Introduction
CONTENTS

1. Introduction
   1.1. Neurodegenerative diseases
   1.2. Alzheimer’s Disease
   1.3. Risk factors
   1.4. Genetics of AD
   1.5. Pathological hallmarks of AD
       1.5.1. Cholinergic hypothesis
       1.5.2. Amyloid beta hypothesis
       1.5.3. Tau hypothesis
       1.5.4. Glutamate excitotoxicity
       1.5.5. Oxidative stress
       1.5.6. Inflammation
       1.5.7. Neurovascular dysfunction
       1.5.8. Metal ions
       1.5.9. Cholesterol
   1.6. Natural products as therapeutics against AD
   1.7. Grewia tiliaeefolia
1. INTRODUCTION

1.1. Neurodegenerative diseases

Neurodegenerative disorder is a term which is used for describing a range of conditions affecting the neurons in the human brain. The loss of structure and function of neurons progressively by various physical, biological, genetic and environmental factors leads to neurodegeneration (Fan et al., 2015). The term neurodegenerative diseases symbolize a range of neurological dysfunction with diverse clinical and pathological symptoms affecting specific areas of brain and halting their normal functions (Przedborski et al., 2003). It is growing as a major concern and public health challenge, as it affects millions of people globally. With increase in the life expectancy of people due to the advancement in medicine and given the correlation between ageing and neurodegeneration, it can be envisaged that the prevalence and incidence of neurodegenerative disorders is increasing progressively. One of the major classifications under neurological disorders is dementia, in which there is disturbance of multiple higher cortical functions including, memory, thinking, orientation, comprehension, calculation, learning capacity, language and judgment (Amit and Vandana, 2013). Dementia is not a unitary condition, but differs vastly among patients reflecting with the regions of brain affected and the nature of biochemical insult. There are several classifications of dementia available based on major clinical symptoms, anatomical regions, cell types affected conformational and biochemical modifications of proteins and cellular and subcellular pathology in which the cell compartments shows deposition of pathological proteins (Kovacs, 2014). Table-1.1 describes the various forms of dementia and their characteristic features.

Table-1.1: Forms of dementia and their characteristic features

<table>
<thead>
<tr>
<th>S.No</th>
<th>Types of dementia</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alzheimer’s disease</td>
<td>Early symptoms include difficulty in remembering names and recent events; apathy and depression. Impaired judgment, disorientation, confusion, behavior changes,</td>
</tr>
</tbody>
</table>
and difficulty in speaking, swallowing, and walking are regarded as later symptoms. Hallmark pathological abnormalities are deposits of the protein fragment amyloid beta (plaques) and neurofibrillary tangles.

| 2 | Vascular dementia | Considered the second most common type of dementia. Impairment is caused by decreased blood flow to parts of the brain, often because of a series of small strokes that block arteries. Symptoms often overlap with those of AD, although memory may not be as seriously affected. |
| 3 | Dementia with Lewy bodies | Pattern of decline may be similar to AD, including problems with memory and judgment as well as behavior changes. Alertness and severity of cognitive symptoms may fluctuate daily. Visual hallucinations, muscle rigidity, and tremors are common. Hallmarks include Lewy bodies (abnormal deposits of the protein alpha-synuclein) that form inside nerve cells in the brain. |
| 4 | Mixed dementia | Characterized by the hallmark abnormalities of more than one type of dementia—most commonly Alzheimer’s combined with vascular dementia, followed by Alzheimer’s with dementia with Lewy bodies, and Alzheimer’s with vascular dementia and dementia with Lewy bodies. |
| 5 | Parkinson’s disease | Many people who have Parkinson’s disease (a disorder that usually involves movement problems) also develop dementia in the later stages of the disease. The hallmark abnormality is the abnormal deposition of the protein alpha-synuclein in the substantia nigra. The aggregates are thought to cause degeneration of the nerve cells that produce dopamine. |
| 6 | Frontotemporal dementia | Includes dementias such as primary progressive aphasia, Pick’s disease, |
corticobasal degeneration and progressive supranuclear palsy. Typical symptoms include changes in personality and behavior and difficulty with language. Nerve cells in the front and side regions of the brain are especially affected. In addition, the upper layers of the cortex typically become soft and spongy and have protein inclusions (usually tau protein or the transactive response DNA-binding protein).

