CHAPTER 1: LITERATURE REVIEW

1.1 Neurodegeneration

Neurodegenerative disease is a term applied to a variety of conditions which result from a chronic breakdown and deterioration of neurons, particularly those of the central nervous system (CNS). These neurons may accumulate aggregated proteins which cause dysfunction (Houghton and Howes, 2005). Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis and spongiform encephalopathy are some of the common forms of neurodegenerative diseases (Chiba et al., 2007). These diseases are commonly found in elderly people and in advanced industrialized societies where life expectancy is long. They are a major cause of morbidity, mortality and impose severe strains on the social welfare systems, and as a result are gaining increased recognition by the World Health Organisation (WHO) (Houghton and Howes, 2005).

Neurodegenerative diseases are characterized by a gradual onset of progressive symptoms including loss of memory and tremor, difficulty in learning or retaining information, inability to handle complex tasks, impaired spatial orientation and abilities, language deficits and behavioral changes. These symptoms have been recognized as a feature of increasing age for a long time and are acknowledged in many traditional medical systems. However, it is only recently that they have been recognized and received attention from mainstream medicine as distinctive diseases (Houghton and Howes, 2005).

1.2 Alzheimer's disease

In 1906, during the 37th Conference of German psychiatrists in Tübigen, the Bavarian neuropsychiatrist, Alois Alzheimer described for the first time the symptoms of "a particular disease of the cerebral cortex", characterized by a gradual and irreversible degeneration of intellectual functions such as memory, orientation, judgement, language and the capacity to acquire new knowledge (Hostettmann et al., 2006). This disease became known as Alzheimer's disease (AD).

AD is the most common age-related neurodegenerative disorder and is also the most common cause of progressive mental deterioration in persons aged 65 or older (Shah et al., 2008). The clinical symptoms result from the deterioration of selective cognitive domains, particularly those related to memory. Memory decline initially manifests as a loss of episodic memory, which is considered as a subcategory of declarative memory. The dysfunction in episodic memory impedes recollection of recent events including autobiographical activities (LaFerla et al., 2007). Patients with AD also suffer from marked reduction of cholinergic neuronal function in those areas of the brain responsible for higher mental functions which partially accounts for the impairments in activities of daily life (Brodaty et al., 1993; Coyle and Kershaw, 2001). The estimated global dementia prevalence in people aged over 60 is approximately 3.9% with regional prevalence being 1.6% in Africa, 3.9% in Eastern Europe, 4.0% in China, 4.6% in Latin America, 5.4% in Western Europe and 6.4% in North America (Qiu et al., 2009). Rates of increase are not uniform; numbers in developed countries are estimated to double between 2001 and 2040, and increase by more than 300% in India, China, south Asia and western Pacific (Ferri et al., 2005).

Hence, the global trend in the phenomenon of population aging has dramatic consequences for public health, healthcare financing and delivery systems in the world and especially in developing countries (Qiu et al., 2007).

1.3 Pathology of Alzheimer's disease

The pathology of AD is complex and three main pathogenic pathways are believed to contribute to the progression of the disease; cholinergic deficit, senile plaque/amyloid-β peptide deposition and oxidative stress (Small and Mayeux, 2005). These three pathogenic pathways are discussed below:

i. Cholinergic hypothesis

In the late 1970s, White and his colleagues discovered that the brains of patients with neurodegenerative diseases including AD were deficient in acetylcholine (ACh) (White et al., 1977), and this became a consistent report (Hollander et al., 1986). As a result, the cholinergic hypothesis was developed, which essentially states that a loss of cholinergic function in the central nervous system contributes significantly to the cognitive decline associated with advanced age (Bartus, 2000; Terry and Buccafusco, 2003; Heinrich and Teoh, 2004) (Figure 1.1).

ACh is critical for an adequately functioning memory. ACh is stored in the nerve terminals in structures called vesicles. The contents of these vesicles are released from the nerve endings when the nerve terminal is depolarized, and the ACh released enters into the synapse and binds to the receptor. The ACh released has a very short half-life due to the presence of large amounts of acetylcholinesterase (AChE), an enzyme which hydrolyses the ester bond in the molecule, thus leading to loss of stimulatory activity. Current therapeutic strategies for the symptomatic

treatment of AD and other related disorders such as vascular dementia, dementia with Lewy bodies, senile dementia and Parkinson's disease are aimed at enhancing the associated cholinergic deficit by inhibiting AChE (Rösler et al., 1999; Brenner, 2000; Rahman and Choudhary, 2001), resulting in a boost in endogenous level of ACh in the brain and an improvement of cognitive function (Elufioye et al., 2010).

