An Isolation, Characterization and In-vitro Evaluation Study of Cholinesterase Inhibitory and Antioxidant Activities of *Mimosa pudica* for the Treatment of Neurodegenerative Disorders

A research paper is submitted to the Department Of Pharmacy, East West University in conformity with the requirements for the degree of Bachelor of Pharmacy

Submitted by

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Dedicated To My Beloved Parents Without Whom I Could Be Here……

Endorsement by the Chairperson

This is to certify that the thesis submitted to the Department of Pharmacy, East West University, Dhaka-1212, in partial fulfilment of the requirement for the Degree of Bachelor in Pharmacy, was carried out by Synthia Sarker, ID: 2013-1-70-077.

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Certificate by the Supervisor

This is to certify that the thesis entitled **"An Isolation, Characterization and In-vitro Evaluation Study of Cholinesterase Inhibitory and Antioxidant Activities of *Mimosa pudica* for the Treatment of Neurodegenerative Disorders"** submitted to the Department of Pharmacy, East West University, in the partial fulfilment of the requirement for the degree of Bachelor of pharmacy was carried out by, **Synthia Sarker, ID: 2013-1-70-077** in 2017, under the supervision and guidance of me. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

Kushal Biswas

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Declaration by the Candidate

I, **Synthia Sarker,** hereby declare that the dissertation entitled **"An Isolation, Characterization and In-vitro Evaluation Study of Cholinesterase Inhibitory and Antioxidant Activities of *Mimosa pudica* for the Treatment of Neurodegenerative Disorder"**submitted by me to the Department of Pharmacy, East West University, in the partial fulfilment of the requirement for the award of the degree Bachelor of Pharmacy is a complete record of original research work carried out by me during 2017, under the supervision and guidance of **Kushal Biswas**, Lecturer, Department of Pharmacy, East West University.The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Abstract

Among the pathologic hypotheses of Alzheimer's disease (AD), cholinergic deficit and oxidative stress have been implicated as two major hallmarks. Therefore, inhibition of cholinesterase and oxidation are the two promising strategies in the development of a drug for AD. *Mimusa pudica* plant extract is used in this research to investigate its anticholinesterase and antioxidant potentials. Anticholinesterase activity was measured by modified Ellman's method. Antioxidant potentials were evaluated by the assay of reducing power, radical scavenging. The Methanolic extract showed strong antioxidant effect. Additionally, the extract exhibited pronounced reducing capacity. The plant extract found moderate inhibitor of cholinesterase (Both Acetylcholinesterase and Butyrylcholinesterase). The tested sample reflects potential antioxidative and moderate anticholin-esterase inhibitory effect which may warrant its effectiveness in the treatment of AD.

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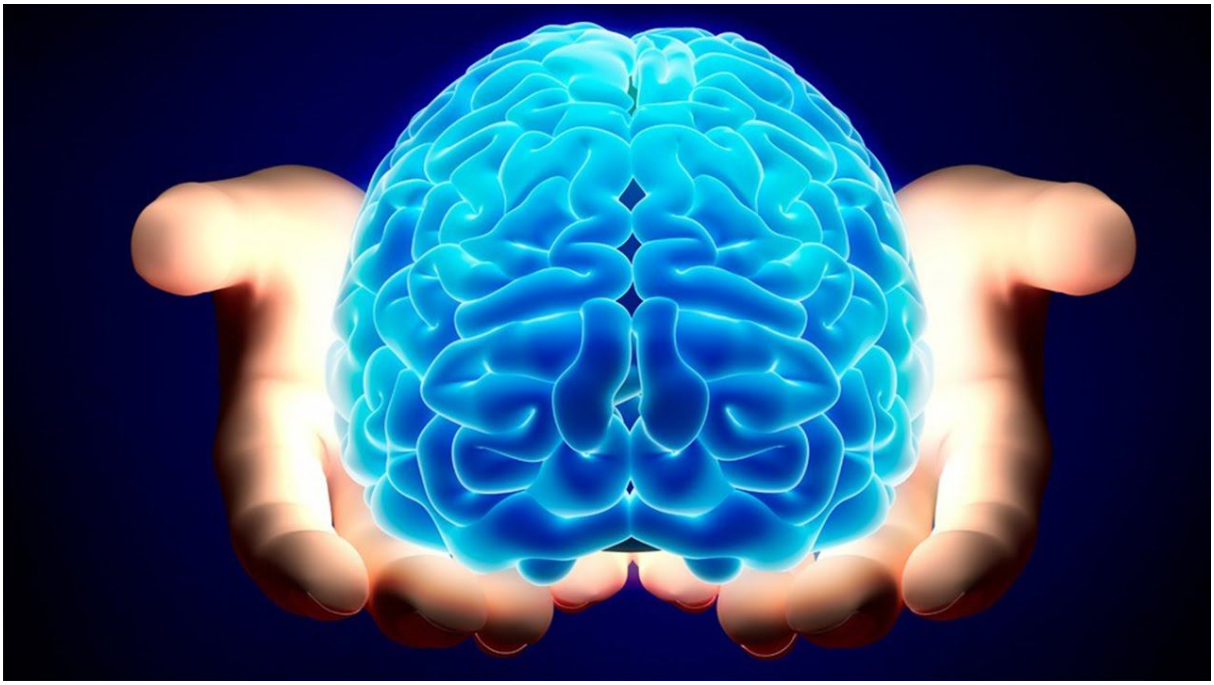
List of Abbreviation

LC	Locus coeruleus
NE	Non epinephrine
FAD	Familial Alzheimer's Disease
BA	Brain Aging
AD	Alzheimers Disease
ABA	Advance Brain Aging
MRI	Magnetic Resonance Imaging
CDC	Centers for Disease Control and Prevention
APP	Amyloid Precursor Protein
NFT	Nutrient Film Technique
AQP	Aquaporins
MCI	Mild Cognitive Impairment
sAAb	Soluble N-terminal Portion of Amyloid Precursor Protein
CTFb	Membrane bound C-terminal portion
apoE4	Apolipoprotein E4
MAP	Microtubule Associated Protein
MIC	Minimum Inhibitory Concentration
CE	Catechine Equivalent
GAE	Gallic Acid Equivalent
CLF	Chloroform Fraction
PET	Petrolium Ether Fraction
BTCI	Butylethiocholine Iodide

BChE	Butycholinesterase Enzyme
AchE	Acetylcholinesterase Enzyme
DTNB	Dithio Bisnitro Benzoic acid
DPPH	1,1-diphenyl-2-picrylhydrazyl radical
FCR	Folin-Ciocalteu Reagent
CME	Crude Methanoic Extract

Introduction



Chapter: 1
Introduction

1. Introduction:

Alzheimer's disease is an irreversible, progressive brain disorder that slowly destroys memory and thinking skills and, eventually, the ability to carry out the simplest tasks. In most people with Alzheimer's, symptoms first appear in their mid-60s. Alzheimer's largest risk factor is aging. However, it is not a normal part of aging. While the majority of people who have Alzheimer's are over the age of 65, it can happen in someone younger. Early-onset Alzheimer's is when the disease becomes noticeable in someone in their 40s or 50s. About 5 percent of people with this disease are in this age group.

This is a disease that progressively gets worse over time. In late stages, someone with Alzheimer's may not be able to communicate or reason with those around them. They will also eventually need full-time care for everyday life. According to the Alzheimer's Association, it is the 6th leading cause of death in the United States. The average life span of someone with the disease is eight years after diagnosis, but they can live up to 20 years depending on other health conditions. There is no cure at this time. However, there are treatments that can slow the progression and improve the quality of life for those with the disease. Alzheimer's disease is the most common cause of dementia. The word dementia describes a set of symptoms that can include memory loss and difficulties with thinking, problem-solving or language. These symptoms occur when the brain is damaged by certain diseases, including Alzheimer's disease.

Alzheimer's disease, named after the doctor who first described it (Alois Alzheimer), is a physical disease that affects the brain. During the course of the disease, proteins build up in the brain to form structures called 'plaques' and 'tangles'. This leads to the loss of connections between nerve cells, and eventually to the death of nerve cells and loss of brain tissue. People with Alzheimer's also have a shortage of some important chemicals in their brain. These chemical messengers help to transmit signals around the brain. When there is a shortage of them, the signals are not transmitted as effectively. As discussed below, current treatments for Alzheimer's disease can help boost the levels of chemical messengers in the brain, which can help with some of the symptoms.

Alzheimer's is a progressive disease. This means that gradually, over time, more parts of the brain are damaged. As this happens, more symptoms develop. They also become more severe.

1.1 Brain & Alzheimer Disease:

Scientists continue to unravel the complex brain changes involved in the onset and progression of Alzheimer's disease. It seems likely that damage to the brain starts a decade or more before memory and other cognitive problems become evident. During this preclinical stage of Alzheimer's disease, people seem to be symptom-free, but toxic changes are taking place in the brain. Abnormal deposits of proteins form amyloid plaques and tau tangles throughout the brain and once-healthy neurons stop functioning, lose connections with other neurons, and die.

The damage initially appears to take place in the hippocampus, the part of the brain essential in forming memories. As more neurons die, additional parts of the brain are affected. By the final stage of Alzheimer's, damage is widespread, and brain tissue has shrunk significantly. Read more about what happens to the brain in Alzheimer's

1.2 Difference between Dementia & Alzheimer's Disease:

Dementia is the decline of memory and other cognitive functions in comparison with the patient's previous level of function as determined by a history of decline in performance and by abnormalities noted from clinical examination and neuropsychological tests. A diagnosis of dementia cannot be made when consciousness is impaired by delirium, drowsiness, stupor, or coma or when other clinical abnormalities prevent adequate evaluation of mental status. Dementia is a diagnosis based on behavior and cannot be determined by computerized tomography, electroencephalography, or other laboratory instruments, although specific causes of dementia may be identified by these means.

Alzheimer's disease is a progressive, dementing disorder, usually of middle or late life. The clinical criteria for the diagnosis are PROBABLE, POSSIBLE, and DEFINITE. Clinical diagnosis of probable Alzheimer's disease can be made with confidence if there is a typical insidious onset of dementia with progression and if there are no other systemic or brain diseases that could account for the progressive memory and other cognitive deficits. Among the disorders that must be excluded are manic-depressive disorder, Parkinson's disease, multi-infarct dementia, and drug intoxication; less commonly encountered disorders that may cause dementia include thyroid disease, pernicious anemia, luetic brain disease and other chronic infections of the nervous system, subdural hematoma, occult hydrocephalus, Huntington's disease, Creutzfeldt-Jakob disease, and brain tumors. A diagnosis of definite Alzheimer's disease requires histopathologic confirmation. A clinical diagnosis of possible Alzheimer's disease may be made in the presence of other significant diseases, particularly if, on clinical judgment, Alzheimer's disease is considered the more likely cause of the progressive dementia. The clinical diagnosis of possible rather than probable Alzheimer's disease may be used if the presentation or course is somewhat aberrant. The information needed to apply these criteria is obtained by standard methods of examination the medical history; neurologic, psychiatric, and clinical examinations; neuropsychological tests; and laboratory studies.

1.3 Brain Changes Associated with Alzheimer's Disease

A healthy adult brain has about 100 billion neurons, each with long, branching extensions. These extensions enable individual neurons to form connections with other neurons. At such connections, called synapses, information flows in tiny bursts of chemicals that are released by one neuron and detected by a receiving neuron. The brain contains about 100 trillion synapses. They allow signals to travel rapidly through the brain's neuronal circuits, creating the cellular basis of memories, thoughts, sensations, emotions, movements and skills. The accumulation of

the protein fragment beta-amyloid (called beta-amyloid plaques) outside neurons and the accumulation of an abnormal form of the protein tau (called tau tangles) inside neurons are two of several brain changes associated with Alzheimer's. Beta amyloid plaques are believed to contribute to cell death by interfering with neuron-to-neuron communication at synapses, while tau tangles block the transport of nutrients and other essential molecules inside neurons. The brains of people with advanced Alzheimer's disease show inflammation, dramatic shrinkage from cell loss, and widespread debris from dead and dying neurons. Research suggests that the brain changes associated with Alzheimer's may begin 20 or more years before symptoms appear. When the initial changes occur, the brain compensates for them, enabling individuals to continue to function normally. As neuronal damage increases, the brain can no longer compensate for the changes and individuals show subtle cognitive decline. Later, neuronal damage is so significant that individuals show obvious cognitive decline, including symptoms such as memory loss or confusion as to time or place. Later still, basic bodily functions such as swallowing are impaired. While research settings have the tools and expertise to identify some of the early brain changes of Alzheimer's, additional research is needed to fine-tune the tools.

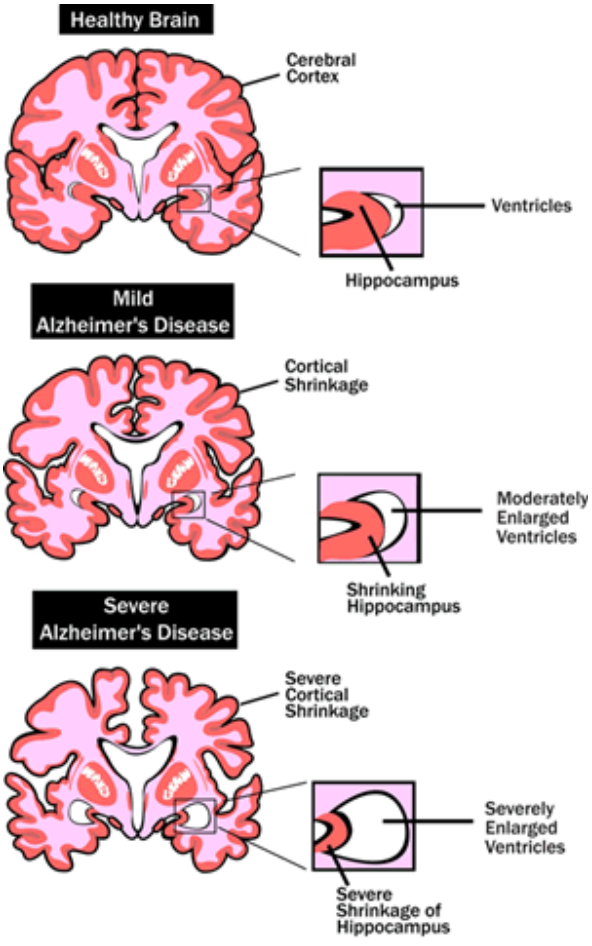


Figure no. 1.1: The changes in brain associated with Alzheimer's Disease.

1.4 Brain where AD Develop:

Research on cognitive aging has focused on how decline in various cortical and hippocampal regions influence cognition. However, brainstem regions play essential modulatory roles, and new evidence suggests that, among these, the integrity of the locus coeruleus (LC)–norepinephrine (NE) system plays a key role in determining late-life cognitive abilities. The LC is especially vulnerable to toxins and infection and is often the first place Alzheimer's-related pathology appears, with most people showing at least some tau pathology by their mid-20s. On the other hand, NE released from the LC during arousing, mentally challenging, or novel situations helps to protect neurons from damage, which may help to explain how education and engaging careers prevent cognitive decline in later years.

In late life, lower LC neural density is associated with cognitive decline. Because of the long unmyelinated axons of its neurons, high exposure to blood flow, and location adjacent to the 4th ventricle, the LC is especially vulnerable to toxins. The tau pathology precursor of Alzheimer's disease emerges in the LC by early adulthood in most people. However, the pathology typically spreads slowly, and only some end up with clinically evident Alzheimer's disease. Norepinephrine helps to protect neurons from factors that accelerate Alzheimer's disease, such as inflammation and excitotoxicity. Activation of the LC–NE system by novelty and mental challenge throughout life may contribute to cognitive reserve.

1.5 Types of AD:

Early-onset Alzheimer's: This type happens to people who are younger than age 65. Often, they're in their 40s or 50s when they're diagnosed with the disease. It's rare -- up to 5% of all people with Alzheimer's have early-onset. People with Down syndrome have a higher risk for it.

Scientists have found a few ways in which early-onset Alzheimer's is different from other types of the disease. People who have it tend to have more of the brain changes that are linked with Alzheimer's. The early-onset form also appears to be linked with a defect in a specific part of a person's DNA: chromosome 14. A form of muscle twitching and spasm, called myoclonus, is also more common in early-onset Alzheimer's.

Late-onset Alzheimer's. This is the most common form of the disease, which happens to people age 65 and older. It may or may not run in families. So far, researchers haven't found a particular gene that causes it. No one knows for sure why some people get it and others don't.

Familial Alzheimer's disease (FAD): This is a form of Alzheimer's disease that doctors know for certain is linked to genes. In families that are affected, members of at least two generations have had the disease. FAD makes up less than 1% of all cases of Alzheimer's. Most people who have early onset Alzheimer's have FAD.

1.6 How Alzheimer's Disease affect the brain:

The changes that take place in the brain begin at the microscopic level long before the first signs of memory loss. The brain has 100 billion nerve cells (neurons). Each nerve cell connects to many others to form communication networks. In addition to nerve cells, the brain includes cells specialized to support and nourish other cells. Groups of nerve cells have special jobs. Some are involved in thinking, learning and memory. Others help us see, hear, smell and tell our muscles when to move. Brain cells operate like tiny factories. They receive supplies, generate energy, construct equipment and get rid of waste. Cells also process and store information and communicate with other cells. Keeping everything running requires coordination as well as large amounts of fuel and oxygen. Scientists believe Alzheimer's disease prevents parts of a cell's factory from running well. They are not sure where the trouble starts. But just like a real factory, backups and breakdowns in one system cause problems in other areas. As damage spreads, cells lose their ability to do their jobs and eventually die.

1.7 Brain and Alzheimer's Disease:

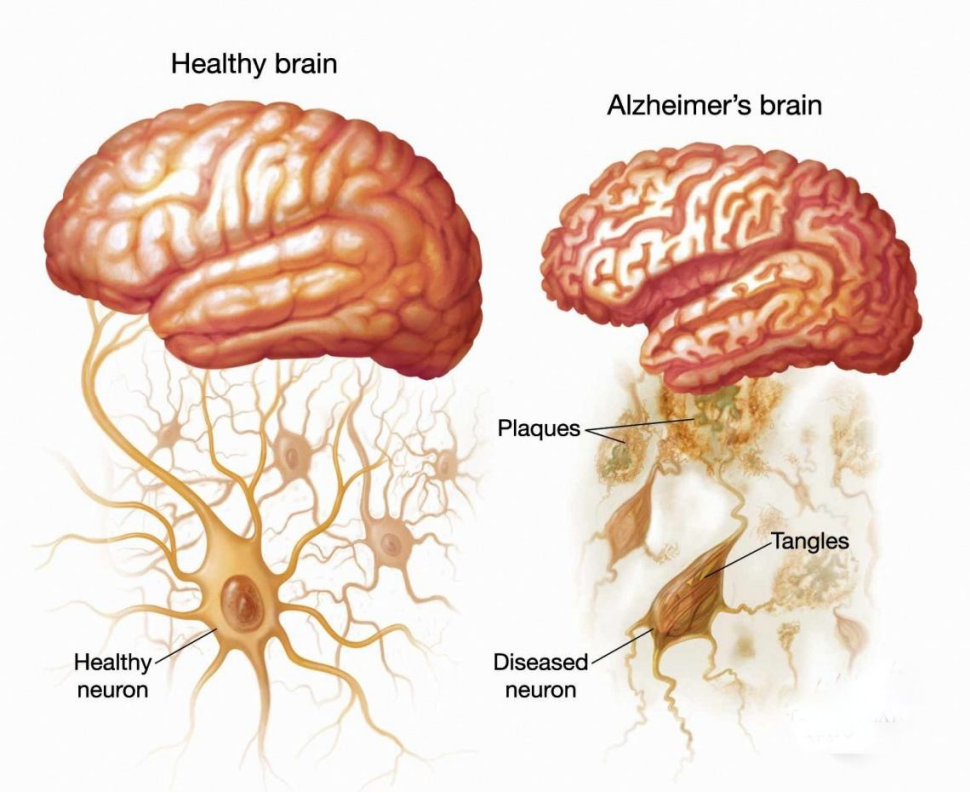


Figure no. 1.2: Cross sections of the brain show atrophy, or shrinking, of brain tissue caused by Alzheimer's disease.

Chapter: 1
Introduction

Scientists continue to unravel the complex brain changes involved in the onset and progression of Alzheimer's disease. It seems likely that damage to the brain starts a decade or more before memory and other cognitive problems appear. During this preclinical stage of Alzheimer's disease, people seem to be symptom-free, but toxic changes are taking place in the brain. Abnormal deposits of proteins form amyloid plaques and tau tangles throughout the brain and once-healthy neurons stop functioning, lose connections with other neurons, and die.

The damage initially appears to take place in the hippocampus, the part of the brain essential in forming memories. As more neurons die, additional parts of the brain are affected, and they begin to shrink. By the final stage of Alzheimer's, damage is widespread, and brain tissue has shrunk significantly.

1.8 Epidemiology:

Aging has been associated with cognitive impairment affecting working memory, processing speed, executive function and episodic memory, but different mechanisms and underlying brain changes have been related to each cognitive domain. Cognitive functions associated with frontal cortex structures and networks, particularly processing speed and working memory, have been associated with 'normal' brain aging (BA) and vascular-related white matter changes. Episodic memory impairment in turn has been attributed to Alzheimer disease (AD), the prevalence of which exponentially increases with age. AD is characterized by tau pathology spreading from the medial temporal lobe and neocortical widespread amyloid beta deposition. Amyloid- and tau-independent mechanisms like mitochondrial dysfunction and oxidative stress have been linked to BA, although this does not exclude that the same aging-related mechanisms can lead to increased AD-related pathology.

Findings from previous studies have shown that BA in individuals without concurrent pathology is associated with pronounced gray matter loss, particularly in frontal and parietal lobes, whereas amnestic mild cognitive impairment and AD subjects have shown atrophy patterns in the temporal lobe, hippocampus and parahippocampalgyrus. In addition, co-morbid conditions such as type 2 diabetes mellitus, hypertension and arteriolosclerosis are also associated with brain atrophy and might have an additive effect on atrophy related to BA. While many studies independently showed spatially specific atrophy patterns occurring with normal aging or due to disease, structural brain changes in advanced BA (ABA), defined as significant deviation from typical BA trajectories, have not been systematically compared with AD-like brain changes in population-based studies. In addition, whether different co-morbid and genetic conditions are associated with BA and AD is still uncovered in the general community.

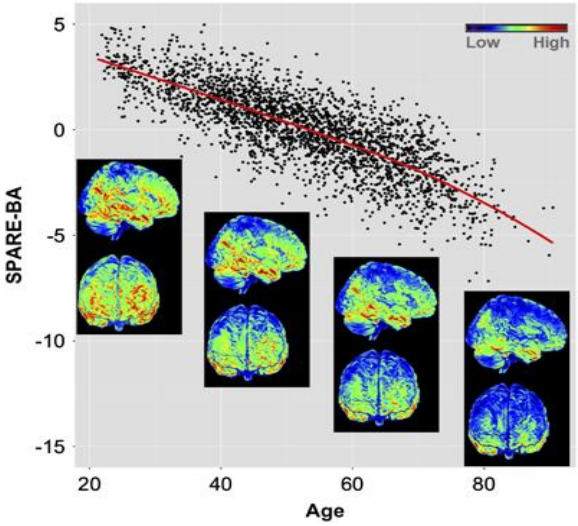
To assess ABA and AD-like patterns a traditional approach using simple radiological measures like hippocampal volume, which is commonly used to investigate brain changes related to aging and AD, might not be able to capture the complex spectrum of changes, and more sophisticated methods are required. Herein, we leverage advanced pattern analysis techniques to derive a new

quantitative index for brain changes as a function of age (Spatial Pattern of Atrophy for Recognition of BA (SPARE-BA)), and to compare those with spatial brain atrophy patterns specifically found in clinically diagnosed AD cases, using the Spatial Patterns of Abnormality for Recognition of Early Alzheimer's Disease (SPARE-AD) index in a large sample from the population-based Study of Health in Pomerania (SHIP) that spanned a wide age range (20–90 years, *n*=2705).

To our knowledge, the current study is the first to employ high-dimensional pattern recognition techniques to assess ABA patterns and to determine similarities and differences with clinical AD patterns in a large population-based cohort spanning almost the entire adulthood age range.

Prevalence of SPARE-BA in SHIP:

Figure 2 shows the SPARE-BA plotted as function of age for all participants (n=2705). There was a strong negative correlation between SPARE-BA and age with Pearson's correlation coefficient of r=−0.800 (P<0.0001). The Pearson's correlation coefficient between SPARE-BA and SPARE-AD was r=−0.491 in the whole sample and r=−0.515 in the sample with age greater than or equal to 65 years old (both with P<0.001).



In subjects older than 65 years, we defined the ABA (n=179) and resilient to aging (RA, n=191) based on the SPARE-BA score being 0.5 s.d. below and above the regression line, respectively (Figure 2). The distributions of the SPARE-AD scores for the subjects in ABA and RA groups are shown in Figure 2. Mean SPARE-AD of the ABA group was higher than the mean SPARE-AD of the RA group (mean±s.d. was −2.051±0.987 and −2.813±0.842, respectively, independent Student's t-test: P<0.0001).

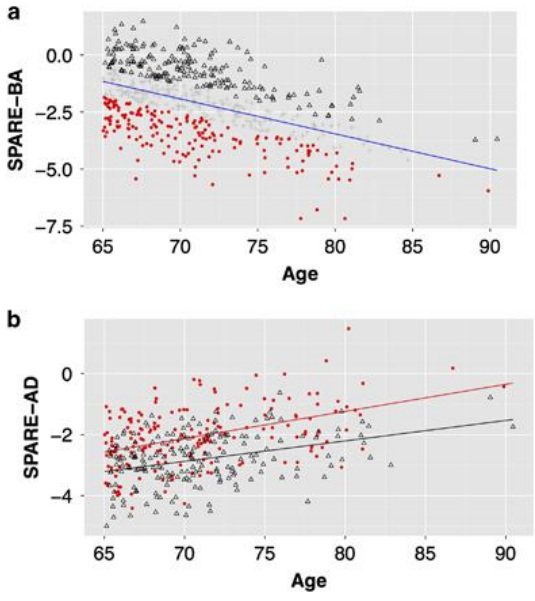


Figure no. 1.3: SPARE-BA of the brain

The relationship between ABA and AD-like structural brain changes has not been systematically studied in population-based studies. In a large neuroimaging cohort, describing atrophy in predefined atlas based-regions, and we quantified atrophy patterns due to aging and AD using summary indices (SPARE-BA and SPARE-AD, respectively) derived from high-dimensional imaging data, leveraging advanced analytical techniques. It is report that ABA individuals display patterns that is different from, albeit partially spatially overlapping with, patterns found in AD. This finding supports the hypothesis that distinct mechanisms might underlie lifetime BA and late-life neurodegeneration. However, by virtue of a partial overlap with AD-affected brain regions, ABA might be a co-morbidity leading to an earlier onset of dementia due to an additive effect of both mechanisms, as has been shown for coincident pathologies.

BA patterns of atrophy and the associations with risk factors

ABA, defined as significant deviation from typical age-related BA trajectories in this population, was associated with significant gray matter volume reduction in widespread frontal and parietal regions and more restricted temporal lobe areas. Our results regarding frontal and parietal age-related atrophy are consistent with observations from Resnick who found that frontal and parietal lobes showed greater decline compared with temporal and lobar regions in cognitively normal aging individuals. Furthermore, we confirm observations of Raz that age is associated with differential shrinkage of frontal regions.

To expand the knowledge regarding BA and the mechanisms underlying the observed MRI patterns, we evaluated the impact of known vascular risk factors on SPARE-BA. Our findings of association between ABA and smoking are consistent with prior reports demonstrating that regional brain volume reductions are associated with smoking.47 It is also observed that anti-hypertensive medication use was associated with ABA patterns. Anti-hypertensive medications could be considered a proxy for chronic hypertension, and thus, our findings are consistent with prior reports that hypertension is associated with brain atrophy,11 particularly in the frontal and temporal lobes.48 Results for waist circumference in men are in line with global loss and regional alterations in gray matter volume in obesity49 and obese men50 The gender-specific result might reflect different fat distribution patterns like the android fat distribution that is more relevant to brain alterations than gynoid fat distribution.51 Our observations regarding ABA patterns in diabetic men compared with men without diabetes are in line with a prior report indicating an association between brain atrophy and diabetes in smaller sample,7, 52 but other studies have also found associations between diabetes and brain atrophy in women.53 Our findings with respect to anti-depressants were only at the trend level and suggest a small effect given the large sample size. It is notable that anti-depressants are prescribed for patients suffering from pain, sleep disturbances and anxiety in addition to conditions associated with

major depressive disorders. Effects of these other chronic diseases and their distress may lead to ABA. In addition, AD and other neurodegenerative diseases have been linked to psychiatric symptoms that can precede the dementia diagnosis.

These results support the hypothesis that ABA patterns are largely associated with several co-morbidities (android fat distribution, hypertension, diabetes, and perhaps, depression or chronic stress). While associations between most of these risk factors and brain atrophy have been reported previously, a contribution of our analysis lies in developing an age-specific index that increases the statistical power to detect BA-related changes, and showing that the studied factors may modify patterns of brain structure in a manner that might 'accelerate' neurodegeneration related to the aging process. In addition, the large sample of this study enabled sex-stratified analyses of risk factors in relation to patterns of age-related atrophy. In addition, only few MR studies have such a statistical power, population-based sample and very high standardization protocol.

Multivariable regression models revealed risk factor associations with SPARE-AD that were different from those associated with SPARE-BA. In women, older age and smoking were associated with SPARE-AD. No significant risk factor other than age was associated with SPARE-AD in men, as reported in Supplementary. In the regression model for SPARE-AD, we found significant association when we included a quadratic age term in the model, indicating a non-linear relationship between age and greater atrophy in AD-related regions. This finding is in line with a previous report in the Baltimore Longitudinal Study of Aging (BLSA) cohort. Overall, the observed differences in risk factors associated with either SPARE-AD or SPARE-BA support the hypothesis that ABA is characterized by pathophysiologic mechanisms that are distinct from clinical AD-related atrophy patterns.

