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# Natural antioxidants in prevention and management of Alzheimer's disease

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## 1. ABSTRACT

Alzheimer's disease (AD) is the most common neurodegenerative disease that causes dementia in the elderly. As the aging population increases, the prevalence of AD has increased remarkably worldwide and AD has become one of the leading causes of disability and death among the elderly. A number of drugs have been approved for the treatment of AD; however, they produce only modest benefits and have a wide range of side effects. Therefore, extensive studies are underway to identify effective drugs that are free of undesirable side effects. As accumulating evidences have implicated oxidative stress in the initiation and progression of AD, the potential of using nature antioxidants for prevention and treatment of AD has attracted considerable attention. The present review discusses the involvement of oxidative stress in the pathogenesis of AD and the neuroprotective effects of natural antioxidants, such as *Ginkgo biloba* flavonoids, soybean isoflavones, theanine and nicotine in cell culture and AD transgenic animal models, specifically, their inhibition on Abeta-induced neurotoxicity and the underlined molecular mechanisms.

## 2. INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disease that causes dementia in the elderly. Patients with AD suffer a gradual deterioration of memory and other cognitive functions, which eventually leads to a complete incapacity and death within 3 to 9 years after diagnosis (1). As the aging population increases, the prevalence of AD has increased remarkably worldwide and is expected to continually rise; it is estimated that there will be 11-16 millions individuals aged 65 and older diagnosed with AD by 2050 in the United States alone (2-5). In fact, this devastating disease has become one of the leading causes of disability and death among the elderly (2-5). The costs of the medical treatments and caregiving for AD patients have caused tremendous financial pressure and emotional stress to the families, and have emerged as a major public health challenge (2, 6-8). Currently, there is no cure for AD, therefore, new and effective strategies for prevention and treatments of AD are urgently needed.

A complicated array of molecular events is involved in the pathogenesis of AD. The major

pathological characteristics of AD are the presence of senile plaques, neurofibrillary tangles (NFT) in the brain and neuronal loss (1, 9). Senile plaques are mainly composed of beta-amyloid peptide (Abeta), which is produced via sequential proteolytic cleavage of the transmembrane amyloid precursor protein (APP) by two membrane-bound proteases, beta-secretase, also known as beta-site APP cleaving enzyme 1 (BACE1), and gama-secretase, a multiprotein complex consisting of presenilin (PS), which is the catalytic subunit of the gama-secretase, nicastrin (NCT), anterior pharynx defective-1 (APH-1) and presenilin enhancer protein-2 (PEN-2) (1, 9, 10). BACE1 cleaves APP at the N-terminal end, producing a 99 amino acid APP C-terminal fragment, which is further cleaved within the transmembrane domain by gama-secretase, resulting in the release of Abeta peptides (1, 9, 11). Cleavage of APP by a third enzyme, alpha-secretase, precludes the formation of toxic Abeta peptides (12). Increased production and/or decreased clearance of Abeta peptides lead to accumulation of Abeta, which stimulates diverse cell signaling pathways, causing impairment of neuronal synapse, oxidative stress and inflammation, and eventually resulting in synaptic degeneration, neuronal loss and decline in cognitive function (9, 11, 13-16). Several peptides of varying length can be generated from the cleavages by beta and gama-secretases, among them, the 42-amino acid form of Abeta (Abeta42) is more toxic than the more abundantly produced 40-amino acid form of Abeta (Abeta40), possibly because of its faster self-aggregation into oligomers (1, 11, 13). Multiple lines of evidence suggest that soluble Abeta oligomers are the most neurotoxic, whose levels correlate with the severity of the cognitive decline in AD (11, 13). Thus, reduction of Abeta production and oligomerization and/or promotion of Abeta clearance are considered one of the strategies for prevention and treatment of AD (13, 14).

NFT is composed of arrays of paired helical filaments (PHFs) structures, which contain mainly self-aggregated hyperphosphorylated tau, a multifunctional protein involved in microtubule assembly and stabilization (9). In normal brain, phosphorylation of tau is tightly regulated. Hyperphosphorylation and abnormal phosphorylation lead to structural and conformational changes of tau, impairing its binding with tubulin and capacity to promote microtubule assembly, and resulting in its self-aggregation into filaments (17). A number of protein kinases and protein phosphatases have been implicated in the abnormal phosphorylation of tau including glycogen synthase kinase-3 beta (GSK-3 beta), cyclin-dependent kinase 5, mitogen-activated protein kinase (MAPK), calcium-calmodulin kinase and protein kinase C (18). Abnormal tau deposition correlates with neurodegeneration and cognitive decline, while treatments targeting tau hyperphosphorylation may be a strategy for treating tau-related neurodegeneration (19-21). Growing evidence suggests that Abeta accumulation appears before the tau pathology and that Aβ aggregates may be one of a cascade of molecular events leading to tau hyperphosphoryaltion and eventually neuronal cell death (22-24).