ii. Amyloid Cascade Hypothesis

Postmortem examinations of the brains of people with AD show characteristic structures termed amyloid plaques and neurofibrillary tangles. Amyloid-β precursor protein (APP) is a type-1 transmembrane protein of unknown function. It is cleaved by two proteases to form amyloid-β (Aβ). Aβ is also secreted by mammalian cells and occurs normally in plasma and cerebrospinal fluid. The Amyloid Cascade Hypothesis, suggests that improper metabolism of APP is the initiating event in AD pathogenesis, leading to the aggregation of Aβ (Shah et al., 2008). It has also been proposed that Aβ peptide deposits, or even the partially aggregated soluble form are responsible for triggering a neurotoxic cascade of events which ultimately results in neurodegeneration (Castro et al., 2002; Dastmalchi et al., 2007). The Aβ peptide is a prime target for developing therapies for neurodegenerative diseases including AD (Jayaprakasam et al., 2010) and so, modulating the chain of events starting from the production of Aβ peptide fragments from APP to its deposition in the form of extracellular plaques are believed to be possible approaches towards the treatment of AD (Dastmalchi et al., 2007).

Recent evidence indicates that AChE may be involved in the pathogenesis of plaques. AChE appears to enhance the aggregation of the Aβ peptide, a major event in the process of plaque formation (Inestrosa et al., 1996). It also increases the toxicity of Aβ (Reyes et al., 2004).

Administration of AChE inhibitors has been shown to interfere with Aβ production, most likely by reducing the levels of the APP from which Aβ is cleaved (Lahiri et al., 1994; Greig et al., 2001; Shaw et al., 2001). This evidence gives rise to the fact that in addition to amelioration of cholinergic deficit, AChE inhibition may also retard the disease process as it may reduce the toxicity of Aβ and subsequently reduce plaque formation (Eskander et al., 2005).

iii. Oxidative stress and antioxidant activity

Aging in most species studied to date is accompanied by the progressive accumulation of oxidative damage in many tissues (Head, 2009). Oxidative stress is the imbalance between cellular production of free radical species and the ability of the cells to eliminate them employing endogenous antioxidant defense mechanisms (Sanvicens et al., 2006). Oxidative stress causes cellular damage and subsequent cell death especially in organs such as the brain. The brain in particular is highly vulnerable to oxidative damage as it consumes about 20% of the body's total oxygen, has a high content of polyunsaturated fatty acids and lower levels of endogenous antioxidant activity relative to other tissues (Halliwell and Gutteridge, 1985; Floyd and Hensley, 2002; Shulman et al., 2004). Oxidative stress is involved in the propagation of cellular injury that leads to neuropathology in several neurodegenerative diseases. It is intimately linked with an integrated series of cellular phenomena, which all seem to contribute to neuronal demise (Andersen, 2004). Several lines of evidence have also suggested that Aβ-induced oxidative stress plays an important role in the pathogenesis or progression of AD (Butterfield et al., 2001). Aβ induces oxidative stress (Hensley et al., 1994), and oxidative stress promotes the production of

