CHAPTER 3

REVIEW OF LITERATURE

3.1 Ageing

Harman has defined ageing is a progressive accumulation of the diverse deleterious changes in cells and tissue allied with progressive increase in the rate of morbidity and mortality. Ageing is found to be the biggest risk factor for many chronic disease including cancer, cardiovascular disease and neurodegenerative diseases. Changes attributed to ageing are development, genetics, environment, disease and ageing process [20, 21]**.** The study of ageing has expanded worldwide due to the following reasons 1). Increase in average human life span, 2). Increase in their percentage worldwide. 3). Health expenditure increase for elderly.

Ageing is not simple process with a single cause in spite it is a complex, multi-factorial and integrated one [22]. Over the years a number of theories have been evolved with the process of ageing, such as evolutionary theory of ageing, oxidative theory of ageing, mitochondrial theory of ageing, molecular inflammatory theory of ageing, gene regulation theory, cellular senescence theory and neuro-endocrine theory. A few important theories have been discussed below.

3.2 Theories of ageing

3.2.1 Evolutionary theory

The theory states that ageing results from decline in the force of natural selection. Evolutionary theory paved way to the mutation accumulation theory of ageing, which proposed that deter mental, late acting mutations may accumulate in the population leading to senescence and pathology [23]. The disposable soma theory of ageing states that

organisms were maintained only for reproductive success, after which they were disposed. The next theory under evolutionary concept was the theory of antagonist's pleitrophy, which suggested that certain genes were selected in early life for beneficial effects which have unselected deleterious effects with age, therefore contributing directly to senescence. The antagonist theory was supported by research in which limiting reproduction by destroying germ cells would extend life span in drosophila and *Elegans sp*. [24, 25].

3.2.2 Molecular theories

The gene regulation theory of ageing states that senescence results from changes in the expression of genes [26]. Many genes show changes in their expression with age [27-29] and it was unlikely that selection could act on genes that stimulate senescence directly [30]. Recent research reports encourage the idea that longevity had genetic component at the locus of chromosome 4, which contain genes that promote longevity [31].

3.2.3 Free radical theory

The theory proposes that free radical reaction is an inevitable action in biological system resulting in damage and senescence. Elevated levels of oxidative stress induced damage to DNA and protein was found in aged organism [32, 33]. Free radical theory was further expanded by mitochondrial theory of ageing where the mutations in mitochondrial DNA enhance free radical damage by producing an altered enzyme component into electron transport chain. This results to elevated free radical leakage ultimately leading to more mt DNA damage and increased oxidant production. This "salvage cycle" of overproduction of oxidant and mutation leads to cellular catastrophe, organ failure and senescence [34]. The reduction in the ability to degrade oxidant damaged protein in ageing may contribute to accumulation of damaged and dys-functioned molecule in the cell.

3.2.4 Inflammation theory of ageing

The role inflammation in several clinical conditions (diabetes, dementia, and atherosclerosis) is well understood, but its importance in ageing has been recently revealed [35]. Inflammation is now considered as one of the major factors involved in the pathogenesis of ageing termed as "infl amm-ageing" [36]. Inflammatory response has been

constituted by cellular and humoral immunity which consists of the following phase 1) intracellular activation; 2) proinflammatory cells in the tissues; 3) increase of vascular permeability; 4) damageing of tissues and cell death. As it is very much essential to understand the mechanism of inflammation and ageing it is also equally important to study relation of inflammation with other theories of ageing. Such closest relationship has been identified between inflammation and oxidative stress. ROS and RNS are implicated with inflammation and overproduction of ROS is the major factor in tissue inflammation.

3.3 Brain Ageing

The processes involved in brain ageing evolution: the metabolism, damage and pathology in the cells [37]. Metabolism is required for sustaining life and it generates toxins which are found more in long living post mitotic cells like neurons and these toxins accumulate in the cells as end products [38]. These toxins are stored at different organelles like lysosomes, phagosomes and proteosomes with different degrading mechanism and kinetics. Lipofuscin is a non degradable intralysosomal polymeric substance produced during ageing process. An interaction between lipofuscin loaded lysosomes and mitochondria has been reported to play a pivotal role in the evolution of cellular senescence [39]. The toxic and degradation products present in the cells disturb mitochondrial turnover, leading to aged mitochondrial deficient in ATP, which release excess reactive oxygen species, manifesting as pathology related to ageing [40]. The above said sequences of events reduce the cellular adaptability and trigger pro–apoptotic pathway, finally leading to cell death. The common age related changes in the brain are summarized below.

The volume and weight of the brain declines with age at a rate of about 5% per decade after 40 yrs of age and the decline increases with age over 70 years as reported. There was a uniform decrease in volume of cerebral white matter; the gray matter of frontal and parietal cortex, and striatum were the most affected regions compared to temporal cortex, cerebellar vermis and hippocampus; and the occipital cortex was least affected [41] thus leading to cognitive changes on ageing [42, 43]. Studies have demonstrated cortical thinning, shrinkage and synaptic loss were the most observed changes that occur in the pre frontal cortex during ageing [44]. Brain shrinkage was found to be region specific and was related with weight and volume [45, 46].

Neuronal shrinkage occurs due to extensive neuronal loss in the aged brain as reported for several years [47]. Neurons are were found to be lost on ageing [48, 49], and that 1,00,000 neurons in the human brain disappear daily resulting in a 19.7% reduction in cell number at the age of 80 as hypothized by Meier-Ruge and co-workers. About 40–50% neuronal loss occur in the hippocampus [50], up to 79% in nucleus basalis [51] and 15–58% in the cortex [52]. It was concluded that there was a definite nerve cell loss rather shrinkage of nerve cells in the ageing brain. These changes, however does not occur in all nerve cell populations in aged brain. Other age related type of structural change was loss of synapses and dendrites loss. Dendritic losses include shortening [53] and fewer dendritic branches.

Electron microscopy studies revealed significant loss of synapses with age in laboratory animals [54] and humans [55]. Dendritic regression and spine loss may probably contribute to the first signs of cognitive decline in learning and memory performance noted in normal ageing.

Increase in the size of astrocytes and microglia was found with the brain ageing [56] and several other studies reported the presence of neurofibrillary tangles (NFT) and senile plaques (SP), which are the hallmarks of Alzheimer‘s disease in brain ageing. These tangles and plaques occur within the same regions, both in normal ageing and Alzheimer‘s disease, with only difference in the severity of lesions [57, 58]. NFT formation and could be considered one of the prime cause of cognitive impairment than just accumulation of Aβ and structural changes [59].

3.4 Dementia

Dementia is a clinical syndrome characterized by global cognitive decline with memory and one other area of cognition that interfere significantly with the person’s ability to perform daily life tasks. Dementia of Alzheimer’s Type is a fatal progressive neurodegenerative disorder with deterioration in cognition and memory, progressive impairment in the ability to carry out day to day living activities along with a number of neuropsychiatric and behavioral symptoms. DAT is found among the elderly subjects and accounts for two third cases of dementia which increases with age. SDAT turns to be a health burden as there is a decline in the cognitive, behavioral and functional activity, therefore it is a growing medical and social and economic problem.

