To study the effect of *Hypericum perforatum* on Alzheimer's disease

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Project report

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CONTENTS

Content	Page no.
Certificate	3
Acknowledgment	4
Summary	5
Chapter 1 • Introduction	6-27
Chapter 2 • Plant background	28-33
Chapter3 • Literature review • Objective and work plan • Work done • Materials and methods	34-39
Chapter4 • Results • Discussion and conclusion	40-42
• References	43-45

CERTIFICATE

This is to certify that the work entitled,**"To study the effect of *Hypericum perforatum* on Alzheimer's disease"** submitted by **Kriti Sharma** in partial fulfillment for the award of degree of bachelor of Pharmacy in Department of Pharmacy, Jaypee University of Information Technology has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

Signature of Supervisor ……

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Date:

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Kriti Sharma

SUMMARY

To study the effect of *Hypericum perforatum* on alzheimer's disease

Alzheimer's disease (AD) is the most common form of dementia. It is an incurable and a degenerative disease. This disease is characterized by impairment of memory and eventually by disturbances in perception, language, reasoning and planning. It is diagnosed over
65 years of age. Early, moderate and last are the three stages of this disease that determine the symptoms of the patient varying from mild to severe. Computed tomography, magnetic resonance imaging can be used to diagnose AD along with patient history and clinical observations. Although there is no way to cure AD or stop its progression encouraging advances is being made by researchers in Alzheimer's treatment including non-drug and drug approaches to improve symptom management. Mainly cholinesterase inhibitors like donepezil and rivastigmine are used in AD as they increase the level of acetylcholine in the brain which plays a key role in learning and memory. These drugs may have certain side effects like tremors, diarrhea, nausea, seizures, fatigue and insomnia. In view of these, the herb used in this study may provide a better alternative for the treatment of AD. Medicinal plants are widely used by traditional medical practitioners for curing various diseases. *Hypericum perforatum* or St john's wort is a flowering plant species and a medicinal herb that is sold over the counter as a treatment for depression. It is also used for tiredness, loss of appetite and in sleeplessness. This study investigated the role of *Hypericum perforatum* extract in treating AD.

Experimental work done:

Collection and drying of plant material (leaves).

Extract preparation using soxhlet apparatus.

Phytochemical screening of plant extract.

Physicochemical tests.

Enzyme assay to check activity of the plant extract.

In-vitro assay of the extract to check its activity on Alzheimer disease.

Chapter-1

INTRODUCTION

What is Alzheimer's disease?

- Alzheimer's disease is a brain disease which is progressive as well as irreversible. It slowly destroys thinking and memory skills and also the ability to carry out the simplest tasks of day to day life.[1]
- It was described by a German psychiatrist and neuropathologist Alois Alzheimer in 1906 and was named after him.
- AD is the most common cause of dementia among older people. It accounts for more than half of all cases of dementia. Symptoms first appear after 60 years of age.[2]
- Dementia is the loss of thinking, reasoning and behavioral abilities, to such an extent that it starts to interfere with an individual's daily activities. It consists of a mild stage, when it starts affecting a person's day to day activities, and then advancing to a severe stage, when the person becomes dependent on others for day to day activities of life.[3]
- The human brain functions well in the later years of life in the absence of this neurodegenerative disease.
- It is still unknown that what exactly causes AD it seems likely that damage to the brain starts a decade or more before problems become evident.[4]
- During the preclinical stage of AD, people are free of symptoms but toxic changes are taking place in the brain.
- AD has an insidious onset, at first the changes are barely perceptible but they gradually lead to serious problems.
- Alzheimer's disease affects people in many ways. The symptoms begin with a gradual worsening ability to remember new information.
- Neurons that malfunction and die are usually neurons in brain regions involved in forming new memories.[5]
- As neurons in other parts of the brain do not function properly and die, individuals experience other difficulties.

How common is AD?

In India about 3.2 million people are suffering from this disease, whereas in America the number is 5.2 million. More than 35,000 people have dementia in Ireland. The risk of developing AD increases with growing age but the disease can also occur in people in their 40's and 50's.Worldwide 44.4 million people suffer from dementia.[5]

Prevalence

- An estimated 5.2 million Americans of all ages have Alzheimer's disease in 2013.
- An estimated 5 million people age 65 and older and approximately 200,000 individuals under age are included in this.
- Who have younger-onset Alzheimer's are 65 in number.
- One in nine people age 65 and older (11 percent) has Alzheimer's disease.
- About one-third of people age 85 and older (32 percent) have Alzheimer's disease.
- Of those with Alzheimer's disease, about 4 percent are under age 65, 13 percent are 65 to 74, 44 percent are 75 to 84, and 38 percent are 85 or older.

Deaths caused by AD

Officially AD is listed as the sixth-leading cause of death in the United States.
It is the fifth-leading cause of death for those of 65 years of age and older than that. However, it may cause even more deaths than official sources recognize. It is difficult to determine how many deaths AD causes each year because of the way causes of death are recorded.[6]

Types of Alzheimer's disease

There are two known types of Alzheimer's disease. Alzheimer's has been categorized by doctors into the following types:

Familial

- Genes directly cause the disease.
- Approximately 10% of early onset AD cases have a familial form of condition, transmitted as an autosomal dominant trait.
- The majority of cases of familial AD are caused due to mutations in three genes:
- Amyloid precursor protein, Presenilin-1 and Presenalin-2.
- This is a form of Alzheimer's disease that is known to be entirely inherited.
- At least two generations of affected family members have had AD.
- It is extremely rare, accounting for less than 1% of all cases of Alzheimer's disease.AD can have a much earlier onset (often in the 40s).[7]

Sporadic

- Genes don't cause the disease directly but they may influence the risk of developing the disease.
- It occurs in fewer family members.
- Sporadic type is the most common form of Alzheimer's disease, accounting for about 90% of cases, and usually occurs after age 65.
- Late-onset Alzheimer's disease strikes almost half of all people over the age of 85 and may or may not be hereditary.

Causes of Alzheimer's disease[8]

- Deficiencies in neurotransmitters like acetylcholine, somatostatin and norepinephrine.
- Plaques-These are the deposits of beta amyloid protein that accumulates in the spaces between nerve cells.
- Tangles-These are the deposits of protein tau that accumulates inside the nerve cells.
- Genetic factors.
- Environmental factors
- Viral factors such as slow growing CNS virus.
- Trauma.

Neurotransmitters involved in AD

Absence of acetylcholine

- The severity of dementia is directly related to the reduction of the amount of neurotransmitter acetylcholine.
- During the course of the normal aging process, concentrations of acetylcholine may decrease, resulting in the short term memory sporadic lapses that many old aged individuals tend to experience frequently.
- This normal, fall or decline in memory, is known as Age-Associated Memory Impairment, or simply forgetfulness, so it should not be related to or confused with Alzheimer's disease a neurodegenerative disorder where the level of acetylcholine can drop by up to 90 percent.
- The brain of Alzheimer's patient may contain as less as 10% of the normal amount of acetylcholine. The gradual death of cholinergic brain cells results in a progressive and significant loss of cognitive and behavioral function.

Hippocampal cells lose their connection to other neurons and die, thus short-term memory falters and, as a result, individuals become easily confused.

