3. REVIEW OF LITERATURE

Literature survey was carried out related to AD, its etiology and pathophysiology, treatment and limitations, alternative treatment strategies, intranasal route of drug delivery and nanomedicine applied to treatment of AD. The most relevant literature related to aforementioned topics is summarized in this section.

3.1 Alzheimer’s disease

AD disease was first described by Alois Alzheimer in 1907 where he described strange behavioral symptoms in a 51 year old patient, Auguste D. He also described gross histopathological changes in the brain. Alzheimer’s studies on the pathology of the cerebral cortex culminated with the publication of his now famous short article providing the first description of the neurofibrillary tangles (Alzheimer, 1907).

Vickers et al., 2000 described that the development of Aβ plaques in the brain may cause physical damage to axons, and the abnormally prolonged stimulation of the neuronal response to this kind of injury ultimately results in the profound cytoskeletal alterations that underlie neurofibrillary pathology and neurodegeneration. They concluded that inhibition of the neuronal reaction to physical trauma may be a useful neuroprotective strategy in the earliest stages of AD (Vickers et al., 2000).

Scorer, 2001 has explained molecular and genetic basis of AD. Mutations in three genes-βAPP and PS-1 and PS- have been shown to cause early-onset FAD. In addition, the ε4 allele of the apoE gene increases susceptibility to AD, and also increases the number of amyloid plaques in humans and in transgenic animal models of AD (Scorer, 2001).

Auld et al., 2002 have provided evidence implicating involvement of the basal forebrain cholinergic system in AD pathogenesis and its accompanying cognitive deficits. The authors also indicated that Aβ is a potent negative modulator of ACh synthesis and release, and interferes with normal signaling mediated by muscarinic and nicotinic receptor subtypes (Auld et al., 2002).

Sambamurti et al., 2002 have described the non-amyloidogenic and amyloidogenic pathways of processing of APP. They explained three important enzymes viz. α, β and γ secretase participate in APP processing (Sambamurti et al., 2002).

Scarpini et al., 2003 have reviewed the clinical features of available drugs for management of AD. They also suggested that potentially disease modifying therapies such as Aβ vaccination, secretase inhibitors, cholesterol lowering agents, metal chelators and
anti-inflammatory agents could be useful treatment strategies as they are more closely targeted to pathogenesis of the disease (Scarpini et al., 2003).

**Barnham & Bush, 2008** have described the role of metals in AD. They explained that AD is characterized by elevated levels of iron and miscompartmentalization of copper and zinc (e.g. accumulation in amyloid plaques). They explained that the ability of metal ions, for example, copper and iron, to accept and donate electrons can lead to radical formation, reactive nitrogen and oxygen species and oxidative attack of tissue components contributing to disease and perhaps aging itself. When coupled to proteins, electrochemistry involving the protein is possible which may lead to oxidation or to catalytic ROS production. $\text{Zn}^{2+}$, on the contrary, maintains its valence, and generally acts as an antioxidant, however has a notorious ability to precipitate $\text{A}\beta$ (Barnham and Bush, 2008).

**Ray et al., 2009** have described basic pathology and role of key molecules like APP, $\beta$-secretase and apoE pathogenesis of AD. They have also highlighted that inflammation involving activated microglia and NFkB plays an important role in progression of AD (Ray and Lahiri, 2009).

### 3.2 Oxidative Stress in AD

**Frautschy et al., 1991** found injections of preparations of mature plaque amyloid isolated from the AD brain into the rat brain exert neurotoxic effects and induce antigens found in the AD brain. The authors injected SDS-isolated amyloid cores into rat cortex and hippocampus. Similarly isolated lipofuscin fractions from control human brains were injected on the contralateral side. Rats were perfused and brains were examined immunohistochemically at 2 days, 7 days, and 1 month after injection. Alz-50, a monoclonal antibody against abnormally phosphorylated tau proteins, stained neurons along the cortical needle track at 2 but not 7 days after injection of either amyloid or lipofuscin. At 1 month, however, ubiquitin, Alz-50 antigen, and silver-positive structures were observed only in response to amyloid. In 7 of 10 animals, there was considerable neuronal loss in the hippocampal layers. In each instance, these effects were in the immediate vicinity of $\beta$-protein immunoreactive material. These results indicate a neuronal response to amyloid.

**Pappolla et al., 1992** provided immunohistochemical evidence of antioxidant stress in AD. While exploring this possibility, tissue sections from five brains with AD and five neuropathologically normal age-matched controls were immunostained with polyclonal antibodies against superoxide dismutase (CuZn- and Mn-forms) and catalase. A standard avidin-biotin-peroxidase method was used for antigen detection. A subgroup of neurofibrillary tangles (15-25%) and senile plaques (50%) showed immunoreactivity for
both enzymes with a staining pattern similar (but not identical) to that usually observed with antibodies against ubiquitin. Senile plaques displayed a granular pattern of immunostaining. Amyloid cores in mature classical plaques remained unstained. In addition, occasional elements with features consistent with reactive glial cells were strongly immunostained. Tangle-free neurons in both diseased and control brains showed weak to absent intracytoplasmic immunoreactivity. The immunoreactivity was totally abolished by preincubation of the primary antibodies with the corresponding purified antigens. These findings support the hypothesis that OS may be involved in the pathogenesis of AD (Pappolla and Omar, 1992).

Koppal et al., 1998 demonstrated that Aβ induces changes in neocortical synaptosomal membrane lipid structure and composition. Aβ caused lipoperoxidation of membranes with increase in level of free fatty acids like palmitic acid, stearic acid, oleic acid, and arachidonic acid that are involved in modifications of proteins by covalent binding. Arachidonic acid can act as a precursor for the synthesis of inflammatory agents like leukotrienes and prostaglandins, through the lipoxygenase and cyclooxygenase pathways, and cause impairment of various cellular functions. In addition, arachidonic acid is hypothesized to be a precursor for 4-HNE, formed in neuronal systems exposed to Aβ. These changes were reversed on treatment with Vitamin E (Koppal et al., 1998).

