pathology through anti-stress, free radical scavenging, neurohormonal regulation and inhibition of neuroinflammation.

II. REVIEW OF LITERATURE

Alzheimer’s type of dementia is one of the foremost progressive neurodegenerative disorders principally persuade memory loss with intra-neuronal fibrillary tangle pattern and cerebral parenchyma accumulation by ß-amyloid(25-35) protein in typical form of plaques. The most primitive remarkable indication is failure of short term reminiscence (amnesia) (Selkoe, 1991). Amnesia occurs in numeral brain disorders, in those the destruction of cognitive aptitude represents a turn down in prior function levels and also hinders the capability to execute habitual activities as well as daily functions. On progression of Alzheimer’s disease (AD), cognition of remote events and acquired information decline. Behavioral conflict comprises agitation, violence, declined mood, insomnia and apprehension.

The range of dementia advances in the group of 60-65 years (Sharma et al., 1997) AD type of dementia may be presenile or senile and is challenging to median age group individuals (Sloane et al., 2002). In 2004 AD was the 7th leading disease which causes death with 65,829 numbers (Hebert et al., 2003). Presently the death rate escalated and it was estimated that 5.4 million Native Americans of variable ages have AD
in 2011 (Alzheimer’s Association, 2011). This survey indicates 5.2 million people at the age of 65 and above, 200,000 patients below the age of 65 those are considered as patients have younger-onset AD. One in eight (constitutes 13%) of the individual has AD type of dementia. Half of the people of 85 years old and more (43 percent) have AD. In United States and other countries, one of the disease which cause high rates of death and risk factors is AD. Indication of death rate for other diseases is presently declined but still AD deaths polls high. Between 2000 and 2008, preliminary data indicates the death rate of AD increased up to 66%.

NEUROPATHOLOGY OF ALZHEIMER’S DISEASE

The major pathogenesis which exist to explain the cause of disease are

AMYLOID HYPOTHESIS

It is well recognized that the principal component of senile plaques stirring in AD was Aß-peptide 1-40 and 1-42 sequenced amino acid, a product of amyloid protein precursor (APP) (Kang et al., 1987). Secretases are the enzyme which nicks the sequence of Aß. Both in the case of \textit{in-vitro} and \textit{in-vivo} Aß produces potent neurotoxicity (Pereira et al., 1999). The primary neurotoxic sequence of amino acid was (25-35) of beta amyloid and is highly neurotoxic for primary cortical neurons (Seubert,
1992). In normal cells of healthy humans Aβ is formed and it can be evidently present in cerebrospinal fluid as well as in plasma as circulating peptide (Rang and Dale, 2005).

Aβ production entails the bifurcation at two dissimilar spots, together with one in transmemberane sphere of APP by both β and γ-secretases. Secretases is a clumsy enzyme, lacks accuracy and cleaves APP in unusual points by identical vicinity, generating Aβ splinters of diverse lengths constituting Aβ-40 and Aβ-42. Amyloid theory points to cytotoxicity through aggregated mature fibrils and are assumed to be potential toxic form of protein accountable for distressing cell calcium ion homeostasis and leads to apoptosis (Yankner et al., 1990). These changes in calcium channels cause excess influx and cascades the neurotoxicity (Mark et al., 1997).

The magnitude of AD has three organized aspects of life cycle with the role as Aβ initiation; construction, its degradation and aggregation. During the process of Aβ in normal cells, in the step of degradation, some molecules of peptides escape from intracellular or extracellular degredation and accumulate to form aggregates of Aβ peptides (oligomers or polymers). These oligomers or polymers are highly cytotoxic.
Cell biology of Amyloid protein precursor (APP)

The specific gene encoded for AD was located in chromosome 21. In this Down’s syndrome AD patients the APP cascade was identified and mapped out (Selkoe, 1999). After the cloning of gene on chromosome 21 it was purified and sequenced to form microvascular fragments of amyloid deposits. Amyloid protein precursor is expressed in heterogeneity because of the alternative splicing and post translational modification in heterogenetic forms, the residue splice of 751 and 770 are expressed throughout the body especially in non-neuronal cells. 695 residue isoform is majorly expressed in neurons of brain and also evidenced in neuronal cell cultures. In 751–770 isoform, an exon is present which encodes to a 56 aminoacid pattern and is homologus to serine protease inhibitors in Kunitz type. This 751-770 isoform was present in platelets, also share and have a role as factor XIa inhibitor in blood clotting mechanism. The “C” terminal of APP has hydrophobic stretch which anchors with Golgi network, endoplasmic reticulum and plasmalemma.

In the secretory pathway, APP cleavage undergoes between 60th and 70th aminoacid of AB region. During the cleavage, enhanced production of phrobol esters increase the shedding of TNF-α, which leads to cascading of inflammation. The principal APP comprising 770 amino
acids and consist of A-17 residue peptide present in N-terminus, an exon of 56 amino acid encoded at 289 residue with a inhibitor for serine protease enzyme. Another regions in APP was 700-723 transmembrane amino acid, constitutes a part of Aβ residue. Upon recognition of 687 residue of amino acid by α-secretase, it induces the secretion of δAPP-α in the medium to produce C83 fragment. These further undergo scission in 711 or 713 residue to release p340 and p342 fragment.

Another alternative scission was at 671 residue by β secretase activity resulting in δAPP-β molecule to produce C99 and also can undergo further cleavage by γ-secretase and 711 and 713 to produce Aβ_{40} and Aβ_{42} peptides

**CHOLINERGIC HYPOTHESIS**

The divergent projections of cholinergic innervations to areas of brain are

1. Cortex, hippocampus, basal forebrain, amygdala and thalamus
2. Lateral segmental neurons connecting to thalamus and cortex called Pedunculopontine.

Cognitive hypofunctions are the prime dysregulated activities in AD (Whitehouse, 1998). The neurons project to hippocampus and cortex, (Geula and Mesulam, 1994) are predominantly lost. Acetylcholine (Ach)
receptors in these discrete areas of the brain are reduced, especially in presynaptic nerve terminals (Nordberg et al., 1990).

In early AD or mild cognitive impairment (MCI), ChAT does not have much impact however; NGF and BDNF are reduced in nbM of myentric neurons and perform reduced repairing mechanisms. The above findings depicts the early treatments or up regulation of cholinergic system (Marta et al., 1988).

**Cerebral blood and cholinergic innervations**

Cerebral blood vessels (CBV) are surrounded by cholinergic system and it controls the microvessel activity. In AD the CBV are degenerated and reduced cerebral flow of blood was observed. These are believed as early manifestation of AD. These are also evidenced by the improvement of cerebral blood flow (CBF) in treatment of AD with acetylcholine esterase inhibition. The innervations starts from the cerebral arteries and travel through dura mater and branches further through pial arteries and produces intracellular arteries as well as microvascular capillaries. These contain acetylcholine and other vascular substances. These vascular subdivisions are innervated as intrinsic and extrinsic vessels (Van and claassen, 2011).
**Intrinsic Supply**

These supplied micro vessels consist of muscarinic and nicotinic receptor mediation. The brain cholinergic innervations are abundantly seen in diagonal band of broca center and other regions of medial septum and mynert’s nucleus basalis. Upon the stimulation of muscarinic receptor, the perivascular nerve terminal of cholinergic system in hippocampus and nucleus basalis dilates. These inturn activates the nitric oxide production and causes activation to release acetylcholine.

**Extrinsic Supply**

These innervations comprise adrenergic and cholinergic nerves of ANS. In perivascular post ganglionic non adrenergic neurons 7-nAch (nicotinic acetylcholine) receptors sub type is present and has a role in neuroprotective effects to enhance the memory. When these receptors are activated, vasodilation occurs. Upon vasodilation, sympathetic post synaptic nerves release nor-adrenaline (NE) which acts on neighbour presynaptic nerve terminals of nitrergic interneurons and produces nitric oxide. Various studies in animals shown that AChE inhibitors, inhibit 7-nAch receptors and inhibits the vasodilation to produce modulatory function between neurons and glia especially in brain injuries.

**ACETYLCHOLINE ESTERASE ENZYME (AChE) IN AD**
In cholinergic and non cholinergic neurons, AChE is predominantly found and it hydrolyzes acetylcholine (Atack et al., 1983). In and around the deposit of amyloid plaques AChE was increased and it promotes, the Aβ-peptides to fibrillay form to enhance the toxicity. It was evidenced with in vitro investigations; AChE enhances the formation of normal Aβ-peptides into aggregatory fibrils and lead to Aβ fibrillary complex with AChE. This has higher toxic effect than Aβ peptide. The $K_m$ and $V_{max}$ values are expressed in high kinetic property for the Aβ-AChE complex. These formed complexes are insensitive to inhibitors of AChE and are highly resistant for low pH. In CT-100 expressing transgenic mice Aβ induces the release of AChE (Sberna et al., 1998). In P-19 cells AChE is induced after the treatment with Aβ due to over expressed entry of calcium, through L-type voltage sensitive calcium channels indicate the changes in calcium homeostasis cause exitotoxicity and neurodegeneration. In tissues of nervous system, muscles, circulating blood, plasma AChE is present.

