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Review

New therapeutic approaches to mild cognitive impairment and mild dementia: the role of Ginkgo Biloba (EGb 761®)

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Abstract: Mild cognitive impairment (MCI) and mild dementia are a clinically relevant health problem in the elderly and Alzheimer's disease being the most common neurodegenerative disorder. Furthermore, MCI and mild dementia are characterized by a deterioration of cognitive function and their diagnosis is mainly based on cognitive examination and, the prognosis of the disease seems to be an essential reason for the diagnosis, because there is a high risk of cognitive decline in the two syndromes. This review describes the effectiveness of Ginkgo biloba (EGb761®) leaf extract for the treatment of dementia syndrome and EGb761® combination therapy with other medications for symptomatic dementia. Tebonin® is a drug of plant origin based on the active ingredient "Ginkgo biloba". This drug has shown encouraging results, improving cognitive function, neuropsychiatric disorders and consequent reduction of caregiver stress and maintenance of autonomy in patients with age-related cognitive decline, MCI and mild dementia. Nowadays, there is little evidence to support the efficacy of EGb761® combination therapy with anti-dementia drugs and, therefore, more evidence is needed to evaluate the role of EGb761® in mixed therapy.

Keywords: mild cognitive impairment (MCI), mild dementia, Alzheimer's disease, Ginkgo biloba (EGb761®), Tebonin, anti-dementia drugs, randomized controlled trials.

1. Introduction

Mild cognitive impairment (MCI) is a clinically relevant health problem in the elderly and it is considered an intermediate state between normal aging and dementia [1]. This state can progress to dementia, and Alzheimer's disease (AD) is the most common form of neurodegenerative disorder [2]. The prevalence of MCI in the population over 60 is approximately 5.9%, it tends to increase with age and is more common in men. The MCI development varies according to numerous risk factors that, beyond age, also include genetics, comorbidities, chronic diseases as vascular risk factors, pulmonary diseases, depression, metabolic risk such as diabetes mellitus, hypertension and also tobacco utilization [3].

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Nowadays, despite pharmacological new findings, there is still no specific drug approved by the Food and Drug Administration (FDA) for the treatment of MCI. The only drugs used, approved by the FDA for the treatment of mild and moderate AD, are Acetylcholinesterase (AChE) inhibitors: AChEIs or memantines, although their effects are not very effective and have numerous side effects such as nausea, bradycardia, fatigue [4]. For this reason, the focus has shifted towards non-pharmacological treatments that involve behavioral interventions, psychosocial support, physical activity, diet and cognitive stimulation [4,5].

Additionally, an important factor involved in the etiology of cognitive decline is inflammation and the subsequent development of oxidative stress.

Consequently, several studies suggested an important role of diet in the prevention of neurodegenerative diseases, showing a protective role against the damaging effects of neuroinflammation and oxidative stress [4,5].

Indeed, the Mediterranean diet has been shown to reduce the incidence of mild cognitive impairment (MCI) and, possibly, the conversion of MCI to dementia [6]. Vitamins, minerals, polyphenols have been associated to the prevention of cognitive impairment due to their antioxidant effects, such as the reduction of free radical species and therefore the oxidative stress development [7-10].

Considering polyphenols deriving from plants, Ginkgo biloba is the oldest living tree species in the world and is one of the most studied herbals for cognitive disorders and AD [11].

In traditional medicine Ginkgo leaves were used mainly for the treatment of respiratory and cardiovascular disorders while in Chinese medicine, Ginkgo biloba seeds were used to treat pulmonary symptoms, alcohol abuse and bladder infections [12]. The modern use of Ginkgo biloba extract is all about leaf-based preparations and numerous health benefits have been attributed to its utilization. Recent studies showed an important role of Ginkgo Biloba in cognitive improvement due to its possible effect as vasodilator and in cerebral vascularization [13].

In particular, it has been observed that Ginkgo biloba extract (EGb761) could play a protective effect in the treatment and prevention of Alzheimer's diseases and other neuro-degenerative disorders [14].

EGb761® mechanisms of action include the antioxidant, anti-inflammatory and anti-apoptotic action and the defense against mitochondrial dysfunction. However, its clinical efficacy is not yet well known.

Tebonin is a vegetal origin drug based on EGb761 active principle, registered as "well established use" and authorized in many European States. Its pharmacological effect is based on several neurobiological mechanisms, including the increase of neurogenesis and synaptogenesis, the prevention of mitochondrial dysfunction and the improvement of neuro-transmission. In clinics, these mechanisms lead to a cognitive improvement, impeding the evolvement of neurodegenerative diseases.

This review describes the main clinical and preclinical human studies highlighting the efficacy of the Ginkgo biloba (EGb761®) leaves extract for the treatment of MCI and dementia syndrome, and EGb761® combination therapy with other symptomatic drugs for dementia.

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1.1. Mild cognitive impairment (MCI) and mild dementia: recent classification criteria, pathogenesis, therapeutic aspects.

Neurodegenerative diseases involve different groups of pathologies of the central nervous system, characterized by a progressive loss of synaptic transmission in some specific neuronal circuits. This information flow deficit, without any clear structural damage, is then followed by progressive neuronal death with loss of volume and obvious accumulation of toxic substances in both extra- (e.g. amyloid beta plaques) and intra-neuronal spaces (e.g. neurofibrillary tangles) [15,16]. Moreover, these disorders are classified considering clinical features (Parkinson or dementia), anatomic distribution of neurodegeneration (frontotemporal degenerations, spinocerebellar degenerations), or as a result of molecular abnormalities [17]. Depending on the type of disease, neuronal deterioration can result in cognitive deficits, dementia, motor alterations, behavioral and psychological disorders [18].

Specifically, mild cognitive impairment (MCI) and mild dementia are neurodegenerative diseases characterized by cognitive degeneration, the most common are Alzheimer's disease (AD), vascular dementia and Lewy Body's disease [19]. Unlike MCI, different cognitive domains are involved in mild dementia and the interference with daily life is evident [2]. In the recent years, MCI prevalence in the over 60 population is estimated to be around 6% - 22% [20], depending on the demographics of the population. MCI is clinically heterogeneous and different factors can increase the risk of MCI development (Figure 1).

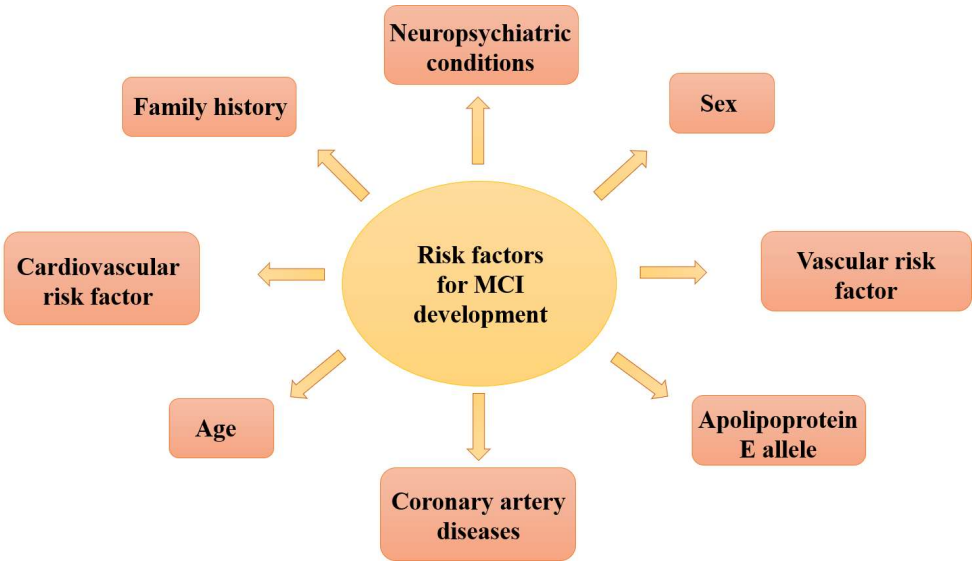

Figure 1: Risk factors for MCI development.

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Diagnostic criteria employed to identify MCI include a slight decrease in the performance of several cognitive functions, in one or more domains, related to memory, orientation or verbal skills [15,16]. This cognitive decline, which is still common in the elderly population, is not necessarily indicative of incipient dementia.

In this regard, an MCI classification has been proposed in amnestic MCI (aMCI, where memory is significantly impaired) and non-amnestic MCI (naMCI, where memory remains intact). Furthermore, MCI may consist on impairment in a single cognitive domain or in multiple cognitive domains (Figure 2). The combination of the several clinical subtype with degenerative, psychiatric or vascular etiology could be an important predictor factor of the type of dementia that the MCI patient would develop (AD, vascular dementia, frontotemporal dementia, Lewy bodies) [21]. The progression of different MCI subtypes in a particular type of dementia has not yet been well understood[22].

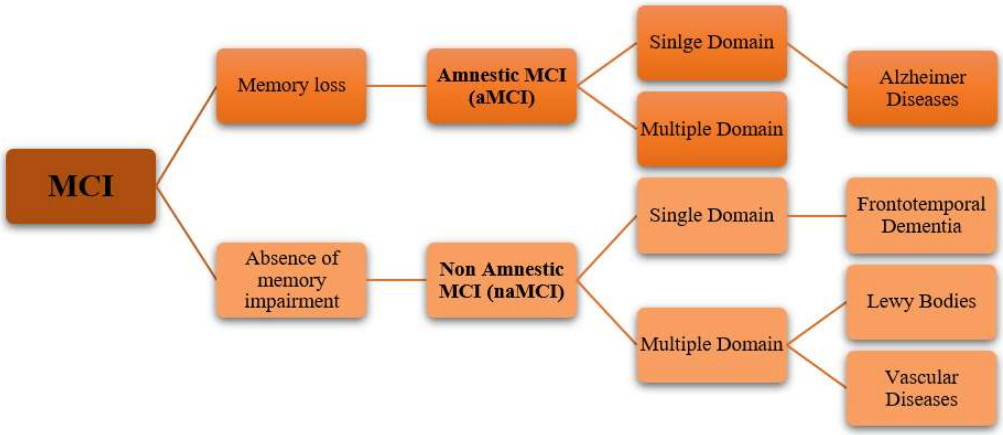

Figure 2: Schematic representation of MCI subtypes.

