Review of Literature
Alzheimer's disease is a progressive, degenerative disease of the brain, which causes thinking and memory impairment. It is the most common form of dementia. Dementia is a syndrome consisting of a number of symptoms that include loss of memory, judgment and reasoning, changes in mood, behaviour and communication abilities. The disease was first identified by Dr. Alois Alzheimer in 1906. He described the two hallmarks of the disease: "plaques" - numerous tiny dense deposits scattered throughout the brain which become toxic to the brain cells at excessive levels and "tangles" which interfere with vital processes eventually "choking" off the living cells. As well, when the brain cells degenerate and die, the brain markedly shrinks in some regions.

Over time, AD was split into 2 clinical conditions depending on the age of onset. AD, because of its initial description in a relatively young woman, was a term reserved for a type of “presenile” dementia affecting individuals younger than 65 years of age, whereas a similar dementia in the elderly, i.e, in individuals over 65 years of age, was referred to as senile dementia of the Alzheimer-type (Roth et al., 1967; Tomilson et al., 1970). AD is now generally recognized as a single entity with a prevalence that increases sharply after the age of 65.

As AD progresses and affects different areas of the brain, various abilities become impaired. AD attacks nerves, neurons and neurotransmitters. Although AD is often associated with increasing age, the exact cause is unknown. AD is a progressive condition, which means that it will continue to get worse as it develops. Mild forgetfulness and memory delays are often part of the normal aging process. Older individuals simply need more time to learn a new fact or to remember an old one. We all have occasional difficulty remembering a word or someone's name; however, those with AD will find these symptoms progressing in frequency and severity. Everyone, from time to time will forget where they placed their car keys; an individual with AD may not remember the purpose of the keys. There has been recent interest in a condition called mild cognitive impairment (MCI). Individuals with “amnesic” MCI, the most common form, have memory impairment (for example, difficulty remembering names and following conversations and pronounced forgetfulness), but are able to perform routine daily activities without assistance. These MCI patients generally have normal judgment, perception and reasoning skills. Many people with MCI are at risk for further cognitive decline, usually caused by AD. However, while all patients who develop some form of dementia go through a period of MCI, not all patients exhibiting MCI will develop AD.
Symptoms of MCI may include:

- Memory problems that are noticed by others
- Poor performance on cognitive tests
- Depression
- Irritability, anxiety and sometimes aggressive or apathetic behaviour

<table>
<thead>
<tr>
<th>Normal</th>
<th>Early Alzheimer's disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Can’t find keys</td>
<td>Routinely place important items in odd places</td>
</tr>
<tr>
<td>Search for casual names and words</td>
<td>Forget names of family and common objects</td>
</tr>
<tr>
<td>Briefly forget conversation details</td>
<td>Frequently forget entire conversations</td>
</tr>
<tr>
<td>Can’t find a recipe</td>
<td>Can’t follow recipe directions</td>
</tr>
<tr>
<td>Forget to write down a cheque</td>
<td>Can no longer manage chequebook</td>
</tr>
<tr>
<td>Cancel a date with friends</td>
<td>Withdraw from usual interests</td>
</tr>
<tr>
<td>Miss an occasional right turn</td>
<td>Get lost in familiar places</td>
</tr>
</tbody>
</table>

Table 1: Comparison between sign of normal changes verses early Alzheimer’s symptoms.
(Source: http://www.helpguide.org/elder/alzheimers_disease_symptoms_stages.htm)

Many conditions can contribute to the development of memory problems and dementia; AD is just one of them. A decline in intellectual functioning that significantly interferes with normal social relationships and daily activities is characteristic of dementia, which is most commonly caused by AD. AD and multi-infarct dementia (a series of small strokes in the brain) cause the vast majority of dementias in the elderly. Other possible causes of dementia-like symptoms include infections, drug interactions, a metabolic or nutritional disorder, brain tumors, depression or another progressive disorder like Parkinson's disease. If memory loss
increases in frequency or severity, makes an impression on friends and family, begins to interfere with daily activities (for example, employment tasks, social interactions and family chores), seek qualified professional advice and evaluation by a physician with extensive knowledge, experience and interest in dementia and memory problems.

Prevalence:

AD constitutes approximately 70% of all dementia cases (Fratiglioni et al., 1999; Small et al., 1997). Incidence of AD increases with age, doubling every 5-10 years. For persons between ages 65 and 69, 70 and 74, 75 and 79, 80 and 84, 85 and older (Zhu and Sano, 2006) the incidence of AD has been estimated to be 0.6%, 1.0%, 2.0%, 3.3%, and 8.4% respectively (Hebert et al., 1995). Prevalence also increases exponentially with age, rising from 3% among those 65-74 to almost 50% among those 85 or older; AD affects 25 million people worldwide. In the USA, prevalence was estimated at 5 million in 2007 and, by 2050, it is projected to increase to 13 million (Zhu and Sano, 2006). Aside from age, other risk factors include family history of dementia, head trauma, genetic factors [e.g., apolipoprotein E (ApoE) ε 4 allele]; being female, low education level, vascular disease, and environmental factors. The above data are in keeping with the projected demographic changes resulting from the "baby boomer" generation reaching old age, and the continued increase in life expectancy.

Among the many diseases and afflictions that we face as we aged, AD remains among the least understood and most frightening. This deadly disease attacks that most essential and complex of organs, the brain and can leave the patient confused, depressed and helpless while challenging the ability of loved ones to cope.

Symptoms & Stages of Alzheimer's Disease

Some common early symptoms of AD include confusion, disturbances in short-term memory, problems with attention and spatial orientation, changes in personality, language difficulties and unexplained mood swings. Normally, these symptoms are very mild, and presence of the disease may not be apparent to the person experiencing the symptoms, loved ones or even health professionals. The three stages listed below represent the general progression of the disease. Although these symptoms will likely vary in severity and chronology, overlap and fluctuate, the overall progress of the disease is fairly predictable. On average, people live for
8 to 10 years after diagnosis, but this terminal disease can last for as long as 20 years.

**Stage 1 (Mild):** This stage can last from 2 to 4 years. They exhibit minor memory loss and mood swings, and are slow to learn and react.

The examples of behaviours that people exhibit in this mild stage include:

- Getting lost
- Difficulty managing money and paying bills
- Repetitive questions and conversations
- Taking longer than usual to finish routine daily tasks
- Poor judgment
- Losing things or misplacing them in odd places
- Noticeable changes in personality or mood

**Stage 2 (Moderate):** This is generally the longest stage and can last 2 to 10 years. In this stage, the person with Alzheimer’s disease is clearly becoming disabled. Individuals can still perform simple tasks independently, but may need assistance with more complicated activities. They forget recent events and their personal history and become more disoriented and disconnected from reality. Memories of the distant past may be confused with the present, and affect the person’s ability to comprehend the current situation, date and time. They may have trouble recognizing familiar people. Speech problems arise and understanding, reading and writing are more difficult, and the individual may invent words. They may experience sleep disturbances and have more trouble eating, grooming and dressing.

**Stage 3 (Severe):** This stage may last 1 to 3 years. During this final stage, people may lose the ability to feed themselves, speak, recognize people and control bodily functions, such as swallowing or bowel and bladder control. Their memory worsens and may become almost non-existent. They will sleep often and grunting or moaning can be common. In a weakened physical state, patients may become vulnerable to other illnesses, skin infections, and respiratory problems, particularly when they are unable to move around.
10 Warning signs for Alzheimer's disease (AD)

To help us know what warning signs to look for, the Alzheimer Society has given the following list to help the Alzheimer's patients:

1. **Memory loss that affects day-to-day function**

   It's normal to occasionally forget appointments, colleagues' names or a friend's phone number and remember them later. A person with Alzheimer's disease may forget things more often and not remember them later, especially things that have happened more recently.

2. **Difficulty performing familiar tasks**

   Busy people can be so distracted from time to time that they may leave the carrots on the stove and only remember to serve them at the end of a meal. A person with Alzheimer's disease may have trouble with tasks that have been familiar to them all their lives, such as preparing a meal.

3. **Problems with language**

   Everyone has trouble finding the right word sometimes, but a person with Alzheimer's disease may forget simple words or substitute words, making her sentences difficult to understand.

4. **Disorientation of time and place**

   It's normal to forget the day of the week or your destination for a moment. But a person with Alzheimer's disease can become lost on their own street, not knowing how they got there or how to get home.

5. **Poor or decreased judgment**

   People may sometimes put off going to a doctor if they have an infection, but eventually seek medical attention. A person with Alzheimer's disease may have decreased judgment, for example not recognizing a medical problem that needs attention or wearing heavy clothing on a hot day.
6. Problems with abstract thinking

From time to time, people may have difficulty with tasks that require abstract thinking, such as balancing a cheque book. Someone with Alzheimer's disease may have significant difficulties with such tasks, for example not recognizing what the numbers in the cheque book mean.

7. Misplacing things

Anyone can temporarily misplace a wallet or keys. A person with Alzheimer's disease may put things in inappropriate places: an iron in the freezer or a wristwatch in the sugar bowl.

8. Changes in mood and behaviour

Everyone becomes sad or moody from time to time. Someone with Alzheimer's disease can exhibit varied mood swings -- from calm to tears to anger -- for no apparent reason.

9. Changes in personality

People's personalities can change somewhat with age. But a person with Alzheimer's disease can become confused, suspicious or withdrawn. Changes may also include apathy, fearfulness or acting out of character.

10. Loss of initiative

It's normal to tire of housework, business activities or social obligations, but most people regain their initiative. A person with Alzheimer's disease may become very passive, and require cues and prompting to become involved.

Causes of Alzheimer's disease:

Till now nobody knows exact cause of Alzheimer's disease. However, scientific researchers have begun to point in several directions. Like several other adult onset neurodegenerative diseases, AD is a multifactorial illness with both genetic (Selkoe, 1997; Lovestone et al., 1999) and non genetic causes (Jellinger, 2004).
Family history and genetics

A very small percentage of people with Alzheimer's disease (5-7%) have familial Alzheimer's disease or FAD (formerly known as "early onset Alzheimer's disease"). At some point in their family history certain genes mutated and developed the abnormal characteristics that cause FAD. These inherited genes have a powerful influence: if one parent has FAD, each child has a 50% chance of inheriting the disease, and with two parents with FAD, 75% of their children will go on to develop Alzheimer's disease in adulthood. These inherited genes differentiate FAD from the more common sporadic form of Alzheimer's disease, but the disease itself is identical. The sporadic form of Alzheimer's disease (which used to be called "late onset Alzheimer's disease"), was formerly assumed to have no family linkages. However, it's now known that a person with a direct relative (parent or sibling) with Alzheimer's disease has a three times greater chance of developing the disease than someone who does not. The risk increases further if both parents have the disease. So aside from the FAD-related genes there are Alzheimer's disease-related genetic factors shared by family members. In fact, the risk associated with any one of these newly discovered genetic risk factors is lower than the risk associated with having a parent with the sporadic form of the disease.

Amyloid Precursor Protein (APP)

The vast majority of AD cases are sporadic, a number of genetic mutations have been identified which result in FAD (Tanzi and Bertram, et al 2005). Cases of FAD typically become apparent clinically at younger ages than cases of sporadic AD. Identification and characterization of the mutations that produce FAD have placed the APP processing pathway centre-stage, consequently shaping subsequent research efforts worldwide. Interestingly, of the known FAD mutations, only 10% of these involve modification in the APP gene itself. The remainder is made up of missense mutations in the genes that encode the presenilins (PSEN1 and PSEN2). Presenilins (PSEN) are integral membrane proteins, the holoprotein forms of which are located in the endoplasmic reticulum (ER). PSEN1 and PSEN2 undergo proteolysis and the cleaved protein associates with nicastrin, Alph-1 and Pen-2 to form the active multiprotein γ-secretase complex responsible for the intramembranous cleavage of APP (De Strooper et al., 1999; De Strooper et al., 1998; Wolfe, 2001).
The APP precursor protein undergoes a series of endoproteolytic cleavages (Selkoe, 1994). One of these is mediated by a putative membrane-associated α-secretase, which cleaves βAPP695 in the middle of the Aβ peptide domain and liberates the extracellular N-terminus of βAPP (Fig.1). The other cleavage pathway involves sequential cleavages by β- and γ-secretases, and generates 40–42 amino acid Aβ peptides. The first cleavage occurs at the beginning of the Aβ domain, and is mediated by β-secretase (BACE 1), a Type 1 transmembrane glycosylated aspartyl protease, resident in post-Golgi membranes and at the cell surface (Vassar et al., 1999). The second set of cleavages occurs at residues +40, 42 by a putative enzyme activity termed γ-secretase. Both γ-cleavage and β-cleavage require the presence of the presenilin proteins. The N-terminal product of γ/β-secretase is Aβ, while the C-terminal product is a labile fragment termed amyloid intracellular domain (AICD), which might act as a signal transduction molecule. The γ/β-secretase cleavage actually generates a mixture of Aβ peptides, containing 39 to 43 residues. Aβ peptides ending at residue 42 or 43 (long tailed Aβ) are thought to be more fibrillogenic and more neurotoxic than Aβ ending at residue 40, which is the predominant isofomi produced during normal metabolism of βAPP (Jarret and Lansbury, 1993; Lorenzo and Yanker, 1994). Aβ1-42 species are the main constituent of amyloid plaques in Alzheimer’s disease patients.

A physiological role for Aβ42 in mediating synaptic plasticity has been suggested (Balducci et al., 2010; Shankar et al., 2008) and the cellular prion protein can act as an Aβ-oligomer receptor (Lauren et al., 2009). Besides the release of Aβ peptides by the cleavage of APP at the γ-site, the APP intracellular domain (AICD) is released by γ-secretase mediated β-cleavage of APP. AICD is thought to regulate gene transcription of several putative target genes (Cao and Sudhof, 2001), although its role in the pathophysiology of AD is debated (Muller et al., 2008). Recently, it has been shown that AICD-overexpressing transgenic mice show hyperphosphorylation and aggregation of tau protein (Ghosal et al., 2009) implying that AICD can contribute to AD pathology independent of Aβ.

The decrease in the ratio of Aβ42/Aβ40 seems more pronounced than the reduction of Aβ42 alone (Hansson et al., 2007). Multiple molecular mechanisms have been proposed to explain the neurotoxic effects of Aβ. These include inducing apoptosis by direct effects on cell membranes and by indirect effects, such as potentiating effects of excitatory amino acids, oxidative stress, and increases in intracellular calcium and free radicals (Matteson et al., 1995).
Fig 1. Proteolytic cleavages of APP: APP processing is initiated by β-secretase, which causes the secretion of the large β-sAPP molecule and the retention of C-terminal fragment (β-CTF). This fragment undergoes further cleavage by γ-secretase to release Aβ peptides terminating at residues 40 and 42, as well as several shorter Aβ isoforms (Source: Hampel et al., 2010).

**Presenilins (PSEN)**

Two separate PSEN genes are identified in human, PSEN1 and PSEN2, which show a high sequence homology. Autosomal dominant mutations in either of the PSEN genes are causative for the hereditary form of FAD (De Strooper, 2007). PSENs are the catalytic subunit of the proteolytic γ-secretase complex, a protease performing intra-membrane cleavage of many different type 1 transmembrane proteins. Two highly conserved aspartates in transmembrane domain 6 and 7 form the core of the catalytic site (Spasic and Annaert, 2008). Besides PSEN, the γ-secretase complex consists of three additional components; nicastrin, anterior pharynx defective 1 (Aph-1) and presenilin enhancer 2 (Pen-2 or Psenen) (De Strooper, 2007). The γ-secretase complex cleaves many substrates, of which APP and Notch are the most well known. Consecutive cleavage of APP by β- and γ-secretases results in the formation of Aβ peptides, which form the main component of the AD plaques (Parks
and Curtis, 2007; Selkoe and Wolfe, 2007). As yet, over 170 autosomal mutations in the PSEN1 gene and more than 10 mutations in PSEN2 have been identified, which lead to early onset of FAD. In the APP gene, over 25 causative mutations are identified for FAD (http://www.molgen.ua.ac.be/ADMutations). The majority of PSEN1 mutations are missense mutations resulting in single amino acid changes in the PSEN1 protein. The mutations are spread throughout the whole length of the PSEN1 protein and exert their effect in a dominant negative manner. Many FAD mutations increase the ratio of more fibrillogenic form of Aβ, the Aβ42 peptide, over Aβ40, which is interpreted as a gain of toxic function. However, this altered ratio can also be derived from a decrease in Aβ40 formation, accompanied by increased accumulation of the γ-secretase substrate APP CTF (Bentahir et al., 2006; Kumar-Singh et al., 2006). Therefore, biochemical loss-of-function of PSEN can result in a gain-of-toxic-function of the APP gene through decreased or alternative γ-secretase cleavage, favouring the generation of the Aβ peptides of 42 amino acids in length. Although the underlying causes leading to sporadic AD and inherited FAD likely differ, they are indistinguishable in terms of neuropathological hallmarks (De Strooper et al., 2007). New insights into PSEN function using PSEN FAD model systems may thus also provide clues regarding the role of PSEN in sporadic AD.

