Section-I

Chapter-1

INTRODUCTION

1. INTRODUCTION

According to the English Dictionaries Dementia is defined as “severe destruction or loss of intellectual capability and personality combination, due to the damage or loss of neurons in the brain.” “Dementia” word is formerly taken from Latin, meaning "madness", from *de-* "without" + *ment*, the root of *mens* "mind" [1]. Dementia is a serious loss of comprehensive cognitive aptitude in a previously normal person, beyond what might be expected from normal ageing. Precisely dementia is not a disease. It's in general term that illustrates a wide range of symptoms related with decline in memory or other thinking skills severe enough to reduce a person's capacity to perform everyday performance [2]. It may be stagnant as a result of distinctive global brain injury or advancement, and ensuing in long-term decline due to damage or disease in the body. Dementia is a distracted syndrome (i.e., set of signs and symptoms). Affected cognitive areas can be attention, language, memory and problem solving. Particularly in later stages of the condition, subjects may be confused in time (not knowing the day, week, or even year), in place (not knowing where they are), and in person (not knowing who they are and/or others around them are). Some of the most universal forms of dementia are: vascular dementia, semantic dementia, Alzheimer's disease, frontotemporal dementia and dementia with Lewy bodies [3, 4].

1.1. Alzheimer's Disease (AD)

AD is the most common neurodegenerative disease. Alois Alzheimer [5], a German psychiatrist in 1906, first described AD and the disease was named after him. Most often, AD is diagnosed in people over 65 years of age, although the less-prevalent early-onset AD can occur much earlier. In 2006, there were 26.6 million sufferers worldwide. AD is envisaged to affect 1 in 85 people globally by 2050 [6, 7]. Even though AD widens differently for every individual, there are many ordinary symptoms [8]. Early symptoms are often misguidly thought to be 'age-related' alarms, or manifestations of stress. The most common symptoms associated with Alzheimer’s are as follow:

- One of the most familiar signs of AD is memory loss, principally not remembering lately learnt information. Other signs of AD are individuals forgetting important dates or events; over and over enquiring for the same information etc.
- A number of persons may experience alterations in their capability to build up and follow a chart or work with numbers. People may have problem following well-known guidelines. They find it hard to concentrate and they take much longer time to do things that they were doing before.

- ❖ Persons with AD can lose way of dates, seasons and the course of time. Persons may also have trouble accepting something if it is not happening immediately. Occasionally they may not remember where they are or how they got there.
- ❖ People suffering from vision problems can be a sign of AD. They may have obscurity in reading, judging distance and deciding colour, which may cause trouble in driving.
- ❖ Persons suffering from AD may have problem in joining a discussion. They may stop in the middle of a talk and have no idea how to continue or they may duplicate themselves. Persons may struggle with words, have difficulty deciding the right word or entitle belongings by the wrong name (e.g., calling a "watch" a "hand-clock").
- ❖ People suffering from AD may place belongings in strange places. They may misplace belongings and they find it hard to get them again. Sometimes, they may blame others of stealing. Eventually this may occur more frequently.
- ❖ People suffering from AD may practice variations in decision? For example, they may use poor decisions when dealing with money, giving large amounts to telemarketers. They may pay less attention to tidiness or keeping themselves clean.
- ❖ People suffering from AD may begin to withdraw themselves from hobbies, social deeds, work projects or sports. They may have difficulty in keeping up with a preferred sport's team or memorizing how to perform a favourite hobby. They may also keep away from being social because of the changes they have practiced.

The cause and development of AD are not well understood. Previous findings suggest that this disease is linked with tangles and plaques in the brain [9-11]. The treatments that are available for AD do not stop or repeal the development of the disease. On or after 2012, about 1050 clinical trials [12, 13] have been conducted to find ways to treat the disease, but it is unidentified if any of the compounds under discovery will work, because AD cannot be alleviated and is degenerative, the patient mainly relies on others for assistance.

On the basis of cognitive and functional impairments and progressive patterns, AD is divided into four stages [14].

- ❖ Pre-dementia: Memory troubles are characteristically one of the first signs of AD. Now and then, other thinking problems, such as difficulty in finding the right phrases or poor decision are well-known early on.
- ❖ Mild AD: As the disease steps forward, memory loss deteriorates, and alterations in other cognitive capabilities are evident. Problems can include:
    - Losing the mind

- Problem in management of money and paying bills
- Replicating queries
- Taking time to finish normal daily tasks
- Pitiable judgment
- Frame of mind and behaviour changes

AD is often diagnosed at this stage.