Aging is a major risk factor for sporadic forms of AD. A widely explored link between aging and neurodegenerative diseases is oxidative stress. Numerous studies have implicated oxidative stress in the initiation and progression of AD and suggested a potential role of nature antioxidants in prevention and treatment of AD (25). The present review discusses the involvement of oxidative stress in the pathogenesis of AD and the neuroprotective effects of natural antioxidants, such as *Ginkgo biloba* flavonoids, soybean isoflavones, theanine and nicotine in cell culture and transgenic animal models, particularly, their inhibition on Abeta-induced neurotoxicity and the underlined molecular mechanisms.

## 3. OXIDATIVE STRESS IS INVOLVED IN THE PATHOGENESIS OF AD

### 3.1. Oxidative stress in AD

Oxidative stress is the condition resulted from the imbalance between oxidant production and endogenous antioxidant defense system when the generation of oxidants exceeds the scavenging capacity of the antioxidant defense system. During oxidative stress, reactive oxygen species (ROS) and reactive nitrogen species (RNS) react with proteins, nucleic acids and lipids in the cells and impair their functions, causing progressive cell damages and eventually cell death (26). Brain is particularly vulnerable to oxidative stress because of its high metabolic rate, high contents of polyunsaturated fatty acids and transition metals, and relatively low levels of antioxidants. Accumulating evidences have shown that the presence of extensive oxidative stress is a characteristic of AD brain in addition to the established pathology of senile plaques and NFT (25). The increased oxidative stress and free radical generation in AD are based on the following facts: first, protein oxidation, demonstrated by increased levels of protein carbonyls and 3-nitrotyrosine, and markers of oxidative damages to DNA and RNA, such as 8-hydroxydeoxyguanosine (8OHdG) and 8-hydroxyguanosine, are prominent in AD brains (27-31). Lipid peroxidation products such as malondialdehyde (MDA), 4-hydroxynonenal and $F_2$-isoprostanes, are also elevated in multiple brain regions and cerebrospinal fluid (CSF) of patients with AD or mild cognitive impairment (MCI) (32-36). Moreover, the accumulation of the products of free radical damages in AD brains is combined with alterations in antioxidant defenses, in both central nervous system and peripheral tissues (37). Expression of superoxide dismutase (SOD) is abnormally increased within the neuropathological lesions in brains of AD patients, perhaps an adaptive response to the increased oxidative damages in those regions; in contrast, most studies show a reduction in the activities of antioxidant enzymes including SOD and catalase in brains of AD patients (36, 38, 39). The discrepancy between the expression and activities of antioxidant enzymes may reflect a redistribution of antioxidant enzymes in neuropathological lesions or an inactivation of the enzymes due to oxidation (38). Furthermore, in AD and MCI brains, increased oxidative damages to lipids and proteins and decline of glutathione and antioxidant enzyme activities are more localized to the synapses and correlate with the

severity of the disease, suggesting an involvement of oxidative stress in AD-related synaptic loss (40). Interestingly, many of above studies show elevations of oxidative stress in MCI, which is proposed as an intermediate state between normal aging and dementia, indicating that the oxidative stress damage in AD may occur preceding the onset of the disease. These evidences suggest that oxidative stress is one of the earliest alterations that occur in the pathogenesis of AD, and prevention of oxidative damages may be a strategy for delaying the onset and progression of AD.

While oxidative stress has emerged as one of the important factors in AD pathogenesis, the mechanisms by which the redox balance is altered and the sources of free radicals remain elusive. Several mechanisms including mitochondrial dysfunction, Abeta-mediated processes, transition metal accumulation and microglia activation have been proposed to play important roles in the redox imbalance (41), understanding of which may provide new therapeutic targets for AD prevention and intervention.

