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Article *in* CNS & neurological disorders drug targets · March 2017

DOI: 10.2174/1871527316666170328113309

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CNS & Neurological Disorders - Drug Targets, 2017, *16*, 000-000

REVIEW ARTICLE

Neuroprotective Effects of Citrus Fruit-Derived Flavonoids, Nobiletin and Tangeretin in Alzheimer's and Parkinson's Disease

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ARTICLE HISTORY

Received: August 29, 2016
Revised: January 31, 2017
Accepted: March 03, 2017

DOI:
10.2174/1871527316666170328113309

Abstract: Neurodegenerative diseases, namely Alzheimer's disease and Parkinson's disease represent a deleterious impact worldwide. Despite extensive preclinical and clinical research in neurodegenerative disorders, therapeutic strategies aimed at the prevention and chronic treatment of neurodegenerative conditions have not been successfully translated to the clinic. Therefore, the identification of novel pharmacological intervention derived from natural products is warranted. Nobiletin and tangeretin are important citrus flavonoids derived from the peel and other parts of *Citrus* L. genus, and have been shown to exhibit neuroprotective effects in several *in vitro* and *in vivo* studies. Apart from there antioxidant and anti-inflammatory effects, nobiletin and tangeretin have been shown to attenuate cholinergic deficits, reduce the abnormal accumulation of neurotoxic amyloid-beta peptides, reverse N-methyl-D-aspartate (NMDA) receptor hypofunction, ameliorate ischemic injury, inhibit hyperphosphorylation of tau protein, enhance neprilysin levels, modulate several signaling cascades, and protect against 1-methyl-4-phenylpyridinium (MPP(+)) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity. Taken together, these naturally occurring phytochemicals may represent beneficial drug candidates for the treatment and prevention of Alzheimer's and Parkinson's disease.

Keywords: Alzheimer's disease, amyloid, nobiletin, Parkinson's disease, tangeretin, tau.

1. INTRODUCTION

Improved medical care and other factors leading to a longer life span have led to an increased incidence of neurodegenerative disorders, such as Alzheimer's disease (AD) and Parkinson's disease (PD) [1-3]. The pathological hallmarks of AD include: (i) extracellular amyloid-β (Aβ) plaques, (ii) neurofibrillary tangles (NFT) containing hyperphosphorylated tau - a microtubule protein, (iii) synaptic impairment, (iv) reduced mitochondrial membrane potential and mitochondrial dysfunction, and (v) cholinergic deficits [4]. At present, only two classes of drugs have been approved for the management of AD. These include acetylcholinesterase (AChE) inhibitors, such as donepezil, galantamine and rivastigmine, and the noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, memantine. While AchE inhibitors mitigate their effects by enhancing synaptic levels of the important neurotransmitter acetylcholine, NMDA receptor antagonists protect against neurodegeneration by attenuating glutamate-mediated excitotoxicity [5]. While these drugs can improve cognitive decline in AD patients, they cannot prevent, stop or reverse disease progression.

Unlike AD, PD is characterized by the progressive and diffuse loss of dopaminergic neurons in the substantia nigra and the accumulation of Lewy bodies containing abnormal α-synuclein aggregates in neuronal cells. PD is characterised clinically by the evidence of tremor at rest, rigidity, bradykinesia and postural instability [6]. In these neurobiological disorders, the etiologies of AD and PD remain to be elucidated. However, it is well understood that these diseases are associated with oxidative stress-induced cell damage [7-9].

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1871-5273/17 $58.00+.00

Oxidative stress leads to many biological effects, including the aggregation of abnormal protein, neuroinflammation, and cell death [10-13]. Activated microglia may initially serve a protective effect in the early phase of these diseases. However, hyperactivation of microglia may lead to the release of pro-inflammatory mediators such as tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), interleukin-6 (IL-6) and interferon gamma (IFN-γ) [14], which may promote degeneration of hippocampal and cortical cholinergic neurons, and substantia nigra pars compacta dopaminergic neurons. As well, these pro-inflammatory mediators can activate astrocytes that are involved in the neuroinflammatory processes associated with the pathogenesis of AD and PD [15]. Despite intensive laboratory and clinical research over the last few decades, effective pharmacological strategies for the long-term treatment and prevention of AD and PD are not yet available currently and, are necessary to overcome the socio-economic burden arising from the incidence of these diseases.

Numerous studies have shown the beneficial effects of dietary flavonoids for health due to their bioactivities including antioxidant action, inhibition of tumorigenesis, reduction of plasma cholesterol and blood sugar levels, reduction in blood pressure, anti-inflammation, and neuroprotection [16, 17]. It is well established that flavonoids may serve as important neuroprotective agents against oxidative stress, by directly scavenging free radicals, inducing prosurvival regulatory pathways, or indirectly enhancing endogenous antioxidant defenses mechanims. Flavonoids have been shown to modulate numerous signaling signalling pathways including protein kinase A (PKA), protein kinase B (Akt/PKB), protein kinase C (PKC), extracellular signal–regulated kinases (ERK1/2), p38, and c-Jun N-terminal kinases (JNK) pathways *via* receptors and upstream/downstream kinases [18]. The neuroprotective effects of flavonoids against oxidative stress and amyloid-derived neurotoxicity may be mediated by their combined antioxidant and anti-inflammatory properties, and signaling responses [19]. Furthermore, extracts of strawberry, spinach, and blueberry, which are rich in flavonoids have been shown to improve cognitive performance in animal and human clinical studies [20].

Recently, citrus-derived flavonoids have been shown to exert beneficial antiallergic, anticancer, and anti-inflammatory activities, thereby promoting cardioprotective and neuroprotective effects [21]. Citrus flavonoids have also been shown to induce chemopreventive effects on bladder, colon, liver, lung, prostate, mammary, and oral cancers [22]. It has been reported that the antiproliferative activity of a citrus flavonoids is dependent on the position and number of hydroxyl groups of the flavonoid A and B rings, number of methoxy groups, and low polar planar structure. The hydroxyl at C-3 and the methoxy residue at C-8 are essential for the antiproliferative effects. Anti-cancer effects of flavonoids may also involve the inactivation of kinases, and kinase inhibition, which also regulate cell cycle arrest and cellular apoptosis [23]. Owing to their limited toxicity in cellular and animal models, but potent anti-inflammatory and chemopreventive characteristics, the potential for health-promoting properties of citrus flavonoids have been explored further in humans [24].

The neuroprotective effects of citrus consumption have been previously reported. One study showed that methanol extract of orange pulps was neuroprotective against oxidative damage in PC12 cells [25]. Similarly, hesperidin protected cortical neurons from oxidative injury *via* modulation of the Akt and ERK1/2 signaling pathways [26]. Hesperidin also protected cortical neurons against Aβ-associated neurotoxicity, and glutamate-induced excitotoxicity, rotenone-induced oxidative stress and apoptosis, and increased the expression levels of antioxidant enzymes [27]. Additionally, another study showed that oral administration of hesperidin slowed the degree of rat brain damage following stroke by attenuating oxidative stress and neuroinflammation [28]. Moreover, naringenin, another flavonoid of *Citrus junos*, attenuated Aβ-associated oxidative damage, and enhanced the memory performance of mice with scopolamine-induced amnesia [29].

Another novel and interesting compound is nobiletin; a bioactive polymethoxylated flavone (5,6,7,8,3',4'-hexamethoxyflavone), that has been isolated from the peel of citrus fruits including *Citrus depressa* (shiikuwasa), *Citrus sinensis* (oranges), and *Citrus limon* (lemons) [30, 31]. The beneficial effects of nobiletin have been previously investigated (Table **1**). For instance, it has been shown to ameliorate 2,4,6-trinitrobenzene sulfonic acid-induced colitis by lowering the expression of inducible nitric oxide synthase and cyclooxygenase-2 enzymes [32]. Another study showed that nobiletin can prevent bone loss by suppressing nuclear factor-κB-dependent prostaglandin E1 synthesis in osteoblasts, and inhibiting lipopolysaccharide-stimulated activation of RAW 264.7 cells [33]. It is well-known that nobiletin has biological activities such as anti-inflammatory effects [34], anti-carcinoma effects [35-40], and ameliorating scratching behavior in mice [41]. Marked increases in the mRNA expression of peroxisome proliferator-activated receptor-γ and regulating expression of glucose transporters 1 and 4 following treatment with nobiletin have been shown to improve insulin resistance in obese diabetic mice [42] and high-fat diet-induced obese mice [43]. In a report, nobiletin attenuated memory decline in AD model rats by restoring β-amyloid-impaired cAMP response element binding protein phosphorylation [44]. In a case study, nobiletin could prevent the progression of cognitive impairment in donepezil-preadministered AD patients using the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-J cog) [45]. In that study, a daily dose of 30 g of nobiletin was mixed with 500 mL of water and decocted until the volume was reduced to 300 mL. Then, 100 mL of aliquot was given to patients three times a day for 1 year. Moreover, other studies have shown that nobiletin restored cognitive deficits in animal models of AD [46, 47] and PD [48]. In a recent study, nobiletin was shown to protect against hydrogen peroxide-induced cell death in HT22 neurons *via* mitogen-activated protein kinases and apoptotic pathways, and prevent the progression of cognitive impairment in donepezil-preadministered AD patients [49].

