# Saikosaponin B2 modulate oxidative stress in scopolamine induced murine model of Alzheimer's Disease

**Submitted By**

**Mehreen Nadeem Malik**

**Reg. No. 00000328047**

**Supervisor**

**Dr. Adeeb Shehzad**

Department of Biomedical Engineering & Sciences

School of Mechanical & Manufacturing Engineering (SMME)

National University of Science and Technology (NUST)

Islamabad, Pakistan

2022

# Saikosaponin B2 modulate oxidative stress in scopolamine induced murine model of Alzheimer's Disease

Submitted By

Mehreen Nadeem Malik

Registration Number: NUST00000328047

A thesis submitted in partial fulfilment of the requirements for the degree

of

MS Biomedical Sciences

Thesis Supervisor: Dr. Adeeb Shehzad

Thesis Supervisor's Signature: _____

Biomedical Sciences

School of Mechanical & Manufacturing Engineering (SMME)

National University of Science and Technology (NUST)

Islamabad, Pakistan

2022

# Thesis Acceptance Certificate

Certified that final copy of MS/ MPhil Thesis written by **Ms. Mehreen Nadeem Malik,** Registration No. **00000328047** of **MS Biomedical Science and Engineering (SMME)** has been vetted by undersigned, found complete in all respects as per NUST Statutes/Regulations, is free of plagiarism, errors and mistakes and is accepted as partial fulfillment for award of MS/MPhil Degree. It is further certified that necessary amendments as pointed out by GEC members of the scholar have been incorporated in the said thesis.

Supervisor: _____

Dr. Adeeb Shehzad

HOD: _____

Dr. Omer Gilani

Principal: _____

Dr. Javed Iqbal

**FORM TH-4**

# MASTER THESIS WORK

We hereby recommend that the dissertation prepared under our supervision by **Mehreen Nadeem Malik (Reg. No. 00000328047)**, Titled **Saikosaponin B2 modulate oxidative stress in scopolamine induced murine model of Alzheimer's Disease** accepted in partial fulfillment of the requirements for the award of MS degree and awarded grade '___'

**Examination Committee Members**

1. Name: Dr. Omer Gilani Signature:_____

2. Name: Dr. Saima Zafar Signature:_____

3. Name: Dr. Asim Waris Signature:_____

Supervisor's name: Dr. Adeeb Shehzad Signature: _____

Date:_____

_____ _____

Head of Department Date

**COUNTERSINGED**

Date _____ Dean/Principal _____

# Declaration

I certify that this research work titled "*Saikosaponin B2 modulate oxidative stress in scopolamine induced murine model of Alzheimer's Disease"* is my own work. The work has not been presented elsewhere for assessment. The material that has been used from other sources it has been properly acknowledged / referred.

Mehreen Nadeem Malik

2022-NUST-MS-BMS-00000328047

# Proposed Certificate for Plagiarism

It is certified that PhD/M.Phil/MS Thesis Titled **Saikosaponin B2 modulate oxidative stress in scopolamine induced murine model of Alzheimer's Disease** by **Mehreen Nadeem Malik (Reg. No. 00000328047)** has been examined by us. We undertake the follows:

a. Thesis has significant new work/knowledge as compared already published or are under consideration to be published elsewhere. No sentence, equation, diagram, table, paragraph or section has been copied verbatim from previous work unless it is placed under quotation marks and duly referenced.

b. The work presented is original and own work of the author (i.e. there is no plagiarism). No ideas, processes, results or words of others have been presented as Author own work.

c. There is no fabrication of data or results which have been compiled/analyzed.

d. There is no falsification by manipulating research materials, equipment or processes, or changing or omitting data or results such that the research is not accurately represented in the research record.

e. The thesis has been checked using TURNITIN (copy of originality report attached) and found within limits as per HEC plagiarism Policy and instructions issued from time to time.

**Name & Signature of Supervisor**

Name: Dr. Adeeb Shehzad

Signature: _____

## Copyright Statement

# Acknowledgement

All praise is to Almighty ALLAH, the most beneficent, the most merciful who bestowed upon me with the sight to observe, the mind to think and the courage to work more. I could complete my research work only because of Allah's help and blessing.

My deepest and sincerest gratitude goes to my Supervisor **Dr. Adeeb Shehzad,** for his knowledge, flexibility, help, supportive criticism and seemingly endless patience. Thank you for being the best mentor.

I would like to thank my mother **Samiah Nadeem** and my father **Nadeem Malik** for their continued support throughout. I would like to acknowledge my siblings **Iqra Abdullah, Omer Nadeem and Saad Nadeem** who always inspired me to be a better person and to achieve more in life.

I would like to pay gratitude to my friend **Moiz Shaikh** in whom I found another brother. Thank you for listening to all my problems and for inculcating positivity in me at every hurdle. Special thanks to my friends **Rimsha Azhar** and **Ijaz ul Haq** for their constant support and for this beautiful journey full of memories I have now. Without their encouragement and constant faith in me, it wouldn’t have been possible.

I would like to acknowledge my friends **Jannat Imran** and **Zuha Sarwar** for being so helpful throughout this journey. My heartfelt appreciation and sincere gratitude extends to the most caring friends **Moizza Abbas** and **Sameen Fatima,** whose unconditional love and strong personality have been a source of strength and inspiration for me.

***Mehreen Nadeem Malik***

## Dedication

*I would like to dedicate this thesis to my brother, Omer Nadeem Malik, as he inspires me to be strong in every path of life and criticizes me to see me grow and face all the challenges in life to achieve every success that comes along. All that I have done is because of his unfailing love and support that gives me motivation to be better than who I am.*

## LIST OF TABLES

# LIST OF FIGURES

# LIST OF ACRONYMS

| | |
|---|---|
| AD | Alzheimer's disease |
| ADAM | A disintegrin and metalloproteinase |
| APLP | Amyloid precursor like protein |
| ApoE | Apolipoprotein E |
| APP | Amyloid precursor protein |
| Aβ | Beta amyloid |
| BACE | β-site APP converting enzyme |
| BBB | Blood brain barrier |
| BMI | Body mass index |
| cAMP | Cyclic adenosine monophosphate |
| CAT | Catalase |
| CDK | Cyclin dependent like kinase |
| CNS | Central nervous system |
| CREB | cAMP response element binding protein |
| CSF | Cerebrospinal fluid |
| FDA | Food and drug administration |
| GSH | Glutathione |
| GSK3β | Glycogen synthase kinase 3β |
| GST | Glutathione-S-transferase |
| HAT | Histone acetyltransferase |
| iGLURs | Ionotropic glutamate receptors |
| LDL | Low density lipoproteins |
| LPO | Lipid peroxidation |
| MDA | Malondialdehyde |

| | |
|---|---|
| MPO | Myeloperoxidase |
| MMP | Matrix metalloproteinase |
| MRI | Magnetic resonance imaging |
| NBM | Nucleus basalis of meynert |
| NFT | Neurofibrillary tangles |
| NGF | Nerve growth factor |
| NMDA | N-methyl D aspartate |
| PET | Positron emission tomography |
| PHF | Paired helical fragment |
| PP2A | Protein phosphatase 2A |
| PSEN | Presenilin |
| RNS | Reactive nitrogen species |
| ROS | Reactive oxygen species |
| sAPPα | Soluble amyloid precursor protein |
| SOD | Superoxide dismutase |
| TACE | Tumor necrosis factor alpha converting enzyme |

# Table of Contents

## Abstract

One of the initial pathological hallmarks of Alzheimer's disease is cognitive decline and memory loss by disruption of cholinergic neurons and oxidative brain damage. A postsynaptic muscarinic receptor blocker, scopolamine impairs cholinergic transmission and impairs cognition. Here, we observed the physiological processes underlying Saikosaponin b2's impact on memory deficits in mice that had been given scopolamine repeatedly. Scopolamine (1 mg/kg) administration for 15 days caused severe deficits in working and short-term memory in mice, as determined by the elevated plus maze, Morris water maze, and novel object recognition tests. However, scopolamine administered mice who were additionally given Saikosaponin b2 did not experience either deficit. This effect was associated with an increase in antioxidant enzymes (superoxide dismutase, Glutathione reductase, glutathione s transferase and catalase), followed by reduction in lipid peroxidation and myeloperoxidase activity.

**Keyword:** Oxidative stress, cognitive impairment, Saikosaponin b2, Scopolamine, neuronal damage

# Chapter 1

## 1. Introduction

The human brain is an intricate assembly of neural tissues. Anatomically, it is widely divided into the frontal, parietal, temporal, and occipital lobes (Ribas, 2010). Most of the time, the larger left and right hemispheres of the brain, also known as hemispheres, are similar to one another. However, some incredibly specialised sections of language, hearing, etc., differ geographically between the two sides (Hutsler & Galuske, 2003). Despite being covered by the skull and the cerebrospinal fluid (CSF), the human brain is frequently vulnerable to physical harm. In addition, it suffers from the degenerative process of its tissues, which causes several incapacitating illnesses including Alzheimer's, Parkinson's, and Huntington's disease. Cognitive impairment resulting to amnesia is a significant characteristic of degenerative disorders.

Alzheimer disease, also known as dementia, can be defined as the permanent gradual loss of one's memory caused due to the gradual demise of the brain's neural cells. Approximately, 10-15% cases of AD are genetic in origin. Where in all cases, the beta amyloid (Aβ) protein observes to form plaques while the Tau proteins form various tangles successfully disrupting the neuronal function of the brain. This disturbs neurotransmission, transport of nutrients and communication between neuron which leads to development of this disease. Dementia caused by Alzheimer's disease (AD), which often develops slowly and worsens over time in elderly persons (Burns & Iliffe, 2009). The prevalence of dementia patients has risen in recent years, which may be linked to an increase in average life expectancy worldwide. The World Health Organization (WHO 2019) estimates that there are over 50 million individuals worldwide who have dementia, and that there are roughly 10 million new cases each year. The frequency of persons with AD could account for 60–70% of all people, and

the number of people with dementia may rise to 82 million in 2030 and 152 million in 2050, according to estimates (Duthey 2013).

Alzheimer's disease AD has been characterized mainly as a nerve damaging ailment through definite structural abnormal changes in protein leading to this illness which is initially occurred by the irregular assembly of otherwise soluble proteins (Theleritis et al., 2017). Some of the factors, prominently evident in dissolvable neuronal protein modifications include mis-folding by virtue of genetic mutation, or through non modifiable factor such as aging that lead to loss of functionality of neural cells. Aloi Alzheimer, a German neuropathologist discovered AD as a nerve damaging disease while analyzing an old female, Auguste Deter who suffered memory loss, had difficulty in language comprehension, remained in a state of confusion and showed delusional behavior. As her postmortem results were studied, they revealed plaques and tangles in the cerebral cortex (Bachurin et al., 2018) that assured of this illness to be different from the obvious dementia. Further examination showed neuritic amyloid β (Aβ) plaques, similar to the dementia patients. Even though it's a rare cause, predisposition of PS1 gene abnormity classifies as a distinction in the beginning of the disease(dos Santos et al., 2018). It is necessary to have an understanding of the diverse pathology and etiology of this disease to have better approaches in prevention and therapy of this worldwide ailment. Objective of this research is to inhibit the oxidative stress in mice that could be related to the activation of parkin mediated mitophagy signaling pathway.

## 1.1 . Objectives

Alzheimer's disease, generally characterized by dementia has been proposed to increase three times by 2050 due to an escalation in life expectancy.

- Drug targeted treatment of AD associated mitochondrial dysfunction.
- To evaluate therapeutic efficacy of Saikosaponin B2 which can prove to be a novel potential drug.
- To underscore the precise pathophysiology of AD.

# Chapter 2

## 2. Literature Review

### 2.1. Memory impairment targets

To understand the diverse nature of AD's pathology, different features have been characterized and the mechanisms on the basis of which current and future medications have been generated that can help in treating the disease. Aβ plaques have been the primary cause of the disease but their removal or decreasing it has not been reported as an effective cure. Hence, tau targeting mechanisms have been in highlight. One of the classified features of this ailment is brain atrophy along with synaptic loss and neural damages. Through microscopic observations, presence of amyloid β plaques on the exterior of cells and neurofibrillary entangling has been reported. These two features, extracellular β amyloid deposits and intracellular neurofibrillary entangles are the primary pathophysiological conditions of AD.

Through histopathological findings, the extracellular clumps of Aβ plaques and internal entangled fibers of neurons containing phosphorylated tau. Primarily, Aβ plaques are formed at the basal, temporal and orbitofrontal neo-cortex parts of the brain and as the disease progresses it forms clumps on the further parts such as hippocampus, amygdala, diencephalon and basal ganglia (Cassano et al., 2019). In more serious patients it can even develop to mesencephalon affecting lower brain and cerebellar cortex as well. These aggregations of β amyloid plaques initiate entangling in the coeruleus and transentorhinal cortex of the brain. If it advances to worse conditions, it can even engulf hippocampus in the temporal lobe and neocortex (Goedert, 2015). Hence, Aβ and NFTs are the major contributors in the progression of Alzheimer's that have been studied.

## 2.2. Cognitive decline and dementia model

Amnesia can occur naturally for a variety of reasons, such as disease or trauma related brain injury. As sedative and hypnotic medication concentrations rise, they can also temporarily impair memory and produce amnesia. Drug injection is used to purposely generate this kind of amnesia, which is known as drug induced amnesia. Due to their use prior to surgical procedures, these medications are frequently referred to as "premedicants." Drugs have the power to block recollections of the days leading up to and following surgery (Walsh et al., 2011). Benzodiazepines like flunitrazepam, lorazepam, and midazolam are examples of amnesic substances (Riss et al., 2008). Anterograde amnesia is caused by these medications; retrograde amnesia is produced by combining medications such pethidine, diazepam, and hyoscine, although these combinations are frequently too depressant for routine clinical use. For research purposes, drugs like Physostigmine, Lorazepam, Clozapine, etc. are administered orally or through injections to animals to produce amnesic models (Li et al., 2007). Memory impairment is also caused by the use of NMDA receptor blockers like MK-801 (De Lima et al., 2005). Atropine, Propofol, and Scopolamine are further potent amnesic medications. Due to its ability to impair memory, scopolamine, muscarinic blocker, is frequently employed in drug testing for memory-enhancing substances.

## 2.3. Scopolamine

Scopolamine, an anticholinergic substance (anti-muscarinic), is a common medication used to impair cognition in both people and animals (Klinkenberg & Blokland, 2010). Alzheimer's disease (AD) individuals who have central muscarinic receptors blocked experience a more profound pattern of cognitive deterioration. A nonselective muscarinic antagonist, scopolamine is a tropane alkaloid medication that results in

"Cholinergic Amnesia." It is very responsive to muscarinic receptors. It has a molecular weight of 303.352905 Da and the chemical formula $C_{17}H_{21}NO_4$ (Falsafi et al., 2012). It has a 4.5-hour half-life. Bioavailability ranges from 10% to 50%. Secondary metabolites from plants in the Solanaceae (nightshade) family, including Jimson weed and henbane, are its source. Its transitory amnestic properties make it a popular paradigm for describing medications that improve cognition (Francis et al., 2006).

