Chapter 1
Introduction

1 Introduction

The fifth most common cause of death for the elderly population worldwide is Alzheimer's disease (AD). It is characterized by alterations in the structure of the brain brought by buildup of extracellular amyloid plaques and intracellular neurofibrillary tangles, which result in the death and eventual shrinking of brain cells (Figure 1) [1]. Memory, thinking, behavior, and social abilities gradually deteriorate as a result of brain damage. The capacity to do daily tasks may be significantly impacted by these changes [2]. Only symptomatic treatment that make up for the neurotransmitter disturbances and assist the patient in managing the symptoms of AD are available as a cure for Alzheimer's disease as of yet [3].

The non-competitive N-methyl-D-aspartate receptor antagonist memantine is a drug of choice for the management of moderate to severe AD, and there are three leading cholinesterase inhibitors (CIs) available and licensed for the treatment of mild to moderate AD [4]. The processes that lead to the development of extracellular amyloid plaques and formation of intracellular neurofibrillary tangles, as well as inflammation, oxidative damage, dysregulation of iron metabolism, and cholesterol, must be stopped in order to control the disease's progression during treatment. The complicated interaction of neuron networks and medications' failure to cross the blood-brain barrier present the largest obstacles to treating aberrant nerve function in Alzheimer's disease [5,6].

The therapeutic advantages and potential use of nanocarriers, more specifically “pegylated memantine-loaded nanoscaffolds" in AD can be better understood as a result of this investigation. Through this study, potential of polymeric nanocarriers to assist in facilitating drug transfer through the blood-brain barrier, providing a microenvironment of the extracellular matrix for the growth and development of neural networks, which will help in improvement of mental health via improved neural signalling can be envisioned. Memantine demonstrates therapeutic and neuroprotective behaviour by limiting excessive glutamate attacks on nerve cells and controlling the levels of neurotransmitters [6–8]. Beyond that, it also contributes to the maintenance of levels of neurochemicals like acetylcholine and butylcholine, which is primarily linked to enhancing memory and learning capacity.

Due to the higher inhibitory effect of the nanoscaffolds on the beta-secretase enzyme, the PEG coated-memantine self-assembled nanoscaffolds demonstrated better efficacy than pure drug memantine in reducing amyloid β plaque accumulation (abnormal breakdown of the amyloid precursor protein by the β-secretase enzyme). In this study, memantine-loaded nanostructured self-assembled PEG (PEG-MEM-PLGA) SANs were created for drug administration across the

blood-brain barrier and sustained action in Alzheimer's disease. They were created, validated, and optimised utilising Box-Behnken design by using response surface methodology to identify the optimum set of variables in order to create better polymeric nanocarriers. The "non-solvent-Induced Thermal Phase Separation (N-TIPS)" approach has been employed for it's simple and effective preparation technique making use of easily available reagents and equipment, and can be scaled up for industrial use [9].

The impact of the independent variables, such as concentration of PLGA, pluronics F127 and rotational speed on the dependent variables (porosity and drug loading) were examined, and the best values were estimated for experimental optimization. To ascertain the link between the dependent and independent variables, the data were statistically evaluated. Researchers from all over the world are working on self-assembled nanocarriers (SANs), which can create nanostructures and nanomaterials with distinctive physical and chemical properties and whole molecular organisation that can interact with living cells and respond to generation in relation to the emergence of cellular life [10,11]. By using the intrathecal route of administration, site-specific delivery was ensured, which concurrently avoids the danger of liver and kidney toxicity by shortening the dosing regimen while improving patient compliance [12].

The building blocks of nanopolymer scaffolds, such as PLGA, assemble into three-dimensional porous structures at physiological pH (5.7–6.8) and imitate extracellular matrix (ECM), assisting in the regeneration of damaged brain tissue. Paracellular nanostructures' possible brain transport processes were investigated and validated using Simultaneous Artificial Membrane Permeability Test (PAMPA) [13]. Pluronics F-127 is used as a surfactant, which acts as a pore-forming agent and increases the surface-to-mass ratio, improves drug loading efficiency and regenerative ability by stimulating cell adhesion, proliferation and differentiation [14]. Encapsulation of stem cells in the carrier matrix provides neuronal regeneration capacity, as stem cell attachment to neural tissue facilitates self-assembly of extracellular matrix-mimicking nanostructures by increasing hippocampal synaptic density and reducing neuronal death. This will help restore memory function and may aid in the treatment of AD amnesia [15,16]. Thermosensitivity, porosity and swelling capability of pluronics F127 enable nanocarriers to hold encapsulated cells in its structure and favor cell adhesion at site of neurodegeneration and promote neurogenesis. Therefore, this may be a promising technique for encapsulating stem cells to promote the regeneration in neurons, cartilage, tendons, epithelial tissues or even adipose and bony tissues.

As there is a progressive b impairment in memory and cognitive function leading to mild cognitive impairment (MCI), AD is a leading contributor of dementia in the aged globally,

mostly because it affects transmission of nerve signals from the brain to the muscles due to the creation of plaques and tangles [17,18]. Self-assembled PEG-coated nanoscaffolds (PEG-MEM-PLGA SANs-BMSc) were administered to scopolamine-induced amnesic Swiss albino mice animals that were loaded with memantine and transplanted with bone marrow stem cells. Animals showed an increase in cognitive function as a result of neural regeneration, indicating its use in regenerative therapy. The findings from the present study could assist the future and on-going research for management of AD.

