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Review Article

ALZHEIMER'S AND CURRENT THERAPEUTICS: A REVIEW

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ABSTRACT

In recent past several efforts have been made to analyze the symptoms, causes, and cure of Alzheimer's disease (AD) and also to reveal biochemical changes and pathogenesis of the brain affected with AD. Several studies indicated the main cause of the disease is deposition of necrotic β-amyloid plaques in the brain. The enzymes β-secretase and γ-secretase catalyze the β-amyloid production. It has also been observed that the cells producing acetylcholine, a major neurotransmitter, are destroyed by two closely related enzymes namely acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in AD progression leading to cognitive disabilities. Hence, the research is going on in finding the inhibitors for these enzymes which will help to either prolong or cure the AD. Discovering the inhibitors for AChE and BChE without side effects remains a major challenge as the AChE and BChE inhibiting drugs available possess several side effects. Several new drugs are being discovered utilizing medicinal plant resources. Uses of flavonoids as plant secondary metabolites are being tried for the treatment of AD. Efforts are being made to apply computational knowledge to streamline the drug discovery process. Nevertheless, blood-brain barrier (BBB) permeation plays a vital role in drug discovery. BBB a physical barrier in the brain through which the central nervous system therapeutic molecule has to permeate for its activity. Finding the potent lead candidates capable of crossing the BBB remains to be a major challenge in neurodegenerative diseases. In the present review, attempts have been made to discuss on all these important aspects.

Keywords: Alzheimer's disease, Amyloid precursor protein, β-amyloid, Blood-brain barrier, Flavonoids, Molecular docking, Multi-enzyme targeting.

INTRODUCTION

Alzheimer's disease (AD) is a neurological ailment and one of the most common dementia in the age group above 60 years. It is estimated from the world population that more than 35.6 million people are living with AD as on today and this may increase to 65.7 million by 2030 and 115.4 million by 2050 [1]. According to WHO report, more than 50% persons with AD live in the developing world and are stated to go up to 70% by 2025.

In AD abnormal changes in the brain worsen over time, eventually interfering with many aspects of brain function. Memory loss is one of the earliest symptoms, along with a gradual decline of other intellectual and thinking abilities, called cognitive functions, and changes in personality or behavior. Numbers of causes have been attributed for the development of AD out of which the formation of amyloid plaques from β-amyloid protein (Aβ) that accumulates in the brain is thought to be major cause [2-4].

While reviewing the role of the β-amyloid protein in AD disease, it has been mentioned that AD is categorized by a series of structural changes and abnormalities in the brain [5]. These are neuronal dysfunction and death, the presence of neurofibrillary tangles on the surface of cell bodies at proximal dendrites [6-8] and presence of extracellular deposits of amyloid proteins in the form of senile plaques.

β-amyloid protein at the majority is thought to be associated with neurodegeneration, and is also observed in neurotoxic *in vitro* and *in vivo* studies, but its exact role in the development of the disease is not fully understood. It has also been linked with an increase in oxidative stress, deregulations of calcium dynamics and inhibition of the activity of some enzymes, possibly through interaction with cellular membrane structures [9]. Therefore, in many cases it has been concluded that the hallmark of AD is due to the presence of amyloid deposits and neurofibrillary tangles in the brain in parenchyma of amygdala, hippocampus, and neocortex regions [10].

Over the last few years, several key proteins have been reported as being involved in Aβ production but further elucidation is required to understand the mechanism involved so that potential drug therapies can be developed to combat AD.

AMYLOID PRECURSOR PROTEIN (APP): BIOLOGY AND FUNCTIONS

β-amyloid plaques, the characteristics of AD, are generated by processing of a much larger transmembrane APP [9,11] through the successive reaction of proteolytic enzymes known as secretases [12], whereas APP is an integral membrane protein in synaptic regions of neurons [13,14].

The functions of APP in the nervous system are still not completely revealed. Some studies have given indirect evidences about the role of APP that it interacts with G-protein and may thereby influence signal transduction pathways [15]. Sisodia and Price (1995) have mentioned that the conservation of APP between species, the abundance of APP in the brain, and the evidence that changes in APP biology influence brain function have been interpreted to indicate that APP and members of APP gene family play important role in the biology of neural cells [5].