### 2.4. Pathophysiological pathways leading to AD

#### 2.4.1. Beta amyloid aggregation in Alzheimer's

These Aβ peptide, produced in result of breakage of amyloid precursor protein APP through β secretase enzymes BACE1 and y-secretases present in the plasma membrane lead to the formation of amyloid fibrils that are resistant to degradation. Aβ molds itself into oligomeric molecules that passes into the synaptic cleft and constrain the signaling pathways. Further polymerization forms these indissoluble amyloid fibrils into plaques which are capable of stimulating kinases that play their role in phosphorylating microtubule associated protein that accumulate into insoluble NFTs. These events of plaque formation and entangling initiation trigger microglia that surrounds these aggregates and initiate inflammation causing neurotoxicity in AD. There has been enormous amount of data supporting the role of Aβ as the initiator of the pathogenic disease but recent studies support the evidence that even though it plays major function in triggering early disease processing, it does not seem to be likely present in the later stages of the disease (Musiek & Holtzman, 2015).

### 2.4.2. Role of Human amyloid precursor protein

These proteins have similarities with the mammalian amyloid precursor proteins such as APLP1 and APLP2 and drosophila. APP is an important transmembrane protein that regulates cellular activities. Through enzymatic reactions, APP enables the breakdown of proteins resulting in the production of amyloidogenic fragments. The investigations have showed biological functions of APP involving cell life, cellular growth and transmission as well as neuritogenesis when the APP degrades and releases diffusible fragments (Tang et al., 2017). Understanding the nature of APP has become essential to study the changes occurring through introducing APP RNAi along with APP ectodomain intracerebral inoculations as they have showed better comprehension abilities and synaptic transmissions (Jo et al., 2020). Type 1 transmembrane glycoprotein is encoded by APP that can degrade either by nonamyloidogenic pathway which is considered non diseased condition or it can be cleaved by amyloidogenic pathways which is a diseased state. The cleavage which is possibly through alternate splicing, glycosylation, phosphorylation or diverse proteolysis that lead to the production of several polypeptides (Hefter et al., 2016). APP itself is composed of 770 amino acids where Aβ involves 28 of them along with 14 other residues of transmembrane part of APP. Under normal conditions, the cleaving within the Aβ area is achieved by α secretase which is the process known as shedding to produce secreted APPsα and a membrane bound C83 molecule which is further spliced by y-secretase producing extracellular fragment P3. On the other hand, under diseased condition, abnormal cleaving through β-secretase produces sAPPβ and a carboxy terminal fragment C99 that remains bound to the membrane. This is further cleaved by y-secretase that forms indiffusible Aβ peptide. У-secretase mediated cleavage of both C83 and C99 occurs in the transmembrane region releasing fragments into the

cytoplasm that diffuses and transfers to the nucleus to finally express gene(Chakraborty & Diwan, 2020).

#### 2.4.2.1. Non-amyloidogenic pathway

APP alternatively metabolizes through α- secretases at specific amino acid precipitates of the Aβ regions and releases soluble fragments which are not toxic. ADAM (a disintegrin and metalloproteinase) family of proteinases has the ability to play α-secretase like functions and in neuronal regions, ADAM10 and ADAM17 also termed as tumor necrosis factor –α converting enzyme TACE were abundantly found. APP processing through α and β secretases result in the production of soluble molecules of P3, that are actively involved in synaptic transmission however their exact role has not been determined yet. These α-secretases have been reported as beneficial for their neuroprotective excitation that helps in subcellular distribution of APP. Interestingly, sAPPα has an important role in synaptic signaling and adequate synaptic plasticity, learning, thinking, psychological aspects of emotions and conscious efforts and neural survival. As mentioned earlier it has the ability to release the fragments intracellularly that further moves into the nucleus where it expresses the gene through nuclear signaling and enhances the metabolic activities.

#### 2.4.2.2. Amyloidogenic pathway

Under diseased condition, APP cleaves in an abnormal manner by BACE-1 which is also termed as membrane bound aspartyl protease. Amyloidogenic pathway is the process of Aβ biogenesis where the β secretase breakdowns APP into soluble fragments β-APP and C-terminal β fragment C99 followed by cleavage from y-secretase enzyme (Tu et al., 2014). This y-secretase splicing produces toxic Aβ fragments. At first, β

secretase makes a break at the N terminal of Aβ and this step is considered as rate limiting reaction where it exterminates massive part of the protein and C terminal of APP remains that is cleaved again leading to the production of the Aβ oligomers, the most toxic form of Aβ peptide. As polymerization of these oligomers takes place, the toxic mass of plaques is formed.

Where non amyloidogenic pathway involves cleavage of APP by α-secretase that forms C83, which remains in the membrane and it also produces sAPPα which is released into the extracellular space. In amyloidogenic pathway, β secretase release sAPPβ into the extracellular medium by proteolysis and then forms C99 fragment that remains in the membrane where it is consequently cleaved by γ-secretase producing Aβ peptides.

The process of plaque formation involves the activity of two distinct types of Aβ polymers; Aβ40 and Aβ42. Where Aβ40 is present in excess compared to Aβ42 and is found to be less pathogenic. On the other hand, Aβ42 is in diffusible, severely neurotoxic and has more capability of producing Aβ aggregates with toxic contents. This aggregate formation is followed by blocked ion gates, abnormal calcium levels, enhanced mitochondrial oxidative stress and reduced energy for metabolism and glucose uptake, eventually resulting in neuronal death by the deprivation of these contributors.

### 2.5. Pathological changes in tau hypothesis

Before the onset of the symptoms many changes occur in the brain which include neutrophil cells and phosphorylated entangling. Tangling of nerve fibers can be observed by the help of Gallyas silver stain and even prior to the generation of tangling, there are many changes taking place through tau which can be differentiated from healthy brain. These changes are generally post translational modification such as

acetylation, N-glycosylation and hyperphosphorylation. These modified tau joining forms highly complexed clumps. This deposition begins in entorhinal cortex and hippocampus, from where it spreads to other parts of the brain (Murray et al., 2011). In upto 25% cases of AD, hippocampal sparing is the most common symptom along with limbic predominancy in a few cases (Crary et al., 2014). Other patients have pre AD tau pathological subtype where these people have limited deposition of tau and they do not exhibit any major impairment. This condition is generally termed as primary age related taupathy.

These complex patterns of aggregative tau polymers are heterogeneous in nature and its composition varies with disease and its stage. Normally, tau can either exist in the form of small oligonucleotides or as paired helical filaments (PHFs) and straight filaments. PHFs have different morphology than NFTs on other hand straight filaments are found to be more prevalent in Pick disease. The heterogenic composition of tau isoforms is different in all taupathies. In the CNS of adults, the neurons express six different forms of tau. These isoforms contain 3 or 4 amino acid repeat sequences in the microtubule associated region and also have differences in exclusion and inclusion of two amino terminal exons (Claeysen et al., 2012).

### 2.6. Important pathological mechanisms

Some important pathological mechanisms are as follows:

#### 2.6.1. Post translational modifications

Generally, tau is a protein in neurons which is responsible for stabilizing microtubules in the cytoskeleton. To prevent the formation of aggregates of tau, it is necessary to target following modifications to help the protein perform its activities in a normal

manner. These mechanisms occur in the prodromal phase so it is possible to prevent spread of the disease by introducing medication at an early phase.

### 2.6.2. Hyperphosphorylation

Tau is considered one of the main components in formation of NFTs related to AD. NFTs consist of tangles due to hyperphosphorylation of the microtubule binded protein. Also these NFTs are interconnected filaments containing paired and helical shaped protein filaments that help in the stabilization of microtubules. But hyperphosphorylation destabilizes the tubules resulting in the production of massive clumps of filaments. This hyperphosphorylation occurs when kinases react with protein and form oligomeric fragments. This is followed by the generation of more NFTs. Structurally, NFTs become straight, fibrillary and extremely non soluble in the neuronal cytoplasm which makes it difficult for the signals to transmit, resulting in abnormal manner of communication following neuronal cell death (Shukla et al., 2012).

Initially, several phosphorylation regions and kinases were reported however in AD the mechanism and sites of phosphorylation conforms as the diseases advances. It can even direct phosphorylation to other sites which enhances the availability of multiple epitopes leading to more structural changes. These phosphorylation events are different for all taupathies. Similarly, for familial taupathies, the pathological events make tau more prone to specific kinases.

Certain kinases are activated in the brain of patients suffering from AD because of either upregulation or dysregulation. For instance, histopathological reports from the post mortem of affected individuals showed that the amount of some kinases such as active cyclin dependent like kinase CDK 5, cyclin dependent kinase 5 activator 1 and p25 which is reduced form of CDK5 regulator, are elevated in the disease. In the same

manner, some of the kinases related to neurofibrillary pathology are over expressed in AD like active glycogen synthase kinase-3β (GSK3β). Since these kinases are capable of phosphorylating at distinct sites, it can be possible to identify the stages at which the activity is increased.

Furthermore, the activated kinases can also aggravate the neurodegeneration through various pathways. As in case of CDK5, it is also responsible for the deposition of Aβ, indirectly reducing nerve growth factor (NGF), increase in oxidative stress and stimulation of cell cycle repetitions. Upregulation of GSK3β lead to activation of inflammatory cells which is eventually followed by apoptic cell death and impairment of axonal channels. Through these studies it is shown that activated kinases have been contributing in the spread of the disease in several ways which can be aimed at while considering the treatment. As compared to kinases, phosphatases have been observed where protein phosphatase 2A (PP2A) is the one which is responsible for about 70% of the overall tau phosphatase activity. Studies and experimentation have shown that disruption in PP2A interactions with tau protein results in the promotion of AD (Wang & Mandelkow, 2012).

### 2.6.3. Acetylation

Tau protein in the individuals affected with AD and related taupathies is greatly acetylated than in the normal individuals. Similar to phosphorylation, tau acetylation can be gained via several mechanisms such as histone acetyltransferase p300 (p300 HAT), cAMP responsive element binding protein (CREB) binding protein, or auto acetylation. The disruption in the processes can lead to neurodegenerative state. Acetylation stimulates tau cleavage thereby rendering ubiquitin binding and preventing

tau movement. Hence tau remains in the cytoplasmic matrix becomes more capable of forming aggregates and thus its removal from the cell becomes challenging.

### 2.6.4. Carboxy terminal truncation

While observing the paired helical fragments PHFs which was obtained from the brains of AD affected patients, tau was seen to be reduced at carboxy terminal by caspase 3. This is similar to the condition of AD in which Aβ triggers caspase activation (Zhao et al., 2016). There are many sites present in tau for caspase cleavage in amino terminal such as (caspase 6) and at Asp314 (caspase 2). Similar to the other pathological events, truncation also hampers the binding of tau to microtubules and promotes the formation of tau aggregates, mitochondrial dysregulation and synaptic deficits (Jadhav et al., 2015).

### 2.6.5. O-GlcNAcylation and N-glycosylation

Recent studies have showed O glycosylation to play an active role in brain glucose metabolism as its impairment leads to the growth of AD. But other than all the post translational modifications, the O-GlcNAcylated proved to be beneficial in contrast to other tauopathies. The tissues from affected patients of AD demonstrated decreased amount of O-GlcNAcylated tau in comparison of healthy controls. On the other hand, N-glycosylation of tau protein which is supposed to trigger phosphorylation and cause physiological abnormalities is seen to be increased in patients with AD (Zhang et al., 2015).

### 2.6.6. Cytoskeletal dysfunction

Cytoskeletal dysfunction is important in stabilizing the structure by maintaining the microtubule symmetry and transport but disturbances of the microtubule associated tau binding domains can affect them. It is an important characteristic among the other neurodegenerative etiology. In AD, the neural fibers show a decrease in the length and amount of microtubules, with less acetylated tubulin which is an important indicator of stable microtubule. It also causes axonal swellings consisting of vesicles and organelles. In addition to this, tau involves the transport of kinesin and dynamin along the axon and reduces the movement of organelles and amyloid precursor protein (APP) (Vazquez, 2013).

### 2.6.7. Tau aggregate formation

Tau proteins combine together and polymerize as the concentration of cytosolic tau in increased because of post translational modifications and less availability of microtubules binding proteins. There are several kinds of tau multimers associated with AD which include the PHFs, linear filaments, helical ribbons and small molecule aggregations. These species have varied characteristics that occur as a result of abnormalities as well as changing mechanisms of post translational modification and the stimulators that enhance the occurrence of polymerizations. Collectively, it is observed in humans that tau aggregates and NFTs are associated with symptomatic conditions and the nerve cell death as compared to Aβ lesions (Shafiei et al., 2017).

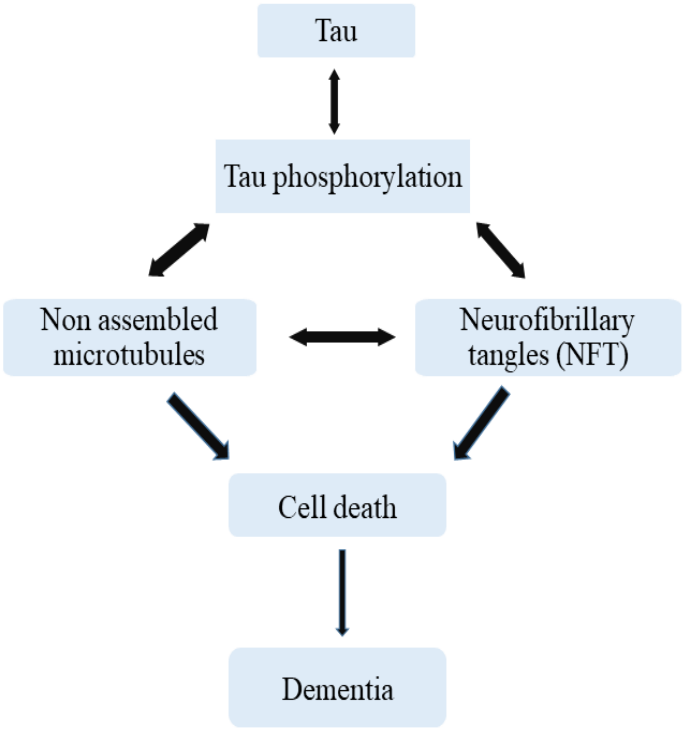



Figure 1: Tau aggregate formation and propagation

In this figure 1, tau protein aggregates in the neurofibrillary tangles are showed. NFTs are considered as a significant feature in the pathology of AD. This tau pathology spreads from one region to another through the neural network and plays major role in the progression of AD thereby gaining attention as a therapeutic target for AD.

It is suggested that large fibrils can cause cellular dysfunction by molecular crowding leading to changes in the activity of metabolism. The neurons without tangling showed elevated levels of synaptophysin mRNA and had more synapses in comparison to neurons with tangles. It has been observed through investigations that small soluble forms of tau are more toxic to cells than the mature filaments (Fagan et al., 2014). The amount of tau in the CSF of affected patients of AD is higher compared to the controls where it decreases when the disease advances to other stages. This can be supported by observing the reduced secretion of tau or formation of tau-tau aggregates into larger

forms with more stabilized microtubule structures in the neurons. We also know that tau levels are not elevated in people with non-AD tauopathies. All of these studies are important in targeting the main changes to find the treatment (Olsson et al., 2016).

