Ginkgolides and Neuroprotective Effects 122

Syed Haris Omar

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S.H. Omar
School of Biomedical Sciences and E.H. Graham Centre for Agricultural Innovation, Charles Sturt University, Wagga Wagga, New South Wales, Australia
e-mail: somar@csu.edu.au; syedharisomar@gmail.com

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Abstract

Ginkgo biloba is commonly known as maidenhair tree, available as a popular herbal supplementary in Asian, European, and American countries. Extensive in vitro, in vivo, and clinical studies demonstrated and confirmed the neuroprotective effects of a commercial standard ginkgo extract formulation known as EGb-761. Terpene trilactone comprises 5–7 % in EGb-761, collectively called as ginkgolides (G-A, G-B, G-C, G-J, G-K, G-L and G-M) and bilobides. Its clinical application gained popularity in herbal medicine due to treatment of early-stage Alzheimer's disease, cerebrovascular disorders, PAF antagonism, and vestibular disorders. In addition, ginkgolides showed potent antioxidant activities via scavenging of reactive oxygen and nitrogen species. The physiological dosage of ginkgo extracts ranges between 120 and 240 mg/day in humans, and it is readily available as an over the counter product/supplementary product. According to the recent clinical findings, EGb-761 did not confirm its effect on long-term cognitive functioning, while it showed effectiveness and enhancement in shor-term cognitive and related activities. Ginkgo is generally well tolerated, but in a high dose, it can cause gastric upset, skin allergy, and increase the risk of bleeding in patients with risk factors (anticoagulant or antiplatelet treatment, surgery, etc.). Neuropharmacology, clinical issues of safety and usage are addressed in this book chapter.

Keywords

Ginkgo • ginkgolides • neuroprotection • PAF antagonism • terpenoids

Abbreviations

5-HT	5-Hydroxytryptamine
8-OHdG	Hydroxyl-deoxyguanosine
ACh	Acetylcholine
AD	Alzheimer's disease
ADDLs	Amyloid beta-derived diffusible soluble ligands
APP	Amyloid precursor protein
ATP	Adenosine triphosphate
Aβ	Amyloid beta
BALF	Bronchoalveolar lavage fluid
BBB	Blood–brain barrier
BN-52021	Ginkgolide B
cAMP	Cyclic adenosine monophosphate
D_2	Dopaminergic receptor 2
DA	Dopamine
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DSC-MRI	Dynamic susceptibility contrast-enhanced magnetic resonance imaging
EGb	*Ginkgo biloba* extract

EGb-761	Standard ginkgo extract
FFA	Free fatty acid
G. biloba	*Ginkgo biloba*
G-A	Ginkgolide A
$GABA_A$	Gamma-amino butyric acid
G-B	Ginkgolide B
G-C	Ginkgolide C
G-J	Ginkgolide J
G-K	Ginkgolide K
G-L	Ginkgolide L
G-M	Ginkgolide M
GSH	Glutathione
H_2O_2	Hydrogen peroxide
HO-1	Heme oxygenase-1
i.p.	Intraperitoneally
IL	Interleukin
iNOS	Inducible nitric oxide synthase
JNK	c-jun N-terminal kinase
KYNA	Kynurenic acid
LI-1370	Standard *Gingko* extract
MAO	Monoamine oxidase
MDA	Malondialdeide
MGluR	Metabotropic glutamate receptor
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mRNA	Messenger ribose nucleic acid
MS	Multiple sclerosis
NCE	Noncontact erection
NE	Norepinephrine
NF-kappa B	Nuclear factor kappa B
NFT	Neurofibrillary tangles
NIDDM	Non-insulin dependent diabetes mellitus
NMDA	*N*-methyl D-aspartate receptor
NO	Nitric oxide
P8A	Standard *Gingko* extract
PAF	Platelet activating factor
PBR	Peripheral-type benzodiazepine receptor
PD	Parkinson's disease
PKA	Protein kinase-A
PVN	Paraventricular nucleus
RAGE	Receptors for advanced glycation end products
RNA	Ribose nucleic acid
RONS	Reactive oxygen and nitrogen species
ROS	Reactive oxygen species

USP	United States Pharmacopeia
VF	Ventricular fibrillation
VSMCs	Vascular smooth muscle cells
VT	Ventricular tachycardia
WHO	World Health Organization

1 Introduction

Ginkgo biloba, maidenhair tree, is one of the ancient tree classified in its own division called, Ginkgophyta which belongs to family Ginkgocea, the only species still surviving and found in Asian countries (China, Japan, and Korea) [1]. There are unequivocal representatives of the genus Ginkgo which have been described as a new species, *Ginkgo yimaensis* and another extinct "species," *Ginkgo adiantoides* [2]. Genus, *Ginkgo* has been derived from the Japanese name Yin-Kwo, meaning silver fruit and the species *biloba*, describes the bilobed shape of the leaves. *G. biloba* is also known as a living fossil because of the finding of the fossil plants quite similar to *G. biloba* which date back to 180 million years ago [3]. In the ancient period, ginkgo seeds were used against cough, asthma, enuresis, alcohol misuse, pyogenic skin infections, and worm infestations in the intestinal tract. This is first mentioned in the great herbal Pen Ts'ao Kang Mu of 1596 by Li Shih-chen [4]. The leaf extracts were used for the improvement of the blood circulation, both peripherally and centrally. This started in the 1960s in Germany [5]. The draft monographs on ginkgo folium and extract mentioned in the USP [6, 7] and ginkgo folium and standardized ginkgo extract are in the European Pharmacopeia [6]. The positive monographs on *G. biloba* were published in the German Commission E, which are available in an English translation as well [8]. In addition to the Commission E, WHO also published a positive monograph on *Ginkgo* leaf extracts, which is in principle comparable [9]. This chapter will summarize the beneficial effects and provide an update on gingkolides, mostly in the form of EGb-761 preparation in the various neurological conditions and diseases.

2 Major Bioactive Constituents and Preparations

The commonly used parts of *G. biloba* are the seeds and leaves, which contains most of their pharmacologically important constituents and makes them medicinally important. The seeds contain mainly polysaccharide and protein (ginkbilobin), which stimulates apoptosis of human hepatoma SMMC-7721 cells and exhibit antifungal activity. However, seed also contains 4-*O*-methylpyridoxine, a ginkgotoxin, a poisonous compound whose primary mode of action is to antagonize the activity of vitamin B_6 [10]. The leaf extract is generally categorized into two types: full extracts and standardized extracts. The full leaf extracts are usually prepared with alcohol and contain all alcohol soluble constituents. The standardized extracts are EGb-761 and LI-1370, but EGb-761 is more common

in use and contains a variety of active constituents. The major bioactive compounds in EGb-761 are classified as flavonol glycosides (24 %), terpene trilactones (5–7 %), proanthocyanidins (7 %), carboxylic acids (13 %), catechins (2 %), non-flavonol glycosides (20 %), alkylphenols (5 ppm), and other (unknown 28 %) shown in Fig. 122.1. Other preparation like ginkgo extract (P8A) that is approximately 10-fold enriched in terpene trilactones and contains bilobalide and the four ginkgolides (G-A, G-B, G-C, G-J) extracted from the leaves of the plant [11]. While the preparation BN-52021 is specific for ginkgolide B and used as ginkgolides activity.

3 Ginkgolides

Ginkgolides were first isolated by Furukawa in 1932 from the root bark of *G. biloba* [12]. Maruyama et al. [13–17] and Nakanishi [18] isolated and elucidated the structure of ginkgolides G-A, G-B, G-C, and G-M from the root bark. Meanwhile, Okabe and Sakabe independently determined the structures of G-A, G-B and G-C from leaves of *G. Biloba* by means of X-ray crystallography [19, 20]. Later, ginkgolide G-J [21] and two new traces ginkgolide G-K and G-L have been identified [22]. From the total of 5–7 % terpene trilactones, 2.8–3.4 % are ginkgolides A, B, and C, and 2.6–3.2 % are bilobalides, ginkgolides contributes 50–60 %. Chemically, ginkgolides (Fig. 122.2) are diterpene trilactones consist of C-20 terpenes, 6 five-membered rings, that is, a spiro[4, 4]-nonane carbocylic ring, three lactones, and a tetrahydrofuran ring. The presence of a tertiary butyl group makes ginkgolides unique among natural products. All are pharmacologically active; however, ginkgolide B is by far the most potent and has received far more attention from researchers due to their distinguishing structure, specific occurrence, and broad pharmacology.

4 Ginkgolides Pharmacology

A number of studies have been conducted on the pharmacological activities of *G. biloba* crude extract, commercial available extract (EGb-761), and leaf extract. G-B and ginkgolide extract (EGb-761) have received more attention due to their higher potency, broad action, and versatile constituents [23]. The in vitro, in vivo, and clinical studies demonstrated the anti-inflammatory, cardioprotective, anticancer, antidiabetic, antioxidant, and gastroprotective activities as shown in Table 122.1. Out of the various beneficial studies, few of them have not been confirm by further researcher, in animal models, and which are confirmed in animals did not further conducted in human (clinical trial).

5 Neuroprotective Activities of Ginkgolides

Neuroprotection refers to maintaining the intactness of cellular interactions/intercellular communication in the brain resulting in an overall undisturbed

