A DISSERTATION ON

Elucidation of *in-vitro* antioxidant and Anti-Acetylcholinesterase Property of Sequentially Extracted Fractions of Khudari (*Phoenix dactylifera L.)*

SUBMITTED TO THE
DEPARTMENT OF BIOSCIENCES
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IN PARTIAL FULFILMENT
FOR THE
DEGREE OF MASTER OF SCIENCE
IN BIOTECHNOLOGY

BY

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CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **Ms. Tarannum Khan** 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 **"Elucidation of *in-vitro* Antioxidant and Anti-acetylcholinesterase Property of Sequentially Extracted Fractions of Khudari *(Phoenix dactylifera* L.)"** 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:**26/June/2022**
Place: Lucknow

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TO WHOM IT MAY CONCERN

This is to certify that the study conducted by **Ms. Tarannum Khan** student of M.Sc. Biotechnology (IV semester), Integral University has completed her four months dissertation work entitled **"Elucidation of *in-vitro* antioxidant and anti-acetylcholinesterase Property of sequentially extracted fractions of Khudari *(Phoenix dactylifera* L.)"** 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.

Dr. Snober S. Mir
Head
Department of Biosciences
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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: **26/June/2022**

Place: **Lucknow** **Ms. Tarannum Khan**

CONTENTS

LIST OF ABBREVIATIONS

DW	Distilled water
DPPH	2,2-diphenyl-1-picryl hydrazyl
AchE	Acetylcholinesterase Enzyme
AchI	Acetylcholine iodide
ROS	Reactive oxygen species
AD	Alzheimer's Disease
NFT	Neurofibrillary tangles
Aβ	Amyloid- Beta
EOs	Essential Oils

AchEIs	acetylcholinesterase inhibitors
IC50	Inhibitory concentration 50
PA	Pathological ageing
NMDA	N-methyl-D-aspartate
DLB	Dementia with lewy bodies
APP	Amyloid precursor protein
DAT	Dopamine transporter
JNK	Jun amino-terminal kinase
CADASIL	Cerebral Autosomal Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy
SPECT	Single-photon emission tomography
COPD	Chronic obstructive pulmonary disease
ApoE	Apolipoprotein
CSF	Cerebrospinal fluid
ABTS	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid
VCP	Valosin containing protein
MAPT	Microtubule associated protein
FUS	Fused in sarcoma
PS-1	Presenilin 1
PS-2	Presenilin 2

INTRODUCTION

Alzheimer's disease (AD) has been considered as the major worldwide health anxietiesas 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 impacton 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 ofAβ, decreased activity of Aβ-degrading enzymes, or alterations in transport processes that shuttle Aβ across the blood–brain barrier. Aβ oligomers impairsynaptic 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 oligomersand can form larger intra-neuronal aggregates, displacing vital intracellular organelles (Mucke, 2009b).

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 oftools. 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 decreasedglucose 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 intensifier 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 of acetylcholinesterase inhibitors, maintaining its activity as a neurotransmitter (Balkiset al., 2015; Knapp et al., 1994).

REVIEW OF LITERATURE

Dementia

Dementia is not a particular disease; it is a general term that describes an ample range of symptoms. In other words, it is a multi-faceted cognitivedemolition that is typically progressive, and constantly involves functional impairments (Ripich & Horner, 2004). With the ageing of the population and the estimated augment of dementia in the upcoming years, it is crucial that we understand the needs of people with dementia in order to furnish proper care (Cadieux et al., 2013). Persons affected by dementia are incapable to engage in daily activities with the similar level of independence as they had enjoyed previously in their life. While symptoms of dementia can vary significantly, at least two of the following core mental functions must be extensively impaired to be considered dementia: (Schneider et al., 2014).

- Memory
- Communication and language
- Ability to focus and pay attention
- Reasoning and judgment
- Visual perception

Diagnosis of dementia

There has been a foremost swing in how scientists, neurologists, neuroradiologists, neuropsychologists, and other health professionals think about the dementias due to major scientific advances during 1990 to 1999 which is well known as the “Decade of the Brain”. These advances have affected diagnostic terms, pharmacologic options, and behavioural interventions. There are two categories of dementia that are; “presenile dementia” (before age65 years) and “senile dementia” (after age 65 years) (Nakamura, 1990). In recent years, several different working groups comprising panels of international experts have developed consensus statements regarding differential diagnosis. By testing patients at periodicintervals, the diagnosis is determined by clinical examination to be “possible”, “probable” or “definite”. Sadly, the gold standard for making a “definite” diagnosis is only autopsy, which is really hazardous for brain (Lin et al., 2013).

Causes of dementia

Dementia is caused by damage to brain cells. This injure interferes with the aptitude of brain cells to communicate with each other. When brain cellscannot communicate normally, thinking, behavior, and feelings can be affected (Ewald & Li, 2010). The brain has many discrete regions, each of which is responsible for diverse functions (forexample, memory, judgment andmovement). When cells in a particular region are damaged, that region cannotcarry out its functions normally. Different types ofdementia are associated withparticular types of brain cell damage in particular regions of the brain. For example, in AD, high levels of certain proteins inside and outside brain cells make it hard for brain cells to stay healthy and to communicate with each other. The brain region called the hippocampus is the centre of learning and memory in the brain, and the brain cells in this region are often the first to be damaged. That's why memory loss is often one of the earliest symptoms ofAD (Liang et al., 2008). While most changes in the brain that cause dementia are permanent and worsen over time, thinking and memory problems caused by the following conditions may improve when the condition is treated oraddressed: like depression, medication side effects, excess use of alcohol, thyroid problems, vitamin deficiencies (Prince et al., 2015).

Types of dementia and their characteristics

There are many different forms of dementia; some are reversible, but the majority are not. Because of the degeneration of brain cells and their interactions, neurodegenerative dementias are gradual and irreversible. Dementia is divided into two main categories: cortical and sub-cortical dementias, depending on which region of the brain is thought to be the originof the disease (Huber et al., 1986).

Cortical Dementia

Cortical dementia come up from a disorder affecting the cerebral cortex, the outer layersof the brain that play a critical role in thinking abilities like memory and language. AD and Creutzfeldt-Jakob disease are two forms of cortical dementia. People with cortical dementia typically show severe memory loss and aphasia: the inability to recall words and understand language (Whitehouse, 1986).

Sub-Cortical dementia

Sub-cortical dementia occurs from dysfunction in the parts of the brain that are beneaththe cortex. The term subcortical dementia was introduced to describe a clinical syndrome believed to be characteristic of progressive supranuclear palsy, normal pressure hydrocephalus, and Huntington's disease. In these conditions, impairments in memory and learning were associated with slowness of intellectual function, inertia, and apathy (Cummings & Benson, 1984). There are cases of dementia where both parts of the brain have a tendency to be affected, for instance multi-infarct dementia or vascular dementia (Battistin & Cagnin, 2010).

Historical perspective of Alzheimer's disease

Alzheimer's disease was discovered in 1906 by Alois Alzheimer, a German neurologist and psychiatrist (Alzheimer's Association 2010). The disease was initially observed in a 51-year-old woman named Auguste D. Her family brought her to Dr. Alzheimer in 1901 after noticing changes in her personality and behavior. The family reported problems with memory, difficulty speaking, and impaired comprehension. Dr. Alzheimer later described Auguste as having an aggressive form of dementia, manifesting in memory, language and behavioral deficits (Khachaturian, 1985). One of his patients died after years of rigorous memory problems, and confusion etc. The doctor observed eminent opaque deposits of neuritic plaques surrounding the nerve cells after her death, while performing a brain autopsy. He also observed twisted bandsoffibers called NFTs (neurofibrillary tangles) inside the nerve cells. This degenerative brain disorder bears his name, and these plaques and tangles indicate a distinct diagnosis of AD. In the 1960s, scientists revealed an association among cognitive decline and thenumber of plaques and tangles in the brain. Since then, AD is formally recognized as a disease by the medical community (Berchtold & Cotman, 1998). In the1970s, Researchers madehuge breakthroughs in comprehending the human body as a whole, andAlzheimer's disease (AD) became a major focus of research. In the 1990s,this increased attention resulted in important discoveries as well as a better understanding of complicated neuron cells in AD brains. Advance research was done on AD propensity genes; also a number of drugs were permitted to

treat the symptoms of the disease (Derouesné, 2008). Scientists have made tremendous progress in identifying potential environmental, genetic, dietary, and other risk factors for Alzheimer's disease during the previous decade. Scientists also uncovered the mechanism of formation of plaques and tangle in the brain region that are pretentious. The genes that are associated with the early onset Alzheimer's disease (EOAD) and Late onset Alzheimer's disease (LOAD) has been identified. However, genetic risk factors of AD are notclearly defined the causes. The researchers are exploring the life style and environment to discover the process which associated with the progression of the disease (Osborn & Saunders, 2010). Various class of drugs have been tested and approved by the FDA for the alleviation of AD but AD is still untreatable. The drugs presently in use can only treat the symptoms, not the cause of the disorder. Hence, they can only deliberate the progression of cognitive decline (Alvi et al., 2019a).

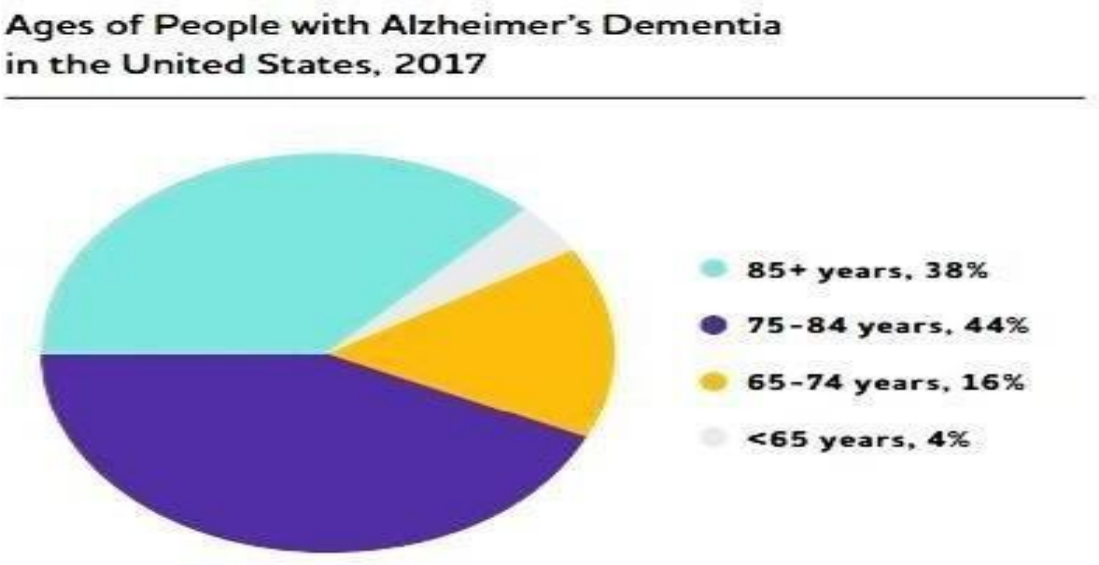

Figure:1. Proportion of people with AD (by age) in the United States. Percentagesmay not total 100 because of rounding, Source: AD Facts and Figures 2014

Alzheimer's Disease

The most familiar category of dementia amongst older people is AD, which primarily involves the parts of the brain that control thought, memory and

language.AD continues to be one of the most complicated human diseases to treat. AD is a genetically intricate and heterogeneous disorder (Bertram et al., 2007). It is an irreversible, progressive brain disease that gradually destroys memory and thinking skills. It is still not apparent whether AD is one disease with a particular foundation ormanifold syndromes with familiar symptoms and/or a common pathology. Even though the possibility of developing AD increases with age; in the majority of people with AD, symptoms initially materialize after age of 60. AD is not a part of normal AG; it is a deadly diseasethat affects the brain (Scodellaro & Pin, 2011). Once considered an exceptionaldisorder, it is now seen as a foremost public health trouble that is critically affecting millions of older and their families. Younger's also get affected by AD but in very few percentages as compare to the older people. An expected 5.2 million Americans of all ages have AD in 2014. This comprises an expected 5 million people of age 65 and older and around 0.2 million individuals under age 65 who have EOAD (Hebert et al., 2013) (**Fig.2**). The brain has billions of neurons, each with an axon andloads of dendrites. To reside healthy, neurons ought to communicate with each other, carry out metabolism, and refurbish themselves. AD disrupts all of these indispensable functions. No one knows what causes AD to begin, but we do know a lot about what happens in the brain once AD takes hold. The most fundamental features of AD incorporate the construction of extracellular protein deposits in the brain that consist of aggregates of beta amyloid protein (Aβ protein) (senile plaques) and NFTs (hyper- phosphorylated tau protein) in the intracellular compartments, turbulence in calcium homeostasis, and disintegration of synapses and neurons. That leads to nerve cell death and tissue loss throughout the brain (Venugopal et al., 2008). Over time, the brain shrinks considerably, disturbing nearly all its functions (**Fig.2)**.