❖ Moderate AD: In this stage, damage arises in areas of the brain which control reasoning, language, conscious thought and sensory processing. Symptoms may include:

- Increased memory loss and confusion
- Difficulty in recognizing friends and family
- Incapability to find out new things
- Complexity in bringing out jobs that involve manifold steps
- Trouble in dealing with new circumstances
- Delusions, hallucinations and fear
- Hasty behaviour

❖ Severe AD: People with severe AD cannot converse and are fully reliant on others for their care. At the end, the individual may be on the bed most or all of the time as the body shuts down. The symptoms often include:

- Incapability to converse
- Weight loss
- Convulsions
- Infections in skin
- Trouble in swallowing
- Moaning, murmuring or groaning
- Increased sleeping
- Not having control on bowel and bladder

The reason behind most AD cases is still fundamentally unknown (apart from 1% to 5% of the cases where genetic variations have been recognized) [14-22]. Quite a few challenging hypotheses exist aiming to describe the reasons of the disease.

1.1.1. Cholinergic hypothesis

It is one of the oldest hypothesis and most of the drug therapies are currently available on the basis of this hypothesis. This hypothesis suggests that AD is caused when there is reduction in concentration of the neurotransmitter viz. acetylcholine across synaptic cleft [23]. The reduction of acetylcholine is due to degradation of acetylcholine by acetylcholinesterase (**Figure 1**). This hypothesis has not maintained widespread support, mainly because medications proposed to treat acetylcholine deficiency have not been very efficient. Other cholinergic effects have also been suggested, for example, when beta amyloid binds with pre-aggregatory site present on acetylcholinesterase, it leads to beta amyloid aggregation which ultimately leads to generalized neuroinflammation. It has been discovered that there are two types of ChE present in brain of AD patients i.e. acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). BuChE is thought to arise at advanced stage of AD [24-26].

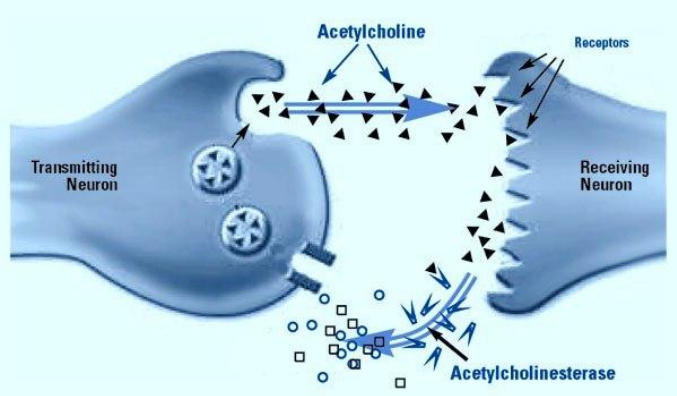

Figure 1: After signalling, acetylcholine is released from receptors and broken down by acetylcholinesterase.

1.1.2. Amyloid hypothesis

This hypothesis came in the year 1991. According to this hypothesis deposition of beta amyloid (Aβ) is the primary cause of the disease. AD has been recognized as a protein misfolding disease (proteopathy). It is mainly caused when there is gathering of abnormally folded beta amyloid (Aβ) proteins in the brain [27]. The plaques that are formed comprise of small peptides; 39–43 amino acids in length and known as beta-amyloid (Aβ). Aβ is a fragment from a larger protein called amyloid precursor protein (APP). APP is a transmembrane protein which penetrates through the neuron's membrane. APP is essential for nerve growth, survival and repairing [28, 29]. In AD, APP is divided into smaller fragments by the action of enzymes [30-34]. One of these fragments forms fibrils of Aβ, which further

forms clumps and these clumps get deposited outside the neurons and are called as senile plaques (**Figure 2**).