Glutamate

- Neuronal death can take place due to malfunctions in the components of the glutamate glutamine cycle.
- Evidence is also there for impaired glutamate uptake due to deficiency of glial transporters in AD.
- The interaction between oxidative radicals and A β, glutamate transporters, is seen in AD.

Norepinephrine

- Norepinephrine is reduced in several areas of the brain.
- This reduction is limited mostly to patients with a greater severity of neuronal death and an earlier age.
- There is neuronal death and loss of α-2 receptor function in AD.
- It has also been shown by studies and findings that noradrenergic system is a prerequisite for the integrity of at least some central cholinergic functions.
- Therefore, multiple lines of evidence implicate norepinephrine in AD.

Dopamine

- Evidence is there that micro molar concentrations of dopamine or L-dopa are sufficient to significantly inhibit fibril formation or disaggregate existing fibrils of A β.
- D1 receptor seems to play a more prominent role in mediating plasticity and specific aspects of cognitive function, including spatial learning and memory processes and D1 agonists are being tried for improving cognition in AD.

Other risk factors

Genetics family history and age are all risk factors that cannot be changed. Research has revealed clues about some other risk factors that may be involved in the process leading to AD. It's important to protect your head by buckling your seat belt, wearing your helmet when participating in sports and fall-proofing your home. There appears to be a strong link between serious head injury and future risk of Alzheimer's. A promising line of research suggested that strategies for overall healthy aging help to keep the brain healthy and may even offer some protection against Alzheimer's. These measures include eating a healthy diet, avoiding tobacco and excess alcohol, staying socially active, and exercising both mind and body. Some of the strongest evidence links heart health to brain health. The risk of developing Alzheimer's appears to be increased by many conditions that damage blood vessels and the heart. These include stroke, high blood pressure heart disease, diabetes, and high cholesterol. Doctors should monitor the patient's heart health and treat any problems that arise. Studies of donated brain tissue provide additional evidence or the heart-head connection. These studies suggest that
Plaques and tangles are more likely to cause Alzheimer's symptoms if strokes or damage to the brain's blood vessels are also present.

Aluminum

During the 1960s, aluminum emerged as a possible suspect in causing Alzheimer's disease. This suspicion led to concerns about everyday exposure to aluminum through sources such as foil, beverage cans, cooking pots, antacids and antiperspirants. Since then, studies have failed to confirm any role for aluminum in causing Alzheimer's. Almost all scientists today focus on other areas of research, and few experts believe that everyday sources of aluminum pose any threat.

Pathophysiology of Alzheimer's disease

Diagnosis of Alzheimer disease made by autopsy examination of a patient's brain revealed gross cerebral atrophy, signifying loss of neurons. The diagnostic lesions found on microscopic evaluation of the brain, revealed the presence of large numbers of extracellular plaques and intracellular neuro fibrillary tangles. Plaques and tangles are found predominantly in the temporal and frontal lobes including the hippocampus. In more advanced cases, the pathology extends to other regions of the cortex, including the occipital lobes and the parietal lobes.

- Plaques are extracellular insoluble deposits, composed of a 40–43 amino acid peptide called β-amyloid.
- β- Amyloid derives from a larger protein, β-amyloid precursor protein (APP).
- There are two types of plaques classical or diffuse.
- Classical plaques contain densely aggregated β-amyloid and cause neuron loss and degeneration.
- It may be possible that plaques arise from raised β-amyloid levels.
- Diffuse plaques are not related with dystrophic or abnormal neurons they are amorphous aggregates of β-amyloid.
- The intracellular deposits of the microtubule associated protein tau (t) found within dystrophic neurons are called tangles.
- Normally tau is found in great abundance in the neurons, inside the neurons it forms stable polymers by binding tubular monomers together. These polymers are necessary for axonal growth and cellular transport.
- Tau becomes hyperphosphorylated in the AD tangles and thus there is less efficient binding to microtubules.
- Then spontaneously the unbound tau aggregates into insoluble paired-helical filaments, these are seen in the neurons as deposits.
- Normal ageing brains do consist of plaques and tangles but they are distributed in brains of patients with Alzheimer disease more widely.[8]

Cell loss in the hippocampus[9]

- The areas of the brain which are most affected by plaques and tangles in AD are hippocampus, parietooccipital cortex, temporal cortex and frontal cortex.
- Small sea-horse-shaped structures present in the temporal lobes are called the hippocampi which play a main role in developing and maintaining memory.
- The earliest changes in Alzheimer disease are shown by the hippocampi and this region also has the greatest concentration of tangles and plaques.
- The early and progressive symptoms of memory loss in patients with Alzheimer disease corresponds to these findings.
- Other clinical findings seen in Alzheimer disease due to the formation of plaques and tangles include problems with skilled tasks and language abnormalities.

Vascular dementia

- Vascular dementia occurs when there is restricted flow of blood to the brain and nerve cells are deprived of oxygen, causing them to die.
- Probable risk factors include high blood pressure, high cholesterol, and advanced age and it is commonly associated with post-stroke patients.
- The symptoms of vascular dementia are similar to Alzheimer's disease.
- However, unlike Alzheimer's disease, vascular dementia affects specific parts of the brain, therefore certain abilities may be affected and some may remain unaffected.
- Unlike Alzheimer's disease, which is steadily progressive, symptoms of vascular dementia may stay the same for some time and then suddenly appear to decline.

Amyloid hypothesis[10]

- Traditionally the amyloid hypothesis points to the accumulation of beta amyloid peptide as the main event triggering neuron degeneration.
- The toxic form of protein that is aggregated amyloid fibrils accumulate and thus cause disruption of the cell's calcium ion homeostasis.
- Thus programmed cell death is also induced.
- It may also be possible that A-beta selectively builds up in the mitrochondria of the cells of Alzheimer's affected brains.
- Many cytokines and inflammatory processes may also have a role in the pathology of AD.
- A series of secretases break down the amyloid precursor protein (APP).
- A non soluble fragment of APP (called A -beta 42) during this process accumulates and is deposited outside the cell.
- The other protein fragments gather into plaques due to the sticky non soluble nature of A-beta42.
- Neuronal death is caused due to the formation of the plaques.