One of the toxic species of tau is oligomeric tau which is in the brain at the primary stage of the disease. These oligomers of tau are more potent in causing toxicity than the tau filaments that lead to neurodegeneration and represent the cognitive phenotypes. Moreover, they are also responsible in the spread of the disease. For instance, the CSF tau levels are reduced with drug therapies which might be through directing extracellular tau at the progressive stages. In addition to these identified features of tau fragments by enzyme linked immunosorbent assays, more facts about tau can be discussed through mass spectroscopy. We have found that the drugs that can prevent formation of tau aggregates can help in the improvement of cellular condition and can stop further spread of the disease to other parts of brain (Cárdenas-Aguayo et al., 2014).

### 2.6.9. Impairment of proteolysis mechanism

Protein degradation is another factor that contributes to the pathology of this neurodegenerative disease through several processes including autophagy. We already know that activated kinases change the pattern of mechanistic target of rapamycin (mTOR) where the associated autophagic proteins are decreased in the affected brain. By observing the damaged neurites and cell organelles of AD suffering individuals, the vesicular formations are visible due to the disrupted autophagy pattern of fusion with the lysosomes. Autophagosomes consists of APP, presenilin and y-secretase which are affected when the autophagosomes are not able to perform their functions in neurons resulting in the elevated generation of beta amyloids which can reverse protein metabolism (Perluigi et al., 2021). Tau is one of the factors of autophagy so if there

occurs any delay in the processing then it could result in increased production of intracellular tau with further misfolding, altered protein formations.

In AD, ubiquitin proteasome pathway is also altered where ubiquinated proteins are formed enormously with tau proteins. Proteasome can affect the CREB pathway as the absorption of regulatory subunit of cAMP dependent protein kinase A (PKA) can lead to more phosphorylation of CREB. Another unique feature of tau pathology is that it remains constant once the aggregates are formed in the neurons as it inhibits the proteasome systems with autophagic degradation (Gong et al., 2016).

### 2.7. Cholinergic hypothesis

This hypothesis was introduced in the 1970s that showed cholinergic deficiency in the AD affected people and associated decrease in the acetylcholine transferase enzyme in the brains. Acetylcholine is responsible for learning and memory aspects of the brain which lead to finding ways that improve cholinergic activities therapeutically. However, cholinergic deficiency symptom is present at a later stage in neurodegenerative pathway. Cholinergic activity is enhanced when the cholinesterase inhibitors play their role by blocking cholinesterase enzyme that result in the breakdown of acetyl choline at synaptic cleft (Deger et al., 2015).

This cholinergic damage is found to be mostly presynaptic than post synaptic even if it is seen at the initial stages or in the latent phases of the disease. This can be explained by the cholinergic loss due to degeneration of nucleus basalis of meynert (NBM) due to innervation at the cortical part of the brain causing severe neurodegeneration. The alteration in nicotinic as well as muscarinic receptors is another effect of this cholinergic lesion where the nicotinic receptors are reduced and the muscarinic receptors become dysfunctional. Through experimentation on rat hippocampal areas,

with administration of cholinergic agonists and antagonists it confirmed the function of acetylcholine as a durable enhancer. So, the changes of synaptic physiology are through modifications in the spread of nicotinic and muscarinic receptor in AD. Different cases exhibit an overexpression of cortical choline acetyl-transferase in the neurons at the prodromal stage with Alzheimer's patients that can support deficiency of basal cholinergic neurons (Ramos-Rodriguez et al., 2013).

In addition to this, an increased amount of 7 nicotine gene expression is also observed in patients with AD as compared to the healthy controls of elderly people. Acetylcholine has several other functions which are associated with neuroplasticity, interconnectivity of neurons and their synchronization.

The cholinergic lesion of AD is multifactorial from damaging the synaptic transmission in the cortical and limbic regions to inducing neuroplasticity and altering the cerebral blood flow processing. The animal models showed that cholinergic deficits are responsible for tau aggregation and Aβ depositions that lead to the delayed responses of speech and learning. For instance, when the rodent models with AD were observed they showed enhanced deposition of beta amyloid and increased activity of tau phosphorylation in the hippocampal and cortical areas of the forebrain where these features are correlated with the lesions in cholinergic neurons (Bergmans & De Strooper, 2010).

### 2.8. Excitotoxicity

It involves the neurotransmitter glutamate which is present abundantly in the central nervous system. N-methyl-D-aspartate (NMDA) which is a receptor gated by the neurotransmitter glutamate gets over stimulated in excitotoxicity. It is important to discuss this factor as it is majorly involved in the progression of AD. The neuronal loss

by cholinergic system is mediated by this aspect which leads to the movement of excessive calcium into the cells.

### 2.8.1. Glutamate excitotoxicity and associated NMDAR function in AD

Glutamate and its receptors are actively involved in neurotransmission specifically ligand-gated ionotropic glutamate receptors (iGluRs). Their key role is found in neuro plasticity affecting the molecular pathway of acquiring and memory. While the neurotransmission occurs, any alteration in the processing through the glutamate mediated receptors such as iGluRs, can lead to many neurological diseases and disorders such as epilepsy, Parkinson's and Alzheimer's disease. Collectively, iGluRs is an essential factor in causing the disease so it is important to direct therapeutic strategies on it.

If there is not proper synaptic NMDAR transmission occurring, then it severely affects the neuron survival. It is followed by excitotoxicity which involves neuron death, nerve cells are destroyed or any injury to the brain, nerve or spine that could result into a stroke. Several observations have been reported about the presence of excitotoxic glutamate activity in late slow responding neurodegeneration. This mechanism basically involves the entry of uncontrolled $Ca^{2+}$ ions mainly by the NMDA receptors. This is because NMDAR are supposedly more permeable to calcium ions than other receptors such as iGluRs (Morales et al., 2014).

This occurs when the $Mg^{2+}$ channels are not activated through depolarization of the postsynaptic membrane and the NMDA receptors are stimulated in a chronic manner allowing the movement of $Ca^{2+}$ into the nerve cell. This excessive inflow on calcium results in the disruption of cellular function and eventually destroys the neuron. This explains the associated impaired memory and learning abilities that are major

symptoms involved with the neurodegenerative diseases and the patterns of identification in examining the neural anatomy of AD patients. For this factor, the proposed treatment via clinical trial was memantine, a NMDAR antagonist which is proved to be beneficial in regards with symptomatology and neuroprotective for AD.

So, NMDARs are responsible in the neuronal cell damage so in order to prevent this it is important to maintain the NMDAR transmission for cell survival. Glutamate accessibility and the alterations in NMDAR signaling are the primary factors involved in AD progression. As glutamate and NMDAR are conversely related, it is important to control glutamate uptake. But this can be badly disturbed in AD patients. This has been provided by the evidence seen in the report of AD patient that glutamate uptake was dysregulated with a decrease in protein expression and specific loss of vesicular glutamate transporter. Other factors that are involved in glutamate uptake process include toxic Aβ proteins as they were examined in various neuronal cell cultures where they showed Aβ peptides to be associated with glutamate increased availability. In this way, excitotoxicity is induced causing neurodegeneration.

Also, the duration of presynaptic signal is associated with glutamate supply. Synaptophysin, syntaxin and synaptotagmin are some of the presynaptic proteins which are under expressed in the presence of Aβ. Their reduction results in the disrupted neurotransmitter signaling. Recent studies show that if there is a deficiency in deliverance of presynaptic vesicle then there will be less glutamate present which would result in less excititoxicity process. Even though, it is not actively involved, it is constant with the spread of the disease with the degenerating neurons.

Thus it is inferred that specific types of Aβ are effective in increasing NMDAR associated synaptic transmissions and stimulation of toxicity. These factors can be prevented by using NMDAR antagonists. Altogether, in AD the pathological changes

due to Aβ also involve this aspect where glutamate accessibility and its receptors are relatively causing neurotoxicity and increasing the progression of this neurodegenerative disease (Jang et al., 2014).

### 2.9. Neuroinflammation

It is an inflammatory reaction which is carried out by the aggregation of glial cells in the CNS. It is also an essential factor in the pathophysiology of AD. Earlier the brain was not considered to be involved by the immune cells but many studies have now proposed the mechanisms of anti-inflammatory drugs that are directed onto the neuro-inflammatory cascades. This could contribute majorly in the treatment of AD.

Furthermore, chronic inflammation is associated with hampering the processes that remove the mutated or altered proteins in elderly people's brains. These abnormal proteins project impairment of axonal activities and aggregation of amyloid precursor protein (APP), production of PHFs and disruption in synaptic transmission. Matrix metalloproteinases (MMPs) are mostly the stimulators of inflammatory responses and are related to AD pathology. The inflammatory regulators or the abnormal proteins consisting of aggregated peptides activate the MMPs. These MMPs are zinc and calcium dependent endopeptidases. If they excessively start activating MMPs then it results in major disorders. It is possible to treat this at the start of the disease as when the symptoms appear the treatment doesn't work properly in the prevention of the disease.

## 2.10. Other causative factors leading to AD

### 2.10.1. Vascular disease

Vascular disease is also considered an important factor in AD pathogenesis. The environmental factors such as the modifiable risk factors involving high BMI, smoking, hypercholesterolemia and hypertension are responsible in the development of AD. Apart from vascular lesions being common, hypertension is also dependent as one of the main components in the formations of NFTs, which is the important causative characteristic of AD. This is triggered with hypertension. Collectively it is reported that vascular disease can target the increased productions of amyloid aggregates or NFTs. In order to reduce the integrity of the disease, it is necessary to limit the risk factors which might induce vascular disease so that the level of cognitive behavior can be slowed in the patients affected by AD.

### 2.10.2. Diabetes and hyperinsulinaemia

Diabetes and AD are correlated where insulin metabolism plays a significant role in the development of this disease. Insulin has the ability to move across the blood brain barrier and it is also produced in the CNS. Some there is evidence that insulin present in CNS is responsible for managing tau phosphorylation and preventing Aβ aggregations through insulin degrading enzyme. In some studies, less amount of insulin was present in patients with AD. The primary mechanism involves the peripheral hyperinsulinaemia which downregulates the movement of insulin across the blood brain barrier. This decreases the production of insulin degrading enzyme in the brain that arbitrates amyloid formations. NFTs and tau aggregations are also affected by reduced insulin transmission as the activity of glycogen synthase kinase GSK3 is increased.

Several studies have proposed the idea of utilizing the medicines that are used in diabetes to treat the pathophysiology of AD.

### 2.10.3. Apolipoprotein gene

The AD pathogenesis is also affected by non-modifiable risk factors involving the genetic factors. In AD, the Apo epsilon 4 (Apoe4) allele is used for the cholesterol metabolism and codes for lipid transport. If an individual has one copy of e4 allele then there is three time more probability of developing AD. On the other hand, those individuals who have two alleles have less chances of developing AD. This also assists in the deposition of Aβ which influence the progression of neuronal death thereby increasing the pathology of AD.

## 2.11. Pharmacological Treatments for Alzheimer's disease

Precise diagnosis is needed for Alzheimer's disease and also appropriate etiological and pathophysiological analysis is necessary for its treatment because it is neurodegenerative disorder having correlation with the age of individual. In order to ameliorate this public health problem therapeutic analysis are conducted for reduced progression but therapies are not the solution to problem so need prevention.

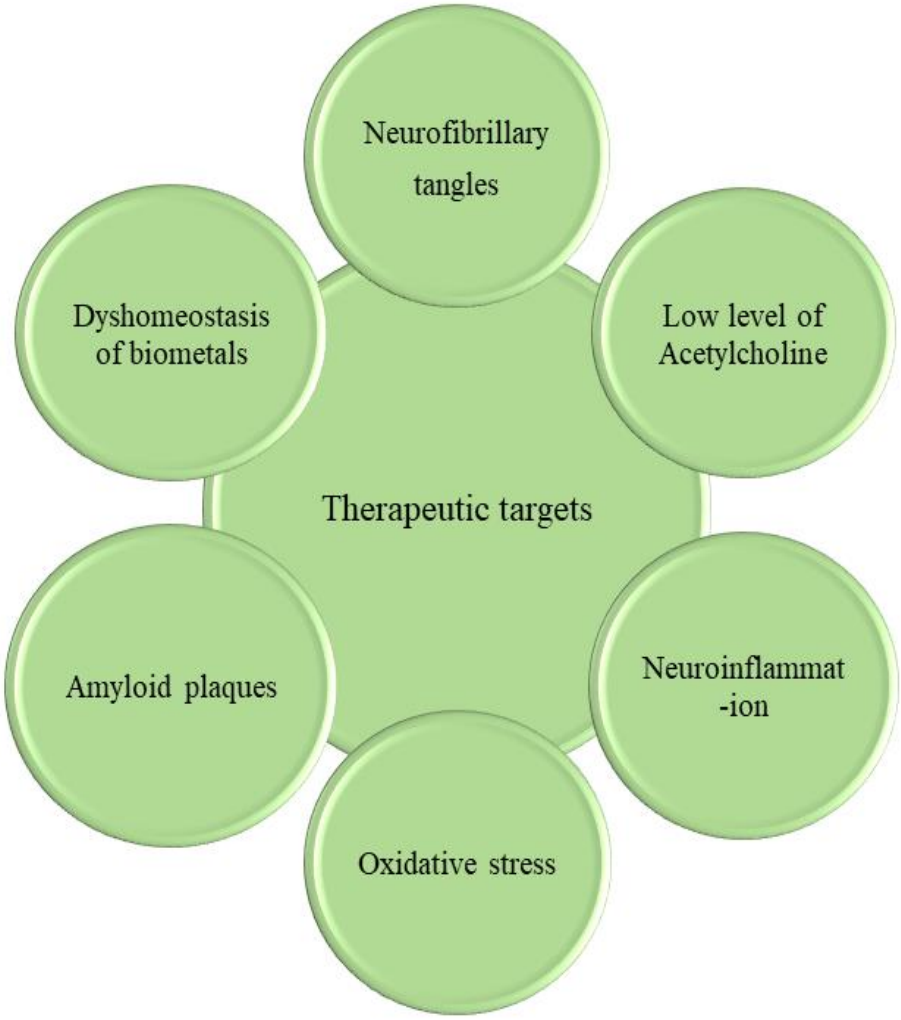



Figure 2: Therapeutic targets in the pathogenesis of AD

This figure typically represents the common targets that affect the pathological features of the disease. It is important to develop new pharmacological treatments regarding these targeted approaches. It has been seen that Ab in the brain are responsible for its progression so those peptides are the target of drugs. Even so specific enzyme and ligands are considered as the target such as the enzyme, cholinesterase, and the ligand named glutamate antagonist helping in relief of symptoms but such dementia associated with more complexity as seen in the studies. Treatment which are based on etiological analysis are still under consideration on the other hand preventive treatment are also studies such as by improving the diet, providing stimulation of concentration and thinking by making small groups.