AChE exist as quartenary structure as a complex polymorphic nature. Molecular AChE exists in two forms such as asymmetric and globular form. Asymmetric forms preferentially distributed in neuromuscular area. Globular form subsists as monomeric, dimeric or tetrameric catalytic forms. These also seen as soluble secreted form. In
mammalian CNS, it’s found as molecular type bound with hydrophobic peptide. In nerves, blood cells and muscles, AChE is linked to the membrane with glycolipid anchor. In human brain, AChE is exceedingly glycosylated through three prospective glycosylation spots (Vincenzo, 2001).

**Role of AChE in Non cholinergic site:**

AChE apart from hydrolysis of acetylcholine, especially implicated in differentiation of cell and proliferation, as well as in stress and for the formation of amyloid peptide.

**Loss of AChE molecular forms in AD**

In AD brain, there is a discriminating loss of dynamic G4 globular type which is mostly seen in cortical and sub-cortical regions. Postmortem AD brains depicted a decline in the ratio of G4/G1 molecular form due to the membrane bound AChE loss. This type of AChE is especially found in cholinergic synapses and is a marker for the site. The G4 type corresponds to 80% of overall AChE action in axons.

*In-vitro* incubation of Aβ peptides with AChE promoted the aggregation and fibril formation and the intensity of formation of amyloid fibrils was three fold increased than Aβ only. This character was also
exerted by other molecular types of (G1, G2 and G4). The domain responsible for the production of Aβ into amyloid fibrils exists in monomer of the structure. In AD patients the excessive glycosylation of AChE is seen in frontal cortex and in cerebrospinal fluid and is due to the G2 and G1 structure.

**MONOAMINE OXIDASE ENZYME IN AD**

One of the main neurotransmitter metabolic enzymes that metabolize catecholamines and serotonin in brain as well as in periphery was MAO. In CNS monoamine oxidase exists in two isoforms such as MAO A and B. Catecholaminergic neurons consist of MAO A isoform and serotonergic neurons and glia consist of MAO B isoform (Westlund et al., 1988).

In Alzheimer’s disease brain both MAO-A and MAO-B have been concerned its etiology. Increment of MAO-A in Alzheimer’s neurons, produces increased neurotoxic metabolic products, activates free radicals and leads to neuron loss. In several areas of brain such as hippocampus, cerebral cortex and platelets of Alzheimer’s patients have elevated level of MAO-B action with increased mRNA for MAO B. Especially MAO-B was appeared to be elevated in coupled with Aβ containing plaques. As like the MAO B, MAO A isoform is also increased in the all parts of cortex in brain with specific mRNA. In locus ceruleus, whole action is dropped
with 31% in AD patients, however was lead with 80% decline in neuronal count, implicating the activity of the enzyme per neuron (Burke et al., 1999a). Evidences indicated that treatment with MAO-A inhibitor be able to progress cognitive function in AD (Burke et al., 2001).

Alteration in MAO-A and B action may influence to produce Alzheimer’s disease with definite genetic variations in promoter region of the enzyme. Genetic variants of these enzymes materialize to influence an extensive range of brain disorder including AD. As cholinergic hypofunction predominates to AD, selective disturbance of cholinergic neurons in hippocampus leads to the resultant elevation of MAO-B. Loss of neurons leads to gliosis and glia illustrate to comprise elevated concentration of MAO-B (Oreland et al., 1980). This elevated MAO-B in AD emulates higher numbers and stimulation of glia and this is observed in cortical areas of brain. As stress is inducted, corticosteroids are elevated in brain causing excess secondary activation of MAO-A and B and are evidenced in old rats and in cultured astrocytes. Another possible factor for increase in MAO levels in AD was aluminium ions and aluminium deposits in brain.

**OXIDATIVE STRESS IN AD**
All cells generate reactive oxygen species, principally superoxide as a product of molecular oxygen reduction in mitochondria. One of the major parts in the human system which is highly affected by free radicals was brain. Of the oxygen supplied to body, 20% is utilized by brain even it composes only 2-3% of body mass. As brain has extreme lipid content, increased metabolic rate with weak free radical scavenging activity and heavily contributes for transition metals, it’s an ideal target for the free radicals.

The oxidative stress due to free radicals and its sequestration in mitochondria are implicated in the various neurodegenerative diseases including Alzheimer’s type of dementia (Xiongwei Zhu et al., 2004). The cerebral metabolism is dysregulated in AD brain and extensively it is concerned with the mitochondrial function (Aliev et al., 2003; Gibson et al., 19981). All mitochondrial enzyme activities are reduced including cytochrome oxidase (COX), α-ketoglutarate dehydrogenase (KGDHC) and pyruvate dehydrogenase (PDHC). The dynamic changes in these enzymes leads to the abnormal production of ROS since COX participates in mitochondrial electron transport chain which interacts with molecular oxygen.
The loss of molecular oxygen activity increases the by-production of superoxide anion radicals which makes the back up of electrons in complex III site, leads to escalation of ROS in mitochondria. Elimination of ROS requires their chemical detoxification with the help of electrons provided through Krebs tricarboxylic acid (TCA) cycle. Consequently, any deficiency in PDHC and KGDHC of TCA cycle, favours the activation of ROS.

In neurodegenerative disease, there was deregulation of cellular iron metabolism which impairs the iron homeostasis in the disease. The iron regulatory protein (IRP) -2, was increased in AD and selectively linked through neurodegeneration (Smith et al., 1998). The concentration of iron is increased in AD and implied a decrease in ferritin (Connor et al., 1995). IRPs are implicated in intracellular iron homeostasis regulation and interaction of protein ferritin with a conserved RNA called as iron-responsive element (IRE). The dysfunctioned interaction of the IRP/IRE complex causes impaired iron homeostasis (Pincro et al., 2000) and leads to the neuronal vulnerability.

- The role of oxidative stress in AD indicates infinite variation in diverse biomacromolecules in neurons. Oxidation of DNA and RNA is indicated by elevated levels of 8OHdG and 8OHG (8-hydroxyl-2-deoxyguanosine and 8-hydroxyguanosine). Repairing of DNA was
higher in AD brain, which is indicated by nicking and fragmentation (Maurer et al., 2000).

- In AD brain, nitration of tyrosine compounds and increased levels of protein carbonyls indicates the susceptibility of oxidation in protein moieties. With the help of proteomic technology some oxidized proteins were identified and they are related to energy dependent enzymes, ex: enzymes involved in ATP synthesis. This indicates the metabolic changes and other oxidative modifications in AD brain (Cras et al., 1995). Thiobarbituric acid reactive substances (TBARS), isoprostane, malondialdehyde (MDA), 4-hydroxy-2-transnonenal (HNE), increased glycation, glycoxidation and altered phospholipid components are expressed in Alzheimer’s patient’s brain and plasma (Castellani et al., 2001).

- The elevated levels of 8OHG and nitrated tyrosine residues in AD with Aβ deposition causes further progression of disease and particularly seen in Down’s syndrome type (Nunomura et al., 2000).

**BIOGENIC AMINES ON AD**

The concentration of biogenic amines (nor-epinephrine, dopamine) and dopamine β-hydroxylase in AD patients are decreased. The biogenic amines improve fluency as well as creativity. In locus cerules, amyloid
deposition causes neuronal degradation and declined level of monoamines (Alan, 1996).

Immense biological, physiological and psychological functions are regulated by balanced concentration of serotonin in brain. This includes behavioural attitudes such as mood, apprehension, excitement, violent behavior and judgment abilities. Even though serotonin was the major neurotransmitter for behavioural effects, Dopamine and nor epinephrine also manipulates mood & arousal. High amounts of serotonin cause relaxation, mild sleep and reduce the ability of sexual drive.

Major mechanism behind the changes of neurotransmitter levels in AD as well as major depression was also linked with serotonergic and cholinergic systems as because the acetylcholine induces the release of serotonin, mediated by nicotinic receptors in neuronal junctions (Curcio and Kemper, 1984). During brain development and in matured brain, serotonin assists synapse configuration and preservation, by this means it transforms total figure of synapse (Lucian et al., 2007). The main region for cognition which associated with serotonergic neural system was found in raphe nuclei’s dorsal and ventral projection, in AD the serotonin signaling was declined with multifactorial pathological conditions. Injury of raphe nuclei induced by 5-HT 7-D subtype impairs
working memory. Serotonergic neurons are important for the acquisition of operational reminiscence and exploratory behavior.

**THE GLUTAMATERGIC SYSTEM**

Principal excitatory transmitter in brain is glutamate and its pathway derived throughout cortex with connections to striatum, hippocampus, thalamal region, substantia nigra and brainstem. Glutamatergic neurotransmission is essential in long-term potentiation and responsible for the learning and memory.