Similarly, tangeretin is one of the most bitter citrus flavonoids with an O-polymethoxylated flavone structure [50{Tripoli, 2007 #14968]]. There are 5 methoxyl moieties in the chemical structure, which lead to a high hydrophobic character [51]. Tangeretin has been widely found in both the flavedo and albedo of different citrus fruits such as grapefruits,

Table 1. Summary of studies examining the neuroprotective effects of nobiletin.

Model	Dose	Effect	Reference
Olfactory bulbectomy (OBX) mice	50 mg/kg	Improved OBX-induced associative memory impairment as measured using the passive avoidance test	[80, 81]
Olfactory bulbectomy (OBX) mice	50 mg/kg	Improved OBX-induced short-term memory impairment in the Y-maze test; improved the density of hippocampal acetylcholinesterase (AChE)-positive fibers	[80, 81]
Rats exposed to chronic infusion of $A\beta_{1-40}$	10-50 mg/kg	Improved reference and working memory using the eight-arm radial maze test	[87]
APP-Swedish/London (SL) 7-5 Tg mice	10 mg/kg	Attenuated the memory impairments, and significantly lowered the levels of guanidine-soluble $A\beta_{1-40}$ and $A\beta_{1-42}$, and Aβ plaques in the brain	[88]
3XTg-AD	30 mg/kg	Improved short-term memory and recognition memory, reduced the levels of soluble Aβ1-40 in the brain these mice, and ameliorated the generation of free radicals in the hippocampus	[90]
Mice exposed to MK-801	10-50 mg/kg	Reversed the MK-801-induced impairment of context-dependent fear memory by 40-70%	[98]
PC12D cells	30-100 μM	Activated ERK signaling and cyclic AMP response element-dependent transcription	[101, 102]
Bilateral common carotid artery occlusion mice	50 mg/kg	Enhanced short-term memory	[105]
Senescence-accelerated mice (SAM)	10-50 mg/kg	Significant improvements in object recognition memory and attenuated context-dependent fear memory impairment	[107]
Senescence-accelerated mice (SAM)	10-50 mg/kg	Reversed the increase in tau phosphorylation at Ser202 and Thr231 residues in the hippo-campus	[107]
HT22 cells exposed to hydrogen peroxide	30-100 μM	Suppressed caspase 3 and Bax expression, but induced Bcl-2 expression	[108]
Senescence-accelerated mice (SAM)	10-50 mg/kg	Attenuate the decline in the GSH/GSSG ratio, and significantly increase the activity of GPx	[109]
SK-N-SH cells	50-100 μM	Significantly increased the activity of neprilysin	[121]
Rats exposed to unilateral injections of MPP(+)	10 mg/kg	Significantly inhibited microglial activation	[122]
Rats exposed to unilateral injections of MPTP	50mg/kg	Ca(2+)/calmodulin-dependent protein kinase II (CaMKII) autophosphorylation and phos-phorylation at Thr-34 of dopamine- and cAMP-regulated phosphoprotein-32 (DARPP-32) in the striatum and hippocampal CA1 region were restored to control levels	[48]

tangerines, mandarins, and oranges [51]. In plants, tangeretin acts as a defensive mechanism against pathogens. Tangeretin possesses a range of known therapeutic effects, including anti-hypercholesterolemic, anti-tumoric and anticarcinogenic activities, as well as neuroprotective effects [52, 53]. In addition, tangeretin shows potent antioxidant activity, which has an important role in its neuroprotective effects (Table **2**) [17]. The objectives of this paper were to discuss the current literature data regarding nobiletin and tangeretin, and their potential roles as neuroprotective agents, to provide a snapshot of the chemistry, pharmacokinetic and metabolism of nobiletin and tangeretin, and to clarify their effects on patient care with neurodegenerative disorders.

2. NOBILETIN

2.1. Chemistry of Nobiletin

Flavonoids (from flavus, means “pure yellow”) comprise the most common group of polyphenolic metabolites found in all vascular plants [54-57]. Structurally, all contain fifteen carbons, with a common benzopyrano (chromano) moiety derivative (C6-C3-C6) [31, 58]. Nobiletin, low molecular weight O-metylated flavones is one of the citrus polymethoxylated flavones (PMFs). They are a class of flavone compounds with two or more methoxyl groups, and without glycosidic linkage, which are more hydrophobic than hydroxylated ones. Presence of six methoxyl groups on basic flavone pattern, 5, 6, 7, 8-positions on A ring and 3’, 4’-positions on B ring, incorporates a kind of extremely lipophilic structure. This lipophilic nature results in extracellular accumulation in oil glands or in bud excretion. It is also correlated with a lot of biological effects, especially the ability to pass through the blood-brain barrier and enter the CNS [59].

In most plants, biosynthetic pathway of flavonoids is complex and involves several enzymes [60]. Limited literature is available regarding the development of PMFs in citrus plants. In general, flavones are synthesized from flavonones by flavone synthase II (FSII). 7-O-methyl transferase; an additional flavonoid modifying enzyme produced O-methylated flavones. It seems that methoxylated flavonones and flavones are precursors in biosynthesis of PMFs in citrus [61].

Table 2. Summary of studies examining the neuroprotective effects of tangeretin.

Model	Dose	Effect	Reference
Rats exposed to 6-OHDA	20 mg/kg	Attenuated the 6-OHDA-induced decline in both TH+ cells and ameliorated the depletion of striatal dopamine	[128]
Chronic MPTP/probenecid (MPTP/P) injection in mice	20 mg/kg	Upregulated the mRNA levels of UPR-target genes in dopaminergic neurons and astrocytes, and facilitated neuronal protection	[129]
HepG2	20-50 μM	Prevented apoptosis	[134]
Ischemia-reperfusion rat model	(200 mg/kg)	Attenuated brain injury	[134]

2.2. Sources of Nobiletin

Nobiletin along with other PMFs (such as tangeretin and sinensetin) are unique flavonoids found in peel, seed, juice and other parts of *Citrus* L. genus (Rutaceae Fam.). They are present particularly in the outer layer of citrus fruit pericarp [62]. These compounds are found incorporated in fresh fruits and their hand-squeezed or industrially processed juices. To the best of our knowledge at least 61 types of PMFs were reported with a variety in the type of isomers and content in different citrus species [63]. Commercial tangerine and orange peel oil contain nobiletin and tangeretin as the most abundant PMFs [64]. The concentration of nobiletin in peel oils of various citrus fruit sample can vary considerably; it has been reported in orange (0.50 g/l), common mandarin (2.00 g/l), king mandarin (0.60 g/l), clementine (0.40) and tangerine (1.50 g/l). In citrus fruits, its estimated range is from 7 to173 mg/kg of dry weight. The PMFs individual content is extremely variable during the fruits development. In some citrus species, Nobiletin, sinensetin and tangeretin are in the highest concentration in unripe fruits, whereas hepatomethoxyflavone is generally found in mature fruits [65]. Several plant growth hormones could regulate the accumulation of PMFs during fruits development. Ortuno et al [66] studied the effects of benzylaminopurine, a growth cytokinin hormone, on the level of PMFs in treated tangelo Nova fruits after 7 days. The amount of PMFs in treated fruits was increased by 20% for nobiletin and sinensetin and 12% for tangeretin.

Traditionally, citrus plants are used to treat the symptoms of mental disorders. *Citrus aurantium*, commonly known as sore orange, has been used for reducing anxiety, insomnia and depression in some countries. The results of several studies in animal model are in agreement with these traditional uses [67]. In Iranian traditional medicine, *Citrus aurantium* leaves' oil (Narranj oil, in Persian old) have also been used for sedative effects, locally [68]. Nobiletin has been isolated from aqueous extract of this plant [69].

In addition, nobiletin has been isolated from different species as a new source in recent years. *Laurus nobilis* (Lauraceae*), Croton caudatus* (Euphorbiaceae), *Murraya exotica* (Rutaceae), *Arisaema franchetianum* Engl. (Araceae), *Selaginella doederleinii* (Selaginellaceae), *Physalis alkekengi* (Solanaceae), *Ilex latifolia* (Aquifoliaceae) [70-76].

2.3. Neuroprotective Effects of Nobiletin

2.3.1. Effects of Nobiletin on Cholinergic Deficits

A significant deficit in olfactory function has been reported in early stages of AD [77, 78]. These changes are accompanied by progressive degeneration of the cholinergic system in the brain leading to impairments in learning and memory [79]. The neuroprotective effects of nobiletin (50 mg/kg, intraperitoneal [*i.p.*], or 50-100 mg/kg, oral [*p.o.*]) have been previously examined using olfactory bulbectomy (OBX) mice following an 11-day treatment regimen [80, 81]. This mice model exhibits impaired learning and memory induced following cholinergic deficits. Nobiletin treatment significantly improved OBX-induced associative memory impairment as measured using the passive avoidance test [80, 81]. Similarly, improved short-term memory impairment in the Y-maze test was also reported after 11 days following treatment with nobiletin (50mg/kg p.o.) [80, 81]. Treatment with nobiletin (50 mg/kg, intraperitoneal [*i.p.*], or 50-100 mg/kg, oral [*p.o.*]) for 11 days also improved the density of hippocampal AChE-positive fibers by up to 32%, and therefore, increased cholinergic septohippocampal innervations [80, 81].

