

NEUROPROTECTIVE ACTIVITIES AND REPAIR MECHANISMS OF *GINKGO BILOBA* EXTRACT EGB 761 ON CNS INJURIES AND NEURODEGENERATIVE DISORDERS

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ABSTRACT

With an increasing number of neurodegenerative diseases and brain injuries, the focus of the research using synthetic and herbal medicines with an objective to treat the existing problems in neuroscience is ever-increasing. The availability of natural resources, knowledge about ancient herbs, and the mechanisms underlying neurodegenerative disorders and brain injuries is essential to developing herbal supplements with the potential to treat brain diseases and injuries. Despite the variety of synthetically manufactured drugs for brain health, the need for herbal and natural supplements is ever-rising due to the adverse long-term side effects of synthetic drugs. *Ginkgo biloba* — an Asian plant that is known to possess various neuroprotective properties — has been used as a medicine, even before 1505 AD. *G. biloba* extract EGB761 has been known to treat and manage impaired nervous system disorders. The current review discusses the neuroprotective properties of *G. biloba* and its repair mechanisms against subarachnoid hemorrhage, central nervous system (CNS) injuries like traumatic brain injury, ischemic brain injury, early brain injury, spinal cord injury, hypoxia, neuronal apoptosis, neurodegenerative diseases like Alzheimer's, Parkinson's, neuroplasticity, neurogenesis, synaptogenesis and its free radical scavenging activity, and neuroleptic properties for a nutritional approach to manage neurodegenerative disorders and CNS injuries.

Keywords: Alzheimer's, Parkinson's, Brain injuries, Neuroplasticity, Free radical scavengers.

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INTRODUCTION

Recent advances in neuroscience and phytotherapy in the association have driven several advancements in the use of complementary medicines, such as plant extracts, in neuro therapies. Not only various ancient worldwide cultures are known to have used plant extracts for their neuroprotective properties but also the scientific advances made recently have added an impetus to the studies of plant extracts and their bioactive compounds' effects on the brain. *Melissa officinalis*, *Centella asiatica*, *Bacopa monnieri*, *Panax ginseng*, *Rosemary officinalis*, and *Ginkgo biloba* are a few of the most common and widely used, studied plants for their neuroprotective properties and nootropic effects on the human brain. From neurodegenerative disorders to central nervous system (CNS) injuries, these plants have been extensively studied, especially *G. biloba*, due to the various bioactive compounds present in them.

The neuroprotective and nootropic properties of the plant extracts are due to the presence of bioactive components such as flavonoids, terpenoids, and antioxidants. Quercetin, kaempferol, and tamarixetin are the components present in the flavonoid extract of plants that possess neuroprotective activities. As most such nootropic plants are rich in flavonoids, terpenoids, and antioxidants that boost brain performance and help in repair mechanisms, *G. biloba* is one of the plants which have additional bioactive components such as ginkgolides and bilobalide as a part of their terpenoid extracts. *G. biloba* extract EGB 761 is one of the most commonly prescribed drugs for dementia in many countries and a leading dietary supplement worldwide.

The acetylcholinesterase inhibition and cholinergic receptor binding properties in *M. officinalis* and *G. biloba* are known to possess memory-improving properties and cholinergic effects. Kennedy *et al.*, 2003 [1]. Antioxidants are the most important free radical scavengers and the oxidative damage-reducing property is due to the antioxidants present in plants such as *C. Asiatica* and *G. biloba* as per research by Kumar *et al.*, 2003 [2]. Both these plants are equally well known for the presence

of rich antioxidants that reduce oxidative damage in the CNS which can be a cause of external injury. The *C. asiatica* extract and EGB 761 are also known to be used in treating a neurodegenerative disorder that is caused due to Aβ plaques. Terpenoids, as well as alkaloids in neuroprotective plants like *B. monnieri* and *G. biloba*, are known as antipsychotic agents. Jeyasri *et al.*, 2020 [3].