The various states of cognitive impairment are– Age Related Cognitive Decline (ARCD) termed for abnormal cognitive function less severe than dementia in persons older than 50 [60], Mild Cognitive Impairment (MCI) which is featured abnormal memory loss relative to one‘s age, but without the other changes which characterize dementia [61] and Alzheimer‘s dementia characterized by progressive loss of the personality and increasing inability to perform even the simplest tasks. Dementia is a progressive cognitive deterioration syndrome caused by various different pathologies [62]. SDAT is one of the commonest forms of dementia affecting the elderly population. Epidemiological studies state that about 60 to 70% of the dementia cases can be attributed to SDAT [63]. In Western countries, SDAT represents one of the most frequent causes of death among the elderly– ranking seventh after cardiovascular diseases, cancer, stroke, chronic diseases of the lower respiratory tract, pneumonia and diabetes [64].

3.4.1 Prevalence

The incidence of DAT increases with age, 1% of people among the age 60-64 are affected with DAT whereas this increase up to 40% for people above the age 80. With increase in the aged population there is a profound emergence of dementia epidemic. Recently the early onset of dementia has increased, whereas dementia is a clinical condition that affects the elderly population leading to disability and dependence [65, 66]. The increase in the number and proportion of dementia is more in India and China and Latin America. It has been forecasted that by 2050 the No. of people above the age 60 will be increased to 1.5 billion accounting to 22% of world’s population of this 79% are those from world’s under developed regions, this is because there is less attention or awareness of dementia and health system in these regions.

The American disease International in 2005 has estimated 24.3 million people with dementia in 2001 with 60% living in low and middle income nations. 4.6 million new cases are predicated each year with the number doubling to 1.1 Million by 2040. The percentage of incidence with various regions is as follows East Asia 5.5 million, South Asia 4.5 million and North America 4.4 million. As estimated in 2010 by country level there were 9 country with highest level of Dementia cases: China (5.4 million), USA (3.9 million), India (3.7 million), Japan (2.5 million), Germany (1.5 million), Russia (1.2 million), France

(1.1 million), Italy (1.1 million and Brazil 1.0 million). The estimated increase of SDAT by 2030 is 65.1 million and 115.4 million by 2050. There is an exponential increase in dementia with age and the prevalence in higher in women than in men. In India, the prevalence rate of dementia in people aged 65 years and above was 33.6 per1000 whereas SDAT was found to have a prevalence rate of 15.5 per 1000 [67].

3.5 Behavioral dsyfunction in ageing and SDAT

Occurrence of behavioral deficits was expressed in several studies as result of alterations in both cognitive [68] and motor functions [69] with normal ageing and SDAT. Researches detected 30% reduction in neuronal number in pre frontal cortex in aged non human primates, which was correlated with poor performance on working memory task [70]. Alterations in memory were reported to occur primarily in the spatial and episodic memory systems and were reflected in the storage of newly acquired information both in ageing and SDAT [71]. Aged rats showed decrement in both reference and working memory in the Morris water maze [72], the radial arm maze [73] and the radial arm water maze [74]. Motor deficits such as decreases in balance, muscle strength, and coordination were also reported in ageing and SDAT in addition to cognitive deficits. These motor deficits were considered as the result of alterations in the striatal dopamine or cerebellar systems, which showed marked neurodegenerative changes with age. Similarly, agitation and aggression were also prevalent in SDAT [75, 76]. A prospective study conducted for ten years in dementia subjects who were measured for physical aggression, aggressive, resistance, physical threats, verbal aggression, refusal to speak, destructive behavior and general irritability showed changes in aggressive behavior throughout the course of disease [77]. It has been suggested that Norepinephrine may be responsible for the non-cognitive behavioral disturbances associated with SDAT [78].

3.6 Alterations in neurotransmission system in ageing and SDAT

Numerous studies have demonstrated age-related variation in cholinergic, monoaminergic, and amino acid neurotransmitter systems. Clinical studies indicate that basal forebrain and rostral forebrain cholinergic pathways including converging projections to the thalamus are damaged [79] with advanced age and SDAT, these regions play

important functional roles in conscious awareness, attention, working memory. The cholinergic hypothesis stated that a loss of cholinergic function in the central nervous system contribute significantly to the cognitive decline associated with advanced age and SDAT [80].

Researches on examination of acetylcholine synthesis in rats from 3 to 30 months of age conclusively reported that the biosynthesis of acetylcholine declined up to 75% in 30 months old animals [81]. Mild hypoxia was also found to contribute to the decrease in acetylcholine synthesis by 90%. Aged cholinergic neurons were more impaired in acetylcholine release than its synthesis through potassium stimulation. Moreover, there are strong evidences on the role of cortical cholinergic transmission in controlling, processing and formation of β-amyloid plaque as well as tau protein phosphorylation. Several researchers have demonstrated a decrease of muscarinic cholinergic ligand binding sites with ageing [82] and it was found that the m1, m3, and m5 receptors also selectively influence the processing of the amyloid precursor protein, such that activation of these receptors increases the secretion of non-amyloidogenic peptides [83]. *In vitro* studies indicated m1 stimulation dephosphorylates tau protein, suggesting that receptor subtypes could potentially alter the hyperphosphorylation of tau proteins and neurofibrillary pathology in SDAT [84]. Besides the acetylcholine transmission recent studies evidenced that dopamine system may also play a relevant role in the mechanisms involved in learning and memory processes, showing strong synaptic interaction with acetylcholine in different brain regions [85-87].

Extra pyramidal signs were observed in patients suffering from SDAT, which was related to cognitive decline and higher mortality [88]. Even in the absence of extra pyramidal signs there is impairment in the dopamine transport in various regions of the brain [89, 90] and it has been noted that there is a relation between dopamine receptor D2 and cognitive dysfunction in SDAT [91]. On ageing there is an endogenous decrease in the levels of dopamine and dopaminergic neurons due to degeneration. This loss was more profound in the corpus striatum while other regions show minor alterations. Senescence rats were reported to show decreased motor performance, lower level of dopamine and dopamine tissue in the ventral striatum and in the midbrain [92]. Scientific evidences support the

occurrence of significant abnormalities in the noradrenergic system in SDAT. significantly lower levels of cortical and sub cortical Nor-Epinephrine levels were found in frontal medial gyrus, temporal superior gyrus, cingulate gyrus, hippocampus, amygdala, thalamus, hypothalamus, caudate, putamen as well as the locus Coerulus [93-96].

Loss of noradrenergic neurons was well established in patients with SDAT [97, 98], but they were not identified in vascular dementia [99]. It has been stated that estimation of nor-adrenergic turnover provides accurate representation of noradrenergic function than measuring the levels of NE concentrations [100]. NE levels were also found to have an inverse relationship with cognitive impairment [101]. As noted by [102] a significant positive relationship was seen between basal plasma 3-Methoxy-4-hydroxyphenylglycol (MHPG) levels and cognitive impairment. Nor adrenergic projections from Locus Coerulus LC was reported to involve in the regulation of arousal, flight-and- fight responses [103], agitation, anxiety [104], sleep-wake cycle, and levels of vigilance and emotion [105] as well as aggressive behaviors [106]. When this system gets interrupted there is a potential abnormal behavioral response to ordinary stimuli. Serotonin (5-hydroxytryptamine, 5-HT) a major biogenic amine is involved in a wide range of physiological functions including sleep, appetite, pain perception, sexual activity, and memory and mood control [107]. Studies have reported loss of both cholinergic [108] and serotonergic amines in the brain of SDAT patients. Cholinergic-serotonergic interactions play an important role in learning and memory [109]. *In vivo* Studies indicated that decrease in cholinergic and serotonergic activity produces a synergic decrement in learning and dementias of the Alzheimer type [110].