Huang X., et al 1999 showed that human Aβ directly produces H₂O₂ by a mechanism that involves the reduction of metal ions, Fe(III) or Cu(II), setting up conditions for Fenton-type chemistry. Spectrophotometric experiments established that the Aβ peptide reduces Fe(III) and Cu(II) to Fe(II) and Cu(I), respectively. Molecular oxygen is then trapped by Aβ and reduced to H₂O₂ in a reaction that is driven by substoichiometric amounts of Fe(II) or Cu(I). In the presence of Cu(II) or Fe(III), Aβ produces a positive TBARS assay, compatible with the generation of the hydroxyl radical (OH). The amounts of both reduced metal and TBARS reactivity are greatest when generated by Aβ 1-42>>Aβ 1-40>>rat Aβ 1-40, a chemical relationship that correlates with the participation of the native peptides in amyloid pathology. These findings indicate that the accumulation of Aβ could be a direct source of OS in AD (Huang et al., 1999).

Cecchi et al., 2002 measured the levels of typical end products of the processes of lipid peroxidation, protein oxidation, and total antioxidant capacity (TAC) in skin fibroblasts and lymphoblasts taken from patients with FAD, SAD, and age-matched healthy controls. Compared to controls, the fibroblasts and lymphoblasts carrying APP and PS-1 gene mutations showed a clear increase in lipoperoxidation products, malondialdehyde (MDA), and 4-HNE. In contrast, the antioxidant defenses of cells from FAD patients
were lower than those from normal subjects. An oxidative attack on protein gave rise to
greater protein carbonyl content in FAD patients than in age-matched controls.
Furthermore, ADP ribosylation levels of poly (ADP-ribose) polymerase (PARP) nuclear
substrates were significantly raised, whereas the PARP content did not differ significantly
between fibroblasts carrying gene mutations and control cells. These results indicate that
peripheral cells carrying APP and PS-1 gene mutations show altered levels of oxidative
markers even though they are not directly involved in the neurodegenerative process of
AD. These results support the hypothesis that oxidative damage to lipid, protein, and
DNA is an important early event in the pathogenesis of AD (Cecchi et al., 2002).

3.3 Curcumin and AD

Lim et al., 2001 evaluated affect of CUR on Alzheimer-like pathology in the transgenic
APPsw mouse model (Tg2576) (Lim et al., 2001). They tested a low (160 ppm) and a
high dose of dietary CUR (5000 ppm) on inflammation, oxidative damage, and plaque
pathology. Low and high doses of CUR significantly lowered oxidized proteins and
interleukin-1β, a proinflammatory cytokine elevated in the brains of these mice. With
low-dose but not high-dose CUR treatment, the astrocytic marker GFAP was reduced,
and insoluble Aβ, soluble Aβ, and plaque burden were significantly decreased by 43-50%.
However, levels of APP in the membrane fraction were not reduced. Microgliosis was
also suppressed in neuronal layers but not adjacent to plaques. The authors concluded in
view of its efficacy and apparent low toxicity, CUR shows promise for the prevention of
AD.

Yang et al., 2005 investigated effect of CUR on formation of amyloid oligomers and
fibrils, binding to plaques and in vivo plaque burden (Yang et al., 2005). Under
aggregating conditions in vitro, CUR inhibited aggregation (IC\textsubscript{50}=0.8 µM) as well as
disaggregated fibrillar Aβ40 (IC\textsubscript{50}=1 µM), indicating favorable stoichiometry for
inhibition. CUR was a better Aβ40 aggregation inhibitor than ibuprofen and naproxen,
and prevented Aβ42 oligomer formation and toxicity between 0.1 and 1.0 µM. Under
EM, CUR decreased dose dependently Aβ fibril formation beginning with 0.125 µM. The
effects of CUR did not depend on Aβ sequence but on fibril-related conformation. AD
and Tg2576 mice brain sections incubated with CUR revealed preferential labeling of
amyloid plaques. In vivo studies showed that CUR injected peripherally into aged Tg
mice crossed the blood-brain barrier and bound plaques. When fed to aged Tg2576 mice
with advanced amyloid accumulation, CUR labeled plaques and reduced amyloid levels
and plaque burden. Hence, CUR directly binds small Aβ species to block aggregation
and fibril formation in vitro and in vivo. These data suggest that low dose CUR effectively
disaggregates Aβ as well as prevents fibril and oligomer formation, supporting the rationale for CUR use in clinical trials preventing or treating AD.

**Ishrat et al., 2009** examined the modulating impacts of CUR against cognitive deficits and oxidative damage in intracerebroventricular–streptozotocin (ICV-STZ) infused rats (Ishrat et al., 2009). Rats were injected bilaterally with ICV-STZ (3 mg/kg), while sham rats received the same volume of vehicle and then supplemented with CUR (80 mg/kg) for three weeks. After two weeks of ICV-STZ infusion, rats were tested for cognitive performance using passive avoidance and water maze tasks and then sacrificed for biochemical and histopathological assays. ICV-STZ rats showed significant cognitive deficits, which were significantly improved by CUR supplementation. CUR supplementation significantly augmented increased 4-HNE and MDA, TBARS, H₂O₂, protein carbonyl (PC) and oxidized glutathione (GSSG); decreased levels of reduced GSH and its dependent enzymes (Glutathione peroxidase [GPx] and glutathione reductase [GR]) in the hippocampus and cerebral cortex; and increased ChAT activity in the hippocampus of ICV-STZ rats. The study suggests that CUR is effective in preventing cognitive deficits, and might be beneficial for the treatment of SAD.