P. ginseng, a nootropic plant, is similar to *G. biloba* 761. Both these plants are well known for their effectiveness in reducing amyloid beta (Aβ) plaques which are senile peptide plaques present in Alzheimer's disease patient's brains. Yang *et al.*, 2009 [4] *G. biloba* EGB 761 is one of the most commonly used herbs to treat age-related disorders in Asian culture. Antinociception is the blocking of the detection of an injurious stimulus by sensory neurons. *R. officinalis* and EGB 761 are known for their antinociceptive properties which are due to the presence of terpenoids, Perry *et al.*, 2009 [5].

Out of all the plants that possess neuroprotective activity, *G. biloba* is the most widely used and most common plant in the history of Asian culture. The reason behind the wide use of *G. biloba* among all neuroprotective plants known is the main components of the leaves of *G. biloba* are flavonoids, constituting the largest group of active compounds such as benzo-γ-pyrone derivatives which include mainly biflavones: ginkgetin, isoginkgetin, bilobetin, scjadopitzuna, amentoflavone, and the flavonols: kaempferol, quercetin, isorhamnetin, rutin, myricetin, flavones: luteoin, apigenin, and their glycosides and flavanonols.

As per Gong *et al.*, (2008) [6], the Maidenhair tree, that is – *G. biloba* is popularly known as a living fossil. It is a plant native to the Asian continent that has been used for more than 5000 years in traditional Asian medicine. *G. biloba* EGB761 is a popular herbal supplement, collected from the plant's dried leaves. *G. biloba* extract of this plant is available as herbal medicine/supplement in the form of capsules, tablets, and liquid extracts. Old people in the Asian continent consume

this plant's leaves in their natural form or in the form of supplements on a large scale. *Ginkgo* has been used for ophthalmological purposes, including glaucoma, age-related macular degeneration, brain-related disorders such as ADHD, autism, other disorders such as high cholesterol, premenstrual syndrome, bloody diarrhea, improving liver and gallbladder function, Lyme disease, controlling blood pressure, to treat allergies, asthma, and bronchitis.

Studies on phytochemicals present in the *G. biloba* leaves have revealed the presence of flavonoids (24%), terpene trilactones (6%), proanthocyanidins, organic acids, and other constituents [7]. Other than the medicinal benefits of *G. biloba*, its usage in neurodegenerative disorders, and brain injuries has been remarkable. The first publication for the medicinal use of *G. biloba* dates back to 1505 AD and studies on its standardized extract form – EGB761 have been conducted ever since the 1990s. Flavonoids are the main components that contribute to the antioxidant activity of the *G. biloba* extract [8,9], whereas the terpene trilactones in it contribute to blood microcirculation and neuroprotective properties [10]. Phytochemicals identified in *G. biloba* are flavonoids, terpenoids, biflavones, organic acids, etc. Flavonoids - Quercetin, Kaempferol, Isorhamnetin, Glycosides [11], Terpenoids like Ginkgolide, Ginkgolide B, Ginkgolide C, Ginkgolide J, Biflavones like Bilobetin, Ginkgolide acid [10], Schneider *et al.* 2007 [12] have identified Ginkgetin, Organic acids like Shikimic acid, Kynurenic acid, Ascorbic Acetate, 3-methoxy-4-hydroxybenzoic acid, 4-hydroxybenzoic acid 3,4-dihydroxybenzoic, 6-hydroxyquinurenic acid and other phytochemicals such as Glucose, Rhamnose, Sterols, Aliphaticketones, Alcohols, Diterpenes, Phenylpropanoids, and Carotenoids in the plant leaves. Research conducted on flavonoids to date has shown that flavonoid-containing foods possess antioxidative activity, free-radical scavenging activity, anticancer activity, anti-human immunodeficiency virus properties, and protect cardiovascular health [13]. Terpenoid ginkgolide B is known to prevent migraine in young individuals [14]. Shikimic acid-containing plants are known to act as analgesic, anti-cancer, anti-inflammatory, anti-microbial, and antioxidative agents [15]. As per Wang *et al.*, [16], *G. biloba* has also been found to have anti-aging activities *in vitro*.