### 3.2. Abeta toxicity, oxidative stress and mitochondria dysfunction are interlinked

Oxidative modifications of cellular components can disrupt the integrity of membranes and alter the function of essential proteins, leading to the disruption of ion homeostasis, mitochondria dysfunction, and eventually the activation of apoptotic pathway and neuronal cell death (42). A great deal of research has implicated oxidative stress in Abeta-induced neurotoxicity (43). *In vitro* experiments using cell models showed that Abeta treatment could increase the levels of hydrogen peroxide and lipid peroxides (44). Consistently, in various AD transgenic mouse models carrying mutants of APP and PS-1, increased hydrogen peroxide and nitric oxide production as well as elevated oxidative modifications of proteins and lipids correlated the age-associated Abeta accumulation, further confirming that Abeta promotes oxidative stress (45-49). In hippocampal neuronal cell cultures, the induction of ROS by soluble Abeta oligomers required activation of N-methyl-d-aspartate (NMDA) receptor and was associated with a rapid increase in neuronal calcium levels, suggesting a possible role of soluble Abeta oligomers as proximal neurotoxins and the involvement of oxidative stress in the synaptic impairment and neuronal loss induced by soluble Abeta oligomers (50). Meanwhile, Abeta was found to be localized to mitochondria in AD patients as well as transgenic mice and neuroblastoma cells stably expressing human mutant APP (48, 51). Mitochondrial respiratory chain is a major site of ROS production in the cell (52, 53). In fact, in isolated mitochondria, Abeta could cause oxidative injury to mitochondrial membrane, disrupted lipid polarity and protein mobility, and inhibited key enzymes of mitochondria respiratory chain, leading to increased mitochondrial membrane permeability and cytochrome c release (54, 55). Consistently, data from transgenic mice showed that the presence of Abeta in mitochondria was associated with impaired mitochondrial metabolism and increased mitochondrial ROS production (48). Furthermore, oxidative stress mediated by Abeta accumulation may result in modification and damages of important cellular components including enzymes critical for antioxidant defense. Manganese superoxide dismutase (MnSOD), a primary antioxidant enzyme protecting mitochondria against superoxide, was found to be a target for nitration and inactivation in a double homozygous knock-in mouse model expressing APP and PS-1 mutants (56). The decreased activity of antioxidant defense enzymes such as MnSOD may further increase ROS levels and compromise mitochondria function, contributing to the loss of mitochondrial membrane potential and eventually caspase activation and apoptosis (56).

Abeta has also been shown to alter other cellular protective mechanisms against oxidative stress. Uncoupling proteins (UCPs) are a family of mitochondrial anion carrier proteins that are located on the inner mitochondrial membrane with diverse physiological functions (57). It has been demonstrated that UCP-2 and UCP-3 can be activated by ROS or products of lipid peroxidation to diminish proton motive force, reduce mitochondrial membrane potential and ATP production, causing mitochondria uncoupling and decrease of ROS generation from mitochondria (58). Therefore, the expression and activation of UCPs are considered to be a protective mechanism in response to oxidative stress. This protective mechanism appears dysfunctional in AD brains where the expression of UCP-2, 4 and 5 is significantly decreased (59). In SH-SY5Y neuroblastoma cells overexpressing APP or APP mutant, it was found that the upregulation of UCP2 and UCP4 protein levels in response to the exposure of superoxide was abrogated; although the mechanisms are unclear, the result suggests that Abeta accumulation may lead to irreversible cellular alterations that render the cell more susceptible to oxidative stress (60). Moreover, the UCP2 and UCP4-dependent upregulation of mitochondrial free calcium in response to superoxide treatment was found to be diminished in cells overexpressing APP or APP mutant, indicating that the Abeta accumulation may be associated with a dysfunction of mitochondria as reserve pool of intracellular calcium which leads to an increased cell sensitivity to the loss of calcium homeostasis (60). Taken together, Abeta toxicity, oxidative stress and mitochondria dysfunction appear to be interlinked in the pathogenesis of AD.

### 3.3. Oxidative stress enhances Abeta production

In addition to mediating Abeta-induced cytotoxity, numerous studies have implicated oxidative stress in the increased Abeta production in AD. It was demonstrated that defects in antioxidant defense system caused elevated oxidative stress and significantly increased Abeta deposition in transgenic mice overexpressing APP mutant (61, 62), while dietary antioxidants such as curcumin lowered the elevation of oxidized proteins and decreased brain Abeta levels and Abeta plaque burden (63). Moreover, the increased Abeta deposition and its associated earlier onset and more severe cognitive dysfunction induced by the defect in antioxidant defense system could be ameliorated by antioxidant supplementation (62). In line with these findings, it was recently reported that overexpression of MnSOD in transgenic mice

overexpressing APP mutant decreased protein oxidation and increased antioxidant defense capability in brains while reduced Abeta plaque burden and restored the memory deficit (64). These evidences suggest that the enhancement of Abeta production/plaque formation by oxidative stress is important for the initiation and development of AD.

Studies on how oxidative stress enhances Abeta production have pointed to a direct relationship between oxidative stress and activation of BACE and gama-secretase, enzymes critical for generation of Abeta from APP (65-68). Moreover, it was found that the induction of BACE1 and PS1 expression and the activation of gama-secretase by oxidative stress was dependent on the activation of *c-jun* N-terminal kinase (JNK)/c-*jun* pathway, a major cell signaling cascade that is stimulated by oxidative stress (69, 70). In fact, the promoter and 5' untranslated region of BACE gene contain binding sites for multiple transcription factors including the redox-sensitive activator protein (AP)1 and nuclear factor (NF)-kappa B, activation of which by oxidative stress may in turn enhances BACE expression (71). In AD brains, both the activation of JNK signaling cascade (72-74) and the elevation of BACE1 and PS1 expression/activity have been detected (75-77), thus it is possible that the increased oxidative stress in AD brain may initiate the activation of a cascade of redox-sensitive cell signal pathways including JNK, which promotes the expression of BACE1 and PS1, eventually enhancing the production of Abeta and deterioration of cognitive function. As JNK has also been implicated in Abeta-induced neuronal apoptosis (78), pharmacological inhibition of the redox-sensitive signaling pathways such as JNK may reduce Abeta accumulation as well as inhibit neuronal apoptosis (78).