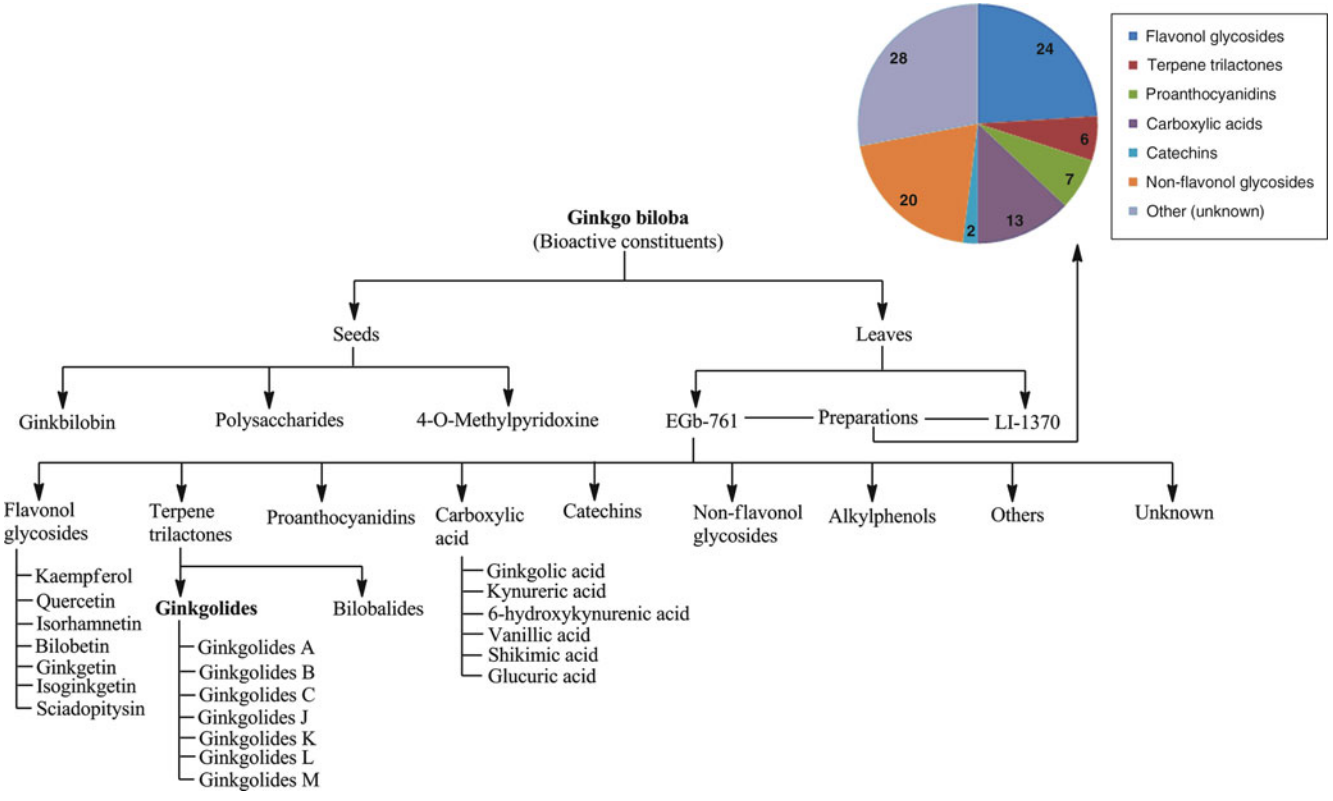
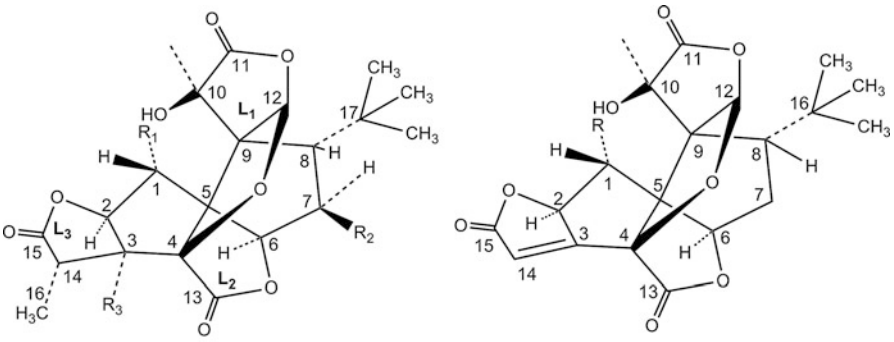


Fig. 122.1 Major bioactive components and their broad classification in *Ginkgo biloba* extract (EGb-761)



	R_1	R_2	R_3
Ginkgolide G-A	H	H	OH
Ginkgolide G-B	OH	H	OH
Ginkgolide G-C	OH	OH	OH
Ginkgolide G-J	H	OH	OH
Ginkgolide G-M	OH	OH	H

	R
Ginkgolide G-K	OH
Ginkgolide G-L	H
	L
Lactone ring	1,2,3

Fig. 122.2 Chemical structures of ginkgolides

function [45]. Both prevention and delayed onset of neurological disorders would have a large impact in terms of reducing both suffering and costs. Nowadays, researcher interest in herbal medicine has grown in several countries. Moreover, efforts have been made to find new therapeutic agents from these natural products for the prevention or treatment of memory disorders, such as the gradual impairment of memory in aging or in neurodegenerative pathology and even lifestyle factors. Ginkgolides from *G. biloba*, especially G-B and preparation EGb-761, have emerged as natural/herbal source of neuroprotective agents. Various in vitro/in vivo and preclinical/clinical studies confer their neuroprotective activities (Fig. 122.3).

6 CNS Effects of *Ginkgo Biloba*

6.1 Cerebral Blood Flow

Interruption or inclusion in cerebral blood flow leads to a decrease in supply of oxygen and nutrient to the brain, which ultimately leads to several neurodegenerative diseases. Table 122.2 showed the protective effect of EGb-761 in cerebral blood flow disorder animal models.

There are various mechanisms involved in decrease in cerebral blood flow. Research studies suggested three major events including increase uptake of glucose or decrease tissue glucose content, leads to increase glucose utilization and cerebral embolism which have cumulative effects on the cerebral blood flow (Fig. 122.4).

Table 122.1 Common pharmacological activities of *G. biloba* (Ginkgolides)

Pharmacological activities	Preparations	Effects	References
Anti-inflammatory	Ginkgolide B, A, C and J	Inhibit the increase of T-helper 2 cytokines, such as interleukin IL-5 and IL-13, in bronchoalveolar lavage fluid (BALF), suppression of extracellular regulating kinase/MAPK (mitogen activated protein kinase) pathway; ginkgolide B, A, C, and J inhibited PAF-mediated aggregation of human platelets at concentrations of 2.5, 15.8, 29.8, and 43.5 μg/mL,	[24, 25]
Antiarrhythmic	EGb-761	Dose-dependently reduction in ventricular fibrillation (VF) and ventricular tachycardia (VT) in the hearts of rats	[26]
Anticancer	EGb-761	Dose-dependent decreases in xenograft growth of both MDA-MB-231 breast cancer and U-87 glioma cell lines in nude mice; suppress proliferation and increase cytotoxicity in HepG2 and Hep3B cells	[27, 28]
Antidiabetic	EGb-761 and G-B	Ingestion of *G. biloba* extract by an NIDDM subject may increase the hepatic metabolic clearance rate of not only insulin but also the hypoglycemic agents; protect beta cells	[29–31]
Anti-ischemic reperfusion	EGb-761	preventive effect on ischemia-reperfusion injury in rat urinary bladder; reduced hydroxyl-deoxyguanosine (8-OHdG) formation in the DNA from liver undergoing ischemia-reperfusion	[32, 33]
Anti-atherosclerotic	EGb-761	Significantly suppressed the proliferation and migration of VSMCs, promoted apoptosis and reduced inflammatory processes	[34]
Antimicrobial	G-A, G-B, and the standardized Ginkgo leaf extracts	Exhibited antimicrobial activity against *Streptococcus pyogenes*; crude extracts of Ginkgo leaves (7.8 μg/mL) possess inhibitory activity against Gram-positive and Gram-negative bacteria	[35, 36]
Antioxidant	*G. biloba* extract; G-A & G-B	Inhibit NF-κB activation induced by H_2O_2; antagonize iNOS-mediated NO production in macrophages stimulatory effect via antioxidation and attenuation of NF-κB activation	[37–39]
Antithrombotic	EGb-761	In a rat model of thrombosis, the antithrombotic effects of EGb-761 combination therapy were more effective than with ticlopidine alone; combinative therapy of G-B and cilostazol enhanced antithrombotic efficacies without increasing side effects	[40, 41]

(*continued*)

Table 122.1 (continued)

Pharmacological activities	Preparations	Effects	References
Gastroprotective	*G. biloba* extract	EGb (25, 50, and 100 mg/kg, ig) inhibit the increase of MDA both in gastric mucosa and in serum; significantly inhibit the ethanol-induced gastric lesions in rats	[42, 43]
Vasodilation	*G. biloba* extract injectable solution	Treatment in healthy elderly adults leads to the increase of LAD (left anterior descending) blood flow and improved endothelium-dependent vasodilatory capacity	[44]

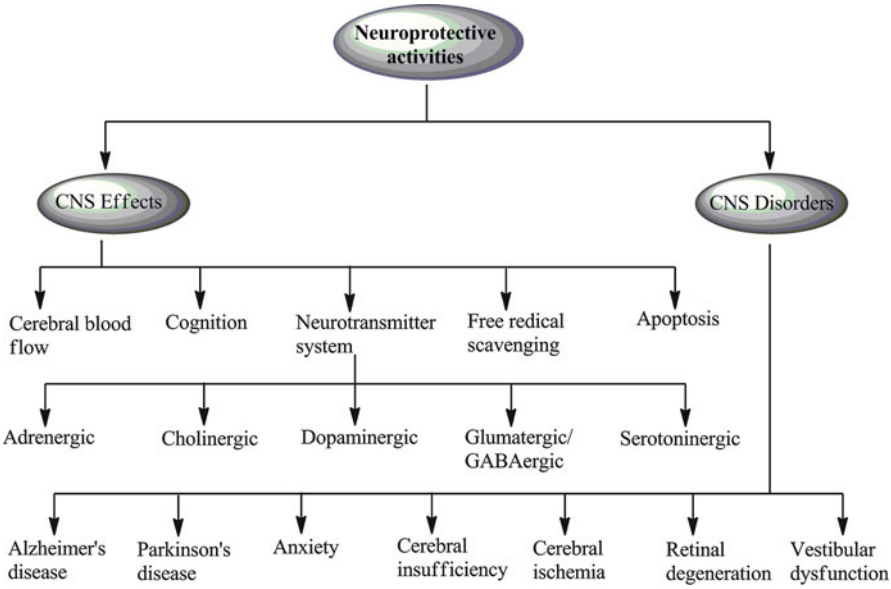


Fig. 122.3 Ginkgolides involved in multiple neuroprotective activities

6.1.1 Clinical Trials

In a double-blind randomized placebo-controlled trial of 72 outpatients with cerebral insufficiency of at least 24 weeks duration, EGb-761 improved mental/mnestic performance [53]. A meta-analysis was conducted to evaluate *G. biloba* (120–240 mg/day) effectiveness in treating cerebrovascular insufficiency [54]. All of the seven studies included in the analysis were double-blind, placebo-controlled

Table 122.2 Protective effect of ginkgolides in animal models of cerebral blood flow disorders

Compound	Study	Action	Reference
EGb-761	Rats	Partially suppressed the effects of the brain embolization	[46]
EGb-761	Rats	(100 mg/kg, i.p.) survived hypobaric hypoxia for a much longer period than controls	[47]
EGb-761	Rats	Dose (10 mg/mL) dependently inhibition of glucose uptake and decrease in the cortical glucose concentration	[48]
EGb-761	In vitro	0.5–1 μg/mL inhibit the ATP decrease induced by hypoxia	[49]
EGb-761	Rats	50 mg/kg/day decreases in glucose utilization in the frontoparietal somatosensory cortex, nucleus accumbens, cerebellar cortex, and pons	[50]
EGB	Dogs	Injection of an extract of *G. biloba*; the cerebral blood flow was increased and decreased in cerebrovascular resistance	[51]
EGb-761	Rats	Reduction in cranial perfusion pressure and regional cerebral blood flow	[52]

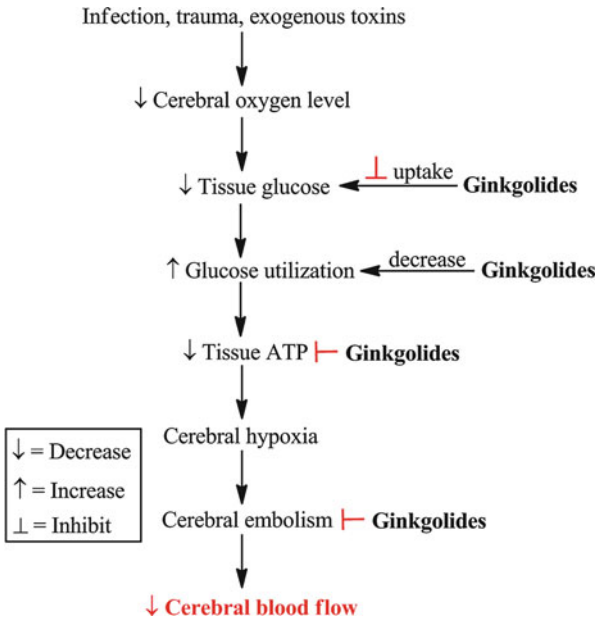


Fig. 122.4 Ginkgolides in cerebral blood flow: inhibits the glucose uptake, decrease glucose utilization, inhibit decrease in ATP contents and inhibits cerebral embolism

trials that showed significant improvements compared to placebo on all of the individual symptoms that were analyzed (one study was inconclusive) [54]. Recently, in a pilot study [55] of 9 healthy subjects, the dynamic susceptibility contrast-enhanced magnetic resonance imaging (DSC-MRI) showed a significant increase of non-normalized cerebral blood flow after *G. biloba* extract capsule (15 % in white and 13 % in gray matter).