3.7 Oxidative stress in brain ageing in SDAT

On ageing there is a morphological and functional modification in the brain affecting the dendrites and its synapses, neurotransmitter levels there circulation and metabolism, motor and sensory system, sleep, memory and learning, and lipofuscin accumulation [111]. On the analysis of these changes it was implicated by many studies that oxidative stress plays major role [112]. There exists a natural equilibrium between antioxidants and pro-oxidants under normal physiological conditions; this homeostasis is

disturbed in case of stress and disease leading to oxidative stress in cells [113]. Oxidative stress also occurs during antioxidant deficiency [114] or excess of ROS/RNS production.

The free radical theory of ageing was proposed in the year 1956 by Harman who postulated that damage to cellular macromolecules through free radical production in aerobic organisms was a major determinant of their life span [115]. Many reviews have been published in the past two decades which contain information in relation to free radical theory of ageing [116-120]. The theory was further been modified as mitochondrial theory of ageing, which hypothesized that mitochondria are the critical sites that control ageing. Electrons leaking from the electron transport chain (ETC) produce ROS which can damage ETC components and mitochondrial DNA, leading to further increase in intracellular ROS levels and a decline in mitochondrial function [121]. Recent studies has supported the mitochondrial theory of ageing and suggested that mitochondrial DNA damage is increased with ageing [122]. ROS was found to play major role in brain ageing [123], and that ROS causes apoptosis and necrosis of neuron [124] and astrocytes [125]. Oxidative stress was related with the release of glutamate and NMDA receptor activation during cerebral ischemia [126], superoxide radicals were produced in the neurons and brain macrophages and in glutamine-induced astrocytes [127].

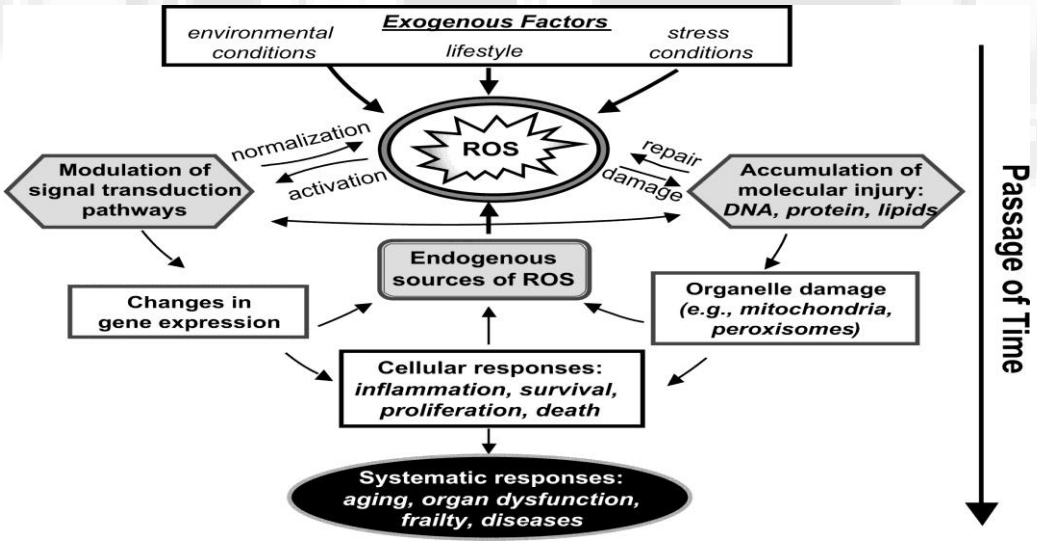

Figure 3.1 A diagrammatic representation of proposed mechanisms by which ROS and oxidative stress could contribute to the process of ageing. Various kinds of exogenous and endogenous factors can stimulate an increase in ROS production at the cellular level.

Oxidative stress has been identified has one of the risk factors for SDAT and ageing, which is found elevated in SDAT subjects [128-130]. Subjects with mild cognitive impairment where found with higher levels of oxidative stress when compared with age–matched controls which indicates that oxidative stress was involved in the progression of SDAT and not a consequence of the disease. Recently study by Lovell [131] reported that oxidative damage occurs in early stages of Alzheimer‘s disease, thus oxidative stress may be identified as an early marker in Alzheimer‘s disease and it also correlates with the advancement of the disease. Oxidative stress associated modifications of bio-macromolecules has been described in association with the susceptible neurons of AD.

The resistance of neurofibrillary tangles to proteolysis may play a major role in the progression of SDAT [132]. Lipid peroxidation is noted by increased levels of thiobarbituric acid reactive substances (TBARS), malondialdehyde (MDA), 4-hydroxy-2-transnonenal (HNE), isoprostanes and altered phospholipids composition [133, 134]. Modifications of tau protein by HNE promote and contribute to the generation of the major conformational properties defining neurofibrillary tangles [135]. Oxidative stress may also trigger the formation of toxic Amyloid beta species, which in turn can exacerbate accumulation of ROS [136, 137]. Immunohistochemical reports in animal have found increased level of oxidative stress in brains of transgenic rat bearing APP mutations [138].

3.8 Inflammation in brain ageing and SDAT

Several studies have demonstrated that ageing was accompanied by up-regulation of the inflammatory response and impaired immune system [139, 140]. *In vivo* studies on aged rat explain a functional decline of monocytes and macrophages, with a low level expression of Toll-like receptors from splenic and peritoneal macrophages, also an altered secretion of several chemokines and cytokines was observed in aged rats [141, 142]. Mitogen activated peripheral blood mononuclear cells was found to produce increased level of pro inflammatory cytokines such as IL-1β, IL-6 and TNF-α in elderly persons when compared with young donors [143]. There is an abnormal elevation of proinflammatory cytokines during inflammatory responses in elderly subjects [144]. Serum IL-6 levels were found to increase in healthy elderly and centenarians compared to young controls [145, 146]. Moreover, activation of the peripheral innate immune system leads to exacerbated neuro-

inflammation in the aged which in turn is likely to be involved in the severe behavioral deficits that frequently occur in older adults [147].

The pathogenesis of SDAT has been linked with inflammation as the damaged neurons and neuritis, highly insoluble Aβ42 peptide deposits and neurofibrillary tangles all acts as stimuli for inflammation. The Senile plaques found in the brains of SDAT subjects are associated with reactive astrocytes and activated microglial cells; and an over expression of cytokines and acute phase proteins were found in microglia and astrocytes surrounding neuro-pathological lesions in SDAT brains. Cytokines, chemokines, complement components and acute phase proteins were over-produced in SDAT brains and activated microglia were found surrounding senile plaques and areas of neurodegeneration [148].

Further, multiple studies suggest that Aβ peptide may promote inflammation by inducing glial cells to release immune mediators. Inflammation plays a major role in ageing and SDAT this was well emphasized by epidemiological studies that the long-term use of non-steroidal anti-inflammatory drugs may protect against SDAT [149]. Inflammatory marker CRP was found associated with cognitive impairment in several studies [150]. Several other studies have shown increased circulatory cytokines, such as IL-1β and IL-6, and acute phase proteins α-1-antichymotrypsin, (ACT) in patients with clinical AD [151-152]. Hence, altered immune responses in the brain and the peripheral circulating system are associated with the disease progression. Plasma levels of ACT is also correlated with the degree of cognitive impairment in SDAT patients from a case-control study suggesting inflammation or impaired immune responses may be used as a disease marker for monitoring its progression. Evidences suggest that inflammation leads to oxidative stress. Activated microglia releases higher levels of superoxide superoxide ions which affect the CNS [153, 154] and this activated microglia was also a source of neurotoxic like free radicals and glutamate [155].