1.1 Alzheimer's Disease (AD)

The most frequent cause of dementia in an ageing world population is Alzheimer's disease (AD). Dementia is a common syndrome defined by a steady deterioration in cognitive functions that make it difficult to carry out essential everyday tasks, including those requiring memory, language, and visual-spatial skills [19]. The assessment of brain tissue, cerebrospinal fluid (CSF), positron emission tomography (PET) scans, and fluid and imaging biomarkers are typically used for clinical diagnosis of AD [20,21]. Symptomatic therapy is currently the mainstay of treatment of AD. Healthcare providers estimate direct and indirect personnel costs at nearly $500 billion per year incurred in the treatment of AD [22–24].

1.2 Diagnostics for AD: labelling and imaging

The concentration of Aβ peptides (Aβ 1-42: Aβ 1-40 ratio) and total and hyper phosphorylated protein (Thr181 and Thr231) in the CSF fluid are two recognized biomarkers that must be monitored in clinical diagnosis of AD. Positron emission tomography (PET) allows for the visualization of amyloid plaque buildup [25,26]. CSF sample, however, is intrusive and isn't always possible or well tolerated in certain elderly people. As a result, non-invasive imaging techniques have been recommended to address this issue [20]. Because impaired brain metabolism (hyper- and hypometabolism) is linked to various stages of AD, fludeoxyglucose PET offers information about brain metabolism, which is highly helpful clinically. Another non-invasive method to detect functional abnormalities is magnetic resonance imaging (MRI), which has improved field strength and resolution [27,28].

1.3 Pathogenesis of AD

The two main pathophysiological characteristics of AD identified are extracellular aggregation of β amyloid (Aβ) plaques and intracellular aggregation of neurofibrillary tangles (NFTs), which are made up of τ-associated hyperphosphorylated microtubules [29]. Aβplaques mostly develop

in the basal, temporal, and orbitofrontal regions of the neocortical brain during the early stages, and in the hippocampus, amygdala, diencephalon, and basal ganglia during the later stages. Yet, in severe cases, Aβ plaque aggregation continues in all parts of the brain, including the cerebellar cortex, lower brain stem, and midbrain. In severe conditions, Aβ plaque aggregation leads to formation of Aβ tangles in the locus coeruleus, transretinal, and entorhinal regions of the brain, which also extend to the hippocampus and neocortex [30–32]. As a result, Aβ plaques and NFTs are seen as the primary causes of neurodegeneration and the primary pathogenic forces behind the emergence of AD.

1.3.1 Amyloid β and AD pathogenesis

The cleavage of plasma membrane's core protein, amyloid precursor protein (APP), occurs by β -secretase (BACE1) and γ -secretase affects the pathogenesis of amyloid, which leads to the production of insoluble Aβ fibrils. When it continues to oligomerize and diffuse into the synaptic cleft, synaptic communication is disrupted, which leads to the polymerization of insoluble Aβ plaques [30,33]. Aβ plaque buildup activates kinases, causing hyperphosphorylation of τ-proteins linked with microtubules and their polymerization into insoluble NFTs. In order to promote microglial activation and local inflammatory response, plaque aggregation and tangling instigate microglial recruitment near the plaque [34,35].

Structure and function of APP

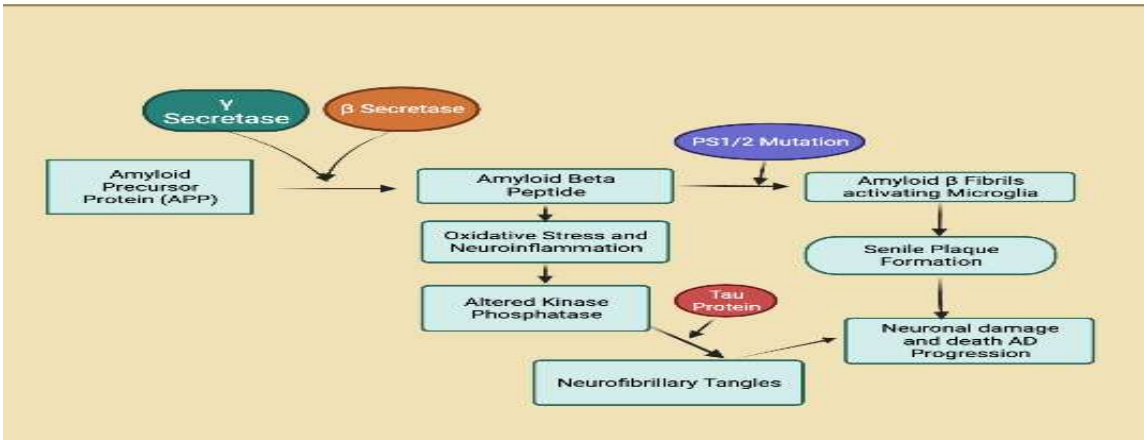
In mammals, the APP protein has an extracellular domain and is a nuclear transmembrane protein (Figure 2). The β-secretase (BACE1) and γ-secretase enzymes cleave APP into amyloidogenic fragments in Alzheimer's disease. BACE-1, a membrane-spanning aspartyl protease with an active site in the lumen, and γ-secretase, an intramembranous aspartyl protease made up of presenilin, nicastrin, anterior pharyngeal defect 1 (Aph1), and the Psen2 complex (Figure 2), are the aspartyl proteases with active sites in the membrane [36,37]. This compound stimulates the action of γ-secretase, resulting in the formation of insoluble and harmful Aβ -fragments. The first and most important step that leads to the N-terminal cleavage of Aβ is the cleavage by β-secretase. Although the physiological role of APP is currently poorly understood, numerous investigations in cell lines that have been transiently transfected have shown that APP has physiological effects [38]. Recent studies have also highlighted the activity of APP in neurological disorders by showing improvements in cognitive function and synaptic density when administered to animals as Amyloid precursor protein RNA interference (APP RNAi) via intravenous injection. APP encodes a type 1 transmembrane glycoprotein, which is cleaved through the non-amyloidogenic pathway (normal state) or through the amyloidogenic pathway