Contribution of high-dimensional pattern classification techniques

An important contribution of our study is the use of advanced methods of high dimensional pattern classification for BA assessment, which allowed us to investigate in detail the spatial patterns of atrophy, and to derive individualized indices that were further correlated with epidemiologic and clinical factors. Our approach utilizes information from all brain regions jointly, thereby capturing the structural abnormality subtleties in BA, which is high dimensional in nature and goes beyond the small number of dimensions represented in one or few volumetric measures. Recently, Janowitzet al.36 analyzed prediction patterns for hippocampal volumes in SHIP. Interestingly the associated risk factors with hippocampal volume were similar to those associated with the aging patterns in the current study but different from the prediction patterns of SPARE-AD. This finding indicates that the hippocampus alone is unlikely to adequately reflect the complexity of neurodegeneration in AD, as previously demonstrated.

Overlap between ABA and AD spatial patterns of atrophy

The regions of the ABA-related spatial patterns of atrophy overlapped only partially with the clinical AD-related patterns. While ABA-like patterns were widespread in the brain (in blue), clinical AD-like patterns were spatially more localized (in red), mostly significant in several (especially medial) temporal lobe regions. The overlap between clinical AD-like and ABA-like regions (in green) existed mainly in parts of the hippocampus and in areas of the temporal lobe. These differences in the spatial distribution of atrophy patterns associated to ABA and to clinical AD suggest that ABA stems from distinct mechanisms, which potentially constitute a co-morbidity for clinical AD largely by virtue of affecting spatially overlapping brain regions.

The AD polygenic risk score associations

Finally, the AD polygenic risk score was not significantly associated with SPARE-BA across the whole age range or in analyses restricted to older subjects, it was only close to significant association in the whole age range sample (r=0.044, P=0.069) and significantly associated with SPARE-AD in older subjects (r=0.124, P=0.016) as reported in Supplementary Analysis. These differences in association with polygenic risk score for SPARE-BA vs SPARE-AD offers additional support for the hypothesis that AD-related genetic risk leads to distinctive atrophy patterns. In fact the increase in prevalence of AD is more pronounced after age 65, 42 which is in line with the significant association we found between SPARE-AD and the polygenic risk score at older age. The underlying disease process may start years before the AD diagnosis in elderly individuals. Singh-Manouxet al.56 showed that the brain function started to deteriorate as early as age 45. However, we did not find a significant association between AD polygenic risk score and SPARE-AD in the whole age range sample, likely reflecting the fact that only a small subset of individuals could be in a preclinical AD stage, or that SPARE-AD captures neurodegenerative changes occurring relatively later in the disease process.

This study has several strengths including the large sample size in a population-based sample and the use of novel pattern analysis approaches to investigate BA. However, this study has also limitations, which include the lack of longitudinal MRI scans and detailed clinical information for the complete SHIP cohort.

In summary, the current study is the first, to our knowledge, to employ high-dimensional pattern recognition techniques to assess BA patterns in a cohort of this size and show that it has a unique spatial pattern of brain atrophy that differs from the one found in AD.

1.9 Morbidity & Mortality:

Alzheimer's disease is officially listed as the sixth-leading cause of death in the United States. It is the fifth-leading cause of death for those ages 65 and older. However, it may cause even more deaths than official sources recognize. Alzheimer's is also a leading cause of disability and poor health (morbidity). Before a person with Alzheimer's dies, he or she lives through years of morbidity as the disease progresses.

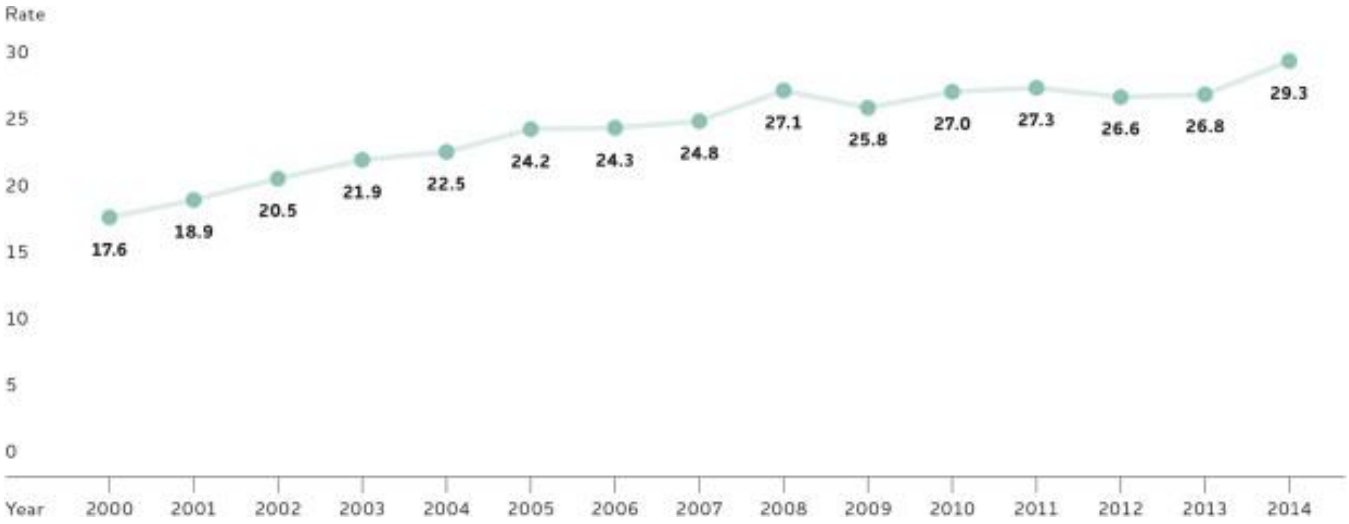


Figure no.1.3: U.S. annual Alzheimer's death rate (per 100,000 people) by year.

1.10 Deaths from Disease:

It is difficult to determine how many deaths are caused by Alzheimer's disease each year because of the way causes of death are recorded. According to data from the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC), 93,541 people died from Alzheimer's disease in 2014. The CDC considers a person to have died from Alzheimer's if the death certificate lists Alzheimer's as the underlying cause of death, defined by the World Health Organization as “the disease or injury which initiated the train of events leading directly to death.”

Severe dementia frequently causes complications such as immobility, swallowing disorders and malnutrition that significantly increase the risk of serious acute conditions that can cause death. One such condition is pneumonia, which is the most commonly identified cause of death among elderly people with Alzheimer's or other dementias. Death certificates for individuals with Alzheimer's often list acute conditions such as pneumonia as the primary cause of death rather than Alzheimer's. As a result, people with Alzheimer's disease who die due to these acute conditions may not be counted among the number of people who died from Alzheimer's disease according to the World Health Organization definition, even though Alzheimer's disease may well have caused the acute condition listed on the death certificate. This difficulty in using death

certificates to accurately determine the number of deaths from Alzheimer's has been referred to as a "blurred distinction between death with dementia and death from dementia."

Another way to determine the number of deaths from Alzheimer's disease is through calculations that compare the estimated risk of death in those who have Alzheimer's with the estimated risk of death in those who do not have Alzheimer's. A study using data from the Rush Memory and Aging Project and the Religious Orders Study estimated that 500,000 deaths among people age 75 and older in the United States in 2010 could be attributed to Alzheimer's (estimates for people age 65 to 74 were not available), meaning that those deaths would not be expected to occur in that year if those individuals did not have Alzheimer's.

The true number of deaths caused by Alzheimer's is somewhere between the number of deaths from Alzheimer's recorded on death certificates and the number of people who have Alzheimer's disease when they die. According to 2014 Medicare claims data, about one-third of all Medicare beneficiaries who die in a given year have been diagnosed with Alzheimer's or another dementia. Based on data from the Chicago Health and Aging Project (CHAP) study, in 2017 an estimated 700,000 people age 65 and older in the United States will have Alzheimer's when they die. Although some seniors who have Alzheimer's disease at the time of death die from causes that are unrelated to Alzheimer's, many of them die from Alzheimer's disease itself or from conditions in which Alzheimer's was a contributing cause, such as pneumonia.

Irrespective of the cause of death, among people age 70, 61 percent of those with Alzheimer's are expected to die before age 80 compared with 30 percent of people without Alzheimer's.

Table no 1.1: U.S. annual Alzheimer's death rates (per 100,000 people) by age and year

Age	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
45-54	0.2	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2
55-64	2.0	2.1	1.9	2.0	1.8	2.1	2.1	2.2	2.2	2.0	2.1	2.2	2.2	2.2	2.1
65-74	18.7	18.6	19.6	20.7	19.5	20.2	19.9	20.2	21.1	19.4	19.8	19.2	17.9	18.1	19.6
75-84	139.6	147.2	157.7	164.1	168.5	177.0	175.0	175.8	192.5	179.1	184.5	183.9	175.4	171.6	185.6
85+	667.7	725.4	790.9	846.8	875.3	935.5	923.4	928.7	1002.2	945.3	987.1	967.1	936.1	929.5	1006.8

1.11 Diagnosis:

There is no single test for Alzheimer's. Instead, physicians, often with the help of specialists such as neurologists and geriatricians, use a variety of approaches and tools to help make a diagnosis. They include the following:

• Obtaining a medical and family history from the individual, including psychiatric history and history of cognitive and behavioral changes.

•Asking a family member to provide input about changes in thinking skills and behavior.

•Conducting cognitive tests and physical and neurologic examinations.

•Having the individual undergo blood tests and brain imaging to rule out other potential causes of dementia symptoms, such as a tumor or certain vitamin deficiencies.

Diagnosing Alzheimer's requires a careful and comprehensive medical evaluation. Although physicians can almost always determine if a person has dementia, it may be difficult to identify the exact cause. Several days or weeks may be needed for the individual to complete the required tests and examinations and for the physician to interpret the results and make a diagnosis.

Current diagnosis methods: The Mayo Clinic (2016) has published a set of diagnostic tests because at this time there is no single medical test to determine if a person is suffering from Alzheimer's disease. Diagnosis is difficult and requires extensive and careful medical evaluation. Because neurons affected by Alzheimer's disease begin to decay the amount of healthy neurotransmitters as much as ten to twenty years before symptoms begin to affect the patient, damage is already pronounced at the mild stage of the disease. Typically, patients are diagnosis in the Mild stage of Alzheimer's disease. Brain areas that have already deteriorated when diagnosis has been made are the hippocampus, the frontal cortex, and occipital lobes and at this stage of degeneration the damage is irreversible.

The Mayo Clinic tests (2016) include a thorough medical history, physical exams, and neurological exams. The physical tests include testing reflexes, coordination, sight and hearing, and muscle tone and strength. These tests determine if testing for Alzheimer's should proceed. Lab tests determine that conditions such as thyroid disorders, vitamin deficiencies, and diabetes are not causes of memory loss and confusion. An extensive mental status assessment is performed to assess cognitive function. The Mayo Clinic suggests the use of brain imaging such as CT scans to rule out tumors, strokes, and head injuries as causes for patient's symptoms. MRI scans are used to assess if brain atrophy implicates Alzheimer's disease as the cause of symptoms. Recently improved PET scans use glucose to show brain activity and can also show brain levels of amyloid plaques and tangles, two brain abnormalities associated with Alzheimer's disease. Unfortunately, the new PET scans are not yet available to all medical doctors. Theses PET techniques are only found in research and clinical trial settings (Mayo Clinic, 2016). Cerebrospinal fluid can be tested for biomarkers that indicate a genetic factor for early on-set Alzheimer's disease (Mayo Clinic, 2016). Only a small fraction of 1% of people worldwide have the rare genetic form of Alzheimer's disease, these people tend to show signs of amyloid plaques in PET scans and in cerebrospinal fluid tests. Another biomarker of interest is tau, which is

implicated in the development of neurofibrillary tangles that build up in the brains of Alzheimer's disease patients. Notably these tangles are found in the hippocampus or memory center of the brain. Tau mimics the shape of healthy cells and destroys them, however genetic testing for this biomarker is implausible at this time as there is no scan to detect the tau in aliving patient (Landau, 2010).

Currently the diagnosis of Alzheimer's disease is difficult because no specific cause has been discovered. With no specific cause identified, there are no reliable treatments to cure or reverse the atrophy that develops in the brain afflicted with Alzheimer's disease. Improved participant numbers in research and clinical trials will lead to a far superior diagnostic system and an opportunity to develop sophisticated tools for earlier diagnosis. In addition to advancingdiagnosis increased research will inevitably lead to scientific breakthroughs in the discovery of acure.

1.12 Expanding Impact of AD:

Binding of acetylcholine from one neuron to another is the very basis of brain activity, and the neurons responsible for producing acetylcholine are affected by Alzheimer's disease. As Alzheimer's disease progresses acetylcholine production is reduced and neurons slowly lose function and begin to die after which neurons and neurotransmitters can no longer be reproduced or repaired. The area in the brain responsible for memory retention is the hippocampus, and it is commonly the first area to be affected by Alzheimer's disease. In a healthy brain the hippocampus becomes active during memory formation and retrieval. In a brain affected by Alzheimer's disease, forming new and retrieving old memories becomes increasingly more difficult due to the loss of acetylcholine-producing neurons in the hippocampus. As hippocampal neurons continue to decay, communication between nerve cells is permanently lost. Most commonly memory loss is one of the first observable symptoms of Alzheimer's disease. Motor activity is generated in the motor cortex, which is located in the frontal lobe. As neurons decay and die in the motor cortex, movement becomes increasingly difficult and proceeds to a total loss of mobility (National Institute on Aging, 2016).The Alzheimer's foundation of America (AFA) identifies three stages of Alzheimer's disease as mild, moderate, and severe. These stages reflect the movement of the disease from the central areas of the brain outward. As the disease progresses the symptoms become more severe. In the mild stage of Alzheimer's disease, the hippocampus appears to be the area affected the most. Patients experience short-term memory loss, most often have trouble remembering what they were doing, saying, or where they were going. Patients often find themselves more confused and find themselves struggling to remember the steps needed to complete a previously normal task. Complaints of memory loss are often the first indication that a person is suffering from Alzheimer's disease. Patients begin to have increased trouble speaking and understanding what other people are saying, this could perhaps be because of the inability to remember words and word meanings. Patients begin to need outside assistance. Family and friends will also begin to notice unexpected behaviors and personality

changes. In the moderate stage of Alzheimer's disease, patients begin to lose long-term memory as well as permanent loss of short term memories. The ability to learn new tasks is extremely limited. In this stage the areas responsible for memories of specific information such as facial recognition rapidly begins to decline, with intermittent bouts of lucidity. The spread of the disease from the central brain regions outward is now noticeable as patients begin to lose the ability to differentiate between and recognize family members. Processing

1.13 Diagnosis of Alzheimer's Disease:

A doctor can diagnose most cases of Alzheimer's. However, nobody can be 100% sure until after death, when a microscopic examination of the brain detects plaques and tangles. There is no basic testing, such as a blood test, urine test, biopsy, or image scan for diagnosing Alzheimer's disease. A brain scan may help identify changes in the brain.

Lund-Manchester Criteria for Frontotemporal Dementia:

The Lund-Manchester diagnostic criteria for frontotemporal dementia require all of the following core components to be present:

1. Insidious onset and gradual progression,
2. Early decline in social interpersonal conduct,
3. Early impairment in regulation of personal conduct,
4. Early emotional blunting,
5. Early loss of insight.

Supportive diagnostic features include:

A. Behavioral disorder:

a. decline in personal hygiene and grooming
b. mental rigidity and inflexibility
c. distractibility and impersistence
d. hyperorality and dietary change
e. utilization behavior

B. Speech and language:

Altered speech output (aspontaneity and economy of speech, press of speech), stereotypy of speech, echolalia, perseveration, mutism)

C. Physical signs:

Primitive reflexes, incontinence, akinesia, rigidity, tremor, low/labile blood pressure.

D. Investigations:

a. Neuropsychology: impaired frontal lobe tests; no amnesia or perceptual deficits
b. EEG: normal on conventional EEG despite clinically-evident dementia
c. Brain imaging: predominant frontal and/or anterior temporal abnormality

Ruling out other conditions:

Doctors will usually carry out some tests to rule out other conditions which typically have symptoms that are also present in Alzheimer's. Below are some examples of diseases and conditions that need to be ruled out:

a. Anxiety
b. Brain tumor
c. Depression
d. Infection
e. Thyroid problems
f. Vitamin deficiency

1.13.1 Tests for AD diagnosis:

A. **Blood tests**:
To see if the patient has a thyroid disorder or vitamin deficiency.

B. **Neuropsychological testing**:
This involves an extensive assessment of cognitive (thinking) and memory skills. It can take several hours. These types of tests are extremely useful in detecting Alzheimer's as well as other dementias early on.

C. **MRI (magnetic resonance imaging) scan**:
A powerful magnetic field is created by passing an electric current through the wire loops. Meanwhile, other coils in the magnet send and receive radio waves. This triggers protons in the body to align themselves. Once aligned, radio waves are absorbed by the protons, which stimulate spinning. Energy is released after "exciting" the molecules, which in turn emits energy signals that are picked up by the coil. This information is then sent to a computer

which processes all the signals and generates it into an image. The final product is a 3-D image representation of the area being examined, which in this case would be the brain.

D. **PET (positron emission tomography) scan:**
This test uses radiation, or nuclear medicine imaging, to produce 3-dimensional, color images of the functional processes within the human body. It is very useful in helping the doctor diagnose Alzheimer's disease. A PET scan that measures uptake of sugar in the brain significantly improves the accuracy of diagnosing a type of dementia often mistaken for Alzheimer's disease, a study revealed.

E. **CT (computerized tomography) scan**:
This device uses digital geometry processing to generate a 3-dimensional (3-D) image of the inside of an object. The 3-D image is made after many 2-dimensional X-ray images are taken around a single axis of rotation - in other words, many pictures of the same area are taken from many angles and then placed together to produce a 3-D image.

F. **Biomarker Tests**

The new criteria and guidelines identify two biomarker categories:

(1) Biomarkers showing the level of beta-amyloid accumulation in the brain

(2) Biomarkers showing that neurons in the brain are injured or actually degenerating.

Many researchers believe that future treatments to slow or stop the progression of Alzheimer's disease and preserve brain function (called "disease-modifying" treatments) will be most effective when administered during the preclinical and MCI stages of the disease. Biomarker tests will be essential to identify which individuals are in these early stages and should receive disease-modifying treatment. They also will be critical for monitoring the effects of treatment. At this time, however, more research is needed to validate the accuracy of biomarkers and better understand which biomarker test or combination of tests is most effective in diagnosing Alzheimer's disease. The most effective test or combination of tests may differ depending on the stage of the disease and the type of dementia.

1.14 Neuropathology in AD:

Alzheimer's disease is characterized by loss of neurons and synapses in the cerebral cortex and certain subcortical regions. This loss results in gross atrophy of the affected regions, including

degeneration in the temporal lobe and parietal lobe, and parts of the frontal cortex and cingulate gyrus. Degeneration is also present in brainstem nuclei like the locus coeruleus. Studies using MRI and PET have documented reductions in the size of specific brain regions in people with AD as they progressed from mild cognitive impairment to Alzheimer's disease, and in comparison with similar images from healthy older adults.

There are two major hallmarks in the brain that are associated with the disease processes of AD.

1. Amyloid Plaques.
2. Neurofibrillary Tangles.

The formation of amyloid plaques and neurofibrillary tangles are thought to contribute to the degradation of the neurons (nerve cells) in the brain and the subsequent symptoms of Alzheimer's disease.

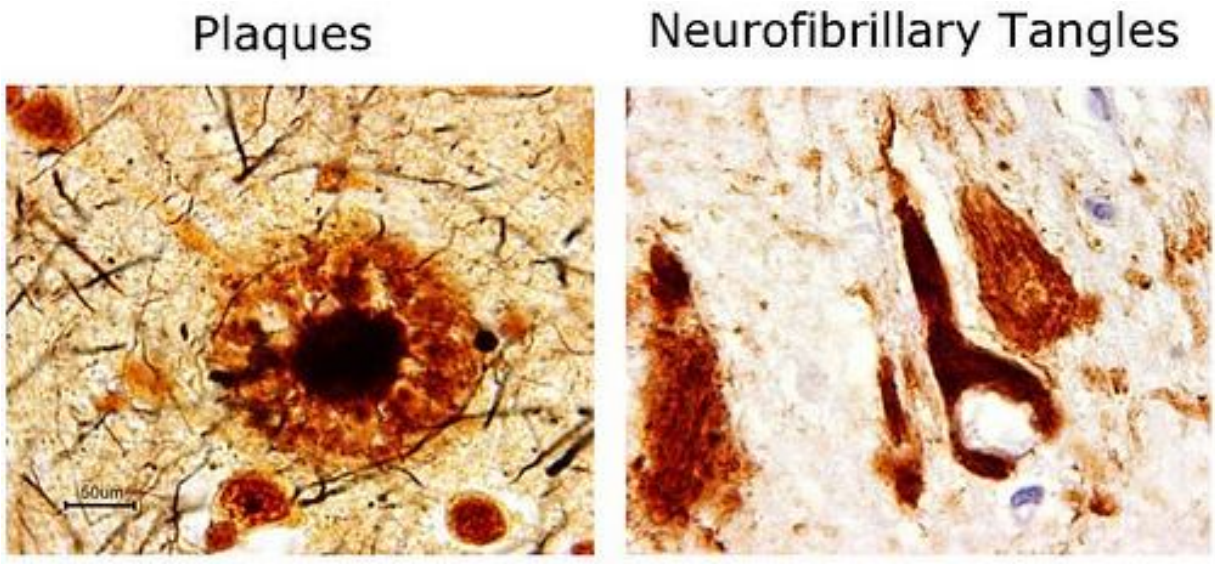


Figure no. 1.4: Plaques and Neurofibrillary tangles in brain.

Amyloid Plaques:

One of the hallmarks of Alzheimer's disease is the accumulation of **amyloid plaques** between nerve cells (neurons) in the brain. **Amyloid** is a general term for protein fragments that the body produces normally. **Beta amyloid** is a protein fragment snipped from an amyloid precursor protein (APP). In a healthy brain, these protein fragments are broken down and eliminated. In Alzheimer's disease, the fragments accumulate to form hard, insoluble plaques.

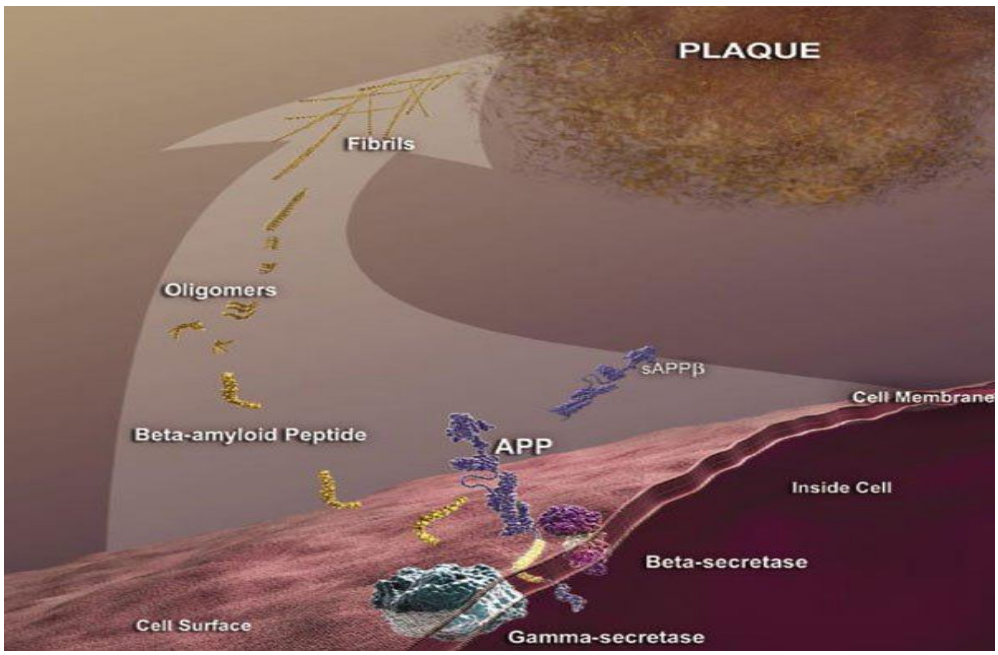


Figure no. 1.5: Amyloid plaques in brain.

Neurofibrillary Tangles:

Neurofibrillary tangles are insoluble twisted fibers found inside the brain's cells. These tangles consist primarily of a protein called **tau**, which forms part of a structure called a microtubule. The microtubule helps transport nutrients and other important substances from one part of the nerve cell to another. In Alzheimer's disease, however, the **tau** protein is abnormal and the microtubule structures collapse.

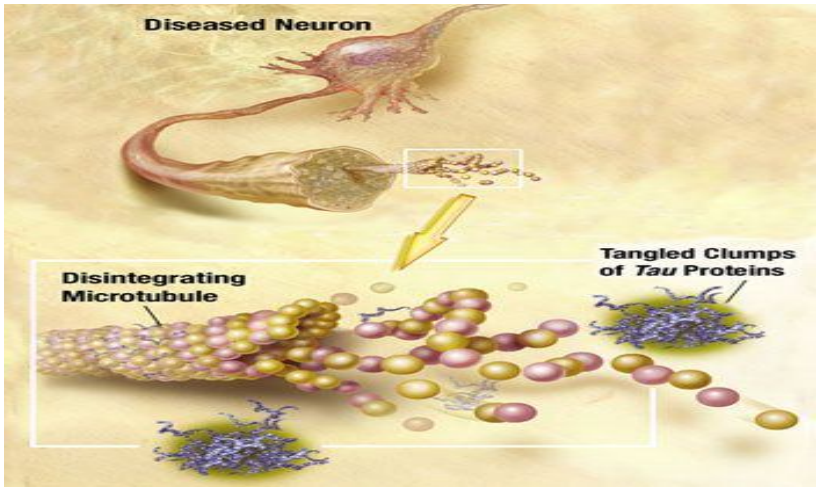


Figure no. 1.6 : Neurofibrillary tangles.

1.15 Changes in the brain during AD:

One of the most consistent changes in Alzheimer's disease is a reduction of the activity of choline acetyltransferase in the cerebral cortex and hippocampus. 3 Selective loss of cholinergic neurons occurs in the cholinergic projection pathway from deep nuclei located in the septum near the diagonal band of Broca to the hippocampus, and from the nearby basal nucleus of Meynert, which provides the major cholinergic input to the neocortex and to the cerebral cortex The basal nucleus of Meynert undergoes profound neuron loss in Alzheimer's disease. The neocortex exhibits a loss of cholinergic fibers and receptors and a decrease of both choline acetyltransferase and acetylcholinesterase enzyme activity. Reductions also occur in the corticotropin-releasing factor and somatostatin, both of which have been identified within degenerating neurites of the neuritic plaque. Glutaminergic neurons are also involved, which account for many of the large neurons lost in the cerebral cortex and hippocampus in Alzheimer's disease.

1.16 Different Hypothesis for the Pathogenesis of Alzheimer's disease:

The pathogenesis of Alzheimer's disease is highly complex. While several pathologies characterize this disease, amyloid plaques, composed of the β-amyloid peptide are hallmark neuropathological lesions in Alzheimer's disease brain. Indeed, a wealth of evidence suggests that β-amyloid is central to the pathophysiology of AD and is likely to play an early role in this intractable neurodegenerative disorder. Many molecular lesions have been detected in Alzheimer's disease, but the overarching theme to emerge from the data is that an accumulation of misfolded proteins in the aging brain results in oxidative and inflammatory damage, which in turn leads to energy failure and synaptic dysfunction. There are several hypothesis are established for the pathogenesis of AD. They are:

a. β-Amyloid cascade hypothesis,
b. Cholinergic hypothesis,
c. Oxidative stress hypothesis,
d. Tau hypothesis,
e. Inflammatory hypothesis.

β-Amyloid Cascade Hypothesis:

Generation of Aβ from APP requires two proteolytic processes by several different proteases.The amyloidogenic pathway results in production of intact Aβ, whereas the non-amyloidogenic pathway precludes intact Aβ-formation. In the amyloidogenic pathway, APP is first cleaved at the β-secratase site by β-site cleaving enzyme (BACE), generating a soluble extracellular fragment of APP (sAPPβ) and a membrane-bound 99 amino acid residue C-terminal fragment

(C99). C99 is further processed by cleavage at the γ-secretase site, located within the APP trans-membrane domain, to generate Aβ-peptides of different lengths of the two main variants formed; Aβ1-40 is the most abundant whereas Aβ1-42 is produced to a lesser. The amyloid cascade hypothesis has been one of the leading theories in the pathogenic events causing AD. This hypothesis postulates a causative role for Aβ deposition in the pathogenesis of AD, and the NFTs, inflammatory response, cell loss, vascular damage and dementia follow as a direct result of this deposition. Evidence that Aβ play a casual role in the development of AD came from the following studies. First, mutations in APP, PS1 and PS2 genes that are linked to the early onset forms of AD increase the production of total Aβ or specifically increase the relative amount of Aβ1-42. Second, individuals with Down's syndrome overproduce Aβ and develop AD-like dementia and neuropathology. Third, the levels of deposited Aβ correlate with cognitive decline and severity of the disease in both AD patients and transgenic mice. Fourth, fibrillar Aβ has been shown to be neurotoxic *in vitro* and is able to mediate neurotoxic effects, inflammatory responses and abnormal tau phosphorylation. Moreover, several reports suggest a casual link between Aβ and impaired neuronal function and cognitive decline. Recently, attention has been focused on which form (monomers, oligomers, protofibrils or fibrils) of amyloid species has the most deleterious effects. Several studies have suggested that the oligomeric and protofibrillar forms of amyloid as the most toxic. Even if the amyloid cascade hypothesis is convincing, it does not fully explain the role of tangles and/or inflammatory response. One argument against the amyloid cascade hypothesis is that the APP/PSI double transgenic mice only develop plaques pathology, but not the NFTs in the brain. Recently, amyloid deposition was shown to precede tangle formation in a triple transgenic mouse model over expressing mutant APP and mutant tau on a PSI mutation Knock-in background.