7 Creutzfeldt–Jakob disease

Rapidly fatal disorder that impairs memory and coordination and causes behavior changes. Caused by the misfolding of prion protein throughout the brain. Variant Creutzfeldt–Jakob disease is believed to be caused by consumption of products from cattle affected by mad cow disease.

8 Normal pressure hydrocephalus

Caused by the buildup of fluid in the brain. Symptoms include difficulty in walking, memory loss, and inability to control urination. Can sometimes be corrected with surgical installation of a shunt in the brain to drain excess fluid.

(Adopted from Alzheimer’s Association, 2014)

1.2. Alzheimer’s disease

Alzheimer’s disease is one of the most common types of dementia, which accounts for an estimated 60 to 80% of all dementia cases. It has been predicted that there will be 115 million cases of AD by the year 2050 (Bros et al., 2015). Though the notable risk factor is increasing age, AD is not a part of normal aging. The symptoms of AD in patients develop slowly and deteriorate over time by hindering the ability to do daily tasks (Kandale et al., 2013). The brain of AD patient shows several changes long before any signs of the disease, which last for years and is termed as pre-clinical AD. Once the symptoms start to appear, AD can be separated into three stages (National Institute of Aging, 2016).
Mild Alzheimer’s disease (Early stage):

Despite the normal independent function and daily activities, persons with mild AD struggle with memory lapses like forgetting familiar words or recent conversations, poor judgment and anxiety.

Moderate Alzheimer’s disease (Mid stage):

As the disease develops, problems with memory loss increases like difficulties in remembering the names of the family members and recognizing them. Also AD patients develop other symptoms like increasing confusion and disorientation, delusion, hallucination, mood swings and difficulty in performing spatial tasks.

Severe Alzheimer's disease (Late stage):

In the later stage of the disease, the symptoms worsen and the individuals lose their ability to converse or control their movements, difficulty in eating and as the memory and cognition deteriorate progressively, the individuals need the help of care takers.

1.3. Risk factors

The following are some of the potential risk factors contributing to AD (National Institute of Aging, 2016).

- Ageing
- Family history
- Factors increasing vascular risk (diabetes, high cholesterol and high blood pressure)
- Educational level
- Race and ethnicity
- Head trauma
- Gender
- Dietary factors
1.4. Genetics of AD

In the context of genetics, AD is regarded as a heterogenous disorder with both familial and sporadic form. Familial AD (FAD) or early-onset AD (EOAD) is an autosomal dominant form that occurs before the age of 65 (Blennow et al., 2006). FAD is comparatively rare with frequency of incidence of less than 0.1%, and occurs mainly due to mutations in the genes APP, Presenilin-1 and 2 (PSEN-1, PSEN-2) (Goate et al., 1991; Sherrington et al., 1995; Levy-Lahad et al., 1995). Sporadic Alzheimer’s disease (SAD) or late-onset AD (LOAD) is considered to be multifactorial and studies have indicated that the heritability of this form is as high as 80% (Gatz et al., 2006). For many years, APOE ε4 allele was believed to be the only gene involved in LOAD, however due to the advancement in technology, several other risk genes involved in cellular pathways like maintenance of synaptic plasticity, immune function, lipid metabolism have been identified. Table-1.2 represents the list of genes involved in EOAD and LOAD.