Another viewpoint of how substantial cell loss changes the whole brain in advanced AD **(Fig.3)**. This figure shows a diagonal “segment” throughout the centre of the brain flanked by the ears. In the AD brain, the cortex shrivels up, damaging areas involved in thinking, planning and remembering. Contraction is particularly rigorous in the hippocampus, a part of the cortex that plays a significant role in the construction of new-fangled memories. Ventricles (fluid-filled places contained by the brain) grow bigger**.**

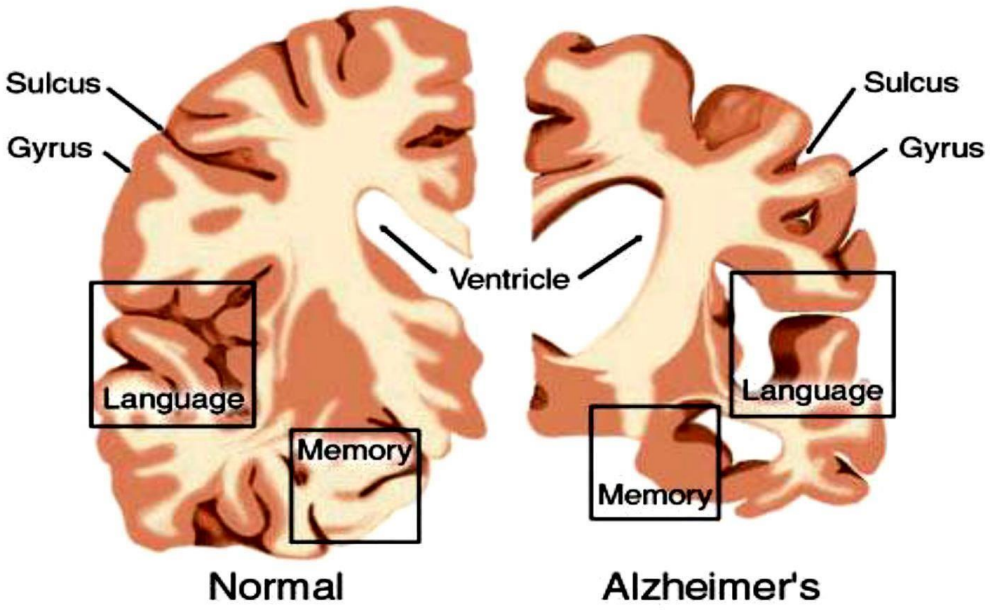

Figure:2 Difference between Normal Brain and AD Affected Brain

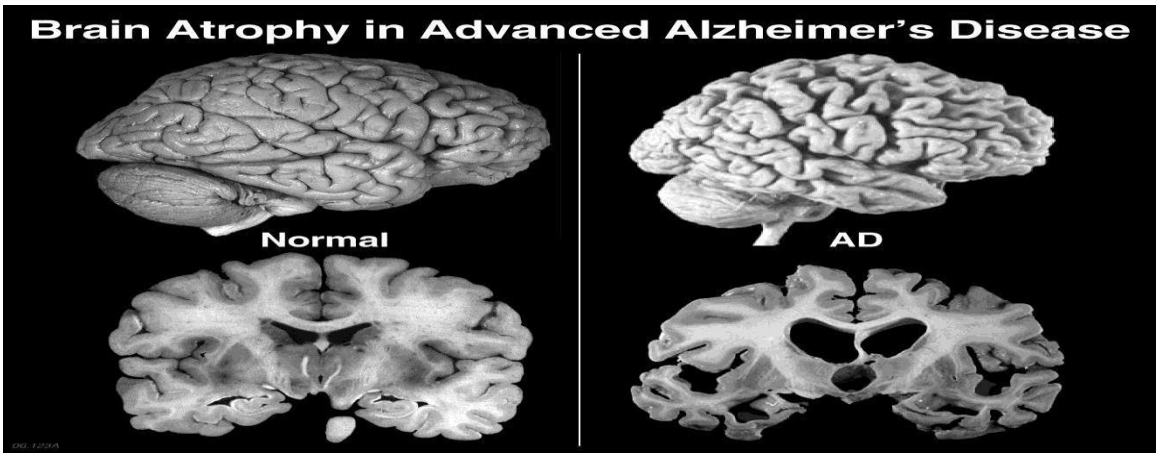

Figure:3. Cross-section of the brain as seen from the front. The cross-section on the left represents a normal brain and the right side represents a brain with Alzheimer’s disease.

As we discussed above, one of the hallmarks of AD is the amassing ofamyloid plaques among nerve cells in the brain. Amyloid is a common expression for protein fragments that the body produces generally. Aβ is a piece of a protein snipped from one more protein named amyloid precursor protein (APP). These protein pieces would collapse and eliminated in case of a healthy brain. However, in case of AD, the splinters mount up to form solid and impenetrable plaques inside the brain. NFTs are insoluble twisted fibers originate in the interior part of the brain's nerve cells. Succession of NFTs pathology is directly associated with both increased neurodegeneration and cognitive decline in AD and other tauopathies, for instance fronto-temporal dementia (AM & M, 2008). Scientists are not completely convinced what causes cell death and tissue loss in the AD brain, but plaques and tangles are key suspects. Scientists can see the dreadful effects of AD when they visualize at brain tissue under the microscope (**Fig.4&5**).

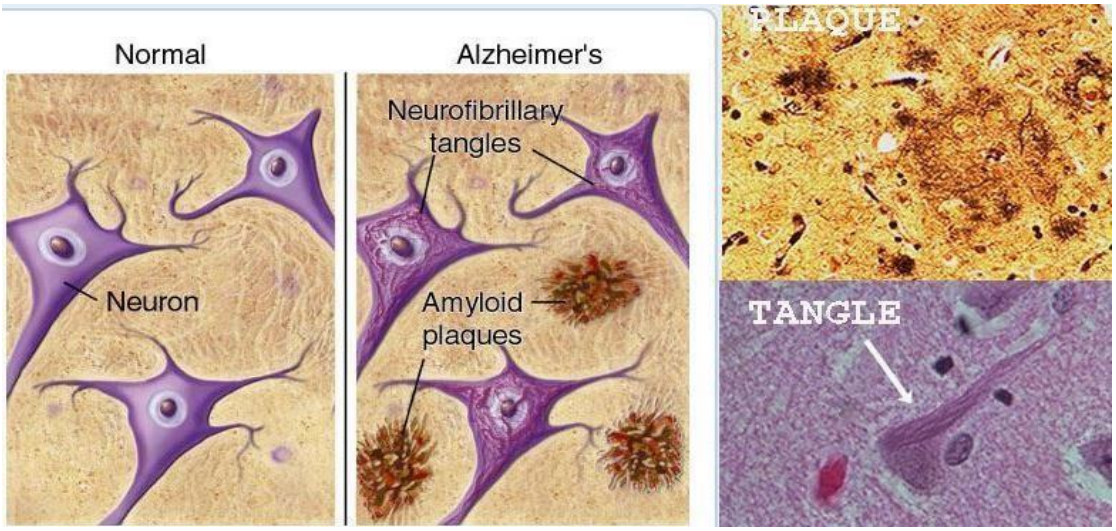

Figure:4. The construction of A*β* plaques and NFTs are thought to contribute to the degradation of the neurons in the brain and the consequent symptoms of AD.

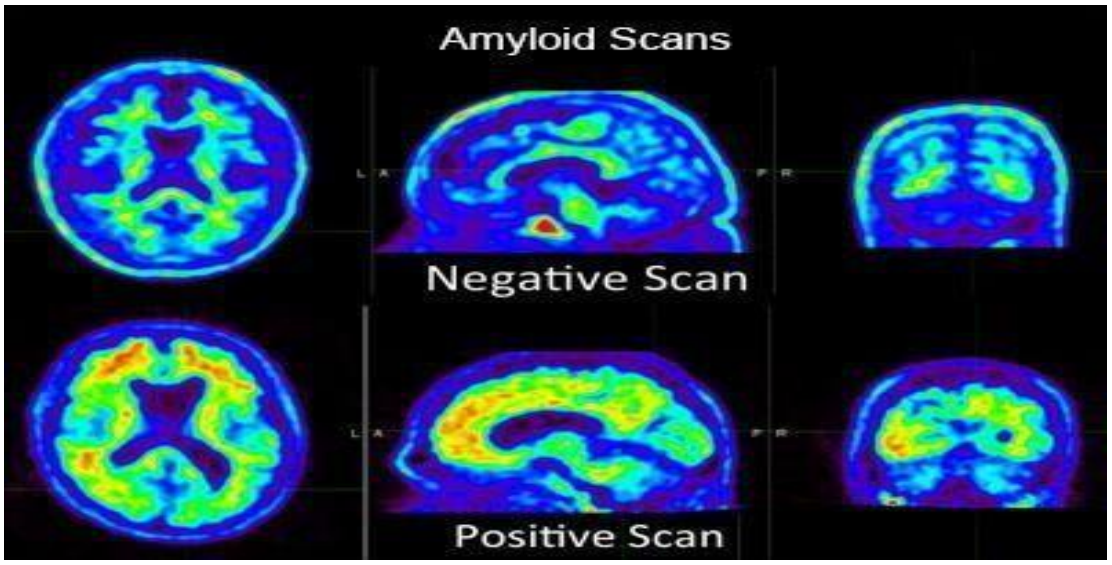

Figure:5. Positive Amyloid plaques Scan shows the bright yellow colour in the brain.

Beta-amyloid Plaques

APP is the precursor to Aβ plaque. It sticks through the neuron membrane. Enzymeslike β- secretase (beta-amyloid cleaving enzyme (BACE or β)) followed by γ- secretase (γ) divide the APP into fragments of protein, principally to form Aβ-42 type aggregations **(Fig.6)** (Hong et al., 2004; Oddo et al., 2003). The construction of extracellular Aβ plaques is described by the amyloid cascade theory of plaque causing pathology (Dam & Deyn, 2006) **(Fig.7).** Aβ fragments come together in clumps to form plaques in vulnerable brain regions, and disrupt the function of neurons. Aβ is chemically "sticky" and progressively builds up into plaques **(Fig8)**. The most detrimental form of Aβ may be groups of a small number of pieces rather than the plaques themselves. The miniature clumps may obstruct cell-to-cell signalling at synapses. They may also activate immune system cells that generate inflammation and devour disabled cells.