In summary, evidences have demonstrated that the accumulation of Abeta promotes oxidative stress, which mediates Abeta-induced neurotoxicity and further enhances Abeta production, forming a vicious cycle in AD pathogenesis. Regardless a primary or secondary event, oxidative stress is an important factor contributing to the development of AD, thus, removal of ROS or prevention of their formation may inhibit the progression of AD.

## 4. NATURAL ANTIOXIDANTS PROTECT NEURON AGAINST AD

Current pharmacotherapies for AD based on inhibition of acetylcholinesterase or antagonization of NMDA receptor activity cause diverse side effects, but produce only modest benefits (79). Therefore, extensive studies are underway to identify effective drugs that are free of undesirable side effects. As accumulating evidences have implicated oxidative stress in the initiation and progression of AD, the potential of using natural antioxidants for prevention and treatment of AD has attracted considerable attention. Here we will review studies on several naturally occurring compounds including *Ginkgo biloba* flavonoids, soybean isoflavones, theanine and nicotine. Specially, we will discuss their neuroprotective effects against AD and the associated molecular mechanisms.

### 4.1. *Ginkgo biloba* flavonoids

EGb 761, a standardized extract of *Ginkgo biloba* leaves containing 24% flavonoids and 6% terpenoids, is taken by the general population to enhance mental focus and by the elderly to delay the onset of age-related loss of cognitive function. During the past decade, *in vivo* and *in vitro* experiments in mammalian systems and clinical studies in humans have demonstrated that EGb 761 exhibits a broad range of biochemical and pharmacological effects including cognition enhancement (80-83). Many studies have shown that the neuroprotective effects of EGb 761 are associated with its antioxidant properties.

In rat cerebellar neurons, hydroxyl radicals induced a wide variety of oxidative damages including lipid peroxidation, decrease of membrane fluidity and alteration of the sulfhydryl group binding sites on membrane proteins and apoptosis (84-86). EGb 761 pre-treatment effectively attenuated above oxidative damages while prevented the decrease of *Bcl-2* expression induced by hydroxyl radicals, and protected the cells from apoptosis (84-86). When different components of EGb 761 were studied, it was found that its flavonoid components scavenged hydroxyl radicals as effectively as EGb761, whereas the terpenoid components did not scavenge hydroxyl radicals or protect the cells against hydroxyl radical-induced apoptosis (86, 87), suggesting that the inhibitory effect of EGb 761 on hydroxyl radicals-induced oxidative stress and apoptosis is mainly contributed by its flavonoid components, and that it is possible that components other than flavonoids and terpenoids also contribute to the inhibitory effect of EGb 761 on hydroxyl radicals-induced toxicity. EGb 761 was also shown to be able to rescue rat hippocampal cells from nitric oxide-induced free radical accumulation and cell death, and this effect was attributable to the flavonoid but not the terpenoid components of EGb 761 (88). Moreover, the flavonoid components of EGb761 also protected hippocampal cells from Abeta-induced oxidative stress and apoptosis (89). It has been indicated that the flavonoids with different structures may have different antioxidant potentials and distinct protective mechanisms against cell toxicity (90). In addition, it was also demonstrated that EGb 761 components other than flavonoids and terpenoid was responsible for the elevation of endogenous glutathione synthesis (91). Thus further purification and experimentation using individual components of EGb761 may provide more insights of the neuroprotective mechanisms of the EGb761.

Using a N2a neuroblastoma cell model which expresses double-mutated human APP and PS1 and exhibits increased secretion and intracellular accumulation of Abeta upon stimulation, Luo *et al* demonstrated that an anti-amyloidogenic effect was apparently involved in the neuroprotection of EGb761. It was shown that EGb761 decreased Abeta aggregation both *in vitro* and in the conditioned medium of this Abeta-producing cell model (92). Meanwhile, EGb761 significantly attenuated mitochondrion-initiated apoptosis, decreased the release of cytochrome c, and reduced the activity of caspase 3, a key enzyme in the apoptotic cell signaling cascade (92, 93). In the N2a APP/PS1 cell model as well as a transgenic