3.9 Other mechanisms

Apart from the pathways discussed above there are several other mechanisms that contribute to the deleterious effect of ageing and SDAT progression. Aberrations in protein aggregation and degradation pathways in ageing and SDAT have been explained in several studies. There is a general decrease in proteosomes level and functioning with age which leads to the formation and accumulation of oxidized proteins [156, 157]. The accumulation of these protein intracellular and extracellular has been strongly linked to ageing-related neurodegeneration like intracellular neurofibrillary tangles that contain tau protein, and extracellular deposits of the amyloid peptide in the most common form of dementia, Alzheimer‘s disease. Impaired proteosome degradation pathway leads to accumulation oxidized and ubiquitinated in age and SDAT cases [158-161]. Improper regulation of calcium homeostasis has been directly related to the ageing process and SDAT progression. Gene expression studies have revealed that expression of Na+/K+, Ca2+ and H+ATPases required for the regulation calcium homeostasis was reduced in both hypothalamus and cortex [162]. The activity of Ca2+ATPase enzyme was also affected by reactive oxygen species produced during ageing. The increase in net intracellular calcium concentration is a major pleiotropic death-signaling event which interfaces with both necrotic and apoptotic mechanisms. In addition, to the above glutamate induced excitotoxicity has been interlinked with Ca2+ influx in ageing and SDAT [163].

3.10 Animal models for ageing and SDAT

In respect to the above contents and discussions that both ageing and SDAT share similar mechanistic pathway therefore, the use of aged rodents are employed for studying the intricate relationship between ageing and SDAT as well as development of therapeutic interventions. There are few studies which have revealed the use of rats and mice of different age as a preliminary model of SDAT. The age of an animal is an important criterion to be known while studying the age associated neurodegenerative disorders like SDAT. On studying the developmental stages of rodents, it was found that both mice and rats showed a similar developmental profile. Ageing rats have been used extensively for determining the efficacy of drugs on age-dependent memory impairment, and the underlying

neurochemical changes. Much focus has been laid on investigating the ageing brains cholinergic system as a model for SDAT. A study by Pepeu [164] noted a statistically significant impairment in the acquisition and retention of a passive avoidance conditioned response in rats of 16 months of age, and the severity of the impairment gradually increased with age. Also a statistically significant impairment in object recognition was determined at 20 to 22 months of age, using a 60-min intertribal time, whereas the 16 to 18 months only showed a slight reduction of the discrimination index in comparison with the 3-month-old rats [165].

Loss of cholinergic neurons has been observed in cognitively impaired rats of 14-18 months age [166] and a large decrease in the level of acetylcholine (ACh) release from the cerebral cortex, hippocampus, and striatum regions have been reported in 19 months old Wistar rats [167] whereas, the motor activity and feeding behavior were similar in 14-19 months old rats and young adult rats.

Studies on age-related behavioral differences in mouse shows that in the passive avoidance test the number of rat failing to reach the end point increased in 9 months old rat than 3 months and that 10 months old rat need a higher number of trials compared to that of 3 months old rat in T maze test [168]. The impairments were found to increase more progressively at 23 and 31 months of age. Saucer et al, found impairment in the acquisition and retention of the water maze task in 18 to 19 months old rat which was associated with a decrease in the volume of cholinergic neurons [169].

Two main limitations have been noted in the ageing animal model. Primarily these animals don not develop the neuro-pathological picture as seen in AD and secondarily the ageing animals easily obtained improvement in cholinergic hypo function and the cognitive deficits on treatment with drug, but this efficacy was difficult to obtain in clinical trials [170-172]. As overseen, that the age-associated loss of cholinergic neurons can be corrected in aged rodents is an important for both the therapy of AD and age associated memory impairment. Finally, the validation of drugs potentially active on AD is determined from the observation that cholinesterase inhibitors (ChEI) correct both cholinergic hypo function and cognitive deficits in ageing animals [173]. For this reason demonstration of

activity in ageing animals is an unavoidable step in the development of new drugs for SDAT.

3.11 Current Treatment strategies for cognitive impairment and SDAT

At the current status there is no drug which cures or acts effectively on cognitive impairment or SDAT. Donepezil approved by the Food Drug Administration (FDA) is the most commonly prescribed acetylcholine esterase inhibitor (AChEI) for the past 14 years. Currently only 5 drugs have been approved by FDA for treating AD, these include four drugs under AChEIs and one N-methyl-daspartate (NMDA) antagonist. The first drug approved by the FDA is the Cholinesterase inhibitors which act by enhancing the cholinergic transmission in SDAT subjects. Though tacrine was effective as an AcHE inhibitor, its use was inhibited due to its high prevalence of hepatotoxicity [174]. Further three other drugs emerged under the AChEIs: Donepezil (1996), rivastigmine (2000) and galantamine (2001) were also approved by FDA in the following years. These drugs were considered the standard and used for the first-line treatment for AD. Several reviews and double-blinded, randomized, placebo-controlled trials (RCT) conducted on these drugs showed benefit on cognitive functions, Daily living activities (ADL) and global function in patients with mild to moderate AD; Significant difference in efficacy was not noticed between individual AchEi drugs [175, 176]. Donepezil was also used in the treatment of severe AD. However, long term efficacy of AChEIs still remains controversial [177, 178] but continuous treatment was found beneficial and suggested for subjects if well-tolerated [179]. The efficacy of AChEI in preventing the subjects with mild cognitive impairment (MCI) from the progression to AD is inconclusive [180].

A new class of drug mentamine was approved in the year 2003 by FDA which is a voltage gated and uncompetitive NMDA antagonist with moderate affinity. This drug was approved for treating the patients with moderate to severe AD. Mentamine protect the neurons from excitotoxicity as demonstrated by double-blinded, parallel-group, RCT studies, it also improved the cognitive function, ADL and behaviors in subjects with moderate to severe AD on treatment for 6 months. This drug was found well tolerated, except in some group of patients who developed agitation. Other systemic review on the

drug indicated that memantine may reduce behavioral and psychological symptoms of dementia in people who was treated for 6 months.

3.12 Healthy brain ageing as a neuroprotective strategy for prevention of age related neurodegeneration.

According to the current drug strategy there are no proper treatment for curing age related neurodegenerative disorders like SDAT therefore there is a need for long term neuroprotection and prevention of disease progression. Multiple studies have suggested that ageing and its associated risk factors may be prevented and reversed in order to gain healthy ageing. Scientists have demonstrated that processes associated with ageing can be slowed, or reversed by modifying the behavioral and lifestyle interventions such as exercise, diet, and/or stress reduction [181]. Similarly, neurobehavioral ageing, including cognitive decline, may not be inevitable. The experts confirm that there is a need of an interdisciplinary approach to transform normal ageing to successful ageing which involves behavioral, biomedical, nutritional and other interventions [182]. Therefore, effective neuroprotective agents are of prime necessary which may promote successful ageing in terms of the optimization of life expectancy and minimizing physical and mental deterioration, thus acts as a preventive measure rather than being used as a curative in elderly population with chronic conditions.

