A DISSERTATION ON

Evaluation of *in-vitro* Antioxidant and Acetylcholinesterase Inhibitory Potential of Night Blooming Jasmine (*Cestrum nocturnum*)

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CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **Ms. Mantasha Ahsaan** during the months Feb–May, 2022 reported in the present thesis was under my guidance and supervision. The results reported by her are genuine and script of the thesis has been written by the candidate herself. The thesis entitled is **"Evaluation of in-vitro Antioxidant and Acetylcholinesterase Inhibitory Potential of Night Blooming Jasmine *(Cestrum nocturnum)*"** therefore, being forwarded for the acceptance in partial fulfilment of the requirements for the award of the degree of Master of Science in Biotechnology, Department of Biosciences, Integral University, Lucknow**.**

Date:**20/June/2022**
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TO WHOM IT MAY CONCERN

This is to certify that the study conducted by **Ms. Mantasha Ahsaan** student of M.Sc. Biotechnology (IV semester), Integral University has completed her four months dissertation work entitled **"Evaluation of in-vitro Antioxidant and Acetylcholinesterase Inhibitory Potential of Night Blooming Jasmine *(Cestrum nocturnum)*"** successfully. she has completed this work from the Department of Biosciences, Integral University, under the guidance of **Dr. M. Salman Khan.** This dissertation was a compulsory part of her M.Sc. Degree.

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Before I present my work, I would like to gratefully acknowledge the contribution of all those people who have helped in the work described in this Dissertation. I am going to try anyway, and if your name is not listed, it is rest assured that my gratitude is not less than for those listed below.

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Date: **20/June/2022**

Place: **Lucknow** **Ms. Mantasha Ahsan**

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ABBREVIATIONS

AD	Alzheimer’s Disease
apoE	Apolipoprotein
ROS	Reactive oxygen species
CSF	Cerebrospinal fluid
AchE	Acetylcholinesterase
NMDA	N-methyl-D-aspartate
COPD	Chronic obstructive pulmonary disease
SPECT	Single-photon emission tomography
CADASIL	Cerebral Autosomal Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy
TDP 43	TAR DNA-binding protein 43
DLB	Dementia with lewy bodies
APP	Amyloid precursor protein
FTLD	Frontotemporal lobar degeneration
FTD	Frontotemporal dementia
DAT	Dopamine transporter
PHF	Paired helical filaments
NFT	Neurofibrillary tangles
SPs	Senile plaques

JNK	Jun amino-terminal kinase
AchEIs	acetylcholinesterase inhibitors
DPPH	1,1-diphenyl-2-picrylhydrazyl
ABTS	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid
IC50	Inhibitory concentration 50
DC	Dense core
ELISA	Enzyme linked immunosorbent assay
PA	Pathological ageing
HIV	Human immunodeficiency virus
PS-1	Presenilin 1
PS-2	Presenilin 2
CBS	Corticobasal syndrome
VCP	Valosin containing protein
MAPT	Microtubule associated protein
FUS	Fused in sarcoma

INTRODUCTION

Alzheimer's disease (AD) has been considered as the major worldwide health anxieties as it shares about 60–80% of the pathologies of dementia (Wortmann, 2012). According to a recent estimation, there are more than 45–50 million subjects suffering with epilepsy and dementia across the globe, and this count is increasing with a rise of 7.70 million newly diagnosed cases annually, whereas the contribution of migraine is also influential among neurodegenerative disorders (Wimo A.& Prince M., 2010). A lot of evidence suggests that neurodegenerative diseases, including AD, stem from the abnormal accumulation of harmful proteins in the nervous system. In AD, these include Aβ peptides, the lipid-carrier protein apolipoprotein E (apoE), the microtubule associated protein tau, and the presynaptic protein α-synuclein, which is also involved in growth factors. A form of apolipoprotein E, apoE4, contributes to the abnormal accumulation of Aβ and tau, but probably also damages mitochondria and the cellular cytoskeleton. Aβ, tau, apoE and α-synuclein interact with many other molecules and modulate diverse signalling cascades that regulate neuronal activity and survival. Genetically modified rodents and other experimental models are being used to tease out this complexity and to determine which biochemical cascades have the greatest impact on the initiation and progression of the disease (Mucke, 2009a). Among distinct pathologies of AD, "amyloid hypothesis" has gained the most attention, which refers to the aggregation of amyloid-beta (A-β) as a major determinant of the continuous death of brain neuronal cells. The most challenging pathologies of AD are erratic emotion, impaired memory, sleep disorders and loneliness which have been linked to A-β-stimulated injury to cholinergic neurons, inflammation, reactive oxygen species (ROS) and excitotoxicity mechanisms (Whitehouse et al., 1982).Aggregation and accumulation of amyloid-β (Aβ) in the brain may result from increased neuronal production of Aβ, decreased activity of Aβ-degrading enzymes, or alterations in transport processes that shuttle Aβ across the blood–brain barrier. Aβ oligomers impair synaptic functions, whereas fibrillar amyloid plaques displace and distort neuronal processes. Aβ oligomers interact with cell-surface membranes and receptors,

altering signal-transduction cascades, changing neuronal activities and triggering the release of neurotoxic mediators by microglia (resident immune cells). Vascular abnormalities impair the supply of nutrients and removal of metabolic by-products, cause microinfarcts and promote the activation of astrocytes and microglia. The lipid-carrier protein apoE4 increases Aβ production and impairs Aβ clearance. When produced within stressed neurons, apoE4 is cleaved into neurotoxic fragments that destabilize the cytoskeleton and, like intracellular Aβ, impair mitochondrial functions. The proteins tau and α-synuclein can also self-assemble into pathogenic oligomers and can form larger intra-neuronal aggregates, displacing vital intracellular organelles (Mucke, 2009b)

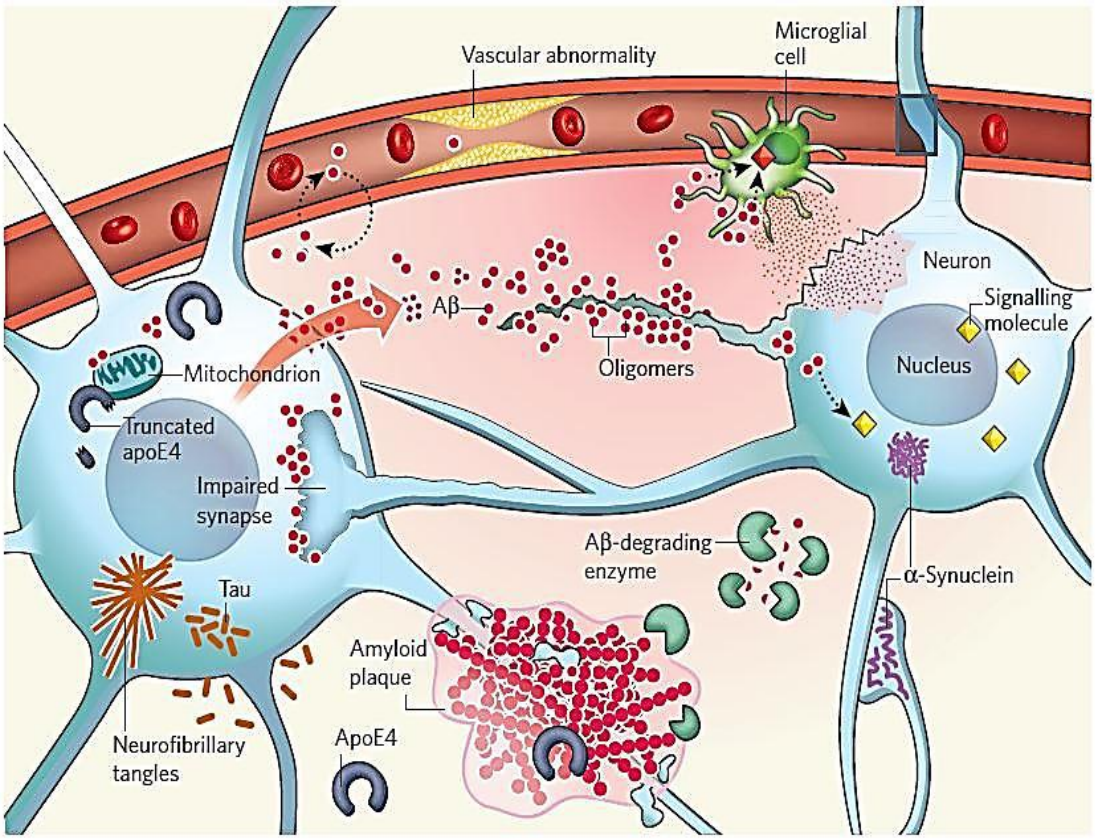


Figure:1 Key elements in the pathogenesis of AD

Diagnosing AD with 100% certainty requires a detailed post-mortem microscopic examination of the brain. But nowadays, AD can be diagnosed with more than 95% accuracy in living patients by using a combination of tools. These include taking a careful history from patients and their families, and

assessing cognitive function by neuropsychological tests. Other causes of dementia must be ruled out, such as low thyroid function, vitamin deficiencies, infections, cancer and depression. It's also crucial to differentiate AD from other neurodegenerative dementias, including frontotemporal dementia, Lewy-body dementia and Creutzfeldt–Jakob disease. Brain imaging and tests of cerebrospinal fluid (CSF) can help to distinguish AD from these conditions. Patients with AD typically show shrinkage of brain regions involved in learning and memory on magnetic resonance images, as well as decreased glucose metabolism and increased uptake of radioligands that detect abnormal protein deposits (amyloid) on positron emission tomography scans. CSF abnormalities include low levels of amyloid-β (Aβ) peptides and increased levels of the protein tau (Khachaturian,1985). The site of the origin of the cortical cholinergic neurons, forebrain region, is a crucial target for most of the AD pathologies, and neuronal loss in this part of the brain reflects the degree as well as severity of AD symptomatology (Whitehouse et al., 1982).Till date, various classes of drugs have been tested to alleviate AD which are the acetylcholinesterase (AChE) inhibitors, e.g. rivastigmine, tacrine and donepezil, and antagonist of N-methyl-D-aspartate (NMDA) receptor such as memantine (Raschetti et al., 2007). Acetylcholinesterase (AChE) which is the main enzyme involved in the hydrolysis of acetylcholine (ACh). Presently, the drugs used to treat patients suffering from the AD act as intensifiers of the acetylcholine level in the brain, accounting for central cholinergic transmission (Wszelaki et al., 2010). Acetylcholine is hydrolyzed to a choline and an acetyl group by the enzyme acetylcholinesterase, after being conveyed across neuronal synapses. Consequently, acetylcholine is not hydrolyzed after applying acetylcholinesterase inhibitors, maintaining its activity as a neurotransmitter (Balkis et al., 2015; Knapp et al., 1994).

Night blooming jasmine, botanically known as *"Cestrum nocturnum"* (*C. nocturnum*) is an evergreen shrub that grows in tropical and sub-tropical regions throughout the world. *"C. nocturnum"* is a popular ornamental plant due to its showy and fragrant white flowers. It is also used as a hedge plant and cultivated as a medicinal plant. The flowers volatile compound was identified as phenylacetylaldehyde and linalool (Li *et al.,*) The leaves of *C. nocturnum* have

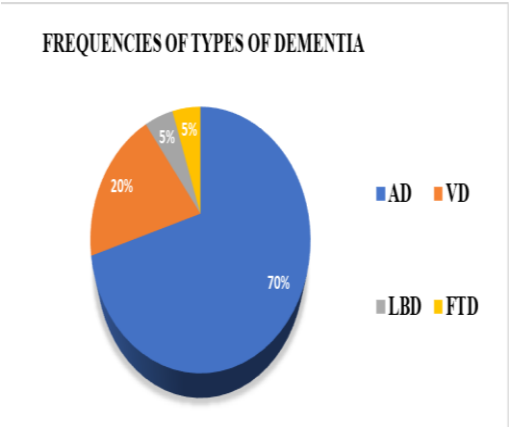
pharmacological significance in Chinese folks' medicine and have been used for the treatment of burns and swelling. The medicinal properties of night blooming jasmine include antioxidant, antihyperlipidemic, hepatoprotective, analgesic, antifungal, anticonvulsant, anti-HIV and larvicidal activities (Rokade *et al.,* 2018).

REVIEW OF LITERATURE

Dementia

Dementia is associated with cognitive memory loss and impairment in physical functions. It does not indicate a specific disease or disorder but rather it is a general term linked with neurological disorders. The characteristic features of dementia are problems with memory, thinking, language, and visual perception. It is common in elderly people and regarded as a progressive condition. The occurrence is nearly 1 percent at the age of 65 and doubles for five consecutive years to attain 50 percent at the age of 85 (Approach, 2015).

The prevalence of dementia is 3 to 6 times higher in patients with stroke. It is seen that the frequency is 32% in the population who experienced a first-time stroke in the age group of 60-80 years. In stroke patients, pre-existing dementia accounts for 7-16% but remains undiagnosed. (Leys *et al*., 2005). The statistics revealed that approximately 1.3% of all age groups in the United Kingdom have dementia. Further global findings revealed a doubling up of dementia cases every 20 years. European findings revealed that women have a higher occurrence of dementia than men (Rocca *et al*., 2014). The key factors contributing to dementia are gender; women are more vulnerable to dementia, lack of education and some professions have emerged as possible risk factors (Katzman & Kawas, 1994). The most common type of dementia is Alzheimer's disease which holds for 50-75% accompanied by vascular dementia, around 20%, Lewy body dementia;5%, and frontotemporal dementia;5% (Holmes & Amin, 2020). It affects memory and social abilities. There are two types of changes. Additional symptoms are as follows;



1. **Cognitive changes**

- Memory loss
- Difficulty in communication

- Hampering of visual abilities
- Loss of motor skills
- Confusion and Difficulty in learning

2. **Psychological changes**

- Depression
- Anxiety
- Hallucinations
- Delusions
- Mood swings Perception (Approach, 2015).

Causes of dementia

Vitamin deficiencies; Folate, B12, and homocysteine

Vitamin B12 and/or folate deficiencies and their clinical repercussions in the elderly have been widely characterised in the post-World War II literature (Cape and Shinton, 1961). Such deficits may have far-reaching consequences due to the action of vitamins on homocysteine (Hcy). Elevated Hcy has been linked to an increased risk of cardiovascular disease and dementia (Seshadri *et al*., 2002).

Niacin (vitamin B3) deficiency

Nicotinic acid and nicotinamide, which constitute niacin, produce two coenzymes, nicotinamide adenine dinucleotide (NAD) and NAD phosphate (NADP), which are vital in the human body's oxidation and reduction processes. Tryptophan, an important amino acid, may also be turned into niacin. Niacin deficiency causes pellagra, which has lately been widely researched (Hegyi *et al*., 2004). The illness was originally documented in 1762, among the impoverished, who ate mostly corn and rarely fresh meat. The three Ds of Pellagra are dermatitis, diarrhoea, and dementia. Laziness, weakness, decreased appetite, anxiety, irritability, and sadness are common early symptoms. Later neurologic abnormalities include psychotic characteristics, psychomotor retardation or agitation, tremor, ataxia, and sleeplessness, all of which can progress to encephalopathy.

