Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Misan

College of Pharmacy

The Therapeutic Efficacy of *Ginkgo Biloba* in Neurological Disorders, Cardiovascular Diseases, and Cancer

Research

Submitted to Council of the College of Pharmacy, University of Misan, as a partial Fulfilment of the Requirements for the BSC Degree in Pharmacy

By

Safa jassim Mohammed

Zeena Bassim Aqeel

Saad hameed neyroz

Shams mohammed sabre

Supervisor

Dr. Widad A. Abed Salman

2025 A.D **1446 A.H**

بسم الله الرحمن الرحيم

﴿ يَرْفَعِ ٱللَّهُ ٱلَّذِينَ ءَامَنُواْ مِنكُمْ
وَٱلَّذِينَ أُوتُواْ ٱلْعِلْمَ دَرَجَٰتٍ ﴾

« سورة المجادلة : 11 »

الاهداء

بفضل الله ونعمته تمكنا من اكمال هذا البحث فالحمد لله.....

الى من كان درعاً للإسلام وكلمات حكمته مناره للأجيال محمد (ص) وال بيته الاطهار...

إلى من علموني أن العلم طريق النجاح، وأهدوني بذور المعرفة التي نمت بفضل دعمهم وتشجيعهم...

إلى أسرتي العزيزة، التي كانت السند الدائم في كل خطوة...

إلى أصدقائي الذين شاركوني الأفكار والمواقف ...

هذا البحث هو ثمرة جهد جماعي، وأهديه إلى كل من ساهم في بناء هذه الرحلة العلمية

SUPERVISOR CERTIFICATION

Certify that this Project (The Medical Applications of *Ginkgo Biloba* in Neurological and Cardiovascular Diseases) was prepared under our supervision at the College of Pharmacy, University of Misan, as graduation research

Supervisor

Signature:

Name: Dr. Widad A. Abed Salman

Specific specialization : Medicinal plant

Date:

Table of contents

List of Figures

ABSTRACT

Ginkgo biloba is an ancient medicinal tree species that has existed for millennia without undergoing modifications due to its resistance to environmental stresses. The tree is slow-growing, adapted to many ecological conditions, and shows numerous adaptations in developmental patterns. Medicinal use of the species is attracting research interest, especially the various parts of the tree that are used in orthodox or traditional medicine to treat diseases due to the many bioactive compounds. The primary compounds receiving increasing research interest are the triterpene lactones and flavonoids Currently, there are different forms of *G. biloba* available in the market, including extracts and isolated compounds such as terpenoids, flavonoids, bioflavonoids, and organic acids; Both its leaves and nuts have been widely prescribed for the treatment of various ailments, including pulmonary diseases, alcohol abuse, bladder inflammation, heart and lung dysfunction, and skin infections In Retrieved studies showed that the extracts and isolated compounds from *G. biloba* have beneficial activities in cerebrovascular, nervous, cardiovascular, endocrine, muscular and skeletal, renal, respiratory, digestive, and immunity systems. In many countries, the cultivation of plantations for the supply of ginkgo leaf-based pharmaceutical formulations is in progress, and efforts to standardize ginkgo leaf extract as an herbal medication for human use are being made.

1. INTRODUCTION

Ginkgo biloba L., known as a "living fossil," has been utilized for over 2000 years as a therapeutically valuable plant for human health [1–3]. The name "*Ginkgo*" is derived from a Japanese word, while the term "*biloba*" refers to the characteristic two-lobed shape of its leaves. The ginkgo tree thrived during the Mesozoic era, with its peak development occurring in the Jurassic and Cretaceous eras [1]. Throughout Chinese herbal medicine history, ginkgo seeds have been recognized for their therapeutic properties, and the leaves have been used in the form of teas for medicinal purposes. The plant contains bioactive compounds such as organic acids (*e.g.*, ginkgolic acid), flavonoids (*e.g.*, kaempferol, quercetin, isorhamnetin), biflavonoids (*e.g.*, sciadopitysin, ginkgetin, isoginkgetin), and terpenoids (*e.g.*, ginkgolides, bilobalides), which have extended its applications in various biological systems [3]. The therapeutic effectiveness of *G. biloba* leaf extracts is believed to be attributed to the presence of flavonoid glycosides and terpene trilactones (ginkgolides and bilobalide). Standardized and commercial ginkgo leaf extracts typically contain 5–7 % terpene lactones and 22–27 % flavone glycosides [3]. In Traditional Chinese Medicine (TCM), *Ginkgo biloba* has been used for centuries to manage stomach pain, cognitive dysfunction, bronchitis, asthma, tuberculosis, and other conditions. It has been clinically evaluated and proven effective as a nutritional supplement and medicine for improving memory, as well as a therapeutic or preventative measure for Alzheimer's disease and other neurological disorders. Additionally, it has shown promise as a therapeutic approach for cardiovascular-related disorders due to its immunomodulatory, anti-inflammatory, neuroprotective, and antioxidant properties [1,4–6]. Ginkgo leaf extracts are now available in various forms, including film-coated pills, oral liquids, and injectable formulations, in Europe and America [3].

These extracts are widely used in herbal pharmaceutical products, food and nutritional supplements, botanical and complementary treatments.

Ginkgo extracts contain active chemicals that have several beneficial effects on the body. These extracts enhance blood circulation, inhibit clot formation, strengthen capillary walls, and protect neural cells during oxygen deprivation [7]. *Ginkgo* leaf extracts are commonly used to manage symptoms of dementia, such as difficulties in concentration and memory-related problems. They also possess anti-asthma properties [8, 9], promote wound healing [2], and exhibit neuroprotective effects, along with improving mental capacities in patients with Alzheimer's disease [10,11]. Numerous pharmacological investigations have been conducted to explore the activity of ginkgo extract [12]. In recent years, there have been several pharmacological studies focusing on exploring new medicinal aspects of ginkgo. This updated study aims to provide a comprehensive review of these recent studies to gain better insights for future clinical investigations. By examining the findings of these studies, researchers can gain a deeper understanding of the potential therapeutic applications of *Ginkgo* and identify areas for further research and clinical trials.