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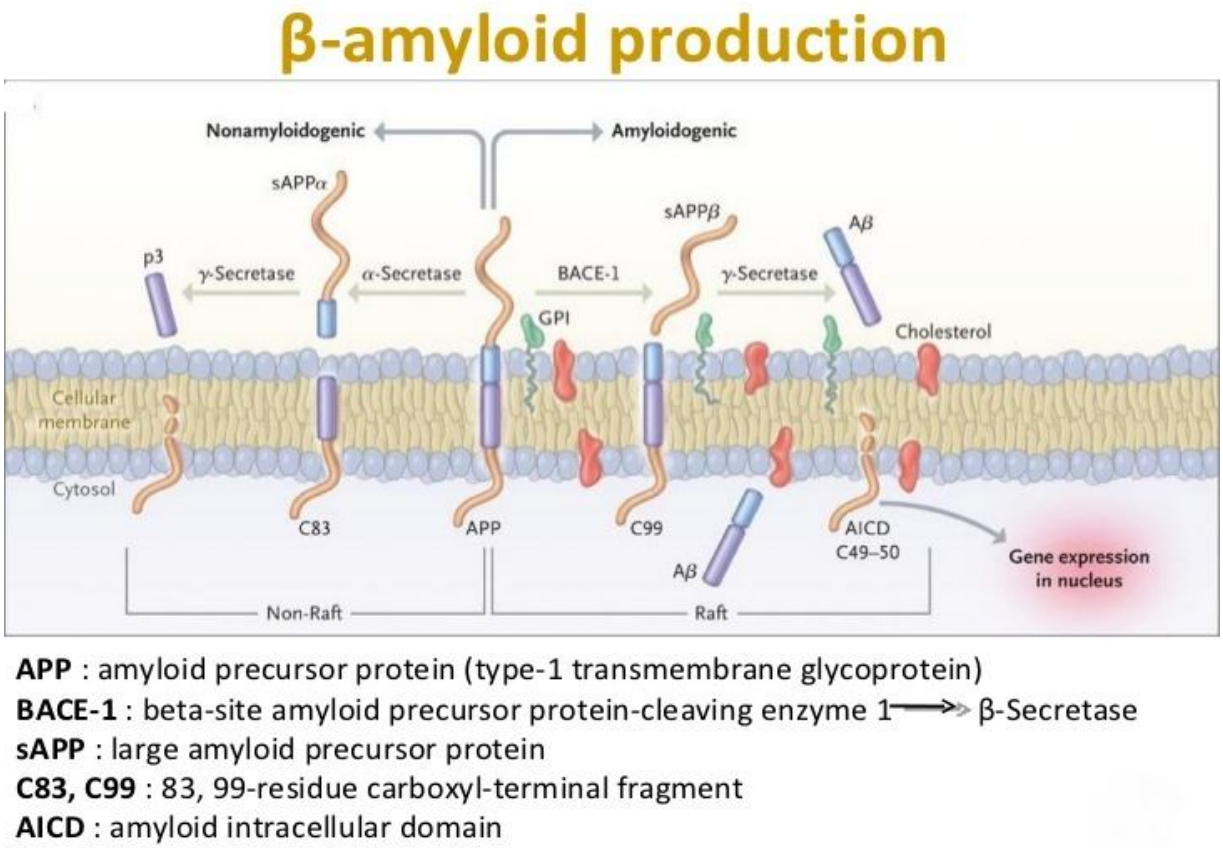


Figure No. 1.7 : Production of beta amyloid in brain

Cholinergic hypothesis:

Acetylcholine is an important neurotransmitter in brain regions involving memory. As expected, loss of cholinergic activity corrrelates with cognitive impairment. A variety of studies in humans indicate that basal forebrain and rostral forebrain cholinergic pathways including converging projections to the thalamus serve important functional roles in conscious awareness, attention, working memory and a number of additional mnemonic processes.

For more than 20 years, studies of the brains of those with advanced age and Alzheimer's disease (AD) have consistently found damage or abnormalities in these pathways (particularly basal forebrain projections) that appeared to correlate well with the level of cognitive decline. As a result, the so-called "cholinergic hypothesis" was developed, which essentially states that a loss of cholinergic function in the central nervous system contributes significantly to the cognitive decline associated with advanced age and AD. In AD, cholinergic abnormalities are the most prominent of neurotransmitter changes, primarily because of the reduced activity of choline acetyltransferase (an enzyme involved in acetylcholine synthesis). By late-stage AD, the number

of cholinergic neurons is markedly reduced, particularly in the basal forebrain. One of the most prominent features observed in AD patients is a deficiency of acetylcholine (ACh), a neurotransmitter found in the synapses of the cerebral cortex. The cholinesterase enzyme exists in two different forms in humans, AChE andbutyrylcholinesterase (BuChE). AChE is the main cholinesterase in the CNS, while BuChE, originated from glial cells, is more common in serum. Decreased AChE activity and stable or increased BuChE activities were detected in the brains of AD. Most of the neocortical AChE activity in AD brain was found associated with NPs, in which it colocalized with Aβ deposits including both the diffuse amyloid deposits and the mature NPs. Further studies showed that AChE promoted the aggregation of Aβ peptides and accelerated the formation of amyloid plaque, suggesting that AChE may play a pathogenic role in AD by influencing Aβ processing. It has also been shown that Aβ which aggregates with AChE is more toxic to cells compared to aggregates of Aβ alone.

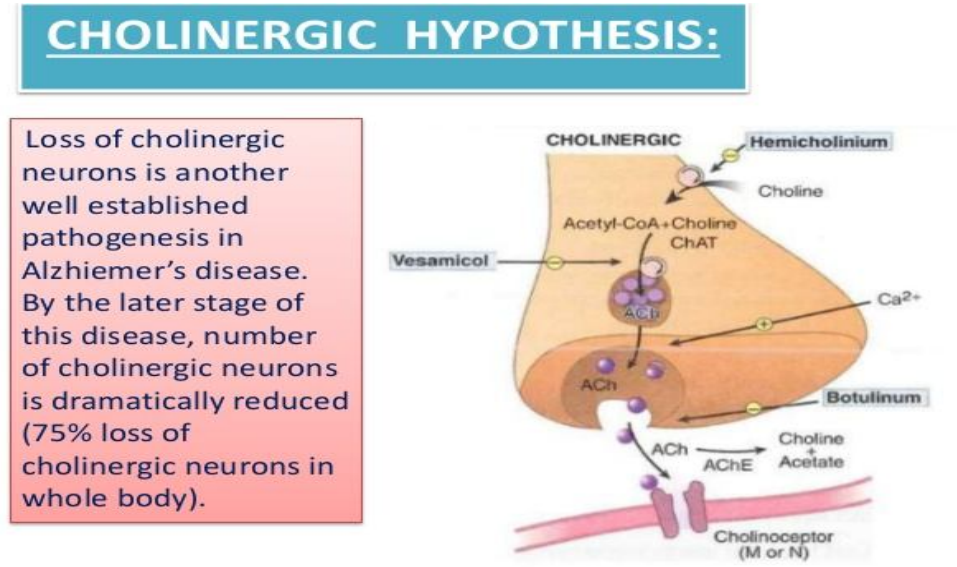


Figure no. 1.8 : Oxidative stress

Oxidative stress hypothesis:

Oxidation can include the combination of a substance with oxygen or free radical damage. The oxidative process of adding an oxygen molecule to a protein can be a normal part of cellular function or may be aberrant, with a resulting change in the protein's form and ability to function properly. Free radical damage occurs when an oxygen or nitrogen molecule containing an unpaired extra electron (termed species) reacts with other molecules to achieve a stable configuration. During this process a high-energy election is thrown off (termed a free radical) that can cause cellular and molecular damage. Either of these oxidative processes can cause oxidative stress with resulting cellular damage from oxidize. The central nervous system (CNS)

is especially vulnerable to free radical damage as a result of the brain's high oxygen consumption rate, its abundant lipid content and the relative paucity of antioxidant enzymes compared with other tissues. AD brain is under extensive oxidative stress as manifested by lipid peroxidation, protein oxidation and DNA oxidation. Aβ might be the central to the pathogenesis of AD. Aβ has been shown to induce protein oxidation and lipid peroxidation *in vitro* and *in vivo*. Many studies have indicated that Aβ-induced oxidative stress is involved in the pathogenesis of AD.

Lipid peroxidation is an important mechanism of neurodegeneration in AD brain. Many studies have shown increased lipid peroxidation in several regions of AD brain, where the histopathologic alterations are very noticeable.[43] It has been shown that there is a strong regional correlation between the thiobarbituric acid reactive substances (TBARS), one indicator of lipid peroxidation, antioxidant enzymes, the presence of NPs and NFT in AD brain. Aβ is widely reported to cause lipid peroxidation in brain cell membranes in a manner that is inhibited by free radical antioxidants.

Aβ leads to an increased level of 4-hydroxy-2-nonenal (4-FINE), one of the major products of lipid peroxidation, in hippocampal and cortical neuronal cells. Increased 4-FINE was found in AD brain and it was proven to be toxic to hippocampal neuronal cells. 4-HNE can also increase the vulnerability of cultured hippocampal neurons to excitotoxicity, as well as an alteration in multiple cellular functions including glucose or glutamate transport.

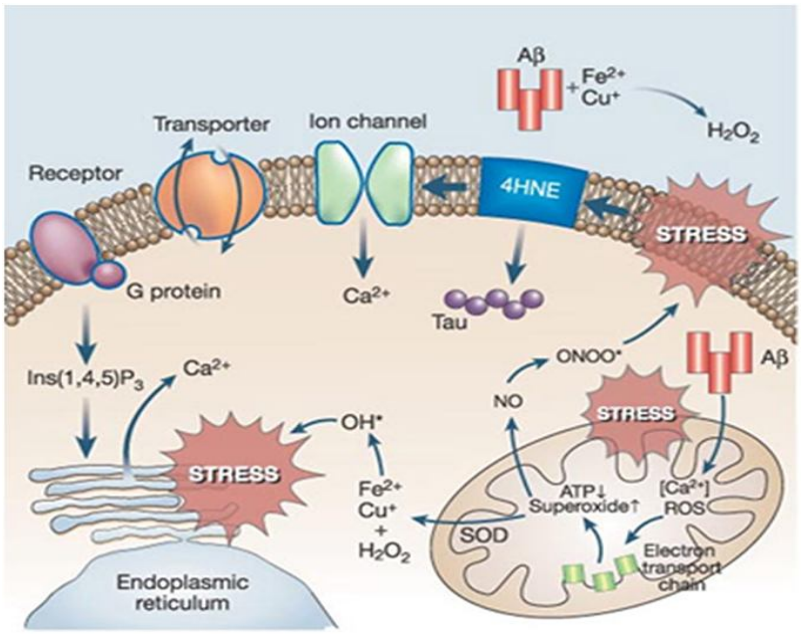
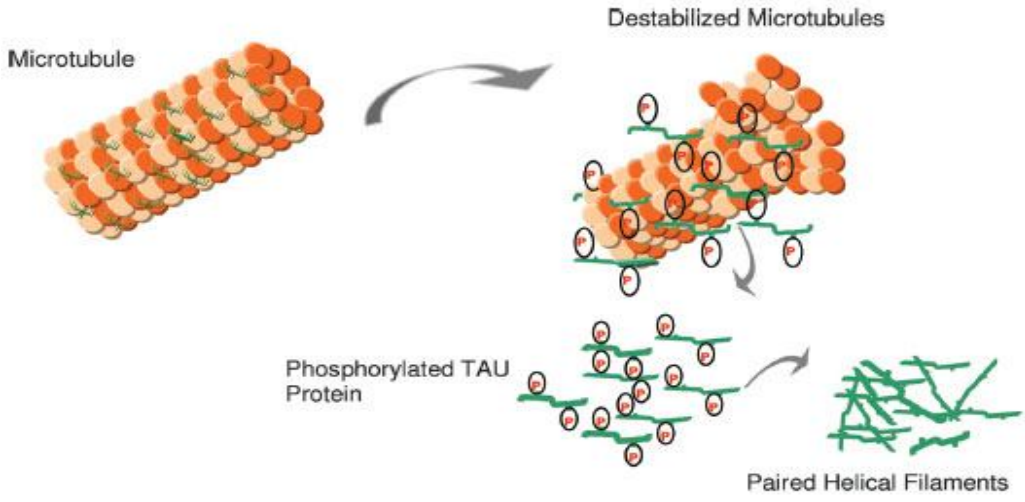


Figure no. 1.9: Oxidative Stress hypothesis in Alzheimer's disease.

Tau Hypothesis:

Neurofibrillary tangles, which are filamentous inclusions in pyramidal neurons, occur in Alzheimer's disease and other neurodegenerative disorders termed tau hypothesis.

The number of neurofibrillary tangles is a pathologic marker of the severity of Alzheimer's disease. The major component of the tangles is an abnormally hyperphosphorylated and aggregated form of tau. Normally an abundant soluble protein in axons, tau promotes assembly and stability of microtubules and vesicle transport. Hyperphosphorylated tau is insoluble, lacks affinity for microtubules and self-associates into paired helical filament structures. Enzymes that add and those that remove phosphate residues regulate the extent of tau phosphorylation. Like Aβ oligomers, intermediate aggregates of abnormal tau molecules are cytotoxic and impair cognition. Insoluble helical filaments may be inert, however, since decreases in axonal transport and neuron number are independent of the burden of neurofibrillary tangles. These helical filaments sequester toxic intermediate tau species, a process that may be protective. More than 30 mutations of *Tau* on chromosome 17 have been detected in front temporal dementia with parkinsonism. By contrast, *Tau* mutations do not occur in Alzheimer's disease, and the extent of neuron loss is out of proportion to the number of neurofibrillary tangles. Nevertheless, increased levels of phosphorylated and total tau in the cerebrospinal fluid correlate with reductions in scores on cognitive examinations. Elevated levels of phosphotau amino acids T181, T231, and total tau in the cerebrospinal fluid together constitute a biomarker test with good accuracy for predicting incipient Alzheimer's disease in patients with mild cognitive impairment. Experimental evidence indicates that Aβ accumulation precedes and drives tau aggregation. Moreover, Aβ-induced degeneration of cultured neurons and cognitive deficits in mice with an Alzheimer's disease–like illness require the presence of endogenous tau.



. **Figure no.1.10** : Tau hypothesis in Alzheimer's disease

Increased oxidative stress, the impaired protein-folding function of the endoplasmic reticulum and deficient proteasome-mediated and autophagic- mediated clearance of damaged proteins-

a. All of which are also associated with aging,
b. Accelerate the accumulation of amyloid and tau proteins in Alzheimer's disease.

Inflammatory Hypothesis:

Neurons are not the only brain cells affected in AD. Microglia, 1 of 3 glial cell types (along with astrocytes and oligodendrocytes) in the CNS, is involved in immune and inflammatory responses to injury or infection within the brain. Several lines of evidence have proposed that inflammation may play a significant role in the pathogenesis of AD. According to the "inflammation hypothesis", the deposition of amyloid in AD brain brings about activation of microglia and astrocytes, initiating a pro-inflammatory cascade that results in the release of potentially neurotoxic substances, cytokines and other related compounds, bringing about degenerative changes in neurons. Reactive astrocytes and activated microglia are often found in and around amyloid plaques in the brains of AD patients and APP transgenic mice.

Other hypotheses:

Herpes simplex virus type 1 has been proposed to play a causative role in people carrying the susceptible versions of the apoE gene. Some have hypothesized that dietary copper may play a causal role.

Another hypothesis asserts that the disease may be caused by age related myelin breakdown in the brain. Iron released during myelin breakdown is hypothesised to cause further damage. Homeostatic myelin repair processes contribute to the development of proteinaceous deposits such as beta-amyloid and tau.

1.17 Risk factors of Alzheimer's disease:

Although a great deal of research has been done and is currently being done on the possible causes of Alzheimer's, experts are still not sure why the brain cells deteriorate. However, there are several factors which are known to be linked to a higher risk of developing the disease. These include:

Genetic Etiology of AD:

a. APP:

So far there are sixteen known mutations within the vicinity of the β amyloid (Aβ) region on the APP gene. These medications include the AP 670/671 double Swedish mutation, the APP692 Flemish mutation, the APP 717 London mutation and the APP693 Arctic mutation. Due to their strategic localization at the enzymatic cleavage sites of the β and δ secretases, all known APP mutations alter APP metabolism and subsequently lead to an accumulation of Aβ, which forms the major components of AD amyloid plaque deposits.

b. Presenilin 1 and 2:

The vast majority of all known familiar AD mutations have been found in the gene encoding PS1. To date, 146 mutations in PS1 on chromosome 14 have been reported to cause autosomal dominant AD in several hundred families worldwide. In contrast to PS1, very few mutations leading to AD have been found in the PS2 gene, with a later age of onset than PS1 mutation carriers. Mutations in both of these genes lead to the increased production of the more fibrillogenic Aβ-1-42 by selectively increasing γ-secretase cleavage of APP. PS1 mutation causes the earliest and most aggressive forms of AD, commonly leading to a clinical onset of AD before the age of 50.

c. APOE:

APOE is a lipoprotein that plays a central role in lipoprotein metabolism and cholesterol homeostasis. APOE has three different isoforms E_2, E_3 and E_4 encoded by the three alleles of varying frequency: APOE ε-2 7-8%, ε-3 77-78%, ε-4 14-16%. APOE ε-4 is identified as a susceptibility gene for late onset AD. The frequency of the APOE ε-4 allele is increased in AD patients and a dose dependent risk for developing AD is observed with one or two copies of the ε-4 allele. The mechanisms by which APOE ε-4 allele contributes to AD pathogenesis is somewhat unclear. Several studies from knockout and transgenic mice suggest that APOE ε-4 facilitates the aggregation and deposition of Aβ.

Non-genetic Etiology of AD:

a. Age:

After the age of 65 the risk of developing Alzheimer's doubles every five years. Although Alzheimer's is predominantly a disease that develops during old age, some younger people may also develop the condition.

b. Type II Diabetes:

There is growing evidence of a link between Alzheimer's disease and type II diabetes. In type II diabetes insulin does not work effectively to convert blood sugar into energy. This inefficiency results in production of higher levels of insulin and blood sugar which may harm the brain and contribute to the progression of Alzheimer's disease.

c. Oxidative Damage:

Free radicals are unstable molecules that sometimes result from chemical reactions within cells. These molecules seek stability by attacking other molecules, which can harm cells and tissue and may contribute to the neuronal brain cell damage caused by Alzheimer's.

d. Inflammation:

Inflammation is a natural, but sometimes harmful, healing bodily function in which immune cells rid themselves of dead cells and other waste products. As protein plaques develop, inflammation results, but it is not known whether this process is damaging and a cause of Alzheimer's, or part of an immune response attempting to contain the disease.

e. Family history:

People who have a close family member who developed Alzheimer's have a slightly higher risk of developing it themselves - just a slightly higher risk, not a significantly higher risk. Only about 7% of all cases are associated with genes that cause the early onset inherited familial form of the disease. Among those who do inherit the condition, it may start at an earlier age.

f. Down's syndrome:

People with Down's syndrome have an extra copy of chromosome 21, which contains a protein that exists in the brain of people with Alzheimer's. As people with Down's syndrome have a larger amount of this protein than others, their risk of developing the disease is greater.

g. Whiplash and head injuries:

Some studies have identified a link between whiplash and head injuries and a higher risk of developing Alzheimer's.

h. Aluminum (UK/Ireland/Australia: Aluminium):

The link here is a theory which most scientists have discarded. Aluminum exists in the plaques and tangles in the brains of Alzheimer's patients. Some have suggested that aluminum absorption by humans could increase the risk. However, studies have failed to find a link. Aluminum exists

in some foods and plants. It is found in some cooking pans, medications and packaging. Scientists doubt there is a link because our bodies absorb minimum amounts and our bodies eliminate it through the urine.

i. Gender:

A higher percentage of women develop Alzheimer's than men. As women live longer than men and Alzheimer's risk grows with age, this may partly explain the reason.

j. Atrial fibrillation:

A study of more than 37,000 patients showed a strong relationship between atrial fibrillation and the development of Alzheimer's disease.

k. Heart disease risk-factors:

People with the risk factors of heart disease - high blood pressure (hypertension), high cholesterol and poorly controlled diabetes - also have a higher risk of developing Alzheimer's. If your high-blood pressure, high cholesterol and or poorly controlled diabetes type 2 is a result of lifestyle, it is called a lifestyle factor. Eating a well balanced diet, doing plenty of exercise, aiming for your ideal body weight and sleeping between 7 to 8 hours each night will probably eliminate these factors. If you cannot eliminate your diabetes II, good diabetes control will help. Sometimes these factors have nothing to do with lifestyle, i.e. if you have high blood pressure for another reason, have diabetes type I, or are susceptible to high blood cholesterol despite being the right weight, exercising, etc. good control and treatment of the condition helps minimize the risk of developing Alzheimer's (and heart disease).

l. Academic level:

There is some data showing a higher risk of developing Alzheimer's among people with lower educational qualifications, compared to highly qualified individuals. However, nobody really knows why.

m. Processed foods and fertilizers (nitrates):

A study carried out by researchers at Rhode Island Hospital found a significant link between increased levels of nitrates in our environment and food, with increased deaths from diseases, including Alzheimer's, diabetes and Parkinson's. The study looked at progressive increases in human exposure to nitrates, nitrites and nitrosamines through processed and preserved foods as well as fertilizers.

1.18 Vascular hypothesis:

Vascular risk factors, such as smoking, obesity and high total cholesterol levels, together with vascular morbidity, such as hypertension, diabetes mellitus and asymptomatic cerebral infarction, are associated with a higher risk of dementia including Alzheimer's disease.

a. Smoking:

Earlier cross-sectional studies often reported a lower prevalence of AD among smokers compared with nonsmokers.

b. Alcohol:

It is well recognized that alcohol abuse causes alcohol dementia. Moreover, middle-aged heavy drinkers, especially apoE4 allele carriers, were found to have a more than 3-fold higher risk of dementia and AD later in their lives. On the other hand, the risk of developing dementia and AD was Overweight and obesity Higher BMI in middle age is a risk factor for AD and other dementias.

c. Nutritional factors:

Several analytical studies showed a decreased risk of AD associated with higher intake of antioxidants such as vitamins E and C, either in the diet or in dietary supplements. However, some studies found a negative effect.

Hypercholesterolemia and statin therapy:

High total serum cholesterol levels in middle age were found to be a risk factor for the development of AD at a later age. High total cholesterol in middle age is a risk factor for the development of AD and other dementias 20 years later but decreasing serum cholesterol levels in late middle age may be due to ongoing disease processes and may represent a marker for later AD and other dementias.

According to several cross-sectional and case-control studies, the use of statins significantly decreases the prevalence of AD. Whereas an analytical study (the Rotterdam Study) showed that the use of statins was associated with a lower risk of AD, other prospective studies found either no beneficial effect or only a slightly decreased risk of AD related to the use of statins. Experimental studies have suggested that statins may decrease the production of beta amyloid both *in vitro* and *in vivo*. Statins also have various other effects that may be beneficial for the CNS and thus may lower the risk of AD.

d. Social network and social engagement:

Longitudinal observational studies suggest that a poor social network or a lack of social engagement is associated with decreased cognitive functions and dementia. The risk of dementia was also higher in elderly persons with increased social isolation and less frequent and unsatisfactory contacts with relatives and friends. Persons with low neuroticism combined with high extraversion had a lower risk of dementia. Low levels of social engagement in late life and decreased social engagement from middle to late life were associated with a two-fold increase in the risk of the development of dementia and AD later in life.

e. Physical activity:

Regular physical exercise is associated with a delay in onset of dementia and AD in the elderly without cognitive impairment. Physical activity in the form of various leisure activities rather than sports or specific physical exercise led to a decrease in the risk of dementia. Even low-intensity physical activity such as walking may lower the risk of dementia and cognitive impairment. A significant protective effect of regular physical activity in middle age, with respect to the development of dementia and AD later in the life, was found especially in persons with the apoE4 allele.

f. Mental activity:

Various activities requiring a mental effort, such as reading, social and cultural activities, knitting, gardening, dancing, tabletop games, playing musical instruments, watching specific TV programs, showed a protective effect against dementia and AD. A study of Swedish twins showed that complexity of work, in particular more complex work with people, may reduce the risk of AD. A recent neuroimaging study suggested that a high level of complex mental activity across the lifespan was correlated with reduced hippocampal atrophy.

In October 2012, researchers from Drexel University College of Medicine in Philadelphia reported in *PLOS ONE* that they discovered a natural anti-cancer mechanism in the human body that may encourage the development of Alzheimer's disease.

1.19 Treatment Strategies of AD:

There is currently no cure for AD, however there are multiple drugs that have been proven to slow disease progression and treat symptoms. When initiating treatment for AD patients, physicians divide the symptoms into “cognitive” and “behavioral and psychiatric” categories. The treatment strategies are given below:

1.19.1 Cholinesterase inhibitors:

- Prevent the breakdown of acetylcholine (a-SEA-til-KOH-lean), a chemical messenger important for learning and memory. This supports communication among nerve cells by keeping acetylcholine levels high.
- Delay worsening of symptoms for 6 to 12 months, on average, for about half the people who take them.
- Are generally well tolerated. If side effects occur, they commonly include nausea, vomiting, loss of appetite and increased frequency of bowel movements.

The cholinesterase inhibitors are commonly prescribed:

a. Donepezil (Aricept) is approved to treat all stages of Alzheimer's.
b. Rivastigmine (Exelon) is approved to treat mild to moderate Alzheimer's.
c. Galantamine (Razadyne) is approved to treat mild to moderate Alzheimer's.
d. Tacrine (Cognex) was the first cholinesterase inhibitor approved.

There are currently three cholinesterase inhibitors commonly prescribed: tacrine, donepezil, galantamine, and rivastigmine.

a. **Donepezil:**

Donepezil is a piperidine derivative that reversibly inhibits acetylcholinesterase. It is often regarded as providing only symptomatic relief without providing neuroprotective effects. However, *in vitro* studies show that donepezil offers neuroprotection by reducing glutamate excitotoxicity, diminishing βA toxicity and consequently increasing cell longevity. It slowed atrophy of the hippocampus in humans, which suggest a neuroprotective effect. It is very well absorbed after oral administration and reaches peak plasmatic concentration (C_{max}) in 3-4 hours. Elimination half-life of donepezil is approximately 70 hours allowing once daily administration. It binds to plasma proteins in a proportion of 96% and is metabolized by isoenzyme 2D6 and 3A4 of cytochrome P450. Starting and minimal effective dose is 5 mg once daily. Maximal recommended dose is 10 mg daily. Overall, both the doses of 5 mg and 10 mg were beneficial, with the higher dose being marginally more effective. More side effects were reported with donepezil than with placebo. Most common side-effects were nausea, vomiting, diarrhoea, muscle cramps, dizziness, fatigue and anorexia and they were dose-dependent.

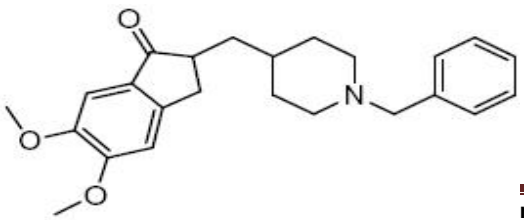


Figure no. 1.11: Donepezil (Chemical Structure).

b. **Galantamine:**

Galantamine is a tertiary alkaloid agent that reversibly inhibits AChE. Galantamine, a natural AChEI (originally derived from the common snowdrop and other plants, but now synthesized), protects neurons and reduced cell death by modulating nicotinic receptors, which are significantly reduced in AD brains. In an animal model, galantamine also increased dopaminergic neurotransmission in the hippocampus, a brain area particularly important in memory.

Galantamine is rapidly absorbed after oral administration and reaches C_{max} in approximately 1 hour. Elimination half-life is between 7 to 8 hours. It binds to plasma proteins in a proportion of 18% and is metabolized by isoenzyme 2D6 and 3A4 of cytochrome P_{450}. Galantamine is commercialized as an extended-release formulation allowing once-daily dosing. Starting dose of galantamine ER is 8 mg once daily. Minimal effective dose is 16 mg daily and maximal dose is 24 mg daily. Galantamine's side effects are comparable to other ChEI's and consist mainly of cholinergic gastrointestinal symptoms.

Figure no. *1.12:* Galantamine (Chemical structure).

c. **Rivastigmine:**

Rivastigmine is a carbamate derivative that reversibly inhibits both acetyl- (AChE) and butyryl- (BuChE) cholinesterase. It is the only ChEI with significant inhibition of BuChE. Butyrylcholinesterase is widely distributed in the central nervous system and may play a role in cholinergic function and neurodegeneration. It is unclear how specific BuChE inhibition relates to rivastigmine's clinical effect.

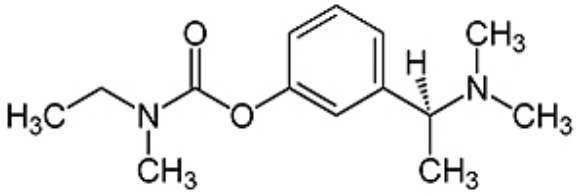


Figure no. 1.13: Rivastigmine (Chemical structure).

Rivastigmine is well absorbed after oral administration and reaches C_{max} in one hour. Its elimination half-life is approximately 1 to 2 hours. It binds to proteins in a proportion of 40%, is hydrolysed by esterases (including cholinesterases) and is excreted in the urine. Cytochrome P_{450} isoenzymes are not involved in the metabolism of rivastigmine hence minimizing drug-drug interactions. Starting dose of rivastigmine is 1.5 mg twice a day and can be gradually titrated to the maximal dose of 6 mg twice a day. The minimal effective dose is 3mg twice a day. A transdermal form of rivastigmine has been developed and is available on most markets since 2008. The main objective of transdermal rivastigmine is to allow titration to the highest (and most therapeutic) doses of the medication while minimizing side effects. This is achieved by slow release of the medication into the circulation as demonstrated by a C_{max} of 8 hours by transdermal route. Starting dose of transdermal rivastigmine is 5 cm^2 and the effective and maximal dose is 10 cm^2. More side-effects were reported with rivastigmine than with placebo and they were dose-dependent. Most common side effects were nausea, vomiting, diarrhoea, anorexia, headache, syncope, abdominal pain and dizziness.

d. **NMDA antagonist (Memantine):**

Memantine is thought to reduce cell damage by reducing excitotoxicity resulting from over activation of NMDA glutamate receptor during synaptic transmission.