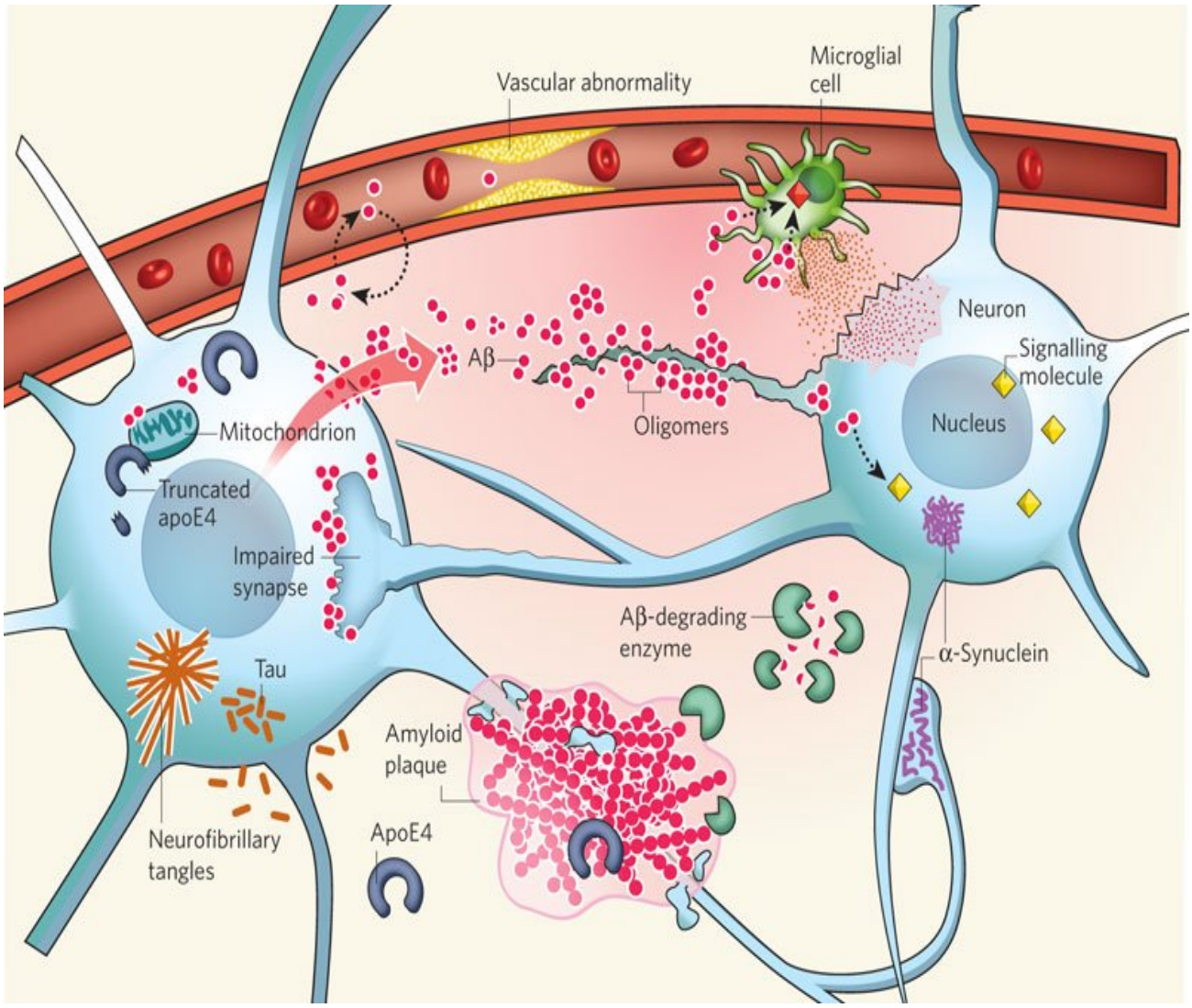
Support for amyloid hypothesis

- It has been indicated by growing evidence that genetic variability in A-beta catabolism and clearance may contribute to the risk of late onset of AD.

Problems with amyloid hypothesis

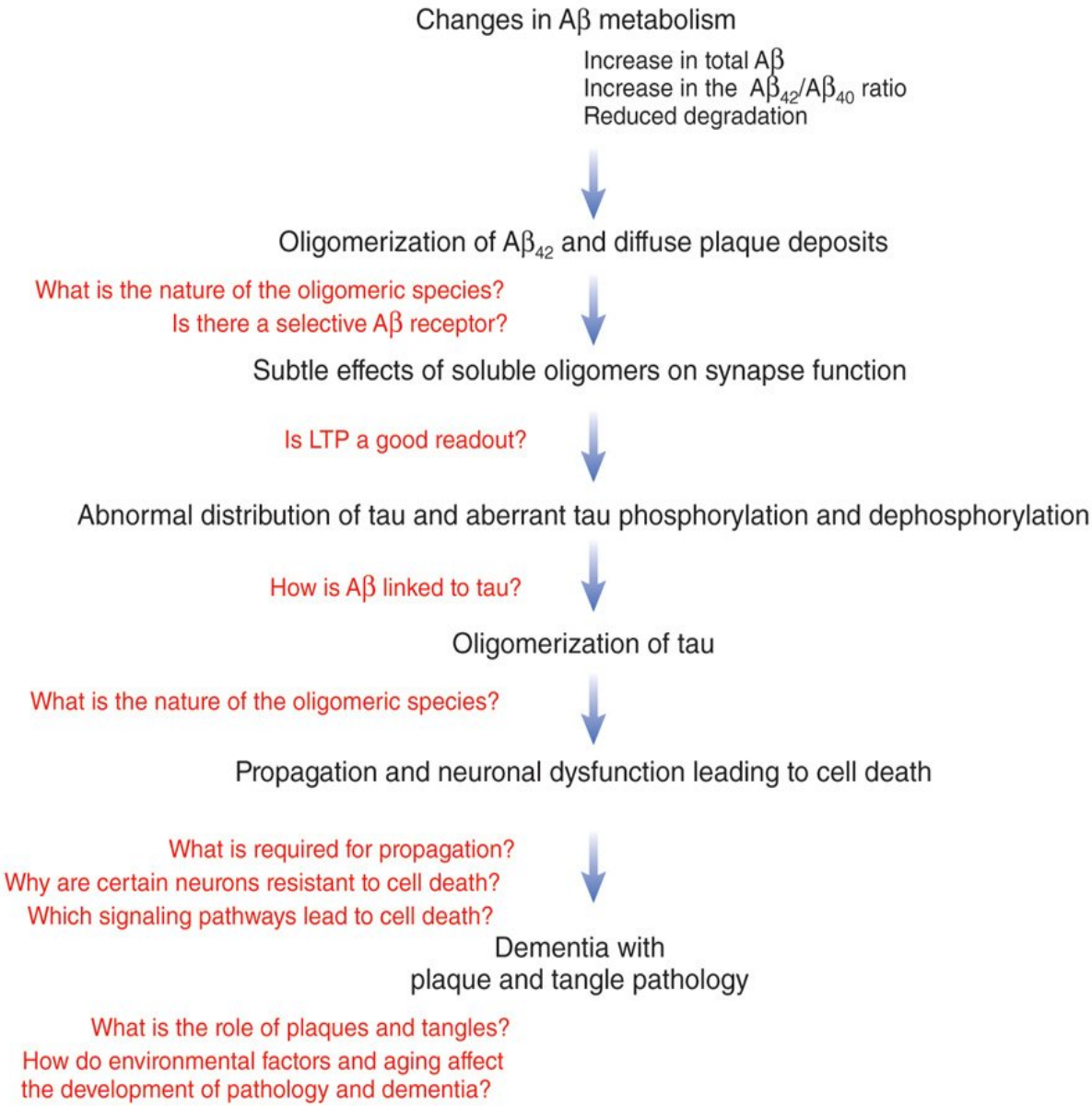
- The amyloid hypothesis remains controversial because a specific neurotoxic species of A-beta and its effects have not been defined in vivo.