Figure 1. Gingko *biloba* tree

2. Chemical Constituents

Several chemical compounds have been derived from *G. biloba* with a wide range of therapeutic activities. In recent years, novel chemical compounds, including new terpenoids and lignans, have been identified in *G. biloba* [13], as described in the following sections [13_23].

2.1 Flavonoid:

Liquid chromatography-mass spectrometry (LC-MS) has been used to identify and separate several flavonoids found in *G. biloba*. To date, 110 flavonoids have been identified, including kaempferol 3-O-*α*-l- [6000-p-coumaroyl(*β*-d)-glucopyranosyl(1,2)-rhamnopyranoside]-7-O-*β*-d-glucopyranoside ,and isorhamnetin 3-O-α-l-[6000-p-coumaroyl(*α*-d)-glucopyranosyl(1,2)-rhamnopyranoside], which were identified in an n-BuOH extract of *G. biloba* leaves. flavonoids exhibit antioxidant properties when they bind with six other flavonol glycosides: quercetin 3-O-*β*-D-glucopyranoside, quercetin 3-O-*β*-rutinoside, quercetin 3-O-*α*-L-[6‴-p-coumaroyl-(*β*-D)-glucopyranosyl-(1,2)-rhamnopyranoside], kaempferol 3-O-*α*-L- [6‴-p-coumaroyl-(*β*-D)-glucopyranosyl-(1,2)-rhamnopyranoside], quercetin 3-O-*β*-D-glucopyranosyl-(1–2)-alpha-Lrhamnopyranoside, and quercetin 3-O-*α*-L-[6‴-p-coumaroyl-(*β*-D)-glucopyranosyl-(1,2)-rhamnopyranoside]-7- O-*β*-D-glucopyranoside [14]. Flavonoids can be characterized into seven groups: flavanones, isoflavones, flavones, biflavones, flavan-3-ols, flavonols, and biginkgosides. Ma et al. first separated and described biginkgosides in 2016, describing the isolation of nine biginkgosides.

2.2 Terpenoids:

Ten diterpenoid lactones have been discovered, known as ginkgolides Q, P, N, M, L, K, J, C, B, and A. Until recently, bilobalide was thought to be the only sesquiterpene lactone in *G. biloba*, but Dong et al. announce a new bilobalide isomer in 2020 [15]. *G. biloba* also contains nor-terpenoids, including three nor-

sesquiterpenoids discovered by Shu et al. in *G. biloba* L.

2.3 Alkylphenols and Alkylphenolic Acids.

Alkylphenols can be divided into five groups: cardols, cardanols, α-hydroxycardanols, urushiols, isourushiols, and alkylphenolic acids. [30]. Although ginkgolic acids are known to be toxic [16], they have also been reported to display potential pharmacological effects.

2.4 Carboxylic Acids

Carboxylic acids that have been identified in *G. biloba* include ferulic acid, p-coumaric acid, protocatechuic acid, caffeic acid, p-hydroxybenzoic acid, m-hydroxybenzoic acid, vanillic acid, isovanillic acid, gallic acid, and sinapic acid [17]. Phenolic acids have been demonstrated to form glycosidic or covalent bonds, except for the free form in GBL.

2.5 Lignans.

Lignans were identified in G. biloba roots in 2015 and in G. biloba seeds in 2018 [33, 34]. Lignans obtained from G. biloba show antioxidant properties [18]. In 2018, lignans were also discovered in GBE [19]. Pinoresinol contains 0.012–0.020 mg/mL diglucoside and 1.05–1.87 mg/ mL total lignan glycosides. Five lignans were isolated from G. biloba by Shu *et al.*

2.6 Proanthocyanidins.

Prodelphinidin and procyanidin are two proanthocyanidins that have been identified in *G. biloba* at a ratio of 85: 15 Prodelphinidin is an epigallocatechin polymer, whereas procyanidin is comprised of epicatechin [20].

2.7 Polyprenols.

Polyprenols, which are active ingredients identified in *G. biloba*, are comprised of long chains of 14–24 isopentenyl units and have a similar structure as S-polyterpene alcohol (dolichols), which can be found in mammals, including people [21].

2.8 Polysaccharides

Okhti et al. examined the chemical structures of polysaccharides found in *G. biloba* and discovered that they were composed of glucose, rhamnose, mannose, arabinose, and galactose [22].

2.9 Others

Toxic component 4′-O-methylpyridoxine (MPN) was first discovered in G. biloba seeds and isolated by Klein et al. [23]. *G. biloba* essential oil contains 68 compounds, which include 42.11% sesquiterpenes.

3. Bioactive Compounds in *Ginkgo biloba*

G. biloba leaves have been extensively investigated as a source of the plant's major medicinal components. Active chemical components found in *G. biloba* leaves include flavonoids and terpenoids, and plant extracts have exhibited a variety of pharmacological activities, including antibacterial, antioxidant, anti-inflammatory, antiallergic, and cytotoxic anticancer activities [4, 11]. Many other bioactive compounds, including bioflavonoids, organic acids, and polyprenols, have been identified in *G. biloba.* Other constituents of *G. biloba* with known pharmacological activities are ginkgolides and bilobalide. Ginkgolides can be divided into five types (A, B, C, J, and M), in which has a unique set of properties. Flavonoids, such as quercetin, kaempferol, and isorhamnetin, are found as glycoside derivatives in *G. biloba* (Figure 2). A standardized leaf extract of *G. biloba*, known as EGb 761, includes 6% terpenoids, 5%–24 % flavonoid glycosides, 10% organic acids, and other bioactive compounds that are known to exert a wide range of beneficial health effects [4, 11].