Fig 2. Schematic representation of human PSEN1 membrane topology. PSEN1 has ten hydrophobic domains (in purple) with nine proposed transmembrane domains (TMD) (Spasic and Annaert, 2008). PSEN undergoes endoproteolytic cleavage within hydrophobic domain 7 forming the stable NTF and CTF fragments. The two aspartate residues in TMD6 and TMD7 making up the PSEN1 catalytic site are shown in red triangles.
The molecular genetics of Alzheimer's disease

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>No. of mutations</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>APP</td>
<td>21</td>
<td>&gt;25</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>PSEN1</td>
<td>14</td>
<td>&gt;170</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>PSEN2</td>
<td>1</td>
<td>10</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>ApoE4 allele</td>
<td>19</td>
<td></td>
<td>Risk increased 4 times with one copy</td>
</tr>
</tbody>
</table>

Table 2: Source: http://www.molgen.ua.ac.be/Admutations

Apolipoprotein E (ApoE)

This gene is the most important genetic risk factor for the sporadic form of Alzheimer's disease. ApoE is a glycoprotein with a molecular weight of 34, 200 Da (Mahley, 1988). ApoE genes regulate the production of a protein that helps to carry cholesterol and other fats in the blood to the cells of the body. The ApoE gene, located on chromosome 19, the three common isoforms, ApoE2, ApoE3 and ApoE4, are encoded by three alleles (e2, e3 and e4, respectively) of a single gene (Mahley, 1988). Therefore, three homozygous (Apoe2/2, e3/3 and e4/4) and three heterozygous (Apoe3/2, e4/3 and e4/2) genotypes occur in humans. In almost all human populations, the ApoE3/3 genotype is the most common (typically 50–70% of the population), and the e3 allele accounts for the vast majority of the ApoE gene pool (typically 70–80%). The e4 allele accounts for 10–15% and the e2 allele for 5–10%. Sequence differences among the ApoE isoforms have significant functional consequences. ApoE4 is the major known genetic risk factor for AD (Corder, 1993; Saunders, 1993). ApoE4 carriers account for 65–80% of all AD cases, highlighting the importance of ApoE4 in AD pathogenesis (Farrer, 1997). ApoE4 associates with an increase in amyloid plaques and neurofibrillary tangles (NFTs) (Namba, 1999; Wisniewski and Fratigione, 1992) that are characteristic of AD. The e4 allele is significantly overrepresented in late onset familial AD patients (50%) compared with age- and sex-matched controls (16%) (Strittmatter, 1993). The e4 allele associates with late-onset familial AD in several populations and in late-onset sporadic AD (Saunders, 1993). However, ApoE play an important role in the transport and
uptake of triglycerides and cholesterol in the brain. This implies that uptake of triglycerides into the brain may be involved in the development of Alzheimer's disease. Furthermore, ApoE4 has a dosage effect on the risk and age of onset of AD (Corder, 1993) as the number of ApoE4 alleles increases from 0 to 2, the risk of developing late-onset AD increases from 20% to 90%, and the mean age of onset decreases from 84 to 68 years (Corder, 1993) by contrast, ApoE2 might protect against AD in some populations (Corder, 1994). In addition, the e4 allele associates with a poor clinical outcome in patients with acute head trauma (Chamelian, 2004). ApoE4 also associates with other types of neurodegenerative disorders. ApoE4 can affect the age of onset, progression or severity of stroke (Alberts, 1995; Slooter, 1997) Parkinson's disease (Li. et al., 2004), multiple sclerosis (Schmidt, 2002; Kantarci et al., 2004) and amyotrophic lateral sclerosis (Drory, 2001).

Age

Age is the most important risk factor for AD (Harman, 2006). As we aged, our body's ability to repair itself becomes less efficient. At 65 to 70 years, the risk is about 1.5% and increases simultaneously at 70 to 74 years. The extent by which the self-repair of our brains diminishes varies from person to person and these differences contribute to an individual's susceptibility to Alzheimer's disease as they aged. As well, many of the other known risk factors for the disease tend to increase with age (such as elevated cholesterol and being overweight). However, risk factors do not cause Alzheimer's disease on their own. The brain has to reach a certain critical age for the disease to occur. The older we become the higher the risk.

Female Gender

Several studies have reported that women have a much higher risk of AD than men. If there is a gender difference, it is likely to be due to estrogen, the primary female hormone, which appears to have properties that protect against the memory loss and lower mental functioning associated with normal aging. Such actions include blocking production of beta amyloid, offering antioxidant protection, and regulating blood sugar (glucose) levels in the brain. The drop in estrogen levels after menopause may explain a higher risk for AD in older women than in men. (Testosterone, the male hormone, converts to estrogen, which may help protect men). Studies have been mixed, however, on the association between the
decline in natural estrogen levels and mental functioning in older women. However, hormonal changes are not the only factor contributing to the increased incidence of AD in women. On average, women live longer than men and age is a risk factor. Women are also more prone to diabetes, which is also a risk factor that appears to somewhat increase the risk for AD.

Cardiovascular Disease

High blood pressure and high cholesterol level are the risk factor for the cardiovascular disease as well as AD and vascular dementia.

Diabetes

Diabetes has long been recognized as a risk factor for vascular dementia — a type of cognitive decline caused by damaged blood vessels in the brain. Many people with cognitive decline have brain changes that are hallmarks of both AD and vascular dementia. Some researchers think that each condition helps fuel the damage caused by the other. Ongoing research focuses on confirming the link between Alzheimer’s and diabetes and understanding why it exists. The link between type 2 diabetes (type 2 diabetes is a metabolic disorder that is characterized by high blood glucose in the context of insulin resistant and relative insulin deficiency) and Alzheimer’s may be especially strong as a result of the complex ways that type 2 diabetes affects the ability of the brain and other body tissues to use sugar (glucose) and respond to insulin. Diabetes may also increase the risk of developing mild cognitive impairment (MCI), a transition stage between the cognitive changes of normal aging and the more serious problems caused by AD and other types of dementia.

Down syndrome

It is clear that there is an increased incidence of early onset dementia of the Alzheimer type among people with Down syndrome (trisomy of chromosome 21). Estimates vary, perhaps due to different diagnostic criteria and population biases, but around 10% of people with Down syndrome may be diagnosed with Alzheimer type dementia between the ages of 40 and 49, rising to around 30% between the ages of 50 and 59. It is important to note, however that not all people with Down syndrome who develop these brain changes will go on to develop
dementia. It seems likely that these people may not yet have developed other age-induced changes that occur in most people with AD.

**Mild Cognitive Impairment (MCI)**

Memory impairment is beyond what is normally expected with aging, but not sufficient to be classified as dementia. It is estimated that 85% of people with mild cognitive impairment will develop AD within 10 years, making MCI an important risk factor for the disease. Brain imaging may make it possible to detect the most at-risk individuals with MCI, and research to this end is ongoing.

**Head injury**

Brain injuries at any age, especially repeated concussions, are accepted by most clinicians as risk factors for the later development of AD.

**Low Levels of Formal Education**

Several studies have shown that people who have less than six years of formal education appear to have a higher risk of developing AD. However, new studies challenge this conclusion, and it may be that factors often associated with low educational background, such as unhealthy lifestyle, account for the risk rather than low educational level itself.

**Depression**

There is a significant overlap between depression and dementia in the elderly. Some evidence suggests that there may even be common genetic factors in people who have both early depression and AD.

**Other Risk Factors**

In addition to the risk factors described above, all of the following have been documented as risk factors for AD: inflammatory conditions (possibly reflecting immune system malfunction), a history of episodes of clinical depression, stress, and inadequate exercising of the brain. Risk factors that are less firmly established include smoking, excessive alcohol consumption and drug abuse.
Aluminium

Most researchers no longer regard aluminium as a risk factor for AD. However, some researchers are still examining whether some people are at risk because their bodies have difficulties in handling foods containing the metals such as copper, iron, and aluminium.

Neuropathology of Alzheimer’s Disease

Nerve cells changed during the progression of Alzheimer’s disease:

There is an overall shrinkage of brain tissue as AD progresses. In addition, the ventricles are noticeably enlarged. In the early stages of AD, short term memory begins to decline when the cells in the hippocampus, which is part of the limbic system, degenerate. The ability to perform routine tasks also decline. As AD spreads through the cerebral cortex judgement declines and emotional outbursts may occur and language is impaired. Progression of the disease leads to the death of more nerve cells and subsequent behavioural changes, such as wandering and agitation. The ability to recognise faces and to communicate is completely lost in the final stages. This stage of completely dependency may last for years before the patient dies. The average length of the time from diagnosis to death is 4-8 years, although it can take 20 years or more for the disease to run its course.

![Brain Cross-Sections](http://drpinna.com/alzheimers-disease-will-you-get-it-6566/alzheimers-disease)

Fig 3: This image represents a cross section of the brain as seen from the front. The cross-section on the left represents a brain from a normal individual and the one on the right represents a brain with AD (Source: [http://drpinna.com/alzheimers-disease-will-you-get-it-6566/alzheimers-disease](http://drpinna.com/alzheimers-disease-will-you-get-it-6566/alzheimers-disease)).
Neuritic plaques

Neuritic plaques are dense, extracellular deposits of amyloid surrounded by dystrophic neurites (damaged axonal and dendritic processes), plus reactive astrocytes and activated microglia, the presence of which indicates chronic low-grade localized inflammation. ‘Amyloid’ is a generic term for an abnormal aggregate of proteins or peptides that specifically adopt a regular β-sheet configuration. These aggregates may form fibrils, 10 nm in diameter, which can be observed radiating from the dense core of the neuritic plaques. In AD, the amyloid deposits are largely spherical, reaching up to 200 μm in diameter, and are prevalent throughout the cortex and hippocampus of brains from affected individuals. The main component of amyloid in AD is the β-amyloid peptide consists of 42 amino acids (Aβ42) (Glenner and Wong, 1984). More recently, early intraneuronal accumulation of Aβ42, the most pathogenic Aβ species, has been described in AD, Down syndrome (Busciglio et al., 2002; Cataldo et al., 2004), and transgenic AD mouse models (Oakley et al., 2006; Van Broeck et al., 2008). Further, transgenic AD mice develop physiological and behavioural abnormalities prior to plaques (Chapman et al., 1999; Holcomb et al., 1998) but concomitant with intraneuronal Aβ peptide accumulation (Bayer and Wirths, 2008; Billings et al., 2005), supporting that intraneuronal Aβ peptides are involved in the initiation of AD pathogenesis (Gouras et al., 2005).

Neurofibrillary tangles (NFTs)

Like neuritic plaques, NFTs are the result of abnormal aggregation of proteinaceous material. However, unlike the extracellular Aβ pathology, NFTs develop within neurons, gradually filling the intracellular space. Eventually, these aggregates (often referred to as ‘inclusions’) are believed to kill the affected neurons. In support of the neurotoxicity of such inclusions, extracellular ‘ghost’ NFTs exhibiting neuronal morphology can also be observed within the AD brain tissue. The main constituent of NFTs is the microtubule-associated protein, tau (Ballatore et al., 2007). In healthy neurons, the chief function of tau is to assist in the assembly and stabilization of microtubules, the fibrous structures largely responsible for communication and transportation within the cell (Braak et al., 1999).
Fig 4: The pathological hallmarks of AD are extracellular deposits of amyloid plaques and intracellular accumulation of hyperphosphorylated tau proteins in the form of neurofibrillary tangles (Source: amyloidbeta.gif).

Tau proteins found in neurofibrillary tangles, however, are abnormally hyperphosphorylated, making them unable to bind to microtubules. Precisely which protein kinases phosphorylate tau in the brain is unknown (Spillantini and Goedert, 1998). However, in AD tau becomes detached from microtubules and aggregates. Notably, this pathology is associated with aberrant phosphorylation of the individual tau molecules, which may be crucial for initiating NFT formation. The presence of ever-larger NFT inclusions combined with destabilization of the microtubules is likely to seriously impair neuronal function long before cell death occurs.

Molecular Mechanisms of Alzheimer's Disease

The Amyloid cascade hypothesis

The β-amyloid cascade is the most prominent hypothesis for development of AD (Tanzi et al., 2004; Schroeder and Koo, 2005) and is thought to be the primary event that triggers the pathological cascade in AD (Selkoe, 1998), although the findings in support of the amyloid hypothesis have become questionable and controversial (Robakis, 2010). It is hypothesized
that the accumulation of β-amyloid in the brain causes the AD pathology and an imbalance between β-amyloid production and clearance results in other hallmarks of the disease. The β-amyloid cascade hypothesis (Hardy and Selkoe, 2002; Tanzi and Bertram, 2005) favours the model that insoluble fibrillar β-amyloid triggers the neuronal degeneration. Evidence is now accumulating that soluble activated monomers, soluble oligomers (dimer, trimer and tetramer), and protofibrils could be responsible for triggering the pathology in AD (Walsh et al., 2002; Canevari et al., 2004). Soluble β-amyloid levels in the cortex correlate with the degree of synaptic loss in dementia, and it becomes more and more clear that AD is primarily caused by dysfunction of nerve axons and synapses (Selkoe, 2002).

Fig 5: The amyloid cascade hypothesis: According to this hypothesis, the central event in AD pathogenesis is an imbalance between Aβ production and clearance, with increased Aβ production in familial AD and decreased Aβ clearance in sporadic AD. Aβ oligomers could directly inhibit hippocampal LTP and impair synaptic function, in addition to the inflammatory and oxidative stress caused by aggregated and deposited Aβ. Tau pathology with tangle formation is regarded a downstream event, but may contribute to neuronal dysfunction and cognitive symptoms (Source: Hampel et al., 2010).
In AD, axonal degeneration may depend on β-amyloid levels, but not on plaque deposition, which means that nerve damage occurs before deposition of plaques. β-amyloid is present in the brain and in the blood and is transported through the blood brain barrier (BBB) via two important receptor transport systems: the receptor for advanced glycosylation end products (RAGE) and low-density lipoprotein-related protein (LRP) (Tanzi et al., 2004; Zlokovic, 2004). The influx of β-amyloid from blood into the brain is mediated via RAGE, while the efflux from the brain into blood is mediated via LRP (Tanzi et al., 2004; Zlokovic, 2004). This clearance from the brain to blood is of pivotal importance for the regulation of β-amyloid levels in the brain (Tanzi et al., 2004; Zlokovic, 2004).

Cholinergic Hypothesis of Alzheimer’s disease

The history of acetylcholine in neuroscience has been one of great advancement of our knowledge in many functions of the nervous system as well as in very harmful neuropathology. The importance of cholinergic neurotransmission is testified by the fact that it has led several investigators to elaborate a “cholinergic hypothesis” for several brain functions and dysfunctions, from affective disorders, depression, schizophrenia and delirium to sleep regulation and the brain traumatic injury (Hshie et al., 2008; Readler et al., 2007). A primary role for cholinergic function in cognition was initially supported by observations indicating that anticholinergic drugs had amnestic effect and reproduced memory deficits similar to those commonly observed in non-demented elderly subjects (Drachman et al., 1974). While these results were corroborated by primate studies, indicating the feasibility of pharmacologically reproducing in young and old animals some of the cognitive dysfunctions seen in elderly humans and AD patients (Berman et al., 2007; Bartus et al., 1982) A specific cholinergic deficit, involving the cholinergic projection from a basal fore brain neuronal population, the nucleus basalis magno cellular is of Meynert, to the cortex and hippocampus was consistently found in autoptic material from Alzheimer’s patients. The activity of the enzyme responsible for the synthesis of acetylcholine, choline acetyl transferase, are liable marker of cholinergic neurons and synapses, was found to be remarkably decreased, sometimes in a rather severe way, in pathological samples from the cortex and hippocampus of Alzheimer’s patients (Davies and Maloney, 1976; Perry et al., 1977). Two other specific markers of the function of cholinergic synapses, depolarization-induced acetylcholine release and choline uptake in nerve terminals to replenish the acetylcholine synthetic machine, were also reduced in the same tissues (Nilsson et al., 1986; Rylett et al., 1983). The parallel
observation of a substantially decreased number of the cholinergic projection neurons in the nucleus basalis of Meynert led to conclude that a major event in the pathogenesis of AD was represented by the degeneration of the cholinergic connection from the nucleus of Meynert to the cortex and hippocampus (Whitehouse et al., 1982). As memory impairment and dementia are primary symptoms of AD, the then emerging role of acetylcholine transmission in cognitive functions supported this conclusion (Drachman and Levit, 1974).