MECHANISM OF APP PROCESSING AND Aβ PRODUCTION

The mechanism of proteolysis of APP (Fig. 1) is catalyzed by three enzymes α-secretase, β-secretase and γ-secretase through two pathways called as amyloidogenic and non-amyloidogenic. In the amyloidogenic pathway, APP is cleaved by β-secretase at β-site producing soluble proteins sAPPβ and a C99 peptide. The C99 terminal is again cleaved by γ-secretase producing APP intracellular domain (AICD) and a peptide with a specific sequence called as β-amyloid protein [10]. The non-amyloidogenic pathway lead by α-secretase and γ-secretase does not produce β-amyloid protein.

PROTEASES INVOLVED IN β-AMYLOID PRODUCTION

The proteases namely β-secretase (beta active site cleavage enzymes [BACEs]) and γ-secretase are mainly involved in Aβ production. All

three proteases belonged to metalloprotease family (A disintegrin and metalloproteinase [ADAM] family) and had been suggested to exert the secretase activity [16].

α-secretase

The non-amyloidogenic pathway is initiated by α-secretase followed by the successive cleavage of γ-secretase. This enzyme belongs to the family ADAM, more specifically to ADAM 10 out of its three members ADAM9, ADAM10, ADAM17. The cleavage by alpha-secretase does not produce beta-amyloid plaques, and it has also been observed that there is a decrease in alpha-secretase activity of APP processing in AD patients [17]. Hence, this pathway is as much important as that of the amyloidogenic pathway but in a different way. If the efforts would be made to stimulate the activity of alpha-secretase, the production of β-amyloid could be prevented and so one of the potential therapeutic strategies to cure or prolong AD is to increase the alpha-secretase level [18].

β-secretase

β-secretase acts as a rate-limiting first step in amyloid generation and has been a focus of intense research as a prime drug target [19]. The gene encoding this enzyme is BACE1. BACE1 plays an important role in the amyloidogenic pathway which generates Aβ peptides whose complex gives rise to β-amyloid plaques.

The studies on BACE1 showed that the deletion of the BACE1 gene abolished the production of β-amyloid [20,21]. Hence, inhibition studies on BACE1 could give insight on reduction in the formation of amyloid plaques which can further lead to disease inhibition. Cole and Vassar (2007) found that BACE1 knockout mice do not produce β-amyloid and are free from Alzheimer's associated pathologies including neuronal loss and certain memory deficits. It was also observed that BACE1 levels are found to be elevated in this particular disease which may provide direct and compelling reasons to develop therapies directed at BACE1 inhibition thus reducing β-amyloid and its associated toxicities [22].

γ-secretase

γ-secretase is a protein complex of high molecular weight responsible for the membrane cleavage of the APP C-terminal remnants after cleavage by either α or β secretase [23]. γ-secretase complex comprises four core components which include presenilin, nicastrin, anteriorpharynx-defective-1, and presenilin enhancer-2 [24,25]. All of these four core components of γ-secretase are necessary for the enzymatic activity of the complex. Besides, the four components of γ-secretase complex, there are some other factors which play a modulatory role in the enzyme activity [26].

Acetylcholinesterase (AChE)

Acetylcholine (Ach), a neurotransmitter in the brain cells helps in delivery of message and when the messages reach at receiving cell, the enzyme AChE breakdown the Ach so as to recycle it again and again (Fig. 2).

One of the prominent features of AD pathology is increased levels of AChE around the amyloid plaques, which is an indicative of investigation to be done in this area. This increased AChE in turn reduces the amount of Ach, a brain neurotransmitter to carry the message which has been considered as a link to the pathogenesis of AD. If acetyl-cholinesterase activity is reduced or by some means inhibited then it may help to maintain the levels of Ach in neuronal cells thus preventing the loss of functioning brain cells. Hence, AChE inhibitors (AChEI) are supposed to be another potential drug targets for controlling AD [27].

Many drugs are now available for controlling AD, which target both AChE and Butyrylcholinesterase (BChE) [28,29]. Salud *et al*. (2011) while reviewing the role of acetylcholinesterase in AD disease mentioned that although our understanding of the relationship between AChE and pathological features of AD is incomplete, there are evidences which suggest that both β-amyloid protein and abnormally hyperphosphorylated tau can influence expression [30]. The following Table 1shows the various types of drugs which are available in the market or in clinical trial phases.

BChE

BChE is another type of cholinesterase found in liver. These are various reports where it has been clearly shown that the genesis of amyloid protein plaques associated with AD is connected to modification of both AChE and BChE, since the plaque is significantly decreased in AD patients using cholinesterase inhibitors [27].