In addition to cholinesterase inhibitors, memantine has also been approved for the treatment of AD. Memantine regulates the activity of glutamate in the brain. Glutamate is an excitatory neurotransmitter involved in learning and memory. Over stimulation of nerves by glutamate may be the cause of the neuron degeneration seen in AD, called Bethune 16 excitotoxicity. Glutamate binds to N-methyl-D-aspartate (NMDA) receptors on the surface of brain cells. Memantine functions by blocking the NMDA receptors and therefore protecting the nerves from excessive glutamate stimulation.

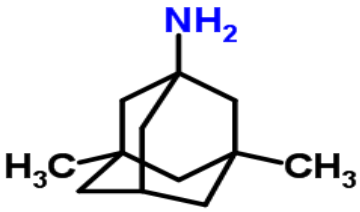


Figure no. 1.14: Memantine (chemical structure)

Memantine is indicated in the treatment of moderate to severe AD and can temporarily delay worsening of cognitive symptoms. It is well absorbed after oral administration and reaches C_{max} in 3 to 8 hours. Elimination half-life is 60-80 hours. It binds to proteins in a proportion of 45% and is almost completely excreted unchanged in the urine. Starting dose is 5 mg daily (in one or two doses). Minimal therapeutic dose is 10 mg daily and maximal dose is 20 mg daily. NMDA antagonists, such as memantine, have generally been regarded as neuroprotective, but they have also demonstrated neurotoxic properties that diminish memory, incite neuron death and even produce psychotic episodes in humans.

1.19.2 Antihypertensive drugs:

Antihypertensive drugs have potential for AD therapy. ACE inhibitors reduce inflammation and mental decline in AD patients by 50%. Mild to moderate AD subjects with high blood pressure had less cognitive decline when given an ACE inhibitor that cross the BBB than when given an ACE inhibitor that did not or a Calcium channel blocker.

A recent study has confirmed that ACE inhibitors slow the progression of AD. A potential downside of ACE inhibitors is that they may block ACE from converting βA_{1-42} to less damaging βA_{1-40}, thereby reducing its protective function.

Possible mechanism by which ACE inhibitors work, include reducing angiotensin II, increasing an enzyme that break βA and increasing acetylcholine.

Calcium channel blockers are another category of antihypertensive drugs. It may be that βA, mutation in presenilin proteins or other factors open channels that permit Ca^{2+} to enter the damage cell.

1.19.3 Anti-inflammatory drugs:

Most research on NSAIDs has focused on prevention rather than treatment of AD. One study has examined 49,349 NSAID user for five years found the risk of AD was clearly reduced by ibuprofen and less so by indomethacin, while salicylate provides no protection.

1.19.4 Secretase inhibitors:

Secretase are the enzymes that break the APP, found in cell membranes, into βA fragments that form plaques. Secretase inhibitors should slow the production of βA. A gamma-secretase inhibitor has been shown to reduce plasma βA about 60 percentin a small 14 week study of mild to moderate AD patients.

B-secretase inhibitors have been shown to reduce βA in animal models and may have fewer adverse effects. Memoquin is a β-secretase inhibitor that also inhibits AChE, reduces βA production, limits tau hyperphosphorylation and fights oxidation, but it is in the early developmental stage.

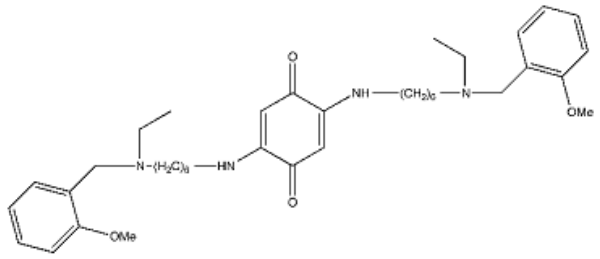


Figure no. 1.15: Memoquin (Chemical Structutre).

Insulin:

Insulin has many roles in normal cell function. Nasal administration of insulin improved several cognitive measures in subjects with early AD or mild cognitive impairment. Insulin resistance can affect the brain as well as other organs, making it difficult for the brain cells to acquire energy for cell maintenance and synaptic connections, thus cell death can occur. Also hyperinsulinemia has been found to increase inflammation and βA_{1-42} in healthy adults.

Etanercept:

Etanercept has recently generated interest because it produced dramatic cognitive improvement. AD brain have elevated lavels of the cytokines TNF-α. Since TNF-α regulates neural transmission, lowering by spinal injections of etenercept might restore the brain to more normal

functioning. A dramatic cognitive imprvement was evedenced in one moderate to severe AD subject within minutes.

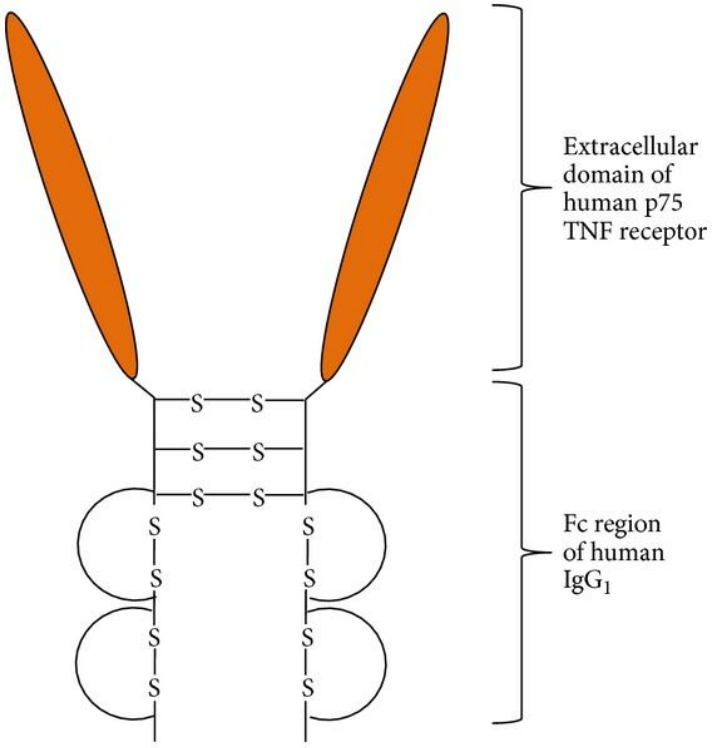


Figure no. *1.16.* Structure of Etanercept.

1.19.5 Brain Derived Neurotrophic factor (BDNF):

BDNF is a protein produced in the brain that help existing neuron survive, facilitate the growth of new neuron and synaps and reverses neuronal atrophy and behavior deficits; intracellular signalling is also facilitated. BDNF is active in the hippocampusand cortex. It stimulates neurogemesis. BDNF level decline with age and are lower in the AD brains than in whose without AD.

1.19.6 Immunization:

βA has been reduced by injecting AD patients with a synthetic form of βZ called AN1792. Although this reduces βA, the effect on AD is unclear. Some people respond to immunization without a showing of disease progression even after 4.6 years.

1.19.7 Antipsycotics and sedatives:

Antipsycotics and sedatives have accelarated the progression of AD, defines as an increase of one or more points in the Global deterioration Scale, and produced a 50% decrease in cortical plasticity in cats. Thus care should be exercrised in using such drugs for AD patients.

1.19.8 Flavanoids and other novel plant constituents:

a. HuperzineA (HupA):

HupA is an extract from the chinese moss Huperzia serrata that has been used for centuries in Chinese folk medicine to treat wide range of diseases.

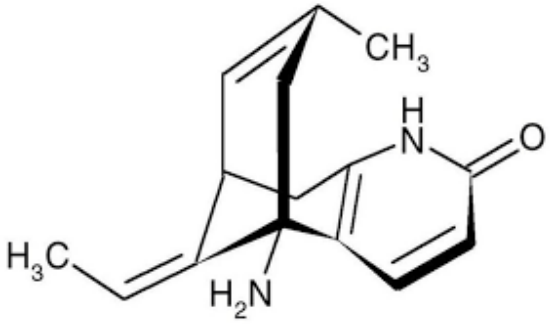


Figure no. 1.17: Huperzine (Chemical Structure)

A review of in vitro studies found HupA preserves Ach longer than Tacrine, Galantamine or Donepezil. HupA reduces βA induced neuronal degenaration in the hippocampus and the cortex, decrease oxidative damage from free radical induced βA plaque, protects neuron from cytotoxins and apoptosis induced by βA and free radicals and inhibit glutamate toxicity.

b. Polyphenols:

Polyphenols are group of plant derived chemical compunds with more than one phenol units. They protect the plant from stress induced by ultraviolet radiation, disease, pests and physical damage. Polyphenols also protect animals by activating a number of intracellular processes that preserve neurons.

c. Curcumin:

Curcumin is extracted from the plant Curcuma longa (turmeric). It may provide promising therapy for AD because it has at least 10 neuroprotective properties including anti-inflammatory,

antioxidant, inhibition of βA formation, clearance of βA formation and copper and iron chelation.

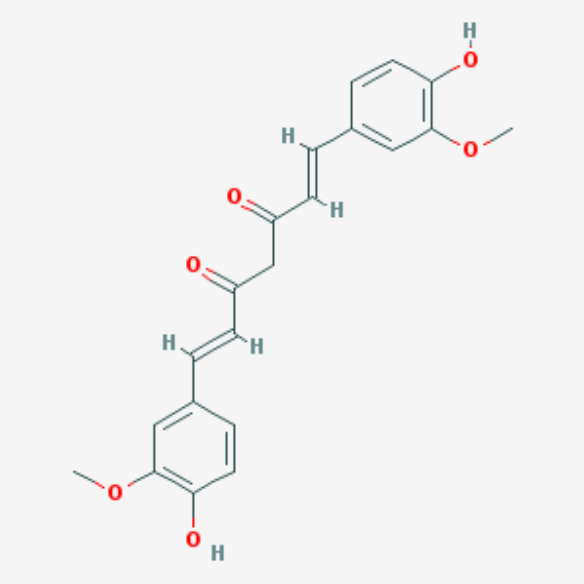


Figure no. 1.18: Curcamine (Chemical Structure)

d. Reveratrol:

Reveratrol is a polyphenol found in red wine, peanuts and other plants which reduce oxidative stress, decrease inflammation, reduce βA formation, protect DNA, decrease cell death and modulates various other systemsthat protect cells. Animal models suggest that reveratrol mimics the effect of caloric restriction on longivity and negates the harmful effects of high fat diet, doubles resestence to muscle fatigue, reduce neurotoxicity, decrease cell death, decrease degeneration of the hippocampus and prevents learning impairments. Several studies has shown thatmoderate consumption of red wine reduces the risk of developing AD.

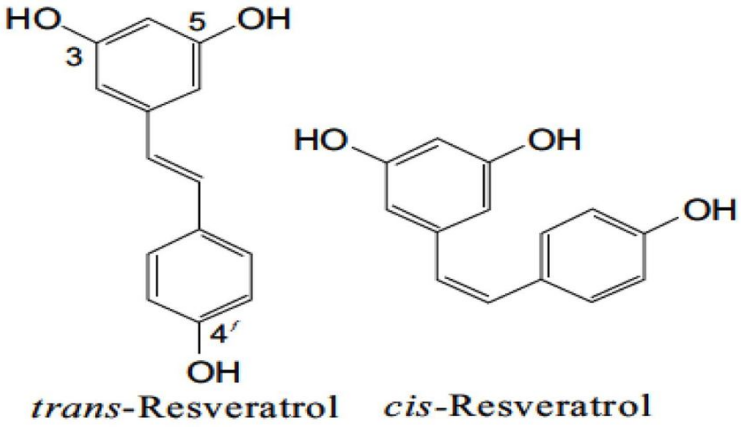


Figure no. 1.19: Reveratrol (Chemical structure)

e. Tacrine (Cognex):

Tacrine was the first cholinesterase inhibitor approved. Doctors rarely prescribe it today because it's associated with more serious side effects than the other three drugs in this class.

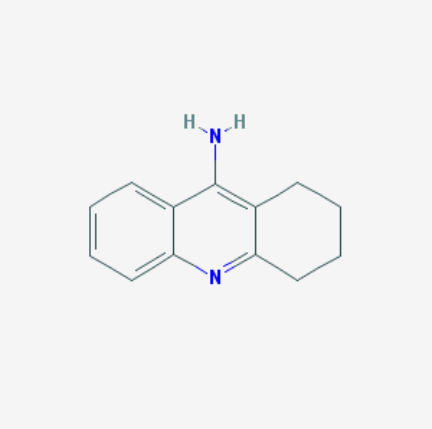


Figure no. 1.20: Tacrine (Chemical structure).

1.19.9 Herbal suplement:

a. Gingko Biloba:

Gingko Biloba contains compounds that have antioxidant and antiinflammatory properties that protects neuron membrane, regulate neurotransmitters and retard cell degeneration. Gingko Biloba extract contains RGb 761that reduce βA and cell death.

b. Panax ginseng:

Panax ginseng has been studied for its effect of cognition. The active componant in ginseng are thought to be steroid like compounds called ginsenosides. Ginsenosides Rg3 reduced βA_{1-42} by 84% invitro and 31% invivo.

c. Withania sominifera:

Withania sominifera, a small evergreen hrub commonly called ashwagandha has been used to treat many diseases. A recent study have been shown that it have many neuroprotective properties including anti-inflammatory, antioxidant, inhibition of βA, inhibition of Calcium, inhibition of AChE and reduction of cell death. In vitro research demonstrates that ir regenate damaged axons, dendrites and synaps.

1.19.10 Nutrients:

a. Posphatidylserine:

Posphatidylserine is important in neurotransmission, mitochondrial function and cell metabolism. It has also been implecated in the enhancement of nerve growth factor. Recent research demonstrate that Posphatidylserine increases Ach and provides neuroprotection by inhibiting βA and inflammation.

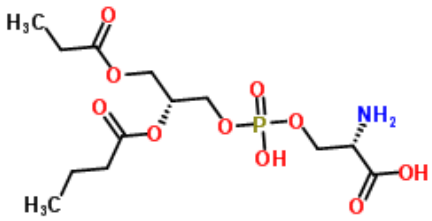


Figure no. 1.21: Posphaidylserin

b. Alpha-Lipoic acid (ALA):

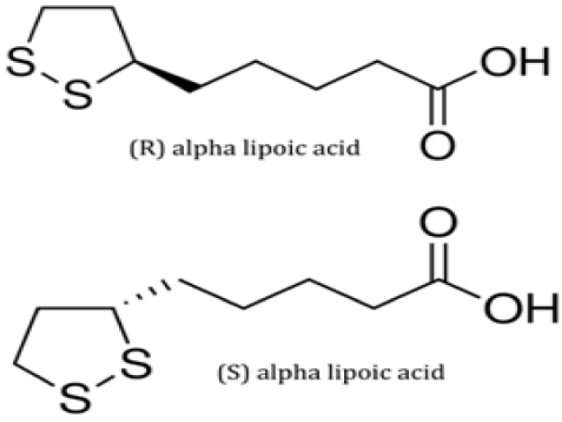


Figure no. 1.22: Alpha-Lipoic Acid (Chemical Structure)

ALA is a fatty acid found in all cells and in some food. It is a powerfull antioxidant that readily penetrates the blood brain barrie, chelate metals, reduce inflammaiton and increase ACh.

c. Omega-3 Fatty Acids:

Omega-3 Fatty Acids have many benificial effectsthat make them investigative prospects of AD. Daily taking of Omega-3 Fatty Acids reduces the risks of developing AD. It has also benificial effects on mild to moderate AD.

d. Acetyl L-Carnitine (ALCAR):

ALCAR derived from amino acid L-carnitine, work synergestically with ALA to transport acyl groups and fatty acids into the mitochondria for energy produciton. IT is a small molecule that readily penetrates the BBB and promotes biosynthesis of Ach. It also clears the toxic fatty acid metabolites. ALCAR also increases nerve growth factors.

e. Coenzyme Q10:

Coenzyme Q10 is essential for mitochondrial energy production. Many mitochondrial dysfunction occurs in AD brains, including disruption of energy production, apoptosis deregulation, altered calcium homeostasis and others. Coenzyme Q10 reduced oxidative stress and tau pathology in mice and metabolized βA and inhibited its formation invitro.

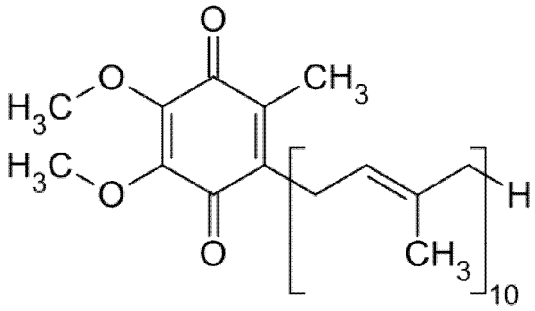


Figure no. 1.23: Coenzyme Q-10

1.19.11 Vitamins and mineals:

a. B vitamins:

Low level of vitamin B12 and folate appear to be associated with an increased rate of cognition decline. Since AD patients typically have high levels of homocysteine, researchers have examined the possibility that lowering homocysteine would be therapeutic. A combination of B12 and B6 and folate lowered homocysteine level in mild to moderate AD.

b. Vitamin A:

Vitamin A is essential for learning, memory and cognition. Vitamin A level decline with age and lower still in individuals with AD. A derivative of vitamin A, retinoic acid is known to slow cell death and offer protection from βA.

c. Vitamin E:

Doctors sometimes prescribe vitamin E to treat cognitive Alzheimer's symptoms. No one should take vitamin E to treat Alzheimer's disease except under the supervision of a physician. Vitamin E is an antioxidant, a substance that may protect brain cells and other body tissues from certain kinds of chemical wear and tear. Its use in Alzheimer's disease is based chiefly on a 1997 study showing that high doses delayed loss of ability to carry out daily activities and placement in residential care for several months. That study was conducted by the Alzheimer's Disease Cooperative Study (ADCS), the clinical research consortium of the National Institute on Aging (NIA). Since the ADCS study was carried out, scientists have found evidence in other studies that high dose vitamin E may slightly increase the risk of death, especially for those with coronary artery disease. No one should take vitamin E to treat Alzheimer's disease except under the supervision of a physician. Vitamin E especially at the high doses used in the ADCS study can negatively interact with other medications, including those prescribed to keep blood from clotting or to lower cholesterol.

d. Lithium:

Lithium is a naturally occuring mineral found in small amounts in many food. It increases the neuroprotective proteinscalled BCL-2 in the rat hippocampus and frontal cortex and inhibit glycogen synthase kinase 3 β, which is implecated in increasing levels of phosphorylated tau and is thought to be a factor leading to βA plaques and cell death. An invitro study found lithium's neuroprotection and resulted from inhibiting Ca2+ influx mediated by NMDA receptors.

1.19.12 Hormones (Melatonin):

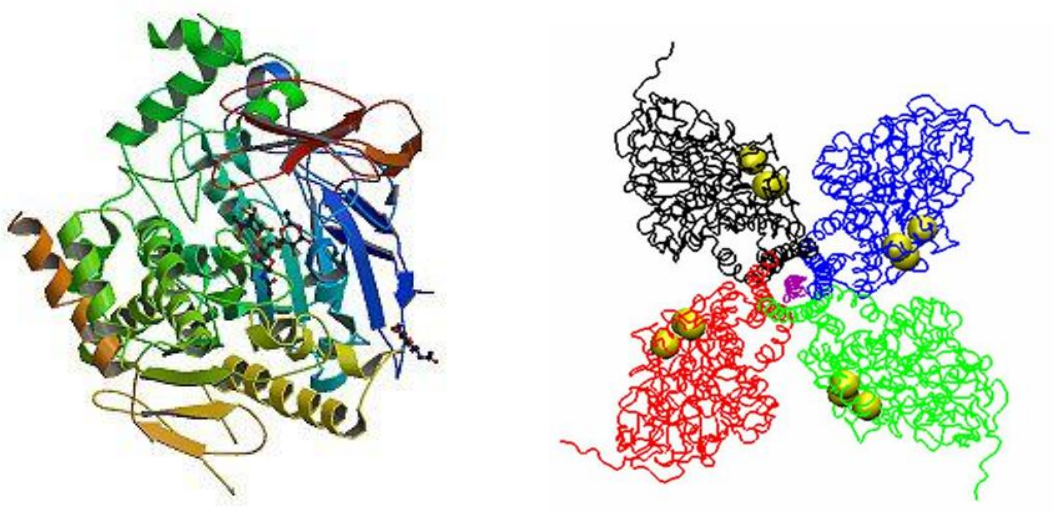
Melatonin is a naturally occuring hormone that is produced in decreasing amounts with age. It is a powerful antioxidants, provides mitochondrial support, protects against tau tangles and βA toxicity. It readily cross the BBB and enters all cell structures.

1.20 Cholinesterase and their mechanism:

1.20.1 Acetylcholinesterase:

Acetylcholinesterase (AChE) is key enzyme in the nervous system of animals. It is ahydrolasethathydrolyzesthe neurotransmitter acetylcholine. AChE is found at mainly

neuromuscular junctions and cholinergic brain synapses, where its activity serves to terminate synaptic transmission. It belongs to carboxylesterase family of enzymes. It is the primary target of inhibition by organophosphorus compounds such as nerve agents and pesticides.



(a) (b)

Figure no. 1.24: (a) 3D structure of AChE, (b) Tetramer of AChE

1.20.2 Enzyme structure and mechanism:

AChE has a very high catalytic activity - each molecule of AChE degrades about 25000 molecules of acetylcholine (ACh) per second, approaching the limit allowed by diffusion of the substrate. The active site of AChE comprises 2 sub sites - the anionic site and the esteraticsubsite. The structure and mechanism of action of AChE have been elucidated from the crystal structure of the enzyme.

The anionic sub site accommodates the positive quaternary amine of acetylcholine as well as other cationic substrates and inhibitor. The cationic substrates are not bound by a negatively-charged amino acid in the anionic site, but by interaction of 14 aromatic residues that line the gorge leading to the active site. All 14 amino acids in the aromatic gorge are highly conserved across different species. Among the aromatic amino acids, tryptophan 84 is critical and its substitution with alanine results in a 3000-fold decrease in reactivity. The gorge penetrates half way through the enzyme and is approximately 20 angstroms long. The active site is located 4 angstroms from the bottom of the molecule.

The esteraticsubsite, where acetylcholine is hydrolyzed to acetate and choline, contains the catalytic triad of three amino acids: serine 200, histidine 440 and glutamate 327. These three amino acids are similar to the triad in other serine proteases except that the glutamate is the third

member rather than asparate. Moreover, the triad is of opposite chirality to that of other proteases. The hydrolysis reaction of the carboxyl ester leads to the formation of an acyl-enzyme and free choline. Then, the acyl-enzyme undergoes nucleophilic attack by a water molecule, assisted by the histidine 440 group, liberating acetic acid and regenerating the free enzyme.

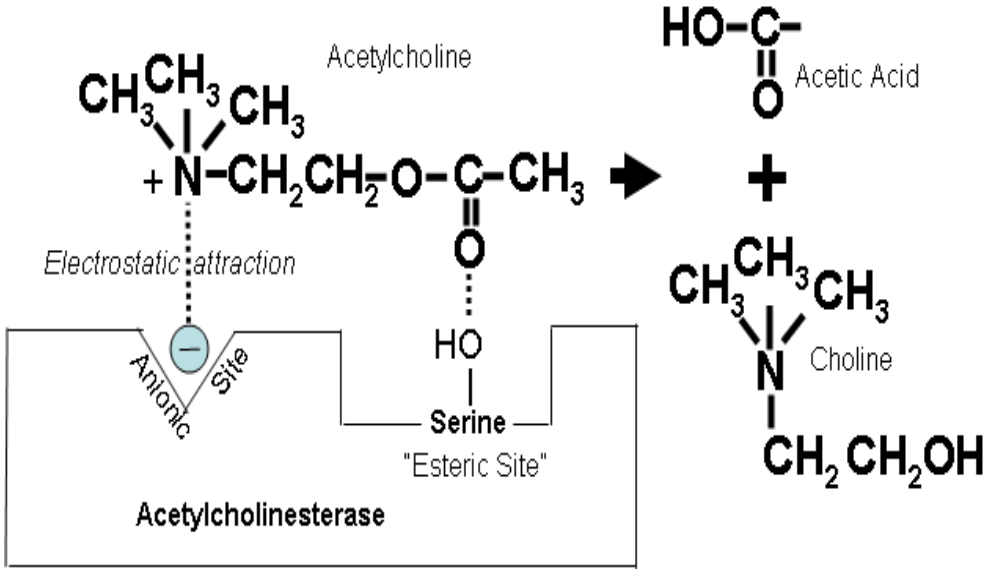


Figure no. 1.25: Mechanism of action of AChE

1.20.3 Distribution of AChE:

AChE is found in many types of conducting tissue: nerve and muscle, central and peripheral tissues, motor and sensory fibers, and cholinergic and noncholinergic fibers. The activity of AChE is higher in motor neurons than in sensory neurons.

Acetylcholinesterase is also found on the red blood cell membranes, where different forms constitute the Yt blood group antigens. Acetylcholinesterase exists in multiple molecular forms, which possess similar catalytic properties, but differ in their oligomeric assembly and mode of attachment to the cell surface.

1.20.4 Biological function of AChE:

During neurotransmission, ACh is released from the nerve into the synaptic cleft and binds to ACh receptors on the post-synaptic membrane, relaying the signal from the nerve. AChE, also located on the post-synaptic membrane, terminates the signal transmission by hydrolyzing ACh. The liberated choline is taken up again by the pre-synaptic nerve and ACh is synthetized by combining with acetyl-CoA through the action of choline acetyltransferase.

1.21 Butyrylcholinesterase enzyme:

Butyrylcholinesteraseis a non-specific cholinesterase enzyme that hydrolyses many different choline esters. It is also known as pseudocholinesteras or plasma cholinesterase. In humans, it is found primarily in the liverand is encoded by the *BCHE* gene.

It is very similar to the neuronal acetylcholinesterase, which is also known as RBC or erythrocyte cholinesterase.The term "serum cholinesterase" is generally used in reference to a clinical test that reflects levels of both of these enzymes in the blood.

Assay of butyrylcholinesterase activity in plasma can be used as a liver function test as both hypercholinesterasemia and hypocholinesterasemia indicate pathological processes.

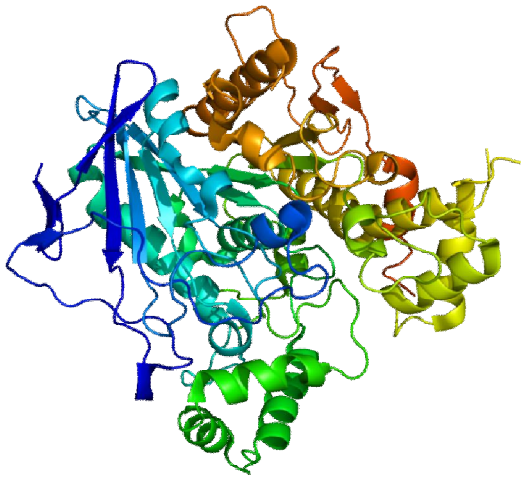


Figure no. 1.26: 3D structure of Butyrylcholinesterase.

1.21.1 Enzyme Kinetics:

Enzyme kinetics is the study of the chemical reactions that are catalyzed by enzyme. In enzyme kinetics, the reaction rate is measured and the effects of varying the conditions of the reaction are investigated. Studying an enzyme's kinetics in this way can reveal the catalytic mechanism of this enzyme, its role in metabolism, how its activity is controlled, and how a drug or an agonist might inhibit the enzyme.

Enzymes are usually protein molecules that manipulate other molecules, like the enzymes substrates. These target molecules bind to an enzyme's active site and are transformed intoproducts through a series of steps known as the enzymatic mechanism. These mechanisms can be divided into single-substrate and multiple-substrate mechanisms. Kinetic studies on enzymes that only bind one substrate, such as triosephosphateisomerase, aim to measure the affinity with which the enzyme binds this substrate and the turnover rate. Some other examples of enzymes are phosphofructokinase and hexokinase, both of which are important for cellular respiration (glycolysis).

1.22 Side Effects of the AD Drugs:

Although, acetylcholinesterase inhibitors are widely used for the treatment of AD, the benefit of cholinesterase inhibitors do not come without unpleasant side effects. The side effects of currently used drugs for AD treatment are given in the table 1.3.

Table 1.2: Side Effects of the AD Drugs

Generic	Side Effects
Donepezil	Nausea, vomiting, dizziness, loss of appetite, weight loss, muscle cramps, tiredness, trouble sleeping and increased frequency of bowel movements.
Galantamine	Nausea, vomiting, loss of appetite and increased frequency of bowel movements.
Memantine	Headache, constipation, confusion and dizziness, drowsiness, headache, insomnia, agitation and hallucination.
Rivastigmine	Nausea, vomiting, loss of appetite and increased frequency of bowel movements.
Tacrine	Possible liver damage, nausea, emesis, diarrhea, vomiting, dyspepsia, rhinitis, myalgia, tremor and excessive urination.
Vitamin E	Can interact with medications prescribed to lower cholesterol or prevent blood clots; may slightly increase risk of death.

1.23 Historical background of Herbal drugs:

Herbal products have been traditionally used as therapeutic agents and dietary supplement in both Eastern and Western cultures. The use of medicinal plants has substantially increased in the last decades and a World Health Organization survey indicated that 70-80% of the world population still relies on herbal based traditional medicine for their primary healthcare. Nature has a cure for all kinds of diseases and dilemmas that occur in people. It is the largest storehouse that includes everything for human survival from the enormous natural resources. The plants are being used for therapeutic purposes since the dawn of civilization. For thousands of years, natural products have played an important role throughout the world in treating and preventing human diseases. Natural product medicines have come from various source materials including terrestrial plants, terrestrial microorganisms, marine organisms and terrestrial vertebrates. The value of natural products in this regard can be assessed using 3 criteria:

- The rate of introduction of new chemical entities of wide structural diversity, including serving as templates for semi synthetic and total synthetic modification.
- The number of diseases treated or prevented by these substances and
- Their frequency of use in the treatment of disease.

An analysis of the origin of the drugs developed between 1981 and 2002 showed that natural products or natural product-derived drugs comprised 28% of all new chemical entities (NCEs) launched onto the market. In addition, 24% of these NCEs were synthetic or natural mimic compounds, based on the study of pharmacophores related to natural products.