Diagrammatic representation of amyloid hypothesis



Neuroscience: Alzheimer's disease
Lennart Mucke
Nature 461, 895-897(15 October 2009)

Amyloid cascade hypothesis



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Tau hypothesis[11]

- The accumulation of amyloid plaques does not alone contribute to neuron loss. There are other factors also that lead to degeneration of neurons.
- Many renal processes including apoptosis and synaptic plasticity are modulated by calcium.
- Intracellular calcium signaling dysregulation has been implicates in the pathology of AD.
- Tau hypothesis is also a contributing factor in causing neuron loss. Tau is a microtubule associated protein which keeps the microtubules stabilized.
- The microtubule is responsible for axonal transport.
- Accumulation of phosphate on the tau proteins leads to the formation of paired helical filaments that accumulate and lead to the formation of neuro fibrillary tangles.
- Abnormal helical filament aggregates may be one of the causes to destabilize this protein.
- Probably impaired axonal transport is the cause of cell death.
- The loss of tau protein which stabilizes the microtubules causes degradation of the cytoskeleton based on this a mechanism for neurotoxicity has been proposed.
- The exact cause behind the hyperphsophorylation of the tau protein is still unknown.
- Taupathies is a disease in which missfolding of the same protein takes place. So provides a support for tau hypothesis.
- However it is believed by a majority of scientists that amyloid hypothesis is the primary cause of AD.
- Tau hypothesis also doesn't explain everything.
- The formation of tangles is often observed in the brain of people as young as thirty years of age. Apparently, there are some processes and mechanisms that can eliminate the tangles or prevent them from affecting the brain functions.
- These days, many researchers tend to consider Alzheimer's an aging-related breakdown of neuronal pathways. It can be accelerated or caused by certain events such as head injury, some diseases and so on. Both plaques and tangles are the products of this process, rather than causative factors. Once they are formed in sufficient quantities, however, they start to spread along the neuronal pathways, eventually causing the observed symptoms of the disease.

Genetics of Alzheimer's disease[12]

- Basically there are three genes which play a role in the pathology of AD:
  APP on chromosome 21
  Presenilin-1 (PS1) gene on chromosome14
  Presenilin-2 (PS2) gene on chromosome1
  Apolipoprotein E on chromosome 19.

Presenilin mutations

- Mutations in the presenilin genes are the most common known cause of familial Alzheimer disease.
- Proteins that are Trans membrane proteins localized primarily in the endoplasmic reticulum and the Golgi apparatus are known as presenilins.
- These are widely expressed proteins but their functions are still unknown.
- Nematode Caenorhabditis elegans has been used to isolate a gene which is similar to presenilins.
- Notch signalling pathway, is a pathway which is important for cell-fate decisions during development. A phenotype is caused by mutations in this gene which causes defects in the notch signaling pathway.
- Mice that lack PS1 also show severe defects resembling a phenotype in which Notch is missing, thus supporting the role of PS1 in this signaling pathway.
- Most of the mutations found to cause familial Alzheimer disease have been in PS1 so far with only two mutations found in PS2, which is 67% homologous toPS1.
- All the mutations increase the amount of Ab42 and Ab43 produced through an unknown mechanism which accelerates β-amyloid aggregation and amyloid plaque formation.

APP mutations

- β amyloid is the main protein component of the extracellular plaque.
- Soluble β-amyloid generated by cleavage of the larger APP by two enzymes called β-secretase and g secretase is a normal constituent of the human brain.
- An alternative proteolytic pathway involving α-secretase prevents A-beta formation.
- β-Amyloid in the brain is heterogeneous, consisting of a series of peptides varying in length from 39 to 43 amino acids.
- β- Amyloid of size 40 amino acids is referred to asAb40, and is normally the most abundant form.
- Ab42 and Ab43 refer to the 42 and 43 amino acid forms, and the proportions of these two forms increase in the amyloid plaques of Alzheimer disease brains.
- Mutations in the APP that are known to cause some forms of autosomal dominant Alzheimer disease appear to alter normal APP processing by causing increased production of Ab42 and Ab43.
- Another type of APP abnormality occurs in patients with Down syndrome, a condition caused by an extra copy of part or all of chromosome 21.
- Patients with Down syndrome are intellectually impaired and have a number of developmental abnormalities noted early in life.
- In mid -adulthood, many go on to develop dementia with widespread deposition of β-amyloid in plaques and tangles similar to those seen in Alzheimer disease.
- APP is critical to injury repair and neuron growth.
- In AD an unknown process leads to the division of APP into smaller fragments by enzyme proteolysis.
- One of these fragments give rise to fibrils of beta amyloid which form clumps that deposit outside neurons in dense formation known as senile plaques.

Apo -lipoprotein E[13]

- The genes whose mutations cause familial Alzheimer disease are among the few known 'causes' of Alzheimer disease, but they are responsible for less than 1% of the total number of cases.
- Of greater public health significance has been the finding that the e4 allele of the apolipoprotein E gene (ApoE-e4) occurs in sporadic cases of Alzheimer disease with increased frequency compared with controls.
- Apo E is a major serum lipoprotein involved in cholesterol metabolism.
- There are three naturally occurring alleles of the ApoEgene, e2, e3 and e4, which differ from one another by a single codon.
- While the e4 allele is over represented among patients with Alzheimer disease compared with control populations, the e2 allele frequency is lower inpatients with Alzheimer disease than in controls, implying that this allele may be protective against developing the condition.
- The ApoE-e4 allele shows a dose-dependent increase in risk for Alzheimer disease, apparently mediated through a decrease in the age of onset, such that individuals with two copies of the e4 allele have an earlier onset than those with one copy, who have an earlier onset than individuals with no e4 allele.
- The molecular mechanism by which the ApoE genotype is involved in Alzheimer disease pathogenesis is unclear, but patients with ApoE-e4 show a significant, dose-dependent increase in the density of b-amyloid deposits.
- The epsilon4 allele of apolipoprotein E (APOE) is the major genetic risk factor for Alzheimer's disease (AD).
- Although there have been numerous studies attempting to elucidate the underlying mechanism for this increased risk, how apoE4 influences AD onset and progression has yet to be proven.
- However, prevailing evidence suggests that the differential effects of apoE isoforms on A-beta aggregation and clearance play the major role in AD pathogenesis.

Progression of the disease[14]

Alzheimer's disease generally progresses through three stages: mild, moderate and severe.

Stage 1: Mild Alzheimer's disease

The mild stage of Alzheimer's disease can last from 2 to 4 years or longer.

Those in this phase of the disease may:

• Say the same thing over and over

• Lose interest in things they once enjoyed

• Have trouble finding names for common items

• Lose things more often than normal

• Seem to experience personality changes

• Have difficulty grasping complex ideas

Stage 2: Moderate Alzheimer's disease

The moderate stage of Alzheimer's disease is often the longest, lasting from 2 to 10 years.

In this stage, a person may:

• Get lost easily, even in places they know well

• Become more confused about recent event

• Argue more than usual

• Believe things are real when they are not

• Experience restlessness and agitation

• Have difficulty sleeping and may wander

Stage 3: Severe Alzheimer's disease

The severe stage can last from 1 to 3 years or longer. People with severe AD cannot:

• Recognize family members

• Care for themselves

• Move around independently

Symptoms of AD

• Forgetting things – names, dates, places, faces, getting stuck for words.
• Loss of interest in starting projects or doing things
• Difficulty in solving problems or doing puzzles
• Difficulty in performing everyday tasks
• Misplacing things regularly
• Poor or decreased judgment
• Changes in mood and behavior
• Disorientation in familiar surroundings
• Changes in personality.