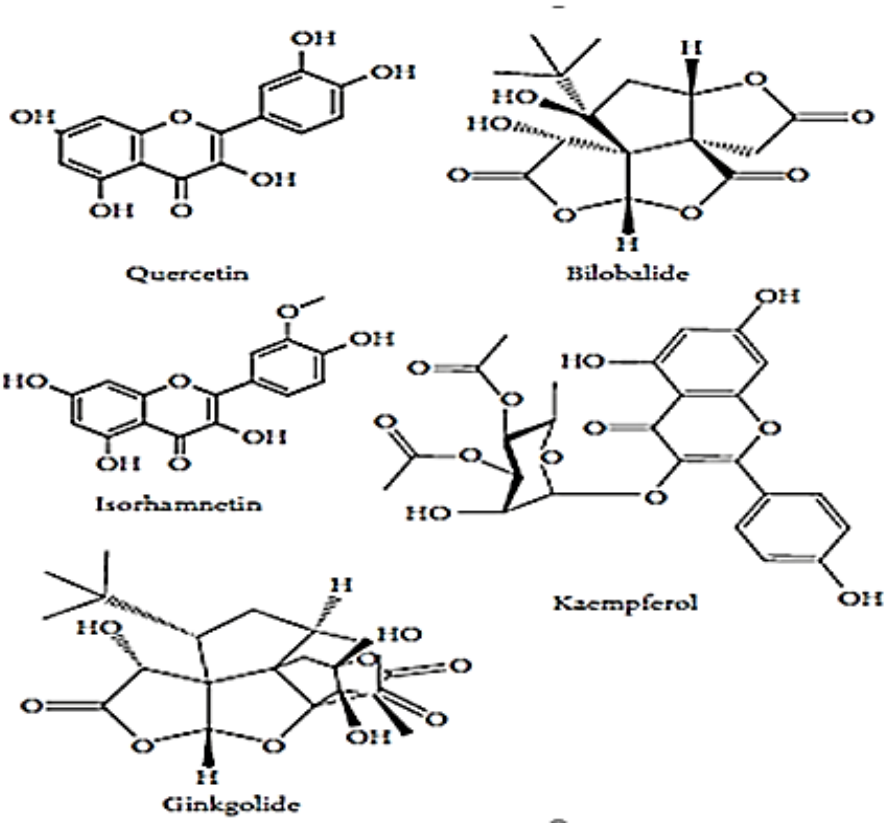

Figure 2: Chemical structures of *Ginkgo biloba*'s key bioactive compounds

4. Pharmacologic properties of *Ginkgo Biloba*

There are plenty of studies showing that *G. biloba* has many beneficial activities on different body organs. We summarized in vitro and in vivo studies of *G. biloba* in the following paragraphs. [24_74]

4.1 Alzheimer's Disease and Dementia:

Evidence from Cellular and In Vivo Studies. In vitro, Ginkgolide A treatment reduced the expression of pro-inflammatory mediators like COX2 and nitric oxide, as well as cytokines like TNF-α, IL-1, and IL-1β in mouse macrophages and differentiated human monocytes [24_61]. Furthermore, it has been shown to significantly reduce neurological deficit scores and brain infarct volume in rats suffering from cerebral ischemia/reperfusion damage [24], as well as repair mitochondrial dysfunction in cell culture [25]. Ginkgolide B has been shown to inhibit glutamate-induced apoptosis in astrocytes in vitro when glutamate metabolism is abnormal in the pathological environment of AD (Figure 3) [26].

According to studies, it improves neurological function by promoting the proliferation and differentiation of neural stem cells in rats with cerebral ischemia/reperfusion injury [27]. Electrophysiological recordings from the brain slices of rats exposed to hypoxia in vivo showed increases in the frequency of spontaneous discharge, the frequency of action potentials, and the magnitude of calcium entries. Pre-treatment with Ginkgolide B, which may regulate Ca2+ influx in hippocampal neurons, suppressed all of these effects of hypoxia [28]. In model rats with middle cerebral artery lusion reperfusion, Ginkgolide C suppresses the CD40/NF-κB pathway to ameliorate cerebral ischemia or reperfusion-induced inflammatory impairments [28]. This compound can inhibit adipogenic factors and enzymes, increase lipolysis in a culture of differentiated adipocytes [29], and exhibit an intriguing anti-neoplastic effect in hepatocellular carcinoma cells [30], suggesting that other tissues may respond better to its application. It has been demonstrated that bilobalide increases neurogenesis and synaptogenesis in the cells of rat fetuses by stimulating the proliferation of hippocampal progenitor cells in a dose-dependent manner [31]. It supports the use of APP processing promoted by alpha-secretase in delaying the onset of AD by reducing the production of beta-amyloid in the human neuroblastoma cell line. Furthermore, studies have shown that bilobalide improves cognitive functions in AD mice [32]. In an in vitro model of AD, resveratrol can reduce oxidative damage to neurons caused by beta-amyloid via the mitophagy pathway [33]. It also had a significant effect on the integrity of the blood–brain barrier in AD-induced rats [34]. This substance was found to have good anticholinergic effects in mice with dementia when combined with other antioxidants such as vitamin E [35]. In a culture of human umbilical endothelial cells, kaempferol was discovered to bind to vascular endothelial growth factor, improving some angiogenic functions [36]. Kaempferol increased dopaminergic and cholinergic neurotransmission in lab rats' prefrontal cortices, improving cognitive function [37].

It has a synergic effect on the learning and memory capabilities of model rats with AD [38]. Isorhamnetin has been shown to stimulate urofilament production, which enhances neurite outgrowth and NGF-induced neurofilament expression.

