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EMERGING MOLECULAR MECHANISM AS THERAPEUTICS FOR ALZHEIMER'S

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CANDIDATE'S DECLARATION

I**, SANYA,** (Roll No.: 2K21/MSCBIO/41) of **M.Sc. Biotechnology,** declare that this work which is presented in this Major Project titled "**"Emerging molecular mechanism as Therapeutics for Alzheimer's**" submitted to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirements for the award of the degree of Master of Science is original and my own, under the supervision of **Prof. Pravir Kumar**. I also declare that this work has not previously formed the basis for the award of any Degree or other similar title or recognition.

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SUPERVISOR CERTIFICATE

To the best of my knowledge, the work titled **"Emerging molecular mechanism as Therapeutics for Alzheimer's**" has not been submitted anywhere else either in part or in full for any Degree or Diploma at this University or elsewhere. I further certify that the publicationand indexing information given by the student is correct.

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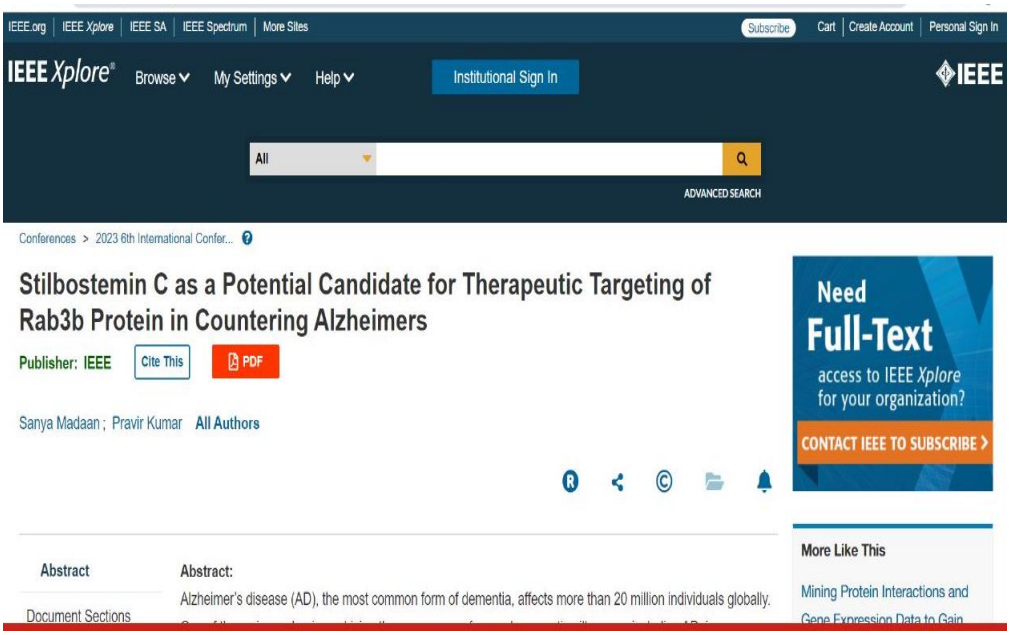

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EMERGING MOLECULAR MECHANISM

AS THERAPEUTICS FOR ALZHEIMER'S

ABSTRACT

Worldwide, there are more than 20 million people who have Alzheimer's disease (AD), a common neurological condition. Dysregulation of neuronal autophagy, a crucial cellular process responsible for protein degradation, has been determined to be a significant criterion in the emergence of AD and other neurodegenerative illnesses. Understanding the interplay between various elements of autophagy, such as Aβ and tau protein metabolism, the mTOR pathway, neuroinflammation, and the endocannabinoid system, holds promise for the creation of fresh treatment methods for AD.

This research has the analysis of protein-protein interactions (PPI) and functional enrichment were both used for utilizing clinical data from the GDC data portal to identify key genes associated with AD. Differential expression analysis allowed us to distinguish genes with altered expression levels between mutant and normal groups. Among the genes identified, we focused on the small protein binding to GTP, RAB3B, a RAB family member implicated in cell autophagy. Disruptions in the RAB3B protein interactome have the potential to impair autophagy and exacerbate AD pathogenesis. To explore potential treatment options, we investigated stilbostemin C, a natural compound with therapeutic potential. In order to clarify the interactions between stilbostemin C and the RAB3B protein, molecular docking studies were carried out. The findings demonstrated stilbostemin C's strong affinity for RAB3B, pointing to its potential as a therapy candidate.

The positive pharmacokinetic profile of stilbostemin C was also revealed by an ADMET study, confirming its potential for further research. In conclusion, this study clarifies the significance of autophagy. dysfunction in AD and highlights RAB3B as a potential target for therapeutic interventions. Moreover, stilbostemin C demonstrates promising interactions with RAB3B, suggesting its potential as a treatment option for AD. The therapeutic effectiveness of stilbostemin C and its suitability for upcoming investigations in the setting of AD both call for more investigation.

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LIST OF ABBREVIATIONS

S.No,	Abbreviation	Full Form
1	AD	Alzheimer's Disease
2	GEO	Gene Expression Omnibus
3	DEGs	Differential expression genes
4	PPI	Protein-protein interaction
5	ADME/T	Chemical absorption, distribution, metabolism, excretion, and toxicity
6	NCBI	National Center for Biotechnology Information Gene Expression Omnibus
7	GO	Gene Ontology
8	KEGG	Kyoto Encyclopedia of Genes and Genomes
9	COCONUT	Collection of Open Natural Products Database
10	SDF	Structure-Data File
11	PDB	Protein Data Bank

CHAPTER-1

Introduction

A neurological condition called Alzheimer's disease (AD) worsens and endangers global health. Recent developments in science have revealed a number of exciting new molecular pathways that have the potential to completely change how AD is treated. The primary causes of AD have long been thought to be plaques in beta-amyloid and neurofibrillary tangles in the brain. However, these methods have not resulted in appreciable therapeutic advantages, necessitating the investigation of other therapy modalities. The goal of research in recent years has shifted to the recognition of novel molecular mechanisms that help in the growth and evolution of AD.

The most prevalent neurological ailment affecting the elderly, Alzheimer's disease (AD), will have a negative social and economic impact as the world's population ages. Tau phosphorylation, neuroinflammation, deregulation of neurotransmitters, and oxidative stress are some of the pathogenic pathways that are thought to contribute to AD. It is distinguished by a progressive and irreversible loss of cognitive function as well as the development of neurofibrillary tangles (NFTs) made of tau protein and senile plaques, which are mainly made of amyloid β(Aβ). The metabolism of Aβ is dysregulated in AD patients' brains, which causes Aβ to build up and aggregate.

One such intriguing topic of study is the connection between neuroinflammation and Alzheimer's disease. Evidence suggests that neuronal damage and cognitive decline are associated with activated microglia and astrocytes, inflammatory substances including cytokines and chemokines, and chronic inflammation in the brain. These factors all

significantly contribute to the development of neurodegeneration.

Alterations in synaptic plasticity and neural network connection have too been connected to cognitive loss in AD, according to recent studies. Researchers and physicians hope to create ground-breaking treatments that slow or stop the disease's course and offer respite to people experiencing the devastating impacts of Alzheimer's disease by investigating these new paths. The metabolism of the A and tau proteins is significantly influenced by autophagy. Organelles and proteins are destroyed during autophagy, a lysosome-dependent, homeostatic process, and then recycled into energy. Thus, defective autophagy is assumed to be the root cause of the buildup of toxic proteins in the AD brain. [1]. Neurodegeneration is caused by excessive or insufficient autophagic activity in neurons, which affects their survival and ability to maintain homeostasis. Axons, dendrites, and synapses, which are intercellular connection structures found in neurons, are essential for the reciprocal transport of proteins, organelles, and autophagosomes over considerable distances from the soma. Inadequate autophagy disrupts cellular communication, which causes neurodegeneration.