2.3.2. Effects of Nobiletin on Amyloid-beta (Aβ) Pathology

The deposition of extracellular Aβ plaques represents an important step in the pathogenesis of AD. In fact, the amyloid hypothesis suggests that the accumulation of Aβ is pivotal to the pathobiology of AD [82]. Acute injection or continuous infusion of Aβ into the brain of rodents has been shown to induce synaptic dysfunction, neuronal loss, and cognitive deficits which mimic clinical AD [83-86]. One study showed that daily administration of nobiletin (10-50 mg/kg, *i.p.*) can improve reference and working memory using the eight-arm radial maze test, in rats exposed to chronic infusion of $A\beta_{1-40}$ into the cerebral ventricle using an osmotic pump [87].

The potential neuroprotective effects of nobiletin have also been examined in several transgenic mice models for AD. Supplementation with nobiletin has been shown to improve cognitive deficits by attenuating Aβ pathology in amyloid precursor protein transgenic APP-Swedish/London (SL) 7-5 Tg [88]. These mice express human APP695 harbouring the double SL mutations. A limited number of Aβ plaques develop in the hippocampus and entorhinal cortex as early as

9 months of age, and the number increased considerably by 12 months of age [89]. Deficits in spatial memory have been reported in these mice from 3-12 months using the Morris Water Maze test [88]. Daily administration of nobiletin (10 mg/kg, *i.p.*) to 9-month-old APP-SL 7-5 Tg mice attenuated the memory impairments after 4 months in APP-SL 7-5 Tg mice, and significantly lowered the levels of guanidine-soluble $A\beta_{1-40}$ and $A\beta_{1-42}$, and Aβ plaques in the brain [88].

Another study investigated the effects of nobiletin treatment on cognitive deficits and amyloid pathology in a triple transgenic mouse model of AD (3XTg-AD). These models have been shown to exhibit both amyloid plaques, intracellular neurofibrillary tangles containing hyperphosphorylated tau protein, and cognitive impairments [90]. Treatment with nobiletin (30 mg/kg) improved short-term memory and recognition memory in 3XTg-AD mice after 3 months, reduced the levels of soluble Aβ1-40 in the brain these mice, and ameliorated the generation of free radicals in the hippocampus of 3XTg-AD and wild-type mice [90].

2.3.3. Effects of Nobiletin on Prolonged Depression of NMDA Receptor-Mediated Neurotransmission

Recent studies have shown that NMDA receptor hypofunction may be associated with age-related brain dysfunction [91, 92]. Numerous studies have shown that the mRNA and protein levels of NR1/NR2B subunits are decreased in patients with severe AD [93, 94]. Moreover, exposure to Aβ has been shown to enhance endocytosis of NMDA receptors in cultured primary murine neurons [95, 96]. The effects of nobiletin on cognitive abnormalities associated with NMDA receptor hypofunction have been previously examined [97]. In that study, MK-801, a noncompetitive NMDA receptor blocker was used to induce alterations in NMDA receptor function in mice. Treatment with nobiletin (10-50 mg/kg *i.p.*) reversed the MK-801-induced impairment of context-dependent fear memory by 40-70% following single daily injections of nobiletin for seven days [98]. Similarly, treatment with 50 mg/kg nobiletin ameliorated memory deficits induced by MK-801 by 90% [98].

It is well established that passive avoidance training can activate extracellular signal-regulated kinase (ERK) signaling which is vital for consolidation of memory [99]. Intraneuronal amyloid-beta (Aβ) oligomers, which are critical in the pathogenesis of AD, can induce impairments in the regulation of ERK signaling [100]. Previous studies have shown that nobiletin can activate ERK signaling and cyclic AMP response element-dependent transcription in PC12D cells *in vitro* [101, 102]. Repeated treatment with nobiletin was reported to attenuate inhibition of hippocampal ERK activation due to MK-801, [98]. Nobiletin may therefore provide renewed insight into the development of novel therapeutic compounds targeting NMDA receptor hypofunction for the treatment and management of symptoms of AD.

2.3.4. Effects of Nobiletin on Ischemic Injury

Ischemic injury has been shown to induce neuropathological changes similar to AD pathology [103]. For instance, arterial carotid occlusion has been shown to induce synaptic dysfunction, memory deficits, and deposition of Aβ oligomers in rats [104]. The effect of nobiletin on cerebral ischemia has been previously investigated [105]. Improvements in 5-min bilateral common carotid artery occlusion (BCCAO)-induced associative memory impairment were reported following treatment with 50 mg/kg nobiletin (*i.p.*) for seven consecutive days pre and post brain ischemia, using the passive avoidance test. Enhanced short-term memory was also reported in the Y-maze test. Improvements were also reported in memory parallel to an increase in the levels of major synaptic protein, including calcium/calmodulin-dependent protein kinase II (CaMKII), microtubule-associated protein 2 (MAP2) and GluR1 in the hippocampal CA1 region [105]. These studies further suggest that nobiletin may exert neuroprotection by activating CaMKII signaling.

2.3.5. Effects of Nobiletin on Hyperphosphorylation of Tau

Senescence-accelerated mice (SAM) has been established as an attractive model to investigate the pathobiology of AD, since it exhibits early onset of learning and memory deficits and several pathological features, common to clinical AD, notably increased oxidative stress, neurofibrillary tangles and amyloid plaques [106]. Recently, treatment with nobiletin (10-50 mg/kg, *i.p.*) administered daily for one month to SAMP8 mice aged 4-6 months prior, showed significant improvements in object recognition memory and attenuated context-dependent fear memory impairment compared to SAMP8 mice fed with a standard chow diet [107]. No significant difference was observed in anxiety-like behaviour between nobiletin-treated, and non-treated SAMP8 mice, suggesting that nobiletin-induced improvements on cognition may be independent of the change into the emotional state [107]. Additionally, treatment with nobiletin (10-50 mg/kg, *i.p.*) reversed the increase in tau phosphorylation at Ser202 and Thr231 residues in the hippocampus of SAMP8 mice [107]. Hyperphosphorylation of tau destabilises microtubules, and inhibits axonal transport, culminating in retrograde neurodegeneration. Therefore, it is likely that nobiletin may be an effective treatment attenuating hyperphosphorylation of tau, and leading to improvements in learning and memory.

2.3.6. Antioxidant Effects of Nobiletin

Chronic oxidative stress plays a key role in the aging process and is associated with the pathobiology of several neurodegenerative diseases. The antioxidant effects of nobiletin have been well characterised [108-110]. Treatment with nobiletin has been shown to inhibit cell death due to hydrogen peroxide in HT22 cells [108]. In particular, nobiletin suppressed hydrogen peroxide, which induced the expression of phospho-Jun N-terminal kinases (p-JNK) and p-p38 without altering the levels of JNK or p38. Nobiletin also suppressed caspase 3 and Bax expression, but induced Bcl-2 expression in HT22 cells [108]. This suggests that the antioxidant effects of nobiletin may be mediated through alterations in the expression of mitogen-activated protein kinases and inhibition of pro-apoptotic protein.

Elevated levels of lipid hydroperoxide and protein carbonyl formation, and reduced levels of endogenous antioxidants such as glutathione (GSH), and antioxidant enzymes like a glutathione peroxidase (GPx), which have been previously reported in the brain of aged SAMP8 mice [111]. Nobiletin has been shown attenuate the decline in the GSH/

GSSG ratio in SAMP8 mice, and significantly increase the activity of GPx in SAMP8 mice [109]. These changes occurred parallel to a reduction in the levels of protein carbonyl; a measure of protein oxidation. The neuroprotective effects of nobiletin may be mediated, at least in part through regulation of endogenous antioxidant defense mechanisms in the brain.

2.3.7. Effects of Nobiletin on Neprilysin

Neprilysin (NEP) is an important enzyme involved in the Aβ degradation. Numerous studies have shown that NEP declines in the brain with age, leading to increased Aβ deposition in AD [112-120]. One study showed that nobiletin significantly increased the activity of NEP in a dose- and time-dependent manner in SK-N-SH cells; a neuroblastoma cell line [121]. The mRNA and protein expression of NEP also increased in a dose and time dependent manner [121]. These findings suggest that modulation of NEP *via* nobiletin may be a potential strategy to prevent the progression of AD.

2.3.8. Effects of Nobiletin on Models for Parkinson's Disease

It has been well established, that treatment with neurotoxin, 1-Methyl-4-phenylpyridinium (MPP(+)) can damage dopaminergic (DA) neurons in several models of PD. Treatment with nobiletin (10 mg/kg bw) significantly protected DA neurons in the substantia nigra (SN) of rats exposed to unilateral injections of MPP(+) [122]. Treatment with nobiletin also significantly inhibited microglial activation. This suggests that nobiletin may protect against MPP(+) toxicity through the suppression of neuroinflammation [122].

