Review

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Research progress on flavonoids isolated from traditional Chinese medicine in treatment of Alzheimer's disease

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Summary **Alzheimer's disease (AD) is a severe condition in aging countries. The currently used drugs including donepezil, rivastigmine, galantamine, and memantine are effective in managing the symptoms. However, they are hardly capable of preventing, halting, or reversing the disease. In the long history of development of traditional Chinese medicine, much experience has accumulated and is summarized in treatment of diseases that correspond to the concept of AD. In recent years, exploration of natural active ingredients from medicinal herbs for treatment of AD has attracted substantial attention. Some flavonoids have been revealed to have a variety of biological actions such as scavenging free radicals, inhibiting neuron apoptosis, and nurturing neuronal cells that constitute the basis for treatment of AD. In this article, we review recent research progress on flavonoids isolated from traditional Chinese medicine against AD and their underlying mechanisms.**

Keywords: Ginkgo flavonoids, soy isoflavones, puerarin, total flavonoids of Baical Skullcap stem and leaf, liquiritin, apigenin

1. Introduction

Alzheimer's disease (AD) is characterized by progressive deterioration in intellect including memory and cognitive functions. It is the most common type of dementia among older people, accounting for 50-75% of all dementia cases (*1*). The number of AD patients was estimated at 36 million in 2010 and will triple in the world by 2050 (*2*). In China, this figure is estimated at 9 million currently and the prevalence rate of AD in the population over the age of 60 years is 2.43% (*3,4*). Proportionate increases over the next forty years in the number of people with AD will be much steeper in China since it is witnessing the aging of society in which the population over the age of 60 years will account for approximately 31% (about 400 million calculated on the current population base) of the whole population by the year of 2050 (*5*). These epidemiological data have painted a less than optimistic outlook in prevention and treatment of this disease in the world, especially in those countries with a rapidly aging society such as China.

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The currently approved drugs for treatment of AD, *e.g.* donepezil, rivastigmine, galantamine, and memantine, aim to either inhibit acetylcholine esterase to increase the levels of the neurotransmitter acetylcholine, or antagonize *N*-methyl-D-aspartic acid (NMDA)-type glutamate receptors to prevent aberrant neuronal stimulation (*6,7*). These medicines, however, exhibit modest and transient effects in improving disease manifestation and could hardly prevent, halt, or reverse the disease (*2*). The typical course of AD lasts for a decade or so, from the mildest stage when the symptoms like memory problems appear to the most severe stage when the patients must depend on others for basic activities of daily living and finally die in a completely helpless state. The long duration of AD and shortage of effective or curative treatments bring an enormous emotional and financial burden on patients, their families and society.

In the past several decades, much research has been done to evaluate the anti-AD effects of natural agents isolated from traditional Chinese medicines from perspectives such as scavenging free radicals, inhibiting lipid peroxidation, suppressing neuronal apoptosis, enhancing the function of cholinergic neurons, and improving behavioral abnormalities in experimental

animal models (*8-10*). Flavonoids are a series of compounds that are spread widely in higher plants and ferns and have attracted much attention due to their various biological actions (*11*). The characteristic chemical structures of these compounds is two benzene rings with hydroxyl groups linked by a three-carbon chain (*11*). The most commonly known biological action of flavonoids is their antioxidant activity, which could be understood from the reduction properties of phenol hydroxyls in the chemical structures. That said, compounds of this type exhibit various pharmacological effects and clinical efficacies that may not be solely related to their anti-oxidative activities, such as effects on the vascular system, inflammatory response, and estrogen-like effects (*11*). These actions of flavonoids constitute the underlying basis for their anti-AD effects. In this article, we review recent research progress on flavonoids isolated from traditional Chinese medicine against AD and their underlying mechanisms.

2. Pathological basis of AD

The presence of extracellular amyloid plaques, intracellular neurofibrillary tangles (NFTs), and loss of neurons and synapses in the cerebral cortex and certain subcortical regions in the brain are the main features of AD (*2*). A great deal of evidence indicates that the onset of AD is probably the consequences of complex interactions among genetic, environmental, and lifestyle factors (*12*). The pathogenesis of AD has been revealed to correlate with the following aspects.