Table 122.3 Beneficial effects of ginkgolides in animals models of cognitive disorders

Compound	Study	Action	References
EGb-761	Mice	Oral administration (100 mg/kg) facilitate memory processes, acquisition, and improved the performance	[56]
EGb-761	Mice	100 mg/kg dose per day showed improvement in short-term memory but only in the aged group mice	[57]
EGb-761	Mice	a daily i.p. injection (40 mg/kg) cause enhancing effect on the performance of a learning task as observed in both group of mice (adult and aged)	[58]
EGb-761	Rats	200 mg/kg dose showed significant improve in continuous learning and delayed nonmatching to position tasks in aged rats	[59]
EGb-761	Rats	Chronic (60 mg/kg/day i.p.) and acute (60 or 120 mg/kg i.p.) injections enhanced olfactory recognition in young rats and facilitative effects in aged rats that received a single 60 mg/kg i.p. injection	[60]
EGb-761	Chicks	(3 mg/mL) dose facilitate memory in chicks with poor long-term retention	[61]
EGb-761	Rats	high doses (150 mg/kg/b.w./day) significantly improved considerable memory, cognitive performance, and exploratory behavior	[62]
Leaf extract (gingkoselect)	Rats	(100 mg/kg/day, orally, 21 days) normalized cognitive deficits in rats chronically treated with corticosterone and improved memory in the chronically stressed rats	[63]
EGb-761	Rats	50 or 100 mg/kg doses per day protect against intermittent hypoxia-induced memory impairment, oxidative stress, and neuronal DNA damage	[64]

6.2 Cognition

Cognitive abilities include perception, memory, judgment, perceptual speed, spatial manipulation, and reasoning. These declines as part of normal aging. Dementia is a loss of cognitive abilities in multiple domains that results in impairment in normal activities of daily living and loss of independence. AD is the most common cause of dementia, responsible for 60–80 % of all dementia. *Gingko* extract has been involved in improvement of cognitive disorder in various animal models (Table 122.3).

6.2.1 Clinical Trials

Numerous clinical studies have been conducted that showed the *G. biloba* has a beneficial effect in age-related decrease in cognition.

A double-blind, crossover comparative trial [65] with healthy female volunteers documented the ability of EGB ability to improve short-term memory. There was no any significant effect observed at lower doses (120 and 240 mg). However, when the women were given 600 mg of EGB and tested 1 h later, the results showed a very significant improvement in short-term memory compared to women taking

a placebo [65]. In a double-blind, randomized placebo-controlled long-term study (24 weeks) involving 72 outpatients with cerebral insufficiency using EGb-761, it showed statistically significant improvement in the short-term memory after 6 weeks and of the learning rate after 24 weeks were observed in the ginkgo group, but not in the placebo group (longitudinal analysis) [53]. In a randomized, double-blind, placebo-controlled study [66] on aged patients receiving either *G. biloba* alcohol/water extract in a high dose (RD), a low dose (LD) or a placebo (PL) for 24 weeks resulted increase in short-term visual memory by 18 %, 26 %, and 11 % in the RD, LD, and PL groves respectively and indicating that the use of ginkgo extracts in elderly individuals with cognitive impairment might be promising. In contrast, another double-blind, placebo-controlled study [67], in 30 healthy male subjects who were receiving 260-mg tablets of Bio Ginkgo (LI-1370) daily for 5 days did not showed any significant results, furthermore another 24-week, randomized, double-blind, placebo-controlled, parallel-group, multicenter trial [68] did not show any statistically significant differences in mean change of scores between ginkgo (either 240 mg/day or 160 mg/day) or placebo. In a 6-week, double-blind, fixed-dose, placebo-controlled trial on intact persons over the age of 55 years who received EGb-761 (180 mg/day) [69], results showed significantly more improvement on a task, assessing speed of processing abilities (i.e., Stroop Color and Word Test color-naming task) and improved overall abilities to remember by the end of treatment as compared to participants who received placebo [69]. A placebo-controlled, multi-dose, double-blind, balanced, crossover trial of 20 healthy subjects who received 120, 240, and 360 mg of a standardized extract of ginkgo (GK501, Pharmaton, SA) or a matching placebo [70] was conducted. The results showed a number of significant changes on the performance measures after administration of ginkgo. The most striking of these was a dose-dependent improvement of the "speed of attention" factor following both 240 mg and 360 mg of the extract, which was evident at 2.5 h and was still present at 6 h. Additionally, there were a number of time and dose-specific changes (both positive and negative) in performance of the other factors [70]. In a very short-term study [71] on healthy postmenopausal, women aged 53–65 years, of one week of ginkgo treatment, showed improved performance in three of the cognitive tasks – the tests of short-term nonverbal recognition memory, mental flexibility, and sustained attention. In another placebo-controlled double-blind design study [72] on young healthy volunteers showed an acute dose of ginkgo significantly improved performance on the sustained attention task and pattern recognition memory task. In a prospective community-based cohort study involved 3,534 subjects aged 65 years and older [73], the results showed that initial consumption of EGb-761 did not modify the risk of dementia, whereas the consumption of other treatments for memory impairment was associated with a higher risk of dementia. Subjects who took *G. biloba* had a significantly lower risk of mortality in the long-term, even after adjustment for potentially confounding factors [73]. In a placebo-controlled, multi-dose, double-blind, balanced-crossover study [74] where combination treatment of 120 mg EGB complexed with phosphatidylserine resulted both in improved secondary memory performance and significantly increased speed of memory task

performance across all of the post-dose testing sessions. In a small randomized, double-blind, placebo-controlled trial [75] of GB, 120 mg significantly improves the cognitive performance of subjects with multiple sclerosis (MS) via the Stroop test. In a meta-analysis study [76], which included 6 randomized placebo-controlled trial. Considering baseline risk in the assessment of treatment effect, EGb was found to be effective for cognitive functions in dementia with the treatment of 6 months [76]. Recently, Kaschel [77] showed that the EGb-761 (240 mg once daily) improves free recall of appointments in middle-aged healthy volunteers, which requires high demands on self-initiated retrieval of learned material.

6.3 Neurotransmitter System

Neurotransmitters are the chemical messengers in the nervous system, which relay information across synapses via excitation or inhibition of the next neuron or effector tissue. Neurotransmitters can be classified into two broad categories, small-molecule neurotransmitters and neuropeptides. Small-molecule neurotransmitters, such as acetylcholine and the monoamines, are synthesized in the axon terminal of the neuron and larger neuropeptides, such as somatostatin and vasopressin, are synthesized in the neuron's cell body. It has been shown that *G. biloba* and its gingkolides produces effect on a number of neurotransmitter systems, including serotonergic, adrenergic, dopaminergic, and cholinergic systems.

6.3.1 Adrenergic Transmission

A neurotransmitter formed in sympathetic postganglionic synapses, known as noradrenaline. Very few studies have been conducted to show the effect of *G. biloba* on adrenergic system. Chronic treatment with *G. biloba* extract on rat cerebral cortex resulted in increase in noradrenaline release along with decrease in the density of cerebral β-adrenoceptors and suggested as an adaptive mechanism (after 27 days or 2 months) [78]. The age-related decrease of α-2-binding-sites in rat cerebral cortex was prevented by EGb-761 treatment, indicating a relative increase of noradrenergic neurotransmission in aged rats, while the reduction in binding affinity was unaffected [79]. Oral administration of *G. biloba* extract at a dose of 90 mg/kg for seven consecutive days on rat brain modulate the β-adrenoceptors and implicated in the favorable effects of *G. biloba* extracts on learning and memory [80]. After 14 days of daily oral treatment with 100 mg/kg of EGb-761, which resulted significantly only in decrease of NE uptake in mice [81].

6.3.2 Cholinergic Transmission

Choline is a precursor for biosynthesis of the neurotransmitter acetylcholine; any modulation in the cholinergic system is known to influence cognitive processes, learning processes, and working memory [82]. Indeed, increases in cholinergic transmission are known to enhance working memory performance [83] and vice versa [84]. *G. biloba* extracts have been shown to enhance cholinergic processes in various cortical regions. In vitro studies indicate that EGb-761 (100 μg/mL)

increases acetylcholine (ACh) release in hippocampal synaptosomes [85]. In vivo studies showed that EGB attenuate the amnesia induced by scopolamine [86], and chronic oral treatment with an extract of *G. biloba* increases the apparent muscarinic receptor population in the hippocampus of the aged rat [87].

6.3.3 Dopaminergic Transmission

Dopamine (DA) is a monoamine neurotransmitter which has a number of important physiological roles and influences on brain function, including playing a role in regulating attention, cognition, movement, pleasure, and hormonal processes. Studies have shown the benefits of *Gingko* extract in the improvement of DA neuron or its physiological function under the influences of neurotoxicity. Administration of EGb-761 (20, 50, 100 mg/kg/day i.p.) for 7 days before or after MPTP treatment effectively protects against MPTP-induced nigrostriatal dopaminergic neurotoxicity and suggesting that the inhibitory effect of EGb-761 on brain MAO may be involved in its neuroprotective effect [88]. Another study showed the significant recovery of rats observed after EGb (50, 100, and 150 mg/kg) treatment for 3 weeks in 6-OHDA induced decrease in the level of DA and its metabolites and an increase in the number of dopaminergic D_2 receptors in striatum [89]. The neuroprotective effect of EGb-761 against MPTP neurotoxicity in mice, receiving EGb-761, had significantly attenuated MPTP-induced loss of striatal dopamine levels and tyrosine hydroxylase immune staining in the striatum and substantia nigra pars compacta. Moreover, the author suggested that the neuroprotection was associated with blockade of lipid peroxidation and reduction of superoxide radical production (indicated by a downregulation of Mn-superoxide dismutase activity) [90]. Chronic (100 mg/kg/14 days/once daily) treatment with EGb-761 showed dose-dependent increases in frontocortical dopamine levels and, to a lesser extent, in the striatum [91]. A recent study results suggest that administration of EGb-761 increases dopaminergic activity in the paraventricular nucleus (PVN) and the mesolimbic system to facilitate noncontact erection (NCE) in male rats [92].