Table-1.2: List of genes involved in early and late onset Alzheimer’s disease

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Inheritance pattern</th>
<th>Proposed function</th>
<th>Implicated pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>APP</td>
<td>19</td>
<td>Autosomal dominant or recessive</td>
<td>Substrate of amyloid β peptide, cell signaling events, tau phosphorylation, GSK-3β activation</td>
<td>Amyloid β pathway, endocytotic receptor trafficking, tau pathway</td>
</tr>
<tr>
<td>PSEN1</td>
<td>14</td>
<td>Autosomal dominant</td>
<td>γ-secretase activity, transmembrane protein processing, intracellular signaling</td>
<td>Amyloid β pathway, synaptic plasticity, neuronal survival</td>
</tr>
<tr>
<td>PSEN2</td>
<td>2</td>
<td>Autosomal dominant</td>
<td>γ-secretase activity, transmembrane protein processing, intracellular signaling</td>
<td>Amyloid β pathway, synaptic plasticity, neuronal survival</td>
</tr>
<tr>
<td><strong>Gene</strong></td>
<td><strong>Risk Category</strong></td>
<td><strong>Function</strong></td>
<td><strong>Pathways Involvement</strong></td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>-------------------</td>
<td>--------------</td>
<td>-------------------------</td>
<td></td>
</tr>
<tr>
<td><strong>APOE</strong></td>
<td>19 Risk gene</td>
<td>Self dominant aggregation and clearance, intracellular signaling through LRP</td>
<td>Lipid transport and metabolism, amyloid β pathway, synaptic plasticity, neuro-inflammation</td>
<td></td>
</tr>
<tr>
<td><strong>CLU</strong></td>
<td>8</td>
<td>Risk gene Molecular chaperone, synapse turnover, amyloid β aggregation, clearance, and toxicity</td>
<td>Amyloid β pathway, lipid metabolism, immune system, inflammation, apoptosis</td>
<td></td>
</tr>
<tr>
<td><strong>CR1</strong></td>
<td>1</td>
<td>Risk gene Complement system activation, amyloid β clearance</td>
<td>Immune system, amyloid β pathway</td>
<td></td>
</tr>
<tr>
<td><strong>PICALM</strong></td>
<td>11</td>
<td>Risk gene Clathrin-mediated endocytosis</td>
<td>Synaptic cell functioning, amyloid β toxic effects, processing of APP</td>
<td></td>
</tr>
<tr>
<td><strong>BIN1</strong></td>
<td>2</td>
<td>Risk gene Synaptic vesicle endocytosis, formation of tubular membrane structures</td>
<td>Synaptic cell functioning, caspase-independent apoptosis</td>
<td></td>
</tr>
<tr>
<td><strong>EPHA1</strong></td>
<td>7</td>
<td>Risk gene Synaptic development and plasticity</td>
<td>Immune system</td>
<td></td>
</tr>
<tr>
<td><strong>ABCA7</strong></td>
<td>19</td>
<td>Riskgene Transportation of substrates across cell membranes</td>
<td>Cholesterol metabolism, immune system, processing of APP</td>
<td></td>
</tr>
<tr>
<td><strong>MS4A4A, MS4A6E</strong></td>
<td>11</td>
<td>Risk gene No known functions</td>
<td>Immune system (MS4A2), cell surface signaling</td>
<td></td>
</tr>
<tr>
<td><strong>CD33</strong></td>
<td>19</td>
<td>Risk gene Clathrin-mediated endocytosis</td>
<td>Immune system, synaptic cell functioning</td>
<td></td>
</tr>
</tbody>
</table>
1.5. Pathological hallmarks of AD

Alteration in the cholinergic system, deposition of β-amyloid (Aβ) plaques, formation of neurofibrillary tangles (NFT), glutamate excitotoxicity, inflammation, dystrophic neuritis and neuropil threads has been identified to be the major pathological hallmarks of AD (Woodhouse et al., 2005).

1.5.1. Cholinergic hypothesis

Alteration in the cholinergic system has been believed to contribute to the initial progression of AD, which is based on the finding of cholinergic disruption in the brain of AD patients and experimental animals. During the progression of AD, many different types of neurons deteriorate, with profound loss of forebrain cholinergic neurons, accompanied by a progressive decline in the level of the neurotransmitter acetylcholine (ACh) (Mufson et al., 2008). Cholinergic neurons, by the action of the enzyme choline acetyl transferase (ChAT), synthesizes large amount of ACh, which are released in the synapse during neurotransmission. ACh signals through metabotropic muscarinic receptors (mAChRs) and ionotropic nicotinic receptors (nAChRs) and is found to maintain synaptic plasticity (Picciotto et al., 2012). Under pathological conditions, a sharp decline in the level of ACh is found due to its hydrolysis mainly by acetylcholinesterase (AChE) and partly by butyrylcholinesterase enzymes (BChE) (Hebert et al., 1995). Under normal conditions these two enzymes maintains the level of ACh in the synaptic cleft, however during AD progression AChE is affected, which vigorously cleaves ACh leading to cognitive deficit. Further, there exists a cross link between cholinergic transmission and amyloid beta pathway (Aβ), and the initiation of either pathway influences the other (Kar et al., 2004).
Fig-1.1: Mechanism of cholinergic dysfunction in neurons

1.5.2. Amyloid beta hypothesis

Amyloid hypothesis proposes the formation and deposition of the protein amyloid beta (Aβ) in the brain of AD patients leading to deterioration of neurons and memory loss. The Aβ peptide is generated from amyloid precursor protein (APP) which is an evolutionarily conserved family of type I transmembrane glycoprotein involved in many biological processes (Muller-Hill and Beyreuther, 1989). APP has been reported to have a role in neurite outgrowth and synaptogenesis, neuronal protein trafficking,
transmembrane signal transduction, cell adhesion and calcium metabolism (Zhou et al., 2011). APP undergoes two types of processing – amyloidogenic and non-amyloidogenic based on the secretases acting upon it.