### 2.11.1. Saikosaponin b2

The main bioactive ingredients of Bupleurum falcatum (Umbelliferae) have been widely used to treat a wide range of diseases. They have a variety of known pharmacological actions (Li et al., 2018). Among those whose chemical compositions have undergone extensive research and are often used in therapies are saikosaponins A, B2, C, and D. (Kuntzen et al., 2008). Saikosaponin B2 (SSB2, Fig. 1) has been studied as a combination therapy for cancer, hepatitis C, and renal fibrosis, but nothing is known about its effects on Alzheimer's disease (Zhao et al., 2019; Lee et al., 2019).

Pentacyclic triterpene saponins known as saikosaponins are predicted to have some pharmacological properties similar to those of steroids. Saikosaponins have been shown to have anti-inflammatory, anti-allergic, immunomodulatory, antiviral, and anticancer properties in numerous scientific studies.

It is understood that SSb2 performs a number of biological processes. According to a study, SSb2 can operate as a natural antagonist of the hepatitis C virus by blocking viral entry/fusion neutralization and inhibiting viral attachment. By triggering apoptosis, SSb2 can also operate as a sensitizer to etoposide-induced cell death in melanoma cells. However, nothing is known about how SSB2 works to reduce inflammation. In LPS-induced macrophages, SSb2's anti-inflammatory activity was discovered for the first time. IKK/IB/NF-B signaling activations and the ensuing rises in the pro-inflammatory chemicals NO, PGE2, and cytokines were both reduced by SSb2.

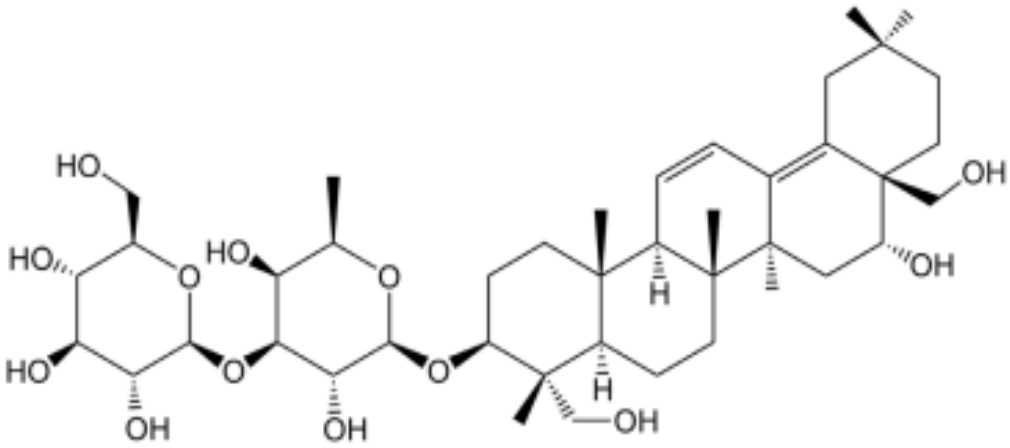



Figure 3: Chemical Structure of SSb2

### 2.11.2. Symptomatic Treatment of Alzheimer's disease

#### 2.11.2.1. Acetylcholinesterase Inhibitors

Acetylcholine is responsible for the mediating learning as well as memory. When Aβ and cholinergic system interact, that results into negative feedback loop. As Ab accumulates at abnormal level and reduces cholinergic transmission effectiveness that also focus on the receptors of alpha-7 nicotinic acetylcholine. In 1976, two Davies and Maloney proposed revealed that the Alzheimer's disease can be treated with cholinesterase inhibitors.

Tacrine, donepezil, rivastigmine, galantamine, xanthostigmine, para-aminobenzoic acid, coumarin, flavonoid, and pyroloisoxazole analogues are only a few of the medications that have been created to treat this disease. Drugs like rivastigmine, denopezil, and galantamine are essential for producing the most acetylcholine, which ultimately promotes the cholinergic function of the brain. It inhibits an enzyme named as acetycholinesterase that functions as the degradation of neurotransmitter. Acetylcholinestrase inhibitors seems well tolerated but tacrine not. There adverse effects depend upon the dosage. Another acetylcholine inhibitor named as ladostigil

(TV3326) passing through a clinical trial. It is also responsible to produce antidepressant effects. It directly inhibits the monoamine oxidases A and B.

#### 2.11.2.2. N-Methyl-D-aspartate Receptor (NMDA) Antagonist

Glutamate-mediated excitotoxicity lead to accumulation of calcium to abnormal levels as well as results mitochondrial dysfunction. It also increases the nitiric oxide generation. It produces oxidants at higher levels and neuronal apoptosis occurs in the result. In 2003, Food and drug administration (FDA) proposed that Alzheimer's disease can be treated by memantine. It can block the receptor "NMDA". It protects the neurons in order to attenuate tau phosphorylation. That leads by decrease in glycogen synthase kinase 3β (GSK - 3β) activity. AD can be treated by administrating the receptor "NMDA" alone as well as it can also be treated in combination with acetylcholinesterase inhibitor. Muscarinic and Nicotinic acetyl cholinesterase receptors are also a target in the therapy of AD. However, clinical experiments with EVP-6124, which is still in testing, were able to overcome the sensitivity of their agonists. Numerous neurotransmitter networks are considered, including the Hippocampus for the cholinergic hypothesis and the involvement of NMDA glutamate in AD. Similar to this, increasing acetylcholine release can be a potential treatment for mild Alzheimer's disease (AD) by targeting serotonin receptors, which are involved in memory and specifically the 5-HT6 serotonin receptors. In the brain's cognition-related regions, histamine receptors like the H3 receptor are present, and their antagonists can be employed to enhance cholinergic neurotransmission.

### 2.11.2.3. Etiology-Based Treatment

Genetic factor for sporadic AD is defect in the ApoE $\varepsilon 4$. This factor account for second most perilous as first one is the age factor. Amyloid cascade is the basis for its treatment in which secretase, amyloid and kinases are targeted so carried out the hyperphosphorylation of tau protein.

### 2.11.2.4. Secretase Inhibitors

APP is first cleaved either by $\alpha$- secretase or by $\beta$-secretase enzymes, and the resulting fragments are processed by $\gamma$-secretase. The proposal of the "over-activation" of $\beta$- and $\gamma$-secretases, or age-related decreased $\alpha$- secretase processing, has led to the use of inhibitors for this amyloidogenic pathway. Anticipation of A$\beta$ have been studied in relation with the upregulation of secretase and another disintegrin unit named metalloproteinase 10 (ADAM10) and this approach is considered better for prevention of AD. There is another substance named melatonin has been studies which regulate the $\alpha$-secretase activity as it stimulates the processing of non-amyloidogenic by transcriptional regulation of ADAM10 and ADAM17 and also by the stimulation of agonist, serotonin 5-HT4. BACE1 has crucial role in the metabolism of myelination proteins and inhibition of it demonstrate less sever effect as compare to ADAM proteases. Aspartyl protease BACE1 approach is also studied and seen that its inhibitor offers the molecular docking mechanism but this trial has not been approved for unsuccessful results.

Few substances are being investigate included MerckSharp&Dohme'sMK-8931 (Verubecestat) and Eli Lilly/Astra-Zeneca's AZD3293 (LY3314814), in phase II/III trials NCT01739348 and NCT02245737, respectively. Myricetin and quercetin,

essential Flavonols and flavones demonstrate the effective inhibitory effects for BACE1.

$\gamma$-secretase consists of, presenilin 1, nicastrin, anterior pharynx defective-1 (APH-1), and presenilin 1 enhancer-2 (PEN- 2), so a transmembrane protease enzyme entail many signaling proteins reside inside the membrane. It has been studied that $\gamma$-secretase inhibitors induce gastrointestinal disorder and skin cancer.

One of its compound is Notch protein regulates the cell differentiation cell, cell progression and maturation. Inhibitor for $\gamma$-secretase has been designed in a study but not effective results been seen. Activation of promotors of ADAM protease can be initiated by several antioxidants which can be accomplished by the dietary changes. In order to prevent A$\beta$, activity of $\gamma$-secretase before the $\beta$-secretase is needed. And any defect in $\gamma$-secretase (PSEN-1 and PSEN-2) considered as the risk factor for AD. Due to the inhibition $\gamma$-secretase trail was not successful but its modulation shows better results.

There are other signaling pathways which are involve in the toxicity of $\gamma$-secretase inhibitors. BDNF axonal trafficking and signaling is affected by the $\gamma$- secretase inhibitor. But the $\gamma$-secretase modulator affects the A$\beta$ cleaving sites and not the complete complex of cleaving region.

Hypercholesterolemia is another risk factor in secretase activity as many steroids which are acidic in nature are act as $\gamma$-secretase modulators and reduced the A$\beta$42 for example cholestenoic acid is act as effective endogenous modulators. Those endogenous metabolites have relation with obesity induced AD.

#### 2.11.2.4. Amyloid Binders

Modifications can be used as a treatment for AD. Formation of A$\beta$ extracellular neurotic plaques can be prevented as displayed by the evidence of correlation between the A$\beta$ markers and cognitive deficits. Various inhibitors of A$\beta$ aggregation have been considered for clinical trial. Amyloid-$\beta$-directed immunotherapy can also be used as an efficient treatment for AD with Microglia-mediated clearance such as Bapineuzumab, Gantenerumab, Crenezumab and BAN2401 currently in clinical trials. However, immunization again AD may have side effects such as neuron-inflammation but this can be reduced by using anti-inflammatory agents in AD treatment.

#### 2.11.2.5. Anti-A$\beta$ Aggregation Compounds

Recent studies focus on the prevention of A$\beta$ peptide formation. Inhibitors molecules such as Tramiprosate, Clioquinol and scylloinositol in clinical trial as anti- A$\beta$ aggregation compounds that have successfully stabilized the A$\beta$ monomers. However, they have important side effects. Azetidine-2-carboxylic acid, 3-phenyl azetidine-2-carboxylic acid and $\beta$-proline are synthetic beta-sheet breaker peptides that can prevent the damage caused by the inhibitor agents by inhibiting fibril formation. The have successfully improved spatial memory. Furthermore, Stemazol curcumin, T718MA, and SK-P C-B70M can also protect the SH-SY5Y cells and other neurons from the inhibitor toxic side effects.

#### 2.11.2.6. Tau Therapies

The paired, helically twisted filaments of hyper phosphorylated tau are prevented from aggregation into tangles in the Tau therapy. Immunotherapy such as AADvac1 vaccine has been developed and is in trail. Cyclic dependent kinase 5 (CDK5) is involved in

hyper phosphorylation of Tau proteins and has been considered a potential target for therapy while Inhibitors of the phosphorylation of tau proteins such as tideglusib, has shown no benefits. Similarly, Methylene blue and its metabolites has shown significant degradation of proteins and has caused inhibition of caspase-1 and caspase-3 activity. Leucomethylthioninium with a counter ion such as LMTX and methylthioninium chloride or MTC has inhibited Tau aggregation and slowed down the progression of AD as shown in mouse model. Other inhibitor includes N-phenyl amines, phenylthiazolyl-hydrazides, rhodanines and phenothiazine.

#### 2.11.2.7. Other Therapies

Alzheimer disease is significantly correlated with chronic degenerative disorders. Various therapies are needed to overcome this. In one therapy, intranasal insulin is used as a treatment for AD as it can easily penetrate the blood brain barrier. Similarly, Statin can also be used as a treatment which LDL can increase the risk of development of disease. Other diseases such as Dyslipidemia, obesity, hyperglycemia, insulin resistance and hypertension are also linked with AD. Statin can reduce cholesterol which affects the receptor signaling neurotransmissions in the diabetic brain thus its use can be done in the initial stage. Drugs such as Amylin and glucagon-like peptide-1 receptor agonist have shown potential for the treatment of AD. Similarly, drugs for the treatment of type 2 diabetes can also be used for the treatment of AD as they have neuroprotective effect. Treatment of AD with reactive oxygen species (ROS) and reactive nitrogen species (RNS) is also considered. The mitochondrial cascade hypothesis creates an imbalance by overproducing the free radicles along with the use of immune cells and NO signaling. Anti-inflammatory drugs are also considered in this treatment. Thus prevention of AD can also prevent obesity and diabetes. Alternative

remedies such as coenzyme Q10, Coral Calcium and omega-3 fatty acids can also be used for the prevention of AD. But no enough research is done on their preventive role.

# Chapter 3

## 3. Material and methodology

### 3.1. Animals

Male balb/c mice having age of 6-7 weeks and weight of 25-30g were used in the current study. All animal activities were carried out in the pathogen free zone of National University of Science and Technology, Islamabad, following procedures outlined in guideline for the care and use of laboratory animals. Approval of the protocols was obtained from the Internal Review Board (IRB) code (Approval no. 04-2021-02/36), Atta-Ur-Rahman School of Applied Biosciences.

### 3.2. Experimental design

Animals were housed in stainless steel cages at standard conditions of 23 ± 1°C and 12h light/dark cycle. Animals were acclimatized for one week before study and fed with standard diet and water. During this study, animals were randomly and double blindly assigned to four groups, each group comprising of 5 mice to avoid experimental biasness such as (1) control group treated with normal saline; (2) Diseased group injected with scopolamine dissolved in 0.9% NaCl; (3) Treatment group was administrated with Saikosaponin b2 (5mg/kg) dissolved in 0.9% NaCl and (4) Standard group was injected 5mg/kg Donepezil. All the mice received scopolamine (1mg/kg) once daily for 15 days except for the Control group.

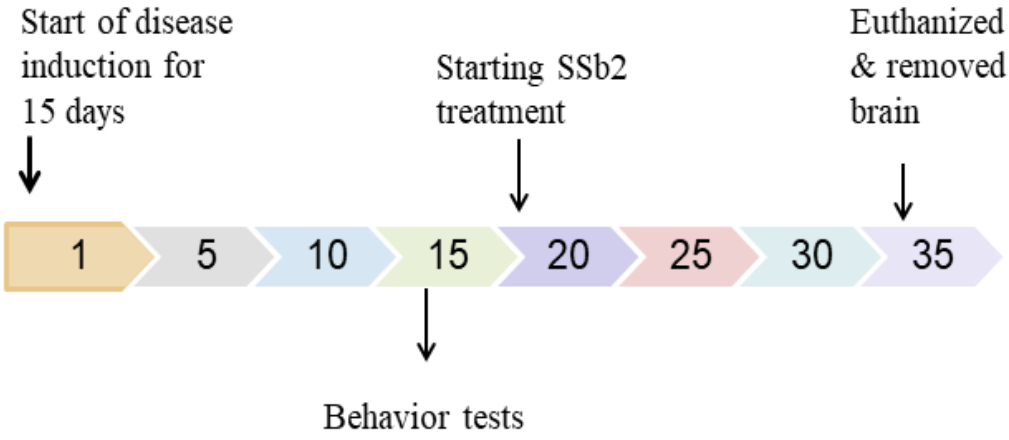



Figure 4: Study plan for the development of Alzheimer's Mice model

### 3.2.1. Handling of mice under experiment

The mice were held with utmost care from the base of the tails at all times. Mice were retained in sound environment where there were no abrupt movements. The intraperitoneal injections were given through insulin syringes. The mice's weights were measured daily and scopolamine dose of 1mg/kg dissolved in normal saline was administered intraperitoneally every day for 15 days. The mice were held meticulously in order to restrain it by positioning its tail into fingers. The needle was then injected, by slowly inserting the chemical. After successfully transmitting the material, the mice were observed for any reactions.