Autophagy is usually recognized as a pro-survival activity because of its historically recognized role in hunger and other severe conditions including oxidative damage and organelle damage that it helps to mitigate. A considerable accumulation of autophagic vacuoles, such as autophagosomes or autolysosomes, has been observed in the affected brain region in a variety of neurodegenerative diseases. However, it is still unclear how exactly autophagy is involved in each neurodegenerative disease. As neurons are particularly vulnerable to protein misregulation due to their postmitotic status, sustained autophagy activation, disruption of the balance between autophagosome synthesis and lysosomal fusion, and impairment of lysosomal components are hypothesized to

contribute to nervous system degeneration.[2]. We aim to change the autophagy mechanism that ultimately leads to Alzheimer's disease by doing this. The Ras superfamily includes a subset of GTPases known as Rab proteins, which have a low molecular weight. Rab GTPases work as molecular switches that switch between GTP-bound active and GDP-bound inactive forms to control vesicle transit to the correct sites by tethering/docking vesicles to the appropriate compartments. It has been determined that RAB3B, a tiny GTP-binding protein linked to drug resistance and cell autophagy, is an essential oncogene in a number of malignancies and neurological disorders.[3]. To stop the buildup of amyloid Aβ plaques, we will target the rab3b gene, which is implicated in autophagy[4], [5].

1.1 Alzheimer's Disease

The maximum mutual source of dementia in the creation is AD, although because of better vascular treatment and greater brain health, the incidence may have declined in the Western world[5]. Amyloid and tau proteins are well-established core cerebrospinal biomarkers, but amyloid oligomers and synaptic markers are novel potential biomarkers[6]. MRI and fluorodeoxyglucose PET are recognized imaging techniques for the diagnosis of AD. Preclinical Alzheimer's disease is characterized by the presence of pathological abnormalities in Alzheimer's biomarkers in people with normal cognitive function. Interventions focusing on lifestyle factors in older adults without dementia and fairly encouraging preliminary outcomes for reducing amyloid in pre-dementia In order to effectively battle the disease, specific anti-Alzheimer's therapy will be paired with dietary changes aimed at improving overall brain health.

CHAPTER-2

DIFFERENT MOLECULAR MECHANISMS

2.1 Autophagy

Autophagy is a highly controlled and important intracellular process that maintains cell homeostasis by removing damaged organelles and misfolded proteins. Neurodegeneration is the result of excessive or insufficient autophagic activity in neurons, which affects their survival and homeostasis. Most neurodegenerative illnesses are marked by the accumulation of abnormal proteins and inclusion bodies because misfolded proteins and damaged organelles cannot be dispersed during cell division and postmitotic neurons rely largely on high basal autophagy relative to non-neuronal cells Axons, dendrites, and synapses, which are intercellular connection structures found in neurons, are required for the reciprocal transport of proteins, organelles, and autophagosomes over considerable distances from the soma. Neurodegeneration is caused by defects in autophagy, which prevent cells from communicating with one another.[8].

Autophagy is usually regarded as a pro-survival activity because of its long-known role in preventing hunger and other extreme conditions including oxidative damage and organelle damage. Autophagic vacuoles, such as autophagosomes or autolysosomes, have been observed to accumulate significantly in the damaged brain region in several neurodegenerative diseases. Autophagy's precise role in each neurodegenerative disease is still unclear, though. As neurons are particularly vulnerable to protein misregulation because of their postmitotic status, it is believed that sustained autophagy activation, disruption of the equilibrium between autophagosome production and lysosomal fusion,

and impairment of lysosomal components contribute to the degeneration of the nervous system.

2.2 Mitophagy

DNA damage or genetic mutation can be caused by mitochondrial metabolic byproducts. As a result, it has been found that maintaining mitochondrial function is crucial for maintaining cellular health. Mitophagy, which is a type of selective autophagy, is critical for mitochondrial health. ATP is the end product of oxidative phosphorylation, which can cause mitochondria to produce reactive oxygen species (ROS). Under certain circumstances, excessive mitochondrial ROS can cause cytotoxicity and cell death. Since mitochondria's DNA is "bare" and they have a low antioxidant capacity, they are particularly vulnerable to ROS damage. [9]. Diseased mitochondria will continue to create ROS and release pro-apoptotic proteins into the cytoplasm, eventually resulting in cell death, if they are not repaired or removed from cells. Mitophagy can partially account for cellular homeostasis mechanisms; as a result, an appropriate mitophagy level is critical for reducing aberrant protein aggregation and stimulating organelle elimination. It is vital to preserve mitochondrial activity and encourage the breakdown of damaged mitochondria to protect neurons. One of the therapeutic methods for several neurodegenerative illnesses has been suggested to be the modulation of mitophagy. Mitophagy activation has been found to improve the phenotypes of neurodegenerative illnesses and provide neuroprotection. Several treatment approaches, including PINK1/parkin regulators, metformin, and resveratrol, have been shown to promote mitophagy[10]. Some medications that target the Sirtuins family have been shown in recent studies to slow

disease progression.

2.3 Necroptosis

Necroptosis is a kind of controlled cell death that takes place under specific circumstances, such as when apoptosis is repressed. Neurons and glial cells in the brain have been found to have dysregulation of necroptosis signaling in Alzheimer's disease. This instability sets off a series of actions that release pro-inflammatory chemicals and attract immune cells, escalating neuroinflammation and neuronal injury in the process.[11] Receptor-interacting protein kinase 3 (RIPK3), tumor necrosis factor-alpha (TNF-), and other death receptor ligands are some of the many factors that activate necroptosis in AD. [12]Necroptosis has effects in AD that go beyond cell death, such as the release of inflammatory cytokines and molecular patterns linked with damage (DAMPs) that can worsen neuronal damage and impair cognitive function. concentrating on certain molecules

2.4 Apoptosis

A physiological process called apoptosis, which is tightly controlled and necessary for development, causes cells to die. as well as maintaining the equilibrium between cell division and death later on. Injures that quickly interrupt cellular metabolism and cause the cells to disintegrate non-physiologically are what causes necrosis. Differentiating between apoptosis and necrosis can be done using a variety of morphological and biochemical traits.[13] Numerous studies have sought to categorize the specific pathway of cell death as either by necrosis or by apoptosis in order to characterize the mechanisms of cell death occurring during neurodegenerative illnesses like Alzheimer's disease

(AD)[14]. The degradation of nerve cells associated with AD can be modeled using a variety of model systems. AD tissue has cells that are apoptotic and necrotic. Certain genes linked to familial AD may make neurons more sensitive to apoptosis, however, A great deal of cases of AD are sporadic and not exclusively hereditary in nature. Under certain circumstances, apoptosis and necrosis may occur concurrently, overlap, and not always be clearly distinguished.

2.5 Linkage Of Various Mechanisms

A neurodegenerative ailment called AD is marked by cerebral decline and memory loss. Numerous molecular processes, like autophagy, mitophagy, necroptosis, and apoptosis, are tangled in the pathogenesis of AD. The accumulation of misfolded proteins, oxidative stress, neuroinflammation, and neuronal injury are all caused by dysregulation of these pathways[15], [16]. By removing harmed organelles and proteins, autophagy is essential in preserving cellular homeostasis. Protein aggregation seen in AD is caused by dysfunctional autophagy. For the health of the mitochondria, a particular type of autophagy known as mitophagy is required. Impaired mitophagy causes damaged mitochondria to accumulate, increasing oxidative stress and harming neurons in AD.