Therefore, patient's history is critical to recognize and detect various clinical signs and making a diagnosis.

Cognitive decline in the elderly is a common problem and healthcare providers are often the first point of contact for patients and their families. Generally only elderly with moderate to severe dementia are subject to medical attention, while the MCI are exposed to continuous challenges in the detection of disease by primary care providers [23].

In this regard, MCI has attracted a lot of interest within the scientific community because it appears to be a transition pathology between normal age-related cognitive function and a clinically

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probable diagnosis of several dementias [24]. It has become the "target" of many recent clinical trials with potentially disease-modifying drugs.

However, one of the scientific and public health problems, lies in the impossibility to distinguish, through neuropsychological tests, those MCI who will never get sick with dementia from those who are in fact already sick, being in a prodromal-to-dementia condition [19].

Nowadays, medical history and mental health examination are the most commonly used tools to determine a diagnosis of MCI or mild dementia [25]. Specifically, through medical history, the clinician determines whether or not there is a decline in the patient's daily functions while, through the mental health examination, the clinician establishes a patient's noticeable cognitive impairment. Clinical judgment is the key to integrate information from these two tools [25]. Another important tool that could be performed for the MCI and mild dementia diagnosis is the general neurological examination whose role in the diagnostic process is to understand the etiology of cognitive disorder and to exclude/confirm the presence of modifiable factors (i.e. subdural hematoma, frontal lobe neoplasm, dysmetabolic syndromes etc.) [23]. Consequently, over the last twenty years, several attempts have been made to draw up general investigations for this disorder [26,27].

Therefore, after a comparison with normal subjects of the same age, a greater degree of memory impairment has been noted in MCI patients with little or absent involvement of common activities of daily living [28]. As the disease progresses, a sub-sequential loss of cognitive function, a loss of functional independence, and the development of behavioral problems occur. Therefore, early diagnosis and treatment may delay the onset of symptoms.

For this reason, the concept of 'biomarkers' is now emerging, as instrumental examinations that, in association with neuropsychological tests, could be used to identify individuals with MCI, dementia or subjects who are already in a prodromal-to-dementia stage [19]. Due to the general interest in this area, it has recently designed - by the Ministry of Health and Italian Drug Agency (AIFA) - the INTERCEPTOR project that aims to compare 6 different biomarkers [Fluorodeoxyglucose (FDG)-Positron Emission Tomography (PET), neuropsychological tests, liquor for beta and tau, electroencephalogram (EEG) for connectivity through graphs, magnetic resonance imaging (MRI) for hippocampal volumetric, genetics for Apolipoprotein E (ApoE)] and an organizational model to validate a sustainable, non-invasive and widely widespread method across the country for the diagnosis of prodromal MCI. This will be important to identify high-risk individuals, to find the resources for early pharmacological and non-pharmacological-treatments, as well as for the delivery of any disease-modifying drugs, which should be effective among several trials.

1.2. Therapeutic approaches in mild cognitive impairment and mild dementia.

Currently, the only modest value pharmacological treatments approved are for mild dementia due to AD, while none it has been yet approved for MCI. In fact, despite numerous randomized clinical trials (RCTs) were conducted in the MCI patients, none have been able to demonstrate the effectiveness at delaying disease progression [27,29]. In addition, it is important to note that (when symptoms are disabling) the delaying or slowing down the onset or the worsening of dementia-

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related symptoms can have a major impact on the social and health costs. An extension of the total/partial autonomy time can reduce the total costs of the disease by about 50% [30].

To date, the only drugs used to prevent the development of symptoms are cholinesterase inhibitors and memantines.

Indeed, several studies identified the cholinergic deficit in subjects with dementia [31]. Evidences showed a reduction in AChE activity and an atrophy of the nucleus basalis of Meynert (the main source of AChE, the origin of cholinergic neurotransmission and projections to the cortical brain areas associated with learning and memory), through Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MRI) respectively. Therefore, actual therapeutic hypothesis in MCI and AD is to restore physiological levels of acetylcholine through the inhibition of acetylcholinesterase enzyme activity [32]. Reversible AChEIs drugs are now available; they are lipophiles enough to overcome the blood-brain barrier to act preferably on the central nervous system [33].

Among these drugs, the most studied are donepezil, physostigmine (not more in use), rivastigmine and galantamine, approved by FDA for the treatment of mild dementia due to AD, but no treatment has yet been approved by the US FDA for MCI.

Another important therapeutic hypothesis involves the use of drugs that act directly on the glutamatergic system, such as memantine [32]. In particular, memantine is a noncompetitive N-methyl-D-aspartate receptor (NMDA) receptors-antagonist drug and it is able to delay the process of dementia by inhibiting the pathological activation of NMDA receptors [34]. Its neuroprotective effects have been demonstrated in several neurological disorders. Indeed, studies conducted in ischemic models showed that an increase in NMDA receptor antagonist drugs, in the blood, led to a decrease in glucose metabolism, thus supporting the memantine neuroprotective effect.

Memantine is used for cognitive and behavioral disorders in patients with mild to moderate vascular dementia and AD. Despite this, no drugs have been approved for the treatment or prevention of these disorders.

Moreover, some studies investigated the use of statins as primary prevention of cognitive impairment, as a link between cholesterol metabolism and the development of AD has been observed [31]. These studies demonstrated that statins led to a reduction in cardiovascular risk but showed no benefit in cognitive functioning. Finally, studies with negative results were also conducted through the use of Nonsteroidal anti-inflammatory drugs (NSAIDs) both in the development of AD and in the secondary prevention of people with MCI.

For this reason, the focus has shifted towards non-pharmacological treatments that involve behavioral interventions, psychosocial support and cognitive training. Such measures are usually supplemented with drug treatment, and their positive effectiveness in the overall clinical patient's management have been demonstrated [19]

Cognitive training (of different types, and with different functional goals: Reality-Orientation Therapy, Validation Therapy, Reminiscence Therapy, various cognitive stimulation therapy programs – Cognitive Stimulation Therapy, etc.) showed results both in stimulating and reinforcing neuro-cognitive abilities, as well as in improving the execution of daily life tasks [19].

The effect of moderate physical and motor activity, especially in the intermediate stages of the disease, seems to be positive for the tone of mood, the physical wellness- and the regularization of behavioral disorders, sleep and nutrition [19].

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Furthermore, inflammation and therefore oxidative stress are important factors involved in cognitive decline [13]. Particularly, oxidative stress appears to be involved in the early phase of AD and MCI and therefore could be considered as a prodromal phase of dementia. For this reason, various antioxidant therapies have been shown to influence the onset and progression of AD [35].

The natural polyphenolic compounds confer an antioxidant effect thus reducing the development of free radicals, restoring the endogenous antioxidant defense and carrying out important neuroprotective effects for the body [13].

1.3. Ginkgo Biloba: from traditional Chinese medicine to anti-dementia drug based on scientific evidence.

Ginkgo biloba is one of the oldest tree species in the planet used especially for its health properties [12].

Ginkgo and derived pharmaceutical formulations have a millenary tradition in the Chinese medicine; at the beginning seeds and then, in the modern phytotherapy, leaves extracts were used [12,36].

In the 60s, Dr Willmar Schwabe pharmaceuticals synthesized a drug based on leaves extract that contained terpenoids and flavonoid glycosides as active constituents and organic acids for greater water solubility. This compound was commercialized as Tebonin and several studies, conducted in cell and animal models, showed neuroprotective effects of this drug.

Later, this product has been modified to improve the good characteristics and decrease side effects; in the 80s it has been proposed with the name of EGb761® with an enrichment in flavonoids, ginkgolides and bilobalide, and a reduction of ginkgolic acids [36]. This standardized extract has been produced in collaboration with the French company Ipsen, which have commercialized the same product with the name Tnakan, known as Tebonin in Germany[36].

Specifically, Tebonin is a vegetal origin drug based on EGb761 active principle, belonging to the potentiation and cognitive drug category (Ginkgo biloba, ATC cod: NO6DX02, pharmacotherapeutic group) and authorized in many European States.

Tebonin indication is approved by EMA report and monograph, as a vegetal drug for the improvement of cognitive deterioration (linked to age) and life quality in the mild dementia. It is commercialized in Italy by Schwabe Pharma Italia S.r.l. - Dr. Willmar Schwabe GmbH& Co. group.

Moreover, EGb761® is available in two formulations: upholster pill (enveloped) and oral solution. Ginkgo biloba L. (*Salisburia adiantifolia Smith, Pterophyllus salisburiensis Nelson*; Family: *Ginkgoaceae*) dry leaves are used to satisfy the European pharmacopeia and specific pharmaceutical companies requisites.

Particularly, EGb761® is a light yellow - brown/yellow-orange bitter powder which has to satisfy all the requisites of European pharmacopeia actual monograph “Dry Ginkgo, purified and titled”. EGb761® contains between the 22-27% of flavonoid glycosides, the 2.8-3.4% of A, B and C ginkgolids, the 2.6-3.2% of bilobalide and contains less than 5 ppm of ginkgolic acids (Figure 3). The Ginkgo flavonoids (kaempferol, quercetin and isorhamnetin) and the terpene lactones (A, B, C ginkgolide diterpenes and the sesquiterpene bilobalide, all provided with three lactone rings) are active markers inside EGb761® ingredients with therapeutic characteristics (Figure 3).