![Fig-1.2: Amyloidogenic pathway and the progression of AD](image)

In the non-amyloidogenic pathway, APP is initially cleaved by α-secretase within the Aβ domain to form soluble sAPPα and C-terminal fragment (αCTF). The subsequent cleavage of CTF by γ-secretases results in the release of P3 peptide and amyloid precursor protein intracellular domain (AICD). However in non-amyloidogenic pathway, APP is cleaved by β-secretase to form sAPPβ and βCTF. βCTF is further cleaved by γ-secretase yielding Aβ peptide and AICD (Dong et al., 2012). Though β-secretase cleaves APP specifically indicating its site-specific activity, the cleavage by γ-secretase is
imprecise resulting in the heterogeneity of peptide populations of varying length, including predominantly formed Aβ1-40/42, and several short peptide forms of 16-22, 22-35 and 25-35 in AD patients (Selkoe, 2001). The monomeric Aβ generated aggregate within them to form oligomer and fibrillar structures and finally deposits in the brain as senile plaques. Aβ induces neurotoxicity in both *in vitro* and *in vivo* systems through oxidative stress mechanisms, which damages cellular lipids, proteins, DNA and eventually lead to neuronal death (Chen et al., 2012). Though in the normal brain a balance between Aβ production and elimination is always maintained by efflux across blood brain barrier (BBB) through receptor for advanced glycation end products (RAGE), low-density lipoprotein receptor-related protein (LRP) and enzymatic degradation by neprilysin, these mechanisms are severely impaired in AD patients contributing to the severity of the disease (Deane et al., 2009).

### 1.5.3. *Tau hypothesis*

Tau is an important protein located in the axonal compartment that helps in the microtubule stabilization by promoting the assembly of tubulin to microtubules and neurite outgrowth (Mandelkow and Mandelkow, 2012).

![Fig-1.3: Tau phosphorylation, destabilization of microtubule assembly in AD](image)

Being a phosphoprotein, the biological activity of tau is regulated by the extent of phosphorylation. Normally tau is found predominantly in hyperphosphorylated form in the neurons during the early developmental stages due to the huge demand for neuroplastic changes (Ribaut-Barassin et al., 2005). However in adults, dephosphorylated tau is present in abundance, which carries out the function of...
maintaining cytoskeletal homeostasis, both structurally and functionally \cite{Lovestone1994}. Phosphorylation of tau is under the control of several kinases and phosphatases, the major enzyme being glycogen synthase kinase-3β (GSK-3β). The anomalous hyperphosphorylation makes the tau protein to resist against proteolysis by proteases, which in turn impairs the capacity of tau to bind to tubulin, causing deterioration of the microtubule structure, destruction of axonal transport and synaptic metabolism \cite{Mietelska-Porowska2014}. These changes ultimately result in the disintegration of the cytoskeleton and neuronal death.

1.5.4. Glutamate excitotoxicity

![Fig-1.4: Mechanism of glutamate excitotoxicity and the progression of AD](image)

Glutamate is an excitatory neurotransmitter involved mainly in maintaining synaptic plasticity, memory and learning \cite{Mattson2008}. It is synthesized through several metabolic pathways and its availability at appropriate levels for cellular signaling
is strictly under the control of glutamate uptake and recycling mechanisms (Danbolt, 2001). The excitatory neurotransmission occurs chiefly through ligand gated ionotropic glutamate receptors (iGluRs) mainly via N-methyl-d-aspartate receptor (NMDAR) (Riedel et al., 2003). Blockade of NMDAR functions results in neuronal apoptosis and degeneration while activation by glutamate triggers Ca\(^{2+}\) dependent transcription factors and leads to the expression of several genes involved in cell survival under normal conditions (Monti and Contestabile, 2000). However, stimulation of excessive signaling results in glutamate excitotoxicity, in which neuronal cells are damaged and degenerated. Increasing evidences suggests that the toxic effects of glutamate excitotoxicity occur due to the prolonged influx of Ca\(^{2+}\) ions into the postsynaptic neuron (Arundine et al., 2003).