This combined percentage (52% of all NCEs) suggests that natural products are important sources for new drugs and are also good lead compounds suitable for further modification during drug development. The large proportion of natural products in drug discovery has stemmed from the diverse structures and the intricate carbon skeletons of natural products. Since secondary metabolites from natural sources have been elaborated within living systems, they are often perceived as showing more "drug-likeness and biological friendliness than totally synthetic molecules" making them good candidates for further drug development.

Scrutiny of medical indications by source of compounds has demonstrated that natural products and related drugs are used to treat 87% of all categorized human diseases (48/55), including as antibacterial, anticancer, anticoagulant, antiparasitic and immunosuppressant agents, among others. There was no introduction of any natural products or related drugs for 7 drug categories (anesthetic, antianginal, anti histamine, anxiolytic, chelator and antidote, diuretic, and hypnotic) during 1981 to 2002. In the case of antibacterial agents, natural products have made significant contributions as either direct treatments or templates for synthetic modification Of the 90 drugs of that type that became commercially available in the United States or were approved worldwide from 1982 to 2002, ~79% can be traced to a natural product origin.

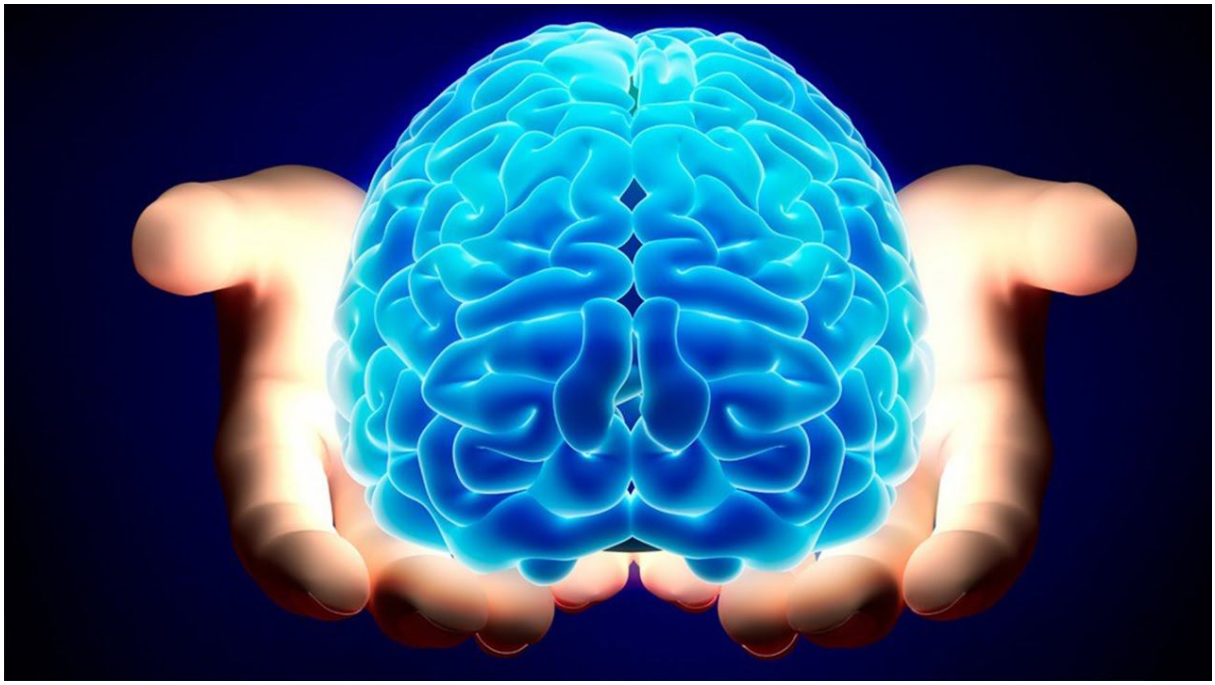
Frequency of use of natural products in the treatment and/or prevention of disease can be measured by the number and/or economic value of prescriptions, from which the extent of preference and/or effectiveness of drugs can be estimated indirectly. According to a study by Grifo and colleagues, 84 of a representative 150 prescription drugs in the United States fell into the category of natural products and related drugs. They were prescribed predominantly as anti-allergy/pulmonary/ respiratory agents, analgesics, cardiovascular drugs, and for infectious diseases. Another study found that natural products or related substances accounted for 40%, 24%, and 26%, respectively, of the top 35 worldwide ethical drug sales from 2000, 2001, and 2002.

1.24 Plants as a source of AD drugs:

None of the pharmacological lines of intervention have so far been able to stop the progression of AD; thus a need for an alternative approach was believed necessary to make progress with particular emphasis on plants. Plants have been used since antiquity in traditional medicinal systems for the treatment of memory dysfunction. Studies carried out on some species have resulted in the identification of compounds which are currently either in clinical use or templates for further drug discovery, e.g. galantamine, an alkaloid isolated from *Galanthusnivalis* L. (Amaryllidaceae). Galantamine was approved by the FDA in 2004 for use an acetylcholinesterae inhibitor in the treatment of AD. It was the traditional use of of *G. nivalis* L. in Bulgaria and Turkey for neurological conditions that lead to the development of this drug.

There have been previous reviews on the plants demonstrating pharmacological and clinical effects of potential interest in AD therapy, including Clement *et al.*, (2003), Howes and Houghton (2003), Howes*et al.*, (2003) and Zhang (2006). By studying the reviews it becomes clear that ethopharmacological screening is one of the main approaches used in drug discovery.

Plant Literature and Literature review



Chapter 2
Plants Reviews and Literature

2.1 Plant Name: Mimasa pudica lin.

2.1.1 Common Names of Mimosa pudica

Mimosa pudica is also known as chuimui or lajwanti in hindi because of its unique property to droop or collapse when touched and opens up a few minutes later. Its other names are Betguen Sosa (Guam), Memege (Niue), Mechiuaiu (Palau), Limemeihr (Pohnpei), Ra Kau Pikikaa (Cook Islands). The Chinese name for this plant translates to"shyness grass". Its Sinhala name is Nidikumba, where 'nidi' means 'sleep'. Its Tamil name is Thottal Sinungi, where 'Thottal' means 'touched' and 'Sinungi' means 'little cry'. Other non-English common names include Makahiya (Philippines, with maka- meaning "quite" or "tendency to be", and -hiya meaning "shy", or "shyness"), Mori Vivi (West Indies). In Bengali, this is known as 'Lojjaboti', the shy virgin. Sanskrit: Samanga, Varakranta, Namaskari. English: Touch-me-not. In Indonesia, it is known as Putri Malu (Shy Princess). In Myanmar (Burma) it is called 'Hti Ka Yoan' which means "crumbles when touched". It has been described as “sparshaat sankochataam yaati punashcha prasruta bhavet” -a plant which folds itself when touched and spreads its leaves once again after a while.

2.1.2 The Plant Family Fabaceae:

Alternative Titles: legume family, Leguminosae, Papilionaceae, pea family

Fabaceae, also called Leguminosae, pea family of flowering plants (angiosperms), within the order Fabales. Fabaceae, which is the third largest family among the angiosperms after Orchidaceae (orchid family) and Asteraceae (aster family), consists of more than 700 genera and about 20,000 species of trees, shrubs, vines, and herbs and is worldwide in distribution. Some of the most important commercial species include soybeans (Glycine max), garden peas (Pisum sativum), peanuts (Arachis hypogaea), and alfalfa (Medicago sativa). Most woody species are tropical; herbaceous (i.e., nonwoody) species occur mainly in temperate regions. The leaves usually are pinnately compound (feather formed), sometimes trifoliate (with three leaflets), or palmate (the leaflets radiating from a common point). The leaves of a few species are simple or reduced to scales. The fruit is typically a legume, or pod, which splits open as it dries, releasing the seeds.

Figure no. 2.1: Fabaceae flower section

2.1.3 Classification of Plant Family:

Plants Name	**Plants Name**
Acacia (genus Acacia)	Locoweed (Astragalus and Oxytropis species)
Alfalfa (Medcago sativa)	Locust (genus Robinia)
Almendro (Dipteryx oleifera)	Logwood (Haematoxylum campechianum)
Bean(genus Phaseolus)	Lupine (genus Lupinus)
Green bean (P. vulgaris)	Mesquite (genus Prosopis)
Lima bean (P. lunatus)	Mimosa (genus Mimosa)
Scarlet runner bean (P. coccineus)	Sensitive plant (M. pudica)
Birds foot trefoil (lotus corniculatus)	Narra (Pterocarpus species)
Bush clover (genus Lespedeza)	Pagoda tree (Styphnolobium japonicum)
Broom (genus Cytisus)	Palo verde (genus Parkinsonia)
Carob (Ceratonia siliqua)	Pea (Pisum sativum)
Chick pea (Cicer arietinum)	Peanut (Arachis hypogaea)

Clover (genus Trifolium)	Redbud (genus Ceris)
Cowpea (Vigna unguliculata)	Rosary pea (Abrus precatprius)
Crown vetch (Sceruigera varia)	Royal Poinciana (Delonix regia)
Fenugreek (Trigonella foenum-graecum)	Senna (genus senna)
Honey locust (Gledistsia species)	Silk tree(genus Albizia)
Indigo (genus indigofera)	Smoke tree (Dalea spinosa)
Jicama (Pachyrhizus erosus)	Soybean (Glycin max)
Kentucky coffee tree (Gymnocladus dioica)	Genus Lathyrus
Kidney vetch (Anthyllis vulnereraria)	Beach pea (L. japonicas)
Kudzu vine (Pueraria Montana)	Sweet pea(L. odoratus)
Laburmum (genus Laburmum)	Lentil (Lens culinaris)
Golden chain (L. anagyroides)	Licorice (Glycyrrhiza glabra)

2.1.4 The plant Genus:

Mimosa (genus Mimosa), large genus of plants in the pea family (Fabaceae), native to tropical and subtropical areas throughout both hemispheres. They are so named from the movements of the leaves in certain species that “mimic” animal sensibility. The well-known sensitive plant, or humble plant (Mimosa pudica), is commonly grown in greenhouses as a novelty for its rapid leaf movements in response to touch. Various other species are cultivated as ornamentals for the beauty of their foliage. Most Mimosa species are herbs or undershrubs, some are woody climbers, and a few are trees. They are often prickly. The leaves of most are bipinnate (i.e., the leaflets of the feather-formed leaves, in turn, bear leaflets). The roots of some species are poisonous; others contain substances irritating to the skin. The plants are characterized by small regular flowers and produce legume fruits. In addition to those responsive to physical stimuli, a few species have leaves that are sensitive to light and droop in response to darkness. Many species of the related genus Acacia are commonly but erroneously called mimosas.

Some other plants: Mimosa dutrae Malme, Mimosa incana (Sprengel) Malme, Mimosa pudica L.

Figure no. 2.2: Mimosa tree

2.1.5 Classification for Down to Genus Mimosa L.

No.	Species-	Common name
1	Mimosa aculeaticarpa Mimosa ul Variety- Mimosa aculeaticarpaortega biuncifera Var. (Benth.) Barneby	Ortega, catclaw mimosa Varietycatclaw Mimosa
2	Mimosa arenosa (Wild),	Poir,elegant Mimosa
3	Mimosa asperata L.	sensitive briar
4	Mimosa borealis A.	Gray,Fragrant Mimosa
5	Mimosa casta L.	graceful Mimosa
6	Mimosa d Mimosa diplotricha	C. wright giant false

		sensitive plant
7	Mimosa ceratonia L.	black ambret
8	Mimosa dysocarpa Benth	velvetpod Mimosa
9	Mimosa emoryana Benth	Emory's Mimosa
10	Mimosa grahamii A.Gray	Graham's Mimosa
11	Mimosa hystricina (Small ex Britton & Rose) B.L. Turner	porcupine Mimosa
12	Mimosa Mi Mimosa latidens (Small) B.L. Turner, briar	Kairn's sensitivebriar
13	Mimosa malacophylla A. Gray	Soft leaf Mimosa
14	Mimosa microphylla Dryand	little eaf sensitivebriar
15	Mimosa nuttallii (DC. ex Britton & Rose) B.L. Turner	Nuttall's sensitivebriar
16	Mimosa pellita Kunth ex Willd., Mimosa pudica L., Variety- Mimosa pudica L.varpudica, Mimosa pudica L. var. Unijuga, (Duchass. & Walp.) Griseb.,shameplant Mimosa quadrivalvis L.,fourvalve mimosaMimosa ", Mimosa quadrivalvis L.	lollipop Mimosa shameplant Urban's Mimosa
17	Mimosa roemeriana Scheele	Roemer's Mimosa

18	Mimosa rupertiana B.L. Turner	eastern sensitive plant
19	Mimosa schomburgkii Benth	Schomburgk's Mimosa
20	Mimosa strigillosa Torr. & A. Gray	Powderpuff
21	Mimosa texana (A. Gray) Small	Texas Mimosa
22	Mimosa turneri Barneby	Desert Mimosa

2.1.6 Mimosa pudica Taxonomy

Scientific Classification:

Kingdom : Plantae

Subkingdom : "Tracheobionta", "Vascular plants"

Super division: Spermatophyta

Division : Magnoliophyta, "Dicotyledons"

Class : Magnoliopsida

Subclass: Rosidae

Order : Fabales

Family : Fabaceae

Subfamily : Mimosoideae

Genus : Mimosa

Species : M. pudica

2.1.7 Plant Description:

A prickly, long-lived (perennial), herbaceous plant or small shrub with a creeping (prostrate or decumbent) or sprawling habit. It usually only grows 15-50 cm tall, but can reach up to 1 m or more in height when supported by other vegetation.

Mimosa pudica is not the only member of the legume plant family (Leguminosae) to move in response to stimuli. More species of Mimosa show sensitivity to touch, known as seismonasty. Other legumes, for example some members of the genera Neptunia, Acacia, Albizia and Samanea, respond to a lesser degree by showing 'sleep movements' (nyctinasty) in their natural habitats. This involves the closing up of the leaves a few hours before dusk, and the re-opening of the leaves a few hours before dawn. It is thought that these 'sleep movements' aid water conservation as well as defence against herbivory.

Sensitive plant is an annual or long-lived (perennial) that normally grows to 50-70 cm tall (but can be up to 1 m tall), and often takes the form of a straggling prickly sub-shrub (Burkill 1995). Its stems have sparse prickles, 2-2.5 mm long, or are sometimes bristly, or can also be almost hairless. The leaves are alternate, twice-compound (bipinnate), do not have prickles and are very sensitive to touch. The rachis (axis of the compound leaf) is 1.5-5.5 cm long, and the pinnae (primary divisions of the compound leaf) are subdigitate (almost finger-like projections). There are 10-26 pairs of leaflets (the smallest segments of the leaf) per pinna, which are 6-15 x 1.2-3 mm and linear-oblong. The flowers are lilac or pink (the colour mainly the stamen filaments) and are held in ovoid, stalked heads of 1-1.3 x 0.6-1 cm. A cluster of 1-5 flower-heads is borne in the leaf axil. The calyx is minute, about 0.2 mm long. The corolla is 2-2.3 mm long, and contains four stamens. The pods are 1.8 cm x 3-5 mm, densely bristly, clustered, and have prickles along their margins.

Origin and geographical distribution: The plant is a native of tropical America and naturalized nearly all through the tropical and subtropical parts of India.

Habitat: Commonly distributed in open-spaces, especially road side, cultivated land, and waste area.

Propagation: By seeds and vegetative methods. Each plant can produce over 700 bristled seeds which can be carried on animal fur, feathers or on people's clothing.

Bending Movement of curiosity plants Mimosa pudica:

Plant leaf movements can be mediated by specialized motor organs, the pulvini or can be epinastic (i.e. based on different growth velocities of the adaxial (facing toward the stem of a plant (especially denoting the upper surface of a leaf) and abaxial(facing away from the stem of a plant) halves of the leaf). Both processes are associated with diurnally regulated increase in the rates of membrane water transport, which in many cases, has been shown to be facilitated by Aquaporins (AQP) are integral membrane proteins that serve as channels in the transfer of water, and in some cases, small solutes across the membrane. They are conserved in bacteria, plants, and animals. Structural analyses of the molecules have revealed the presence of a pore in the center of each aquaporin molecule). Rhythmic leaf movements are known from many plant species but more recently a promising model plant to study pulvinus-mediated leaf movements is M. pudica. The contribution of both plasma membrane and tonoplast localized aquaporins to the seismonastic leaf movements in M. pudica has been analyzed. The bending movement of the pulvinus of M. pudica is caused by a rapid change in volume of the abaxial motor cell, in response to various environmental stimuli. The bending of the pulvinus is retarded by treatments with actin-affecting reagents and calcium channel inhibitors. The actin filaments in the motor cells are fragmented in response to electrical stimulation. Hence the study demonstrated that depolymerization of the actin cytoskeleton in pulvinus motor cells in response to electrical signals results in increased levels of calcium.

The seismonastic movement (rapid responses to vibration, touch, and flexure) of M. pudica is triggered by a sudden loss of turgor pressure. On comparing the cell cytoskeleton by immunofluorescence analysis before and after movement and evaluation of the effects of actin and microtubule targeted drugs by injecting them into the cut pulvinus (an enlarged section at the base of a leaf stalk in some plants that is subject to changes of turgor, leading to movements of the leaf or leaflet), it is seen that fragmentation of actin filaments and microtubule occurs during bending, although the actin cytoskeleton and not the microtubules are involved in the regulation of the movement.

TEM (Transmission electron microscopy- in which a beam of electron is transmitted through the specimen to form an image) reveals that actin cables become loose after bending. On injecting phosphatase inhibitors into several pulvinus to examine the effects of such inhibitors, it is seen that changes in actin isoforms, fragmentation of actin filaments and the bending movements are all inhibited after injecting a tyrosine phosphatase inhibitor. Special red cells are found on the adaxial surface of the tertiary pulvini of M. pudica. Using anatomical (light, scanning and transmission electron microscopy) and electrophysiological techniques it has been demonstrated that these red cells are the real mechanoreceptor cells. They can generate receptor potential following mechanical stimuli and they are in connection with excitable motor cells (through

plasmodesmata). These red cells are derived from stomatal subsidiary cells and not the guard cells.

Figure no. 2.3: a) open leaves, b)close leaves

Mimosa pudica symbionts

Bacteria isolated from Mimosa nodules in Taiwan, Papua New Guinea, Mexico, and Puerto Rico were identified as belonging to either the alpha- or beta-proteobacteria. The beta-proteobacteria Burkholderia and Cupriavidus strains formed effective symbiosis with the common invasive species Mimosa diplotricha, M. pigra, and M. pudica, but the alpha-proteobacterial Rhizobium etli and R. tropici strains produced a range of symbiotic phenotypes from no nodulation through ineffective to effective nodulation, depending on Mimosa species.

The largest significant effect was for M. pudica, in which LMG19424 formed 57% of the nodules in the presence of 0.5 mM potassium nitrate. In the host, ammonium also had a similar,

but lesser, effect. Comparable results were also found using an N-containing soil mixture, and environmental N levels are therefore suggested as a factor in the competitive success of the bacterial symbionts in vivo. The ability of Burkholderia phymatum STM815 to effectively nodulate Mimosa species, and to fix nitrogen ex planta, was compared with that of the known Mimosa symbionts Cupriavidus taiwanensis LMG19424. Both strains were equally symbionts of M. pudica, but nodules formed by STM 815 had greater nitrogenase activity. STM 815 was shown to have a broader host range across the genus Mimosa than LMG 19424 nodulating 30 out of 31 species, 21 of these effectively. LMG 19424 effectively nodulated only nine species.

Figure no. 2.4: Nodules of M. Pudica

2.1.8 Botanical Features:

MACROSCOPY

Root

Cylindrical, tapering rependant, with secondary and tertiary branches, varying in length up to 2-cm thick, surface more or less rough or longitudinally wrinkled; grayish-brown to brown, cut surface of pieces pale yellow, fracture hard, woody, bark-fibrous; odor, distinct; taste, slightly astringent.

Stem

Cylindrical, up to 2.5 cm in diameter; sparsely prickly, covered with long, weak bristles longitudinally grooved, external surface light brown, internal surface grey, bark fibrous; easily separable from wood.

Leaf

Digitately compound with one or two pairs of sessile, hairy pinnae, alternate, petiolate, stipulate, linear lanceolate; leaflets 10–20 pairs, 0.6–1.2-cm long, 0.3–0.4-cm broad, sessile, obliquely narrow or linear oblong; obliquely rounded at base, acute, nearly glabrous; yellowish green.

Flower

Pink, in globose head, peduncles prickly; calyx very small; corolla pink, lobes 4, ovate oblong; stamens 4, much exerted; ovary sessile; ovules numerous.

Fruit

Lomentum, simple, dry, 1–1.6-cm long, 0.4–0.5-cm broad, with indehisced segments and persistent sutures having —two to five seeds with yellowish spreading bristle at sutures, 0.3-cm long, glabrous, and straw colored.

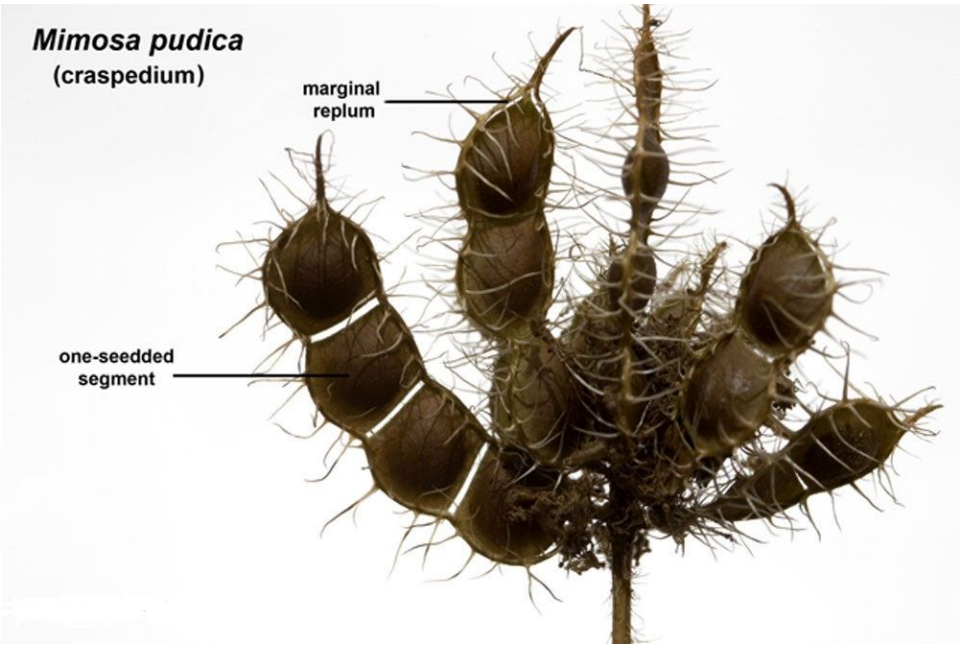


Figure no. 2.5: Fruits of Mimosa Pudica

Seed

Compressed, oval-elliptic, brown to gray, 0–0.3-cm long, 2.5-mm broad, having a central ring on each surface.

MICROSCOPY

Root

Mature root shows cork 5–12 layered, tangentially elongated cells, a few outer layer crushed or exfoliated; secondary cortex consisting of 6–10 layered, tangentially elongated thin-walled cells; secondary phloem composed of sieve elements, fibers, crystal fibers, and phloem parenchyma traversed by phloem rays, phloem fibers, single or in groups, arranged in tangential bands; crystal fibers thick walled, 3–25 chambered, each with single or two to four prismatic crystals of calcium oxalate; phloem rays uni-to-multi-seriate, —two to three seriate more common; secondary xylem consists of usual elements traversed by xylem rays; vessels scattered throughout secondary xylem having bordered pits and reticulate thickenings; crystal fibers containing one or rarely two to four prismatic crystals of calcium-oxalate in each chambers; parenchyma, thick walled, scattered throughout secondary xylem; xylem rays uni-to-bi-seriate; rarely multi-seriate, wider toward secondary phloem and narrow toward center; starch grains, prismatic crystals of calcium oxalate and tannin present in secondary cortex, phloem and xylem rays, and parenchyma; starch grains both simple and compound having two to three components, rounded to oval measuring 6–20 mm and 16–28 mm in diameter, respectively.

Stem

Mature stem shows four to eight layered, exfoliated cork of tangentially elongated cell filled with reddish brown contents; secondary cortex wide, consisting of large, moderately thick walled, tangentially elongated to oval, parenchymatous cells, filled with reddish brown contents, a few cells contain prismatic crystals of calcium oxalate, a number of lignified, fibers single or in groups, scattered throughout; secondary phloem consisting of usual elements, two to five transversely arranged strips of fibers occur alternating with narrow strips of sieve elements and parenchyma, crystal fibers elongated, thick-walled, containing single crystal of calcium oxalate in each chamber; phloem rays thick walled radially elongated; secondary xylem composed of usual elements traversed by xylem rays, vessels, drum shaped with spiral thickenings, tracheids pitted with pointed ends, fibers of two types, shorter wide lumen and longer with narrow lumen; xylem rays radially elongated, thick walled, 1–6 cells wide and 3-30 cells high; pith consisting of polygonal, parenchymatous cells with intracellular spaces.

Leaf

Petiole shows single layered epidermis, covered with thin cuticle; cortex four to seven layered of thin walled, parenchymatous cells; pericycle arranged in a ring four central vascular bundles present with two smaller vascular bundles arranged laterally, one in each wing.

Midrib

Shows a single-layered epidermis, covered with thin cuticle, upper epidermis followed by a single-layered palisade, spongy parenchyma single-layered, pericycle same as in petiole; vascular bundle single.

Lamina

Shows epidermis on both surfaces, palisade single-layered; spongy parenchyma, three to five layers consisting of circular cells; rosette crystals and few veins present in spongy parenchyma.

Fruits

Shows single-layered epidermis with few nonglandular, branched, shaggy hair; mesocarp five to six layers of thin walled, parenchymatous cells; some amphicribral vascular bundles found scattered in this region; endocarp of thick-walled lignified cells followed by single-layered thin-walled, parenchymatous cells.

Seed

Shows single-layered radially elongated cells; followed by five- to six-layered angular cells filled with dark brown contents; endosperm consists of angular or elongated cells, a few containing prismatic crystals of calcium oxalate; cotyledons consist of thin-walled cells, a few cells containing rosette crystals of calcium oxalate; embryo straight with short and thick radical.

Powder

Reddish brown, shows reticulate, pitted vessels, prismatic and rosette crystals of calcium oxalate, fibers, crystal fibers, yellow or brown parenchymatous cells, palisade cells, nonglandular, branched, shaggy hair, single and compound starch grains, measuring 6–25 mm in diameter with two to three components.

2.1.10 Traditional Uses

Ayurveda has declared that its root is bitter, acrid, cooling, vulnerary, alexipharmic, and used in the treatment of leprosy, dysentery, vaginal and uterine complaints, inflammations, burning sensation, asthma, leucoderma, and fatigue and blood diseases. Unani Healthcare System its root is resolvent, alternative, and useful in the treatment of diseases arising from blood impurities and bile, bilious fevers, piles, jaundice, and leprosy etc. Decoction of root is used with water to gargle to reduce toothache. It is very useful in diarrhea (athisaara), amoebic dysentery (raktaatisaara), bleeding piles and urinary infections. It arrests bleeding and fastens the wound healing process. It is mainly used in herbal preparations for gynecological disorders. It has been

said to have medicinal properties to cure skin diseases. It is also used in conditions like bronchitis, general weakness and impotence. It is also used to treat neurological problems. The content of M. pudica has a capacity of arresting bleeding and it fastens the process of healing of wounds. It is recommended in diarrhea, amoebic dysentery and bleeding piles. It is also used in herbal preparations of gynecological disorders. Its extract can cure skin diseases. Some herbal doctors recommend it for bronchitis, general weakness and impotence. All the five parts of the plant leaves, flowers, stems, roots, and fruits are used as medicines in the traditional healthcare systems. In India, different parts of the plant have been in popular use for treating various ailments since long. Recent researches show that the extract of this plant can be used for checking child birth. Some authors have reported that this herb can replace contraceptive pills if researches are done properly. According to different researches done so far, Mimosa tenuiflora bark is used to relax the mind, and relieve depression, mental distress, irritability, severe palpitations, and amnesia. It is a mood enhancer and improves circulation of the blood. Some believe Mimosa can reduce the onset of baldness. Due to its ability to promote healthy cell growth, Tepezcohuite is used in shampoos, creams, capsules, and soaps. In Ayurvedic and Unani medicine, Mimosa pudica root is used to treat bilious fevers, piles, jaundice, leprosy, dysentery, vaginal and uterine complaints, inflammations, burning sensation, fatigue, asthma, leucoderma, and blood diseases. In Western medicine, Mimosa root is used for treating insomnia, irritability, premenstrual syndrome (PMS), menorrhagia, hemorrhoids, skin wounds, and diarrhea. It is also used to treat whooping cough and fevers in children, and there is some evidence to suggest that Mimosa is effective in relieving the symptoms of rheumatoid arthritis. All parts of the Mimosa plant are reportedly toxic if taken directly. Its consumption is not recommended to pregnant or nursing ladies. Due to these reports, it seems to be best to consult a physician before using Mimosa internally. Researches regarding safety in young children or those with severe liver or kidney disease have not been found.

2.1.12 Medicinal uses of the plant Mimosa pudica:

Mimosa Pudica Wound Healing Activity:

Traditionally the leaf extract made by grinding the leaves with little water and extracting the juice is used for treating wounds. This remedy has been proven scientifically now! For the study, both the methanolic and water extract was used in 3 different concentrations (0.5 %, 1 % and 2 %) in a basic ointment base. The ointment containing 2 % of both methanolic and water extract showed significant wound healing activity.

Mimosa Pudica Anti Venom Activity:

An interesting study was done on the anti venomous activity of mimosa pudica and that too cobra venom! The study which was done on the water extract of the mimosa pudica dried root (made by boiling the dried root in water) proved that it is very good at inhibiting the activity of the snake venom. But this remedy has to done under the observation of an experienced healer or herbalist.