**Agrawal et al., 2010** investigated the effect of CUR on memory functions, brain insulin receptors, acetylcholinesterase (AChE) activity and oxidative stress in ICV-STZ induced dementia in rats (Agrawal et al., 2010). Rats were injected with STZ (3 mg/kg, ICV) bilaterally twice, on day 1 and 3 and CUR (200 mg/kg, po) was administered in pre- and post-treatment schedules. Pre-and post treatment of CUR in STZ (ICV) treated rats significantly restored the memory deficit and insulin receptor protein level in hippocampus and cerebral cortex. CUR was also found to attenuate the OS induced by STZ in both the regions.

**Awasthi et al., 2010** investigated the effect of CUR on cerebral blood flow (CBF), memory impairment, oxidative stress and cholinergic dysfunction in ICV-STZ induced memory impairment in mice (Awasthi et al., 2010). To study the preventive effect, CUR (10, 20 and 50 mg/kg, PO) was administered for 21 days starting from the first dose of STZ. In another set of experiment, CUR was administered for 7 days from 19th day after confirming STZ induced dementia to observe its therapeutic effect. Biochemical parameters of oxidative stress and cholinergic function were estimated in brain on day 21. CUR dose dependently improved CBF in STZ treated mice together with amelioration of memory impairment both in preventive and therapeutic manner.
Xiao et al., 2010 investigated protective effects of CUR and its derivatives in SK-N-SH model induced by Aβ1-42 (Xiao et al., 2010). The viability of cells was significantly increased by CUR and CUR1, and the expression of MAP-2 protein was up-regulated in immunocytochemical staining and Western blot. The cell morphologies, including the number of neurites, neurite growth and neurite extension, were significantly improved. CUR1 showed more significant protective effect on SK-N-SH cells than CUR. This study revealed for the first time that the neuroprotective effect of CUR and CUR derivatives not only directly depends on their special chemical constitution, but they can resist to Aβ damage by up-regulation of MAP-2 expression.

Qin et al., 2010 explored the effects of CUR on the intracellular Aβ (iAβ) induced toxicity in primary cultured rat prefrontal cortical neurons (Qin et al., 2010). The cell viability of primary cultured prefrontal cortical neurons decreased significantly after virus driven transfection of Aβ from 1 day to 7 days. Interestingly, administration of 1 µM, 10 µM or 20 µM of CUR significantly inhibited the iAβ-induced toxicity in primary cultured rat prefrontal cortical neurons tested by MTT and lactate dehydrogenase (LDH) release assays. They further studied the involvements of apoptotic or neuroprotective pathway proteins in CUR protection against iAβ-induced cytotoxicity in primary cultured rat prefrontal cortical neurons. The results demonstrated that the contents of activated caspase-3 increased significantly by iAβ, while CUR significantly inhibited the iAβ-induced increases of activated caspase-3 tested by Western blot. And the contents of p-AKT (also called protein kinase B) decreased significantly after iAβ treatment, while administration of CUR significantly inhibited the iAβ-induced decreases in the contents of p-AKT. The results suggest that CUR may play a protective effect in primary cultured rat prefrontal cortical neurons against iAβ-induced cytotoxicity, and both AKT and caspase-3 are involved in the CUR-induced protective effects.

Mourtas et al., 2011 investigated CUR decorated nanoliposomes with high affinity for amyloid-β1-42 peptide (Mourtas et al., 2011). Herein, a click chemistry method was used to generate nanoliposomes decorated with a CUR derivative, designed to maintain the planar structure required for interaction with Aβ, as directly confirmed by Surface Plasmon Resonance experiments. Another type of liposomes was formed starting from CUR-phospholipid conjugate, in which the planar structure of CUR is disrupted. Both types of generated CUR-decorated vesicles had mean diameters in the nano range (131-207nm) and slightly negative zeta-potential values according to their lipid composition, and were stable for periods up to 20 days. They also demonstrated high integrity during incubation in presence of plasma proteins. Surface Plasmon Resonance experiments,
measuring the binding of flowing liposomes to immobilized Aβ 1-42, indicated that the liposomes exposing the CUR derivative (maintaining the planarity) have extremely high affinity for Aβ 1-42 fibrils (1-5 nM), likely because of the occurrence of multivalent interactions, whereas those exposing non-planar CUR did not bind to Aβ1-42.

Taylor et al., 2011 investigated effect of various types of nanoliposomes (associated with CUR, phosphatidic acid, cardiolipin, or GM1 ganglioside) on the aggregation of the amyloid-β1-42 (Aβ1-42) peptide (Taylor et al., 2011). Nanoliposomes incorporating CUR (CUR-liposomes) were prepared by adding CUR in the lipid phase during liposome preparation, whereas CUR surface-decorated liposomes were prepared by using a CUR-lipid conjugate (lipid-S-CUR liposomes) or by attaching a CUR derivative on preformed liposomes by click chemistry (click-CUR liposomes). The lipid ligands (phosphatidic acid, cardiolipin, or GM1) were also incorporated into nanoliposomes during their formation. All nanoliposomes with CUR, or the CUR derivative, were able to inhibit the formation of fibrillar and/or oligomeric Aβ in vitro. Of the three forms of CUR liposomes tested, the click-CUR type was by far the most effective. Liposomes with lipid ligands only inhibited Aβ fibril and oligomer formation at a very high ratio of liposome to peptide. CUR-based liposomes could be further developed as a novel treatment for AD.

