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ALZHEIMER'S DISEASE AND ITS THERAPY BY WITHANIA SOMNIFERA PHYTOCHEMICALS

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Abstract

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder, which is the most common form of dementia found in the older population. The etiological hallmarks of AD are an extracellular agglomeration of Aβ protein (Aβ plaques) and intracellular accumulation of tau protein (Neurofibrillary tangle). Aβ plaques and neurofibrillary tangles are colocalized/joined together with astrocytes and microglia, releasing neuroinflammatory mediators in the brain of an AD patient. The available drugs (AchE inhibitors, NMDA receptor antagonists, and monoclonal antibodies) are associated with various adverse drug reactions including GI problems, anorexia, tremor, and found to be ineffective in a section of AD patients, demanding an alternative better therapy for AD. Interestingly, Withania somnifera (W. somnifera), a natural plant has been found to play a crucial role in the management of various diseases including AD, owing to the various medicinal properties of its phytoconstituents such as anti- inflammatory, and antioxidant. Numerous studies have been conducted in the context of the potential role of W. somnifera in AD therapy. This chapter discusses the role of cellular mediators (astrocytes, microglia, and oligodendrocytes) and chemical mediators (cytokine, chemokine, and cyclooxygenase) involved in the AD pathogenesis. Further, the significant contribution of various in-silico, in-vitro, and in-vivo studies pertaining to the potential role of various phytoconstituents of W. somnifera in AD therapy has been discussed.

Keywords: Alzheimer's disease, Withania somnifera, Phytoconstituents

1. Introduction

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Alzheimer's disease (AD) has become common in older adults and usually begins after age 65 (late-onset AD) [1]. Currently, there are more than 55 million people are living with dementia and nearly 10 million new cases adding per year. The most common form of dementia is AD and may take part of 60-70% of total cases [2]. AD Patients have impairment of cognitive function with other symptoms. The most affected part of the brain in AD is the medial temporal lobe, entorhinal cortex, and hippocampus. Aβ and tau protein agglomerate in AD patient's brain. The etiological hallmarks of AD are an extracellular agglomeration of Aβ protein (called Aβ plaques) and intracellular accumulation of tau protein (called as Neurofibrillary tangle) [3][4]. Plaques and neurofibrillary tangles cause the damage and death of neurons by blocking neuron to neuron transmission. They also block the flow of nutrients and essential molecules [4][3],. Different kinds of genes are also responsible for AD, in which the most common gene is apolipoprotein E(APOE), which encodes lipid carrier protein apoE. A person with the Ɛ4 allele of APOE is three times more susceptible to AD. The other genes are PSEN1 and PSEN2 encoding presenilin1 and presenilin2 respectively [4]. Aβ plaques and neurofibrillary tangles are colocalized/joined together with astrocytes and microglia, neuroinflammatory mediators in a patient with AD, signalling that neuroinflammation might be a principal component of AD pathogenesis. As AD progress, plaques and tangles stimulate astrocyte and microglia following the migration of astrocyte and microglia and encircle the plaque and tangles, resulting in disposing of inflammatory mediator cytokines, chemokines in the brain [5].

The drug which is used to treat AD gives only symptomatic relief. They delay disease progression by 6-12 months, after which deterioration resumes. Currently, there is no curative treatments are available [4][6]. There are mainly two mainstays for AD treatment i.e. first cholinesterase inhibitor and second is NMDA receptor antagonism. Donepezil, rivastigmine, and galantamine (reversible anticholinesterase) are used for cognitive impairment. These are well tolerable with some common adverse drug reactions (ADRs) like GI distress, muscle spasms [4]. Memantine (NMDA receptor antagonist) delays the clinical deterioration in Alzheimer's disease. Some other drugs are also used to treat AD-like citalopram for agitation, antipsychotics such as risperidone, olanzapine, and quetiapine are effective in agitation and psychosis for Alzheimer's. The use of these drugs is limited as these drugs are associated with some mild to severe side effects-sedation, falls, dizziness [4].