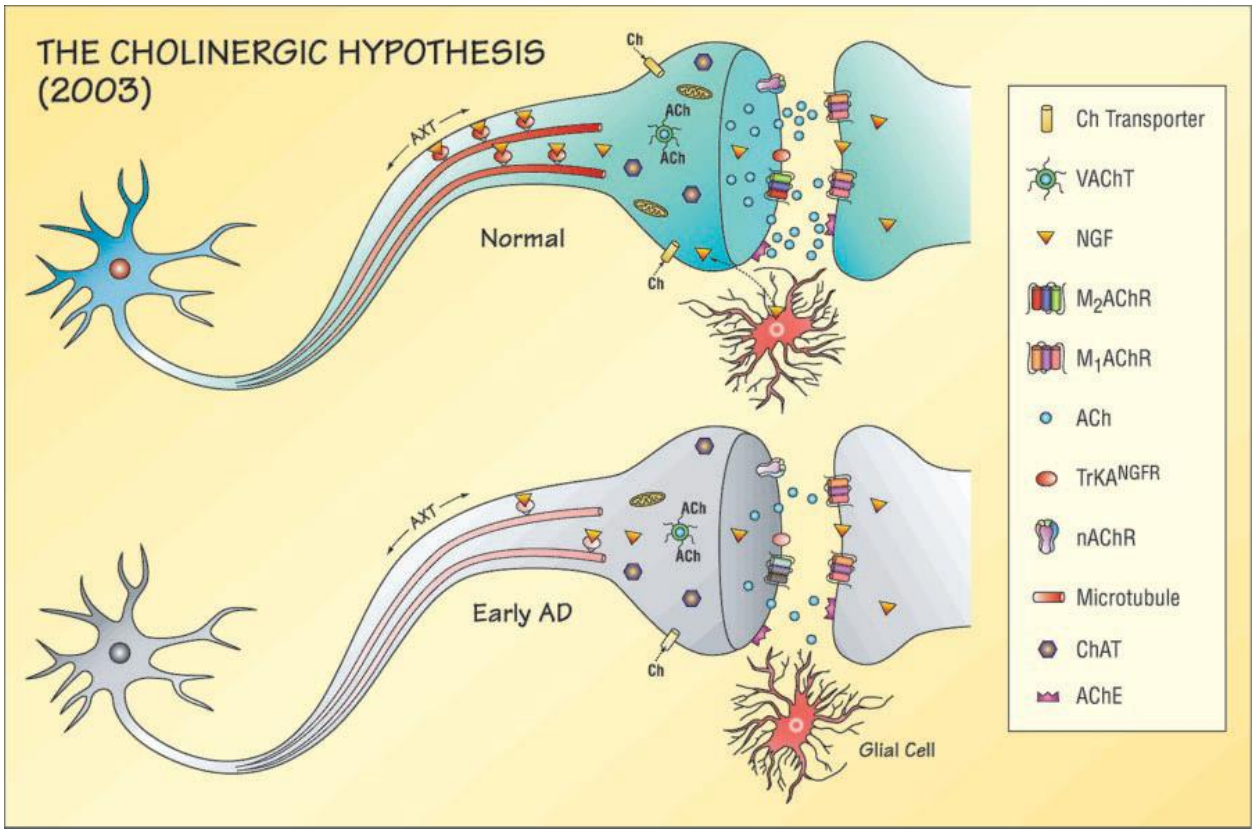

Figure 1.1 Schematic representation of the known and proposed changes in cholinergic neurons that occur in the aged and early AD brain compared with healthy young neurons. Alterations in high-affinity choline uptake, impaired acetylcholine release, deficits in the expression of nicotinic and muscarinic receptors, dysfunctional neurotrophin support (i.e., NGF receptors), and deficits in axonal transport are represented in the early AD neuron either by a decrease in the number of symbols presented or by reduced color intensity (From Terry and Buccafusco, 2003).

Aβ (Tamagno et al., 2008). The relevance of oxidative stress to the progression of many neurodegenerative disorders has generated an interest in the potential use of radical scavengers and their natural biological counterparts for protecting cells and tissues from oxidative damage (Behl, 2000; Chen et al., 2003; Prokai et al., 2003; Deng et al., 2004). The radical scavengers help to scavenge free radicals before they can bring about their deleterious effects (Dastmalchi et al., 2007). *In vitro* studies have demonstrated the neuroprotective activities of antioxidants (Sanvicens et al., 2006), and data from clinical trials appear very promising and have shown potential benefit from treatment with radical scavengers in pathologies such as AD (Grundman, 2000; Doraiswamy, 2002; Shults, 2003; Nagayama et al., 2004). The deleterious effect of oxidative stress and reactive oxygen species in neurodegeneration is provided in Figure 1.2.

It is thus evident that the cholinergic hypothesis, amyloid cascade hypothesis and oxidative stress are three very important pathologic pathways which contribute to the progression of several neurodegenerative diseases. These three pathways occur simultaneously and form a chain reaction leading to neurodegeneration (Figure 1.3). These pathways are the target for drug development.

1.4 Current therapeutic approaches for AD

AD sufferers have a longer life expectancy if they are not institutionalized and if properly cared for by spouses or family members in a known environment. It is therefore ideal to delay institutionalization for as long as the spouse and/or family members can cope and within the borders of self-dignity of the patient and overall affordability (Greeff, 2009). Drug development for AD is challenging, however, several therapeutic approaches have been adopted. Drugs that are currently in use and the role of dietary factors in the treatment of AD are discussed in detail.