Fig 6: Schematic representation of the known and proposed changes in cholinergic neurons that occur in the aged and early AD brain compared with healthy young neurons. Alterations in high-affinity choline uptake, impaired acetylcholine release, deficits in the expression of nicotinic and muscarinic receptors, and disrupted axonal transport were seen in early AD neurons compared to the healthy neurons. (Source: Terry and Buccafusco, 2003).

Inflammation in Alzheimer’s Disease

Inflammation is an important trigger of neurodegeneration during aging (“Inflammaging”) (Franceschi et al., 2000) and considered as a major factor of neurodegeneration in AD. Inflammation is a potential target for AD therapy and anti-inflammatory drugs may delay AD (Moore and O’Banion, 2002). In AD oxidation of DNA, proteins and fatty acids occur in different brain areas. Some of the oxidation products have been found in the neurofibrillary tangles and senile plaques (Markesbery and Carney, 1999) and these oxidative modifications are closely associated with an inflammatory process in the AD brain (Butterfield et al., 2002). These inflammatory processes include activation of microglia and subsequent neuroinflammatory processes (Gonzalez-Scarano and Baltuch, 1999). Evidence from post-mortem analysis implicates the involvement of microglia and astrocytes in the
neurodegenerative process of numerous degenerative neurological diseases, including AD (Liu and Hong, 2003). Microglia, considered as the macrophages of the central nervous system (CNS), are found in cluster around Aβ deposits and may even be a driving force in the evolution of diffuse non-neuritic plaques into condensed neuritic plaques (Sheng et al., 1997). When microglia becomes activated, they undergo morphological changes including enlargement and show increased expression of major histocompatibility complex type II, cytokines, chemokines and complement. Neuroinflammation in AD is thought to be triggered by Aβ and/or by the substances released by dying neurons, causing microglia activation (Eikelenboom et al., 2010; Wyss-Coray, 2006). This neuroinflammatory component of AD is further characterized by a local cytokine accumulation mediating acute-phase response, activation of the complement cascade and induction of inflammatory enzyme systems such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) (McGeer and McGeer, 2010). Tumor necrosis factor-α (TNF-α) is a pleiotropic cytokine involved in a broad range of biological activities, including inflammation, cell survival, cell proliferation, and paradoxically cell death. Notably, improper TNF-α signaling is implicated in the pathogenesis of many human diseases, including AD. Increased TNF-α level in the brain and plasma, as well as a TNFR1 up regulation in the brain has been detected in AD patients (Fillit et al., 1991; Li et al., 2004). In most tissues, cyclooxygenase-1 (COX-1) is constitutively expressed, whereas COX-2 is induced in response to injury (Mitchell et al., 1993). In brain, however, COX-2 is expressed in neurons during both physiological and pathological processes. In AD brain, COX-2 mRNA is up regulated in the cortex and hippocampus in the early stages of the disease. Conversely, it has been shown a decline in the COX-2 expression in the advanced stages of AD. Notably, the stage-dependent change in the COX-2 expression correlates with prostaglandin (PG) E2 levels in the cerebrospinal fluid of AD patients (Minghetti et al., 2004; Combrinck et al., 2006). Overall, the role of COX-2 in neurodegenerative diseases is very controversial and conflicting data exist in the literature. For instance, while some studies have demonstrated that the raise in COX-2 activity greatly contributes to the progression of AD, other reports have suggested an entirely opposite effect (Cakala et al., 2007; Jantzen et al., 2002; Koitlinek et al., 2008). The reason for such discrepancy is still unknown; hence, additional studies are necessary to clarify the role mediated by COX-2 in the AD brain. NF-κB is widely known for its ubiquitous roles in inflammation and immune responses, as well as in control of cell division and apoptosis. NF-κB in its inactive form is present in the cytosol as a three-subunit complex, with the prototypical components being p65 and p50 (transcription factor dimer) and IκBα (inhibitory
NF-κB is activated by signals that activate IκB kinase (IKK), resulting in phosphorylation of IκBα; this targets IκBα for degradation in the proteosome and frees the p65-p50 dimer, which then translocates to the nucleus and binds to consensus κB sequences in the enhancer region of κB-responsive genes.

![Diagram of NF-κB activation and inflammation](image)

**Fig 7:** Schematic diagram shows the sequence of events initiated by Aβ, results in the generation of ROS/RNS that induce neurotoxicity. These free radicals activate NF-κB which in turn induce the transcription of iNOS, IL-6, IL-1β, TNF-α and COX-2 that work in concert to induce inflammation and finally leads to neuronal cell death.

Diverse signals can induce NF-κB activation, including TNF-α, sAPPα, NGF, and glutamate; increases in levels of intracellular Ca\textsuperscript{2+} and reactive oxygen species such as H\textsubscript{2}O\textsubscript{2} can be potent activators of NF-κB. This transcription factor play important role in the pathogenesis of oxidative stress associated neurodegenerative diseases. NF-κB is one of the most important transcriptional regulators of proinflammatory gene expression. Synthesis of cytokines, such as TNF-α, IL-1β, IL-6, and IL-8, are mediated by NF-κB. These roles are apparent in the
nervous system, but neurons and their neighbouring cells employ the NF-κB pathway for distinctive functions as well, ranging from development to the coordination of cellular responses to injury of the nervous system and to brain-specific processes such as the synaptic signalling that underlies learning and memory. Studies of post-mortem brain tissue from patients with Alzheimer's disease (AD) have revealed increased NF-κB activity in cells involved in the neurodegenerative process. p65 immunoreactivity increases in neurons and astrocytes in the immediate vicinity of amyloid plaques in brain sections from AD patients, consistent with NF-κB activation in those cells (O'Neill et al., 1997). In addition, immunohistochemical studies suggest that levels of NF-κB are increased in cholinergic neurons in the basal forebrains of AD patients (Boissiere et al., 1997); dysfunction and degeneration of cholinergic neurons is believed to contribute greatly to cognitive impairment in AD.

Poly (ADP-ribose) polymerase-1 (PARP-1; also known as poly (ADP-ribose) synthetase or poly (ADP-ribose) transferase) is an abundant nuclear enzyme consisting of three domains: N-terminal DNA binding domain containing two zinc fingers, auto-modification domain and catalytic domain (de Murcia et al., 1994). PARP-1 functions as a sensor of DNA damage and are able to bind to DNA breaks/nicks through Zn finger domains (de Murcia and Menissier de Murcia, 1994). Activated PARP-1 rapidly cleaves substrate NAD+ molecules to form ADP-ribose polymers on histones and other cellular proteins as part of the cellular defence and DNA repair programme (Petermann et al., 2005). Massive damage of DNA leads to over-activation of PARP-1, resulting in cell death due to its rapid depletion of cellular energy sources such as NAD+ and ATP (Szabó et al., 2006). Autopsy samples from Alzheimer patients showed poly (ADP-ribose) (PAR) accumulation, a sign of PARP activation in cortical pyramidal neurons and in few astrocytes, but no PAR accumulation was detected in microglia (Love et al., 1999). The cellular distribution of PAR in these disease was somewhat surprising, since microglial PARP-1 activation was not the most prominent as one might expect in disease with inflammatory nature. However both diseases showed astrocytic PARP activity suggesting that these major glial cells of brain might have been underestimate and may really significantly contribute to inflammatory responses in chronic neurodegenerative disorders.
response to genotoxic stress (Xiang et al., 1998; Chong et al., 2000). These findings suggest that some forms of neuronal injury invoke a common pathway involving signal transduction through p53, Bax, mitochondrial dysfunction, cytochrome c release and caspase activation. However, other forms of injury have been shown to induce neuronal cell death by stimulating Bax translocation and caspase activation independently of p53 (Ghatan et al., 2000).

Fig 8: Apoptotic stimuli like reactive oxygen species trigger the release of apoptogenic factors from the mitochondrial intermembrane space to the cytosol, such as cytochrome c, which induces the formation of the apoptosome and the activation of procaspase-9. By the action of cytochrome c and dATP the Apaf-1 protein adopts a confirmation that allows the formation of a heptameric wheel-like structure, the apoptosome. Procaspase-9 molecules can bind to the inner "hub" region of the apoptosome and are activated by dimer formation. Active caspase-9 dimers further mediate activation of effector caspases that may execute the neuronal cell death.
Apoptosis in Alzheimer's disease:

The demonstration that p53 promotes apoptosis, has important implications for the central nervous system (CNS), in response to injury, and in neurodegenerative disorders such as Alzheimer's and Huntington's disease (Raff et al., 1993; Stefanis et al., 1997; Friedlander et al., 1998). Neuronal injury, especially damage mediated by excitotoxicity, has been associated with increased production of reactive oxygen species, (Ankarcrona et al., 1995; Dugan et al., 1995) and accumulation of single-strand DNA breaks (Reynolds and Hastings, 1995). DNA strand breaks are capable of inducing p53 accumulation (Jayaraman and Prives, 1995; Huang et al., 1996) which has prompted investigators, to begin examining p53 for a role in regulating neuronal cell death. p53 promotes apoptosis by modulating the expression of select target genes. Numerous pro-apoptotic genes are susceptible to regulation by p53 including Bax (Miyashita et al., 1994a) IGF-binding protein-3 (Buckbinder et al., 1995) and p53-inducible genes (PIG's) (Polyak et al., 1997). p53 may also induce apoptosis through transcriptional repression although the mechanism for repression is not understood. Genes down regulated by p53 include bcl-2 (Miyashita et al., 1994b) the IGF-I receptor (Prisco et al., 1997) the microtubule associated protein (MAP4) (Murphy et al., 1996) and presenilin-1 (Roperch, 1998). Disruption of the mitochondrial membrane potential and increased production of reactive oxygen species have been defined as early events in the process of neuronal apoptosis (Deckwerth et al., 1993; Vayssiere et al., 1994). The mechanism by which p53 specifies the neuronal response to injury is poorly understood. However, few studies published to date utilizing neurons are in agreement with the idea that Bcl-2 family member, Bax, is essential for p53-mediated cell death in neurons. Bax deficient neurons are protected from cell death induced by DNA damaging agents (Xiang et al., 1998) and adenovirus-mediated p53 over-expression (Xiang et al., 1998; Cregan et al., 1999). A relationship between Bax and alterations in mitochondrial function is substantiated by the recent demonstration that cell damage promotes Bax translocation from the cytosol to the mitochondria in neurons (McGinnis et al., 1999; Putcha et al., 1999). Bax activation has been associated with a reduction in mitochondrial membrane potential, mitochondrial release of cytochrome 'c' and activation of caspases (Xiang et al., 1996). This suggests that caspases may also be a component of the p53-induced cell death pathway sitting downstream of Bax activation. The relationship between p53 and caspase activation has recently been examined in neurons. Recent studies indeed demonstrated that p53 is required for caspase activation in
Molecular mechanisms of oxidative Stress in Alzheimer's disease

Oxidative stress has been implicated in the pathogenesis of a number of diseases including neurodegenerative disorders, cancer, ischemia, etc. Under physiological conditions, there is a balance between the pro-oxidant and anti-oxidant levels. However, certain environmental factors, stressors, or disease may cause an imbalance leading to increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS may react with biomolecules including proteins, lipids, carbohydrates, DNA and RNA (Halliwell et al., 2006) leads to oxidative damage of these biomolecules. Oxidative modification of biromolecules has been shown to cause cellular dysfunction (Aksenova et al., 2002; Butterfield et al., 2001). The markers of oxidative stress that are commonly studied to determine the level of oxidative stress in biological samples include protein carbonyls, thiobarbituric acid reactive substance (TBARS), free fatty acid release, acrolein and 4-hydroxy-2-nonenal (HNE) (Dalle-Donne et al., 2006; Zarkovic, 2003)

The products of lipid peroxidation such as HNE are highly reactive and accumulates in membranes in response to oxidative insults (Esterbauer et al., 1991) and invokes a wide range of biological activities, including inhibition of protein and DNA synthesis, disruption of Ca\(^{2+}\) homeostasis, membrane damage, activation of stress signaling pathways and cell death (Tamagno et al., 2003).

Since the cell membrane is rich in polyunsaturated fatty acid, increased levels of ROS can lead to increased production of toxic lipid peroxidation products, which can mediate oxidative stress-induced death in many cell types (Tamagno et al., 2003). Among all the body organs, the brain is particularly vulnerable to oxidative damage because of high levels of polyunsaturated fatty acids in addition to its high oxygen consumption and high levels of redox transition metal ions and low levels of antioxidant enzymes. Oxidative stress in AD brain is manifested by decreased levels of antioxidant enzymes and also by increased protein oxidation, lipid peroxidation, DNA oxidation and ROS formation (Lauderback et al., 2001).

In MCI patients, plasma levels of non-enzymatic antioxidants and activities of antioxidant enzymes appeared to be decreased when compared to those of controls (Sultana et al., 2008) with no change in the levels of antioxidant protein levels (Guidi et al., 2006).
Aβ coordinates the metal ions Zn, Fe and Cu, which induces aggregation and neurotoxicity leading to the generation of H\textsubscript{2}O\textsubscript{2} resulting oxidative-nitrisative stress. These stresses can initiate a number of different events, including lipid peroxidation, protein oxidation, DNA and RNA oxidation and consequently leads to neuronal cell death.

Further, studies showed increased levels of oxidative damage in MCI indexed by elevated levels of markers such as 5-hydroxycytosine, HNE, malonaldehyde (MDA), protein carbonyls, 3-nitrotyrosine (3-NT) etc. (Butterfield et al., 2007; Cenini et al., 2008; Keller et al., 2005). These are indicative of oxidative stress in MCI (Keller et al., 2005; Butterfield et al., 2007) and further supporting the notion that oxidative stress could be an early event in the progression of MCI to AD.

The amyloid β-cascade hypothesis, suitably updated, postulates that Aβ is likely central to the pathogenesis of AD (Butterfield, 1997; Hardy, 2002). The mechanisms involved in Aβ-mediated neurotoxicity are unknown, but there is evidence suggesting that oxidative stress plays a key role (Hardy, 2002; Butterfield, 2002). Growing attention has focused on oxidative
mechanisms of Aβ toxicity as well as the search for novel neuroprotective agents. The ability of toxic Aβ peptides to induce protein oxidation and to inhibit the activity of oxidation-sensitive enzymes is consistent with the hypothesis that Aβ can induce severe oxidative damage. Therefore, increased oxidative stress caused by Aβ may lead to increased oxidative modification of proteins and lipids, leading to impaired cellular function and cell death, and consequently to cognitive impairment and AD-like pathology.

**Free Radical Production**

Of all the organs in the body, the central nervous system is especially sensitive to free radical oxidative damage. Its high consumption of oxygen, rich content of easily oxidizable fatty acids, relatively low content of antioxidant enzymes and antioxidants, and the presence of high levels of iron make it a prime substrate for damage by free radicals (Floyd et al., 1992). Compared with other organs, the brain consumes a large fraction (20%) of the oxygen that the body takes in, suggesting a high metabolic rate. This high oxygen consumption and possibly metabolic rate may increase the amount of free radicals produced in the brain (Floyd et al., 1992). The brain contains membranes that are composed of proteins and an abundant amount of phospholipids. These phospholipids contain oxidizable polyunsaturated fatty acids (PUFAs), such as arachidonic acid and docosahexaenoic acid. These PUFAs are vulnerable to attack by free radicals because they contain hydrogen ions held together by weak double bonds that serve as a target for reactive oxygen radicals and the generation of oxidative damage. It has been suggested that oxidative damage resulting in alterations of brain phospholipids may play a role in AD (Hazel and Williams, 1990). Elemental iron has also been found to play a role in the genesis of free radicals. Iron is an element that can catalyze oxidative reactions and can generate oxygen radicals. By way of the Fenton's reaction (Markesbery, 1999), molecules of iron may transition from the Fe^{2+} state to the Fe^{3+} state by donating a loosely bound electron to hydrogen peroxide, resulting in the generation of hydroxyl radicals. It has been shown that iron, ferritin, and transferrin are found in the senile plaques of patients with AD (Loeffler et al., 1995). By use of histochemical methods, the distribution of iron in the brain of patients with AD was found to match the distribution of neurofibrillary tangles and senile plaques (Smith et al., 1995). It is proposed that alterations in iron homeostasis may be a factor in the disease process resulting in AD.
The importance of increased oxidative stress, lipid peroxidation, and lipid peroxidation products has consistently been shown in the pathogenesis of AD (Markesbery, 1999). Moreover, significantly increased levels of the lipid peroxidation products, 4-hydroxynonenal and acrolein, are found in the hippocampus/parahippocampal gyrus, superior and middle temporal gyrus, and cerebellum of subjects with mild cognitive impairment and early AD compared to age matched controls, suggesting that lipid peroxidation occurs as an early event in the pathogenesis of AD. This is consistent with evidence from animal models of AD, where increased oxidative stress is also found in the several model of AD as well as streptozotocine model of cognitive impairment (Ishrat et al., 2006). Cerebral cortical and hippocampal homogenates were found to have higher levels of lipid peroxidation than those from wild-type mice, and lipid peroxidation preceded amyloid plaque formation (Pratic’ et al., 2001).