(pathological state) to produce a variety of polypeptides by splicing, glycosylation, phosphorylation or a complicated alternative.

APP consists of 770 amino acids, including 28 Aβ residues and additional 14 residues from the transmembrane domain of APP. At the cleavage site, α-secretase cleaves and releases a large soluble ectodomain, which is further cleaved by γ-secretase at residue 711, releasing soluble P3 peptide. Alternatively, in the diseased state, abnormal cleavage occurs by β-secretase which releases the cleaved APPsβ and the C-terminal C99 fragment is retained in the membrane and further cleaved by γ-secretase, releasing the insoluble Aβ peptide and the intracellular domain of APP is released in the cytoplasm , which dissolves and moves to the nucleus where it is polymerized, forms plaque aggregates and affects gene expression [39,40].

1.3.2 Hyperphosphorylation of τ and Microglial infiltration leading to neurodegeneration

AD is also characterized by hyperphosphorylation-induced production of neurofibrillary tangles (NFT) and accumulation of microtubule-associated τ-proteins. The τ-protein associates with tubulin through its microtubule-binding domain to form mature and stable microtubules that maintain microtubule integrity by forming bridges between adjacent microtubules to hold them together [41]. Under AD conditions, the abundance of Aβ plaques leads to the release of kinase enzymes, including glycogen synthase kinase 3 (GSK3β) and dependent kinase 5 (CDK5), when the microtubule-associated τ-protein interacts with kinases, it hyperphosphorylates and oligomerizes to form unstable microtubular subunits which immediately dissociate to produce τ strands, and then aggregate into the form of NFTs. These NFTs are straight, fibrillar and highly insoluble patches in the cytoplasm and neural processes, causing abnormal loss of communication between neurons and signal processing, and ultimately leads to apoptosis in the neurons [42] (Figure 2). Extracellular and intracellular plaques and Aβ plaques cause increased toxicity, leading to synaptic damage and increased reactive oxidative stress, leading to microglial infiltration around the plaque area. Microglia, found as resident phagocytes in the central nervous system, play an important role in maintaining neuronal plasticity and synapse remodeling. The accumulation of Aβ plaques and tangles activates microglia that initiate an innate immune response and the secretion of proinflammatory cytokines and chemokines [43].



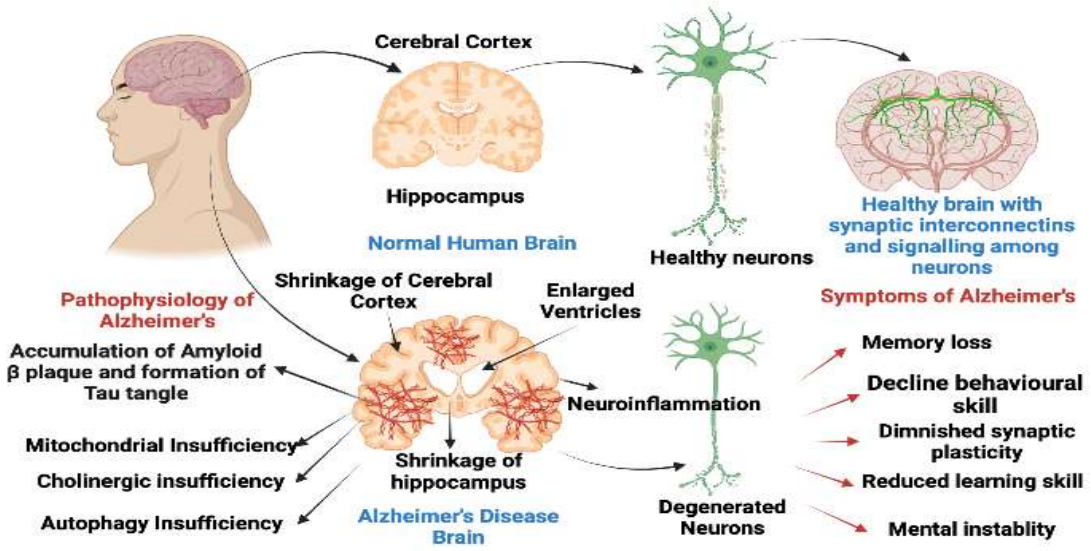


Figure 1: Mechanism of neuronal damage and Alzheimer's disease (AD) progression.