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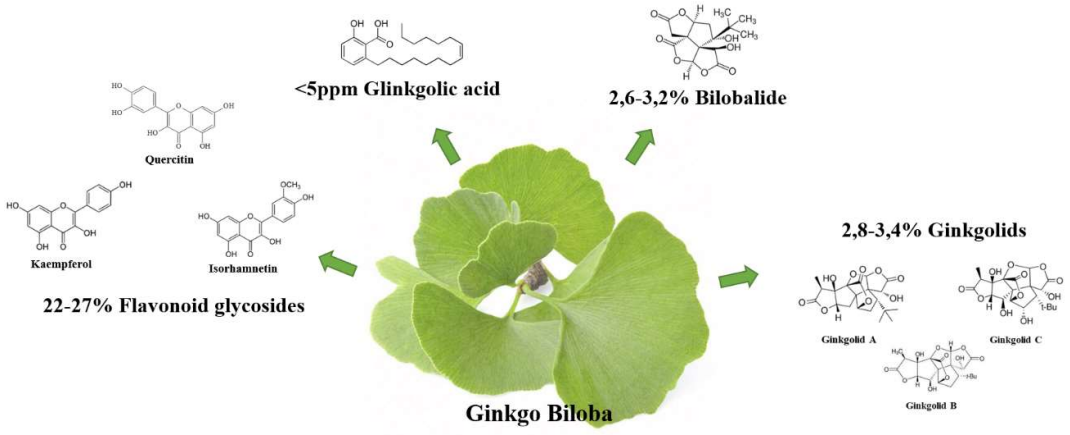

Figure 3: EGb761 active components.

At the first beginning, EGb761® has been used for peripheral and central vascular Binge Eating Disorder (BEDs) diseases [37]. Nowadays, EGb761® utilization has been abandoned in the peripheral vascular diseases, as well as Raynaud syndrome, the intermitted claudication and the peripheral arteriopathy, despite the beneficial evidences obtained in placebo-controlled trials [37,38]. Positive effects have been observed also for the cerebrovascular diseases, above all for vascular cognitive impairment.

Later, it has been used also for cognitive impairment associated with the aging, demonstrating beneficial effects for patients enrolled in different clinical trials, in line with the international diagnostic criteria modification but with several limitation about the most updated guidelines for Alzheimer disease drugs development [6,12,39,40].

In Germany, the *E commission* has approved the monograph which defines the use of Ginkgo Biloba undefined leaves preparation, for cerebral and arterial blood circulation, for the vertigo and the reinforcement of vascular system, (i.e. veins), for the stimulation of blood circulation (i.e. after psychotropic and neurotropic therapy). The specific monograph of the purified and titrated product, extracted by Ginkgo biloba leaf (DER 35-67:1), described its use in the symptomatic treatment of mental performances cerebral insufficiency, as a part of a general therapeutic strategy for dementia with the following principal symptoms: deficit of memory, concentration and mood disturbance, vertigo, tinnitus and migraine [41,42].

The main target group includes primary degenerative dementia and/or vascular dementia patients. Moreover, it has been used to improve the free from pain motor skills in patients with second stage of peripheral occluding arteriopathy, according to Fontaine classification (claudication intermittent).

The 2003 European Scientific Cooperative On Phytotherapy (ESCOP) monograph [43] describes the Ginkgo biloba leaf utilization for the symptomatic treatment of moderate and mild dementia,

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involving the degenerative primary dementia, the vascular dementia, the mixed forms and the cerebral impairment; for neurosensorial impediments as well as dizziness/vertigo and tinnitus; for cognitive performances improvement; for the symptomatic treatment of occlusive peripheral arteries disease (claudication intermittent).

The 2006 British Herbal Compendium [44] lists the following indications for Ginkgo biloba leaves: symptomatic treatment of moderate and mild dementia, including primary degenerative dementia, as Alzheimer, multi-infarct dementia and mixed forms; treatment of symptoms of cerebral vascular impairment and concentration/memory problems, confusion, deficit of energy and initiative, anxiety and depression; improvement of cognitive performances; neuro-sensorial disturbance as vertigo and visual dysfunction.

1.3.1. EGb761® in basic research: the mechanism of action

EGb761® Ginkgo Biloba pharmacological effect is based on 3 main neurobiological mechanisms: 1. increase of neurogenesis and synaptogenesis, 2. mitochondrial DNA oxidation prevention, followed by stabilization of mitochondrial membranes which slows down the aging, by improving the functions and 3. neuro-transmission improvement.

The different extract active components act simultaneously, in the cerebral contest with different mechanisms in order to enhance beneficial effects on the cognitive functions and improve neuro-protection. Several preclinical experimental studies showed that EGb761® has antioxidant proprieties due to its flavonoids [45-47]. These components neuro-protect against several neurotoxic agents and in a specific manner against the neurotoxicity from amyloid beta oligomers [41]. The antioxidant action can be explicated by a direct reactive oxygen species (ROS) scavenging or through the modulation of specific mechanisms of signaling and transcription factors, able to stimulate the cellular repairer system and to amplify the endogenous antioxidant defenses [42,48,49].

A peculiar EGb761® activity is to improve the mitochondrial dysfunction during the aging and in cerebral cognitive impairment [47,50-54]. This mechanism underlines EGb761® neuroprotection activity. Thus, several studies demonstrated that functional mitochondrial homeostasis plays a critical role in the neuro degeneration and the cognitive deterioration associated with dementia [42,55].

EGb761® reduces mitochondrial ROS production and protects mitochondria and mitochondrial complexes of the respiratory chain from ROS, it increases the energetic metabolism and the ATP availability [47,51,56-59].

Preclinical studies demonstrated that EGb761® has a positive role also in neuroinflammation [60,61], including a specific inhibitory action of inflammatory molecules transcription factors at neuronal level and in the microglia [62]. Moreover EGb761® can inhibit the aggregation and production of amyloid beta [60,63-71]. Recently it has been observed that EGb761®, and in particular its A ginkgolid (but not B and C ginkgolid), bilobial and flavonoid components, enhanced autophagic activity and degradation of phosphorylated TAU protein in lysosomes and neuron [72]. Moreover, EGb761® stimulated the dopaminergic and noradrenergic neurotransmission [73,74], by improving cognitive functions in the elder [75].

Cellular and animal studies demonstrated that EGb761® helps also synaptic functions and neuronal plasticity, including the neuritogenesis, the spinal cord density, the long-term potentiation,

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and the neurogenesis. These effects are stronger in experimental model of hypoglycemia, hypoxia, amyloid beta exposition, oxidative stress [52,76,77].

1.3.2. EGb761® in preclinical and clinical dementia: evidence of efficacy

Although it is already used in the treatment of neuro-cognitive disorders, sometimes with successful results, for several years, the expert evaluation about EGb761® has been very prudent or negative because of the contradictory results of clinical studies, due to the heterogeneity of enrolled patients, the imprecise methods and drug dosages. Moreover, there has always been a negative consideration of herbal medications by medical doctors. In the last years, scientific reviews and meta-analysis studies results have been pivotal in the achievement of EGb761® as a drug for dementia, as underlined in the guidelines of cognitive disorders treatments.

As for other dementia disease modifying treatments, clinical trials about Tebonin have criticized that clinical evaluation have been done with uneven neuro-psychological methods, despite the scientific community guide lines. Moreover, Alzheimer diagnosis was wrong between 20-30% of the cases due to the lack of evaluation of amyloid beta and neurofibrillary tangles accumulation (trough radio ligands PET or lumbar puncture).

Likewise, preclinical studies of Mild Cognitive Impairment haven't used specific biomarkers.

1.3.3. EGb761® in vMCI and MCI early treatment and neurocognitive disorders and dementia prevention

Prospective observational studies, such as the clinical study conducted by Amieva et al., 2013 [78] which involved 3612 patients, aged over 65, from the South of France, highlighted a slower cognitive impairment in the EGb761® group of patients than in Piracetam ones. This difference has been confirmed also through a multiple-choice visual memory test (Benton Visual Retention Test (BVRT)) and language skills test (Isaacs Set Test (IST)). Moreover, EGb761® patients showed a significant reduction of psychotropic drugs assumption, considering that, in dementia experimental clinical trials, ADAS-Cog is the more used formulation (Table 1).

Experimental clinical studies focusing on subjective memory disorders and neuro-cognitive impairment and dementia prevention, conducted by Grass-Kapanke et al., in 2011 [79], observed EGb 761® positive effects with very mild cognitive impairment (vMCI). In this study, 300 vMCI patients, aged 45 to 65 years, received EGb761® (240mg/die) or placebo, for 12 weeks. These patients showed an improvement of memory performances, measured through Wechsler Memory Scale III (human face recognition in pictures) and a significant attention improvement, by using the Vienna Test System Work Performance Series (a computerized math test to keep concentration) (Table 1).

Differently, a randomized, double blind, placebo controlled, multicentric study (GuidAge), including French people with subjective memory deficit and enrolled for primary clinical care for Alzheimer evaluation, prospective followed for more than 5 years [80], showed that EGb761® treatment couldn't prevent dementia insurgence.

However, a post-hoc analysis of a subgroup of people on going EGb761® for at least 4 years, showed a significant reduction of dementia development (50% more than placebo group).

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This long-term effect made scientists doubt and confirm the failure of the statistical models used [81]. Nowadays, it is not possible to evaluate if EGb761® could potentially prevent dementia yet.