### 3.3. Behavioral analysis

#### 3.3.1. Morris water maze test

Morris water maze (MWM) test is a test used to access spatial learning and memory. The experimental mice subjected to this test can be assessed with a few alterations. For five days, the mice underwent five trials per day for a minimum of 10 min inter-trial interval. For every trial, the starting point was selected according to the table 1. The mice had to locate the platform submerged in the southeast quadrant of water maze tank before the 90 sec cut off. It was allowed to rest on it for 5 sec. If mice couldn't locate the platform, they were manually guided towards it and had to remain on it for 20 sec. The escape latency for each trial was recorded and analyzed. On $6^{th}$ day, probe trial was conducted where the platform was removed. The time spent in the target quadrant was recorded.

**Table 1:** Direction of release for Morris Water Maze Test

| No of Days | Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 |
|---|---|---|---|---|---|
| Day 1 | West | South | North | East | South |
| Day 2 | North | West | East | West | South |
| Day 3 | North | East | West | South | North |
| Day 4 | East | South | West | East | North |
| Day 5 | West | South | North | East | South |
| Day 6 | Single Trial Without Platform<br>Release Direction, West. | | | | |

Figure 5: Morris water maze tank filled with opaque water divided into four quadrants.

### 3.3.2. Elevated Plus Maze test

The elevated plus maze test was executed to investigate the anxiety level of mice group under study. (Walf and Frye, 2007). The apparatus of maze constituted of two open and two closed arms, perpendicular to each other. Primarily, the mice were positioned at the center of the maze for a single trial and allowed to explore the maze for a duration of 5 min. The trial was video recorded and the data extracted was for the determination of time spent in open and closed arms.

### 3.3.3. Novel Object Recognition test

The novel object recognition test was carried out in accordance with the instructions provided by (Farhat et al., 2017). The experiment was carried out using an equipment that was a 40 by 40 by 40 cm open field box. The mice spent five minutes in the open field cage during the habituation phase. After that, the mice were allowed to investigate

two identical objects for ten minutes during the familiarisation phase. The mice underwent training phase after a 30-minute break between trials. The mice spent another 10 minutes exploring the open field box during this phase after one of the objects was substituted with novel object. The amount of time the mice spent sniffing and engaging with the objects was observed. The index of discrimination was computed by the following formula:

$$DI=\frac{TN}{TF+TN}\times 100$$

The discrimination index is defined as the mice curiosity towards the novel object, where TN is the time spent with the novel object and TF is the time spent with the familiar object.

**Table 2**: Novel Object Recognition test. Comparison of all groups in familiarization and training phase.

| Treatment / dose (mg) | Familiarization Phase | | Training Phase | | DI% |
|---|---|---|---|---|---|
| | Identical Object | Identical object | Familiar Object | Novel Object | |
| | A | B | A' | N' | |
| Control | 48.25±7.292 | 51.7±4.384 | 48.2±15.700 | 52.25±12.735 | 26.35±0.11 |
| Scop | 46.75±7.395 | 55.1±9.475 | 27.5±14.585 | 33.7±2.687 | 6.607±0.11 |
| SSb2 | 24.5±10.61 | 32.5±12.757 | 17.5±13.219 | 33.20±11.03 | 25.35±0.12 |
| DPZ | 22.1±0.707 | 22.25±6.609 | 13±6.964 | 19.60±3.111 | 9.599±0.18 |

### 3.4. Harvesting Brain Tissue

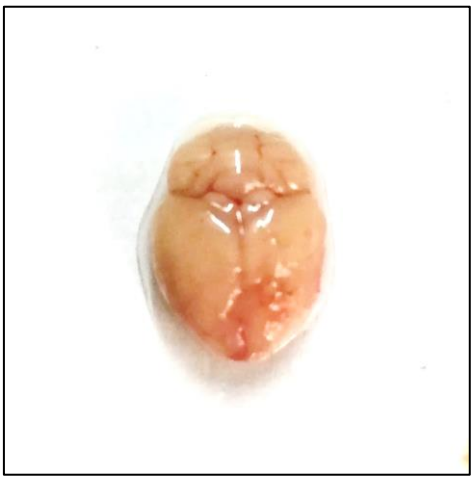


Figure 6: Dorsal view of whole brain harvested from mice

### 3.5. Histopathological Analysis of Brain tissues

#### 3.5.1. Hematoxylin and Eosin staining

After mice were euthanized, brain samples were stored in ice cold 4% paraformaldehyde and stained for slide preparation. Further, organ embedded paraffin plates were prepared from Ali pathology lab, Islamabad. Slides were photographed using a light microscope with a 100x magnification.

#### 3.5.2. Preparing Tissue Homogenate

We prepared the homogenate using the brain samples that had been frozen at -80 degrees. Then, brain samples were thawed and put through a tissue homogenizer at 1000 rpm for two minutes to create the homogenate. Supernatants were obtained after homogenates were centrifuged at 8000 g for 10 minutes at 4 °C.

### 3.6. Biochemical assessment

#### 3.6.1. Determination of anti-oxidant levels

Brain tissue homogenates were analyzed for antioxidant activity. GSH, GST, SOD and Catalase are the oxidative stress factors that were assessed. To evaluate the internal defenses, antioxidant enzymes levels were examined against the oxidative stress predominantly in the hippocampus and prefrontal cortex of the brain.

Catalase activity was calculated at 340 nm, measured by the estimation of decomposition rate of $H_2O_2$ followed by the incorporation of supernatant from the sample, according to the conventional technique described by Aebi et al. Likewise, SOD assay was conducted by the method described by Lowry et al, which was measured at 450 nm, by combining 20 µl of sample in 50 mM Tris-EDTA buffer and 100µl pyrogallol to form the ultimate volume of 200µl. GST activity was assessed by (Warholm et al., 1985) procedure where, 2,4- dinitrochlorobenzene (CDNB) was taken as the substrate. In the reaction mixture of reduced glutathione and supernatant, CDNB was added and absorbance was estimated at 450nm by microplate reader. Additionally, the reduced glutathione (GSH) activity was estimated by Ellman's method (Ellman, 1959). The absorbance was measured at 450 nm and expressed as per µM.

#### 3.6.2. Determination of lipid peroxidation

Thiobarbituric acid reactive substances (TBARS) were quantified in tissue homogenates of brain samples as an essential part of the formation of ROS, and lipid peroxidation (LPO) was estimated. LPO levels in the sample were measured using a microplate reader. At 535 nm, absorbance was quantified and represented as µM of LPO.

### 3.6.3. Estimation of myeloperoxidase for neutrophilic infiltration

To assess Saikosponin b2's inhibitory impact on scopolamine-induced neutrophil infiltration, MPO assay was carried out. The absorbance was measured at 450 nm, and it was calculated using the Ohkwa et al CTAB's and o dianiside technique.

## 3.7. Statistical Analysis

For statistical analysis, Graph Pad Prism software (version 9.0) was used. Following a One Way ANOVA, the Bonferroni Multiple Comparison test was used to assess the data. P values under 0.05 were regarded as significant. Data were presented as mean SD.

# Chapter 4

## 4. Results

### 4.1. Neurobehavioral Assessment

#### 4.1.1. Effect of Saikosaponin b2 on Morris Water Maze Test

Assessment of cognitive and behavioral functioning was done using this test. It was investigated how Saikosaponin b2 affected spatial learning and reference memory. The development of an escape response to the hidden platform is the product of repeated training.

The typical escape latency of mice to reach a concealed platform is a clear indicator of Saikosaponin b2's impact on spatial memory. All of the groups improved during acquisition, with the control and Saikosaponin b2 groups regularly finding the platform at almost 20 seconds on days four and five. Scopolamine-induced cognitive impairment (Scop) model animals learn and remember spatial information more slowly than control mice, with escape latency of 39 seconds on day 4 and nearly 26 seconds on day 5. On days 4 and 5, the scopolamine + Saikosaponin b2 (Scop + Ssb2) group demonstrated better spatial memory with a latency time of almost 10 seconds.

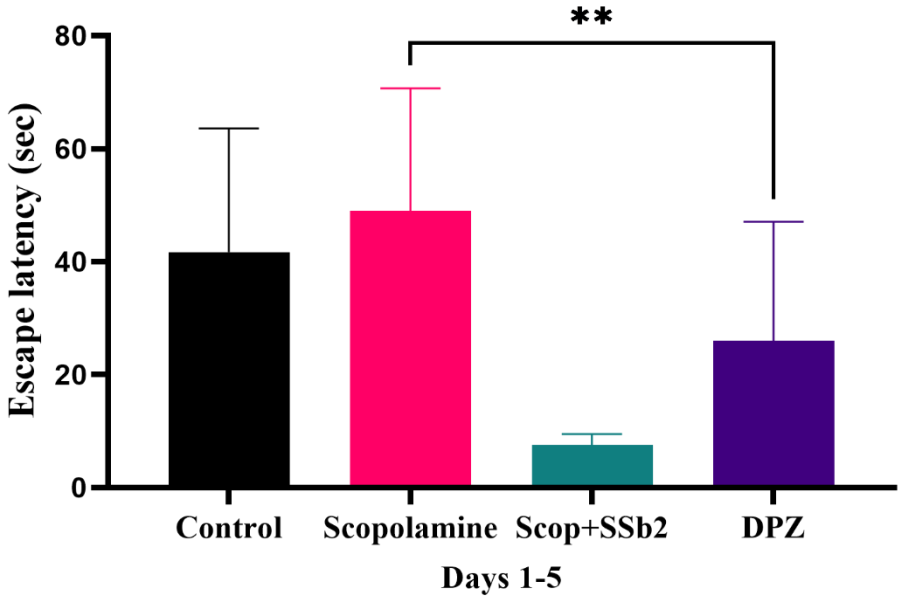



Figure 7: Using the Morris water maze (MWM) test, to determine the impact of Saikosponin b2 on learning and memory. The graph compares the escape latencies (in seconds) between the groups for Control, Scopolamine (Scop), Scopolamine and Saikosaponin b2 (Scop + SSb2), and Donepezil. Mice given scopolamine acquire and forget spatial information more slowly than control group. According to the results, the scopolamine + Saikosaponin b2 group locates the place far more quickly than the scopolamine group. Error bars depict mean ± SEM; n=05

A probing trial was conducted on day six to evaluate reference memory. To check the exploration duration for the previously placed concealed platform, time spent in the objective quadrant was recorded. As they spent less time in the target quadrant than the control (41.68±9.815) and Saikosaponin b2 (7.544±0.8617) groups, the scopolamine group (30.00±2.500) demonstrated a substantial ($p<0.001$) reduction in reference memory. The scop + SSb2 group, on the other hand, spent considerably more time ($p<0.01$) in the goal quadrant than the scopolamine group (49.02±9.694), demonstrating that Saikosaponin b2 overcame the amnesic impact of scopolamine and developed reference memory.

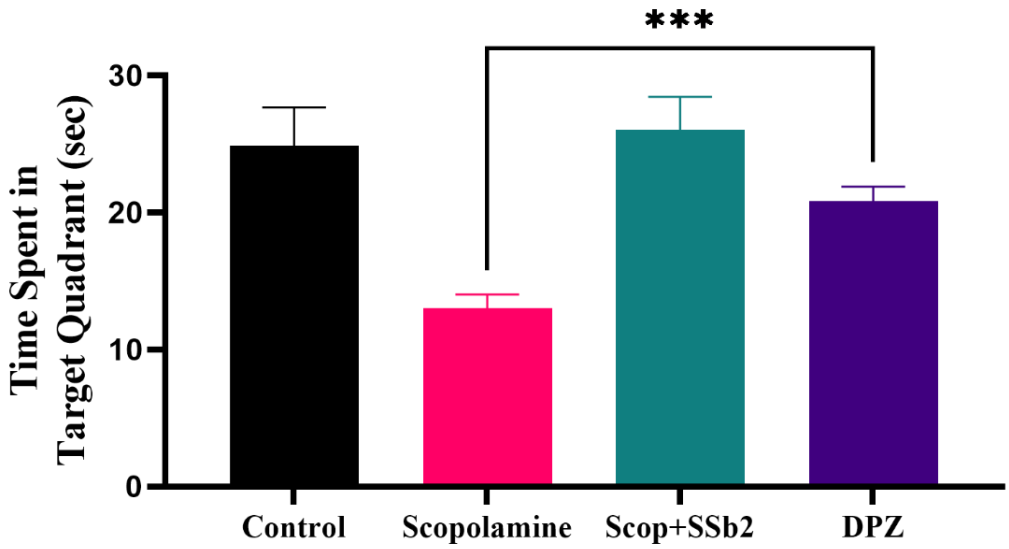


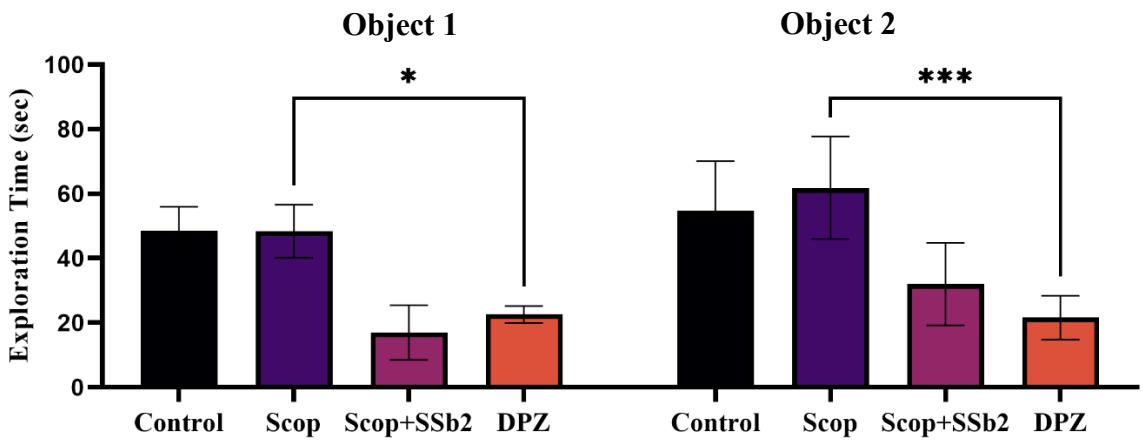

Figure 8: Saikosaponin b2's impact on reference memory (MWM Test, Probe Trial). The time the test animals spent in the platform's goal quadrant is depicted in the bar diagram. Between the control and Scopolamine (Scop) groups, there is a significant difference ($p<0.001$) that shows how the reference memory of amnesic animals is affected. The time spent by the Scop + SSb2 group in the target quadrant is longer than that of the Scop group, demonstrating a significant difference between the two groups ($p<0.01$) with Scopolamine + Saikospaonin b2 (Scop + SSb2). This finding suggests that reference memory is restored by the consumption of Saikosaponin b2. Error bars depict mean SEM; n=05; A one-way ANOVA was used, followed by the Bonferroni Multiple Comparison Test.