Another study investigated the effect of nobiletin on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced motor and cognitive deficits [48]. Treatment with nobiletin (50mg/kg *i.p.*) improved motor deficits seen in MPTP-induced Parkinsonian model mice by 2 weeks. The effect was maintained for an additional 2 weeks following drug withdrawal. However, treatment with nobiletin failed to rescue dopaminergic neurons from MPTP-mediated toxicity, and no significant effect was reported on striatal or hippocampal tyrosine hydroxylase (TH) protein levels. Despite this, the levels of Ca(2+)/calmodulin-dependent protein kinase II (CaMKII) autophosphorylation and phosphorylation at Thr-34 of dopamine- and cAMP-regulated phosphoprotein-32 (DARPP-32) in the striatum and hippocampal CA1 region were restored to the levels reported in sham-operated mice. Nobiletin treatment also attenuated decline in dopamine levels in the striatum and hippocampal CA1 region [48].

2.4. Bioavailability of Nobiletin

Bioavailability represents a critical factor for the development of therapeutic compounds for the treatment of neurodegenerative disorders such as AD and PD. The ability for drugs to permeate the blood brain barrier (BBB) can limit the therapeutic application of drugs used to treat disorders of the central nervous system. Studies using high-performance liquid chromatography (HPLC) and positron emission tomography (PET) have shown that nobiletin can cross the BBB [123]. Maximal concentrations in plasma and brain (1.78 and 4.20 μg/ml) have been achieved after 1 hour following single oral dosing (50 mg/kg, >97% purity) [123]. The Parallel Artificial Membrane Permeability Assay (PAMPA) of nobiletin was high at both pH 4.0 and 7.0. Moreover, an important urinary metabolite of nobiletin, 4'-demethylnobiletin, can ameliorate memory and learning deficits in rats and mice [124]. Taken together, these pharmacokinetic studies suggest that the rapid and significant brain permeability of nobiletin is satisfactory to induce significant biological activity to produce a beneficial therapeutic effect.

3. TANGERETIN

3.1. Chemistry of Tangeretin

Tangeretin (Fig. **1**) is a low molecular weight (average mass 372.369 Da and molecular formula, $C_{20}H_{20}O_7$) secondary metabolite belonging to the flavonoid class of polyphenols. The simplest structural analogue of tangeretin (3) within flavone structural subclasses is apigenin (1). As shown in Fig. (**1**), the characteristic structural features of flavones are the presence of two aromatic rings (ring A and B) joined together by the three carbon chain that cyclises to form ring C; the presence of double bond at C2/C3 position; and the lack of oxygenation at the C-3 position. Biosynthetically, tangeretin is likely to derive from its closest structural analogue, 5,6,7,8,4'-pentahydroxyflavone (nortangeretin, 2). Methoxylation of all the hydroxyl groups of nortangeretin can lead to the polymethoxy flavone, tangeretin (5,6,7,8,4'-pentamethoxyflavone, 3).

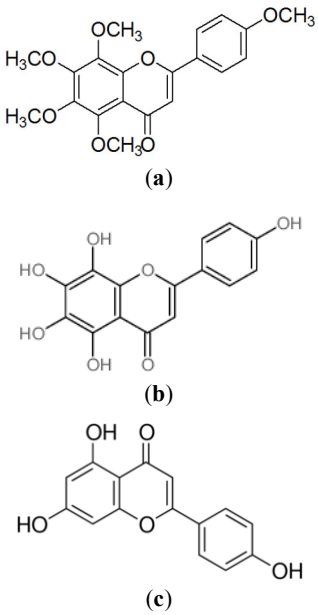

Fig. (1). The structure of (**a**) tangeretin and its structural analogues (**b**) nortangeretin and (**c**) apigenin.

3.2. Sources of Tangeretin

Tangeretin and many other polymethoxylated flavonoids are abundantly found in the genus *Citrus* where they are particularly concentrated within fruit peels. Many other natural sources of tangeretin in lower quantities have been reported, such as *Fructus species* [125]. Due to their high prevalence

in citrus fruits and their varying degree of concentration between *species*, tangeretin and other related polymethoxylated flavonoids are routinely used for quality control of citrus juices. Hence, the main source of tangeretin to humans, to date, is *via* the dietary intake of citrus products. Tangerine (*Citrus tangerina*), sweet orange (*Citrus sinensis*) and bitter orange (*Citrus aurantium*) fruit peels are particularly known for their high tangeretin content, along with the 4'-methoxylated derivative of tangeretin and nobiletin.

The numerous pharmacological activities of tangeretin and related compounds reported in the past few decades have prompted many laboratories to focus on the development of extraction methodologies for tangeretin and related polymethoxylated derivatives. The high lipophilicity of these compounds means that they are readily extractable with organic solvents and supercritical carbon dioxide [126]. In view of obtaining a reliable supply of these compounds through chemical synthesis, a complete synthetic approach has also been designed [127].

3.3. Neuroprotective Effects of Tangeretin

3.3.1. Parkinson's Disease

The neuroprotective effects of tangeretin, a citrus flavonoid, have been evaluated in models for PD [128]. One study showed that a unilateral infusion of 6-hydroxydopamine (6-OHDA; 8 μg), the dopaminergic neurotoxin, onto the medial forebrain bundle substantially lowered the number of tyrosine hydroxylase positive (TH+) cells in the substantia nigra and reduced striatal dopamine content in control vehicle treated rats [128]. Treatment with high doses of tangeretin (20 mg/kg/day for 4 days; p.o.) attenuated the 6-OHDA-induced decline in both TH+ cells and ameliorated the depletion of striatal dopamine [128]. This represents the first *in vivo* study to suggest that tangeretin can cross the blood-brain barrier effectively, mediating a neuroprotective effect in the brain.

Another study examined the effect of tangeretin against chronic MPTP/probenecid (MPTP/P) injection in mice [129]. This murine model for PD has been previously shown to induce damage to dopaminergic neurons, and a hyperactivation of the unfolded protein response (UPR), and ATF6α and PERK/eIF2α/ATF4 in particular. Moreover, neuronal degeneration and ubiquitin accumulation are enhanced following MPTP/P injection in ATF6α -/- mice, in line with a decrease in astroglial activation, impaired synthesis of the brain-derived neurotrophic factor (BDNF), and lowered the mRNA expression of heme oxygenase-1 (HO-1) and xCT, two important antioxidant genes. These changes have been associated with reduced expressions of both GRP78, an ATF6α-dependent molecular chaperone in the endoplasmic reticulum, and the ATF4-dependent pro-apoptotic gene CHOP [130]. Oral administration of tangeretin upregulated the mRNA levels of UPR-target genes in dopaminergic neurons and astrocytes, and facilitated neuronal protection following MPTP/P injections [129]. These data suggest that the neuroprotective effects of tangeretin on the maintenance of striato-nigral integrity may be mediated at least partially through activated astrocytes.

3.3.2. Neurogenesis and Cognition

It is well established that the cAMP response element (CRE) transcription is dysregulated in several neurodegenerative disorders [131]. Tangeretin, along with five other citrus-derived flavonoids: nobiletin, 5-demethylnobiletin, sinensetin, 6-demethoxytangeretin, and 6-demethoxynobiletin, has been reported to stimulate CRE-dependent transcription and promote neurite outgrowth in PC12D cells [81] and hippocampal neurons [132]. Moreover, the cAMP/PKA/ERK/CREB signalling pathway is crucial for learning and memory [133]. These findings suggest that tangeretin may mediate neuroprotection through CRE-mediated transcription linked to the upstream cAMP/PKA/ERK/CREB pathway in neuronal cells.

3.3.3. Ischemic Stroke

It is well established that ischemic stroke occurs in response to cerebral injury following prolonged ischemia by occlusion of the cerebral arteries [134]. Tangeretin, the main bioactive compound of *Aurantii Immatri Pericarpium* (HY5356), has been shown to improve cell viability and prevent apoptosis of human hepatocellular carcinoma cells (HepG2) exposed to hypoxic conditions [134]. Moreover, intraperitoneal injection of HY5356 (200 mg/kg) or tangeretin (200 mg/kg) before occlusion attenuated brain injury in a rat model of ischemia-reperfusion [134].

3.4. Bioavailability of Tangeretin

The absence of free hydroxyl groups in the structure of tangeretin suggests high lipophilicity and low water solubility, resulting in low bioavailability. As this physicochemical characteristic is a major limiting factor for the application of tangeretin in medicine and as part of nutraceutical preparations, various formulations need to be investigated to enhance its bioavailability. By using viscoelastic emulsion, Ting *et al.* [135] have shown that the oral bioavailability of tangeretin in experimental animals increased 2.3 fold when incorporated in unformulated oil suspension. The authors have also demonstrated through *in vitro* lipolysis studies that emulsified tangeretin was digested and became bioaccessible much faster than an oil suspension of unprocessed tangeretin. Once absorbed, tangeretin is known to be converted in to demethylated and hydroxylated products [136]. The occurrence of glucuronide products at substantially higher concentrations than aglycone metabolites in rat blood serum and the detection of low levels of tangeretin and its metabolites 24 h after treatment have also been reported [137].