Chronic Obstructive Pulmonary Disease

Despite occasional reports of disorientation and tiredness caused by anoxia and hypercapnia, chronic obstructive pulmonary disease (COPD) has received little attention until lately. Recent research compared the cognitive performance and single-photon emission tomography (SPECT) of COPD patients with and without hypoxemia to those of AD patients (Incalzi *et al*., 2003). COPD patient's neuropsychological abnormalities include impairments in attention, verbal memory, and deductive reasoning, with hypoxemic patients performing worse than nonhypoxic subjects (Grant *et al*.,1982). Although motor speed, strength, and coordination were all reduced in half of the patients, abstracting ability and sophisticated perceptual-motor integration were the most significantly impaired (Heaton *et al*., 1983).

Hypoglycaemia and hyperglycaemia

Cohort studies have found a link between diabetes mellitus and incident instances of dementia, which appears to indicate an elevated risk for both AD and VaD (Ott *et al*., 1999; Yoshitake *et al*., 1995). The causative mechanism for this connection is unclear, although the length of insulin therapy is a significant factor, which may imply either more severe or protracted hyperglycaemia, prolonged hyperinsulinemia, macrovascular brain injury, microvascular brain damage, or recurrent hypoglycaemia. There is experimental evidence that hypoglycaemia impairs all cognitive processes evaluated in insulin-dependent diabetes mellitus individuals, including sensory-perceptual processing, basic motor abilities, attention, learning, memory, language, and temporal and conceptual abilities. (Draelos *et al*., 1995) Even at an early stage in life, impairments in learning, memory, problem-solving, and mental and motor agility are more typical among type I diabetics. Type II diabetics have special difficulties in storing and retrieving new knowledge, as well as doing poorly in abstract thinking and complicated psychomotor functioning. (Sima *et al*., 2004)

Hypothyroidism

Even though hypothyroidism has long been known as one of the "reversible dementias," a review of the evidence (Wahlund *et al*., 2002) has demonstrated that:

1. There have only been a few reports of hypothyroid dementia.
2. Patients with hypothyroidism have cognitive abnormalities, although these effects are only partially reversible following thyroxine supplementation.
3. There is scant evidence that hypothyroidism, whether reversible or irrevocably, causes dementia.
4. There have been no reports of randomised studies with impacts on cognition.

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL)

A tiny proportion of VaD patients are caused by underlying genetic disorders that enhance the risk of stroke. This is a broad category of ailments that includes hemoglobinopathies, coagulation disorders, mitochondrial disorders, connective tissue diseases, and angiopathies, among others, and can result in small- and large-artery disease, embolic or hemorrhagic strokes. (Schmidt and Schmidt, 2002) CADASIL is a rare hereditary nonamyloid systemic angiopathy caused by notch 3 gene mutations on chromosome 19. It was just recently recognized as a rare cause of familial VaD, with around 100 families worldwide identified. (Dichgans, 2004; Tournier-Lasserve *et al*., 1993) Migraine with aura, epileptic seizures, transient ischemic episodes (TIAs), strokes, vascular cognitive impairment, and dementia are all examples of this condition (Chabriat *et al*., 1995; Dichgans *et al*., 1998) Cognitive decline has been documented in 60% of cases, with two-thirds achieving the clinical criteria of dementia syndrome by the age of 65. Dementia syndrome is largely subcortical. (Dichgans *et al*., 1998)

Missense mutations cause a gain or deletion of cysteine residues in the transmembrane notch 3 protein's extracellular region. The damaged region of

the protein is known as the epidermal growth factor (EFG)-like domain. (Dichgans, 2004). Angiopathy damages the cerebral small arteries and causes substantial damage to the smooth muscle cell medium and endothelium. One theory is that this angiopathy reduces the ability of blood arteries to autoregulate, resulting in decreased brain perfusion. (Singhal *et al*., 2004) The bulk of CADASIL genotype-phenotype correlation studies found no effect of the mutation location on the phenotype. One possible explanation is that lacunar strokes occur as a result of an abrupt interruption in blood vessel perfusion, which necessitates the existence of additional modifying variables in addition to the genotype to worsen the situation. Smoking was found as one of these environmental variables in a recent genotype-phenotype investigation of 65 CADASIL families from the United Kingdom. (Singhal *et al*., 2004) The authors hypothesised that smoking might adversely influence vasomotor function (Iida *et al*., 1998) and cause a prothrombotic condition (Hioki *et al*., 2001) in CADASIL patients.

Role of oxidative stress in Alzheimer's disease

Oxidative stress, a process that worsens with age, is caused by a redox imbalance, which involves the production of excess reactive oxygen species (ROS) or the failure of the antioxidant system. At the cytochrome oxidase complex, the mitochondrial electron transport chain uses over 98 percent of molecular oxygen, and the remaining oxygen is converted to hydrogen peroxide and superoxide radicals. The oxygen radical superoxide (O2•) and the non-radical oxidant hydrogen peroxide (H2O2), as well as hypochlorous acid, are created during normal metabolism and numerous activities. When (O2 •) and (H2O2) production becomes excessive, it can cause tissue damage, which frequently includes the creation of the highly reactive hydroxyl radical (OH•) and other oxidant molecules in the presence of catalytic iron or copper ions. As a result, because metal catalysed redox reactions, one of the most important forms of antioxidant defence is storing and transporting iron in forms that do not catalyse the formation of reactive radical, as is the case during tissue injury, when there is an increase in iron availability that can accelerate free radical reactions.

Oxidative stress refers to a wide range of compounds and free radicals generated from molecular oxygen. These are chemical species with one unpaired electron in their outer shell.In its ground state, molecular oxygen is a bi-radical with two solitary electrons in the outer shell that share the same spin. As a result, because the oxygen molecule can only react with one electron at a time, oxygen is not very reactive with the electrons in a chemical bond. When one of the two electrons is energised, it spins. The two electrons have opposite spins and can react fast with other pairs of electrons, especially double bonds. The resultant singlet oxygen is a potent oxidant. The reduction of oxygen by one electron results in the development of very stable intermediates that lead to the synthesis of a superoxide anion (O2•), the precursor of most ROS and a mediator in oxidative stress chain reactions. Molecular oxygen is a bi-radical in its ground state, with two lone electrons in the outer shell that share the same spin. As a result, oxygen is not highly reactive with the electrons in a chemical bond since the oxygen molecule can only react with one electron at a time. One of the two electrons spins when it is energised. Because the two electrons have opposite spins, they can react quickly with other pairs of electrons, particularly double bonds. The resulting singlet oxygen is a very powerful oxidant. When oxygen is reduced by one electron, extremely persistent intermediates form, leading to the formation of a superoxide anion (O2•), the precursor of most ROS and a mediator in oxidative stress chain reactions.

The brains of Alzheimer's disease patients show severe oxidative damage coupled with abnormally marked A buildup and neurofibrillary tangle formation. A growing body of research reveals that biometals like as iron, zinc, and copper play a crucial role in A and neurodegeneration.Copper and zinc have high affinity binding sites on the N-terminal metal-binding domains of A and its precursor APP, while copper is a potent mediator of the highly reactive hydroxyl radical (OH•), and thus contributes to the increase in oxidative stress characteristic of the AD brain due to the high concentration of copper found in amyloid plaques. High zinc concentrations were linked to memory and cognitive brain areas such as the neocortex, amygdala, and hippocampus, all of which are damaged in AD disease. This zinc binding has a highly organised conformational state of Aβ (1-40), resulting in the creation of poisonous,

fibrillary Aβ aggregates. Uncontrolled zinc or A build up causes zinc-induced and A-mediated oxidative stress and cytotoxicity. Furthermore, protein oxidation by free radicals may be important in AD because oxidation of brain proteins might impair enzymes necessary to neuron and glial activities. This is the case for two enzymes that are especially sensitive to oxidative modification, glutamine synthetase and creatine kinase, which are significantly reduced in AD brains, reflecting changes in glutamate concentrations and increased excitotoxicity, whereas oxidative impairment of creatine kinase may cause decreased energy metabolism in AD. Protein pathologic aggregation results in fibril formation and insolubility.

Therefore, Overproduction of ROS, resulting in oxidative stress may have a negative effect and be a significant mediator of damage to cell structures, and hence different disease states and ageing (Huang *et al*., 2016).

Etymology of Dementia

The term "dementia" first appeared in human history in approximately 600 A.D and was coined by Saint Isidore (560–636 A.D.), archbishop of Seville, in his work "Etymologies.". The name is derived from the Latin prefix 'de,' which denotes a deprivation or loss, the root 'ment,' which means mind, and the suffix 'ia,' which signifies a condition. In a nutshell, dementia is defined as a "state of mind" (Yang *et al*., 2016).

History of dementia

Dementia existed throughout human history before it was identified. The ancient Egyptians were well aware that memory decreases with ageing around 2000 B.C. 3 Pythagoras (570–495 B.C.), a Greek doctor and mathematician, categorised a human existence into six stages: infancy (years 0–6), adolescence (ages 7–21), adulthood (ages 22–49), middle-age (ages 50–62), senescence (ages 63–79), and old age (age 80 or older). Senescence and old age were considered as a deteriorating phase of the mind and body, and those who lived to this age were anticipated to deteriorate mentally to the level of a suckling baby and eventually become foolish. Hippocrates (460–370 B.C.), a Greek physician, thought that brain damage causes cognitive dysfunction,

whereas Plato (428–347 B.C.), a Greek philosopher, stated that the primary cause of dementia in old age itself because the mental ability is destined to deteriorate. On the contrary, Marcus Tullius Cicero (106–43 B.C.), a Roman philosopher, statesman, and lawyer, observed that ageing does not necessarily result in a reduction in mental capacity, especially in persons with weak will. In summary, he stated that dementia is not an unavoidable result of ageing. Around the 2nd century A.D., Aretheus, a Turkish physician, classified dementia as delirium, a reversible acute loss of cognitive function, and dementia, an irreversible chronic condition.

Classification of dementia

Their underlying diseases are primarily defined by the buildup of abnormal protein aggregates in neurons. The broad majority of non-vascular dementia is classified into six foremost groups of neurodegenerative proteinopathy: amyloid-β (Aβ), microtubule-associated protein tau, TAR DNA-binding protein 43 (TDP-43), fused in sarcoma (FUS), α-synuclein, and prion protein. In-vivo functional studies show that the majority of neurodegenerative diseases start in susceptible neurons, and follow spread throughout the brain (Elahi & Miller, 2018).

Alzheimer’s Dementia

Alzheimer’s dementia accounts for roughly 60% of all types of dementia worldwide. The main symptoms include aphasia and apraxia. (Elahi & Miller, 2018) AD resembles other prevalent disorders such as type 2 diabetes mellitus or hypertension. (Lautenschlager & Martins, 2005) Extrapyramidal symptoms affect 35% of individuals with Alzheimer's disease, while cognitive impairment affects up to 40% of patients with Parkinson's disease (Galvin *et al*., 2001). It is marked by a decline in cognition and memory, and gradual impairment in the capacity to carry out daily tasks. Visuospatial skills are frequently affected. Patients with this type of dementia experience disorientation. The early signs of AD signify impairment in facial recognition. Imaging revealed defusal atrophy. The average life expectancy is 8 to 10 years, though this varies greatly. The ability to focus attention and remember distant events may be slightly impaired

and gradually deteriorate with time. The language of the patient is often imprecise with increased usage of automatic phrases and cliches. Anomia which is an impaired ability to name objects is often observed in Alzheimer's patients. Although the capability to repeat phrases is usually preserved, verbal comprehension begins to deteriorate. Complex deficits include difficulties in recognizing faces and misperceptions. Breakdowns in fundamental visual-processing abilities, such as processing of contrast and spatial connections, detection of motion, and figure-ground discrimination, may impede the capacity of the patient to execute daily tasks, including driving. Apraxia, a disability of skilled movement, may also impede one's capacity to perform daily chores. Noncognitive or social symptoms are a significant but frequently overlooked issue in Alzheimer's disease. They cause caregivers more stress than the cognitive impairment itself. Delusions impact up to 50% of Alzheimer's sufferers (Approach, 2015). After age 65, the proportion of dementias assigned to Alzheimer's disease rose (logistic regression, p 0.051), although the increases found after age 85 were not statistically significant. Probable AD cases with concomitant illness accounted for 30% of all possible AD cases (Disorders, 1994).

Vascular Dementia

In most areas of the globe, dementia caused by cerebrovascular illness is the second most frequent kind of dementia. The frequency of vascular dementia varies by demographic, although it accounts for 10 to 20% of all dementia cases. Urinary difficulties and gait abnormalities have been proposed as early indicators of the illness. (Approach, 2015) VD was the second most prevalent kind of dementia in the research population. The total prevalence of VD in the 85+ group was 3.8 percent. (Disorders, 1994) Imaging investigations of periventricular white-matter abnormalities are frequently regarded as indications of ischemia illness. One radiographic indication of vascular dementia is central atrophy or enlargement of the third ventricle. (Approach, 2015) Laukka *et al*. (2012)'s study shows that no brain imaging was available, and around 70% of patients had a diagnosis of VaD, which was predominantly a diagnosis of dementia after stroke. The fraction of dementias related to VD

declined considerably with age after 65 (logistic regression, p 0.051), although the declines after 85 years did not achieve statistical significance. Vascular dementia (VaD) is associated with a high incidence of psychosis; delusions may develop in up to 50% of patients, and VaD is also more likely to produce depression (45%) than Alzheimer's disease (AD) (17 %). The clinical characteristics of vascular lesions are determined by their location, number, size, and aetiology. Dysarthria lowered pace, and disruption of melody and pitch are examples of motor speech disorders. (Pasquier & Disease, 2014) Diagnosis includes MRI for illness subtype and severity, and atrophy pattern indicative of mixed disease. If clinical characteristics or atrophy patterns imply mixed illness, molecular biomarkers (CSF or PET) may be used. If a family illness is suspected or has unusual symptoms, such as white matter pathology in the anterior temporal lobes, then genetic testing (for example, NOTCH3 for CADASIL) is recommended. (Elahi & Miller, 2018)

Lewy Body Dementia

Visual hallucinations, variations in cognitive function, frequent falls, vulnerability to antipsychotic drug extrapyramidal side effects, and parkinsonism are all symptoms of DLB. (McKeith *et al*., 1996) Cortical and subcortical-frontal dysfunctions describe this kind of dementia. Recent research found extreme but comparable degrees of impairment in assessments of attention/short-term memory (digit span), frontal lobe function (verbal ability, category, and Nelson card-sort test), and motor sequencing in both Lewy body dementia and AD groups as well as Parkinson's disease patients and controls. In the clock face assessment, increased performance in the "copy" vs "draw" component of the test was observed in normal patients with Alzheimer's disease, and those with Parkinson's disease, but not in patients with Lewy body dementia, who scored particularly poorly in both parts of the test. The clock face test evaluates executive and spatial cognition skills, both of which may be severely affected in Lewy body dementia. Variations in performance from one test to the next are a notable hallmark of Lewy body disease (Pasquier & Disease, 2014). Autopsy investigations revealed that DLB was present in 15–20% of dementia patients. (McKeith, 2002) However, the distinction between AD pathology is contentious,

leading to the creation of subgroups such as "DLB with senile plaques," "pure DLB," and "plaque-only AD with or without Lewy body" (Ince *et al*., 1998). It is a type of α-synucleinopathy. It is distinguished by distinctive intracellular -synuclein aggregates (Lewy bodies) that induce cerebral neural network failure. The majority of DLB patients have mixed pathology, with vascular disease and/or AD pathology coexisting. To some extent, the genetics of AD pathogenesis and LBD overlap. The APOE*4 genotype is more common in sporadic LBD, but the APOE*2 allele appears to be protective against both DLB and sporadic AD. To some extent, the genetics of AD pathogenesis and LBD overlap. DLB is distinguished by fluctuating cognition, recurring visual hallucinations, and parkinsonism. The variations in mental state, referred to as low-grade chronic delirium, might be caused by significant cholinergic deficiencies as well as neocortical Lewy body disease. Other characteristics are anxiety and depression, autonomic symptoms (including constipation and hyper sialorrhea), olfactory dysfunction, and extreme neuroleptic sensitivity, which causes parkinsonism aggravation. The relative sparing of the posterior cingulate cortex in DLB – the so-called posterior cingulate 'island sign' – is one differentiating characteristic between DLB and AD. DLB is indicated by low dopamine transporter (DAT) uptake in the basal ganglia, as detected by SPECT or PET (Elahi & Miller, 2018).