As the level of Ca\(^{2+}\) ions builds up inside the cell, it gradually leads to loss of synaptic plasticity and cell death (Gleichmann and Mattson, 2011). Therefore, maintaining the physiological level of glutamate at the synapse is essential for preventing excitotoxicity and sustaining normal signal transmission. Removal of excess glutamate is accomplished largely by transporter-mediated uptake by glutamate transporters (GLTs) (Foran and Trotti, 2009). Studies indicate that glutamate uptake is highly reduced after A\(\beta\) generation and deposition, due to which excitotoxic condition prevails (Fernández-Tomé et al., 2004).

### 1.5.5. Oxidative stress

Oxidative stress is a complex process caused by an imbalance between the formation of free radicals and the dysfunction in the antioxidant system. Brain is constantly vulnerable to oxidative stress because of its enrichment with polyunsaturated fatty acids (PUFA) that can be easily oxidized due to the high demand of oxygen (Wang and Michaelis, 2010). Mitochondria are the main source of oxidative stress, wherein the free radicals are produced during the mitochondrial respiratory chain. It has been identified that the dysfunctional mitochondria produces ATP less efficiently while generating ROS predominantly, leading to oxidative imbalance (Gibson et al., 1998). Oxidative stress in the neurons injure the brain through several cross-linked mechanisms, with oxidation of lipids, proteins, nucleic acids and the formation of toxic by-products, intracellular Ca\(^{2+}\) increase, release of excitatory molecules, inflammation and modulation.
of several other signaling pathways (Uttara et al., 2009). These factors contribute to the neuronal dysfunction and eventually cell death. To combat the oxidative stress during AD, the neurons modulates its endogenous antioxidant ability through the activation of several transcription factors including, Nuclear factor (erythroid-derived 2)-like 2 (Nrf-2), which controls antioxidant response element (ARE) genes (Nguyen et al., 2009). Under normal condition the half life of expressed Nrf-2 is very less and is degraded by ubiquitin-proteosome system. Whereas in AD, Nrf-2 translocates to the nucleus and induce the transcription of ARE genes including catalase, superoxide dismutase, thioredoxin, NAD(P)H quinone oxidoreducase-1, hemeoxygenase-1, and glutathione, which are involved in cytoprotection from various oxidative damage mediated cell injuries (Itoh et al., 2003; Joshi and Johnson, 2012). Numerous reports have implicated the role of oxidative stress in Aβ mediated neurotoxicity both in vitro and in vivo. Aβ treatment to cell models has showed a significant increase in hydrogen peroxide, lipid peroxide, protein carbonyls, ROS and RNS (Suganthy and Devi, 2016). Similar concomitant results are seen in mice administered with Aβ intracerebroventrally and transgenic mice expressing Aβ (Suganthy et al., 2016; Resende et al., 2008). It has been identified that during AD there is deterioration in the antioxidant defense system causing oxidative stress. Also, oxidative stress enhances the production of Aβ, which could be due to the reduction in the α-secretase activity and increased expression and activation of β,γ-secretases during stress condition (Oda et al., 2010). Further, prolonged oxidative stress initiate the activation of several cell signaling pathways including JNK, the activation of which further enhances β-secretase expression and activation leading to the generation of Aβ and degeneration of neurons (Fukumoto et al., 2002). Increased production of Aβ also causes mitochondrial dysfunction and affects mitochondrial biogenesis, a process involved in maintaining a healthy mitochondria as well as responding to stress stimuli through PGC-1α-NRFTFAM pathway (Sheng et al., 2012). In Aβ induced pathogenesis the expression of Pgc-1α is highly downregulated, correlating with the impairment of mitochondrial biogenesis (Zhu et al., 2012). In addition, Aβ mediated oxidative stress acts along with the mitochondrial matrix protein cyclophilin-D (Cyp-D) and causes mitochondrial membrane permeability transition pore
(mPTP) resulting in the loss of mitochondrial membrane potential, release of apoptotic factors and eventually cell death (Rao et al., 2014).