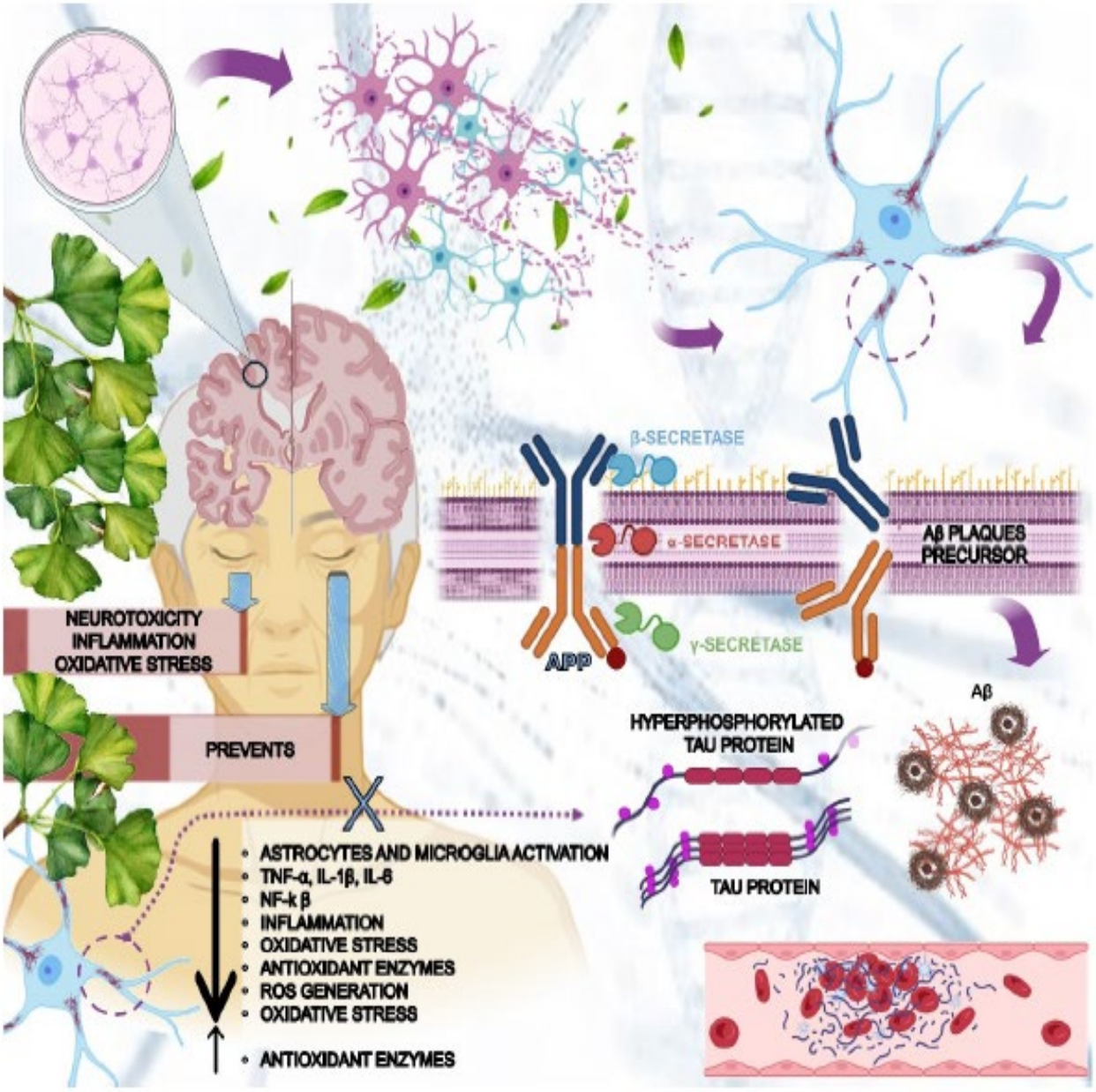

Figure 3. Protective mechanisms of *Ginkgo biloba* against Alzheimer's disease (AD). Gingko biloba and its extracts can play protective roles against AD and can interfere with the formation of βA plaques.

4.1.1 *Ginkgo Biloba* and Neurotransmitters Related to Alzheimer's Disease

4.1.1.1 Acetylcholine

Several studies indicate that GB may have a direct and indirect impact on cholinergic function [39_58]. Scopolamine, a muscarinic receptor antagonist, has been shown to cause memory dysfunction in models, supporting this theory. Memory and cognitive function have been demonstrated to be negatively impacted by its administration's transient blockade of cholinergic muscarinic receptors [39]. In rodents, GB treatment reduces scopolamine-induced amnesia, indicating improved cognition. This condition was associated with GB's direct action on cholinergic receptors. This plant appears to directly affect presynaptic cholinergic nerve terminals, inhibiting choline absorption, which is a precursor to acetylcholine synthesis [40]. Other learning and memory models applied to rodents demonstrated the effectiveness of GB in memory acquisition and retention in the face of prolonged treatment [41], as well as an improvement in the animals' memory while using a maze [42]. The study found that using GB in healthy young and elderly humans improved both short-term [43] and long-term [44] memory. In Alzheimer's patients, a 3-to-6-month treatment with 120 to 240 mg of GB extract has a small but significant effect on GB's clinical efficacy [45].

4.1.1.2. Glutamate and Dopamine

Ginkgolides, biologically active terpene lactones in GB, regulate glutamate transmission in the cortex and hippocampus. Glutamate binding to receptors activates a short-term modulation pathway that allows ions to enter and exit the cell. The flow of calcium ions triggers the release of glutamate [46_53]. Ginkgolides influence phospholipase A2 and prevent kinase C activation. In turn, kinase C has a significant effect on calcium circulation by influencing endoplasmic reticulum calcium release. When this pathway is inhibited, calcium flow is blocked, and glutamate cannot be released into the synapse. Thus,

Ginkgolides' inhibitory function is demonstrated by their ability to reduce glutamate-induced damage in hippocampal neuronal cells in the face of cerebral ischemia [46]. Normal excitatory neurotransmission requires glutamate to be removed from the synapse by its transporters [47]. Failure to withdraw causes toxicity that can cause acute neurodegenerative diseases like epilepsy and hypoxia, as well as chronic neurodegenerative diseases like Alzheimer's and Huntington's Syndrome [48]. Similarly, the presence of Ginkgolides appears to modulate the neurotransmitter dopamine, which regulates cognition, voluntary movements, and the activation of the punishment and reward system [49]. Chronic Ginkgolide administration increases dopaminergic and noradrenergic transmission in the frontal cortex of the brain [50]. Furthermore, Ginkgolides boost dopaminergic activity in rats' paraventricular nuclei [51]. This effect can be explained by the inhibitory effect of Ginkgolides on MAO (monoamine oxidase). MAO is responsible for the elimination of norepinephrine at synapses. Yoshaitatake et al. demonstrated that a single oral dose did not affect monoamine concentration levels [52]. Dysregulation of dopamine homeostasis is associated with the pathogenesis of neurodegenerative diseases. For example, growth hormone receptor 1α (GHSR1α)-induced disruption of dopamine D1 receptor (DRD1) function exacerbates the pathophysiology of AD [53].