### 4.1.2. Effect of Saikosaponin b2 on Novel Object Recognition Test

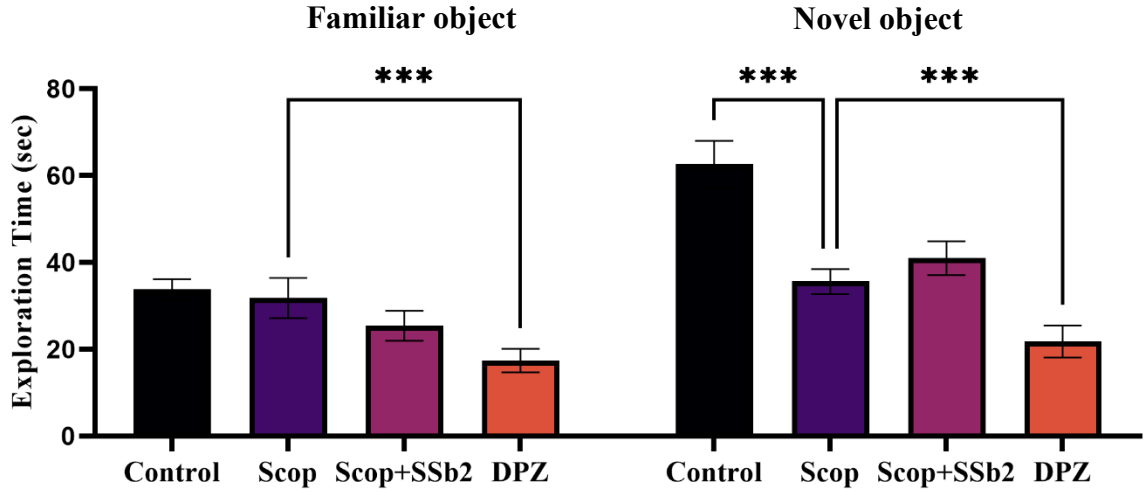
As Scopolamine group spent less time with novel objects (33.7±1.9) than the Control group (48.2±14.4), the data shows memory impairment in this group. After the illness was induced, the group that received scopolamine demonstrated reduced recognition for novel objects. The discrimination index for familiar and novel objects served as

evidence for this, exhibiting that the control group and Saikosaponin b2 showed better recognition for novel objects than the scopolamine group.

Session I




Session II



Discrimination index

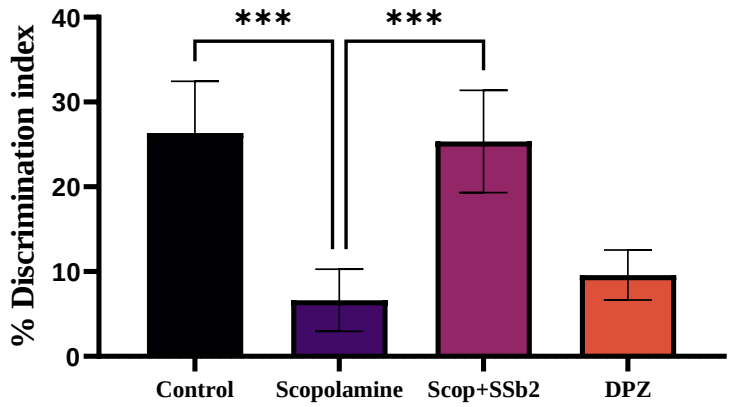



Figure 9: Effect of Saikosaponin b2 on the discrimination index. Each bar represents the mean ± SD (n=5). The discrimination index decreases in the Scopolamine group (p<0:0001vs. control), and the administration of Saikosaponin b2 increases this discrimination index (∗∗∗p<0:0001vs. Scopolamine). One-way ANOVA followed by the Bonferroni multiple comparisons test.

### 4.1.3. Elevated Plus Maze Test

Results of EPM exhibit increased anxiety in the mice treated with scopolamine (23.75, p<0.001), where control and Saikosaponin b2 groups spent significantly more time in open arms (74.75, 45.5, p<0.001). Similarly, time spent in closed arms by scopolamine group (276.3, p<0.001) is significantly increased.

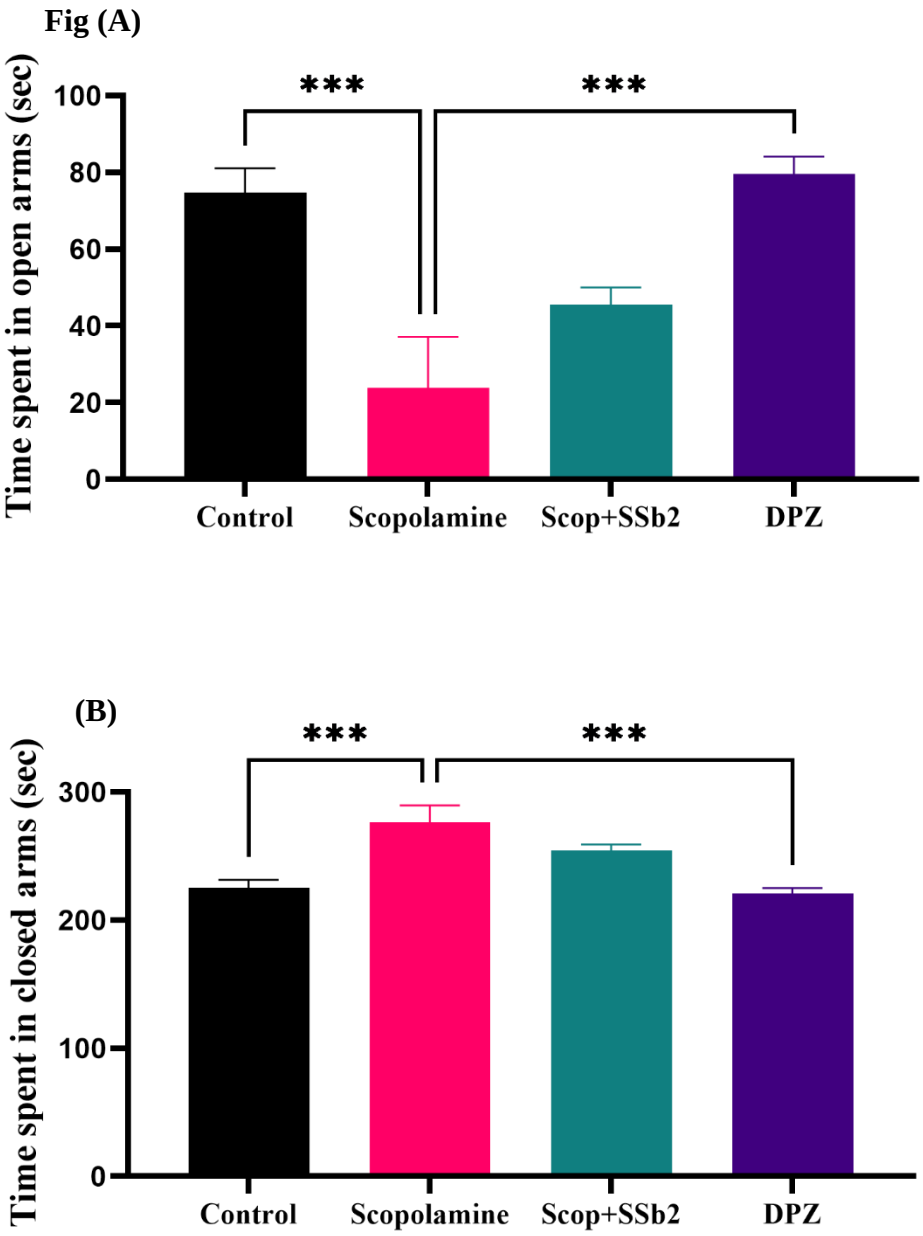
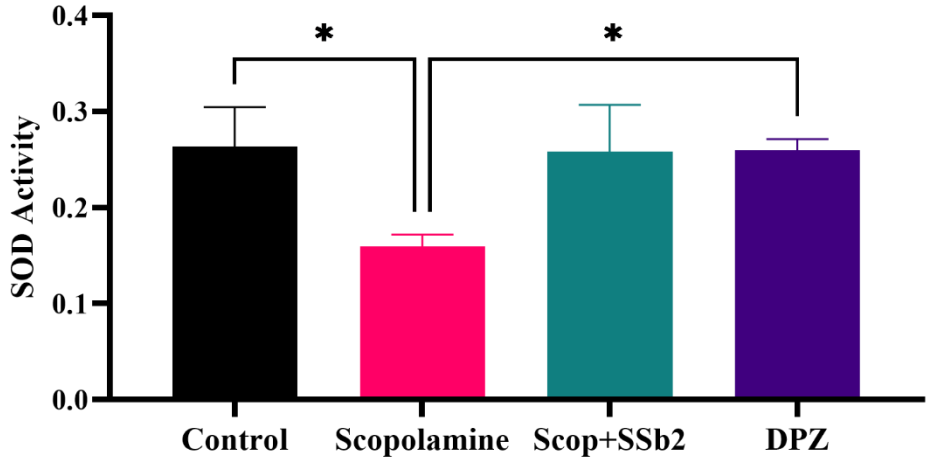



Figure 10: Effect of Saikosaponin b2 on anxiety in elevated plus maze test. (A) graph depicts time spent by control and Scop+SSb2 group compared to the diseased Scopolamine group. (B) graph depicts significantly increased time spent by scopolamine group compared to the control and Scop+SSb2 group.

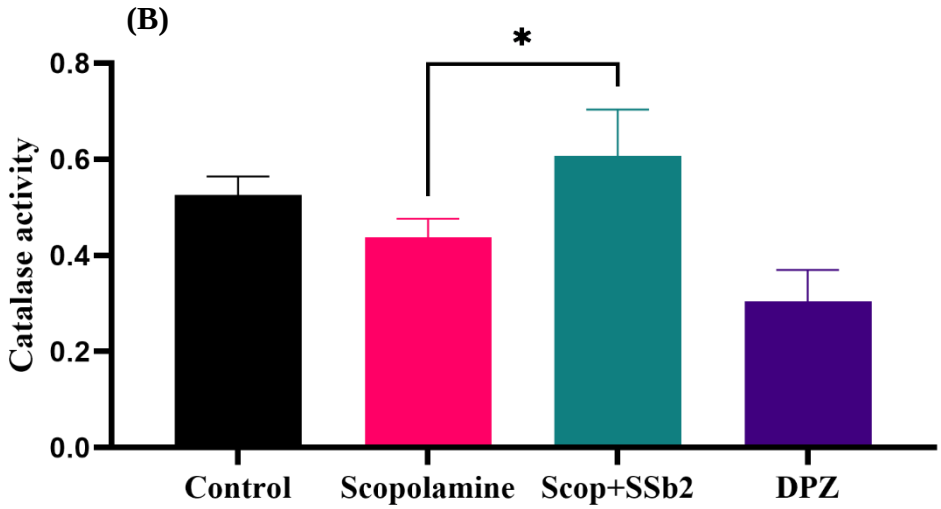
### 4.2. Effect of Saikosaponin b2 on Antioxidants

Effect of treatment with Saikosaponin b2 (5mg/kg) on levels of antioxidant enzymes such as (A) SOD, (B) CAT, (C) GSH, (D) GST in brain tissue homogenate.

**Fig(A)**




**(B)**



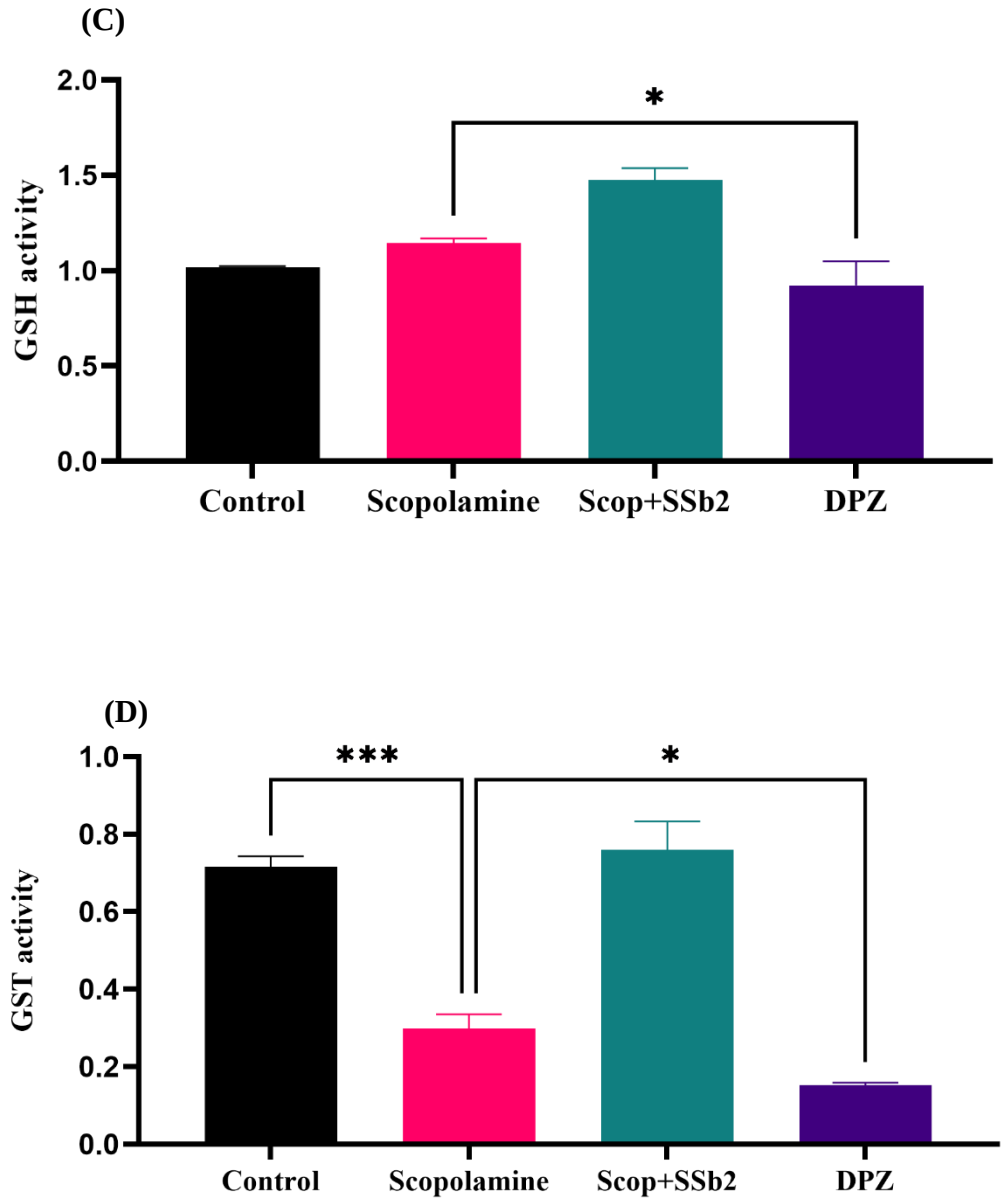
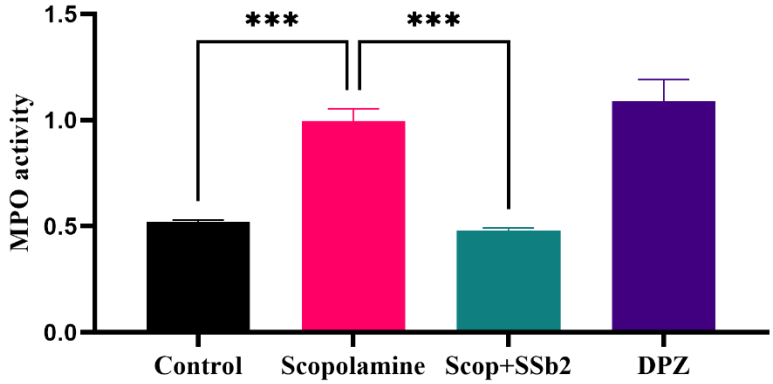



Figure 9: The level of these antioxidants and oxidative stress parameters were markedly compromised. However, Saikosaponin b2 significantly enhanced antioxidant enzymes such as GSH, GST, Catalase, SOD. All data were expressed as mean (n = 5) ± SD. (*) $p < 0.05$, (**) $p < 0.01$ and (***) $p < 0.001$, indicates significance compared to scopolamine group with all the groups and difference between control and scopolamine group.