2.1. *Genetic factors*

AD has been demonstrated to be related to mutations or polymorphisms of at least four genes, including *amyloid precursor protein* (*APP*), *presenilin* (*PS*)-*1*, *PS-2*, and *apolipoprotein E4* (*APOE4*) located at chromosomes 21, 14, 1, and 19, respectively (*13*). Early-onset (< 60 years) familial AD, which probably accounts for less than 1% of AD cases, was found to be caused by mutations in *APP*, *PS-1*, and *PS-2* genes (*14,15*). It was demonstrated that genetic abnormality occurred in at least one of these three genes in the early-onset familial AD. Late-onset (> 60 years) familial and sporadic AD, which accounts for most AD cases, has been genetically linked to *APOE4* which has a gene-dosage effect on increasing the risk and lowering the age of onset of the disease (*16,17*). In addition, genetic defects of *PS-1* and *APOE4* were usually discovered in sporadic AD (*12*).

2.2. *Aggregation and accumulation of amyloid-β (Aβ) in the brain*

The amyloid plaques of AD brains largely consist of Aβ protein, which is a 39-42 amino acid protein derived from its parent protein, APP, by proteolytic cleavage at the β- and γ-secretase cleavage sites (*12*). The amyloid cascade hypothesis suggested Aβ is the pathogenic factor and drives the progression of this disease. The aggregation and accumulation of Aβ, which may result from increased production of Aβ, decreased degradation by Aβ-degrading enzymes, or reduced clearance across the blood-brain barrier, gave rise to plaques which induced neurodegeneration and finally led to the clinical dementia syndrome typical of AD (*2*). It was found that nonfibrillar assemblies of Aβ such as Aβ dimers, trimers, and larger oligomers are more pathogenic than insoluble Aβ fibrils found in amyloid plaques and monomeric Aβ (*2*). The neurotoxic activities of Aβ were expressed through a mechanism that induces intracellular generation of reactive oxygen species (ROS), lipid peroxidation, calcium overload, and eventually neuronal death (*18-22*).

2.3. *Formation of NFT in neurons*

Besides the abnormal accumulation of amyloid plaques, another pathologic feature of AD is intracellular formation of NFT which are primarily made up of aggregated tau protein bearing abnormal posttranslational modifications, including increased phosphorylation and acetylation (*23-25*). Tau protein is abundant in neurons with a function of stabilizing microtubules. The progressive accumulation of abnormal tau protein may lead to instability of the microtubular structure and the consequent loss of effective intracellular transport, and ultimately, neuronal death (*26,27*).

2.4. *Disequilibrium of calcium homeostasis*

Overload of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is one of the key factors that leads to neuron damage or death (*28*). Ca^{2+} is a major intracellular messenger that mediates many physiological responses of neurons to chemical and electrical stimulation. A regulated rise in $[Ca^{2+}]_i$ could trigger many physiological events, while an unregulated elevation in $[Ca^{2+}]_i$ can alter cell viability or induce cell apoptosis through activating proteases (*i.e.* calpains), reinforcing signals leading to caspase activation, or triggering other catabolic processes mediated by lipases and nucleases (*29*).

2.5. *Free radical oxidative damage*

Much evidence supported that free radical induced oxidative damage may play a role in the pathogenesis of AD (*30,31*). Features of brain, including a high content of readily oxidized fatty acids, high use of oxygen, and low levels of antioxidants, make it especially sensitive to oxidative damage. Both postmortem and living patients with AD demonstrated evidence of oxidative damage in brain tissue. Free radicals may attack and damage lipids, proteins, and DNA, lead to

change in structure and function of these molecules, and consequently result in cellular damage, dysfunction and cell death (*32*). Besides, oxidative stress could also enhance Aβ production, which further induces nerve tissue damage (*33*).

2.6. *Mitochondrial impairments*

Mitochondrial dysfunction has a certain impact on the pathogenesis of AD as indicated by impaired mitochondrial respiration observed in brain, platelets, and fibroblasts of AD patients (*34*). Energy failure, increased oxidative stress, and accumulation of Aβ could be caused by dysfunction of mitochondria, which would damage neurons and could explain many of the biochemical, genetic, and pathological features of sporadic AD (*35*).

