

TRACING ANTIOXIDANT AND ANTI-INFLAMMATORY MECHANISMS OF PHYTOCHEMICALS IN ALZHEIMER'S DISEASE

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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative illness and the main reason for dementia. It is accompanied by memory disturbances, cognitive impairment, and neuronal cell death. The pathology of Alzheimer's disease is considered multivariate and includes amyloid- β plaque accumulation, tau hyperphosphorylation, mitochondrial dysfunction, oxidative stress, and chronic neuroinflammation. Among those factors, oxidative stress and inflammation are interconnected and significantly contribute to the development of the disease. Uncontrolled generation of reactive oxygen species (ROS) leads to the emergence of lipid peroxidation, protein oxidation, oxidative damage of the DNA molecules, and mitochondrial dysfunction. At the same time, activation of microglia and astrocytes contributes to the increase in the secretion of pro-inflammatory cytokines. Phytochemicals are biologically active substances naturally present in the plants that have attracted much attention due to their antioxidant, anti-inflammatory, and neuroprotective properties. Various groups of phytochemicals – polyphenols, flavonoids, terpenoids, alkaloids, etc. are able to affect numerous pathogenic pathways of AD. Antioxidant effects include direct free radical scavenging, reduction of ROS and RNS, decrease in lipid peroxidation, and stimulation of endogenous antioxidants such as superoxide dismutase, catalase, glutathione, and glutathione peroxidase. Phytochemicals can activate antioxidant signal transduction pathways like pathway and thus increase cellular resistance to the oxidative damage. Apart from the antioxidant effects, phytochemicals exert anti-inflammatory influence by suppressing the microglial activation and reducing the generation of inflammatory mediators. Phytochemicals can regulate signalling pathways involved in the regulation of the inflammation like inflammasome. In that way, they might suppress the synthesis of pro-inflammatory cytokines and protect neurons from the inflammatory damage.

Key words: Alzheimer's disease (AD), reactive oxygen species (ROS), polyphenols, flavonoids, terpenoids, alkaloids.

Introduction

Alzheimer disease is a Neurodegenerative disease which increases slowly in the brain which later affects our memories, learning capacity, thinking and understanding capacity and also our behaviour. It is a type of dementia which especially seen in old aged people. In Worldwide as the population is increasing with that the Alzheimer disease patient is also increasing. As a result it is not only a disease but also became a social and financial challenge. This disease reduces the quality of patient and also increases the mental stress, financially and sentiments problem. Although the medical research is in continuous progress then also the old age people are suffering from disability and leading to death due to the reason of Alzheimer disease[1,3,4]

Due to the several changes of biological in brain is the only reason for developing the Alzheimer disease. The main characteristics of Alzheimer disease is the unwanted accumulation of Amyloid- β protein at the outer side of nerve cells and also inside the nerve cells the entangled clumps i.e Neurofibrillary Tangles of Tau protein is formulated. Along with it other reason is also there like oxidative pressure, Neuroinflammation for long time, lack of mitochondrial function, lack of neurotransmitter, and also the progressive loss of nerve cells leads to the loss of ability of cognitive and loss of memory. Since the several changes of biological in Alzheimer disease are affected equally. Therefore the medicine which are working only on single target to treat the Alzheimer disease cannot often handle the progress of the Alzheimer disease.[2,11]

The main characteristics of the medicine which are available currently helps to handle the symptoms of Alzheimer disease for short period of time. Two factors that can help some patient in the improvement of memory capacity and ability of cognitive are cholinesterase inhibitors and NMDA receptors but they don't have the ability to stop the progressive degeneration of brain nerve cells. Also the use of medicines/medication for long period of time causes adverse effects and also the high cost of treatment and limited effectiveness arises. This is the only reason the need of safe and effective treatment can be felt that can work equally on multiple disease at same time.[12]

The natural products are used from long period of time in traditional medical field/research and still it is important in new drug discovery. The plant- parts derived or phytochemicals which are rich in medicinal properties which are the bioactive compounds attract the attention of scientist due to their medicinal properties. The bioactive compounds are having the antioxidant, anti-inflammatory which is used to treat inflammation, and the anti- apoptotic which prevents the cell death and neuroprotective which prevents the nerve cells all these are very important and benefit for the treatment of neurodegenerative disorder. The main characteristics of this compound is that it can work equally on multiple molecular pathways, due to which it increases the importance of their in the treatment of multifactorial disease like Alzheimer.[22]

The main phytochemical constituents i.e curcumin, tamanolides, ginkgolides are having the ability of neuroprotective potential which are extensively studied. Curcumin are the phytochemical constituents which are derived from curcuma longa (turmeric) which has the ability to decrease the oxidative stress, control the inflammation, and decreases the unwanted accumulation of Amyloid- β protein and Tau protein. Withanolides are the primary active constituents of Ashwagandha (Withania Somniferous) which have the ability in protecting and repairing the nerve cells , ability of improving the synaptic function and increasing the memory . Another active constituents, Ginkgolides are derived from Ginkgo Biloba which plays a very important role in the improvement of cerebral blood flow, reduces the oxidative damage, regulates the inflammatory process and helps in protecting nerve cells from injury. Due to their multi properties or actions or beneficial properties are most important in the treatment of Alzheimer disease. [9,25,26]

Standardized phytochemical constituents have the most significant role in the development of scientific herbal medicines. The quantity of active constituent in each herbal product remains consistent and defined and it is ensured by standardization. Standardization maintains the uniformity, safety and efficacy of product. It also makes the research and findings more reliable. In this review article the detailed mechanism of neuroprotective of curcumin, withanolides, ginkgolides are studied. Also the studies of oxidative stress, mitochondrial damage, Amyloid beta protein, Tau protein are also studied which are used for the management of Alzheimer disease [8]

Background , Rationale and Scope

There is a lot of interest in finding phytochemicals from plants. This has led to a lot of research on phytochemicals. However, we still do not know how these phytochemicals work on Alzheimers disease. Most of the research on phytochemicals only looks at the -inflammatory effects of phytochemicals. We need to study how phytochemicals affect pathways in the body. The problem is that the way we extract and prepare

phytochemicals can affect their composition and dosage. This is why we need to review the existing research on phytochemicals.[15]

Another important issue is that some phytochemicals, like curcumin have bioavailability issues. This means that curcumin is not easily absorbed by the body. Curcumin has shown promise in laboratory tests. Curcumin is not very effective in practice. This is because curcumin is not well absorbed and is broken down quickly. The same problem exists with phytochemicals like withanolides and ginkgolides. The effect of phytochemicals like withanolides and ginkgolides depends on the quality of the preparation and the dosage of phytochemicals. Recent advances in sciences have led to the development of drug delivery systems. These systems can improve the bioavailability and delivery of phytochemicals to the brain.[16]

In addition to improving bioavailability researchers are also studying the signaling pathways affected by phytochemicals. Phytochemicals like curcumin, withanolides and ginkgolides have been shown to affect pathways. These pathways are involved in neuroprotection and cerebral function. Phytochemicals like curcumin, withanolides and ginkgolides can inhibit stress, suppress neuroinflammation and prevent the formation of amyloid-beta. Phytochemicals like curcumin, withanolides and ginkgolides can also improve function enhance plasticity and regulate apoptotic pathways. The fact that phytochemicals like curcumin, withanolides and ginkgolides can target pathways makes phytochemicals like curcumin, withanolides and ginkgolides promising for the treatment of diseases like Alzheimers disease.[90]

Many pre-clinical studies have shown that standardized phytochemicals have effects. Standardized phytochemicals can improve memory and learning protect neurons reduce amyloid plaque load and reduce stress. While there have been studies it appears that standardized herbs can have a positive impact on cognitive abilities and quality of life. However clinical studies are needed to confirm the safety and efficacy of phytochemicals. There is a need for affordable drugs that can target multiple pathways. This has led researchers to look for alternatives or adjuvants to existing Alzheimers disease medications. Standardized herbal extracts have benefits, including acceptability by patients reduced side effects and natural source. Standardized herbal extracts can also target pathways making herbal extracts a promising option for the treatment of Alzheimers disease.[91]

The aim of this review is to provide an overview of the research on the neuroprotective effects of standardized phytochemicals. We will discuss the actions of phytochemicals, mechanisms of action of phytochemicals and influence of phytochemicals on stress, neuroinflammation and amyloid-beta pathology. We will also review the studies, standardization, bioavailability, formulations, safety and future research areas of phytochemicals. Alzheimers disease is a condition that requires agents that can target multiple pathways. Phytochemicals like curcumin, withanolides and ginkgolides have effects meaning phytochemicals like curcumin, withanolides and ginkgolides can interact with cellular pathways. This makes phytochemicals like curcumin, withanolides and ginkgolides a promising option for the treatment of Alzheimers disease. Recent advances in biology and neuropharmacology have improved our understanding of the processes involved in neuronal injury.

Oxidative stress is one of the features of Alzheimers disease. Oxidative stress can damage lipids, proteins and nucleic acids leading to dysfunction. Brain tissues are particularly sensitive to oxidative stress due to oxygen consumption and low levels of antioxidants. Persistent oxidative stress can lead to dysfunction amyloid-beta aggregation and neuroinflammation. Compounds with activity like phytochemicals may be able to prevent this cycle. Neuroinflammation is another aspect of Alzheimers disease. The activation of microglia and astrocytes can lead to the secretion of cytokines which can aggravate damage. Phytochemicals with -inflammatory properties, like curcumin, withanolides and ginkgolides may be useful in this context.

Mitochondrial dysfunction is also a pathology associated with Alzheimers disease. Mitochondria are involved in ATP production and cellular metabolism. Damage to mitochondria can lead to energy deficiency, production of oxygen species and disruption of calcium homeostasis. Certain plant compounds, like phytochemicals may

be able to promote biogenesis strengthen their membranes and reduce damage. Synaptic dysfunction is another pathology associated with Alzheimers disease. Amyloid beta oligomers can disrupt transmission decrease the release of neurotransmitters and impair plasticity. Phytochemicals like curcumin, withanolides and ginkgolides may be able to increase the number of proteins normalize communication and improve synaptogenesis.

Autophagy and protein clearance mechanisms are also important in Alzheimers disease. The accumulation of proteins like hyperphosphorylated tau and amyloid-beta peptides can lead to toxicity. Standardized phytochemicals may be able to regulate and clear misfolded proteins reducing toxicity. In addition to their effects on neurons standardized phytochemicals may also affect blood flow and vasculature. Phytochemicals like ginkgolides may be able to improve microcirculation, platelet disaggregation and delivery of oxygen to brain tissues.

The gut-brain axis is another area of increasing significance. Changes in the composition of gut microbiota may impact neuroinflammation, regulation, oxidative stress and amyloid pathology. Some phytochemicals may have properties ensuring a proper intestinal microbiome balance and promoting brain functions. Despite the research results the clinical implementation of treatment is difficult. The variability of plant growth conditions, harvesting, extraction procedures and preparation can affect the composition and biological properties of phytochemicals. The absence of quality control and non-standardized production can complicate data comparison.