The fundamental role of the enzyme BChE at the cholinergic synapse is to terminate neurotransmission by rapid hydrolysis of the substrate, Ach, into choline, and acetic acid [32]. Hence, BChE has been identified as potential targets in the treatment of AD. The following Table 2 shows various BChE inhibitor drugs available in the market.

Blood brain barrier (BBB)

BBB is the specialized system of brain microvascular endothelial cells that shields the brain toxic substances in the blood, supplies brain tissues with nutrients, and filters harmful compounds from the brain back to the blood stream. BBB separated the brain and the central nervous system (CNS) from the blood stream [35] and acts as physical barrier.

BBB permeability is regulated by brain capillary endothelial transport properties. Microvascular biology in the CNS is regulated by paracrine interactions between the capillary endothelium in brain and its neighboring cells (Fig. 3a and b), the pericyte (which shares capillary

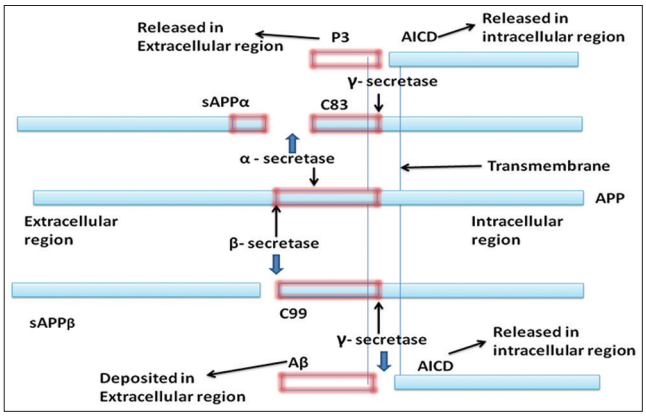

Fig. 1: Schematic representation of amyloid precursor protein proteolysis

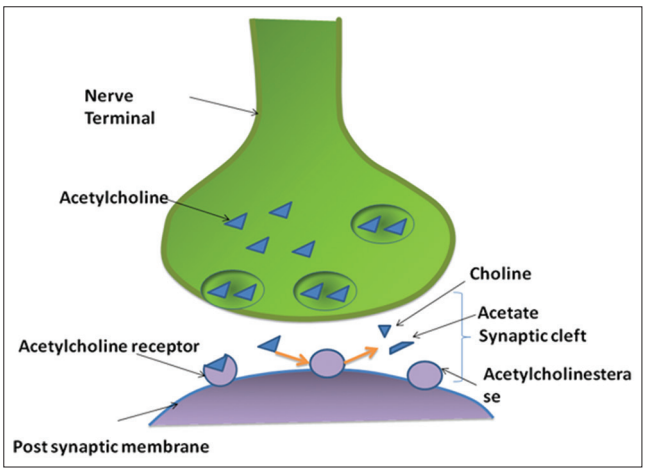

Fig. 2: Acetylcholinesterase activity in Alzheimer's disease

Table 1: The AChEI drugs for AD currently available in the market and their status in clinical trial phases

Drug	Drug origin	Side effects	FDA approval	References
Tacrine	Synthetic	Possible liver damage, nausea, and vomiting	1993	[31]
Donepezil	Synthetic	Nausea, vomiting, loss of appetite, and diarrhea	1996	[31]
Rivastigmine	Semi-synthetic	Nausea, vomiting, loss of Appetite, and increased frequency of bowel movement	2000	[31]
Galantamine	Plant derived	Nausea, vomiting, loss of appetite, and increased frequency of bowel movement	2001	[29]
Ganstigmine	Synthetic	No published reports available	Phase II and but company discontinued development of the drug candidate	[28]
P58(PYM-50028)	Plant derived	No published reports available	Phase II	[28]
Phenserine	Synthetic	No published reports available	Abandoned in Phase III	[29]
RU 47213	Pro-drug	No published reports available	Phase II	[28]
ZT-1	Pro-drug	No published reports available	Phase II	[28]
NS2330 (Tesofensine)	Synthetic	NA	Trials discontinued in 2008	[29]
Tolserine	Synthetic	No published reports available	NA	[29]
Esolerine	Synthetic	No published reports available	NA	[29]
HupA	Plant derived	GI side effects	Phase II	[29]

AD: Alzheimer's disease, GI: Gastrointestinal, AChEI: Acetylcholinesterase inhibitor