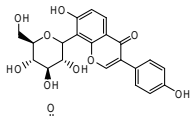
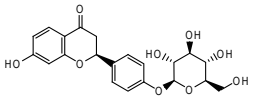
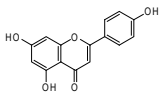
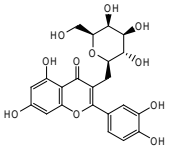
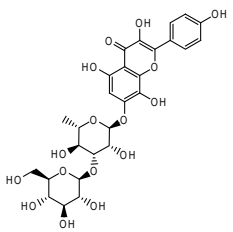
3. Flavonoids as anti-AD agents

Thus far, flavonoids including ginkgo flavonoids, soy isoflavones, puerarin, total flavonoids of Baical Skullcap stem and leaf, liquiritin, apigenin, rhodosin, and hyperoside were reported to have potent effects against AD (Table 1).

3.1. *Ginkgo flavonoids*

Ginkgo flavonoids are the main constituents in the extract of *Ginkgo biloba* (EGB). Ginkgo flavonoids consist mainly of flavonols such as quercetin, kaempferol, and isorhamnetin and biflavonoids like ginkgetin, isoginkgetin, and amentoflavone (*36,37*). These ginkgo flavonoids have free radical scavenging effects and could inhibit lipid peroxidation. Studies demonstrated that mitochondrial DNA from brain of old rats exhibited oxidative damage that is significantly higher than that from young rats (*38*). In addition, mitochondrial glutathione was more oxidized and peroxide formation in mitochondria was higher in old than in young rats (*38*). Treatment with EGB could partially prevent the indices of oxidative damage in brain from old animals (*38*). Other studies demonstrated that ginkgo flavonoids exhibited neuroprotective effects *via* antioxidant activity in brain damaged mice caused by ischemia-reperfusion (*39*). One randomized, double-blind, placebo-controlled, and multicenter clinical trial indicated that EGB was safe and capable of stabilizing and improving the cognitive performance and the social functioning of AD patients for 6 months to 1 year (*40*). Currently, EGB is used in clinics as a medical drug for treatment of AD in China, France, and Germany.

Table 1. Flavonoids isolated from traditional Chinese medicine in treatment of AD

Agents	Structures or contents	Typical origin	Reference
Gingko flavonoids	Mixture: mainly including quercetin, kaempferol, isorhamnetin, and biflavonoids like ginkgetin, isoginkgetin, and amentoflavone	*Ginkgo biloba* L. leaves	*36,37*
Soy isoflavones	Mixture: mainly including daidzin, daidzein, genistin, genistein, and glycitin, glycitein	*Glycine max*	*41,42*
Total flavonoids of Baical Skullcap stem and leaf	Mixture: mainly including scutellarin, baicalin, and chrysin	*Radix puerariae* roots	*64*
Puerarin		*Scutellaria baicalensis* Georgi stems and leaves	*69*
Liquiritin		*Glycyrrhiza uralensis* Fisch. roots	*73*
Apigenin		*Apium graveolens*	*76*
Hyperin		*Hypericum perforatum* L.	*81*
Rhodosin		*Rhodiola rosea*	*83*

3.2. *Soy isoflavones*

Soy isoflavones attracted much interests in recent years due to its estrogen-like effects and role in influencing sex hormone metabolism. The main constituents of isoflavones are demonstrated to be daidzin, daidzein, genistin, genistein, glycitin, and glycitein (*41,42*). It is thought that soy isoflavones intake is a "natural" way to replenish the aging body's declining estrogen levels and thus relieve menopausal symptoms. A previous study demonstrated that postmenopausal women who undertook estrogen-replacement therapy had a significantly lower risk for the onset of AD than women who did not (*43*). These facts suggested the possible benefits of soy isoflavones in AD prevention and treatment.