3.13 Plants as neuroprotective agents

Plants are always a rich source of biologically active compounds which can be used as a source for therapeutic interventions for the prevention and management of age related neurodegenerative disorders like SDAT. Medicinal plants were used as remedy both in traditional and folklore medicine system in India. Ayurveda is one of the oldest systems of medicine in India. “Rasayana chikitsa” in ayurveda portrays the preservation and promotion of health by rejuvenating the whole functional dynamics of the body organs. “Rasayana” are class of drugs which act in the biological system by modulating the neuro-endocrine-immune systems and have been found to be a rich source of antioxidants [183]. The properties of plants used in the Rasayan class of drugs are anti-ageing, re-establish youth, strengthen life, brain power and prevent diseases [184, 185]. Several researches have

been conducted on the plants used in the rasayana class of drugs to modern context. Puri et al accounted on the plants used in preparations of rasayan drugs [186-189]. Udupa studied the effects of 'Rasayana' drugs on psychosomatic stress [190]. Singh & Murthy, studied the effect of drugs to treat epilepsy [191], convulsive disorders [192] and to reduce anxiety, apprehension and keep the mind calm and cool [193].

Plenty of studies has been undertaken to provide scientific evidence to the Rasayana drugs as immunomodulators and adaptogens. Wagner after a detailed study concluded that rasayana preparations, which act both as herbal immunostimulant and adaptogens, regulate the immunological and endocrine systems with relatively low doses, without damageing the auto regulative functions of the organisms [194]. Rasayan drugs have been reported to treat generalized weakness [195] and provide protection from cyclophosphamide-induced leucopenia [196]. Many traditional medicinal plants are used as crude samples and mixture with a reputation of alleviating or preventing symptoms of neurodegeneration [197].

3.13.1 Some of the common and popular drug used in the rasayana drugs and their activities

Centella asiatica was reported for its cognitive enhancement property which was due to its antioxidant property [198], its immunomodulatory property was also reported, and the acetylcholinesterase inhibition and antidepressant activity of *centella asiatica* in rat brain [199]. The major compound Asiatic acid was found to impart protection against glutamate-induced cytoxicity [200].

Bacopa monnieri is widely known in the Ayurvedic system of medicine it helps to prevent ageing and degeneration. It is also a brain stimulant and help in improving memory and cognitive abilities [201].

Ashwagandha *Withania somnifera* noted to improve the behavioral dysfunction in scopolamine induced rats [202]. Withaferin A, a major component in *W. somnifera* was found to inhibit acetylcholinesterase activity in brain and in cholinergic markers of cognition in rats [203]. Other components of Withania were found to prevent Amyloid beta peptide

(25-35) induced neurodegeneration and helps in regaining neuronal functions by inducing neurite outgrowth [204]. Root sample of *W. somnifera* act as a potent immunoregulator with confounded anti-inflammatory activity [205].

Celastrus paniculatus (CP), an indigenous plant of India has been extensively used for its cognitive enhancement property [206]. The seed oil of *C. paniculatus* was found effective in reversing the effect scopolamine-induced memory loss [207] and exhibited significant exhibited antioxidant properties.

Nardostachys jatamansi- increases the levels of monoamines and inhibitory amino acids viz. NE, DA, 5-HT, 5-HIAA, and GABA [208]. The root sample of this plant has been used clinically in Ayurveda for their anti-ischemic, antioxidant, anticonvulsant, and neuroprotective activities [209]. Several researches have noted the neuroprotective activity of polyphenols.

The evidence based for the efficacy of the medicinal plants were based on their a) literary and conceptual evidence, b) evidences based on long traditional use and c) new scientific evidence which was being attempted recently with the help of selected experimental and clinical studies.

In the present study the use of polyherbal formulation which consists of *B. monnieri*, *H. rhamnoides* and *D. bulbifera* were comprehensively studied for their neuroprotective mechanism of action and were reviewed further.

3.14 *Bacopa monnieri*

Figure 3.2 ***Bacopa monnieri***

3.14.1 Family: Scrophulariaceae

3.14.2 Synonyms: Bramia monnieri Pennell, Moniera cuneifolia Michx. Herpestis monneira (Linn.), Herpestis spathulata Blume, Gratiola monniera Linn, Lysimachia monniera Linn

3.14.3 Vernacular names: Water Hyssop, brahmi, whole plant jal brahmi, and nir-brahmi.

Bacopa monnieri (L), belong to the family Scrophulariaceae, it is an indigenous plant found throughout the Indian subcontinent. The plant has been employed in ancient system of medicine. The plant is commonly known as Brahmi which originates from the word "Brahma", the mythical "creator" in the Hindu religion. Since, the brain is the region of creativity and as the compounds present in *B. monnieri* improves the brain health; hence it is called as Brahmi. The plant is used as a revitalizing agent in ayurvedic system of medicine since 3000 yrs. The plant is classified under medhyarasayana class of drugs, i.e. drugs that act on the Brain and cognition. The pharmacological use of *B. monnieri* is recorded in the ancient ayurvedic text "charaka samhita" (6^{th} centaury AD). The drug is also used in management of mental conditions including anxiety, poor cognition, lack of concentration, and the Bravprakash, Var-Prakarana (16th century A.D.).

3.14.4 Morphology

The genus consists of more than 100 species distributed throughout the tropical region of the world. It is an annual creeping plant found throughout the Indian in wet, damp and marshy areas [210]. *B. monnieri* is a small herb having purple flowers, numerous branches and small fleshy, oblong leaves. Flowers and fruits appear in summer. Stem is prostrate, succulent and herbaceous. Leaves are decussate, simple, oblong, 1-0.4 cm, succulent, punctuate, penninerved, margin entire, apex obtuse, sessile. Flower is axillary, solitary, bracteate, linear, pedicel to 0.5 cm, purple in color. Calyx is 5 lobes (unequal), outer 2 lobes larger, oval, 7-3.5 mm; inner 2 lobes linear, 5.5-0.7 mm; median 1 lobe oblong, 5.5-2 mm, imbricate, (sub)succulent, punctuate, obtuse, acute. Corolla is white with violet and green bands inside the throat, 0.8 cm across, 5mm tube; 5 lobes, obscurely 2-lipped, 2+3, (sub) equal, obtuse or emarginated. Stamens are 4, didynamous; filament pairs 1 and 2.5mm anthers oblong, contiguous, 1.5 mm; ovary: oblong-globose, 2 mm; style slightly deflexed, 5.5 mm; stigma flat capsule, oblong-globose, 5-2.5 cm septicidal or locilicidal or 4 valved.