Table 2: The BChE inhibitor drugs for AD currently available in the market

Drug name	Drug origin	Side effects	References
Rivastigmine	Semisynthetic	Nausea, vomiting, loss of appetite, and frequency of bowel movement	[33]
Tacrine	Synthetic	Possible liver damage, nausea, and omitting	[29]
Huperzine A	Plant Derived (Huperzia Serrata)	GI side effects	[34]

BChE: Butyrylcholinesterase, AD: Alzheimer's disease, GI: Gastrointestinal

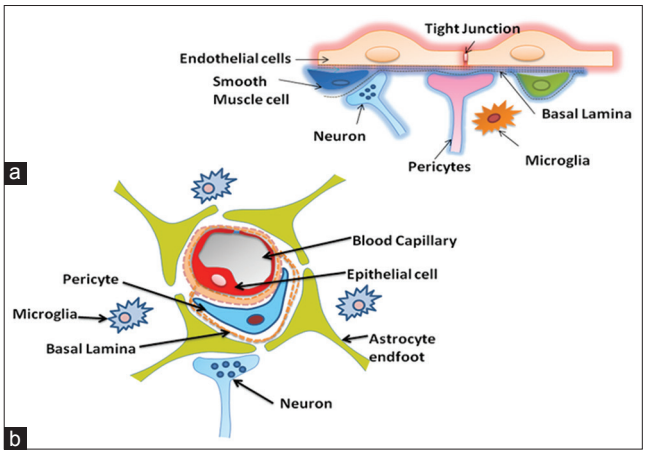

Fig. 3: (a) Blood-brain barrier structure; (b) Cellular components of blood-brain barrier

basement membrane with the 99% of the albuminal surface of the endothelium), the astrocyte foot process (which invests 99% of the albuminal surface of the brain capillary), the smooth muscle cells (which invests the endothelium of precapillary arterioles), and neuronal endings (which directly innervate either the capillary endothelium or the astrocyte foot process investing the capillary endothelium) [36,37].

Small lipophilic substances such as O_2 and CO_2 diffuse freely across plasma membranes along their concentration gradient [38]. Nutrients including glucose and amino acids enter the brain via transporters; whereas receptor-mediated endocytosis mediates the uptake of larger molecules including insulin, leptin, and iron transferrin [39,26].

Brain blood barrier and drug discovery

BBB permeability plays very important role in drug discovery of CNS diseases. The drugs aimed to target CNS to cure AD must be able to cross this barrier to exert their therapeutic effects. The secondary metabolites or the small molecules which would cross the BBB and would have inhibition ability for either of the enzymes AChE, BChE, and BACE1, may be the potential drug targets for AD. Ballabh *et al*. (2004) reviewed about BBB system, its structure, regulation, and also clinical applications [40]. In their review, they have focused on intraventricular hemorrhage in premature infants which may involve dysfunction of the eight junctions seal as well as immaturity of the BBB in the germinal matrix. It also describes the pathogenesis of increased BBB permeability in hypoxia-ischemia and an inflammatory mechanism involving the BBB in septic encephalopathy, HIV-induced dementia, multiple sclerosis, and AD.

Mode of action of AD drugs

The attempts of correcting Ach deficiency in the brain of AD affected individuals produced the development of first medication for the symptomatic treatment of AD in the form of AChEI. Although the benefits of these agents are modest, three drugs donepezil, rivastigmine, and galantamine are been licensed in the stabilization of cognitive decline. There is evidence that AChEI may slow down the disease progression and hippocampal atrophy and may have disease-modifying effects [41].

In addition, symptomatic improvement in AD patients is not restricted to agents that enhance Ach function in the brain, as is the case for Memantine, which acts on another neurotransmitter. However, further research is needed to establish an anti-inflammatory role for Memantine [42].

In the cell cultures and animal studies, as well as in human epidemiological surveys, agents known to dampen down inflammation such as vitamin antioxidants, herbal extracts with antioxidant properties, and long-term use of non-steroidal anti-inflammatory drugs have shown some protective effects against AD pathology. There is no current interest in statins for the treatment of AD [43]. Significantly, their suspected role in cognitive enhancement appears to be mediated through an anti-inflammatory effect, independent of their cholesterol-lowering properties [44].

Mentenopopulos (2003) while mentioning pharmacotherapy of AD has indicated that both the enzymes AChE and BChE play an important role in addition to their role in the formation of amyloid protein plaques [45].