Standardization is necessary to provide composition reproducible properties, enhanced safety and improved efficacy of preparations. Recent advances in pharmaceutical formulation science have improved the effectiveness of phytochemicals. Nanotechnologies, including nanoformulation, polymeric nanoparticles, solid lipid nanoparticles, nanoemulsion, liposomes, phytosomes and other drug delivery systems have been developed to increase solubility stabilize drug molecules and improve permeability of the blood-brain barrier. These novel methods can circumvent the challenges related to herbal medicines and help in the successful management of neurodegenerative diseases.

Alzheimers disease is a problem with causes. Because of this scientists are looking at things to understand Alzheimers disease better. They are looking at things like biology and pharmacology. They are also looking at phytochemistry and clinical medicine. By combining all this information we can learn about all the things that are involved in Alzheimers disease. This will help us make medicines from plants that are based on science. We will also learn things that will be useful for studies on people. So we need to keep doing research on how to make these plant medicines in a way. We need to figure out the way to give phytochemicals to people and how phytochemicals work. We also need to make sure phytochemicals are safe and effective for people, with Alzheimers disease. Alzheimers disease is the thing we are trying to understand and treat.

Epidemiology, Pathophysiology and Current Treatment

1. Epidemiology of Alzheimer's disease

Alzheimer's disease (AD) is the most prevalent type of dementia and is a progressively worsening disease that involves deterioration of memory, cognition, behavior, and activities of daily living. According to the World Health Organization, an estimated 57 million people were living with dementia globally in 2021, with about 10 million more cases developing every year. Alzheimer's disease causes about 60-70% of dementia cases. More than 60% of dementia sufferers are from low- and middle-income countries. It is predominantly a condition affecting women not only due to the high prevalence among females but also because of the significant number of female caregivers.[17,18,19]

Old age is the main risk factor identified for AD and the number of patients will be on the rise with the increasing life expectancy. Genetic susceptibility, specifically the APOE ϵ 4 allele, family history,

cardiovascular disease, high blood pressure, diabetes, obesity, lack of exercise, smoking, depression, loneliness, and traumatic brain injuries are other significant risk factors for AD.[20]

2. Causes and pathophysiology

The exact etiology of AD is complicated and not well known. It is now viewed as a multifactorial disorder where the genetic, aging, metabolic, vascular and environmental factors interact with each other. The main pathological hallmarks of AD include:

- Deposition of amyloid- β ($A\beta$) plaques
- Hyperphosphorylated tau and neurofibrillary tangles
- Oxidative stress
- Mitochondrial dysfunction
- Neuroinflammation
- Activation of microglia and astrocytes
- Synaptic dysfunction and neuronal loss
- Impairment of cholinergic neurotransmission

and neuronal energy and insulin signaling abnormalities.

The deposition of $A\beta$ peptides leads to oxidative stress and inflammatory signaling whereas hyperphosphorylation of tau results in neuronal microtubule destabilization and dysfunction of neurons. The pathological events are interconnected rather than independent. Thus, AD cannot be explained only through one mechanism which is one of the reasons why the multitarget phytochemicals are now studied. [21]

3. Risk factors/causes associated with Alzheimer's disease

Risk factor	Role in Alzheimer's disease
Advanced age	Most important risk factor; risk increases substantially with ageing
Family history	Increases susceptibility to AD
APOE $\epsilon 4$	Major genetic risk factor for late-onset AD
Hypertension	Can contribute to cerebrovascular and neuronal injury
Diabetes mellitus	Associated with metabolic dysfunction and increased dementia risk
Obesity	Associated with inflammation and vascular/metabolic abnormalities
Physical inactivity	Associated with increased cognitive decline
Smoking	Promotes oxidative stress and vascular damage
Traumatic brain injury	Can increase later-life dementia risk
Depression/social isolation	Associated with cognitive decline and dementia risk
Vascular disease/stroke	Can increase neuronal and cognitive damage

Age, cardiovascular disease, diabetes, obesity, previous head injury and low physical, mental or social activity are recognized risk factors by the FDA and other major health authorities.

4. Drugs currently used in Alzheimer's disease

The current pharmacological approach may be broadly categorized into symptomatic medications and anti-amyloid disease modifying agents.[23,24]

4.1 Cholinesterase inhibitors

The main cholinesterase inhibitors include:

1. Donepezil
2. Rivastigmine
3. Galantamine

These medications have the ability to inhibit acetylcholinesterase and or butyrylcholinesterase therefore the degradation of acetylcholine is inhibited. The enhancement of cholinergic neurotransmission may lead to temporary improvement or stabilization of the clinical picture. Donepezil is prescribed for mild to severe forms of AD while rivastigmine and galantamine are used only for mild-to-moderate disease, in accordance with the recommendations.[38]

Adverse effects

Main adverse effects:

- Nausea
- Vomiting
- Diarrhoea
- Abdominal pain
- Decreased appetite
- Weight loss
- Dizziness
- Headache
- Muscle weakness
- Sleep disturbances

Since cholinergic activity is increased by these medications gastrointestinal side effects are especially common. Application site adverse reactions may occur as a result of using rivastigmine as a transdermal patch. [39]

4.2 Memantine

Memantine is a type of NMDA-receptor antagonist mostly used for moderate to severe AD. Overactivation of glutamatergic system may cause neuronal excitotoxic damage. Memantine controls NMDA receptor overactivation. It may be taken either separately or together with a cholinesterase inhibitor. [40,41]

The possible side effects may be:

- Dizziness
- Headache
- Constipation
- Confusion
- Sleep disorders
- Some patients experience agitation or hallucinations

4.3 Anti-amyloid monoclonal antibodies

Recently more disease-modifying medications for targeting amyloid pathology were developed. They include lecanemab and donanemab which target amyloid- β pathology in selected cases of early-stage symptomatic AD. It should be noted that the goal of such medication is not improving symptoms, but reducing amyloid pathology and slowing the development of clinical symptoms of the disease. Nevertheless, there are safety concerns about these medications, e.g., lecanemab causes amyloid-related imaging abnormalities (ARIA) in form of cerebral edema and microhemorrhage as well as infusion reactions. Thus, we see why there is a need in developing multi-targeting compounds, which could have a neuroprotective effect on oxidative stress, inflammation, A β and neuronal survival.[42]

5. Why investigate phytochemicals in Alzheimer's disease?

Conventional pharmaceuticals generally target specific pathways such as cholinergic neurotransmission or amyloid pathology. However, AD is characterized by multiple pathological features. Thus, phytochemicals have been under consideration since there are compounds that have more than one biological activity such as:

Anti-oxidant → Anti-inflammatory → Anti-amyloid → Cholinesterase inhibitors → Mitochondrial protection → Synaptic/neurotrophic support

This multifunctionality explains why curcumin, withanolides and ginkgolides are being studied in AD. However, the evidence of the activity of compounds in cell and animal models does not guarantee their effectiveness in treating AD in humans.

6. Curcumin in Alzheimer's disease

Curcumin is a major polyphenolic compound of *Curcuma longa* (turmeric). It is among the most well studied dietary phytochemicals in AD.

Neuroprotective mechanisms

There were a number of suggested mechanisms of the action of curcumin:

6.1 Anti-amyloid activity

The mechanism of interaction of curcumin with A β peptides and its inhibiting effect on the A β aggregation process and disaggregation of pre-formed fibrils has been shown in the experimental studies. It is significant since the aggregation of A β peptides is one of the main features of AD.[27]

6.2 Antioxidant activity

Another critical factor contributing to neuronal injury in AD is oxidative stress, which can be scavenged by curcumin via direct and indirect mechanisms.

6.3 Anti-inflammatory activity

As curcumin can affect various signaling pathways related to inflammation, such as NF- κ B pathway, and possibly decrease inflammatory mediators induced by activation of microglia and astrocytes, it becomes another important factor that helps to inhibit neuroinflammation.

6.4 Effect on tau pathology

Although in experiments curcumin can help to decrease abnormal phosphorylation of tau and regulate mechanisms of tau removal, there is a lack of significant clinical evidence for its effect on this type of pathology in comparison with its antioxidant and anti-inflammatory properties.

6.5 Cholinesterase inhibition

In addition to the above, curcumin has been shown to have acetylcholinesterase inhibitory activity experimentally.

6.6 Major limitation

One of the biggest problems of curcumin in relation to its neuroprotective properties is its poor oral bioavailability, which makes it quite difficult to achieve clinically relevant concentrations in the brain. That is why in an NTCC review, curcumin should be considered as a promising investigational neuroprotective phytochemical rather than a well-proven drug against Alzheimer's disease.

7. Withanolides in Alzheimer's disease

Withanolides are lactone derivatives of steroids that have been identified primarily in *Withania somnifera* (Ashwagandha). Key components of withanolides include withanolide A, withanolide B, withaferin A, and various withanosides.

Importance in Alzheimer's disease research is associated with their ability to affect various pathological processes involved in this disease.[29,30,31]

7.1 Anti-amyloid activity

In vitro experiments have been conducted using the components isolated from *Withania* to determine their capacity to affect the process of A β 42 fibrillation. Withanolide A, withanolide B, withanoside IV and withanoside V have been studied experimentally in relation to their capacity to affect A β 42 fibrillation. [28]

7.2 Antioxidant activity

Oxidative stress plays an essential role in neuronal damage. The components of *Withania* extracts possess antioxidant activity, which may help protect neurons from oxidative stress. [32]

7.3 Anti-inflammatory activity

The *Withania* ingredients might have an effect on inflammatory pathways such as NF- κ B and other inflammatory mediators. This may contribute to reducing chronic inflammation in the brain, which is thought to cause neurodegeneration.[31]

7.4 Cholinergic and cognitive actions

Some studies have shown that withanolides have a possible action on cholinergic pathways, as well as acetylcholinesterase activity. Human studies of standardized extracts from *Withania somnifera* have demonstrated improvements in certain cognitive tests, but this was not necessarily conducted specifically for Alzheimer's patients. A 2025 randomized pilot study of adults with mild cognitive impairment found improvements in certain memory and cognitive tests when given a standardized extract of Ashwagandha

compared to placebo. It is an intriguing clinical study; however, it does not constitute proof that withanolides cure Alzheimer's disease. [32]

7.5 Neurotrophic and neuronal protection

There is a suggestion from pre-clinical studies that *W. somnifera* components could affect neuronal growth, synaptic function and cell survival pathways.

Important limitation

The research base for standardized withanolides for AD patients is much less developed than research on existing AD medications. The majority of scientific evidence comes from animal or cellular experiments and/or with extracts, not with standardized withanolides.