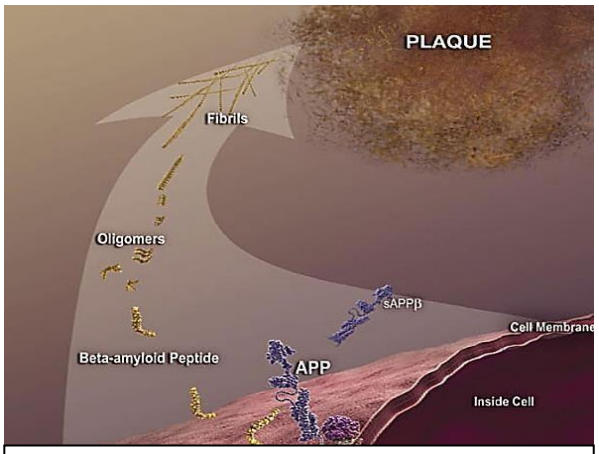

Figure:8 Amyloid plaque

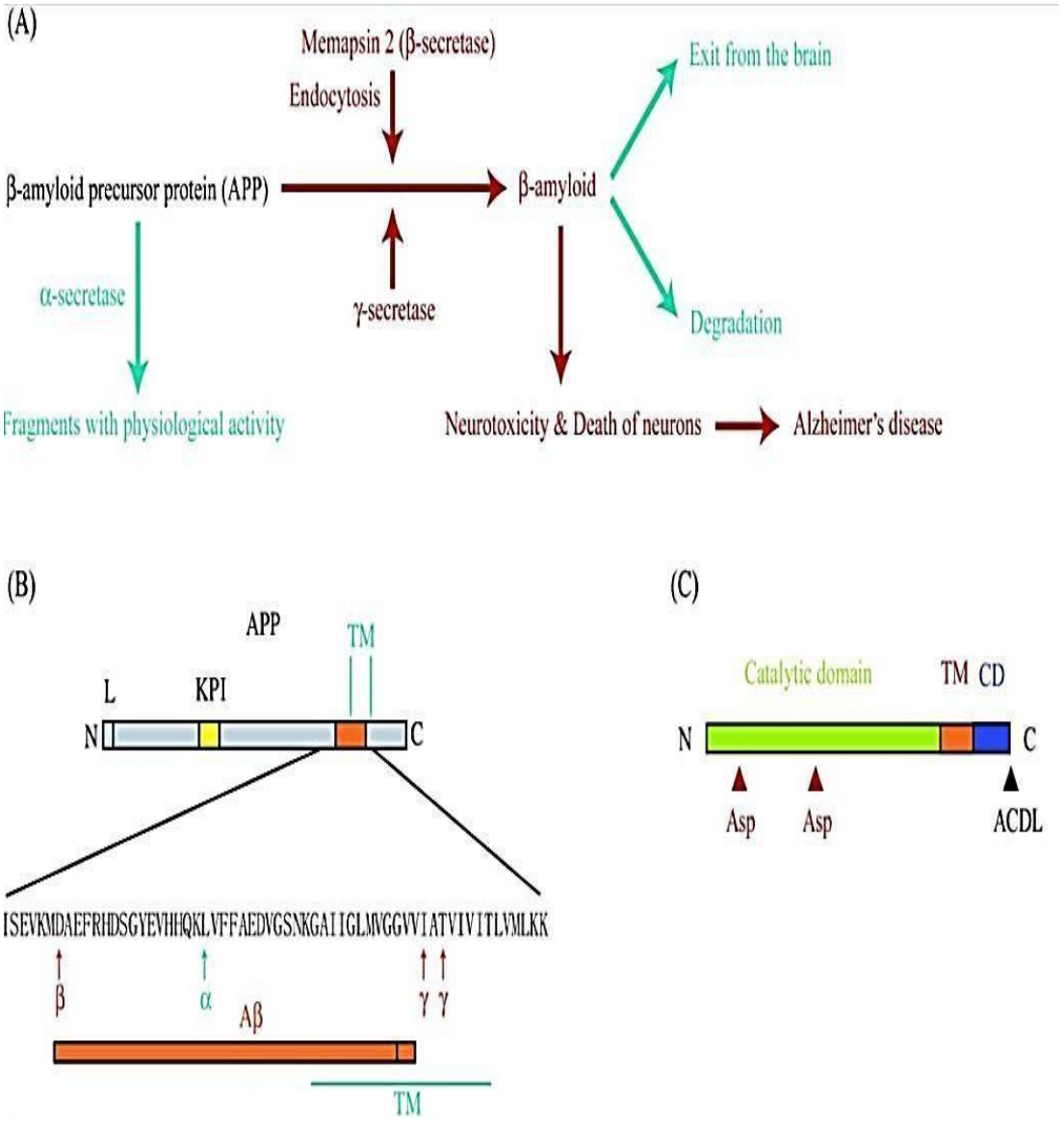

Figure:6. (A) Graphic presentation of the Aβ formation and role of memapsin 2 (β) in AD progression. Pathway to AD in red and competing pathways that reduce Aβare in blue. (B) Processing sites of APP by α, β, and γ-secretase. (C) Structural domains in β. The catalytic transmembrane (TM) and cytosolic domains (CD) are shown. Red triangles stain the positions of active site aspartic acids and the black one stain the position of ACDL motif. KPI, Kunitz protease inhibitordomain; L, leader sequence. Adapted from (Hong et. al, 2004).

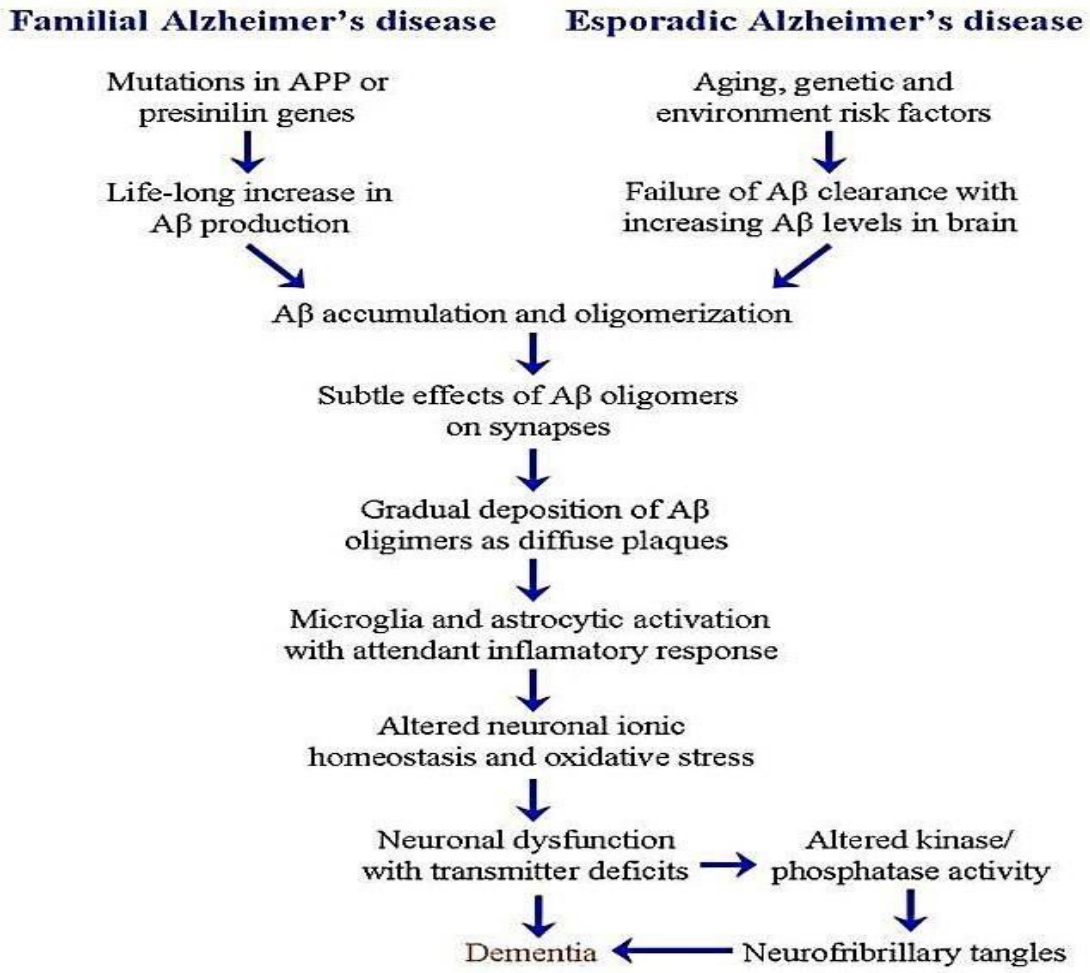

Figure:7. The Amyloid cascade hypothesis.

The Amyloid cascade hypothesis proposes that there is a primary inequality among Aβ production and its subsequent clearance, with amplified Aβproduction in familial disease and decreased Aβclearance in sporadic disease. Aβoligomers may reduce hippocampal function and damage the synaptic function, as well as leading to inflammation and oxidative stress caused by the aggregation and deposition of Aβ. Both the processes unite to damage neuronal and synaptic function through ensuing neurotransmitter deficits and cognitive symptoms.

Neurofibrillary Tangles

The subsequent major hallmark of AD associated changes in the brain is intracellular formations named NFTs. NFTs are principally composed of paired helical filaments (PHF). The essential constituent of the NFTs is the tau protein, a microtubule associated protein (MAP), which binds with microtubulin to supply structural stability to a cell. In AD, the tau protein is abnormal and the microtubule structures collapse or dissociation of the tau protein from the

microtublin leads to unbounded tau protein aggregation. The cause for the aggregation is elucidated by the tau hypothesis (Su et al., 1996). Tau, which is a soluble protein, undergoes phosphorylation and dephosphorylation, hence forming insoluble aggregates, under normal conditions. An inequality in this dynamic function, results in increased levels of unusually hyperphosphorylated tau (P-tau 181, P-tau 199, P-tau 231, P-tau 396, and P- tau 404), which in turn sequesters normal tau and other MAPs (MAP1 and MAP2) (Blennow et al., 2007). Hyperphosphorylated tau associates into PHF, and tangle construction. Corresponding to the progression of tangle construction is the disassembly of microtubules. The collective results of both tangle formation and disassembly of microtubules is that they disrupt normal neuronal and synaptic function (Blennow et al., 2007). According to the amyloid cascade hypothesis, it is the raise in concentration levels of Aβ that stimulate the changes in tau,therefore leading to the formation of NFTs. Alim etal. (2004), revealed that the protein α-synuclein (aberrant forms of which are core components of Lewy body-based pathologies, recognized as synucleinopathies), having the same function like tau, which is engaged in microtubule assembly, serving as a fastening for the tubulin 25 protein. However, this fastening capability is vanished when α-synuclein becomes mutated, ensuing in tubulin aggregation. The microtubule helps transport nutrients and other crucial substances from one part of the nerve cell to another. Since microtubule function is essential for regular neuronal and synaptic function, dysfunction of the microtubules may be critical in neurodegeneration (Alim et al., 2004). AD tissue has smaller quantity of nerve cells and synapses than a healthy brain. More specific comparison can be givenas:

In healthy brain:

- The transport system is organized in orderly parallel strands somewhat like railroad tracks. Food molecules, cell parts and other crucial materials travel along the “tracks”.
- Tau protein helps the tracks reside straight.

In brain areas where tangles are forming:

- Tau collapses into twisted strands named tangles.
- The tracks can no longer reside straight. They collapse and disintegrate.
- Nutrients and other necessary supplies can no longer travel through the cells, which ultimately die.

Aβ and its association with tau

The important question at this point is how Aβ and tau are interrelated during the disease. Even though the association between these two proteins remains indistinct, data has lent support to the hypothesis that phosphorylation of tau protein could be the key linking mechanism. Few years ago it was found that Aβ fibrils hasten the construction of abnormally phosphorylated NFTs in a tau transgenic mouse (Götz et al., 2001). Recently, it has been revealed that Aβ oligomers cause abnormal tau phosphorylation and morphology changes of spines by missorting of endogenous tau into dendrites. Aβ self- aggregates into oligomers of various sizes and forms disperse and neuritic plaques in the blood vessels and parenchyma. Aβ oligomers and plaques are potent synaptotoxins, block proteasome function, stimulate inflammatory processes, inhibit mitochondrial activity and alter intracellular Ca^{2+} levels. Aβ interacts with the signalling pathways that control the phosphorylation of the tau. Hyperphosphorylation of tau disrupts its regular function in regulating axonaltransport and directs the accumulation of NFTs and toxic species of soluble tau (Zempel et al., 2010). Moreover, degradation of hyperphosphorylated tau by the proteasome is inhibited by the actions of Aβ. These two proteins and theirconnected signalling pathways consequently signify imperative therapeutic targets for AD. Some evidence has shown that Aβ might not be the single constituent involved in the pathogenesis of AD. There is one other cleavage fragments of APP, called amyloid C-terminal fragment(βCTF), which might involvein the pathophysiology of AD. The βCTF shows higher neurotoxicity than Aβ, including endosome dysfunction, neurodegeneration, and synaptic deficits (Chang & Suh, 2005; H. Chen et al., 2014; Choi et al., 2001; Lee et al., 2006).

Symptoms and stages of Alzheimer's disease

In AD patient's brain, the ventricles, which are chambers within the brain that restraincerebrospinal fluid, are noticeably enlarged. In the early stages of AD, short-term memory begins to decline when the cells in the hippocampus

region disintegrate. As AD extends throughout the cerebral cortex, judgment get worse, emotional outbreaks may takes place and language is damaged. In the final stages, people may lose the capability to feed themselves, speak, recognize people and control bodily functions. Memory worsens and may become approximately non-existent. Constant care is usually compulsory. On standard, individuals with AD live for 8 to 10 years after diagnosis, but this incurable disease can last for as long as 20 years. The three stages listed below characterize the universal succession of the disease. Even though these symptoms will likely differ in severity, chronology, overlap, and swingthe overall progress of the disease is reasonably conventional; however, AD doesn't affect every person in a similar way (Förstl & Kurz, 1999).

Stage 1 (Mild)

Early in the sickness, persons with AD have a tendency to be less lively and spontaneous. This stage can last from 2 to 4 years. They may becomeintrovert, circumvent people and are lethargic to learn and respond. They also have difficulty performing everyday duties, and face difficulty in communicating and understanding written material. A number of specific examples of behaviours that people exhibit in this mild phase comprise [Alzheimer's Association, 2010; Mayo Clinic Medical Information and Tools for Healthy Living, 2010]:

- Getting lost
- Trouble managing money and paying bills
- Repetitive questions and conversations
- Deprived judgment
- Losing things or misplacing them in unusual places
- Noticeable changes in individuality or temper

Stage 2 (Moderate)

This is normally the longest phase and can last from 2 to 10 years. Individuals with AD are noticeably becoming disabled, in this period. Persons can still complete simple tasks autonomously, however may require support with other convoluted activities. They fail to remember current events and their individual history, and happen to more perplexed and disjointed from reality. Verbal

communication problems occur, reading and writingare more complicated, and the person may instigate terminology. They may no longer be secure single-handedly and can wander. Since AD patients become attentive to this loss of control, they may become disheartened, short-tempered and impatient or apathetic and introvert. Individuals may experience sleep turbulence and have more problems such as; ingestion, grooming and dressing [Alzheimer's Association, 2010; Mayo Clinic Medical Information and Tools for Healthy Living, 2010].

Stage 3 (Severe)

During this phase patients may last 1 to 3 years. Individuals in this period may mislay the capability to feed themselves and manage bodily functions, for example bowel andbladder control. They will sleep frequently and grunting or moaning can be ordinary. During this destabilized physical phase, individuals may become susceptible to additional illnesses, skin infections, and respiratory troubles, predominantly while they are incapable to move around [45; Mayo Clinic Medical Information and Tools for Healthy Living, 2010].

Types of Alzheimer's disease

There are primarily three types of AD, and they are as follows [(Alzheimer's Disease Education and Referral (ADEAR) Centre), [*http://www.nia.nih.gov/alzheimers/about- adear-center*]:

Early Onset Alzheimer's Disease (EOAD)

EOAD is a rare form of AD that affects individual beneath 65 years of age. The symptoms start to emerge in the 40-50 age groups. This accounts for below 10% of overall AD patients. Individual with Down's syndrome, who experience premature AG seems more prone to develop EOAD. The majority EOAD is sporadic, although about 5% of patients with EOAD have an extremely penetrant genetic mutations in amyloid pathway genes including APP on chromosome 21, presenilin 1 (PSEN1) on chromosome 14, andpresenilin 2 (PSEN2) on chromosome 1. These mutations lead to the accumulation of Aβ plaques (Bertram, 2009).