1.4.1 Acetylcholinesterase inhibitors

Acetylcholinesterase inhibitors (AChEIs), are the best developed therapy currently used in the treatment of mild to moderate AD (Shah et al., 2008). AChEIs slow the progression of the disease by decreasing levels of Aβ protein. Tacrine was the first widely used AChEI (Summers, 2006). A 30-week randomized control clinical trial showed a significant dose related improvement in cognitive function with tacrine (Whitehouse, 1993; Knapp et al., 1994). However, subsequent studies were less impressive and a short half-life, hepatotoxicity and cholinergic side-effects, have limited the use of the drug. Second generation AChEIs, including donepezil, galanthamine and rivastigmine have since been developed. These drugs have fewer side effects, longer half-lives and greater efficacy (Shah et al., 2008).

AChEIs are usually started at low doses to minimize side effects such as facial flushing, dyspepsia, nausea, vomiting and diarrhea. The dose is then titrated up to the maximum tolerated dose (Mulugeta et al., 2003).

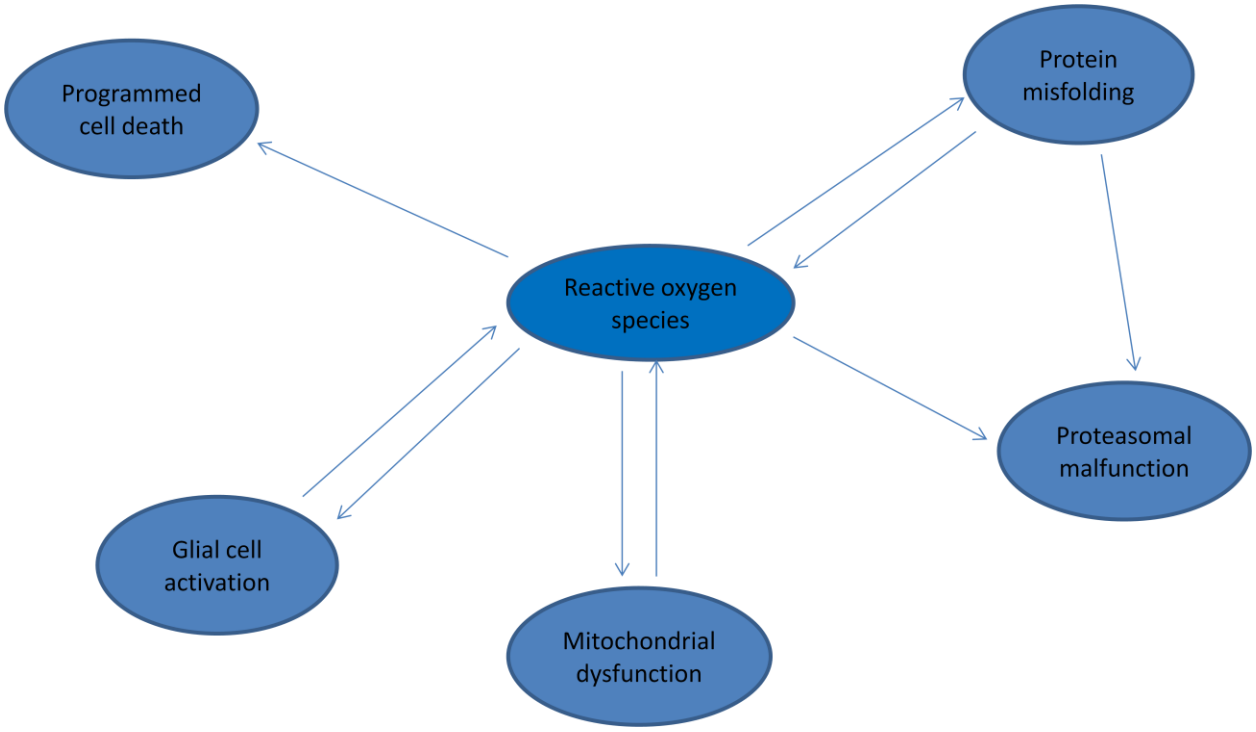

Figure 1.2 ROS production as a player in the cycle of events leading to neurodegeneration (From Anderson, 2004).