The causal mutation, E46K in the -synuclein gene on chromosome 4, was discovered (Zarranz *et al.,* 2004). This mutation appears to disrupt the normal function of the presynaptic-synuclein protein. Misfolded -synuclein is the key element of Lewy bodies, often seen in the substantia nigra, the basal nucleus of Meynert, locus coeruleus, and grey matter in DLB. It may be engaged in lipid binding and have a function in the control of dopamine release at the presynaptic terminal (Galvin *et al.,* 2001).

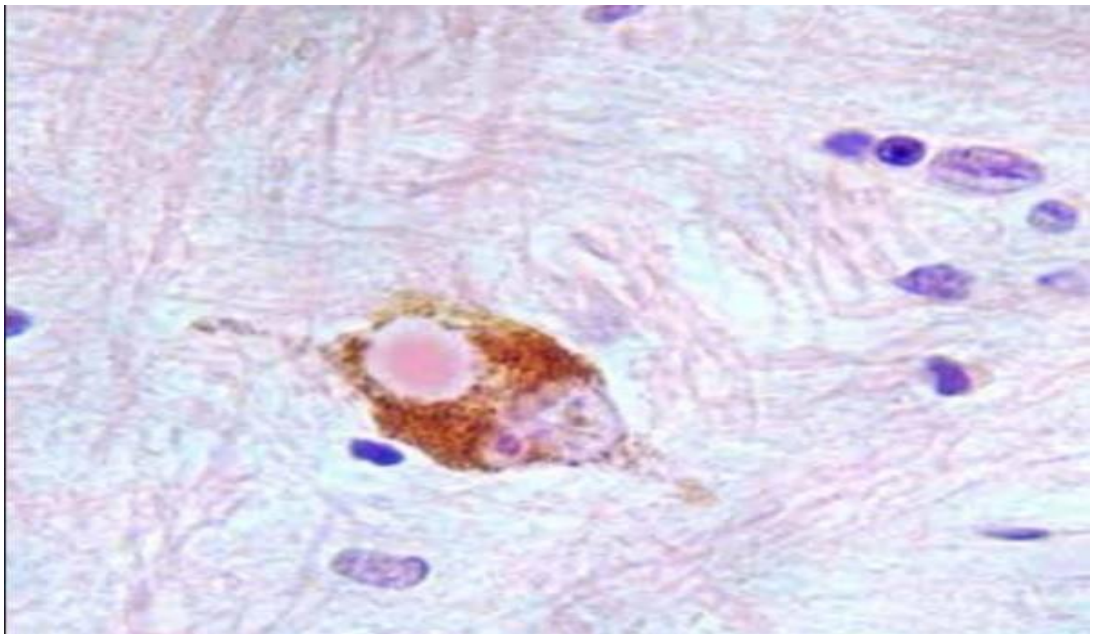


Figure:2. A neuron from the substantia nigra that is pigmented. There is only one Lewy Body present. This patient also had diffused cortical Lewy bodies, which is a sign of Pure Lewy Body Dementia (El *et al*., 2015).

Frontotemporal Dementia (FTD)

Pick's disease, FTD without particular neuropathological symptoms, corticobasal degeneration, dementia with parkinsonism connected to chromosome 17 mutations, and dementia associated with motor neuron disease are all included in this category (Lovestone *et al*., 2002). FTD usually appears before the age of 65. Recent prevalence surveys found a mean age of onset of 52.8 8.7 years in the United Kingdom and 57.9 9.0 years in the Netherlands (Ratnaavalli *et al*., 2002; Rosso *et al*., 2003). Research conducted in the United Kingdom discovered an incidence of 15 incidents per 100,000 people aged 45–64 years (Ratnaavalli *et al*., 2002). FTD has a larger percentage of family cases than AD and DLB. In a study of 345 FTD patients, Rosso *et al*., (2003) discovered tau gene mutations on chromosome 17 in 32% of individuals with additional afflicted first-degree familial relatives and 43 percent of all FTD patients. (Lautenschlager & Martins, 2005) FTD syndromes are caused by frontotemporal lobar degeneration (FTLD). It is a broad clinical term that refers to the degradation of cortical and subcortical structures in the frontal and temporal regions of the brain. Fronto Insular cortices, anterior

temporal poles, basal ganglia, brainstem, and thalamus are among the regions affected, as is the cerebellum in certain familial types of the condition. Neuronal networks that support personality, behaviour, executive processes, language, and motor abilities are progressively destroyed, leaving memory and visuospatial capabilities relatively unscathed. Intracellular clusters of tau, TAR DNA-binding protein 43 (TDP-43), or fused in sarcoma (FUS) induce frontotemporal lobar degeneration (FTLD). (Elahi & Miller, 2018) Stanford *et al*. (2004) discovered a tau gene mutation in 25% of individuals with familial FTD but only 4% of those who did not have a positive family history. (Van Swieten *et al*., 2004) More than 25 distinct tau gene mutations have been discovered, with fibril formation being a common clinical characteristic in the majority of these "mutation cases" of FTD. (Pickering-Brown, 2004) About 90-95% of cases are caused by tau or TDP-43 and the remaining cases are caused by FTLD-FUS, caused by intracellular FUS inclusions. Tau splicing is alternative splicing of the microtubule-associated protein (MAPT) pre-mRNA that generates tau isoforms with three or four microtubule-binding domain repetitions (3R and 4R). As a result, FTLD-tau is further classified as 3R, 4R, and 3R/4R tauopathies). There are three fundamental FTD syndromes, including the most frequent, behavioural variation FTD (bvFTD), and two linguistic syndromes, non-fluent/agrammatic form PPA (nfvPPA) and semantic form PPA (svPPA). Furthermore, three FTD motor syndromes have been identified: FTD with motor neuron disease (FTD–MND) and two parkinsonian forms, cortico basal syndrome (CBS) and PSP syndrome (PSP-S). However, in familial FTLD, the defective genes are linked with consistent pathology signs. TDP-43 disease is related to mutations in the C9orf72, progranulin (GRN), valosin-containing protein (VCP), and TDP-43 (TARDBP) genes. A familial history of dementia affects around one-third of FTD patients. The most prevalent genetic cause of FTLD-TDP is an intronic hexanucleotide repeat expansion in C9orf72. The molecular diseases of FTLD cause degeneration in susceptible neural networks that serve cognitive, behavioural, and sensorimotor processes. Despite severe deficiencies in social and interpersonal connections, self-awareness and insight frequently decline. Global hemispheric abnormalities with right-predominant patterns of atrophy cause prominent behavioural symptoms in bvFTD and right-sided svPPA

(svPPA-R), whereas individuals with left-predominant atrophy, such as nfvPPA and svPPA-L, present with language-related difficulties. The mesiotemporal and parietal lobes get implicated as the illness progresses. The reported age of onset of FTD spans from the early twenties to the late nineties (Elahi & Miller, 2018).

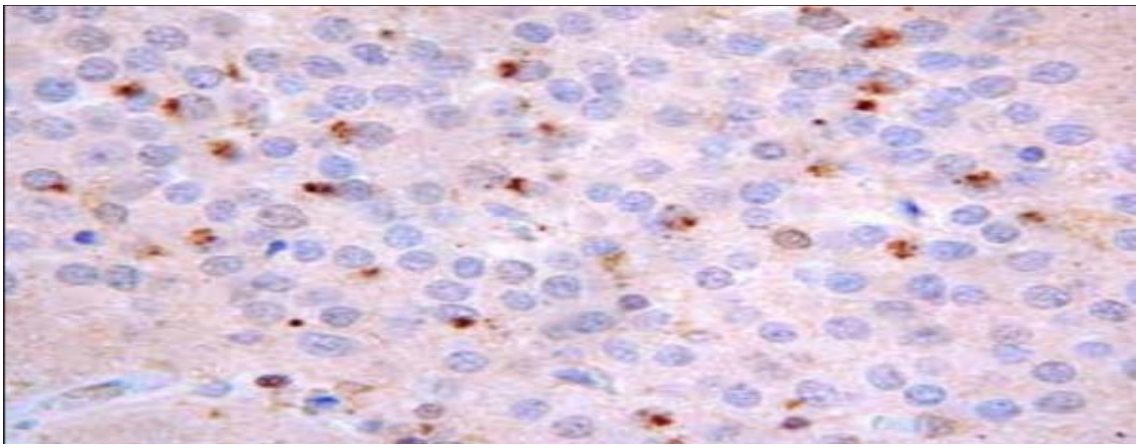


Figure: 3. A section of the hippocampal dentate fascia showing dot-like ubiquitin deposition.This is typical of Frontotemporal Lobar Dementia with Ubiquitinated Inclusions (now called TDP) (El *et al*., 2015).

The sequence of memory breakdown is consistent with "frontal-type" amnesia, with memory failures developing after failures of attentional, retrieval methods, organisational, and regulatory variables rather than a basic impairment of storage. FTD can be clinically diagnosed based on history (psychological and behavioral changes precede and stay significant throughout the disease), the form of the behavioral problem, normalcy on EEG, and the preponderance of frontal or anterior temporal abnormalities on brain scans, and neuropsychology. Patients with disjoined accounts may achieve normal results on the Wechsler Memory Scale's logical memory subtest. (Pasquier & Disease, 2014)

1. **Behavioural variant frontotemporal dementia**

The early indications of bvFTD are gradual changes in mood, attitude, and behavior, particularly in interpersonal relationships and social behavior. Executive function impairment is exacerbated by dysfunction of the dorsolateral prefrontal cortices. Disinhibition; apathy or lethargy; stereotypic behavior;

changes in dietary preferences and eating habits; and dysexecutive are additional signs.

TDP-43-related hippocampus degeneration and sclerosis are assumed to represent the fundamental neuropathology in amnestic FTD. It might be difficult to distinguish behavioral characteristics from basic psychiatric diseases such as depression, borderline personality disorder, bipolar disorder, and schizophrenia. Around 20% of patients experience delusions and 10% experience hallucinations. These symptoms are more prevalent in hereditary disorders caused by mutations in genes.

2. Language-centred frontotemporal dementias

The language-centered FTDs usually demonstrate focused advent but, with illness persistence, impairments ultimately occur in all language functions. Neurodegenerative illnesses affecting the language circuitry generally appear asymmetrically, with dominant (often left) hemisphere pathology showing as language syndromes. Speech becomes less fluent, more telegraphic, and less melodic (prosodic). Apraxia develops, with inconsistent sound mistakes and aberrations, particularly when pronouncing consonant clusters. Grammar deficiencies, which are first noticeable in writing, subsequently restrict understanding. Anomia is not a core weakness, although it is commonly associated with delayed naming, hesitations, and word-finding pauses. Repetition has less of an impact than spontaneous speech. Most patients have motor impairments, including asymmetric parkinsonism and/or pyramidal symptoms. Markers of neurodegeneration and neuronal dysfunction evaluated by MRI, PET, or SPECT in the left posterior frontoinsular area support the diagnostic criteria for nfvPPA. (Elahi & Miller, 2018)

It is distinguished by a significant loss of meaning for both words and things. They are also severely deficient in nonverbal activities that require matching semantically linked photographs of items. In contrast, well-preserved memory for everyday occurrences, such as recalling recent personal events and schedules, is retained. (Pasquier & Disease, 2014)

Dysfunction of the front temporal lobes in svPPA impairs access to semantic memory. Importantly, the loss of semantic information in svPPA affects all sensory modalities, including visual, tactile, olfactory, and gustatory, in addition to auditory. Furthermore, the problems extend to reading and writing, with the onset of surface dyslexia and dysgraphia. Communication gets increasingly difficult when content is depleted and speech becomes increasingly hazy. TDP-43 type C164 is usually invariably the neuropathological basis of svPPA, however TDP-43 type B and Pick disease are also possibilities.

3. Motor frontotemporal dementias

The FTD range includes disorders characterized by pyramidal and/or extrapyramidal dysfunction. These 'motor FTD syndromes' can manifest sooner than nonmotor syndromes, with an average beginning age in the seventh decade of life. FTD and MND are increasingly seen as part of a clinicopathological spectrum with the same underlying biology. TDP-43 pathology is closely attributed to ALS and FTD–MND, while C9orf72 mutations are linked with FTD–MND. Approximately 60% of people with FTD show electromyographic indications of MND, with 10–15% acquiring clinical indicators of MND. Early bulbar impairment is more common in FTD–MND than in solitary ALS, and people with FTD–MND have the lowest lifespan among persons with FTD syndromes. (Elahi & Miller, 2018)

Dementia with Parkinsonism

The diagnosis of dementia with parkinsonism is complicated. Rigidity and postural instability develop in around 30% of Alzheimer's patients. The same proportion of Parkinson's disease patients develops dementia as a result of Alzheimer's disease or other factors. Lewy bodies have been detected in parts of the brain where they do not normally appear in idiopathic Parkinson's disease, such as the cerebral cortex, in many instances of dementia with parkinsonism. These bodies can appear with or without the obvious alterations associated with Alzheimer's disease (diffuse Lewy-body disease). Dementia with parkinsonism as an early characteristic sometimes advances faster than uncomplicated Alzheimer's disease. General slowing of cognition and activity

(psychomotor slowness) and disrupted executive function are typical cognitive abnormalities. Delusions and hallucinations are common early in the course of the illness and can be aggravated by parkinsonian medication. (Approach, 2015)

Alzheimer’s Disease

Alzheimer's disease (AD) is one of the most common neurodegenerative conditions in humans, affecting memory and cognitive judgment and accounting for more than 80% of dementia patients worldwide. Alzheimer's disease brains show a reduction in overall size as well as a reduction in glucose absorption, indicating decreased neuronal activity. According to United Nations statistics/predictions, the world's aging population (60 years or older) will grow from 900.9 million in 2015 to 2092 million in 2050, increasing the number of people with Alzheimer's disease and other dementia from 46.8 million presently to 131.5 million in 2050. As a result, it is vital to resolve the perplexing questions surrounding Alzheimer's disease, such as its genesis, causation, prevention, and early and exact diagnosis. The phrases "dementia" and "Alzheimer's illness" are sometimes used interchangeably. Dementia is a clinical illness characterized by memory impairment; it is caused by Alzheimer's disease in more than 75% of cases. Alzheimer's disease, on the other hand, is a neuropathological entity distinguished by a lengthy preclinical phase followed by the beginning of slowly increasing dementia. (Eschweiler *et al*., 2010).