4.1.1.3. Serotonin—5HT

Changes in behavior, as in the case of depression, can be identified in individuals with AD [54], suggesting functional changes in the monoaminergic system, not only in the cholinergic system [54_58]. Some studies have shown that serotonin receptors, in addition to increasing cholinergic neurotransmission, also improve neurogenesis processes and neuronal plasticity, as well as reduce amyloid load in the brain [55,56]. In relation to serotonin 5-HT1A receptors a decrease in their expression is observed in the aging phase [57]. However, this condition can be reversed with treatment with GB [58].

Although the mechanisms of action of GB in neuroprotective events are not well understood, the modulation of serotonin levels was observed, as well as the increase in dopamine levels due to the reduction in monoamine oxidase (MAO) activity in the prefrontal cortex in the presence of this substance [59–60]. In rats that received GB treatment for 3 weeks with an average consumption of 50–300 mg of GB, a slight increase in serotonin levels was observed in brain regions such as the prefrontal cortex and hippocampus [61]. The evaluations of the study show that 5-HT has an important role in neurodegenerative diseases, and the understanding of its mechanisms can bring new insights into AD treatment.

4.2 Cardiovascular system

Studies show that *G. biloba* and its isolated compounds have benefits on cardiovascular problems, including cardiotoxicity, arrhythmia, heart failure, and other problems [62_68] .

Through the production of prostaglandins and NO, G. biloba extracts were reported to increase the blood flow, enhance blood rheology, inhibit platelet aggregation and hypoxia, and decrease capillary permeability [62]. Nevertheless, a double-blind trial conducted revealed that G. biloba was not proven to reduce total mortality or the cardiovascular disease-related death rate or cardiovascular events [63]. In another study, EGb761 showed inhibitory activity on myocardial cell apoptosis. It could activate the Akt/Nrf2 pathway, elevate HO-1 expression, reduce oxidative stress, and inhibit inflammatory responses to protect the myocardium [64]. The extract showed efficacy in viral myocarditis by lowering myocardial damage and fibrosis through S100A4 and MMP-3 suppression [64]. Based on the literature, *G. biloba* biflavones might be the active cardioprotective constituents of *G. biloba*. As an example, *G. biloba* biflavones have been natural inhibitors for human thrombin, and these chemicals might be utilized for the creation of new thrombin inhibitors with better effectiveness and excellent safety profiles [65].

Additionally, studies show that *G. biloba* extract is a beneficial therapeutic compound for cardiovascular and ischemic diseases by exerting vascular-protective function. The *G. biloba* extract, ginkgolide B, and ginkgo leaf tablets showed activity against atherosclerosis via elevating the production of clopidogrel active metabolite, increasing endothelial dysfunction, and decreasing inflammatory factors [66]. An in vivo study showed that nanosuspensions from ginkgo lactones could decrease platelet aggregation [67] . Furthermore, *G. biloba* showed benefits in acute myocardial infarction through decreasing inflammation, apoptosis, and increasing endogenous antioxidant enzymes [68].

4.3 Anticancer Activity

Bai *et al.* have investigated the effects of *G. biloba* extract on cell apoptosis and G0/G1 cycle in gastric cancer cells [69_74]. The anticancer activity of Tebonin Egb761 is widely used in the treatment of various cancers. According to this study, after 48 hours of treatment, the data revealed that Tebonin Egb761 significantly suppressed the proliferation of human gastric cancer AGS cells in a dose-dependent manner. Tebonin Egb761 at a concentration of 80 mg/L increased the number of cells in the G0/G1 phase and decreased the cells in the G2/M and S phases. In addition, Tebonin Egb761 treatment significantly increased the apoptosis rate of AGS cells. In conclusion, Tebonin Egb761 can induce apoptosis in human gastric cancer cells through various mechanisms and exhibit anticancer activity by causing cells to remain in G0/G1 phase [69]. investigated the activity of *G. biloba* extracts on inducing apoptosis in Lewis Lung Cancer Cells (LLC) involving MAPK signaling pathways. This study has shown that GBEE (50-200 mg/kg) has dose-dependent inhibitory effects on the growth of LLC-transplanted tumors. As a result, GBEE induces apoptosis in LLC cells through the mitochondrial- mediated intrinsic pathway and the death receptor-mediated extrinsic pathway, which may be closely related to the regulation of MAPK signaling pathways by various mechanisms.[70] .

Wang et al. have investigated the anticancer effect of Tebonin Egb761 on hepatocellular carcinoma (HCC) cell lines in their study. They found that Tebonin Egb761 inhibited cancer cell growth, reduced cell viability, and supported apoptosis in hepatocellular carcinoma cells. Additionally, Tebonin Egb761 dose-dependently reduced the proliferation of human hepatocellular carcinoma (HepG2) cells and increased their apoptosis. In addition, it has been observed that Tebonin Egb761 exerts an anticancer effect on HepG2 cells by activating p53 and inhibiting nuclear factor (NF)-κB signaling pathways. In conclusion, Wang et al. determined that Tebonin Egb761 inhibited the proliferation and induced apoptosis of hepatocellular carcinoma cells through the NF-κB/p53 signaling pathway [71] . DeFeudis et al. investigate the activity of G. biloba extracts on cancer in their study. In this study, ginkgolide B inhibited the growth of a highly aggressive human breast cancer cell line in mice. In addition, exposure of bladder cancer cells to a Ginkgo extract has elicited an adaptive transcriptional response that prevents DNA damage. In humans, Ginkgo extracts also inhibit the formation of oxidative stress effects caused by radiation-induced clastogenic factors and ultraviolet light, which may be associated with anticancer activity [72]. Liu et al. investigated the anti-metastatic effect of Tebonin Egb761 on colorectal cancer cells in their study. The treatment of colorectal cancer cells with Tebonin Egb761 has been shown to induce inhibition of cell migration and invasion ability in a concentration-dependent manner. As a result, Tebonin Egb761 has been shown to upregulate LincRNA-p21 expression in a dose- and time-dependent manner. Therefore, Tebonin Egb 761 may be a promising treatment regimen for colorectal cancer [73] .Pretner et al. aimed to determine the role of PBR in cancer and the possible anticancer effects of Tebonin Egb761 against it in their study. Treatment with Tebonin Egb761 reduced PBR mRNA levels and inhibited the proliferation of breast, glioma, and hepatocarcinoma cell lines. As a result, treatment with Tebonin Egb761 is believed to be beneficial in preventing or

treating cancer metastasis by reducing PBR overexpression [74] .