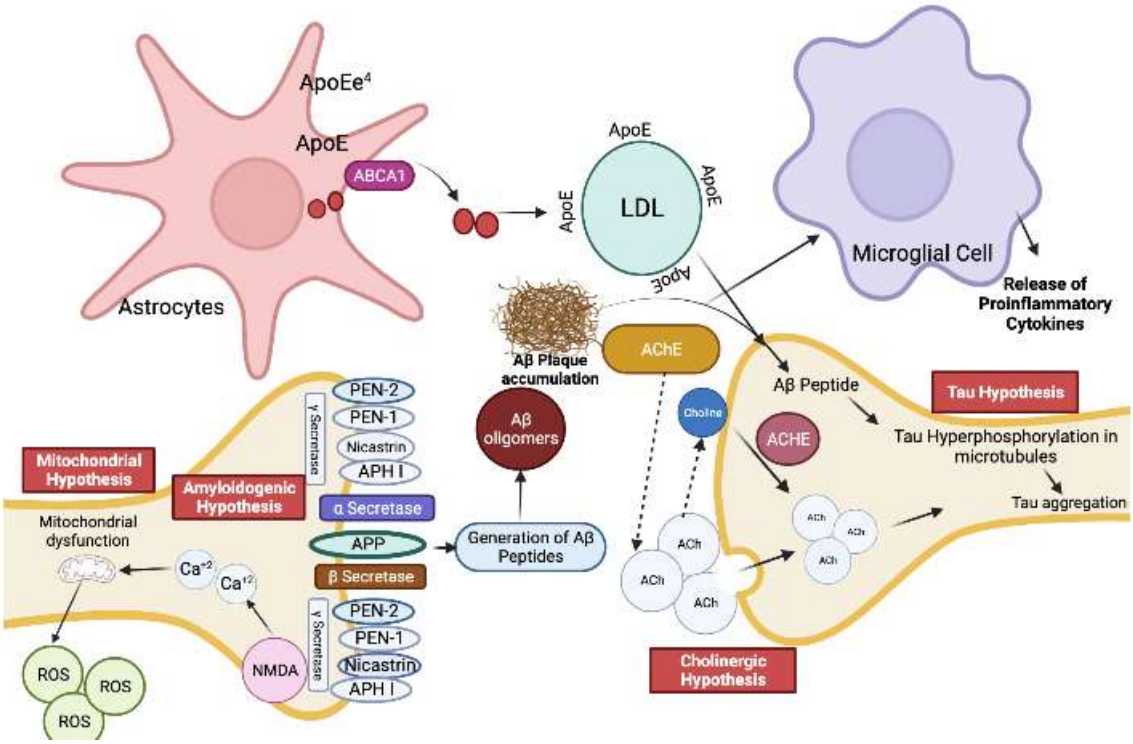


Figure 2: Hypothesis of developing Alzheimer's Disease.

Amyloid β and extracellular and intracellular adhesions cause increased toxicity, causing synaptic damage and increased reactive oxidative stress, leading to microglial infiltration around the plaque area.

1.4 Nanotechnology as a therapeutic tool for drug delivery across the BBB

While there are several possible pharmacological options for the treatment of Aβ neurotoxicity, penetration of medication across the central nervous system is major obstruction in anti-AD therapy. Neurotherapeutic compounds (such medications, peptides, transporters, and molecules) are not able to move freely into the brain due to the presence of semipermeable BBB supported by its selective transport mechanisms [44,45]. Nevertheless, nanoparticle systems have the potential to solve such issues and can be employed as Trojan horse systems to transport active compounds over the BBB, lowering toxicity and boosting therapeutic efficacy. Besides lowering the toxicity, nanocarriers can help improve the pharmacokinetics and pharmacodynamics of the drugs. The development of nanomedicine must consider the targeted and regulated delivery of medications to the target site. [46]. Nanocarriers can prove to be indispensable alternative for efficient diagnosis and therapeutic application, as shown by recent studies on nanotechnology breakthroughs. Drug bioavailability through BBB to the CNS can be drastically increased by use of nanocarriers, with supposedly little to no side effects. PEGylation, which is the

covalent/noncovalent aggregation of polyethylene glycol (PEG) to the surface to impart special physicochemical features, is one of the methods for surface modification of nanocarriers that promotes drug transport across the BBB [47,48]. charge alteration increases the drug-loading capacity of both hydrophilic and hydrophobic drugs within NPs and aids in on-demand release of the drugs for sustained release across the BBB. Through surface modification of nanocarriers, surface charge can be altered which facilitate movement across BBB.

1.5 Therapeutics in AD

The U.S. Food and Drug Administration has approved memantine and cholinesterase inhibitors as current medications for treatments of AD. Nevertheless, this is only symptomatic treatment and has no impact on control of the disease progression. Modern therapeutic approaches are therefore required to treat this condition. Unfortunately, identifying effective treatment is extremely difficult due to the numerous processes involved in the pathogenesis of AD [49].

1.5.1 Modulating neurotransmission

Loss of synaptic transmission in the brain and basal forebrain cholinergic pathology in AD is predominantly caused by the cholinergic group of nervous system chemicals. These neurons support the development of the cerebral cortex, maintenance of cerebral blood flow, modulation of cognition, learning, activity-related activity and memory, and control of sleep-wake cycles. Reduced choline acetyltransferase activity, decreased choline absorption, decreased acetylcholine production, and altered acetylcholine receptor (AChR) levels contribute to cholinergic neuronal dysfunction in AD [50].