Traditional medicine also offers effective drugs for cognitive impairment in Alzheimer's disease as well as in neurodegenerative disorder. Withania somnifera (WS) also known as ashwagandha (Indian ginseng) used as a nootropic agent (nerve tonic and memory enhancer), antistress, anticancer, debility, and anti-

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inflammation [7][8]. Phytochemical studies have shown Withania somnifera contains alkaloidal constituents and withanine as the main constituent and somniferine, tropine, pseudotropine, present in minor quantities in a different region of the plant [7]. Withanolide (steroid lactone) is present in leaves. WS has shown promising effects against Alzheimer's disease, Parkinson's disease, and Huntington's disease [8]. This article discussed the role of Withania somnifera particularly in AD. Table 1 shows the computational studies targeting Alzheimer disease with Withania Somnifera.

Table 1: Screening of various phytoconstituents of Withania Somnifera against target of AD using computational studies

Sr.No.	Author's name	Methods	Findings
1	Choudhary et.al 2005	Molecular docking study	Withanolide 1,3,5 are predicted to be a competitive inhibitors whereas Withanolide 2,4 are predicted to be a non-competitive inhibitors of acetylcholinesterase (AChE)
2	Grover et.al. 2012	Molecular docking and dynamics simulation	Withanolide A has had a high binding affinity with the AChE
3	Ahmad et.al. 2021	Molecular docking and dynamics simulation	Out of 27 phytochemicals, chlorogenic acid showed the best docking score

2. Molecular mediators

Various mediators such as proinflammatory, inflammatory are involved in the development and progression of AD [5][9]. Mediators can be cellular as well as chemical, they can be beneficial and harmful at the same time. Usually, cellular mediators include astrocytes, microglia, oligodendrocytes whereas chemical mediators include cytokines, chemokines, and cyclooxygenases. The detail about individual mediators is mentioned below.

2.1 Cellular mediators

Astrocytes and Microglia are the main cells that are responsible for the release of different kinds of neuroinflammatory mediators in the brain of AD patients [9].

2.1.1 Astrocytes

Astrocyte is a type of glial cell present in the CNS playing an important role in neuroprotection and brain maintenance. Astrocytes are activated by pro-inflammatory mediators and respond

in a pleiotropic manner. Further, astrocytes result in activation of early gene response, cause expression of various chemokines, cytokinin, and intensify inflammatory response in the brain [5][9],. Evidence also suggests that AD astrocytes release ectoenzymes which plays role in the degradation of Aβ plaque. Activated astrocytes cause upregulation of glial fibrillary acidic protein (GFAP) expression, hypertrophy, gliosis, swelling of astrocytes. Astrocytes are present around plaques, tangles, dark neurons [9].

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2.1.2 Microglia

Microglia cells consist of about 10-15% of the brain as a cellular population, present as a phagocyte of the brain. These cells act as the first line of defense towards any foreign particle and any type of brain injury [10]. Cells are supposed to be in quiescent stat and show no macrophage activity in resting stat. In a normal person, microglia have neurotrophic roles in the brain [9]. It also contributes to brain development by regulating apoptosis of neurons and maturation [5]. When microglial cells are activated generate inflammatory mediators that are chemokines, cytokines, ROS, COX-2, oxidative stress, or other response, which ultimately disrupts the neuronal function and cause damage to the neuron. It also produces some neuroprotective agents such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) that protects the brain from any injury [10]. The binding of microglial cell with Aβ fibrils causes the fixation of the cells and produce reactive oxygen species (ROS) [9]. At the beginning of AD development, the microglial immune response causes the Aβ clearance suggesting immune response can favor the regulation of AD pathologies. In contrast longer (chronic) activation of glial immune response results in enhancement of AD pathologies [5]. Aβ stimulates intracellular signaling cascade in glial cells, which affects the generation of ROS by NADH oxidase and discharging of neurotoxins and excitotoxins. Excitotoxins can cause serious damage to dendrites, as these agents act preferably on weaker subcellular synaptic and dendrites. Notably, synaptic damage is an important correlative for cognitive loss in AD patients[9].

2.1.3 Oligodendrocytes

Oligodendrocytes are a type of glial cell, that plays a crucial role in neurotransmission and maintaining the proper functionality of neurons. Studies have suggested that oligodendrocytes are associated with immune response in various neurodegenerative diseases, especially in sclerosis. However, less information is available regarding oligodendrocytes' involvement in the advancement of AD. Studies have been reported that in transgenic mice as well as in AD

patients, demyelination was associated with Aβ plaques in the gray matter of the brain. Studies also suggested that microglial proliferation can be induced by Aβ injections [5].