1.5.6. Inflammation

Inflammation is a process that occurs to eliminate cell injury and necrotic cells that are formed from the original insult and helps in restoring tissue health. Inflammation becomes a chronic condition when the tissue health is not restored and leads to the deterioration of surrounding tissues (Heppner et al., 2015). Though neuroinflammation protects and defends the CNS from infection, injury and damage, it is considered as one of the underlying causes of AD. Growing number of evidences suggests that Aβ deposits plays important roles in the initiation of neuroinflammation in AD by the activation of astrocytes and microglia (Zhang et al., 2011). These cells activates NF-κB pathway that subsequently leads to the secretion of several proinflammatory factors including, inducible nitric oxide synthase (iNOS), interleukin-1β (IL-1β) and cyclooxygenase-2 (COX-2), which eventually contributes to neuronal damage and death (Choi et al., 2009). Apart from the direct neurotoxic effect, studies indicate that activated inflammatory cells can help in Aβ deposition (Guo et al., 2002). Further, the secreted cytokines upregulates the expression of β-secretase and induces its enzymatic activity resulting in increased production of Aβ (Sastre et al., 2003).

1.5.7. Neurovascular dysfunction

In the CNS, the delivery of essential nutrients and the removal of metabolic waste products are efficiently done by the blood vessels circulating in the brain (Upadhyay, 2014). For brain health and survival, regulation of proper cerebral blood flow (CBF) is much essential and is maintained by neurovascular units (NVU), that consist of blood brain barrier (BBB), vascular cells, glial cells and neurons (ElAli et al., 2014). The BBB is much unique and allows only selected molecules in and out of the membrane. Studies from the past decade indicate that abnormalities in neurovascular dysfunction contributes to the onset and progression of the pathological processes associated with AD, including microvascular reductions, BBB breakdown and failure in the clearance of Aβ from the brain (Zlokovic, 2011). Degeneration of brain endothelial wall occurs during AD, which
leads to the ionic imbalance and Aβ deposition on the outer side of basement membrane (Kalaria, 2010; Viswanathan and Greenberg, 2011). These abnormal events provoke neuroinflammation, which in turn restrains the CBF and amplifies cellular stress contributing to cognitive dysfunction.

1.5.8. Metal ions

The dynamic nature of metal ions (copper, zinc and iron) is vital for many of the physiological functions including enzyme catalysis, maintaining structural stability, transport of oxygen and signaling (Duce and Bush, 2010). Several metal ion transporters like ATP7A, Ctr1, ZnT and ferritin helps in the transport of these metals across the BBB and maintains the normal brain functions. Under normal conditions, the Zn transporter (ZnT3) helps in the release of Zn$^{2+}$ from the presynaptic neuron, as a result of which, the concentration of Zn$^{2+}$ in the synapse increases, which further activates the Cu$^{2+}$ transporter ATP7A by binding to NMDAR. Activated ATP7A further releases Cu$^{2+}$ and there exists a pool of Cu$^{2+}$ and Zn$^{2+}$ ions in the synaptic region (Sensi et al., 2009). These metal ions are uptaken by postsynaptic neuron against concentration gradient by transporters leads to cellular signaling. Metal ions cause degeneration of neurons due to their toxic exposure, or a collapse in the way that compartmentalize and regulate their homeostasis (Wong et al., 2014). Dysregulation of metal ion homeostasis initiates the cascade of pathological processes in the brain leading to neurodegeneration. Metal ions assist in the generation of Aβ by interfering and modulating secretase-dependent APP processing. The α and β- secretase enzymes have Zn$^{2+}$ and Cu$^{2+}$ binding sites and their catalytic activity depends on these metals (Cross et al., 2002; Angeletti et al., 2005). Zinc binding to α-secretase inhibits its enzymatic activity whereas, copper binding to β-secretase interferes and inhibits the antioxidant activity of superoxide dismutase (SOD) (Buckley et al., 2005; Angeletti et al., 2005). Also during AD, the reuptake of Zn$^{2+}$ from the synapse is reduced after neuronal signaling, which leads to the prolonged activation of NMDAR and the release of more Cu$^{2+}$ ions into the synapse (Jakob-Roetne and Jacobsen, 2009). The metal ions have a greater tendency to interact with the generated Aβ. It has been reported that the Aβ contain specific binding sites for metal ions, that facilitate the peptide to coordinate with Cu$^{2+}$ and Zn$^{2+}$ and promote their aggregation.
(Miller et al., 2010; Syme et al., 2004). As the aggregation proceeds, the metal ions are sequestered inside the Aβ fibrils making them deficient in the other regions of neurons steering to miscompartmentalization, inducing oxidative stress and neuronal apoptosis (Savelieff et al., 2013).