6.3.4 Glutamateric and GABAergic Transmission

Glutamate is the major excitatory neurotransmitter of the cortex and hippocampus, released from vesicles in presynaptic terminals by a Ca^{2+}, and is involved in many aspects of higher mental function. In particular, loss and dysfunction (hyperactivity) of both the pre- and postsynaptic glutamatergic system have been linked to neurodegenerative disorders. *Gingko* extract treatments have shown the positive effect in the glutamate transmission either from loss or excitotoxitcity. Ginkgolide B reduced excitotoxic damage in cultured chick embryo telencephalic neurons overexposed to glutamate [93]. Furthermore, BN-52021 (100 mM) showed protection against glutamate toxicity when it was added to rat neuronal cultures 24 h after glutamate exposure [94]. EGb (2.5 mg/L) and its constituent G-B (2 mg/L) protected the neuronal viability against glutamate-induced injury and prevented the glutamate-induced elevation in the intracellular free calcium (Ca^{2+}) concentration. EGb (3–10 mg/kg) attenuated the decrease of nucleus areas in arcuate nuclei induced by glutamate (1 g/kg, s.c.) [95]. In contrast, another study showed an

increase in protein kinase A (PKA) activation by G-B, which subsequently enhances the Ca^{2+} entry through voltage-dependent N and P/Q-type Ca^{2+} channels to cause increase in evoked glutamate release from rat hippocampal nerve terminals [96]. Recently, EGb-761 showed neuroprotection at a concentration 200 μg/mL to the spinal cord neurons from glutamate excitotoxicity and oxidative stress-induced cell death [97]. Kynurenic acid (KYNA) belongs to the group of low-affinity metabotropic glutamate receptor (mGluR) antagonists and interferes with the glycine B site of the NMDA receptor. An earlier study showed that EGb-761 modulating the glutamatergic systems are KYNA and 6-hydroxykynurenic acid (6-HKA) [98]. Ginkgolides are selective and potent antagonize at a concentration 10 μM the glycine receptor action and at IC_{50} 73 μM inhibit gamma-aminobutyric acid ($GABA_A$) receptors activity [99]. Ginkgolides A, B, and C noncompetitive inhibit the direct action of α, β, and γ $GABA_A$ receptor [100]. In vivo treatment of rats with EGb and its bioactive components G-A and G-B reduces the ligand-binding capacity, protein, and messenger RNA expression of the adrenocortical mitochondrial peripheral-type benzodiazepine receptor (PBR) [101].

6.3.5 Serotoninergic Transmission

Serotonergic neurotransmission plays a pivotal role in the etiology and expression of stress and anxiety disorders. In vitro EGb-761 (4–16 μg/mL) significantly increase the 5-HT uptake (23 %) and also similar effects have been found in ex vivo synaptosomes preparation from the cortex of mice treated orally (100 mg/kg/day) with EGb-761 [102]. It was found that an age-related decrease in 5-HT_{1A}-receptor binding density in human cerebral cortex. Intraperitoneally administration of EGb-761 (5 mg/kg) resulting significantly (33 %) increase the binding density in aged rats [103]. These findings indicate that changes in 5-HT_{1A} receptors may reflect changes in the brain that are responsible for the impaired cognition that occurs with aging. *G. biloba* extract (14 mg/kg p.o.) restored restraint stress-induced elevation in whole brain levels of catecholamines (NE, DA), 5-HT, and plasma corticosterone to near normal levels [104]. The administration of EGb-761 (50 mg/kg per o.s./14 days) antistress property via enhancing the stimulation of 5-HT_{1A} receptors and preventing their desensitization after subchronic cold stress [105]. In addition another study showed the anti-aggressive effect of EGb-761 may be mediated by 5-HT_{2A} receptors in the MAO-A deficient mice [106]. EGb-761 also induces a stimulus control similar to that of 5-HT_{1A} receptor agonists, and indeed, changes in behavior induced by EGb-761 (10 mg/kg i.p.) were antagonized by the selective 5-HT_{1A} receptor antagonist WAY 100635 [107].

6.4 Free Radical Scavenging

Oxidation and reduction process is one of the cellular activity occurred in each and every kind of cells, whereas reactive oxygen and nitrogen species (RONS) are the major by product formed. Whenever the rate of formation of RONS is more than the

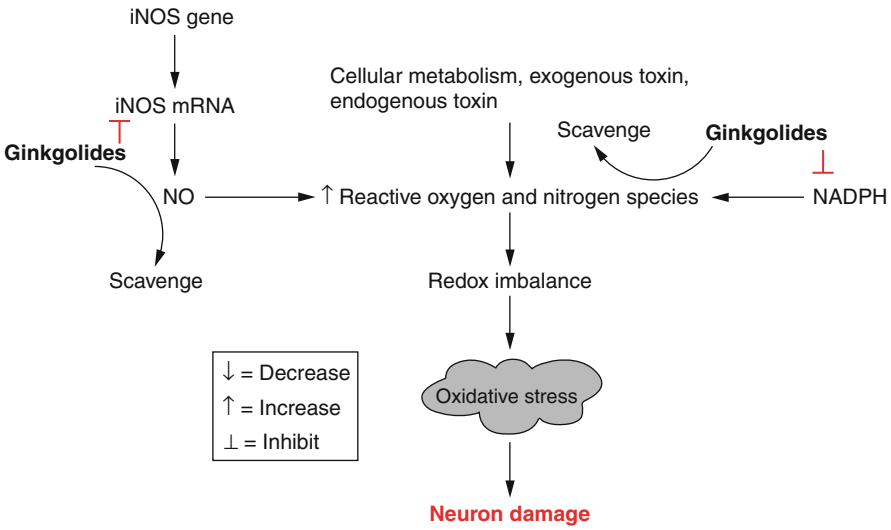


Fig. 122.5 Antioxidant activity of ginkgolides: inhibits NADPH and scavenge RONS activities, inhibits iNOS and scavenge NO

rate of clearance from the cells then the oxidative stress environment created, which cause a number of neurodegenerative disorders including cerebral ischemia, neuronal hypoxia, and AD. Extensive research have been conducted and shown the antioxidant activity of *G. biloba* constituent (Fig. 122.5). In vitro, EGb-761 is a potent free radical scavenger via inhibition of NADPH-oxidase, decreased in the concentration of superoxide anion (O^{-2}) and hydrogen peroxide (H_2O_2) along with the reduction of hydroxyl radical generation ($OH^{\cdot}$) at concentrations as low as 15.6 μg EGb/mL [108, 109]. EGb-761 showed the dose-dependently inhibition of nitric oxide (NO) production in lipopolysaccharide/gamma interferon (LPS/IFNγ)-activated macrophages by concomitantly scavenging NO and inhibiting inducible nitric oxide synthase (iNOS) mRNA and enzyme activity [110, 111]. Excessive iron deposition and mitochondrial insufficiency are responsible for aging and degenerating nervous system. Heme oxygenase-1 (HO-1) is the rate-limiting enzyme that degrades heme to biliverdin, free iron, and carbon monoxide; its immunoreactivity is enhanced greatly in neurons and astrocytes of the hippocampus and cerebral cortex of Alzheimer subjects and co-localizes to senile plaques and neurofibrillary tangles (NFT) [112]. A study showed that EGb-761 induces HO-1 in a dose-dependent manner (0, 10, 50, 100 and 500 μg/mL) and suggested the protective activity in ischemia [113]. In an in vitro study, EGb-761 also displayed protective effects against toxicity produced by either H_2O_2 or nitric oxide which possibly mediate Aβ toxicity and completely blocked Aβ-induced events, such as reactive oxygen species accumulation and apoptosis [114]. Recently, EGb-761 pretreatment (100 mg/kg/o.s.) significantly increased the protein expression levels of Nuclear factor E_2 (Nrf2), HO-1, GAPDH, β-actin,

$CRMP_2$, and histone H_3 during t-BuOOH-induced oxidative stress and showed antioxidant as well as neuritogenic potential and suggesting the beneficial effect of extract in stroke and ischemic brain injury [115]. EGb-761 has been shown to increase the protein level and activity of antioxidant enzymes such as superoxide dismutase (60 %) and catalase (22 %) in rat hippocampus [116] and rat ileum [117] as well as of glutathione (GSH) reductase in mouse liver specifically from G-A [118]. Similarly, the activity of γ-glutamylcysteinyl synthetase, the rate-limiting enzyme of GSH synthesis, was enhanced by EGb-761 (200 μg/mL) in a dose-dependent manner [119]. In vitro study on human neuroblastoma cell line (SK-N-SH), where G-A, and G-B inhibit the NO-induced cytotoxicity in SK-N-SH cells via scavenging [120].

6.5 Apoptosis

EGb-761 (100 mg/L), G-J (100 mM/L), and G-B (10 mM/L) reduces apoptosis from staurosporine-induced apoptotic chick embryonic neurons to 24 %, 62 %, and 31 %, respectively [121]. It is well established that ROS may trigger apoptosis in various types of cells including T cells and neuronal cells. Mice were treated daily with 100 mg/kg EGb-761 per o.s. over a period of 2 weeks showed significantly reduction in ROS-induced apoptosis and protects spleen T-lymphocyte [122]. In vitro study showed that EGb-761 (100 mg/mL) prevented the hydroxyl radical-induced thymocyte apoptosis [123]. In contrast, in vivo study using EGb-761 (250 μg/mL) shown to effectively decrease oral cavity tumors by inducing apoptosis via caspase-3 activation [124]. In addition, another study also support and showed that treatment of mouse blastocysts with 5–10 μM G-A and G-B dose-dependently induced five- to eightfold increases in apoptosis and suggesting their pro-apoptotic activity [125]. Moreover, a recent animal study revealed after administration of 10 and 20 mg/kg G-B significantly suppress gene expressions of TLR-4 and NF-κB, lessen concentrations of TNF-α, IL-1β and IL-6 as well as reduce number of apoptotic neuronal cells in haemorrhagic rat brain tissues [126].