5. Conclusion

In this study, we conducted focusing on the phytochemical, and pharmacological aspects of *G. biloba.* Ginkgo biloba is known to contain various bioactive constituents, including flavonoids, biflavonoids, terpenoids, polyphenols, and phenolic acids, which contribute to its medicinal properties. Among these compounds, terpene lactones such as ginkgolides, bilobalide, and their derivatives, as well as flavonoids, particularly biflavonoids, are the predominant constituents that exhibit significant biological and pharmacological activities. Quercetin, kaempferol, and isorhamnetin are the major flavonoids found in *G. biloba*, occurring both as glycoside derivatives and aglycone forms. It is important to note that ginkgolic acids present in the plant are considered toxic and allergenic. Therefore, a standardized ginkgo leaf

extract should contain approximately 22 % to 27 % flavonoid glycosides, 5 % to 7 % terpene lactones, and no more than 5 parts per million of ginkgolic acids, as recommended by the German government. Our review of the literature revealed that *G. biloba* extracts and isolated compounds exhibit pharmacological and protective activities against various diseases, including cerebrovascular and nervous system disorders, cardiovascular conditions, endocrine disorders, muscular and skeletal issues, renal diseases, respiratory problems, digestive disorders, anticancer and immune-related ailments. However, further studies are warranted to evaluate the claimed benefits of *G. biloba* on human health, to obtain more robust findings regarding its efficacy and safety.

6. References

[1] T. Belwal, L. Giri, A. Bahukhandi, M. Tariq, P. Kewlani, I.D. Bhatt, R.S. (2019). Rawal, Chapter 3.19 - Ginkgo biloba, in: S.M. Nabavi, A.S. Silva (Eds.), Nonvitamin and Nonmineral Nutritional Supplements, Academic Press, pp. 241–250.

[2] E.T. Noor, R. Das, M.S. Lami, A.J. Chakraborty, S. Mitra, T.E. Tallei, R. Idroes, A. A. Mohamed, M.J. Hossain, K. Dhama, G. Mostafa-Hedeab, T.B. (2022). Emran, Ginkgo biloba: a treasure of functional phytochemicals with multimedicinal applications, Evid. Based on Complement. Alternat. Med, 8288818.

[3] Y. Liu, H. Xin, Y. Zhang, F. Che, N. Shen, Y. Cui (2022). Leaves, seeds, and exocarp of Ginkgo biloba L. (Ginkgoaceae): a comprehensive review of traditional uses, phytochemistry, pharmacology, resource utilization and toxicity, J. Ethnopharmacol. 298, 115645.

[4] J. Hort, T. Duning, R. Hoerr (2023). Ginkgo biloba extract EGb 761 in the treatment of patients with mild neurocognitive impairment: a systematic review, Neuropsychiatr. Dis. Treat. 19 647–660.

[5] L. Xie, Q. Zhu, J. Lu (2022). Can we use *Ginkgo biloba* extract to treat Alzheimer's disease? lessons from preclinical and clinical studies, Cells 11 (3).

[6] Z. Kuli´c, M.D. Lehner, G.P.H. Dietz (2022). *Ginkgo biloba* leaf extract EGb 761. as a paragon of the product by process concept, Front. Pharmacol. 13, 1007746.

[7] S.W.Barth, M.D. Lehner, G.P.H. Dietz, H. Schulze)2021(. Pharmacologic treatments in preclinical tinnitus models with special focus on Ginkgo biloba leaf extract EGb 761, Mol. Cell. Neurosci. 116: 103669

[8] M.A. Ibrahim, H.H. Ramadan, R.N. Mohammed (2021). Evidence that Ginkgo Biloba could use in the influenza and coronavirus COVID-19 infections, J. Basic Clin. Physiol. Pharmacol. 32 (3) 131–143.

[9] T. Akanchise, A. Angelova (2023). Ginkgo Biloba and long COVID: in vivo and in vitro models for the evaluation of nanotherapeutic efficacy, Pharmaceutics 15 (5) 1562.

[10] Q. Li, L. Rubin, M. Silva, S. Li, C. Yang, P. Lazarovici, W. Zheng (2022). Current progress on neuroprotection induced by Artemisia, Ginseng, Astragalus, and Ginkgo traditional Chinese medicines for the therapy of Alzheimer's disease, Oxid. Med. Cell. Longev. 3777021.

[11] C. Villegas, R. Perez, L.L. Petiz, T. Glaser, H. Ulrich, C. Paz (2022). Ginkgolides and Huperzine A for complementary treatment of Alzheimer's disease, IUBMB Life 74(8) 763–779.

[12] S.M. Barbalho, R. Direito, L.F. Laurindo, L.T. Marton, E.L. Guiguer, R.A. Goulart, R.J. Tofano, A.C.A. Carvalho, U.A.P. Flato, V.A. Capelluppi Tofano, C.R. P. Detregiachi, P.C.S. Bueno, R.S.J. Girio, A.C. Araújo (2022). *Ginkgo biloba* in the aging process: a narrative review, Antioxidants 11 (3) 525 (Basel).

[13] Droy-Lefaix MT, Cluzel J. Menerath JM, Bonhomme B, Doly M. (1995). Antioxidant effect of a Ginkgo biloba extract (EGb761) on the retina. Int. J. Tissue React ;17:93-100.

[14] Rojas P, Serrano-García N, Mares-Sámano JJ, Medina-Campos ON, Pedraza- Chaverri J. (2008). Ogren SO. EGb 761 protects against nigrostriatal dopaminergic neurotoxicity in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinsonism in mice: role of oxidative stress. Eur J. Neurosci: 28:41-50.

[15] DeFeudis FV, Drieu K. (2000). *Ginkgo biloba* extract (EGb 761) and CNS functions: basic studies and clinical applications. Curr Drug Targets:1:25-8.

[16] Gerlach M, Ben-Shachar D, Riederer P, Youdim MB (1994)**.** Altered brain metabolism of iron as a cause of neurodegenerative diseases? J Neurochem :63:793- 807.

[17] Jenner P, Olanow CW. **(1996).** Oxidative stress and the pathogenesis of Parkinson's disease. Neurology;47(suppl 3):5161-70.

