, lower cholesterol, bind copper, inhibit acetylcholinesterase, modify microglial activity, and mediate the insulin signaling pathway. Fisetin has been reported to maintain the cognitive function in AD transgenic mice by reducing the levels of the cyclin-dependent kinase 5 activator p35 cleavage product, p25, which is closely related to neuroinflammation and neurodegeneration. Moreover, fisetin stimulated autophagy degradation of phosphorylated tau by activating TFEB and Nrf2 transcription factors. Icariin reduced Aβ contents, amyloid plaques, and APP and BACE-1 levels. Furthermore, icariin reduced neuronal apoptosis, as well as suppressing endoplasmic reticulum stress, and protected the structure and function of mitochondria in AD neurons.

Ginsenoside could inhibit tau phosphorylation by reducing expression of glycogen synthase kinase 3beta, the most important kinase involved in tau phosphorylation, and enhanced the activity of protein phosphatase 2A, a key phosphatase involved in tau dephosphorylation. Ginsenosides could also increase γ-aminobutyric acid, acetylcholine, dopamine, glycine, and serotonin levels, decrease Aβ, glutamate, and aspartic acid levels, and repair the damage of the hippocampus. Moreover, all these compounds have functions of antioxidation, anti-inflammation, and antiaging, which have been implicated in the intervention of AD. Therefore, we have made an attempt to explore the multitarget treatment of AD by 4 groups of CH, CL, FH, and FL consisting of either curcumin, piperine, icariin, and ginsenosides or of fisetin, piperine, icariin, and ginsenosides.

Several studies have shown that curcumin inhibits the formation of Aβ oligomers and fibrils, and reduces amyloid levels and plaque burden. Many researchers have also reported that curcumin ameliorates cognitive decline and improves synaptic functions in mouse models of AD. Similarly, we found that CH and CL including curcumin have reduced the level of Aβ oligomers and increased the levels of synapse-associated proteins PSD95, Syn1, and Syna, which can indicate synaptic functions. Piperine was added to increase the bioavailability of curcumin in the CH and CL group, and as a control in the FH and FL group. Fisetin is a small, orally active molecule, which can act on many of the target pathways involved in AD. Recent research revealed that fisetin could reduce cognitive deficits, restore multiple markers associated with impaired synaptic function, and decrease levels of phosphorylated tau. All these results coincide with our findings that FH and FL have decreased the expression of pS404-tau protein, as well as the contents of NFT, and restored the reduction of synaptic protein levels and neuronal death of AD mice. Icariin, a main constituent of Epimedi Herba and a well-known tonic crude drug, has been found to have a remarkable effect on AD in the past 10 years. Researchers found that icariin could reduce the Aβ burden and amyloid plaque deposition in the hippocampus of AD transgenic mice, and preserve the expression of synaptic functional proteins and improve synaptic function. Ginsenoside could attenuate pathological tau phosphorylation and ameliorate hippocampal Aβ deposition in AD mice. In this study, CH, CL, FH, and FL that all contained icariin and ginsenoside exhibited all of these similar effects.

The current study has thought-provoking results, but still has certain limitations. We have only used the combinations of these natural drugs that can improve locomotor and exploring activity as well as ameliorating AD-related neuropathologies. However, it is not clear whether one of the herbal medicines has exerted the effect or whether these 4 drugs have played a synergistic role. In addition, we do not know the specific receptor/enzyme or specific cellular pathway on which the combinations work during neuroprotection. Thus, our future study will focus on the comparison of drug combinations and individual drugs on AD, and the specific targets and pathways that the drug combinations work on. This may underline the merits of combinations of natural products acting through a polytherapeutic approach over a monotherapy option of AD therapy.
Conclusions
In the present study, the effects of CH, CL, FH, and FL on AD mice were investigated and the results showed that all these combinations lowered the level of serum lipids and improved the locomotor and exploring ability of APP/PS1 transgenic mice by mitigating Aβ pathology, reducing tau pathology, and reversing synaptic deficits without inducing toxicity to the liver and kidneys. The overall goal of the study was to test whether these herbal medicines could provide protective effects against AD when used in combination, and, if positive, could be useful for future AD drug development.

Patent
Chinese patent no. 201910613202.6 has resulted from the work reported in this manuscript.

Acknowledgment
The authors would like to acknowledge Dr Xin Liu of the drug dosage recommendation and experimental design.

Declaration of Conflicting Interests
The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: XD, XL, and JN are the inventors and owners of Chinese patent no. 201910613202.6.