One study compared the pharmacokinetics of both nobiletin and tangeretin in rats by oral gavage and intraperitoneal injections [138]. Using high performance liquid chromatography electrospray ionization mass spectrometry (HPLC-ESI-MS) to measure blood serum concentrations, the study showed a 10-fold higher absorption of nobiletin compared to tangeretin at the same oral dose (50 mg/kg). The maximum levels of glucuronidated metabolite were reported in the blood serum at 5-8 hours, and at higher levels than aglycone metabolites. This suggests that nobiletin may be more bioavailable than tangeretin [138]. The bioavailability profile of tangeretin in humans remains to be explored, as do

the various options for enhancing its pharmacological effects through formulation studies.

CONCLUSION AND FUTURE PROSPECTS

The cytoprotective effects of nobiletin and tangeretin against AD and other neurodegenerative disorders have been clearly demonstrated, although the exact molecular targets remain unclear. Therefore, further studies aimed at elucidating the primary molecular targets of nobiletin and tangeretin in the central nervous system using -*omics*-based approaches are warranted. Additionally, phase II clinical trials in patients with neurodegenerative disorders are necessary to confirm whether the neuroprotective effects of nobiletin and tangeretin can be successfully translated to the clinic. A recent pilot clinical study showed that treatment with nobiletin-rich *C. Reticulate* peel extracts can slow down cognitive decline in AD patients treated with donepezil, and no adverse effects were reported [139]. Nevertheless, larger clinical trials are necessary to confirm the efficacy of nobiletin and/or tangeretin as safe and effective therapeutic strategies for AD and related neurodegenerative disorders.

LIST OF ABBREVIATIONS

AChE	=	Acetylcholinesterase
AD	=	Alzheimer's Disease
Akt/PKB	=	Protein Kinase B
Aβ	=	Amyloid-β
cAMP	=	Cyclic Adenosine Monophosphate
ERK1/2	=	Extracellular Signal-Regulated Kinases
IFN-γ	=	Interferon Gamma
IL-1β	=	Interleukin 1 Beta
IL-6	=	Interleukin-6
JNK	=	c-Jun N-terminal Kinases
MPTP	=	1-Methyl-4-phenylpyridinium
(MPP(+))	=	Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NMDA	=	N-methyl-D-aspartate
PD	=	Parkinson's Disease
PKA	=	Protein Kinase A
PKC	=	Protein Kinase C
TNF-α	=	Tumor Necrosis Factor Alpha

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Ahmed T, Abdollahi M, Daglia M, Nabavi SF, Nabavi SM. Berberine and neurodegeneration: A review of literature. Pharmacol Reports 2015.

[2] Orhan I, Daglia M, Nabavi S, Loizzo M, Sobarzo-Sanchez E, Nabavi S. Flavonoids and Dementia: An Update. Currt Med Chem 2015; 22(8): 1004-15.

[3] Nabavi SF, Sureda A, Habtemariam S, Nabavi SM. Ginsenoside Rd and ischemic stroke; A short review of literatures. J Ginseng Res 2015.

[4] Sarkar A, Irwin M, Singh A, Riccetti M, Singh A. Alzheimer's disease: The silver tsunami of the 21st century. Neural regeneration research. 2016; 11(5): 693-7. P

[5] Huang YD, Mucke L. Alzheimer mechanisms and therapeutic strategies. Cell 2012 16; 148(6): 1204-22.

[6] Wirdefeldt K, Adami HO, Cole P, Trichopoulos D, Mandel J. Epidemiology and etiology of Parkinson's disease: A review of the evidence. Eur J Epidemiol 2011; 26: S1-S58. P

[7] Allen C, Bayraktutan U. Oxidative stress and its role in the pathogenesis of ischaemic stroke. Int J stroke 2009; 4(6): 461-70.

[8] Gackowski D, Rozalski R, Siomek A, *et al*. Oxidative stress and oxidative DNA damage is characteristic for mixed Alzheimer disease/vascular dementia. J Neurol Sci 2008; 266(1): 57-62.

[9] Zuo L, Motherwell MS. The impact of reactive oxygen species and genetic mitochondrial mutations in Parkinson's disease. Gene 2013; 532(1): 18-23.

[10] Nabavi SM, Nabavi SF, Eslami S, Moghaddam AH. In *vivo* protective effects of quercetin against sodium fluoride-induced oxidative stress in the hepatic tissue. Food Chem 2012; 132(2): 931-5.

[11] Zhao ZY, Luan P, Huang SX, *et al*. Edaravone protects HT22 neurons from H2O2-induced apoptosis by inhibiting the MAPK signaling pathway. CNS Neurosci Ther 2013; 19(3): 163-9.

[12] Nabavi SF, Nabavi SM, Habtemariam S, *et al.* Hepatoprotective effect of gallic acid isolated from Peltiphyllum peltatum against sodium fluoride-induced oxidative stress. Ind Crop Prod 2013; 44: 50-5.

[13] Nabavi SF, Daglia M, Moghaddam AH, Habtemariam S, Nabavi SM. Curcumin and liver disease: from chemistry to medicine. Compr Rev Food Sci Food Saf 2014; 13(1): 62-77.

[14] Norden DM, Muccigrosso MM, Godbout JP. Microglial priming and enhanced reactivity to secondary insult in aging, and traumatic CNS injury, and neurodegenerative disease. Neuropharmacology 2015; 96: 29-41.

[15] Takano T, He W, Han XN, *et al*. Rapid manifestation of reactive astrogliosis in acute Hippocampal brain slices. Glia 2014; 62(1): 78-95.

[16] Hwang SL, Shih PH, Yen GC. Neuroprotective effects of citrus flavonoids. J Agric Food Chem 2012; 60(4): 877-85.

[17] Datla KP, Christidou M, Widmer WW, Rooprai HK, Dexter DT. Tissue distribution and neuroprotective effects of citrus flavonoid tangeretin in a rat model of Parkinson's disease. Neuroreport 2001; 12(17): 3871-5.

[18] Spencer JPE, Rice-Evans C, Williams RJ. Modulation of pro-survival Akt/protein kinase B and ERK1/2 signaling cascades by quercetin and its *in vivo* metabolites underlie their action on neuronal viability. J Biol Chem 2003; 278(37): 34783-93.

[19] Kang SS, Lee JY, Choi YK, Kim GS, Han BH. Neuroprotective effects of flavones on hydrogen peroxide-induced apoptosis in SH-SY5Y neuroblostoma cells. Bioorg Med Chem lett 2004; 14(9): 2261-4.

[20] Joseph JA, Shukitt-Hale B, Denisova NA, *et al*. Long-term dietary strawberry, spinach, or vitamin E supplementation retards the onset of age-related neuronal signal-transduction and cognitive behavioral deficits. J Neurosci 1998; 18(19): 8047-55.

[21] Gonzalez-Molina E, Dominguez-Perles R, Moreno DA, Garcia-Viguera C. Natural bioactive compounds of Citrus limon for food and health. J Pharmaceut Biomed 2010; 51(2): 327-45.

[22] Benavente-Garcia O, Castillo J. Update on uses and properties of Citrus flavonolds: New findings in anticancer, cardiovascular, and anti-inflammatory activity. J Agric Food Chem 2008; 56(15): 6185-205.

[23] Benavente-Garcia O, Castillo J, Alcaraz M, Vicente V, Del Rio JA, Ortuno A. Beneficial action of Citrus flavonoids on multiple cancer-related biological pathways. Curr Cancer Drug Tar 2007; 7(8): 795-809.

[24] Manthey JA, Guthrie N, Grohmann K. Biological properties of citrus flavonoids pertaining to cancer and inflammation. Curr Med Chem 2001; 8(2): 135-53.

[25] Heo HJ, Kim DO, Shin SC, Kim MJ, Kim BG, Shin DH. Effect of antioxidant flavanone, naringenin, from Citrus junos on neuroprotection. J Agric Food Chem 2004; 52(6): 1520-5.

[26] Vauzour D, Vafeiadou K, Rice-Evans C, Williams RJ, Spencer JPE. Activation of pro-survival Akt and ERK1/2 signalling pathways underlie the anti-apoptotic effects of flavanones in cortical neurons. J Neurochem 2007; 103(4): 1355-67.

[27] Tamilselvam K, Braidy N, Manivasagam T, *et al*. Neuroprotective effects of Hesperidin, a plant Flavanone, on Rotenone-Induced oxidative stress and apoptosis in a cellular model for Parkinson's disease. Oxid Med Cell Longev 2013; 2013: 102741.

[28] Raza SS, Khan MM, Ahmad A, *et al*. Hesperidin ameliorates functional and histological outcome and reduces neuroinflammation in experimental stroke. Brain Res 2011; 1420: 93-105.

[29] Heo HJ, Lee CY. Strawberry and its anthocyanins reduce oxidative stress-induced apoptosis in PC12 cells. J Agric Food Chem 2005; 53(6): 1984-9.