3.4 Nanomedicine in AD

Zhang et al., 2007 prepared donepezil microparticles (DM) as a sustained release delivery system with subcutaneous injection once a month (Zhang et al., 2007). DM was prepared using PLGA by an oil-water emulsion solvent evaporation technique. DM showed the loading ratio 13.2% (w/w) and yield 54.8% with mean particle size about 75mm. In vitro release of DM showed that donepezil completely released within 28 days in water, but the cumulative release percentages up to day 30 were 98.4% and 49.1% for phosphate buffer saline (PBS, pH 5.8) and PBS (pH 7.4), respectively. The in vivo experiment demonstrated that DM (90 mg/kg) produced a sustained release process in rats, and reached steady-state concentration at day 8 and maintained until day 27 with steady-state levels of donepezil between 130.3±7.8 and 121±9.8 ng/ml, which was accordance with that of free donepezil by oral application route (3 mg/kg day). DM (90 mg/kg) by subcutaneous infusion in rats produced the same pharmacological role as free donepezil (3 mg/kg day) by oral application route. These results implicated that DM as a sustained release delivery strategy could substitute for its oral formulation for therapy of AD and come true its administration once a month.
Wilson et al., 2008a made an attempt to target the anti-Alzheimer's drug rivastigmine in the brain by using poly (n-butylcyanoacrylate) nanoparticles (Wilson et al., 2008a). The drug was administered as a free drug, bound to nanoparticles and also bound to nanoparticles coated with polysorbate 80. In the brain a significant increase in rivastigmine uptake was observed in the case of poly (n-butylcyanoacrylate) nanoparticles coated with 1% polysorbate 80 compared to the free drug. The study demonstrated that the brain concentration of intravenously injected rivastigmine can be enhanced over 3.82 fold by binding to poly(n-butylcyanoacrylate) nanoparticles coated with 1% nonionic surfactant polysorbate 80.

Wilson et al., 2008b investigated tacrine loaded poly(n-butylcyanoacrylate) nanoparticles for targeted delivery to brain (Wilson et al., 2008b). In the brain a significant increase in tacrine concentration was observed in the case of poly(n-butylcyanoacrylate) nanoparticles coated with 1% polysorbate 80 compared to the uncoated nanoparticles and the free drug. A higher concentration of drug tacrine was observed in liver, spleen and lungs with the nanoparticles in comparison to the free drug. The accumulation of drug tacrine in the liver and spleen was reduced, when nanoparticles were coated with 1% polysorbate 80.

Mistry et al., 2009 studied effect of physicochemical properties of nanoparticles on transit into murine olfactory epithelium upon IN administration (Mistry et al., 2009). The authors used fluorescence microscopy and stereology to track intranasally administered chitosan-coated polystyrene (C-PS) or polysorbate-coated polystyrene (P80-PS) nanoparticles (100 nm or 200 nm in diameter) in olfactory and respiratory nasal epithelia and olfactory bulbs in mice. Chitosan coating caused particles to adhere to the extracellular mucus which could provide useful modality for paracellular drug transport. Nanoparticle transport was exclusively transcellular. None of the nanoparticle formulations showed preference for uptake into olfactory axons over other nasal epithelial cells. Both 100 nm PS and 100 nm P80-PS were observed in olfactory epithelial cells but were absent from the olfactory bulbs; therefore, it is speculated that an optimal nanoparticle diameter for axonal transport is <100 nm in mice.

Joshi et al, 2010 prepared rivastigmine loaded PLGA and PBCA [poly(n-butylcyanoacrylate)] nanoparticles for brain targeting (Joshi et al., 2010). PLGA nanoparticles were prepared by nanoprecipitation technique, while PBCA nanoparticles were prepared by emulsion polymerization technique. Effect of key formulation variables on particle size and entrapment of nanoparticles was studied by using factorial design. Nanoparticle formulations released the drug in biphasic pattern, initial burst release
followed by prolonged release. Pharmacodynamic study demonstrated faster regain of memory loss in amnesic mice with both PLGA and PBCA nanoparticles when compared to drug solution. This indicates rapid and higher extent of transport of rivastigmine into the mice brain and thus shows the suitability of both nanoparticleless as potential carriers for providing sustained brain delivery of rivastigmine.

3.5 Lipid based nanocarriers

Cavalli et al., 1997 prepared diazepam solid lipid nanoparticles (SLN) by microemulsion technique (Cavalli et al., 1997). The chosen lipid was melted, then surfactant, cosurfactant and warm water were added successively. A clear microemulsion was obtained under gentle stirring at a temperature close to the melting point of the lipid used. For drug-loaded SLN, diazepam was added to the melted lipid before the other components. SLN were obtained by dispersing the warm o/w microemulsions in a cold aqueous medium (about 2°C) under mechanical stirring a ratio of 1:25 (micromulsion/aqueous medium). The aqueous media used were aqueous solutions of trehalose or Pluronic F68 at 2%, or distilled water.

Shafiq-un-Nabi et al., 1997 explained the basis for calculations and construction of pseudoternary phase diagrams and to give an idea for selection of nanoemulsion formulations from the phase diagrams, to avoid metastable formulations in the least possible time (Shafiq-un-nabi et al., 2007). Pseudoternary phase diagrams were developed using the aqueous titration method. Slow titration with the aqueous phase was performed for each combination of oil and Smix (surfactant and co-surfactant mixture) separately.