7 *Ginkgo biloba* in CNS Disorders

7.1 Alzheimer's Disease

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, pathologically characterized by deposition of amyloid beta (Aβ) plaques and neurofibril tangles ultimately leads to decline cognitive function and memory. A number of researches have been done on anti-Alzheimer's activity of ginkgo extract, and most of them have shown significant beneficial effect (Fig. 122.6). But also there were some studies which do not show any beneficial activity of ginkgo extract. Even though the results of nonsignificant, we cannot neglect the plenty of significant outcomes from the ginkgo extract. EGb-761 (100 μg/mL) was even able to protect (up to 8 h)

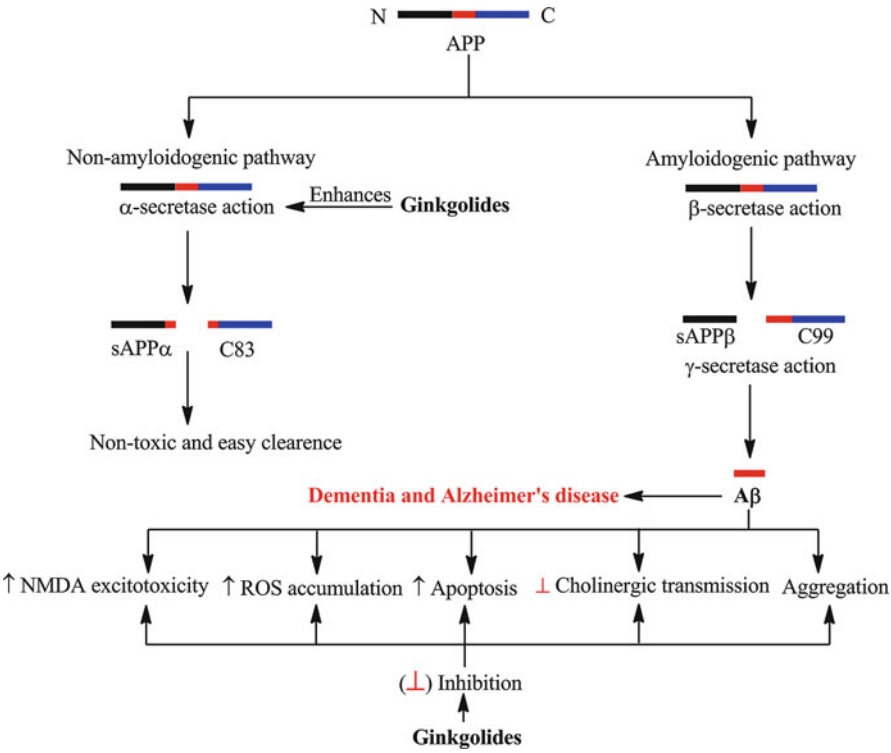


Fig. 122.6 Alzheimer's disease pathogenesis and ginkgolides effect: amyloid precursor protein found in the cytosol and upon action of various enzymes forms soluble nontoxic amyloid and toxic amyloid beta via nonamyloidogenic and amyloidogenic pathway. Secretase involved in non-toxic amyloid formation and ginkgolides enhances this enzyme activity and protect the brain. While amyloid beta from the amyloidogenic pathway induces toxicity by increasing NMDA excitotoxicity, increase in ROS accumulation, increase in apoptosis, inhibition of cholinergic transmission and aggregation. These all events are inhibited ginkgolides and showed neuroproecion against demetia and AD

hippocampal cells against toxicity induced by $A\beta_{25–35}$ and $A\beta_{1–40}$ [127]. Diffusible, nonfibrillar ligands derived from $A\beta_{1–42}$ are potent central nervous system neurotoxins, and these ADDLs soluble oligomers of Aβ have been found in AD brains. An in vitro study [128] showed that EGb-761 inhibits the formation of amyloid beta-derived diffusible neurotoxic soluble ligands (ADDLs) in a dose-dependent manner. Alpha-secretase (α-secretase), the enzyme regulating the non-amyloidogenic processing of APP (cuts within the Aβ segment) and the release of α-APPs, EGb-761, enhance the effect on the α-secretase pathway observed at low concentrations (5 and 25 μg/mL) in hippocampal slices could be counterbalanced by an α-secretase PKC-dependent pathway at higher concentration (100 and 200 μg/mL) [129]. Free cholesterol may be involved in the production of APP and Aβ peptide, key events in the development of AD. EGb-761 (50 mg/kg) lowered circulating free cholesterol

and inhibited the production of brain APP and Aβ after 28 weeks of treatment, as compared with controls rats [130]. As Aβ elicit its neurodegenerating effects by interfering with the central cholinergic system, therefore an in vitro study [131] results showed that G-B (0.01–10 μM) caused a concentration-related reversion of the inhibitory effect elicited by the effective concentration of Aβ (1 μM) and suggesting its anti-amnesic effect by minimizing the inhibitory effect of Aβ peptides on cholinergic transmission. Chronic *G. biloba* extract (similar to EGb-761) treatment (70 mg/kg/day) block an age-dependent decline in spatial cognition without altering Aβ levels and without suppressing protein oxidation in a transgenic mouse model of AD [132]. EGb-761 (100 μg/mL) directly inhibits amyloid fibril formation in solution in vitro and in the cell culture medium, moreover G-J also inhibit (72 %) the Aβ aggregation [133]. In a (SH-SY5Y) neuroblastoma cell line study [134], ginkgolides (A and B) inhibit PAF and that platelet-activating factor antagonists block the toxicity of amyloid-β_{1-42} or sPrP106. The results suggested that PAF antagonists such as the ginkgolides may be relevant treatments for prion or AD [134]. Furthermore, pretreatment with ginkgolides A or B protects neurons against $A\beta_{1-42}$-induced synapse damage, reduced the effects of PAF, and suggested that the ginkgolides are active components of *G. biloba* preparations and may protect against the synapse damage and the cognitive loss seen during the early stages of AD [135]. There is also evidence which showed suppression of Aβ-related pathological behaviors. Among six single components of EGb-761, only G-A and G-J (100 μg/mL) exhibited a statistically significant delay of Aβ-induced paralysis in transgenic worms [136]. Transthyretin plays an important role in hormone transport in the brain and possibly a neuroprotective role by Aβ sequestration. The only gene on the array whose expression was upregulated more than threefold that encodes transthyretin in the hippocampus by dietary supplementation with EGb-761 in a dose of 36 mg/kg [137]. $A\beta_{1-42}$ induces cell apoptosis, reactive oxygen species (ROS) accumulation, mitochondrial dysfunction and activation of c-jun N-terminal kinase (JNK), extracellular signal-regulated kinase 1/2 (ERK1/2), and Akt signaling pathways. In a double-transgenic mouse model (TgAPP/PS1) study [138], EGb-761 (100 mg/kg) significantly increases cell proliferation in the hippocampus of both young (6 months) and old (22 months) TgAPP/PS1 mice, and the total number of neuronal precursor cells in vitro in a (0–120 μg/mL) dose-dependent manner. Furthermore, Aβ oligomers inhibit phosphorylation of cAMP response element-binding protein (CREB) and cell proliferation in the hippocampus of TgAPP/PS1 mice. Administration of EGb-761 (100 mg/kg) reduces Aβ oligomers and restores CREB phosphorylation in the hippocampus of these mice and suggesting therapeutic potential for the prevention and improved treatment of AD [138]. In other transgenic mice for human APP (Tg2576) model study [139], long-term treatment (16 months) with EGb-761 (300 mg/kg diet) significantly lowered human APP protein levels by approximately 50 % as compared to controls in the cortex but not in the hippocampus. However, APP levels were not affected by EGb-761 in young mice, suggesting the potential neuroprotective properties of EGb-761 may be, at least partly, related to its APP lowering activity [139]. An in vitro study [140] revealed that EGb-761 prevent the activation of NF-κB, ERK1/2, and JNK pathways induced by Aβ in

neuroblastoma cell line N2a. Another human neuroblastoma SH-SY5Y cell line study [141] showed that EGb-761 (50–200 μg/mL) constituents G-B (5–20 μg/mL) along with quercetin (1.5–6 μg/mL) involved in the inhibitory effects on $A\beta_{1-42}$ induces cell apoptosis, reactive oxygen species (ROS) accumulation, JNK, ERK1/2, and Akt signaling pathways. While only G-B helped to improve mitochondrial functions. Decreased clearance of Aβ from brain is the main root cause of their deposition in sporadic AD. However, the mechanisms underlying ischemia-mediated AD pathogenesis remain unclear. The receptors for advanced glycation end products (RAGE) and low-density lipoprotein receptor-related protein-1 (LRP-1) expressed at blood–brain barrier (BBB) are actively involved in Aβ clearance. In vitro study [142] suggested that EGb-761 favor clearance of Aβ via regulating the expression of RAGE and LRP-1 during brain ischemia. Synaptic dysfunction is likely to occur at early stages of AD. Low levels of oligomeric Aβ alter mechanisms underlying the excitatory response at single synapses producing synaptic dysfunction before synapse loss, cell death, and a complex series of events including inflammation, deposition of Aβ in senile plaques and within the walls of the cerebral microvasculature and appearance of neurofibrillary tangles. A new *G. biloba* extract P8A, 70 % enriched with terpene trilactones, prevents $A\beta_{1-42}$ induced inhibition of long-term potentiation in the region I of hippocampus proper (CA1) in mouse hippocampal slices and also capable of inhibiting cell death of rodent hippocampal neurons caused by $A\beta_{1-42}$, which is attributed in large part to G-J (1–5 μM) that completely replicates the effect of the extract [143]. EGb LI-1370 (100 μg/mL) significantly improved oxidative phosphorylation system performance and was able to restore Aβ-induced mitochondria failure [144]. An in vitro study [145] showed that G-B (40 μg/mL) significantly dampens $A\beta_{25-35}$-induced apoptosis, and the neuroprotective effects may be intimately associated with brain-derived neurotrophic factor upregulation caused by G-B. The *N*-methyl D-aspartate receptor (NMDA) plays a pivotal role in the process of glutamate-induced excitotoxicity associated with many neurological disorders including AD. Studies in isolated rat hippocampal neurons indicated that the modulatory effects of EGB on NMDA-activated currents may contribute to the neuroprotective effects of two solvent preparations that is mEGB (0.1 mg/mL, dissolved in DMSO) and nEGB (0.1 mg/mL) either dissolved in DMSO or dissolved in standard extracellular solution where the modulatory effect of nEGB on NMDA-activated current was greater than that of mEGB [146].