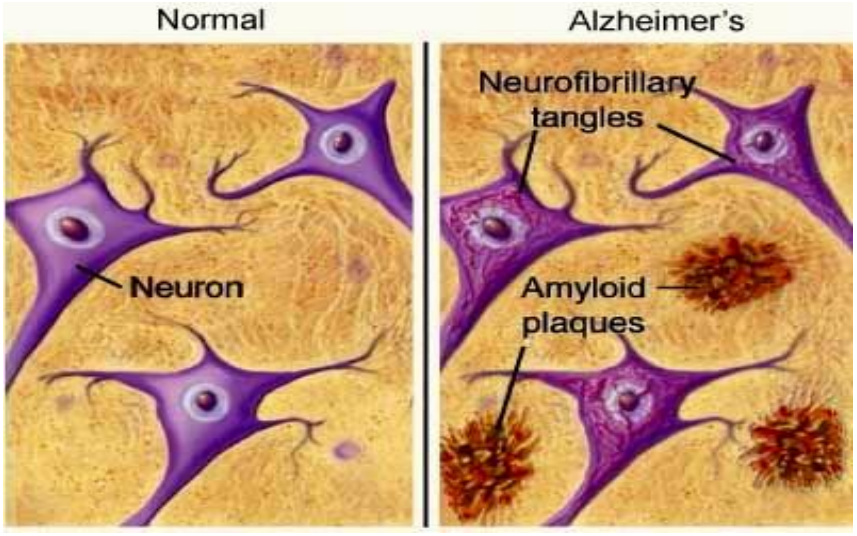

Figure 2: Senile plaques formed get deposited outside the neurons in AD.

1.1.3. Tau hypothesis

According to this hypothesis the disease cascade is initiated by abnormalities in tau proteins. Firstly, a tau protein undergoes hyperphosphorylation and this hyperphosphorylated

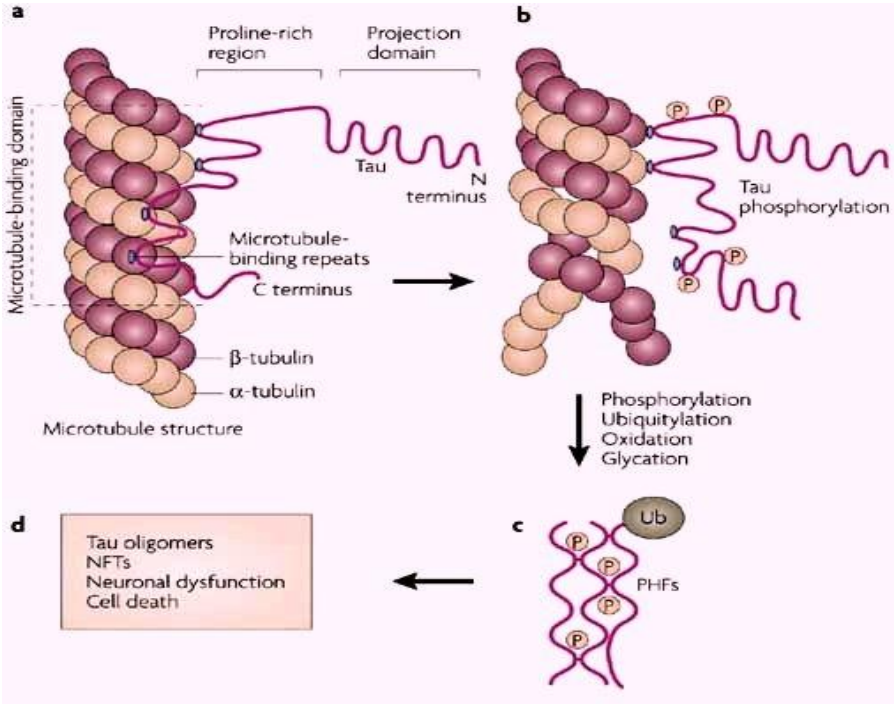

Figure 3: In AD, changes in tau protein lead to the disintegration of microtubules in brain cells.

tau protein is further paired with other tau protein threads [35]. Finally, neurofibrillary tangles are formed in nerve cell bodies. This results in disintegration of microtubules leading

to failure of neuron's transport system [36]. This chain of events leads to malfunctioning in biochemical communication between neurons, and finally results in cell death. (**Figure 3**).

1.2. AD pathogenesis

In view of the fact that AD is a non-curable disease, there is a decline in cognition for one year by current treatments that are available [37]. The agents used to treat AD have limited effectiveness. This led to discovery of more causative pathogenic targets. Since last decade, three major hypotheses on the pathogenesis of AD have appeared which focussed on diverse description of the disease. These challenging hypotheses are:

- The amyloid cascade hypothesis
- The metal ion hypothesis and
- The oxidative stress hypothesis [38].