8. Ginkgolides in Alzheimer's disease

Ginkgolides are terpene lactones isolated from *Ginkgo biloba*. They are present in standardized preparations of *Ginkgo biloba*, e.g. *Ginkgo biloba* extract EGb 761, together with flavonoid glycosides and other terpenes[33,34,35].

8.1 Antioxidant activity

Ginkgo-derived constituents can decrease oxidative stress and protect cellular membranes from damage by free radicals. It is important in view of strong correlation between AD pathology and oxidative stress. [33]

8.2 Anti-inflammatory activity

Ginkgo constituents can regulate inflammation and reduce its effect on neuronal cells.[34]

8.3 Mitochondrial and neuronal protection

Ginkgo preparations have been shown to possess neuroprotective activity in experimental studies, including protection from oxidative and metabolic insults.[35]

8.4 Platelet-activating factor antagonism

One notable feature of ginkgolides, especially of ginkgolide B, is that it acts as an antagonist of the PAF (platelet activating factor) receptor.

8.5 Cognitive function

The clinical evidence that has been accumulated is related mostly to standardized extracts of *Ginkgo biloba* than to individual ginkgolides. Previous meta-analysis of EGb 761 had indicated benefits in cognition, activities of daily living, and global assessment when compared with placebo in selected patients with dementia.

However, there is some inconsistency in the available data. According to the 2026 Cochrane review, it is important to take into consideration the heterogeneity of the evidence. Therefore, the review must clearly differentiate between evidence related to standardized *Ginkgo* extracts and individual ginkgolides.

Side effects and limitations of the phytochemicals

One should be careful not to say that herbal substances are inherently "safe".

Curcumin

Among the possible adverse events are:

- Gastrointestinal symptoms

- Nausea and/or diarrhea
- Varying absorption
- Drug interactions
- Low systemic/central nervous system bioavailability
- Concerns about chronic, high-dose supplementation

Withania/withanolides

The reported adverse effects of preparations containing Ashwagandha are as follows:

- Gastrointestinal symptoms
- Drowsiness
- Headache
- Maybe some drug interactions
- Concerns related to quality of preparations.

As most of the scientific research deals with the whole preparation and not isolated withanolides, the safety of the latter cannot be considered the same as that of the whole preparation.

Ginkgo/ginkgolides

Among the possible adverse effects are:

- Gastrointestinal symptoms
- Headache
- Dizziness
- Hypersensitivity
- Bleeding risk due to interaction with antithrombotic and anticoagulant drugs.

Therefore, standardization and pharmacovigilance are very important.

Pharmacological Therapy and Investigational Phytochemicals

1. Pharmacological Therapy and Investigational Drugs in Alzheimer's Disease

Alzheimer's disease is an irreversible neurodegenerative condition in which management is done for the purpose of amelioration of cognitive symptoms, behavior, and functional impairment, as well as in some selected early cases, alteration of the pathological process itself through amyloid modification. Currently available symptomatic pharmacological treatments are donepezil, rivastigmine, and galantamine, which are acetylcholinesterase inhibitors, and memantine, which is a N-Methyl-D-Aspartate (NMDA) antagonist. Though the above treatments have modest effects on cognitive improvement, performance of daily activities, and overall functionality, they do not cure the disease and halt neurodegeneration.

1.1 List of drugs used in Alzheimer's disease

Donepezil is a cholinesterase inhibitor, which is mostly used to treat mild to severe Alzheimer's disease. It enhances the levels of acetylcholine in the synaptic cleft and, thus, improves cholinergic neurotransmission.

Studies have shown the positive effect of donepezil on cognitive and global function in patients with mild to moderate Alzheimer's disease. [1,2]Rivastigmine blocks acetylcholinesterase and butyrylcholinesterase enzymes and is prescribed to treat mild to severe Alzheimer's disease. The drug is taken orally and transdermally. Galantamine blocks acetylcholinesterase enzyme and also modulates nicotinic acetylcholine receptors, hence improving the cholinergic transmission. [3] Memantine is an NMDA receptor antagonist used to treat moderate to severe Alzheimer's disease. It works via inhibition of pathological glutamate excitotoxicity. Memantine may be combined with cholinesterase inhibitor.

Besides symptomatic therapy, disease-modifying anti-amyloid drugs represent another significant advance in the treatment of Alzheimer's disease. Lecanemab is an anti-amyloid monoclonal antibody aimed at amyloid- β aggregates and indicated for the appropriate patients with early symptomatic Alzheimer's disease. CLARITY-AD trial showed significant slowing of the clinical decline in comparison with placebo.[6] Another anti-amyloid monoclonal antibody, which targets deposited amyloid plaques, is Donanemab. Phase III TRAILBLAZER-ALZ 2 trial revealed that Donanemab significantly slowed the clinical decline in people with early symptomatic Alzheimer's disease; however, the treatment is associated with possible risks such as amyloid-related imaging abnormalities.

Another amyloid-directed monoclonal antibody is aducanumab, which got FDA accelerated approval in 2021 because of its capacity to remove amyloid plaques; however, the clinical effect from the treatment is controversial, and the use of the drug is rather limited. Consequently, aducanumab needs to be considered as a major achievement in the treatment of Alzheimer's disease only, not as the routine treatment modality.

1.2 Therapy used in Alzheimer's disease

Alzheimer disease management can therefore be subdivided into symptomatic, disease-modifying and supportive/non-pharmacological therapies. Symptomatic management in mild Alzheimer disease includes the use of acetylcholinesterase inhibitors, namely donepezil, rivastigmine or galantamine to improve or stabilize cognitive symptoms. If Alzheimer disease progresses to moderate or severe stage, memantine can be used both independently and in combination with acetylcholinesterase inhibitors. This combination is based on the mechanisms of cholinergic facilitation and inhibition of glutamate-induced excitotoxicity.

For a suitable group of patients with mild cognitive impairment or mild dementia due to Alzheimer disease and confirmed amyloid pathology, disease-modifying management with lecanemab or donanemab may be considered. Unlike typical symptomatic medications, these drugs aim at reduction of amyloid pathology and slowing down of disease progression. Nevertheless, the appropriate diagnosis and continuous monitoring are required owing to side effects such as ARIA, cerebral edema and microhemorrhage.

Along with pharmacological treatment, Alzheimer disease management involves cognitive stimulation, physical exercise, management of cardiovascular/metabolic risk factors, nutritional therapy, management of sleep, education of caregivers and treatment of behavioral and psychological symptoms of disease. This approach is necessary as pharmacological therapy is unable to cope with functional, behavioral and social consequences of progressive Alzheimer disease.

1.3 Drugs and phytochemicals on which research is ongoing

Alzheimer disease still remains a complicated disease with involvement of many pathological processes including amyloid- β deposition, tau pathology, oxidative stress, neuroinflammation, mitochondrial dysfunction, synaptic dysregulation, cholinergic dysfunction and neuronal apoptosis, despite the development of symptom-based and anti-amyloid therapies. This explains the increase in attention to multitarget drugs with a potential ability to interfere with many pathways at once. This is the rationale for the study of standard phytochemical components like curcumin, withanolides and ginkgolides.

Curcumin

Curcumin is one of the main polyphenolic components of *Curcuma longa*, which is one of the most widely studied natural components in the context of Alzheimer disease. Preclinical research shows that curcumin is able to prevent the formation of amyloid- β and stimulate its clearance, to decrease hyperphosphorylation of tau proteins, to reduce neuroinflammation, to inhibit acetylcholinesterase activity and to diminish oxidative stress.

However, translating curcumin into clinical practice is not an easy task, since clinical trials usually yield inconsistent data, and poor aqueous solubility, rapid metabolism, low systemic exposure and poor brain availability are among the disadvantages of curcumin. Consequently, today's research concentrates on formulations and modified preparations of curcumin, as well as nanoparticles, liposomes, phospholipid complexes and other advanced delivery systems. In turn, curcumin should be mentioned in the NTCC review as an investigational neuroprotective phytochemical and possible adjunctive therapy, but not as an approved therapy for Alzheimer disease.

Withanolides

Withanolides are steroidal lactones, which are mainly found in *Withania somnifera*. Some of the thoroughly studied compounds include withanolide A, withanolide B, withanoside IV and withanoside V. Research focused on their neuroprotective, antioxidant and anti-inflammatory properties with respect to Alzheimer disease. Experimental studies showed that withanolides might impact amyloid- β aggregation and provide protection of neurons from pathological and oxidative injury. Research involving *Withania somnifera* and withanolides is mainly preclinical; further research is needed to evaluate the proper dose and other characteristics of these phytochemicals. Therefore, withanolides are promising experimental agents, but not Alzheimer disease drugs.

Ginkgolides

The term 'ginkgolides' refers to diterpene lactones found in *Ginkgo biloba*, especially ginkgolides A, B and C. Research attention has been drawn to ginkgolides because of their antioxidant, anti-inflammatory and neuroprotective actions and also their ability to regulate platelet-activating factor-mediated signaling pathways. Research related to standardised preparations of *Ginkgo biloba*, especially EGb 761, has focussed on their possible actions on cognition and behavioural symptoms in dementia. There is inconclusive clinical evidence regarding the preparations of *Ginkgo biloba*, and there is no conclusive evidence with respect to isolated ginkgolides for Alzheimer disease compared to conventional Alzheimer disease medications. Hence, it is necessary that the NTCC review must differentiate between the research on standardised extracts of *Ginkgo biloba* and research on individual ginkgolides. Currently, research focus is on the possible role of ginkgolides as neuroprotective agents.

1.4 Therapeutic relevance of the three phytochemicals

The scientific justification for selecting the NTCC topic is due to the multi-pathways action mechanism of curcumin, withanolides, and ginkgolides simultaneously. The conventional medications like donepezil, rivastigmine, and galantamine treat cholinergic dysfunction, whereas memantine treats glutamate-mediated excitotoxicity and anti-amyloid antibodies treat the amyloid pathology. However, these phytochemicals might affect amyloid- β aggregation, tau pathology, oxidative stress, neuroinflammation, mitochondrial dysfunction, apoptosis, and synaptic dysfunction. Hence, due to this multitarget action mechanism, they can be considered promising candidates for further studies on Alzheimer disease.

Hence, the therapeutic approach can be visualized as follows:

Conventional treatment

→ Donepezil / Rivastigmine / Galantamine

→ Cholinergic enhancement

Memantine

→ NMDA receptor modulation

→ Excitotoxicity inhibition

Lecanemab / Donanemab

→ Amyloid- β clearance

→ Disease modification in selective early Alzheimer disease patients

Phytochemical under investigation

→ Curcumin: antioxidant + anti-inflammatory + anti-amyloid + anti-tau

→ Withanolides: neuroprotective + antioxidant + anti-inflammatory + anti-amyloid activity

→ Ginkgolides: antioxidant + anti-inflammatory + PAF modulation + neuroprotective activity

Therefore, the treatment strategy in the future might be that of multitarget therapy and/or combined treatments, although the phytochemicals discussed in your review article should be considered as candidates for further research rather than alternative medicines for treating Alzheimer's disease. The current scientific data is continually highlighting the necessity to develop standard formulations and clinical trials before using curcumin, withanolides or ginkgolides to treat Alzheimer disease patients.