In AD patient’s severe dementia is caused due to the loss of neurons in this principal excitatory neurotransmitter system (Francis et al., 1999). In different neurodegenerative diseases, extreme activation of NMDA receptors may lead to the neuronal injury and death. The NMDA receptors are divided into three main types according to their selective agonist: α-amino-3-hydroxy-5methyl-4-isoxalopropionate (AMPA), N-methyl-D-aspartate (NMDA), as well as kainate. In the NMDA receptor, one of the regulations of cations (calcium and sodium) influx is dependent upon redox state, during which it reacts with oxidized form of nitric oxide to form S-nitrosothiol. Intracellular calcium is vital normal physiological processes of human brain, but disproportionate and abnormal quantity contribute to excitation of normal activity and damages the neurons. Neuronal amplification of calcium can trigger a
sequence of enzymes with protein kinase C, xanthine oxidase, nNOS, proteases, phosphatases and phospholipases (Lipton and Rosenberg, 1994). The phospholipase generates reactive oxygen species (ROS) and reactive nitrogen species (RNS) by triggering cascade of arachidonic acid. To sustain low intracellular free calcium amounts, the cationic exchange pumps in the endoplasmic reticulum in plasma membrane and mitochondria of the neurons have to function with considerable metabolic energy. Mitochondrial dysfunctions leading to condensed ATP levels generate excitotoxic lesions \textit{in vivo} (Beal, 2000).

In ordinary physical conditions, exitatory neurotransmission was magnitude to avoid excitotoxicity. Certainly, the glutamate concentration in neurons is more or less 10 mmol/l in intracellular area, whereas the glutamate concentration in extra cellular area was estimated to be 0.6mmol/l. Considerable excitotoxic disruption to cortical or hippocampal neuronal cells in intact tissue is predicted to happen during the concentration of extra cellular glutamate escalates to 2-5 mmol/l. Hence, it is essential to prevent excitotoxicity by compartmentalization of the extra cellular glutamate concentration. With the involvement of sodium-dependent high affinity system glutamate (and aspartate) is cleared from extra cellular space and transported to astrocytes and neurons. This high efficient glutamate uptake system operates for
transporting glutamate from micromolar concentration in extra cellular fluid into millimolar concentration in cells.

Any abrupt changes that impairs the cell sodium gradient due to ATP depletion, i.e. mitochondrial dysfunction causes neurotoxicity through enlargement of astrocytes and liberates of glutamate. The astrocytes take part in a fundamental function in the fate of glutamate in extra cellular space of neuronal cells by glutamate-glutamine cycle. Astrocytes acquire up glutamate and convert it to glutamine by the action of glutamine synthetase with the consumption of ATP, and release for uptake in neurons. Neurons then transfer glutamine back to glutamate with the help of glutaminase. This sequence can be interrupted by blockers of glutamine synthetase, an enzyme which is easily denatured by oxidative stress (Levine, 1983).

The translation of glutamate to glutamine in astrocytes might be impeded with oxidative stress, leads to intracellular glutamate accumulation and its discharge in extra cellular space. Finally, disproportionate response to glutamate-receptor-mediated activation occurs intracellularly (Lipton and Rosenberg, 1994). These leads to the abnormal production of proteolytic enzymes, ROS, RNS and leads to lipid peroxidation and trigger’s neuronal death for neurologic diseases. So in
the current treatment possibly, those drugs which act on glutamergic system (Memantine a weak NMDA antagonist) recover the cognition, through reducing exitotoxicity by excess calcium. Other drugs endorse glutamate receptor association (AMPAkines and co-agonists) also effective in the treatment of AD.

INFLAMMATION IN AD

In AD brain, local inflammation was noted with enhanced pro-inflammatory cytokines. Reactive astrocytes, activated microglia and complement system are notably participated to the progression of Alzheimer’s associated cognitive impairment. Further inflammatory process was marked by increased free radicals and these inflammatory mediator acts as co-factors to escalate the production of Aβ deposits (Mark et al., 1995). The mediators, astrocytes principally established in neuroglial cells of CNS, produce other chemokines and effector molecules to mediate various immunological responses (Raivich et al., 1999).

In neurodegeneration, triggering of inflammation causes activation of microglia for enhancing APP (Amyloid protein precursor) in-turn enhances Aβ production to make a cruel circle of amyloid progression. Interleukin-6 is over-expressed in alzheimer’s brains and elevates fibrillation of Aβ and forms plauque. IL-6 appears to play multiple roles in a variety of brain functions, since this cytokine may affect cell to cell
signaling, coordination of neuroimmune responses and neuronal differentiation, growth and survival. In interleukins, IL-1 was highly neurotoxic element in AD. IL-8 expressed in pro-inflammatory cytokines. IL-8 is low-molecular-weight proinflammatory protein formed by wide variety of cells to amplify chemotactic substances including microglia and astrocytes.

Aβ peptide stimulates the assembly of cyclooxygenase (COX-2) in neuroglial cells. Elevation of COX-2 expression in astrocytes adjoining Aβ coordinates to neurotoxicity and damages the brain. The microglia plays an important function in cellular response during neuropathological lesions, such as neuritic plaques and tau proteins (Streit, 2004). Aβ peptide has a property to sequester and stimulate the microglia to leading to deposit around its site in the brain. Microglia can communicate the scavenger receptor which mediates union of the microglia to coated surfaces Aβ fibrills, this in turn leads to secretion of ROS and cell immobilization (El Khoury et al., 1996). Activation of ROS can lead to promote the neurodegeneration through the free radical oxidative pathway. During the exposure of microglia to Aβ, expression of major histocompatibility complex II (MHC II) was increase in the neuronal cell surfaces causes and increases the secretion of the pro-inflammatory cytokines interleukin-1, 6 (IL-1 and IL-6), chemokines
interleukin-8 (IL-8), macrophage inflammatory protein-1 (MIP-1), as well as monocyte chemo-attractant protein-1 and tumor necrosis factor (TNF) (Rogers & Lue, 2001).

Cascading of Aβ causes microglia activation through advanced glycation end products receptors (RAGE) and colony stimulating factors (M-CSF). M-CSF itself can induce microglial chemotaxis, proliferation, increased macrophage scavenger receptor expression, and enhanced cell survival (Lue et al., 2001)

The expression of mRNA responsible for COX-2 and its protein are higher in AD brain and it up regulates neurodegeneration through activation of harmful prostanoids and other free radicals (Rohrenbeck et al., 1999).

**COMPLEMENT ACTIVATION IN AD BRAIN**

Complement activation structure constitutes 30 proteins which plays an important function in the host resistance and targets the regulation of inflammation (Morgan and Gasque, 1996). The activation of complement takes place into three pathways; through Mamalian binding lectin, classical complement and alternative complement pathways. These are activated by various activators. Altogether these all merges to generate an enzyme, called C3 (complement factor 3) which will be the liable factor
for production of the activation products that leads to opsonisation and pathogenolysis, also enhances phagocytes and other peptide mediators of inflammation to activate the Membrane Attack Complex (MAC) (Murphy et al., 2008).

In AD brain, the complement was found to be expressed in affected regions and indicated the up regulated levels of classical pathway (Eikelenboom and Stam, 1982). The high source of complement was neurons, in addition to it, complement is abundantly found in glial cells. The complement receptors are expressed in microglia, astrocyte and oligodendroglial, astrocytoma culture cells (Hosokawa et al., 2003).

The complement protein concentrations were highly present in plasma of blood (Kang et al., 2009) and the passage of complement in to the brain from blood plasma was restricted by the blood-brain barrier (BBB). Even though, the BBB prevents the entry, in neurodegenerative disease especially on AD it compromises the entry. In neurodegenerative diseases the initial process was the breakdown of BBB in concern with the secondary effect of complement. In brain the activation of complement was carried out through classical complement pathway. Especially in Alzheimer’s type of neurodegeneration it was confirmed that the neurofibrillary tangles and neuritic plaques are have a potential role
for the activation of complement system (Mc Geer et al., 1989). In the absence of antibody, the Aβ plaques are accomplished for triggering classical complement pathway in AD brain (Rogers et al., 1992).

The Amyloid-β peptide which deposits in AD brain, binds on the particular site surrounded by collagen-like domain present in the C1q. The primary component responsible for the activation of classical complement pathways are C1q. Aβ Fibrills and the tau protein also been indicated to interact on C1q through one of the 3 spherical heads of C1q, ghB and leads to the activation of both classical and alternative C3 pathways (Kishore et al., 2003).

The activation of both complement pathways may leads to the chronic mild inflammation during the progression of disease. The Amyloid β peptide deposition which are colocalized along with C1q, appear during the period of cognitive dysfunction in AD. These are also evidently ruled out in C1q deficient mouse which expressed decreased neuropathological symptoms when compared with satisfactory C1q mice. The cross breed of C1q deficient mice with APP swedish mutation mouse model, produced decreased Aβ constituted AD mouse, which implicates that the influence of complement. (Fonseca et al., 2004). The C1q was also claimed to damage the neuronal integrity, because of reduced
amounts of synaptophysin and MAP-2. At chromosome 1 in the 1q32 location on the cluster complement genetic proteins, CR1 (complement receptor 1) was found.