[18] Dexter DT, Holley AE, Flitter WD, Slater TF, Wells FR, Daniel SE (1994). Increased levels of lipid hydroperoxides in the parkinsonian substantia nigra: an HPLC and ESR study. Mov. Disord: 9:92-7.

[19] Zhang J. Perry G, Smith MA, Robertson D, Olson SJ, Graham DG, Montine TJ. (**1999).** Parkinson's disease is associated with oxidative damage to cytoplasmic DNA and RNA in substantia nigra neurons. Am J Pathol:154:1423-9.

[20] Yao Z, Wood NW. (2009). Cell death pathways in Parkinson's disease: role of mitochondria. Antioxid Redox Signal: 11:2135-49.

[21] Perier C, Bové J. Vila M(2012). Mitochondria and programmed cell death in Par- kinson's disease: apoptosis and beyond. Antioxid Redox Signal;16: 883-95.

[**22] DeFeudis FV (1998).** Ginkgo biloba extract (EGb761); from chemistry to the clinic. 1st ed. Wiesbaden, Germany: Ullstein Medical.

[23] Drieu K. (1986). Preparation and definition of *G. biloba* extract. Press Med ;15:1455-7.

[24] Kleijnen J. Knipschild P (1992). *Ginkgo biloba*. Lancet ;340:1136-9.

[25] Bilia AR. (2002). *Ginkgo biloba* L. Fitoterapia 73:276-9.

[26] Diamond BJ, Shiflett SC, Feiwel N, Matheis RJ. Noskin O, Richards JA, Schoenberger NE. (2000). *Ginkgo biloba* extract: mechanisms and clinical indications. Arch Phys. Med Rehabil 81:668-78.

[27] Rojas P. Garduño B, Rojas C. Vigueras RM, Rojas-Castañeda J. Ríos C. Serrano-Garcia N. (2001). EGb 761 blocks MPP+-induced lipid peroxidation in mouse corpus striatum. Neurochem Res. 26:1245-51.

[28] Ernst E. (2002). The risk-benefit profile of commonly used herbal therapies: Ginkgo, St John's wort, ginseng. Echinacea, saw palmetto and kawa. Ann Intern Med.136:42-53.

[29] Tansey MG, Goldberg MS. (2010). Neuro-inflammation in Parkinson's disease, its role in neuronal death and implications for therapeutic intervention. Neurobiol Dis 37:510-8.

[30] Chan LW, Wang YQ, Wei LC, Shi M, Chan YS. (2007). Chinese herbs and herbal extracts for neuroprotection of dopaminergic neurons and potential therapeutic treatment of Parkinson's disease. CNS Neurol Disord Drug Targets, 6:273-81.

[31] Hynes N. Bishai 1, Lees J, Coceani F. (1991). Leukotrienes in brain: natural occurrence and induced changes. Brain Res,553:4-13.

[32] Kunievsky B, Yavin E. (1992). Platelet-activating factor stimulates arachidonic acid release and enhances thromboxane B2 production in intact fetal rat brain ex vivo. J Pharmacology Exp. Ther. 263:562-8.

[33] Shi C, Zhao L, Zhu B, Li Q, Yew DT, Yao Z, Xu J. (2009). Dosage effects of EGb761 on hydrogen peroxide-induced cell death in SH-Sy5Y cells. Chern Biol. Interact,180:389-97.

[34] Du ZY, Li XY. (1998). Effects of ginkgolides on interleukin-1, tumor necrosis factor- alpha and nitric oxide production by rat microglia stimulated with lipo- polysaccharides in vitro. Arzneim Forsch :48:1126-30.

[35] Marcocci L, Packer L, Droy-Lefaix M, Sekaki A, Gardes-Albert M. (1994). Antioxidant action of Ginkgo biloba extract EGb 761. Methods Enzymol; 234:462-75.

[36] Gardés-Albert M, Ferradini C, Sekaki A, Droy-LeFaix MT. (2020). Oxygen-centered free radicals and their interactions with EGb 761 or CP

[37] Saija A, Scalese M, Lanza M, Marzullo D, Bonina F, Castelli F. (1995). Flavonoids as antioxidant agents: importance of their interaction with biomembranes. Free Radic Biol Med. 19:481-6.

[38] Shi C, Zhao L, Zhu B, Li Q, Yew DT, Yao Z. (2009). 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, 181:115-23.

[39] Sastre J. Millán A, García J. Plá R, Juan G, Pallardó FV. (1998). A *Ginkgo biloba* extract (EGb 761) prevents mitochondrial aging by protecting against oxidative stress. Free Radic. Biol. Med ,24:298-304.

[40] Bridi R, Crossetti FP, Steffen VM. Henriques AT. (2001). The antioxidant activity of standardized extract of Ginkgo biloba (EGb 761) in rats. Phytother Res. 15:449-51.

[41] Pardon MC, Joubert C, Perez-Diaz F. Christen Y, Launay JM, Cohen-Salmon C. (2000). In vivo regulation of cerebral monoamine oxidase activity in senescent controls and chronically stressed mice by long-term treatment with Ginkgo biloba extract (EGb 761). Mech. Ageing Dev. 113:157-68.

[42] Eckert A, Keil U, Kressmann S, Schindowski K, Leutner S, Leutz S. (2003). Effects of EGb 761 *Ginkgo biloba* extract on mitochondrial function and oxidative stress. Pharmacopsychiatry , 36:515-23.

[43] Winter JC. (1998). The effects of an extract of *Ginkgo biloba*, 761, on cognitive behavior on longevity in the rat. Physyiol Behav ,63:425-33.

[44] Liu TJ, Yeh YC, Ting CT, Le WL, Wang LC, Lee HW. (2008). Ginkgo

biloba extract 761 reduced doxorubicin- induced apoptotic damage in rat hearts and neonatal cardiomyocytes. Cardiovasc Res, 80:227-35.

[45] Yeh YC, Liu TJ, Wang LC, Lee HW, Ting CT, Lee WL. (2009). A standardized extract of Ginkgo biloba suppresses doxorubicin-induced oxidative stress and p53-mediated mitochondrial apoptosis in rat testes. Br J Pharmacology,156:48-61.