Mimosa Pudica For Piles:

Mimosa Pudica is very good for treating bleeding piles and has been used as a remedy for it for many many years. For the remedy, crush the leaves into a fine paste and apply as a poultice, it will greatly ease the burning and bleeding. This is due to it's amazing wound healing properties. Toxic alkaloid, such as L-mimosine is reported to be present in higher proportion in leaves than bark and roots of plant and is also responsible for depilatory effect & it was reported that depilatory activity of mimosine noted during the 1st hair growth cycle of the mouse at the dose of 10ìl which was injected via s.c route causes significant loss of hairs from body surface. It was reported that mimosine bears a structural resemblance to L-tyrosine & mimosine probably acts as a tyrosine analogue or tyrosine antagonist which inhibits protein biosynthesis in the living body and causes toxic symptoms including retardations of growth. It was reported that in rats, growth inhibition caused by mimosine also the metal chelating power of mimosine could possibly disturb the action of metal containing enzymes, especially those containing iron cations.

Mimosa Pudica For Ulcers:

Another very important study on mimosa pudica was its effect on ulcers. The study done on rats with artificially induced ulcers proved that 100 mg of ethanolic extract very effectively reduced the ulcers.

Mimosa Pudica For Diarrhea:

Mimosa pudica is very good in treating diarrhea. A study done on albino rats by inducing them to diarrhea using castor oil and treating them with ethanolic extract proved to be very effective in controlling the diarrhea.

Mimosa Pudica Anti inflammatory Properties:

Another study proved its anti inflammatory properties. The study done on rats with artificially induced paw odema proved its anti inflammatory properties. The results were very effective and significant. In village sides, we do boil the leaves of mimosa pudica and use the warm liquid as a compress, happy to know that it has been proven scientifically.

Mimosa Pudica Anti Diabetic Activity:

Mimosa pudica's anti diabetic activity has been proven through research. The research was done using the ethanolic extract but usually the leaf powder or the root powder is taken daily for bringing down the blood sugar levels.

Liver Protecting Activity of Mimosa Pudica:

Another important medicinal use it's protection of liver against toxins . When rats were given toxic ethanol along with mimosa pudica extract, it proved to be very effective in protecting the liver from toxicity.

Anti microbial, Anti Fungal & Anti Viral Properties Of Mimosa Pudica:

Mimosa pudica has been proven for its anti microbial, anti fungal and anti viral properties. The research was done using different concentrations of the mimosa pudica ethanol extract on various fungus and bacteria and it proved to be very effective in controlling them.

Anti fertility Activity of Mimosa Pudica:

Mimosa pudica has proven to have anti fertility properties. so if you are trying for pregnancy, never consume mimosa pudica in any form.

CNS depressant activity

The open field test (OFT) is a commonly used for measure of general locomotor activity and also to assess anxiety, depression. In addition, repeated exposure or extended session length provides a method for assessing habituation to the increasingly familiar chamber environment. It has been suggested that two factors influence anxiety-like behavior in the open field. The first is social isolation resulting from the physical separation from cage mates when performing the test. The second is the stress created by the novel test environment by using open field test and hole cross test it has been reported that, number of movements of animals and number of passage from chamber were significantly decreased in dose dependant manner at doses of 100 mg/kg and 200 mg/kg.

Anticonvulsant activity

Epilepsy is a major neurological disorder and up to 5% of the world population develops epilepsy in their life time. The current therapy of epilepsy with modern antiepileptic drugs is associated with side effects, dose-related and chronic toxicity, as well as teratogenic effects, and approximately 30% of the patients continue to have seizures with current antiepileptic drugs therapy. Traditional systems of medicine are popular in developing countries and up to 80% of the population relies on traditional medicines or folk remedies for their primary health care need. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effect. The electroshock assay in mice is used primarily as an indication for

compounds which are effective in grand mal epilepsy. Tonic hind limb extensions are evoked by electric stimuli which are suppressed by anti-epileptics but also by other centrally active drugs. It has been reported that ethanolic extract of Mimosa pudica exhibit significant protection against tonic seizures in dose dependant manner, and maximum effect was observed at 200mg/kg.

Diuretic activity

It was reported that the Lipschitz test was employed for assessment of diuretic activity of petroleum ether, ethanolic and aqueous extracts of Mimsoa pudica was based on water and sodium excretion in test animals and compared to rats treated with a high dose of urea. The "Lipschitz- value" is the quotient between excretion by test animals and excretion by the urea control. The ethanolic and aqueous extract of Plant was reported to be tested for evaluation of diuretic activity by using Furosemide (20 mg/kg) as standard. Among the two extracts ethanolic extract was reported to produce significant diuretic activity at doses of 100 and 200 mg/kg. Extract caused increase in total urine volume and ion concentration of Na+, Cl-, k+. at these doses.

Anthelminthic activity

It was reported that helminthes infections, repeatedly entitled helminthiasis are among the most pervasive infection and a foremost degenerative disease distressing a large proportion of world's population. In developing countries, they pose a large threat to public health and contribute to the prevalence of malnutrition, anemia, eosinophilia and pneumonia. The helminthes parasites mainly subsist in human body in intestinal tract. Development of resistance in helminthes against conventional anthelmintics is a foremost problem in treatment of helminthes diseases. Hence medicinal plants were screened for their anthelmintic activity. Anthelminthic activity has been reported for seeds of M. pudica. In a study undertaken for evaluation of anthelmintic activity, different successive extracts namely petroleum ether, ethanol and water using Pheretima posthuma as a test worm to the different concentrations (100, 200, 500mg/kg) were tested for bioassay which involved determination of paralysis and time of death of the worms, however, Petroleum Ether was reported to have weak anthelminthic activity as compared to other two extracts.

Immuno modulatory activity

The concept of immunomodulation relates to nonspecific activation of the function and efficiency of macrophages, granulocytes, complement, natural killer cells and lymphocytes and also to the production of various effector molecules generated by activated cells. It is expected that theses nonspecific effects give protection against different pathogens including bacteria, viruses, fungi. Immune functions are indispensable for defending the body against attack by pathogens or cancer cells, and thus play a pivotal role in the maintenance of health. However, the immune functions are disturbed by malnutrition; aging, physical and mental stress or undesirable

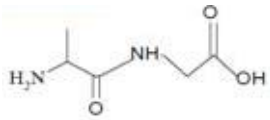
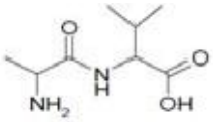
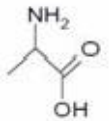
lifestyle immuno modulatory effect is associated with compounds capable of modifying or regulating immune function. The Immuno modulatory effect of plant is reported for alcoholic extract of the various aerial parts of Mimosa pudica. The assessment of immuno modulatory activity was carried out by various hematological and serological test. Further, immune modulatory activity was studied by Cell Mediated Immune Response (CMIR) measured by delayed type of hypersensitivity reaction to SRBC and humoral immune response (HIR) was measured by hemagglutination antibody titer. The alcoholic extract was reported to be significantly enhancing humoral as well as cell mediated response thus indicating the Immuno modulatory potential of Mimosa pudica (Linn).

2.1.13 Principal Constituents of Mimosa plant:

M. pudica contains Mimosine, which is a toxic alkaloid. Adrenalin like substance has been identified in the extract of its leaves. Some workers have reported the presence of Crocetin dimethyl Easter in the extract of the plant. Roots contain tannin up to 10 per cent. Seeds contain a mucilage which is composed of d-xylose and d-glucuronic acid. The plant extract contains green yellow fatty oil up to 17 per cent. The plant is reported to contain tubuline and a new class phytohormone turgorines is found to be active in the plant. The periodic leaf movement factors are reportedly the derivatives of 4-α-(b-D-glucopyranosyl-6-sulphate)gallic acid. The preliminary phytochemical screening of the M. pudica leaf extract showed the presence of bioactive components such as terpenoids, flavonoids, glycosides, alkaloids, quinines, phenols, tannins, saponins, and coumarins.

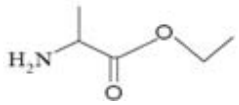
Phytochemical constituents in Mimosa pudica oil-

Amino acid derivatives

Compound name	**Structure**
N-dl-Alanylglycine, (C5H10N2O3)	
dl-Alanyl-dl-Valine, (C8H16N2O3)	
d-Alanin, (C3H7NO2)	

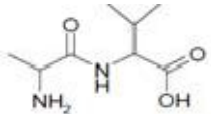
dl-Alanin ethyl ester,

(C5H11NO2)



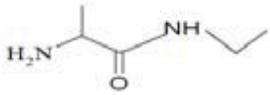
dl-Alanyl-dl-Valine,

(C8H16N2O3)



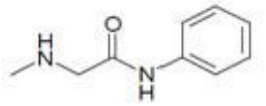
1-Alanine ethyl amide

(C5H12N2O)

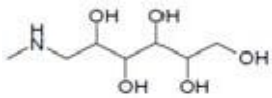
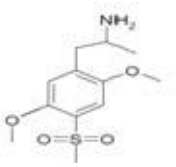


2-methylamino-N- phenylacetamide,

(C9H12N2O)



Carbohydrates

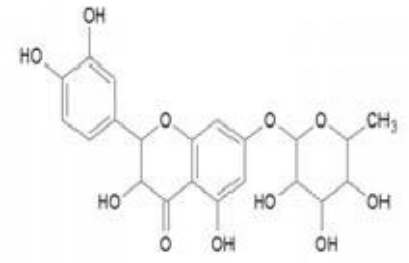
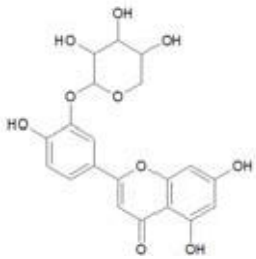
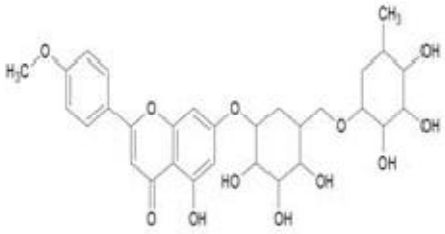
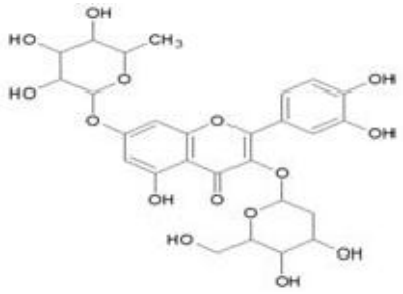
Compound name	**Structure**
Meglumine, (C7H17NO5)	
5-Dimethoxy-(methylsulphonyl) amphetamines, (C12H19NO4S)	

Fatty acid derivatives-

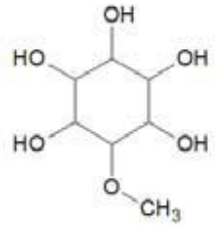
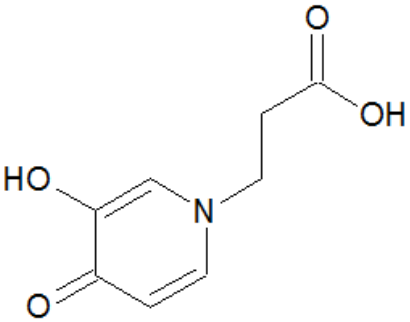
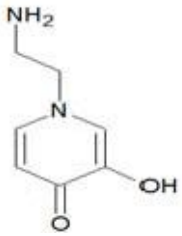
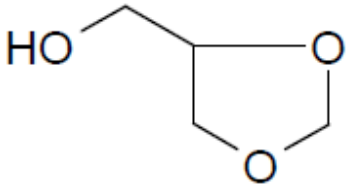
Compound name	**Structure**
1)9, 12-Octadecadienoic acid (Z, Z), methyl ester	(C19H34O2)
2) 12-Octadecadienoic acid,methyl ester	(C19H34O2)
3) 13-Eicosadienoic acid,	(C21H38O2)

methyl ester

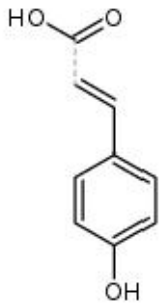
Flavonoid glycosides

Compound name	Structure
Quercetin-7-rhamnoside	
Leutolin-3xyloside	
Acacetin-7-rutinoside	
Quercetin-3glucoside-7-rhmnoside	

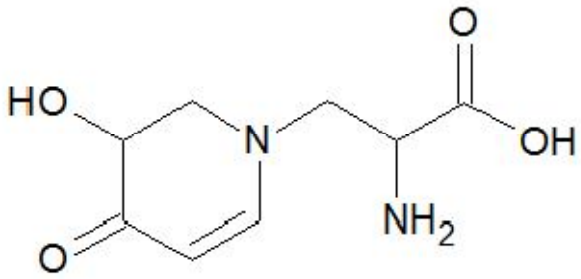
Miscellaneous

Compound name	**Structure**
D-Pinitol	
Mimosainic acid	
Mimosinamine	
In the oil of whole plant, 1, 3-Dioxolane-4-methanol, (C4H8O3) In the oil of p	

P-coumaric acid



L-Mimosine



2.2 Literature Review:

Antiulcer activity of the leaf ethanolic extract of Mimosa pudica in rats found in parts like whole plant, leaves, and roots.The methanolic extract exhibited good wound healing activity probably due to phenols constituents Dnyaneshwar et al 2009.

Hypolipidemic Activity of Chloroform Extract of Mimosa pudica Leaves Rekha 2010.

Regeneration of sciatic nerve: An extract administered in a dose of 1.6 mg/100 g parenterally every 4th day up to 120 days in rats having experimental injury of sciatic nerve, exhibited 30–40% higher results in the process of regeneration of sciatic nerve as compared to the hydrocortisone group.Khare et al 2014.

Antidepressant action Results showed that clomipramine (1.25 mg/kg, I.P.), desipramine (2.14 mg/kg, I.P.), and M. pudica (6.0 mg/kg and 8.0 mg/kg, I.P.) reduced immobility in the forced swimming test and increased the rate of reinforces received in the DRL-72 s test; these data suggest that M. pudica produces antidepressant effects in the rat. Diazepam increased the open-arms exploration time in the elevated plus-maze test, but M. pudica did not show any comparable action at any tested dose. M. pudica therefore produced an anti-depressant like profile similar to two tricyclic anti- depressants. Moline et al 1999.

Anticonvulsant action: The decoction of M. pudica leaves given intraperitoneally at a dose of 1000-4000 mg/kg protected mice against pentylenetetrazole and strychnine-induced seizures. M. pudica had no effect against picrotoxin-induced seizures. It also antagonized N-methyl-D-aspartate-induced turning behavior. These properties could explain its use in African traditional medicine. Bum 2004.

Hyperglycemic effect: Ethanolic extracts of M. pudica leaves given by oral route to mice at a dose of 250 mg/kg showed a significant hyperglycemic effect.Amalraj et al 2002.

Diuretic effect: Decoction of leaves of M. pudica in doses of 200, 500, 1000, and 2000 mg/kg in rats and dogs exhibited diuretic activity (considering urinary output Na+–K+–Cl- excretion). The activity in rats at 250 mg/kg dose was found to be 82% of standard diuretic (hydrochlorthiazide 2.5 mg/kg) treated group of rats. There was significant reduction (above 50%) of Na+ and Cl- excretion without affecting K+ excretion. The drug can be combined as a moderate diuretic with any modern synthetic diuretic causing K+ loss.

Effect on uterine bleeding: Aqueous extracts of root powder in pilot studies on patients with dysfunction uterine bleeding gave promising results.Khare et al. 2004.

Antifertility activity: *Mimosa pudica* is one of the folk medicinal plants commonly used as antifertility agent in some places in India. Air-dried roots of *M. pudica* were extracted using

methanol. The dried methanol extract of the root was administered orally to Swiss albino mice for 21 consecutive days. Estrous cycle, reproductive hormones (LH, FSH, prolactin, estradiol, and progesterone) and number of litters produced were studied in both control and extract administered groups by using standard methods. Phytochemical studies of the methanolic root extract were carried out using qualitative and TLC methods. The root extract of *M. pudica* has antifertility effect as it prolongs the estrous cycle and disturbs the secretion of gonadotropin hormone in albino mice. The decrease in FSH levels in the proestrous and estrous stages in the extract administered group compared with those of control animals indicates the disturbance of estrous cycle and ovulation through suppression of FSH.

M. pudica root powder (150 mg/kg body weight) when administered intragastrically, altered the estrous cycle pattern in female Rattus norvegicus. Nucleated and cornified cells were absent in all rats. The smear was characterized by leucocytes only, as in diestrus, which persisted for 2 weeks. There was a significant reduction in the number of ova in rats with the root powder compared with the control rats, and a significant increase in the number of degenerated ova. Valsala et al. 2002.

Antifertility effect: The root extract of M. pudica has antifertility effect as it prolongs the estrous cycle and disturbs the secretion of gonadotropin hormones in albino mice. The decrease in FSH level in the proestrus and estrus stages in the extract-administered group compared with those of control animals indicates the disturbance of estrous cycle and ovulation through suppression of FSH.Ganguly et al 2007.

Spasmogenetic potential Ethanol extracts (50%) of the whole plant exhibited spasmogenetic activity in isolated guinea pig ileum.Khare 2004

Antihepatotoxic and antioxidant potential: Reactive oxygen species (ROS) are believed to be responsible for pathogenesis of various diseases affecting tissues and the main organ, the liver. Hence, in this study, the extent of lipid peroxidation (LPO) and ROS elimination and its defense mechanisms by the enzymic and nonenzymic antioxidants in liver and serum was investigated. Hepatotoxicity was manifested by significantly decreased ($P < 0.05$) levels in the activities of the enzymic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase, and the non-enzymic antioxidants such as glutathione and vitamin-C in rats with induced hepatic damage by ethanol. Simultaneous administration of the leaf extract M. pudica along with the toxin ethanol in rats showed a considerable protection against the toxin-induced oxidative stress and liver damage as evidence by a significant increase ($P < 0.05$) in antioxidant activities. The study reveals that the co-administration of the M. pudica aqueous extract significantly lowered the level of lipid peroxidation in alcohol-fed mice.Nazeema et al. 2009.

Antivenom activity: The aqueous root extract of M. pudica dose dependently inhibited the hyaluronidase and protease activities of Indian snakes (Naja naja, Vipera russelii, and Echis

carinatus) venom.Aqueous and alcoholic extracts of dried roots of M. pudica were tested for their inhibitory activity on lethality, myotoxicity, and toxic enzymes of Naja kaouthia venom. The aqueous extract, particularly the normal water extract, displayed a significant inhibitory effect on the lethality, myotoxicity, and tested enzyme activities of venom compared with alcoholic extracts. The present findings suggest that an aqueous extract of M. pudica root possesses compound(s), which inhibit the activity of cobra venom.Mahanta et al 2001.

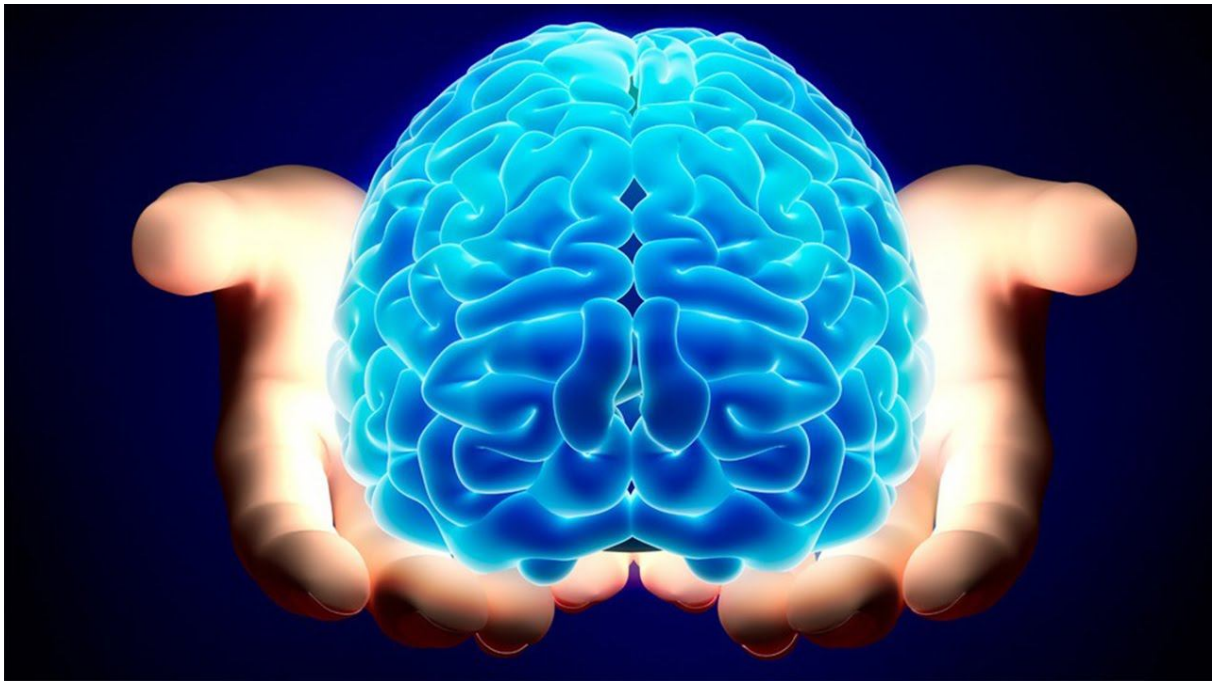
Antimicrobial properties: Antimicrobial activity of the successive extracts of M. pudica whole plant in petroleum ether, chloroform, ethyl acetate, methanol, and water was studied against various Gram positive and Gram negative bacterial strains using the zone of inhibition. Both the agar well diffusion method and agar disc diffusion method were used to evaluate the antibacterial efficacy of the said plant extracts. The microorganisms used in the test were: Escherichia coli, Staphylococcus aureus, Staphylococcus albus, Proteus vulgaris, Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B, Shigella flexneri, Klebsiella pneumonia, and Pseudomonas aeruginosa. The minimum inhibitory concentration (MIC) of the methanolic extract of said plant was determined by the agar well diffusion method. The reference antibiotics chloramphenicol and ampicillin were also tested against the said microorganisms used in the assay and the results were compared with that of the plant extracts. The results of the study revealed that the M. pudica whole plant extract possesses good antimicrobial activity between the range of 7–18 mm against the pathogens used for screening.Pawaskar et al. 2006.

Antifungal activity: The methanolic extract and aqueous extract of 100, 200, and 500 mg were tested against different fungal pathogens, Aspergillus fumigates for their antifungal activity. It was demonstrated by a well diffusion assay.Gandhiraja et al 2009.

Antiviral properties: Four of the seven tested medicinal plants exhibited antimicrobial activity against Vibrio cholerae. These seven plants are: Ficus capensis, Mitragyna stipulosa, Entada Africana, Piliostigma reticulatum, Terminalia avicennoides, M. pudica, and Lannea acid. M. pudica showed antimicrobial activity. Potential of these herbs in the control of cholera needs to be determined.Akinsinde et al. 1995.

Aphrodisiac property: Ehanolic extract of roots of M. pudica Linn. (Mimosae) produced a significant and sustained increase in the aphrodisiac activity of normal male mice, without any adverse effects.Pande et al. 2009.

Materials and Method



Materials and Methods

3.1. Chemical study:

Generally the following methods are used throughout the experimental work-

- Collection and proper identification of the plant sample,
- Preparation of the plant material,
- Extraction,
- Solvent-solvent partitioning of the crude extract,
- Determination of total phenolics,
- Determination of total flavonoids,
- Determination of Cholinesterase inhibition,

3.1.1. In-vitro studies:

a. In- vitro antioxidant studies
b. In- vitro acetyl cholinesterase inhibitory studies

3.1.2. Material:

The fresh parts of the plant was selected for the chemical and biological investigations.

3.1.3. Collection of Plant Materials:

Since the plant is parasitic, the whole plant was collected from Dhaka an other districts of Bangladesh, in October, 2016 and identified by an expert taxonomist. A voucher specimen was submitted to the herbarium of the Department of Pharmacy, East West University.

Figure no. 3.1 : Collection of Plant.

3.1.4: Preparation of Plant Material:

The collected barks were first washed with water to remove adhering dirt and then shade dried for several days with occasional sun drying. These were then dried in an oven for 24 hours at considerably low temperature for better grinding. The dried barks were ground into coarse powder by a grinding machine in the Department of Pharmacy, Rajshahi University.

3.1.5. Cold extraction of the plant materials:

Powdered plant materials (barks) having a weight of about 1.6 kg were taken in an amber colored reagent bottle and soaked in 6.0 liter of methanol. The bottle with its contents were sealed and kept for a period of about 7 days with occasional shaking and stirring. The whole mixture was then filtered through cotton and then through Whatman No.1 filters paper and was concentrated with a rotary evaporator under reduced pressure at 50°C temperature to afford crude methanolic extract (CME).

Figure no. 3.2 : Cold Extraction of the extract.

3.1.6. Solvent-solvent partitioning of crude extract:

An aliquot (55.0 gm) of the concentrated methanolic extract was fractionated by modified Kupchan method and the resultant fractions that are petroleum ether, chloroform, Ethyl acetate, Aqueousfractions were obtained and used for the experiment purpose.

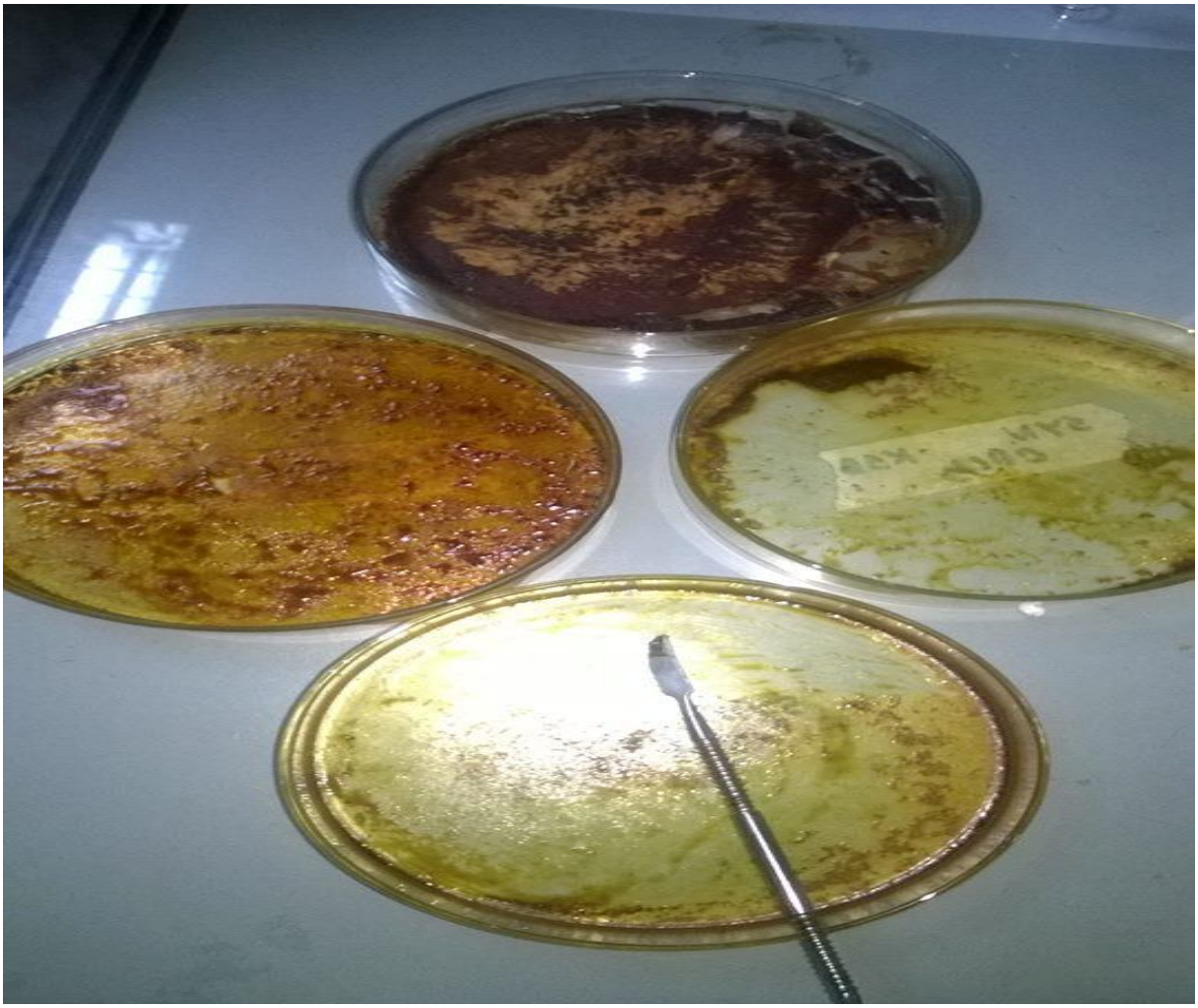


Figure no. 3.3 : Solvent-solvent partitioning of crude extract.

3.2: Determination of Total Phenolics:

Total phenolic content of the different extractives of L. globosuswere determined employing the method as described by Singleton in 1965 involving Folin-Ciocalteu reagent as oxidizing agent and catechin as standard.

3.2.1. Principle:

The content of total phenolic compounds of different fractions in the plant was determined by Folin–Ciocalteu Reagent (FCR). The FCR actually measures a sample's reducing capacity. The exact chemical nature of the FC reagent is not known, but it is believed to contain heteropolyphosphotungstates–molybdates. Sequences of reversible one or two-electron reduction reactions lead to blue species, possibly $(PMoW_{11}O_{40})_4$. In essence, it is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Mo (VI):

$$\mathbf{Mo\ (VI) + e^- \rightarrow Mo\ (V)}$$

3.2.2. Materials:

- Folin – ciocalteu reagent (Sigma chemical company, USA),
- Sodium carbonate (Sigma chemical company, USA),
- Methanol (Sigma chemical company, USA),
- Gallic acid (Wako pure chemicals Ltd., Japan),
- Micropipette (10-100 µl),
- Pipette (1-10 ml),
- UV-spectrophotometer (Shimadzu, USA).