Under normal physiological conditions, it is estimated that up to 1% of the mitochondrial electron flow leads to the formation of superoxide (O$_2^-$), the primary oxygen free radical produced by mitochondria; and interference with electron transport can dramatically increase O$_2^-$ production. While these partially reduced oxygen species can attack iron sulfur centers in a variety of enzymes, O$_2^-$ is rapidly converted within the cell to hydrogen peroxide (H$_2$O$_2$) by the superoxide dismutase. However, H$_2$O$_2$ can react with reduced transition metals, via the Fenton’s reaction, to produce the highly reactive hydroxyl radical ('OH), a far more damaging molecule to the cell. In addition to forming H$_2$O$_2$, O$_2^-$ radicals can rapidly react with nitric oxide (NO) to generate cytotoxic peroxynitrite anions (ONOO$^-)$). Peroxynitrite can react with carbon dioxide, leading to protein damage via the formation of nitrotyrosine and lipid oxidation.

![Redox cycling which maintain GSH pool to fight against the oxidative stress.](image)
The generation of ROS in normal cells, including neurons, is under tight homeostatic control. To help detoxify ROS, biological antioxidants, including glutathione, α-tocopherol (vitamin E), carotenoids, and ascorbic acid, will react with most oxidants. In addition, the antioxidant enzymes catalase and glutathione peroxidase (GPx) detoxify H$_2$O$_2$ by converting it to O$_2$ and H$_2$O. During detoxification, oxyradicals are reduced by GPx at the expense of GSH to form glutathione disulfide (GSSG). GSH is regenerated by redox recycling, in which GSSG is reduced to GSH by glutathione reductase (GR) with the consumption of one NADPH. GST catalyse the conjugation of GSH via sulphydryl group to the electrophilic centre of peroxides which can alleviate damage from lipid peroxidation. A reduction in the level of GSH may impair H$_2$O$_2$ clearance and promotes formation of OH, the most toxic free radical to the brain leading to more oxidant load and consequently oxidative damage. The enzyme superoxide dismutase (SOD) catalyzes the dismutation of superoxide into O$_2$ and H$_2$O$_2$, which is one of the most toxic molecules in the brain.

**High Level of Cholesterol as a Risk Factor of Alzheimer’s Disease**

Controversy exists regarding the specific relationship between obesity and AD (Luchsinger et al., 2008). Whereas mid-life obesity is considered a risk factor for dementia (Whitmer, 2008), other reports indicate no association (Stewart, 2005). Age could represent a conditioning factor in this regard, because higher waist circumference relates to AD risk only in the youngest, whereas in older individuals even a low body-mass index has been related to higher AD risk (Atti, 2008). In addition, weight loss often precedes the onset of AD, supporting the presence of an underlying metabolic disorder (Stewart, 2005; Buchman, 2005). Increased lipolysis occurs in obesity and is partly responsible for enhanced free fatty acid (FFA) concentrations, eventually leading to elevated very-low-density lipoprotein (VLDL). A contribution in this sense is also provided by concomitant insulin resistance. Both high FFA and VLDL, as well as high fat-feeding, have been associated with increased amyloid burden (Pimplikar, 2009). Other dyslipidemic conditions such as elevated postprandial LDL (low-density lipoprotein) or low levels of HDL (high-density lipoprotein) are associated with AD. Oxidized LDL levels are significantly higher in plasma and cerebrospinal fluid of AD patients, and treatment of cultured neurons with oxidized LDL potentiates Aβ-induced toxicity and increases Aβ deposition in cerebral vessels (Smith, 2007). FFAs can also affect AD pathogenesis by stimulating amyloid and tau filament formation (Patil and Chan, 2005) and by inhibiting insulin-degrading enzyme (IDE), a metalloprotease that contributes to Aβ
clearance. Lipoprotein lipase present in endothelial cells hydrolyzes triglycerides from chylomicrons and VLDL to provide FFA for energy use. Lipoprotein lipase is found in amyloid plaques in the AD brain, and mutations in its gene are associated with increased risk of AD (Blain, 2006), however, it is to be noted that its function in the CNS remains unknown, although its expression increases in mouse brain following injury (Paradis, 2004). It has been known for over a decade that hypercholesterolemia and high incidence of coronary artery disease Aβ-synthesizing enzymes, the β- and γ-secretases that are located in cholesterol-rich lipid rafts, is positively modulated by high cholesterol concentrations whereas the opposite occurs with α-secretase (Kojro, 2001). Conversely, reducing cholesterol in membranes causes a decrease of Aβ (Grimm, 2007). It is then suggested that the brain cholesterol levels directly influence Aβ formation through stimulation of the amyloidogenic pathway.

Modelling in Alzheimer's disease type Dementia in Animals:

There is no animal model available that can mimic all the cognitive, behavioural, biochemical, and histopathological abnormalities observed in patients with AD. However, partial reproduction of AD neuropathology and cognitive deficits has been achieved with pharmacological and genetic approaches.

(1). Cholinergic dysfunction-related animal models:

Since degeneration of the basal forebrain cholinergic neurons occurs early in the course of AD and is correlated with cognitive deficits (Coyle et al., 1983; Winkler et al., 1998). Cholinergic lesion paradigms have been used to study the role of the cholinergic system in cognitive function (McDonald & Overmier, 1998). Many types of acute manipulations, including electrocoagulation, use of excitotoxins, transection 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 (ICV) infusion of quinolinic acid was also developed to simulate the slow evolution of the neurodegenerative diseases, including AD (Yamada et al., 1990, 1991). It is suggested that lesions of the medial septal nucleus produce behavioural deficits that are most similar to the cognitive impairments in the earliest stage of AD (McDonald and Overmier, 1998). Although none of these animal models shows the neuropathological characteristics, such as senile plaques and NFT, found in the
brains of AD patients, they have been widely used for assessing the validity of therapeutic interventions with cholinergic drugs (Murray and Fibiger, 1986; Itoh et al., 1997).

(2). **Amyloid β-peptide-related animal models**

Aβ related animal models for AD are divided into two groups: transgenic and non transgenic animal models.

(i). **Amyloid β-peptide-related non transgenic animal models**

A growing number of studies have demonstrated that acute injection or continuous infusion of Aβ into the brain causes brain dysfunction, as evidenced by neurodegeneration and an impairment of learning and memory (Pepeu et al., 1996; Nabeshima and Itoh, 1997b). An in vivo model for the neurodegenerative effects of Aβ1-40, including neuronal loss and degenerating neurons and neurites with an induction of Alz50-immunoreactive proteins, was first established by Kowall et al. (1991). Similarly, insoluble amyloid core from the AD brain produces neurotoxic effects when injected into the rat brain (Frautschy et al., 1991). It is also demonstrated that fibrillar Aβ neurotoxicity is age-dependent and highly species-specific (Geula et al., 1998). Microinjection of plaque-equivalent concentrations of fibrillar, but not soluble, Aβ in the aged rhesus monkey cerebral cortex results in profound neuronal loss, tau phosphorylation, and microglial proliferation, although it is not toxic in the young animal. The effects of Aβ fragments on learning and memory were first examined in mice (Flood et al., 1991). Many studies subsequently have demonstrated that various Aβ fragments, such as Aβ1-40, Aβ1-42, and Aβ25-35, cause learning and memory impairment in mice and rats (Harkany et al., 1998; Oka et al., 1999).

(ii). **Amyloid β-peptide related transgenic animal models**

To generate AD animal models exhibiting senile plaques and Aβ-associated neuropathology, different types of transgenic mice that express human APP (Quon et al., 1991), Aβ (LaFerla et al., 1995), the C-terminal fragment of APP (Nalbantoglu et al., 1997), and the APP genes carrying familial AD mutations (Reaueme et al., 1996; Lamb et al., 1997) have been created. Transgenic mice overexpressing human APP751, which develop early AD-like histopathology with diffuse deposits of Aβ and aberrant tau protein immunoreactivity in some cases (Higgins et al., 1994), exhibit age-dependent deficits in spatial learning in a water
cognitive impairment (Janus et al., 2000). These properties have prompted a number of studies into the characteristics of mice expressing the human versions of presenilins and of APP. Doubly transgenic mice from a cross between Tg mice expressing HuAPP Swedish mutation, Tg (HuAPP695, K670N–M671L) 2576, and Tg mice expressing a mutant HuPS1 gene, Tg(M146L) develop visible Aβ deposits in hippocampus and cerebral cortex at earlier ages (6–16 weeks) than their single Tg2576 littermates (Holcomb et al., 1998).

In the period preceding overt Aβ deposition, doubly transgenic mice show a selective 41% increase in Aβ 42(43) in the brain. Both doubly and singly transgenic Tg2576 mice showed reduced spontaneous alternation performance in a Y-maze before substantial Aβ deposition was apparent, indicating that some behavioural alterations in these mice may be related to an event that precedes plaque formation (Holcomb et al., 1998).

(5). Model used in the present study

Structure and chemical properties of Streptozotocin:

Streptozotocin (STZ) a glucosamine nitrosourea compound, has a chemical name of 2-deoxy2-(3-methyl-3-nitrosoureido)-D glucopyranose (C₉H₁₅N₃O₇). The structure is composed of a nitrosourea moiety with a methyl group attached at one end and a glucose molecule at the other as shown in Fig. 11. The structure has been determined to be the nitrosamide methyl nitrosourea (MNU) linked to the C2 position of D-glucose.

![Fig 11: Structure of Streptozotocin](image-url)
Intracerebroventricular Injection of Streptozotocin in rats/Mice: Provided a Relevant Model of Sporadic Dementia of Alzheimer's type (SDAT):

Availability of reliable animal models of progressive decline in cognitive function resembling dementia e.g. sporadic dementia of Alzheimer's type (SDAT) in human being is not only a basic requirement for the better understanding of the cellular and molecular mechanisms underlying their deficits. It is also for improved changes of the success in the search for the therapeutic agents potentially useful for the management of such a devastating AD. Brain glucose utilization and metabolism are basic to the function of learning and memory (Benton and Ownes, 1993; Benton et al., 1994). SDAT in particular characterized by a progressive deterioration of both cognitive function and energy metabolism (Hoyer, 1991). Besides diminished glucose utilization insulin receptor signal transduction cascade is severely impaired in hippocampus and hypothalamus of AD brain (Steen et al., 2005). Another feature of SDAT pathophysiology is oxidative stress, which can affect all classes of macromolecules (Lipids, sugars, proteins and DNA), leading inevitably to neuronal dysfunction (Polidori and Mecocci, 2002). Moreover impaired insulin signalling has already been linked to increased oxidative stress and mitochondrial dysfunction in neuronal cells (Hoyer and Lannert, 1999).

The glucose in the brain supply the energy essential for the maintenance of the nervous system, the deficiency of glucose in the cell can trigger neuronal injury (Seo et al., 1999). A major imbalance between glucose and oxygen consumption has been found in the incipient stage of AD, whereas in the advanced stage, both glucose and oxygen consumption diminished. There is a close relationship between cortical and hippocampal cholinergic transmission and glucose utilization. It is reported that impaired cerebral glucose metabolism have been found in learning and memory impaired rats (Hoyer, 1991; Cao et al., 2003). Reduction in glucose below a critical level can decrease the rate of acetylcholine synthesis by lowering concentration of acetyl-coenzyme A, which is derived from glucose. On the other hand, cerebral glucose utilization is reduced by lesion of the nucleus basalis of Meynert, which decrease cortical glucose metabolism may have a causal role in the loss of cognitive function in the SDAT (Hoyer, 1991).

To test this hypothesis, Hoyer and his colleagues (Lannert and Hoyer, 1998) attempted to disrupt brain glucose utilization in rats by intracerebroventricular (ICV) injection of STZ.
Section II

Review of Literature

The ICV-STZ injected rat causes desensitization of insulin receptors and biochemical changes similar to that of AD and described an appropriate animal model for SDAT (Nitsch and Hoyer, 1991; Lannert and Hoyer, 1998; Sonkusare et al., 2005).

Amyloid β and Tau protein are regarded as the main pathological features of AD and both of them are responsible for pathological cascade responsible for dementia, neuropsychiatric changes and finally death of neurons. ICV administration of STZ leads to an increase in the total tau protein and amyloid β in the brain and increase in the β amyloid formation enhance inflammatory process and free radical formation (Baluchnejadmojarad and Roghni, 2006), gradually increasing the levels of malondialdehyde (MDA) – the end product of lipid peroxidation. An increase in the levels of lipid peroxidation in nerve cells results in apoptosis and cell death (Grunblatt et al., 2006).

It is well documented that ICV-STZ injection to rats has increased the oxidative stress, neuroinflammation, neuronal cell death and produce neuropathological and biochemical alterations similar to those observed in sporadic Alzheimer's disease. It is now considered to be a valid model of the early pathophysiological changes in AD (Grunblatt et al., 2007; Javed et al., 2011).

**Diagnosis of Alzheimer's disease and Dementia**

There are number of diagnostic tests available to establish a reliable diagnosis of dementia. Neurological signs and symptoms are usually associated with particular types of dementia. AD is one type of dementia. Testing the individual looks to identifying the type of dementia the person’s has.

4. Psychological and Neurological Tests for Dementia

Clinical interview

1. The mini mental state examination (MMSE).

The mini mental state examination (MMSE) is the most commonly used to test memory problems and contributes to a possible diagnosis of dementia. The MMSE test is used to screen for the presence of cognitive impairment over a number of areas. The great thing about
the MMSE is that it is quick and simple to use. Based on a series of question and tests, points are achieved correctly. A maximum 30 points is possible with scores of 26 or less generally being reported by people with AD.

(i). Areas tested by the mini mental examination in MMSE
(a). Orientation: 10 points can be scored for answering question about date and location.
(b). Memory: This test recall. Immediate memory scores 3 point (one point for each of 3 objects). People are asked to remember three words e.g. pen, ball and ring. The three objects names will be asked for the later in the test.
(c). Attention and calculation: The next part of the MMSE test the ability to concentrate. One test scoring a maximum of 5 points requires the person to subtract 7 from 100 and continue. The answers are 93; 86; 79; 72; 65. Some clinicians ask for 5 to be serially subtracted from 100. The person may also be asked for to spell a 5-letter word backwards. One of the two scores is included in the final score.

Language writing and Drawing
The final 9 points of the MMSE test spoken and written language, the ability to write copy and remember name objects. This includes naming objects e.g. brush and a pen. This scores 1 point for each correct answer
Carry out a three step process-3 points
Repeat a sentence- 1 point. Copy a figure or shape 1 point. Write a sentence on a piece of paper 1 point. The score are then added up to give a result of the MMSE.

2. Clock drawing test:
This is a simple test that can be used as a part of a neurological test or as a screening tool for AD and other types of dementia. The person undergoing testing is asked to:
1. Draw a clock
2. Put in all the numbers
3. Set the hands at ten past eleven

Fig. 12: Clock drawing is another commonly used screening for the cognitive impairment (Source: Joung, J., et al 2011)
(i) Scoring system for clock drawing test (CDT).

There is a number of scoring systems for this test. The AD cooperative scoring system is based on a score of five points.
1 point for the clock circle
1 point for all the numbers being in correct order
1 point for the number being in the proper special order
1 point for the two hands of the clock
1 point for the correct time
A normal score is four or five points

(3). Mental status examination diagnostics test for Alzheimer’s disease:

One of the key diagnostic tests for dementias such as AD is the mental status examination (MSE). The doctor/clinicians makes judgement on the way the patient is functioning in a number of key areas. These components of the MSE cannot be judged in isolation. Each aspect will contribute to a diagnosis. There are many forms of mental illness and disorder that will show abnormalities in these areas but it is the combination of abnormality, together with other diagnostic that lead to a 90% to 95% probability of AD.

Mental status examination evaluates:

(i). Affect mood: Affect is about the emotion behind the action, the emotional state of an individual. The doctor will be looking at mood as well as the appropriateness of emotion that the person is expressing. People with AD may show inappropriate affect and mood.

(ii). Appearance attitude, behaviour, the mental status examination in Alzheimer’s diagnosis:

People with AD may show signs of their inability to carry out basic tasks such as washing, dressing appropriately combing their hair etc. The person may walk in a particular way, be unsteady, be restless apathetic and anxious pace or wander about the consulting area.
(iii). Cognition mental status examination in Alzheimer's disease:
Cognition is the ability to think. Attention reasoning, decision making and dealing with concepts are all affected in AD as is the ability to carry out tasks in logical and sequential way.