1.5.9. Cholesterol

Brain is the most cholesterol-rich organ in the body and cholesterol plays a vital function in neuronal development, regulation of signaling pathways and preservation of synaptic plasticity. Cholesterol offers rigidity to lipid bilayers and plays an instrumental role in the function of several membrane associated proteins (Maxfield and Tabas, 2005). The region of membrane rich in cholesterol is termed as lipid raft and a range of proteins including APP and β-secretase were found to be present in this region (Hattori et al., 2006). Lipid rafts are highly responsive to distorted cholesterol metabolism, which in turn results in lipid raft destabilization (Marquer et al., 2011). Cholesterol homeostasis is synchronized by the balance between its synthesis, uptake, breakdown and release. The genes Apolipoprotein E (ApoE) and ATP-binding cassette transporter-1 (ABCA-1) along with the control of the Liver X receptors (LXR) acts as major regulators of cholesterol by switching on the efflux and excretion mechanisms (Fan et al., 2009). During AD progression, the level of genes in maintaining cholesterol is highly altered leading to the accumulation of cholesterol in the membrane. Impaired cholesterol metabolism induces the APP processing through amyloidogenic pathway further leading to the generation of Aβ peptides (Refolo et al., 2000).

1.6. Natural products as therapeutics against AD

Plant based medicines for the livelihood of human race has been extensively used and documented. The first record of traditional medicinal systems was found in Mesopotamia that dates back to 2100 BC, reports around 1000 plant derived substances used in day-to-day life (Cragg and Newman, 2005; Fakim, 2006). Similarly, Egyptian pharmaceutical practices have been recorded in Ebers papyrus dating from 1500 BC, which documents around 700 drugs (Nakanishi, 1999; Brahmachari, 2012). Also, the Chinese material medica documented in Wu Shi Er Bing Fang, Shennong Herbal, Tang
Herbal and the Indian Ayurvedic system documented in Charaka and Sushruta Samhitas also indicates the importance of natural plant based products (Newman and Cragg, 2001; Cragg and Newman, 2013; Spainhour, 2005). The plant-based medicinal system is still in play in the modern healthcare, with 80% of the human population depending on traditional medicine according to the report by World Health Organization (Campbell-Tofte et al., 2012). More than half of all the pharmaceuticals currently in existence have been identified from plants, which might be due to the diversity in chemical structures and remarkable biological activities of the compounds. Many of the biologically active natural products became important not only for their use directly as therapeutic agents or prototype lead compound for the development of new drugs, but also as biochemical probes to unravel the principles of human pharmacology (Clark, 1996). Starting from the isolation and commercialization of morphine (Papaver somniferum); digitoxin (Digitalis purpurea), quinine (Cinchona succirubra), pilocarpine (Pilocarpus jaborandi), taxol (Taxus brevifolia) are some of the noteworthy leads identified from plants approved by Food and Drug Administration for the treatment of various ailments (Gurnani et al., 2014).

![Graph](image)

**Fig-1.5: Schematic representation of approved drugs between 1984-2014 (Newman and Cragg, 2014)**

According to available reports, in the years spanning 1981-2014, 1562 natural product based drugs have been approved for a range of complications including,
infectious diseases, cancer, neurological disorders, and cardiovascular diseases (Newman and Cragg, 2014). The plant kingdom comprising of 3 to 4 million higher species is one of the major sources of new chemical entities for potent pharmaceutical ingredients and lead molecules.

Plants have often been used to treat CNS disorders and retain mental ability for centuries. *Huperzia seratia* is being traditionally used in China to treat schizophrenia and its active compound Huperzine-A has been approved by FDA as an anti-AD drug (Ma et al., 2007). The prescribed anti-cholinesterase drug for AD, Galantamine has been identified from the plants *Galanthus nivalis, Lycoris radiate, Lycoris aruea*, and *Lycoris squamigeric* belonging to Amaryllidaceae family, which are being used as herbal medicines by traditional medicinal practitioners in China (Tsvetkoval et al., 2016). *Ginkgo biloba* is a noteworthy plant used in traditional Chinese medicine for the treatment of various neurological disorders. Apart from them *Panax ginseng, Uncaria rhynchophylla, Polygala tenuifolia, Salvia miltiorrhiza, Salvia officinalis, Radix polygalae* are used as memory enhancers in various Chinese medicinal formulations (Wu et al., 2011). In the context of Indian Ayurvedic system of medicine, around 85 plant formulations for CNS disorders are categorized into neuroleptics, hypnotics, anxiolytics, memory enhancers and so on (Kumar, 2006). The following table indicates some of the important plants used in Ayurveda for boosting the memory and cognitive function.