Neuroprotective Mechanisms of Selected Phytochemicals

A. Curcumin, Withanolides, and Ginkgolides in Alzheimer's Disease Management

The three standard phytochemicals: curcumin, withanolides, and ginkgolides are of special interest in relation to Alzheimer's disease due to their ability to impact different pathological processes and not a single one. The multitarget mechanisms of action proposed for the mentioned phytochemicals include inhibition of aggregation, tauopathy, oxidative stress, neuroinflammation, mitochondrial dysfunction, neuronal apoptosis, and cholinergic dysfunction. However, not all the evidences concerning the effectiveness of these phytochemicals are strong, and there are many preclinical data available.

1. Curcumin

Curcumin is a main polyphenolic compound of the plant *Curcuma longa* (turmeric). Curcumin is widely studied as a multitarget phytochemical in AD because it is able to simultaneously impact amyloid pathology, tau phosphorylation, oxidative stress, neuroinflammation, and neuronal survival. Experimental studies show that curcumin is able to inhibit aggregation and oligomerization, decrease tau hyperphosphorylation, reduce acetylcholinesterase activity and modulate different intracellular signaling pathways.

1.1 Anti-amyloidogenic activity

One of the main proposed mechanisms of curcumin is the impact on peptides. The studies have shown that curcumin is able to disrupt aggregation and oligomerization and can also influence the pathways involved in

production. Curcumin can decrease amyloidogenic processing and formation of plaques through modulation of signaling pathways.

1.2 Reduction of tau hyperphosphorylation

Formation of neurofibrillary tangles caused by the tau hyperphosphorylation is the main pathological characteristic of AD. Curcumin can modulate the activity of different kinases and signaling pathways related to tau phosphorylation, including . The reduction of abnormal tau phosphorylation and aggregation can prevent the destruction of neuronal structure and function.

1.3 Antioxidant mechanism

Oxidative stress significantly causes the injury of neurons in AD. Curcumin can act as a direct scavenger of reactive oxygen species and can increase the activity of endogenous antioxidants. Curcumin decreases the damage to lipids, proteins, mitochondria, and neuronal DNA caused by oxidative stress.

1.4 Anti-neuroinflammatory activity

Activation of glia and astrocytes in AD leads to the release of inflammatory mediators like TNF- α , IL-1 β , IL-6, COX-2, and inducible nitric oxide synthase. Curcumin is able to inhibit important inflammatory pathways, especially mediated pathways, and to reduce the generation of pro-inflammatory mediators.

1.5 Other neuroprotective actions

Curcumin is able to modulate the pathways related to cholinergic neurotransmission by inhibiting acetylcholinesterase, as well as neurotrophic and neurogenesis-related pathways, and also affect mitochondrial and apoptotic signaling. All these actions make curcumin an interesting multitarget phytochemical candidate and not just a monotherapeutic agent.

1.6 Limitation

Although the preclinical data are promising, the main limitation of curcumin is the low bioavailability, rapid metabolism, and insufficient brain penetration. For this reason, the research is being conducted to find the way to increase the therapeutical properties of this phytochemical using standardized forms and brain-targeting delivery systems.

Mechanism flow:

Curcumin → aggregation and amyloidogenic processing → Tau hyperphosphorylation → ROS → inhibition of mediated neuroinflammation → improvement of neuronal survival and synaptic function → improvement of memory and cognition.

2. Withanolides

Withanolides are natural C28 steroidal lactones mainly extracted from *Withania somnifera* (Ashwagandha). Among the known constituents, withanolides A, D, withaferin A, and withanosides are to be highlighted. Literature data indicate the possible neuroprotective effects of withanolides on the basis of antioxidant, anti-inflammatory, anti-apoptotic, mitochondria-protective, and protein-aggregation-preventing activity. However, most of the evidence regarding withanolides' role in AD pathogenesis is preclinical.

2.1 Reduction of amyloid- β pathology

In experimental studies, it was shown that *Withania somnifera* components such as withanolides and withanosides could inhibit -related neurotoxicity and induce neuroprotective response. The attention was drawn to the possible effect of withaferin A as a component that can decrease pathological accumulation and aggregation in AD.

2.2 Modulation of tau pathology

Pathological accumulation and phosphorylation of tau cause neurodegeneration leading to neurofibrillary tangle formation. The experiments show that withanolides, in particular withaferin A, can affect pathological tau accumulation and cellular stress response pathways helping to preserve neuron integrity in AD.

2.3 Antioxidant and mitochondria protection

Mitochondrial dysfunction and ROS generation represent one of the major reasons for neurons' degeneration. Extracts of *Withania somnifera* and its active constituents possess antioxidative properties providing protection of mitochondria. Reducing the oxidative stress and maintaining cellular energy balance, withanolides can increase neuronal resilience.

2.4 Anti-inflammatory activity

Neuroinflammation is another target of withanolides. It was reported that the components of *Withania somnifera* can influence inflammation-related signaling pathways including related pathway reducing cytokines' production and neuronal damage caused by microglia activation.

2.5 Anti-apoptotic and neuroregenerative effects

Withanolides can increase the survival rate of neurons by inhibiting apoptosis and promoting neurite outgrowth and repair processes. According to the experiments, *Withania somnifera* increased mitochondria functionality and neuroprotection under various neurodegenerative conditions.

2.6 Cholinergic mechanism

Some studies suggested that withanolide A has an impact on acetylcholinesterase-related targets. As cholinergic dysfunction is related to memory deficit in AD, the modulation of this pathway can be the additional mechanism of standard withanolide preparations.

2.7 Limitations

Despite the promising neuroprotective properties of *Withania somnifera* and its components, there is not enough clinical evidence to prove the efficacy of withanolides as therapeutic agents for AD treatment. It is important to develop standardized extracts and identify the components more accurately for future clinical use.

Mechanism flow:

Withanolides → Aβ-related toxicity → modulation of tau pathology → oxidative stress and mitochondria dysfunction → inhibition of neuroinflammation → neuronal apoptosis + promotion of neuroregeneration → increased neuronal survival and cognitive function.

3. Ginkgolides

Ginkgolides are trilactones of diterpenes obtained from *Ginkgo biloba*. Mainly the following ginkgolides are used: A, B, C, J, and M. One of the most investigated ginkgolides is ginkgolide B. Terpene lactones and flavonoids occur together in the standardized *Ginkgo biloba* preparation; hence, all the evidence should not automatically be attributed only to ginkgolides.

3.1 Protection against amyloid- β neurotoxicity

In experimental studies, the use of ginkgolides protects against the neurotoxicity caused by A β . Particularly, ginkgolide B has been found to reduce the damage associated with β -amyloid and help neuronal cells to survive.

3.2 Anti-inflammatory mechanism

It is possible to say that ginkgolides can affect neuroinflammatory signaling. The experiments have shown that the substances can inhibit inflammatory signaling that involves Toll-like receptors, MyD88, and NF- κ B, thus decreasing production of such cytokines and Moreover, the reduction in microglial activation will additionally protect from neurotoxic injury.

3.3 Antioxidant and anti-excitotoxic effects

The substances and their derivatives have shown antioxidant and anti-excitotoxic properties during experimental studies. The decrease in oxidative injury and excitatory signaling can help protect neurons from degenerative diseases.

3.4 Neurotrophic and synaptic effects

In experimental studies, it has been found that ginkgolide B stimulates expression of brain-derived neurotrophic factor (BDNF) and helps in learning and memory process. Furthermore, experimental evidence suggests that the substances and their derivatives can preserve synaptic proteins and influence neuronal survival pathway associated.

3.5 Standardized Ginkgo biloba extract and clinical relevance

The most famous standardized Ginkgo biloba preparation is EGb 761, which consists of several components and does not include isolated ginkgolides. The clinical evidence about the effect of Ginkgo biloba preparations on cognitive impairment and dementia is heterogeneous. There were some trials and reviews showing the positive effect on cognition, neuropsychiatric symptoms or functional capacity and there were other studies without any effect. Thus, all the results should be evaluated carefully, especially when separating evidence of the whole extracts from evidence about ginkgolides.

Mechanism flow:

Ginkgolides $\rightarrow$ reduced A β -induced neuronal toxicity $\rightarrow$ reduced microglial activation and inflammatory cytokines $\rightarrow$ reduced oxidative stress and excitotoxicity $\rightarrow$ stimulation of neuronal survival and neurotrophic pathways $\rightarrow$ preservation of synapses and neurons $\rightarrow$ improvement of cognition.

Advanced Formulations and Brain- Targeted Delivery

1. Introduction to Advanced Formulation :-

Alzheimer's disease (AD) is a neurodegenerative condition associated with A β aggregation, hyperphosphorylated tau, oxidative stress, inflammation, dysfunction of mitochondria, and progressive damage to neurons. Natural bioactive compounds, such as curcumin, withanolides, and ginkgolides, might prove valuable due to their capacity to influence the abovementioned pathological conditions. Nevertheless, the use of these substances in clinics is problematic due to their pharmacological and pharmacokinetic limitations, including poor solubility, instability, incomplete absorption, fast metabolism, and insufficient penetration through the BBB. Therefore, advanced drug delivery systems are required to optimize stability, bioavailability, and brain delivery of the neuroprotective agents. The main goal of advanced formulation does not consist only in increasing the amount of administrated dose but in the enhancement of quantity of

pharmacologically active compound delivered to the target tissue. The major approaches currently developed to facilitate delivery through the blood-brain barrier (BBB) in case of AD are associated with nanoparticles, lipid carriers, liposomes, polymeric delivery systems, and intranasal delivery.

2. Advanced Formulation for Curcumin

The application of curcumin as neuroprotective phytochemical is hampered by extremely low aqueous solubility, poor absorption, instability, and limited permeability through the BBB. It has led to the development of numerous nanoformulations and brain-targeting delivery systems.

2.1 Polymeric Nanoparticles

Polymeric nanoparticles, in particular, those based on poly(lactic-co-glycolic acid) (PLGA) and other biodegradable polymers, can encapsulate the substance and protect it from degradation. Research data prove the possibility of increasing the delivery of the substance across the BBB using the nanoparticles rather than free curcumin. Chitosan-coated and PLGA-based nanoparticles were used to improve brain delivery, especially intranasally.

Proposed formulation pathway:

Curcumin → Polymeric encapsulation → Improved stability and dispersion → Increased absorption and systemic circulation → Increased possibility of brain delivery → Neuroprotective effect.

2.2 Lipid Nanoparticles

As curcumin belongs to lipophilic drugs, solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) can be applied to incorporate the substance. The lipid matrix of these nanoparticles improves the drug loading and protects the active compound from degradation. In experimental AD models, curcumin-loaded lipids nanocarriers have shown an improvement of bioavailability and efficacy in amelioration of pathological characteristics, including reduction of A β pathology in preclinical research.