The gene was encoded on a four co-dominant alleles, constitutes various ranges and the variations are because of genetic multiplications or deletions. This progression was considered to arise due to imprecise crossing over of chromosomes (Holers et al., 1987). The changes in chromosomes are pre-translational, and this has been indicated by observation of similar variation in the unglycosylated transcripts (Lublin et al., 1986). These roles of genetic polymorphisms indicates the involvement of Aβ cascade which leads CR1 to induce the etiology of AD.

**NEUROENDOCRINE CHANGES IN AD**

Neuronal cells are specific to be affected by stress. Stress can impair the process of learning and memory and causes associated cognitive dysfunctions (Walesiuk et al., 2009). Hippocampal cells are predominantly affected by stress. As stress targets brain cells, the integrity of neurons are lost and the performance of spatial and declarative memory are affected (Sapolsky et al., 1986). In mammalian brain glucocorticoid receptors are abundantly distributed in
hippocampus and mediate the negative feedback of stress through HPA (Hypothalamus-Pituitary-Adrenal) axis.

In stress condition the HPA axis is activated and produces an escalated level of corticotrophin releasing factor (CRF). This CRF release from the pituitary activates adrenal cortex to secrete the glucocorticoids. Thus in plasma increased levels of glucocorticoids were seen which produces stressful condition. Through glucocorticoid receptors and mineralocorticoid receptors (GRs and MRs) the events of gene expression were sequenced in CVS, lymphatic system and nervous system. This gene expression maintains the homeostasis in stressful condition (De Kloet et al., 1998). The major quantity of secreted glucocorticoid have higher affinity towards the MRs and the occupies the receptors, in contrast the remaining level of hormone binds to the GRs. Histologically high densities of GRs-expressing units of cells were found in anterior pituitary, hypothalamus, amyglada and hippocampus and are found to be involved in stress (Morimoto et al., 1996). Glucocorticoids are also plays an important role in survival and senescence of neurons. At physiological concentration, glucocorticoid protects the neuronal cells from excitotoxicity.
Higher plasma corticosterone concentration activated by stress response or exogenous administration of glucocorticoid were suggested to impairs gene expression levels and impairs the neuropeptide Y (Conrad and McEwen, 2000) which is responsible for brain development. Other factors such as BDNF and NGF are also dysregulated (Hansson et al., 2003; Murakami et al., 2005). It is also suggested that the prenatal exposure of corticosterone in rodents impair the behavioural activity through altering mRNA expression (Welberg and Seckl, 2001).

Corticosterone is the principal hormone of glucocorticoids which regulates the genomic expression, metabolism in neurons and alteration in cell morphology. During chronic stress normal physiological function of hippocampus was lost with inability to perform long-term potentiation as well as primed-burst potentiation (PBP) (Diamond et al., 1996) and leads to amnesia. Unceasing stress or unceasingly increased concentration of corticosterone, generate neuronal deterioration and death in hippocampus. In addition they induce various changes in the concentration of neurotransmitters (Adrenaline, nor-Adrenaline, 5HT and Gamma-Aminobutyric acid (GABA). Corticosterone causes neurodegeneration in Alzheimer’s brain through reduced activity of GABA<sub>A</sub> receptors especially in hippocampus. Therefore benzodiazepines are used to hinder stress-induced dendrite deterioration.
The nitric oxide synthases commencing endothelial cells (eNOS) and neuron of brain (nNOS) were inspired by raise in intracellular \( \text{ca}^{2+} \). Inducible NOS (iNOS) mediates the immune function by calcium-independent manner. These three nitric oxide synthase utilize NADPH for electron donation and take up 5 cofactors of enzymes to produce NO from arginine by production of citrulline. As a messenger role, NO enhances the neuronal plasticity in various brain regions like hippocampus and cerebellum.

NO involves in long-term potentiation of hippocampal tissue indicates the synaptic plasticity and is anchored for the spatial learning. Spatial information is processed in the CA1 region of hippocampus and plays an important role in the cognition (Morris, 2007). Memory process involved with NO in hippocampus was evidenced in rats by inhibiting NOS, impaired the memory of working in runway task (Ohno et al., 1993) and also indicated in inhibitory avoidance with impaired memory retention (Bernabeu et al., 1995; Fin et al., 1995).

Together with hippocampus Nitric oxide (NO) is considered to involve for synaptic plasticity for the memory in numerous brain regions. The NO/ cGMP pathway provide a varied implications in human physiological system. NO involvement in excitatory tissues needs rapid and restricted
release of nitric oxide to target cells. This controlled and restricted release of NO indication is primarily synchronized at the biosynthetic level of NO. There are three different nitric oxide synthetase (NOS) isozymes which has their own corresponding genes. The NOS from endothelial cells (eNOS) and neurons (nNOS) is constitutively indicated enzymes, whose functions are stimulated through activation of intracellular calcium. Immune properties of nitric oxide are carried out by Ca\(^{2+}\)-independent inducible nitric oxide (iNOS). In neurons the highest NO levels were found which involved in the long term potentiation in hippocampal cells through retrograde messenger system (Hawkins et al., 1998). The treatment with NOS inhibitors are mediate to generate the memory deficits in a passive avoidance task in rats (Kopf et al., 2001), and impaired the olfactory learning in rats. It also has been indicated that antagonism of NOS prior to training significantly despaired the food-finding aptitude of snail *Helix pomatia* (Teyke, 1996).

Nitric oxide plays a key role between its function and glutamate receptor mediated increases of cytoplasmic Ca\(^{2+}\) which was essential for the long term potentiation and synaptic function for the formation of short and long term memory (Yun et al., 1999). Consequently, these imply that the activation of eNOS and nNOS isoforms is essentially
MEMBRANE BOUND ATPase IN AD

Action of Na⁺/K⁺-ATPase pump plays a pivotal function of neuronal cells. Na⁺/K⁺-ATPase regulates and controls membrane potential, controls the volume of cell. It re-establish Na⁺ and K⁺ ion gradients following the neuronal excitation to afford energy (through Na⁺ gradient) for supplementary transportors (Na⁺/Ca²⁺ replace and re-uptake of neurochemicals). In AD brain, thalamal region and nbM has impaired function of neuronal Na⁺/K⁺-ATPase. Amyloid beta protein has the capability to reduce the activity of Na⁺/K⁺-ATPase by changing the structure of enzyme, leaving irreversible and also increases the intracellular calcium. The increase in intracellular calcium leads to further neurodegeneration (Bores et al., 1998).

NITRIC OXIDE IN AD

Nitric oxide was synthesized in mammalian cells from L-arginine (L-Arg), oxygen and NADPH in the presence of NO synthases (NOS) and other enzymes from cytochrome family. Existence of nitric oxide synthases in three isoforms with their appropriate gene functions in various aspects of physiological role (Schmidt et al., 1994).
involved in the retrieval and memory formation through cGMP-dependent increase of glutamate with excitatory amino acid.

**PATHOGENESIS INVOLVING ALIPOPROTEIN**

ApoE is a constituent of high density lipoprotein (HDL) and very low density lipoprotein (VLDL) and its composites arbitrate cellular uptake and cholesterol metabolism. ApoE take part in regulation of lipid metabolism after injury of axon in the CNS and PNS (central nervous system and pherephreal nervous system) (Ignatius et al., 1986).

During the axonal injury, the released cholesterol was sequestered and scavenged by ApoE. After the neuronal uptake of free cholesterol with ApoE, it produces complex to form ApoE–lipoprotein complex which in-turn furthers cyclized to release cholesterol and is transported to terminals of neurons for neuronal growth and synaptogenisis. ApoE3 and ApoE4 have different action in neurite growth and development. In case of complex with ApoE3 and VLDL neurite outgrowth was increased, but with ApoE4 an inhibitory activity is exerted.

In AD, aged people with ApoE4 allele are most vulnerable in disease progression, have abnormal neuronal loss with decreased repairing capacity (compensatory outgrowth of neurons and
synaptogenesis). In ApoE4 allele, only amyloid plaques deposition causes dementia and other pathological characters comprising AD are least contributed (Schmechel et al., 1993). When compared to ApoE3, ApoE4 have a potential property to bind with Aβ and promotes the fibrillation of amyloid protein. Immunohistochemical studies with ApoE revealed the association of ApoE in the presence of Aβ plaques.

**GENETIC BASIS OF AD**

25% cases of all patients affected by AD are due to genetic factors. As it is a familial inherited pattern, it can be determined by genetic tests and familial history. In common, these types of AD are hereditarily encoded as an autosomal dominant disorder and have a chance of 50% transmission of mutated gene from the affected individuals to their offsprings during every pregnancy. The types of genetically based AD are classified as familial forms of late onset (AD2) and three early-onset (AD1, AD3, AD4), respectively and a type of AD related to Down syndrome.