Managing symptoms

• Try to maintain daily routines.
• Allow time to complete tasks and don't be rushed.
• Label presses and shelves with contents.
• Keep a checklist beside the door of things to do before leaving the.
House e.g. turn off the oven, switch off lights, lock the door.
• Consider using appliances that have an automatic shut-off device.
• Use a pill box to help organize and remind when to take medicines.
• Place important phone numbers beside the telephone.
• Leave a spare set of keys with a trusted neighbor.
• Keep a collection of photos of friends and family members,
labeling with names if necessary.

Diagnosis of AD

- To diagnose Alzheimer's, doctors may ask questions about overall health, past medical problems, ability to carry out daily activities, and changes in behavior and personality.
- Conduct tests of memory, problem solving, attention, counting, and language.
- Carry out standard medical tests, such as blood and urine tests, to identify other possible causes of the problem.
- Perform brain scans, such as computed tomography (CT) or magnetic resonance imaging (MRI), to distinguish Alzheimer's from other possible causes for symptoms, like stroke or tumor doctors information about how the person's memory is changing over time.
- Early, accurate diagnosis is beneficial for several reasons. It can tell people whether their symptoms are from Alzheimer's or another cause, such as stroke, tumor, Parkinson's disease, sleep disturbances, side effects of medications, or other conditions that may be treatable and possibly reversible.
- Beginning treatment early on in the disease process can help preserve function for some time, even though the underlying disease process cannot be changed.
- Having an early diagnosis also helps families plan for the future, make living arrangements, take care of financial and legal matters, and develop support networks.
- In addition, an early diagnosis can provide greater opportunities for people to get involved in clinical trials.
- In a typical clinical trial, scientists test a drug or treatment to see if that intervention is effective and for whom it would work best.
- Doctor's information about how the person's memory is changing over time.

Treatment of Alzheimer's disease[15]

- The most important class of drugs used in the specific treatment of Alzheimer disease was developed for the ability to increase acetylcholine levels in the central nervous system.
- Not only are acetylcholine levels reduced in Alzheimer disease brains, but memory and cognitive impairment can be induced in healthy young persons and animals whose cholinergic transmission systems are pharmacologically blocked.
- There are now two classes of compounds that can increase brain acetylcholine levels:
- Acetyl cholinesterase inhibitors (ACEIs), which increase synaptic concentrations of acetylcholine.
- Muscarinic agonists, which mimic acetylcholine by directly stimulating the muscarinic acetylcholine receptor.
- ACEIs have been shown to be of modest clinical benefit in Alzheimer disease, and some ACEIs have been commercially available to treat the condition for several years.
- Since acetylcholinesterase breaks down acetylcholine, ACEIs act to increase the concentration of acetylcholine in the synapse.
- This provides more acetylcholine to interact with the brain's cholinergic receptors, the most important of which are thought to be the muscarinic cholinergic receptors.
- These receptors, when activated , have effects on learning, memory and behavior; they may also be involved in the processing of APP.
- While ACEIs do modestly decrease the rate of cognitive decline in patients with Alzheimer disease, the dementia remains progressive and the benefits of these medications, while measurable, are small.
- The muscarinic agonists currently being developed are specific for the muscarinic M1 receptor subtype. The M1receptors are localized in the cortex and hippocampus, whereas other muscarinic receptors are also found in smooth muscle and glandular tissue.
- The latter may be responsible for the uncomfortable side effects – namely, salivation, sweating, nausea and vomiting – seen when trying to manipulate the cholinergic system pharmacologically.

Other treatments include

- Reducing calcium toxicity with calcium channel blockers, which protect neurons from calcium ion-induced injury by limiting calcium ion entry.
- Using cholesterol-lowering drugs to lower the brain concentrations of ApoE.
- Reducing the chemical changes in the tau protein.
- Preventing the development of plaques, neuritic dystrophy.
- Preventing the formation of β-amyloid by inhibiting the secretases that release it from APP or by preventing β-amyloid from aggregating into its toxic form.
- Other studies have been done using vitamin E or other antioxidants, which can protect the neurons against free radical damage.
- Experiments in cell culture have shown that b-amyloid neurotoxicity may be due to its ability to increase production of hydrogen peroxide in nerve cells.
- Hydrogen peroxide is a chemical that releases hydroxyl radicals, which can in turn damage cell membrane lipids and other cell components.
- Alzheimer disease in women who have taken estrogen replacement therapy.
- Estrogen has a number of well documented effects on the cardiovascular system, including protection against atherosclerosis and vascular injury.
- Estrogen may also interact with ApoE to reduce the development of atherosclerotic lesions in the cerebral blood vessels, and it may be this effect that lowers the risk of Alzheimer disease.
- Estrogen has other beneficial effects, which include increasing levels of choline acetyltransferase, the enzyme needed to synthesize acetylcholine, in the basal forebrain, and promoting the growth of axons and dendrites, the projections that nerve cells send out to communicate with one another, in injured neurons.
- Several epidemiological studies have demonstrated that chronic use of nonsteroidal antiinflammatory drugs (NSAIDs) reduces the risk of Alzheimer disease, supporting the hypothesis that development of the condition involves inflammation.

Treatment of mild moderate AD

- Choline-esterase inhibitors
  - Donepezil
  - Rivastigmine
  - Galantamine

Treatment of severe AD

- NMDA antagonists
  - Memantine
- Slows intracellular Ca accumulation and delay nerve damage
- Used in combination with Donepazil

Current status of cholinesterase inhibitors

- Effective in 6 month & 12 month trials
- Early initiation of therapy
- Delay institutionalization
- Decrease troublesome behavior

Dosage and Side Effects

Doctors usually start patients at low drug doses and gradually increase the dosage based on how well a patient tolerates the drug. There is some evidence that certain patients may benefit from higher doses of the cholinesterase inhibitors. However, the higher the dose, the more likely are side effects. Patients should be monitored when a drug is started

Summary of drugs used in the treatment of AD[16]

DRUG NAME	DRUG TYPE AND USE	HOW IT WORKS	COMMON SIDE EFFECTS
Namenda® (memantine)	N-methyl D-aspartate (NMDA) antagonist prescribed to treat symptoms of moderate to severe Alzheimer's	Blocks the toxic effects associated with excess glutamate and regulates glutamate activation	Dizziness, headache, constipation, confusion
Razadyne® (galantamine)	Cholinesterase inhibitor prescribed to treat symptoms of mild to moderate Alzheimer's	Prevents the breakdown of acetylcholine and stimulates nicotinic receptors to release more acetylcholine in the brain	Nausea, vomiting, diarrhea, weight loss, loss of appetite
Exelon® (rivastigmine)	Cholinesterase inhibitor prescribed to treat symptoms of mild to moderate Alzheimer's (patch is also for severe Alzheimer's)	Prevents the breakdown of acetylcholine and butyrylcholine (a brain chemical similar to acetylcholine) in the brain	Nausea, vomiting, diarrhea, weight loss, loss of appetite, muscle weakness
Aricept® (donepezil)	Cholinesterase inhibitor prescribed to treat symptoms of mild to moderate, and moderate to severe Alzheimer's	Prevents the breakdown of acetylcholine in the brain	Nausea, vomiting, diarrhea

*Available as a generic drug.