2.3 Liposomes

Liposomes are phospholipid-based vesicles, which are able to incorporate hydrophobic compounds, like curcumin. The liposomal systems containing curcumin were developed for brain targeting, amyloid interaction, and treatment. The surface modification of the liposomes with targeting ligands could enhance the interaction with transport mechanisms at the BBB and neural cells.

2.4 Nanoemulsions and Other Lipid-Based Systems

Nanoemulsions and similar lipid-based systems can improve the dispersion of hydrophobic compounds in water solutions and could enhance the gastrointestinal absorption. Such methods are more relevant for oral delivery, since they can improve the dissolution and bioavailability of water-insoluble phytochemicals, in particular, curcumin.

2.5 Intranasal Delivery

Intranasal delivery is one of the most significant ways of non-invasive delivery of CNS drugs. Curcumin-loaded nanocarriers administrated intranasally have shown improved brain delivery in experimental research. This method allows some part of the compound to be delivered to the brain using olfactory and trigeminal pathways without the necessity of BBB crossing.

3. Advanced Formulation for Withanolides

Withanolides are steroidal lactones mostly found in *Withania somnifera*. Their development as agents for treating neurodegenerative disorders must take into account such aspects as standardization, physicochemical

properties, and consistent delivery. In contrast to curcumin, there is relatively less amount of formulation work published specifically for individual withanolides used for Alzheimer's disease (AD).

3.1 Standardized Extract Formulations

Standardization is an important step for botanical formulations since the concentration of withanolides may vary depending on the plant, its growing conditions, extraction process and other factors. Formulations based on defined concentrations of chosen withanolides would contribute to achieving batch-to-batch reproducibility and allow establishing dose-exposure-response relationship.

3.2 Nanoencapsulation and Lipid-Based Delivery

The techniques of nanoencapsulation and lipid-based carriers may help to improve the stability and delivery of low-soluble phytochemicals. Polymeric nanoparticles, liposomes, solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) might be adapted for withanolide delivery. However, this field needs additional formulation-specific pharmacokinetics and brain distribution studies before any conclusions about their clinical applicability in AD could be made.

Proposed pathway:

Withanolide → encapsulation or lipid-based carrier → increased formulation stability → improved systemic exposure → possible brain delivery improvement → neuroprotective activity assessment

3.3 Controlled-Release Approaches

Controlled-release systems could provide continuous exposure to withanolides and help to avoid variations in systemic levels of the compounds, which is especially important in case of chronic neurodegenerative diseases. But prolonged systemic release does not mean at all the improved brain exposure to drugs; therefore, pharmacokinetic and blood-brain barrier (BBB) transport studies are required during the process of formulation development.

4. Advanced Formulation for Ginkgolides

Ginkgolides are diterpene lactones found in Ginkgo biloba. Among them, ginkgolide B received much attention for its neuroprotective effects. But the formulation of ginkgolides faces many difficulties connected with low solubility, stability, and insufficient brain delivery.

4.1 Nanoparticle-Based Delivery

The use of nanoparticles as a delivery system represents an approach that might increase the stability, biodistribution, and controlled-release properties of ginkgolides. Recent CNS nanomedicine literature provides an example of nanoparticle delivery systems for ginkgolide B, which are designed to increase its BBB delivery. This information is a good basis for further research on the delivery of ginkgolides using nanoparticles for neurodegenerative diseases, even though the direct evidence of its efficacy in AD is still absent.

4.2 Liposomal Formulations

Another potential approach that could be used for ginkgolide delivery is liposomes. One recent study has developed a DHA-conjugated ginkgolide B liposomal system that improves solubility, stability and brain targeting of the drug in cerebral ischemia model. The proposed formulation has shown a higher brain hemisphere accumulation compared to the free drug and better neuroprotective activity. Even though this study was performed not on AD but on cerebral ischemia, it gives a valuable example of how brain-targeted ginkgolide formulations can be developed.

4.3 Future Potential in AD

Future research should be devoted to the determination of whether targeted ginkgolide nanocarriers could reach therapeutic brain concentrations in validated AD animal models. Important endpoints in such experiments would include β -amyloid and tau-related biomarkers, neuroinflammatory mediators, oxidative stress, cognitive performance, pharmacokinetics, and long-term safety.

5. Targeted Delivery to the Blood–Brain Barrier

One of the major issues related to effective CNS drug delivery is the presence of the blood-brain barrier. In turn, advanced nanocarriers might be modified regarding their size, surface properties, or ligand composition to optimize the interactions with the BBB-associated transport mechanisms. The phytochemical-loaded liposomes and other nanocarriers might be additionally equipped with targeting molecules for better receptor-mediated uptake or transcytosis.

Mechanism:

Phytochemical-loaded carrier



Surface modification or targeting ligand



Interaction with BBB-associated receptors/transport systems



Increased uptake or transcytosis



Increased brain exposure



Potentially modulated pathological pathways of AD

As an alternative method, intranasal delivery can be used for the purposes of the nose-to-brain delivery. Nowadays, the use of lipid nanocarriers, polymeric nanoparticles, liposomes, and other nanocarriers for the purposes of intranasal CNS delivery has been proved, yet the questions of safety, tolerability, and reproducibility remain to be important.

6. Multifunctional and Combination Delivery Systems

Since the Alzheimer's disease includes a lot of interconnected pathological mechanisms, the future delivery systems might include the delivery of more than one agent. Multifunctional carriers might theoretically contain the agents that are able to affect each other regarding the antioxidant, anti-inflammatory, anti-amyloid, and neuroprotective activities.

Curcumin + Withanolides + Ginkgolides



Multifunctional or combination delivery system



Improvement of formulation properties and multifunctionality

↓
Modulation of oxidative stress, neuroinflammation, and neuronal dysfunction

↓
Perspective multi-target strategy for AD
Still, combination formulations need thorough evaluation regarding the compatibility, release, pharmacokinetics, toxicity, and potential interactions.

7. Challenges to Clinical Translation

7.1 Safety and Nanotoxicity

Nanocarriers can largely change the biodistribution of the active substance; therefore, safety assessment needs to be performed separately regarding both phytochemical and carrier. Some of the concerns include the immunogenicity, complement activation, accumulation, and toxicity after the repetitive administration.

7.2 Manufacturing and Scaling Up

From laboratory formulations to pharmaceutical products, it is necessary to control particle size, drug loading, release characteristics, stability, and reproducibility. All these factors are the major challenges for many experimental nanomedicines.

7.3 Standardization of Natural Products

Standardization is especially important for botanical products. Content of withanolides and ginkgolides can differ according to the botanical origin, cultivation methods, extraction procedures, and manufacturing processes. Therefore, it is important to characterize the chemical components to make the clinical development reproducible.

7.4 Limited Clinical Evidence

The major problem is the difference between promising preclinical findings and clinical efficacy. Improvement of the solubility or increase of brain exposure do not necessarily imply clinical efficacy. Future studies need to involve the research of formulation, pharmacokinetics, biomarkers, and clinical endpoints.

8. Future Perspectives

Future research needs to focus on developing standardized and reproducible formulations. Regarding curcumin, it is necessary to optimize the nanoparticles, lipid carriers, and intranasal systems. Regarding withanolides and ginkgolides, the emphasis should be put on the generation of the strong evidence on formulations, including the studies of brain distribution and efficacy in AD models.

Some potential future directions include:

1. BBB-targeted nanocarriers with targeting by receptors or transporters.
2. For the non-invasive Central Nervous System delivery purposes the Formulation of Intranasal is done.
3. Multifunctional carriers with the integration of therapeutic and diagnostic properties.
4. Combination formulations targeting multiple AD-related pathways.
5. Stimuli-responsive delivery systems that respond to the particular conditions of the disease (e.g., oxidative stress or inflammation).
6. Biomimetic and nanovesicle-based systems for enhancing the biocompatibility and targeting.

7. Biomarker-guided clinical studies for the purpose of defining if the enhanced performance of formulation results in some biological and cognitive benefits.

Standardization and Characterization

Standardization of herbal medicines is very important in order to assure their identity, purity, quality, safety and reproducible therapeutic efficacy. In contrast to synthetic pharmaceutical products, plant materials possess considerable variability in the composition of their chemical components due to different genotypes of plants, their origin, cultivation, harvesting, processing and many other factors. That is why prior to the evaluation of neuroprotective effects of phytochemicals in Alzheimer's disease (AD) complete characterization and testing are necessary. Curcumin, withanolides and ginkgolides are three different classes of phytochemicals. Curcumin is the polyphenolic diarylheptanoid, withanolides are steroidal lactones and ginkgolides are diterpene lactones. Due to the different structures of these molecules it is necessary to use different approaches to their identification and quality evaluation. Standardized preparation of phytochemicals is expected to be characterized in botanic, physicochemical, chromatographic, spectroscopic, quantitative in terms of marker compounds, tests for contaminants, stability and biological tests.

CHARACTERIZATION

1. Curcumin Characterization

1.1 Source and Chemical Structure

Curcumin is the main bioactive substance of the rhizomes of *Curcuma longa* L. which is also known as turmeric. The rhizome of turmeric plant comprises a mixture of similar substances called curcuminoids. The main curcuminoids are:

- Curcumin
- Demethoxycurcumin
- Bisdemethoxycurcumin

Among these curcumin is the major component and is usually used as the marker component.

Curcumin is the hydrophobic polyphenolic molecule composed of two aromatic rings which are linked to each other by a linker consisting of seven carbons with α,β -unsaturated diketone group. This molecule possesses phenolic hydroxyl groups and conjugated double bonds that provide antioxidative and free radicals scavenging properties. The structure of curcumin makes it poorly soluble in water and prone to degradation in some environmental conditions.

1.2 Organoleptic and Physical Characterization

Preliminary characterization of curcumin involves physical parameters evaluation. The pure curcumin usually exists in the form of bright yellow to orange-yellow crystalline powder.

Parameters include:

- Color
- Appearance
- Odor
- Taste

- Crystallinity
- Particle size
- Solubility

It has low solubility in water and relatively high solubility in organic solvents such as ethanol, methanol, acetone, DMSO and some others. The profile of solubility is especially important for the development of formulations due to the low aqueous solubility and restricted oral availability in neurodegenerative diseases. The determination of melting point can be useful in preliminary characterization since the narrow melting range is usually the sign of high purity of the isolated substance.

1.3 Physicochemical Characterization

Physicochemical characterization is very important for evaluation of the quality and stability of curcumin preparations. The following parameters are usually estimated:

- Loss on drying
- Moisture content
- Ash value for crude turmeric
- Extractive values
- Solubility
- pH
- Melting point
- Particle size distribution

The loss on drying and moisture content are especially important since moisture can promote growth of microorganisms and degradation of curcumin. Extractive values are helpful in standardization of crude herbal material and give indirect information about the quantity of soluble components which were extracted by different solvents. The physicochemical characterization of purified curcumin includes solubility, melting behavior, crystallinity and stability. Differential Scanning Calorimetry (DSC) can be used in the study of thermal behavior and possible interactions with formulation excipients.