Funding
The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was funded by the National Natural Science Foundation of China (21771126), the Shenzhen Bureau of Science, Technology and Information (JCYJ20180305124000597, JCYJ20170818142241972), and Shenzhen-Hong Kong Institute of Brain Science-Shenzhen Fundamental Research Institutions (2019SHIBS0003).

ORCID ID
Xiubo Du https://orcid.org/0000-0003-4907-074X

References
1. Patterson C. World Alzheimer Report 2018. Alzheimer’s Disease International; 2018.
2. Scheltens P, Blennow K, Breteler MMB, et al. Alzheimer’s disease. Lancet. 2016;388(10043):505-517. doi:10.1016/S0140-6736(15)01124-1
3. Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer’s disease at 25 years. EMBO Mol Med. 2016;8(6):595-608. doi:10.15222/emmm.201606210
4. Zhang H, Cao Y, Chen L, et al. A polysaccharide from Polygonatum sibiricum attenuates amyloid-β-induced neurotoxicity in PC12 cells. Carbohydr Polym. 2015;117:879-886. doi:10.1016/j.carbpol.2014.10.034
5. Caruso G, Fresta C, Musso N, et al. Carnosine prevents Aβ-induced oxidative stress and inflammation in microglial cells: a key role of TGF-β1. Cells. 2019;8(1):E64:64. doi:10.3390/cell s8010064
6. Avila J, Lucas JJ, Perez M, Hernandez F. Role of tau protein in both physiological and pathological conditions. Physiol Rev. 2004;84(2):361-384. doi:10.1152/physrev.00024.2003
7. Alonso AC, Grundke-Iqbal I, Iqbal K. Alzheimer’s disease hyper-phosphorylated tau sequesters normal tau into tangles of filaments and disassembles microtubules. Nat Med. 1996;2(7):783-787. doi: 10.1038/nm0796-783
8. Dixit R, Ross JL, Goldman YE, Holzbaur ELF. Differential regulation of dynein and kinesin motor proteins by tau. Science. 2008;319(5866):1086-1089. doi:10.1126/science.1152993
9. Petrucelli L, Dickson D, Kehoe K, et al. Chip and Hsp70 regulate tau ubiquitination, degradation and aggregation. Hum Mol Genet. 2004;13(7):703-714. doi:10.1093/hmg/ddh083
10. Mandelkow E. Alzheimer’s disease. The tangled tale of tau. Nature. 1999;402(6762):588-589. doi:10.1038/45095
11. Takashima A. Significance of tau in the development of Alzheimer’s disease. Brain Nerve. 2010;62(7):701-708.
12. Zhang A, Sun H, Wang P, Han Y, Wang X. Future perspectives of personalized medicine in traditional Chinese medicine: a systems biology approach. Complement Ther Med. 2012;20(1-2):93-99. doi:10.1016/j.ctim.2011.10.007
13. Tao F, Zhang Y, Zhang Z. The role of herbal bioactive components in mitochondria function and cancer therapy. Evid Based Complement Alternat Med. 2019;2019:1-12. doi:10.1155/2019/3868354
14. Li K, Wei Q, Liu F-F, et al. Synaptic dysfunction in Alzheimer’s disease: Aβ, tau, and epigenetic alterations. Mol Neurobiol. 2018;55(4):3021-3032. doi:10.1007/s12035-017-0533-3
15. Zeng H, Sun H, Wang P, Han Y, Wang X. Future perspectives of personalized medicine in traditional Chinese medicine: a systems biology approach. Complement Ther Med. 2012;20(1-2):93-99. doi:10.1016/j.ctim.2011.10.007
16. Wanninger S, Lorenz V, Subhan A, Edelmann FT. Metal complexes of Cys-Pro-His-(His)2 in mitochondria function and cancer therapy. Evid Based Complement Alternat Med. 2019;2019:1-12. doi:10.1155/2019/3868354
17. Jarrell JT, Gao L, Cohen DS, Huang X. Network medicine for Alzheimer’s disease and traditional Chinese medicine. Molecules. 2018;23(5):1143. doi:10.3390/molecules23051143
18. Howes M-JR, Fang R, Houghton PJ. Effect of Chinese herbal medicine on Alzheimer’s disease. Int Rev Neurobiol. 2017;135:29-56. doi:10.1016/bs.irn.2017.02.003
19. Yang W-T, Zheng X-W, Chen S, et al. Chinese herbal medicine for Alzheimer’s disease: clinical evidence and possible mechanism of neurogenesis. Biochem Pharmacol. 2017;141:143-155. doi:10.1016/j.biopha.2017.07.002
20. Wanninger S, Lorenz V, Subhan A, Edelmann FT. Metal complexes of curcumin—synthetic strategies, structures and medicinal applications. Chem Soc Rev. 2015;44(15):4986-5002. doi:10.1039/C5CS00888B
21. Kunnumakkara AB, Bordoloi D, Harsha C, Banik K, Gupta SC, Aggarwal BB. Curcumin mediates anticancer effects by modulating...
multiple cell signaling pathways. Clin Sci. 2017;131(15):1781-1799. doi:10.1042/CS20160935