7.1.1 Clinical Trials

In a randomized, double-blind, placebo-controlled, multicenter study (24 weeks) on 156 (222 patients at entry) outpatients with presenile and senile primary degenerative dementia of the Alzheimer type (DAT) and multi-infarct dementia (MID) [147], where patients received a daily oral dose of 240 mg of EGb-761 or placebo. There was a significant difference in the number of responders at the end of the treatment (28 % for EGb-761 compared with 10 % for placebo), suggesting that EGb-761 is of clinical efficacy in the treatment of outpatients with dementia. Furthermore a placebo-controlled, randomized, double-blind clinical trial [148] of 40 patients with moderate dementia received intravenous infusions of either

EGb-761 or placebo 4 days per week for 4 weeks. The result showed in an improvement of psychopathology and cognitive performance, which is reflected in an increased ability to cope with the demands of daily living. In addition, placebo-controlled, double-blind, randomized trials [149], 137 patients (327 patients at entry) were treated for 52 weeks with 120 mg of EGb-761. The patients showed significant improvement in learning, memory, visual and spatial orientation, and social behavior. A meta-analysis of effect size on the results of the 4 studies of more than 50 studies considered that met the author's inclusions criteria [150]. This analysis revealed a highly significant overall effect of ginkgo compared with placebo. The results showed that there is a small but significant effect of 3- to 6-month treatment with 120–240 mg of *G. biloba* extract on objective measures of cognitive function in AD [150]. In a long-term (26 weeks) study [151] on mild to severe AD patient, EGb-761 treatment with a 120-mg dose (40 mg t.i.d.) and the placebo group showed a statistically significant worsening in all domains of assessment, while the group receiving EGb-761 was considered slightly improved on the cognitive assessment and the daily living and social behavior. A retrospective analysis [152] explored whether the therapeutic effect of EGb-761 (120 mg) in AD depends on baseline severity. Treatment effect favorable for EGb-761 could be observed with respect to cognitive performance and social functioning, regardless of the stage of dementia. However, improvement was observed only in the group of patients with very mild cognitive impairment, while in more severe dementia, the mean effect of EGb-761 should be considered in terms of stabilization or slowing down of worsening [152]. An interesting result of a 6-week study [153] on healthy volunteers (203 completed out of 230) indicates that ginkgo (40 mg, t.i.d.) did not facilitate performance on standard neuropsychological tests of learning, memory, attention, and concentration or naming and verbal fluency in elderly adults without cognitive impairment. The ginkgo group also did not differ from the control group in terms of self-reported memory function or global rating by spouses, friends, and relatives. These data suggest that ginkgo provides no measurable benefit in memory or related cognitive function to adults with healthy cognitive function [153]. In a randomized controlled trial [154] of 359 dementic patient aged 50 years or above, treated with EGb-761 (240 mg/day) or placebo for 22 weeks. Their short syndrome test (SKT) score improved by −3.0+/−2.3 and −3.4+/−2.3 points in patients with AD and VaD, respectively, whereas the patients on placebo deteriorated by +1.2+/−2.5 and +1.5+/−2.2 points [154]. Another, randomized, double-blind exploratory trial [155] of 96 outpatients, aged 50 years or above, where EGb-761 (240 mg/day), donepezil (initially 5 mg, after 4 weeks 10 mg/day) or EGb- 761 and donepezil combined (same doses) were administered for 22 weeks. The results showed no significant difference in the efficiency between EGb-761 and donepezil, but a combination therapy will be superior to a mono-therapy with one of both substances with fewer side effects under a combination therapy than under monotherapy with donepezil [155]. In a German study [156], it was reported that the efficacy of EGb-761 has its place in the treatment of dementia. EGb-761 in the treatment of dementia (AD and VD) had been studied in 10 randomized controlled, double-blind clinical trials. In 3 of the

4 large trials conducted in accordance with recent recommendations, EGb-761 was significantly superior to placebo with respect to cognitive performance and one or more further (global, functional, or behavioral) outcomes demonstrating the clinical relevance of the findings. The findings from the 6 smaller trials were in line with those of the large trials [156]. Recently, published systematic review and meta-analysis [157] using 9 relevant trials were found statistically significant advantage of *G. biloba* extract compared to placebo in improving cognition for the whole group of patients with AD, vascular, or mixed dementia. While regarding activities of daily living, there was no significant difference for the whole group. However, in the subgroup of patients with AD, there was a statistically significant advantage of *G. biloba* extract compared to placebo [157]. Moreover, a recent multicenter trial [158] of 410 outpatients with mild to moderate dementia (AD, VD, or mixed form) with neuropsychiatric features, where the patients were treatment with 240 mg of EGb-761 or placebo once daily for 24 weeks. The results showed significantly superior to placebo in the treatment of patients with dementia with neuropsychiatric symptoms [158]. More recently, in a multicenter, double-blind, randomized, placebo-controlled, 24-week trial [159] with 410 outpatients, treatment with EGb-761 at a once daily dose of 240 mg was safe, confers the previous study findings, which is resulted in a significant clinically relevant improvement in cognition, psychopathology, functional measures, and quality of life of patients and caregivers.

7.2 Parkinson's Disease

Parkinson's disease (PD) is characterized by the degeneration of the dopaminergic nigrostriatal pathway, as indicated by the severe loss of substantia nigra neurons and by the decrease in striatal dopamine (DA) concentration. N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA) selectively causes degeneration of the nigrostriatal dopaminergic neuronal pathway and used as lesion model in several studies to investigate the possible protective effect of EGb-761. Semi-chronic ingestion of EGb-761 for 17 days (100 mg/kg/day) prevents MPTP-induced reduction (approximately 25 %) in the striatal dopaminergic nerve endings in mice [160]. The 2 mono-amino oxidase (MAO) isoforms (A and B) regulate levels in the brain of most biogenic amines (e.g., dopamine, serotonin, and norepinephrine), and it is also well established that MAO-B activity increased with age and that some MAO-B inhibitors may improve quality of life in the elderly. Interestingly, 2 *G. biloba* extracts different from EGb (dried leaved extract) have been reported to induce reversible inhibition of both MAO-A and MAO-B activity in rat brain mitochondrial extracts [161]. Moreover, EGb-761 administered before (20, 50, 100 mg/kg/d i.p.) or after (50 mg/kg/d i.p.) MPTP treatment effectively protects against MPTP-induced nigrostriatal dopaminergic neurotoxicity and that the inhibitory effect of EGb-761 on brain MAO may be involved in its neuroprotective effect in mice [88]. EGb (100 mg/kg/d) decreased the duration and frequency of the rotation of rats ($P < 0.05$, $n = 10$) while EGb (50 or 100 mg/L) inhibited the decreases of dopamine (DA) and superoxide dismutase (SOD) and the

increase of malondialdehyde (MDA) induced by MPTP [162]. 6-OHDA-induced rat models of PD study suggested that levodopa (50 mg/kg/day for 3 days, 5 days, 7 days, L-dopa group) had neurotoxic effect, and EGb (100 mg/kg/day) decreases the toxicity of levodopa [163]. EGb-761 (100 mg/kg/day) was investigated on 6-OHDA-induced neurotoxicity in the nigrostriatal dopaminergic system of the rat brain and reduces the behavioral deficit in 6-OHDA lesions in rat and also indicates a possible role for the extract in the treatment of PD [164]. Rats were treated with 50, 100, and 150 mg/kg EGb for 3 weeks showed dose-dependent protection against 6-OHDA-induced Parkinsonism in rats [89]. EGb-761 attenuates MPTP-induced neurodegeneration of the nigrostriatal pathway and suggesting that an inhibitory effect against oxidative stress possibly partly responsible for its observed neuroprotective effects [90]. Recently, chronic treatment with EGb-761 (100 mg/kg/14 days/once daily) showed dose-dependent increases in frontocortical dopamine levels [91].

7.2.1 Clinical Trials

The in vitro and animal data suggesting the beneficial effect of EGb-761 in PD, but there is no any clinical trial conduct to investigate the protective effect in human. Therefore, there is a need of conduction of such trials.

7.3 Anxiety

Anxiety is a condition which describes a normal feeling person experience when faced with threat, danger, or when stressed and the anxious condition makes a person upset feeling, uncomfortable, and tense. EGb-761 (100 mg/kg in 5 % ethanol) treatment group inhibited the development of polydipsia in rats due to the stress of daily handling and intubation [165]. In a 20 days of oral treatment with an extract EGb-761 (50 or 100 mg/kg/day) showed that auditory perturbation (stress) during the discriminative phase of learning decreased the percentage of correct responses and increased the number of errors in young as well as old rats [166]. Chronic administration of EGb-761 (50 or 100 mg/kg p.o. daily for 14 days) inhibits stress-induced corticosterone hypersecretion through a reduction in the number of adrenal peripheral benzodiazepine receptors [101]. Another, long-term EGb-761 (50 or 100 mg/kg p.o. daily for 14 days) administration study [167] on rats resulted in a decreased basal corticosterone secretion and an attenuation of the related increase in corticotropin releasing hormone (CRH) and arginine vasopressin (AVP) gene expression, while chronic i.p. injection of G-B reduced basal corticosterone secretion without alteration in the subsequent CRH and AVP increase. Under intense surgical stress CRH, ACTH, and corticosterone plasma concentrations were markedly elevated in control animals but significantly less so in EGb-761 treated animals. These findings confirm that the administration of EGb-761 and G-B reduces corticosterone secretion suggest that EGb-761 interferes with the regulation of the activity of the hypothalamic-pituitary-adrenocortical (HPA) axis [167]. There were few studies conducted and showed the anxiolytic effect of EGb-761 in

combinational therapy. Hasenohrl et al., in the year 1996 and 1998 [168, 169], showed the anxiolytic effect of a combination preparation of standardized extracts of *G. biloba* and *Zingiber officinale*. Ginkgolic acid conjugates (GAC), isolated from the *G. biloba* leaf, having similar profile with EGb-761, GAC (0.6 mg/kg) significantly increased ambulation and reduced the immobility time, suggesting the anxiolytic activity [170]. EGb-761 (100 mg/kg/day) serve as an antistress buffer, showed attenuating the increase in anxiety in mice after cold water exposure [171]. EGB (0.125 g/kg, p.o.) produces a significant anxiolytic-like effect following repeated administration and that G-A (1 or 2 mg/kg, p.o.) is most likely responsible for this effect [172]. It was found that *G. biloba* extract (14 mg/kg p.o.) restored restraint stress-induced elevation in whole brain levels of catecholamines (NE, DA), 5-HT, and plasma corticosterone to near normal level [104]. In prevention and treatment of the post-stress memory dysfunctions study [173], repeated administration of EGb-761 (100 mg/kg) prevents stress and corticosterone-induced impairments of information retrieval in rats.

7.3.1 Clinical Trials

A few clinical studies have conducted to evaluate the anxiolytic activity of ginkgolides. An early German studies, patients enrolled with cerebrovascular disease induced cognitive impairment, in which anxiety was one of the noncognitive symptoms relieved significantly by EGb-761 treatment [174]. In a pilot study [175], EGb (240 mg/day) significantly improved sleep pattern by an increase of sleep efficiency and a reduction of awakenings especially in the treatment of the depressive syndrome with sleep disturbance. In a trial on healthy volunteers [176], single treatment with EGb-761 (120 mg) reduced stress-induced rise in blood pressure without affecting the heart rate and influence salivary cortisol release in response to some stress stimuli. A randomized, double-blind, placebo-controlled trial [177] suggested that EGb-761 (240 and 480 mg/day) has a specific anxiolytic effect that is dose-dependent and significantly exceeds the placebo effect commonly seen in trials of psychoactive drugs.