Late Onset Alzheimer's Disease (LOAD)

LOAD is a frequent form of AD that appears in individual having 65 years of age and over. LOAD accounts roughly 90% of overall AD cases. It strikes approximately half of all individual over 85 years of age. Various low-penetrant genetic risk factors conferring a modest increase in possibility of disease have been recognized for LOAD,the most studied one is the apolipoprotein ε4 allele (APOE ε4). The overall population occurrence of APOE-ε4 is 22%, although roughly 60% of LOAD cases carry at least one allele. Large, multi-centre genome-wide association studies (GWAS) approximate the population attributable threat for APOE variants is 19-35% (Ertekin-Taner, 2010).

There are extra polymorphisms connected with LOAD risk including genes, recognized by GWAS those are ABCA7, BIN1, CD2AP, CD33, CLU, CR1, EPHA1, MS4A, and

PICALM. Further APOE ε4 dose amendment reveals 50% of the population attributable threat for LOAD is accounted for by identified single nucleotide polymorphisms (SNPs). Although these variants are significant for both risk judgment and discovery of novel mechanisms of pathogenesis, still they are neither essential nor satisfactory for the progress of LOAD (Naj et al., 2011).

Familial Alzheimer's Disease (FAD)

This category makes up less than 1% of cases, and is noticeably evidenced by multiple patients over 3 or more generations being diagnosed with the disease. FAD seems to show up in the patients of age 40. All FAD known has an EOAD. FAD is also known as EOFAD i.e. early onset familial AD. Some patients with EOFAD, however, may lack a known family history or may have deficient penetrance. For these causes, it is central to consider evaluating EOAD patients with an extremely early age of onset or anomalous neurological findings for genetic factors (Lladó et al., 2010).

Risk factors for Alzheimer’s disease:

Non-Genetic Risk

FactorsAge:

Right now, age is the distinct utmost risk factor for developing AD, along with family history. More women have AD than men, although this is estimated because women normally live longer than men. Most cases of AD are seen in

older i.e. ages 65 years orbeyond. Between the ages of 65 and 74, roughly 5 to 10 percent of people have AD. For individuals over 85, the threat raises to 50% ("2010 Alzheimer's Disease Facts andFigures," 2010).

Education:

There might be a correlation among educational level and the possibility of developing AD. Individuals having fewer years of education appear to be at a superior threat. It is theorized that a greater education level directs to the construction of more synaptic connections in the brain, however the accurate reason for this correspondence is unidentified. This generates a "synaptic preserve" in the brain, that enabling patients to reimburse for the loss of neurons as the disease progresses ("2010 Alzheimer's Disease Facts and Figures," 2010).

Concomitant Health Problems

Earlier history of head trauma is also frequently agreed upon as plausible risk factors for AD. There is a sturdy connection among cardiovascular health and brain health. Having heart disease, high blood pressure or high cholesterol can amplify the risk of developing AD. This is caused by damage to blood vessels in the brain, resulting in less blood flow and possible brain tissue death. Type 2 diabetes may also increase the risk for AD. Inefficiency of insulin to convert blood sugar to energy may cause higher levelsof sugar in thebrain, causing harm. The use of certain groups of drugs, including non- steroidal anti-inflammatory drugs (NSAIDs) and cholesterol-lowering drugscalled statins, may also impact AD risk, according to a number of studies (Shobab et al., 2005). Compelling new evidence is now indicating that other "lifestyle aspects", such as one's dietary habits may impact one's risk for developing AD. The rationales why these factors enhance AD risk are unclear. Hypercholesterolaemia, hypertension, coronary heart disease, obesity, atherosclerosis, diabetes and smoking are all allied to AD, since these are all considered to be vascular risk factors, distressing the effectual supply of blood. Evidence implies that countering risks that incline a person to dementia consist of benefits resulting through diet modification i.e. escalating an intake of homocysteine- related vitamins (vitamin B12 and folate); antioxidants, like vitamin C and E; unsaturated fatty acids etc (Blennow et al., 2007). In a large

populace-based twin study, heritability for sporadic AD was high i.e. 79% by the similar genetic factors being as significant, irrespective of sex and other non-genetic risk factors (Gatz et al., 2006).

Genetic Risk Factors*:*

Familial Alzheimer's disease

All FAD recognized so far has an early onset and as many as 50% of the cases are nowknown to be caused by defects in three genes situated on threedifferent chromosomes as discussed above. Even though one of these mutations is present in only one of the two copies of a gene inherited from a person's parents, the person will inevitably develop that form of EOAD. However, the overall known number of these cases is very few (between 100 and 200 worldwide), and there is as yet no evidence that any of these mutations play a major role in the more common, sporadic form of LOAD. Scientists are working to divulge the common function of APP and presenilins (PSEN1 and PSEN2) and to find out how mutations of these genes cause the onset of FAD (Mudher& Lovestone, 2002).

Sporadic Alzheimer's disease

Genetics appear to play a significant role in the development of the more frequent form of AD; LOAD. Scientists have found an increased threat for LOAD in populace who inherit one or two copies of a particular variation of gene, APOE ε4. The variations in the APOE gene that directs the manufacture of APOE, a protein that helps carry blood cholesterol all through the body, among other functions. It is found in neurons and further supportive brain cells(known as glia) of healthy brains; however, it is also connected in surfeit amounts with the plaques found in the brains of AD people. Researchers are mainly interested in three frequent alleles of the APOE gene that are; ε2, ε3 and ε4. The finding that increased threat is linked with inheritance of the APOE ε4 allele, the more APOE ε4 alleles one inherits, the lower the age of disease onset (Mattson, 2004). The comparatively uncommon APOE ε2 allele may protect some populace against the disease. APOE ε3 is the most frequentversion found in the general population and may perform as a neutral role in AD threat. An individual can have oneor two APOE ε4 alleles and still not get the disease, and an individual who develops the disease may not have any

APOE ε4 alleles. That means it increases the threat of developing AD, however it does not cause the disease. The mechanism how, APOE ε4 increases the probability of developing AD are not known with conviction, but one possible mechanism is that it facilitates Aβ build up in plaques. Supplementary theoriesengross interactions with cholesterol levels and effects on nerve cell death that are independent of its effects on plaque build-up (Corder et al., 1993).

Important Facts of Alzheimer's disease

- AD is a distressing neurodegenerative disorder with a relentless progression.
- One person in the U.S is diagnosed with AD approximately every 69 seconds. According to data from the CDC, in 2010, over 82,000 deaths were traced as beingcaused by AD.
- AD is the 6th leading cause of death in the U.S. It is anticipated that there were 35.6million people living with dementia worldwide in 2010, and will rise to 65.7 millionby 2030 and 115.4 million by 2050.
- It is expected that more than one in three Americans 85 year and older have AD. The lifetime threat of AD among those who reached the age of65 is just about 1 in5 for women and 1 in 10 among men.
- Around 5.1 million Americans are age 85 years or older, and this age group is one of the fastest growing segments of the population. It is also the group with the highest threat of AD. It is anticipated that at least 19 million people in America willbe age 85 and older by the year 2050.
- Patients with AD live for approximately 8 to 10 years after diagnosis, but this fataldisease can last as long as 20 years, or as little as 3 to 4 years if the patient is over 80 years old when diagnosed.
- Just about 70% of AD patients receive care at home. In terms of health care expenses and lost wages of both patients and their caregivers, the cost of AD nationwide is estimated at $100 billion per year. For an individual with AD, the annual cost of home care is estimated at $76,000, including medical expenses and indirect costs such as a caregiver's time and lost wages.
- 58% of populace with dementia worldwide lives in low or middle-income countries.One third of those whose lives have been touched by AD supply

support to their loved ones.

- One third fears about getting AD, among those who do not personally have AD. Those who have a parent or parent-in-law with the disease are even more concerned.
- Approximately 50% of all caregivers are between the ages of 18 and 49, with the average age of the typical caregiver being 48.

Oxidative stress and Alzheimer's disease

Oxidative stress, a process increased in the brain with aging, is induced by an imbalance in the redox state, involving the generation of excess reactive oxygen species (ROS) or the dysfunction of the antioxidant system (Andreyev et al., 2005). The mitochondrial electron transport chain consumes almost 98% of molecular oxygen at the cytochrome oxidase complex and the remaining oxygen is reduced to hydrogen peroxide and superoxide radicals. During normal metabolism and various functions, the oxygen radical superoxide ($O_2^{\bullet-}$) and the non-radical oxidant hydrogen peroxide (H_2O_2) andhypochlorous acid are produced (Leeuwenburgh & Heinecke, 2012). The brain of patients suffering AD present a significant extent of oxidative damage associated with the abnormal marked accumulation of Aβ and the depositionof neurofibrillary tangles(Y, 2000).The brain of patients suffering AD present a significant extent of oxidative damage associated with the abnormal marked accumulation of Aβ and the deposition of neurofibrillary tangles (Kozlowski et al., 2009). . In concordance with those findings, there are high affinity binding sites for copper and zinc on the N-terminal metal-bindingdomains of Aβ and itsprecursor APP (Barnham et al., 2003; Takashi Miura et al., 2000). Therefore, whereas the brain membrane phospholipids are composed of polyunsaturated fatty acids, this organ is particularly vulnerable to free radical attacks. Their double binds allow the removal of hydrogen ions (Tsaluchidu et al., 2008) and increased lipid peroxidation, which is the most prominent feature in which degenerativechange is most pronounced in the AD brain (Markesbery, 1997). In addition, the oxidation of proteins by free radicals may be significant in AD as the oxidation of brainproteins can affect enzymes critical to neuron and glialfunctions. This is the case for two enzymes especially sensitive to oxidative modification, that of glutamine synthetase and creatine kinase, which aremarkedly reduced in AD brains (Butterfield et al., 1997), reflecting the

alteration of glutamate concentrations and enhancement of excitotoxicity, whereas oxidative impairment of creatine kinase may cause decreased energy metabolism in AD (Moreira et al., 2005). The pathologic aggregation ofprotein leads to fibril formation and insolubility (Koo et al., 1999). Thus, neurofibrillary tangles are characterized by the aggregation and hyperphosphorylation of the τ protein into paired helical filaments. Phosphorylation is linked to oxidation through the microtubule-associated protein kinase pathway and through activation of the transcription factor nuclear factor-κB, thus potentially linking oxidation to the hyperphosphorylation of τ proteins (Markesbery, 1997) Protein oxidation is also capable of inducing advanced glycation end products as a post- translational modification of proteins that are formed when amino group of proteins react non- enzymatically with monosaccharides (Bonfili et al., 2018). Furthermore, oxidation of the brain can affect DNA, producing strand breaks, sister chromatid exchange, DNA- protein crosslinking, and base modification (Cooke et al., 2003). Thus, the overproduction of ROS resulting in oxidative stress may have a deleterious effect and can be an important mediator of damage to cell structures and consequently various disease states and aging. However, antioxidant treatments have demonstrated that AD is associated with oxidative stress, being a more complex disease.

Oxidative stress is recognized to bring damage of diverse biological macromolecules in an unrestrained mode. It is also considered to be a characteristic of neurodegenerative diseases. One deliberation is that the plaques and tangles rather than being critical in the beginning or pathology of the disease are potentially acting as an antioxidant defence, to facilitate a protective action. Consequently the subsequent appearance of Aβ deposits and tau hyperphosphorylation is a result of this defence (Smith et al., 2002). It has shown in animal model that, oxidative damage precedes the pathological modifications connected with AD (Nunomura et al., 2001).

Inflammation

It is known that, brain regions which are affected by AD are containing augmented neuroinflammatory mediators (cytokines and microglia) through increased inflammatory cascades. Whether this is an innate response to

control inflammation or an out-of-control immune procedure is anonymous. Cyclooxygenase (COX) a prime mediator of the inflammatory cascade is targeted by Nonsteroidal anti-inflammatory drugs (NSAIDs), affecting COX levels. The use of NSAIDs does not diminish the threat or setback the onset of AD (Van Gool et al., 2001). Microglia activation is thought to be an untimely event in the AD pathogenesis and may be important in synaptic disorder and hence early memory impairment. The AD Anti-inflammatory Prevention Trial (ADAPT) looked into the function of NSAIDs in people vulnerable to dementia, using COX-1 and COX-2 drugs. The testing was cancelled because of cardiovascular risks (ADAPT, 2006). COX-1 targeting NSAIDs are thought to be a better preference than COX-2 inhibitors (McGeer & McGeer, 2007).

Diagnosis of Alzheimer's disease

The only technique to definitively diagnosing AD is through a brain autopsy. On the other hand, mental and behavioural tests and physical evaluations allow physicians to make a correct diagnosis of AD in 90% of cases. The decisive factor for detecting mental disorders can be found in the Diagnostic and Statistical Manual of Mental Disorders (DSM-III), published by the „American Psychiatric Association". In this manual, AD falls into the group of principal degenerative dementia. The diagnostic criterion includes dementia (diagnosis of dementia includes loss of intellectual capabilities severe enough to obstruct with social or occupational functioning, memory impairment etc.), insidious onset with progressive deterioration and exclusion of all other types of dementia by history and physical inspection [American Health Assistance Foundation (AHAF)]. The initial step in finding a diagnosis is obtaining the patient history. Through this time, the physician will verify symptoms; those are present at the beginning stage, and how they have progressed over time. The family history of sickness is also significant. To rule out additional potential reasons of dementia, such as hormone imbalance, urinary tract infections and vitamin deficiency (vitamin B12), physician should perform a physical examination (blood tests and urinalysis etc).