3.14.5 Active constituents

The compounds responsible for the biological activities of the plant are alkaloids, saponins and sterols. The first reported compound isolated from *B. monnieri* is an alkaloid Brahmine [211]. This was followed by the isolation of other alkaloid compound such as nicotine and herpestin [212]. Isolation of D- mannitol and saponin, herpasopnin ad potassium salt was carried out by Shastri M.S. [213] The major effect neuropharmacological compound responsible for the nootrophic activity of *B. monnieri* is bacoside A, assigned as 3-(a-L- arabinopyranosyl)-O-b-D-glucopyranoside-10,20-dihydroxy-16-keto-dammar-24-ene [214], this compound usually occurs within the Bacoside B which is an artifact compound produced during process of isolating bacoside A [215]. Bacoside A yields into mixture of aglycones on acid hydrolysis, Bacogenin A1, A2, A3 [216-218]. Which are artifact of two genunine sapogenins, jujubogenin and pseudojujubogenein and bacogenine, A4 is a lactone pseudojujubogenin was then isolated [219]. Further three dammarene-type triterpenoid saponins such as bacosaponins A, B, C, pseudojujubiogenin were isolated followed by bacoside D [220]. Two new pseudobiogenin glycoside was then isolated and desiginated as bacopaside I and II which were isolated from glycosidic fraction in methanol [221]. Subsequently newer saponins bacopasides III, IV and V were isolated. [222]. Later in addition to these three new phenylthnoid gylcosides, monnierasides I-III along with plantainoside B was isolated from the glycoside fraction of *B. monnieri*.[223]. Lastely another new saponin jujubiogenin named bacopasaponin G was reported [224].

3.14.6 Neuroprotective effect of *Bacopa monnieri*

The cognition-facilitating effect of *B. monnieri* was due to two active saponins, bacosides A and B, present in the ethanol fraction of the sample [225]. These compounds facilitate learning and memory in rats and it also inhibits the amnestic effects caused by scopolamine, electric shock and stress. The bacosides induce membrane de-phosphorylation, with a concomitant increase in Protein and RNA turn over in brain tissues. The protein kinase activity is enhanced in the hippocampus on treatment with *B. monnieri*, which could also contribute to its nootropic action [226]. The administration of *B. monnieri* for two weeks, on Colchicines induced rats, reversed the depletion of acetylcholine, and reduced the enzyme acetylcholinesterase activity and decrease in muscarninc receptor binding in the

hippocampus and frontal cortex regions of the brain [227]. The plant also lowered the noradrenaline level and increased the 5HT in hippocampus, hypothalamus and cerebral cortex. The sample produced an anxiolytic effect which was much better compared to the standard benzodiazepine, since it promoted memory in both animals and humans. *B. monnieri* sample was found to have significant antidepressant activity in rodents which was assessed through forced swim and learned helplessness paradigms of depression [228].

The anticonvulsive action is one of the traditional use this plant [229]. The beneficial effect of *B. monnieri* against AD was studied in PSAPP transgenic rats, where both short and long term administration of the extract reduced the Aβ (amyloid beta) 1-40 and 1-42 levels in the cortex of rats brain and also reversed the behavioral deficits in PSAPP rat [230].

The protective effects of bacoside A on cigarette-smoking-induced changes in brain was studied by Anbarasi and co-workers. Bacoside A inhibited the lipid peroxidation, and improved the activity of ATPases [231] and also prevented the structural and functional impairment of mitochondrial membranes in the brains [232] of rats exposed to chronic cigarette smoke. Moreover, bacoside A prevented cigarette-smoking-induced Hsp70 expression and neuronal apoptosis [233]. It was also found to prevent the cigarette-smoke-induced activity of creatine kinase (CK) and its isoforms (CK-MM, CK-MB, CK-BB), sensitive markers used in the assessment of cardiac and cerebral damage through its antioxidant activity [234]. Administration of bacoside A improved the antioxidant status, in chronic smoke exposed rats which was evidenced by increased levels of reduced glutathione, vitamins C, E, and A, and activities of SOD, catalase, glutathione, glutathione peroxidase. Based on animal studies results, bacosides were shown to have anti–stress property [235]. It was suggested that the adaptogenic properties of the herb would be beneficial in the management of stress related conditions [236]. An increase in the activity of CYP 450 dependant enzymes 7–pentoxyresorufin-odealkkylase (PROD) and 7–ethoxyresorufin–o-deethylase (EROD) was observed in all the brain regions after exposure to stress whose magnitude was reduced on *B. monnieri* sample treatment.

3.14.7 Other biological effects

The Ayurvedic text has also mentioned the use of *B. monnieri* in other physiological conditions besides memory enhancing. The anti-inflammatory, cardiotonic and other pharmacological effects of *B. monnieri* samples was evaluated and studies revealed that *B. monnieri* effectively suppressed inflammatory reactions by inhibiting prostaglandin synthesis and partly by stabilizing lysosomal membranes without causing gastric irritation in inflammation induced rats [237]. The juice of *B. monnieri* is reported for its anti-ulcerogenic activity [238]. *B. monnieri* extract in the dose of 1000mg/ml showed anti-helicobacter pylori activity [239]. The anticancer activity of plant sample was reported in Walker carcinoma and it also inhibited Sarcoma-180 cell growth *in vitro* [240].

B. monnieri extract possessed relaxant properties in blood vessels and tracheal preparations from rabbit and guinea-pigs with a partial contribution by β-adrenoreceptors and prostaglandins [241], it also produced bronchodilation in anaesthetized rats which supports its folklore medicinal property as respiratory disease healer [242]. The ethanol sample of *B. monnieri* acts as a calcium antagonist as assessed by inhibited spontaneous movements of both guinea-pig ileum and rabbit jejunum [243].

The plant possess a direct effect on the influx of calcium ions into cells which was demonstrated by the calcium chloride-induced responses in rabbit blood vessels and jejunum were attenuated in the presence of plant sample (10–700 mg/ml) [244].

3.14.8 Clinical studies

B. monnieri has been found to improve various aspects of cognitive function in children and adults as seen in many clinical trial reports. The learning, memory, perception and reaction times was improved in 20 primary school children who were given Bacopa syrup of a dosage of 350mg three times daily for three months [245]. Children with attention deficit hyperactivity disorder (ADHD) were found to benefit from Bacopa administration as reported by a randomized, double blind, placebo-controlled trial for a period of 12 weeks which was assessed through tests of sentence repetition, logical memory and paired associate learning tasks [246].

In an open trial 12 gm of *B. monnieri* was given to 35 adults with anxiety neurosis for 4 weeks and the anxiety level, concentration and memory span were all significantly improved with no significant side effects [247]. A recent study conducted in Australia demonstrated that *B. monnieri* may be effective for enhancing cognition in longer term of administration rather than short term.

The speed of information processing, learning rate and memory consolidation and state anxiety levels was improved in forty six healthy adults between 18-60 years of age treated with *B. monnieri*, as seen in a randomized, double-blind, placebo-controlled design [248].

The ability to retain information over time was improved on *B. monnieri* treatment as measured by a task requiring delayed recall of word pairs this may be due to the decrease in forgetfulness. A double-blind, placebo-controlled study in which bacosides were administered in various single doses (ranging from 20mg to 300mg) as well as multiple doses (100mg and 200mg) to healthy male volunteers for one month demonstrated an absence of any side-effects as noted by singh & Dawan. The plant is approved by TGA in Australia, and it is considered safe with no adverse reactions as recorded in the literature. The traditional recommended dosage regimen is 5-10g of the powdered dried herb daily [249].

3.15 *Dioscorea bulbifera*

Figure 3.3 ***Dioscorea bulbifera***

3.15.1 Family: Dioscoreaceae

3.15.2 Synonyms: True yam, Air potato

3.15.3 Vernacular names: Varahi, kaachil, gonth, kolkand and dukkarkhand

Dioscorea bulbifera (L); belong to the family dioscoreaceae or true yam. It is a native species of Asia, Africa and Australia. The perennial vine has broad leaves with two storage organs system bulbils and tubers and grows up to 150 feet [250, 251]. About 50 species of dioscorea is found in India, many in wild state which are bitter in taste.