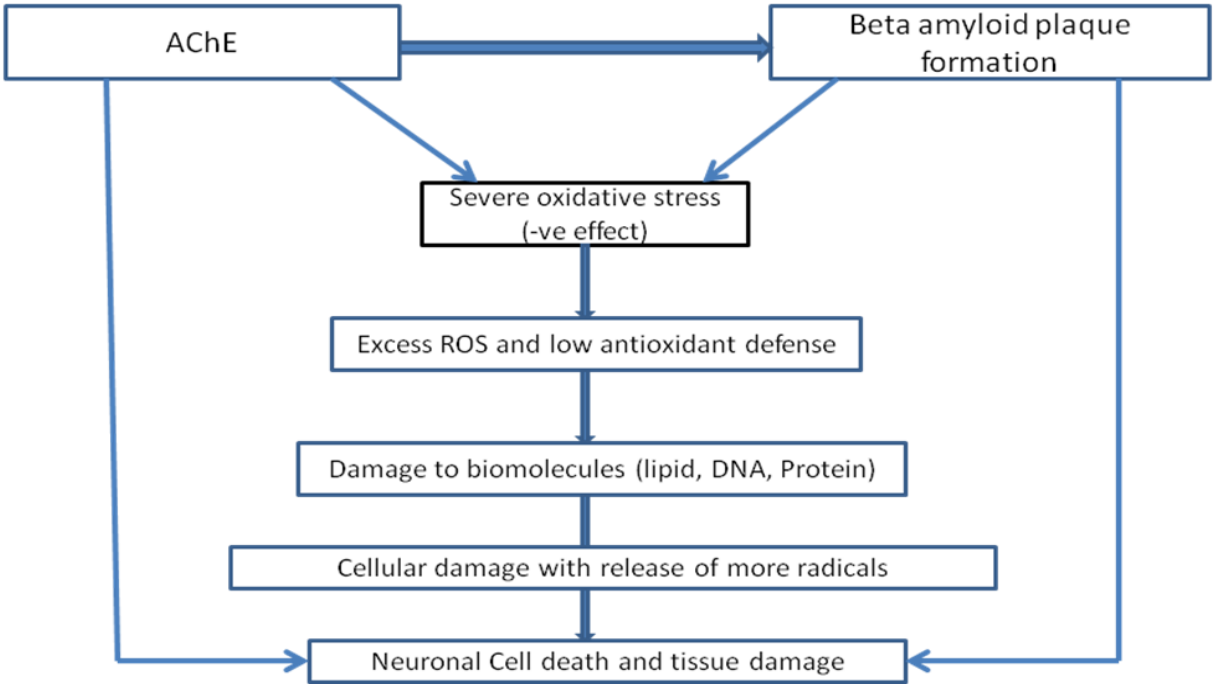

Figure 1.3 Acetylcholinesterase, beta amyloid plaque formation and oxidative stress as part of a chain reaction leading to neurodegeneration.

1.4.2 Antioxidants

Multiple lines of evidence indicate that oxidative stress is an important pathogenic process associated with aging and AD. In addition, markers of oxidative stress have been shown to precede pathological lesions in AD, including senile plaques and neurofibrillary tangles (Sayre et al., 1997; Nunomura et al., 1999; Nunomura et al., 2001; Castellani et al., 2006). Antioxidants act by slowing the progression of the disease or preventing cognitive decline (Shah et al., 2008). Vitamin E in combination with vitamin C is reported to be associated with a decrease in the prevalence and incidence of AD (Zandi et al., 2004; Shah et al., 2008). Also, in controlled clinical trials conducted with α-tocopherol, AD patients with moderate impairment taking high doses of the antioxidant were observed to display some beneficial effect with respect to the rate of deterioration of cognitive functions (Sano et al., 1997; Ramassamy, 2006).

1.4.3 Statins

Cerebral Aβ levels have been shown to be decreased *in vivo* with simvastatin (Fassbender et al., 2001). The first study to show neuropathologic change in statin use was published by Li et al. (2007). Several epidemiologic studies have indicated that the use of statins significantly reduces the risk of AD (Jick et al., 2000; Wolozin et al., 2000; Rockwood et al., 2002; Yaffe et al., 2002). These studies suggest that statins may slow progression of AD, but may not be able to reverse neuronal degeneration once it has occurred (Shah et al., 2008)

1.4.4 Non-steroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs (NSAIDs), act by down-regulating pro-inflammatory signals, microglia and astrocytes and may reduce the risk of AD by lowering Aβ production (Breitner et al., 1994). The Baltimore Longitudinal Study of Aging showed reduced risk for AD

with NSAID use proportional to the duration of use (Stewart et al., 1997). In addition, case control studies of individuals taking NSAIDs for arthritis, a small clinical trial of indomethacin, and a number of other similar studies also indicated protection from development of AD or progression of the disease (Rogers et al., 1993; Andersen et al., 1995). However, a randomized controlled primary prevention trial of NSAIDs in AD, the AD Anti-inflammatory Prevention Trial (ADAPT), was terminated in 2004 due to concerns regarding cardiovascular risks (Shah et al., 2008). As a result of the associated risks, cyclooxygenase-II (COX-2) inhibitors and NSAIDs are currently not recommended for the treatment or prevention of AD.