Brain scans may also be performed to exclude other possible causes of dementia, including brain tumours, stroke, blood accumulation on the brain surface etc. These scans are also helpful in identifying the characteristic tangles and plaques seen in AD. Structural imaging scan includes; magnetic

resonance imaging (MRI) and computed tomography (CT) which will provide information about the shape and volume of the brain. Functional imaging allows the physician to find out how efficiently the brain cells are functioning. A functional MRI or positron emission tomography (PET) scan can be used (Dr. Gérard Emilien Dr. Kenneth Lloyd Minaker, Professor Bengt Winblad, Professor Serge Gauthier, Professor Jean-Marie Maloteaux (auth.)-, 2004). Physicians may direct an electroencephalogram (EEG) to determine the electrical activity in the brain. Rarely, spinal fluid may be tested throughout a lumbar puncture.

Neuropsychological tests also identify cognitive symptoms associated with brain injuryor abnormal brain function. Physicians usually start with a concise screening tool, for instance the Mini-Mental Status Examination (MMSE), to verify that the patient is experiencing tribulations with intellectual functions. In MMSE, the physician begins by asking a series of questions premeditated to check the patient's aptitude to recall andname a list of objects, perform simple arithmetic and follow instructions. The patient is then assigned a score out of 30 probable points, with a score of less than 12 indicating severe dementia. AD patient's usually scores 2 to 4 points. Further neuropsychological testing beyond the MMSE is typically not needed, if a patient has severe dementia. Conversely, for patients with mild intellectual deficits, further tests may be necessary to decide whether the patient is just showing signs of advanced age or is developing AD. The physician may also use the Alzheimer's disease Assessment Scale (ADAS) to determine the severity of the disease. The ADAS evaluates the patient's orientation. memory, language and reasoning on a scale of "0 to 70". A higher score represents a higher level of cognitive impairment. ADAS is sensitive to a broad array of symptoms and evaluates many cognitive skills, including spoken language ability, ability to find correct words, recall of instructions, following commands and orientation to surroundings and time (Dr. Gérard Emilien Dr. Kenneth Lloyd Minaker, Professor Bengt Winblad, Professor Serge Gauthier, Professor Jean-Marie Maloteaux (auth.)-, 2004). Besides mental tests, the doctor may perform a neurological test to evaluate the function of the patient's brain and nervous system. This will test reflexes, coordination and balance, muscle strength, speech, sensation, and eye function. Scientists are also looking at changes occurring in the blood and cerebrospinal fluid that may specify the progression

of AD. Additionally, they are developing sophisticated brain imaging methods that helps in measuring the slightest changes in brain function or structure to detect AD prior to any noticeable symptoms occur.

Cholinergic hypothesis

Cholinergic hypothesis of AD proposes that destruction of the cholinergic pathway in the basal forebrain consequences 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.10)**. 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).

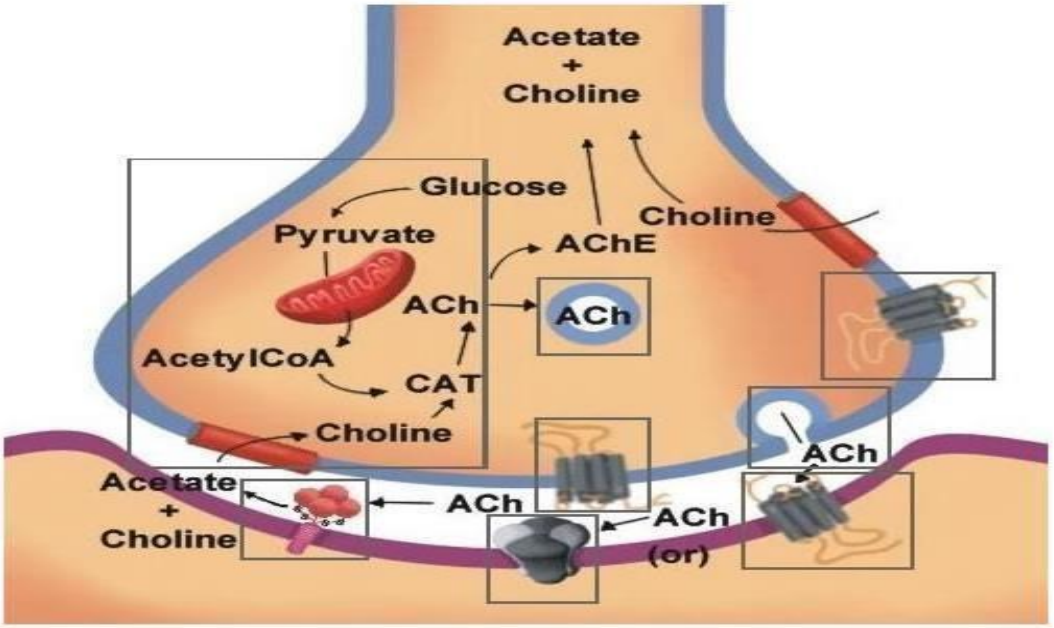

Figure 8: Scheme summarizing the role of ACh and AChE in cholinergic activities

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 hypotheses' 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 theuse of statins does not impact on enhancement in recorded cognitive impairment (Jones et al., 2008). APOE is involved in the transporting ofcholesterol and APOE ε4 allele is a universally recognized marker which enhances AD risk. A high cholesterol level throughout a person's mid-life 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 andmolecular 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, level of dementia show a relationship with the extent ofneuronal death caused by surplus of glutamate, the most widespread excitatory neurotransmitter in the brain (Alvi et al., 2019a; Wenk et al., 1996) **(Fig.11)**.

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 bythe 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 ofpatients. 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).

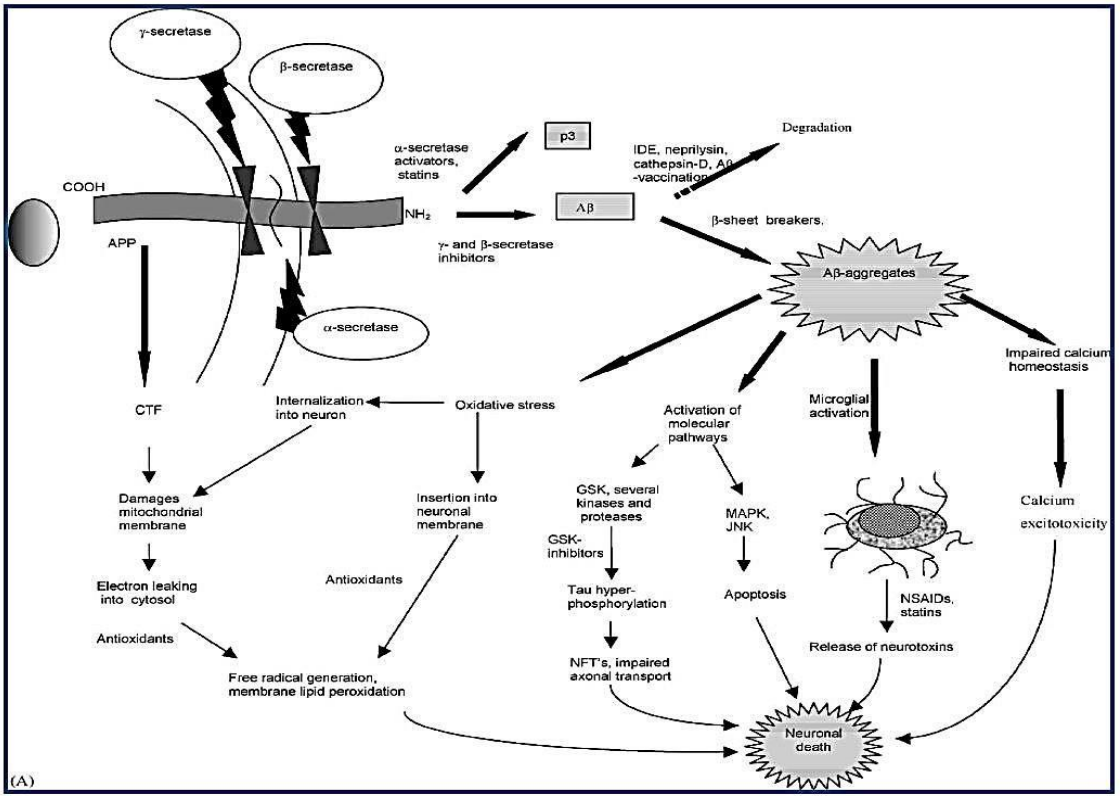

Figure:9. Steps involved in neurodegeneration of AD and targets there in for novel treatment strategies. GSK: glycogen synthase kinase; CTF: C-terminal fragment; MAPK: mitogen activated protein kinase (A: calcium channel).

Since the last decade, AD patients are being treated with substitute 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 beentested for the inhibition of acetylcholinesterase which is main enzyme involvedin the synthesis of choline (Baum et al., 2008; White & Levin, 1999; Wilson et al., 1995; Zhu et al., 2018). Plants are thefull of natural antioxidant source that are effective to reduce oxidative stress raised during AD (Alvi et al., 2019b).

Vitamins have been described as therapeutic compounds

for AD. 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 the neuroinflammation (A. Ahmad 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).

Essential oils (EOs) are natural, and mixture of volatile substances that 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). It is well known for their antioxidative, anti-inflammatory and neuroprotective effect (Prasad & Muralidhara, 2017; Rekha & Selvakumar, 2014).

DATE PALM

Kingdom:	Plantae
Order:	Arecales
Family:	Arecaceae
Genus:	Phoenix L.
Species:	*Phoenix dactylifera L*.

Dates (Phoenix dactylifera) are one of the members of the palm family

Arecaceae, or Palmae (Zohary D. et al 1993). The species name dactylifera "date-bearing" originate from two words; one from greek dáktulos "date"(Rahmani, A et al 2014) and the stem of the Greek verb ferō (Lewis and Short 1996). The date palm (Phoenixdactylifera L.) is one of oldest cultivated plants of human kind and used as food for 6000 years (Sulieman et al, 2012). There are more than two hundred varieties (Amer 1979) of dates available worldwide.

It is the main crop in Egypt, Saudi Arabia, and Middle Eastern countries. It is thought that the native origin of dates is around the Persian Gulf, and has been cultivated from Mesopotamia to prehistoric Egypt as early as 4000 BCE. Due to the old historical prospective of date, the exact date of origin is very difficult to identify (Chao CT et al 2007). Most likely it originated 4000 BC from the ancient Mesopotamia area (southern Iraq) or western India. Another report regarding the origin of dates is pre-Islamic archaeology; south-eastern Arabia was predicated upon the domestication of the date palm in 2500 BC (Wrigley G. et al, 1995) Over all the origin of dates is very old as per the information from the religious books and literature reports. Another support of the ancient times of the date palm is Egypt's Nile Valley where it was used as the symbol for a year in Egyptian hieroglyphics and its frond as a symbol for a month (Dowson VHW 1982). Earlier report also showed that old background of dates as date cultivation in Mehrgarh around 7000 BCE and in the Indus Valley around 2600 to 1900 BCE (Kenoyer JM e al, 2013). The fruits of dates have important place in religion. In Islam dates fruits are used to break the day long fast during the holy month of Ramadan (Al-Shahib et al, 2003). The Jews believe the date as one of the seven holy fruits and they celebrate Palm Sunday.

Prophet Muhammed (Peace Be Upon Him) said that the best asset is date palm, dates cure several disorders, and he suggested Muslims to eat the date and have a tendency the date palm (Zaid, A., & De Wet, P. F. 1999). The

importance of dates has been documented in the Qur'an in Surah Maryam. One significant role of dates comes as when Mary gave birth to the Prophet Jesus (may peace be upon Him) under a palm tree, she heard a voice telling her: "Shake the trunk of the palm tree towards thee: it will drop fresh, ripe dates upon thee. Eat, then, and drink, and let thine eye be gladdened!"(Qur'an 19: 25-26). Ajwa is a type of dates, cultivated only in Saudi Arabia/Al- Madinah Al-Munawara and have significant value in diseases cure. The healthbenefit of Ajwa dates has been documented in hadith as Saud (R.A) narrated that I heard Allah's Apostle saying, "If Somebody takes seven Ajwa dates in the morning, neither magic nor poison will hurt him that day (Al-Bukhari, M. I., & Sahi, A. B. 1976).

Numerous studies have confirmed the ability of date palm (Phoenix dactylifera L.) to reduce parameters associated with cardiovascular disease. A brief description of some of these studies is given below.