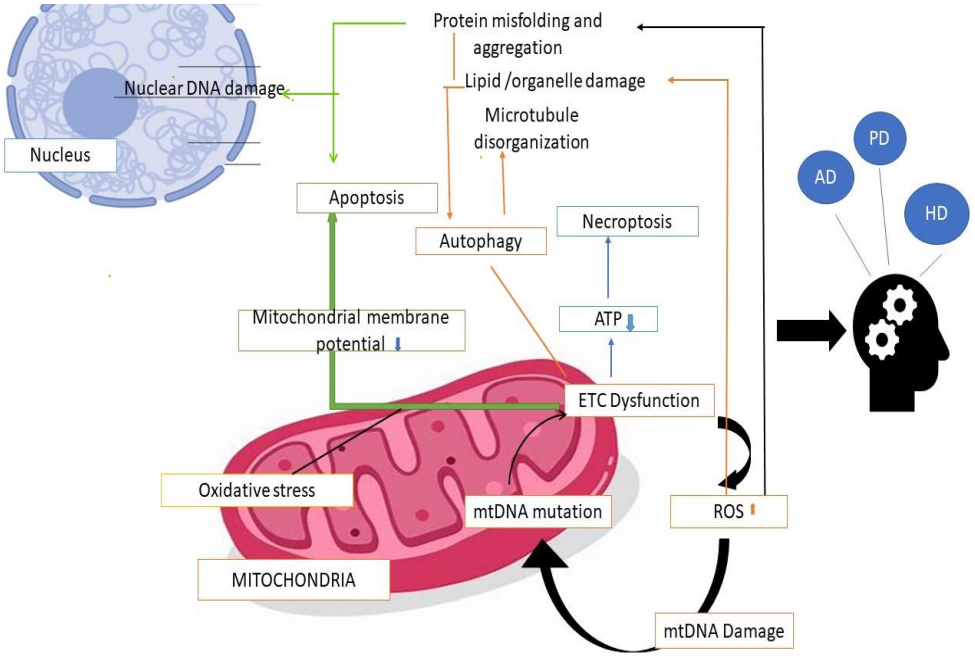

Figure 2.1: Linkage Of Various Molecular Mechanisms

CHAPTER- 3

SELECTION OF AUTOPHAGY AS A MECHANISM FOR ALZHEIMER'S

In AD, misfolded proteins such as beta-amyloid and tau accumulate, which results in the formation of plaques and neurofibrillary tangles. Cells destroy and recycle damaged or unneeded cellular components, such as protein aggregates and organelles, through the tightly regulated process of autophagy.[17] Autophagy dysregulation has been linked to the etiology of AD, which makes it a desirable molecular target for therapeutic intervention. Neurons and glial cells in the brain, as well as other cell types, have been found to have impaired autophagy in AD patients and animal models. There are various benefits to using autophagy as a therapeutic target for AD, including facilitating the removal of harmful protein aggregates, lowering their load, and minimizing their negative effects on neuronal function.

By treating a significant pathological feature of AD, this strategy may be able to delay or stop disease development. In AD patients, restoring correct autophagic activity can enhance cognitive results, retain synaptic connections, and promote neuronal survival[3]. While lifestyle changes like calorie restriction and exercise have been found to activate autophagy and enhance cognitive function, pharmacological therapies like rapamycin and its analogs have shown promising outcomes in preclinical investigations. To guarantee the best treatment results, autophagy regulation must be precisely modulated[18]. A prospective therapeutic strategy to improve protein clearance, reestablish cellular homeostasis, and maintain neuronal function targets autophagy as a biological mechanism. There is an enormous promise for developing disease-modifying therapeutics for AD with further investigation and development of secure and efficient autophagy

modulators.

3.1 Therapeutic Approaches And Clinical Trials On Autophagy

AD, an extremely frequent brain condition affecting the elderly, will have a negative social and economic impact as the world's population ages. The N-methyl-D-aspartic acid receptor antagonist memantine and the cholinesterase inhibitors donepezil, rivastigmine, and galantamine, which are now licenced for the treatment of Alzheimer's disease, only treat symptoms without slowing the course of the disease.[19]. We reviewed current advances in Alzheimer's disease medicines that control neurotransmitters, inflammation, autophagy, microbiota, circadian rhythm, and genes altered by the disease. Macro autophagy dysfunction may increase α-secretase activity, which raises Aβ by cleaving amyloid- β -precursor protein (APP).

Furthermore, the AD’s medication galantamine hydrochloride inhibits autophagy. Autophagy defects and autophagosome accumulation are attributable to a reduction in berlin-1 expression. As a result, inducing autophagy with tiny compounds that may critically regulate autophagy may be a superior beneficial target for treating AD, Resveratrol is a phenolic chemical found in red wine, grapes, and a variety of foods. Resveratrol reduces the quantities of secreted and intracellular Ab peptides generated by several cell types. Resveratrol's Aβ-lowering action is based not on the prevention of Aβ synthesis, but the stimulation of its intracellular breakdown via a proteasome-based mechanism. Lithium influences multiple intracellular pathways involved in neuroplasticity and neural damage resistance. These features, which include down-regulation of apoptosis, oxidative stress, and inflammation and up-regulation of the neurotrophic response and autophagy, have repeatedly been seen in experimental studies.

Future controlled investigations are needed to clarify the therapeutic implications of the current findings, especially in light of the potential use of lithium as a disease-modifying therapy for some neurodegenerative diseases like Alzheimer's. One of the Neuronal mitochondrial dysfunction is one of the most obvious signs of AD.

Recent research suggests that the malfunctioning of mitochondria in AD may be significantly influenced by mitochondrial miRNAs, particularly those that target the synapse. An extensive review of the literature was carried out to pinpoint and discuss the function of mitochondrial miRNAs that control synaptic and mitochondrial functions, the contribution of synapse damage and mitochondrial dysfunctions, and various therapeutic approaches that enhance synaptic and mitochondrial functions in AD[22]. ATP synthesis, oxidative stress, mitophagy, bioenergetics, mitochondrial dynamics, synaptic activity, synaptic plasticity, neurotransmission, and synaptotoxicity in AD neurons have all been demonstrated to be altered by variations in mitochondrial miRNA expression. Additionally, the precise processes by which these modifications are mediated have been found.

3.2 RAB3B Protein

A complicated neurodegenerative ailment (AD) is characterized by the buildup of protein clumps, neuroinflammation, and dysfunctional neurons. The cellular process of autophagy, which breaks down and recycles damaged or undesirable cellular components, has been linked to the pathophysiology of AD. Recent research has revealed a potential function for the RAB3B protein in controlling autophagy and its relationship to AD, illuminating a unique molecular mechanism with therapeutic implications. RAB3B is a

member of the RAS-related protein family and is essential for exocytosis and vesicle trafficking.[20] RAB3B has been shown to accumulate in dystrophic neurites around amyloid plaques, and studies have shown that its expression levels and localization are altered in the brains of people with AD. RAB3B may play a function in controlling the autophagic process as evidenced by its interactions with important autophagy-related proteins as ATG16L1 and LC3. RAB3B affects autophagy in the context of AD, but the precise methods by which it does so are not known. In the regulation of autophagy and its connection to Alzheimer's disease, the RAB3B protein has come into focus as a potential participant. Although it has been discovered in preclinical investigations and experimental models, more study is required to confirm and build on these discoveries. Additional research into the function of RAB3B in autophagy dysregulation may open the door to the creation of cutting-edge treatment approaches meant to improve the pathogenic aspects of AD and restore autophagic activity. RAB3B's altered expression and location in AD brains