1.4 UV-Visible Spectroscopic Characterization

UV-Vis spectroscopy is a simple and widely used method for identification and preliminary quantitation of curcumin. Curcumin possesses the large conjugated system which makes it a strong absorber in the visible region. The absorption profile is based on conjugated aromatic rings and diketone group; so, UV-Vis spectra can be used for identification of the presence of curcumin and possible changes due to the degradation or interactions with other compounds. The applications include:

- Preliminary identification
- Quantitative estimation
- Monitoring degradation
- Dissolution studies
- Stability studies
- Formulation analysis

But UV-Vis spectroscopy cannot differentiate curcumin from all structurally similar compounds; so, it is usually accompanied by chromatographic methods for more precise characterization.

1.5 FTIR Characterization

Fourier Transform Infrared (FTIR) spectroscopy is used for identification of functional groups of curcumin and confirmation of its chemical structure. The characteristic bands of absorption correspond to:

- Phenolic hydroxyl (O–H)
- Carbonyl (C=O)
- Aromatic carbon–carbon double bonds (C=C)
- Ether linkages (C–O–C)
- Alkene groups

FTIR analysis can be useful not only for confirmation of curcumin structure but also for detection of possible chemical interactions during formulation development. When curcumin is incorporated into nanoparticles, liposomes, phospholipid complexes or any other delivery systems the FTIR analysis is useful in order to find out if there are any interactions between curcumin and formulation components. Thus, FTIR is the important instrument both for structural characterization and preformulation studies.

1.6 Chromatographic Characterization

Chromatographic methods are one of the most reliable methods for curcumin characterization and separation from other curcuminoids.

TLC (Thin-Layer Chromatography)

It is a simple preliminary method used for identification of curcumin in plant extract. The value of R_f of the sample is compared with that of the authentic standard. The applications include:

- Preliminary identification
- Detection of adulteration
- Monitoring extraction
- Rapid screening

HPTLC (High-Performance Thin-Layer Chromatography)

HPTLC provides better resolution and reproducibility than conventional TLC and can provide the characteristic chemical fingerprint of turmeric extracts and detection of several curcuminoids. HPTLC is widely used in routine standardization of herbal drugs since several samples can be analyzed simultaneously.

HPLC (High-Performance Liquid Chromatography)

It is the main method of quantitative characterization of curcuminoids providing separation and quantitation of:

- Curcumin
- Demethoxycurcumin
- Bisdemethoxycurcumin

The chromatographic fingerprint of HPLC analysis helps to establish the batch-to-batch consistency.

LC-MS/MS (Liquid Chromatography-Tandem Mass Spectrometry)

LC-MS/MS is used for highly specific and sensitive identification of curcuminoids providing confirmation of molecular masses and fragmentation pattern and identification of degradation products or impurities.

1.7 Structural Characterization by NMR

Nuclear Magnetic Resonance (NMR) spectroscopy gives the detailed information about curcumin structure. Proton and Carbon spectroscopies can be used for confirmation of the position of hydrogen and carbon atoms in the molecule. The applications of NMR analysis include:

- Structural confirmation
- Impurity identification
- Characterization of isolated compounds
- Detection of structural modifications

Since curcumin can be in different tautomers NMR analysis is important for its structural investigations.

2. Characterization of Withanolides

2.1 Source and Chemical Identity

Withanolides are naturally occurring steroidal lactones and are primarily found in *Withania somnifera* (Ashwagandha). Roots and leaves of the plant contain numerous withanolides and withanosides. Some of the important withanolides are:

- Withaferin A
- Withanolide A
- Withanolide B
- Withanone
- Withanoside IV
- Withanoside V

Withanolides possess a steroidal structure which contains a lactone ring attached to the steroidal nucleus. Structural differences between various withanolides occur due to the presence of hydroxylation, oxidation and different side chains. Concentration of withanolides may vary significantly based on different parts of the plants, geographical location, culture and isolation processes. Hence, it becomes necessary to carry out chemical and molecular characterization and standardization based on markers.

2.2 Pharmacognostic characterization of *Withania somnifera*

Prior to the chemical evaluation, the identity of the crude herbal material should be established. Macroscopic analysis involves:

- Shape of the root
- Colour
- Surface features
- Smell
- Texture

Pharmacognostic identification through microscopic methods can help in identification of any plant tissues and cellular structures. Powder microscopy can help in identification of any kind of adulteration or contamination. It is very important to identify the plant as wrong identification will affect the phytochemistry and pharmacological properties of the formulation.

2.3 Physicochemical characterization

Physicochemical analysis of the crude drug *Withania somnifera* involves:

- Foreign organic matter
- Loss on drying
- Moisture content
- Total ash
- Acid insoluble ash
- Water soluble extractive value
- Alcohol soluble extractive value

It helps in providing a baseline quality data of the crude herbal materials. In case of purified withanolide fractions, it includes:

- Appearance
- Solubility
- Melting point
- Stability
- Molecular weight

2.4 Spectroscopic Characterization

UV Spectroscopy

It can potentially give some preliminary data about the absorption properties of withanolides. However, because of the spectral similarity of structurally related withanolides, UV spectroscopy is usually not sufficient for the definitive identification.

FTIR Spectroscopy

Fourier-transform infrared (FTIR) spectroscopy is useful for the detection of characteristic functional groups, such as:

- hydroxyl groups,
- carbonyl groups,
- lactone function,
- bonds characteristic of steroidal skeleton.

The unique FTIR fingerprint may assist in distinguishing withanolide-containing plant extracts from other plant extracts.

NMR Spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is an essential tool for the structural elucidation of individual withanolides. Proton and carbon spectroscopies give data about:

- steroidal skeleton,
- lactone ring,
- hydroxyl substitution,
- double bonds,
- side chain modifications.

2.5 Chromatographic Characterization

HPTLC

High-performance thin-layer chromatography (HPTLC) is widely used for the fingerprint profiling of Ashwagandha extracts. Withaferin A and Withanolide A may be recognized and quantified as markers.

Advantages of HPTLC include:

- quick analysis,
- simultaneous analysis of several samples,
- cost-effectiveness,
- suitability for herbal fingerprinting.

HPLC

High-performance liquid chromatography (HPLC) allows better separation and quantification of individual withanolides. HPLC is efficient for the analysis of markers in standardized extracts.

The most common substances include:

- Withaferin A,
- Withanolide A,
- Withanolide B,
- Withanone.

UHPLC

Ultra-high-performance liquid chromatography (UHPLC) gives faster separation and higher resolution. UHPLC becomes highly important when several structurally similar withanolides and withanosides should be characterized.

LC-MS/MS

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is used for molecular-level identification and discrimination of compounds by molecular mass and fragmentation patterns. UHPLC-QTOF-MS is one of the modern approaches to comprehensive phytochemical profiling and identification of minor components.

3. Characterization of Ginkgolides

3.1 Source and Chemical Identification

Ginkgolides are unique diterpene lactones extracted from the leaves of *Ginkgo biloba*. Terpene lactones consist of:

- Ginkgolide A
- Ginkgolide B
- Ginkgolide C
- Ginkgolide J
- Bilobalide

Ginkgolides have unique complex cage-like structures with several lactone rings and oxygen-containing functional groups. Among ginkgolides, Ginkgolide B is worth mentioning due to its strong antagonist activity against platelet-activating factor (PAF).

3.2 Pharmacognostic Characterization

Material obtained from *Ginkgo biloba* leaves should be authenticated botanically as the first step. Macroscopically one should examine:

- Fan-shaped leaves
- Typical bilobed shape
- Venation
- Color
- Texture

Additional microscopic examination may confirm the identity of the plant. It is important to authenticate raw materials since the quality and chemical composition of commercial preparations made of *Ginkgo biloba* are extremely variable.

3.3 Physicochemical Characterization

The quality of *Ginkgo biloba* material is characterized by the following parameters:

- Moisture content
- Loss on drying
- Total ash
- Acid-insoluble ash
- Extractive values
- Foreign matter

In case of standardized extracts, additional characteristics should be considered:

- Solubility
- Stability

- Molecular weight
- Chemical fingerprint

3.4 Spectroscopic Characterization

FTIR

FTIR (Fourier-transform infrared spectroscopy) can reveal characteristic functional groups of the lactone structure.

NMR

NMR (nuclear magnetic resonance) spectroscopy provides valuable information about the complicated structures of ginkgolides:

- Carbon skeleton
- Lactone rings
- Hydroxyl groups
- Molecular structure

Mass Spectrometry

Mass spectrometry provides accurate data of the molecular mass and fragmentation pattern. Considering the complicated structure of ginkgolides and their low content, high-resolution mass spectrometry will be especially useful in the identification process.

3.5 Chromatographic Characterization

HPLC

HPLC (high-performance liquid chromatography) technique is extensively used for characterization of Ginkgo biloba extracts and individual terpene lactones quantitation.

HPLC-ELSD

Since ginkgolides have rather poor absorption in UV range, ELSD (evaporative light scattering detection) will enhance the sensitivity of detection.

LC-MS/MS

LC-MS/MS (liquid chromatography tandem mass spectrometry) will enable to identify and quantitate individual ginkgolides and bilobalide.

GC-MS

GC-MS (gas chromatography-mass spectrometry) can be used after appropriate derivatization.

It is necessary to provide the complete chromatographic fingerprint since Ginkgo biloba extract contains both flavonoid glycosides and terpene lactones.

Testing And Evaluation

TESTING AND EVALUATION

1. Testing of Curcumin

1.1 Identity Testing

Identity testing confirms the identity of the substance being used and ensures it is not any other coloring substance. The procedures involved in identity testing include;

- UV-Visible spectra comparison
- FTIR fingerprint
- TLC comparison with a reference standard
- HPLC retention time comparison
- Mass spectrometry confirmation

Identity testing is the first essential step before proceeding to pharmacology testing.

1.2 Purity Testing

It is vital to test the purity of products with curcumin to ascertain any contamination or adulteration in the preparation. Some tests include.

Heavy Metal Test

Testing for toxic metals like lead, cadmium, mercury, and arsenic.

Pesticides Test

Since plants have pesticide residues from agricultural activities, the pesticide residues can be detected.

Residual Solvents Test

Solvents used in the process of isolation have to be tested since their presence may pose dangers to the health of the consumer.

Microbiological Testing

Total aerobic microorganism count

Yeast and mould count

Pathogenic microorganism count

Adulteration Testing

Turmeric and curcumin can be adulterated with artificial colours, and this is done using spectroscopic and chromatographic procedures.