[30] Nogata Y, Sakamoto K, Shiratsuchi H, Ishii T, YANO M, Ohta H. Flavonoid composition of fruit tissues of citrus species. Biosci Biotechnol Biochem 2006; 70(1): 178-92.

[31] Devi KP, Rajavel T, Nabavi SF, *et al*. Hesperidin: A promising anticancer agent from nature. Ind Crop Prod 2015; 76: 582-9.

[32] Xiong Y, Chen D, Yu C, *et al*. Citrus nobiletin ameliorates experimental colitis by reducing inflammation and restoring impaired intestinal barrier function. Mol Nutri Food Res 2015; 59(5): 829-42.

[33] Harada S, Tominari T, Matsumoto C, *et al*. Nobiletin, a Polymethoxy Flavonoid, suppresses bone resorption by Inhibiting NF. KAPPA. B-dependent prostaglandin E synthesis in Osteoblasts and prevents bone loss due to Estrogen deficiency. J Pharmacol Sci 2011; 115(1): 89-93.

[34] Guo S, Qiu P, Xu G, *et al*. Synergistic anti-inflammatory effects of nobiletin and sulforaphane in lipopolysaccharide-stimulated RAW 264.7 cells. J Agric Food Chem 2012; 60(9): 2157-64.

[35] Yoshimizu N, Otani Y, Saikawa Y, *et al*. Anti-tumour effects of nobiletin, a citrus flavonoid, on gastric cancer include: antiproliferative effects, induction of apoptosis and cell cycle deregulation. Aliment Pharmacol Ther 2004; 20(s1): 95-101.

[36] Chien S-Y, Hsieh M-J, Chen C-J, Yang S-F, Chen M-K. Nobiletin inhibits invasion and migration of human nasopharyngeal carcinoma cell lines by involving ERK1/2 and transcriptional inhibition of MMP-2. Expert Opin Ther Targets 2015; 19(3): 307-20.

[37] Shi M-D, Liao Y-C, Shih Y-W, Tsai L-Y. Nobiletin attenuates metastasis *via* both ERK and PI3K/Akt pathways in HGF-treated liver cancer HepG2 cells. Phytomedicine 2013; 20(8): 743-52.

[38] Morley KL, Ferguson PJ, Koropatnick J. Tangeretin and nobiletin induce G1 cell cycle arrest but not apoptosis in human breast and colon cancer cells. Cancer lett 2007; 251(1): 168-78.

[39] Miyamoto S, Yasui Y, Tanaka T, Ohigashi H, Murakami A. Suppressive effects of nobiletin on hyperleptinemia and colitis-related colon carcinogenesis in male ICR mice. Carcinogenesis 2008; 29(5): 1057-63.

[40] Lien LM, Wang MJ, Chen RJ, *et al*. Nobiletin, a Polymethoxylated Flavone, inhibits Glioma cell growth and migration *via* arresting cell cycle and suppressing MAPK and Akt pathways. Phytother Res 2015.

[41] Jang S-E, Ryu K-R, Park S-H, *et al*. Nobiletin and tangeretin ameliorate scratching behavior in mice by inhibiting the action of histamine and the activation of NF-κB, AP-1 and p38. Int immunopharmacol 2013; 17(3): 502-7.

[42] Lee Y-S, Cha B-Y, Saito K, *et al*. Nobiletin improves hyperglycemia and insulin resistance in obese diabetic ob/ob mice. Biochem Pharmacol 2010; 79(11): 1674-83.

[43] Lee Y-S, Cha B-Y, Choi S-S, *et al*. Nobiletin improves obesity and insulin resistance in high-fat diet-induced obese mice. J Nut Biochem 2013; 24(1): 156-62.

[44] Matsuzaki K, Yamakuni T, Hashimoto Y, *et al*. Nobiletin restoring β-amyloid-impaired CREB phosphorylation rescues memory deterioration in Alzheimer's disease model rats. Neurosci Lett 2006; 400(3): 230-4.

[45] Seki T, Kamiya T, Furukawa K, *et al*. Nobiletin-rich Citrus reticulata peels, a kampo medicine for Alzheimer's disease: A case series. Geriatrics Gerontol Intl 2013; 13(1): 236-8.

[46] Nakajima A, Aoyama Y, Shin E-J, *et al*. Nobiletin, a citrus flavonoid, improves cognitive impairment and reduces soluble Aβ levels in a triple transgenic mouse model of Alzheimer's disease (3XTg-AD). Behav Brain Res 2015; 289: 69-77.

[47] Onozuka H, Nakajima A, Matsuzaki K, *et al*. Nobiletin, a citrus flavonoid, improves memory impairment and Aβ pathology in a transgenic mouse model of Alzheimer's disease. J Pharmacol Exp Ther 2008; 326(3): 739-44.

[48] Yabuki Y, Ohizumi Y, Yokosuka A, Mimaki Y, Fukunaga K. Nobiletin treatment improves motor and cognitive deficits seen in MPTP-induced Parkinson model mice. Neuroscience 2014; 259: 126-41.

[49] Cho HW, Jung SY, Lee GH, Cho JH, Choi IY. Neuroprotective effect of Citrus unshiu immature peel and nobiletin inhibiting hydrogen peroxide-induced oxidative stress in HT22 murine hippocampal neuronal cells. Pharma Mag 2015; 11(Suppl 2): 284.

[50] Drewnowski A, Gomez-Carneros C. Bitter taste, phytonutrients, and the consumer: a review. Am J Clin Nutr. 2000; 72(6): 1424-35.

[51] Nielsen SE, Breinholt V, Cornett C, Dragsted LO. Biotransformation of the citrus flavone tangeretin in rats. Identification of metabolites with intact flavane nucleus. Food Chem Toxicol 2000; 38(9): 739-46.

[52] Arafa ESA, Zhu QZ, Barakat BM, *et al*. Tangeretin Sensitizes Cisplatin-Resistant Human Ovarian Cancer Cells through Downregulation of Phosphoinositide 3-Kinase/Akt Signaling Pathway. Cancer Res 2009 1; 69(23): 8910-7.

[53] Roza JM, Zheng XL, Guthrie N. Effect of Citrus Flavonoids and Tocotrienols on Serum Cholesterol Levels in Hypercholesterolemic Subjects. Altern Ther Health M 2007; 13(6): 44-8.

[54] Alinezhad H, Azimi R, Zare M, Ebrahimzadeh MA, Eslami S, Nabavi SF, *et al*. Antioxidant and antihemolytic activities of ethanolic extract of flowers, leaves, and stems of Hyssopus officinalis L. Var. angustifolius. Int J Food Prop 2013; 16(5): 1169-78.

[55] Nabavi SF, Nabavi SM, Moghaddam AH, Naqinezhad A, Bigdellou R, Mohammadzadeh S. Protective effects of Allium paradoxum against gentamicin-induced nephrotoxicity in mice. Food Func 2012; 3(1): 28-9.

[56] Nabavi SF, Nabavi SM, Ebrahimzadeh MA, Eslami B, Jafari N. *In vitro* antioxidant and antihemolytic activities of hydroalcoholic extracts of Allium scabriscapum Boiss. & Ky. aerial parts and bulbs. Intl J Food Prop 2013; 16(4): 713-22.

[57] Nabavi SM, Marchese A, Izadi M, Curti V, Daglia M, Nabavi SF. Plants belonging to the genus Thymus as antibacterial agents: From farm to pharmacy. Food Chem 2015; 173: 339-47.

[58] de Oliveira MR, Nabavi SM, Braidy N, Setzer WN, Ahmed T, Nabavi SF. Quercetin and the mitochondria: A mechanistic view. Biotechnol Adv 2016; 34(5): 532-49.

[59] Singh M, Kaur M, Silakari O. Flavones: An important scaffold for medicinal chemistry. Eur J Med Chem 2014; 84: 206-39.

[60] Koes RE, Quattrocchio F, Mol JN. The flavonoid biosynthetic pathway in plants: function and evolution. BioEssays 1994; 16(2): 123-32.

[61] Manthey JA, Grohmann K, Berhow MA, Tisserat B. Changes in citrus leaf flavonoid concentrations resulting from blight-induced zinc-deficiency. Plant Physiol Biochem 2000; 38(4): 333-43.

[62] Gattuso G, Barreca D, Gargiulli C, Leuzzi U, Caristi C. Flavonoid composition of citrus juices. Molecules 2007; 12(8): 1641-73.

[63] Zhang H, Xi W, Zhou Z, Wang HL, Bai Z. Bioactivities and structure of polymethoxylated flavones in citrus. J Food Agr Env 2013; 11(2): 237-42.

[64] Manthey J, Buslig B. Flavonoids in the living system: Springer Science & Business Media Work; 1998.

[65] Shahidi F, Naczk M. Phenolics in Food and Nutraceuticals: CRC Press; 2003. 576 p.

[66] Ortuno A, Arcas MC, Botia JM, Fuster MD, Del Rio JA. Increasing resistance against Phytophthora citrophthora in tangelo Nova fruits by modulating polymethoxyflavones levels. J Agr Food Chem 2002; 50(10): 2836-9.