(iv). Insight Judgment, mental status examination (MSE) in Alzheimer's diagnosis: Key elements in the MSE are whether an individual has insight into their condition and circumstances. An ability to make judgment about things and act on them deteriorates significantly in dementia.

(v). Speech language mental status examination in Alzheimer's diagnosis: People with AD may show particular abnormalities in their speech and language. Aphasia is a major symptom of AD and dementia. Also of interest to clinicians will be the way in which people talk, the volume and tone.

(vi). Thought content, thought processes mental status examination in Alzheimer's Diagnosis: The way in which people with dementia such as Alzheimer's think and their thought content will be disrupted. This may be a direct result of the brain damage that occurs in Alzheimer's or it could be because of associated conditions such as psychosis or depression, suicidal idealation.

Standard medical test for Alzheimer's disease and dementia:
1. Physical exam:
The physical exam is part of the patient care process. The exam enables the doctor to assess the overall physical condition of the patient. If the patient has a medical complaint, the physical exam provides the doctor with more information about the problem, which helps him determine an appropriate plan of treatment. The physical exam includes an examination of the following:
   - Vital signs (temperature, blood pressure, pulse)
   - Height and weight
   - Skin
   - Head, eyes, ears, nose
   - Throat/neck
2. Chest X-ray:

An X-ray is a test in which an image of the body is created by using low doses of radiation. X-rays can be used to diagnose a wide range of conditions, from bronchitis to broken bones. When viewing X-ray images of the chest, doctors can view the structures inside the chest, including the heart, lungs, and bones. This test may be used by the doctor to help rule out other disorders that may be causing symptoms similar to those of AD.

4. Laboratory tests:

These tests can help to identify problems and diseases. There are hundreds of laboratory tests available to help doctors make a diagnosis. The most common are blood tests and urinalysis. Blood tests involve a series of tests routinely done on blood to look for abnormalities associated with various diseases and disorders. Blood tests may also be used to look for the presence of a specific gene that has been identified as a risk factor for AD. A urinalysis is a test in which a urine sample is evaluated to detect abnormalities, such as abnormal levels of sugar or protein. This test may be used by the doctor to help rule out other disorders that may be causing symptoms similar to those of AD.

5. Electrocardiogram (ECG):

An electrocardiogram (ECG) is a recording of the heart's electrical activity. This activity is registered as a graph or series of wavy lines on a moving strip of paper. This gives the doctor important information about the heart. For example, it can show the heart's rate and rhythm. It can also help to show decreased blood flow, enlargement of the heart, or the presence of
damage due to a current or past heart attack. This test may be used by the doctor to help rule out other conditions that may be causing symptoms similar to those of AD.

6. Electroencephalography (EEG):

Electroencephalography (EEG) is a medical technique that measures brain function by analyzing the electrical activity generated by the brain. This activity is measured through special electrodes applied to the scalp. EEG can be used repeatedly in adults and children with virtually no risks and is helpful in diagnosing seizures, which may be contributing to behavioural changes seen in the patient.

7. Computed tomography (CT) scan:

CT scanning is a technique in which multiple X-rays of the body are taken from different angles in a very short period of time. These images are then fed into a computer, which creates a series of images that look like "slices" through the body. CT scans can show certain changes that are characteristic of AD in its later stages. These changes include a reduction in the size of the brain, referred to as atrophy.

8. Magnetic Resonance Imaging (MRI):

Magnetic resonance imaging, usually called MRI, is a test that produces very clear pictures, or images, of the human body without using X-rays. Instead, MRI uses a large magnet, radio waves, and a computer to produce these images. MRI is beneficial in ruling out other causes of dementia, such as tumors or strokes. It may also help to show the structural and functional changes in the brain that are associated with AD.

9. Positron Emission Tomography (PET) Scan:

PET scanning is a three-dimensional imaging technique that allows a doctor to examine the heart, brain, or other internal organs. PET scans also can show how the organs are functioning; unlike X-ray, CT, or MRI, which show only body structure. PET is particularly useful for the detection of cancer and coronary artery disease and can provide information to
pinpoint and evaluate diseases of the brain. PET imaging can show the region of the brain that is causing a patient to have seizures and is useful in evaluating brain diseases like Alzheimer's, Huntington's, and Parkinson's. PET scans can show the difference in brain activity between a normal brain and one affected by AD; it can also help differentiate AD from other forms of dementia.

10. Single photon emission computed tomography (SPECT) scan:

SPECT is a technique for creating very clear, three-dimensional pictures of a major organ, such as the brain or heart. SPECT scans involve the injection of a very small amount of a radioactive substance. Energy from the radioactive substance in the body is detected by a special camera, which then takes the pictures. SPECT can be used to see how blood flows in certain regions of the brain and is useful in evaluating specific brain functions. This may reveal abnormalities that are characteristic of AD.

11. Magnetic resonance spectroscopy imaging (MRSI):

MRSI is a test that allows the doctor to observe certain substances throughout the brain without the use of radioactive materials. MRSI is an imaging technique that is used to study changes caused by brain tumours, strokes, seizure disorders, AD, depression, and other diseases affecting the brain.

Treatment of Alzheimer's disease

Currently there is no complete cure for AD. Medication and care giving are the primary treatment for the patients.

(I) Medications: Current medications for the AD can't stop or reverse the underlying disease process. But they may slow it down, lessening signs and symptoms. Medications commonly recommended for people with mild to moderate AD include:

(I). Memantine: Glutamate is the principal excitatory neurotransmitter in the brain. Glutamatergic overstimulation may result in neuronal damage, a phenomenon that has been termed excitotoxicity. Such excitotoxicity ultimately leads to neuronal calcium overload and has been implicated in neurodegenerative disorders. Glutamate stimulates a number of
postsynaptic receptors, including the NMDA receptor, which has been particularly implicated in memory processes, dementia, and the pathogenesis of AD (Cummings, 2004). Memantine is a relatively new drug specially developed for use in moderate-to-severe dementia (Reisberg et al., 2003). It is a non-competitive NMDA receptor antagonist and reduces glutamatergic excitotoxicity. The neurobiological basis for the therapeutic activity of memantine in AD is not fully understood. However, it is not acetylcholine esterase inhibitors and therefore, is different from other drugs currently used for AD treatment. Memantine's mechanism of action is a voltage-dependent, low-moderate affinity, uncompetitive NMDA receptor antagonism with fast blocking/unblocking kinetics. Memantine blocks the effects of abnormal glutamate activity that may lead to neuronal cell death and cognitive dysfunction.

(ii). Antioxidants

Various antioxidants, free radical scavengers, calcium channel blockers, metal chelators, or modulators of certain signal transduction pathways might protect neurons from the downstream effects of accumulation of Aβ protein. One problem found with such approaches may be that neurons respond to Aβ protein and its associated inflammatory process in several ways. Blockade of one or two of these response pathways might not substantially decrease overall neuronal dysfunction and loss. A chelator of copper and zinc ions that may decrease cerebral β-amyloid protein levels is being tested in patients with AD (Cherny et al., 2001). Some studies suggest that high intake of vitamins C, E, B6, and B12, and folate, unsaturated fatty acids, and fish are related to a low risk of AD, but the results of different reports are inconsistent. Modest to moderate alcohol intake, particularly wine, may be related to a low risk of AD (Luchsinger and Mayeux, 2004).

(iii). Selegiline

Selegiline, a monoamine oxidase inhibitor, similarly to alpha-tocopherol may have beneficial effects in patients with AD. Selegiline also increases levels of catecholamines, and adrenergic stimulation may improve cognitive deficits associated with AD. A number of studies have examined evidence for the use of selegiline, a selective monoamine oxidase inhibitor, in the treatment of AD (Sano et al., 1996). In 2000, the authors of a meta-analysis of 15 clinical trials concluded that there was not enough evidence to recommend selegiline as a treatment for AD. Because of the risk of stupor, rigidity, severe agitation, and elevated temperature,
selegiline therapy is contraindicated in patients who are taking meperidine, and this precaution often is extended to other opioids. Concurrent use of selegiline with tricyclic antidepressants and selective serotonin reuptake inhibitors also should be avoided. These restrictions may limit the use of selegiline in patients with AD (Standridge, 2004).

(II). Anti-inflammatory agents

Another approach to treating AD is to administer anti-inflammatory drugs that could interfere with aspects of the microglial, astrocytic, and cytokine responses found in the brain of a patient with AD. The epidemiologic evidence that prolonged use of certain NSAIDs (specifically inhibitors of cyclooxygenase-1) is associated with a lower risk for AD could be explained on this basis. Observational studies have found that persons who regularly use NSAIDs have a decreased incidence of AD. Thus, NSAIDs likely have some neuroprotective effect. However, several investigations of anti-inflammatory drugs do not show a benefit for treatment (Cummings, 2004).

(III). Cholinesterase inhibitors

Symptomatic treatment of AD is based on cholinergic neurotransmission enhancement obtained by pharmacological means. The cholinergic agonists have been found to facilitate learning, which lends support to the important physiologic role of acetylcholine in attention and learning (Gauthier et al., 2002). Among the different compounds that can modify cholinergic neurotransmission, the only class of drugs that have been obtained from regulatory authorities the indication for the symptomatic treatment of AD is the cholinesterase inhibitors (ChEIs). These drugs act by slowing the biochemical breakdown of acetylcholine and thereby, at least theoretically, prolonging cholinergic neurotransmission. These enzymes are found in neuritic plaques, and their inhibition with cholinesterase inhibitors may modify the deposition of Aβ, a key component of the pathophysiology of AD. Three ChEIs are commonly used to treat patients with mild to moderate AD: donepezil, rivastigmine and galantamine. Donepezil and galantamine are selective acetylcholine esterase inhibitors (AChEIs). ChEIs are indicated in patients with mild to moderate AD, although some studies suggested a small benefit also in patients suffering from more advanced stages of AD (Doody et al., 2001; Cummings, 2004).
(IV). Inhibitor of Aβ aggregation

The rationale of this strategy is to block the aggregation of Aβ to insoluble fibrils and thus to enhance Aβ clearance from the brain. This can be achieved in several different ways. Beta-sheet breakers have been designed as small synthetic peptides which bind to Aβ oligomers and fibrils and lead to disruption of the β-sheet conformation (Soto et al., 1998; Wisniewski and Sadowski, 2008). An example of this strategy which proceeded to human trials is tramiprosate, a modification of the amino acid taurine (Aisen et al., 2008). It binds preferentially to soluble Aβ peptide, interferes with the β-sheet formation of amyloid, maintains Aβ in a non-fibrillar form, and thereby inhibits amyloid formation and deposition. Since the aggregation of Aβ is enhanced by the presence of metal ions such as copper, iron, and zinc, metal ion scavengers have a potential of reducing the fibrillisation of Aβ (Bush, 2003). A compound with metal-attenuating properties that has been tried in AD is clioquinol, an antifungal and antiprotozoal drug with neurotoxic effects at large doses. A pilot phase II study demonstrated less clinical decline in patients on active treatment as compared to patients receiving placebo (Ritchie et al., 2003).

(V). Tau hyperphosphorylation and aggregation

Hyperphosphorylation appears to be an early and central event in tau-related neurodegradations including AD (Sergeant et al., 2008). There is evidence that phosphorylation is involved in regulatory processes associated with tau affinity for microtubules (Reynolds et al., 2008) and that tau hyperphosphorylation by kinases including glycogen synthase kinase 3 (GSK3) is related to disrupted microtubule function and interferes with cargo transport to pre- and postsynaptic sites resulting in synaptic malfunction and cell death (Spires-Jones et al., 2009). Due to the critical role of tau hyperphosphorylation in AD pathogenesis several strategies are currently being tested to prevent phosphorylation (Hanger et al., 2009). These strategies include kinase inhibitors such as lithium, which is a weak GSK3 inhibitor and has been shown to reduce tau levels in cultured neurons (Martin et al., 2009) and transgenic mice (Caccamo et al., 2007). However, in a 10-week phase 2 study involving patients with mild AD, lithium treatment did not result in decreased lymphocyte GSK3 activity, reduced cerebrospinal fluid phosphorylated tau concentration, or cognitive improvement (Hampel et al., 2009). In addition to interventions directed against hyperphosphorylation, anti-Tau vaccination may be a promising approach. In transgenic
mice, immunization with a derivate of phosphorylated tau reduced the aggregation of tau and also slowed the progression of the tangle-related behavioural phenotype (Sigurdsson, 2008).

**Prevention of Alzheimer's disease:**

Right now, there is no way to prevent the onset of AD. Currently approved medications for AD have a discouragingly small effect on cognition and on disease progression. Pharmacotherapy will hence need to be directed at primary prevention strategies because neurodegeneration may be impossible to reverse. MCI is therefore a logical target for early intervention against AD, and age-related cognitive impairment (not amounting to MCI) may hold even greater promise. Dietary measures, such as high intake of fish, fruit and vegetables suggest a positive role for omega-3 fatty acids (docosahexaenoic acid and eicosapentaenoic acid), antioxidants (vitamin E and flavonoids), and B group vitamins such as folate, B6, and B12.

(i). **Fish, fruit and vegetable consumption may lower the risk of Alzheimer's disease**

Epidemiologic studies suggest that a diet rich in fish is associated with less cognitive decline (Morris et al., 2005) and with a decreased risk of AD (Morris et al., 2003). Regular fruit and vegetable intake, even in the form of juices, has also been associated with a decreased risk of AD (Dai et al., 2006). It is also reported that, in men and women aged 65 years and above, the daily intake of fruit and vegetables and the weekly intake of fish are associated with a 30%-40% decrease in the risk of dementia and AD across a 4-year follow-up period.

(ii). **Tea intake may protect against cognitive decline in the elderly**

The regular intake of tea has been associated with a number of health benefits, including a reduced risk of cardiovascular, cerebrovascular, cognitive, and other disorders as reported by Ng et al. (2008).
(iii). Physical exercise may reduce the risk of dementia

A large body of epidemiological literature suggests that higher levels of intelligence, education, occupational attainment, and participation in intellectual and leisure activities independently reduce the risk of AD (Stern et al., 2006). Much evidence suggests that higher levels of physical activity also reduce the risk of dementia.

(iv). Benefits of cognitive training in the elderly

Epidemiological studies suggest that persons who are mentally active are at a lower risk of cognitive decline and dementia in old age (Friedland et al., 2001; Wilson et al., 2002).

(v). Lithium may protect against dementia in bipolar patients

Bipolar disorder is associated with increased risk of dementia (Kessing et al., 2003). Lithium has been shown to stimulate neuroplasticity in the brain (Muñoz-Montaño et al., 1999). Lithium also inhibits the formation of both beta amyloid and hyper phosphorylated tau protein (Lovestone et al., 1999; Phiel et al., 2003).

(vi). The Alzheimer’s vaccine

Approaches towards the development of a vaccine against AD have been based on two strategies: active immunotherapy using the pre-aggregated synthetic Aβ42 preparation AN1792 vaccine (QS-21) and passive immunization using injections of already prepared polyclonal anti-beta amyloid antibodies (intravenous immunoglobulin). The candidate Alzheimer’s vaccine AN1792 was found to reduce Aβ42 plaque burden and preserve cognitive function in amyloid precursor protein transgenic mice. The phase IIA trial in humans was discontinued because of the occurrence of meningoencephalitis in 6% of the patients. However, the analysis of data revealed that the performance of patients who received the vaccine improved significantly on the neuropsychological test battery (Gilman et al., 2005).
(vii). Herbal medicines may attenuate cognitive impairment

Many studies have investigated the efficacy of herbal medicines in cognitive deficit disorders. In a systematic review and meta-analysis, it was observed that many studies have found cognitive benefits with standardized extract of Ginkgo biloba after intermediate- to long-term administration to patients with AD; however, as much of the literature is of poor methodological quality, it was concluded that the clinical value of Ginkgo is unconvincing. A large number of Indian herbal preparations and formulations have been suggested to have precognitive properties in animal models (Andrade et al., 2008).