Chapter-2

Hypericum perforatum[17]

Common name- St. John's Wort

Family- Hypericaceae

Synonyms-Hypericum deidesheimense, Hypericum vulgare, Hypericum lineolatum, Hypericum mixtum

Description

- St. John’s wort is a five-petalled, yellow-flowered perennial weed common to the western United States, Europe, and Asia.
- Close examination of the flowers reveals small black dots that, when rubbed between the fingers, produce a red stain.
- This red pigment contains the constituent hypericin. Held up to light, the leaves of the plant display a number of bright, translucent dots. This perforated look led to the species name perforatum.
- The plant is currently cultivated in Europe, North and South America, Australia, and China.

Active constituents present in *Hypericum perforatum*

Biochemical class	% Fresh plant[a]	Active constituent
Naphthodianthrones	0.03–3.0 (Flowers/buds)	Hypericin Pseudohypericin
Phloroglucinols	2–5 (Flowers/buds)	Hyperforin Adhyperforin
Flavonoids	12 (Leaves) 7 (Stalk) 2–4 (Buds)	Quercetin Hyperoside Quercitrin Isoquercitrin Rutin Campferol Myricetin Amentoflavone I3,II8-Biapigenin
Procyanidins	12 (Aerial parts) 8 (Flowers/buds)	Procyanidin Catechin Epicatechin polymers
Tannins	6–15	Tannic acid
Essential oil	0.06–1.0 (Flowers/leaves)	Terpenes, alcohols
Amino acids	0.01	GABA Cysteine Glutamine Leucine Lysine Ornithine Proline Threonine
Phenylpropanes	0.1	Caffeic acid Chlorogenic acid
Xanthones	0.01 (Roots) 0.0004 (Leaves/stem)	Kielcorin, norathyriol
Other water-soluble components	0.5	Organic acids, peptides, polysaccharides

Mechanism of action

- A number of proposed mechanisms exist for St. John's wort's antidepressant effect, involving several neurotransmitters and hormones. Initially, inhibition of monoamine oxidase (MAO) was believed to be the primary mode of action.
- More recent research indicates constituents of St. John's wort exert MAO inhibition only at concentrations higher than those typically found in commercially available extracts.

Preparations

- Dried or fresh herb for infusion, liquid extract and tablets for internal use.
- Infused oil of hypericum is made by mixing the flowers in a good-quality fixed oil (such as olive oil) in a well-sealed vessel in the presence of sunlight over several weeks.
- The action of the sunlight produces a red oil containing hypericin derivatives, hyperforin, xanthones, flavonoids and the breakdown products of hyperforin.

Effects

- Mild antidepressant activity; useful for wound healing; antiviral activity with application to disorders caused by enveloped viruses.

Traditional view

- Hypericum was considered primarily for the nervous system, particularly for nervous afflictions (excitability, menopausal neurosis, hysteria) and disorders of spine, spinal injuries, neuralgia, sciatica and muscular rheumatism.
- It was also used for its supposed diuretic and astringent properties and to treat urinary problems, diarrhoea, dysentery, parasitic nfestations, jaundice, haemorrhages, and bed wetting.

Dosage

- 2–5 g of dried herb per day or the equivalent of 1.0–2.7 mg of total hypericin per day.
- Hypericum tablets (1.5 g, standardized to contain 0.9 mg): 2–3 tablets per day.
- The volume of liquid extract prescribed depends upon the level of TH in the extract; typical doses are 3–6 ml of 1:2 liquid extract per day, 7.5–15 ml of 1:5 tincture per day.
- Doses at the higher end of this range have been utilized in the treatment of depression, HIV infection and other viral infections.
- For the short-term treatment of acute viral infections, even higher doses may be necessary.

Pharmacokinetics

- Pharmacokinetic studies in humans have shown that, at clinically relevant doses (900 mg and 1800 mg), peak plasma levels of totalhypericin was observed approximately 4 h after dosage.
- The elimination of hypericin has been shown to be slow with an approximate half-life of 25 h.
- Similarly, hyperforin concentrations have been observed in the nM range, peaking at 3.5 h after dosage.
- Compared to hypericin, hyperforin has been shown to have a shorter half-life of 9 h. Steady-state levels of hypericin and hyperforin are achieved after 3–4 days and concentrations are comparable to the plasma levels achieved by other antidepressants.
- Recently, a number of pharmacokinetic interactions between hypericum extracts and other drugs have been reported.
- Interaction may be due to an effect of hypericum on liver cytochrome (CYP) P-450 enzymes.
- Reduced plasma concentrations of the ophylline, cyclosporin, wafarin, anaesthetics and the oral contraceptives ethinyloestradiol/desogestrel have been noted with combination with hypericum extracts.

Pharmacological activity of *Hypericum perforatum*

- **Antidepressant activity** – Hypericin is a standardized extract has shown significant antideprerssant activity by inhibiting enzyme monoamino oxidase .The other mechanisms of antidepressant activitiy are inhibition of dopamine β-hyroxylase *in vitro* inhibition of uptake of serotonin and dopamine, inhibition of catechol o-methyl transferase. The antidepressant activity of hyperforin is attributed to its inhibition of neuronal uptake of serotonin, norepinephrine, and dopamine like many other antidepressants.
- **Antiviral activity**- Hypericin is a well known photosensitizing agent used in the photodynamic therapy of cancer and viral infections.
- **Anticancer activity**- Hypericin have been found active against human leukemia squamous cell carcinoma , nasophyrngial carcinoma, mouse mammary carcinoma**.**

Side effects

- Generally few side effects and quite mild
- Not a single recorded hypericum-related death in 2,400 years
- All side effects are reversible (stops when the herb is no longer being used)
- Photosensitivity (sensitive to sunlight)
- Fair skinned people should avoid, if possible, prolonged sunlight exposure
- Constipation
- Gastrointestinal problems (nausea, vomiting, diarrhea)
- Fatigue
- Dry mouth
- Dizziness
- Allergic reactions
- Confusion
- To help combat these side effects it is suggested that St. John's wort be taken with food**.**

Drug interactions

- It is advised to be cautious when taking other medications with St. John's Wort as it has shown to decrease their effectiveness this includes:
- Antidepressants
- Immunosuppressive medications
- Especially in those with heart or kidney transplants as it can cause the body to reject the transplanted organs
- Indinavir and other protease inhibitors (treats HIV)

Cautions

- Should not be used during pregnancy or while breast feeding as there is no information on its safety under these conditions
- Should not be used for severe depression or bipolar disorder as not enough research is yet available to show effectiveness in these circumstances
- Do not take St. John's wort in combination with MAOI's, can produce a dangerous rise in blood pressure.
- Should not be taken in combination with prescription SSRI's as this can lead to serotonin syndrome – too much serotonin. Few side effects

Advantages

- Mild side effects
- Reversible side effects
- Do not need a prescription
- Sexual dysfunction (as a side effect) has never been reported, whereas this is a common problem with other antidepressants.

Chapter-3

Objectives and work plan

- Literature review
- Collection and drying of plant material (leaves).
- Extract preparation using soxhlet apparatus.
- Phytochemical screening of plant extract.
- Physicochemical tests.
- Enzyme assay to check activity of the plant extract.
- In-vitro assay of the extract to check its activity on Alzheimer disease.