[67] Akhlaghi M, Shabanian G, Rafieian-Kopaei M, Parvin N, Saadat M, Akhlaghi M. Citrus aurantium blossom and preoperative anxiety. Revista brasileira de Anestesiologia 2011; 61(6): 707-12.

[68] Hamedi A, Zarshenas MM, Sohrabpour M, Zargaran A. Herbal medicinal oils in traditional Persian medicine. Pharmaceutical Biol 2013; 51(9): 1208-18.

[69] Wu M, Zhang H, Zhou C, Jia H, Ma Z, Zou Z. Identification of the chemical constituents in aqueous extract of Zhi-Qiao and evaluation of its antidepressant effect. Molecules 2015; 20(4): 6925-40.

[70] Tanaka R, Sakano Y, Shimizu K, Shibuya M, Ebizuka Y, Goda Y. Constituents of Laurus nobilis L. inhibit recombinant human lanosterol synthase. J Nat Med 2006; 60(1): 78-81.

[71] Zou GA, Su ZH, Zhang HW, Wang Y, Yang JS, Zou ZM. Flavonoids from the stems of Croton caudatus Geisel. var. tomentosus Hook. Molecules 2010; 15(3): 1097-102.

[72] Peng A, Qu X, Li H, Gao L, Yu B, Yang H. Isolation and purification of flavones from Murraya exotica L. by high-speed counter-current chromatography. Chinese J chromatograp 2010; 28(4): 383-7.

[73] Su Y, Xu JJ, Bi JL, *et al*. Chemical constituents of Arisaema franchetianum tubers. J Asian Nat Prod Res 2013; 15(1): 71-7.

[74] Zhao Q, Wang CX, Li YL, Liu CY, Rong YH. Chemical constituents from Selaginella doederleinii and their bioactivities. Chinese Trad Herbal Drugs 2013; 44(23): 3270-5.

[75] Peng X, Wu Q, Gao C, Gai C, Yuan D, Fu H. Chemical constituents from the fruit calyx of Physalis alkekengi var. francheti. J Chinese Pharm Sci 2015; 24(9): 600-6.

[76] Wang CQ, Wang L, Li BJ, Fan CL, Huang XJ, Ye WC. Chemical constituents from leaves of Ilex latifolia. Zhongguo Zhongyao Zazhi 2014; 39(2): 258-61.

[77] Makowska I, Kloszewska I, Grabowska A, Szatkowska I, Rymarczyk K. Olfactory deficits in normal aging and Alzheimer's disease in the polish elderly population. Arch Clin Neuropsychol 2011; 26(3): 270-9.

[78] Bahar-Fuchs A, Moss S, Rowe C, Savage G. Awareness of olfactory deficits in healthy aging, amnestic mild cognitive impairment and Alzheimer's disease. Int Psychogeriatr 2011; 23(7): 1097-106.

[79] Potter PE, Rauschkolb PK, Pandya Y, *et al*. Pre- and post-synaptic cortical cholinergic deficits are proportional to amyloid plaque presence and density at preclinical stages of Alzheimer's disease. Acta Neuropathol 2011; 122(1): 49-60.

[80] Nakajima A, Yamakuni T, Haraguchi, *et al*. Nobiletin, a citrus flavonoid that improves memory impairment, rescues bulbectomy-induced cholinergic neurodegeneration in mice. J Pharmacol Sci 2007; 105(1): 122-6.

[81] Nagase H, Omae N, Omori A, *et al*. Nobiletin and its related flavonoids with CRE-dependent transcription-stimulating and neuritegenic activities. Biochem biophy Res Commun 2005; 337(4): 1330-6.

[82] Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 2002; 297(5580): 353-6.

[83] Butterfield A, Swomley AM, Sultana R. Amyloid beta-Peptide 1-42-induced Oxidative stress in Alzheimer disease: Importance in disease pathogenesis and progression. Antioxid Redox Signaling 2012.

[84] Osborne C, West E, Nolan W, McHale-Owen H, Williams A, Bate C. Glimepiride protects neurons against amyloid-beta-induced synapse damage. Neuropharmacology; 101: 225-36.

[85] Bate C, Williams A. Amyloid-beta-induced synapse damage is mediated *via* cross-linkage of cellular prion proteins. J Biol Chem 2011; 286(44): 37955-63.

[86] Minano-Molina AJ, Espana J, Martin E, *et al*. Soluble oligomers of amyloid-beta peptide disrupt membrane trafficking of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor contributing to early synapse dysfunction. J Biol Chem 2011; 286(31): 27311-21.

[87] Matsuzaki K, Yamakuni T, Hashimoto M, *et al*. Nobiletin restoring beta-amyloid-impaired CREB phosphorylation rescues memory deterioration in Alzheimer's disease model rats. Neurosci lett 2006; 400(3): 230-4.

[88] Onozuka H, Nakajima A, Matsuzaki K, *et al*. Nobiletin, a citrus flavonoid, improves memory impairment and Abeta pathology in a transgenic mouse model of Alzheimer's disease. J Pharmacology Exper Thera 2008; 326(3): 739-44.

[89] Shin RW, Ogino K, Shimabuku A, *et al*. Amyloid precursor protein cytoplasmic domain with phospho-Thr668 accumulates in Alzheimer's disease and its transgenic models: a role to mediate interaction of Abeta and tau. Acta Neuropathol 2007; 113(6): 627-36.

[90] Nakajima A, Aoyama Y, Shin EJ, *et al*. Nobiletin, a citrus flavonoid, improves cognitive impairment and reduces soluble Abeta levels in a triple transgenic mouse model of Alzheimer's disease (3XTg-AD). Behav brain Res 2015; 289: 69-77.

[91] Dawson N, Xiao X, McDonald M, Higham DJ, Morris BJ, Pratt JA. Sustained NMDA receptor hypofunction induces compromised neural systems integration and schizophrenia-like alterations in functional brain networks. Cerebral Cortex 2014; 24(2): 452-64.

[92] du Bois TM, Huang XF. Early brain development disruption from NMDA receptor hypofunction: relevance to schizophrenia. Brain Res Rev 2007; 53(2): 260-70.

[93] Hynd MR, Scott HL, Dodd PR. Selective loss of NMDA receptor NR1 subunit isoforms in Alzheimer's disease. J Neurochem 2004; 89(1): 240-7.

[94] Hynd MR, Scott HL, Dodd PR. Glutamate(NMDA) receptor NR1 subunit mRNA expression in Alzheimer's disease. J Neurochem 2001; 78(1): 175-82.

[95] Kurup P, Zhang Y, Xu J, *et al*. Abeta-mediated NMDA receptor endocytosis in Alzheimer's disease involves ubiquitination of the tyrosine phosphatase STEP61. J Neurosci 2010 28; 30(17): 5948-57.

[96] Goto Y, Niidome T, Akaike A, Kihara T, Sugimoto H. Amyloid beta-peptide preconditioning reduces glutamate-induced neurotoxicity by promoting endocytosis of NMDA receptor. Biochem Biophyl Research Commun 2006; 351(1): 259-65.

[97] Gilmour G, Dix S, Fellini L, *et al*. NMDA receptors, cognition and schizophrenia--testing the validity of the NMDA receptor hypofunction hypothesis. Neuropharmacology 2012 ; 62(3): 1401-12.

[98] Nakajima A, Yamakuni T, Matsuzaki K, *et al*. Nobiletin, a citrus flavonoid, reverses learning impairment associated with N-methyl-D-aspartate receptor antagonism by activation of extracellular signal-regulated kinase signaling. J Pharmacol Exper Thera 2007; 321(2): 784-90.

[99] Alonso M, Viola H, Izquierdo I, Medina JH. Aversive experiences are associated with a rapid and transient activation of ERKs in the rat hippocampus. Neurobiol Learn Mem 2002; 77(1): 119-24.

[100] Zhang L, Xie JW, Yang J, Cao YP. Tyrosine Phosphatase STEP61 negatively regulates amyloid beta-Mediated ERK/CREB signaling pathways *via* alpha 7 Nicotinic Acetylcholine receptors. J Neurosci Res 2013; 91(12): 1581-90.

[101] Kimura J, Nemoto K, Yokosuka A, Mimaki Y, Degawa M, Ohizumi Y. 6-Demethoxynobiletin, a Nobiletin-Analog citrus flavonoid, enhances extracellular signal-regulated Kinase Phosphorylation in PC12D Cells. Biol Pharm Bull 2013; 36(10): 1646-9.

[102] Nagase H, Yamakuni T, Matsuzaki K, *et al*. Mechanism of neurotrophic action of nobiletin in PC12D cells. Biochem 2005; 44(42): 13683-91.

[103] Sagare AP, Bell RD, Zlokovic BV. Neurovascular dysfunction and faulty amyloid beta-peptide clearance in alzheimer disease. Csh Perspect Med 2012; 2(10).

[104] Wang XJ, Xing AF, Xu CL, Cai Q, Liu H, Li LA. Cerebrovascular hypoperfusion induces spatial memory impairment, synaptic changes, and Amyloid-beta oligomerization in Rats. J Alzheimers Dis 2010; 21(3): 813-22.