22. Tang M, Taghibiglou C. The mechanisms of action of curcumin in Alzheimer’s Disease. J Alzheimers Dis. 2017;58(4):1003-1016. doi:10.3233/JAD-170188

23. Abrahams S, Haylett WL, Johnson G, Carr JA, Bardien S. Anti-oxidant effects of curcumin in models of neurodegeneration, aging, oxidative and nitrosative stress: a review. Neurosciences. 2019;40(6):21-21. doi:10.1016/j.neuroscience.2019.02.020

24. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: problems and promises. Mol Pharm. 2007;4(6):807-818. doi:10.1021/mp700113r

25. Ishige K, Schubert D, Sagara Y. Flavonoids protect neuronal cells from oxidative stress by three distinct mechanisms. Free Radic Biol Med. 2001;30(4):433-446. doi:10.1016/S0891-5849(00)00498-6

26. Jia S, Xu X, Zhou S, Chen Y, Ding G, Cao L. Fisetin induces autophagy in pancreatic cancer cells via endoplasmic reticulum stress- and mitochondrial stress-dependent pathways. Cell Death Dis. 2019;10(2):142. doi:10.1038/s41419-019-1366-y

27. Cur rais A, Prior M, Dargusch R, et al. Modulation of p25 and inflammatory pathways by fisetin maintains cognitive function in Alzheimer’s disease transgenic mice. Aging Cell. 2014;13(2):379-390. doi:10.1111/acel.12185

28. Khan N, Syed DN, Ahmad N, Mukhtar H. Fisetin: a dietary antioxidant for health promotion. Antioxid Redox Signal. 2013;19(2):151-162. doi:10.1089/ars.2012.4901

29. Rengarajan T, Yaacob NS. The flavonoid fisetin as an anticancer agent targeting the growth signaling pathways. Eur J Pharmacol. 2016;789:8-16. doi:10.1016/j.ejphar.2016.07.001

30. Chen Y-J, Zheng H-Y, Huang X-X, et al. Neuroprotective effects of Icariin on brain metabolism, mitochondrial functions, and cognition in triple-transgenic Alzheimer’s disease mice. CNS Neurosci Ther. 2016;22(1):63-73. doi:10.1111/cnst.12473

31. Zheng M, Xin Y, Li Y, et al. Ginsenosides: a potential neuroprotective agent. Biomed Res Int. 2018;2018:1-11. doi:10.1155/2018/8174345

32. Liu Q, Kou J-P, Yu B-Y. Ginsenoside Rg1 protects against hydrogen peroxide-induced cell death in PC12 cells via inhibiting NF-κB activation. Neurochem Int. 2011;58(1):119-125. doi:10.1016/j.neuint.2010.11.004

33. Wong AST, Che C-M, Leung K-W. Recent advances in ginseng as cancer therapeutics: a functional and mechanistic overview. Nat Prod Rep. 2015;32(2):256-272. doi:10.1039/C4NP00080C

34. Teter B, Morihara T, Lim GP, et al. Curcumin restores innate immune Alzheimer’s disease risk gene expression to ameliorate Alzheimer pathogenesis. Neurobiol Dis. 2019;127:432-448. doi:10.1016/j.nbd.2019.02.015

35. Kim S, Choi KJ, Cho S-J, et al. Fisetin stimulates autophagic degradation of phosphorylated tau via the activation of TFEB and Nrf2 transcription factors. Sci Rep. 2016;6:24933 doi:10.1038/srep24933

36. Zhang L, Shen C, Chu J, Zhang R, Li Y, Li L. Icariin decreases the expression of APP and BACE-1 and reduces the β-amyloid burden in an APP transgenic mouse model of Alzheimer’s disease. Int J Biol Sci. 2014;10(2):181-191. doi:10.7150/ijbs.6232