Work done

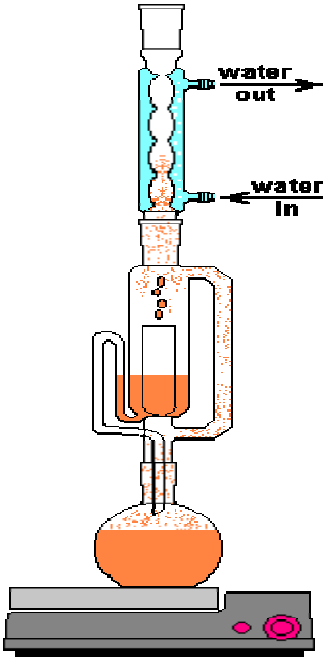
- Plant collection – whole plant of *Hypericumperforatum* was collected from Jaypee herbal garden.
- Drying of plant – air drying.
- Then the leaves of the dried plant were collected, sieving was done to reduce their size.
- Extraction using soxhlet
- Phytochemical screening of the plant: It was done to test the extract for its chemical constituents. The plant extract was tested using various reagents for different tests.
- Physicochemical tests were performed to check the extract for impurities.
- In vitro assay was done to check activity of plant: anti-inflammatory, antioxidant
- In vitro assay to check inhibition of acetylcholinesterase.

Materials

The plant *Hypericum perforatum* was collected from Jaypee University of Information Technology. Extraction was performed using oxhlet assembly. Rotatory evaporator was used to remove the solvent and then lypholysation was done using a lypholyser. Defa tting of the extract was done with petroleum ether. Then ethanol and water in the ratio of 1:1 were used for the hydroalcoholic extraction.

Methods

Soxhlet extraction



- The powdered leaves of *Hypericum perforatum*were packed in the thimble using some cotton. Organic solvent (petroleum ether) was placed in the flask which was placed on heating mantle(temperature maintained at 20 to 30 °C), continuous water supply was provided to the condenser to keep the system cool and extraction was done as follows:
- Defatting of plant – it was done with petroleum ether for 48 hours; the leaves were placed in the assembly of the soxhlet along with the solvent (petroleum ether).Temperature maintained was 20 °C.
- Methanolic extraction of plant – it was done for 10 days. Solvent used was methanol temperature maintained was 30 °C.
- The solvent was removed from the plant extract using rotator evaporator at 50 °C .
- Lypholysation of the plant extract was done and stored at 4 °C.

Phytochemical Tests:

The test for Carbohydrates (Molisch Test): Few drops of α-napthol was added to each portion dissolved in distilled water, this was then followed by the addition of 1 ml of conc. H_2SO_4 by the side of the test tube. The mixture was then allowed to stand for 2 min. Formation of a red or dull colour at the interphase of the two layers was a positive test.

The test for Tannins: About 0.01g of each extract was stirred with 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2ml of the filtrate occurrence of blue-black, green or blue –green precipitate indicates the presence of tannins.

The test for Terpenoids (Liebermann-Burchard test): To 0.02g of each, 2ml of acetic acid was added; the solution was cooled well in ice followed by the addition of conc. H_2SO_4 carefully. Colour development from violet to blue or bluish-green indicated the presence of terpenoids.

The test for Saponins*:* Some portion of each extract was boiled with 5ml of distilled water, filtered. To the filtrate, about 3ml of distilled water was further added and shaken vigorously for 5 min. Frothing which persisted on warming was taken as an may be the presence of saponins.

The test for Flavonoids: Few quantity of the each portion was dissolved in water and filtered. To 5ml of each of the filtrate, 3ml of Lead ethanoate solution was then added. Appearance of buff-colored ppt indicates the presence of flavonoids.

The test for Alkaloids: Few quantity of the each portion was stirred with 5ml of 1% aq. HCl on water bath and then filtered. Of the filtrate, 1ml was taken individually into 2 test tubes. To 1ml, Mayer's Reagent was added and appearance of buff-colored ppt will be an indication for the presence of alkaloids.

The test for inulin- to the test extract, a solution of α- napthol and sulphuric acid was added. Brownish red solution indicate the presence of inulin.

The test for cardiac glycosides (legals test) -the test extract was treated with pyridine followed by the addition of sodium nitropuside solution, red colour indicate the presence of cardiac glycosides.

Physicochemical tests

Ash test-

Ignite a crucible of platinum, quartz or porcelain at 500-550˚C for 1 hour,

↓

Allow it cool in a desicator and weigh it accurately

↓

Place few gm of sample in the crucible and weigh it accurately

↓

Take off or slide the lid of crucible if necessary, heat the crucible gently first then raise the temperature gradually.

↓

Ignite at 500-550˚C for not less than 4 hours to incinerate until it is free from charred material, cool in a desiccator and weigh accurately. Incinerate the residual to constant weigh, cool in a desicator and weigh accurately.

Acid insoluble ash-

Add carefully 25 ml of dil. Hcl to the ash (obtained as directed under the ash limit test), boil gently for 5 min, collect the insoluble matter on the filter paper for quantitative analysis, wash thoroughly with hot water and dry the residue together with the filter paper.

Ignite it for 3 hours in a crucible of quartz which has been prepared as directed in the ash limit test and whose weight is already known.cool it in a desiccators and weigh accurately.

If the measured amount is larger than the specified value ignite until a constant weight is obtained.

Water soluble ash

To crucible with total ash add 25ml of water and boil for 5 min followed by collection of insoluble matter in sintered glass crucible/ filter paper .wash with hot water and ignite in crucible for 15 minutes at a temperature not exceeding 450˚C. Subtract weight of the residue in mg from weight of total ash.

Moisture content

The most convenient procedure for determining the mass of the sample before and after drying is to place it in a tared container where it will remain throughout the test. The mass of the container and sample are determined and the mass of the container subtracted. If the mass of the test sample is not determined immediately after preparation, place the moisture-tight cover on the container to prevent evaporation. Dry to constant mass at 110 ± 5°C.

In vitro assay

- Ileum tissue of chicken was obtained from a local meat shop in Waknaghat.
- All chemicals and materials were collected.
- To one piece of ileum the thread was tied to top and bottom ends without closing the ileum, and mounted the tissue in the organ bath containing PSS maintained at 32-35˚C and bubbled with air.
- The magnification from 5-7 folds and bath volume of about 25 ml was maintained, and the tissue was allowed to equilibrate for 30 min before adding Acetylcholine to the organ bath.
- The Acetylcholine induces the contraction in the ileal smooth muscles which were recorded on Kymograph by using frontal writing lever. Contact time of 30 sec, and 5 min time cycle was kept for proper recording of the responses.
- The CRC was recorded till ceiling effect to Acetylcholine was obtained.
- Various parameters were changed and responses were taken
- All physiological salt solution were tried and responses were taken.
- Height of response was measured.