7.4 Cerebral Insufficiency/Ischemia and PAF

Cerebral insufficiency refers to describe the people with age-related decline in mental function and decrease blood flow to the brain cause by clogged arteries. Platelet activating factor (PAF) is an inflammatory mediator, plays an important role in allergy, inflammatory processes, coronary, and cerebral vasoconstriction [178]. The production and release of PAF in the brain under various pathological conditions, including oxidant stress-induced ischemic injury [179]. The efficiency of EGb-761on cerebral circulation and metabolism has been demonstrated in various models of cerebrovascular insufficiency and showed beneficial effect like cerebral edema in rats intoxicated with triethyltin chloride (TET) [180]. Moreover, oral or intravenous administrations EGb decrease cerebral edema development in gerbils [181]. In an animal study [182], EGb-761 and an extract of local ginkgo

leaf (LGb) improved spatial memory function with chronic cerebral insufficiency (produced by bilateral common carotid artery ligation) from the second week after operation, but only EGb-761 delayed deterioration of motor functions from the fifth week after operation.

EGb enhanced the local cerebral blood flow as well as the blood glucose level dose-dependently but decreased the cortical glucose concentration without other substrate levels being changed in rat [180]. EGb-761 (110 mg/kg/day) could prevent the ischemia-induced impairment of the Na,K-ATPase activity [183]. Pretreatment (15 days) with oral administration of *G. biloba* extract (Ph-GB; 37.5–150 mg/kg) significantly and in a dose-dependent way reduced post-ischemic brain MDA (malondialdeide) levels and post-ischemic brain edema in gerbils [184]. Oral administration of EGb-761 at a dose of 25, 50, and 100 mg/kg/day protects against ischemia-induced neuron death and reductions in mitochondrial gene expression in gebrils [185]. In addition, EGb-761 (100–200 mg/kg) protects against transient and permanent focal cerebral ischemia and was effective after a prolonged reperfusion period even when therapy is delayed up to 2 h in rats [186]. EGb-761 (100 mg/kg) also showed protection of neuronal cells against ischemic brain injury by preventing injury-induced decreases in p70S6 kinase and S6 phosphorylation in rats [187]. In contrast, a recent study showed that after p.o. administration of G-B (6 mg/kg) once daily for 7 days does not cause acute increase in cerebral blood flow after reperfusion, especially in hyperglycemia condition but effective in reactive oxygen species or MDA control in hyperglycemia ischemic rats [188]. G-B protects against cerebral ischemic injury by inhibiting excitotoxicity by modulating the imbalance of excitatory amino acids versus inhibitory amino acids, which may support the traditional use of *G. biloba* leaves for the treatment of stroke [189]. Pretreatment with G-K (2, 4, and 8 mg/kg (i.v.) once a day for 5 days) significantly diminished the volume of infarction and brain water content and improved neurological deficit score [190]. Moreover, G-K markedly reversed the level of MDA, NO, NOS, and SOD to their normal state in serum or cerebral ischemic section. Another recent in vitro (brain slices) and in vivo study [191] revealed that administration of EGb-761 (300 mg/kg) strongly reduces cellular edema formation and neurodegeneration under conditions of ischemia possibly via reduction of excitotoxicity because ischemia-induced release of glutamate was strongly suppressed.

Administration of ginkgolide B (BN-52021) in dose of 10 mg/kg/day via i.p. or oral-improved stroke index scores (determined by symptoms ranging from piloerection to seizures) and protect brain against hypoxic damage with their PAF antagonistic properties following bilateral carotid artery occlusion in the gerbil [192]. Free fatty acids, diacylglycerols, and polyphosphoinositides were accumulated in ischemic condition. BN-52021 (10 mg/kg, i.p.) inhibited the maturation of ischemic injury; increased cerebral blood flow and increased free fatty acid levels were reduced likely by inhibition of phospholipase A [193]. Moreover pretreatment with BN52021 (10 mg/kg, i.p.) reduces the injury-induced activation of phospholipase A2 and lysophospholipase, which mediate the accumulation of FFA in mice brain [194]. A temperature-controlled model of transient forebrain ischemia in the rat receiving BN-52021 (25 mg/kg, s.c.), 1 h before and 1 h after the induction of

transient forebrain ischemia, exhibited a significant reduction in hippocampal and neocortical damage and proposed that PAF plays an important role in the pathophysiology of ischemic/excitotoxic neuronal injury via a direct action on neurons [93]. A comparative study [25] confirmed that induction of aggregation of human platelets by PAF requires at least 200 times higher concentration when compared to rabbit cells. Under the chosen experimental conditions, PAF-mediated aggregation of human platelets was half-maximally inhibited by ginkgolide B, A, C, and J at concentrations of 2.5, 15.8, 29.8, and 43.5 μg/mL, respectively [25]. In an animal study [195], both pre- and posthypoxic treatment with BN 52021 (25 mg/kg/dose, two serial doses) decreased the incidence of cerebral infarction from 90 % to about 30 %. The result suggested either prophylactic or rescue administration of PAF antagonists decreases the incidence and severity of brain injury associated with an episode of perinatal cerebral hypoxia-ischemia [195]. In a photochemically induced thrombotic cerebral ischemia in tree shrews model, G-B (5 mg/kg, i.v.) 6 h after photochemical reaction, cortical NA, DA, and 5-HT contents recovered to control levels and water, and calcium contents decreased significantly [196]. The results suggested that PAF may play an important role in inducing calcium overload, brain edema, and secondary brain damage in penumbra and that G-B produces its neuroprotective effects by inhibiting the pathological manifestation of PAF [196]. A recent animal study [197] showed that administration of G-B (10 or 20 mg/kg) before ischemia reduced the ischemia-induced elevation of levels of glutamate, aspartic acid, and glycine, increased the elevation of extracellular GABA, decreased the excitotoxic index, and diminished the volume of cerebral infarction. The results suggested the protection against cerebral ischemic injury by G-B-induced inhibition excitotoxicity by modulating the imbalance of excitatory amino acids versus inhibitory amino acids [197].

7.4.1 Clinical Trails

There were numerous clinical trial conducted between 1980s and 1990s, and most of them were shown a significant result. In an open one year German trial [198], *G. biloba* extract at a dose of 120 mg/day showed a statistically significant regression of the major symptoms of vertigo, headache, tinnitus, short-term memory, vigilance, and mood disturbance in 112 outpatient with chronic cerebral insufficiency. In a multicentric, double-blind, EGb versus placebo French trial [199] involving 166 patients confirmed that *G. biloba* extract is effective in 3 months against cerebral disorders due to aging. Moreover, in double-blind, randomized placebo-controlled study [53] of 24 weeks duration in outpatients with cerebral insufficiency showed statistically significant improvement in the short-term memory after 6 weeks and of the learning rate after 24 weeks in the test substance group. A critical review identified 40 such trials included small patient numbers [200]. Nevertheless, 8 out of 40 trials were found to be well performed, and the qualities of trials were sufficient enough to make credible conclusions. In these 8 trials, patients were typically given 120- to 160-mg *G. biloba* extract daily for at least 4–6 weeks. All 8 trials reported positive results and supported the conclusion that *G. biloba* extracts reduce the symptoms of cerebral insufficiency to the extent that is clinically

relevant [200]. A meta-analysis including 11 clinical trials revealed the usefulness of the *G. biloba* extract, Kaveri (LI-1370), in cerebral insufficiency [54]. Three studies were excluded due to methodological inadequacies. In one study, the findings were inconclusive, but the pooled data from the remaining trials confirmed the effectiveness of the extract compared to placebo controls. Patients received Kaveri (150 mg per day, oral) for a period of 12 weeks, and the results support the clinical use of *G. biloba* extracts for cerebral insufficiency [54]. The results suggested the further need of clinical evaluation investigation in healthy as well as cerebral insufficiency/ischemic patient.

7.5 Retinal Degeneration and Glaucoma

The lens of the eye focuses an image of an object on a portion of the retina called the *macula*, the area of finest visual perception. *Gingko* extract have shown beneficial effect in macular degeneration, reduced intraocular pressure, and reduced ocular blood flow and glaucoma. Early studies showed that EGb reduces ischemia-reperfusion injury in rat retina [201, 202] as well as inhibits the preretinal proliferation in experimental tractional retinal detachment [203]. In an isolated rat retina model study suggested the existence of PAF-acether-specific receptors inside the retina. Simultaneous administration of ginkgolide B (BN 52021; 2×10^{-5} M) inhibited an irreversible decrease of the electroretinogram β-wave amplitude [204]. EGb-761 (50 mg/kg, per o.s.) was administered in a daily dose for 10 days showed significantly reduction in the maldistribution of ions induced by ischemia and reperfusion in rat retina obtained from normotensive and spontaneously hypertensive rats [205]. It was reported that EGb-761 (40 mg/kg) protects against susceptibility of rabbit retinal cells from proteolytic enzymes [206]. EGb have a protective effect against the progression of diabetic retinopathy and neuropathy [207]. Pretreatment and early posttreatment with EGb-761 protect and effective against neurotoxicity of retinal ganglion cells of rats with chronic moderately elevated intraocular pressure (IOP) [208]. Intraperitoneal injection of EGb-761 enhances the antioxidation ability of retina and partially inhibits the apoptosis of photoreceptors and exerts a protective effect on photoreceptors [209]. Intragastral administration of a *G. biloba* extract applied after an experimental and standardized optic nerve crush in rats were associated with a higher survival rate of retinal ganglion cells in a dosage-dependent manner [210]. Intraperitoneal injections of a *G. biloba* extract given prior to and daily after an experimental and standardized optic nerve crush in rats were shown associated with a higher survival rate of retinal ganglion cells [211]. In a recent study [212], pretreatment with EGb-761 prevented the focal cerebral ischemic injury-induced decrease in PEA-15 (phosphoprotein enriched in astrocytes 15) expression in rats.

7.5.1 Clinical Trials

In a 6-month, double-blind, placebo-controlled study of 10 people with macular degeneration, use of ginkgo at a dose of 160 mg daily resulted in a statistically

significant improvement in long-distance visual acuity [213]. In a French double-blind trial, in 29 diabetic subjects with an early diabetic retinopathy (6 months period) showed an improvement tendency was evidenced in EGb treated subjects [214]. Phase I crossover trial [215] of with either EGb 40 mg or placebo 3 times daily orally in 11 healthy volunteers was treated for 2 days showed significantly increased end diastolic velocity (EDV) in the ophthalmic artery (OA). EGb-761 was investigated in a controlled, double-blind trial involving 99 patients with impaired vision due to senile, dry macular degeneration for 6 months [216]. Both the dosages (240 mg/day or 60 mg/day) of EGb-761 results in increase therapeutic efficacy of EGb-761 in patients with senile, dry macular degeneration, with obvious benefits in everyday life [216]. A small double-blind, placebo-controlled trial found that use of ginkgo extract at a dose of 120 mg daily for 8 weeks significantly improved vision in people with glaucoma [217]. In a randomized, double-masked, placebo-controlled, two-way crossover study [218] included 15 healthy male volunteers, before and up to 3 h after oral intake of 240 mg EGb-761 cause significantly decreased retinal venous diameters, but there was no significant difference between the two groups. The optic nerve head blood flow was significantly increased in response to *G. biloba*, but this effect was not significant compared with that of placebo. However, the results suggesting the drug may influence ocular blood flow in patients with ocular vascular disease after long-term treatment [218]. In a case study [219], 11 months after commencing *G. biloba* (120 mg/day) treatment, visual acuity improved to 20/80 OD and 20/40 OS, and subsequently at 30 months follow-up, his visual acuity improved further to 20/40 OD and 20/30 OS.