Moreover, glutamate is crucial for cognition, memory, and learning. It is a key excitatory neurotransmitter that binds postsynaptically to the N-methyl-D-aspartate (NMDA) receptor, which is present in the hippocampus and neocortical regions of the brain. According to reported literature, high extracellular glutamate release in Alzheimer's disease causes increased presynaptic glutamate release and reduced reuptake, which causes tonic activation of NMDA receptors. Hence, using medications to modulate neurotransmission is still the best way to treat the symptoms [51].

1.5.2 Cholinesterase system

Tacrine, donepezil, rivastigmine, and galantamine are the four acetylcholinesterase inhibitors (AChEIs) that the U.S. Food and Drug Administration have licensed for use in AD. Tacrine, however, is currently infrequently used because of its hepatotoxicity. Blocking of acetylcholinesterase (AChE), lowers the breakdown of acetylcholine, by which AChEI enhances cholinergic neurotransmission. It is evident that there is an increase in long-term potentiation

when cholinergic transmission takes place or when receptors are active. Increased amounts of acetylcholine in the synaptic cleft may have a bidirectional effect because most of the hippocampus contains vast inhibitory interneurons that express cholinergic AChRs both pre- and postsynaptically [52].

Studies have demonstrated that AChEI decreases oxidative stress in both animals and , and cholinergic transmission also plays a role in modifying the pathways involved in adult neurogenesis. Clinically significant and encouraging improvements in cognitive function, a slower rate of functional decline or clinical worsening when compared to placebo, and a decrease in behavioral symptoms have all been seen in short-term studies of AChEI monotherapy in patients with mild-to-moderate and moderate-to-severe AD [53]. The gastrointestinal adverse effects that have been observed may be explained by the brief rise in plasma drug levels caused by oral dosages of AChEI. Transdermal patches, on the other hand, can offer prolonged release with little variation in plasma drug concentration. The rivastigmine patch (9.5 mg/24 hours) has a better safety and tolerability profile than the oral dose. Also, the patch considerably lessens discomfort [52].

Moreover, several novel AChEI compounds have recently been developed. Galantamine benzoyl ester product Memogain (GLN-1062; Galantos Pharma) is offered as an intravenous formulation and has been found to be effective with good bioavailability in the central nervous system (CNS) in the preclinical animal trials [54]. Natural alkaloid huperzine A was discovered in Chinese moss (Huperzia serrata) and exhibits AChE inhibitory activity with mild effects on APP metabolism and neuroprotection [55]. Phase I and Phase II trials of the medication have revealed a favorable safety profile. In patients with mild to severe AD, this medication was able to enhance cognition results by 2.27 points at a dose of 400 mg twice daily [56]. The prodrug of huperzine, ZT-1, has shown a promising pharmacokinetic profile in a recent phase I study [56]. Methanesulfonyl fluoride (SNX-001) is an irreversible AChE inhibitor first reported in 1999 for its therapeutic value in AD [57]. There has recently been interest in the molecule following preclinical studies showing its cognitive benefits. Phase I trials have investigated the extent of AChE inhibition in healthy subjects with promising results by showing direct modulation of the cholinergic AChR. In AD, presynaptic M2 AChR levels are decreased, but postsynaptic M1 AChR levels are unchanged. A number of partial M1 agonists such as AF102B, AF150(S), AF267B and AF292 and allosteric agonists such as 77-LH-28-1, LY-593093 and Lu AE51090 are available; ML 169 has recently been identified with positive allosteric modulation of M1 [58]. M1 agonists appear to play a role in APP processing and thus indirectly in other processes

such as tau phosphorylation; Studies have shown that ablation of M1 AChR leads to increased amyloid b (Ab) formation [59].

M1 agonists have a role in processing of APP and hence indirectly in other processes, such as tau (τ) phosphorylation. Further, AF150(S) and AF267B presented encouraging outcomes in the preclinical settings. Phase I/IIa trials are currently underway for ANAVEX 2-73, a mixed muscarinic/s1 agonist. This substance exhibits partial agonist effect on the muscarinic proteins AChR and s1, which are chaperone proteins in the endoplasmic reticulum that are activated by the unfolded protein response and which, in turn, cause tau hyperphosphorylation. The function of nicotinic AChR in AD is still unclear, and the therapeutic potential of muscarinic AChR in AD is still under debate [60]. These receptors have varied functional roles in cognition, memory processes, neuroprotection of trophic processes, and memory and are expressed by nervous system [61]. Cotinine is weak agonist of the α7 receptor and primary metabolites of nicotine has shown neuroprotective action with improvement of memory in primates as well as prevents memory loss, and lowers amyloid-beta (Aβ)) burden in AD mice. In AD, cotinine's positive effect on memory is associated with the inhibition of Aβ aggregation, the stimulation of pro-survival factors such as Akt, and the inhibition of pro-apoptotic factors such as glycogen synthase kinase 3 beta (GSK3β) leads to stimulation of α7 nicotinic acetylcholine receptors (α7nAChRs) which positively modulates these factors and memory, could be due to the involvement of these receptors in cotinine's mechanism of action. Alternatively, the beneficial effects of cotinine on cognitive abilities have been substantiated by another mechanism in which cotinine desensitizes Neuronal nicotinic acetylcholine receptors (nAChRs) located on inhibitory GABAergic neurons of the hippocampus. The sensitized neurons further stimulate the excitatory glutamate receptors in this region of the brain, thereby stimulating cognitive functions. This hypothesis is interesting; however, direct evidence that cotinine desensitizes the hippocampal α7 receptor is still under investigation [62]. The properties that make cotinine a better ligand is its low toxicity profile, non-addictive nature and good clearance across the blood-brain barrier. In addition to receptor modulation, this drug inhibits ABS aggregation. The pharmacokinetic and safety profile of cotinine has been studied in humans, but its role in AD has not been documented. Several novel ligands for the nicotine a7 AChR are under development [63]. EVP-6124 is a novel selective α7 partial agonist that improves memory performance in animals; the compound has passed a phase II trial (NCT01073228) [64]. MT-4666 is another nicotine agonist currently in Phase II trials (NCT01764243). Among other novel compounds, ABT-384 (NCT01137526) has completed Phase II studies and studies on ABT-126 (NCT01527916) are ongoing. In