Bondì et al., 2009 investigated Ferulic acid (FA), a phenolic compound with significant antioxidant activity in AD, loaded solid lipid nanoparticles (SLN and NLC) prepared using microemulsion technique (Bondì et al., 2009). Stable SLN and NLC formulations having mean size ranging between 94-140 nm and high zeta potential were obtained. The SLN obtained using Compritol 888 ATO as lipid matrix was chosen for further characterization because of high loading capacity and the best characteristics in terms of size, polydispersity, and drug release profile. Empty SLN showed no cytotoxicity on human neuroblastoma cells (LAN 5) at tested concentrations and the ability to penetrate into these cells. Moreover, cells treated with FA-loaded SLN showed a higher reduced ROS production than cells treated with free FA. These findings demonstrate that FA-loaded SLN possess a higher protective activity than free FA against oxidative stress induced in neurons and suggest that SLN are excellent carriers to transport FA into the cells.
**Gobbi et al., 2010** lipid-based nanoparticles with high binding affinity for amyloid $\beta_{1-42}$ peptide (Gobbi et al., 2010). The authors formulated and characterized two types of NPs (liposomes and SLNs, 145 and 76 nm average size, respectively) functionalized to target $\text{A}\beta_{1-42}$ with high affinity. Preliminary immunostaining studies identified anionic phospholipids [phosphatidic acid (PA) and cardiolipin (CL)] as suitable $\text{A}\beta_{1-42}$ ligands. PA/CL-functionalized, but not plain, NPs interacted with $\text{A}\beta_{1-42}$ aggregates. Surface Plasmon Resonance studies indicated that, when exposed on NPs surface, PA/CL display very high affinity for $\text{A}\beta_{1-42}$ fibrils (22-60 nM), likely because of the occurrence of multivalent interactions which markedly decrease the dissociation of PA/CL NPs from $\text{A}\beta$. The PA/CL NPs described in this work are endowed with the highest affinity for $\text{A}\beta$ so far reported.

**Ismail et al., 2013** investigated efficacy of rivastigmine loaded liposomes in aluminium chloride (AlCl$_3$) induced model of AD (Ismail et al., 2013). Liposomes were prepared by film hydration and heating methods which gave liposomes having particle size of 67.51nm and 528.7nm respectively. Liposomal formulations had a superior effect in improving deterioration of spatial memory induced by aluminium chloride compared to drug solution. Rivastigmine liposomes succeeded in normalization of AChE and Na+/K+ ATPase activities. Gene-expression profile showed that treatment with rivastigmine liposomes to AlCl$_3$-treated rats succeeded in exerting significant decreases in BACE1, AChE, and IL-1B gene expression. The profound therapeutic effect of rivastigmine liposomes over solution was evidenced by nearly preventing amyloid plaque formation, as shown in the histopathological examination of rat brain.

### 3.6 Intranasal strategy in AD

#### 3.6.1 Conventional dosage forms

Physostigmine, an AChEI, and arecoline, a muscarinin agonist, have shown to improve Alzheimer presenile dementia when administered parenterally. Both the compounds undergo extensive first pass metabolism and are ineffective orally. IN delivery of these drugs was investigated as an alternative route for parenteral delivery. The nasal bioavailability (BA) of physostigmine was 100% compared to IV administration and that of arecoline was 85% compared to intramuscular administration (Hussain and Mollica, 1991).

**Gozes et al., 1996** synthesized a potent lipohilic analogue of VIP [stearyl-norleucine$^{17}$] VIP ([St-Nle$^{17}$]VIP), that has been found to exhibit neuroprotection in AD models (Gozes et al., 1996). It was found to completely prevent $\text{A}\beta$ induced cell death in rat cerebral cortical cultures with several fold greater potency than VIP exhibiting maximal
potency at $10^{-14}$M. The ability of [St-Nle$^{17}$]VIP to improve learning and memory capacities were tested in cholinotoxin AF64A treated animals by Morris water maze test. Daily ICV injections of [St-Nle17]VIP completely prevented the learning impairment in animals treated with the cholinergic blocker. The authors also studied the effect of intranasally administered [St-Nle$^{17}$]VIP on learning and memory. IN administration significantly improved performance of animal in Morris water maze test compared to animals treated with AF64A alone.

Frey et al., 1997 demonstrated non invasive delivery of unconjugated NGF for first time in rats (Frey et al., 1997). IN administration of $^{125}$I-labeled NGF in rats showed rapid appearance in olfactory bulb within 20 min of administration. The authors suggested that $^{125}$I-NGF was transported via intercellular clefts in the olfactory epithelium and extracellular transport along the olfactory neural pathway rather than uptake by olfactory neurons and subsequent intracellular axonal transport.

NXX-066, a physostigmine analogue, is a potent inhibitor of AChE. It is well absorbed orally but oral BA is poor due to its presystemic metabolism. Dahlin and Bjork, 2001 have studied uptake of NXX-066 into CSF after IN and IV administration in Sprague-Dawley rats (Dahlin and Bjork, 2001). It was absorbed rapidly and completely into systemic circulation after nasal administration with $t_{\text{max}}$ of 1.5 min and 100% BA which was similar to nasally administered physostigmine as reported earlier. $t_{\text{max}}$ value for NXX-066 was lesser than physostigmine which had $t_{\text{max}}$ value of $6.5\pm1.7$ min. Authors suggested this difference could be because of difference in log P value of two drugs. However, the concentration of drug in CSF was very low after IN and IV administration indicating that uptake into CSF was not enhanced by nasal administration. The transport of drugs to CNS via nasal administration may be significant for poorly soluble drugs and insignificant for drugs which are completely and rapidly absorbed into systemic circulation.

IN administration of insulin in humans was first studied by Benedict and colleagues (Benedict et al., 2004). Delayed recall of words was significantly improved after 8 weeks of IN insulin (4 x 40 IU/d) administration. Moreover subjects showed marked improvement in mood and self-confidence with reduced anger. These benefits were observed without any systemic side effects which could be of relevance in AD patients.