(viii). Drugs used in the present study:

S-allyl cysteine (SAC), a sulfur-containing amino acid derived from garlic, has been reported to have antioxidant activity (Yamasaki et al., 1994; Numagami et al., 2001) anti-cancer promoting activity (Imada et al., 1992), antihepatopathic activity (Nakagawa et al., 1989) and neurotrophic activity (Nishiyama et al., 2001). SAC content in the intact garlic is small (not more than 30 μg/g-fresh weight), however, this compound is produced in a soaking preparation through hydrolysis of γ-glutamyl-S-allylcysteine which exists in raw garlic as a precursor of SAC (Koch et al., 1996). Among these constituents, SAC, the most abundant organosulfur molecule with reported antioxidant properties (Kim et al., 2001),

\[
\begin{align*}
\text{S-allyl cysteine} & \\
\text{SAC} & \\
\text{S-allyl cystein}
\end{align*}
\]

SAC is one of the chemically and biologically remarkable sulfur-containing compounds and exerts its protective actions through its ability to scavenge \( \text{O}_2^- \) (Kim et al., 2001) and \( \text{H}_2\text{O}_2 \) (Ide and Lau, 2001), thus preventing \( \text{H}_2\text{O}_2 \)-induced endothelial cell damage and lipid peroxidation, as well as low-density lipoprotein oxidation (Ide and Lau, 2001). As a food, garlic shows very low toxicity, and aged garlic extract exhibits no histopathological changes in various organs and tissues (Sumiyoshi and Wargovich, 1990). At a central nervous system
Section II  Review of Literature

(CNS) level, SAC reduces edema formation in ischemic rat brain through the inhibition of lipid peroxidation, ameliorates learning deficits in senescence-accelerated mice (Nishiyama et al., 2001) and evokes neurotrophic actions in cultured rat hippocampal neurons (Moriguchi et al., 1997). Moreover, two of the most remarkable protective effects of SAC are those produced on Aβ peptide-induced apoptosis (Peng et al., 2002) and neurotoxicity in organotypic hippocampal cultures (Ito et al., 2003).

Taurine is a non-essential amino acid and is found in high concentrations in the white blood cells, skeletal muscles, central nervous system as well as the heart muscles. In adults, but not children, this nutrient can be manufactured from methionine in the body and from cysteine in the liver, but vitamin B₆ must be present. It is a key ingredient of bile, which in turn is needed for fat digestion, absorption of fat-soluble vitamins as well as the control of cholesterol serum levels in the body. This nutrient is also used in the proper use of potassium, calcium as well as sodium in the body, and for maintaining cell membrane integrity. It is thought to be helpful with anxiety, hyperactivity, poor brain function and epilepsy as well as hydrating the brain. Taurine, together with zinc is also required for proper eye health and vision. Children with Down's syndrome may benefit from taurine, and women being treated for breast cancer as well as people with metabolic disorders, since metabolic disorders can cause loss of this nutrient via urine. Diabetics may also benefit from this nutrient, since this disease increase the need for this nutrient.

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\text{Taurine}
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Taurine crosses the blood-brain barrier (Tsuji et al., 1996; Salminen et al., 2003.) and has been implicated in a wide array of physiological phenomena including inhibitory neurotransmission, (Olive et al., 2002) long-term potentiation in the striatum/hippocampus, (Dominy et al., 2004) membrane stabilization, (Birdsall et al., 1998) It also acts as an antioxidant and protects against toxicity of various substances (such as lead and cadmium). Taurine is involved in a number of crucial physiological processes. However, the role of taurine in these
processes is not clearly understood and the influence of high taurine doses on these processes is uncertain. A substantial increase in the plasma concentration of growth hormone was reported in some epileptic patients during taurine tolerance testing, suggesting a potential to stimulate the hypothalamus and to modify neuroendocrine function.

Rutin is a member of bioflavonoid, a class of water-soluble plant pigments. Rutin has several pharmacological properties such as antioxidant, anti-inflammatory, antiallergenic, antiviral, anticarcinogenic and potent scavenger of superoxide radicals (La Casa et al., 2006; Kamalakkannan and Prince, 2006; Bishnoi et al., 2007). Rutin is a glucoside of quercetin and contains approximately 50% of quercetin. It is a flavonol glycoside comprised of the flavonol backbone and the glycon rutinose. It is a solid substance, pale yellow in appearance and is much more soluble in water than its aglycone quercetin. Rutin is the basis of a whole group of bioflavonoid. Rutin is a bioflavonoid that works with vitamin C and assists in reducing pain and intraocular pressure. Bioflavonoids are important nutrients due to their ability to strengthen and modulate the permeability of the walls of the blood vessels including capillaries.

Rutin is used by animal feed, cosmetic, and chemical industries as a natural pigment, stabilizer, food preservative, and UV absorbent (Pu et al., 2005). It is reported that rutin supplementation from natural food sources, such as “soba” noodles or groats, might improve memory impairment and decreased hippocampal pyramidal neurons death such as in Alzheimer’s disease. Rutin has the ability to suppress the microglial activation and pro-inflammatory cytokines (Koda et al., 2008). Rutin can chelate metal ions, such as ferrous cations which are involved in Fenton’s reaction, which generates reactive oxygen species. Rutin may also modulate the respiratory burst of neutrophils. The in vivo antioxidant activity.
of rutin is most likely due to its aglycone quercetin to which it is metabolized following ingestion. Rutin is most abundant in apricots, buckwheat, cherries, prunes, rose hips, the whitish rind of citrus fruits, and the core of green peppers. Buckwheat is rich in proteins (12-15%) and essential amino acids such as lysine (5-7%) that are deficient in major cereal crops, and is also abundant in lipids, minerals (iron, phosphorus, and copper), vitamins (B₁ and B₂) and rutin. Rutin is not found in other grains such as rice, wheat or beans.

Hesperidin (hesperetin 7-rutinoside) is a flavanone glycoside, comprising of an aglycone, hesperetin, and an attached disaccharide, rutinose. Oranges (Citrus sinensis) and tangelos are the richest dietary sources of hesperidin. Hesperidin is abundantly found in citrus fruits (family Rutaceae) and has also been reported to occur in many plants other than Citrus, such as genera Fabaceae, Betulaceae, Laminaceae and Papilionaceae. Hesperidin demonstrate strong antioxidant properties (Zhang et al., 2007). This antioxidant activity is through their ability to quench oxidative radical chain reactions (capable of oxidizing and nitrating cellular proteins, nucleic acids, and lipids), and can thus help preserve neuronal health.

Hesperidin also exhibits significant anti-inflammatory activity by modulating the prostaglandin synthesis and COX-2 gene expression pathways (Hirata et al., 2005). Hesperidin has been reported to possess analgesic (Galati et al., 1994), hypolipidemic (Monforte et al., 1995), anti-hypertensive and diuretic activity (Galati et al., 1994). The possible hypolipidemic effect of hesperidin is due to its important role in lipid lowering activity. Hesperidin may reduce plasma cholesterol levels by inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, as well as acyl coenzyme A (Park et al., 2001). Inhibition of these enzymes by hesperidin has been demonstrated in rats fed a high cholesterol diet. A deficiency of hesperidin in the diet has been linked with abnormal
capillary leakiness as well as pain in the extremities causing aches, weakness and night leg cramps. Another potential therapeutic application of hesperidin is its anticancer activity mediated through the suppression of cell proliferation (Tanaka et al., 1997a; Tanaka et al., 1997b). Currently hesperidin is used as a dietary supplement for improving blood flow and for its vasoprotective properties, and is available as an oral dosage form.
Materials and Methods
Materials

Chemical and reagents
Glutathione oxidized (GSSG), glutathione reduced (GSH), glutathione reductase (GR), nicotinamide adenine dinucleotide phosphate reduced form (NADPH), 5,5'-dithio bis-2-nitrobenzoic acid (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), thiocholine iodide, paraformaldehyde, proteinase K, RNase A, agarose, thiobarbituric acid (TBA), trichloroacetic acid (TCA), ethylene diamine tetraacetic acid (EDTA), N-acetyl neuraminic acid, taurine, rutin, resorcinol, hesperidin, streptozotocin (STZ), monoclonal mouse antibodies against IL-8, inducible nitric oxide synthase (iNOS) and phospho-p65 NF-κB, hematoxylin, eosin, and cresyl violet were purchased from Sigma-Aldrich, Chemicals Pvt. Ltd., India. Ethyl alcohol, acetone, chloroform, n-butyl acetate, n-butanol and methanol were purchased from SD Fine Chemicals Ltd, India. Monoclonal mouse antibodies against Bcl-2 and p53 were purchased from Bio Vision, U.S.A; COX-2 was purchased from Santa Cruz Biotechnology, USA. Polyclonal rabbit antibody anti ChAT was purchased from Abcam USA. Biotinylated secondary anti-mouse IgG and FITC labelled anti-mouse IgG were purchased from Jackson Immuno Research Laboratories Inc., West Groove, PA. Alexa flour 488 anti-rabbit was purchased from Invitrogen, USA. Monoclonal mouse anti GFAP, Avidin biotin complex (ABC kit) and DAB were purchased from Vector laboratories Ltd UK. S-allylcysteine (SAC) was purchased from LGC Prochem, Bangalore, India. Other chemicals were of analytical reagent grade.

Animals:
Male Wistar rats weighing 450–500 g and Swiss male albino mice weighing 35–40 g each one year old were used in the study. They were kept in the Central Animal House of Jamia Hamdard (Hamdard University). They were kept in colony cages and maintained under standard housing conditions (room temperature of 25±2 °C and relative humidity 45–55%) with 12 h light/dark reverse cycles. The standard rodent pellet diet and water were available ad libitum. Experiments were conducted in accordance with the Animal Ethics Committee of the University.

Methods

Experimental Design
Experiments were designed to check the efficacy of s-allyl cysteine, taurine, rutin and hesperidin on streptozotocin model of cognitive impairment.
Experiment I was carried out to evaluate the pre-treatment effect of \textit{s-allyl} cysteine (SAC) [30 mg/kg body weight intraperitonally in normal saline] supplementation for 15 days on intracerebroventricular injection of streptozotocin (ICV-STZ) induce neurodegeneration in mice. The mice were divided into four groups of 10 animals each. Group I was sham operated vehicle treated control (S) group. Group II was sham operated and pre-treated with SAC (SAC+S) group. Group III was ICV-STZ infused and pre-treated with SAC (SAC+L). Group IV was ICV-STZ infused and vehicle treated lesion (L) group. The SAC dose used in this experiment was determined from previous study (Mostafa et al., 2000).

Mice were pretreated with SAC or normal saline for 15 days and then infused bilaterally with ICV-STZ (2.57 mg/kg, body weight in saline and 2 µl/injection). Two weeks after the surgery, ICV-STZ mice demonstrated altered cognitive behaviour in memory deficits as tested by Morris water maze test. After three weeks of surgery, animals were sacrificed and their brains were taken out quickly on ice to dissect hippocampus for biochemical (TBARS content, GSH level, activities of GR and GPx) parameters.

Experiment II was carried out to evaluate the pre-treatment effect of SAC supplementation on DNA fragmentation, histopathological analysis and immunostaining for Bcl-2 and p53. The mice were divided into four groups as in experiment I and each group has 14 mice.

Experiment III was carried out to evaluate the pre-treatment effect of taurine (50 mg/kg body weight orally in normal saline) supplementation for 15 days on ICV-STZ injected rats. The rats were divided into four groups of 10 animals each. Group I was sham operated vehicle treated control (S) group. Group II was sham operated and pre-treated with taurine (T+S) group. Group III was ICV-STZ infused and pre-treated with taurine (T+L). Group IV was ICV-STZ infused and vehicle treated lesion (L) group. The taurine dose used in this experiment was determined from previous study conducted by Sankar Samipillai et al 2010.

Rats were pre-treated with taurine or normal saline for 15 days and then infused bilaterally with ICV-STZ (3.0 mg/kg, body weight in saline and 5 µl/injection). Two weeks after the surgery, ICV-STZ rats demonstrated altered cognitive behaviour in memory deficits as tested by Morris water maze test. After three weeks of surgery, animals were sacrificed and their
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Brains were taken out quickly on ice to dissect hippocampus for biochemical (TBARS content, GSH level, activities of GR, GPx, Catalase, SOD, GST and AchE) parameters.

Experiment IV was carried out to evaluate the pre-treatment effect of taurine on histopathology, immunofluorescence staining of ChAT and cresyl violet staining in ICV-STZ-infused rats. The rats were divided into four groups as in experiment II and each group has 8 rats.

Experiment V was carried out to evaluate the pre-treatment effect of rutin (25 mg/kg body weight orally in normal saline) supplementation for 3 weeks on ICV-STZ injected rats. The rats were divided into four groups of 10 animals each. Group I was sham operated vehicle treated control (S) group. Group II was sham operated and pre-treated with rutin (R+S) group. Group III was ICV-STZ infused and pre-treated with rutin (R+L). Group IV was ICV-STZ infused and vehicle treated lesion (L) group. The rutin dose used in this experiment was determined from previous study conducted in our laboratory (Khan et al., 2009).

Rats were pretreated with rutin or normal saline for 3 weeks and then infused bilaterally with ICV-STZ (3.0 mg/kg, body weight in normal saline and 5 μl/injection). Two weeks after the surgery, ICV-STZ rats demonstrated altered cognitive behaviour in memory deficits as tested by Morris water maze test. After three weeks of surgery, animals were sacrificed and their brains were taken out quickly on ice to dissect hippocampus for biochemical (TBARS content, GSH level, nitrite level, poly ADP-ribose polymerase activity, and activities of antioxidant enzymes like GR, GPx, and Catalase) parameters.

Experiment VI was carried out to evaluate the pre-treatment effect of rutin on histopathology and immunofluorescence staining of inducible nitric oxide synthase (iNOS) and interleukin-8 (IL-8) and immunohistochemistry (ABC method) of glial fibrillary acidic protein (GFAP), cyclooxygenase-2 (COX-2) and nuclear factor κB (NF-κB) in ICV-STZ-infused rats. The rats were divided into four groups as in experiment V and each group has 8 rats.

Experiment VII was carried out to evaluate the pre-treatment effect of hesperidin (100 and 200 mg/kg b.wt. orally in normal saline) supplementation for 15 days on ICV-STZ injected
mice. The mice were divided into five groups of 10 animals each. Group I was sham operated vehicle treated control (S) group. Group II was sham operated and pre-treated with hesperidin 200 mg/kg (H200+S) group. Group III was group ICV-STZ infused and pre-treated with hesperidin 100 mg/kg (H100+L). Group IV was ICV-STZ infused and pre-treated with hesperidin 200 mg/kg (H200+L). Group V was ICV-STZ infused and vehicle treated lesion (L).

Mice were pretreated with hesperidin or normal saline for 2 weeks and then infused bilaterally with ICV-STZ (2.57 mg/kg, b. wt. in normal saline and 2 µl/injection). Two weeks after the surgery, ICV-STZ mice demonstrated altered cognitive behaviour in memory deficits as tested by Morris water maze test. After three weeks of surgery, animals were sacrificed and their brains were taken out quickly on ice to dissect hippocampus for biochemical estimation viz: contents of TBARS and GSH and activity of AchE.

Experiment VIII was carried out to evaluate the pre-treatment effect of hesperidin on total cholesterol, triglycerides, phospholipids and ganglioside. The rats were divided into four groups as in experiment VII and each group has 8 rats.

Intracerebroventricular injection of streptozotocin to rats and mice:

The animals were anesthetized with chloral hydrate rat's 400 mg/kg b.wt. and mice 685.71 mg/kg b.wt. intraperitoneally (i.p.) and placed on a stereotaxic frame (dual manipulator model 51600 Stoelting Co., IL, USA) and the skin overlying the skull was cut to expose it. The coordinates of rats for the lateral ventricle (Paxinos and Watson, 1986) were measured accurately as antero-posterior −0.8 mm, lateral, 1.5 mm and dorso-ventral −4.0 mm and the coordinates of mice for the lateral ventricle were measured accurately as antero-posterior −0.8 mm, lateral, 1.0 mm and dorso-ventral −3.0 mm relative to bregma and ventral from dura with the tooth bar set at 0 mm. Burr hole was made in skull by automatic micro drilling machine attached on stereotaxic apparatus. Through the hole, a 28-gauge Hamilton® syringe of 10 µl attached to a micro-injector unit and piston of the syringe was lowered manually into each lateral ventricle. The lesion groups received a bilateral ICV injection of STZ (3 mg/kg, body weight in saline, 5 µl/injection sites for rats and 2.57 mg/kg b.wt in saline for mice, 2µl/injection sites). The sham groups underwent the same surgical procedures, but same
volume of saline was injected instead of STZ. After surgery, the animals were housed individually and had access to food and water *ad libitum*.

**Behavioural testing:**
The behavioural tests were started 2 weeks after ICV-STZ infusion. The experiment was performed between 9.00 a.m. to 4.00 p.m. at standard laboratory conditions. Behavioural tests were performed and analyzed by a researcher blind to the experimental conditions.