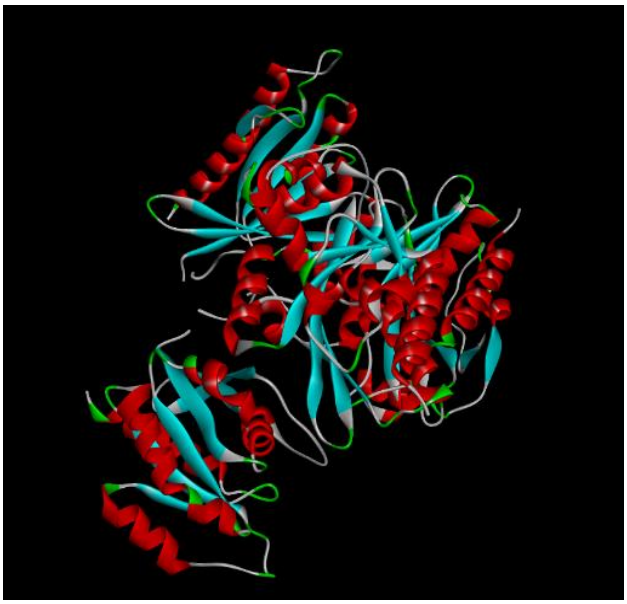

Figure 3.2 Structure of RAB3B Protein

3.3 Huperzine A

A naturally occurring substance called huperzine A is obtained from the Chinese club moss plant, Huperzia serrata. Due to its potential as a treatment for cognitive impairments, studying neurology and AD has attracted a lot of interest.. It is known to have acetylcholinesterase (AChE) inhibitory activity, which means it can stop the neurotransmitter acetylcholine from being broken down. Huperzine A's effects in various cognitive disorders, including Alzheimer's disease, have been investigated in several preclinical and clinical investigations.[21] Huperzine A has demonstrated effective and specific AChE inhibition, which raises acetylcholine levels in the brain. Huperzine A has shown potential cognitive advantages in both animal models and clinical trials. Effects on neuroprotection: By lowering oxidative stress, preventing apoptosis, and guarding against neurotoxic assaults, huperzine A demonstrates neuroprotective capabilities. Huperzine A, derived from a plant called Chinese club moss, has shown acetylcholinesterase inhibitory activity and possible cognitive advantages in preclinical and clinical tests.[22] It also has anti-inflammatory properties. Its capacity to raise acetylcholine levels, defend against neurotoxicity, and control inflammation raises the possibility that it could be effective in treating cognitive diseases like Alzheimer's disease. Before it can be widely recommended for clinical usage, additional research is required to determine its efficacy, safety, and long-term consequences.

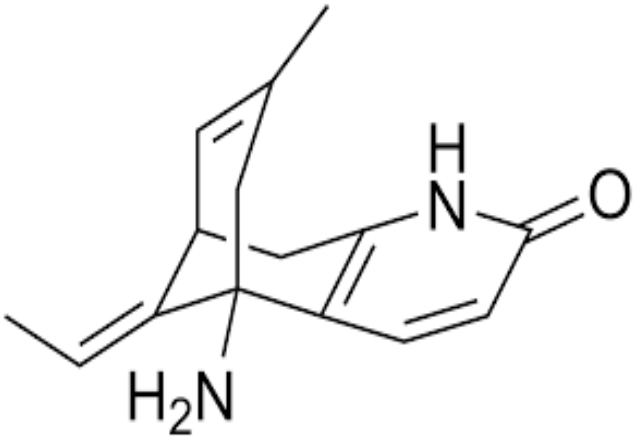

Figure 3.3: Structure of Huperzine A

CHAPTER- 4

MATERIALS & METHODS

4.1 Collection of Data Set

The present investigation utilized microarray gene expression datasets obtained from the NCBI-GEO (National Centre for Biotechnology Information-Gene Expression Omnibus) database to explore the molecular basis of AD. A total of 31 microarrays were employed to analyze gene expression patterns in a cohort consisting of nine healthy controls and 22 AD patients with varying degrees of disease severity This study's goal was to evaluate the gene expression profiles. and examine their associations with AD indicators. By contrasting the degrees of each gene's expression across all 31 subjects, irrespective of disease status, significant correlations were established between gene expression and the severity of AD. The analysis revealed a robust transcriptional response, with thousands of genes displaying significant associations with AD indicators. However, to focus on early-stage AD, only control subjects and participants in the early stages of the disease were considered for further analysis and correlation with several hundred of these genes. To identify differentially expressed genes (DEGs) associated with AD, strict selection criteria were employed, requiring a |log(foldchange)| greater than 2 and a p-value less than 0.01 The findings of this research provide valuable insights into the transcriptional changes occurring in AD and their potential relevance to disease progression. By examining gene expression patterns, researchers were able to identify a large number of genes that exhibited significant associations with AD indicators. These DEGs represent potential targets for further investigation and may offer valuable clues regarding the underlying

molecular mechanisms and pathways involved in AD's growth and development. This is significant to analyse that the utilization of microarray gene expression datasets from the NCBI-GEO database allowed for a comprehensive examination of gene expression profiles in a relatively large cohort of healthy controls and AD patients. The inclusion of multiple microarrays ensured robustness and statistical power in the analysis, providing a comprehensive view of gene expression changes associated with AD[23]. Overall, this investigation highlights the transcriptional response in AD and provides evidence for the involvement of specific genes in disease progression. The identification of DEGs associated with AD markers represents a significant step towards understanding the molecular mechanisms underlying the disease and may contribute to the development of novel diagnostic tools and therapeutic interventions. However, further research is required to validate these findings and elucidate the functional significance of the identified genes in AD pathogenesis. The National Centre for Biotechnology Information's NCBI-GEO (https://www.ncbi.nlm.nih.gov/gds) database provided the microarray gene expression datasets utilized in this study. In order to arrive at these conclusions, we used 31 different microarrays to look at the gene expression in 22 AD patients of varying severity as well as nine healthy controls. The expression of each gene was then compared to neurofibrillary tangle (NFT) and Mini-Mental Status Examination (MMSE) scores in all 31 patients, regardless of disease. The results of this research showed that thousands of genes had a significant transcriptional response that was closely correlated with AD indicators. The connection of hundreds of these genes with AD markers only included controls and patients with early-stage AD. The criterion for selection were P 0.01 for $|\log(\text{foldchange})| > 2$ and P 0.01 for DEGs.

4.2 Functional Enrichment Analysis

In this study, the differentially expressed genes (DEGs) identified from the microarray AD datasets were further subjected to the pathway and gene ontology (GO) investigations utilising the web-based Enrich tool for gene set enrichment analysis (GSEA) EnrichR, available at https://maayanlab.cloud/Enrichr/, provides a comprehensive platform for gene list annotation and functional enrichment analysis.

We aimed to gain insights into the biological processes (BP) and pathways associated with the shared DEGs in AD. To accomplish this, the biological process Gene Ontology (GO) database was utilized as an annotation source in the EnrichR analysis.[24] The significance threshold for GO analysis was set at a p-value of 0.05, ensuring the identification of enriched functional categories relevant to AD

Additionally, pathway enrichment from the KEGG (Kyoto Encyclopaedia of Genes and Genomes) was analyzed with a statistical significance cutoff set at the highest p-value of 0.05. KEGG pathways provide valuable information about the molecular interactions and networks involved in specific biological processes or diseases, allowing us to understand the underlying mechanisms and potential therapeutic targets. By utilizing EnrichR and the GO database, the researchers aimed to uncover the enriched biological processes associated with the shared DEGs in AD. This analysis offers important insights into the functional roles and molecular mechanisms of these genes in the context of AD pathology.[25]

The KEGG pathway enrichment analysis, on the other hand, offers a comprehensive overview of the specific pathways that are significantly enriched among the shared DEGs. By identifying the pathways associated with AD, this analysis provides a more focused understanding of the molecular processes and networks that support the onset

and development of the disease.

By conducting these analyses, the researchers aimed to uncover the biological processes and pathways that are dysregulated in AD, shedding light on the underlying molecular mechanisms. This information can potentially contribute to the the discovery of novel treatment targets and the development of more targeted interventions for AD.

It is crucial to remember that the utilization of EnrichR and the GO and KEGG databases provides a systematic and comprehensive approach to functional enrichment analysis. The web-based nature of EnrichR allows for easy access to these resources, facilitating the analysis of gene expression data and enabling researchers to gain insights into the biological relevance of their findings.

In summary, the shared DEGs from the microarray AD datasets were subjected to pathway and GO analyses using EnrichR. The utilization of EnrichR and the GO and KEGG databases allowed to identify enhanced biological pathways and activities connected to AD. This comprehensive analysis provides valuable insights into the functional roles of the shared DEGs and contributes contribute to our knowledge of the cellular mechanisms driving AD pathogenesis. The findings from these analyses may guide future research efforts and the development of novel therapeutic strategies for AD.