1.3 Quantitative Assay

Quantitative analysis provides the amount of the active ingredient. HPLC can be used for quantification of:

- Curcumin
- Demethoxycurcumin
- Bisdemethoxycurcumi

Validation of quantitative methods involves ensuring:

- Accuracy
- Precision
- Specificity
- Linearity
- Robustness
- Limit of detection
- Limit of quantification

Quantified methods will ensure the consistency of batches.

1.4 Stability Testing

Under certain conditions, curcumin is chemically unstable and degrades. Stability testing tests for

Effect of Light

Since curcumin is photosensitive, studies on the effect of light are necessary

Effect of pH

It is essential to test the effects of pH because of the poor stability at high pH.

Effect of Temperature

High temperatures increase degradation.

Oxidative stability

The oxidative conditions affect the chemical structure and biological activity.

Stability testing is very important in identifying:

- Shelf life
- Conditions of storage
- Suitable packaging
- Formulation stability

1.5 In Vitro Neuroprotective Testing

Within the realm of Alzheimer's disease research, standardized curcumin may be tested through several existing biological tests.

Antioxidant Tests

Tests include:

- DPPH
- ABTS
- FRAP
- Reactive oxygen species assay

This testing evaluates curcumin's ability to reduce oxidative stress.

Acetylcholinesterase Inhibition Test

The acetylcholinesterase inhibition test assesses curcumin's ability to reduce the hydrolysis of acetylcholine.

Amyloid-beta Protein Aggregation Tests

This test assesses curcumin's ability to interfere with the aggregation of beta amyloid proteins.

Tau Protein Related Tests

Assessments may include evaluations of:

- Tau phosphorylation
- Tau protein aggregation
- Microtubule integrity

Tests in Neuronal Cells

Testing may be done using neuronal cells exposed to oxidative stress or β -amyloid protein toxicity in order to determine curcumin's ability to:

- Increase cell viability
- Enhance mitochondrial functioning
- Promote antioxidant enzyme activity
- Reduce apoptosis

1.6 In Vivo Testing

Tests on animals may be used in order to assess cognitive and neuroprotective effects. Models include:

- Morris water maze
- Y-maze
- Novel object recognition test
- Passive avoidance test

Tests on biochemical parameters include:

- Brain acetylcholinesterase activity
- Oxidative stress markers
- Cytokine production
- Amyloid protein deposition

2. Testing of Withanolides

2.1 Identity Testing

Withanolides identity testing consists in comparing with reference standards. Some common ways to do it are:

- HPTLC fingerprinting
- HPLC retention time

- FTIR spectrum

In view of the fact that extracts of Ashwagandha contain multiple withanolides, chemical fingerprinting is very important in this case.

2.2 Purity and Contaminants Testing

Extracts which are standardized need to be tested for:

- heavy metals
- pesticides
- microorganisms
- residual solvents
- mycotoxins
- adulteration

These tests ensure that the effects which are observed are caused not by contaminants but by standardized phytochemicals.

2.3 Quantitative Assay

In quantitative assay, the concentration of certain marker substances is determined. Markers can be:

- withaferin A
- withanolide A
- withanolide B
- withanone

HPTLC and HPLC are widely used for routine quantitative assays. The analytical technique must be validated in terms of:

- accuracy
- precision
- repeatability
- specificity
- linearity
- robustness

Multiple markers-based standardization can be considered more accurate than one-compound-based standardization because of the presence of numerous biologically active withanolides in Ashwagandha.

2.4 Stability Testing

Stability study will help in estimating the effect of environmental factors on the concentration and quality of withanolides. These factors include:

- temperature
- humidity

- light exposure
- oxidation
- storage time

Long-term and accelerated stability testing help to establish shelf life of standardized extracts.

2.5 In Vitro Neuroprotective Testing

Withanolides can be tested in various neuroprotective assays.

Antioxidant Testing

The ability of the substance to neutralize oxidative stress can be established using such tests as:

- DPPH assay
- ABTS assay
- ROS assay
- lipid peroxidation assay

Acetylcholinesterase Testing

Inhibiting activity of acetylcholinesterase can be evaluated.

Amyloid-Beta Neurotoxicity Testing

Neuronal cells are treated with β -amyloid peptides and then with withanolides to test protection from:

- cell death
- oxidative stress
- mitochondrial dysfunction
- apoptosis

Neurite Outgrowth Studies

An interesting property of Ashwagandha-derived compounds is ability to promote neurite growth and maintaining of neuronal networks. Thus, neurite outgrowth studies are very relevant for neuroregenerative evaluation.

2.6 In Vivo Testing

It is possible to use animal models to evaluate the effects on cognition of standardized withanolides. Cognitive behavior tests include

- Morris water maze
- Y-maze
- radial arm maze
- novel object recognition
- passive avoidance test

In brain tissues, the following parameters can be evaluated:

- acetylcholine levels
- oxidative stress
- inflammatory mediators
- amyloid pathology
- neuronal survival

3. Testing of Ginkgolides

3.1 Identity Testing

Identity testing of ginkgolides is done using the following procedures:

- HPLC retention profiling
- LC-MS/MS
- FTIR
- NMR
- Comparative with certified reference standards

Given the fact that standardized Ginkgo biloba extracts contain several phytochemical classes, chromatographic fingerprinting becomes extremely important in identity testing.

3.2 Purity and Safety Testing

One of the key issues in the analysis of Ginkgo biloba extracts involves checking for presence of undesirable components. The analyses involved include the following:

Heavy Metals

Tests for:

- Lead
- Cadmium
- Mercury
- Arsenic

Pesticide Residue

Use GC-MS or LC-MS/MS to detect the presence of pesticides.

Microbial Contamination

Detection of microbial limits and any pathogenic microbes.

Ginkgolic Acid Analysis

Ginkgolic acids represent undesirable alkylphenolic compounds which might be toxic or allergic; consequently, standardized Ginkgo biloba extracts require careful monitoring of ginkgolic acid content. The detection and management of ginkgolic acids become one of the most important indicators of the quality of the extract.

3.3 Quantitative Assay

Quantification refers to measurement of concentration levels of main terpenoid lactones, specifically:

- Ginkgolide A
- Ginkgolide B
- Ginkgolide C
- Ginkgolide J
- Bilobalide

The techniques used are:

- HPLC
- HPLC-ELSD
- LC-MS/MS

Validation of the assay should show satisfactory performance in terms of:

- Accuracy
- Precision
- Specificity
- Linearity
- Robustness

3.4 Stability Testing

Stability tests measure the change in the chemical composition of ginkgolide preparations during storage.

Key factors include:

- Temperature
- Humidity
- Light
- Oxidative condition
- Long-term storage

The use of chromatographic analysis before and after storage helps detect degradation and change in the concentration of bioactive components.

3.5 In Vitro Neuroprotective Testing

Antioxidant Testing

The potential of ginkgolides and standardized Ginkgo extracts can be tested against:

- Generation of reactive oxygen species
- Lipid peroxidation
- Oxidative neuronal injury

Platelet-Activating Factor Antagonism

It is well known that ginkgolide B exhibits antagonistic properties against platelet-activating factor receptors. This property can be tested in receptor- and cell-based assays.

Amyloid-Beta Neurotoxicity Testing

Neuronal cells treated with β -amyloid can be treated with ginkgolides to determine their impact on:

- Cell viability
- Apoptosis
- Oxidative stress
- Mitochondrial dysfunction

Mitochondrial Dysfunction Testing

Mitochondrial dysfunction is one of the key pathological mechanisms in Alzheimer's disease. Such testing might include

- Mitochondrial membrane potential
- ATP production
- REC generation
- Cytochrome-c release

3.6 In Vivo Testing

Animal experiments can help to determine if the standardized ginkgolide preparations have a positive effect on cognitive function.

Behavioral testing includes:

- Morris water maze
- Y-maze
- Novel object recognition
- Passive avoidance test

The biochemical and histological testing can evaluate:

- Amyloid plaques formation
- Oxidative stress markers
- Cholinergic function
- Neuroinflammation
- Neuronal apoptosis
- Synaptic protein expression

Pharmacological Effects of Phytochemicals

Pharmacological Effects of Phytochemicals on Alzheimer's Disease

Alzheimer's disease (AD) is an irreversible neurodegenerative disorder with multiple pathological processes developing concurrently, including the formation of β -amyloid plaques, hyperphosphorylation of tau protein, oxidative stress, mitochondrial dysfunction, apoptosis of neurons and inflammation. Because of the multiplicity of pathologies involved in AD development, phytochemicals are considered multi-target agents. Compounds like curcumin, withanolides, and ginkgolides possess antioxidant, anti-inflammatory, anti-amyloidogenic, anti-apoptotic, and neuroprotective effects. All these features together could preserve neuronal integrity and function under AD conditions. Some reviews suggest that phytochemicals affect several signaling pathways involved in AD, related signaling pathways.

1. Antioxidant effects and protection against oxidative stress

Oxidative stress plays an important role in neuron destruction during AD. An increased ROS production can negatively affect neuron membranes, proteins, DNA and mitochondria. Phytochemicals are capable of reducing the oxidative stress both through the scavenging of reactive species and up-regulation of endogenous antioxidant systems. For example, curcumin has been shown to activate pathway that leads to an increased expression of antioxidant enzymes (SOD, catalase). Activation of this pathway promotes redox balance in cells and protects them from oxidative damage. In addition, curcumin reduces mitochondrial dysfunction and neuronal apoptosis in neurons.

2. Anti-amyloidogenic effects

Formation of aggregates and their accumulation in AD patients brains is one of the main pathological features of this disease. Some phytochemicals are able to interfere with generation, aggregation, and toxicity. Curcumin, for example, has demonstrated an ability to bind protein and inhibit its aggregation. Moreover, this compound is able to promote degradation of toxic protein aggregates by means of autophagy-related pathways. Thus, reducing the related toxicity, phytochemicals are likely to reduce microglia activation, oxidative stress and neuronal injury caused by them. The importance of these effects lies in the fact that related toxicity initiates cascades of oxidative damage, inflammation, mitochondrial dysfunction and cell death.

3. Tau inhibitors and cytoskeletal protectors

Abnormal tau hyperphosphorylation causes neurofibrillary tangles and disturbance of the neuronal transport system. According to available studies, curcumin is able to reduce -induced tau hyperphosphorylation due to alteration of signaling cascades such as pathway. Modulation of the above-listed pathways can be helpful for stabilization of cytoskeleton and maintenance of its integrity and function. Thus, the pharmacological activities of phytochemicals in addition to elimination can include neuroprotection from tau-related damage.

4. Cholinergic and synaptic protective effect

The decrease in cholinergic function is one of the reasons of memory deficits observed in AD. Some phytochemicals show the ability to inhibit acetylcholinesterase and thus increase the concentration of acetylcholine in the synaptic cleft. Moreover, there are some phytochemicals which can affect the neurotrophic signaling and synaptic plasticity. The impact of withanolides on the neuronal signaling, the synaptic density and neurogenesis has been examined. Withanolide A was shown to interact with signaling pathway that is very important for neuronal survival, dendritic development and synaptic plasticity.