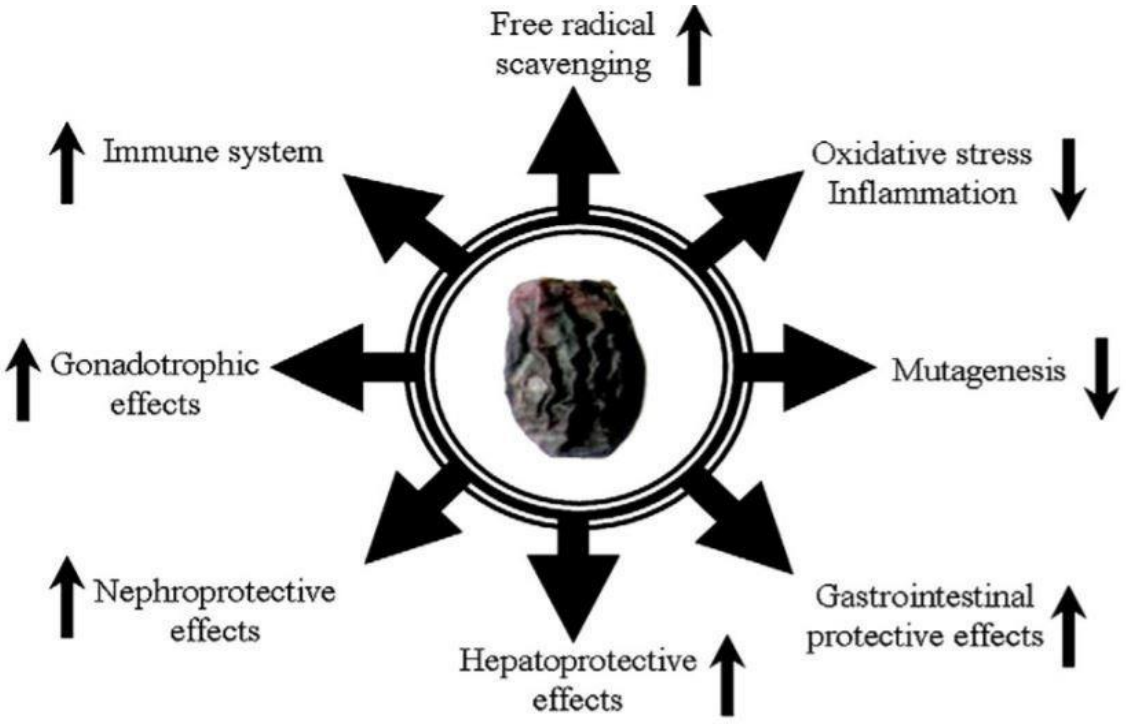

Figure:10. **Biological activity of dates**

Antioxidant activity of *phoenix dactylifera* L.

Multiple factors are involved in the development of cardio-vascular disease (CVD). Pathological changes or dysregulation of physiological functions increase performance of immune cells which lead towards systemic inflammation characterized by high levels of reactive oxygen species (ROS)

(Zorov et al., 2014). In patients suffering from inflammatory diseases antioxidants levels are very low due to poor intake of antioxidant rich foods (Mangge et al., 2014). Antioxidants are substances that remove potentially damaging oxidizing agents in a living organism (Forman et al., 2014). Excessive production of reactive oxygen species (ROS) increases the chance of cardiovascular disease. A study showed that Ajwa extract inhibit cyclooxygenase (COX), COX 1 and COX 2 these enzymes are responsible to initate oxidative stress. Similarly, it had proved that "Ajwa," a variety of date fruits, consumed by rats prevented the depletion of superoxide dismutase (SOD) and catalase (CAT). SOD and CAT both are endogenous antioxidants which prevent lipid oxidation and inflammation diseases including cardiac hypertrophy, atherosclerosis, cardiomyopathy, hypertension, heart failure and myocardial infarction. It is Important to prevent these free radicals production by consuming a significant amount of antioxidants (Bonner and Arbiser, 2014). Different antioxidants such as beta carotene, quercetin, CoQ10, reserveterol, lycopene, vitamin E and vitamin C have shown therapeutic benefits in several types of CVD (Jain A et al., 2015). Not only CVD antioxidants also reduce the chance of other metabolic and inflammatorydiseases such as diabetes and cancer (Zhang et al., 2015).

Anti-hypercholesteraemic and hypolipidemic activity

Hypercholesterolemia is a major threat for the development of cardiovascular diseases. Currently, many studies have been reported the hypocholestolemic effect of phoenix dactylifera (Sureka et al., 2016). In a study, conducted by Vayalil et al in 2012 hamsters were induced with cholesterol supplements to increases cholesterol and lipids levels in blood. One specific group of cholesterol fed hamsters was fed with date fruit supplement. After sometime measurements showed a considerably reduction in total plasma cholesterol levels, organ weights, triglycerides and LDL levels which were increased by cholesterol-induced supplements. This study shows that date fruit supplementation has a potential to change the absorption or metabolism of cholesterol. In this way date fruit supplement may prevent the chance of atherosclerosis and other heart diseases. Different mechanisms are used to explain hypocholestrolemic effect of dates. First, date fruit contains small amount of fats and this fat is available in the form of small fatty acids which are easily absorbable. Second, as Dates are good source of dietary fiber

(Berry et al., 2011) and fiber content of dates reduces the absorption and reabsorption bile acids in gastrointestinal track. Dietary fibre also inhibits the biosynthesis of cholesterol by producing small chain fatty acids on fermentation. Third, the date fruit also rich in phytosterols (plants sterols)which work similar to cholesterol lowering drugs (AL Saif et al., 2007). Phytosterols inhibit cholesterol absorption in small intestine by preventing the attachment of cholesterol with micellar bindings.in this way phytochemicals in date fruit lower cholesterol and lipids levels in blood (John et al., 2007). β- sitosterol is a phytosterol that have an obvious effect in lowering cholesterol levels in human beings. It inhibits bio synthesis of cholesterol by restricting gene expression of HMG-CoA reductase enzyme required for cholesterolsynthesis (Batta et al., 2006). Plasma triglycerides levels were also decreasedin hamsters fed with date fruit supplementation (Vayalil, 2012).

Anti-Inflammatory activity of *Phoenix dactylifera* L.

A study was performed in 2017 by Kehili et al to find out anti-inflammatory activity of date fruit (Phoenix dactylifera). About 50 mg/kg extract of the P. dactylifera was given to formalin-induced edema mice. At end the inflammation level in mice was measured by the size of edema, level of C- reactive protein (CRP) and homocysteine content in the blood. There was a significant decrease in the edema size and reduction of CRP and homocysteine levels in blood. This study suggests that date fruit extract have power to reduce inflammation because inflammatory disorders causes a secondary immune cell activation, which result in heart diseases and the atherogenesis (Kehili et al., 2016). Flavonoids and phenolic contents in dates vary depending upon their type. According to Mohammad Al-Mamary et al. (2014) Rotab date syrup had more antioxidant capacity then Saudi syrup and Iraq-syrup because it had more flavonoids and phenolic contents. These flavonoids had ability to scavenge free radicals or ions to prevent the production of free radicals (Al-Mamary et al., 2014).

Atherosclerosis

Oxidation of accumulated fat cells causes atherosclerosis which is the leading cause of cardiovascular diseases. As mentioned in previous studies Phenolic compounds and flavonoids, are effective natural nutritional antioxidants which are capable of scavenging free radicals, metal ions and preventing lipid

peroxidation. Epidemiological studies showed that high consumption of diet rich in polyphenols directly linked with reduced morbidity and mortality rate from cardiovascular disease. Borochov-Neori et al determined the atherogenic property and polyphenolic contents in nine different types of date fruit. They examined atherogenic properties by measuring free radical induced oxidation, its effect on LDL levels in serum and phenolic content was examined by reverse phase high pressure liquid chromatography (RP-HPLC), Common phenolic compounds were hydroxycinnamates, hydroxybenzoates and flavonols. There was a clear difference between phenolic contents of all varieties of date fruit and all types had shown the inhibition of cholesterol and lipid peroxidation. Phenolic content of all types was varied and most varieties also showed the athrogenic property. According to this study soluble phenolic compounds in date fruit had atherogenic property to prevent cardiac diseases (Borochov-Neori et al., 2013)

Myocardial injury

Date fruit also help to prevent cell damage and improve cell induced injury to make healthy organs. The cells protective effect of date fruit determined by Asadi-Shekaari M et al in a study in 2008. In this study they showed that aqueous extract of date fruit had protective effect against hydrogen peroxide (H2O2) induced cytotoxicity In addition, the total antioxidant capacity of aqueous extract of date fruit was very high about 1.97±0.04 mmol on measuring. The results investigated that date fruit extract inhibited H2O2 induced cell damage. In this study two aqueous date fruit extract were used 0.1% and 10%. Findings showed that both percentages of aqueous solutions had preventive effect but 10% date fruit solution showed more protective capacity than 0.1% because it had more concentration and apoptotic features. So these results show that aqueous date fruit extract has protective and proliferative effect against H2O2induced cytotoxicity (Asadi-Shekaari et al., 2008).

Doxorubicin (Dox), is an antibiotic (anthracycline) most commonly used for the treatment of cancer. High administration of Doxorubin causes cardiotoxicity. Cardiotoxicity is determined by high levels of LDL, VLDL and decreased HDL level in blood. A study was conducted by MUBARAK et al. (2018) to determine the protective effect of date fruit extract on Dox-induced cardiotoxicity. In this study 40 female albino rats were used and divided into

four groups including control, date fruit extract, Dox, and treated date palm fruit extract groups. Doxorubin produced increase in creatine kinase-MB and lactate dehydrogenase activities. It also reduced the activities of cardiac glutathione peroxidase and superoxide dismutase but increased levels of cardiac malondialdehyde. High different Histopathological studies showed the alteration of cardiac tissue structure by Doxorubicin. Treatment with date palm fruit extract recovered the cardiac tissue injury caused by Doxorubincin. So it can be said that phoenix dactylifera have a cardioprotective effect on the heart tissue against cardiotoxicity induced by Dox (Mubarak et al., 2018). Alhaider et al. (2017) investigated the potential of date fruit fruit extracts in repairing tissue injury such as myocardial infarction by increasing circulating progenitor cells. Extract of four different types of date fruit had been used, all extracts were rich in flavonoids and phenolic compounds which were involved in antioxidant activities and protection of cardiac tissues damaged by myocardial infraction. All date fruit extract showed the ability to improve cardiac muscles and increase the number ofprogenitor cells from bone marrow to the place of myocardial infraction. So, date fruit extract showed the ability to promote tissue repairing. Blood control Hypertension is one of the major causes of the onset of CVD (De Puala et al., 2012). Daily dietary consumption of phytochemicals reduces the chance of hypertension and other coronary diseases (Cassidy et al.,2011). It is well known that the Phoenix dactylifera fruit has a considerable number of phytochemical compounds. Now a day's natural remedies are usedto treat human diseases because some modern drugs have potential drawbacks. Most commonly cardiovascular diseases are controlled with anticoagulants like aspirin and warfarin. Basic aim to prevent coagulation main cause of strokes, heart attacks and ischemic heart disease. Due to someclinical complications these drugs should be replaced with some natural source. That is why a study was conducted in 2018 by Hasson et al. to check the anticoagulant ability of date fruit. In that study the efficacy of differenttypes of *Phoenix dactylifera* was determined by evaluating PT (prothrombin time) and BT (bleeding time) activities. There was a significant prolongation in Prothrombin time. The results were further confirmed by platelet aggregation and platelet mass which were low (Hasson et al., 2018). Dates contain a significant amount of S potassium, magnesium, calcium, iron and sodium

these minerals help to maintain electrolyte balance for example Potassium control sodium concentration and prevent hypertension It also helps to regulate heartbeat and maintain heart rhythm (Tahraoui et al., 2007; El Fouhil et al., 2013).

HYPOTHESIS

Based on the above review of literature, we hypothesized that the *Phoenix dactylifera* (Khudari cultivar) fruit extract may have significant role against Alzheimer's disease via impeding Acetylcholinesterase activity or by the anti-oxidant activity.

OBJECTIVES

I. collection and preparation of *Phoenix dactylifera* L. (Khudari Cultivar).

II. To perform solvent based extraction and phytochemical screening of *Phoenix dactylifera* L.

III. In-vitro antioxidative studies of different fractions of *Phoenix dactylifera L.* (Khudari cultivar) extract by DPPH radical scavenging assay and FRAP assay.

IV. To perform *in-vitro* Acetylcholinesterase inhibitory activity of various *Phoenix dactylifera* L. fruit extract and reference standard (Tacrine).

V. To determine the *Phoenix dactylifera* fruit extract-mediated mode of enzyme inhibition by enzyme kinetics studies.

MATERIAL AND METHODS

Chemicals

Chemicals such as Methanol (MeOH) were obtained from Merck, India.1,1-diphenyl-2- picrylhydrazyl (DPPH), ascorbic acid, were 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.

Collection and preparation of date extract

Dates are purchased from the local market of Lucknow, India. Pulp and seeds of dates were separated and shed dried and made in coarse powder, avoiding sun dried due to the Signature modification of the biochemicals.

Processing of plant materials

Fresh leaves, stems and fruits of plant were shed dried at room temperature (25-35ºC) for 4-6 days.The dried leaf, stem and fruits were coarse powered in a grinder, avoiding sun dried due to the signature modification of the biochemicals and weighed before extraction for calculating the yield.