Galantamine is an AChEI which has been associated with dose-limiting GI-mediated side effects such as nausea and vomiting, the most common adverse events leading to discontinuation of treatment. However, IN administration of galantamine hydrobromide salt is limited due to its poor aqueous solubility. Leonard et al., 2005 investigated
different techniques like addition of co-solvents, cyclodextrins and counter-ion exchange in order to enhance solubility of galantamine (Leonard et al., 2005). Among these, replacement of bromide with lactate resulted in 12 fold increase in drug solubility. Galantamine lactate formulations showed better permeation across epithelial membrane than hydrobromide salt. In vivo pharmacokinetic studies revealed that IN galantamine had comparable blood levels compared to oral route.

Reger et al., assessed the acute effects of different doses of IN insulin (10, 20, 40, 60 IU) on hippocampus dependent memory function in memory impaired subjects (early stage AD) in comparison to age-matched healthy controls (Reger et al., 2008, 2006). IN insulin treatment produced significant memory improvement, with the patients showing maximal benefits at dose of 20 IU.

Huperizin A (Hup A), an unsaturated sesquiterpene alkaloid, extracted from a club moss (Huperzia serrata) is a powerful and reversible inhibitor of AChE. It easily penetrates the BBB and is a promising therapeutic agent for AD. However, it influences peripheral cholinergic system leading to side effects and alternate delivery systems are required to specifically target the drug to brain. To overcome these limitations Zhao et al., 2007 have investigated Hup A nasal delivery by means of in situ gel of gellan gum (Zhao et al., 2007). The authors studied the brain uptake of Hup A after IN administration of in situ gel to rats and compared the pharmacokinetic parameters with intravenous (IV) and peroral route. The results indicated that concentration of the drug after 6 hour in the cerebrum, hippocampus, cerebellum, left olfactory bulb and right olfactory bulb were 1.5, 1.3, 1.0, 1.2, and 1.0 times of those after IV administration, and 2.7, 2.2, 1.9, 3.1, and 2.6 times of those after oral administration. The AUC brain_{0→6h}/AUCplasma_{0→6h} of drug in the cerebrum, hippocampus and left olfactory bulb following the IN administration was significantly higher than the IV administration. The results revealed that IN route is a viable option for improving the brain targeting efficiency of HupA and also to reduce the side effects to peripheral tissues.

Peroral administration of Tacrine, a potent, centrally active, reversible AChEI is associated with low BA due to an extended hepatic metabolism, short elimination half-life and hepatotoxicity. Jogani et al., 2007 have investigated direct nose-to-brain delivery of tacrine to improve its BA, avoid first-pass effect and minimize hepatotoxicity (Jogani et al., 2007). Tacrine was labeled with 99mTc (technetium) and administered in BALB/c mice intranasally and intravenously. Intranasally administered tacrine was transported quickly to brain (T_{max} 60 min) compared to IV administration (T_{max} 120 min). The drug targeting efficiency and drug transport efficiency following IN administration was found
to be 207.23% and 51.75% respectively. The results showed tacrine was directly transported to brain from nasal cavity resulting in higher BA of drug with reduced distribution to non-target sites.

Leonard et al., 2007 studied IN formulations of galantamine containing methylated-β-cyclodextrin as stabilizer, L-α-phosphatidylcholine didecanoyl as lipid surfactant and disodium edetate as a chelator (Leonard et al., 2007). An *in vitro* tissue model EpiAirway™ system consisting of human upper airway epithelia was used to assess permeation and toxicity of the developed formulation. The presence of three permeation enhancers resulted in about 3-fold greater permeation of galantamine HBr. MTT and LDH assays showed that cell viability was high and formulations were non-toxic to the membrane. Transepithelial electrical resistance (TER) measurements showed dramatic decrease in TER in formulations containing permeation enhancers which is related to opening of tight junctions. Pharmacokinetic studies of the galantamine formulations were carried out in Sprague-Dawley rats by IN route at dose of 1.75mg/kg. IN galantamine HBr dosed alone or in presence of permeation enhancers had absolute BA of 22% and 41% respectively, resulting in 86% increase in BA with permeation enhancers. Addition of permeation enhancers to IN formulation resulted in 78% increase in BA compared to oral dosing. The *in vitro*-in *vivo* correlation between *in vitro* permeation and *in vivo* availability suggested that *in vitro* data is reasonably predictive of *in vivo* behavior ($R^2=0.986$). Authors further carried out series of experiments using design of experiment to optimize concentration of selected penetration enhancers and their effect on permeation of galantamine lactate, a more soluble salt of galantamine. The optimized formulation of galantamine lactate had 4-fold increase in permeation of galantamine in *in vitro* model. Galantamine lactate formulations exhibited similar results in term of cell viability, toxicity and TER measurements. The *in vivo* emetic response of galantamine lactate formulations was studied in ferrets. Ferrets were dosed orally or nasally at dose of 20mg/kg and emesis or retching was observed for 4h after dosing. A separate pharmacokinetic study was conducted at same dose level to compare the BA in 2 dosing routes. The emesis studies revealed significant decrease in emesis or retching by IN route with only 3 observed events compared to 34 events with oral administration. The IN route not only reduced emesis but increased $C_{\text{max}}$ more than 4-fold with AUC$_{0-60}$ and AUC$_{0-120}$ of 200% and 128% respectively compared to oral dosing.