**Table-1.3: List of common plants used in Ayurveda as memory enhancers**

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ashwagandha</td>
<td><em>Withania somnifera</em></td>
<td>Nervine tonic, aphrodisiac, and adaptogen (Singh et al., 2011)</td>
</tr>
<tr>
<td>Brahmi</td>
<td><em>Bacopa monnieri</em></td>
<td>Nervine tonic, therapeutic agent against epilepsy, memory enhancer (Aguiar and Borowski, 2013)</td>
</tr>
<tr>
<td>Shankhpushpi</td>
<td><em>Convolvulus pluricaulis</em></td>
<td>Nervine tonic for improvement of memory and cognitive function (Rao et al., 2012)</td>
</tr>
</tbody>
</table>
**Gotu kola** *Centella asiatica* Rejuvenating herb for nerve and brain cells, memory enhancer *(Gohil et al., 2010)*

**Jyotishmati** *Celastrus paniculatus* Memory enhancer and improves cognitive function *(Bhagya et al., 2016)*

**Jatamansi** *Nardostachys jatamansi* Memory enhancer *(Kulkarni et al., 2012)*

Recent scientific evidences under both *in vitro* and *in vivo* conditions substantiate the use of these plants against AD by acting as inhibitors of cholinesterase enzyme, Aβ aggregation, β-secretase enzyme and improving the cognitive deficits.

### 1.7. *Grewia tiliaefolia*

*Grewia* genus, comprising around 150 species, belongs to the family Tiliaceae and is distributed widely in tropical and sub-tropical regions *(Ullah et al. 2012)*. Approximately 40 species of this genus are reported to be present in India *(Hiwale 2015)*, many of which have medicinal properties. One among them is *Grewia tiliaefolia* Vahl. *G. tiliaefolia*, which is a subtropical tree found in Western and Eastern Ghats of India *(Ahamed et al., 2007)*. This plant has been used in traditional medicine in India by various tribes and traditional medical practitioners. *G. tiliaefolia* has been used as astringent, expectorant, antipruritic, and aphrodisiac by the traditional medicinal practitioners and local tribes *(Selvam et al. 2010)*. It is also used for the treatment of rhinopathy, skin diseases and general debility. The stem bark is used to heal wounds, ulcer, dysentery, jaundice and urinary infections *(Kirthikar and Basu, 1975)*. The plant is also reported to show antioxidant, antiproliferative and hepatoprotective activity *(Badami et al., 2003; Khadeer Ahamed et al., 2010; Selvam et al., 2010)*. Lupeol isolated from the stem bark of *G. tiliaefolia* has been reported to show cytotoxic activity against Vero, Hep-2 and B16F10 cell lines. Methanol extract of bark has potent antiproliferative activity against MCF7, A549, and HepG-2 cell lines *(Selvam et al., 2010)*. Two γ-lactones, d-erythro 2- hexenoic acid γ-lactone and gulonic acid γ-lactone
isolated from the stem bark of *G. tiliaefolia* has shown potent hepatoprotective activity in CCl₄ intoxicated rats (*Khadeer-Ahamed et al. 2010*). Leaves of this plant are reported to be consumed as vegetables (*Patil and Patil, 2006*).

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**Fig-1.6: Grewia tiliaefolia**

**Taxonomy of *Grewia tiliaefolia***

- **Kingdom**: Plantae
- **Phylum**: Magnoliophyta
- **Class**: Magnoliopsida
- **Order**: Malvales
- **Family**: Tiliaeaece
- **Genus**: *Grewia*
- **Species**: *tiliaefolia*
In the present study, the neuroprotective potential of the terrestrial plant *Grewia tiliaefolia* and its active constituent vitexin against Alzheimer’s disease was evaluated by *in vitro* and *in vivo* systems.

The objectives of the study are:

- **Screening of *G. tiliaefolia* for antioxidant, anticholinesterase and anti-aggregation property and identification of active principle**
- **Evaluation of safety profile of *G. tiliaefolia* and vitexin**
- **In vitro evaluation of neuroprotective potential of *G. tiliaefolia* and vitexin against Aβ_{25-35} and glutamate induced toxicity in Neuro-2a cell line**
- **Evaluation of neuroprotective effect of *G. tiliaefolia* in transgenic *Caenorhabditis elegans* model of Alzheimer’s disease**