He found that the activity of BChE substantially increases in the affected areas of the brain and the reason for this increase is under research.

There are total four drugs (Fig. 4) currently available in the market for treating AD. All these have been approved by the FDA to primarily treat the symptoms of AD [46].

Tacrine is the first drug approved for the treatment of AD in 1993, which is an inhibitor of both AChE and BChE [47]. The use of tacrine was limited as it was poorly tolerated due to a number of side effects including nausea, vomiting, dizziness, diarrhea, toxicity which has thought to be caused by the affinity for BChE and because less toxic, better tolerated drugs with easier dosing schedule were approved [47]. Donepezil is also an AChEI which prevents the breakdown of Ach in the brain [29]. It is assumed that donepezil in addition to the role as a neurotransmitter also act at molecular and cellular level in stages of pathogenesis of AD-like blocking excitotoxic cascade induced by glutamate, alleviation of effects of oxidative stress, and reducing the expression of inflammatory cytokines [48]. Rivastigmine also has BChE and AChE inhibitory properties. It has BBB permeability since it is a small molecule. The therapeutic action of galantamine has been reported to be mainly produced by its sensitizing action on AChE rather than by general cholinergic enhancement due to cholinesterase inhibition [29].

Molecular docking as a computational tool in drug discovery

It is generally recognized that drug discovery and development are very time and resources consuming processes. There is an ever growing effort to apply computational power to the combined chemical and biological space in order to streamline drug discovery, design, development, and optimization. Commonly used computational approaches include ligand-based drug design (drug-target docking), and quantitative structure-activity relationships (QSAR) and quantitative structure-property relationships. Regulatory agencies as well as pharmaceutical industries are actively involved in the development of computational tools that will improve effectiveness and efficiency of drug discovery and development process, decrease the use of animals, and increase predictability. It is expected that the power of Computer aided drug discovery will grow as technology continues to evolve [49-52].

While carrying out studies on computational analysis of AD drug targets Gupta *et al*., (2010) have designed potential candidates using molecular docking and reported that the strategy was to identify multi-target directed drugs which are inhibitors of AChE and BACE1 enzymes [53]. Further, it is also mentioned that three dimensional QSAR model for 4,3-hydroxy ethylamine derivatives of BACE1 inhibitors were developed using comparative molecular field analysis and comparative molecular similarity analysis techniques. From these studies, it was concluded that the development of this model of pharmacophore shed some light on the effects of the substitution pattern of the drugs that are related to the biological activity of anti-Alzheimer compounds.

Da Silva *et al*., (2006) while studying molecular modeling, docking, and ADMET which are being applied to the design of a novel hybrid drug for the treatment of AD found that the molecular hybrids of tacrine with donepezil would be a useful proposal for future treatment of AD [54].

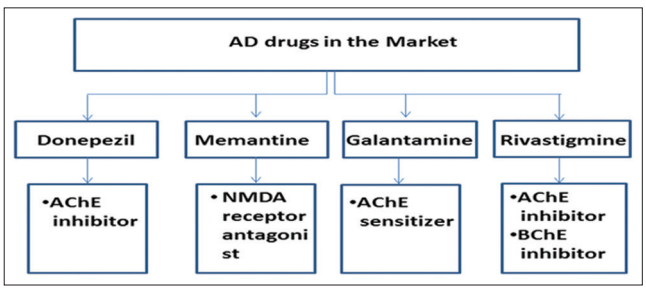

Fig. 4: Alzheimer's disease drugs available in the market

These kinds of molecular modifications based on the molecular hybridization have permitted the elaboration of new therapeutic derivatives, potentially more active, by the optimization of a prototype Camps *et al*., 2000 [55]. Jose *et al*., (2007) attempted studies on docking and quantum mechanics on cholinesterase and their inhibitors in relation [56].

Plant secondary metabolites as potential therapeutics prospects in AD treatment

Plants produce a vast and diverse assortment of organic compounds, the great majority of which do not appear to participate directly in growth and development. These substances, traditionally referred to as secondary metabolites, often are differentially distributed among limited taxonomic groups within the plant kingdom [57].

Secondary metabolites carry out a number of protective functions in human body. Many secondary metabolites have evolved as bioactive compounds that interfere with nucleic acid or protein and show antimicrobial or insecticidal and pharmacological properties. Secondary metabolites are therefore of interest in medicine as therapeutics to treat various health disorders, illness [58].