**Morris water maze test:**
Spatial learning and memory of animals were tested in Morris water maze (Morris, 1984). It consisted of a circular water tank (132 cm diameter and 60 cm height) that was filled 30 cm with water (25±2 °C). A non-toxic white paint was used to render the water opaque. The pool was divided virtually into four equal quadrants, labelled north–south–east–west. An escape platform (10 cm in diameter) was hidden 2 cm (1 cm in case of mice) below the surface of water on a fixed location in one of the four quadrants of the pool. The platform remained in the same quadrant throughout experiment. Before the training started, animals were allowed to swim freely into the pool for 60 s without platform. They were given four trials (once from each starting position) per session for 5 days, each trial having a ceiling time of 60 s and a trial interval of approximately 30 s. After climbing on to the platform, the animal remained there for 30 s before the commencement of the next trial. If animal failed to reach the escape platform within the maximum allowed time of 60 s, the animal was gently placed on the platform and allowed to remain there for the same interval of time. An overhead video camera was connected to a video monitor and computer software was used to track the animal's path and to calculate the escape latency and travelled distance (path length).

On 6th day, a probe test was conducted by removing the platform. Mice were allowed to swim freely into the pool for 60 s. The time spent in the target quadrant, which had previously contained hidden platform was recorded. The time spent in the target quadrant indicated the degree of memory consolidation taken place after learning.

**Biochemical analysis:**

**Tissue preparation for biochemical estimation:**
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After 3 weeks of ICV-STZ infusion, animals were sacrificed and their brains were taken out quickly on ice to dissect hippocampus. The dissected brain part were homogenized at 4°C in 10 mM phosphate buffer (PB, pH 7.0) having 10 μl/ml protease inhibitor to get 5% w/v homogenate. The homogenate was centrifuged at 800g for 5 min at 4 °C to separate the nuclear debris. The supernatant S1 was used for estimation of lipid peroxidation in terms of TBARS content and acetylcholine esterase activity. The remaining S1 was further centrifuged at 10,500g for 30 min at 4 °C to get the post-mitochondrial supernatant (PMS) which was used for estimation of reduced glutathione, nitrite estimation, poly ADP-ribosyl polymerase and antioxidant enzymes.

Tissue preparation for the estimation of lipid content:

The method of Folch et al. (1951) as described by Islam et al. (1980) was used for the extraction of lipids from the hippocampus. Hippocampus was homogenized in test tube with 7 ml chloroform: methanol (2:1, v/v) in ultraturrax homogenizer with and allowed to stand at 40°C for 1 h with occasional shaking. Thereafter, it was filtered through sintered glass funnel (G-4) in graduated stopper tubes under vacuum. Further the test tubes were rinsed thrice times with 1 ml fresh chloroform: methanol (2:1, w/v) each time and makeup the volume of 10 ml. Thereafter 2.5 ml of saline solution was added to each stopper tube and vortexes thoroughly and placed at -10°C in deep freezer overnight for the separation of two layers. After the complete separation, the junction of the two layers in each tube was noted. The upper layer (1 ml) in duplicate was collected in test tubes for the estimation of gangliosides and remaining upper layer was discarded and the junction of aqueous layer was wiped with filter paper to absorb saline. The lower layer was stored at -20°C for the estimation of cholesterol, triglycerides and phospholipids.

TBARS content:

TBARS content was estimated by the method of Utley et al. (1967) as modified by Islam et al. (2002). Homogenate 0.1 ml was pipette into a 15×100mm test tube and incubated at 37°C in a metabolic shaker for 1 h. An equal volume of homogenate was pipette into a centrifuge tube and placed at 0°C and marked at 0 h incubation. After 1 h of incubation, 0.45ml of 5% (w/v) chilled TCA and 0.45 ml 0.67%TBA were added after that centrifuged at 4000g for 10 min. Thereafter, supernatant was transferred to other test tubes and placed in a boiling water
bath for 10 min. The absorbance of pink colour produced was measured at 535 nm. The TBARS content was calculated by using a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ and expressed as nanomoles of TBARS formed per hour mg$^{-1}$ of protein.

**Reduced glutathione (GSH) content:**

GSH content was measured by the method of Jollow et al. (1974) with slight modification. PMS was mixed with 4.0% sulfosalisylic acid (w/v) in 1:1 ratio (v/v). The samples were incubated at 4 °C for 1 h, and centrifuged at 4000g for 10 min at 4 °C. The assay mixture contained 0.1ml of supernatant, 1.0 mM DTNB and 0.1 M phosphate buffer pH 7.4 in a total volume of 3.0ml. The yellow colour developed was read immediately at 412 nm. The GSH content was calculated as μmoles of GSH mg$^{-1}$ protein, using a molar extinction coefficient of $13.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$.

**Estimation of glutathione peroxidase (GPx):**

GPx activity was determined by the method of Mohandas et al. (1984). The reaction assay consisted of phosphate buffer (0.05 M, pH 7.0), EDTA (1mM), sodium azide (1mM), glutathione reductase (1 EU/ml), glutathione (1mM), NADPH (0.2 mM), hydrogen peroxide (0.25mM) and 0.1 ml of PMS in the final volume of 2 ml. The disappearance of NADPH at 340 nm was recorded at room temperature. The enzyme activity was calculated as nmol NADPH oxidized/min/mg protein by using molar extinction coefficient of $6.22 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$.

**Glutathione reductase (GR):**

Glutathione reductase activity was measured by the method of Carlberg and Mannervik (1975) as modified by Mohandas et al. (1984). The reaction mixture consisted of phosphate buffer (0.1 M, pH 7.6), NADPH (0.1mM), EDTA (0.5mM) and oxidized glutathione (1mM) and 0.05 ml of PMS in total volume of 1 ml. The enzyme activity was quantified at room temperature by measuring the disappearance of NADPH at 340 nm and calculated as nmol NADPH oxidized/min/mg protein by using molar extinction coefficient of $6.22 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$. 

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Glutathione-S-transferase (GST):

GST activity was measured by the method of Habig et al., 1974 in a reaction mixture consisting of 0.1 M phosphate buffer (pH 6.5), 1.0 mM reduced glutathione, 1.0 mM CDNB and 0.1 ml of PMS in a total volume of 3 ml. The changes in absorbance were recorded at 340 nm, and the enzymatic activity was calculated as nmol CDNB conjugate formed /min/mg protein.

Superoxide dismutase (SOD):

Superoxide dismutase activity was measured spectrophotometrically as described previously by Stevens et al. (2000) by monitoring the auto oxidation of (-)-epinephrine at pH 10.4 for 3 min at 480 nm. The reaction mixture contained glycine buffer (50 mM, pH 10.4) and 0.2 ml of PMS. The reaction was initiated by the addition of (-)-epinephrine. The enzyme activity was calculated in terms of nmol (-)-epinephrine protected from oxidation/min/mg protein using molar extinction coefficient of 4.02×10³ M⁻¹ cm⁻¹.

Catalase (CAT):

Catalase activity was assayed by the method of Claiborne (1985). Briefly, the assay mixture consisted of 0.05 M phosphate buffer (pH 7.0), 0.019 M H₂O₂, and 0.05 ml PMS in a total volume of 3.0 ml. Changes in absorbance were recorded at 240 nm. Catalase activity was calculated in terms of nmol H₂O₂ consumed/min/mg protein using molar extinction coefficient of 43.6×10³ M⁻¹ cm⁻¹.

Acetylcholinesterase (AchE) activity:

AchE activity was determined by a modified method of Ellman et al (1961). Briefly 2.6 ml of PB (0.1 M, pH 8.0), 40 μl of 0.075M acetylthiocholine iodide and 0.1 ml of buffered Ellman’s reagent (DTNB 10 mM, NaHCO₃ 15 mM) were mixed and allowed to incubate for 5 min at room temperature. Enzyme sample (20 μl) was added and optical density was measured at 412 nm within 5 min. AchE activity was expressed as nmol thiocholine formed/ min/mg protein.

Nitrite level:

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide (NO), was determined by a colorimetric assay using Greiss reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulphanilamide and 2.5% phosphoric acid) as
described by Green et al. (1982). Equal volumes of supernatant and Greiss reagent were mixed, the mixture incubated for 10 min at room temperature in the dark and the absorbance determined at 540 nm spectrophotometrically. The concentration of nitrite in the supernatant was determined from sodium nitrite standard curve and expressed as μmol/mg protein.

Poly ADP-ribosyl polymerase assay:

Poly (ADP-ribosyl) polymerase (PARP) activity was estimated by the method of Masnioudi et al. (1988) using 0.1 mM [3H] NAD⁺ as substrate in reaction mixture containing 10 mM Tris/HCl (pH 8.0), 0.4 mM dithiothreitol, 4.0 mM MgCl₂ and 25 μl of PMS in a total volume of 125 μl. The reaction was carried out at 37°C for 10 min and terminated by the addition of 10% trichloroacetic acid containing 0.02 M sodium pyrophosphate. After 30 min at 4°C, the precipitate was collected on Whatman GF/B glass fiber paper, dried for 15 min at 100°C. Thereafter 10 ml of scintillation fluid was added to each vial and radioactivity was determined on WALLAC-1410 Liquid Scintillation Counter.

DNA isolation and fragmentation:

Genomic DNA was extracted from a modified method of Simantov et al. (1996). Briefly, tissue samples (50 mg) were homogenized in 0.5 ml of digestion buffer (10mM Tris–HCl, 10mM NaCl, 25mM EDTA, 1% SDS, 1mg/ml proteinase K, pH 7.4). Then, samples were incubated at 37°C for overnight. At the end of incubation, 50 μl of 5M NaCl was added to samples. DNA was extracted twice with a mixture of phenol: chloroform: isoamyl alcohol (25:24:1 v/v) and centrifuged at 3000g for 4 min. After extensive washing with ethanol (70%), DNA was recentrifuged at 3000g for 4 min and resuspended in buffer (10mM Tris–HCl, 1mM EDTA, pH 7.4) and incubated with RNase (0.5 mg/ml) for 30 min. Thereafter, DNA(10 μl) from each sample was loaded onto 1% agarose gel, containing 0.5 μg/ml ethidium bromide and electrophoresis for the analysis of DNA fragmentation which was detected with UV transilluminator.

Hematoxylin and eosin (H&E) stain:

The animals were anesthetized with chloral hydrate on 22nd day of lesioning and perfused transcardially through ascending aorta with 100 ml ice cold phosphate buffered saline (PBS
0.1 M pH 7.4) followed by 4% paraformaldehyde in cold PBS (0.1 M pH 7.4). Brains were removed quickly, post fixed in the paraformaldehyde solution for 48 h, and embedded with wax. Coronal sections having hippocampus 5 μm thickness was dewaxed and stained with hematoxylin and eosin.

**Staining procedure:**

1. The gelatine coated slides containing sections were dewaxed in two changes of xylene 10 minutes each and process in gradient ethyl alcohol 5 min each, and finally in distill water for 2 min.
2. Stained in haematoxylin for 5-10 min
3. Washed the sections with running tap water till the sections become blue.
4. Rinsed the slide in acid-alcohol for 20-30 sec.
5. Again washed the sections in running tap water for 2-5 min
6. Stained with eosin for 1-2 min.
7. Sections were dehydrated with gradient ethyl alcohol and finally clear in xylene.
8. Sections were mounted in DPX.

**Cresyl violet stain:**

Adjacent sections were taken for the staining of cresyl violet to identify the pyramidal neurons degeneration in the CA1 region of hippocampus. Cresyl violet is a dye which stains nuclei, Nissl bodies and neurons in histological tissue. Briefly, sections were dewaxed in two changes of xylene 10 minutes each and process in gradient ethyl alcohol 5 minutes each, and finally in distill water for 2 minutes. Sections were then placed for 2 minutes into the cresyl violet solution (0.5% cresyl violet solution: 2.5 g cresyl violet (Sigma®, USA) and 1.5 ml glacial acetic acid was added to 500 ml distilled water. Thereafter, the sections were placed into a basin with tap water in which a constant flow of fresh water replaced the cresyl violet solution over a period of 5 minutes. In order to dehydrate, the sections were subjected to increasing ethanol concentrations and placed two times for 3 minutes each into 95% and 100% ethanol and finally clear with xylene. Sections were cover-slipped with DPX mounting medium (Merck, Germany).

**Immunoflourescence staining:**
Immunofluorescence staining was performed to detect the expression of choline acetyltransferase (ChAT), inducible nitric oxide synthase (iNOS) and interleukin-8 (IL-8). Coronal sections (5 μm thick) at the level of hippocampus were dewaxed by the gradient ethyl alcohol and processed for immunofluorescence staining. The sections were blocked in 10% normal goat serum for 45 min at room temperature thereafter washed the slides with PBST 0.05% and then incubated with primary antibodies anti ChAT polyclonal rabbit (dilution 1:200), anti IL-8 monoclonal mouse (dilution 1:100) and anti iNOS monoclonal mouse (dilution 1:100) at 4°C overnight. Then section were incubated with Alexa flour 488 goat anti rabbit IgG dilution 1:200 and FITC labelled goat anti mouse IgG dilution 1:200 for 1 h. Thereafter slides were washed 3 times with PBS 0.01 M, pH 7.4 and coverslipped with 10% glycerol in PBS and sealed by nail polish and store in dark at 4°C.

Immunohistochemistry (ABC method):

Immunohistochemistry was performed to detect the expression of Bcl-2, glial fibrillary acidic protein (GFAP), cyclooxygenase-2 (COX-2), phospho-p65 NF-kB and p53 proteins. Coronal sections (5 μm thick) at the level of hippocampus were dewaxed and processed for immunohistochemical staining. The gelatin coated slides containing sections were placed in 3% H2O2 in methanol for 20 min at room temperature to eliminate the endogenous peroxidase activity. Slides were washed with PBS (0.01 M, pH 7.4) for several times and pre-incubated in 1% bovine serum albumin for 45 min at room temperature; thereafter, the slides were incubated with primary antibodies, anti Bcl-2 monoclonal mouse (dilution 1:200), anti-GFAP monoclonal mouse (1:1000), anti COX-2 monoclonal mouse (1:500) anti phospho p65-NF-kB monoclonal mouse (1:500) and anti p53 monoclonal mouse (dilution 1:200) at 4 °C for overnight. Then, section was incubated with biotinylated donkey anti-mouse IgG (dilution 1:500) for 1 h. Thereafter washed the slides three times with PBS (0.01 M, pH 7.4) and then incubated with avidin biotin complex for 1 h. After that slides were treated with 3,4-diaminobenzidine and counter stained with haematoxylin for 30 sec (where needed). Slides were dehydrated with different gradient of alcohol and finally clear in xylene. Slides were then cover slipped by using DPX mountant media and observed under light microscope.

Ganglioside estimation:

Ganglioside was estimated according to the method of Pollet et al. (1978) as modified by Islam et al. (1980). To 1.0 ml upper layer of the lipid extract in test tubes, 2.0 ml resorcinol
reagent (10 ml of 3% resorcinol in DDW, 80 ml Conc. HCl, 0.25 ml of 0.1 M CuSO₄ make up volume up to 100 ml) was added. The test tubes were heated in boiling water bath for 30 min. After cooling 5.0 ml of a mixture of butyl acetate: n-butanol (85:125 v/v) was added to each tube. Tubes were shaken thoroughly and stand for 15 min to separate to organic phase. About 3 ml of the organic phase was taken and absorbance was measured at 580 nm against reagent blank. A standard curve with different concentration of N-acetyl neuraminic acid (5-30 μg) having 1.0 ml final volume of water was prepared by treating similarly.

**Phospholipids estimation:**

The phospholipids were estimated by the method of Fiske and Subbarow (1925) as described by Merinetti (1962). Lipids extract 0.2 ml were pipette in duplicate test tubes. Thereafter, 1.0 ml perchloric acid (70%) was added to each test tube and digested for 30 min or until samples becomes clear. After the complete digestion, the tubes were cooled at room temperature. Thereafter, 1.5 ml ammonium molybdate, 0.5 ml reducing reagent 1-amino-2-naphthol-4-sulphonic acid (ANSA) and 2.3 ml DDW were added to make the total volume 5.0 ml. The test tubes were heated in boiling water bath for 10 min. After cooling the colour intensity was read at 700 nm. A calibration curve was drawn with 1.0 μg to 6.0 μg of phosphorus using the same procedure as described above. The values were plotted and concentration of the phosphate was calculated from the calibration curve.

**Cholesterol estimation:**

Cholesterol was estimated by the method of Henly (1957). Lipid extract 0.5 ml was taken in the test tube containing 1.0 ml acetic acid. The test tubes were kept at room temperature for 2-3 h to evaporate the chloroform. To this, 3.0 ml ferric chloride acetic acid solution and 1.0 ml conc. H₂SO₄ was added and mixed at each step. The tubes were kept for 10-15 min. The colour intensity was read at 560 nm against reagent blank. A calibration curve with different conc. of cholesterol (50-400 μg) was drawn according to the same procedure as described above. The values were plotted and concentration of the cholesterol was calculated from the calibration curve.

**Triglyceride estimation:**

Triglyceride was measured in lower layer of lipid extract by commercially available kit (Span diagnostic Ltd. Surat, India.) as per manufacturer's instruction.
Protein content:

Protein content was determined by the method of Lowry et al. (1951) using bovine serum albumin (BSA) as a standard.