Chapter-4

Results

Phytochemical tests

Tannins	positive
Alkaloids	negative
Saponins	positive
Flavnoids	negative
Terpenoids	negative
Volatile oils	positive
Carbohydrates	positive
Amino acids(ninhydrin test)	positive
Inulin	Negative
Cardiac glycoside (legals test)	Negative

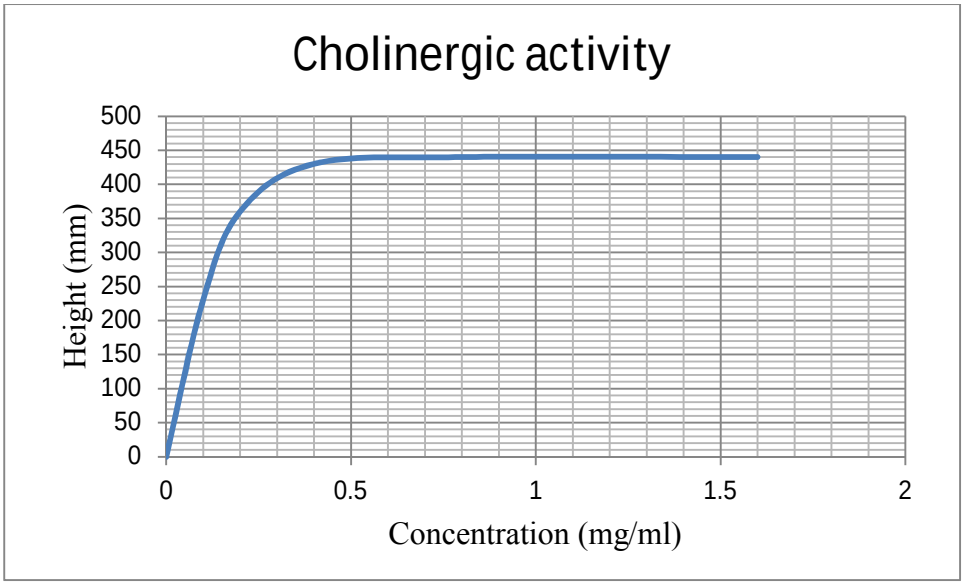
Physicochemical tests

Test	Experimental value	Standard value(as per WHO)
Moisture content	7.5%	Not more than 10%
Total ash content	5,8%	Not more than 7%
Acid insoluble ash	2.2%	Not more than 2.5%
Water soluble ash	11.8%	Not more than 12

Heights in mm of different concentration of the acetylcholine in chicken ileum bioassay.

S No	Dose	Height (cm)	Height (mm)	**Bath volume**: 25ml
1	0.1	2.3	230	**Concentration of acetylcholine used** : 1mg/ml
2	0.2	3.6	360	**Extract concentration:** 1mg/ml.
3	0.4	4.3	430	
4	0.8	4.4	440	
5	1.6	4.4	440	

1g hydroalcholic extract of Hypericum Perforatum have anti-cholinergic activity equivalent to 2.17mg of Acetylcholine



Standard curve for Acetylcholine

Discussion and conclusion

Alzheimer disease is the single most common cause of late life dementia in the industrialized world. The prevalence of the disorder rises with age after the age of 65 years. While some genetic mutations are known to cause a few cases of Alzheimer disease, the etiology for the vast majority of cases is unknown. It is likely that Alzheimer disease represents the final degenerative pathway initiated by a number of genetic and environmental factors. So far, the most important risk factor seems to be the ApoE-e4 allele, which occurs disproportionately in patients with Alzheimer disease compared with the healthy elderly population. Treatment of Alzheimer disease slows the cognitive decline to a modest degree, but severe dementia eventually occurs.

As yet, there is no treatment that can reverse the effects of Alzheimer disease, and there is no effective prevention. In this study *Hypericum perforatum* was used to check its anticholinesterase activity on isolated chicken ileum and the result obtained showed that 1g hydroalcholic extract of Hypericum Perforatum have anti-cholinergic activity equivalent to 2.17mg of Acetylcholine.

References

1.Alagarsamy, V.; Rajesh, R.; Ramaseshu, M.; Vijaykumar, S.; Ramseshu, K. V.; Duraianandakumar, T., Synthesis, analgesic, anti-inflammatory and antibacterial activities of some novel 2-methylthio-3-substituted quinazolin-4-(3H)-ones. *Biological & pharmaceutical bulletin* 2004, *27* (5), 652-656.

2.Hefti, F., Is Alzheimer disease caused by lack of nerve growth factor? *Annals of neurology* 1983, *13* (1), 109-110.

3.Hanin, I.; Reynolds III, C. F.; Kupfer, D. J.; Kopp, U.; Taska, L. S.; Hoch, C. C.; Spiker, D. G.; Sewitch, D. E.; Martin, D.; Marin, R. S., Elevated red blood cell/plasma choline ratio in dimentia of the Alzheimer type: Clinical and polysomnographic correlates. *Psychiatry research* 1984, *13* (2), 167-173.

4.Denecke, R., Preparation and method for the treatment and prevention of dimentia disorders. Google Patents: 2001.

5.de la Monte, S. M.; Wands, J. R., Review of insulin and insulin-like growth factor expression, signaling, and malfunction in the central nervous system: relevance to Alzheimer's disease. *Journal of Alzheimer's Disease* 2005, *7* (1), 45-61.

6.Mölsä, P. K.; Marttila, R.; Rinne, U., Survival and cause of death in Alzheimer's disease and multi-infarct dementia. *Acta Neurologica Scandinavica* 1986, *74* (2), 103-107.

7.Probst, A.; Ulrich, J.; Heitz, P. U., Senile dementia of Alzheimer type: astroglial reaction to extracellular neurofibrillary tangles in the hippocampus. *Acta neuropathologica* 1982, *57* (1), 75-79.

8.Blass, J. P., Brain metabolism and brain disease: is metabolic deficiency the proximate cause of Alzheimer dementia? *Journal of Neuroscience Research* 2001, *66* (5), 851-856.
9.Falkai, P.; Bogerts, B., Cell loss in the hippocampus of schizophrenics. *European archives of psychiatry and neurological sciences* 1986, *236* (3), 154-161.

10.Hardy, J.; Selkoe, D. J., The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 2002, *297* (5580), 353-356.

11.Maccioni, R. B.; Farías, G.; Morales, I.; Navarrete, L., The revitalized tau hypothesis on Alzheimer's disease. *Archives of medical research* 2010, *41* (3), 226-231.

12.Pareés, I.; Edwards, M. I., Psychogenic Movement. *Oxford Textbook of Movement Disorders* 2013, 339.

13.Cayatte, A. J.; Du, Y.; Oliver-Krasinski, J.; Lavielle, G.; Verbeuren, T. J.; Cohen, R. A., The Thromboxane Receptor Antagonist S18886 but Not Aspirin Inhibits Atherogenesis in Apo E–Deficient Mice Evidence That Eicosanoids Other Than Thromboxane Contribute to Atherosclerosis. *Arteriosclerosis, thrombosis, and vascular biology* 2000, *20* (7), 1724-1728.

14.Fox, N. C.; Cousens, S.; Scahill, R.; Harvey, R. J.; Rossor, M. N., Using serial registered brain magnetic resonance imaging to measure disease progression in Alzheimer disease: power calculations and estimates of sample size to detect treatment effects. *Archives of Neurology* 2000, *57* (3), 339-344.

15.Potyk, D., Treatments for Alzheimer disease. *Southern medical journal* 2005, *98* (6), 628-635.

16.O'Brien, J. T.; Ballard, C. G., Drugs for Alzheimer's disease. *Bmj* 2001, *323* (7305), 123-124.

17.Johne, A.; Brockmöller, J.; Bauer, S.; Maurer, A.; Langheinrich, M.; Roots, I., Pharmacokinetic interaction of digoxin with an herbal extract from St John's wort (Hypericum perforatum)*. *Clinical Pharmacology & Therapeutics* 1999, *66* (4), 338-345.