In vitro technology has given new insight to explore the potency of plant cell tissue culture to produce valuable chemical compounds as that of parent plant [59]. The advancement in plant tissue culture method for secondary metabolite production has bloomed expectations. Plant tissue culture is an aseptic technique whereby proper manipulation of the nutrients, culture conditions, phytohormone supply, one may be able to produce desired compounds in levels comparable to that of the plant.

Dastmalchi *et al*., (2007) has reviewed plants as potential sources for drug development against AD [60]. In their review, they screened plants belonging to 21 families. Phytochemicals substances such as alkaloids, biphenolic liganans, curcuminoids, caffeic acid derivatives diterpenes, triterpenoid saponins, triter lactones, stilbenes and withanolides with pharmacological activities relevant to AD treatment are discussed and these phytochemicals were found to show AChE inhibition ability.

Jager and Saaby (2011) while dealing with flavonoids in detail in relation to CNS have emphasized the importance of dietary flavonoids in AD therapy [61]. They found that flavonols, flavanones, and anthocyanins may act in protective ways, increasing the cerebral blood flow and protecting neuron against inflammatory process leading to cell injury. Further, it is mentioned that plants which are rich in flavonoids and categorized as common food products can be used as functional food for treatment of AD besides the use of other medicinal plants.

MECHANISM OF ACTION OF FLAVONOIDS

Flavonoids have attracted not only for the CNS activities but also as free radical scavengers with antioxidant activity. Until now more than 6,000 flavonoids are known and more are being explored [61].

The recent studies on different plant metabolites have been shown that flavonoids may perform a key role in enzyme and receptor systems of the brain, exerting significant effects on the CNS like prevention of the neurodegeneration associated with Alzheimer's and Parkinson's disease [61].

Flavonoids are capable to inhibit the enzymes as the evidences says about number of inhibitory enzymes such as aldose reductase, xanthine oxidase, phosphodiesterase, Ca^{2+} ATPase, lipooxygenase, and cyclooxygenase.

Considerable work has been carried out to search suitable and new flavonoids for therapeutic use in AD by using the technique of molecular docking. Hu *et al*., (2009) have designed new series of flavonoids and evaluated as potent AChEIs [62]. Most of them showed more potent inhibitory activities to AChE than Rivastigmine [62]. Further, it was mentioned that isoflavone skeleton would be a

promising structural template for the development of novel AChEI. Khan (2009) has examined AChE and BChE inhibitory activities of four flavonoids derivatives-quercetin, rutin, kaempferol galactoside, and macluraxanthone displayed a concentration-dependent inhibition of AChE and BChE. Khan (2009) has worked on number of flavonoids to lower Alzheimer's Aβ production using molecular docking studies [27]. They found that there existed a strong correlation between inhibitions of NFkB related mechanism. While doing work on molecular docking of Flavones as BACE1 inhibitors, it has been found that the flavonoids potently inhibit the BACE1 activity and the interactions of flavonoids with the BACE1 catalytic center [63].

Multienzyme targeting

The current trend in drug discovery relies on the computational methods. This acceptance has started to bring new approaches by thinking in a different way may be to bring serendipitous discoveries. One of the revolutionary thinking has been demonstrated by Russo, who described the current tendency in drug design and discovery. Russo *et al.,* (2013) discussed the AD pathology and the designing and discovery of new drug entities challenging multiple targets [64]. Azam *et al.,* (2014) studied the interactions of bioactive compounds with anti-Alzheimer drug targets through the computational molecular docking studies and found that these bioactive compounds showed the inhibition capability, thus provided the new novel potential lead molecules for the treatment of AD [65].

CONCLUSION AND DISCUSSION

The proteases alpha-secretase, beta-secretase, AChE and BChE are proving to be potential drug targets for AD. Computational approaches like molecular modeling and molecular docking are now a day are molecular docking are now a days being used to find novel inhibitors for AD. These methods are helping to give insight on the probable drugs with inhibition abilities, further reducing the efforts as well as being time and cost effective.

BBB remains to be an unbeatable challenge for every new candidate molecule for the treatment of AD. Varieties of approaches like *in vivo, in vitro, in silico* are utilized to check the BBB permeability of the lead molecules. Flavonoids due to their average small molecular weights and properties are considered to be potential lead molecules with an increased probability of crossing the BBB to exhibit their action. The trend of finding the multi-targeting drugs is now being tried. The efforts are going in this direction so as to achieve the cure instead of just prolonging the disease.

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