4.3. Network analysis of Protein-Protein Interactions (PPI)

An overview of the common protein-protein interactions (PPI) network DEGs was made using the web-based visualisation tool STRING (https://string-db.org/). A database called the PPI network provides correlations between the structural and functional characteristics of proteins. To investigate the frequent differentially

expressed genes (DEGs) discovered in the study and their protein-protein interactions, By using STRING, I aimed to gain insights into the physical and functional associations among the common DEGs in the context of (AD)[26]. PPI networks are valuable tools for understanding the intricate relationships and interplay between proteins, as they can reveal potential protein complexes, functional modules, and signalling channels involved in disease processes. The construction of the PPI network through STRING allowed to visualize the interactions between the proteins encoded by the common DEGs. The tool utilizes a mixture of investigational data, text mining, and computational predictions to generate the network, providing a comprehensive view of the protein interaction .The PPI network created using STRING can assist in identifying key hub proteins that play crucial roles in AD pathogenesis. Hub proteins are highly connected within the network and often serve as central regulators or mediators of various biological processes. Analyzing the network topology, including degree centrality and betweenness centrality measures, can help prioritize specific DEGs for further investigation and potential therapeutic targeting. Moreover, STRING offers additional functional annotations and features for the proteins within the PPI network. These annotations may include gene ontology (GO) terms, protein domains, protein families, and pathways in which the proteins are involved. By exploring these annotations, researchers can gain a deeper understanding of the biological functions pathways associated with the common DEGs in AD. Overall, the utilization of the STRING database and visualization tool allowed for building a network of protein-protein interactions, providing insights into the physical and functional associations among the common DEGs in AD. This network-based approach facilitates the documentation of key proteins and signaling ways involved in the disease, offering valuable clues for further research and potential therapeutic

interventions It is important to note that while the STRING database provides a comprehensive resource for PPI information, the generated network should be interpreted in the context of the specific study and additional experimental validation is often necessary to confirm the interactions.[27] Nonetheless, the utilization of STRING enhances our understanding of the complex protein interactions and networks underlying AD, contributing to the overall knowledge of the disease and potentially aiding in the advancement of targeted therapies.

4.5 Data Collection For Molecular Docking

In this study, a web-based open-source project called COCONUT (Collection of Open Natural Products) was utilized as a valuable resource for storing, searching, and analyzing natural products. The COCONUT platform, accessible at https://coconut.naturalproducts.net/, offers a comprehensive database of natural compounds and facilitates their exploration for various applications, including drug discovery and development. In the context of AD, I focused on identifying natural products with potential therapeutic properties. To establish reference standards for their investigation, they selected 31 natural compounds known for their anti-inflammatory capabilities. Additionally, the well-known natural chemical Huperzine A, which has shown promise in AD research, was included as a reference compound.

To facilitate the analysis and computational modeling of these natural compounds, the researchers obtained the SDF (Structure-Data File) structures of the selected medicines from the PubChem database. These structures provide a representation of the chemical composition and three-dimensional arrangement of the compounds, enabling further analysis and virtual screening.[28] For molecular docking, the RAB3B gene was chosen as the target of interest. The obtained PDB (Protein Data Bank) structure

corresponding to the RAB3B protein, which served as the receptor for the docking studies. The PDB structure contains detailed information about the three-dimensional structure of the protein, enabling the researchers to simulate the interactions between the protein and the natural compounds.

To prepare the ligands (the natural compounds) and the protein for the docking simulations, We used Discovery Studio, a software package widely employed in computational drug discovery. The ligands were translated into the mol2 format, which is compatible with the docking software. To execute the docking simulations, the researchers employed SwissDock, a web-based docking server that utilizes molecular docking algorithms to predict the binding interactions between the ligands and the protein receptor. SwissDock performs a thorough analysis of the ligand-protein interactions and generates docking scores to evaluate the binding affinity and potential efficacy of the natural compounds. By utilizing COCONUT, PubChem, Discovery Studio, and SwissDock, I was able to conduct virtual screening and molecular docking studies of 31 natural compounds, along with Huperzine A, against the RAB3B protein. These computational methods provide insights into the potential interactions and binding affinities of the natural compounds with the target protein.

It is important to note that while computational docking studies offer valuable insights, they are preliminary in nature and require further experimental validation to confirm the actual binding and biological activity of the identified natural compounds. Nonetheless, these computational approaches serve as valuable tools , aiding in the identification of potential lead compounds for further investigation.

In summary, the integration of COCONUT, PubChem, Discovery Studio, and SwissDock enabled the researchers to explore and analyze natural compounds for their

potential as treatments for AD. The use of molecular docking simulations allowed for the evaluation of the binding interactions between the selected natural compounds and the RAB3B protein. These computational methods provide a starting point for further investigation and the identification of potential lead compounds with therapeutic potential against AD.

4.6 ADMET Analysis

In our research, we utilized the web-based tool SwissADME to predict ADME (Absorption, Distribution, Metabolism, and Excretion) parameters, which are crucial in drug discovery and development. SwissADME, available at http://www.swissadme.ch/, is a valuable platform for assessing various physicochemical properties and pharmacokinetic parameters of small molecules, providing insights into their drug-likeness and potential suitability as therapeutic agents. To conduct the analysis, we retrieved the Canonical SMILES (Simplified Molecular Input Line Entry System) representations of the fifty ligands from PubChem. The Canonical SMILES provides a concise and standardized illustration of the molecular structure, facilitating further computational modeling and analysis. Using SwissADME, we evaluated several important parameters related to drug discovery. One of these parameters was water solubility, which indicates the compound's ability to dissolve in water. Water solubility is a crucial consideration in drug formulation and delivery, as compounds with higher water solubility are often more easily absorbed and distributed within the body.

We also assessed lipophilicity, which reflects the compound's affinity for lipid environments. Lipophilicity plays a substantial role in the compound's capability to

permeate biological membranes, impacting its absorption and distribution in the body. Evaluating lipophilicity helps us understand how well the compound can traverse biological barriers and reach its target site. Furthermore, SwissADME provided insights into various pharmacokinetic parameters, including absorption, distribution, metabolism, and excretion. These parameters influence the compound's bioavailability, tissue distribution, metabolic stability, and elimination from the body. Analyzing these parameters aids in predicting the compound's efficacy and safety profile.

In addition to ADME parameters, SwissADME allowed us to assess drug-likeness parameters. Drug-likeness refers to the compound's resemblance to known drugs and their likelihood of successful development. By analyzing drug-likeness parameters such as molecular weight, number of rotatable bonds, and the presence of specific functional groups, we gained insights into the compound's potential as a drug candidate.

By employing SwissADME, we were able to analyze and evaluate the virtual physicochemical properties and pharmacokinetic parameters of the fifty ligands. This analysis provided us with valuable information about the compounds' drug-like characteristics, solubility, permeability, and potential suitability for further development

as therapeutic agents. It is important to acknowledge that while computational tools like SwissADME offer valuable insights, they serve as initial assessments and require experimental validation to confirm the actual ADME properties of the compounds.[29] Nevertheless, these computational approaches are essential in the early stages , enabling rapid screening and prioritization of potential drug candidates based on their predicted ADME profiles. In conclusion, the utilization of SwissADME in our

research allowed us to predict and analyze the ADME parameters, physicochemical properties, and drug-likeness characteristics of the fifty ligands. This assessment provided valuable insights into their potential as drug candidates, aiding in the identification of lead compounds for further investigation in the drug discovery process.

CHAPTER-5

RESULTS

5.1 Differently Expressed Genes (DEGs)

We compared samples from people with AD to those from people without the condition in an analysis of two microarray datasets. With the use of this research, we were able to pinpoint a group of 31 genes that showed notable variations between healthy samples and AD cases.