1.4. MCI, neurocognitive deficit and dementia

Gavrilova and colleagues, in 2014 [82], evaluated EGb761® (240mg/die) versus placebo for 24 weeks in 160 patients with amnesic MCI (age> 55 years old). All the neuropsychiatric symptoms, measured through Neuro psychiatrics Inventory score (NPI), improved in patients treated with EGb761®. They reached a better diagnosis also for anxiety, depression and visual-motor and cognitive aspect (Table 1).

In addition, in another study, named the Gingko Evaluation of Memory (GEM - USA), involving 3069 patients with normal cognitive function and MCI (age ≥ 75), EGb761 (240mg/die) versus placebo has been utilized [83].

Unfortunately, GEM didn't demonstrate any significant benefit to prevent dementia development with EGb761® treatment versus placebo, because of the lack of information about mechanisms involved in the pathology and the low adhesion at the trial (about 60% with 40% of dropout) (Table 1).

1.4.1. EGb761® efficacy in mild and moderate dementia with or without neuropsychiatric disorders

The older controlled clinical trials using EGb761® versus placebo, have demonstrated a moderate cognitive functions and activities of daily living (ADL) improvement, as consequence of patient's heterogeneity, the EGb761® dosage and its effect.

In the last 10 years, randomized and controlled trials and sub sequential meta-analysis, considering people affected by AD and MCI associated with neuro-psychiatric symptoms (NPS), have renewed interest on EGb761® benefits at the dosage of 240 mg/die.

In particular, neuro-psychiatric symptoms, known as "behavioral and psychological dementia symptoms (BPSD)" represent a various not-cognitive symptoms groups and behavioral attitude of dementia patients, which dramatically increase during the development of pathology, compromising also "caregivers" life quality. In the last years, EGb761® has been largely studies in BPSD; in addition to neuro psychiatric symptoms improvement, also the recovery of behavioral ability enhancing has been detected. Indeed, three EGb761® studies [83-85] have demonstrated neuro psychiatric improvement for Neuropsychiatric Inventory (NPI) symptoms not only for Alzheimer but also on vascular dementia and mixed forms. AD and Vascular Dementia (VaD) have been included in these studies, where the Caregiver Distress Score was improved in patients on going EGb761®, by showing a stress reduction for patient's relatives (Table 1).

It has been also studied EGb761® in BPSP in MCI, as in Neuropsychiatric Inventory (NPI) [82]. These results underline a possible EGb761® efficacy for dementia, correlated with aging and neuro-psychiatric symptoms.

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Table 1: Clinical studies on EGb761 in patients with MCI, vMCI, neurocognitive deficit and dementia.

Inclusion criteria	Treatment groups	Results	References
EGb761® in vMCI and MCI early treatment and neurocognitive disorders and dementia prevention			
Non-demented patients	*EGb761® or Piracetam and placebo, data collected on cognitive function over a period of twenty years*	Patients treated with EGb761® highlighted a slower cognitive impairment than in Piracetam group. Moreover, EGb761® patients showed a significant reduction of psychotropic drugs assumption.	**[78]**
Patients with very mild cognitive impairment and low functioning	*EGb761® (240mg/die) or placebo, for 12 weeks*	Patients treated with EGb761® showed an improvement of memory performances, measured through Wechsler Memory Scale III (human face recognition in pictures) and a significant attention improvement, by using the Vienna Test System Work Performance Series (a computerized math test to keep concentration).	**[79]**
EGb761® MCI, neurocognitive deficit and dementia			
Patients with amnesic MCI	*EGb761® (240mg/die) or placebo, for 24 weeks*	Patients treated with EGb761 showed improvement in all the neuropsychiatric symptoms, measured through Neuro psychiatrics Inventory score (NPI).	**[82]**
Patients with normal cognitive function or MCI	*EGb761® (240mg/die) or placebo, patients were evaluated every 6 months*	This study didn't demonstrate any significant benefit to prevent dementia development with EGb761® treatment versus placebo.	**[83]**

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Outpatients with mild to moderate dementia	*EGb761® (240mg/die) or placebo, for 24 weeks*	Patients treated with EGb761® demonstrated neuro psychiatric improvement for Neuropsychiatric Inventory (NPI) symptoms.	**[84]**
Outpatients 24-week with mild to moderate dementia (Alzheimer's disease or vascular dementia) associated with neuropsychiatric symptoms	*EGb761®(240mg/die) or placebo, for 24 weeks*	Treatment with EGb761® led to a significant and clinically relevant improvement in patients' cognition, psychopathology, functional measures and quality of life.	**[85]**

1.4.2. EGb761® efficacy and combined (AChEIs and EGb761® association) or compared (AChEIs versus EGb761®) therapy in mild or moderate dementia.

EGb761® in association with anti-dementia drugs studies have been performed. The first result derived from GINDON study [86], which analyzed the potential benefit of a combined therapy with EGb761® and donepezil, after 22 weeks in 96 Alzheimer patients with neuro psychiatric disorders (figure 4).

The results of this study have showed a moderate but not significant benefit at the EGb761® 240 mg/die + donepezil 5/10 mg/die dosage, in monotherapy, versus the single treatment with EGb761® 240 mg/die or the single treatment with donepezil 5/10 mg/die, considering cognitive, physic and functional outcomes and neuro psychiatric disorders (Figure 4).

Further evidences about EGb761® and AChEIs combination cognitive performances benefits derive from a prospective ICTUS study, involving 828 patients with mild and moderate Alzheimer, for 1 year [14]. These patients were treated with donepezil (55%), rivastigmin (27%) or galantamin (18%) with or without co-administration of EGb761® (120 mg/die). After 12 months, patients ongoing also EGb761® showed better results of Mini Mental State Examination (MMSE) than patients using only AChEIs (+1.9 points on MMSE, p=0.005).

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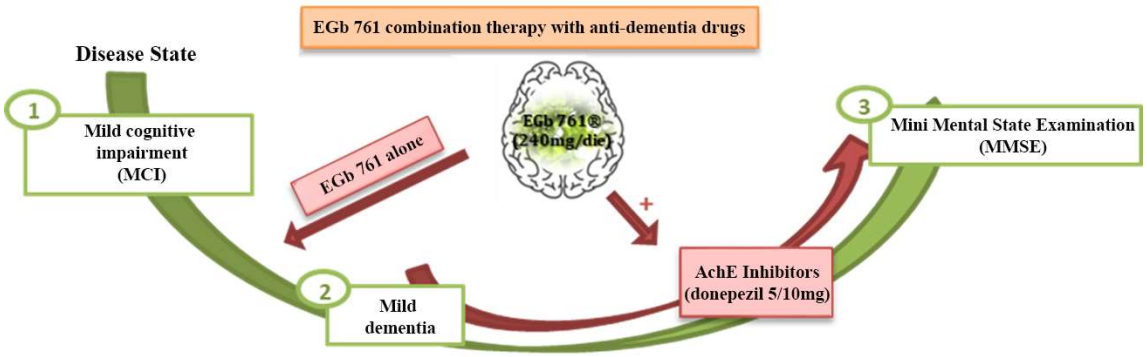

Figure 4: EGb761 in association with anti-dementia drugs.

1.4.3. EGb761® efficacy verified through RCTs meta-analysis in MCI, mild and moderate dementia with or without behavior deficits.

In the last 10 years, meta-analysis and RCTs results on patients affected by dementia, associated with psycho-behavioral deficits, have led to a new interest for EGb761®. Tan and colleagues [39] estimated EGb761® effect on 2561 patients with cognitive deficit and dementia (9 RCTs) after 22-26 weeks (Table 2).

The results of this work demonstrated EGb761® benefits on cognitive decline stabilization or slowing down, on ADL and neuro psychiatric deficit for MCI, Alzheimer and dementia (with/without neuro psychiatric problems) patients. Other analysis in the same work, showed differences in the efficacy of EGb761® different dosages, underling the best treatment with 240 mg/die. Tan and colleagues, 2015, [39], as in the case of Cochrane meta-analysis [87,88], considered only randomized studies of patients with cognitive function compromised or dementia, with the same EGb761® dosage and similar follow up timing, with the only difference in the selection of the best trials (better dementia diagnosis). Moreover, Tan and coworkers identified and included three RCTs published in that period [82,84,85] and showing an EGb761® benefit at the dosage of 240 mg/die, for dementia, AD and MCI associated with NPS. After that, a Gauthier and Schlaefke, 2014, [89] meta-analysis confirmed EGb761® efficacy and tolerability in patients with dementia (Table 2).

This is a well-designed meta-analysis, involving good quality placebo-controlled studies with at least 200 randomized patients. EGb761® efficacy has been demonstrated on cognitive functions ($P=0.03$), ADL ($P<0.001$) and on the clinical impression scales ($P=0.01$).

The best result in term of EGb761® best dosage on cognitive functions has been obtained with 240 mg/die, in studies including significant neuro psychiatric deficits patients. In the 7 selected studies of this meta-analysis, 4 of them enrolled only patients with significative neuro psychiatric symptoms, 2 of them patients with neuro psychiatric symptoms, 1 study excluded patients with significant neuro psychiatric deficits.

A 2015 meta-analysis [90], demonstrated EGb761® benefits versus placebo for cognitive deficits, through Syndrom-Kurztest [SKT] analysis, in AD and AD+VaD patients (Table 2).

Another meta-analysis on patient with dementia and neuropsychiatric deficits [91] showed that 240 mg/die of EGb761® significantly improved the cognitive functions, NPS, caregiver stress

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associated with NPS, ADL and clinical impression, versus placebo in AD, VaD and AD+ Cardio Vascular Disease (CVD) ($p<0.001$ for all the analysis) out patients.