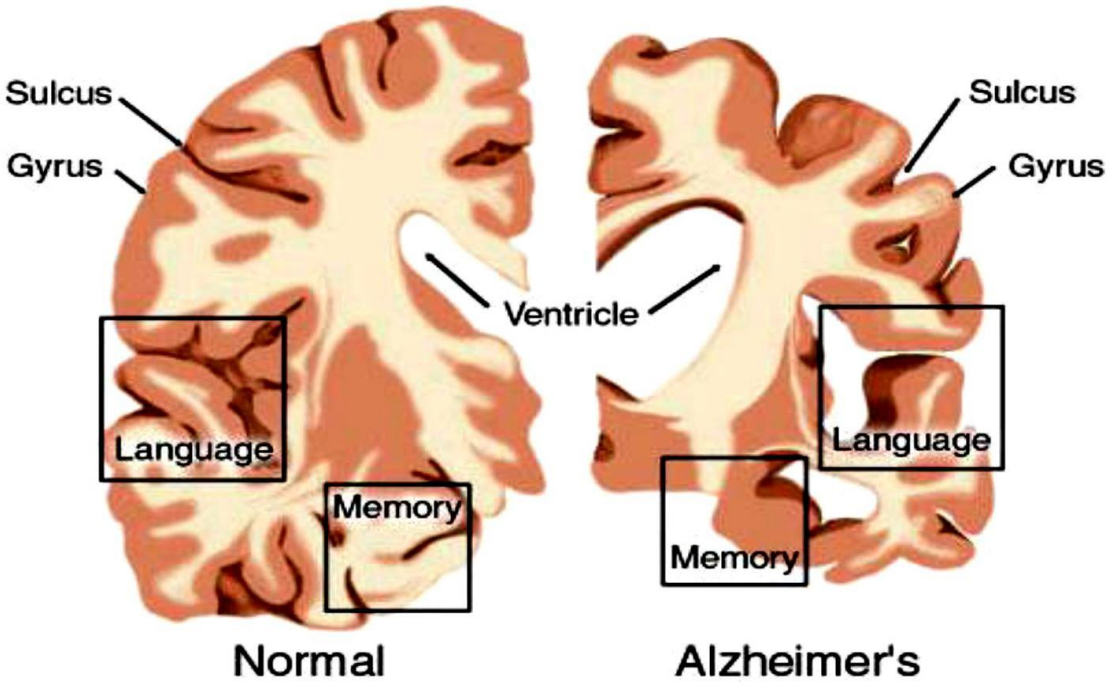


Figure:4. Difference between Normal Brain and AD Affected Brain.

The neuropathology of Alzheimer's disease

60 percent of demented people have the classic pathological features of Alzheimer's disease—amyloid deposits and neurofibrillary tangles—without any additional abnormalities in the brain, whereas the remaining 15 percent have these findings accompanied by vascular brain damage. Dementia caused solely by vascular lesions is uncommon, accounting for less than 15% of cases (e2). Lewy-body dementia (often associated with parkinsonism and substantial variations in awareness) and frontotemporal lobar degeneration (FTLD) both account for roughly 5% of dementia cases. According to epidemiological statistics, dementia is caused by another condition in less than 5% of cases; these reasons include endocrine abnormalities such as hypothyroidism and hyperparathyroidism.

Amyloid plaques and neurofibrillary tangles are the two visible lesions of Alzheimer's disease; they were originally described by Alois Alzheimer at a speech in Tübingen, Germany, in 1906. Amyloid plaques are made up of a pathologically processed amyloid protein known as the amyloid beta-peptide (Abeta). The aggregation of hyperphosphorylated tau proteins into paired

helical filaments (PHF) results in neurofibrillary tangles. Both of these processes are connected with synaptic loss and, eventually, cell death. Activated microglia (also known as "small glial cells" by Alzheimer's) are located in and around amyloid plaques (Eschweiler *et al*., 2010).

Neurofibrillary Tangles and Other Cytoskeletal Pathology

Neurofibrillary degeneration, and more especially neurofibrillary tangles (NFTs), are the hallmark pathological characteristic of Alzheimer's disease that has been recognized from Alzheimer's initial descriptions. Biochemical, molecular biology and current neuropathological approaches have improved our understanding of the paired helical filaments (PHFs), which are the most important ultrastructural component of NFTs. Biochemical investigations of NFT-affected brains have revealed that the predominant protein component of PHF is an altered version of tau (t) protein known as A68 or PHF-t. PHF-t varies from normal t in various ways, the most noticeable being a greater molecular weight and a more acidic isoelectric charge, both of which are caused by enhanced, and likely aberrant, phosphorylation. Antibodies that respond with phosphorylated epitopes in t reveal severe cytoskeletal abnormalities in the AD brain, including not just NFTs but also a broad plexus of aberrant neurites, or "neuropil threads," in afflicted parts of the cortex, hippocampus, and amygdala (Dickson, 1997).

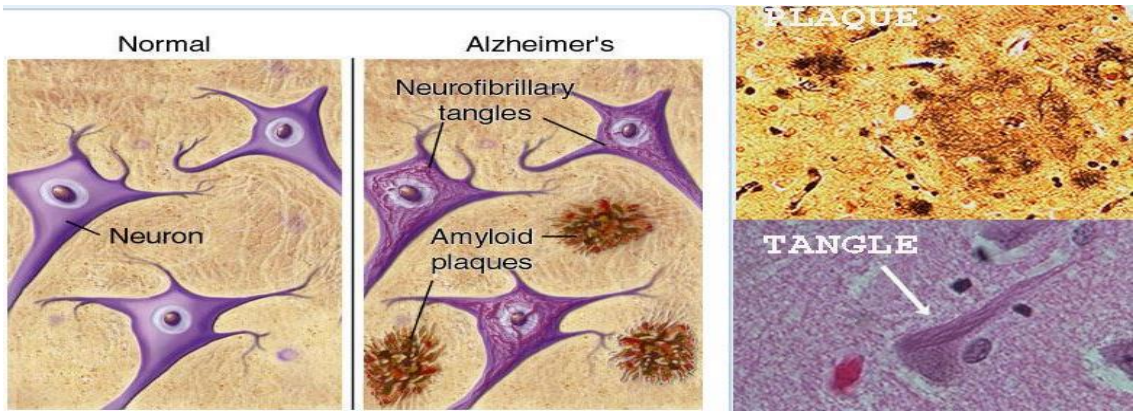


Figure: 5.The construction of Aβ plaques and NFTs are thought to contribute to the degradation of the neurons in the brain and the consequent symptoms of AD.

Cerebral Amyloid and Senile Plaques

Senile plaques (SPs), in contrast to NFTs, are significantly more complex and diverse lesions that incorporate cellular components, extracellular amyloid, and amyloid-associated compounds. SPs include both neuronal processes (neurites) and glial cells. SP neurites are diverse; some have degenerate synaptic components with the buildup of membranous lamellar material and lysosomal dense bodies ("dystrophic-type" neurites), while others have PHFs comparable to those seen in NFTs and neuropil threads ("PHF-type" neurites). Although SPs with dystrophic-type neurites are appropriately referred to as "neuritic plaques," based on our clinicopathological findings, we believe it is critical to distinguish the type of neurite in the SPs. Previous morphological investigations of SPs in the brains of normal aged individuals revealed the presence of dystrophic neurites in SPs. Similar SPs have been observed in older animals (including dogs and monkeys), as well as transgenic mice overexpressing a mutant type of human amyloid precursor protein. SPs with dystrophic neurites have also been seen in the brains of old people suffering from non-AD degenerative dementia, the most frequent of which is Lewy body dementia. In this and subsequent talks, we limit the term "neuritic plaque" to SPs with PHF-type neurites. Several common diagnostic procedures (e.g., silver stains and thioflavin-S fluorescence microscopy) can identify the latter neurites, but immunocytochemistry using antibodies to t protein is the most specific and sensitive way of detecting neuritic SP. (Dickson, 1997)

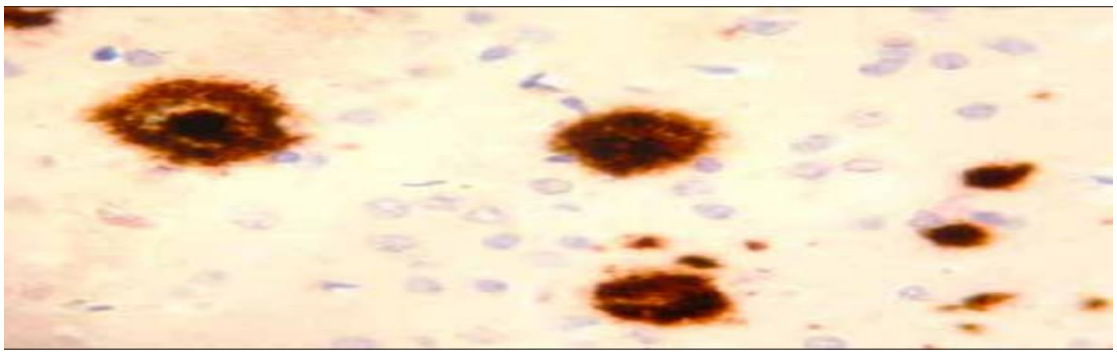


Figure: 6. In a case of AD, an Immunohistochemical section of the cortex was taken. An antibody to Beta A4 amyloid is applied to the tissue, which detects the antigen and stains it brown. This demonstrates dense amyloid deposition

throughout the cortex as dense core (DC) and diffuse (D) plaques (El *et al*., 2015).

Neuronal and Synaptic Loss

Terry and co-workers have proposed that the physical basis for dementia is the loss of synapses, either structurally or functionally. Anatomical and immunological approaches have been used to establish synaptic integrity loss in AD brains after death. The former includes electron microscopy or Golgi methods for morphometric examination of synapses, while the latter includes densitometric measurement of immunoreactivity for synapse-related chemicals in tissue sections or immunoblots, as well as spectrophotometric analysis of enzyme-linked immunosorbent assays (ELISAs). The most important finding of these investigations is that synapse loss occurs in the AD neocortex**.**

Although neuronal and synaptic degeneration and loss are undoubtedly important in cognitive decline in Alzheimer's disease, they are often overlooked in regular histological preparations. Neuritic degeneration and NFTs, on the other hand, can be detected using simple and inexpensive histochemical approaches.

Furthermore, data from clinicopathological research, including quantitative histopathology analyses and ELISAs for PHF proteins, has shown that PHF-type neuritic degeneration is strongly associated with cognitive failure in Alzheimer's disease. Amyloid deposition, on the other hand, has a lower correlation with cognitive impairment. Based on this knowledge, we believe that amyloid is necessary but not sufficient for the diagnosis of Alzheimer's disease (Dickson, 1997).

Cerebral Amyloidosis and Pathological Ageing

The principal constituent protein (Ab) of amyloid in aged persons' brains is biochemically comparable to that of Alzheimer's disease and is produced from a bigger precursor protein. In ageing and AD, sensitive methods for identifying amyloid, such as thioflavin-S fluorescence microscopy and Ab antibodies, show a wide variety of amyloid deposits. Furthermore, cross-sectional and

longitudinal investigations of elderly people have shown that amyloid deposits are highly common in the brain of the elderly. Based on prospective neuropsychological tests and most recently confirmed in an analysis of longitudinal measures of rates of change in psychological tests, some elderly people with extensive cerebral amyloid deposits are cognitively normal and indistinguishable from elderly people with few or no amyloid deposits. This age-related cerebral amyloidosis has been dubbed "pathological ageing" (PA). Instead of being compact, most of the amyloid in PA is diffuse or amorphous. In AD, however, a higher percentage of the deposits feature dense reticular or cored amyloid.

Although A beta is a component of amyloid plaques in the brain and blood vessels, Ab is also a result of normal cellular metabolism; soluble A beta (mainly Ab1-40) has been found in culture supernatants, plasma, and cerebrospinal fluid of healthy people. The processes that lead to Ab deposition in tissue and the ensuing chain of events that lead to mature SP are the most important problems in SP in ageing and AD.

Immunocytochemical studies have recently revealed that the amyloid in SP is heterogeneous. We have discovered that apolipoprotein-E immunoreactivity in SP in AD is higher than in PA (Dickson, 1997).

Neurofibrillary Lesions and Staging of AD Pathology

Several investigators have demonstrated that the medial temporal lobe structures are especially vulnerable to neurofibrillary degeneration in ageing and that such changes can be seen in clinically normal people through detailed neuroanatomical analysis of lesions in the brains of normal and early dementia cases.

Most studies have failed to find a similar strong clinical correlation between cognitive impairment and SP, especially when no attempt has been made to distinguish neuritic SPs from non-neuritic SPs. These findings are consistent with previous research on the distribution of neurofibrillary degeneration in ageing and Alzheimer's disease. Independent studies have shown that neurofibrillary degeneration is a nearly stereotypic pattern of vulnerability that

progresses in severity and extent from normal ageing to Alzheimer's disease. Methods for successfully staging SP in any meaningful way, on the other hand, are less satisfactory (Dickson, 1997).

Pathological Ageing: A Benign Senile Cerebral Amyloidosis

Amyloid deposition is a common (and possibly inevitable) finding in ageing brains. It should be noted that some clinically normal people have significant neocortical amyloid deposits. Although amyloid deposits in PA may contain a few ubiquitin-immunoreactive dystrophic neurites, argyrophilic, PHF-type neurites are absent. Other immunocytochemical studies have found a link between amyloid deposition and neurofibrillary degeneration in a variety of neurodegenerative diseases. Because the lesions are neuroanatomically separate, it is anatomically unreasonable to suggest a direct causal relationship between amyloid deposition and cytoskeletal pathology in AD (Dickson, 1997).

Changes in Brain Structure in Early AD

The damage to the hippocampus formation found in patients is the most plausible explanation for the problems in memory that define the early stages of AD. Neuronal loss and aberrant cell formations (e.g., neurofibrillary tangles and neuritic plaques) are largely detected in the entorhinal cortex and subiculum, the key routes that transmit information into and out of the hippocampus. It has been proposed that anomalies in these areas result in hippocampal functional isolation. These findings imply that neuropathological damage to medial temporal lobe structures may be to blame for the severe memory impairment seen in the early stages of Alzheimer's disease. The fact that the entorhinal cortex exhibits neuronal loss of around 60% and 40% in layers 2 and 4 of the entorhinal cortex, respectively, is very noteworthy. Because this region is known to be vital for new information acquisition and retention, abnormalities here are likely to be responsible for the significant anterograde memory loss seen early in the course of AD. Measures of the hippocampal formation, the para-hippocampal gyrus, the amygdalo–hippocampal complex, and the temporal horn of the lateral ventricles have shown substantial differences between minimally impaired AD patients and

controls in a number of investigations using a variety of methodologies. It is crucial to emphasise that the alterations in the brain found in early Alzheimer's differ significantly from those seen in age-related change. Age-related neuronal loss in monkeys and humans appears to be very selective within the hippocampal formation. The subiculum, for example, exhibits a large age-related loss in humans and a similar tendency in monkeys; nevertheless, the CA1, CA2, and CA3 fields, as well as the entorhinal cortex, show little indication of age-related neuronal loss (Albert, 1996).