Despite its vast biological benefits, *G. biloba* has been seen mostly in the field of neuroscience due to its neuroprotective properties. In the field of neuroscience, *G. biloba* is also known to be effective against subarachnoid haemorrhage (SAH), and neuronal apoptosis, Kim *et al.*, 2016 [17] and improve cognitive function, regulate inflammatory responses [18], cerebral palsy, TBI, early brain injury (EBI), ischemic stroke, nerve regeneration, diabetic neuropathy, chemotherapy-induced peripheral neuropathy, neurodegenerative disorders, and its anxiolytic effect. Our review paper focuses on the neuroprotective properties of *G. biloba* mentioned above.

SAH

SAH is bleeding caused within the subarachnoid space. Several times, SAH is bleeding caused in the subarachnoid space of the brain due to a ruptured aneurysm and has a morbidity and mortality rate higher than 50 %. It accounts for 5% of strokes of all types. EBI is the immediate acute phase pathological process that occurs during the first 72 h after suffering SAH. Clazosentan is one of the widely used standard synthetic drugs for SAH. It is an endothelial receptor antagonist that is known to treat SAH by inhibiting endothelin-mediated cerebral vasospasm. Despite its common use, a study with clazosentan has indicated that it has no significant effect on SAH, and patients administered clazosentan exhibit pulmonary complications, anemia, and hypotension [19].

In a study, standardized extract EGB761 was administered to SAH model rats with an objective to explore potential mechanisms of action of the extract. On inducing SAH to the model organism, variable doses of EGB761 were administered intraperitoneally after 2 h, followed by measuring the mortality, SAH grade, neurological score, and water content (edema) 24 h after inducing SAH. For assessing the expression of apoptosis-related proteins - Bax, Bcl-2, cleaved caspase-3, Akt, and

p-Akt, along with TUNEL and NeuN double immunofluorescence staining for detection of apoptotic neurons was done, Western blot assay was performed, which indicated that the standardized extract of *G. biloba* EGB761 could ameliorate SAH-induced EBI in rats that had suffered serious neurological deficiencies before being treated with EGB761. The standardized *G. biloba* extract EGB761 had attenuated neurological dysfunction, decreased brain water content, TUNEL, and NeuN positive cells, activated Akt signaling that was followed by an increased level of Bcl-2 and a decreased level of Bax, cleaved caspase-3. Despite its beneficial effects on SAH-induced EBI, Akt MK2206 was shown to eliminate the beneficial effects of EGB761. This study conducted by Yu *et al.*, [20] indicated that the beneficial neuroprotective properties of EGB761 on SAH were exerted through the activation of the Akt signaling pathway through suppressed neuronal apoptosis. The Akt signaling pathway is also known as the PI3K-Akt signaling pathway which is a signal transduction pathway in which PI3-kinase (phosphatidylinositol 3-kinase) and Akt (protein kinase B) play a key role in promoting survival and growth in response to extracellular signals.

Endothelin-1 (ET1) is a peptide secreted by vascular endothelial cells in response to stimuli such as stress and neurohormones. Endothelin-1 causes smooth muscle contraction through the intracellular protein kinase pathway, when present in the subarachnoid space of the brain. This eventually gives rise to vasospasm and causes smooth muscle contraction via the intracellular protein kinase pathway. This process is interceded by an increase in intracellular calcium. Following the release of free oxygen radicals, excitatory amino acids play a role by influencing the trigger zone during ischemia. During these physiological events, the administration of an antioxidant may prevent the massive ischemia that occurs in the brain. EGB761 has also been known to affect free radicals [21]. Anti-vasospastic effects of *G. biloba* extract EGB761 have also been studied in experimental SAH in Sprague–Dawley rats by Kotil *et al.*, 2008 [22].