**Down’s syndrome and Alzheimer’s Disease**

These patients generally affected by AD after 40 years of old. The gene responsible for APP, which initiates the cascade to produce different sequence of Aβ is present in chromosome 21. The type of AD occurs, when there is a presence of 3 copies of genes established on chromosome
21 and this defect is called Down syndrome also called as trisomy 21. Because of this trisomy excess protein was cascaded and increases the progression and establishment of the disease (Glenner et al., 1984). 1% population affected by Down’s syndrome is due to the chromosomal defect.

**Early-onset familial AD**

Early onset familial AD constitutes 2% of diseased population. These types of early onset familial AD (AD1, AD3 & AD4), show the progression of disease and severe cognitive dysfunction at the age of 60. By the age 40-50, onset of symptoms usually occurs, but the same symptoms can also be elicited as in early 30 years. Clinically these types of AD patients exhibit the same manifestations as in the adult-onset type of dementia, with short term memory loss and cognitive impairment. AD type 1 (AD1) roughly constitutes 10-15% and its involvement in disease progression is through a protein called Presenilin 1. This Presenilin 1 encodes a gene named PSEN1 present in chromosome 14, which gets mutated to exaggerate the disease. AD3 records about 20–70%, expressed due to the mutation of Amyloid protein precursor present in chromosome 21 where amyloid beta A4 protein was encoded. Another type, AD4 is the mostly rare type, which is expressed due to the mutation in Presenilin2 protein
and encoded with a gene called PSEN2 located on chromosome 1 (Vetrivel et al., 2006).

**Late-onset familial AD**

AD2 (late-onset familial) type records more or less 15–25% of dementia cases. The cases of AD2 are apparently in differentiable as of sporadic cases during clinical observation but they can be predictable on the basis of molecular genetic evaluation. Conversely, in these cases there is no prominent chromosomal location of the gene is accountable. Genetic mapping implicated the involvement of multiple genes for the initiation of this type of AD; but chromosome 19 contains ApoE-e4 gene associated with AD2 in familial cases and elicits dementia through unknown mechanism. Over all there are many genes implicated in AD2 induces the risk of symptoms which are located in various chromosomal positions in case of different families (Selkoe et al., 1997).

**INSULIN SIGNALING**

Brain insulin receptor (IRs) transmits signals for neuronal activity and is implicated to participate AD. Neurohormonal changes with insulin, in case of type 2 diabetes mellitus initiates the development of AD. In AD brain, expression of insulin was high, especially in hippocampus and cortex. Insulin signaling increases the release of acetylcholine, however it
stimulates intracellular trafficking and decreases intracellular insulin levels and worsens to AD progression. In AD brain, insulin reduces the activity of enzyme called insulin degrading enzyme. This enzyme has a pivotal role to metabolize monomeric acetylcholine in neural networks. Loss of brain IRs is linked to pathogenesis of age-related neurodegenerative diseases (Frolich et al., 1998).

IR in brain has faintly low mol. weight when compared with the receptors in periphery and they do not endure down-regulation even after exposing them to high insulin concentrations, rather they undergo modulatory role for glucose metabolism in neuronal through signal transduction mechanisms and synaptic functions (Zhao et al., 2004). Cholinergic hypofunction is expressed by IR desensitization due to condensed energy metabolism & acetyl CoA synthesis (Biessels et al., 2002). Present theoretical targets for the treatment of AD include up-regulating insulin-degrading enzyme (Eric et al., 2005).

**NEUROPEPTIDES AND NEUROTROPICS IN AD**

Neurotropics like vasopressin as well as adrenocorticotropic hormone are involved in hippocampal learning and memory (Pittman et al., 1982). In AD brain the concentration of vasopressin is decreased. Other neurochemical modulators such as somatostatin are also decreased in AD patients. Cerebral atrophy results in reduced release of neurotropic
factors which is required for endurance of cholinergic neurons. Administration of Nerve Growth Factor (NGF) improved the learning and memory properties of brain (Perry et al., 1992). Proper function of other neurotropic factors such as cerebrolysins, Epidermal Growth Factor (EGF) and Brain Derived Neurotropic Factor (BDNF) decreases the dreadful conditions of neurons and also they enhance the sprouting healthy cortical neuronal cells.

**ESTROGEN ON AD**

A well-known neural activity of steroids has a protecting role against any type of neuronal vulnerability and they are involved in the sprouting of neurons. Estrogen is a neuroprotective hormone against a wide nature of insults in different neuronal cultures in *in-vitro* and shows a neuroprotective role in various experimental models of rodent brain injury.

In age related memory diseases such as Alzheimer’s dementia, estrogen plays an important role to protect against Aβ-induced neurotoxicity in different pathways of neurodegenerative process. In the pathology of AD, assembly of Aβ sheet converts the amyliod peptides into the form of insoluble fibrils and soluble oligomers. These transformation leads to the neuronal death by over loading calcium ions, synaptic
disruption and promotes the pro-inflammatory pathways inducing gliosis (El Khoury et al., 1996)

The majority of indications in AD suggest that an up-streamed signaling flow produced by Aβ. Particularly the Aβ elicited neuronal death involves the establishment of JNK signaling and following the impairment of apoptosis-related proteins which belongs to Bcl-2 (Yao et al., 2005). In this the regulation of apoptosis, estrogen plays a key function to protect the vulnerability from Aβ.

Depend upon this hypothesis; the estrogen was thought to implicate the regulation of Bcl-2 family related proteins in neuronal cells (Stoltzner et al., 2001). There are two types of Bcl-2 proteins

- Bcl-2 proteins which promotes the survivalence of the cell - e.g., Bcl-2, Bcl-xL, as well as Bcl-w
- Bcl-2 proteins which antagonize the cell survivalence - e.g., Bax, Bad, Bak, Bik, Bid, BNIP3, as well as Bim

It was hypothesized that physiological concentration of estradiol inhibit the apoptosis of neurons through escalating the articulation of anti-apoptotic factor Bcl - xL and also Bcl - w during the down-regulating process of pro-apoptotic Bim (Yao et al., 2007). The pragmatic property of estradiol on Aβ elicited apoptosis were mediated through estrogen
receptor dependent mechanisms, and was well correlated by the anti-apoptotic action of oestradiol which are blocked by pre-treatment of estrogen receptor antagonist 

α and β receptors of estrogen are demonstrated to be crucially involved in the regulation on Bcl-2 expression as well as in neuronal survival (Zhang et al., 2001). Estradiol also enhances the Bcl-2 by Akt -dependent cyclic AMP responsive binding element activation. The estrogen regulations by Bcl-2 proteins are also evidently involved in protection of neurons through decreasing the excitotoxicity which are seen in Alzheimer’s type of dementia.

Substantial evidences imply that during the pathogenesis of AD, Aβ toxicity with excitotoxicity produced by glutamate considerately triggers the pathways leading to neurodegeneration. Excitotoxicity initiated by over loading of calcium ions in neurons during the imbalance of excitatory transmission. Estrogen diminishes the excitotoxic neurodegeneration produced by agonist of glutamate in cortical neuronal cell cultures. In neuronal cortical murine cells the estrogen protected insult from excitotoxicity and it was blocked pharmacologically with tamoxifen, an estrogen receptor antagonist. Furthermore, estrogen was found to enhance the intracellular Ca²⁺ in physiological doses of
glutamate in neural cells, but it inhibits the same on high concentration of glutamate at exitotoxic dose (Nilsen et al., 2002)

The regulation of Bcl-2 family proteins are involved in estrogen mediated protective mechanism on glutamate related neurodegeneration. Estrogen stimulates an ER-dependent Src / ERK / CREB signaling orientation which may leads to up-regulation of Bcl-2.

In account to the regulation of Bcl-2 family protein, the steroids are also implicated in neuroprotective activity against more well-known pathological conditions of AD including reduction of inflammatory cascade and reducing the oxidative stress, and accumulation of Aβ. Large supports specify that estrogen is a potent inhibitor for oxidative neurodegeneration (Vedder et al., 1999) and H₂O₂ mediated neurodegeneration.

Estrogen elicits the free radical scavenging activity through estrogen receptor-independent system. Pluri-potent property of the hormone was seen in glial function and its activation. During traumatic condition and neuronal degradation estrogen down regulates the reactive gliotic property (Garcia-Segura et al., 1999), also promotes the sprouting
of astrocytes through enhancing arborization and activating the synaptogenesis (Del Cerro et al., 1995).

The estrogen also inhibits the tau hyper-phosphorylation to protect the neurons in AD and other neurodegenerative disease. Progesterone and estrogen modulates the properties of phosphatases and kinases which are involved for the regulation of phosphorylation in tau protein. Especially estrogen and progesterone controls the phosphorylation of tau protein via the pathway of glycogen synthase kinase-3b enzyme (GSK-3b) (Goodenough et al., 2005). The activity GSK-3b can be reduced by estrogen and the expression of GSK-3b and tau are reduced by progesterone.