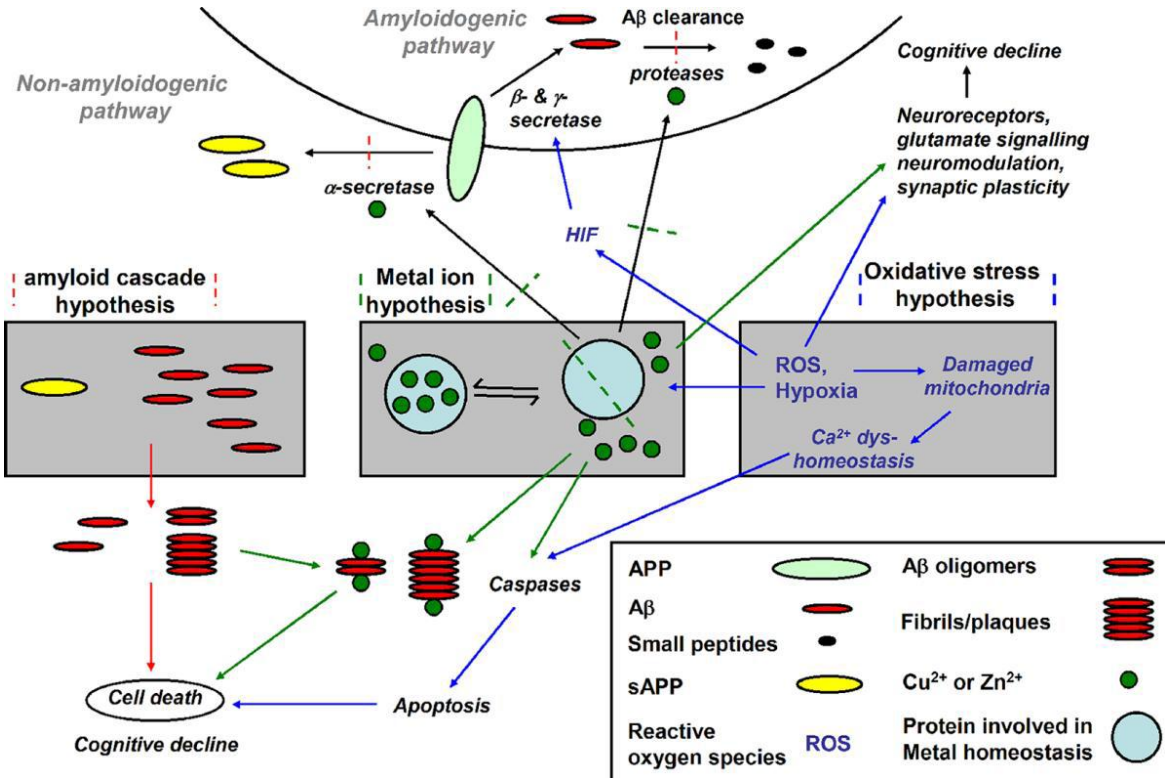

Figure 4: An overview of three hypotheses of Alzheimer's disease: (i) amyloid cascade hypothesis, with Aβ accumulation (red) being a main pathogenic event; (ii) metal ion hypothesis, with metal ion (green) dyshomeostasis leading to amyloid imbalance and (iii) oxidative stress hypothesis, with oxidative and general stress (blue) leading to mitochondrial damage, metal ion dyshomeostasis, apoptosis and Aβ imbalance.

The amyloid cascade hypothesis states that there is impaired balance between Aβ clearance and Aβ production which leads to development of AD. In AD the main neurotoxic substance is Aβ [39]. Subsequently, the treatments which can enhance Aβ clearance and inhibit Aβ production are supported by this hypothesis.

The metal ion hypothesis states that the impaired metal homeostasis is the main reason for AD. The metals viz. Cu, Zn, and Fe with Aβ imbalance could be a result of this [40]. This hypothesis supports treatments such as chelators that deal with the metal ion differences evidently causing amyloid gathering.

According to **the oxidative stress hypothesis**, genetic and age related factors or environmentally and genetically induced oxidative stress lead to gene defects and there is decline in mitochondrial function. Ultimately these consequences result in neurological disorder, either slowly or when reaching a significant threshold that initiates apoptosis in nerve cells [41, 42]. Wide variety of neurological disorders have been attributed by apoptosis, an array of pathways that can be activated, for example, by misfolded proteins, oxidative stress, lesions, Ca^{2+} dyshomeostasis or excitotoxicity (**Figure 4**).