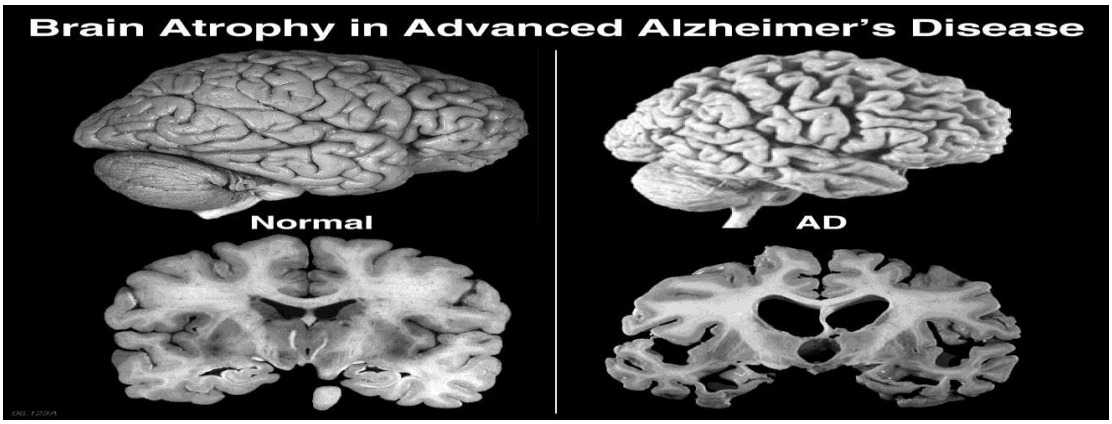


Figure:7. Cross-section of the brain as seen from the front. The cross-section on the left represents a normal brain and the right side represents a brain with Alzheimer's disease**.**

Histopathological Diagnosis of AD

The Cardinal Lesions

Senile Plaques. SPs are critical and must be plentiful, particularly in higher order association cortices. In cases of definite AD, the Khachaturian criteria are usually easily superseded. However, we disagree with the Khachaturian paper's proposal of AD without NFTs. Hansen and colleagues' research has revealed that the majority of such cases are caused by Lewy body disease. The density of SPs is inevitably lower in the primary cortices (motor and visual), which are routinely sampled to determine the typical pattern of cerebral degeneration.

Histopathological diagnosis of Alzheimer's disease should consider not only the lesions (with special attention to specific types of lesions), but also their distribution. The absence or scarcity of lesions in specific regions can aid in the histopathological diagnosis of Alzheimer's disease. In the neocortex, at least some of the SPs must be neuritic (i.e., PHF-type neurites). Although such lesions are rare in the frontal cortex, they are unavoidable in the temporal and parietal association cortices.

Neurofibrillary Tangles. The hippocampus, amygdala, entorhinal cortex, and neocortex must all be examined for NFTs. The Braak staging scheme has proven particularly useful in analysing early stages of AD pathology. Although there may be few NFTs in the neocortex, particularly the frontal lobe, they must be present in multiple cortical areas to be considered AD. It would be unusual to consider a diagnosis of Alzheimer's disease if NFTs were only found in one cortical section. Although it could be argued that these criteria would rule out cases with early onset, this has not been our experience. Clearly, more studies of early-onset AD cases dying in the early stages of disease are required (Dickson, 1997).

Identification of dementia and diagnostic workup

Although largely aimed at senior patients with cognitive complaints, they are also relevant to younger patients with illnesses that might impair cognition, such as multiple sclerosis or human immunodeficiency virus (HIV) dementia, and are divided into two rounds of evaluation.

The first step is to determine whether cognitive impairment occurs and whether that cognitive impairment fits dementia criteria (figure). When awareness is disturbed or conditions impede an effective examination of mental state, a de novo diagnosis of dementia cannot be established.Metabolic and other causes of delirium might emerge in a more chronic manner and must be recognised. If dementia is discovered, the second phase often comprises the evaluations required to ascertain the cause of the dementia and to stage/assess its severity (Corey-Bloom *et al*., 1995).

Specific factors influencing disease expression and course

Cognitive function decline in Alzheimer's disease may not proceed at the same rate in each individual. Rates of deterioration in Alzheimer's disease research vary significantly according to neuropsychological measurements. Even on the broad screening Folstein MMSE test, reported rates of deterioration differ between research, ranging from 2.7 to 4.5 points each year. Significant diversity in baseline values and rates of decrease has been documented for neuroimaging measurements such as hippocampus volume, brain volume, or regional hypo-perfusion. Premorbid state or cognitive reserve, education, age of onset, gender, stage of disease, apoE genotype, alternative genetic factors, and concomitant systemic (e.g cardiac, pulmonary, rheumatologic, or orthopaedic) or brain disorders (e.g cerebrovascular disease and parkinsonism), among others, may be the basis of such disease variability.

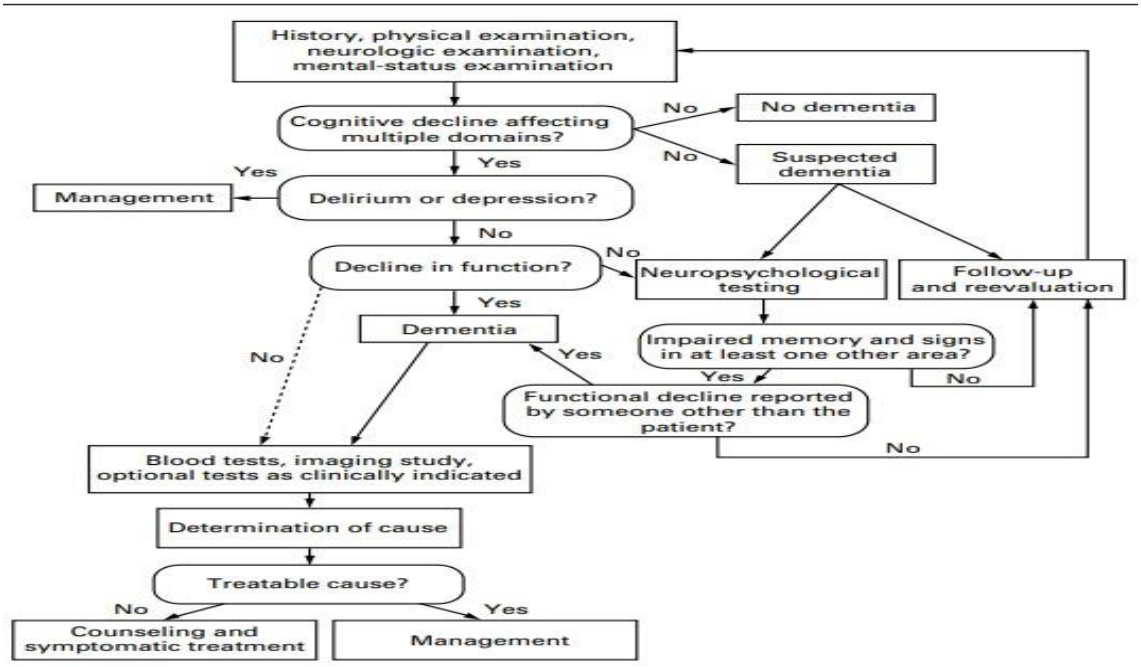


Figure:8. "Suspected dementia" denotes a concerning history or symptoms that jeopardise work, but no clear abnormalities on mental-status tests. The dashed line denotes the absence of neuropsychological testing; some practitioners prefer to exclude such testing from the workup of patients who have no functional deterioration (Corey-Bloom et al., 1995).

Age

Alzheimer's disease (AD) is the most prevalent neurodegenerative illness, affecting 1-2 percent of the total population in Western Europe and North America. However, the prevalence of this illness varies according to age. It is extremely rare in people under the age of six. It becomes more frequent beyond the age of 60, with incidence rates as high as 5-12 percent every year. While death rates in those with Alzheimer's disease are higher, growing incidence rates result in population prevalence of around 50% in people over the age of 85. Some individuals with illness onset at a young age have an autosomal dominant genetic aetiology, and so may have various characteristics.

Gender

Both men and women are affected by Alzheimer's disease. Many studies demonstrate that women have a greater frequency of Alzheimer's disease. This might be due to differences in lifespan (women live longer), and only some studies show a greater incidence rate in women, while others show the opposite, and most currently show equivalent incidence rates. Women often present to medical treatment at a later stage of Alzheimer's disease than males. This is likely due to male carers' unwillingness to seek medical help for these afflicted females, as well as female caregivers' overall greater medical system utilisation for male patients. In terms of disease progression, some data suggests that AD may advance more quickly or end in death earlier in males than in women.

Genetics

Alzheimer's disease risk is strongly influenced by genetic factors. This is covered in further detail elsewhere in this issue. The disease is an inherited condition with a tight autosomal dominant pattern in a tiny number of AD patients (less than 1% overall). These are often early-onset instances (at age less than 60-65). Mutations in three separate genes are to blame: -amyloid precursor protein (APP), presenilin-1 (PS-1) and presenilin-2 (PS-2) (PS-2). These genes can be found on chromosomes 21, 14, and 1. For the vast majority of apparently “sporadic” AD, there are only two generally agreed upon proven

factors: (a) the presence of a positive family history for disease in a first-degree relative, and (b) the presence of the apoE gene 4 allele. There are only two commonly accepted proven causes for the great majority of seemingly "sporadic" AD: (a) the existence of a positive family history of illness in a first-degree relative, and (b) the presence of the apoE gene 4 allele.

Geography

Early epidemiologic investigations revealed a substantial difference in the incidence and prevalence of AD around the globe. Regional variances have been noted even within the same nation. The higher frequency of AD in rural areas compared to metropolitan areas may be due to lower education levels in these areas. Much of the extra heterogeneity is likely due to diagnostic method discrepancies, comorbidities (e.g., vascular illness), case-ascertainment or referral biases, and pockets of inherited genetically-based AD. Recent research has found comparable age-specific incidence rates in other parts of the world. The course of AD appears to be relatively similar in different areas. However, cultural influences influence attitudes regarding cognitive symptoms; these factors determine the stage of disease at which patients may seek medical attention, the likelihood of home-care vs. institutionalisation, and the apparent duration of disease survival.

Education and occupation

A vast number of studies have found that individuals with greater education may have a later age of beginning of AD, but a more precipitous decline. The best explanation is that this represents the "cognitive reserve" of more educated people. In people with a greater premorbid level of cognitive functioning, more disease pathology is thought to be necessary for the same degree of total impairment.

Other environmental factors

Environmental influences have also been identified, although these have yet to be confirmed. The most common include head trauma, smoking, and the use of estrogens and nonsteroidal anti-inflammatory medicines. Other potential risk

factors, such as aluminium and mercury exposure, and food, have been substantially ruled out as variables impacting the onset or course of Alzheimer's disease.

Parkinsonism

The development of parkinsonism, also known as "extrapyramidal signs and symptoms," is typical in Alzheimer's disease. There are two possible explanations for such symptoms: (a) presynaptic dopaminergic losses due to nigral degeneration from concurrent Lewy body involvement, as in Parkinson's disease or "Lewy Body Dementia," and (b) postsynaptic striatal dopaminergic decline due to retrograde losses secondary to AD involvement of the neocortex. With a greater understanding of the amount of overlapping Parkinson's and Alzheimer's disease, it is likely that the former mechanism is more widespread than the latter. Parkinsonism, also known as "extrapyramidal symptoms," has been demonstrated to have a negative impact on the course of Alzheimer's disease to incapacity and death.

Cerebrovascular disease

Cerebrovascular disease is quite frequent among the elderly, depending on the community. Some individuals exhibit hypertension or atherosclerotic abnormalities; others have substantial radiological alterations in the brain that are compatible with "microvascular" white matter damage, frank lacunes, or even "silent" cortical infarcts. Fewer people have suffered definite clinical cerebral infarctions in addition to their gradual degenerative disease. Because of the difficulties of clinically identifying AD in the presence of strokes, evidence on the natural history of AD with and without strokes is somewhat weaker than in previous papers, unless autopsy confirmation is given. Some investigations have found that individuals with concurrent vascular disease have an earlier start and a quicker progression (Honig & Mayeux, 2001).

Cholinergic hypothesis

Cholinergic hypothesis of AD proposes that destruction of the cholinergic pathway in the basal forebrain results in diminish of cholinergic neurons, which

discharge the neurotransmitter acetylcholine (ACh). These neurons project to the hippocampus and neocortex, which are concerned in both memory interruption and cognitive symptoms (Bartus, 2000). ACh is disintegrated by the enzyme acetylcholinesterase (AChE) (Fig.9). Concentration of this enzyme is reduced in moderate and severe AD patients. Cholinesterase inhibition progresses neurotransmitter function and provides relief to AD symptoms (Terry & Buccafusco, 2003).

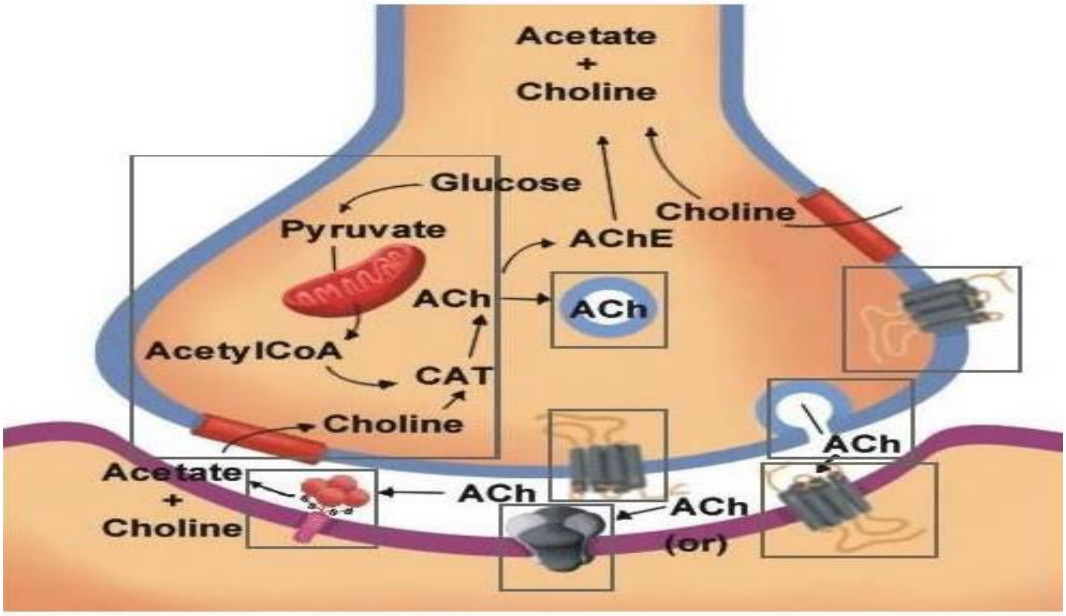


Figure:9. summarising the role of ACh and AChE in cholinergic activities

For a quarter of a century, the pathogenesis of Alzheimer's disease (AD) has been linked to a deficiency in the brain neurotransmitter acetylcholine. This was based on observations that correlated cholinergic system abnormalities with intellectual impairment (Perry et al., 1978). Subsequently, the 'cholinergic hypothesis of AD gained considerable acceptance. It stated that a serious loss of cholinergic function in the central nervous system contributed to cognitive symptoms (RT et al., 1982).

Cholesterol Metabolism

The function of lipid/cholesterol metabolism and AD pathogenesis is gaining adequacy. Cholesterol is known to influence the activity of enzymes implicated in the metabolism of APP in the construction of Aβ. As we have discussed

above, cholesterol-lowering drugs statins associated with a lower threat of developing dementing illness; however, a recent trial has revealed that the use of statins does not impact on enhancement in recorded cognitive impairment (Jones et al., 2008). APOE is involved in the transporting of cholesterol and APOE ε4 allele is a universally recognized marker which enhances AD risk. A high cholesterol level throughout a person's midlife is considered a threat factor for AD (Corder et al., 1993).