1.4.5 Diet

Diet may play an important role in the causation and prevention of AD (Solfrizzi et al., 2003; Luchsinger and Mayeux, 2004). The Mediterranean diet (MeDi), has received increased attention in recent years because of converging ecological and interventional evidence relating it to lower risk for cardiovascular disease, several forms of cancer, and overall mortality (Lagiou et al., 1999; de Lorgeril et al., 1999; Singh et al., 2002). The diet is characterized by high intake of vegetables, legumes, fruits, and cereals; high intake of unsaturated fatty acids (mostly in the form of olive oil), low intake of saturated fatty acids; a moderately high intake of fish; a low-to-moderate intake of dairy products (mostly cheese or yogurt); a low intake of meat and poultry; and a regular but moderate amount of ethanol, primarily in the form of wine and generally during meals (Trichopoulou et al., 2003). Therefore, the MeDi appears to include many of the components reported as potentially beneficial for cognitive performance.

1.5 Traditional Medicine

The use of plants as medicine predates written human history and almost all cultures in the world have a body of expertise concerned with the therapeutic properties of local flora (Houghton, 1995). Herbal medicines are an important part of the culture and traditions of people, as many in urban and rural communities, are reliant on them for their health care needs. This is because in addition to their cultural significance, herbal medicines are generally more accessible and affordable (Mander, 1998; Fennell et al., 2004). As a result, there is an increasing trend worldwide, to integrate traditional medicine with primary health care.

Interaction between different cultures has resulted in the expansion of the pharmacopoeia of each group due to the adoption of the plants used by the other. Thus, northern European herbal medicines use many plants that originate from North America (Houghton, 1995). Also, renewed interest in these pharmacopoeias has meant that researchers are concerned not only with determining the scientific rationale for the plant's usage, but also with the discovery of novel compounds of pharmaceutical value. This has enabled scientists to target plants that may be medicinally useful (Cos and Balick, 1994). The last two decades have witnessed remarkable change in attitude toward plants as a source of pharmaceuticals within the scientific and industrial communities, which have been primarily concerned with the search for molecules with new structures and biological activity. By the 19th century, an estimated 122 drugs from 94 plant species had been discovered through ethno-botanical leads (Fabricant and Farnsworth, 2001).

1.6 Plants used traditionally to treat age-related/neurological disorders

Plants have been used since antiquity in traditional medicinal systems for the treatment of memory dysfunction and several other age-related diseases. An ethno-pharmacological approach

and bio-assay guided isolation have provided leads in identifying compounds which are potential AChE inhibitors, inhibitors of Aβ induced cell death, and antioxidants from plant sources, including those for memory disorders which are currently either in clinical use or templates for further drug discovery (Dastmalchi et al., 2007; Mukherjee et al., 2007).

Celastrus paniculatus seeds and oil have been used in Ayurvedic medicine for stimulating intellect and sharpening memory (Nadkarni, 1976; Warrier et al., 1995). Oral administration of the seed oil to rats resulted in a decrease in levels of noradrenaline, dopamine and 5-hydroxytryptamine (5-HT) in the brain, and this correlated with an improvement in the learning and memory process (Nalini et al., 1995). In addition, an antioxidant effect in the CNS observed with the aqueous seed extract, may explain the reputed benefits on memory since it enhanced cognition *in vivo* (Kumar and Gupta, 2002a). Studies on the inflorescences of the plant have shown the methanol extract to have anti-inflammatory effect which may also have some relevance in the management of neurodegenerative disorders (Ahmad et al., 1994).

The leaves of *Centella asiatica* have been used in Ayurvedic medicine for revitalizing and strengthening nervous function and memory. An ayurvedic formulation composed of four herbs, including *C. asiatica*, is used as a restorative and for the prevention of dementia (Manyam, 1999). An alcoholic extract of the plant has been shown to possess tranquilising and potentially cholinomimetic activities *in vivo*, which may be due to the presence of the triterpenoid brahminoside (Sakina and Dandiya, 1990). Also, aqueous extracts of the whole plant enhanced cognitive function in rats, which was associated with the *in vivo* antioxidant activity of the extract (Kumar and Gupta, 2002b). In addition, the essential oil from the plant is reported to contain monoterpenes including β-pinene and γ-terpinene (Brinkhaus et al., 2000), which have demonstrated AChE inhibitory activity (Perry et al., 2000).