1.3. Current treatments and existing clinical research on AD

Public health benefits in AD can be significantly achieved by alteration in the course of AD. For example, an involvement that could impede the commencement of AD by 2 years would decrease the occurrence in such a way that in 50 years there would be nearly 2 million less cases than the proposed number. One can envisage that modifying the course of the disease extensively would roughly reduce the need for nursing home residency and could help patients stay efficient for much longer.

Food and Drug Administration has so far approved only 5 medications to treat AD. Amongst these five, four are acetylcholinesterase inhibitors (donepezil, galantamine, rivastigmine and tacrine), and the fifth mimantine [43] is *N*-methyl-*D*-aspartate (NMDA) receptor antagonist.

Functioning of the AD patients can be improved by amelioration of the symptoms by these medications, but these medications are not curative, nor do they significantly alter the course of the disease. The most broadly studied treatments over the last century mean to deal with the neuropathological effects and focus on inflammatory markers, acetylcholine, tau-based neurofibrillary tangles and amyloid plaques.

In the subsequent section, the agents which are under investigation for the treatment of AD are discussed.

1.3.1. Interferences targeting amyloid

Researchers who are investigating AD have identified five strategies as possible involvements in opposition to amyloid:

1.3.1.1. β-Secretase (BACE) (also identified as β-APP cleaving enzyme) inhibitors

The BACE inhibitors block the first cleavage of APP outside the neuron. The description of a number of BACE inhibitors have been illustrated using various animal models. Targeting the BACE cleavage site of APP by using novel antibodies is one of the approaches that is being used recently. CoMentis Inc, a biotech company's β-secretase inhibitor's human data (CTS-21166) was first reported in April 2008, by Gerald Koelsch, at the Oklahoma City site [44]. This study showed that the compound under investigation appeared safe and reduced plasma amyloid-β levels considerably for an extensive period of time. In April 2012 Merck reported phase I results for MK-8931 [45]. Merck began Phase II/III trials of MK-8931 in December, 2012.

1.3.1.2. Gamma secretase (γ-secretase) inhibitors

The γ-secretase complex is remarkable among proteases in having a "shoddy" cleavage site at the C-terminal position in generation of Aβ; the cleavage of APP can be done by γ-secretase at any of the multiple positions to generate a peptide from 39 to 42 amino acids long chain length of Aβ, with Aβ40 the most familiar isoform and Aβ42 the most vulnerable one to conformational transformation leading to amyloid fibrillogenesis. Blockade of the second cleavage of APP has been done by γ-secretase inhibitors in the cell membrane and this ultimately stops the successive formation of Aβ and its noxious fragments. Eli Lilly and Élan developed Semagacestat (LY450139) [46], a contender drug of this class. Clinical trials were carried out by Eli Lilly. Phase III trials were conducted for Semagacestat that involved over 3000 patients, but unfortunately in August 2010, the disappointing results came out, in which it was found that semagacestat was no better than the placebo. This led to an end of the clinical trials.

1.3.1.3. Selective $A\beta_{42}$ lowering agents

These agents alter γ-secretase to decrease Aβ42 production in support of other (shorter) Aβ translations. Myriad Genetics developed Tarenflurbil [47], a single enantiomer of the racemate NSAID flurbiprofen. They investigated its prospects for the treatment of AD. The company stopped its investigation in June 2008. After Phase III testing conducted in 1,700 patients with mild AD, it was concluded that the drug did not recover thinking capability or the ability of patients to carry out daily activities significantly better than those who were given placebo [48].

1.3.1.4. Immunotherapy

The therapy arouses the host immune system to distinguish and hit Aβ or supply antibodies that either check plaque deposition or increase clearance of Aβ plaques or Aβ oligomers. Active or passive Aβ immunization can prevent oligomerization of Aβ. Aβ antibodies are used in this course to reduce cerebral plaque levels. This can be achieved by supporting microglial clearance and/or relocating the peptide from the brain to systemic circulation. CAD106 is one such Aβ vaccine that is presently in clinical trials [49]. Immunization with synthetic $A\beta_{1-42}$ has been revealed to be beneficial in mice and demonstrates low toxicity; however no significant differences have been found in clinical trials. Thus, it is not yet successful in humans and needs advance research. Recent research has shown that SDPM1, a 20 amino acid protein binds with tetramer forms of Aβ(1-40) and Aβ(1-42) amyloids and this binding results in blockage of successive Aβ amyloid aggregation. It is significant to note that this study was undertaken in mice. Although it prohibited further advancement of neuropathology it did not show any significant improvement in cognitive function. $A\beta_{42}$ immunization leads to clearance of Aβ plaques in patients with AD but does not stop progressive neurodegeneration.