5.2 Functional Enrichment Analysis

The biological process (BP) GO and KEGG pathway structural enrichment investigation was conducted utilizing 31 DEGs to demonstrate the molecular pathways and functions related to AD. The picture displays a bar graph of KEGG pathways linked to AD and the central nervous system

TABLE I. KEGG Human Pathway

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Genes
GDP binding (GO:0019003)	Jan-67	0.089736	0.162024	0	0	11.17059	RAB3B
ligand-gated cation channel activity (GO:0099094)	Jan-80	0.106224	0.180764	0	0	9.326301	GRIN1
amyloid-beta binding (GO:0001540)	Jan-80	0.106224	0.180764	0	0	9.326301	GRIN1
calcium channel activity (GO:0005262)	Jan-84	0.111239	0.180764	0	0	8.875056	GRIN1
ion channel activity (GO:0005216)	Jan-84	0.111239	0.180764	0	0	8.875056	GRIN1
voltage-gated cation channel activity (GO:0022843)	Jan-97	0.127351	0.194363	0	0	7.66821	GRIN1
cation channel activity (GO:0005261)	Jan-98	0.128579	0.194363	0	0	7.588774	GRIN1
thiol-dependent deubiquitinase (GO:0004843)	1/106	0.13834	0.199825	0	0	7.00776	USP9Y
deubiquitinase activity (GO:0101005)	1/112	0.145592	0.203015	0	0	6.62696	USP9Y
guanyl ribonucleotide binding (GO:0032561)	1/214	0.260232	0.327905	0	0	3.43575	RAB3B

TABLE II. GO MOLECULAR FUNCTION

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Genes
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5.3 PPI NETWORK ANALYSIS

By contrasting normal samples with AD cases, the two chosen microarray datasets were examined. We discovered 31 genes.

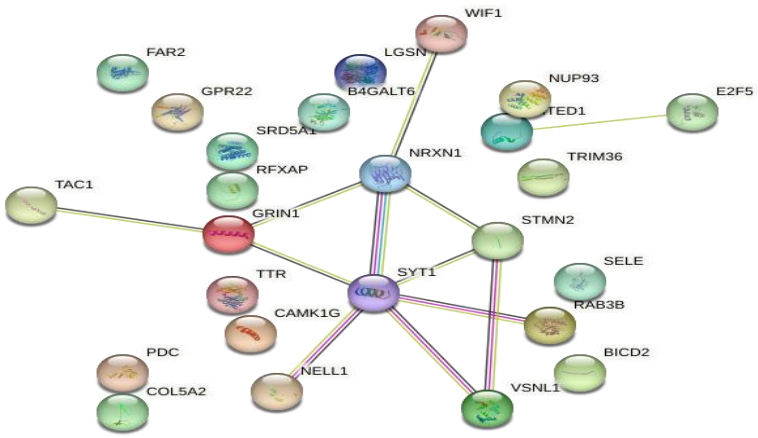

Fig 4. The PPI network of 31 genes

5.4. Molecular Docking Result

SwissDock was used in studying the natural compounds with interactions of RAB3B protein. Out of 40 natural compounds docked, 6 compounds met the required parameters binding energy less than - 7.12 Kcal/mol (energy of standard compound). Out of these six, Stilbostemin C has the most negative binding energy which suggests it has the best binding affinity with the protein[30].

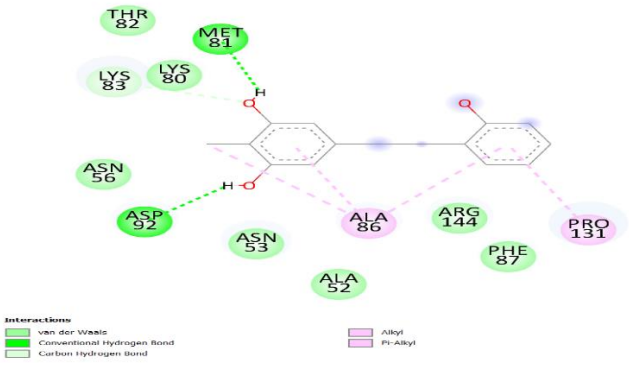

Figure 5. Structure of Stilbostemin C

5.4 ADMET Analysis

ADMET analysis was performed using SwissADME and Stilbostemin C was found to have high GI absorption capacity and BBB permeant. Lipinski rule is one of the most popular parameters used to evaluate the drug-likeness of small molecules. With a bioavailability score of 0.55 and 0 violations, Stilbostemin C satisfies with Lipski rule's drug-likeness standards for bioavailable drugs.

TABLE III. MOLECULAR DOCKING AND ADME/T ANALYSIS

Name	Cluster	Element	FullFitness (kcal/mol)	Estimated ΔG (kcal/mol	GI absorption	Lipinski	BBB permeant	Log Kp (skin permeation)
Huperzine A (standard)	0	0	-5215	-7.12	High	Yes; 0 violation	Yes	-7.77 cm/s
Dipeptidyl peptidase I (DPPI) \	38	1	-5166	-5.31	High	Yes; 0 violation	Yes	-5.66 cm/s
Helenalin	12	0	-5210	-7.19	high	Yes; 0 violation	Yes	-7.47 cm/s

Spiramine U	0	0	-5013	-6.98	High	Yes; 0 violation	Yes	-6.97 cm/s
Glaucocalyxin A	11	0	-5199	-7.36	High	Yes; 0 violation	Yes	-7.10 cm/s
Stilbostemin c	0	0	-5253	-6.88	High	Yes; 0 violation	Yes	-5.35 cm/s
Ribaliprenylene	0	0	-5198	-7.63	High	Yes; 0 violation	Yes	-5.99 cm/s
Jerantinine E	0	3	-5195	-7.21	High	Yes; 0 violation	Yes	-6.44 cm/s
Toddaliopsin A	16	0	-5172	-7.18	High	Yes; 0 violation	Yes	-5.99 cm/s

CHAPTER-6

CONCLUSION

Alzheimer's disease (AD) is a common neurodegenerative condition marked by the buildup of aberrant proteins, such as tau and amyloid-beta (Aβ), which impairs memory and causes cognitive decline. As a major factor in the onset and progression of AD, dysfunction in neuronal autophagy, a cellular process in charge of the breakdown and recycling of cellular components, has been suggested. The poor clearance of tau and Aβ proteins in AD adds to their buildup in the brain via dysregulating autophagy. By affecting the activity of γ- and α-secretases and regulating the recycling of amyloid precursor protein (APP), autophagy plays a critical role in controlling the development of Aβ. These aberrant proteins are less likely to be cleared when autophagy is compromised, which exacerbates their accumulation. In order to treat AD, there is considerable interest in using the autophagy-lysosome pathway as a therapeutic approach. The progression of the disease may be slowed or stopped by improving autophagy, which will increase the removal of soluble and aggregated tau and Aβ proteins. In this work, a cohort of AD patients and healthy controls underwent gene expression profiling utilizing microarrays. RAB3B, one of the genes under investigation, was discovered to be connected to the autophagy-lysosomal pathway that contributes to the buildup of aberrant proteins in AD. This shows that RAB3B may be useful as a predictive indicator for assessing autophagy failure in AD. Additionally, molecular docking studies were carried out to investigate new AD treatment options that target RAB3B.

Among the examined substances, stilbostemin C demonstrated promise as a potential Alzheimer's disease treatment. Additional research, including ADMET experiments on absorption, distribution, metabolism, excretion, and toxicity, revealed that stilbostemin C follows the Lipinski rule, can cross the blood-brain barrier, and exhibits drug-like qualities. The results of this work demonstrate the importance of autophagy failure in Alzheimer's disease and imply that targeting the autophagy-lysosome pathway, perhaps by modulating RAB3B, could be a useful therapeutic strategy. Due to its anticipated molecular interactions with RAB3B and advantageous ADMET features, stilbostemin C emerges as a promising option for more research

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STILBOSTEMIN C AS A POTENTIAL CANDIDATE FOR THERAPEUTIC TARGETING OF RAB3B PROTEIN IN COUNTERING ALZHEIMERS