37. Li L, Liu Z, Liu J, et al. Ginsenoside Rd attenuates beta-amyloid-induced tau phosphorylation by altering the functional balance of glycogen synthase kinase 3β and protein phosphatase 2A. Neurobiol Dis. 2013;54:320-328. doi:10.1016/j.nbd.2013.01.002

38. Habtemariam S. Natural products in Alzheimer’s disease therapy: would old therapeutic approaches fix the broken promise of modern medicines? Molecules. 2019;24(8):E1519:1519. doi:10.3390/molecules24081519

39. May BH, Feng M, Zhou IW, et al. Memory impairment, dementia, and Alzheimer’s disease in classical and contemporary traditional Chinese medicine. J Altern Complement Med. 2016;22(9):695-705. doi:10.1089/acm.2016.0070

40. Chao J, Dai Y, Verpoorte R, et al. Major achievements of evidence-based traditional Chinese medicine in treating major diseases. Biochem Pharmacol. 2017;139:94-104. doi:10.1016/j.bcp.2017.06.123

41. Li F, Zhang Y, Lu X, Shi J, Gong Q. Icariin improves the cognitive function of APP/PS1 mice via suppressing endoplasmic reticulum stress. Life Sci. 2019:234:116739 doi:10.1016/j.lfs.2019.116739

42. Chen Y, Han S, Huang X, Ni J, He X. The protective effect of Icariin on mitochondrial transport and distribution in primary hippocampal neurons from 3x Tg-AD mice. Int J Mol Sci. 2016;17(2):E163 doi:10.3390/ijms17020163

43. Zhang Y, Pi Z, Song F, Liu Z. Ginsenosides attenuate d-galactose- and AlCl3-induced spatial memory impairment by restoring the dysfunction of the neurotransmitter systems in the rat model of Alzheimer’s disease. J Ethnopharmacol. 2016;194:188-195. doi:10.1016/j.jep.2016.09.007

44. Yang F, Lim GP, Begum AN, et al. Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid in vivo. J Biol Chem. 2005;280(7):5892-5901. doi:10.1074/jbc.M404751200

45. Reddy PH, Man czak M, Yin X, et al. Protective effects of Indian spice curcumin against amyloid-β in Alzheimer’s disease. J Alzheimers Dis. 2018;61(3):843-866. doi:10.3233/JAD-170512

46. Dasilva KA, Shaw JE, Laur jin M. Amyloid-Beta fibrillogene sis: structural insight and therapeutic intervention. Exp Neurol. 2010;232(3):311-321. doi:10.1016/j.expneurol.2009.08.032

47. Reddy PH, Man czak M, Yin X, et al. Protective effects of a natural product, curcumin, against amyloid β induced mitochondrial and synaptic toxicities in Alzheimer’s disease. J In vestig Med. 2016;64(8):1220-1234. doi:10.1136/jim-2016-000240

48. Cur rais A, Farrokhi C, Dargusch R, et al. Fisetin reduces the impact of aging on behavior and physiology in the rapidly aging SAMP8 mouse. J Gerontol A Biol Sci Med Sci. 2018;73(3):299-307. doi:10.1093/gerona/glx104

49. Nabavi SF, B rady N, Habtemariam S, Sureda A, Manayi A, Nabavi SM. Neuroprotective effects of fisetin in Alzheimer’s and Parkinson’s diseases: from chemistry to medicine. Curr Top Med Chem. 2016;16(17):1910-1915. doi:10.2174/15680266166616020414J275

50. Zhu T, Zhang F, Li H, et al. Long-term icariin treatment ameliorates cognitive deficits via CD4+ T cell-mediated...
immuno-inflammatory responses in APP/PS1 mice. *Clin Interv Aging.* 2019;14:817-826. doi:10.2147/CIA.S208068

51. Zhang Z-Y, Li C, Zug C, Schluesener HJ. Icariin ameliorates neuropathological changes, TGF-β1 accumulation and behavioral deficits in a mouse model of cerebral amyloidosis. *PLoS One.* 2014;9(8):e104616 doi:10.1371/journal.pone.0104616

52. Li N, Liu Y, Li W, et al. A UPLC/MS-based metabolomics investigation of the protective effect of ginsenosides Rg1 and Rg2 in mice with Alzheimer’s disease. *J Ginseng Res.* 2016;40(1):9-17. doi:10.1016/j.jgr.2015.04.006