CNS injuries

Traumatic brain injury (TBI) is the cause of an external force or blunt force trauma such as a violent blow or jolt to the head or body that causes brain dysfunction. TBI is seen in victims of motor vehicle accidents, falls, combat blasts, violence, sports injuries, etc; whereas ischemic brain injury (IBI) is another kind of injury to the brain that is caused due to decreased blood flow to the brain. TBI can also be a cause of IBI, both being from a challenging domain to assess and treat in the field of neuroscience. Spinal cord injuries are the ones that affect the spinal nerves, thereby causing the individual to suffer from paraplegia or quadriplegia. In general, CNS injuries can affect the physiological as well as the psychological function of the brain and spinal cord by affecting neurons, which eventually gives rise to torn tissues, causes bleeding, and results in other physical damage to the brain. CNS injuries can result in stroke, cerebral palsy, brain anoxia, and sometimes even death.

Convulsions or seizure attacks, pupil dilation in one or both the eyes, loss of consciousness, repeated vomiting or nausea, draining of clear fluid from the nose or ears, lack or loss of coordination, numbness of fingers and toes are physical symptoms of a brain injury. Brain injuries also affect the cognitive functions of individuals in a way that they experience profound confusion, show the presence of slurred speech, go into a coma, and display other disorders of consciousness, exhibit agitation, combativeness, or other unusual behavior. If not treated effectively, brain injuries can result in prolonged or permanent changes in an individual's consciousness and responsiveness. A brain injury can result in a coma, where patients cannot respond to stimuli. Furthermore, coma can cause the individual to end up in a vegetative state, on mechanical support. Brain death can also occur due to coma, where there is no measurable activity in the brain and brainstem.

IBI

Early brain injuries can be a cause of SAH as mentioned earlier in this paper. Injuries to the brain are fatal and can cause death or result in a coma, if not taken care of at the earliest. Apart from the availability

of drugs to treat brain injuries, herbal supplements are also seen as possible medicines, without side effects. According to studies by Sung *et al.*, (2012) [23], *G. biloba* extract EGB761 possesses neuroprotective potential against IBI. This property of *G. biloba* 761 is exerted through an anti-apoptotic mechanism. The proteomic approach in this study has revealed that with EGB761 pre-treatment, there is an alleviation of the ischemic injury-induced decrease in parvalbumin expression. Parvalbumin is a calcium-binding protein that plays an essential role in maintaining the calcium homeostasis of the CNS by decreasing the intracellular concentration of Ca^{2+} . It is similar to calmodulin and troponin C and is found in the brain and some endocrine tissues. An increase in the intracellular Ca^{2+} concentration leads to neuronal death, leading to nervous system disorders [24]. A study conducted by Sung *et al.*, 2012 [23] showed that the number of parvalbumin-positive cells in the vehicle-treated animals was lower than in sham-operated animals and that it was due to *G. biloba*, that helped avoid this decrease. Thus, the study indicates that the maintenance of parvalbumin expression is associated with the neuroprotective potential of *G. biloba* extract EGB761 against ischemia-induced neuronal damage.

Tulsukar *et al.*, 2013 [25] reported the neuroprotective effect of *G. biloba* EGB761 in male mouse models of brain ischemia. The extract's mechanism of action was due to the upregulation of the heme oxygenase 1 (HO1)/Wnt pathway. As there are gender differences seen in clinical stroke with respect to brain damage, Tulsukar *et al.*, 2015 [26] ovariectomized female mice to remove the protective effect of estrogen, followed by treatment with *G. biloba* EGB 761, 7 days before inducing permanent middle cerebral artery occlusion (pMCAO) and was allowed to survive for another 7 days. This study indicated that OVX female mice that were administered EGB761 showed significantly reduced infarct size as compared to the Veh/OVX animals. This study also indicated that EGB761 treatment in female mice was capable of preventing caspase-3 cleavage, thereby blocking the extrinsic apoptotic pathway and inhibiting apoptosis. Thus, this study denoted that EGB 761 pre-treatment significantly enhances the neurogenesis process in OVX mice and upregulated androgen receptor expression with no changes in HO1/Wnt signaling. The experiment concluded that EGB761 prevents brain damage in OVX female mice, improves grip strength and neurological deficits, and blocks the extrinsic apoptotic pathway.