*Caenorhabditis elegans (C. elegans)* AD model that constitutively expresses human Abeta, EGb 761 treatment significantly attenuated the basal as well as the Abeta-induced production of hydrogen peroxide-related ROS (94). The inhibition of EGb 761 on Abeta oligomerization and deposition were further demonstrated in the transgenic *C. elegans* AD model in which EGb 761 treatment also alleviated Abeta-induced pathological behaviors such as paralysis, reduced chemotaxis behavior and 5-HT hypersensitivity (95). Moreover, mechanisms other than reduction of oxidative stress was proposed for the suppression of Abeta-induced paralysis by EGb 761 since other antioxidants such as L-ascorbic acid was not effective in suppressing paralysis in this model but could reduce intracellular levels of hydrogen peroxide to the same extent as EGb 761 (95). In AD transgenic mouse model expressing mutants of human APP and PS1 (TgAPP/PS1), Abeta oligomers impaired cell proliferation, while administration of EGb 761 reduced Abeta oligomers and increased cell proliferation in the hippocampus of these mice. The enhanced neurogenesis by EGb 761 was proposed to be mediated by the activation of cAMP response element binding protein, which was inhibited by Abeta oligomers (96). In another AD transgenic mouse model that overexpresses a human APP mutant (Tg2576), *Ginkgo biloba* extracts was found to block an age-dependent decline in spatial cognition without altering Abeta levels and without suppressing protein oxidation (97). It was speculated that Ginkgo biloba extracts might affect the oligomerization of soluble Abeta in the early stages of amyloidosis in this mouse model to prevent the progressive impairment of cognitive function (98). Recently, it was reported that chronic administration of EGb 761 to rats increased dopaminergic transmission in the frontocortical brain areas, which might be another mechanism that contributes to the beneficial effects of Ginkgo biloba extracts on cognitive function (99).

Supplementation of EGb 761 to cognitively intact older adults and patients with dementia has been shown to have some benefits on cognitive function as reported in several studies (100-104). However, the recently published Ginkgo evaluation of memory study, a randomized double-blind placebo-controlled clinical trial, demonstrated that EGb 761 supplementation was not effective in reducing the incidence of Alzheimer dementia or dementia overall for the elderly with normal cognition or those with MCI (105, 106); therefore, whether supplementation of *Ginkgo biloba* extract can prevent the development of AD is still inconclusive.

**4.2. Soybean isoflavones**

Estrogen, a steroid hormone well known for its classic effects on the female reproductive system, has also been demonstrated to have neuroprotective and neurotrophic properties through actions both dependent and independent of the nuclear estrogen receptors (107-109). Epidemiological studies suggest that post-menopausal women using estrogen replacement therapy have a decreased risk of developing dementia (110). Despite the benefits, long-term hormone therapy has been associated with increased risks of breast cancer and stroke, therefore, a great amount of research has been focused on alternative approaches that can mimic the beneficial impacts of estrogen while have less adverse effects.

Soybeans contain a large amount of isoflavones, including genistein, daidzein, glycitein and their glycosides (111). The soybean isoflavones have been demonstrated to have affinity to estrogen receptor (112) and have diverse physiological functions including reduction of oxidative stress, enhancement of antioxidant defense and inhibition of protein tyrosine kinase (PTK) (113-117). The existing data strongly suggest that the soybean isoflavones have protective effects against several chronic diseases including the diseases associated with postmenopausal estrogen deficiency such as atherosclerosis (118), and hormone-dependent breast and prostate cancers (119). It was reported that genistein protected rat brain synaptosomes from ROS overproduction induced by Abeta oligmers (120), and rescued rat cortical neuronal cells from Abeta-induced oxidative stress and cell death through inhibition of p38 activity (121). In hippocampal neuronal cells, genistein reversed Abeta-induced ROS accumulation, activation of caspase-3 and apoptosis (122). The elevation of intracellular calcium triggered by Abeta, which may act as a sensor in the apoptotic process, was also markedly attenuated by genistein, though the underlined mechanisms are still not clear (122). The increase in intracellular calcium could result in tau hyperphosphorylation through a glycogen synthase kinase 3 beta dependent pathway (123). From this point of view, blocking the elevated intracellular calcium by genistein may reduce tau-associated pathology (122). It was also suggested that the neuroprotective effect of genistein might involve both antioxidative effects and the estrogen receptor-mediated pathways, dependent on the dose of genistein used (122).

Further evaluation on the neuroprotective effects of soybean isoflavones (genistein, daidzein and glycitein) in *C. elegans* AD model revealed that only glycitein, which accounts for 5–10% of the total isoflavones in soy foods, reduced the formation of Abeta and alleviated the paralysis induced by Abeta in the worm, an action correlated with an effective reduction of hydrogen peroxide in the model. The study demonstrated that glycitein might suppress Abeta toxicity through combined reduction of ROS and inhibition of Abeta deposition, thus, the potential of using this soybean isoflavone for prevention of Abeta associated neurodegenerative disorders is worth to explored further (124).