Preparation of plant extracts

Dates are purchased from the local market local market of Lucknow, India. Pulp and seeds of dates were separated and shed dried and made in coarse powder, avoiding sun dried due to the Signature modification of the biochemicals. The dried powder (25 g) of the date was extracted using polar solvents successively with the required amount of each of n-hexane, EtOAc, DCM, MeOH and water solvents in Soxhlet apparatus until it turned colourless. The solvent was removed, filtered, and dried at room temperature and residues were scratched out and stored at −20°C for future use. The percentage yield of different fractions was calculated by using the formula.

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

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 theextraction 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, siphon arm and round boiling flask.

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

Extraction chamber- It allows the sample of solvent that 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 semi-solids. 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 system

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

Phytochemical screening of plant extract

Phytochemical screening is qualitative assay consists of test for phenols, alkaloids, tannins, flavonoids, saponins and triterpenoids, steroids, cardiac glycosides.

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 amylalcohol 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 followed some drop of FeCl3. Formation of brownish green or blue-black coloration 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 amyl alcohol layer indicates flavonoids.

Test for saponins: 10 mg sample was added into 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 anhydrate acetate acid and strong H_2SO_4 (2:1). Formation of red-green colour indicates triterpenoids.

Test for steriods: Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H_2S04. The colour changed from violet to blue orgreen in some samples indicating the presence of steroids.

Test for cardiac glycosides (Keller-Killani test): Five ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violetring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

DPPH Radical Scavenging Activity.

The DPPH radical scavenging capacity of the Geraniol was determined by the method of Brand-Williams et al. 2005. Ascorbic acid was used as a reference standard. Percent (%) scavenging of DPPH free radical was measured using the following equation:

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

Ferric Reducing Antioxidant Potential

A modified method of Benzie and Strain (1996) was adopted to determine the ferric reducing antioxidant potential (FRAP) of various extracts of *Khudari*. Briefly, the FRAP reagent was freshly prepared by mixing sodium acetate buffer (300mM,pH 3.6), 10mM TPTZ solution (in 40mM HCl) and 20mM Fe (III) chloride solutionin a volume ratio of 10:1:1, respectively. 100 µl of the different extracts of *Khudari* was added to 3mL of the FRAP reagent and incubated for 5 minutes at 37ºC.The absorbance was measured after 30min at 593nm. The standard curve was plotted by using various concentration (ranging from 5nmol to 100nmol) of $FeSO_4$ solution, and results were expressed as μmole Fe (II)/g dry weight of plant material.

Acetylcholinesterase Inhibition Assay

Acetylcholinesterase test was prepared according to Ingkaninan et al (2003) with slightmodification. 100µl of 10 mM-DTNB, 100µl of 15 mM-AChI, 700 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, 25µl of buffer was substituted by equal volume of AChE-enzyme-solution containing 0.28 U ml^{-1}. The standard drug tacrine were 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 3replicates. We calculatedthe percent-inhibition of acetylcholinesterase enzyme-activity by the equation given below:

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

Spectrometric Study of the Enzyme Kinetic Assay

The varied concentrations of substrate, acetylcholine-iodide or AChI (i.e., 0.5 1, 1.5 mM), were used for the analysis of kinetic study of AChE activity and its inhibition by varied concentration of tacrine as reference drug and Khudari at room temperature. Kinetic analysis of acetylcholine iodide hydrolyzed by AChE in the absence and presence of inhibitors was observed spectrophotometrically at a wavelength of 405 nm for a total of 20 min, and the absorbance values were recorded at 1 min intervals. Lineweaver Burk and Dixon plots were used to determine the kinetic parameters, such as Ki, Vmax, and Km values.

RESULTS

Phytochemical Estimation

Our results show significant presence of carbohydrates, tannins, saponins, alkaloids, flavonoids, glycosides, phenols, terpenoids, cardiac glycosides, flavonoids, and steroids in methanolic as well as in aqueous extract of Khudari (Table 1).

Table 1.

Phytochemicals	n-hexane	EtOAc	DCM	MeOH	Aqueous
Carbohydrates	+	+	++	++	+++
Tannins	+	-	+	+	+
Saponins	-	-	+	-	-
Alkaloids	-	+	-	+	++
Flavonoids	+	+	-	++	+++
Glycosides	+	-	-	+	++
Phenols	+	+	++	++	+++
Terpenoids	-	+	+	+	-
Cardiac glycosides	-	-	-	-	++
Steroids	-	-	-	-	-

Total Antioxidant Activity.

Antioxidant activities of Both methanol and aqueous extracts were assessed by FRAP assay, which is based on their ability to reduce ferric ions to ferrous form. The results illustrated that water extract has significantly higher FRAP values (331.81±4.56μmol Fe(II)/g) as compared to methanol extract (51.57± 1.183 μmol Fe(II)/g) (**Fig 11**).

Total phenolic content

The total phenolic content of both extracts was determined by the by the standard procedure Folin-Ciocalteu method. The result shows the aqueous extract has higher TPC (17.77±8.21 µg GA/mg Extract) value than MeoH (7.70±0.45 µgGA/mg Extract). Linear relationship between TPC and FRAP was also performed that shows the positive association between them (**Fig.12**).

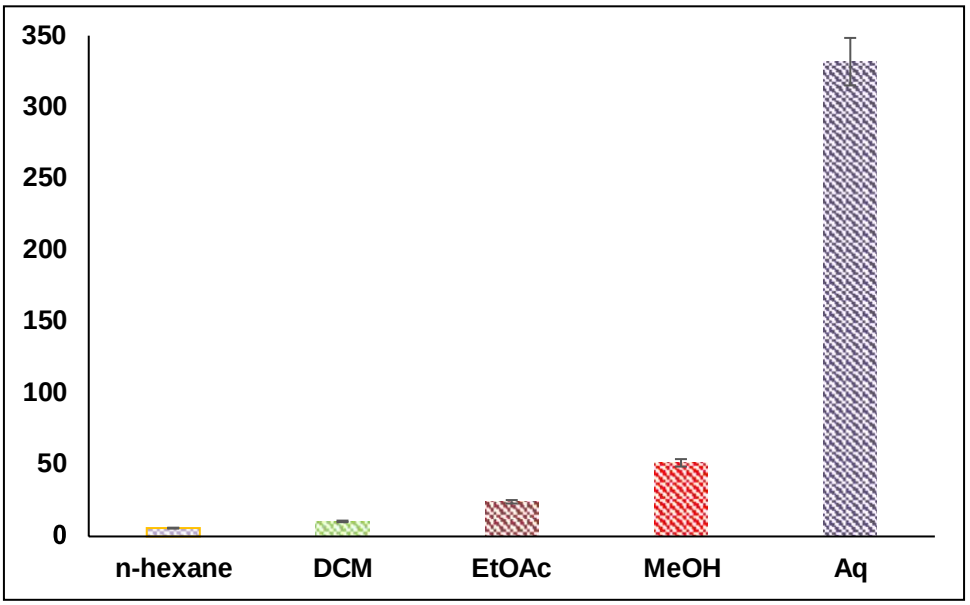

Figure: 11. FRAP Value of various fractions of Khudari. The results are mean ± S.D. of three parallel measurements**.**

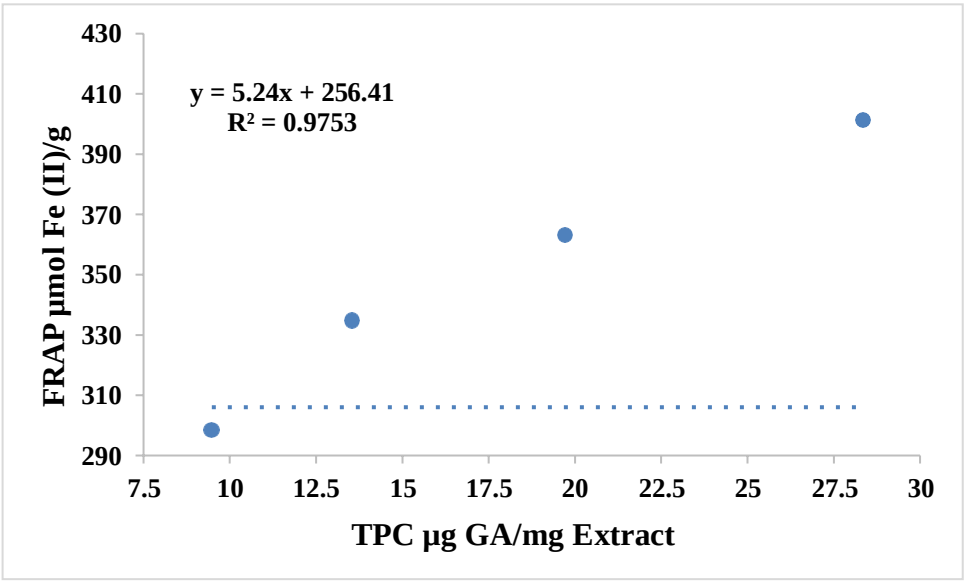

Figure. 12: Linear correlation between the amount of TPC and antioxidant capacity (FRAP) of Khudari in various solvent systems at different concentration.

DPPH Radical Scavenging Activity

The relatively stable DPPH radical is widely used to evaluate the free radical scavenging activity of various natural antioxidants including fruit extract. The data present in Figure 13,14,15 showed the percent inhibition of DPPH radical scavenging activity of different fractions of Khudari dates. The aqueous fraction of Khudari exhibited higher antioxidant activity with an IC_{50} value 235.84 μg/ml. From

the data, we observed that DPPH radical scavenging activity was increased as the concentration increased for each individual extract, with marked increase in water extract. The reference standard ascorbic acid was used. The IC_{50} value for standard was 15.58 μg/ml.

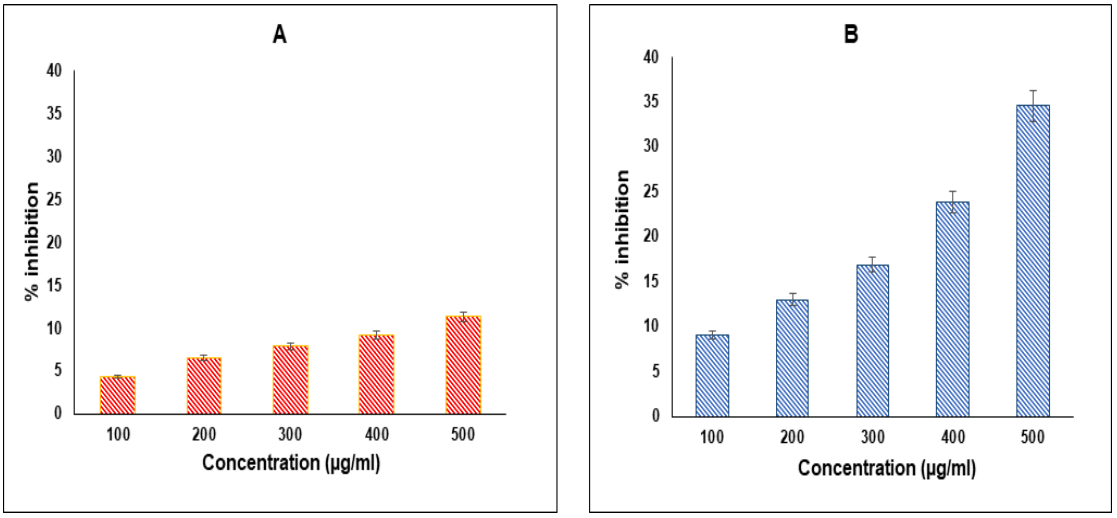

Figure:13. DPPH radical scavenging activity of n-hexane (A) and EtOAc (B) extracts of Khudari. The data represent percent scavenging of DPPH radicals. The results are mean ± S.D. of three parallel measurements.

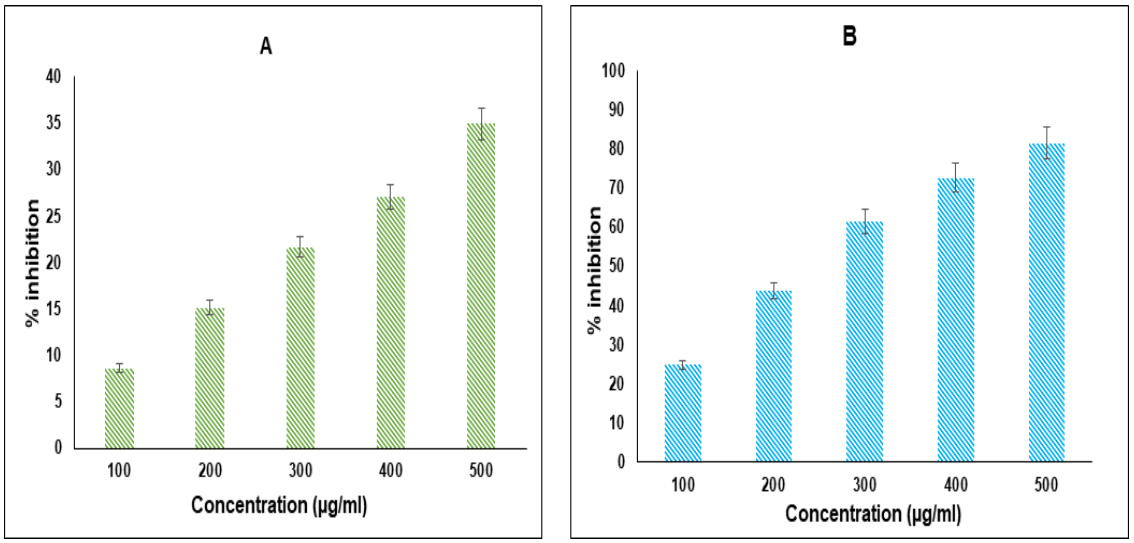

Figure:14. DPPH radical scavenging activity of MeOH (A) and Aqueous (B) standard ascorbic acid. The data represent percent scavenging of DPPH radicals. The results are mean ± S.D. of three parallel measurements.