Traumatic encephalopathy

In an open-label pragmatic clinical intervention study conducted by Amen *et al.*, 2011 [27], 30 football players who had suffered from chronic traumatic encephalopathy (CTE) (i.e. – A type of TBI) during the game were advised supplements, that included *G. biloba*. Brain SPECT scans taken after 6 months of prescribing the supplements, which included *G. biloba* showed increased brain perfusion in the prefrontal cortex, parietal lobes, occipital lobes, anterior cingulate gyrus, and cerebellum. This study not only demonstrated improvements in the cognitive and cerebral blood flow pattern but also manifested the value of a natural nutritional approach to improving brain functioning.

Concussion

Concussion is an event that starts by an excessive release of neurotransmitters and excitotoxins following a mild TBI. The signaling molecules overwhelm the brain's ability to function and repair itself, and thus, damage the brain cells' mitochondria. A concussion can eventually lead to a stroke. Li *et al.*, 2018 [28] conducted a study using *G. biloba* extract in combination with aspirin for the treatment of acute ischemic stroke; which indicated that the *G. biloba* extract significantly reduces the neurological and cognitive deficits that occur due to acute ischemic stroke, without increasing the incidence of vascular events. Thus, incorporating *G. biloba* as a nutritional approach to treat stroke due to concussion seems possible.

Spinal cord injury

Spinal cord injury is another neurological injury that is seen after damage to any part of the spinal nerves or spinal cord in the CNS. Spinal cord

injury (SCI) often leads to permanent changes in an individual, thereby affecting the strength, sensation, and other body functions below or near the injury site. It can result in paraplegia or quadriplegia. The neuroprotective effects of *G. biloba* extract have revealed significantly increased superoxide dismutase activity, a reduced malondialdehyde content, and apoptotic index, and has also shown inducible nitric oxide synthase expression. EGB761 has also relieved neural cell apoptosis, inhibited inducible nitric oxide synthase expression, suppressed lipid peroxidation following SCI, and thus exerted protective effects on SCI [29].

Lacour *et al.*, 1991 [30] administered *G. biloba* EGB761 to unilateral vestibular neurectomized cats for 30 days and observed strongly accelerated postural, locomotor balance recovery, spontaneous neck muscle activity, vestibulo-collic reflexes, the spontaneous firing rate of vestibular units. This indicated that the extract possesses neurotrophic, as well as/or neurogenic properties which improve functional recovery after CNS injury.

Hypoxia

Hypoxia occurs when the human body and brain are devoid of adequate oxygen supply at the tissue level. It may be classified as generalized hypoxia - which affects the whole body, or local hypoxia - thereby affecting a specific body region. Both conditions are dangerous. Intermittent hypoxia (IH) can result in increased hippocampal oxidative stress, DNA damage, carbon monoxide poisoning, obstructive sleep apnea, and memory impairment. Gale *et al.*, (2004) [31]; Basel *et al.*, 2012 [32] conducted research on the effect of *G. biloba* extract EGB761 on rats with IH. This research indicated that the extract is capable of reversing IH-induced memory deficits. The serum and hippocampal levels of 8-OHdG (8-Hydroxy-2'-deoxyguanosine) increased. This study showed that EGB761 can protect against IH-induced memory impairment, oxidative stress, and neuronal DNA damage.

A study by Oberpichler *et al.*, 1988 [33] indicated that the flavone and also the non-flavone fraction of EGB increase the survival time of mice under lethal hypoxia. EGB761 acts against hypoxia by retarding the breakdown of brain energy metabolism in the hypoxic artificially ventilated rat. According to this study by Oberpichler *et al.*, 1988 [33], the non-flavone fraction of EGB761 carries anti-hypoxic activity.

Neuronal apoptosis

Oxidative stress affects the brain in a way that can cause hearing damage. Neural stem cells (NSCs) are known to have great therapeutic potential in the treatment of hearing loss. In a study by Wang *et al.*, 2016 [34], the anti-apoptotic role of EGB 761 was moderated by antagonizing the intrinsic mitochondrial apoptosis. The study showed that *G. biloba* EGB 761 could reverse the changes in key intrinsic apoptosis pathway factors, thus indicating that EGB 761 protects cochlear NSCs, by attenuating oxidative stress-triggered intrinsic apoptosis.