There is some evidence that soybean isoflavone administration may have a positive effect on selective cognitive activities, the clinical trials studying the effects of soybean isoflavones on cognitive function, however, generated mixed results. The variation in the phytoestrogen composition used in the studies and the heterogeneous profile of the study populations are suggested to contribute to the discrepant outcomes across studies (125).

**4.3. Nicotine**

Smokers have a less risk to develop degenerative diseases such as AD and PD that are both associated with increased oxidative stress, although the mechanisms are not

totally understood (126). Nicotine, a major component of cigarette smoke, has been shown to have protective effects against Abeta-induced neurotoxicity. Study using electron paramagnetic resonance (ESR) spectroscopy techniques demonstrated that the scavenging effects of nicotine on hydroxyl radicals and superoxide free radicals were higher than that of vitamin C, indicating that nicotine is a potential antioxidant (127). In cultured hippocampal neuronal cells, nicotine effectively inhibited Abeta-induced caspase activity and apoptosis meanwhile suppressed the accumulation of free radicals and increase of intracellular calcium. Cholinergic antagonist mecamylamine abrogated the protection of nicotine against Abeta-induced caspase-3 activation and ROS accumulation, suggesting that the neuroprotective effect of nicotine is partly mediated by nicotinic receptors (128). In contrast, the inhibition of nicotine on mitochondria swelling and cytochrome c release from mitochondria induced by calcium and neurotoxins such as N-methyl-4-phenylpyridine ($MPP^+$) and 6-hydroxydopamine was found to be a receptor-independent effect (129). Further investigation revealed that nicotine decreased the electron leak at the site of respiratory chain complex I and preserved intra-mitochondria redox state. These results suggest that the neuroprotective effect of nicotine may involve its protection against mitochondrial dysfunction and oxidative stress (129).

It has been reported by several groups that nicotine treatment effectively lower Abeta deposits in the brains of AD transgenic mice (130-132), an action perhaps partly mediated by the alpha 7 nicotinic receptor, however, the exact mechanisms are still not clear. Transition metals such as copper, zinc, and iron have been detected within the amyloid deposits in AD brains (133) and the abnormal interactions of Abeta with the metal ions are implicated in the process of Abeta deposition in AD brains (134-138). When the effect of nicotine was investigated in APPV717I transgenic AD model, it was found that nicotine treatment significantly lowered the copper and zinc concentration in senile plaques and a subfield of the hippocampus CA1 region in the brains of these mice (139). Consistently, in SH-SY5Y cells overexpressing the Swedish mutant form of human APP (APPsw), nicotine treatment decreased the intracellular copper concentration and attenuated the copper-facilitated neurotoxicity induced by Abeta, effects which were found to be independent of the activation of nicotinic acetylcholine receptor (139). These results suggest that nicotine can reduce beta-amyloidosis by regulating metal homeostasis (139).

Along with the decreased accumulation of Abeta, the production of NO, the activity and expression of iNOS were also found to be down-regulated in the cortex and hippocampus of APPV717I transgenic mice by nicotine treatment, suggesting an attenuation of oxidative stress (132). The decreased iNOS expression might be resulted from a decreased NF-kappB activation by nicotine treatment. The expressions of p65 and p50 subunits of NF-kapp B as well as the DNA binding activity of the transcription factor in cortex and hippocampus of the mice were found to be significantly decreased by nicotine. In addition, the expression and nuclear binding activity of c-Myc, which plays a major role in transcriptional regulation of apoptosis and cell cycle, were reduced by nicotine in the cortex and hippocampus. This decreased c-myc expression might involve the down-regulation of NF-kappa B pathways, which have diverse functions including regulating cell cycle and apoptosis. Agreed with these findings, nicotine was found to modulate apoptosis and cell cycle progression in APPV717I mice in a manner that favored its neuroprotective effect. Furthermore, the activation of MAP kinases was decreased in the nicotine-treated animals, which might have a role in mediating the inhibition of c-Myc expression and NF-κB activation by nicotine (132). It is also demonstrated that alpha7 subunit mRNA expression was significantly increased after chronic nicotine treatment in the hippocampus of APPV717I mice and RNA interference experiments showed that the nicotine-mediated effects required alpha 7 nAChR (132). More recently, it was shown in a rat model of AD that chronic nicotine treatment prevented Abeta-induced reduction of alpha 7 nAChR and prevented Abeta-induced impairment of learning and short-term memory (140). These evidences point to the involvement of alpha 7 nAChR in the neuroprotective effect of nicotine. Since nicotine is a toxic chemical that is known for its psychoactivity, these studies on the molecular mechanisms of the neuroprotective effects of nicotine may shed light on developing new compounds that have similar neuroprotective actions as nicotine but are less toxic for future AD therapy.