A 2016 meta-analysis [92] showed an important activity of Ginkgo extract in improving cognition, behavior and activities of daily living in MCI and dementia. One of the most important outcomes was that the efficacy was dose-dependent and only convincing with a daily dose of 240 mg. Its utilization was safe and the adverse events (AEs) were at placebo level. In the sub-group of Alzheimer patients, less AEs arose compared to placebo. Cases of dizziness, Angina pectoris and headache were also less frequent in the active substance group.

Zhang 2016's publication [92] is the result of an analysis of ten systematic reviews and meta-analyses, in which the efficacy of Ginkgo special extract for cognitive disorders was assessed (Table 2).

At the end, in a recent meta-analysis of Savaskan and colleagues, in 2018 [93], it has been demonstrated that EGb761® versus placebo is more efficient for neuro psychiatric symptoms, measured through NPI in AD, VaD or AD+CVD patients, except for delirium, hallucination and euphoria (Table 2).

Moreover, the basal EGb761® assumption reduced also the risk of new neuro psychiatric symptoms onset, as the caregiver stress.

Table 2: Meta-analysis studies on the efficacy of EGb761® in patients with MCI, mild and moderate dementia with or without behavioral deficits.

Meta-Analysis Studies (Inclusion criteria)	Treatment groups	Results	References
Patients affected by dementia, associated with psycho-behavioral deficits	*EGb761® (240mg/die) or placebo*	Patients treated with EGb761 showed benefits on cognitive decline stabilization or slowing down, on ADL and neuro psychiatric deficit for MCI, Alzheimer and dementia (with/without neuro psychiatric problems) patients.	**[39]**
Patients with a diagnosis of AD, VaD, or mixed dementia	*EGb761® (120mg/die) or EGb761® (240mg/die) or placebo*	EGb761® (240 mg/die; best dosage) has been demonstrated efficacy on cognitive functions, including significant neuro psychiatric deficits in patients with dementia.	**[89]**
Patients with AD or AD+VaD patients	*EGb761® (120mg/die) orEGb761® (240mg/die) or placebo*	Patients treated with EGb761® (240mg/die) showed benefits versus placebo for cognitive deficits, through Syndrom-Kurztest [SKT] analysis.	**[90]**

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Patients with the diagnosis of AD, VaD or mixed dementia with behavioral and psychological symptoms (BPSD)	*EGb761® (240mg/die) or placebo*	EGb 761® ginkgo biloba extract (240 mg / day) improved the patients' cognitive performance, BPSD, functional abilities and general condition.	**[91]**
Patients with MCI and dementia (AD,VaD or AD+VaD, mixed dementia)	*EGb761® (240mg/die) or placebo*	EGb761® showed benefit versus placebo for cognition, behavior and activities of daily living in patients with MCI and dementia.	**[92]**
Patients with dementia (probable AD, VaD or AD +CVD)	*EGb761® (240mg/die) or placebo*	EGb761® improved neuro-psychiatric symptoms, in AD, VaD or AD+CVD patients, except for delirium, hallucination and euphoria.	**[93]**

2. Discussion

MCI and mild dementia are important diseases affecting our society and, nowadays, no drug treatment for MCI has been approved [94]. Specifically, MCI represents an intriguing clinical entity and, until now, the only clinically diagnosed pre-dementia stage.

This review describes the efficacy of the Ginkgo Biloba leaves extract in the treatment of dementia syndrome with the following main symptoms: memory deficit, concentration disorder, mood disorders, dizziness, tinnitus and headache.

The main target group includes patients with dementia syndrome, presenting primary degenerative dementia, vascular dementia or a combination of both the forms [27].

Data from clinical trials showed positive effects of EGb761® in people with very mild (vMCI), mild cognitive impairment (MCI) or mild dementia [78]. EGb761® treatment has also been studied in patients with psychological and behavioral symptoms of dementia" (BPSD), highlighting an improvement in neuro psychiatric symptoms as well as a clear recovery in behavioral abilities. There is also further evidence of the benefit of the combination of EGb761® with AChEIs in cognitive performance [14,86]. The exact mechanism of action is still unclear. Human pharmacological data show increased potency of EEG in older subjects, a reduction in blood viscosity and improved brain perfusion in specific areas in healthy male subjects (60-70 years) and a reduction in platelet aggregation.

In addition, more evidences are needed to assess the role of EGb761® in combination therapy with AChEIs or memantine or triple, EGb761®, AChEIs and memantine together.

These compounds combination, in the future, may have a role in the therapy and in the drug management of dementia, improving patient rehabilitation, too. Importantly, EGb761® does not appear to increase the hemorrhagic risk [95,96], neither interacting with anti-aggregators or with anticoagulants [97].

Additional data are also needed to face a hemorrhagic risk evaluation in some sub-groups of patients, with high risk of bleeding such as those with gastrointestinal bleeding or anemic for moderate-to-severe kidney failure that are usually excluded from clinical trials.

Finally, future studies will need neuroimaging criteria to better understand the mechanism of action of EGb761®. Neuroimaging utilization will also allow the evaluation of the brain hemorrhage risk among patients with cognitive impairment resulting from micro-bleeding.

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References

1. Jongsiriyanyong, S.; Limpawattana, P. Mild Cognitive Impairment in Clinical Practice: A Review Article. *Am J Alzheimers Dis Other Demen* **2018**, *33*, 500-507, doi:10.1177/1533317518791401.
2. Bondi, M.W.; Edmonds, E.C.; Salmon, D.P. Alzheimer's Disease: Past, Present, and Future. *J Int Neuropsychol Soc* **2017**, *23*, 818-831, doi:10.1017/S135561771700100X.
3. Ma, L. Depression, Anxiety, and Apathy in Mild Cognitive Impairment: Current Perspectives. *Front Aging Neurosci* **2020**, *12*, 9, doi:10.3389/fnagi.2020.00009.
4. Vlachos, G.S.; Scarmeas, N. Dietary interventions in mild cognitive impairment and dementia. *Dialogues Clin Neurosci* **2019**, *21*, 69-82.
5. McGrattan, A.M.; McEvoy, C.T.; McGuinness, B.; McKinley, M.C.; Woodside, J.V. Effect of dietary interventions in mild cognitive impairment: a systematic review. *Br J Nutr* **2018**, *120*, 1388-1405, doi:10.1017/S0007114518002945.
6. Olivera-Pueyo, J.; Pelegrin-Valero, C. Dietary supplements for cognitive impairment. *Actas Esp Psiquiatr* **2017**, *45*, 37-47.
7. Ilari, S.; Giancotti, L.A.; Lauro, F.; Dagostino, C.; Gliozzi, M.; Malafoglia, V.; Sansone, L.; Palma, E.; Tafani, M.; Russo, M.A., et al. Antioxidant modulation of sirtuin 3 during acute inflammatory pain: The ROS control. *Pharmacol Res* **2020**, *157*, 104851, doi:10.1016/j.phrs.2020.104851.
8. Lauro, F.; Giancotti, L.A.; Ilari, S.; Dagostino, C.; Gliozzi, M.; Morabito, C.; Malafoglia, V.; Raffaeli, W.; Muraca, M.; Goffredo, B.M., et al. Inhibition of Spinal Oxidative Stress by Bergamot Polyphenolic Fraction Attenuates the Development of Morphine Induced Tolerance and Hyperalgesia in Mice. *PLoS One* **2016**, *11*, e0156039, doi:10.1371/journal.pone.0156039.
9. Lauro, F.; Ilari, S.; Giancotti, L.A.; Ventura, C.A.; Morabito, C.; Gliozzi, M.; Malafoglia, V.; Palma, E.; Paolino, D.; Mollace, V., et al. Pharmacological effect of a new idebenone formulation in a model of carrageenan-induced inflammatory pain. *Pharmacol Res* **2016**, *111*, 767-773, doi:10.1016/j.phrs.2016.07.043.
10. Ilari, S.; Giancotti, L.A.; Lauro, F.; Gliozzi, M.; Malafoglia, V.; Palma, E.; Tafani, M.; Russo, M.A.; Tomino, C.; Fini, M., et al. Natural Antioxidant Control of Neuropathic Pain-Exploring the Role of Mitochondrial SIRT3 Pathway. *Antioxidants (Basel)* **2020**, *9*, doi:10.3390/antiox9111103.
11. Liu, H.; Ye, M.; Guo, H. An Updated Review of Randomized Clinical Trials Testing the Improvement of Cognitive Function of Ginkgo biloba Extract in Healthy People and Alzheimer's Patients. *Front Pharmacol* **2019**, *10*, 1688, doi:10.3389/fphar.2019.01688.
12. Bonassi, S.; Prinzi, G.; Lamonaca, P.; Russo, P.; Paximadas, I.; Rasoni, G.; Rossi, R.; Ruggi, M.; Malandrino, S.; Sanchez-Flores, M., et al. Clinical and genomic safety of treatment with Ginkgo biloba L. leaf extract (IDN 5933/Ginkgoselect(R)Plus) in elderly: a randomised placebo-controlled clinical trial [GiBiEx]. *BMC Complement Altern Med* **2018**, *18*, 22, doi:10.1186/s12906-018-2080-5.