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***Abstract*— Alzheimer's disease (AD), the most common form of dementia, affects more than 20 million individuals globally. One of the main mechanisms driving the emergence of neurodegenerative illnesses, including AD, is dysfunction in neuronal autophagy. Autophagy is the only route for organelle turnover in cells and is crucial for breaking down aggregated and normal proteins, especially in stressful or injured situations**[1]**. The endocannabinoid system, the mTOR pathway, neuroinflammation, and the metabolism of Aβ and tau protein are all important aspects of autophagy that may all work together to mediate its impact on AD**[2]**. As a result, drugs that target autophagy may lead to the development of novel therapeutic strategies for the treatment of AD. Tau protein tangles inside of cells and amyloid-β containing neuritic plaques are two features of Alzheimer's disease (AD). This kind of pathology demonstrates unequivocally that AD compromises the systems of neuronal housekeeping and protein quality regulation. Growing data suggest that autophagosome-lysosomal degradation can be hindered, which could interfere with the processing of Aβ plaques and cause AD pathogenesis. The GEO datasets provide publicly available gene expression and clinical data on AD patients to study the prognosis of patient death rates and thereby identify AD biomarkers. In this study, we used the GDC data portal to analyze linked genes with clinical data to determine the most significant AD genes using functional enrichment analysis and protein-protein interaction (PPI). In order to distinguish the genes with different expression levels between the mutant and normal groups, we identified differential expression genes (DEGs). RAB3B, a member of the small GTP-binding protein RAB family that is involved in cell autophagy, is being used. There are several potential mechanisms that could inhibit the function of the RAB3B protein interactome and thus impair autophagy and promote AD pathology. Furthermore, we used the GDC Data Portal to estimate their significance in AD. A natural substance called Stilbostemin C might be evaluated as a therapy option. For a better understanding of the interactions between the bioactive substances and the RAB3B protein, molecular docking research was performed. According to the molecular docking analysis, Stilbostemin C has a stronger affinity for RAB3B. The ADME/T study also revealed the potential of Stilbostemin C for further research.**

Keywords*— *Alzheimer's, Datasets, Differential expression genes, Biomarkers, RAB3B protein, Stilbostemin C

I. Introduction

As the world's population ages, Alzheimer's disease (AD), the most common neurological condition affecting the elderly, will have an adverse societal and economic impact. AD is hypothesized to be caused by a number of pathogenic mechanisms, including tau phosphorylation, neuroinflammation, neurotransmitter dysregulation, and oxidative stress. It is characterised by the development of neurofibrillary tangles (NFTs) made of tau protein and senile plaques, which are largely made of amyloid β (Aβ), as well as a progressive and irreversible loss of cognitive function[3].
The metabolism of Aβ is dysregulated in AD patient's brains, which causes Aβ to build up and aggregate. Autophagy has a significant impact on how the Aβ and tau proteins are metabolized. Organelles and proteins are destroyed during autophagy, a lysosome-dependent, homeostatic process, and then recycled into energy. The accumulation of harmful proteins in the AD brain is therefore thought to be caused by autophagy which is dysfunctional[4].

Too much or too little autophagic activity in neurons impairs their homeostasis and survival rate, resulting in neurodegeneration. Moreover, neurons feature intercellular connection structures like axons, dendrites, and synapses that need the reciprocal transport of proteins, organelles, and autophagosomes over significant distances from the soma. Neurodegeneration is caused by defects in autophagy, which disrupt intercellular communication[5]. Due to its historically known involvement in hunger and other mitigating harsh circumstances including oxidative damage and organelle damage, autophagy is widely regarded as a pro-survival activity.

In numerous neurodegenerative illnesses, a significant buildup of autophagic vacuoles, including autophagosomes or autolysosomes, has been seen in the affected brain region.

However, the precise involvement of autophagy in each neurodegenerative illness is still unknown. Sustained autophagy activation, disturbance of the balance between autophagosome production and lysosomal fusion, and impairment of lysosomal components are thought to contribute to nervous system degeneration, as neurons are more prone to protein mis-regulation due to their postmitotic condition[6].
In doing so, we hope to alter the autophagy pathway that ultimately results in Alzheimer's disease. A subset of the Ras superfamily are the low-molecular-weight GTPases known as Rab proteins. By tethering/docking vesicles to their appropriate compartments, Rab GTPases function as molecular switches that alternate between GTP-bound active and GDP-bound inactive forms to regulate vesicle transit to the proper destinations[7].

Small GTP-binding protein RAB3B, a member of the RAB family that has been linked to drug resistance and cell autophagy, has been described as a key oncogene in a number of malignancies and neurodegenerative illnesses.
To stop the buildup of amyloid Aβ plaques, we will target the rab3b gene, which is implicated in autophagy.

II. Materials and Methods

A. *Data set*

The microarray gene expression datasets used in this investigation were obtained from the NCBI-GEO (https://www.ncbi.nlm.nih.gov/gds) database maintained by the National Centre for Biotechnology Information. For these results, we used 31 different microarrays to examine the gene expression in nine healthy controls and 22 AD patients of differing severity. The expression of each gene was then compared across all 31 subjects, independent of illness, to Mini-Mental Status Examination (MMSE) and neurofibrillary tangle (NFT) scores. The results of these studies showed a substantial transcriptional response including thousands of genes that were significantly linked with AD indicators. Only control and participants with early-stage AD were included in the correlation of several hundred of these genes with AD markers. The selection criteria were |log(foldchange)| > 2 and P 0.01 for DEGs.

B. *Functional Enrichment Analysis*

All of the DEGs that were shared by the microarray AD datasets were subjected to the pathway and GO analyses using the web-based gene set enrichment analysis (GSEA) tool EnrichR (https://maayanlab.cloud/Enrichr/). The biological process (BP) Gene Ontology (GO) was employed as an annotation source in this research, and its threshold was set at P 0.05. The analysis of KEGG pathway enrichment was declared statistically significant at the highest p-value of 0.05. do not revise any of the current designations.

C. *Protein-Protein Interactions (PPI) Network Analysis*

The web-based visualization tool STRING (https://string-db.org/) was used to create a protein-protein interactions (PPI) network of the common DEGs. The PPI network is a database that offers correlations between proteins' physical and functional properties.

D. *Data Collection For Molecular Docking*

A web-based open-source project called COCONUT (Collection of Open Natural Products) is used to store, search for, and analyze natural products (https://coconut.naturalproducts.net/) . 31 natural compounds with anti-inflammatory capabilities and the well-known natural chemical Huperzine A were used as standards for reference when identifying natural products as a treatment for AD. We downloaded the SDF structures of these medicines from PubChem. For molecular docking, the RAB3B gene was chosen, and the PDB structure was obtained. RAB3B was ready to use Discovery Studio to perform docking. Using Discovery Studio, 40 ligands were translated into mol2 format. After the ligands and proteins were ready, SwissDock was used to execute docking.

E. *ADMET Analysis*

SwissADME which is a web-based tool allowed us to predict ADME parameters for drug discovery (http://www.swissadme.ch/). The Canonical SMILES were taken from PubChem and the virtual physicochemical properties of all fifty ligands are analyzed using the Swiss ADME tools, including water-solubility, lipophilicity, pharmacokinetics, and drug-likeness parameters.

III. RESULTS

A. *Differently Expressed Genes (DEGs)*

The two selected microarray datasets were analysed by comparing normal samples with AD cases. We identified 31 genes.

B. *Functional Enrichment Analysis*

The biological process (BP) GO and KEGG pathway structural enrichment investigation was conducted utilising 31 DEGs to demonstrate the molecular pathways and functions related to AD. The picture displays a bar graph of KEGG pathways linked to AD and the central nervous system.