Available Treatment of Alzheimer's disease

There are varied hypotheses proposed for the pathogenesis of AD. These comprise glutamate excitotoxicity as a result of obstruct of glutamate uptake into the astrocytes by Aβ aggregates, oxidative stress and membrane lipid peroxidation induced by Aβ aggregates, membrane lipid peroxidation due to C-terminal fragment of APP, microglial activation by Aβ aggregates and molecular pathways activated by Aβ induced stimulation of various kinases including MAP kinases and JNK (Jun amino-terminal kinase) (Suh & Checler, 2002). A discrepancy of ACh in an AD brain is well identified. At the same instant, levels of dementia show a relationship with the extent of neuronal death caused by surplus of glutamate, the most widespread excitatory neurotransmitter in the brain (Alvi et al., 2019a; Wenk et al., 1996).

Acetylcholinesterase Inhibitors

Over the years, both evidence for and challenges to the relationship between acetylcholine dysfunction and AD have been put forward (Terry & Buccafusco, 2003). In essence, it has been argued that acetylcholine dysfunction is not a primary pathological cause for AD but rather a consequence of the disease. Hence, in addition to cholinergic dysfunction, a role for β-amyloid deposition, oxidative stress and inflammation have been investigated in the aetiology of AD, and currently, trials are underway to test disease-modifying agents. Nevertheless, attempts at correcting acetylcholine deficiency in the brain of affected individuals produced the first licensed medication for the symptomatic treatment of AD in the form of acetylcholinesterase inhibitors (AChEIs). Although the benefits of these agents are modest, three (Tacrine, donepezil,

rivastigmine and galantamine) are licensed in the UK. Current guidelines by the National Institute of Clinical Excellence support the use of these agents, although possible changes to the guidelines are presently awaited. AChEIs are widely available for the treatment of mild-to-moderate AD, and they are well tolerated in the majority of patients. Although their main use has been in the stabilisation of cognitive decline, there is evidence linking them with improvement in behavioural and psychological symptoms of dementia (SI, 2004).

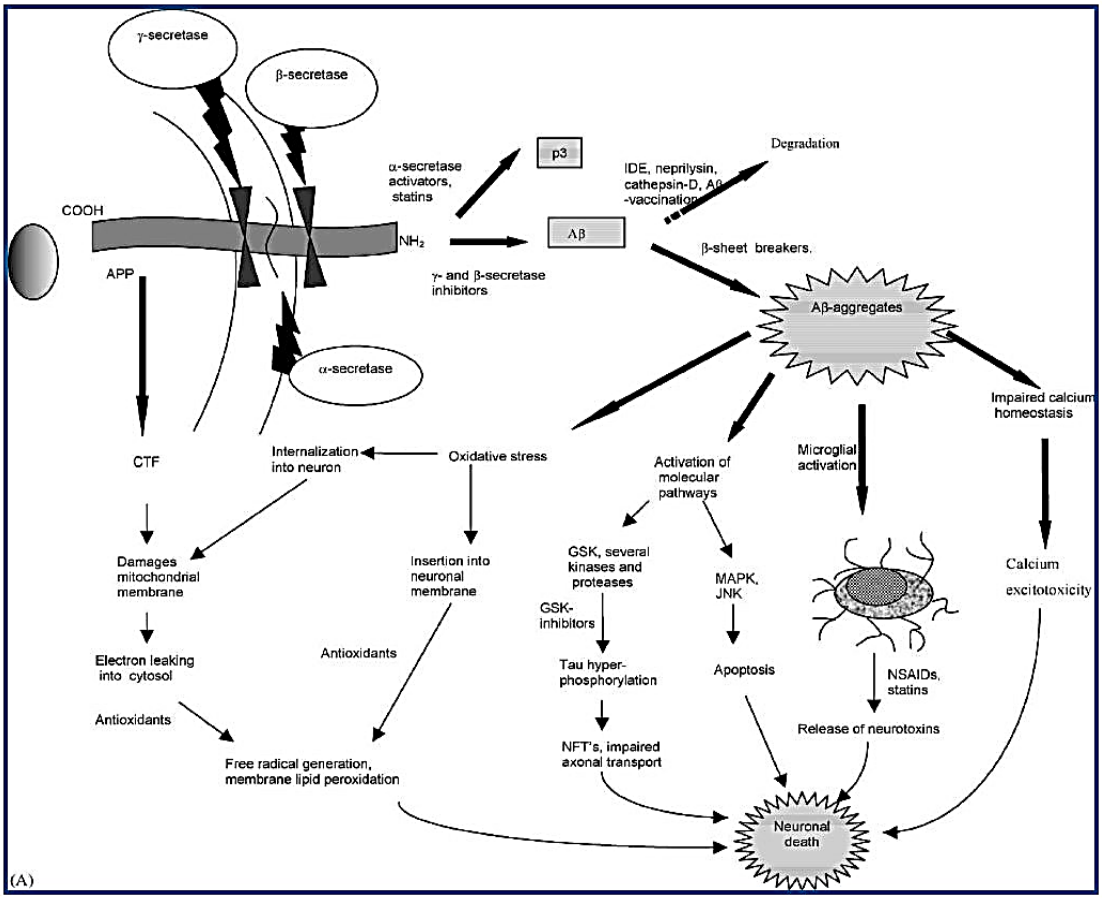


Figure:10. Steps involved in neurodegeneration of AD and targets there in for novel treatment strategies. GSK: glycogen synthase kinase; CTF: C-terminal fragment; MAPK: mitogen activated protein kinase (A: calcium channel).

Since the last decade, AD patients are being treated with substitutes of neurotransmitters that are deficient in AD brain, based on „cholinergic hypotheses". Presently standard drugs for AD treatment are cholinesterase

inhibitors that comprise tacrine (Cognex®), rivastigmine (Exelon®), donepezil (Aricept®) and galantamine (Reminyl®).

Natural compounds as inhibitor of acetylcholinesterase

There are number of compounds that have anticholinesterase activity isolated from the plant sources (Murray et al., 2013). Various plant extract and their compounds such as melatonin, curcumin, resveratrol, nicotine have been tested for the inhibition of acetylcholinesterase which is main enzyme involved in the synthesis of choline (Baum et al., 2008; White & Levin, 1999; Wilson et al., 1995; Zhu et al., 2018). Plants are full of natural antioxidant sources that are effective to reduce oxidative stress raised during AD (Alvi et al., 2019b). Vitamins have been described as therapeutic compounds. Among them, vitamin C, E and D have aroused great interest. Vitamin C is found in several vegetables and fruits, mostly citrus fruits. In vivo studies reported that vitamin C prevented neuroinflammation (A. *et al*., n.d.) and the brain oxidative damage due to its potent antioxidant activity (Sil et al., 2016). Beyond cholinesterase inhibition, these natural chemicals frequently provide a wide range of health advantages, including antioxidation and anti-inflammation. These natural compounds are posing an exciting prospect for the identification of effective treatment of AD since they have the ability to improve symptoms of AD by reducing the inflammation and reactive oxygen species (Heneka et al., 2015).

Plants are natural, and a mixture of various flavonoids, terpenoids, glycosides and phenol that are extracted from different plant organs. Oxidative stress which is mainly induced by the free radicals such as hydroxyl radical (HO•), superoxide (•O−2) etc. has been reported to be involved in several diseased conditions such as diabetes mellitus (Nabi et al., 2019, 2021), neurodegenerative disorders such as Parkinson’s disease and Alzheimer’s disease (Alvi et al., 2019b), cardiovascular diseases (atherosclerosis and hypertension) (P. Ahmad et al., 2020) respiratory diseases (asthma) (Loperena & Harrison, 2017).

Cestrum nocturnum

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Solanales
Family	Solanaceae
Genus	Cestrum
Species	*C.nocturnum*

There are more than 300 species contain genus Cestrum mostly in Asia, Europe, Africa as well as most of them are grown in warm 'subtropical' as well as 'tropical regions'. *Cestrum nocturnum* (*C. nocturnum*) is a member of the family Solanaceae. It is a strongly scented flower that blooms at night thus alternatively known as 'lady of the night', 'Queen of the night' and 'night blooming Jasmine'. Jasmines generally grow in all types of soils. However, they are better adapted to rich loamy or dry sandy and irrigated soil. n soil with more clay, the vegetative growth is vigorous but flower production is lest in amount, while in soil with gravel, the plants exhibit stunted growth. *C. nocturnum* is used as a remedy for different health disorders. This sprawling shrub has glossy simple leaves, vine like stems, greenish-creamy white tubular flowers and fleshy berries. The berries are marfil white or aubergine in colour. The species name 'nocturnum' refers to the species' habit of opening its small, heavily-scented flowers at night. The flowers release powerful sweet perfume at night. It is made into a rare attar (raat ki rani) which is used in Indian and Middle East perfumery. It is said to be the world's strongest smelling plant. Indeed, the scent can reach up to 165 feet away from the location of plant (Ratsch and Christian 1998; Sarkar et al., 2016).

According to WHO, more than 80% of developing country's population depends on plant-based medicines for their health care needs. From the time immemorial, this shrub is used as a traditional medicine. In India the Malasar people use its juice for cataracts. It contains secondary metabolites such as saponins, flavonoids, cardiac glycosides, alkaloids, steroids and tannins which have biological activity, kindling scientific interest. Optimal growth occurs at

about 80˚F. Though night blooming jasmine blooms in night, it requires at least 6 hours of sunlight and partial shade every day to bloom.

Traditional uses

CN flowers are presented as offerings to Shiva and Ganesh in Kathmandu. Nepalese shamans create a ritual incense from the leaves and fresh flowers, eat the fresh flowers and smoke them when dried to increase the spiritual healing energies. The plant is also used as a stupefying charm medicine in West Indies. The Yucatec Maya use CN leaves and flowers in hot baths as a treatment for night sweats. The plant is occasionally added to liquor in Kalinchok, a region north of Kathmandu. CN has long been used in traditional Chinese medicine (TCM) to treat digestive diseases for centuries (Illustrated Handbook of Chinese Advanced Plant). Numerous studies have identified that CN has a great deal of pharmacological actions, including analgesic action, central inhibitory action, and antidiabetic activity (Kamboj et al., 2013)

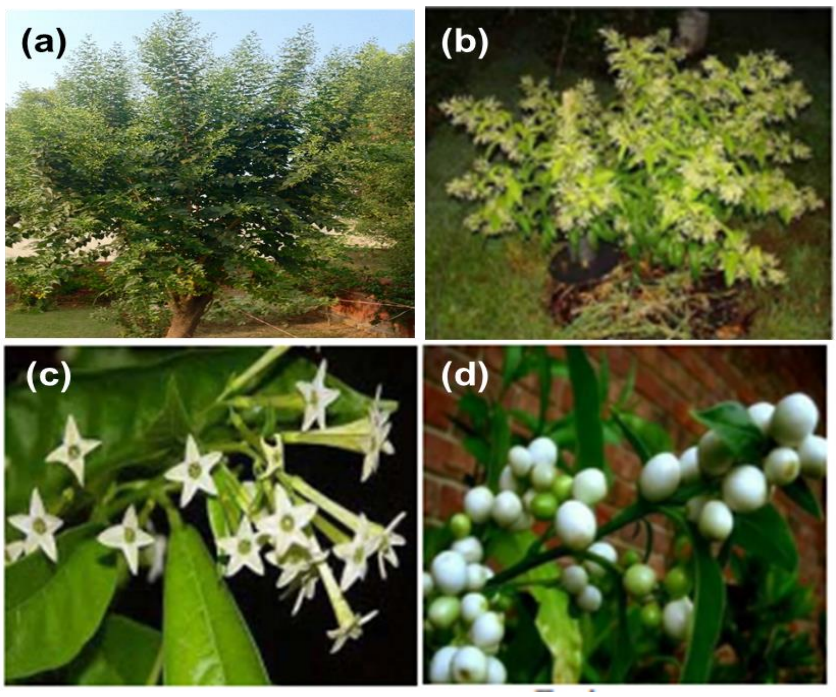


Figure 11: *Cestrum nocturnum* A*:* Whole Tree, B: Leaf C: Flower, D: Fruits

Medicinal uses

In traditional medicine, leaves of *Cestrum nocturnum* have been used for their pharmacological significance in burns and swellings. It is also used for treating epilepsy (He´ctor and Marı´a 2008). Pharmacological studies on the plant proved that the leaves have significant analgesic and bactericidal activity (Zeng

et al., 2003; Avijit et al., 2013). The volatile oil is known to be mosquito-repellent and hence *C. nocturnum* is used to prevent malaria in several African Nations (Jawale at al., 2010). Local anaesthetic effect, inhibitory effect on central nervous system and cardiac arrythmia effect of plant are also documented. Zhong et al., in 2008 reported that n-butanol and polysaccharide extracts from *C. nocturnum* has tumor inhibition ability. respectively.

C. nocturnum possesses many pharmacological properties such as anti-inflammatory, analgesic (Mazumder et al. 2010), antimicrobial (Khan et al. 2011), anti-epileptic (Perez-Saad and Buznego, 2008), anti-cancer (Zhong et al. 2008) and insecticidal (Savchenko et al. 2000) activities. Plant is also being used as a local anesthetic and CNS depressant agent (Zeng et al. 2002). Important phyto-constituents including many flavonoids, alkaloids and phenols have been reported in *C. nocturnum* (Prasad et al. 2013). Most of these flavonoids have hepatoprotective activity (Ali et al. 2013).

Anti-cancerous activity of *C. nocturnum*

Lu et al. performed the n-butanol part isolated from the flowers of *C. nocturnum* produced an inhibitory effect on the proliferation of human hepatocellular carcinoma Bel-7404, human gastric carcinoma SGC-7901, and cervical cancer HeLa cells in a dose-dependent manner (Lu et al., 2010). However, the fractions responsible for the antiproliferation effect of n-butanol part from *C. nocturnum* flowers and related mechanisms remain unknown. The fraction C4 and C5 extracted from the n-butanol part of *C. nocturnum* flowers showed significant cytotoxic potential towards a wide range of human malignant cell lines with low cytotoxicity to immunocytes and exhibited strong antitumor activities against Bel-7404 cells. These antitumor activities include attenuation of cancer cell proliferation as well as induction of apoptosis at the G0/G1 and G2/M phases through enhancement of DNA damage and inhibition of topoisomerase II relaxation activity (Wu et al., 2017).

Anti-Bacterial and anti-fungal activity of *C. nocturnum*

The crude MeOH extract of plant of *C. nocturnum* (Solanaceae) and its subsequent fractions were tested against various bacterial and antifungal

strains with the exception of *Pseudomonas aeruginosa, Staphylococcus aurous, Bacillus subtilis, Escherichia coli and Shigella flexenari*. The zone of inhibition ranged from 19 to 280 µg/ml. The crude extract and fractions were also susceptible to *Candida* species and *Asper* species. The zone of inhibition for various fungi ranged from 170 to 290 µg/ml (Rokade et al., 2016).