summary, the advantage of M1 agonists over AChEI may be their potential role as disease modifying agents along with their symptomatic benefit. AChEI is a drug currently used in the clinical setting; directly active ligands would require more convincing evidence [65]. Because nerve dysfunction begins early in the disease course, the benefit of AChEI is that it temporarily relieves symptoms and preserves available nerve function; As nerve damage progresses, the effectiveness of AChEI therapy gradually decreases. As to how long the drug remains active or how long it takes to treat a patient with AChEI, may vary; reports indicate that benefits can last up to four years [66]. The benefits of the behavioral properties of AChEI in AD and their synergistic role in combination therapy with memantine are discussed in the following sections.

1.5.3 N-methyl D-aspartate antagonism

The glutamatergic system also contributed to the pathology in AD, but this occurs later in the course of the illness. Synaptic plasticity, neuronal growth and differentiation, cognition, learning, and memory are all regulated by glutamatergic neurons. The quantity of synaptic glutamate that is accessible to the receptors is determined by a process known as a "glutamate loop" that occurs between pre- and postsynaptic neurons and astrocytes. Different rates of cyclic errors contribute to excitotoxicity, extracellular glutamate buildup, and enhanced NMDA receptor activation in AD. The relevance of the glutamatergic system in AD is supported by studies showing that stimulation of the NMDA receptor results in the synthesis of Aβ and Oligomeric Aβ attaches to and activates the NMDA receptor. Memantine is a non-competitive, voltage-dependent NMDA antagonist that blocks channels by trapping them in an open conformation and has a fast blocking kinetics and moderate affinity [67]. NMDA channels are blocked by Mg^{2+} ions when the body is at rest; when glutamate is present, the block is removed, allowing passage of Ca^{2+} through the NMDA channels. Even during rest, NMDA activation status is low and persistent in diseased states like AD. Under these circumstances, Mg^{2+} ions are withdrawn from the channels, permitting a constant flow of Ca^{2+} across the membrane [68]. Memantine is useful in AD due to its ability to prevent persistent NMDA activation and its modest affinity and voltage dependence. Also, there is proof that the synaptic cleft of memantine-mediated blocking contains a significant amount of glutamate. Because of this, when a physiological impulse occurs, glutamate overrides the illness barrier and the physiological transmission can proceed as usual. According to experimental data, memantine therapy enhances synaptic degeneration, reduces free radical damage, decreases apoptosis, shields neurons from Ab-induced toxicity, and enhances spatial learning in animal models of AD [69].

Memantine has been reported to exert its antagonistic effect on other receptors such as a7 and

a4b2, 5-HT3, a3b2, a3b2, 5-HT2A nicotinic AChRs, dopamine D2 receptors and histaminergic neurons. Thus, the therapeutic benefit of memantine may not only be due to its effect on NMDA alone, but also on nicotinic α7-AChRs, however there is no strong evidence to suggest that effects on other receptors occur at therapeutically administered concentrations in AD [70,71]. Memantine is currently the only drug approved for clinical use in moderate to severe AD in the United States and Europe. Cholinergic neurons may be impacted early in the disease, while glutamatergic system harm and excitotoxic degradation happen later on. Memantine is also efficient in inhibiting nicotinic α7-AChRs at higher therapeutic concentrations; this blockage may hamper neurotransmission early in the disease when functional cholinergic neurons are still present. Memantine has only been used, thus far, in advanced stages of the illness. The potential advantages of mixing memantine with AChEIs in AD have been extensively studied [71,72]. The majority of research' findings suggest that combining memantine with AChEIs could enhance therapeutic benefits and clinical results for patients. In contrast, combination therapy is currently not recommended; two recent systematic evaluations have suggested considerable positive effects. Ongoing research is being done on memantine and donepezil for moderate-to-severe AD (NCT00866060). The aforementioned drugs are currently in their third phase of testing, with another trial testing the fixed daily dose combination of donepezil and memantine (ADS-8704; Adamas Pharmaceuticals) [73].