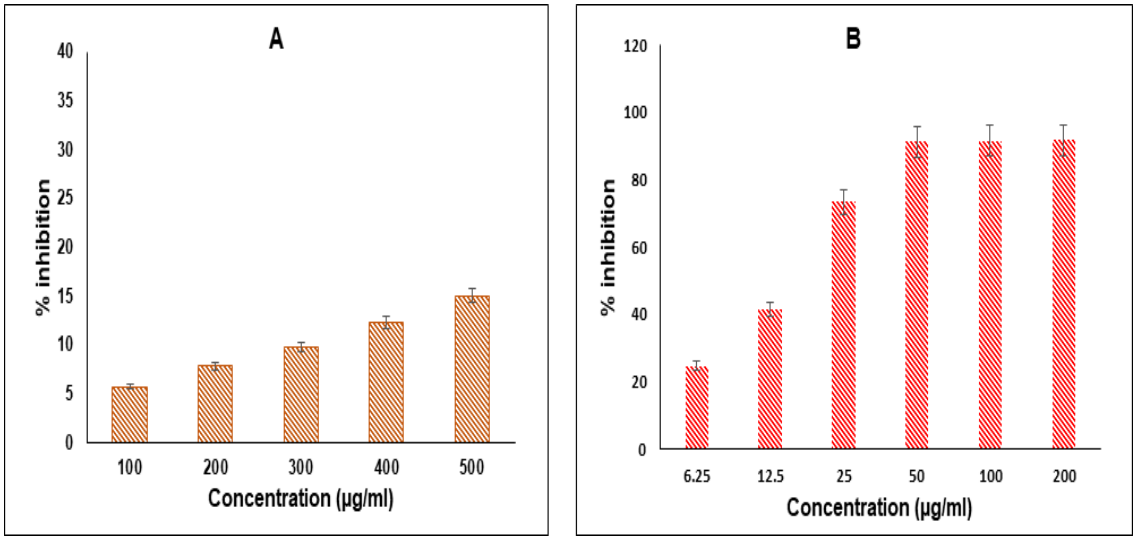

Figure: 15. DPPH radical scavenging activity of DCM (A) and Standard Ascorbic acid (B) standard ascorbic acid. The data represent percent scavenging of DPPH radicals. The results are mean ± S.D. of three parallel measurements.

AChE inhibition activity

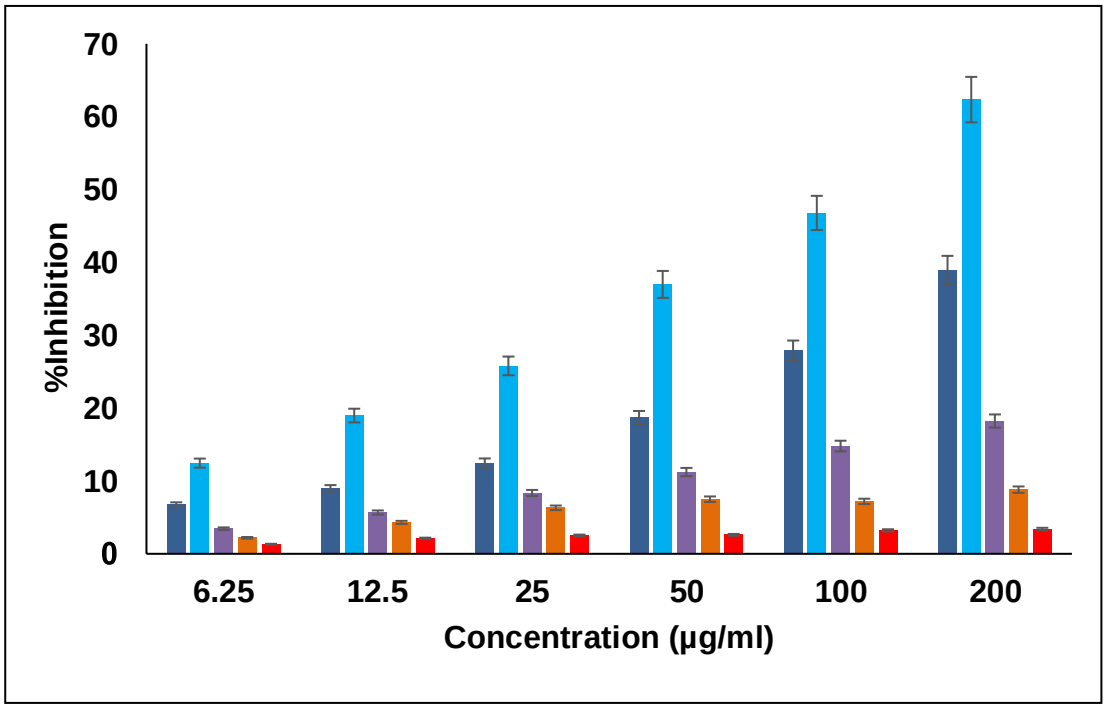

Figure:16. Percent inhibition graph of AchE activity of different Date palm extract of Khudari. The IC_{50} Value of the Aqueous extract was 146.45±4.56. The results are mean ± S.D. of three parallel measurements.

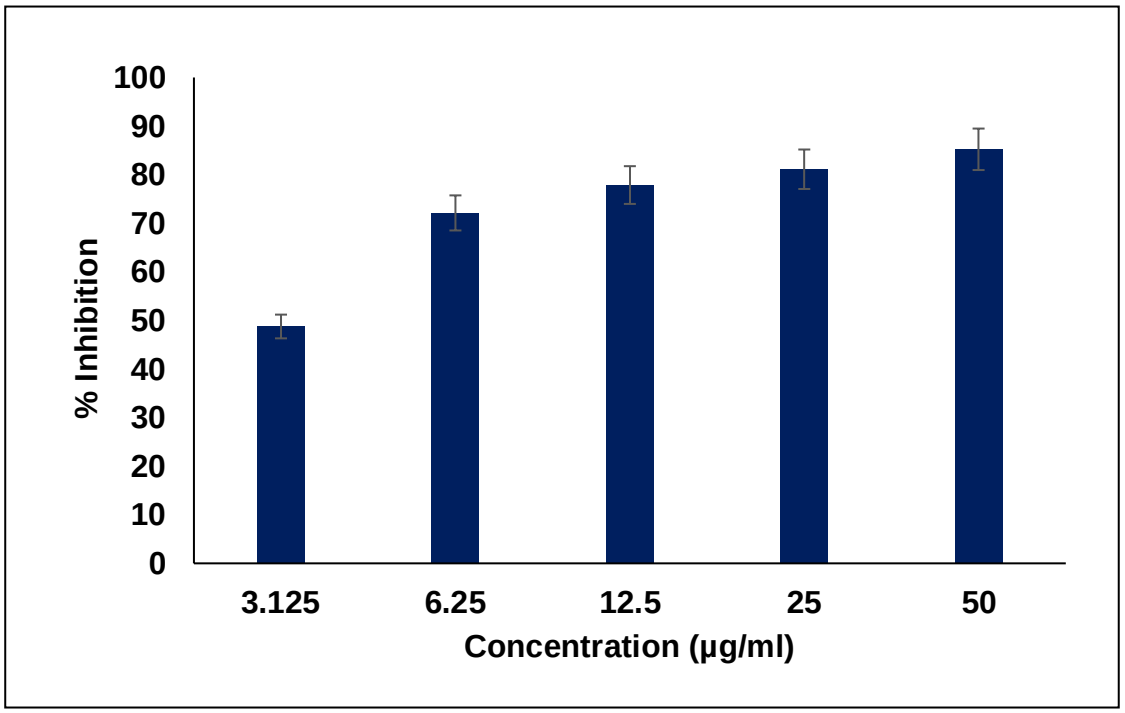

Figure:17. Percent inhibition graph of AchE activity by standard drug Tacrine. The IC_{50} value of the standard is 4.36±2.30 µg/ml.The results are mean ± S.D. of three parallel measurements.

Enzyme inhibition kinetic studies

Our enzyme kinetics results were analyzed through a Lineweaver–Burk double reciprocal plot that is 1/V vs. 1/[S], where "V" is denoted as the velocity (change in absorbance), which represents the enzyme activity, and "[S]" is denoted for substate concentration. This linear regression curve plot gives the idea for the variations in Km (Michaelis constant) and Vmax (maximum enzyme activity). All trendlines of different concentrations of Khudari at various concentrations of the substrate do not intersect at the same location on the Y-axis (Y-intercept =1/Vmax), showing different Km values. Thus, its showed the mixed type of inhibition while in standard drug tacrine the lines cut behind the Y axis but did not change Km values that showed the non-competitive inhibition.

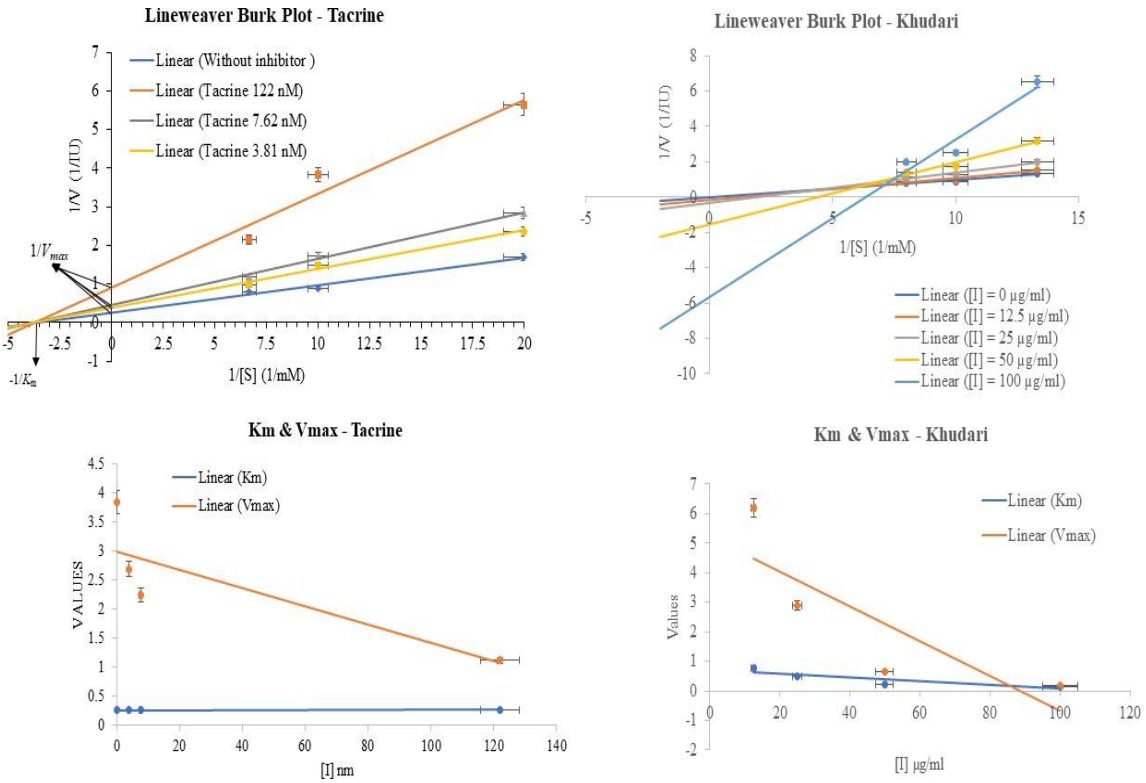

Figure:18. Lineweaver-Burk double reciprocal and Km & V_{max} plot of 1/v versus 1/[S] of Tacrine (a) and Khudari aqueous extract against AchE

CONCLUSION

Within two and half months of my primary study like literature searching, screening of effective natural plant extracts that play a curable role in neurodegenerative disorder. Screening of different natural compounds found in plant extract. There are Various plants that produce variety of phytochemicals. that are helpful to treat oxidative stress, glycation, inflammation, neurological disorders like Parkinson's disease, Alzheimer's disease. Here, we selected the plant on the basis of their antioxidant and anti-inflammatory activity. Our results showed the very strong antioxidant activity of Aqueous fraction of Khudari extract which is confirmed by the DPPH and FRAP assay. The total phenolic content was also investigated, which showed a good correlation with Ferric ion reducing power of aqueous extract. All other fractions of sequentially extracted Khudari extract were tested for their antioxidant activity but the aqueous extractwas the best. On the basis of the antioxidant power, we selected the aqueous fraction of Khudari to perform AChE inhibitory potential. Our results demonstrated the Kinetics studies of aqueous fraction of Khudari extract, that confirms the mixed type of inhibition via this fraction. We cab say the it will helpin the management of Alzheimer's disease and oxidative stress. For the better understanding of the mechanism of action of this extract on Acetylcholinesterase enzyme a full fledged in-vivo study is needed.

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