It have been well recognized that the both estrogen receptors (α and β) subtypes are extensively dispersed in brain, especially in discrete areas of brain such as frontal cortex, hippocampus, and amygdale region. (Hartley et al., 1999). In AD there was a subcellulular changes in the ERs. Further principally, the transfer of ER-α from nucleus towards the cytoplasm reduces the risk factor for development of Alzheimer’s type f dementia. Taken collectively, this study implies the expression of ER-α/β in AD and participates to an important role in neuroprotective function of estrogen.
TRACE ELEMENTS IN AD

Inorganic trace element aluminium is a metal plentifully present in earth. It’s easy to get access for the human body from the environment through respiratory system and gastrointestinal system. As in the cooking utensils, medicines of antacids and other food additives contain aluminium; it’s estimated that the daily intake is nearby 10-20mg (Edwardson et al., 1992). Accumulation of aluminium in brain was involved in the pathophysiology of AD, Guam-Parkinson’s dementia, amyotrophic lateral sclerosis Parkinson’s dementia complex (ALSPD) etc (Kurland, 1998).

Aluminium produces networks within the cholinergic neurons and intensifies the neurofibrally tangle formation to inhibit the conduction and generation of nerve impulse, ultimately leads to dementia (Gulya et al., 1990). Aluminium promotes the potentiation of free radicals in cell, leading to peroxidative damage to proteins and lipids. Oxide form of aluminium is an insoluble one and it does not produce acute toxicological response. Aluminium enhances the Aβ toxicity and it produces fibrillar tangles to impair the learning ability with increased acetylcholinesterase and MAO activity which is related to that expressed in AD (Zatta et al., 1999).
CURRENT APPROACHES IN THE TREATMENT OF AD

The Acetylcholinesterase inhibitors (ACIs) decrease the progression of neurodegeneration by reducing the levels of amyloid-β protein precursor (A-β PP), its assembly and amyloidogenic complexes (Mori et al., 1995). AD is not curable and currently available drugs in the market exert some symptomatic relief but they do not reduce the progression of disease. American Association for Geriatric Psychiatry suggested the statement for Alzheimer’s therapy during 2006 (Lyketsos et al., 2006).

**Acetylcholinesterase inhibitors**

Acetylcholinesterase (AChE) inhibition is primarily used all cases of AD and it is efficient and is important as it counteracts the reduction in acetylcholine indirectly by inhibiting AChE. These AChE-inhibitors decreases the activity at which acetylcholine (ACh) is hydrolyzed down and upregulates the concentration of ACh in brain.

AChE-inhibitors recover moderate symptoms of AD, but they do not modify or inhibit the underlying progression of disease. Four drugs in the category of ACIs approved by USFDA for treatment of Alzheimer’s dementia are Tacrine, Rivastigmine, Donepezil and Galantamine, out of the four drugs tacrine produces hepatotoxicity and so it is rarely used.
Other three drugs are devoid of hepatotoxicity. Donepezil was marketed as Aricept. Galantamine was marketed as Razadyne and in U.S it is marketed as Reminyl and Nivalin other parts of the world. Rivastigmine was marketed as Exelon.

**NMDA antagonists**

Current hypothesis of AD with the association of glutamatergic neuronal excitotoxicity was implicated in etiology of AD led to investigation, development and introduction of NMDA antagonist (Memantine). Memantine is a specific NMDA antagonist, which has moderate affinity towards the receptor. It is used for treatment in the cases with moderate to severe AD (Reisberg et al., 2003; Winblad et al., 1999; Tariot et al., 2004). Memantine blocks the NMDA receptors to prevent abnormal influx of calcium excitatory ion to inhibit the higher firing rates of neurons. After binding to the receptors memantine blocks the calcium channel in open state and allows slow neurotransmission at least stimulation rates. Memantine also well tolerated without much side effects and does not produce any adverse effects.

**Neurotropins**

Various studies investigated on the growth factor effects in adult CNS indicated positive approach for the treatment of Alzheimer’s disease. The
infusion of nerve growth factor (NGF) in adult rat brain completely prevented the death of basal forebrain cholinergic neuronal cells after injury as well as spontaneously (Fischer et al., 1987).

**Vitamin supplements and other antioxidants**

Several lines of supports specify that oxidative stress involves an important pathogenic development linked to aging and AD with senile plaques and NFTs (Castellani et al., 2001). Oxidative damage was concerned in etiology of neurodegenerative disorders and the treatment with antioxidants has been used as therapeutic agents in various types of brain disorders and diseases (Ahmad et al., 2005; Ansari et al., 2004). It was observed that the dietary improvements and consumption of fruits and vegetables which possess antioxidants as well as neuroprotective agents in natural origin may decreases the memory deficits such as AD type (Weinstock and Shoham, 2004). Currently Coenzyme Q10 (CoQ10), and vitamins such as lipophilic antioxidants, had reported to improve cognition and neurochemical alterations in hippocampal cells and cerebral cortex region of brain. Vitamin E was an essential nutrient, which functions as antioxidant in human body (Burton and Ingold, 1989). The antioxidative effect of α-tocopherol was markedly escalated by co-supplementation of vitamin C. Vitamin C is a hydrophilic antioxidant in human CSF (Schippling et al., 2000). This was the first antioxidant
consumed in plasma and cerebrospinal fluid during oxidative stress conditions (Frei et al., 1989) and gives first line of antioxidative defence mechanism for human body.

**Statins**

Recent studies proposed that the inhibitors of 3-hydroxy-3-methylglutaryl CoA reductase (HMG Co-A), also called as statins drugs, are efficient in reducing the prevalence of AD. In the brain, the effect of statins was not due to the local inhibition of cholesterol synthesis or reduced levels of circulatory cholesterol, but the synthesis of cholesterol through *de novo* pathway is thought to be down-regulated in the brain. Cerebral Aβ component are reduced *in-vivo* with simvastatin treatment and also the reduction in NFTs were noted in statin treatment (Fassbender et al., 2001)

**Non-steroidal anti-inflammatory drugs (NSAIDs)**

Epidemiological investigations indicate a protective action of NSAIDs on neurodegenerative diseases. In the management of AD, Aβ accumulation and plaque deposition were linked with innate immunity response which includes activation of complement, increasing of pro-inflammatory cytokines, appearance of chemokines, and excretion of NO which leads to apoptosis. NSAIDs down-regulates the pro-inflammatory signals,
astrocytes and microglia which may reduce risk of AD by lowering amyloid production (Breitner et al., 1994).

**Other drugs**

A plant extract *Ginkgo biloba* (EGb 761) is majorly used by old aged Alzheimer’s patient and it improves the cognition in AD patients. *Ginkgo biloba* is also used to treat all types of dementia and it also has the property to inhibit the aggregation of amyloid-β peptide (Le Bars et al., 1997).

**ANIMAL MODELS FOR EVALUATION OF AD**

For the screening of AD, different animal models were developed to reproduce the similar neuropathological, biological and behavioral alternations. Even though a wide variety of animal models in different species were developed, there is no single model which shows all the pathological changes but by means of different experimental tools (toxins) the required pathological condition can be produced in rodents, monkeys and hamsters (Wenk et al., 1992).

**Lesions of cholinergic basal forebrain nuclei models**

Generally cholinergic dysfunctional models were used for the evaluation of AD type of dementia, because the primary neuropathological change in
AD was degeneration of cholinergic nuclei. Experimentally the basal forebrain cholinergic system was destructed to produce AD model in rats and mice which express the cognitive impairment in behavioural and biochemical aspects (D’Amato et al., 1987).

- The neurotoxicity are produced by electrocoagulation, administration of cholinotoxin, AF64A are been implicated to decrease the cholinergic function. Chronic intracerebroventricular injection of quinolic acid induces similar behavioural and biochemical pattern exhibited in AD type of dementia. Mice, rats and hamsters are used for this neurodegeneration model.

- Lesions of cholinergic system in basal forebrain induce neurotoxicity in rats and monkeys (Voytko et al., 1994).

**Models of lesions of cholinergic basal forebrain nuclei**

In general, cholinergic system impairment plays a relevant role in cognitive dysfunction typical of AD. The degeneration of some cholinergic brain nuclei as those located in basal forebrain, is a main change affecting a specific neural system in the brain of patients with AD. Experimental animal models have been based upon the assumption that destruction of basal forebrain cholinergic neurons may generate cognitive impairment associated with dementia of AD.
Many types of acute manipulations, including electrocoagulation, use of excitotoxins, transaction of the fimbria-fornix and treatment with a cholinotoxin, AF64A, have been applied to reduce cholinergic activity. A chronic animal model with a continuous intracerebroventricular (i.c.v.) infusion of quinolinic acid was also developed to simulate the slow evolution of the neurodegenerative diseases including AD.