2.2 Chemical mediators

Cytokines (TNF, IL), chemokines, and cyclooxygenases are the main neuroinflammatory mediators secreted by activated astrocytes and microglial cells due to some immune response in the brain [5].

2.2.1 Cytokines

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Cytokines play an important role in the development of the CNS. Cytokines are primarily released by glial cells and astrocytes in CNS. A high level of cytokines such as TNF and IL has been observed in patients with neurodegenerative diseases. Several studies have reported that an increased level of cytokines is correlated with glial cells and shows an effect on cognition and neurodegeneration. There are two main types of cytokines, first one is TNF-α which is an important pro-inflammatory having good and bad effects on the different neurons. The elevated level of TNF-α was recorded in patients with AD. Glial cells also produce TNF- α through activation of transcription factor NF-κB due to stimulation by Aβ. TNF-α plays an important role in the activation of β-and γ- secretase, an enzyme responsible for the production of Aβ from amyloid precursor protein in AD progression. Second, IL-1 (major), IL-1α, IL-1β, IL-6, IL-12, and IL-23 are proinflammatory found in the early stage of Aβ deposition amidst AD development [5][11]. The main resource of cytokines is microglia and astrocytes in AD. Cytokines play a part in every behalf of inflammation, bystander neuronal injury, and the response of microglia to Aβ plaques [11].

2.2.2 Chemokines

Chemokines belong to a family of chemoattractant small cytokines. They are mainly released from astrocyte and microglial cells, regulate their movement to inflamed areas and increase local inflammation in AD development [5][11]. Chemokines are divided into four types based on cysteine residue-CXC, CC, CX3C, and C[12]. There has been reported a significant change in chemokines and their receptor in an individual's brain and in body fluid with AD than in healthy individuals [5]. CNS produced different kinds of chemokines CCL2, CXCL8, CXCL10, CCL5, and CCL3 also known as macrophage inflammatory protein-1α(MIP-1α) in response to various inflammatory and foreign agents. Cytokines can enhance Tau phosphorylation and show an impact on its function as well as enhance neurofibrillary tangles

(NFTs) formation. Cytokines protein also act on Tau kinases in the brain and enhance their activity which leads the Tau hyperphosphorylation [12].

2.2.3 Cyclooxygenases

Inflammatory mediators and Alzheimer's disease have well-known relationship pathogenesis. COX is an enzyme responsible for the synthesis of prostaglandins and other mediators. COX enzyme is of two types: COX1 and COX2. The expression of COX1 and COX2 varies according to Alzheimer's disease development. COX1 is mainly expressed in microglia that are involved in fibrillar Aβ plaques in late stages of AD [13][14] whereas COX-2 generate due to inflammation and causes pro-inflammatory prostanoid synthesis. COX2 is mainly

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expressed in neurons and is colocalized with the cell cycle of protein due to low tau tangles and Aβ plaques in early Alzheimer's disease [14][13]. Studies have suggested that cyclooxygenase (COX) inhibitor non-steroidal anti-inflammatory drugs (NSAIDs) can be a drug of choice for Alzheimer's disease. Several clinical studies have been conducted for the effectiveness of NSAIDS or selective COX2 inhibitors in AD. Most clinical trials have failed to show effectiveness. Failure of the study suggests that participation of COX in AD progression is much more complex and needs more study[12] [13]. The various chemical and cellular mediators involved in AD are presented in Figure 1.

3. Role of Withania somnifera in AD

Withania somnifera (WS) is a natural plant used as a nootropic agent from antiquity. In this chapter, we have discussed the role of WS in AD on the basis of available computational, In- vitro, In-vivo studies.

3.1 Computational studies

Choudhary et.al reported in a computational study that W1,3,5 are competitive inhibitors and W2,4 are non-competitive inhibitors of acetylcholinesterase (AChE) which is an important enzyme in the development of AD. The study also indicates the inhibition of AChE by WS would reduce the Aβ aggregating property of AChE in the early development of AD [15]. Grover et.al found ligand (WA) has had a high binding property to the receptor (AChE) in the molecular docking simulation study. WA is quite a promising ligand for the inhibition of human AChE [16]. Ahmad et.al have also performed a computational study and reported that chlorogenic acid (phytochemical screened from WS) has good docking scores (-8.856 and - 8.645) and free binding energies (-49.84 and -50.67 kcal/mol) in chain AB and CD of

NMDARs respectively. It could be a good inhibitor of GLUN2B subunit of NMDARs and block excitatory as well as the neurodegenerative effect of NMDARs [17].