1.3.1.5. Anti-aggregation agents

These agents work by preventing the aggregation of Aβ fragments or by clearing the previously formed aggregates. According to recent research, a nonselective dopamine agonist viz. apomorphine was found out to be an inhibitor of Aβ fibril formation, hence it could be therapeutically used to treat AD. The other attention-grabbing agent in phase 2 clinical trials is apomorphine scyllo-inositol [50]. It is sponsored by Elan Pharmaceuticals [51]. This agent appears to be focussed at the Aβ oligomers as it binds with Aβ oligomers and thus prevents synaptic damage. Scyllo-inositol is a small molecule that easily crosses the blood-brain barrier by active transport. It has received fast track designation from the U.S. FDA.

1.3.2. Advanced Glycation End (AGEs) products

Advanced glycation end products are composed endogenously during glycation and can additionally be ingested in a variety of foods. These AGEs have been implicated in aging through a variety of mechanisms, including incremented protein crosslinking, incremented free radical formation and as proinflammatory mediators. Receptor for cutting edge glycation finished items (RAGE) is an immunoglobulin supergene family communicated on the cell surface of different cell types all through the cerebrum and on the blood-brain obstruction. In AD, RAGE is upregulated on cells in the hippocampus, for example, astrocytes and microglia. Amyloid is known to tie to these receptors. This may be a way in which the

inflammatory cascade is fortified and hence may prompt cell death. Preclinical studies have proposed that hindering this receptor against amyloid tying secures the phone by diminishing plaque formation and irritation. Pfizer and the Alzheimer's Disease Cooperative Study are cooperating on a stage 2 trial of Pf04494700 [52], an oral RAGE antagonist.

1.3.3. NMDA receptor antagonist

Latrepirdine (Dimebon) [53], once utilized as a nonselective antihistamine within Russia, was contemplated in creature models of AD95 and was discovered to be useful in an evasion moulding standard. The component of activity of latrepirdine is indistinct on the grounds that it might additionally tweak α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid and NMDA receptors and pitifully restrain acetylcholinesterase

1.3.4. Nerve Growth Factor (NGF)

The misfortune of acetylcholine neurons is thought to be a noteworthy reason for the memory misfortune of AD. NGF is a trophic variable for acetylcholine neurons. In a mouse model of Down's syndrome, it was seen that expanded APP diminished retrograde transport of NGF, and this prompted the misfortune of cholinergic neurons. It is proposed that this may be an instrument of cholinergic cell misfortune in AD [54]. NGF has been indicated to keep the demise of cholinergic neurons in rats in both matured and injury models. In a quest for a more compelling approach to target NGF at suitable zones, hereditarily changed autologous fibroblasts are a centered region in clinical trial studies.

1.3.5. Targeting Tau protein

Hyperphosphorylated tau protein is the principle part of the other neuropathological sign of AD, the neurofibrillary tangle. Robotically, hyperphosphorylated tau is additionally known to meddle with microtubule gathering, which may push neuronal system breakdown [55]. Various boulevards have been investigated in creature models to address tau. One is restraining tau kinases and others include supporting microtubule get together. An alternate method that has right now passed a phase 2 trial is blocking whole tau with methylene blue (Mtc) [56].

1.3.6. Serotonin receptors

The finding of the serotonin 5-hydroxytryptamine-4 (5-HT_4) receptor in the previous 5 years has given experience into the indicating pathways and the physiological parts of G protein–coupled receptors in neurons. Pre-clinical research has showed the contribution of 5-HT_4 receptors in cognitive processes, the assurance of neurons by means of expanded discharge of the soluble form of APP and some proof of cholinergic incitement, and these all are conceivably restorative in AD. Late 2-week clinical trials of PRX-03140 [57] in people

propose that agonists at the 5-HT_4 receptor may have a cognitive upgrading impact. PRX-03140 was in a stage 2 clinical trial, however after moderate enrolment and inadequate financing, the organization Epix, suspended its advancement and left this as an unexplored entity. Other serotonin receptor modulators have been or are likewise being investigated, for example, 5-HT_{1A} (xaliproden) [58] and 5-HT_6 (SB-742457) [59], which have quite recently experienced stage I clinical trial.