The roots of the Indian medicinal plant *Clitoria ternatea*, have been reported to promote intellect (Warrier et al., 1995; Misra, 1998). A study investigating both the aerial parts and the roots of *C. ternatea,* showed its alcoholic root extracts to be more effective in attenuating memory deficits in rats compared to its aerial parts (Taranalli and Cheeramkuzhy, 2000). An aqueous extract of the root was observed to increase ACh levels in rat hippocampus following oral administration, and it was hypothesized that this effect may be due to an increase in ACh synthetic enzymes (Rai et al., 2002; Howes and Houghton, 2003).

Aqueous and ethanol extracts of several plants including *Malvia parviflora*, *Albizia adianthifolia, A. suluensis* and *Crinum moorei*, used in southern Africa to treat memory loss, have been screened for AChE inhibitory activity. The extracts of *C. moorei* showed good activity (Risa et al., 2004; Stafford et al., 2008). Several other species of *Crinum* including *C. campanulatum*, *C. graminicola*, *C. macowanii* and *C. variabile* have also been investigated for inhibition of AChE. Leaves of these plants were found not to be very active while bulbs and roots, were observed to contain several compounds with inhibitory activity (Jäger et al., 2004; Bay-Smidt et al., 2011).

Among the natural phytochemicals identified from plants, flavonoids represent one of the most important and most interesting classes of biologically active compounds. Evidence suggests that flavonoids are effective in the protection of various cell types from oxidative injury (Zou et al., 2010). It has been reported that the antioxidant activities of flavonoids such as quercetin, luteolin and catechins are stronger than the antioxidant nutrients; vitamin C, vitamin E and β-carotene. Flavonoids from *Scutellaria baicalensis,* including baicalein and baicalin, have been reported to reduce the cytotoxicity of Aβ by a reduction of oxidative stress (Heo et al., 2004).

Galanthus species have been used traditionally in Bulgaria and Turkey for neurological conditions (Mukherjee et al., 2007). Galanthamine is an Amaryllidaceae alkaloid first isolated in the 1950s from *Galanthus nivalis* (Shu, 1998). It also occurs in other genera of the Amaryllidaceae family, *Narcissus* spp. and *Lycoris* spp. Galanthamine increases the availability of ACh in the cholinergic synapse by competitively inhibiting the enzyme responsible for its breakdown, AChE. The binding of galanthamine to AChE slows down the catabolism of ACh and, as a consequence, ACh levels in the synaptic cleft are increased (Thomsen et al., 1991a, b; Bores et al., 1996; Heinrich and Teoh, 2004). In addition to amelioration of cholinergic deficit, galanthamine also helps prevent neurodegeneration due to its inherent antioxidant activity, by reducing the toxicity of Aβ and subsequently reducing plaque formation (Eskander et al., 2005). This effect was determined in human neuroblastoma cell cultures which were exposed to Aβ peptide, and where galanthamine (300 nM) reduced cell death significantly (Arias et al., 2004; Geerts, 2005).

Physostigma venenosum has been used traditionally in Africa as a ritual poison, claimed to determine the guilt or innocence of a person accused of a crime. Treatment with the indole alkaloid physostigmine, an AChE inhibitor isolated from *P. venenosum* has been shown to improve cognitive function in several *in vivo* studies (Mukherjee et al., 2007). The chemical structure of physostigmine has provided a template for the development of rivastigmine (Foye et al., 1995), a carbamate derivative that reversibly inhibits the metabolism of AChE in the CNS (Williams et al., 2003). It binds to both the esteratic and ionic sites of AChE, preventing the enzyme from metabolizing ACh (Polinsky, 1998).

Huperzia serrata (Lycopodiaceae) has been used in Chinese traditional medicine for memory impairment (Hostettmann et al., 2006). Huperazine A, a lycopodium alkaloid related to the

quinolizidines and isolated from *H. serrata* is a potent inhibitor of AChE with a long duration of action (Ashani et al., 1992). In a multi-center, double blind trial, huperazine A significantly improved memory and behavior in AD patients, and was reported to be a more selective inhibitor for AChE and less toxic than the synthetic AChE inhibitors, donepezil and tacrine (Raves et al., 1997; Mukherjee et al., 2007). Huperzine A is also a strong antioxidant that has been demonstrated to inhibit Aβ-induced neurotoxicity (Xiao et al., 2002). Studies using cell cultures have shown that huperazine A decreases neuronal death and protects neurons against Aβ-induced apoptosis (Xiao et al., 2002; Hostettmann et al., 2006).