3.2 In-vitro studies

Kurapati et.al have evaluated a protective effect of WS in the neuronal cells, toxicity induced by β-amyloid, and HIV infection. The author has reported a significant decrease in cell death when AS was added to pretreated Aβ cells which suggests its neuroprotective effects in Aβ induced toxicity cells [7]. AS has also significantly decreased the activity of acetylcholinesterase enzymes responsible for the breakdown of acetylcholinesterase [7]. The pretreated cells with WA have shown a significant reduction in ROS generated by Aβ.

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WA and WB are more potent constituents of WS. Recently another In-vitro study have conducted by Dubey et.al in neuronal cell line SK-N-SH and found a preventive role of derivatives of WS like WA, WB, W4, W5 against neuron degeneration [18]. Another study Singh and Ramassamy has also reported that WS extract prevents neuronal toxicity induced by Aβ and acrolein. The study has also reported that the WS extract increase anticholinesterase activity and a significant reduction in ROS [19]. Atluri et.al have reported a low level of Aβ in cells SH-APP treated with WA as compared to control. Cells are observed by staining them with Congo-red methachrome dye [20]. WA significantly inhibits the expression of nuclear factor κB (NF-kB) in microglial cells. Nair et.al have reported the In-vitro study which indicates WA is more effective against Aβ in PC-12 cells. As the dose of WA increases, the inhibition of Aβ enhances. Cells that are treated with 50 and 100 µg/ml, showed 100% survival which indicates WA can protect PC-12 cells completely [21]. Pandey et.al reported PC-12 (Pheochromocytoma) cells show maximum survival when treated with WS 35.17% than the control (7.82%) [22]. In vitro studies with cell line and dose are compiled in Table 2.

Table 2: Role of Withania somnifera in treatment of AD under In-vitro conditions

Sr.No.	Author's name	Composition	Cell line	Dose	Duration	Finding
1	Kurapati et.al 2014	Dried extract (Methanol:Chloroform)	SK-NMC	0.16µg/ml	48 hrs	extract was neuroprotective against Aβ induced cytotoxicity and HIV-1 infection
2	Dubey et.al 2021	WA,WB,W4,W5 procured fromNatural remedies pvt.ltd	SKN-SH	10, 20, 40, 60, 80, 100 µM	48hrs	there is a reduction in the β-sheet structures of amyloid.
3	Singh and Rammaswamy 2017	Standardised extractof WS	SK-N-SH	25µl	48hrs	protects SK-N-SH cells against Aβ peptide- and acrolein-induced toxicity
4	Alturi et.al 2020	WS commercialy purchased	SH-SY5Y	1µM	96hrs	WA inhibited the expression of NF-κB subunit-2
5	Nair et.al 2009	Methanolic extract of WA and WC	Rat pheochromocytoma (PC-12) cells		50µg/mL	protected PC-12 cells from cell-death caused by BAP

3.3 In-vivo studies

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Sehgal et.al have conducted an In-vivo study in transgenic APP/PS1 mice and reported that Withania somnifera effectively reverses behavioral deficits and decreases the Aβ plaques from the cortex and hippocampus by 77% and 78% respectively. However, the author also reported that elimination was less in old mice. The plaques density significantly decreases after 14 days of treatment with WS extract and no reduction in activated microglia [9]. Another study by Pandey et.al found that the WS-2, a constituent of WS, greatly improved cognitive function and interrupt the production of Aβ in the AD brain which Indicates WS could have a neuroprotective effect [22]. An In-vivo study by Konar et.al have reported WS extract can reduce the damage of neurons in scopolamine-induced neuronal toxicity and regrowth the lost dendrites. WS extract augments the neurite growth factor expression in the cortex and hippocampal region, which could induce the growth of healthy dendrites [23]. The detailed information related to animal model, dose, route of administration is compiled in Table 3.