7.6 Vestibular Dysfunction

Vertigo is a type of dizziness characterized by "spinning" sensation in the head and is usually brought on by sudden changes in position. Ginkgo extract have shown significant beneficial influence on the vestibular system, particularly on compensation after vestibular lesions in experimental animals. In a chemical-induced labyrinthectomy rats, EGb-761 (50 mg/kg per day, i.p.) administration for 73 days post-surgery significantly accelerated compensation of static postural symptoms and spontaneous nystagmus compared with non-treated controls [220]. But there was a limitation of all of these studies that control animals have not received vehicle injections. Due to missing in conduction of a dose–response analysis, there was no evident whether 50 mg/kg/day i.p. was the optimal dose [220]. In an animal study, postoperative administration of EGb-761 (50 mg/kg/day, i.p.) for 30 days following UVD in cats has been shown to accelerate the compensation of postural, locomotor dysequilibrium, and oculomotor symptoms [221]. EGb-761 was administered over 30 days at daily doses of 50 mg/kg i.p. in cat strongly accelerated postural and locomotor balance recovery and demonstrated that EGb-761 acts on

recovery mechanisms considered as key processes in vestibular compensation [222]. Guinea pig vestibular nuclei perfused with EGb-761 has a direct excitatory effect on the lateral vestibular nuclei (LVN) neurons and also i.p. administration of EGb-761 led to a reversible, dose-dependent decrease of the horizontal vestibule-ocular reflex (HVOR) gain without affecting the phase of the reflex [223]. In a comparative study [224] of EGb-761 components (terpenes vs. flavonoids) contained extract, examining on equilibrium function recovery in the unilateral vestibular neurectomized cat. Administration of EGb-761 orally (p.o./2 groups; 40 mg and 80 mg/kg) or intraperitoneally (i.p/2 groups; 50 mg and 25 mg/kg), whereas the 2 others received only a special extract that did not contain the terpenes (i.p. administration: 25 mg and 10 mg/kg) significantly improved the locomotor balance recovery in all the experimental groups as compared to the control groups [224]. There was also significant pharmacological activity of the extract when given i.p. as compared to the p.o. route of administration, and dose-dependent effects were evidenced with the i.p. administration of the special extract without the terpenes, with a lower efficacy for the lowest dose (10 mg/kg) [224]. In an animal study [225], G-B was investigating for the behavioral recovery process (vestibular compensation) which occurs following surgical removal of the vestibular receptor cells in one labyrinth (unilateral labyrinthectomy, UL). Guinea pigs received a single i.p. injection of G-B at the time of the UL (25, 50, or 100 mg/kg), and the effects were evaluated on the compensation of the UL symptoms, spontaneous ocular nystagmus (SN), yaw head tilt (YHT), and roll head tilt (RHT). A single i.p. injection of G-B (25 mg/kg) at the time of the UL produced an acceleration of SN compensation [225].

7.6.1 Clinical Trials

In a randomized, placebo-controlled, double-blind trial in patients (out of 50, 33 completed) with vertigo and ataxia symptoms using EGb-761 (120 mg/day) for 12 weeks showed lateral sway amplitude in the cranio-corpography (CCG) and proportion of subjective improvement [226]. In another randomized, placebo-control, double-blind trial on 35 patients with peripheral vestibular vertigo treated with EGb-761 (160 mg/day) for 12 weeks resulted in sway amplitude in posturography and suggesting combinational therapy with *G. biloba* [227]. In a double-blind trial extending over a 3-month period, the patient (out of 70, 67 completed) with vestibular vertigo were given either EGb-761 (160 mg/day) or a placebo. At the end of trial 47 % of the patients treated were rid of their symptoms as against 18 % of those who received the placebo [228]. In a non-vestibular group of trial, 80 patient receiving EGb-761 (160 mg/day) for 12 weeks showed proportion of the patients free from the symptoms and greatly improved [229]. An open, randomized study of 45 patients suffering from vertigo induced by peripheral vestibular lesions is interesting [230]. All patients participated in a physical training program, 23 patients received EGb-761 in addition. In these patients, posturographic investigations showed a more rapid reduction in sway amplitude [230]. A systematic review published in 2007 showed the beneficial effect of EGb-761

on vestibular compensation in various preclinical and clinical studies [231]. The author suggested the presence of efficacy of EGb-761 for the treatment of vertiginous syndromes in the available studies [231].

7.7 Other Neuroprotective Activities

Neuroinflammation is characterized by activation of local glial cells and production of various pro-inflammatory mediators which lead to the abnormalities in neurons and astrocytes. Cytokine IL-1β has been implicated in the extensive inflammation and progressive neurodegeneration that occurs after ischemia. Brain ischemia induces production of both TNF-α and IL-1β which may disrupt phosphatidylcholine homeostasis by increasing its hydrolysis and inhibiting the synthesis. In a trial of 79 patients suffering from chronic, age-related neurological disorders, treatment with 9.6 mg of EGB (ginkgo extract) twice daily for 8 weeks shown a statistically significant decline IL-6 level to near normal values, but there were no significant changes observed in serum levels of IL-1β and TNF-α [232]. In a pilot study [233] of 10 multiple sclerosis patients in acute relapse were treated with a 5-day course of intravenous G-B. 8 patients had improvement of their neurological score, beginning 2–6 days after the initiation of therapy of G-B [233]. In contrast, a randomized double-blind placebo-controlled trial [234] on 104 multiple sclerosis patients, 43 received placebo, 29 received 240 mg/day G-B, and 32 received 360 mg/day ginkgolide B for 7 days. The result does not showed any significant result, and suggesting G-B is not an effective treatment of exacerbations of multiple sclerosis [234]. Treatment with BN-52021 (10 mg/kg) attenuates the development of early posttraumatic cerebral edema in rats subjected to a mild traumatic insult [235]. The author suggested that PAF may be involved in the pathogenesis of posttraumatic cerebral edema [235]. Moreover, administration of BN-52021 (1 or 10 mg/kg i.v.) 15 min prior to, and 120 min after, fluid percussion-induced traumatic brain injury resulted in the reduction of neurological deterioration due to traumatic brain injury [236].

8 Ginkgolides Pharmacokinetics

Ginkgolide B showed about 50 % of metabolism in vivo and suggesting its hydroxyl metabolites its principal metabolites [237]. A dosage of 40-mg G-B twice daily (every 12 h) is accompanied by a significantly longer half-life ($t_{1/2}$) and mean residence time (MRT) than a single 80-mg dose, even though the latter causes a higher concentration peak (C_{max}). The maximum concentration time (T_{max}) is 2.3 h after administration in both treatments [238]. After each single dose of G-A, G-B and bilobalide ranging from 0.90 mg to 3.36 mg, blood and urine samples were collected for up to 36 h and 48 h, while fasting, the extents of bioavailability are high, as shown by bioavailability coefficients (FAUC) mean (+/− SD) values equal to 0.80 (+/− 0.09) and 0.88 (+/− 0.21) for G-A and G-B respectively [239]. In short, G-A and G- B are nearly completely bioavailable [240].

9 Side Effect

As *G. biloba* is one of the very popular herbal supplementary in dose range of 120–240 mg/day due to its versatility in human health benefits and safer efficacy. But, there were few cases have been reported the side effect of *G. biloba* including minor (stomach upset, skin reaction and headache or dizziness) and severe (haemorrhage). At a higher dose of ginkgo extract in initial supplementary or therapy dose leads to stomach upset. It is recommended to start with a lower dose and titrate as tolerated to minimize or ovoid the gastrointestinal side effects. In some cases, allergic skin reactions is caused by the herbal remedy *G. biloba* extract and that resolve after discontinuing ginkgo therapy [241]. There is also a case of Stevens-Johnson syndrome reported in a patient after their second dose of a combination herbal product containing EGB [242]. Ginkgo seeds contain ginkgo toxin, which can cause seizures, difficulty breathing, loss of consciousness, and shock when consuming more than 10 roasted seeds daily. It is also found that the availability of ginkgo toxin is much higher concentrations in ginkgo seeds than in ginkgo leaf extract. There is an evidence of about 20 detailed reports of hemorrhage (usually cerebral, ocular, or postsurgical) in patients using *G. biloba* extracts have been published [243]. One third of these patients were also taking drugs that increase the risk of bleeding (anticoagulants or antiplatelet drugs). Therefore there is a cautionary recommendation established in practice, patients with risk factors for bleeding (anticoagulant or antiplatelet treatment, surgery, etc.) should avoid using *G. biloba* extracts [243].

10 Conclusion

Aging, which is a fate of all creatures, is a challenge to every living being. There is a cellular, structural, and functional changes occur in the brain during aging. Nerve cells may respond to these changes adaptively, or they may succumb to neurodegenerative cascades that result in disorders such as AD and PD. Neurodegenerative diseases have drawn a lot of attention due to their irreversibility, lack of effective treatment, and accompanied social and economic burdens. *G. biloba* showed various pharmacological effects, among them multiple researches have been demonstrated their neuroprotective effect in the prevention and recovery of cognition, ischemic, antioxidant, anti-amyloidogenic, and PAF inhibitory abilities. It maintains blood vessel health, reduces blood viscosity, and enhances blood supply to the brain, which has recently been implicated as a causative factor for stroke.

At the present time, there are no definitive prevention and treatments for dementias or age-related cognitive decline. The significance of these neurological disorders mandates that every therapeutic option should be investigated with rigorous scientific methodology. There is a major portion (28 %) of unknown or unidentified compounds in ginkgo extract which are need to be investigating because it may be possible that they exhibit the same effects like ginkgolides or other active constituents. Moreover the basic pharmacology and therapeutic potential of *G. biloba* extracts (EGB-761) in

neuroprotection have understood and also the activity of ginkgolide has also been evaluated separately in neurological disorders. But there is a lack of clinical research in PD which is one of the major neurodegenerative disorders. The question is EGb-761 does not contains only ginkgolides at a major constituents, it contributes about 6–7 % of the total components. Moreover, it may be possible that the other bioactive constituents also participating in neuroprotection activities. Even though the gingkolides were used combination and fewer in the total content, we cannot underestimate their role in neuroprotection. Therefore, the complete neuropharmacological potential of ginkgolides will be understood through coordinated in vitro/in vivo animal investigations and should be confirmed with human placebo-controlled, double-blinded research designed to objectively measure relevant functional parameters including their safety parameters.

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