Alzheimer's disease is characterized by the accumulation and deposition of A β peptides over time, in the brain. Activities of apoptosis-related genes and proteins - SOD (superoxide dismutase) and GSH (glutathione S-transferase) and the apoptosis rate of the hippocampus were detected by Tian *et al.*, 2013 [35]. This research found increased activity of SOD and GSH with a reduction in MDA levels, Bax expression, cytochrome c release, and the activity of caspase-9/3, in rat hippocampal tissue. This study thus showed that the *G. biloba* extract EGB761 blocks mitochondria-mediated apoptosis signaling in AD, thereby reducing cell toxicity and oxidative stress.

Silver nanoparticles' (AgNPs) exposure enhances lipid peroxidation activities and increases brain tissue's silver and iron contents. AgNPs up-regulate TNF- α and IL-1 β transcript levels, while simultaneously over-expressing the caspase-3 protein in the cerebrum and cerebellum, thereby inducing neuronal apoptosis. A study by Lebda *et al.*, 2018 [36] showed that *G. biloba* improves the adverse effects of silver nanoparticles on the blood-brain barrier function and tight-

junction proteins. This is due to the presence of antioxidants, and anti-inflammatory, anti-apoptotic capabilities present in EGB 761. *G. biloba* extract also alleviates the neurotoxic side effects of AgNPs.

Bastianetto *et al.*, 2000 [37] showed that excess of the free radical nitric oxide (NO) has a deleterious effect on the CNS; and the protective and rescuing abilities of EGB761 are due to the antioxidant properties of its flavonoid constituents with the ability to inhibit NO-stimulated protein kinase C (PKC) activity [37].

Neurodegenerative disorders

A neurodegenerative disorder is the degeneration of neurons that impairs their physiology, structure, and function. These disorders include Alzheimer's, Parkinson's, and Huntington's disease. These disorders involve pathological phase transitions that can develop over time, affecting the quality of life. They arise due to the misfolding of toxic proteins; their accumulation can lead to memory loss, eventually affecting people's quality of life. Often, it is seen that patients experience disturbance in their sleep during the early stages of neurodegenerative diseases. During sleep, the misfolded proteins associated with neurodegeneration are cleared from the brain via the glymphatic system; loss of sleep is associated with neurodegenerative disorders.

Alzheimer's disease (AD)

G. biloba leaves are effective against age-related neurodegenerative disorders, including AD. Alzheimer's is a progressive neurodegenerative disorder where brain cells degenerate and die due to the accumulation and deposition of A β peptides. Alzheimer's is the most common cause of dementia that results in a continuous decline in thought-processing ability, and impaired behavioral and social skills that disrupt an individual's ability to function independently. AD is characterized by two major physiological hallmarks –the first being intracellular, followed by extracellular A β peptide accumulation, and later, intracellular neurofibrillary tangles (NFT). NFTs are composed of hyperphosphorylated tau protein. Several nutrients and medicinal drugs are shown to slow down the progression of cognitive decline related in AD patients. Such nutrients and chemicals include natural antioxidants such as Vitamin E, monoamine oxidase inhibitors (MAOIs), anti-inflammatory agents/drugs, cholinergic agents, estrogens, or neurotrophic factors. A β peptide or plaque build-up has been suspected to be one of the most prominent mechanisms underlying the pathophysiology of AD. In three studies on the efficacy of *G. biloba* on AD, it was observed that EGB761 protects against A β -induced neurotoxicity by blocking A β -induced events of ROS accumulation, glucose uptake, mitochondrial dysfunction, activation of AKT, JNK, and ERK 1/2 pathways, apoptosis and prevents amyloidogenesis Gerlai *et al.*, (2001) [38], Bastianetto *et al.*, 2000 [39], and Smith *et al.*, 2003 [40].

Due to recent failures in treating AD using amyloid β treatment, studies have been conducted extensively on mitochondrial dysfunction as a major pathophysiological mechanism of cognitive decline in aging. In a study by Stockburger *et al.*, 2018 [41], mitochondrial dysfunction *G. biloba* extract 761 was shown to have reduced mitochondrial dysfunction, improved cognition, impaired neuronal plasticity, and inhibited opening events of mitochondrial permeability transition pore (mPTP).