### 4.4. Theanine

L-theanine is a major amino acid derivative component in green tea that is also widely used as a food additive to reduce anxiety (141). It is suggested that L-theanine may be involved in cognitive performance (142). As a natural antagonist of glutamate, L-theanine can inhibit the re-uptake of glutamate from the synaptic cleft and block the glutamate receptors in the hippocampus (143). Over-stimulation of NMDA subtype of L-glutamate receptors, which causes calcium influx, ROS production and Abeta-induced neuronal death, also accelerates Abeta production (144, 145). According to these findings, it is possible that L-theanine may have protective effects against neurotoxicity induced by L-glutamate and Abeta. Results from studying the neuroprotective effects of L-theanine using an AD cell model overexpressing APPsw (APPsw) support this hypothesis (146). In this cell model, the expression of APPsw rendered cells more susceptible to glutamate-induced excitotoxicity. It was found that the cell viability was decreased by L-glutamate treatment, which was significantly improved by L-theanine. Meanwhile, apoptosis and caspase-3 activation induced by L-glutamate was suppressed by L-theanine. Further, L-theanine ameliorated glutamate-induced apoptosis in a way similar to that of the NMDA receptor inhibitor MK-801 and the NOS inhibitor L-NMMA, indicating that L-theanine may protect APPsw cells from glutamate-induced apoptosis via inhibition of NMDA receptor overactivation, NO overproduction, and the related pathways. In fact, excessive NO formation can be caused by stimulation of the NMDA receptor (147). In APPsw cells, L-glutamate

significantly increased the generation of NO, while pretreatment of cells with L-theanine prevented the increase of NO production, an effect likely resulted from the down-regulation of iNOS and neuronal nitric oxide synthase (nNOS) protein levels by L-theanine. Overactivation of the NMDA receptor by L-glutamate stimulation may cause increase of intracellular calcium and disturbance of calcium homeostasis that have been indicated in the neuronal loss seen in AD (147). Pretreatment of L-theanine significantly prevented the elevation of intracellular calcium level and the disturbance of calcium homeostasis induced by L-glutamate stimulation. Last, L-theanine treatment also inhibited the increase of Abeta secretion induced by L-glutamate. These results indicate that the inhibition of the NMDA subtype of glutamate receptors and its related pathways is the crucial point of the neuroprotective effect of L-theanine in this AD cell model, and support the notion that L-theanine may provide effective prophylaxis and treatment for Alzheimer's disease (146).

Consistently, it was shown that oral treatment of L-theanine dose-dependently reduced Abeta42 levels and the Abeta42-induced neuronal cell death in the cortex and hippocampus of the brain and significantly attenuated Abeta42-induced learning and memory impairment in an AD mouse model, in which mice were subjected to a single intracerebroventricular injection of Abeta peptides. Moreover, L-theanine caused elevation of glutathione levels and significantly reduced oxidative products of proteins and lipids in brains of these mice. It was also found that L-theanine inhibited Abeta42-induced extracellular signal-regulated kinase (ERK) and p38 MAPK as well as the activity of NF-kappa B, suggesting that the positive effects of L-theanine on memory and learning may be contributed by the suppression of these signaling pathways as well as the reduction of macromolecular oxidative damages (148).

### 4.5. Other natural antioxidants

In addition to above mentioned studies, a wide range of natural antioxidants including vitamins, fruits extracts and herb components, have been reported for their neuroprotective effects, several of them have been reviewed in details elsewhere including resveratrol, curcumin and green tea catechins (149-154).

EGCG, the main catechins constituent of green tea, was reported to reduce Abeta generation in both N2a overexpressing human Swedish mutant APP and in primary neurons derived from Tg2576 through promotion of the nonamyloidogenic alpha-secretase proteolytic pathway (155). Interestingly, it was reported that oral co-treatment with fish oil and EGCG led to a synergetic effect on inhibition of cerebral Abeta deposits in Tg2576 mice. As the comparable effective dose of EGCG in humans may exceed clinical convenience and/or safety, the study provides a solution by co-treatment with fish oil, which enhanced bioavailability of EGCG, allowing supplementation with moderate dose to achieve significant therapeutic effects (156).