Lesion of cholinergic system in basal forebrain (BFCS) is related in rats and monkeys with cognitive dysfunction. In general, these models have been implied on behavioural transformation of neuronal cell degradation after the injection of cytotoxins which produce discrete lesions on BFCS of rodents and non-human primate. Ibotenic acid produces excitotoxicity on injection through i.c.v injection and causes extensive degeneration of cholinergic neuronal cells. The cholinergic degeneration was found in medial septum, nucleus basalis magnocellularis (NBM) and broca regions of brain (Coyle et al., 1983).

The exact neuropathological conditions of AD were generated by the animal models expressing senile plaques of Aβ peptide. Aβ linked mice and rats expressing human amyloid protein precursor were developed as transgenic models. In this APP
coded familial AD mice, the studies related to apolipoprotein mutation linked cognitive dysfunction are suitable. Transgenic mice models expressing APP751 indicates the histopathological symptoms of diffuse plaques and tau proteins similar to AD (Quon et al., 1991). These all animal models exhibited selectively loss of hippocampal memory and spatial learning on working memory. In APP mutated animals, the APP695 protein which are abundantly found in Swedish family of Early Onset Alzheimer’s Disease (EOAD) were found and are used for the screening mild cognitive impairment. The amyloid deposits in Tg 2576 mice exhibiting the prompt gliosis as well as the dystrophy of neurons, without the loss of CA1 neurons and with loss of synaptophysin activity in hippocampal region. In this model microglial activation were also found which provisions to evaluate the pro-inflammatory activity in AD brain (Frautschy et al., 1998).

- Injection of okadaic acid, phosphatase 1/2A inhibitor through chronic i.c.v. infusion induces the hyperphosphorylation in tau protein leads to neurofibrillary tangles (NFTs) and produces the memory impairment (Arendt et al., 1994).
- PS2 wild-type transgenic mice indicates the abnormal levels of Aβ_{42(43)} peptide. They were generated by mutation or knockout
in PS2 gene. In knockout mice the accumulation of amyloid β peptide -42 was noted on a dose dependent manner of gene responsible for PS1 and this mutation exhibits significant neurodegeneration with AD linked to familial mutation of PS1 (Oyama et al., 1998).

- The screening of drugs for neurodegeneration also done in senescence accelerated rodents and monkeys, which exhibits the amnesia similar to that of AD.
- Anoxia or hypoxia produced by ischemic reperfusion in rodents resembles the dementia of AD. Even though anoxic or hypoxic models does not show any neurodegeneration in specific brain regions, it shows a significant symptoms neurochemically and behaviorally and exhibit the despaired memory.
- Scopolamine is a muscarinic antagonist that causes blockade of central cholinergic system. It produces reversible blockade of the cholinergic neurons and produces impairment in attention maintenance, causes loss of acquisition in memory and learning. It is also reported to associate the memory impairment with oxidative stress and leads to the degeneration of neurons in basal forebrain cholinergic neurons. (Beatty et al., 1986)
- Administration of aluminum in rodents produces cognitive dysfunction as exhibited in AD. Aluminum ions displace the
other cation in the biological system from their binding sites and produces disruption of metabolic pathway. Aluminum is thought to be interacting with cholinergic neurons, intensifies the inflammation in brain and promotes reactive oxygen species to increase the peroxidative damages. Aluminum chloride 50 mg/kg/day oral dose produces the dementia and significantly increases AChE enzyme. It also has the property to disrupt the synaptic plasticity through interrupting Na/K\(^+\)ATPase and enhances the NFTs formation. The formation of NFTs inhibits conduction and generation of prime burst potential for learning and memory. In aluminum treated animal model loss of hippocampal memory in water maze and other biochemical changes resembles to the AD. Direct injection of aluminum into the brain also causes exitotixicity and leads to neurodegeneration in rodents. In rabbits and monkeys the application of aluminum paste in cerebral cortex also exhibited the neurooxicity produced as such in the Alzheimer’s disease (Syed et al., 2009)

- Intra hippocampal injection of A\(\beta_{25-35}\), scopolamine, ibotenic acid, glutamate and other cytotoxins causes the specific hippocampal memory loss which highly resembles the cognitive defect associated with spatial learning and memory, because
the major degeneration of neurons were found hippocampus of the AD brain. The injections were made by using atlas of rats and mice stereotaxic coordinates. Bilateral injection of the cytotoxins in to the striatum causes 25% of cholinergic hypofunction.

> Oxidative stress induced models are produced by i.c.v administration of streptozotocin (STZ) to rodents in a sub-diabetogenic amount produces extended energy metabolism in brain and its glucose transportations. This impaired energy metabolism causes oxidative damage and leads to cognitive impairment by hindering the production of ATP and acetyl-CoA. This eventually leads to cholinergic hypofunction allied with reduced choline acetyltransferase (ChAT) property in hippocampus (Hoyer et al., 2000) and provide a remarkable model with symptoms of sporadic dementia related to Alzheimer’s type (SDAT).

**REPORTED PLANTS STUDIED FOR AD**

1. The seeds of *Celastrus paniculatus* water extract (CP) had been described to progress the memory in rats and also shown a higher antioxidant properties with reduction of lipid peroxidation in rat brain. The study also reported that the Pre-treatment of rat fore-brain
neuronal cells with aqueous extracts *Celastrus paniculatus* significantly protected the glutamate induced neurotoxicity in neuronal cells (Godkar et al., 2004).

2. *Bambusae concretio Salicea* a therapeutic herbal plant used for treating the symptoms of hypertension and brain disorders. The pharmacological evaluations done in this plant indicates the protective effect on Aβ(25-35) peptide-induced neurotoxicity. There was a significant increase in antioxidative enzymes and reduction in lipid peroxidation activity on astrocyte cells incubated with the water extracts of this plant (Jeong et al., 2005).

3. The ethanolic extract of *Bacopa monniera* (BMEE) leaf was investigated on serotonergic system in post-natal rats for cognitive function. In this study the rats were treated with the ethanolic extract in the dose of 40mg/kg body weight from the post natal day of 15-29. Behavioural studies were done with Y-maze, passive avoidance and hole-board experiments. Biogenic amines were estimated using ELISA method. In this study the treatment of BMEE enhanced the learning and memory significantly on behavioural studies and the level of 5-HT was increased. These results indicates that the BMEE treatment increases the cognitive function in postnatal rats and may through regulating expression of serotonergic neuronal system (Charles et al., 2011).
4. *Huperzia serrata* is a traditional medicinal plant used in Chinese medicine and its folklore utility in brain function made attention to evaluated scientifically for the acetylcholinesterase inhibitory activity and Huperzine A was isolated from the whole plant which exhibited acetylcholinesterase inhibitory action (Ma et al., 2007).

5. The roots of *Polygonum multiflorum* Thunb and leaves of *Convolvulus pluricaulis* was extracted with ethyl alcohol-70% and concentrated with rotary vacuum evaporator. The extracts were screened for acetylcholinesterase inhibitory activity and also investigated for the reduction of amyloid peptide formation and inflection of amyloid protein cascading. The study was carried out in mouse neuroblastoma cells expressing Swedish APP. The cells were treated in high and low doses of extracts for 24 h and the attenuation of amyloid production was quantified by using ELISA. In this study the extracts of *Polygonum multiflorum* and *Convolvulus pluricaulis* demonstrated intense inhibition of amyloid production (Liu et al., 2011).

6. The protective action of *Scutellaria baicalensis* and *Bupleurum scorzonerifolium*, contains the most active flavonoids which are responsible for antioxidant effects and are used in the traditional medicine for learning and memory. The action of both extracts were determined in iron-induced neurodegeneration was in the
nigrostriatal dopaminergic connections of the rat brain and was found to be more potent than melatonin (Lin et al., 2011).

7. The boiled extract of 2 medicinal plants such as mushroom and Panax ginseng called Jangwonhwan is very useful for the patients with cognitive dysfunction. Mice of transgenic model (Tg-APPswe/PS1dE9) were treated with Jangwonhwan at 400mg/kg/day of for three months from four an half months of age. The accumulation of Aβ plaque in the brain was assessed with immune-histological and ELISA estimations. The studies in in-vitro and in-vivo estimations were performed to determine the underlying mechanism. Jangwonhwan reduced the Aβ (1–42) and Aβ (1–40) levels, reduced the oxidative stress and prevented the down-regulation of calbindin and phospho-CREB which is present in the hippocampus of AD brain (Seo et al., 2010).

8. Tabernaemontana divaricata extract (TDE) were investigated in animal models for the determination of cortical and circulating acetylcholinesterase (ChE) enzyme activity, and Fos immunochemistry to determine the neuronal function in cerebral cortex, after the administration of TDE in different doses (250, 500 and 1000mg/kg) at different time intervals. The treatment of TDE 2h after administration, significantly reduced the cortical AChE concentration and increased the neuronal activity in cerebral cortex (Chattipakorn et al., 2007).
9. *Illicium verum* is a known spicy material in Indian traditional medicine, which possess extensive therapeutic potential. The study in regards with the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) revealed an inhibitory action. Especially the oil content anithole present contributed to the anticholinesterase activity of *I. verum* (Bhadra et al., 2011).