OBJECTIVES

I. Collection and preparation of Plant material.

II. Solvent based extraction and phytochemical screening of *C. nocturnum.*

III. In vitro antioxidative studies of different fractions of *C. nocturnum* by DPPH radical scavenging assay.

IV. In vitro antioxidative studies of different fractions of *C. nocturnum* by ABTS radical scavenging assay.

V. In vitro anti-acetylcholinesterase study by *C. nocturnum* extract.

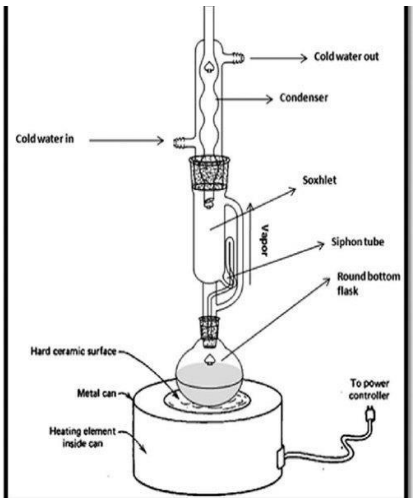
MATERIAL AND METHODS

Chemicals

Chemicals such as n-hexane, ethyl acetate, Dichloromethane, Methanol (MeOH) were obtained from Merck, India.1,1-diphenyl-2-picrylhydrazyl (DPPH), ABTS was purchased from the Hi Media Laboratories, Mumbai, India. DTNB (5,5-dithio-bis-(2-nitrobenzoic acid), Acetylcholine iodide (AchI), 9-Amino-1,2,3,4-tetrahydroacridine hydrochloride (Tacrine hydrochloride) and acetylcholinesterase were purchased from Sigma Aldrich USA. All chemicals were of analytical grade**.**

Instruments

Soxhlet apparatus



A Soxhlet extractor is a type of laboratory glassware invented in 1879 by Franz Von Soxhlet. It was originally designed for the extraction of lipid from a solid test material, but can be used whenever it is difficult to extract any compound from a solid. The key advantage of this type of extraction; only clean warm solvent is used to extract the solid in the thimble. This increases the efficiency of the extraction when compared with simply heating up the solid in a flask with the solvent. In the soxhlet extractor, there are five main components. The components are condenser, extraction chamber, thimble, syphon arm and round boiling flask.

Condenser- It is placed at the top of the soxhlet extractor body. It is converted from vapour into a liquid that trickles into the extraction chamber containing the sample.

Extraction chamber- It allows the sample of solvent that is used during the extraction process. The solvent which condenses at the condenser drips down through the extraction chamber.

Extraction thimble- Cellulose and glass microfiber extraction thimbles are known for their purity and consistent high quality. The thimbles are widely used in soxhlet extraction units providing a safe, convenient and efficient method of solvent extraction of solids and semisolids. Cellulose extraction thimbles are produced from high quality alpha cellulose cotton linter and have excellent mechanical strength and retention.

Round Bottom Flask- It contains a solvent that was used in the extraction. The capacity is 500 ml.

Percentage yield of sequentially extracted plants in different solvent systems was calculated by using the formula.

C. nocturnum whole plant was collected from the local area around Integral University, Lucknow, India, in the months of february. The plant was botanically identified and authenticated by Dr. Mohd. Tariq, National Botanical Research Institute, Lucknow, India. *C. nocturnum* whole plant were shed dried and made in coarse powder, avoiding sun dried due to the signature modification of the biochemicals.Thedried powder (25 g) of the plants was extracted using nonpolar, partially polar, and polar solvents successively with the required amount of each of n-hex, DCM, EtOAc, MeOH, and water solvents in soxhlet apparatus until it turned colourless. The solvent was removed, filtered, and dried at room temperature,

$$\%\text{yield} = \frac{\text{Weight of crude extract}}{\text{Weight of raw material}} \times 100$$

Phytochemical screening

Phytochemical screening is a qualitative assay consisting of tests for phenols, alkaloids, tannins, flavonoids, saponins and triterpenoids, steroids, cardiac glycosides (harborne et. al., 1973).

Test for phenols: The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. 5 ml of the extract was pipetted into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for colour development.

Test for tannins: 10 mg sample was boiled in 50 mL of distilled water and then filtered. A quantity (5 mL) of test solution was added into a test tube following some drop of FeCl3. Formation of brownish green or blue, black colouration indicates presence of tannins.

Test for flavonoids: 10 mg sample was mixed with 10 mL of distilled water. The mixture was heated for 5 minutes and filtered. The filtrate was mixed with Mg powder, 1 mL of strong HCl and 1 mL of amyl alcohol. Formation of colour in the amyl alcohol layer indicates flavonoids.

Test for saponins: 10 mg sample was added into the test tube and 10 mL of boiling water was added and then cooled. The mixture was agitated vertically for 10 seconds. For 10 minutes formation of foam indicates saponins.

Test for triterpenoids: 10 mg sample was mixed with 5 mL of ether solution and evaporated. Test solution was mixed with anhydrous acetate acid and strong H2SO4 (2:1). Formation of red-green colour indicates triterpenoids.

Test for steroids: Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H2S04. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for cardiac glycosides (Keller-Killani test): Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride

solution. This was underlaid with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxy-sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer

Antioxidant assay

DPPH Radical Scavenging activity

The DPPH (1, 1-diphenyl-2- picrylhydrazyl) radical scavenging capacity of the various extract of C. nocturnum was determined by the method of Brand-Williams et al.(1995). Briefly the free radical scavenging activity based on the scavenging activity of the stable DPPH free radical. DPPH molecule determines with the occurrence of a purple colour.DPPH solution (132mM) was prepared in methanol in a dark reagent bottle. 100µl of the leaf, stem and fruit extracts from C. nocturnum and ascorbic acid (Concentration ranging from 7.81 to 1000µg/ml) was added to 2ml of DPPH solution and the reaction mixture was incubated for 15 minutes at 27ºC in a water bath and absorbance was measured at 517 nm. The reduced form of DPPH was generated, accompanied by the disappearance of the violet colour. Ascorbic acid was used as a reference standard. Percent (%) scavenging of DPPH free radical was measured using the following equation.

$$\%\text{DPPH} = \frac{\Delta\text{Absorbance of control} - \Delta\text{absorbance of test sample}}{\Delta\text{Absorbance of control}} \times 100$$

Further, IC50 value represented the concentration of the extract that caused 50% inhibition of DPPH radicals and was calculated by interpolation of linear regression analysis.

ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay ASSAY

Radical-scavenging activity of *C. nocturnum* was determined according to Re et al., radicals were pre generated by adding 5 mL of a 4.9 mM potassium

persulfate solution to 5 mL of a 14 mM ABTS solution and kept for 16 h in the dark. Different concentrations of extract (50–700 *μ*g/mL) were added to the above activated pre-generated ABTS solution. This solution was suitably diluted with distilled water to yield an absorbance of 0.70 at 734 nm and then used for antioxidant assay. Ascorbic acid (50 *μ*g/mL) was used as a reference compound. 50 *μ*L was added to 950 *μ*L of ABTS solution and vortexed for 10 s and after 6 min and then reduction in absorbance was recorded at 734 nm, using distilled water as a blank, on ELICO (SL-150) UV-visible spectrophotometer (Sanathnagar, Hyderabad, Andhra Pradesh, India). Same volume of test solutions of each extract was also taken in a similar manner. The result was compared with control (only ABTS solution) having absorbance 0.712 ± 0.032 (Re et. al., 1999)

Acetylcholinesterase Inhibition Assay

Acetylcholinesterase test was prepared according to Ellman et al (1961) with slight modification (Ellman et. al., 1961). 33µl of 10 mM-DTNB, 100µl of 1mM-AChI, 767µl of 50 mM-Tris HCl having a pH value of 8, and 100µl of varied concentrations of extract were added into a 2 ml cuvette. We used the aforementioned cuvette as a 'blank', while in another cuvette, 300µl of buffer was substituted by equal volume of AChE-enzyme-solution containing 0.28 U ml^{-1}. The standard drug tacrine was used for the competitive analysis. This reaction was observed for 20 min at a wavelength of 405 nm. Values given here represent the average of 3 replicates. We calculated the percent-inhibition of acetylcholinesterase enzyme-activity by the equation given below:

$$= \frac{\Delta\text{Absorbance of control} - \Delta\text{absorbance of test sample}}{\Delta\text{Absorbance of control}} \times 100$$

RESULTS

Table 1: %Yield of phytochemicals in various extracts of *C. nocturnum* leaf

Extract	%Yield leaf extract
n-Hexane	1.44
Dichloromethane	1.9
Ethyl acetate	0.71
MeOH	7.63
Aqueous	6.38

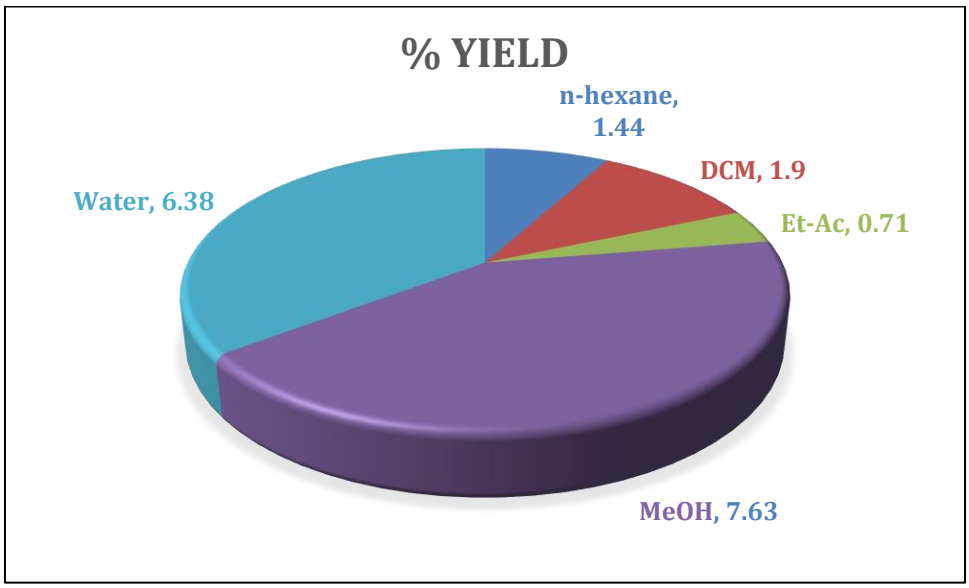


Figure 12: Pie chart representation of %yield leaf extract of *C. nocturnum*

Phytochemical screening of plant

Our result illustrated the significant presence of phenols, alkaloids, tannins, flavonoids, saponins and triterpenoids, steroids, cardiac glycosides in sequentially extracted various fractions of *C. nocturnum*.

Table:2. Phytochemical constituents of sequentially extracted C. nocturnum fractions.

	n-Hexane	EtOAc	DCM	MeOH	Aqueous
Cardiac glycosides	-	+++	-	+	-
Steroids	-	-	-	-	-
Phenols	++	+++	-	-	-
Flavonoids	+++	-	-	+	+
Tannins	++	+++	-	-	-
Saponins	-	-	-	+++	++
Terpenoids	-	-	-	-	-
Quinone	++	+++	-	+++	-
Coumarins	+++	-	-	+	+
Phlobatannins	-	-	-	-	-
Anthocynin	-	-	-	-	-

DPPH Radical Scavenging activity

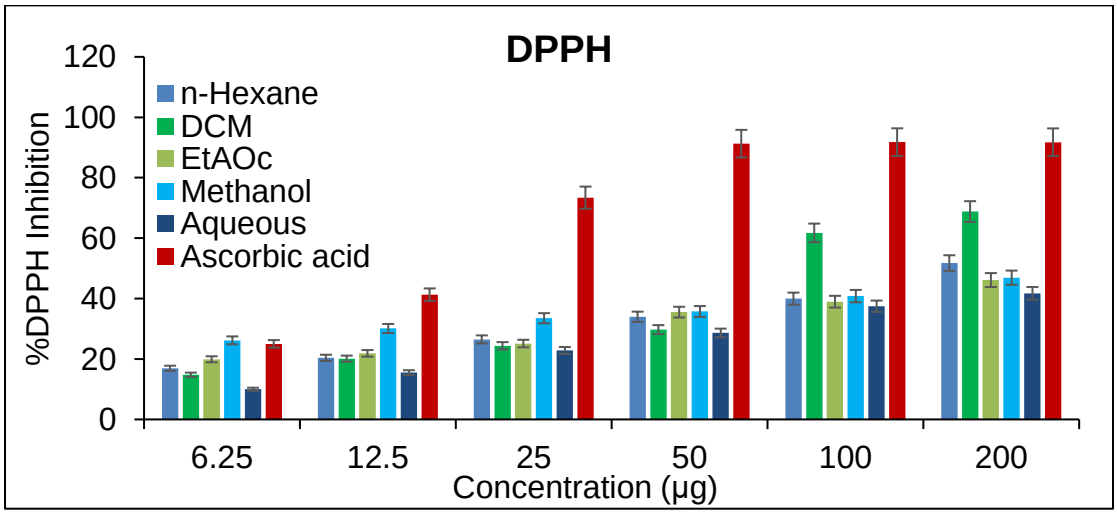


Figure:13. Percent DPPH radical scavenging activity different leaf extract of *C. nocturnum* and standard ascorbic acid. The results are mean ± S.D. of three parallel measurements.

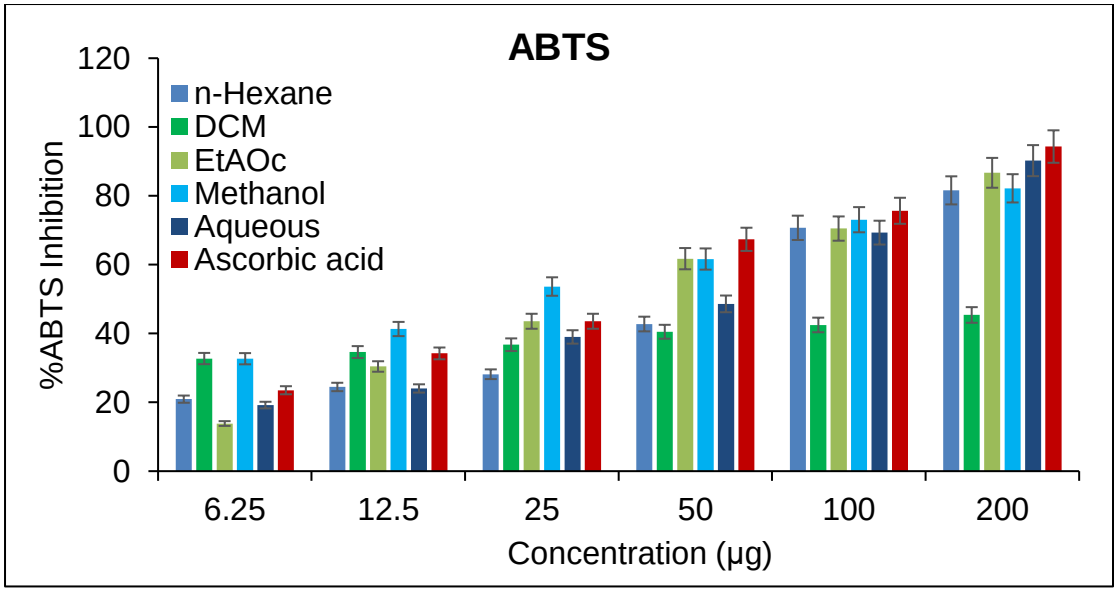


Figure:14. Percent ABTS radical scavenging activity of different leaf extract of *C. nocturnum* and standard ascorbic acid. The results are mean ± S.D. of three parallel measurements.