1.5.4 Amyloid Targeted strategies

Evidence reveals that peptides have various physiological functions, despite the fact that Aβ fibers are one of the pathological characteristics of AD. Although the circumstances under which Aβ becomes a pathogenic molecule are unclear, and probably might be influenced by the peptide's concentration. Aβ start to accumulate and rise in concentration to dangerous levels when Aβ production outpaces their ability to be eliminated. The oligomer-monomer Aβ fibrils and more hazardous oligomeric species are in a dynamic equilibrium. Oligomeric molecules may be vulnerable to destruction when Aβ is present in large quantities. Many facets of APP metabolism are targeted by amyloid-based treatments. Reduced synthesis of the modulator of the secretase Aβ peptide during secretase processing leads to the transmembrane protein APP. Under physiological circumstances, the major enzyme working on APP is α -secretase, followed by γ-secretase [74].

APP undergoes an amyloidogenic process when influenced by alternative β-secretase enzymes instead of α-secretase that increases the activity of secretase and converts APP into a nontoxic by-product, while inhibition of β and γ secretases showed reduction in amyloidogenic processing

of APP [75]. Ligand binding to cell surface receptors (usually muscarine/GABA agonists) and activation of signaling cascades such as protein kinase C regulate α-secretase activity and inhibits β-secretase activity to regulate amyloidogenic processing of APP as shown in figure 2. Epigallocatechin gallate, a polyphenolic substance found in green tea, has been found to activate α-secretory activity and subsequently process non-amyloidogenic APP [75,76].

Research is being done on bryostatin 1, a powerful protein kinase C activator and anticancer drug (Blanchette Rockefeller Institute of Neurosciences). The results of various *in vitro* and animal investigations point to bryostatin's medicinal potential; phase II clinical trials are currently being conducted on the medication. In conjunction with APP, the enzyme g-secretase mostly breaks down different kinds of proteins. The Notch protein, one of the \ molecules, controls how cells divide, develop, differentiate, grow, communicate, and survive. Drugs that affect enzymes mostly fall into two categories: modulators and γ-secretase inhibitors. While γ-secretase modulators have limited impact, inhibitors entirely block the enzyme and prevent it from digesting other proteins; despite these, no class of drugs has shown particularly high clinical efficacy [77,78].

The γ-secretase is modulated by a number of non-steroidal anti-inflammatory medication (NSAID) compounds. Flurbiprofen's enantiomer tarenflurbil, which controls g-secretase activity, lowers Aβ levels. The drug's Phase II studies showed promise, but its Phase III trials were so disappointing that it was put on hold. The cause of tarenflurbil's failure was discovered through further analysis of the study's findings. The study showed that the concentration of tarenflurbil in cerebrospinal fluid was substantially lower than that indicated by preclinical evidence, and CSF antibody levels were also unaffected. In fact, tarenflurbil's apparent effect in a phase II trial was brought on by the placebo group's increased cognitive impairment [79]. The observed failure may be caused by tarenflurbil's anti-inflammatory properties. Another g-secretase inhibitor that gained popularity was semagacestat that made its way into the clinical setting, but in a large phase III trial it produced disappointing results and was discontinued before completion [80].

1.5.5 Modulating Aβ transport

Although Aβ cannot pass the blood-brain barrier, apolipoproteins are crucial for the metabolism and transport of Aβ. They control the flow of Aβ between the central and peripheral neurological systems. The flow of Aβ from the blood to brain is increased by a protein called apoprotein E 34 (ApoE 34). Low-density lipoprotein receptor (LRP) protein is a key component of this receptor-mediated transport mechanism [81,82]. Aging affects LRP expression, which hinders Aβ efflux

and contributes to Aβ staying in the brain for an extended period of time. The infusion of peripheral soluble LRP to promote brain antibody evolution has also been proposed as a potential AD therapeutic method. Anti-LRP antibodies also lessen brain antibody efflux.

Aβ binds to the Receptor for Advanced Glycation End products (RAGE) at the blood-brain barrier, which increases the amount of Aβ infiltrating the central nervous system, causing inflammation, and causing neuronal death. RAGE is a multibinding receptor that binds Aβ with high affinity and enhances Aβ's entry into the central nervous system. The expression of RAGE is elevated in AD [83–85]. The first oral antagonist of the small molecule RAGE, PF-04494700 exhibited a passable safety profile in a phase I trial but failed in a phase II trial (NCT00566397) [86]. An effective strategy is to develop a soluble RAGE receptor analogue that functions as a receptor and minimizes ligand binding. A novel multimodal RAGE receptor called FPS-ZM1, administered to Transgenic mice improved its cognition and cerebrovascular parameters, with significant enhancement in permeability of blood-brain barrier , reduction in amyloid deposition, and improvement in blood flow [87].