3.2.3. Experimental procedure:

The amount of total phenolics in extract was determined according to the Folin-ciocalteu procedure. Samples (500µl) were introduced into test tubes. 2.5mL of Folincio-calteu reagent and 2.5 ml of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absobance at 760 nm was measured. The total phenolic content was expressed as Gallic acid equivalents (GAE) in milligrams per gram extract as calculated from standard Gallic acid graph by the following formula.

$$C = (c \times V)/m$$

Where,

C = total content of phenolic compounds, mg/g plant extract, in GAE;
c = the concentration of gallic acid established from the calibration curve, mg/ml;
V = the volume of extract, ml;
m = the weight of different pure plant extracts, gm.

Figure no. 3.4: Determination of total Phenolic compounds.

3.3. Determination of Total Flavonoids (TF):

Total flavonoid content of the different extractives of *L. globosus*was determined by aluminum chloride colorimetric method. Catechin was used as standard and the flavonoid content of the extractives was expressed as mg of catechin equivalent/gm of dried extract.

3.3.1. Principle:

The content of total flavonoids in different extractives of plant extract was determined by the well-known aluminum chloride colorimetric method.[181] In this method aluminum chloride forms complex with hydroxyl groups of flavonoids present in the samples. This complex has the maximum absorbance at 510 nm.

3.3.2. Materials:

- Aluminum Chloride (Sigma chemical company, USA)
- 5% $NaNO_2$
- 1 mMNaOH
- Methanol (Sigma chemical company, USA)
- Catechin (Wako pure chemicals Ltd., Japan)
- Micropipette (10-100 µl)
- Pipette (1-10 ml)
- UV-spectrophotometer (Shimadzu, Japan)

3.3.3. Experimental procedure:

Total flavonoid (TF) was determined using the procedure by Dewanto, Wu, Adom, and Liu, (2002). One milliliter of extract containing 0.1 g/mL of dry matter was placed in a 10 mL volumetric flask and then 5 mL of distilled water added followed by 0.3mL of 5% $NaNO_2$. After 5 min, 0.6 mL of 10% $AlCl_3$ was added. After another 5 min 2 mL of 1M NaOH was added and volume made up with distilled water. The solution was mixed and absorbance measured at 510 nm. TF amounts were expressed as catechin equivalents per dry matter. All samples were analyzed thrice and result averaged.

The total content of flavonoid compounds in plant extracts in catechinequivalents was calculated by the following formula equation

$$C = (c \times V)/m$$

Where,

C = total content of flavonoid compounds, mg/g plant extract, in catechin equivalent (GAE);

c = the concentration of catechin established from the calibration curve, mg/ml;

V = the volume of extract, ml;

m = the weight of pure plant extracts, gm.

3.4 Total Flavanol Content Determination

Total Flavanol content of the methanol extract of *D. blancoi* is determined by a method named aluminum chloride colorimetric method. This test requires gallic acid was as standard. The flavanol content of the extractives was denoted by mg of Gallic acid equivalent/gm of dried extract.

3.4.1 Principle

The amount of total flavanols in methanoic extract of *D. blancoi* was determined by the popular aluminum chloride colorimetric method. In this process, aluminum chloride forms complex with hydroxyl groups of flavanolswhich may be present in the samples. This formed complex has the highest absorbance at 440 nm.

Figure no. 3.5: Determination of total flavanol compounds.

3.4.2 Materials

- Aluminum Chloride 2% solution (Sigma chemical company, USA)
- Sodium acetate 5% solution
- Gallic acid
- Micropipette (20-200 µl, 100-1000 µl)
- Pipette (1-10 ml)
- UV-spectrophotometer (Shimadzu, Japan)

3.4.3 Procedure

Total flavanol content was identified by using aluminum chloride. As a standard Gallic acid was used. Initially, 300 µl sample was taken from the stock solution. This was made upto 1ml by adding methanol. Then 1ml of 2% aluminium chloride solution which was made with ethanol is added with the sample. After that 1.5 ml 5% sodium acetate solution was added. This mixture was incubated at room temperature for 2.5 hours. And finally, absorbance was taken at 440 nm.

The total content of flavonoid in plant extracts in Gallic acid equivalents was calculated by the following formula

$$C = (c \times V)/m$$

Where,

C = total content of flavonoid compounds, mg/g plant extract, in catechin equivalent (GAE);

c = the concentration of catechin established from the calibration curve, mg/ml;

V = the volume of extract, ml;

m = the weight of pure plant extracts, gm.

3.5 DPPH (1, 1-diphenyl-2-picrylhydrazyl) Free Radical Scavenging Assay:

DPPH was used to evaluate the free radical scavenging activity of various fractions, isolated pure compounds and column subfractions.

3.5.1 Principle:

The 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) has been widely used to evaluate the free radical scavenging capacity of antioxidants. DPPH free radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors. DPPH can make stable free radicals in aqueous or methanol solution. With this method it was possible to determine the antiradical power of an antioxidant activity by measurement of the decrease in the absorbance of DPPH at 517 nm. Resulting from a color change from purple to yellow the absorbance decreased when the DPPH was scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH molecule. In the radical form this molecule had an absorbance at 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule.

3.5.2 Materials:

- DPPH (Sigma chemical company, USA)
- Methanol (Sigma chemical company, USA)
- Catechin
- Pipette (1-10 ml)
- UV spectrophotometer (Shimadzu, Japan)

3.5.3 Experimental procedure:

The free radical scavenging activity of the extracts, different subcolumn fractions and isolated compounds of L. globosuswas detected based on the method described by Braca et al. (2001, J. Nat. Prod., 64, 892-895). Sample (2 ubml) will be added to 3ml of a 0.004% methanol solution of DPPH. Absorbance at 517 nm will be determined after 30 mins and the percentage inhibition activity was calculated from

$$\mathbf{I\% = [(A_0 – A_1)/A_0] \times 100,}$$

Where,

I% is the percentage of scavenging activity
A_0 is the absorbance of the control, and
A_1 is the absorbance of the extract/standard.

Then % inhibitions were plotted against concentration and from the graph IC_{50} was calculated.

3.6 Determination of Total Antioxidant Capacity:

Total antioxidant capacity of the different extractives, column subfractions and the isolated compounds of L. globosus was determined by the method of Prieto et al., (1999) with some modifications. [182]

3.6.1 Principle:

The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, α-tocopherol and carotenoids. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and subsequent formation of a green phosphate/Mo (V) complex at acidic pH.In essence, it is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Mo (VI) and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm.

$$\mathbf{Mo\ (VI) + e \rightarrow Mo\ (V)}$$

3.6.2 Materials

- Sulphuric acid (Merck, Germany)
- Sodium Phosphate (Sigma chemical company, USA)
- Ammonium Molybdate (Sigma chemical company, USA)
- Ascorbic acid (Analytical or Reagent grade)
- Methanol (Sigma chemical company, USA)
- Water bath
- Micropipette (100-1000 µl)
- Pipette (1-10 ml)
- UV-spectrophotometer (Shimadzu, Japan)

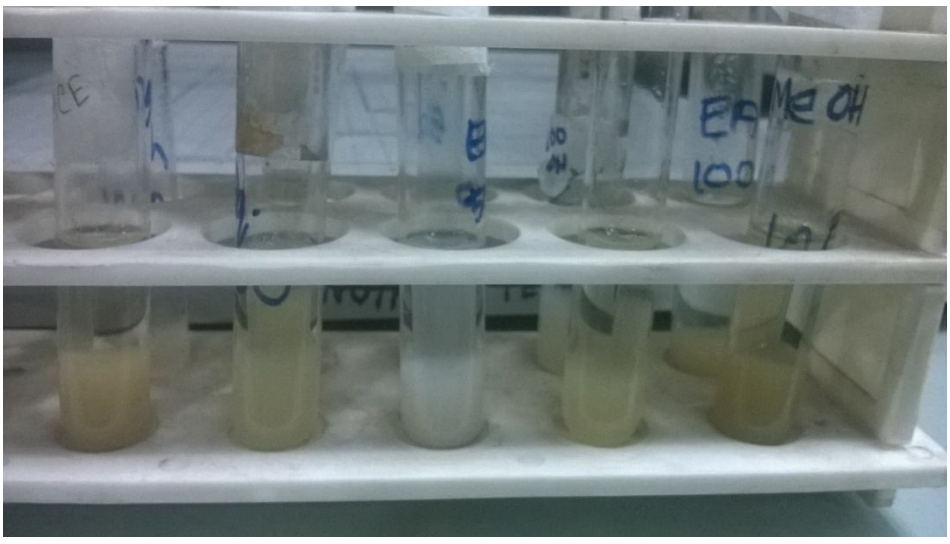


Figure no. 3.6: Determination of Total antioxidant assay.

3.6.3 Experimental procedure:

The sample (0.5 mL) was mixed with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) . The tubes were incubated at 95^0C for 90 min. The mixture was cooled to room temperature, then the absorbance of the solution was measured at 695 nm against blank. A typical blank solution contained 3 mL of reaction mixture and the same volume of solvent used for the sample, and it is incubated under the same conditions as the rest of the sample solution. The total antioxidant activity was expressed as compared with ascorbic acid .

3.7 Hydroxyl Radical Scavenging Assay:

Hydroxyl radical scavenging activity of different extractives, column subfractions and isolated compounds of *L. globosus* was determined by the method as described by Chung et al., (1997) with a slight modification.

3.7.1 Principle:

Hydroxyl radical was generated by the Fenton reaction. When Trichloro Acetic acid (TCA and H_2O_2 was incubated with Fe^{2+}-EDTA system at P^H 7.4, hydroxyl radicals were generated, and reacted with 2-deoxy-2-ribose to generate a malondialdehyde (MDA)-like product. This compound forms a pink chromogen upon heating with thiobarbituric acid (TBA) at low P^H. Samples with antiradical property can remove the hydroxyl radicals from 2-deoxy-D-ribose and prevent the formation of pink chromogen. In the radical form the pink chromogen had an absorbance at 532 nm. As the hydroxyl radicals are scavenged by antioxidant compound, the pink chromogen becomes decolorized and the intensity of color can be quantitatively measured at 532 nm.

3.7.2 Materials:

- 2-deoxy-D-ribose (Sigma-Aldrich, Japan)
- Thiobarbituric acid (Sigma-Aldrich, Japan)
- Phosphate buffer (Sigma-Aldrich, USA)
- EDTA (Sigma chemical company, USA)
- Hydrogen peroxide (Merck, Germany)
- $FeSO_4.7H_2O$ (Sigma chemical company, USA)
- Trichloro Acetic acid (Sigma-Aldrich, USA)
- (+)-Catechin (Sigma-Aldrich, Japan)
- Water bath
- UV spectrophotometer (Shimadzu, Japan)

3.7.3 Experimental Procedure:

The Fenton reaction mixture containing 200 µL of 10mM FeSO4.7H2O, 200 µL of 10mM EDTA and 200 µL of 10mM 2-deoxyribose was mixed with 1.2mL of 0.1M phosphate buffer (pH 7.4) containing 200 µL of samples. Thereafter, 200 µL of 10 mM H_2O_2 was added to the mixture before incubation for 4h at 37°C. Later, 1mL of 2.8% TCA and 1mL of 1% TBA were added and placed in a boiling water bath for 10min. Then, the resultant mixture was allowed to cool up to room temperature and centrifuged at 395g for 5 min. Absorbance was recorded at 532 nm in a UV-VIS spectrophotometer. The percentage (%) inhibition activity was calculated from the following equation.

$$\% I = \{(A_o - A_1)/A_o\} X 100$$

Where,

I% is the percentage of scavenging activity

A_0 is the absorbance of the control, and

A_1 is the absorbance of the extract/standard.

Then % inhibitions were plotted against concentration and from the graph IC_{50} was calculated.

3.8 Reducing Power Capacity Assessment:

The reducing power of the different extractives, compounds and column subfractions of*L. globosus*was evaluated by the method of Oyaizu (1986).

3.8.1 Principle:

In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The presence of reductants such as antioxidant substances in the samples causes the reduction of the Fe^{3+}-ferricyanide complex to the ferrous form by donating an electron. The amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm.

$$Fe^{3+}\text{-ferricyanide} + e^- \longrightarrow Fe^{2+}\text{-ferricyanide}$$

3.8.2 Materials:

- Potassium ferricyanide (Merck, Germany)
- Trichloro Acetic acid (Merck, Germany)
- Ferric Chloride (Sigma chemical company, USA)
- Phosphate buffer (Sigma-Aldrich, USA)
- Ascorbic acid (Sigma chemical company, USA)
- Water bath
- Centrifuge machine
- Pipette (1-10 ml)
- UV spectrophotometer (Shimadzu, USA)

3.8.3 Experimental Procedure:

Reducing power was investigated using the method developed by Oyaizu (1986). A 2.5 mL fraction of L. globosuswas mixed with 2.5 mL of phosphate buffer (200mM, p_H 6.6) and 2.5 mL 1% potassium ferricyanide. The mixture was placed in a water bath for 20 min at 50^0C. The resulting solution was cooled rapidly, mixed with 2.5 mL of 10% trichloroacetic acid and centrifuged at 3,000 rpm for 10 min. A 5.0 mL fraction from the supernatant was mixed with 5mL of distilled water and 1mL of ferric chloride. Absorbance of the resultant mixture was measured at 700 nm after 10 min. The higher the absorbance value the stronger the reducing power.

3.9 In-Vitro Acetyl Cholinesterase Inhibitory Studies:

3.9.1 Principle:

The acetylcholinesterase inhibitory activity of different extractives, column subfractionsand isolated compounds of L. globosus was determined by Ellman's method (Ellmanet al., 1961).[183] This method estimates AchE using acetylcholine iodide (substrate) and dithiobisnitro benzoic acid (DTNB). The enzymatic activity was measured by the yellow color compound produced by thiocholine when it reacts with dithiobisnitro benzoate ion.

Acetylcholine Thiocholine + Acetate $\longrightarrow$ Enzyme

Thiocholine + dithiobisnitro $\longrightarrow$ Benzoate yellow color

The color intensity can be measured on a spectrophotometer and the enzyme activity expressed as the rate of reaction per minute.

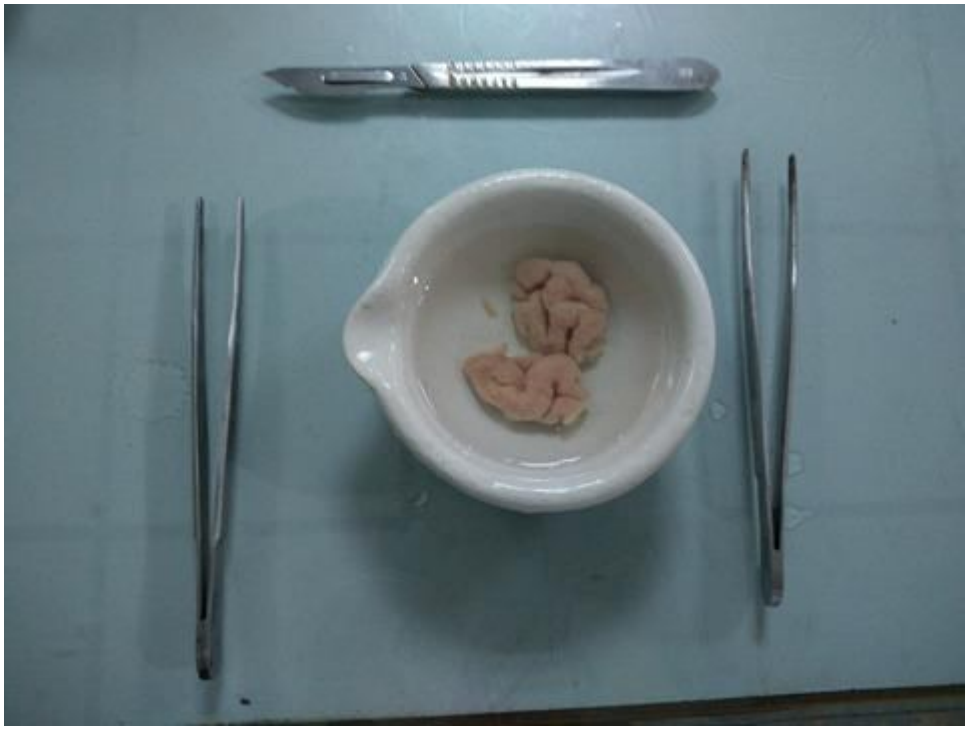


Figure no. 3.7: Brain (before extraction)

3.9.2 Materials:

- 5, 5´-dithio-bis-(2-nitro) benzoic acid (DTNB) (Sigma-Aldrich, Japan)
- Acetylthiocholine iodide (Sigma-Aldrich, Japan)
- Rat brain homogenate (Crude enzyme)
- Tris-Hcl buffer (Merck, Germany)
- Triton X-100 (Sigma chemical company, USA)
- BCA kit (bicinchoninic acid; Sigma Co., USA)
- Bovine serum albumin (Merck, India)
- Donepezil (Sigma-Aldrich, Japan)
- Micropipette (100-1000 µl)
- UV spectrophotometer (Shimadzu, USA)

3.9.3 Experimental Procedure:

The acetylcholinesterase (AChE) inhibitory assay was performed according to the colorimetric method of Ellman using acetylthiocholine iodide as a substrate. For the enzyme source, the rat brains were homogenized in a homogenizer with 5 volumes of a homogenation buffer [10 mMTris-HCl (pH 7.2), which contained 1 M NaCl, 50 mM $MgCl_2$

and 1% Triton X-100] and centrifuged at 10,000 rpmfor 30 min. The resulting supernatant was used as an enzyme source. All of the extraction steps were carried out at 4°C. Protein concentration was determined using the BCA kit (bicinchoninic acid; Sigma Co., USA) with bovine serum albumin (BSA) as a protein standard. The rates of hydrolysis by acetylcholinesterase were monitored spectrophotometrically. Each sample or standard (500 µl) was mixed with an enzyme solution (500 µl) and incubated at 37°C for 15 min. Absorbance at 405 nm was read immediately after adding an Ellman's reaction mixture [3.5 ml; 0.5 mMacetylthiocholine, 1 mM 5, 5´-dithio-bis (2-nitro benzoic acid)] in a 50 mM sodium phosphate buffer (pH 8.0) to the above reaction mixture. Reading was repeated for 10 min at 2 min intervals to verify that the reaction occurred linearly. The blank reaction was measured by substituting saline for the enzyme.

4.0 In- VitroButyryicholinesterase Inhibitory Studies:

4.0.1 Principle:

The butyrylcholinesterase inhibitory activity of different extractives, column subfractions and isolated compounds of L. globosus was determined by Ellman's method. This method estimates BChE using butyrylcholine iodide (substrate) and dithiobisnitro benzoic acid (DTNB). The enzymatic activity was measured by the yellow color compound produced by thiocholine when it reacts with dithiobisnitro benzoate ion.

The color intensity can be measured on a spectrophotometer and the enzyme activity expressed as the rate of reaction per minute.

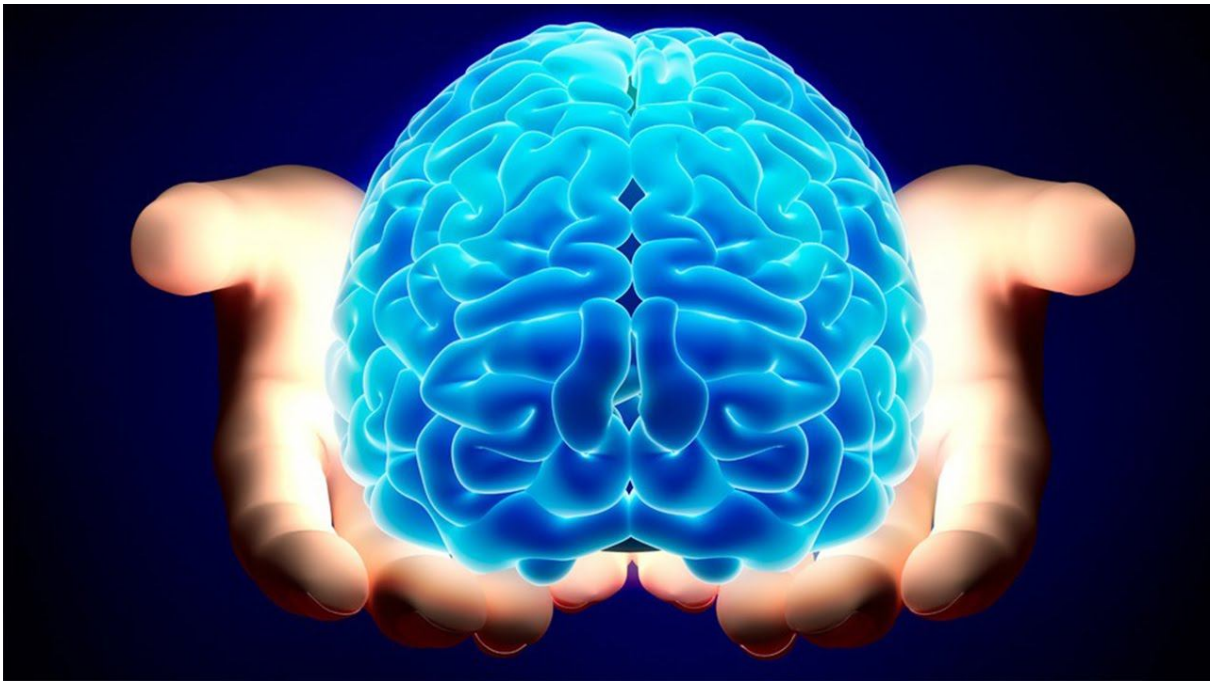
4.0.2 Materials:

- 5, 5´-dithio-bis-(2-nitro) benzoic acid (DTNB) (Sigma-Aldrich, Japan),
- Butyrylthiocholine iodide (Sigma-Aldrich, Japan),
- Human blood plasma (Crude enzyme),
- Tris-Hcl buffer (Merck, Germany),
- Triton X-100 (Sigma chemical company, USA),
- BCA kit (bicinchoninic acid; Sigma Co., USA),
- Bovine serum albumin (Merck, India),
- Donepezil (Sigma-Aldrich, Japan),
- Micropipette (100-1000 µl),
- UV spectrophotometer (Shimadzu, Japan),

4.0.3 Experimental procedure:

The butyrylcholinesterase (BuChE) inhibitory assay was performed according to the colorimetric method of Ellman using butyrylthiocholine iodide as a substrate. For the enzyme source, human blood are collected and centrifuged at 4000 rpm for 5 min. The resulting supernatant was used as an enzyme source. All of the extraction steps were carried out at 4°C. Then 50 µl enzyme, extraction buffer and plants extracts are incubated for 2 hours at room temperature. The rates of hydrolysis by butyrylcholinesterase were monitored spectrophotometrically. After 2 hours 200 µl DTNB (0.7mM) and 400 µl BTCI (0.35mM) added respectively. Heat this for 40 minutes at 37^0C. For measuring the background BTCI was avoided. Reading was taken at 412nm. From the difference between BTCI positive and negative data the activity of extract was measured. The blank reaction was measured by substituting saline for the enzyme.

Results



4.1Determination of Total Phenolics

Phenolic content of the crude methanolic extract, pet ether and chloroform fraction were determined using Folin-Ciocalteu reagent. Phenolic content of the samples were calculated on the basis of the standard curve for gallic acid as shown in Table 3.2 and in figure 3.1. The results were expressed as mg of gallic acid equivalent (GAE)/gm of dried extractives.

Table 4.1: Absorbance of gallic acid at different concentrations after treatment with FCR.

Concentration (µg/ml)	Absorbance			Mean ±STD
	A	b	c	
1	0.098	0.103	0.096	0.099 ± 0.003606
2	0.176	0.179	0.182	0.179 ± 0.003
4	0.403	0.411	0.401	0.405 ± 0.005292
8	0.785	0.789	0.792	0.789 ± 0.003512
16	1.452	1.456	1.432	1.447 ± 0.012858
32	2.654	2.664	2.659	2.659 ± 0.005

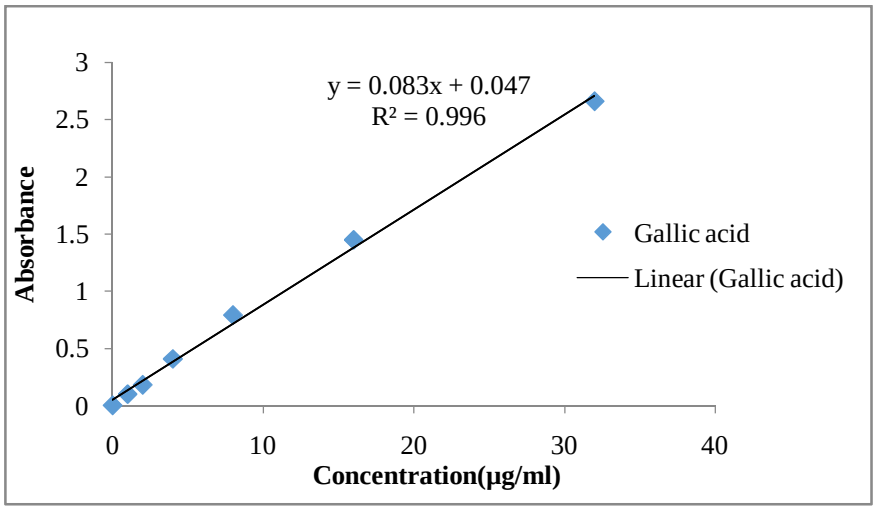


Figure 4.1: Standard curve of gallic acid for the determination of total phenolics.

Table 4.2: Determination of total phenolic content

Plant Name	Sample	Conc. (µg/ml)	Absorbance	GAE/gm of dried sample
Whole Plant	**CME**	200	1.066	71.63
Whole Plant	**CME**	500	2.184	147.30

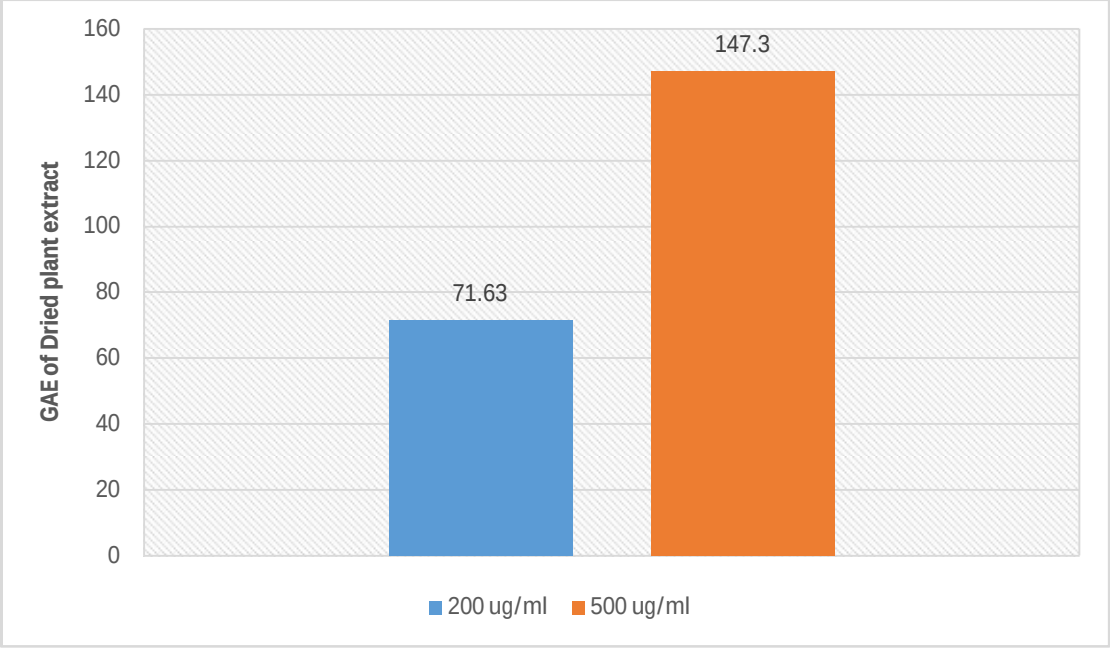


Figure 4.2: Total phenolic content (mg/gm plant extract in gallic acid equivalent)

4.2 Determination of Total Flavonoids

Total flavonoids content of crude methanol extract (CME), pet ether and chloroform fractions were determined using much known aluminum chloride colorimetric method. Flavonoid content of the samples was calculated on the basis of the standard curve for catechin as shown in Table and in Fig. The results were expressed as mg of catechin equivalent (CE)/gm of dried sample.