As a major component of the phospholipids in neuronal membranes, polyunsaturated fatty acids (PUFAs), especially docosahexaenoic acid (DHA, 22:6, n-3), play important roles in maintaining the proper neuronal and brain functions (157). Epidemiological studies have demonstrated that the prevalence of AD is lower in people who regularly consume fish or foods rich in n-3 PUFAs, while the plasma DHA concentration is inversely correlated with the risk of AD (158, 159). Although not effective for patients with mild to moderate AD, supplementation of n-3 PUFAs for 6 months caused a slower decline in cognitive functions in patients with very mild AD, suggesting the possibility that supplementation of n-3 PUFAs may be beneficial for preventing the initial progression of AD (160). Studies using AD transgenic mice showed that supplementation of DHA not only decreased the Abeta production and deposition in the brains of the animals, but also inhibited the activation of caspase (161-164). Multiple mechanisms have been implicated in the prevention of AD by n-3 PUFAs, including anti-apoptosis, anti-inflammations, and promotion of neurogenesis (165-167). Although n-3 PUFAs can react with oxygen free radicals leading to lipid peroxidation, enrichment of n-3 PUFAs in cell membrane has been shown to improve the antioxidant defense capability of the cell manifested by increased catalase activity and glutathione level, and decreased iNOS expression and NO production (168, 169). Consistently, deficiency of n-3 PUFAs in AD transgenic mouse model (3xTg-AD) caused significant increase in protein oxidation (164). Therefore, enhancing the antioxidant defense, reduction of oxidative stress and modulation of cell signals that eventually breaking the vicious cycle forming by Abeta and oxidative stress may be an important mechanism involved in the neuroprotection of n-3 PUFAs.

## 5. CONCLUSIONS AND PERSPECTIVES

As the aging population increases, the prevalence of AD has increased remarkably worldwide and is expected to continually rise, unless effective prevention and/or treatment strategies are developed (2-5). A number of drugs have been approved for the treatment of AD, however, they produce only modest benefits and cause a wide variety of side effects (79). Therefore, there is an urgent need for new AD prevention and treatment strategies with better efficacy and fewer side effects. Oxidative stress is one of the earliest alterations occurring in the pathogenesis of AD and has been implicated in the Abeta-induced synaptic dysfunction and neuronal loss (25, 32, 35). The accumulation of Abeta stimulates oxidative stress, which in turn leads to an enhancement of Abeta production, thus forming a vicious cycle that promotes the initiation and progression of AD. Therefore, it is possible that the prevention of oxidative stress may delay the onset of AD and slow down the disease progression.

Many studies have demonstrated that natural antioxidants can attenuate the neurotoxicty induced by Abeta such as oxidative stress, mitochondrial dysfunction and neuronal apoptosis. As AD pathogenesis involves multiple molecular events (1), the neuroprotective effects of natural antioxidants appear to involve multiple

mechanisms as well. In addition to the reduction of oxidative stress, prevention of apoptosis and promotion of neurogenesis, natural antioxidants have also been shown to inhibit Abeta accumulation and aggregation, restore calcium homeostasis and reduce the transition metal overload in the brain. The anti-inflammation properties of many antioxidants, which are not discussed in the current review, are also implicated in their neuroprotective mechanisms. Most of the evidences suggest that supplementation of natural antioxidants or intake of foods rich in natural antioxidants may provide benefit to the elderly individuals who are at the risk of developing AD and patients with AD. Such foods as fresh fruits and vegetables, soybean and tea are associated with decreased risk of developing high serum cholesterol and glucose intolerance that are also known risk factors for AD and dementia (1).

In the future, more effort should be spent on evaluating the efficacy of the nature antioxidants on AD prevention and intervention using clinical trials. Since most natural antioxidants have complex composition, a standardized formulation may be used for individual antioxidants. A well-characterized study population is also required to avoid the discrepancies among different trials. The absorption and metabolism of individual antioxidants as well as their transport to brain need to be characterized to determine the effective dose and facilitate the evaluation of the efficacy of the clinical trials. In addition, most of the studies have been focused on Abeta mediated pathology. As some natural antioxidants may act differentially on Abeta and tau pathology, it is important to test their effects in a model that develop both Abeta and tau neuropathological lesions. Isolation and purification of natural antioxidants, which have complex compositions, may provide further insights of the protective mechanisms of the natural antioxidants against AD.

## 6. ACKNOWLEDGEMENT

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**Abbreviations:** Alzheimer's disease: AD, neurofibrillary tangles: NFT, beta-amyloid peptide: Abeta, amyloid precursor protein: APP, presenilin: PS, nicastrin: NCT, anterior pharynx defective-1: APH-1, presenilin enhancer protein-2: PEN-2, paired helical filaments: PHFs, glycogen synthase kinase-3beta: GSK-3 beta, mitogen-activated protein kinase: MAPK, reactive oxygen species: ROS, reactive nitrogen species: RNS, 8-hydroxydeoxyguanosine: 8OHdG, malondialdehyde: MDA, cerebrospinal fluid: CSF, mild cognitive impairment: MCI, superoxide dismutase: SOD, N-methyl-d-aspartate: NMDA, uncoupling proteins: UCPs, *c-jun* N-terminal kinase: JNK.

**Key Words**: Oxidative stress, Natural antioxidants, Neurodegenerative diseases, Alzheimer's disease, Free radicals, Review

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