1.5.6 Decreasing Aβ aggregation by passive immunization therapy

Bapineuzumab is a humanized form of the murine monoclonal antibody (mAb), targeting the N-terminus of Aβ, to induce plaque binding and Fc receptor-mediated microglial phagocytosis. This treatment, first developed by Élan and Wyeth and later by Janssen and Pfizer, progressed to phases 1, 2 and 3 clinical trials. After full safety and tolerability was established in phase 1, the phase 2 trial tested four doses of the antibody or placebo in patients with mild to moderate AD were investigated and it showed reduction of AD risk factor allele, ApoE4. Post-hoc analysis showed some cognitive benefit in ApoE4 non-carriers, guiding bapineuzumab in a phase 3 study. A phase 2 studies, with 28 patients assigned to bapineuzumab treatment and 8 patients assigned to placebo treatment, showed that Aβ plaque burden was decreased when measured by PET imaging, which identifies cortical fibrillar Aβ burden. Several phase 3 studies were initiated between 2007 and 2009. However, four of these studies were discontinued after the failure of the first two trials because there was no significant treatment effect on cognitive outcome in ApoE4 carriers or non-carriers. Amyloid-related imaging abnormalities (ARIA) have been observed in a subgroup of bapineuzumab-treated AD patients, which worsened with increasing dose and ApoE4 carrier status. Although biomarker results showed that the antibody reduced Aβ accumulation in patients carrying the ApoE4 risk allele but it did not improve clinical function [88,89]. Cerebrospinal fluid (CSF) Biomarker studies in the animal group treated with bapineuzumab showed significant lowering in the levels of phosphorylated tau (τ) rather than in

the placebo group but Aβ levels in cerebrospinal fluid did not change [90,91]

However, there was an increase in soluble Aβ deposits with a low Aβ 42: Aβ 40 ratio, showing that bapineuzumab impacts Aβ dynamics. There was no difference in plaque density or distribution between immunized and non-immunized participants. Solanezumab anti-Aβ antibody is also being studied for AD [92]. Solanezumab, was the subject of a phase II research that did not provide any therapeutic benefit but did lead to dose-dependent elevations in Aβ 42 concentrations in the blood and cerebral fluid. A phase II study of solanezumab showed no treatment benefit, but did result in dose-dependent increase in plasma and cerebrospinal fluid Aβ42 concentrations. The co-primary endpoint results were not significant in either study, but pooled data analysis showed a significant reduction in cognitive decline in immunocompromised patients; Solanezumab is entering several new Phase III trials, including the Asymptomatic Alzheimer's Disease Trial (A4) (Corbyn, 2013) [92,93]. Gantenerumab (Hoffmann-LaRoche) is another monoclonal antibody that has shown promise in preclinical studies [94].

1.6 Memantine for the treatment of moderate to severe Alzheimer's

Memantine (Ebixa, Axura, Namenda, Akatinol) is a voltage-dependent, moderate affinity NMDA receptor antagonist that prevents excessive calcium influx brought on by long-term overstimulation of NMDA receptors. Memantine is approved for the treatment of mild to moderate Alzheimer's disease in US and EU [93]. Oral memantine, alone or in combination with stable dosages of acetylcholinesterase inhibitors, has been shown in well-designed clinical trials to be effective for up to 52 weeks in the treatment of mild to severe Alzheimer's disease. Overall, memantine reversed the progressive worsening of symptoms in global status, cognition, function, and behavior in patients with moderate to severe Alzheimer's disease in four studies with durations ranging from 12 to 28 weeks. One of the meta-analysis has showed a positive impact on general status and cognition, in a data from three 24-week studies in patients with mild to severe Alzheimer's disease [94,95].

1.7 Self-Assembled Nanoscaffolds

Arrays of nanostructures make up self-assembled nanocarriers (SANs), which interact with one another via non-covalent forces to confer special features. Applications of self-assembled nanomaterials in nanotechnology, health sciences, biosensors, and imaging techniques are numerous. Lipids, proteins, and nucleic acids that are involved in a variety of cellular processes, molecular transport, and cellular contacts with external compartments are typically used to build self-assembled structures. Self-assembled structures' functional behaviour can be improved by

changing their physical and chemical characteristics, as well as their size and shape. Additionally, by controlling the release of the payload in response to exogenous stimuli (such as changes in pH, temperature, redox potential, and enzymes, the pharmacokinetic profile of the loaded medicine can be enhanced [96]. Nanostructures can be created by self-assembly techniques in spherical, polyhedral, elliptical, elongated, disc, shell, nanoscale, and central shell shapes [97,98]. These particles display a weak force-mediated brownian motion, the intensity of which is modulated by variations in temperature, solution pH, adhesion, substrate, etc., as well as additional particle assembly control [99,100]. Self-assembled nanostructures are also simpler to create than mechanically structured nanocarriers because they do not require high pressure, high temperature, or high electrical potential for structuring, which are essentially needed for hydrothermal or solvo-thermal engineering and electrospinning [101]. Self-assembled nanostructures have the potential to be inexpensive nanostructured particles because they don't need expensive or complicated processing ingredients or equipment. These nanostructures have offered a viable solution to the issues with the present treatment methods due to their biocompatibility, biodegradability, and regulated molecular dynamics [102].

Addition of medicinal stem cells with memantine-loaded nanoscaffolds, can have the potential to increase medication effectiveness and nerve regeneration activity [103]. Memantine-loaded nanoscaffolds are biocompatible and biodegradable, which makes them safe to use and reduces the risk of liver, heart, and kidney malfunction [104,105]. By boosting BBB penetration, enhancing drug delivery, increasing drug retention in the brain, and promoting brain microvasculature, nerve regrowth, and maintenance of nerve signals, memantine-loaded nanostructures administered via the intrathecal route can aid in reducing problems of AD. In addition to its many advantages, it also helps in the therapeutic treatment of AD by altering dosing schedules and reducing dosage frequency.