5. Apoptosis and mitochondria protective effects

Progressive neurodegeneration in AD is associated with mitochondria dysfunction and apoptosis. Phytochemicals can prevent the mitochondria damage due to the reduced ROS production, stabilization of mitochondrial membrane potential and apoptosis regulators. Curcumin has been reported to prevent the progression of mitochondrial dysfunction and apoptosis in neurons, while ginkgolides have shown their anti-apoptotic activity in AD cellular models. Ginkgolides reduced the expression of Bax and increased the expression of which resulted in the improvement of cell viability in AD cells.

6. The overall pharmacological effect of neuroprotection

The pharmacological effect of phytochemicals in AD is not limited to just one molecular target. It includes the reduction of oxidative stress, prevention of aggregation, regulation of the tau protein phosphorylation, the improvement of the cholinergic signaling, the protection of the mitochondria, the reduction of apoptosis and the enhancement of neuronal plasticity. This wide range of activities is especially valuable considering the complexity of AD pathophysiology. However, the majority of the studies are preclinical, and there are limitations in relation to the bioavailability, metabolism and achievement of brain concentration levels for curcumin and other phytochemicals.

Anti-inflammatory Effects of Phytochemicals

Phytochemical Anti-inflammatory Pharmacological Effects in Alzheimer's Disease

Neuroinflammation is one of the major aspects of AD and is closely associated with production, oxidative stress, and neural injury. stimulates microglia and astrocytes and activates the release of pro-inflammatory substances, this process leads to synapse and neuronal damage and results in acceleration of AD progression. As a consequence, phytochemicals are being studied for their ability to modulate a number of inflammatory pathways related to AD development, inflammasome pathways.

1) Microglial activation inhibition

Microglia cells are considered the main immune cells of the central nervous system. Despite the fact that initially microglial activation helps to remove and damaged cells, over time it leads to overproduction of pro-inflammatory mediators and neuronal injury. Thus, phytochemicals can help in the inhibition of excessive microglial activation and reduction of cytokines production. Curcumin has shown its anti-inflammatory potential due to regulation of microglial and astrocytic processes, while ginkgolides inhibited microglia-dependent inflammatory signaling in experiments.

2) NF- κ B pathway inhibition

The nuclear factor-kappa pathway plays a crucial role in the development of neuroinflammation. Activation of this pathway contributes to the increased production of many pro-inflammatory mediators, It was shown that curcumin exerts anti-inflammatory effects due to the inhibition of I κ B kinase/NF- κ B pathway and reduction of inflammatory mediators production. In this way, NF- κ B pathway inhibition is a key pharmacological effect that explains the anti-inflammatory and neuroprotective effects of curcumin. It was observed that ginkgolides also affect the NF- κ B signaling pathway. According to a study on an AD cellular model, ginkgolide treatment significantly decreased NF- κ B p65 protein level and decreased level. This study has also found an increase in cell viability and Bax level reduction, which means that inhibition of NF- κ B-associated inflammation might be associated with anti-apoptosis neuroprotection.

3. Inhibition of NLRP3 inflammasome

The NLRP3 inflammasome is another significant inflammatory pathway involved in AD pathology. The activation of NLRP3 results in caspase-1 activation, and subsequently, the maturation. In a preclinical model

of AD, ginkgolide reduced the levels of NLRP3, ASC and caspase-1, along with reduced ROS levels. Also, ginkgolide treated APP/PS1 mice demonstrated lower levels of accumulation, inflammation, and neurodegeneration, including improvement in learning and memory. It was assumed that the inhibition of the NLRP3/caspase-1 pathway could contribute to the neuroprotective effect of ginkgolides.

4. Decreasing the level of inflammatory cytokines

The overproduction of can cause synaptic dysfunction and neuronal apoptosis. Phytochemicals may inhibit the levels of these mediators through affecting the upstream pathways involved in inflammation. Ginkgolides were found to decrease the levels of an experimental AD-related model. Likewise, curcumin has been found to attenuate the inflammation process due to its inhibitory effects on the NF- κ B and NLRP3 pathways. Such modulation of the signaling processes might be helpful in creating a better neurogenic microenvironment.

5. Antioxidant-anti-inflammatory interconnection

The antioxidant and anti-inflammatory effects of phytochemicals are very connected. Overproduced ROS can activate inflammatory pathways, and in turn, inflammatory response can cause more oxidative stress. Curcumin can break this vicious circle by stimulating antioxidant responses through suppressing the inflammatory signaling through NF- κ B and NLRP3 pathways. Thus, the pharmacological effects of phytochemicals should be understood as a complex response including the reduction of oxidative stress, inflammatory signaling and neuronal apoptosis.

6. Pharmacological meaning of anti-inflammatory effects caused by phytochemicals

In summary, the anti-inflammatory pharmacological effects of phytochemicals include regulation of microglia activation, inhibition of pathways, inhibition of inflammatory cytokines and the suppression of oxidative-stress-induced inflammation. Curcumin has mostly demonstrated antioxidant, pathways modulating and anti-apoptotic effects, while ginkgolides were found to have caspase-1-related effects in experimental models of AD. Also, withanolides offer a new neuroprotective strategy through the modulation of neurotrophic and synaptic pathways. The above information suggests the potential of phytochemicals as multitarget agents for AD; however, the experimental data cannot be considered as evidence of their clinical efficacy, and further human studies are necessary.

Conclusion And Research Limitations

Conclusion

Thus, AD is a neurodegenerative pathology associated with cognitive impairment and multiple pathomechanisms: β -amyloid deposits, hyperphosphorylation of tau proteins, oxidative stress, mitochondrial dysfunction, neuronal death, and neuroinflammation. The review provided in WPR 1–10 demonstrates that all these mechanisms are interrelated and contribute to the progressive degeneration of the nervous system. Hence, it can be assumed that the action aimed at addressing one of these mechanisms is unlikely to be sufficient; therefore, multi-target strategies are increasingly being considered in the treatment of AD.

Phytochemicals can be viewed as potential agents due to the multiple biological activities and possibility to affect different mechanisms of AD development. Such substances as curcumin, withanolides, and ginkgolides were found to have neuroprotective, antioxidant, anti-inflammatory, and antiapoptotic effects. It was shown that phytochemicals can modulate oxidative stress, inhibit pro-inflammatory pathways (NF- κ B), regulate the Nrf2-dependent antioxidants response, protect the mitochondria from the dysfunction, inhibit apoptosis and amyloid/tau pathology. Such wide spectrum of mechanisms makes phytochemicals potentially more effective than the agents which can influence only one mechanism.

At the same time, the review provides the information that the efficacy of phytochemicals is largely determined by their physicochemical properties, bioavailability, metabolism, and penetration through the BBB. Limited

solubility, rapid metabolism, poor bioavailability, and insufficient penetrability through the blood–brain barrier still remain major issues. It can be supposed that the advanced formulation techniques and the use of nanotechnology will facilitate the improvement of the stability, bioavailability, and targeted delivery of phytochemicals. In addition, botanical identification, standardization, and characterization of the active components of phytochemicals are critical for achieving reproducible results.

While the existing evidence is quite promising, the majority of data about the therapeutic potential of phytochemicals remains preclinical. Moreover, the heterogeneity of the models used, dosing regimens, formulations, and types of phytochemicals complicates their comparison. The need for clinical trials to determine the safety, pharmacokinetics, effective dose and therapeutic efficacy of phytochemicals in patients with Alzheimer's disease is obvious. Furthermore, the future research should pay attention to the standardization of the phytochemicals, multi-target approach, brain delivery systems, and interactions of phytochemicals with various pathways of AD development.

In general, the WPR 1–10 have provided evidence of the vast potential of phytochemicals in being used as complementary or even future treatment for Alzheimer's disease due to their capacity to simultaneously affect neuroinflammation, oxidative stress, neuronal survival, mitochondrial dysfunction, and many others related to the disease process. However, their potent biological actions do not necessarily equate to proven clinical effectiveness. The translation of the results into practice through proper research, standardization, clinical trials, and safety testing is essential. Consequently, the study of phytochemicals could potentially lead to the development of a safe, multi-target, and standardized approach to treating Alzheimer's disease.

Summary and Future Directions

Summary

All WPR reports together cover potential role of phytochemicals in Alzheimer's disease considering disease mechanisms, pharmacology, standardization and advanced drug delivery methods. The reports started from the knowledge of Alzheimer's disease and its major pathological mechanisms, such as β -amyloid deposition, tau phosphorylation, oxidation, mitochondrial dysfunction, neuroinflammation, apoptosis, and neurodegeneration.

Further on, focus was done on phytochemicals as potential multitarget agents, among which curcumin, withanolides and ginkgolides were highlighted due to reported antioxidant, anti-inflammatory, neuroprotective, anti-amyloidogenic and anti-apoptotic actions. Also, the therapeutic use of phytochemicals is associated with various challenges, which include low solubility, poor bioavailability, fast metabolism, variations in natural materials, non-standardization, contamination, instability and difficulty to translate experimental results into practice.

Advanced drug-delivery technologies, including nanoparticles, liposomes, lipid-based systems, polymeric drugs and intranasal formulations were considered as the possible ways to overcome mentioned difficulties. Furthermore, standardization and characterization of phytochemicals are the key aspects to pay attention to. Analytical techniques as HPLC, HPTLC, FTIR, NMR, LC-MS/MS, UV-Visible spectroscopy and other validated approaches should be used to ensure the identity, purity and strength of substances. Pharmacological reports also show that phytochemicals are capable to impact the multiple linked mechanisms of AD pathogenesis rather than working through a single target. It is especially important that their antioxidant and anti-inflammatory properties affect the mechanisms of AD development, namely amyloid pathology, oxidative stress, mitochondrial dysfunction and neuronal injury.

So, all WPR reports provide evidence that phytochemicals demonstrate promising multitarget neuroprotective and anti-inflammatory potential in Alzheimer's disease; however, to achieve success in their development, standardization, characterization, formulation, safety, pharmacokinetics studies and clinical investigations are

required. Completed work (WPR-1 to WPR-10) serves as a solid base for the preparation of the review article "Neuroprotective and Anti-inflammatory Mechanisms of Phytochemicals in Alzheimer's Disease." The progression of work started from knowledge of disease and its mechanisms, followed by the consideration of selected phytochemicals, their mechanisms of action, advanced drug delivery systems, standardization and characterization, pharmacological and anti-inflammatory effects.

Overall findings prove the experimental potential of curcumin, withanolides and ginkgolides as multitarget phytochemicals; however, additional work is needed to address such problems as standardization, formulation, safety and pharmacokinetics of these compounds. Therefore, completed WPR work provides the scientific background for the further development of review article and consideration of future perspectives, as well as for preparing the abstract and conclusion.

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