Table: 3. IC_{50} values of different extract of *C. nocturnum* against DPPH and ABTS radicals.

Solvents	DPPH	ABTS
N- hexane	184.70 ± 3.4	62.60 ±2.61
DCM	82.67 ± 2.89	NS
EtOAc	NS	33.31 ± 1.84
MeOH	NS	20.59± 0.99
Aqueous	NS	53.20 ± 2.89

Acetylcholinesterase inhibition Assay

Our results illustrated that the DCM extract of *C. nocturnum* significantly inhibits the AchE with an IC_{50} value of 119.15 ± 3.67. Which is quite higher than the standard drug tacrine (**Fig.15**).

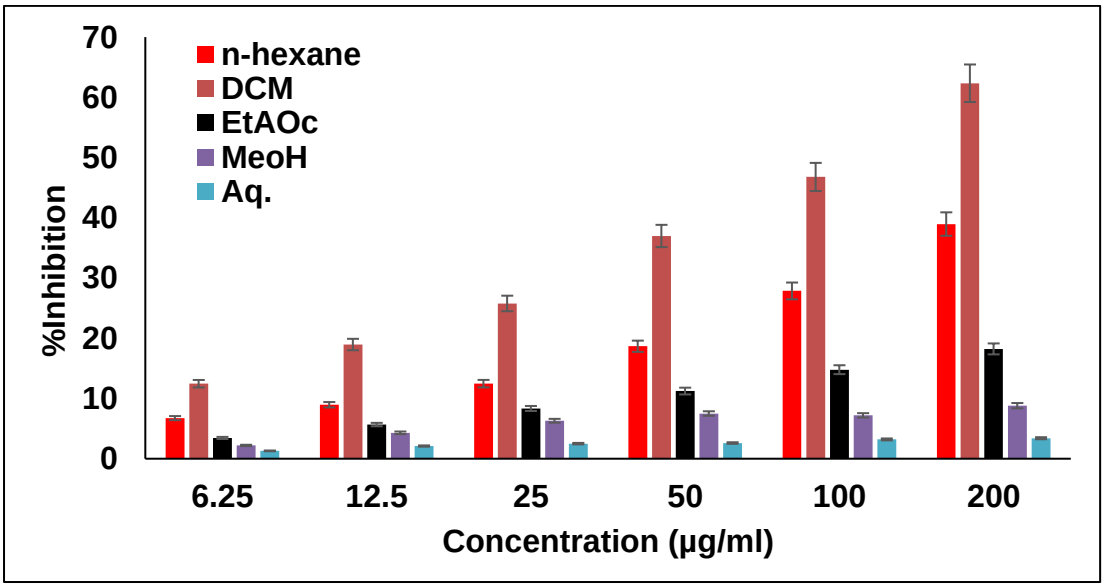


Figure:15. Percent inhibition graph of AchE activity of different leaf extract of *C. nocturnum*. The results are mean ± S.D. of three parallel measurements.

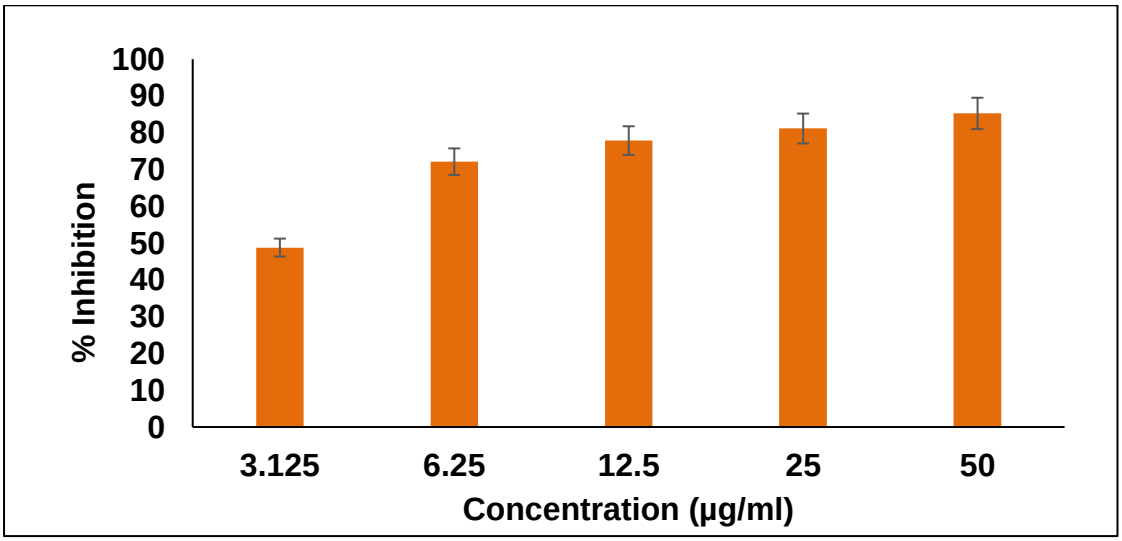


Figure:16. Percent inhibition graph of AchE activity by standard drug Tacrine. The results are mean ± S.D. of three parallel measurements.

Kinetics Study

Kinetics study of methanolic extract of *C. nocturrnum* showed the competitive type of inhibition while aqueous extract showed the non- competitive inhibition. **(Fig.15).**

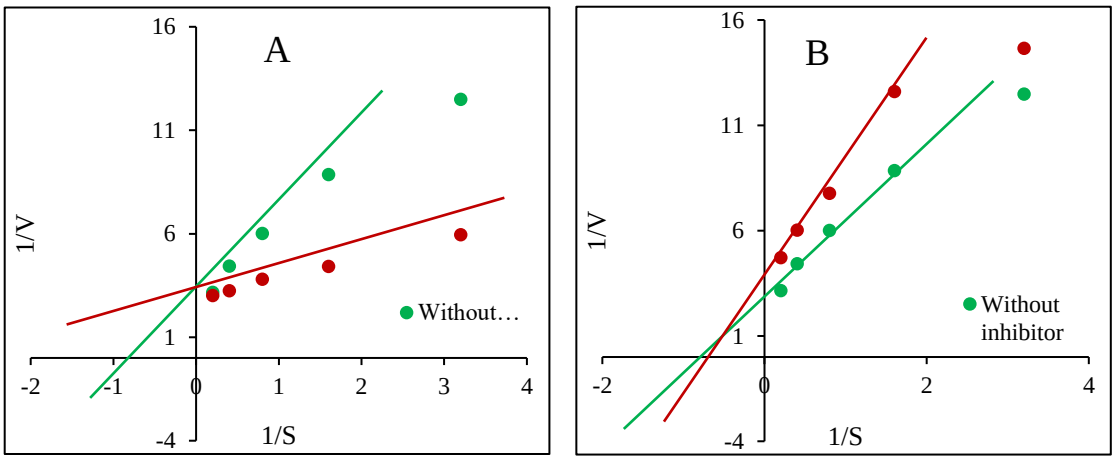


Figure:15. Line weaver burk plot for the inhibition of AchE in the presence of DCM (A) and n-hexane (B) of *C. nocturnum***.**

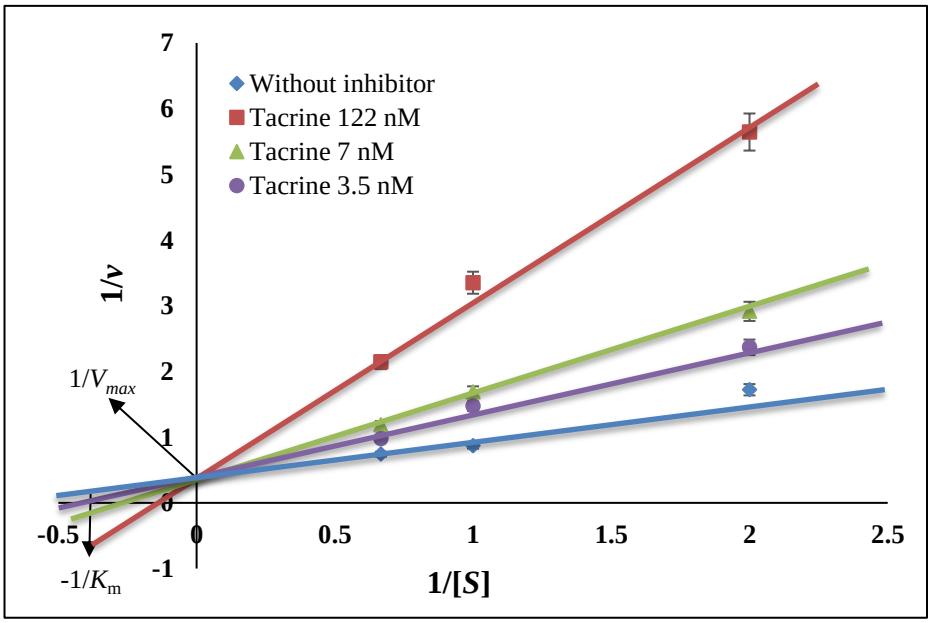


Figure:16. Line weaver burk plot for the inhibition of AchE in the presence of varied concentration of Tacrine.

DISCUSSION

Oxidative stress and inflammation are connected with numerous pathological conditions. Synthetic drugs accessible for curing these disorders cause numerous undesirable effects. A number of studies are being conducted worldwide to assess natural sources for the active or lead compounds with best safety profiles. Phytochemical analysis of *C. nocturnum* extract was done in order to identify the presence of bioactive compounds such as flavonoids, phenols, tannins, and saponins. This is supported by (Samia Shahid 2013) which revealed that *C. nocturnum* were rich in bioactive compounds including phenols, sterols, carotenoids, anthocyanins, procyanins, flavonoids, tannins, carotenoids, alkaloids, and polyphenols which are good sources of antioxidant. The potential health benefits of *C. nocturnum* have been partially attributed to their phenol contents, especially flavonoids that have received much attention from the literature over the past decade for its biological effects. The flavonoids and phenolic acids are known to possess antioxidant activities due to the presence of hydroxyl groups in their structures, and their contribution to the defense system against the oxidative damage due to endogenous free radicals is extremely important (Saggu et al., 2014). Phenolic compounds or polyphenols and alkaloids are secondary plant metabolites that are ubiquitously present in plants and their products. Most of them have been proven to have high levels of antioxidant activities (Razali et al., 2008). Due to their redox properties, these compounds such as flavonoids, tannins, and alkaloids contribute to the overall antioxidant activities. The tabulated data was supported with findings done by (Shahid S. et al., 2013). Medicinal plants are accepted as a vital source of new compounds having therapeutic potential. The research on folkloric usage of plants as pain relievers, anti-inflammatory and hepatoprotective agents should therefore be viewed as a fruitful research strategy for search of new analgesic, anti-inflammatory and hepatoprotective drugs. DPPH radical scavenging activity is widely used to evaluate antioxidant activities in a relatively short time. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The effects of phenolic compounds on DPPH radical scavenging are thought to be due to their hydrogen donating ability (M. N. Siddaraju & S. M. Dharmesh 2007).

It is reported that the decrease in the absorbance of DPPH radical caused by phenolic compound is due to the reaction between antioxidant molecules and radicals, resulting in the scavenging of the radical by hydrogen donation and is visualized as a discoloration from purple to yellow (S. J. Meir et al., 1995). DPPH is a preformed stable radical used to measure radical scavenging activity of antioxidant samples. This method is based on the reaction of DPPH radical that is characterized as a stable free radical with deep violet colour and any substance that can donate hydrogen atom to DPPH thus reduces it to become stable diamagnetic molecule (K. G. Lee and T. Shibamoto 2001). Reduction of DPPH radical was observed by the decrease in absorbance at 517 nm whereas colour changes from purple to yellow. The various fractions of *C. nocturnum* significantly reduced DPPH radicals. It was found (Table-2) figure 12. that activity increases by increasing the concentration of the fractions in the assay. The various concentrations of DCM and n-hexane fractions exhibited the highest percent of inhibition of DPPH radical as compared to other fractions. The various concentrations of the fractions which showed percent inhibition greater than 50% were found to be significant ($p < 0.05$) when compared with negative control i.e. blank. IC_{50} value is defined as the concentration of substrate that causes 50% loss of the DPPH activity and was calculated by linear regression of plots of the percentage of antiradical activity against the concentration of the tested compounds. IC_{50} is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process. A lower value would reflect greater antioxidant activity of the fraction Riaz T. et al., 2012). The IC50 values of the studied fractions were calculated (Table.2). It was found that plants with AChE inhibitory and antioxidant activity may help in preventing or alleviating patients suffering from AD (Ferreira et al., 2006). In this present study, DCM and n-hexane extract of *C. nocturnum* was tested on its inhibitory activity toward AChE due to its high oxidation reducing power. Our results show the inhibitory potential of tacrine and different fraction of *C. nocturnum* in which the n-hexane and DCM showed the highest inhibition against AChE. It is worth mentioning that tacrine in 1993 received FDA approval as the first approved drug for Alzheimer's disease and acts as an AChE inhibitor; however, due to its various

side effects, such as hepatotoxicity, it was discontinued in 2013 for disease treatment (Sharma K, 2019; watkins et. al., 1994). Similarly, the other approved medications have several side effects, and due to the economic and safety of natural products, there is a continuous need to search for bioactive potential of the natural products or compounds derived from them. Prasad and Muralidhara found that co-administration of geraniol with curcumin has an inhibitory effect on AChE activity Prasad & Murlidhara 2014) . Previous reports suggested that natural plant-based products, such as essential oils containing geraniol, showed neuroprotective effects through the inhibition of AChE (Oboh G et. al., 2014) With regard to these earlier reports and our enzyme inhibition results, which suggest the bioactive potential of *C. nocturnum*. It was found that plants with AChE inhibitory and antioxidant activity may help in preventing or alleviating patients suffering from AD (Ferreira et al. 2006).

CONCLUSION

On the verge of side effects of synthetic acetylcholinesterase inhibitors, which are causing serious implications in Alzheimer patients, we need new concepts, new theories and new points of view rather than standard drugs because of their side effects.

Our results for the first time reported the phytochemical screening, antioxidative studies and anti-acetylcholinesterase activity of *C. nocturnum.* The DCM fraction of *C. nocturnum* extract showed the maximum DPPH radical scavenging activity. Therefore, a study was conducted by us to explore the anti-acetylcholinesterase activity of *C. nocturnum*. Naturally occurring inhibitors are more preferred because of their antioxidant and anti-inflammatory properties. They help to reduce inflammation and reactive oxygen species that are responsible for development of various diseases such as, Alzheimer's disease, parkinson disease, cancer, DNA damage, Hyperglycaemia, hypercholesterolaemia, cardiovascular diseases. In our primary study, we performed literature searching, selection of effective medicinal plant as well as the collection and the extraction of *C. nocturnum* extracts in various solvents i.e., n-hexane, Dichloromethane, Ethyl acetate, Methanol, and Aqueous. In vitro antioxidative analysis of five fractions of *C. nocturnum* by DPPH and ABTS assay was carried out. It was inferred that the DCM fraction exhibited the highest antioxidant activity among all. It is well comparable with the standard ascorbic acid. DCM fraction of *C. nocturnum* significantly inhibits the AchE. For the better understanding of anti-acetylcholinesterase activity of the extract, the *in-vivo* study is needed.

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