Statistical analysis:

Results are expressed as mean±S.E.M. Statistical analysis of the data was done by applying the analysis of variance (ANOVA), followed by Tukey's test. Behavioural parameters were analysed by applying the two-way analysis of variance (ANOVA) followed by Bonferroni test. The p-value<0.05 was considered statistically significant.
Chapter I

S-allyl cysteine attenuates oxidative stress associated cognitive impairment and neurodegeneration in mouse model of streptozotocin-induced experimental dementia of Alzheimer's type
Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by a progressive deterioration of memory and cognitive function. It affects millions of people and has become a major medical and social burden for developing countries. The formation of extracellular deposits of amyloid-β-peptide (Tabner et al., 2002) leading to the formation of neuritic plaques and neurofibrillary tangles in brain, is a prominent pathological feature of AD. Current AD therapeutics provides mainly symptomatic short term benefit, rather than targeting disease mechanisms. Numerous studies suggest that oxidative stress, an imbalance between free radicals and the antioxidant system, is a prominent and early feature in the pathogenesis of neuronal damage in AD (Huang et al., 2004; Ishrat et al., 2009).

The brain is very susceptible to the damage caused by oxidative stress, due to its rapid oxidative metabolic activity, high polyunsaturated fatty acid content, relatively low antioxidant capacity, and inadequate neuronal cell repair activity (Halliwell, 2001; Cassarino and Bennett, 1999; Ishrat et al., 2009). Oxidative damage to lipid (lipid peroxidation) and protein (protein carbonyl formation) can lead to structural and functional damage of the cell membrane, inactivation of enzymes, and ultimately cell death. Oxidative stress resulting from reactive oxygen species production is also implicated in apoptosis.

Intracerebroventricular (ICV) injection of streptozotocin (STZ) in mice impairs brain biochemistry, cerebral glucose and energy metabolism, cholinergic transmission, and increases generation of free radicals, ultimately leading to cognitive deficits (Hoyer and Lannert, 1999, 2008; Ishrat et al., 2009). Collectively, these effects are similar to sporadic dementia of Alzheimer's type in humans (Hoyer et al., 1991).

Since oxidative damage is implicated in the etiology of neurological complications, treatment with antioxidants has been used as a therapeutic approach in various types of neurodegenerative disease. S-allyl cysteine, the most abundant organo-sulfur compound with antioxidant properties (Geng et al., 1997; Kim et al., 2001; Numagami and Ohnishi, 2003), exerts its protective actions through its ability to scavenge $O_2^-$ (Kim et al., 2001) and $H_2O_2$ (Ide and Lau, 2001), thus preventing $H_2O_2$-induced endothelial cell damage and lipid peroxidation, as well as low density lipoprotein oxidation (Ide and Lau, 2001). It ameliorates learning deficits in senescence-accelerated mice (Nishiyama et al., 2001) and evokes
neurotrophic actions in cultured hippocampal neurons of rat (Moriguchi et al., 1997). Moreover, the most remarkable protective effects of *S*-allyl *cysteine* are those produced against Aβ peptide-induced apoptosis (Peng et al., 2002) and neurotoxicity in organotypic hippocampal cultures (Ito et al., 2003).

To the best of our knowledge, there is no evidence available on the protective effect of *S*-allyl *cysteine* on in vivo streptozotocin model of cognitive impairment. Therefore, the effects of a systemic administration of *S*-allyl *cysteine* were tested on different markers of learning/memory deficits evoked by STZ administration in mice.

**Materials and Methods:** As described in section III.

**Results**

**Behavioral observation**

**Effect of SAC on performance in Morris water maze task**

**Latency:**

The animals of all groups have improved Morris water maze acquisition performance. S and SAC+S group shows decreased latency to find the platform from the second to fifth day of experiment. However, L group animals presented a significantly (*p*<0.001) higher latency to find the platform than S group, but SAC+L group has shown a significant (*p*<0.001) improvement as compared to L group (Fig. 1).

**Path length:**

Acquisition performance was improved by all group animals in Morris water maze test. S and SAC+S group shows decreased path length to find the platform from the second to fifth day of experiment. However, L group animals showed a significantly (*p*<0.001) higher path length to find the platform than sham, but SAC+L group has shown a significant (*p*<0.001) improvement as compared to L group (Fig. 2).
Fig. 1: Effects of SAC supplementation on escape latency to find the platform in Morris water maze test in ICV-STZ mice. Values are expressed as mean±S.E.M. (n=10). Swimming times of four trials per day for 5 days to each group animals are shown. Average escape latency to find submerged platform was significantly (***p<0.001) prolonged in the L group animals when compared to the S group animals. Pre-treatment with SAC has lessen it significantly (###p<0.001) in SAC+L group animals as compared with the L group animals.

Fig. 2: Effects of SAC supplementation on path length to find the platform in Morris water maze test in ICV-STZ mice. Values are expressed as mean±S.E.M. (n=10). Swimming distance of four trials per day for 5 days to each group animals are shown. Average distance travelled to find the submerged platform was prolonged in L group animals when compared to S group animals. Pre-treatment with SAC lessen significantly (###p<0.001) the learning deficits in SAC+L group animals as compared with L group animal.
Biochemical Observations

Effect of SAC on TBARS content in hippocampus

The effect of SAC on TBARS content was measured to demonstrate the oxidative damage on membrane, in hippocampus of ICV-STZ mice. There was no significant alteration in TBARS content in SAC+S group animals in hippocampus while it was elevated significantly (p < 0.01) in L group animals as compared to S group animals and was protected significantly (p < 0.05) by SAC administration in SAC+L group animals (Fig. 3).

Effect of SAC on GSH in hippocampus:

The GSH level was significantly (p<0.05) decreased in hippocampus of L group as compared to S group. Pre-treatment of SAC has protected the GSH level significantly (p<0.05) in SAC+L group animals as compared with L group animals. SAC pre-treated group alone has exhibited no significant changes in GSH level as compared to S group animals (Fig 4).

Fig. 3: Effect of SAC pre-treatment on TBARS content in the hippocampus of ICV-STZ infused mice. Values are expressed as mean ± SEM. TBARS content was significantly increased in the L group as compared to S group (**p<0.01 L vs. S group). SAC pre-treatment significantly decreased TBARS content in the SAC+L group animals compared with the L group animals (#p<0.05 L vs. SAC+L group).
Effect of SAC pre-treatment on GSH level in the hippocampus of ICV-STZ infused mice. Values are expressed as mean±S.E.M. GSH level was significantly decreased in the L group as compared to S group (* p<0.05 L vs. S group). SAC pre-treatment significantly increased GSH level in the SAC+L group animals compared with the L group animals (# p<0.05 L vs. SAC+L group).

**Effect of SAC on the activity of antioxidant enzymes in hippocampus:**

The activity of antioxidant enzymes (GPx and GR) decreased significantly in L group as compared to S group. On the other hand, the activities were protected significantly by SAC administration in SAC+L group when compared with L group animals. The activity of these enzymes in SAC+S group was not attenuated significantly, compared as to its sham group (Table 1).

**Effect of SAC on the histochemical changes in hippocampal neuron:**

Histopathological changes in neurons following ICV-STZ injection were investigated by haematoxylin-eosin staining on the sections of hippocampal CA1 region. The haematoxylin and eosin has stained the nuclear structure in dark blue, and practically all cytoplasmic and intersubcellular substances were stained with varying shades of pink. The loss of pyramidal neurons was not detected in the sham group. On the other hand, L group showed the degenerative and abnormal neurons. SAC pre-treatment ameliorated hippocampal neuronal abnormalities in SAC+L group as compared to L group animals (Fig. 5).
Table 1: Values are expressed as mean ± S.E.M. STZ infusion leads to significant alterations on the activities of antioxidant enzymes (GPx and GR) in hippocampus in L group animals as compared to S group animals. Administration of SAC has significantly attenuated the activity of these enzymes in SAC+L group animals as compared to L group animals. Values in parentheses show the percentage increase or decrease with respect to their control. (*p<0.05, L vs. S group; ##p<0.01, SAC+L vs. L group).

Effect of SAC on Bcl-2 and p53 expression:

To demonstrate the neuroprotective effect of SAC on pro-apoptotic and neuroprotective proteins, the expression of Bcl-2 and p53 were assessed by immunohistochemistry. In L group the expression of p53-immunopositive cells in CA1 region of hippocampus increased compared to S group. The ICV-STZ induced increased p53 expression was prevented by pretreatment with SAC in SAC+L group animals (Fig. 7). Bcl-2 expression was exceedingly low in the CA1 region of hippocampus of lesion group as compared to sham group. SAC pre-treatment increased the Bcl-2 staining in SAC+L group animals as compared to lesion group animals (Fig. 6).
Fig 5: Histopathological changes in the CA1 region of hippocampus. Section were stained with hematoxylin and eosin. Black arrows indicate the normal pyramidal neuron in sham group (B) and white arrows indicate the degenerated pyramidal neuron in lesioned group (D) while lesioned group pretreated with SAC shows normal pyramidal neuron staining (F). Magnification 10X (A, C, E) and 40X (B, D, F).
Fig 6: Immunostaining of Bcl2 in CA1 region of hippocampus. Normal expression of Bcl2 was found in Sham group (A and B) compared to lesioned group (C and D), while the lesioned group pretreated with SAC (E and F) has shown a moderate staining of Bcl2. Magnification 10X (A,C,E) and 40X (B,D,F).
Fig 7: Immunostaining of p53 in CA1 region of hippocampus. Overexpression of p53 was found in lesioned group (A and B) compared to sham group (C and D), while the lesioned group pretreated with SAC (E and F) has shown a moderate staining of p53. However, the sham group has shown almost negligible staining. Magnification 10X (A,C,E) and 40X (B,D,F).
Effect of SAC on DNA fragmentation

DNA damage is a hallmark of apoptotic cell death. Fig. 8 shows the electrophoretic migration of genomic DNA isolated from hippocampus of various groups. It was observed that ICV injection of STZ caused marked DNA damage in L group when compared with S group DNA, where no fragmentation was observed. SAC pretreated animals showed a marked decrease in DNA fragmentation in SAC+L group when compared with L group.

Fig. 8: Genomic DNA agarose gel electrophoresis; No DNA laddering was observed in hippocampal tissue of the sham group or SAC+S group (Fig. 8 not shown). DNA laddering was detected in the hippocampal tissue of lesion group (L) which was attenuated by the pre-treatment with SAC in SAC+L group animals. Molecular weight (MW) lane showed 1kb DNA standard.

Discussion:

The present study examined the pre-treatment effect of SAC on cognitive deficits, oxidative stress, neuronal morphology and apoptotic parameters in intracerebroventricular streptozotocin (ICV-STZ) induced model of memory impairment in mice. It is well documented that ICV-STZ rodent model is an appropriate animal model used for the study...
of sporadic dementia of Alzheimer's type (Nitsch and Hoyer, 1991; Lannert and Hoyer, 1998; Agrawal et al. 2009; Ishrat et al., 2009). In the present study, SAC pretreatment significantly assuages cognitive behavior, and biochemical and histopathological alterations in ICV-STZ-infused mice. Neuroprotective potential of SAC suggests that it is a powerful antioxidant, corroborating previous studies (Pérez-Severiano et al., 2004; Gracia et al., 2008; Afif et al., 2009).

The Morris water maze test was used to evaluate the spatial learning and memory deficit in mice. A decreased escape latency and path length in Morris water maze task in repetitive trials demonstrates intact learning and memory function. ICV-STZ infused mice showed significant elevation of escape latency and path length as compared to sham group. While SAC+L group animals significantly decreased time and distance travelled to reach the hidden platform was observed. The data showed the conformity of memory impairment indicating the beneficial effect of SAC in enhancing these behavior test induced by STZ.

Oxidative stress is defined as a cytological consequence caused by imbalance between the production of free radicals and the ability to scavenge them. Free radical-induced damage to macromolecules (lipid, sugar, protein and nucleic acids) plays an important factor in the acceleration of aging and age-related neurodegenerative disorders such as AD (Liu et al., 2001; Wickens, 2001). Lipid peroxidation indicates neuronal membrane degeneration. It is reported that lipid peroxidation in the brain occurs in early Alzheimer's disease (Williams et al., 2006). Aging also increases lipid peroxidation in the brain of senescence-accelerated mouse (Petursdottir et al., 2007). Further results from the biochemical estimation indicate the significant increase in TBARS content and noticeable decrease in the level of reduced glutathione, and its dependent enzyme glutathione peroxidase and glutathione reductase in the brains of ICV-STZ infused mice compared to sham (S) group animals.

The antioxidant system requires reduced glutathione (GSH) a tripeptide and an essential antioxidant, which is responsible to accept free radicals in the brain tissue (Dringen et al., 2000). It eliminates H₂O₂ and organic peroxides by glutathione peroxidase (GPx) (Meister et al., 1988). During free radical clearance oxy-radicals are reduced by glutathione peroxidase at the cost of reduced glutathione to form glutathione disulfide (GSSG). GSH is further produced by redox recycling, in which GSSG is reduced to GSH by glutathione reductase (GR) with an expenditure of one NADPH molecule. Reduced level of GSH impairs H₂O₂ clearance and endorse formation of OH⁻, the most toxic molecule to the brain, leading to more free radical level and oxidative stress (Sun et al., 1990; Dringen et al., 2000). There are several reports about modulatory effect of SAC on lipid peroxidation and antioxidant
enzymes following injuries like hypoxia/ischemia and brain injuries (Pérez-Severiano et al., 2004; Gracia et al., 2008; Atif et al., 2009). In agreement with these findings, we also found that SAC significantly reduced the TBARS level and increased in the activity of antioxidant enzymes in hippocampus portion following STZ induction. This response of SAC could be attributed to its potential antioxidant property (Kim et al., 2001). Changes observed in oxidative stress parameters with cognitive dysfunction in STZ infused mice parallels with the earlier reports (Saxena et al., 2008) suggesting that STZ induced learning and memory impairment is associated with oxidative stress in mice.

Several protein families highly conserved during evolution are considered to be specifically involved in regulating apoptotic cell death such as Bax and Bcl-2, which form homo- and heterodimers in vivo. The relative expression of these two proteins determines cell death or survival. When Bcl-2 is in excess, the Bcl-2 homodimer predominates and promotes cell survival. It has been reported that overexpression of Bcl-2 protects a variety of mammalian cells from apoptosis induced by diverse death stimuli, including chemotherapeutic agents, radiation, tumor necrosis factor and glutamate (Zhong et al., 1993; Hartmann et al., 1999; Domen et al., 1998; Howard et al., 2002). Bcl-2 has been proposed to prevent apoptosis by regulating an antioxidant pathway (Hockenbery et al., 1993). In the agreement of above fact, we observed down expression of Bcl-2 protein in ICV-STZ infused mice which is protected by the pre-treatment of SAC.

The tumor suppressor and nuclear transcription factor p53 is a tetramer phosphoprotein that can regulate several major cellular functions including gene transcription, DNA synthesis, DNA repair, cell cycle regulation, senescence, and cell death (Sherr et al., 2002; Hofseth et al., 2004). The upregulation of p53 in response to a diverse array of cellular insults ranging from ischemia/hypoxia and excitotoxicity to oxidative stress in multiple neuronal populations suggests that p53 is a key factor involved in neuronal death in response to different forms of acute insults and chronic neurodegenerative conditions. The regulation of p53 in a variety of neurodegenerative disorders raised the possibility that inhibitors of p53 might prove effective in suppressing the associated mechanisms of neuron cell death. Our findings proposed that SAC reduced the expression of p53 positive cells. This view is supported by the down-regulation of p53 protein expression by SAC pre-treatment.

In neuropathological situations, apoptosis might contribute to neuronal degeneration through a number of factors such as ischemia/hypoxia, radiation, oxidative stress, excitatory, neurotoxicity, and so on (Thompson et al., 1995). It is well recognized that the DNA of apoptotic cells is cleaved into multiple bands activated by endonucleases and pro-apoptotic
molecules like apoptotic protease activating factor-1 (Apaf-1) and pro-caspase-9 which recruits caspase-3 leading to the formation of 'DNA ladders'. However, these ladders are not the only criterion for identifying apoptosis (Collins et al., 1992). The DNA ladders do not appear in some conditions because the apoptotic cells are easily and rapidly cleared by neighbouring cells; alternatively, as little as 2% of apoptotic cells amongst necrotic cells can be detected as a ladder. In our study the DNA extracted from hippocampus of S, L and SAC+L groups was separated onto agarose gel electrophoresis showing fragmentation in L group whereas DNA fragmentation was not found in S group and this fragmentation was almost completely prevented by pre-treatment of SAC in SAC+L group.