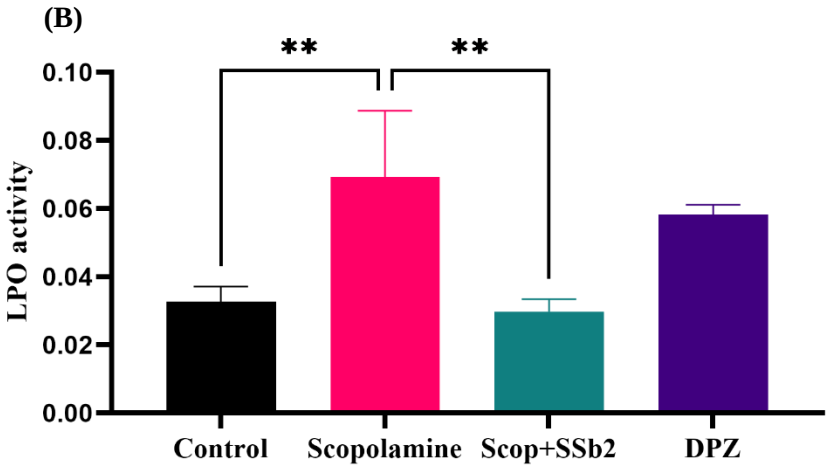
### 4.3. Effect of Saikosaponin b2 on oxidative stress

The scopolamine induced dementia resulted in the elevation of lipid peroxidation, myeloperoxidase and erythropoietin, due to increased reactive oxygen species (ROS). Effect of Saikosaponin b2 showed reduction in (A) LPO, (B) MPO and (C) EPO levels in brain tissue homogenates as compared to control and diseased groups.

**Fig (A)**




**(B)**



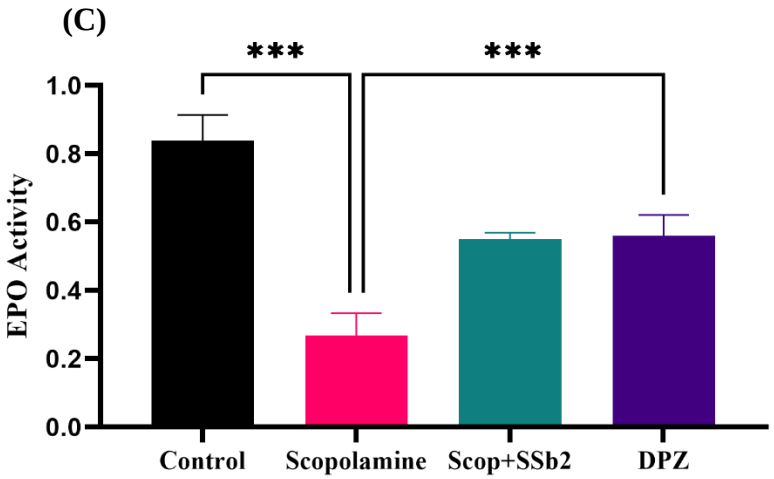



Figure 10 Effect of Saikosaponin b2 on oxidative stress in brain tissue homogenate of balb/c mice (A) LPO (B) MPO (C) EPO. The data were represented as mean ± standard deviation (n = 5). (*) $p<0.05$, (**) $p<0.01$, (***) $p<0.001$ indicates significance and shows comparison with the Scopolamine group.

### 4.4. Effect of Saikosaponin b2 on histopathology

In the prefrontal cortex and hippocampus of the control group and the Saikosaponin b2 treated groups, the H & E staining revealed stained sections of the neuronal cells. When compared to the scopolamine group, they showed a defined and organised morphology. The morphology of the diseased group was changed. The granular area of the hippocampal was showing signs of neuronal degeneration. The preventive effect of

Saikosaponin b2 (5 mg/kg) treatment against the neuronal damage brought on by scopolamine-induced Alzheimer's disease was significantly considerable.

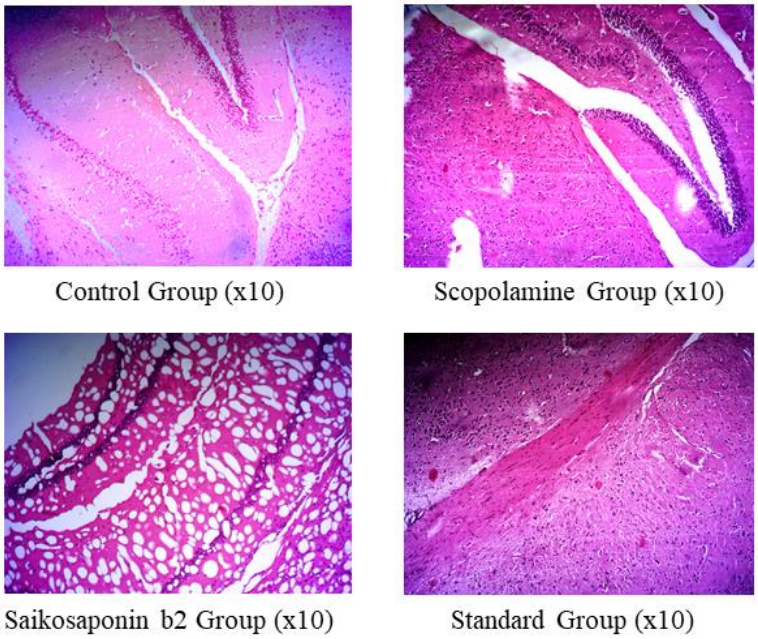



Figure 11: Histopathological analysis of H & E stained slides for Saikosaponin b2 treatment in Scopolamine induced Alzheimer's disease. (a) Control group (b) Scopolamine group (c) Saikosponin b2 group and (d) Donepezil observed at 10X magnification.

### 4.4.1. Effect of Saikosaponin b2 on histopathological analysis of Zone of Apoptosis

Control group exhibits regular cellular organization with no indications of apoptosis and additionally no cells with darkened compacted nuclei. A layer of enlarged, disordered granule cells with a higher number of apoptotic cells is present in some diseased group. In animals administered with Saikosaponin B2 5mg/kg, neurons are noticeably structured with a reduced appearance of apoptotic cells. With the irregular

emergence of apoptotic cells in the donepezil group, the organization and arrangement of neurons are almost completely returned to normal.

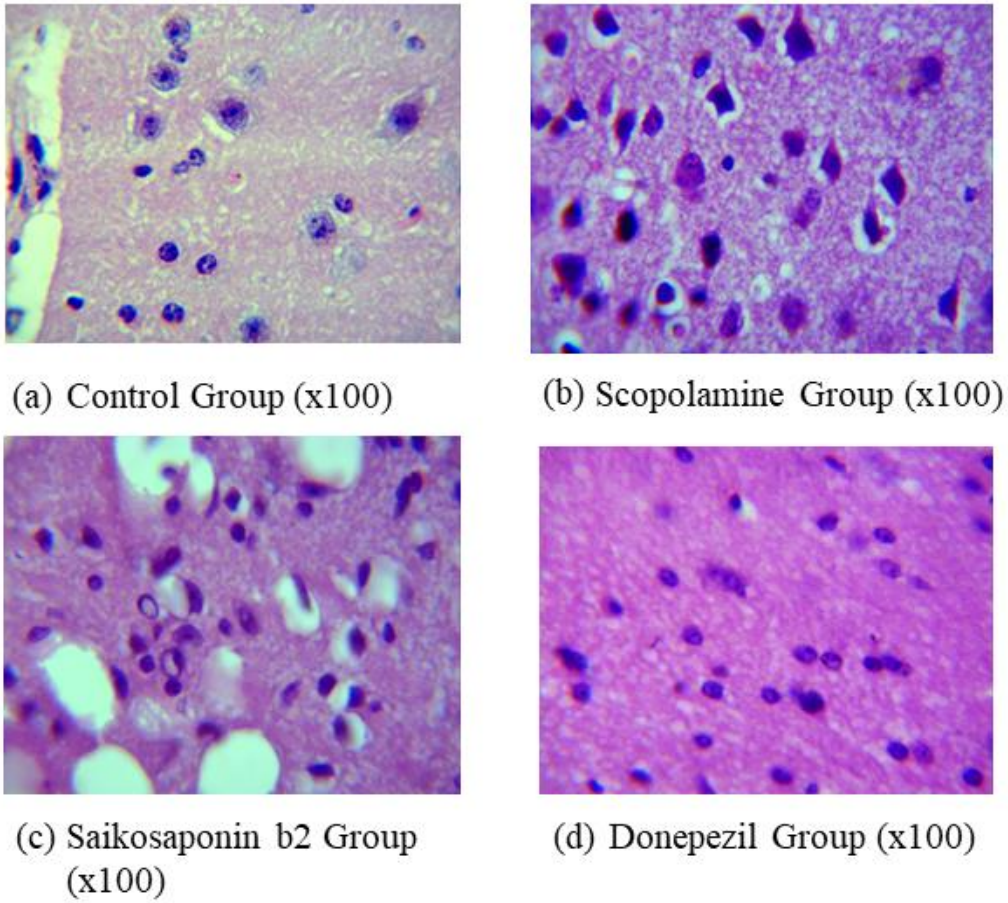



Figure 12: Histopathological Analysis of Zone of apoptosis through H & E staining. (a) Control group, (b) Scopolamine group, (c) Saikosaponin b2 and (d) Donepezil group observed at 10X magnification.

# Chapter 5

## 5. Discussion

The current study focuses on the impact of Saikosaponin b2's neuroprotective qualities on scopolamine-induced impairment of cognitive functioning, a well-known model for memory loss. Due to its high affinity for muscarinic receptors, scopolamine induces a pattern of cognitive deterioration in AD patients by blocking central muscarinic receptors non-selectively. Therefore, scopolamine's blockage of muscarinic receptors is reported to impair learning and memory, and muscarinic receptor antagonists' slowing of cholinergic neurotransmission causes severe deficiencies in attention, perception, and memory. A well-established model system for researching, analyzing, and formulating therapeutic strategies for human neurodegenerative illnesses is the use of scopolamine in animal models to generate the cognitive loss seen in AD.

Scopolamine can be injected intraperitoneally (i.p.), intramuscularly, subcutaneously or intravenously. For scientific objectives, various scopolamine doses have been administered to both experimental animals and human volunteers. Scopolamine at a dose of 1 mg/Kg is enough to have sedative effects that impair memory and learning.

In this study, the effect of Saikosaponin b2 in scopolamine induced dementia was assessed by different behavioral, biochemical and histopathological analysis. Through behavioral data, it is suggested that scopolamine induced Alzheimer's in comparison to the results acquired from biochemical assays. Memory impairment was assessed through Morris water maze test, Novel Object Recognition test and Elevated Plus Maze test. Administration of scopolamine developed a dose dependent increase in the anxiolytic behavior in mice as compared to the control group. Primarily in the MWM, the scopolamine treated mice consistently demonstrated deficits in learning and memory impairment at 7 weeks of age after 15 days of administration (Vorhees &

Williams, 2006). While SSb2 treated mice significantly improved performance by finding the platform with shortened escape latencies and prevented the memory impairment. This result demonstrates that SSb2 improves memory and cognitive functioning in mice model of Alzheimer's disease (Kim et al., 2009).

The latency to recognize the familiar object as opposed to the novel object is delayed by scopolamine, according to neurobehavioral experiments that extensively used the novel object recognition paradigm. It also showed a decrease in exploration time and a discrimination index, which indicated that the memory and recognition processes of the scopolamine-receiving group were compromised. The elevated plus maze is an effective test for determining an animal's level of anxiety. Scopolamine-treated mice showed increased anxiety, less time spent in open arms, and fewer head dips, according to the data. Therefore, scopolamine acts as an anticholinergic drug by blocking the muscarinic receptor, which has been shown to impair both human and animal's memory and learning. Elevated oxidative stress in the brain, which is damaging reactive oxygen species' side effect of increased MDA concentration, is linked to scopolamine-induced Alzheimer's. The findings of the current study demonstrate a breakdown in the brain's antioxidant defense system, as seen by elevated MDA levels and decreased levels of SOD, catalase, glutathione-s-transferase, and glutathione. The ability of Saikosaponin b2 and donepezil to reduce scopolamine-induced changes in brain neurotransmitters may be explained by its effects on lowering increased oxidative stress indicators, which are important hallmarks in dementia pathophysiology. Myeloperoxidase, a potent catalyst for the generation of cytotoxic oxidants is observed to be upregulated and become enzymatically active in AD brain. This increased myeloperoxidase level and associated production of oxidants contribute to tissue injury in chronic inflammation which is inhibited in this study, as a result of effect of SSb2. Similarly, EPO levels show

ameliorated scopolamine induced oxidative stress and stabilizes mitochondrial membrane potential. It demonstrates increased resistance of neurons to subsequent damage derived from the pro inflammatory function of microglial and macrophage cells. The histopathological findings showed normal healthy neurons in control group of H & E stained brain tissues, while the scopolamine group depicted neuronal damage with gliosis and increased apoptosis. However, scop+SSb2 showed markedly improved results, with minimized apoptotic appearance, and lessened neuronal degeneration.

## Conclusion

The current study evaluated the neuroprotective effect of Saikosaponin b2 against Scopolamine induced oxidative stress in Alzheimer's Disease. The Saikosaponin b2 markedly attenuated the oxidative stress markers and increased the antioxidant activities by restoring the anti-oxidant defense system. It also significantly improved the histopathological parameters and reduced the neuronal damage induced by Scopolamine. These findings show the neuroprotective effects of Saikosaponin b2 that could reduce the neurodegenerative effects considering Saikosaponin b2 as a favorable therapeutic agent of potential interest.