G. biloba is known for the improvement in memory and cognitive functions. EGB 761 is known for the increased blood flow in the brain, effect against peroxidation of brain lipids, reduction of amyloid plaque deposition Wan *et al.*, 2016 [18], lowering the level of A β oligomers and APP levels, Tchanchou *et al.*, 2007 [42], Augustin *et al.*, 2009 [43]. *G. biloba* is known to affect several neurotransmitter pathways, as demonstrated by studies in rats administered EGB 761 for 14 days, by Marcilhac *et al.*, 1998 [44].

Dementia

Efficacy of *G. biloba* extract EGB761 against cognitive impairment and dementia studied by Solfrizzi *et al.*, 2015 [45] showed clinical benefits

in cognition and behavior in patients with dementia, AD, and mild cognitive impairment (MCI) who displayed neuropsychiatric symptoms (NPS). Research conducted by Kanowski *et al.*, 1996 [46] on the efficacy of EGB761 in patients with pre-senile and senile primary degenerative dementia of the Alzheimer type (DAT) and multi-infarct dementia (MID) confirmed the efficacy of the extract.

Parkinson's disease

Parkinson's is another neurodegenerative disease in which an individual experiences tremors, rigidity, slowness of movement, and difficulty with walking, cognitive, and behavioral problems. The protective effects of *G. biloba* extract against 6-hydroxydopamine-induced PC12 cell apoptosis were seen in a model of Parkinson's disease. The extract decreased Bax: Bcl-2 and inhibited the activation of p53 and caspase-3. This indicated that the potential effects of *G. biloba* extract might be greater compared to levodopa in treating Parkinson's disease [29].

Cognitive ability

An individual's working memory is a part of their cognitive system that has a limited capacity to hold information temporarily. Object-Location Memory task assesses cognition, particularly spatial memory, and discrimination. *G. biloba* extract was tested on healthy middle-aged male participants by Silberstein *et al.*, 2011 [47], which indicated that steady state visually evoked potential (SSVEP) amplitude at occipital and frontal sites and showed an increase in SSVEP latency at left temporal and left frontal sites, during the hold component of the working memory task.

Neuroplasticity, Neurogenesis, and synaptogenesis

Neuroplasticity is the process and ability of synapses to structurally adapt in response to functional demand or dysfunctions in case of impaired neuronal structures.

In a study by Abder-Kadel *et al.*, 2007 [48], mitochondrial abnormalities during aging were imitated using external factors such as nitrosative stress, serum deprivation, and complex inhibitors, with an altered mitochondrial ability of energy metabolism. Markers for the function of mitochondria, ATP levels, and mitochondrial membrane potential were measured and it was observed that EGB 761 ameliorated mitochondrial functions *in vitro* at concentrations as low as 0.01 mg/ml. This study indicated that EGB 761 protected the mitochondrial membrane potential. A similar study conducted by Eckert *et al.*, 2006 [49] showed that EGB 761 could reverse the decrease in ATP production when administered after damage by sodium nitroprusside (nitric oxide donor) of PC12 cells.

Adult neurogenesis

The discovery of neurogenesis in restricted areas in the adult brain has revolutionized neuroscience research, especially the field of neurodegeneration. Tchanchou *et al.*, 2009 [50] studied EGB761-associated neurogenesis. Among the constituents tested from EGB 761, bilobalide and quercetin significantly increased cell proliferation in the hippocampal neurons in a dose-dependent manner of the AD mouse model. This study reported that bilobalide and quercetin from the extract also enhanced the phosphorylation of cyclic-AMP Response Element Binding Protein (CREB) in these cells. It also elevated pCREB levels and brain-derived neurotrophic factor in mice brains and enhanced neurogenesis and synaptogenesis by bilobalide and quercetin. This study indicated that enhanced neurogenesis and synaptogenesis may share a final signaling pathway that is common and is mediated by the phosphorylation of CREB. In a study by Leuner *et al.*, 2007 [51], it was observed that EGB761 protects the mitochondria by reducing oxidative stress and thus plays a key role as a neuroprotective agent.