TABLE 1. KEGG Human Pathway

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Cell adhesion molecules	2/148	0.018127	0.257672	0	0	10.44573	41.89082	NRXN1; SELE
Calcium signalling pathway	2/240	0.044179	0.257672	0	0	6.378151	19.89669	CAMK1G; GRIN1
African trypanosomiasis	Jan-37	0.05056	0.257672	0	0	20.51029	61.21495	SELE
Primary immunodeficiency	Jan-38	0.051892	0.257672	0	0	19.95495	59.03873	RFXAP
Cocaine addiction	Jan-49	0.066422	0.257672	0	0	15.37346	41.68871	GRIN1
Malaria	Jan-50	0.067732	0.257672	0	0	15.05896	40.54171	SELE
Neuroactive ligand-receptor interaction	2/341	0.081967	0.257672	0	0	4.454958	11.14381	TAC1; GRIN1
Steroid hormone biosynthesis	Jan-61	0.082028	0.257672	0	0	12.29136	30.73698	SRD5A1
Long-term potentiation	Jan-67	0.089736	0.257672	0	0	11.17059	26.93099	GRIN1
Amyotrophic lateral sclerosis	2/364	0.091619	0.257672	0	0	4.167021	9.959667	NUP93; GRIN1
TNF signaling pathway	1/112	0.145592	0.260474	0	0	6.62696	12.76979	SELE
Huntington disease	1/306	0.350793	0.367498	0	0	2.388221	2.5018	GRIN1
Alzheimer disease	1/369	0.406537	0.415991	0	0	1.973027	1.775885	GRIN1
Pathways of neurodegeneration	1/475	0.490071	0.490071	0	0	1.523519	1.086583	GRIN1

II. TABLE 2. GO MOLECULAR FUNCTION

Term	Overlap	P-value	Adjusted P-value	Old P-value	Old Adjusted P-value	Odds Ratio	Combined Score	Genes
GDP binding (GO:0019003)	Jan-67	0.089736	0.162024	0	0	11.17059	26.93099	RAB3B
ligand-gated cation channel activity (GO:0099094)	Jan-80	0.106224	0.180764	0	0	9.326301	20.91147	GRIN1
amyloid-beta binding (GO:0001540)	Jan-80	0.106224	0.180764	0	0	9.326301	20.91147	GRIN1
calcium channel activity (GO:0005262)	Jan-84	0.111239	0.180764	0	0	8.875056	19.49027	GRIN1
ion channel activity (GO:0005216)	Jan-84	0.111239	0.180764	0	0	8.875056	19.49027	GRIN1
voltage-gated cation channel activity (GO:0022843)	Jan-97	0.127351	0.194363	0	0	7.66821	15.80269	GRIN1
cysteine-type peptidase activity (GO:0008234)	Jan-98	0.128579	0.194363	0	0	7.588774	15.56619	USP9Y
cation channel activity (GO:0005261)	Jan-98	0.128579	0.194363	0	0	7.588774	15.56619	GRIN1
cysteine-type endopeptidase activity (GO:0004197)	1/105	0.137126	0.199825	0	0	7.075499	14.05799	USP9Y
thiol-dependent deubiquitinase (GO:0004843)	1/106	0.13834	0.199825	0	0	7.00776	13.86161	USP9Y
deubiquitinase activity (GO:0101005)	1/112	0.145592	0.203015	0	0	6.62696	12.76979	USP9Y
GTPase binding (GO:0051020)	1/201	0.246492	0.320439	0	0	3.661481	5.12764	BICD2
guanyl ribonucleotide binding (GO:0032561)	1/214	0.260232	0.327905	0	0	3.43575	4.625147	RAB3B

C. PPI NETWORK ANALYSIS

By contrasting normal samples with AD cases, the two chosen microarray datasets were examined. We discovered 31 genes.

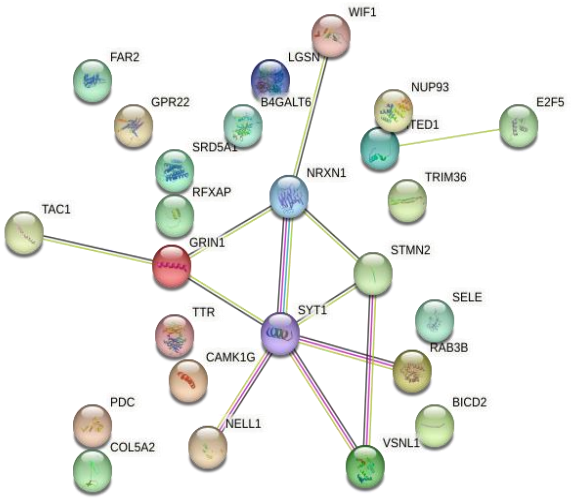

Fig 1. The PPI network of 31 genes

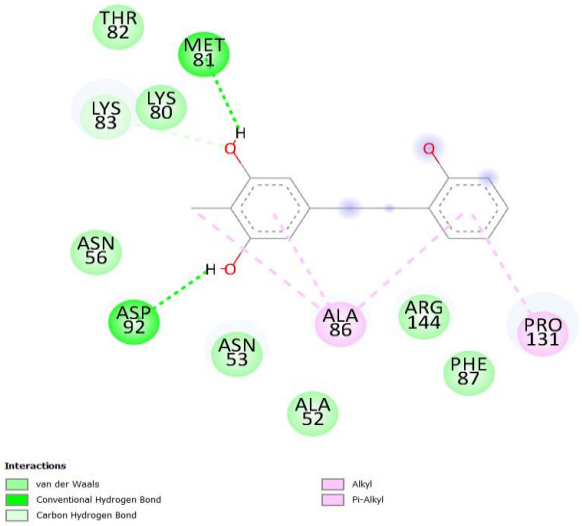

Fig 2. Structure of Stilbostemin C

D. MOLECULAR DOCKING RESULT

SwissDock was used in studying the natural compounds with interactions of RAB3B protein. Out of 40 natural compounds docked, 6 compounds met the required parameters binding energy less than - 7.12 Kcal/mol (energy of standard compound). Out of these six, Stilbostemin C has the most negative binding energy which suggests it has the best binding affinity with the protein[8].

E. ADMET ANALYSIS

ADMET analysis was performed using SwissADME and Stilbostemin C was found to have high GI absorption capacity and BBB permeant. Lipinski rule is one of the most popular parameters used to evaluate the drug-likeness of small molecules. With a bioavailability score of 0.55 and 0 violations, Stilbostemin C satisfies with Lipski rule's drug-likeness standards for bioavailable drugs

III. TABLE 3. MOLECULAR DOCKING AND ADME/T ANALYSIS

Name	Cluster	Element	FullFitness (kcal/mol)	Estimated ΔG (kcal/mol	GI absorption	Lipinski	BBB permeant	Log Kp (skin permeation)
HuperzineA (standard)	0	0	-5215	-7.12	High	Yes; 0 violation	Yes	-7.77 cm/s
Dipeptidyl peptidase I (DPPI) \	38	1	-5166	-5.31	High	Yes; 0 violation	Yes	-5.66 cm/s
Helenalin	12	0	-5210	-7.19	high	Yes; 0 violation	Yes	-7.47 cm/s
Spiramine U	0	0	-5013	-6.98	High	Yes; 0 violation	Yes	-6.97 cm/s
Glaucocalyxin A	11	0	-5199	-7.36	High	Yes; 0 violation	Yes	-7.10 cm/s
Stilbostemin c	0	0	-5253	-6.88	High	Yes; 0 violation	Yes	-5.35 cm/s
Ribaliprenylene	0	0	-5198	-7.63	High	Yes; 0 violation	Yes	-5.99 cm/s

CONCLUSION

A highly controlled, universal, and conserved eukaryotic transportation mechanism called autophagy transports cytoplasmic cargo to lysosomes for degradation. Autophagy contributes significantly to Aβ production by influencing β - and γ-secretases and controlling APP recycling. When autophagy is impaired, tau and Aβ protein clearance is reduced, and their accumulation is accelerated. The clearing of soluble and aggregated Aβ and tau proteins is improved by the induction of autophagy. As a result, regulating autophagy-lysosome protein degradation is becoming a key therapeutic focus for AD. In contrast to this approach, in this study, 31 different microarrays were used to examine the gene expression of 22 AD patients and nine healthy controls with varying degrees of the disease. Out of 31, it was discovered that the RAB3B gene is connected to the autophagy lysosomal pathway in the build-up of aberrant proteins that cause Alzheimer's disease. RAB3B may serve as a reliable prognostic indicator for controlled autophagy. Stilbostemin C may be an effective Alzheimer's drug, based on the molecular docking of the RAB3B genes with 40 natural compounds. Following the Lipinski rule, having BBB that permeates, and being more drug-like are all conclusions drawn from the ADMET study of Stilbostemin C.

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