## References

Bachurin, S. O., Gavrilova, S. I., Samsonova, A., Barreto, G. E., & Aliev, G. (2018). Mild cognitive impairment due to Alzheimer disease: Contemporary approaches to diagnostics and pharmacological intervention. *Pharmacological Research*, *129*, 216–226. https://doi.org/10.1016/J.PHRS.2017.11.021

Bergmans, B. A., & De Strooper, B. (2010). gamma-secretases: from cell biology to therapeutic strategies. *The Lancet. Neurology*, *9*(2), 215–226. https://doi.org/10.1016/S1474-4422(09)70332-1

Cárdenas-Aguayo, M. D. C., Gómez-Virgilio, L., DeRosa, S., & Meraz-Ríos, M. A. (2014). The role of tau oligomers in the onset of Alzheimer's disease neuropathology. *ACS Chemical Neuroscience*, *5*(12), 1178–1191. https://doi.org/10.1021/CN500148Z

Cassano, T., Calcagnini, S., Carbone, A., Bukke, V. N., Orkisz, S., Villani, R., Romano, A., Avolio, C., & Gaetani, S. (2019). Pharmacological treatment of depression in Alzheimer's disease: A challenging task. *Frontiers in Pharmacology*, *10*(SEP), 1067. https://doi.org/10.3389/FPHAR.2019.01067/BIBTEX

Chakraborty, A., & Diwan, A. (2020). Alzheimer and It's Possible Therapy: A Review. *Journal of Experimental Neurology,2020;1*(4), 115–122. https://doi.org/10.33696/NEUROL.1.019

Claeysen, S., Cochet, M., Donneger, R., Dumuis, A., Bockaert, J., & Giannoni, P. (2012). Alzheimer culprits: cellular crossroads and interplay. *Cellular Signalling*, *24*(9), 1831–1840. https://doi.org/10.1016/J.CELLSIG.2012.05.008

Crary, J. F., Trojanowski, J. Q., Schneider, J. A., Abisambra, J. F., Abner, E. L., Alafuzoff, I., Arnold, S. E., Attems, J., Beach, T. G., Bigio, E. H., Cairns, N. J., Dickson, D. W., Gearing, M., Grinberg, L. T., Hof, P. R., Hyman, B. T., Jellinger, K., Jicha, G. A., Kovacs, G. G., … Nelson, P. T. (2014). Primary age-related tauopathy (PART): a

common pathology associated with human aging. *Acta Neuropathologica*, *128*(6), 755. https://doi.org/10.1007/S00401-014-1349-0

De Lima, M. N. M., Laranja, D. C., Bromberg, E., Roesler, R., & Schröder, N. (2005). Pre- or post-training administration of the NMDA receptor blocker MK-801 impairs object recognition memory in rats. *Behavioural Brain Research*, *156*(1), 139–143. https://doi.org/10.1016/J.BBR.2004.05.016

Deger, J. M., Gerson, J. E., & Kayed, R. (2015). The interrelationship of proteasome impairment and oligomeric intermediates in neurodegeneration. *Aging Cell*, *14*(5), 715–724. https://doi.org/10.1111/ACEL.12359

dos Santos, M. M., de Macedo, G. T., Prestes, A. S., Loro, V. L., Heidrich, G. M., Picoloto, R. S., Rosemberg, D. B., & Barbosa, N. V. (2018). Hyperglycemia elicits anxiety-like behaviors in zebrafish: Protective role of dietary diphenyl diselenide. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *85*, 128–135. https://doi.org/10.1016/j.pnpbp.2018.04.017

Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*, *82*(1), 70–77. https://doi.org/10.1016/0003-9861(59)90090-6

Fagan, A. M., Xiong, C., Jasielec, M. S., Bateman, R. J., Goate, A. M., Benzinger, T. L. S., Ghetti, B., Martins, R. N., Masters, C. L., Mayeux, R., Ringman, J. M., Rossor, M. N., Salloway, S., Schofield, P. R., Sperling, R. A., Marcus, D., Cairns, N. J., Buckles, V. D., Ladenson, J. H., … Holtzman, D. M. (2014). Longitudinal change in CSF biomarkers in autosomal-dominant Alzheimer's disease. *Science Translational Medicine*, *6*(226). https://doi.org/10.1126/SCITRANSLMED.3007901

Falsafi, S. K., Deli, A., Höger, H., Pollak, A., & Lubec, G. (2012). Scopolamine Administration Modulates Muscarinic, Nicotinic and NMDA Receptor Systems. *PLoS ONE*, *7*(2). https://doi.org/10.1371/JOURNAL.PONE.0032082

Francis, S. T., Head, K., Morris, P. G., & Macdonald, I. A. (2006). The effect of flavanol-rich cocoa on the fMRI response to a cognitive task in healthy young people. *Journal of Cardiovascular Pharmacology*, *47 Suppl 2*(SUPPL. 2). https://doi.org/10.1097/00005344-200606001-00018

Goedert, M. (2015). NEURODEGENERATION. Alzheimer's and Parkinson's diseases: The prion concept in relation to assembled Aβ, tau, and α-synuclein. *Science (New York, N.Y.)*, *349*(6248). https://doi.org/10.1126/SCIENCE.1255555

Gong, B., Radulovic, M., Figueiredo-Pereira, M. E., & Cardozo, C. (2016). The Ubiquitin-Proteasome System: Potential Therapeutic Targets for Alzheimer's Disease and Spinal Cord Injury. *Frontiers in Molecular Neuroscience*, *9*(JAN). https://doi.org/10.3389/FNMOL.2016.00004

Hefter, D., Kaiser, M., Weyer, S. W., Papageorgiou, I. E., Both, M., Kann, O., Müller, U. C., & Draguhn, A. (2016). Amyloid Precursor Protein Protects Neuronal Network Function after Hypoxia via Control of Voltage-Gated Calcium Channels. *The Journal of Neuroscience*, *36*(32), 8356. https://doi.org/10.1523/JNEUROSCI.4130-15.2016

Hutsler, J., & Galuske, R. A. W. (2003). Hemispheric asymmetries in cerebral cortical networks. *Trends in Neurosciences*, *26*(8), 429–435. https://doi.org/10.1016/S0166-2236(03)00198-X

Jadhav, S., Katina, S., Kovac, A., Kazmerova, Z., Novak, M., & Zilka, N. (2015). Truncated tau deregulates synaptic markers in rat model for human tauopathy. *Frontiers in Cellular Neuroscience*, *9*(FEB). https://doi.org/10.3389/FNCEL.2015.00024

Jang, B. G., In, S., Choi, B., & Kim, M. J. (2014). Beta-amyloid oligomers induce early loss of presynaptic proteins in primary neurons by caspase-dependent and proteasome-dependent mechanisms. *Neuroreport*, *25*(16), 1281–1288. https://doi.org/10.1097/WNR.0000000000000260

Jo, D. S., Park, N. Y., & Cho, D. H. (2020). Peroxisome quality control and dysregulated lipid metabolism in neurodegenerative diseases. *Experimental & Molecular Medicine 2020 52:9*, *52*(9), 1486–1495. https://doi.org/10.1038/s12276-020-00503-9

Kim, S., Kim, D. H., Choi, J. J., Lee, J. G., Lee, C. H., Park, S. J., Jung, W. Y., Park, D. H., Ko, K. H., Lee, S. H., & Ryu, J. H. (2009). Forsythiaside, a constituent of the fruits of Forsythia suspense, ameliorates scopolamine-induced memory impairment in mice. *Biomolecules and Therapeutics*, *17*(3), 249–255. https://doi.org/10.4062/BIOMOLTHER.2009.17.3.249

Klinkenberg, I., & Blokland, A. (2010). The validity of scopolamine as a pharmacological model for cognitive impairment: a review of animal behavioral studies. *Neuroscience and Biobehavioral Reviews*, *34*(8), 1307–1350. https://doi.org/10.1016/J.NEUBIOREV.2010.04.001

Li, N., Wu, X., & Li, L. (2007). Chronic administration of clozapine alleviates reversal-learning impairment in isolation-reared rats. *Behavioural Pharmacology*, *18*(2), 135–145. https://doi.org/10.1097/FBP.0B013E3280D3EE83

Morales, I., Guzmán-Martínez, L., Cerda-Troncoso, C., Farías, G. A., & Maccioni, R. B. (2014). Neuroinflammation in the pathogenesis of Alzheimer's disease. A rational framework for the search of novel therapeutic approaches. *Frontiers in Cellular Neuroscience*, *8*(1 APR). https://doi.org/10.3389/FNCEL.2014.00112

Murray, M. E., Graff-Radford, N. R., Ross, O. A., Petersen, R. C., Duara, R., & Dickson, D. W. (2011). Neuropathologically defined subtypes of Alzheimer's disease with distinct clinical characteristics: a retrospective study. *The Lancet. Neurology*, *10*(9), 785–796. https://doi.org/10.1016/S1474-4422(11)70156-9

Musiek, E. S., & Holtzman, D. M. (2015). Three dimensions of the amyloid hypothesis: time, space and "wingmen." *Nature Neuroscience*, *18*(6), 800–806.

https://doi.org/10.1038/NN.4018

Olsson, B., Lautner, R., Andreasson, U., Öhrfelt, A., Portelius, E., Bjerke, M., Hölttä, M., Rosén, C., Olsson, C., Strobel, G., Wu, E., Dakin, K., Petzold, M., Blennow, K., & Zetterberg, H. (2016). CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *The Lancet. Neurology*, *15*(7), 673–684. https://doi.org/10.1016/S1474-4422(16)00070-3

Perluigi, M., Di Domenico, F., Barone, E., & Butterfield, D. A. (2021). mTOR in Alzheimer Disease and Its Earlier Stages: Links to Oxidative Damage in the Progression of this Dementing Disorder. *Free Radical Biology & Medicine*, *169*, 382. https://doi.org/10.1016/J.FREERADBIOMED.2021.04.025

Ramos-Rodriguez, J. J., Pacheco-Herrero, M., Thyssen, D., Murillo-Carretero, M. I., Berrocoso, E., Spires-Jones, T. L., Bacskai, B. J., & Garcia-Alloza, M. (2013). Rapid β-amyloid deposition and cognitive impairment after cholinergic denervation in APP/PS1 mice. *Journal of Neuropathology and Experimental Neurology*, *72*(4), 272–285. https://doi.org/10.1097/NEN.0B013E318288A8DD

Ribas, G. C. (2010). The cerebral sulci and gyri. *Neurosurgical Focus*, *28*(2). https://doi.org/10.3171/2009.11.FOCUS09245

Riss, J., Cloyd, J., Gates, J., & Collins, S. (2008). Benzodiazepines in epilepsy: pharmacology and pharmacokinetics. *Acta Neurologica Scandinavica*, *118*(2), 69–86. https://doi.org/10.1111/J.1600-0404.2008.01004.X

Shafiei, S. S., Guerrero-Muñoz, M. J., & Castillo-Carranza, D. L. (2017). Tau oligomers: Cytotoxicity, propagation, and mitochondrial damage. *Frontiers in Aging Neuroscience*, *9*(APR), 83. https://doi.org/10.3389/FNAGI.2017.00083/BIBTEX

Shukla, V., Skuntz, S., & Pant, H. C. (2012). Deregulated Cdk5 activity is involved in inducing Alzheimer's disease. *Archives of Medical Research*, *43*(8), 655–662.

https://doi.org/10.1016/J.ARCMED.2012.10.015

Tang, M., Taghibiglou, C., & Liu, J. (2017). The Mechanisms of Action of Curcumin in Alzheimer's Disease. *Journal of Alzheimer's Disease : JAD*, *58*(4), 1003–1016. https://doi.org/10.3233/JAD-170188

Theleritis, C., Sakkas, P., Paparrigopoulos, T., Vitoratou, S., Tzavara, C., Bonaccorso, S., Politis, A., Soldatos, C. R., & Psarros, C. (2017). Two Versus One High-Frequency Repetitive Transcranial Magnetic Stimulation Session per Day for Treatment-Resistant Depression: A Randomized Sham-Controlled Trial. *The Journal of ECT*, *33*(3), 190–197. https://doi.org/10.1097/YCT.0000000000000387

Tu, S., Okamoto, S. ichi, Lipton, S. A., & Xu, H. (2014). Oligomeric Aβ-induced synaptic dysfunction in Alzheimer's disease. *Molecular Neurodegeneration*, *9*, 48. https://doi.org/10.1186/1750-1326-9-48

Vazquez, A. (2013). Metabolic States Following Accumulation of Intracellular Aggregates: Implications for Neurodegenerative Diseases. *PLoS ONE*, *8*(5). https://doi.org/10.1371/JOURNAL.PONE.0063822

Vorhees, C. V., & Williams, M. T. (2006). Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nature Protocols*, *1*(2), 848. https://doi.org/10.1038/NPROT.2006.116

Walsh, P., Elsabbagh, M., Bolton, P., & Singh, I. (2011). In search of biomarkers for autism: scientific, social and ethical challenges. *Nature Reviews. Neuroscience*, *12*(10), 603–612. https://doi.org/10.1038/NRN3113

Wang, Y., & Mandelkow, E. (2012). Degradation of tau protein by autophagy and proteasomal pathways. *Biochemical Society Transactions*, *40*(4), 644–652. https://doi.org/10.1042/BST20120071

Warholm, M., Guthenberg, C., von Bahr, C., & Mannervik, B. (1985). [62] Glutathione

transferases from human liver. *Methods in Enzymology*, *113*(C), 499–504. https://doi.org/10.1016/S0076-6879(85)13065-X

Zhang, F., Su, B., Wang, C., Siedlak, S. L., Mondragon-Rodriguez, S., Lee, H. gon, Wang, X., Perry, G., & Zhu, X. (2015). Posttranslational modifications of α-tubulin in alzheimer disease. *Translational Neurodegeneration*, *4*(1), 1–9. https://doi.org/10.1186/S40035-015-0030-4/FIGURES/5

Zhao, X., Kotilinek, L. A., Smith, B., Hlynialuk, C., Zahs, K., Ramsden, M., Cleary, J., & Ashe, K. H. (2016). Caspase-2 cleavage of tau reversibly impairs memory. *Nature Medicine*, *22*(11), 1268–1276. https://doi.org/10.1038/NM.4199

ORIGINALITY REPORT

| 13% | 9% | 11% | 3% |
| --- | --- | --- | --- |
| SIMILARITY INDEX | INTERNET SOURCES | PUBLICATIONS | STUDENT PAPERS |

PRIMARY SOURCES

| | | |
| --- | --- | --- |
| 1 | www.hindawi.com<br>Internet Source | 2% |
| 2 | Syeda Mehpara Farhat, Aamra Mahboob, Touqeer Ahmed. "Oral exposure to aluminum leads to reduced nicotinic acetylcholine receptor gene expression, severe neurodegeneration and impaired hippocampus dependent learning in mice", Drug and Chemical Toxicology, 2019<br>Publication | 1% |
| 3 | www.nature.com<br>Internet Source | 1% |
| 4 | Abdul Basit, Tao Shutian, Ashrafullah Khan, Shah Masaud Khan, Raheem Shahzad, Abid Khan, Salman Khan, Madeeha Khan. "Anti-inflammatory and analgesic potential of leaf extract of Justicia adhatoda L. (Acanthaceae) in Carrageenan and Formalin-induced models by targeting oxidative stress", Biomedicine & Pharmacotherapy, 2022<br>Publication | 1% |