Mechanisms of anti-AD effects of estrogen lie in the following aspects (*44,45*). *i*) Estrogen reduces the production of Aβ (*46*). Estrogen is capable of regulating the metabolism of APP to enhance the production of soluble APP and decrease the accumulation of Aβ, thus exerting neuroproductive effects. *ii*) Estrogen antagonizes the toxicity of Aβ (*47*). Aβ is capable of promoting lipid peroxidation at the membrane of neuronal cells, leading to production of ROS which further impairs the membrane proteins and breaks the homeostasis of ion balance. The membrane depolarizes and thereby Ca^{2+} influx occurs *via* NMDA receptor channels, which enhances the damage of DNA and lipids and finally leads to neuronal death. Studies indicated that estradiol is a natural anti-oxidant for membrane lipid peroxidation, thereby alleviating the toxicity of Aβ to neurons (*48*). *iii*) Estrogen promotes Ca^{2+} outflow (*49*). Estrogen is capable of releasing intracellular Ca^{2+} *via* non-genomic mechanisms, which is not affected by the concentration of extracellular Ca^{2+}. It was found that estrogen could inhibit the elevation of intracellular Ca^{2+} concentration induced by glutamic acid and antagonize the disequilibrium of calcium homeostasis caused by Aβ. *iv*) Estrogen inhibits inflammation mediated by the transcription factor nuclear factor κB (NF-κB) which is involved in the pathological process of AD (*50*). *v*) Estrogen promotes synaptic growth and expressions of nerve growth factor (NGF) and its receptor (*51*). NGF was demonstrated to be a cytokine that could increase the mRNA levels of choline acetyltransferase, enhance the activities of choline acetyltransferase, and promote the release of acetylcholine. Thus, estrogen is capable of enhancing the effects of NGF. *vi*) Estrogen prevents excessive phosphorylation of tau protein (*52*). Although estrogen exhibits the various above potential actions, its application in clinics for treatment of AD is dismal since it also causes side effects to non-neuronal cells, such as increasing the incidence of breast and endometrial cancer (*53-55*).

Previous studies found that phytoestrogens such as genistein, one of the main ingredients of soy isoflavones, exerted pharmacological effects in a tissue specific manner (*56*). They selectively act on non-reproductive tissues to a certain degree and thus reduce the risk of side effects. Animal studies indicated that soy isoflavones were capable of improving learning and memory abilities through influencing the brain cholinergic system and reducing age-related neuron loss especially in female rats (*57-59*). The underlying mechanisms of favorable effects of soy isoflavones on cognitive function were thought to relate to their potential to mimic the actions and functions of estrogens in the brain (*60*), and promote the synthesis of acetylcholine and neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and NGF in the hippocampus and frontal cortex (*61,62*). A randomized, double-blind, cross-over, and placebo-controlled trial revealed that soy isoflavones were safe and had positive effects on cognitive function, especially verbal memory, in postmenopausal women (*63*). These studies provided evidence of the potential usefulness of soy isoflavones in treatment of AD patients.

3.3. *Puerarin*

Puerarin is an isoflavanone glycoside extracted from species in the family Leguminosae such as *Radix puerariae* and is currently used to treat ischemic cerebrovascular disease and other vascular dysfunctions in China (*64*). Studies found that puerarin had potent effects in improving learning and memory disorders induced by scopolamine or D-galactose in a mouse model (*65*). Yan *et al.* reported that puerarin protected neurons against apoptosis in the cortex and hippocampus of AD rats caused by $Aβ_{25-35}$ through downregulating $Aβ_{1-40}$ and Bax expression in brain tissues, therefore alleviating the spatial learning and memory impairment of diseased animals (*66*). The anti-AD effects of puerarin were also suggested to be related to its abilities in decreasing the lipid peroxidase levels and increasing superoxide dismutase levels in brain tissues, enhancing cerebral blood flow, and improving brain microcirculation (*67,68*).

3.4. *Total flavonoids of Baical Skullcap stem and leaf*

Baical Skullcap is a frequently used traditional Chinese medicine in China. Studies on its active ingredients revealed that the total flavonoids extracted from the stem and leaf, mainly including scutellarin, baicalin, and chrysin, exhibited a series of pharmacological effects such as anti-inflammation, prevention from myocardial damage induced by ischemia-reperfusion, and improved cerebral ischemia (*69,70*). Regarding its effects against AD, Zuo *et al.* found that total flavonoids of Baical Skullcap stem and leaf were capable of protecting hippocampal neurons against damage induced by injection of $Aβ_{25-35}$ in hippocampus in rat (*71*). The underlying mechanisms were related to its actions of decreasing the accumulation of lipid peroxide and proliferation of glial cells induced

by $A\beta_{25-35}$ (*71*). Another study conducted by Ye *et al*. demonstrated that the total flavonoids alleviated memory and learning injury and protected morphological change of hippocampal neurons in AD rats induced by $A\beta_{25-35}$ injection (*72*). These studies suggested the potential efficacies of total flavonoids of Baical Skullcap stem and leaf against AD.