Melatonin, an indole amide neurohormone, secreted by pineal gland has been found to protect neurons against Aβ toxicity and inhibit the progressive formation of β-sheets and amyloid fibrils (Pappolla et al., 1997; Pappolla, 1998). However, it has been found to have low oral BA, short biological half life (45 min) and erratic pharmacokinetic profile.
Jayachandra Babu et al., 2011 have studied IN transport of melatonin using polymeric gel suspensions prepared with carbopol, carboxymethyl cellulose (CMC) and PEG 400 (Jayachandra Babu et al., 2011). In vitro permeation of formulations was studied using EpiAiway™ tissue model confirmed significantly higher permeation of polymeric suspensions. The concentration of melatonin in olfactory bulbs after IN administration were 9.22, 6.77 and 4.04-fold higher for carbopol, CMC and PEG400, respectively, than that of IV melatonin in rats.

Vaccination with Aβ1-42 has been found to prevent Aβ accumulation and clearance of pre-existing amyloid plaques. Several studies have been carried out to study active and passive immunization using anti-Aβ antibodies (Bard et al., 2000; Schenk et al., 1999). Cattepoel et al., 2011 studied immunization of APP transgenic mice with single-chain variable fragment (scFv) derived from full IgG antibody raised against C-terminus of Aβ. scFv was found to enter brain after IN application and bind to amyloid plaques in cortex and hippocampus of APP transgenic mice. It was also found to inhibit Aβ fibril formation and Aβ mediated neurotoxicity. Chronic IN administration of scFv was found to reduce congophilic amyloid angiopathy (CAA) and Aβ plaques in cortex of APPswe/PS1dE9 mice. The authors suggested that reduction of CAA and plaque pathology was associated with a redistribution of brain Aβ from the insoluble fraction to the soluble peptide pool (Cattepoel et al., 2011).

Yang et al., 2012 have investigated tissue distribution and pharmacodynamics of rivastigmine after IN and IV administration at dose of 2mg/kg (Yang et al., 2012). The drug was rapidly and completely absorbed into systemic circulation followed by rapid decline in plasma concentration. IN administration showed higher concentration in CNS and longer inhibition of AChE and butyrylcholinesterase (BuChE) compared to IV route. The inhibitory action on these two enzymes was more pronounced in CNS than peripheral tissues.

A recent clinical trial on IN insulin has shown to improve memory, attention and functioning in patients with the disease. AD was also characterized by reduced uptake and utilization of glucose in the brain that has been documented by positron emission tomography with fludeoxyglucose F 18 (FDG-PET)(Craft et al., 2012).

Recently, Guo et al., 2013 used APP and PS 1 double transgenic mice as a model system to investigate the effects and potential mechanisms of IN administration of DFO on iron induced abnormal tau phosphorylation (Guo et al., 2013). It was shown that high-dose iron treatment markedly increased the levels of tau phosphorylation at a variety of serine and threonine residues, whereas highly induced tau phosphorylation was abolished by
IN administration of DFO in APP/PS1 transgenic mice. The results also indicated that DFO IN administration decreases Fe-induced activities of cyclin-dependent kinase 5 (CDK5) and GSK3b. This in turn suppresses tau phosphorylation in neurons and inhibits the formation of intracellular NFTs in the cortical and hippocampal regions of the brain in AD thus improving the deficits of spatial learning and memory in diseased patients. The authors concluded that IN DFO treatment shows its antagonistic effects on iron induced cognitive dysfunctions and tau phosphorylation via CDK5 and GSK3b pathways. These findings suggest that IN DFO treatment is a potential therapeutic approach against AD neuropathology in the brain.

3.6.2 Novel drug delivery system

Jogani et al., 2008 reported mucoadhesive microemulsion for targeting tacrine to the brain after IN administration in mice. Mucoadhesive microemulsion of tacrine showed 2-fold higher BA in brain compared to drug solution. Rat brain scintigraphy studies were carried out using 99m Tc as a marker. The results revealed higher uptake in brain after IN administration. Pharmacodynamic studies of the developed formulations were carried out in scopolamine induced amnesia model in mice and showed faster regain of memory loss in mucoadhesive microemulsion treated group. Their results suggest a possible role of IN tacrine delivery in treating Alzheimer’s patients (Jogani et al., 2008).

Arumugam et al., 2008 have investigated multilamellar liposomes for IN delivery of rivastigmine using soy lecithin and cholesterol by lipid layer hydration method (Arumugam et al., 2008). The developed liposomes had particle size of 10.0±2.8µm with encapsulation efficiency of 80.0±5.0%. The in vitro release studies showed an initial burst release followed by a log phase with 56.0±2.3% release in 6h. Pharmacokinetic studies in wistar rats revealed five-fold higher AUC (36.13±1.87 µg min ml\(^{-1}\)) for IN liposomes compared to oral treated group (6.58±0.26 µg min ml\(^{-1}\)) and three fold higher value compared to free drug administered intranasally (12.99 ± 0.87 mg min ml\(^{-1}\)). IN liposomal formulation had \(C_{max}\) of 0.60 µg ml\(^{-1}\) which was 1.7-fold and 10-fold higher than intranasally administered free drug (0.35 µg ml\(^{-1}\)) and oral administration (0.06 µg ml\(^{-1}\)) respectively. Nasally administered free drug reached peak within 5 min compared to IN liposomal formulation which had \(t_{max}\) of 45 min. The authors suggested that free drug reached systemic circulation rapidly via nasal route whereas liposomal formulations accumulated in nasal mucosa and released the drug slowly delaying \(t_{max}\). The concentration of rivastigmine in brain was 5.6 times higher following IN administration compared to oral administration. IN liposomes had 20-fold and 3.2-fold higher AUC in
brain compared to free drug orally and intranasally respectively. IN free drug attained $t_{\text{max}}$ in 15 min whereas IN liposomes reached $t_{\text{max}}$ in 60 min.