Table 3: Role of Withania somnifera in the treatment of AD under In-vivo conditions

Sr. No.	Author's name	Composition	Animal model	Group	Cell line	Dose	Route of administration	Duration	Finding
1	Sehgal et.al 2011	Chloroform ethanol extract	Transgenic mice B6C3-Tg (APPswe,PSEN1)	2		1g/kg	Oral	30days	WS Reverses Behavioral Deficits and Plaque Pathology in APP/PS1 and APPSwInd J20 Mice
2	Pandey et.al 2017	Ethanolic extract	Male Wistar rats+female balb/c mice	6		5, 10 and 20 mg/kg	Oral	21days	Administration of WS-2 at graded doses decreased the Aβ concentration
					PC 12 cells				WS-2 shows significant protective effect against Aβ toxicity
3	Konar et.al 2019	alcoholic extract of WS	Male Swiss albino strain mice			200 mg/kg	Oral	7 days	Extract showed remarkable dendrite growth potential in scopolamine-challenged neurons.

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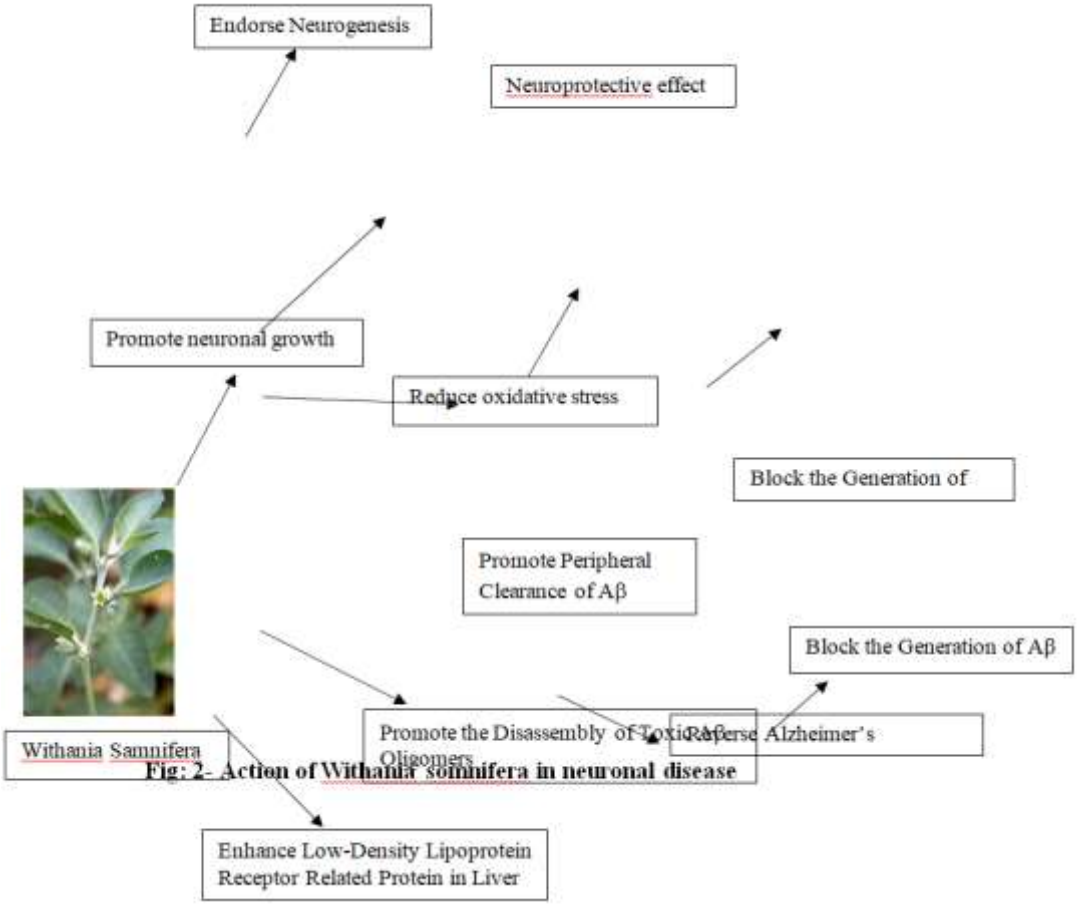

Fig: 2- Action of Withania somnifera in neuronal disease

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