3.5. *Liquiritin*

Liquiritin is an extract from the root of *Glycyrrhiza uralensis* Fisch. (*73*). Yang *et al*. investigated the protective effects of liquiritin on primary cultured rat hippocampal neurons (*74*). They found that pre-treatment with liquirtin for 6 h decreased the elevated levels of intracellular Ca^{2+} concentration and neuron apoptosis caused by $A\beta_{25-35}$. On the other hand, liquirtin is capable of enhancing the effects of nerve growth factor in extending neuraxons (*74*). It is worth noting that liquirtin could also specifically inhibit the activity of acetylcholinesterase and promote the differentiation of neuronal stem cells into cholinergic neurons (*74,75*). The neuroprotective and neurotrophic effects make liquirtin a promising agent against AD.

3.6. *Apigenin*

Apigenin is a flavone usually obtained from *Apium graveolens* (*76*). It is a potent chelating agent that could decrease the metal ions participating in radical reactions and therefore reduce the creation of free radicals (*77*). In addition, apigenin could serve as an anti-oxidant to scavenge free radicals such as oxygen, nitric oxide (NO), and superoxide anion. On the other hand, apigenin possesses estrogen-like effects which are similar to the actions of estradiol (*78*). Due to these biological actions, apigenin was reported to protect human neuroblastoma cells SH-SY5Y against apoptosis induced by oxidative stress *in vitro* (*79*). *In vivo*, apigenin was found to improve the memory and learning disorders of aging mice induced by D-galactose (*80*).

3.7. *Other flavonoids*

Hyperoside is a flavonol isolated from species of *Hypericum* (*81*). In the mouse ischemia-reperfusion injury model, hyperoside was capable of inhibiting lactate dehydrogenase activity decline in brain tissues and obviously improve memory and learning disorders of model mice (*82*). Rhodosin is also a flavonol obtained from the root of Rhodiola rosea (*83*). Rhodosin functions as an anti-oxidant which scavenges free radicals, reduces the content of lipid peroxide, and inhibits degeneration of mitochondria in cerebrum cells and hippocampal pyramidal cells (*68*). Administration of rhodosin was reported to be capable of improving the memory and learning abilities of aging or AD mice (*84*).

4. Conclusion and prospects

AD is a chronic neurodegenerative disease in the central nervous system characterized by progressive memory loss and damage of cognition function. The pathogenesis underlying AD is complicated and not yet well clarified. The currently used medications for treatment of AD are mainly symptom-management drugs. Although they do improve symptoms such as memory disorders and play a key role in treatment of AD at present, these drugs are not capable of reversing the progress of AD. Disease-modifying drugs that aim at root causes of AD are the current research focus and represent the future direction of new drugdevelopment.

In light of the pathogenic complexities of AD, it is probably unlikely that single-target drugs will achieve satisfactory curative effects. The main reasons include the following points. *i*) The onset of this disease involves abnormalities of multiple genes such as *APP*, *PS-1*, *PS-2*, and/or *APOE4*. *ii*) The current targets are multifunctional and strong inhibition or activation of one target may lead to undesired side effects. For example, acetylcholinesterase inhibitors may cause accumulation of peripheral acetylcholine, resulting in peripheral acetylcholine responses such as nausea and vomiting. *iii*) The single-target theory overlooks possible molecular interactions which may constitute cross-talk. Intervention in one of them may not finally affect cell functions or status due to compensatory mechanisms. Given these considerations, development of multiple-target drugs that have both neuroprotective and neurotrophic efficacies are rational strategies in treatment of AD.

Flavonoids reviewed in this article exhibit a series of biological actions against AD including increasing the functions of cholinergic neurons, suppressing typical pathology changes such as neuronal apoptosis, and/or regulating neurotrophy and regeneration relevant mechanisms. These pharmacological effects suggest that more flavonoids may be translated into a new type of anti-AD drugs in the future.

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