13. Singh, S.K.; Srivastav, S.; Castellani, R.J.; Plascencia-Villa, G.; Perry, G. Neuroprotective and Antioxidant Effect of Ginkgo biloba Extract Against AD and Other Neurological Disorders. *Neurotherapeutics* **2019**, *16*, 666-674, doi:10.1007/s13311-019-00767-8.
14. Canevelli, M.; Adali, N.; Kelaiditi, E.; Cantet, C.; Ousset, P.J.; Cesari, M.; Group, I.D. Effects of Gingko biloba supplementation in Alzheimer's disease patients receiving cholinesterase inhibitors: data from the ICTUS study. *Phytomedicine* **2014**, *21*, 888-892, doi:10.1016/j.phymed.2014.01.003.
15. D'Amelio, M.; Rossini, P.M. Brain excitability and connectivity of neuronal assemblies in Alzheimer's disease: from animal models to human findings. *Prog Neurobiol* **2012**, *99*, 42-60, doi:10.1016/j.pneurobio.2012.07.001.
16. Fernandez-Perez, E.J.; Gallegos, S.; Armijo-Weingart, L.; Araya, A.; Riffo-Lepe, N.O.; Cayuman, F.; Aguayo, L.G. Changes in neuronal excitability and synaptic transmission in nucleus accumbens in a transgenic Alzheimer's disease mouse model. *Sci Rep* **2020**, *10*, 19606, doi:10.1038/s41598-020-76456-w.
17. Dugger, B.N.; Dickson, D.W. Pathology of Neurodegenerative Diseases. *Cold Spring Harb Perspect Biol* **2017**, *9*, doi:10.1101/cshperspect.a028035.
18. Matej, R.; Tesar, A.; Rusina, R. Alzheimer's disease and other neurodegenerative dementias in comorbidity: A clinical and neuropathological overview. *Clin Biochem* **2019**, *73*, 26-31, doi:10.1016/j.clinbiochem.2019.08.005.
19. Rossini, P.M.; Di Iorio, R.; Granata, G.; Miraglia, F.; Vecchio, F. From Mild Cognitive Impairment to Alzheimer's Disease: A New Perspective in the "Land" of Human Brain Reactivity and Connectivity. *J Alzheimers Dis* **2016**, *53*, 1389-1393, doi:10.3233/JAD-160482.
20. Costa, A.; Bak, T.; Caffarra, P.; Caltagirone, C.; Ceccaldi, M.; Collette, F.; Crutch, S.; Della Sala, S.; Demonet, J.F.; Dubois, B., et al. The need for harmonisation and innovation of neuropsychological assessment in neurodegenerative dementias in Europe: consensus document of the Joint Program for Neurodegenerative Diseases Working Group. *Alzheimers Res Ther* **2017**, *9*, 27, doi:10.1186/s13195-017-0254-x.
21. Lam, C.L.M.; Yiend, J.; Lee, T.M.C. Imaging and neuropsychological correlates of white matter lesions in different subtypes of Mild Cognitive Impairment: A systematic review. *NeuroRehabilitation* **2017**, *41*, 189-204, doi:10.3233/NRE-171471.
22. Guan, H.; Liu, T.; Jiang, J.; Tao, D.; Zhang, J.; Niu, H.; Zhu, W.; Wang, Y.; Cheng, J.; Kochan, N.A., et al. Classifying MCI Subtypes in Community-Dwelling Elderly Using Cross-Sectional and Longitudinal MRI-Based Biomarkers. *Front Aging Neurosci* **2017**, *9*, 309, doi:10.3389/fnagi.2017.00309.
23. Yokoi, Y.; Takano, H.; Sakata, M.; Maruo, K.; Nakagome, K.; Matsuda, H. Discrete effect of each mild behavioural impairment category on dementia conversion or cognitive decline in patients with mild cognitive impairment. *Psychogeriatrics* **2019**, *19*, 591-600, doi:10.1111/psyg.12447.
24. Wahlman, C.; Doyle, T.M.; Little, J.W.; Luongo, L.; Janes, K.; Chen, Z.; Esposito, E.; Tosh, D.K.; Cuzzocrea, S.; Jacobson, K.A., et al. Chemotherapy-induced pain is promoted by enhanced spinal adenosine kinase levels through astrocyte-dependent mechanisms. *Pain* **2018**, *159*, 1025-1034, doi:10.1097/j.pain.0000000000001177.
25. Langa, K.M.; Levine, D.A. The diagnosis and management of mild cognitive impairment: a clinical review. *JAMA* **2014**, *312*, 2551-2561, doi:10.1001/jama.2014.13806.
26. Petersen, R.C. Mild cognitive impairment as a diagnostic entity. *J Intern Med* **2004**, *256*, 183-194, doi:10.1111/j.1365-2796.2004.01388.x.

27. Petersen, R.C. Mild Cognitive Impairment. *Continuum (Minneap Minn)* **2016**, *22*, 404-418, doi:10.1212/CON.0000000000000313.
28. Morris, J.C.; Price, J.L. Pathologic correlates of nondemented aging, mild cognitive impairment, and early-stage Alzheimer's disease. *J Mol Neurosci* **2001**, *17*, 101-118, doi:10.1385/jmn:17:2:101.
29. Sanford, A.M. Mild Cognitive Impairment. *Clin Geriatr Med* **2017**, *33*, 325-337, doi:10.1016/j.cger.2017.02.005.
30. Khachaturian, Z. The five-five, ten-ten plan for Alzheimer's disease. *Neurobiol Aging* **1992**, *13*, 197-198; discussion 199, doi:10.1016/0197-4580(92)90030-2.
31. Karakaya, T.; Fusser, F.; Schroder, J.; Pantel, J. Pharmacological Treatment of Mild Cognitive Impairment as a Prodromal Syndrome of Alzheimer s Disease. *Curr Neuropharmacol* **2013**, *11*, 102-108, doi:10.2174/157015913804999487.
32. Vanacore, N.; Bianchi, C.; Da Cas, R.; Rossi, M. Use of antiparkinsonian drugs in the Umbria Region. *Neurol Sci* **2003**, *24*, 221-222, doi:10.1007/s10072-003-0140-0.
33. Ibach, B.; Haen, E. Acetylcholinesterase inhibition in Alzheimer's Disease. *Curr Pharm Des* **2004**, *10*, 231-251, doi:10.2174/1381612043386509.
34. Ilhan Algin, D.; Dagli Atalay, S.; Ozkan, S.; Ozbabalik Adapinar, D.; Ak Sivrioz, I. Memantine improves semantic memory in patients with amnestic mild cognitive impairment: A single-photon emission computed tomography study. *J Int Med Res* **2017**, *45*, 2053-2064, doi:10.1177/0300060517715166.
35. Dominguez, L.J.; Barbagallo, M. Nutritional prevention of cognitive decline and dementia. *Acta Biomed* **2018**, *89*, 276-290, doi:10.23750/abm.v89i2.7401.
36. Caesar, W. [Of Ginkgo EGb on GBL - a long path to rational phytopharmacy]. *Pharm Unserer Zeit* **2009**, *38*, 400-405, doi:10.1002/pauz.200900327.
37. DeFeudis, F.V. *Ginkgo Biloba Extract (EGb 761): From Chemistry to the Clinic*; Ullstein Medical: 1998.
38. Gardner, C.D.; Taylor-Piliae, R.E.; Kiazand, A.; Nicholus, J.; Rigby, A.J.; Farquhar, J.W. Effect of Ginkgo biloba (EGb 761) on treadmill walking time among adults with peripheral artery disease: a randomized clinical trial. *J Cardiopulm Rehabil Prev* **2008**, *28*, 258-265, doi:10.1097/01.HCR.0000327184.51992.b8.
39. Tan, M.S.; Yu, J.T.; Tan, C.C.; Wang, H.F.; Meng, X.F.; Wang, C.; Jiang, T.; Zhu, X.C.; Tan, L. Efficacy and adverse effects of ginkgo biloba for cognitive impairment and dementia: a systematic review and meta-analysis. *J Alzheimers Dis* **2015**, *43*, 589-603, doi:10.3233/JAD-140837.
40. Cave, A.E.; Chang, D.H.; Munch, G.W.; Steiner, G.Z. Efficacy of Cognition Support Formula(R) on cognitive function in older adults with subjective cognitive impairment: a protocol for a 26-week, randomised, double-blind, placebo-controlled trial. *Trials* **2019**, *20*, 345, doi:10.1186/s13063-019-3431-3.
41. Liu, L.; Zhang, C.; Kalionis, B.; Wan, W.; Murthi, P.; Chen, C.; Li, Y.; Xia, S. EGb761 protects against Abeta1-42 oligomer-induced cell damage via endoplasmic reticulum stress activation and Hsp70 protein expression increase in SH-SY5Y cells. *Exp Gerontol* **2016**, *75*, 56-63, doi:10.1016/j.exger.2016.01.003.
42. Smith, J.V.; Luo, Y. Elevation of oxidative free radicals in Alzheimer's disease models can be attenuated by Ginkgo biloba extract EGb 761. *J Alzheimers Dis* **2003**, *5*, 287-300, doi:10.3233/jad-2003-5404.
43. Escop; Phytotherapy, E.S.C.o. *ESCOP Monographs: The Scientific Foundation for Herbal Medicinal Products*; European Scientific Cooperative on Phytotherapy: 2003.
44. Bone, K.; Mills, S. *Principles and Practice of Phytotherapy - E-Book: Modern Herbal Medicine*; Elsevier Health Sciences: 2013.

45. Haramaki, N.; Aggarwal, S.; Kawabata, T.; Droy-Lefaix, M.T.; Packer, L. Effects of natural antioxidant ginkgo biloba extract (EGB 761) on myocardial ischemia-reperfusion injury. *Free Radic Biol Med* **1994**, *16*, 789-794, doi:10.1016/0891-5849(94)90194-5.

46. Bridi, R.; Crossetti, F.P.; Steffen, V.M.; Henriques, A.T. The antioxidant activity of standardized extract of Ginkgo biloba (EGb 761) in rats. *Phytother Res* **2001**, *15*, 449-451, doi:10.1002/ptr.814.