10. *Bacopa monnieri* has been used in the traditional system of Ayurvedic medicine to enhance the intelligence and memory in traditional Indian medicine for decades. The effect of ethanolic extract from *Bacopa monnieri* in cognitive impairment and neurodegeneration was studied in animal model of Alzheimer’s dementia induced by ethyl-choline aziridinium ion (AF64A). Male Wistar rats were treated orally Bacopa monnieri extracts at the doses of 20mg/kg, 40mg/kg and 80mg/kg body weight through oral feeding needle until 2 weeks before and 1 week after the intra-cerebroventricular injection of AF64A. The determination of hippocampal memory using Morris water maze and intensity of neurons and cholinergic neurons were determined using histological methods after the 7 days AF64A administration. The findings suggests that the treatment of *Bacopa monnieri* indicate that the time taken to escape on the escape platform was decreased and enhanced the intensity of neuronal cells and revealed the potential activity for the treatment Alzheimer’s disease (Uabundit et al., 2010).
11. *Alium sativam* is commonly called as garlic used in indigenous preparation for various ailments. In Swedish double mutant mice the anti-amyloidogenic, anti-tangle and anti-inflammatory effects of 2% dietary aged garlic extract and was compared with its important components, such as S-allyl-cysteine and di-allyl-disulfide. Possible cholesterol-dependent and cholesterol-independent mechanisms of actions of AGE, SAC and DADS in exerting anti-amyloidogenic, anti-inflammatory and anti-tangle effects were proved (Chauhan NB, 2006)

12. The dichloromethane extract of *Ferulago campestris* roots fractionated and was screened for the acetylcholinesterase inhibitory activity. Through bioassay guided fractionation components such as anisate 4, 1-acetyl-5-angeloyl, ferutinin 5 umbelliprenin 1, coladin, epielmanticine 8, 2-epilaserine are isolated and found to possess AChE inhibitory activity (DallAcqua et al., 2010).

13. Various species of *Mentha arvensis* extract were evaluated for inhibiting activity of acetylcholinesterase and butyrylcholinesterase. Enzyme assay was carried out in 96-well plate and found to be inhibiting the enzymes (Oinonen et al., 2006).

14. The *in-vitro* properties of *Iris pseudopumila* rhizomes and flowers extracts and their constituents for AChE inhibitory activity. isovitexin and Isoorientin revealed the most promising effect in *in-vitro* against
acetylcholinesterase with IC$_{50}$ of 26.8 and 36.4 μM respectively and exhibited butyrocholinesterase inhibition activity (Conforti et al., 2009)

15. Conypododiol is isolated from *Asparagus adscendens* methanolic extract and studied for acetylcholinesterase and butyrylcholinesterase inhibition effect by molecular docking and found to possess the property of inhibiting the neurotransmitter metabolic enzymes (Khan et al., 2010)

16. *Radix Angelicae sinensis* was a Chinese herbal medicine used for the treatment of learning and memory. Z-ligustilide was a compound isolated from this plant and screened for cognitive improvement on scopolamine-induced amnesia in ICR mice. 2.5 mg/kg of ligustilide was orally administrated 26 days. Y-maze and Morris water maze behavioral test was carried out after scopolamine administration. AChE level was estimated in the brain sample of the treated animals. Ligustilide significantly enhanced the spatial long-term memory and short-term memory, inhibited AChE enzyme activity (Cheng et al., 2011).

17. Tenuifolin was extracted from *Radix Polygalae* and evaluated for the learning and memory effect in aged and dementia induced mice. Mice were treated with 20, 40 and 80 mg/kg daily doses and were evaluated the behavioural parameters in step-down inhibitory
avoidance and Y maze. Administration of tenuifolin markedly enhanced the latency and reduced the number of errors. The intensity of cortical AChE was reduced and neurotransmitters in hippocampal tissue were increased by the treatment of tenuifolin (Zhang et al., 2008)

18. *Huperzia saururus* was extensively used in Argentinian traditional medicine for its aphrodisiac and memory property. Aqueous extracts from the leaf was decoctioned and found to have of alkaloids. In human erythrocytes membrane the AChE inhibitory activity was measured. The results illustrated a noticeable inhibition of acetylcholinesterase and pseudocholinesterase (Ortega et al., 2004).

19. The roots of *Ptychopetalum olacoides* (PO) are used native Amazonian population for the treatment of various CNS conditions where free radicals are implicated. The PO ethanolic extract (POEE) was evaluated in aging mice of 14 months with 100mg/kg body wt. After the 60mts of the treatment, the hippocampus, hypothalamus, cerebral cortex and cerebellum were dissected out and antioxidant enzyme activities were measured. The treatment of POEE reduced significantly the lipid peroxidation in brain parts and glutathione peroxidase enzyme was escalated in the hippocampal region of the brain and this study suggests that the treatment with POEE
enhanced the cognitive function through cellular antioxidant network efficiency in brain (Siqueira et al., 2007)

20. *Thespesia populnea* is a large tree predominantly found in tropical regions of India. The bark of *T. populnea* was evaluated for cognitive functions, cholinesterase activity and total cholesterol in mice. For different groups of young and aged mice *T. populnea* ethanolic extract (TPE) was given orally in three doses (100, 200 and 400mg/kg) for 7 days. The activity of mice in Passive avoidance and plus maze illustrated significant memory improvement in young and aged mice. Furthermore TPE also overturned the amnesia persuaded by scopolamine and diazepam (Vasudevan and Parle, 2006)

21. *Salvia lavandulaefolia* essential oil at two dosage levels was administered to the rats for 5 days. After the treatment, cholinesterase activity was determined in three areas of brain. And found to be positive in cholinesterase inhibition assay (Perry et al., 2002).

**III. SCOPE AND PLAN OF WORK**

A key revolution in clinical and experimental neuropharmacology as well as behavioural pharmacology was heralded throughout the ancient periods. The natural products have produced a noteworthy role in the treatment and rejuvenation for neuropsychiatric disorders.
A wide variety of Indian medicinal herbs have been applied for thousands of years in folklore system of medicine (including Ayurveda, Siddha, Unani and Arabic). Exclusively diverse types of potential plants are used to treat neurodegenerative diseases such as Alzheimer's memory loss, inflammatory neural degeneration and other neuronal disorders by Ayurvedic practitioners. Despite the fact that etiology of neurodegenerative diseases remains inscrutable, substantial evidences that indicates that malfunctioned energy metabolism, oxidative damage and excitotoxicity may be fundamental reasons. The element of the Ayurvedic coordination that provides an approach to prevention, management and treatment of degenerative diseases is recognized as *Rasayana*, in addition to plants used for this intention, other herbs classed as rejuvenators are used. These groups of plants generally possess strong antioxidant property and exert anti-stress potential.

The pathological changes in AD by accumulation of Aβ indicate the cholinergic hypofunction, increased AChE and MAO with reduced turnover of biogenic amines. Oxidative stress in AD induces elevation of corticosterone and produce subsequent neuroimmune changes. Neuroimmune system engages a bi-directional neural-immune communication together with neuroendocrine (principal hormonal direction, Hypothalamic-Pituitary-Adrenal axis) & neuronal (sympathetic
connection of lymphoid organs) pathways controls humoral and cellular immune response.

The existing drugs for treating AD are AChE enzyme inhibitors, which indirectly enhances the ACh level in the receptor site and other category of drugs including NMDA antagonist and Vitamin E. All these drugs existing for the present treatment neither offer direct stimulation of ACh receptor nor produce neural regeneration and these afford a little symptomatic relief and exerts various adverse effects. Other drug used in herbal preparations approved by FDA was Ginkgo (Le Bars et al., 1997); even though it has less efficacy in treatment of dementia the formulation is safer. Post marketing surveillance of Ginkgo has shown that the herb is relatively safe with little range (1.7%) of adverse effects. Recently attempts had been made for the drug development from natural origin as the herbal drugs satisfactorily give the therapeutic effect with negligible side effects. From the literature survey it has been found that only limited number of medicinal plants have been explored for the treatment of AD.

One of the folklore claims of the plants Alpinia galanga (L.) Willd and Pseudarthria viscida Wight & Arn is used as rejuvenator in the treatment of neurodegeneration and in memory loss. In order to contribute the medicinal potential of these plants which is not yet proved scientifically, we have planned to investigate scientifically by Aβ(25-35)
induced amnesia in mice. Experimentally the present study was designed to determine the folklore effect of the selected plant drugs, fractions and the bioactive molecule in the involvement of AChE, MAO, ROS (reactive oxygen species), neuroimmune and neuroendocrine pathways for the learning and memory process.

**PLANT INTRODUCTION**

**Description of Alpinia galanga (L.) Willd**

**Botanical Name:** *Alpinia galanga* (L.) Willd.

**Family:** *Zingiberaceae*

![Figure 1](image)

**Figure: 1**

**HABIT:** Herb

**USED IN:** Ayurveda, Folk, Unani and Sidha