Quercetin, a flavonoid, is one of the most prominent dietary antioxidant. It is claimed to improve learning, memory ability and reduce the incidence of certain age related neurological disorders including macular degeneration and dementia (Bastianetto and Quirion, 2002). However, its therapeutic efficacy has been hampered by poor absorption, rapid metabolism and limited ability to cross the BBB (Manach et al., 2004). Tong-Un et al., 2010 have evaluated the effects of nasally administered quercetin liposomes on cognition and biochemical alterations in ethylcholine aziridinium (AF64A) model of AD in rats (Tong-Un et al., 2010). Rats were treated with quercetin liposomes, containing 0.5 mg of quercetin in 20 μL via IN route once daily 2 weeks before and 1 week after AF64A administration. Cognitive function was assessed 7 days after AF64A administration by Morris water maze test. Animals treated with quercetin liposomes showed significant decrease in acquisition time, increase in retention time and decrease in AChE activity compared to negative control. The authors suggested cognitive enhancing effects of quercetin may be attributed to its antioxidant property and inhibition of AChE in hippocampus. Biochemical estimation of OS markers revealed decrease in lipid peroxidation and increase in level of antioxidant enzymes superoxide dismutase and glutathione peroxidase. In an another study, same authors reported IN administration of quercetin liposomes significantly increased the survival of neurons and cholinergic neurons density in hippocampus of AF64A model of AD (Phachonpai et al., 2010). This suggests that in addition to neuroprotective effect quercetin has neurotrophic effect and stimulates neurogenesis in hippocampus.

Luppi et al., 2011 prepared and evaluated the albumin nanoparticles carrying beta cyclodextrins and its hydrophilic derivatives for nasal delivery of tacrine hydrochloride (Luppi et al., 2011). Bovine serum albumin nanoparticles were prepared by coacervation method, followed by thermal cross-linking. The prepared nanoparticles had a mean size and polydispersity lower than 300nm and 0.33, respectively. The authors also reported that the presence of the different beta cyclodextrins in the polymeric network affected drug loading and could differently modulate nanoparticle mucoadhesiveness and drug permeation behavior through the nasal mucosa. The ex-vivo drug permeation studies across sheep nasal mucosa indicated that tacrine standard solution showed 100% drug permeation in 100 min whereas nanoparticles exhibited sustained permeation profiles and in particular nanoparticles containing the different cyclodextrins revealed enhanced drug permeation with respect to nanoparticles based on albumin alone. The lower permeation and better mucoadhesion of nanoparticles in comparison to drug solution is

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suitable for improvement of nasal drug BA. A decrease in lag time and an increase in the flux was observed and correlated to beta cyclodextrins ability to interact with the lipophilic components of biological membranes changing their permeability. Though tacrine has been discontinued due to hepatotoxicity IN administration could be a useful strategy to minimize its distribution to non-target sites and re-introduce the drug as a new treatment strategy.

**Fazil et al., 2012** investigated rivastigmine loaded chitosan nanoparticles for IN delivery (Fazil et al., 2012). The brain/blood ratio of rivastigmine for different formulations were 0.235, 0.790 and 1.712 of rivastigmine (IV), rivastigmine (IN), and rivastigmine nanoparticles (IN) respectively at 30 min indicative of direct nose to brain transport bypassing the BBB. The brain concentration achieved from IN administration of nanoparticles was significantly higher than those achieved after IV and IN administration of drug solution. The higher drug transport efficiency (355±13.52%) and direct transport percentage (71.80± 6.71%) were found with rivastigmine loaded chitosan nanoparticles as compared to other formulation. Thus, IN administration of AChEIs could overcome limitations of oral therapy such as nausea and vomiting.

**Chen et al., 2013** have investigated CUR thermosensitive hydrogel for IN delivery (Chen et al., 2013). The hydrogel was composed of Pluronic F127 and Poloxamer 188 had short gelation time, longer mucociliary transport time and prolonged residence in nasal cavity of rats. Nasal mucociliary toxicity studies of the developed formulations revealed that they did not cause any toxicity and integrity of mucocilia was maintained upto 14 days. *In vitro* release studies revealed that hydrogel release was diffusion controlled by dialysis bag technique and dissolution controlled by membraneless method. *In vivo* pharmacokinetic studies revealed that drug targeting efficiency for drug after IN administration in cerebrum, cerebellum, hippocampus and olfactory bulb were 1.82, 2.05, 2.07 and 1.51 times compared to IV administration of CUR solution respectively. There was significant increase in distribution of CUR into cerebellum and hippocampus.

### 3.7 Design of Experiment

**Singare et al., 2010** identified formulation and process variables affecting the physical properties and scale up of nanosuspension, on bead mill using RSM employing BBD (Singare et al., 2010). Design Expert software (M/s Stat-Ease, Minneapolis, USA) was used to conduct the study. Perturbation graphs were plotted to find those factors that most affect the response. The relationship between the dependent and independent variables were further elucidated using contour plots. The graphical optimization method helped in finding the design space to get nanosuspension with desired physicochemical
properties. The present study highlighted the use of BBD approach for optimization of pharmaceutical nanoformulations.

Rahman et al., 2010 determined the main and interaction effects of compositional variables on the CQA of risperidone SLN by BBD (Rahman et al., 2010). Authors had described the concept of QBD and DoE, which offers the advantage over conventional method of quantitation in that multicomponent of a formulation can be estimated instantaneously and simultaneously after the construction of model.

Zhang et al., 2010 formulated DHA-NLC using RSM to optimize the design space having ideal entrapment efficiency, drug loading and particle size (Zhang et al., 2010). CCD model was constructed to optimize NLC formulation using Design Expert software (M/s Stat-Ease, Minneapolis, USA). Authors showed that RSM-CCD could efficiently be applied for modeling of DHA-NLC and thus highlighted the DoE approach in pharmaceutical formulation development.

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