47. Eckert, A.; Keil, U.; Kressmann, S.; Schindowski, K.; Leutner, S.; Leutz, S.; Muller, W.E. Effects of EGb 761 Ginkgo biloba extract on mitochondrial function and oxidative stress. *Pharmacopsychiatry* **2003**, *36 Suppl 1*, S15-23, doi:10.1055/s-2003-40449.

48. Jiang, X.; Nie, B.; Fu, S.; Hu, J.; Yin, L.; Lin, L.; Wang, X.; Lu, P.; Xu, X.M. EGb761 protects hydrogen peroxide-induced death of spinal cord neurons through inhibition of intracellular ROS production and modulation of apoptotic regulating genes. *J Mol Neurosci* **2009**, *38*, 103-113, doi:10.1007/s12031-008-9140-0.

49. Martin, R.; Mozet, C.; Martin, H.; Welt, K.; Engel, C.; Fitzl, G. The effect of Ginkgo biloba extract (EGb 761) on parameters of oxidative stress in different regions of aging rat brains after acute hypoxia. *Aging Clin Exp Res* **2011**, *23*, 255-263, doi:10.3275/7229

10.1007/BF03337752.

50. Sastre, J.; Millan, A.; Garcia de la Asuncion, J.; Pla, R.; Juan, G.; Pallardo; O'Connor, E.; Martin, J.A.; Droy-Lefaix, M.T.; Vina, J. A Ginkgo biloba extract (EGb 761) prevents mitochondrial aging by protecting against oxidative stress. *Free Radic Biol Med* **1998**, *24*, 298-304, doi:10.1016/s0891-5849(97)00228-1.

51. Abdel-Kader, R.; Hauptmann, S.; Keil, U.; Scherping, I.; Leuner, K.; Eckert, A.; Muller, W.E. Stabilization of mitochondrial function by Ginkgo biloba extract (EGb 761). *Pharmacol Res* **2007**, *56*, 493-502, doi:10.1016/j.phrs.2007.09.011.

52. Muller, W.E.; Heiser, J.; Leuner, K. Effects of the standardized Ginkgo biloba extract EGb 761(R) on neuroplasticity. *Int Psychogeriatr* **2012**, *24 Suppl 1*, S21-24, doi:10.1017/S1041610212000592.

53. Kumar, A.; Singh, A. A review on mitochondrial restorative mechanism of antioxidants in Alzheimer's disease and other neurological conditions. *Front Pharmacol* **2015**, *6*, 206, doi:10.3389/fphar.2015.00206.

54. Leuner, K.; Hauptmann, S.; Abdel-Kader, R.; Scherping, I.; Keil, U.; Strosznajder, J.B.; Eckert, A.; Muller, W.E. Mitochondrial dysfunction: the first domino in brain aging and Alzheimer's disease? *Antioxid Redox Signal* **2007**, *9*, 1659-1675, doi:10.1089/ars.2007.1763.

55. Muller, W.E.; Eckert, A.; Eckert, G.P.; Fink, H.; Friedland, K.; Gauthier, S.; Hoerr, R.; Ihl, R.; Kasper, S.; Moller, H.J. Therapeutic efficacy of the Ginkgo special extract EGb761((R)) within the framework of the mitochondrial cascade hypothesis of Alzheimer's disease. *World J Biol Psychiatry* **2019**, *20*, 173-189, doi:10.1080/15622975.2017.1308552.

56. Janssens, D.; Michiels, C.; Delaive, E.; Eliaers, F.; Drieu, K.; Remacle, J. Protection of hypoxia-induced ATP decrease in endothelial cells by ginkgo biloba extract and bilobalide. *Biochem Pharmacol* **1995**, *50*, 991-999, doi:10.1016/0006-2952(95)00227-q.

57. Eckert, A.; Keil, U.; Scherping, I.; Hauptmann, S.; Muller, W.E. Stabilization of mitochondrial membrane potential and improvement of neuronal energy metabolism by Ginkgo biloba extract EGb 761. *Ann N Y Acad Sci* **2005**, *1056*, 474-485, doi:10.1196/annals.1352.023.

58. Rhein, V.; Giese, M.; Baysang, G.; Meier, F.; Rao, S.; Schulz, K.L.; Hamburger, M.; Eckert, A. Ginkgo biloba extract ameliorates oxidative phosphorylation performance and rescues abeta-induced failure. *PLoS One* **2010**, *5*, e12359, doi:10.1371/journal.pone.0012359.

59. Baliutyte, G.; Trumbeckaite, S.; Baniene, R.; Borutaite, V.; Toleikis, A. Effects of standardized extract of Ginkgo biloba leaves EGb761 on mitochondrial functions: mechanism(s) of action and dependence on the source of mitochondria and respiratory substrate. *J Bioenerg Biomembr* **2014**, *46*, 493-501, doi:10.1007/s10863-014-9590-8.
60. Liu, X.; Hao, W.; Qin, Y.; Decker, Y.; Wang, X.; Burkart, M.; Schotz, K.; Menger, M.D.; Fassbender, K.; Liu, Y. Long-term treatment with Ginkgo biloba extract EGb 761 improves symptoms and pathology in a transgenic mouse model of Alzheimer's disease. *Brain Behav Immun* **2015**, *46*, 121-131, doi:10.1016/j.bbi.2015.01.011.
61. Wan, W.; Zhang, C.; Danielsen, M.; Li, Q.; Chen, W.; Chan, Y.; Li, Y. EGb761 improves cognitive function and regulates inflammatory responses in the APP/PS1 mouse. *Exp Gerontol* **2016**, *81*, 92-100, doi:10.1016/j.exger.2016.05.007.
62. Gargouri, B.; Carstensen, J.; Bhatia, H.S.; Huell, M.; Dietz, G.P.H.; Fiebich, B.L. Anti-neuroinflammatory effects of Ginkgo biloba extract EGb761 in LPS-activated primary microglial cells. *Phytomedicine* **2018**, *44*, 45-55, doi:10.1016/j.phymed.2018.04.009.
63. Ahlemeyer, B.; Mowes, A.; Krieglstein, J. Inhibition of serum deprivation- and staurosporine-induced neuronal apoptosis by Ginkgo biloba extract and some of its constituents. *Eur J Pharmacol* **1999**, *367*, 423-430, doi:10.1016/s0014-2999(98)00903-0.
64. Schindowski, K.; Leutner, S.; Kressmann, S.; Eckert, A.; Muller, W.E. Age-related increase of oxidative stress-induced apoptosis in mice prevention by Ginkgo biloba extract (EGb761). *J Neural Transm (Vienna)* **2001**, *108*, 969-978, doi:10.1007/s007020170016.
65. Luo, Y.; Smith, J.V.; Paramasivam, V.; Burdick, A.; Curry, K.J.; Buford, J.P.; Khan, I.; Netzer, W.J.; Xu, H.; Butko, P. Inhibition of amyloid-beta aggregation and caspase-3 activation by the Ginkgo biloba extract EGb761. *Proc Natl Acad Sci U S A* **2002**, *99*, 12197-12202, doi:10.1073/pnas.182425199.
66. Colciaghi, F.; Borroni, B.; Zimmermann, M.; Bellone, C.; Longhi, A.; Padovani, A.; Cattabeni, F.; Christen, Y.; Di Luca, M. Amyloid precursor protein metabolism is regulated toward alpha-secretase pathway by Ginkgo biloba extracts. *Neurobiol Dis* **2004**, *16*, 454-460, doi:10.1016/j.nbd.2004.03.011.
67. Blasko, I.; Kemmler, G.; Krampla, W.; Jungwirth, S.; Wichart, I.; Jellinger, K.; Tragl, K.H.; Fischer, P. Plasma amyloid beta protein 42 in non-demented persons aged 75 years: effects of concomitant medication and medial temporal lobe atrophy. *Neurobiol Aging* **2005**, *26*, 1135-1143, doi:10.1016/j.neurobiolaging.2005.03.006.
68. Longpre, F.; Garneau, P.; Christen, Y.; Ramassamy, C. Protection by EGb 761 against beta-amyloid-induced neurotoxicity: involvement of NF-kappaB, SIRT1, and MAPKs pathways and inhibition of amyloid fibril formation. *Free Radic Biol Med* **2006**, *41*, 1781-1794, doi:10.1016/j.freeradbiomed.2006.08.015.
69. Shi, C.; Zhao, L.; Zhu, B.; Li, Q.; Yew, D.T.; Yao, Z.; Xu, J. Protective effects of Ginkgo biloba extract (EGb761) and its constituents quercetin and ginkgolide B against beta-amyloid peptide-induced toxicity in SH-SY5Y cells. *Chem Biol Interact* **2009**, *181*, 115-123, doi:10.1016/j.cbi.2009.05.010.
70. Wan, W.B.; Cao, L.; Liu, L.M.; Kalionis, B.; Chen, C.; Tai, X.T.; Li, Y.M.; Xia, S.J. EGb761 provides a protective effect against Abeta1-42 oligomer-induced cell damage and blood-brain barrier disruption in an in vitro bEnd.3 endothelial model. *PLoS One* **2014**, *9*, e113126, doi:10.1371/journal.pone.0113126.
71. Xie, H.; Wang, J.R.; Yau, L.F.; Liu, Y.; Liu, L.; Han, Q.B.; Zhao, Z.; Jiang, Z.H. Quantitative analysis of the flavonoid glycosides and terpene trilactones in the extract of Ginkgo biloba and evaluation of their

inhibitory activity towards fibril formation of beta-amyloid peptide. *Molecules* **2014**, *19*, 4466-4478, doi:10.3390/molecules19044466.