Ginkgo biloba has been used for the improvement of memory loss associated with abnormalities in blood circulation (Samuelsson, 2004). Administration of plant extracts to both AD and non-AD patients in various randomized, double-blind, placebo controlled, multicentre trials resulted in improvement of cognitive function (Hofferberth, 1994; Kanowski et al., 1997; Le Bars et al., 1997; Rigney et al., 1999). Since early pharmacological data revealed that the flavonoids from *G. biloba* modulated contractile motion of vascular smooth muscles, attempts were made to prepare a standardized extract rich in flavonoids, the outcome of which was EGb 761 (Kumar, 2006). EGb 761 showed cognitive enhancing activity in a number of clinical studies (Dastmalchi et al., 2007). The extract also showed neuroprotective effect against Aβ and nitric oxide (NO) induced toxicity in neuronal cell cultures (Bastianetto et al., 2000a, 2000b). Furthermore, apoptosis was reduced both *in vitro* and *in vivo* (Schindowski et al., 2001; Yao et al., 2001) and antioxidant activities were reported (Barth et al., 1991; Marcocci et al., 1994; Topic et al., 2002).

Hypericum perforatum commonly known as St. John's Wort has been used for treatment of neurological disorders (Ross, 2001). The dried crude herb standardized to hypericins was shown to improve memory and learning dysfunction (Widy-Tyszkiewicz et al., 2002; Trofimiuk et al.,

2005). Lu et al. (2001), reported that a standard extract of the plant possessed neuroprotective activity. Hydro-alcoholic extracts of the aerial parts of the plant demonstrated nootropic activity *in vivo*, which may be due to adrenergic (α and β receptor) and serotonergic (5HT1A) antagonistic activity (Khalifa, 2001; Kumar et al., 2000, 2002). In addition, the hydro-alcoholic extract of the plant have also been reported to reduce the rate of degradation of ACh (Re et al., 2003).

Salvia lavandulaefolia (Spanish Sage), has been used for the enhancement of memory (Perry et al., 1998). Volatile oil from the plant showed strong AChE inhibitory activity (Perry et al., 1996), which is likely due to the presence of the cyclic monoterpenes; 1,8-cineole and α-pinene, and other constituents which act synergistically (Perry et al., 2001). Administration of the volatile oil also decreases AChE activity *in vivo* (Perry et al., 2001). An ethanol extract of the plant showed *in vitro* anti-inflammatory activity, while the essential oil resulted in mood elevation and improvement of memory in clinical studies (Perry et al., 2001; Tildesley et al., 2005; Dastmalchi et al., 2007).

Several other plants have been shown in literature to contain cholinesterase inhibitory activity and a list of these plants together with their scientific names, plant part, solvent extract, percentage inhibition and concentration at which the enzyme is inhibited, have been reported in a review article (Adewusi et al., 2010; Appendix A).

Problem Statement

Selective cholinesterase inhibitors, free of dose-limiting side effects, are not currently available, and current compounds may not allow sufficient modulation of acetylcholine levels to elicit the full therapeutic response (Felder et al., 2000). In addition, some of the synthetic medicines used

have been reported to cause gastrointestinal disturbances and problems associated with bioavailability (Melzer, 1998; Schulz, 2003). Therefore, the search for new AChE inhibitors, particularly from natural products, with higher efficacy continues.

1.7 Study Aim

To determine the *in vitro* acetylcholinesterase, antioxidant activity, cytotoxicity and amyloid-β inhibition of selected medicinal plants.

1.8 Objectives

1. To screen selected medicinal plants for AChE inhibitory activity using a TLC and microtiter plate assay based on Ellman's method.
2. To evaluate the antioxidant activity of selected medicinal plants using the DPPH and ABTS radical scavenging assays.
3. To determine the level of phenols and flavonoids in various extracts from the different selected medicinal plants with good antioxidant activity.
4. To isolate active compounds from the plant with promising AChE inhibitory activity using bio-assay guided fractionation.
5. To determine the effect of the isolated compounds and most promising plant extracts on SH-SY5Y cells.
6. To determine the effect of the isolated compounds and most promising plant extracts with good antioxidant activity, on inhibition of β-amyloid induced cell injury in SH-SY5Y cells using the neutral red uptake and MTT assays.