3.15.4 Habitat and Morphology

It is one of the largest genuses, containing 600-800 species. Tubers of Dioscorea have been used throughout the world as a food and herbal medicine. *D. bulbifera* is an annual twining herb, distributed throughout the moist tropics of world and extending into warm temperate regions. They are found growing at elevations of 2438.4 to 4572 m. in Himalayas. It is a large unarmed climber with stems twining to the left. Leaves are alternate, simple, broadly ovate cordate. The leaves are triangular and bitter. Tuber solitary, variable, globose to pysiform, usually small and round but large under cultivation, skin earth colored usually coated with abundant small feeding roots; Inner part of the tuber is white to lemon yellow. The tubers are used as food. Those of the wild forms are bitter and acrid, but can be rendered edible by coursing with ashes and steeping in cold water; the tubers are used for the preparation of starch. The tuber is used by the tribal population of central India as a food particularly in Madhya Pradesh, Chhattisgarh, Jharkhand and Orissa. Fruits are large, tuberculate, bright pinkish orange, seeds 1-4, immersed in pulp and ripe fruits are edible.

3.15.5 History of Human Use

The plant Varahikand (*D. bulbifera*) is not mentioned in Vedas. In Ayurvedic literature Varahikand is mentioned as one of the ingredients of Chywanprash in Charak Samhita (C.Ci 1/69). Later on various therapeutic uses of this drug is mentioned in Susruta samhita. According to Sushruta, Varahikand is used to alleviate pitta (S.S.Ci 7/10). In the form of Varahi ghrita it improves life span and relief sinus (S.S. Ci 27/11). It works as Rasayana (S.S. Ci 30/5).

According to Astang Hridya, Varahikand is used as Medadi ghruta for treatment of Pittaj Kash (A.H. Ci 3/38). It is used as Amritprash ghrita in Kash Chikitsa (A.H. Ci 3/94). Varahikand is used as Dhatri ghrita for Rajyakshma and apasmar chikitsa (A.H. Ci. 3/108). The drug is also used in the treatment of Dah, Atisar and Pradar (Leucorrhea) in form of Rasnadi kalp (A.H. K. 4/12). In Kaideva Nighantu, Vrinda-Madhav (12^{th} Century AD) and Gada-Nigraha the use of *D. bulbifera* has been mentioned as a rasayana agent. Sushruta has also mentioned the rasayana property of *D. bulbifera*.

3.15.6 Active constituents and its biological effects

Many active phyto-chemical constituents have been isolated from the plant *D. bulbifera* and their therapeutic action has been identified. The anti-oxidant and immunomodulatory activity of this plant is also evaluated by Bhandari RM [252]. *D. bulbifera* contains phyto-estrogen and thus it prevents the early decline of estrogen level in females. The active constituents of *D. bulbifera*, diosbulbin B and diosbulbinoside D enhances the adiponetic level in case of insulin resistance, dyslipidemia and obesity [253, 254] associated with various clinical conditions, hence it has potential in the prevention and management of various neurodegenerative and cardiovascular disorders.

D. bulbifera contains many phytomolecules acting on different bio-chemical parameters. Diosogenin a steroidal sapogenin is the product of hydrolysis by acids, strong base or enzymes of saponins sampled from rhizome of *D. bulbifera*. This sugar free aglycone diosogenin; is used as natural phytoestrogen. The presence of phytoestrogen can prevent the early estrogen deficiency, dyslipidemia, atherosclerosis and cardiovascular incidents among menopausal women [255, 256].

Diosbulbinoside isolated from *D. bulbifera* is responsible for reducing inflammation in the body and also act as a potent anti-inflammatory and anti-oxidant drug [257]. *D. bulbifera* is also responsible for reducing glycemic index as well as reducing hyperlipidemia [258].

D. bulbifera has been used in Ayurveda and folk medicine as purgative, deflatulent, aphrodisiac, rejuvenating and anthelmintic tonic and also as a medicine for hematological disorders, scrofula, hemorrhoids, flatulence, conjunctivitis, diarrhea,

dysentery, worm infestations, general debility, diabetic disorders, polyuric and skin disorders [259, 260]. It is also seen that species of Dioscorea has also been used traditionally for treatment of memory-related diseases such as AD and other neurodegenerative diseases [261].

3.16 *Hippophae rhamnoides*

Figure 3.4 *Hippophae rhamnoides*

3.16.1 Family: Elaeagnaceae

3.16.2 Synonyms: Seabuckthorn

3.16.3 Vernacular names: Badripal

Hippophae rhamnoides (L) belongs to Elaeagnaceae family widely known for its nutritional and medicinal value. *H. rhamnoides* are nitrogen fixing thorny deciduous shrub which are found in cold arid region of Eurasia. The plant is now being domesticated in many parts of the world [262].

3.16.4 Plant Description

The plant is deciduous dioecious, branched shrub covered by thorns, usually growing up to 3.4 m in height. They have nitrogen fixing root showing Frankia actinorhizal symbiotic associations. Leaves are alternate, narrow, sliver gray color and lanceolate. Male bud consists of 4-6 apetalous flowers and female consists of single flower with an ovary and one ovule. The female plants produces berry like fruit of 6-9mm in diameter, soft, juicy and rich in oils, the berries are orange/red in color when ripe, with single seed surrounded by soft fleshy outer tissue. Seeds are ovoid in shape, glossy, dark brown of 2.8-4.2mm [263].

The number of species under Hippophae is not known, whereas 7 species have been considered. The species of Hippohae which have been found in India are *H. rhamnoides*, *H. salicifolia* and *H. tibetiana* of which the major one is *H. rhamnoides*. They are usually distributed in cold and arid deserts of north east Himalayas (2590-4175m above sea level). *H. rhamnoides* is distributed in the regions including china, Mongolia, India, Nepal, Pakistan, Russia, Lativa, Romonia, Great Britain, France, Denmark, Netherland, Germany, Poland, Finland, Sweden, Norway and Canada. The plant has 8 subspecies [264].

3.16.5 Active constituents

The plant contains various bioactive compounds both in berries and leaves, which are of special interest and the plant material has being screened for selected compounds. Content such as carotenoids, tocopherols, tocotrienols, essential polyunsaturated fatty acids and other bioactive components in the berries and polyphenols in the leaves are of great interest to many researchers [265, 266].

3.16.5.1 Fruits

The berries of *H. rhamnoides* have a unique composition, combining a number of components usually only found separately. Variation of the active components occurs with fruit maturity, fruit size, species, geographic locations, climate and methods of separating [267, 268]. The berries are orange-yellow to red color on ripping are a rich source of valuable compounds such as vitamins (C and E), carotenoids (beta-carotene, lycopene, lutein and zeaxanthin), flavonoids (isorhamnetin, quercetin, isorhamnetin-3-betad-glucoside; isorhamnetin-3-beta-d-glucosaminide; kaempferol, etc.) organic acids, amino acids, micro and macronutrients [269, 270]. Bioactive compounds isolated from the berries of *H. rhamnoides* are Hippophae cerebroside, oleanolic acid, ursolic acid, 19-alpha-hydroxyursolic acid, dulcioic acid, 5-hydroxymethyl-2-furancarbox-aldehyde, cirsiumaldehyde, octacosanoic acid, palmitic acid and 1-Ohexadecanolenin. The compound Isorhamnetin, isolated from *H. rhamnoides*, showed significant antioxidant activity [271]. Zeaxanthin and betacryptoxanthin esters which are used as food additives, cosmetic ingredients or nutraceuticals were identified on chromatographic analysis [272, 273]. In addition to the plethora of antioxidants, the berries are also rich in fatty acids (saturated

13.7% and 86.3% unsaturated) including palmitic acid, oleic acid (omega-9), palmitoleic acid (omega-7), linoleic acid (omega-6), and linolenic acid (omega-3); and phytosterols.