Scavenging free radicals

Reactive oxygen species (ROS), hydroxyl radical (OH \cdot), free radicals such as superoxide anion (O $_2$ \cdot^-), or intermediate species such as hydrogen peroxide (H $_2$ O $_2$) damage all cell components and organelles, eventually leading to cell death. These free radicals are also responsible for

degrading the cell membrane phospholipids through lipid peroxidation by affecting the macromolecular components of the cell.

The effect of *G. biloba* EGB761 was researched by Da Silva *et al.*, 2011 [52] on the neurons of the myenteric and submucosal plexuses in the jejunum and ileum of streptozotocin-diabetic rats (STZ-diabetic rats). This study found that the extract significantly decreased the neuronal density of myenteric neurons in the ileum and has a neuroprotective effect on the ileum myenteric plexus and on the jejunum submucous plexus of STZ-diabetic rats.

Calapai *et al.*, 2000 [53] have shown that *G. biloba* can reduce post-ischemic brain malondialdehyde (MDA) levels and post-ischemic brain edema, delaying neuronal death in the hippocampus, reducing nitric oxide stress, and thus playing a role of a neuroprotective agent by inhibitory action on nitric oxide formation.

A study by Kobuchi *et al.*, 1997 [54] demonstrated that the standardized extract EGB761 directly acts as a scavenger of nitric oxide by inhibiting nitric oxide production in lipopolysaccharide plus interferon- γ (LPS/IFN- γ) activated macrophages by associated inhibition of induction of iNOS mRNA and the enzyme activity of inducible nitric oxide synthase (iNOS). Thus, this study has shown that EGB761 may act as a potent inhibitor of Nitric oxide production under tissue-damaging inflammatory conditions.

Neuroleptic properties of *G. biloba*

Neuroleptic agents are antipsychotic agents used for psychosis, reducing confused states, delusion, hallucinations, and psychomotor agitation in psychotic patients. Refugees and war survivors are at an increased risk of anxiety, stress, post-traumatic stress disorder (PTSD), and depression. A study on random refugees by administering *G. biloba* and measuring anxiety levels among them, using the Hamilton anxiety rating scale (HAMA) showed that the extract improved anxiety and mental fatigue Alsmadi *et al.*, (2018) [55]; Montes *et al.*, 2015 [56].

Another study by Atmaca *et al.*, 2005 [57] showed that EGB761 enhances the efficiency of antipsychotics in patients with schizophrenia when administered along with antipsychotic haloperidol. The extract of *G. biloba* reduced serum superoxide dismutase (SOD) levels in patients.

CONCLUSION

Although drugs used against neurodegenerative disorders and CNS injuries are effective and work fast, compared to other herbal medicines and supplements, they come with unforeseen side effects that differ in all patients. It is clearly known that *G. biloba* has been used as an effective natural medicine to cure a number of ailments other than those of the nervous system, and considering several studies conducted on the leaf extract EGB761 of the *G. biloba* plant, it is clear that EGB761 has neuroprotective benefits as mentioned in this review paper. Due to the fatal nature and urgency of CNS injuries, it is understandable that drugs are chosen over herbal medicines; but after injury recovery, temporary drug prescriptions can be either accompanied or replaced by herbal supplements such as *G. biloba* to avoid long-term adverse side effects. However, in the case of neurodegenerative disorders, herbal supplements prove more beneficial and effective as compared to synthetically derived drugs. Since researchers have studied several benefits of *G. biloba* extract EGB 761, it can be successfully used as a nutritional approach in treating neurodegenerative disorders and CNS injuries.

DECLARATION OF CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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AUTHOR CONTRIBUTIONS

RSJ conceived, conceptualized, the study, and designed the review paper structure. RSJ collected the data. RSJ contributed to the data and analysis tools. RSJ, NNP, and BS discussed the conclusion. RSJ wrote the manuscript. NNP and BS proofread the manuscript. All authors contributed to the article and approved the submitted version.

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