Table 4.3: Absorbance of catechin at different concentrations for quantitative determination of total flavonoids

Concentration (µg/ml)	Absorbance			Absorbance Mean ± STD
	a	b	c	
31.25	0.241	0.238	0.244	0.241 ± 0.003
62.5	0.380	0.378	0.382	0.38 ± 0.002
125	0.726	0.720	0.732	0.726 ± 0.006
250	1.476	1.472	1.480	1.476 ± 0.004
500	2.667	2.657	2.677	2.667 ± 0.007

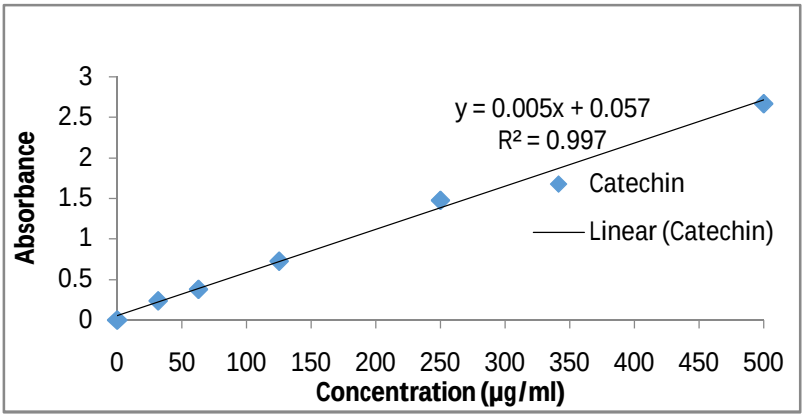


Figure 4.3: Standard curve of catechin for the determination of total flavonoids

Table 4.4: Determination of total flavonoid content

Plant Name *D. blancoi*	Sample	Conc. (µg/ml)	Absorbance	CE/gm of dried sample
Whole Plant	**CME**	200	0.136	5.71
Whole Plant	**CME**	500	0.231	24.26

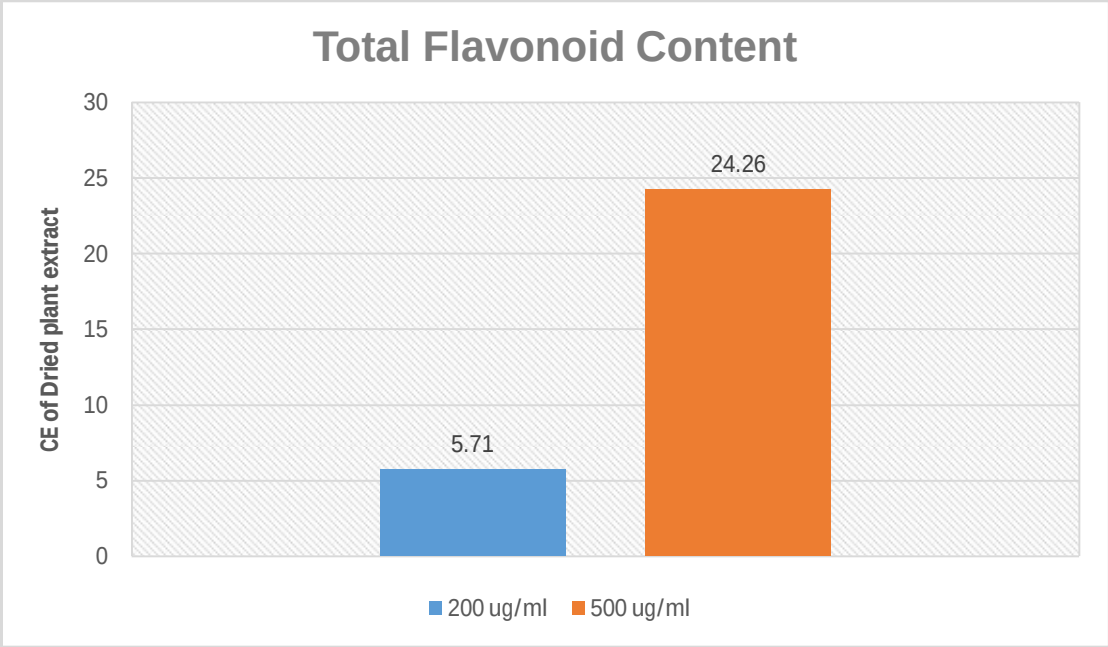


Figure 4.4: Total flavonoid content (mg/gm plant extract in catechin equivalent) of CME, PET, CLF

4.3 Determination of total flavanol

Flavanol content of the crude methanolic extract, pet ether and chloroform fraction were determined using reagents. The results were expressed as mg of gallic acid equivalent (GAE)/gm of dried extractives.

Table 4.5:Absorbance of gallic acid at different concentrations

Concentration (µg/ml)	Absorbance			Mean ±STD
	A	b	c	
1	0.098	0.103	0.096	0.099 ± 0.003606
2	0.176	0.179	0.182	0.179 ± 0.003
4	0.403	0.411	0.401	0.405 ± 0.005292
8	0.785	0.789	0.792	0.789 ± 0.003512
16	1.452	1.456	1.432	1.447 ± 0.012858
32	2.654	2.664	2.659	2.659 ± 0.005

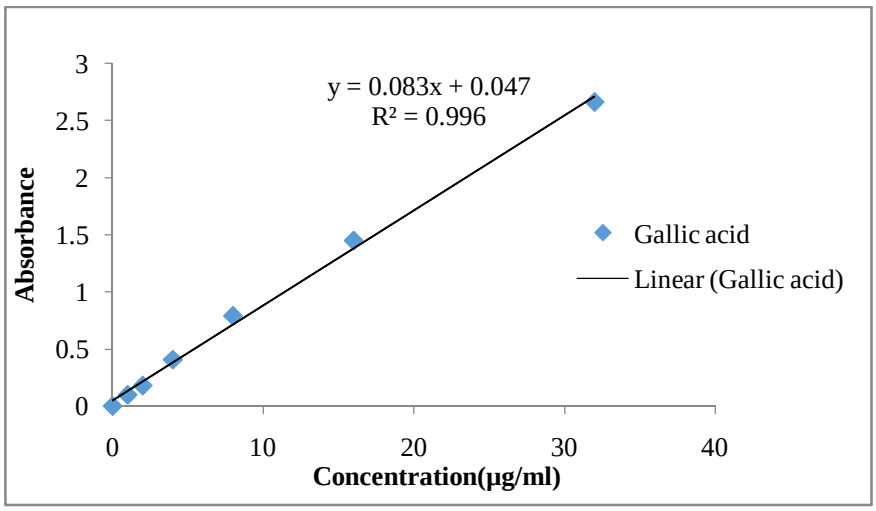


Figure 4.5: Standard curve of gallic acid for the determination of total flavanols.

Table 4.6: Determination of total flavanol content

Plant Name ***D. blancoi***	**Sample**	**Conc. (µg/ml)**	**Absorbance**	**GAE/gm of dried sample**
Whole plant	**CME**	200	1.207	32.25
Whole Plant	**CME**	500	2.663	81.13

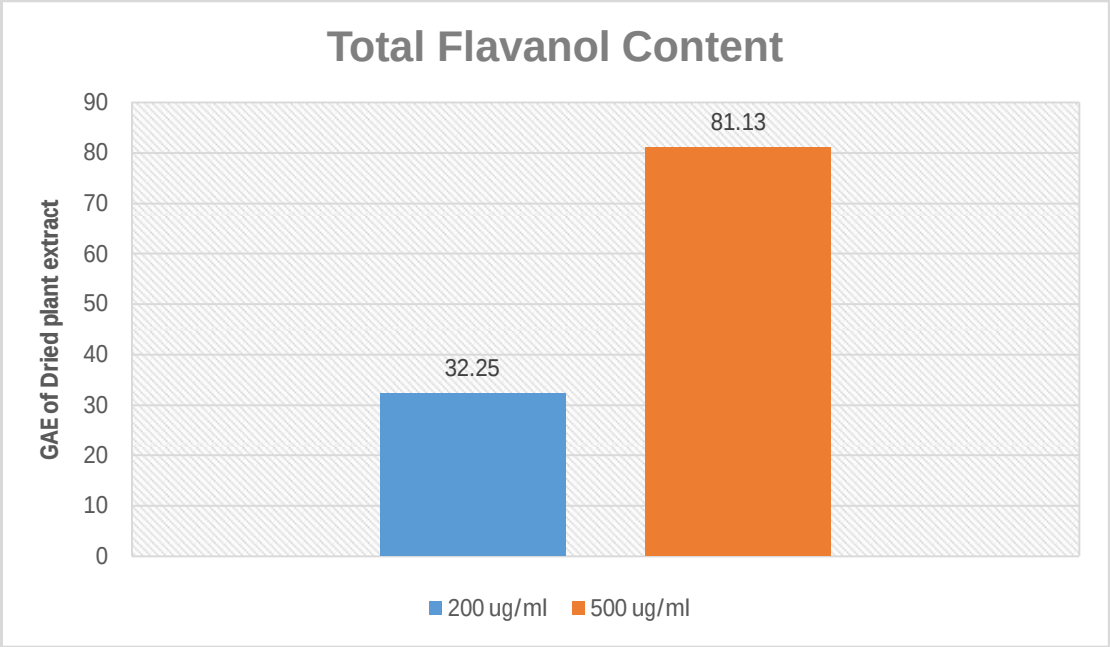


Figure 4.6: Total flavonol content (mg/gm plant extract in gallic acid equivalent)

4.4DPPH Radical Scavenging Activity

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples including plant extracts. DPPH antioxidant assay is based on the ability of 1, 1 diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the change in absorbance and % of scavenging activity is calculated.

Table 4.7: % of inhibition of different parts of the plant

Name of Plant Part	Concentration (µg/µg)	Absorbance	% of Inhibition
Whole Plant	20	1.303	51.67

CME	50	0.933	65.31
	100	0.268	90.03
	200	0.059	97.37
Catechin (Standard)	20	0.19	87.39
	50	0.14	93.87
	100	0.11	94.16
	200	0.09	95.77

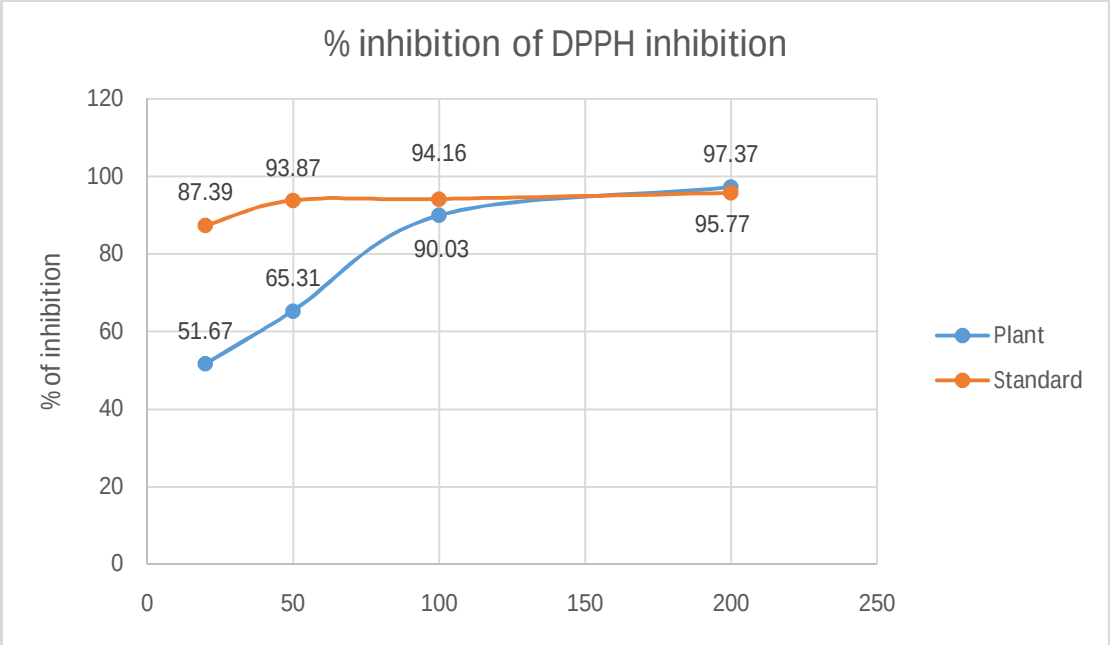


Figure 4.7: % of inhibition of different parts of plants by DPPH radical scavenging activity

4.5 Reducing Power Capacity

Cfractions was determined by the method of Oyaizu (1986) with slight modification. The reductive capabilities of crude methanol extract (CME) and its four fractions and the reference standard catechin are shown in Table.

Table 4.8: Chart of absorbance for reducing power capacity

Name of Plant	Concentration (µg/µg)	Absorbance
Whole Plant (CME)	100	0.230
Catechin	100	2.660

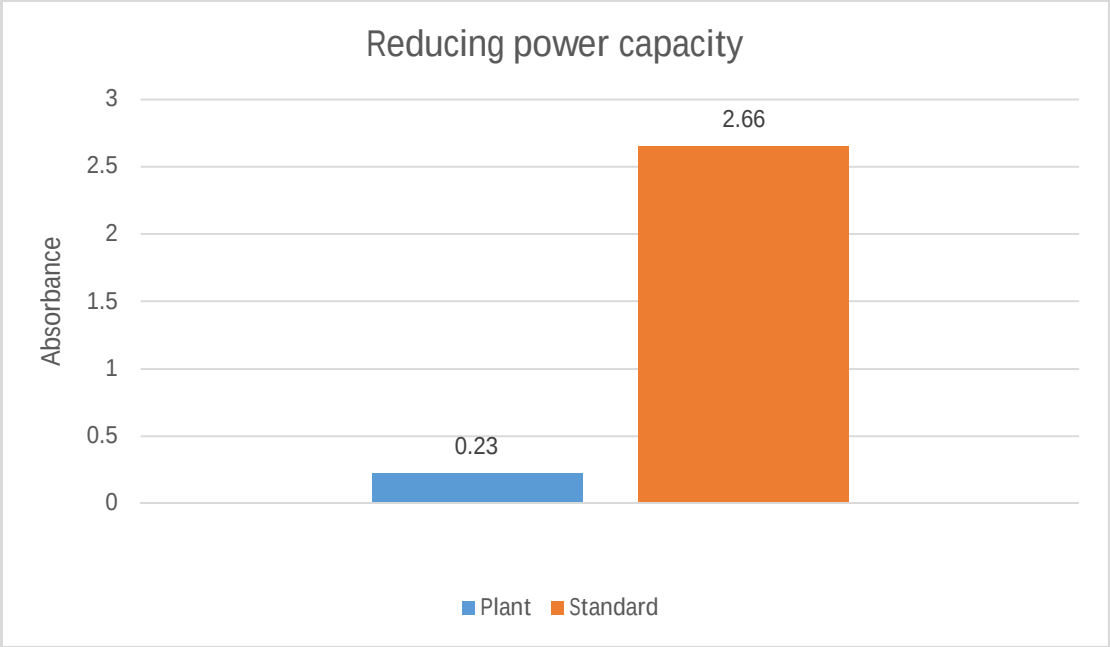


Figure4.8: **Reducing power capacity of CME extract**

4.6Acetyl cholinesterase inhibitory activity assay

Inhibition of acetylcholinesterase, which enhances cholinergic transmission by reducing the enzymatic degradation of acetylcholine, is a widely accepted strategy for the development of AD drug. In this study, the acetylcholinesterase inhibitory activity of the crude methanol extract was assessed by modified Ellman'smethod and

compared with the reference standard donepezil. This method estimates acetylcholinesterase (AChE) using acetylcholine iodide (substrate) and dithiobisnitro benzoic acid (DTNB). The enzymatic activity was measured by the yellow color compound produced by thiocholine when it reacts with dithiobisnitro benzoate ion.

Table 4.9: % of inhibition for acetylcholinesterase inhibitory activity assay

Name of sample	Conc. (µg/ml)	% of inhibition Mean
Donepezil (Std)	20	78.34
	50	86.12
	100	91.44
	200	92.14
Whole Plant (CME)	20	4.78
	50	12.11
	100	26.22
	200	32.35

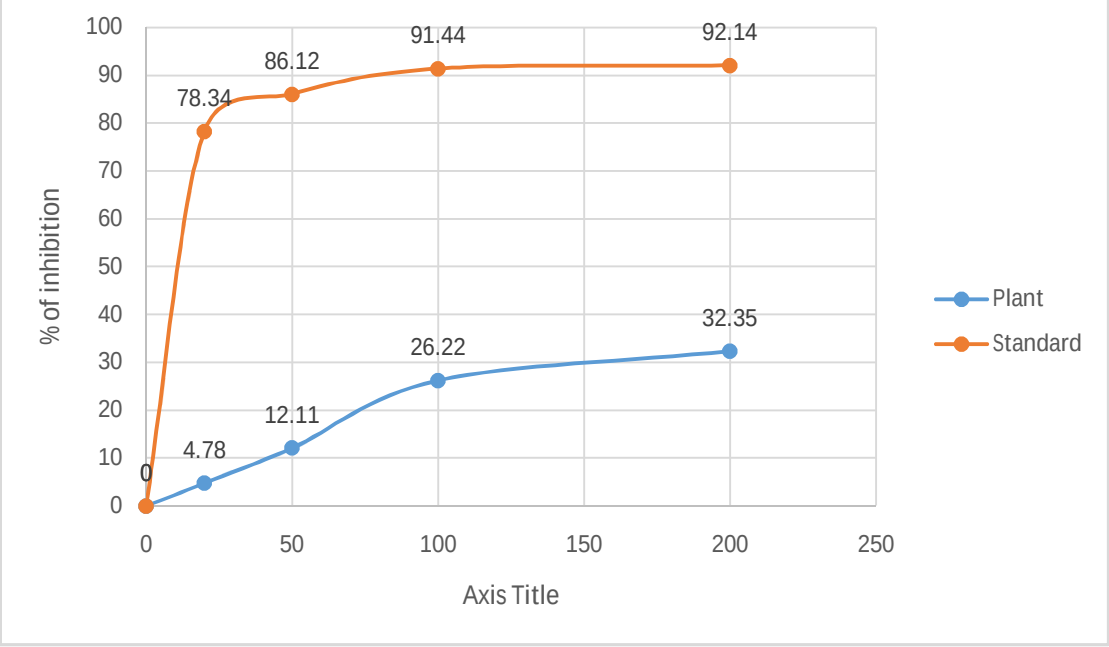


Figure 4.9: % of inhibition for acetylcholinesterase inhibitory activity assay

4.7 Butyrylcholinesterase inhibitory activity of enzymes

Butyrylcholinestserase enhances cholinergic transmission by reducing the enzymatic degradation of both acetylcholine and butyrylcholine. Thus inhibition of butyrylcholinesterase increases the neurotransmission not only in brain but also in other neuronal junctions. This strategy is a widely accepted most advance strategy for the development of AD drug. Butyrylcholinesterase inhibitors have synergistic activity of acetylcholinesterase inhibitory activity.

Table 4.10: % of inhibition for butyrylcholinesterase inhibitory activity assay

Name of sample	Conc. (µg/ml)	% of inhibition Mean
Galantamine (Std)	20	72.43
	50	86.17
	100	88.42
	200	91.41
Whole Plant (CME)	20	5.22
	50	10.11
	100	22.09
	200	36.19

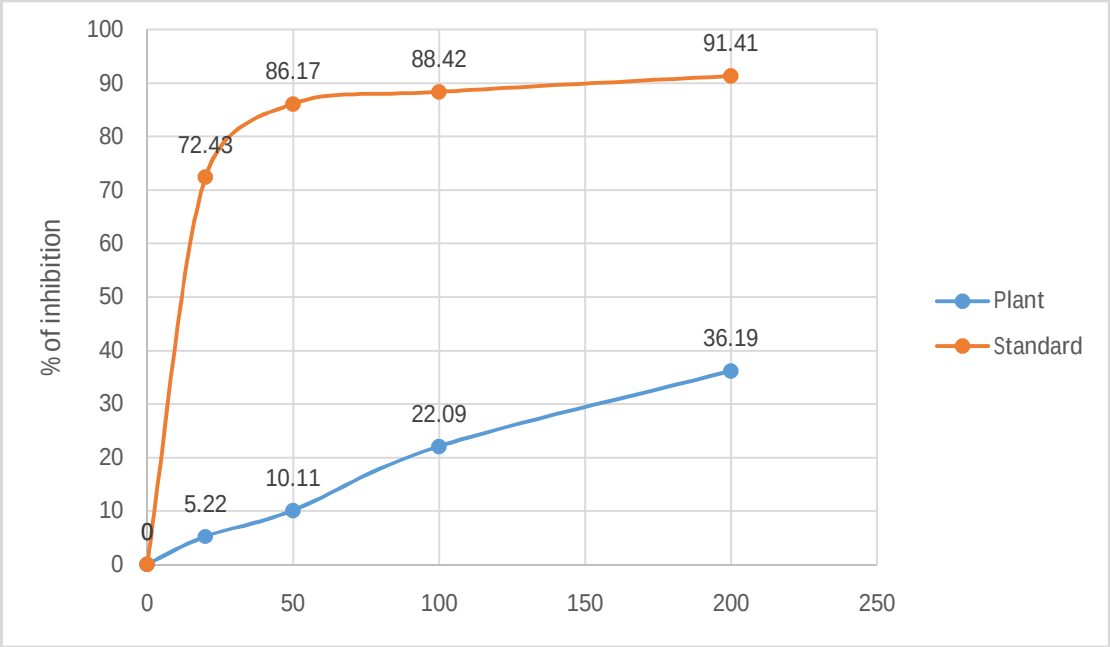
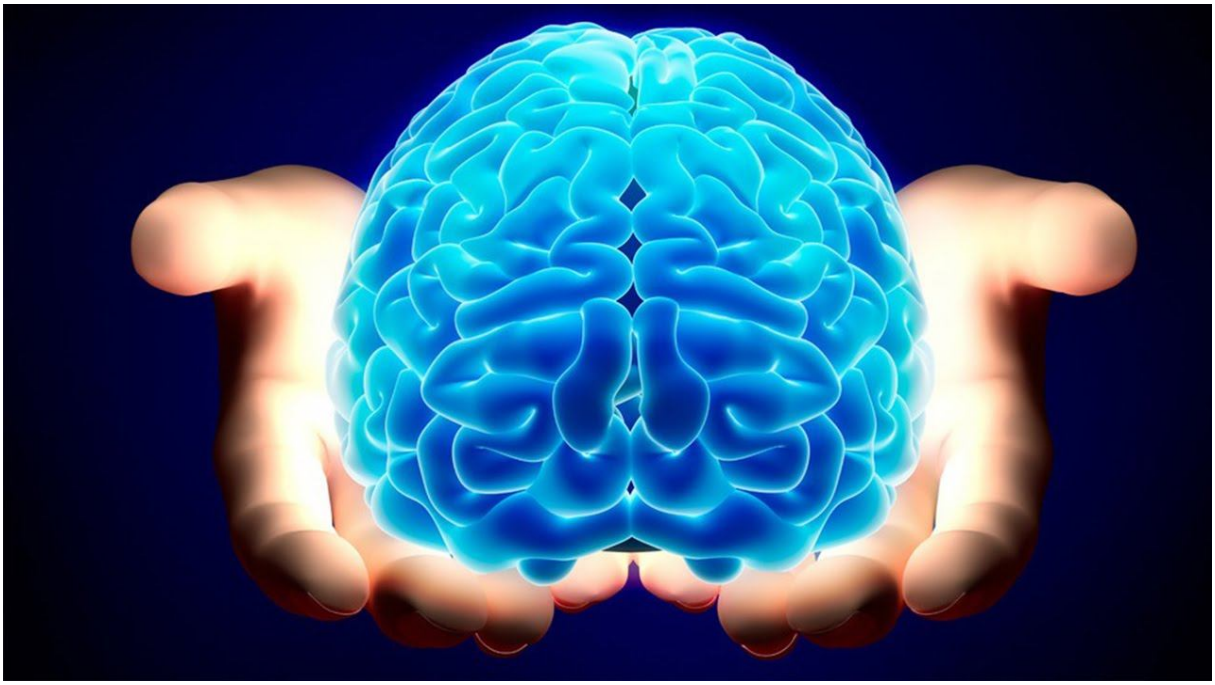


Figure 4.10:% of inhibition for acetylcholinesterase inhibitory activity assay

Discussion and Conclusion



Chapter 5
Discussion and Conclusion

5.1 Determination of Total Phenolics:

The Total Phenolic content of the crude extract of *Mimosa pudica* can be determined by using Folin-Ciocalteu reagent. The crude methanolic extract, pet ether and chloroform fraction were tested. From the table of the result, the crude methanolic extract of *Mimosa pudica* in concentration of 200µg/ml gives absorbance of 1.066 for which Gallic acid equivalent per gram (GAE/gm of dried sample) of dried sample is 71.63. On the other hand, the crude methanolic extract of *Mimosa pudica* in concentration of 500µg/ml shows 2.184 in absorbance and GAE/gm of dried sample is 147.30. So it is clear that, phenolic compounds are increased according to increased concentration of crude extract. These also indicates the presence of high phenolic compound in the molecule.

5.2 Determination of Total Flavonoids:

Total flavonoids content were calculated by using aluminuim chloride colorimetric method. The crude methanolic extract, pet ether, chloroform and n-hexane fraction were tested. From the table of total flavanoid content, it can be said that the crude methanolic extract of *Mimosa pudica* gives absorbance of 0.136 in concentration of 200µg/ml where the catechin equivalent/gm (CE/gm of dried sample) of dried sample is 5.71. Additionally another concentration of 500µg/ml of methanolic extract of *Mimosa pudica* shows absorbance at 0.231 for which the catechin equivalent/gm (CE/gm of dried sample) of dried sample is 24.26. That means the crude extract is rich in flavanoid compounds as CE/gm of dried sample increases with increased concentration. The fraction of the plant is not the rich source of flavonoids.

5.3 Determination of Total flavanol:

The total content of flavanol was assayed by using aluminium chloride colorimetric method. The crude methanolic extract, pet ether and chloroform fraction were tested. From the table of total flavanol content is observed that *Mimosa pudica* having crude methanolic extract gives absorbance of 1.207 in concentration of 200µg/ml for which Gallic acid equivalent/gm of dried sample (GAE/gm of dried sample) is 32.25. Crude Methanolic sample having concentration of 500µg/ml gives absorbance of 2.663 with a calculated GAE/gm of dried sample 81.13. The plant can be a prominent source of flavanol as 200µg/ml CME contain 32.25 GAE/gm of dried sample. Similar to flavonoids this plant is a poor source of favanol but in comparison with flavonoids, contain more flavonols than flavonoids.

5.4 DPPH Radical Scavenging Activity:

The evaluation of free radical scavenging activities is done by using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). From the table of DPPH radical scavenging activity testof methanolic crude extract of *Mimosa pudica* gives percent of inhibition 51.67, 65.31, 90.03, 97.37 for the concentration of 20µl, 50µl, 100µl, 200µl respectively with the absorbance of 1.303, 0.933, 0.268, 0.059 accordingly. Standard Catechin has the percent of inhibiton 87.39, 93.87, 94.16, 95.77 in the concentration of 20µl, 50µl, 100µl, 200µl having the absorbance of 0.19, 0.14, 0.11, 0.09 respectively. *Mimosa pudica* has moderate inhibition capacity. Compared to the percentage of inhibition of Catechin standard in same concentration crude methanolic extract of the plant has less inhibitory activity. Due to higher purity of the standard, extracts how less activity or it may contain other antagonistic or agonistic compounds. From this test we can see this plant is a promising source of antioxidants as it contain similar activity to standard.

5.5 Reducing Power Capacity:

The assessment of reducing power capacity was done by the method of Oyaizu (1986). Reducing power capacity table shows that the crude methanolic extract of *Mimosa pudica* gives an absorbance of 0.230 in the concentration of 100µg/ml. The absorbance of standard catechin is 2.660 in the concentration of 100µg/ml. However, comparing with Catechin standard the highest reducing power capacity is not present in the plant. The activity of the sample plants are much less because the purity of standard is higher than the crude extract may contain many other agonistic or antagonistic compounds. So, there is a chance to form more active molecules from those plants.

5.6 Acetyl cholinesterase inhibitory activity assay:

The evaluation of acetylcholinesterase inhibitory activity is done by Ellman's method and compared with the reference standard donepezil. Table of Acetylcholinesterase inhibitory activity represents that *Mimosa pudica* shows percent of inhibition 4.78, 12.11, 26.22, 32.35 for the concentration of 20µg/ml, 50µg/ml, 100µg/ml, 200µg/ml respectively. Dopenzil has present inhibition values of 78.34, 86.12, 91.44, 92.14. Compare to standard, the drug of choice, dopenzil, the crude extract shows mild acetylcholinesterase enzyme inhibitory activity.

5.7 Butyrylcholinesterase inhibitory activity of enzymes:

The determination of Butylcholinesterase enzyme inhibitory activity is done by Ellman's method. Butylcholinesterase inhibitory activity table presents that *Mimosa pudica* in the concentration of 20µg/ml, 50µg/ml, 100µg/ml and 200µg/ml shows the percentage of inhibition 5.22, 10.11, 22.09, 36.19. Galanthamine used as a standard having the percentage of inhibition of 72.43, 86.17, 88.42, 91.41 in the same concentration respectively. The crude extract has mild inhibitory activity that the standard drug.

Conclusion

The plant is found moderate active in inhibiting Acetylcholinsterase enzyme. Its total phenolic content, total flavonoid content and also its total flavanol content is also promising, so do the reducing power of the plant. As it is only crude methyl extract of the plant, so further research is need to find out the most effective molecule from the mixture of molecule that is biologically active.

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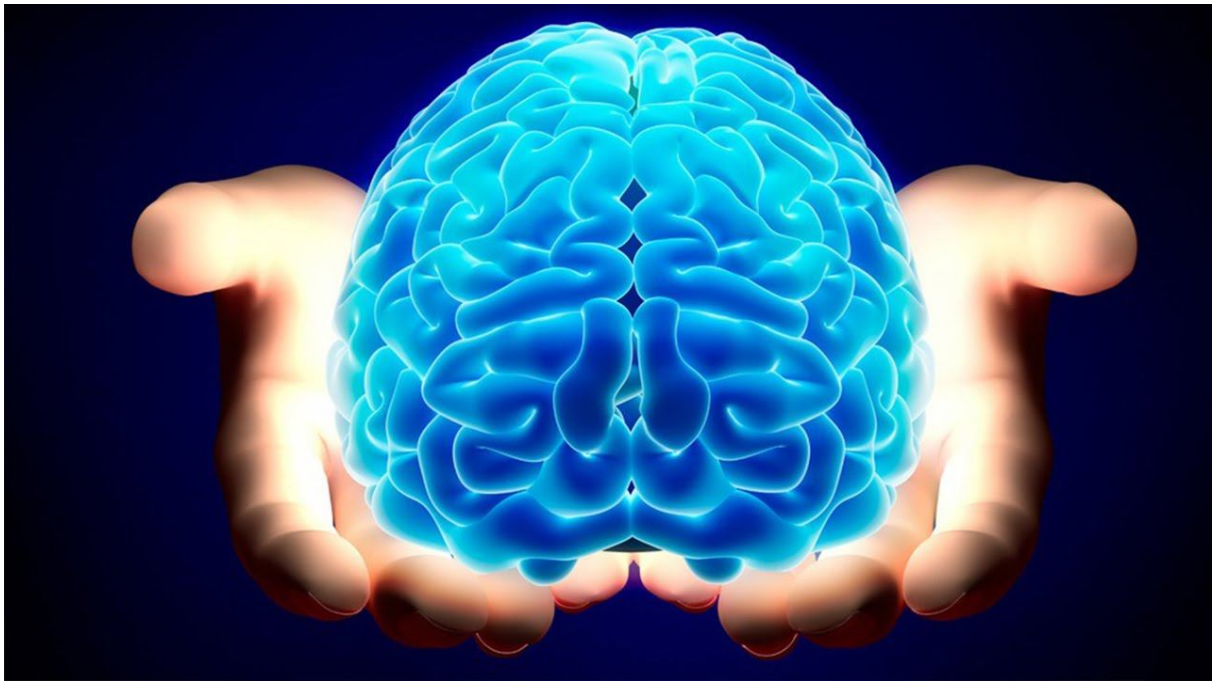

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