Seed oils are the most recognized product of Hippophae, that is enriched in essential fatty acids (omega-3 and 6) and pulp oil that contains high levels of omega-7 [274]. The oil of *H. rhamnoides* is the only oil that naturally provides a 1:1 ratio of omega- 3: omega-6 (linolenic and linoleic acid respectively). The major component of the phyto-sterols identified from the seed oil is sitosterol [275, 276]. Carotenoids lipoprotein rich complexes have also been extracted from the berry of *H. rhamnoides* pulp and several studies showed that carotenoids and fatty acid esters are more stable in their supra-molecular lipoproteic complexes, which are stored in oleosome vesicles where their physiological functions are better kept [277-279]. In most European and Eastern countries, Beta-carotene content acts as quality indicator in *H. rhamnoides* oils. Oils extracted from the fruit pulp and seeds of *H. rhamnoides* absorb ultraviolet light and promote healthy skin and act as raw material for the pharmaceutical and cosmetic industries.

3.16.5.2 Leaves

The leaves of *H. rhamnoides* contain nutrients and bioactive substances which include flavonoids, carotenoids, free and esterified sterols, triterpenols, and isoprenols. The leaves contain equal amount of important antioxidants including beta-carotene, vitamin E, catechins, elagic acid, ferulic acid, folic acid and significant values of calcium, magnesium and potassium. The polyphenolic compounds in the leaves are flavonols, leucoanthocyanidins, (−) epicatechin, (+) gallocatechin, (−) epigallocatechin and gallic acid. The study conducted by Shipulina et al. [280], found that the tannin fraction isolated from leaves had principal components which were hydrolysable gallo- and ellagi-tannins of monomeric type like strictinin, isostrictinin, casuarinin, casuarictin. Few bioactive phenolic constituents, such as quercetin-3-O-galactoside, quercetin-3-O-glucoside, kaempferol and isorhamnetin were quantified from the aqueous and hydroalcoholic samples of the leaves by RP-HPLC [281]. In recent studies these compounds showed antioxidant, cytoprotective and anti-bacterial effects as studied by using various *in vitro* systems and analysis of marker compounds was done using by RP-HPLC.

The plant is drought and cold resistance and hard in nature. It protects farmland system by its vigorous reproduction and strong, complex nitrogen fixing roots nodules [282]. All parts of the plant are rich in bioactive substance like vitamins (A, C, E, K riboflavin, folic acid) carotenoids (alpha, beta, gamma-carotene, lycopene) phytosterols (egosterol, stimasterol, lansterol, amyin) organic acids (malic acid, oxalic acid) polyunsaturated fatty acid and essential amino acids for human use.

3.16.6 History of human use

The word *H. rhamnoides* has been derived from the Latin word "Hippo" meaning horse and "Phaos" meaning shine. It was used as a feed for animals to increase the body weight and improve coat texture especially in horse. Its pharmacological effects have been recorded in the classics of Yidian from Tang dynasty and Jing Zhu Ben Cao from Quing dynasty. As early as 900 AD, Tibetans have been using this plant. Its reference has been found in the ancient Tibetan medical texts including "The Rayud Bgi" dated to Tang dynasty (618-907 AD) [283]. Each part of *H. rhamnoides* has traditional value.

Since the beginning of tang dynasty, the Chinese have been using *H. rhamnoides* as medicine dating back to 1000 yrs. The plants are been also used by many ethnic groups of Asia, Nordic countries and Baltic region as food, fuel, medicine, agriculture, bio fencing. The plant has been traditionally used for treating asthma, skin disease, gastric ulcer and lung disorders.

3.16.7 Biological effects

The berries were used as herbal medicine, food supplement and skin care agent in Europe and Asia. Mongolians and Tibetans used the berries for the treatment of sputum and cough and to improve blood circulation and digestion. Hippophae was used for the treatment of skin disease, jaundice, asthma, rheumatism and gastro-intestinal treatment by Russians and people of Himalaya [284]. In central Asia people used *H. rhamnoides* as a drug for hypertension, ulcer, uterus erosion and digestive disorder and skin disease. The oil was found beneficial in treating gastritis, ulcer, uterus erosion and inflammation, also the dried berries infusion were used for skin disorder [285, 286]. The plant was used for

rehabitation and to control soil erosion by the Germans [287]. Current researches have now supported many of the traditional use of *H. rhamnoides*. The plant has been recently reported for its pharmacological activities like anti-oxidant, immunomodulatory, anti-atherogenic, anti-stress, hepatoprotective, radioprotective and tissue repair.

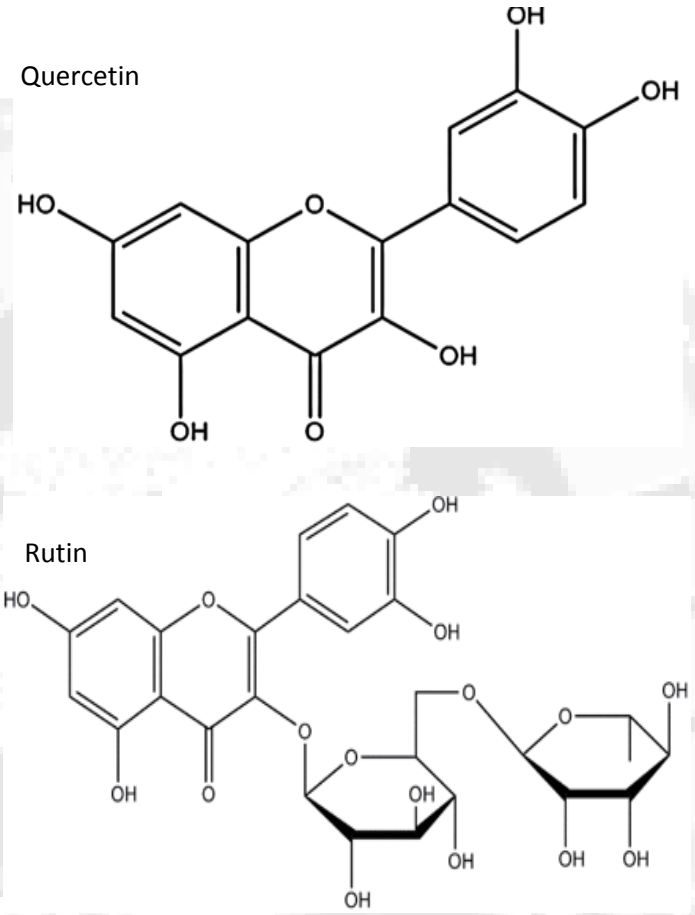

Figure 3.5 Structures of Quercetin and Rutin

Thus with the above known properties of the plants from the literatures a novel polyherbal formulation was developed by combining the hydroalcoholic extract of the three plants to assess its neuroprotective mechanism of action using *in vitro, in vivo* and clinical studies.