[46] Nevado J. Sanz R, Sánchez- Rodríguez C, Garcia-Berrocal JR, Martín-Sanz E, González-Garcia JA. (2010). *Ginkgo biloba* extract (EGb 761) protects against aging-related caspase-mediated apoptosis in rat cochlea. Acta Otolaryngology ,130:1101-12.

[47] Lu G, Wu Y, Mak YT, Wai SM, Feng ZT, Rudd JA, Yew DT. (2006). Molecular evidence of the neuroprotective effect of Ginkgo biloba (EGb 761) using bax/bcl-2 ratio after brain ischemia in senescence-accelerated mice, strain prone-8. Brain Res,1090:23-8.

[48] Koh PO. (2009). *Gingko biloba* extract (EGb 761) prevents increase of bad-bcl. X interaction following cerebral ischemia. Am J Chin Med. 37:867-76.

[49] Li S, Tang D. Xue Z, Zhang Z, Sun X, Liu Y. (2011). Biphasic effect of EGb 761 on simulated ischemia-induced rat BMSC survival in vitro and in vivo. Life Sci. 88:853-63.

[50] Lim S, Yoon WJ, Kang SK, Choi SC, Cho BJ, Kim M. (2011). EGb761, a *Ginkgo biloba* extract, is effective against atherosclerosis in vitro, and in a rat model of type 2 diabetes. PloS ONE,6:1-9.

[51] Wang J. Cheng Y, Yin J, Lu Q, Xu X, Yin X. (2011). Protective effects of Ginkgo biloba extract on 6-hydroxydopamine-induced apoptosis in PC12 cells. Neural Regeneration Res ,6:2565-72.

[52] Yang SF, Wu Q, Sun AS, Huang XN, Shi JS. (2001). Protective effect and mechanism of Ginkgo biloba leaf extracts for Parkinson disease induced by 1-methyl-4- phenyl-1,2,3,6-tetrahydropyridine. Acta Pharmacol Sin ;22:1089-93.

[53] Tsang AH, Chung KK. (2009). Oxidative and nitrosative stress in Parkinson's disease. Biochim Biophys Acta ,1792:643-60.

[54] Halliwell B. (2011). Free radicals and antioxidants Vadis? Trends Pharmacol Sci :32:125-30.

[55] Danielson SR, Andersen JK. (2008). Oxidative and nitrative protein modifications in Parkinson's disease. Free Radic Biol. Med. 44:1787-94.

[56] Andersen JK. (2004). Oxidative stress in neurodegeneration: cause or consequence? Nat Med. 10: S18-25.

[57] Lenaz G, Bovina C, D'Aurelio M, Fato R, Formiggini G, Genova MI. (2002). Role of mitochondria in oxidative stress and aging. Ann NY Acad. Sci. 959:199-213.

[58] McLennan HR, Degli Esposti M. (2000). The contribution of mitochondrial respiratory complexes to the production of reactive oxygen species. J Bioenerg Biomembr :32:153-62.

[59] F. Eisvand, B. M. Razavi, and H. Hosseinzadeh (2020). Effects of Ginkgo biloba on metabolic syndrome: a review," Phytotherapy Research, vol. 34, no. 8, pp. 1798–1811.

[60] S. Mahadevan and Y. Park (2008). "Multifaceted therapeutic benefits of Ginkgo biloba L.: chemistry, efficacy, safety, and uses," Journal of Food Science, vol. 73, pp. R14–9.

[61] Sayre LM, Perry G, Smith MA.(2008). Oxidative stress and neurotoxicity. Chem Res Toxicol ; 21:172-88.

[62] Hussain S, Slikker W Jr, Ali SF. (1995). Age related changes in antioxidant enzymes, superoxide dismutase, catalase, glutathione peroxidase and glutathione in different regions of mouse brain. Int J Dev Neurosci .13:811-7.

[63] Dexter DT, Carter CJ, Wells FR, Javoy-Agid F. Agid Y, Leesz A. (1989). Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. J Neurochem ,52:381-9.

[64] Yoritaka A, Hattori N, Uchida K, Tanaka M. Stadtman ER, Mizuno Y. (1996). Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson disease. Proc Natl Acad Sci USA :93:2696-701.

[65] Floor E, Wetzel MG. (1998). Increased protein oxidation in human substantia nigra pars compacta in comparison with basal ganglia and prefrontal cortex measured with an improved dinitrophenylhydrazine assay. J Neurochem ,70:268-75.

[66] Nakabeppu Y, Tsuchimoto D, Yamaguchi H, Sakumi K. (2007).Oxidative damage in nucleic acids and Parkinson's disease. J Neurosci Res.,85:919-34.

[67] Bender A, Krishnan KJ, Morris CM, Taylor GA, Reeve AK, Perry RH. (2006). High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. Nat Gene, 38:515-7.

[**68] Saggu H, Cooksey J, Dexter D, Wells FR, Lees A, Jenner P. (1989).** A selective increase in particulate superoxide dismutase activity in parkinsonian substantia nigra. J Neurochem, 53:692- 7.

[69] Amnabi LM, Van Woert MH, Murphy S. **(1975).** Brain peroxidase and catalase in Parkinson's disease. Arch Neurol, 32:114-8.

[70] Cohen G. (1986). Monoamine oxidase, hydrogen peroxide, and Parkinson's disease. In: Yahr MD, Bergmann KJ, editors. Advances in neurology: Parkinson's disease. New York: Raven Press; p. 119-25.

[71] Riederer P, Jellinger K. (1983). Neurochemical insights into monoamine oxidase inhibitors, with special reference to deprenyl (selegiline). Acta Neurol Scand Suppl , 95:43-55.

[72] Knoll, J. (1995). Rationale for (-) deprenyl (selegiline) medication in Parkinson's disease and in prevention of age-related nigral changes, Biomed Pharmacotherapy, 49:187-95.

[73] Schapira AH, Jenner P. (2011). Etiology and pathogenesis of Parkinson's disease. Mov Disord , 26:1049-55.

[74] Lipski J. Nistico R. Berretta N, Guatteo E, Bernardi G, Mercuri NB. **(2011).** L-DOPA: a scapegoat for accelerated neurodegeneration in Parkinson's disease? Prog Neurobiol; 94:389-407.