72. Qin, Y.; Zhang, Y.; Tomic, I.; Hao, W.; Menger, M.D.; Liu, C.; Fassbender, K.; Liu, Y. Ginkgo biloba Extract EGb 761 and Its Specific Components Elicit Protective Protein Clearance Through the Autophagy-Lysosomal Pathway in Tau-Transgenic Mice and Cultured Neurons. *J Alzheimers Dis* **2018**, *65*, 243-263, doi:10.3233/JAD-180426.
73. Fehske, C.J.; Leuner, K.; Muller, W.E. Ginkgo biloba extract (EGb761) influences monoaminergic neurotransmission via inhibition of NE uptake, but not MAO activity after chronic treatment. *Pharmacol Res* **2009**, *60*, 68-73, doi:10.1016/j.phrs.2009.02.012.
74. Yoshitake, T.; Yoshitake, S.; Kehr, J. The Ginkgo biloba extract EGb 761(R) and its main constituent flavonoids and ginkgolides increase extracellular dopamine levels in the rat prefrontal cortex. *Br J Pharmacol* **2010**, *159*, 659-668, doi:10.1111/j.1476-5381.2009.00580.x.
75. Beck, S.M.; Ruge, H.; Schindler, C.; Burkart, M.; Miller, R.; Kirschbaum, C.; Goschke, T. Effects of Ginkgo biloba extract EGb 761(R) on cognitive control functions, mental activity of the prefrontal cortex and stress reactivity in elderly adults with subjective memory impairment - a randomized double-blind placebo-controlled trial. *Hum Psychopharmacol* **2016**, *31*, 227-242, doi:10.1002/hup.2534.
76. Tchantchou, F.; Xu, Y.; Wu, Y.; Christen, Y.; Luo, Y. EGb 761 enhances adult hippocampal neurogenesis and phosphorylation of CREB in transgenic mouse model of Alzheimer's disease. *FASEB J* **2007**, *21*, 2400-2408, doi:10.1096/fj.06-7649com.
77. Tchantchou, F.; Lacor, P.N.; Cao, Z.; Lao, L.; Hou, Y.; Cui, C.; Klein, W.L.; Luo, Y. Stimulation of neurogenesis and synaptogenesis by bilobalide and quercetin via common final pathway in hippocampal neurons. *J Alzheimers Dis* **2009**, *18*, 787-798, doi:10.3233/JAD-2009-1189.
78. Amieva, H.; Meillon, C.; Helmer, C.; Barberger-Gateau, P.; Dartigues, J.F. Ginkgo biloba extract and long-term cognitive decline: a 20-year follow-up population-based study. *PLoS One* **2013**, *8*, e52755, doi:10.1371/journal.pone.0052755.
79. Grass-Kapane B., B.A., Lasmanis A., Hoerr R., Kaschel R. Effects of Ginkgo biloba special extract EGb761(R) in very mild cognitive impairment (vMCI). *Neuroscience & Medicine* **2011**, *2*, 48-56, doi:10.4236/nm.2011.21007.
80. Vellas, B.; Coley, N.; Ousset, P.J.; Berrut, G.; Dartigues, J.F.; Dubois, B.; Grandjean, H.; Pasquier, F.; Piette, F.; Robert, P., et al. Long-term use of standardised Ginkgo biloba extract for the prevention of Alzheimer's disease (GuidAge): a randomised placebo-controlled trial. *Lancet Neurol* **2012**, *11*, 851-859, doi:10.1016/S1474-4422(12)70206-5.
81. Scherrer, B.; Andrieu, S.; Ousset, P.J.; Berrut, G.; Dartigues, J.F.; Dubois, B.; Pasquier, F.; Piette, F.; Robert, P.; Touchon, J., et al. Analysing Time to Event Data in Dementia Prevention Trials: The Example of the GuidAge Study of EGb761. *J Nutr Health Aging* **2015**, *19*, 1009-1011, doi:10.1007/s12603-015-0582-0

10.1007/s12603-015-0661-2.
82. Gavrilova, S.I.; Preuss, U.W.; Wong, J.W.; Hoerr, R.; Kaschel, R.; Bachinskaya, N.; Group, G.I.S. Efficacy and safety of Ginkgo biloba extract EGb 761 in mild cognitive impairment with neuropsychiatric symptoms: a randomized, placebo-controlled, double-blind, multi-center trial. *Int J Geriatr Psychiatry* **2014**, *29*, 1087-1095, doi:10.1002/gps.4103.

83. DeKosky, S.T.; Williamson, J.D.; Fitzpatrick, A.L.; Kronmal, R.A.; Ives, D.G.; Saxton, J.A.; Lopez, O.L.; Burke, G.; Carlson, M.C.; Fried, L.P., et al. Ginkgo biloba for prevention of dementia: a randomized controlled trial. *JAMA* **2008**, *300*, 2253-2262, doi:10.1001/jama.2008.683.

84. Ihl, R.; Bachinskaya, N.; Korczyn, A.D.; Vakhapova, V.; Tribanek, M.; Hoerr, R.; Napryeyenko, O.; Group, G.S. Efficacy and safety of a once-daily formulation of Ginkgo biloba extract EGb 761 in dementia with neuropsychiatric features: a randomized controlled trial. *Int J Geriatr Psychiatry* **2011**, *26*, 1186-1194, doi:10.1002/gps.2662.

85. Herrschaft, H.; Nacu, A.; Likhachev, S.; Sholomov, I.; Hoerr, R.; Schlaefke, S. Ginkgo biloba extract EGb 761(R) in dementia with neuropsychiatric features: a randomised, placebo-controlled trial to confirm the efficacy and safety of a daily dose of 240 mg. *J Psychiatr Res* **2012**, *46*, 716-723, doi:10.1016/j.jpsychires.2012.03.003.

86. Yancheva, S.; Ihl, R.; Nikolova, G.; Panayotov, P.; Schlaefke, S.; Hoerr, R.; Group, G.S. Ginkgo biloba extract EGb 761(R), donepezil or both combined in the treatment of Alzheimer's disease with neuropsychiatric features: a randomised, double-blind, exploratory trial. *Aging Ment Health* **2009**, *13*, 183-190, doi:10.1080/13607860902749057.

87. Birks, J.; Grimley, E.V.; Van Dongen, M. Ginkgo biloba for cognitive impairment and dementia. *Cochrane Database Syst Rev* **2002**, 10.1002/14651858.CD003120, CD003120, doi:10.1002/14651858.CD003120.

88. Birks, J.; Grimley Evans, J. Ginkgo biloba for cognitive impairment and dementia. *Cochrane Database Syst Rev* **2007**, 10.1002/14651858.CD003120.pub2, CD003120, doi:10.1002/14651858.CD003120.pub2.

89. Gauthier, S.; Schlaefke, S. Efficacy and tolerability of Ginkgo biloba extract EGb 761(R) in dementia: a systematic review and meta-analysis of randomized placebo-controlled trials. *Clin Interv Aging* **2014**, *9*, 2065-2077, doi:10.2147/CIA.S72728.

90. Hashiguchi, M.; Ohta, Y.; Shimizu, M.; Maruyama, J.; Mochizuki, M. Meta-analysis of the efficacy and safety of Ginkgo biloba extract for the treatment of dementia. *J Pharm Health Care Sci* **2015**, *1*, 14, doi:10.1186/s40780-015-0014-7.

91. von Gunten, A.; Schlaefke, S.; Uberla, K. Efficacy of Ginkgo biloba extract EGb 761((R)) in dementia with behavioural and psychological symptoms: A systematic review. *World J Biol Psychiatry* **2016**, *17*, 622-633, doi:10.3109/15622975.2015.1066513.

92. Zhang, H.F.; Huang, L.B.; Zhong, Y.B.; Zhou, Q.H.; Wang, H.L.; Zheng, G.Q.; Lin, Y. An Overview of Systematic Reviews of Ginkgo biloba Extracts for Mild Cognitive Impairment and Dementia. *Front Aging Neurosci* **2016**, *8*, 276, doi:10.3389/fnagi.2016.00276.

93. Savaskan, E.; Mueller, H.; Hoerr, R.; von Gunten, A.; Gauthier, S. Treatment effects of Ginkgo biloba extract EGb 761(R) on the spectrum of behavioral and psychological symptoms of dementia: meta-analysis of randomized controlled trials. *Int Psychogeriatr* **2018**, *30*, 285-293, doi:10.1017/S1041610217001892.

94. Knopman, D.S.; Petersen, R.C. Mild cognitive impairment and mild dementia: a clinical perspective. *Mayo Clin Proc* **2014**, *89*, 1452-1459, doi:10.1016/j.mayocp.2014.06.019.

95. Halil, M.; Cankurtaran, M.; Yavuz, B.B.; Ozkayar, N.; Ulger, Z.; Dede, D.S.; Shorbagi, A.; Buyukasik, Y.; Haznedaroglu, I.C.; Arogul, S. No alteration in the PFA-100 in vitro bleeding time induced by the Ginkgo biloba special extract, EGb 761, in elderly patients with mild cognitive impairment. *Blood Coagul Fibrinolysis* **2005**, *16*, 349-353, doi:10.1097/01.mbc.0000172695.62363.57.

96. Bal Dit Sollier, C.; Caplain, H.; Drouet, L. No alteration in platelet function or coagulation induced by EGb761 in a controlled study. *Clin Lab Haematol* **2003**, *25*, 251-253, doi:10.1046/j.1365-2257.2003.00527.x.

97. Wolf, H.R. Does Ginkgo biloba special extract EGb 761 provide additional effects on coagulation and bleeding when added to acetylsalicylic acid 500 mg daily? *Drugs R D* **2006**, *7*, 163-172, doi:10.2165/00126839-200607030-00003.