[105] Yamamoto Y, Shioda N, Han F, *et al*. Nobiletin improves brain ischemia-induced learning and memory deficits through stimulation of CaMKII and CREB phosphorylation. Brain Res 2009; 1295: 218-29.

[106] Jeong SJ, Kim K, Suh YH. Age-related changes in the expression of Alzheimer's beta APP in the brain of senescence accelerated mouse (SAM)-P/10. Neuroreport 1997; 8(7): 1733-7.

[107] Nakajima A, Aoyarna Y, Nguyen TTL, *et al*. Nobiletin, a citrus flavonoid, ameliorates cognitive impairment, oxidative burden, and hyperphosphorylation of tau in senescence-accelerated mouse. Behav Brain Res 2013; 250: 351-60.

[108] Cho HW, Jung SY, Lee GH, Cho JH, Choi IY. Neuroprotective effect of Citrus unshiu immature peel and nobiletin inhibiting hydrogen peroxide-induced oxidative stress in HT22 murine hippocampal neuronal cells. Pharmacogn Mag 2015; 11(Suppl 2): S284-9.

[109] Nakajima A, Aoyama Y, Nguyen TT, *et al*. Nobiletin, a citrus flavonoid, ameliorates cognitive impairment, oxidative burden, and hyperphosphorylation of tau in senescence-accelerated mouse. Behav Brain Res 2013; 250: 351-60.

[110] Murakami A, Nakamura Y, Torikai K, *et al*. Inhibitory effect of citrus nobiletin on phorbol ester-induced skin inflammation, oxidative stress, and tumor promotion in mice. Cancer Res 2000; 60(18): 5059-66.

[111] Nomura Y, Wang BX, Qi SB, Namba T, Kaneko S. Biochemical changes related to aging in the senescence-accelerated mouse. Exper Gerontol 1989; 24(1): 49-55.

[112] Bauer C, Pardossi-Piquard R, Dunys J, Roy M, Checler F. gamma-Secretase-Mediated Regulation of Neprilysin: Influence of Cell Density and Aging and Modulation by Imatinib. J Alzheimers Dis 2011; 27(3): 511-20.

[113] Morisaki N, Moriwaki S, Sugiyama-Nakagiri Y, Haketa K, Takema Y, Imokawa G. Neprilysin Is Identical to Skin Fibroblast Elastase its role in skin aging and uv responses. J Biol Chem 2010; 285(51): 39819-27.

[114] Garcia-Espinosa MA, Gallagher PE, Ferrario CM, Diz DI. Neprilysin and ACE2 regulation during aging in rats with low brain angiotensinogen. Hypertension 2005; 46(4): 830-1.

[115] Wang DS, Lipton RB, Katz MJ, *et al*. Decreased neprilysin immunoreactivity in Alzheimer disease, but not in pathological aging. J Neuropath Exp Neur 2005; 64(5): 378-85.

[116] Wang DS, Iwata N, Hama E, Saido TC, Dickson K. Oxidized neprilysin in aging and Alzheimer's disease brains. Biochem Biophy Res Commun 2003; 310(1): 236-41.

[117] Wang D, Younkin SG, Davies P, Decreased neprilysin in frontal gray matter in Alzheimer's disease, but not in pathological aging. J Neuropath Exp Neur 2003; 62(5): 546.

[118] Apelt J, Ach K, Schliebs R. Aging-related down-regulation of neprilysin, a putative beta-amyloid-degrading enzyme, in transgenic Tg2576 Alzheimer-like mouse brain is accompanied by an astroglial upregulation in the vicinity of beta-amyloid plaques. Neurosci lett 2003 ; 339(3): 183-6.

[119] Iwata N, Takaki Y, Fukami S, Tsubuki S, Saido TC. Region-specific reduction of A beta-degrading endopeptidase, neprilysin, in mouse hippocampus upon aging. J Neurosci Res 2002; 70(3): 493-500.

[120] Iwata N, Tsubuki S, Takaki Y, *et al*. A decline of neprilysin, A beta-degrading enzyme, activity in mouse brain with aging. Japanese J Pharmacol 2002; 88: 94p-p.

[121] Fujiwara H, Kimura J, Sakamoto M, *et al*. Nobiletin, a flavone from Citrus depressa, induces gene expression and increases the protein level and activity of neprilysin in SK-N-SH cells. Can J Physiol Pharm 2014; 92(5): 351-5.

[122] Jeong KH, Jeon MT, Kim HD, *et al*. Nobiletin protects dopaminergic neurons in the 1-methyl-4-phenylpyridinium-treated rat model of Parkinson's disease. J Med Food 2015; 18(4): 409-14.

[123] Singh SP, Wahajuddin, Tewari D, Patel K, Jain GK. Permeability determination and pharmacokinetic study of nobiletin in rat plasma and brain by validated high-performance liquid chromatography method. Fitoterapia 2011; 82(8): 1206-14.

[124] Al Rahim M, Nakajima A, Saigusa D, *et al*. 4 '-Demethylnobiletin, a bioactive metabolite of nobiletin enhancing PKA/ERK/CREB signaling, rescues learning impairment associated with NMDA receptor antagonism *via* stimulation of the ERK Cascade. Biochemistry 2009; 48(32): 7713-21.

[125] Chen HF, Zhang WG, Yuan JB, Li YG, Yang SL, Yang WL. Simultaneous quantification of polymethoxylated flavones and coumarins in Fructus aurantii and Fructus aurantii immaturus using HPLC-ESI-MS/MS. J Pharmaceut Biomed 2012; 59: 90-5.

[126] Lee YH, Charles AL, Kung HF, Ho CT, Huang TC. Extraction of nobiletin and tangeretin from Citrus depressa Hayata by supercritical carbon dioxide with ethanol as modifier. Ind Crop Prod 2010; 31(1): 5 9-64.

[127] Wang QA, Wu Z, Liu L, Zou LH, Luo M. Synthesis of Citrus Bioactive Polymethoxyflavonoids and Flavonoid Glucosides. Chinese J Org Chem 2010; 30(11): 1682-8.

[128] Datla KP, Christidou M, Widmer WW, Rooprai HK, Dexter DT. Tissue distribution and neuroprotective effects of citrus flavonoid tangeretin in a rat model of Parkinson's disease. Neuroreport 2001; 12(17): 3871-5.

[129] Hashida K, Kitao Y, Sudo H, *et al*. ATF6alpha promotes astroglial activation and neuronal survival in a chronic mouse model of Parkinson's disease. PloS one 2012; 7(10): e47950.

[130] Ghribi O, Herman MM, Pramoonjago P, Savory J. MPP+ induces the endoplasmic reticulum stress response in rabbit brain involving activation of the ATF-6 and NF-kappaB signaling pathways. J Neuropathol Exp Neurol 2003; 62(11): 1144-53.

[131] Chalovich EM, Zhu JH, Caltagarone J, Bowser R, Chu CT. Functional repression of cAMP response element in 6-hydroxydopamine-treated neuronal cells. J Bioll Chem 2006; 281(26): 17870-81.

[132] Kawahata I, Yoshida M, Sun W, *et al*. Potent activity of nobiletin-rich Citrus reticulata peel extract to facilitate cAMP/PKA/ERK/CREB signaling associated with learning and memory in cultured hippocampal neurons: identification of the substances responsible for the pharmacological action. J Neural Transmission 2013; 120(10): 1397-409.

[133] Ljungberg MC, Ali YO, Zhu J, *et al*. CREB-activity and nmnat2 transcription are down-regulated prior to neurodegeneration, while NMNAT2 over-expression is neuroprotective, in a mouse model of human tauopathy. Human Mol Genetics 2012; 21(2): 251-67.

[134] Yang EJ, Lim SH, Song KS, Han HS, Lee J. Identification of active compounds from Aurantii Immatri Pericarpium attenuating brain injury in a rat model of ischemia-reperfusion. Food Chem 2013; 138(1): 663-70.

[135] Ting Y, Jiang Y, Lan Y, *et al*. Viscoelastic emulsion improved the bioaccessibility and oral bioavailability of crystalline compound: A mechanistic study using in *vitro* and in *vivo* models. Mol Pharm 2015; 12(7): 2229-36.

[136] Nielsen SE, Breinholt V, Cornett C, Dragsted LO. Biotransformation of the citrus flavone tangeretin in rats. Identification of metabolites with intact flavane nucleus. Food Chem Toxicol 2000; 38(9): 739-46.

[137] Manthey JA, Cesar TB, Jackson E, Mertens-Talcott S. Pharmacokinetic study of nobiletin and tangeretin in rat serum by high-performance liquid chromatography-electrospray ionization-mass spectrometry. J Agr Food Chem 2011; 59(1): 145-51.

[138] Manthey JA, Cesar TB, Jackson E, Mertens-Talcott S. Pharmacokinetic study of Nobiletin and Tangeretin in rat serum by high-performance liquid chromatography-electrospray ionization-mass spectrometry. J Agr Food Chem 2011; 59(1): 145-51.

[139] Seki T, Kamiya T, Furukawa K, *et al*. Nobiletin-rich Citrus reticulata peels, a kampo medicine for Alzheimer's disease: A case series. Geriatrics Gerontol Int 2013; 13(1): 236-8.