Chapter-V

NOVEL THERAPEUTIC STRATEGIES FOR ALZHEIMER'S DISEASE

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Abstract--- Alzheimer's disease (AD) is an advanced stage of dementia that gradually worsens memory loss, impairs thinking abilities, and makes it harder to complete basic tasks. The disease is called Dr. Alois Alzheimer, in the year 1906. The loss of connection between neurons in the brain, which transmits information to the body's organs, is another effect of AD. The hippocampus, which stores memories, is first affected by AD. Later, it spreads to other parts of the brain and causes it to shrink. The brain would have considerably decreased by the time AD reached its most severe stage. It has been noted that not every memory loss issue results in AD. The majority of AD symptoms vary from patient to patient and are not universal. Not all patients with moderate cognitive impairment (MCI) develop Alzheimer's disease (AD), despite MCI being a warning indication of AD. Because AD patients may not be able to perform their own responsibilities, the majority of them get anxious, and some of them become more aggressive. These drugs simply stop the decline of cognitive capacities and momentarily relieve symptoms. In this article, we first address the drawbacks of anti-Aβ treatments, such as their low effectiveness and possible side effects, which emphasize the need to look into different targets and processes. We next go over intriguing non-Aβ-based approaches such gene and stem cell therapy, neuroinflammation management, and tau-targeted therapeutics. These approaches offer new therapeutic possibilities for AD by tackling downstream consequences and other disease markers besides Aβ buildup.

Keywords--- Alzheimer's Disease, Dementia, Neuroinflammation, Amyloid, Tau, and Aducanumab.

1. INTRODUCTION

Alzheimer's disease (AD) is the most common type of dementia among the elderly, accounting for up to 70% of cases. In 2010, there were about 36 million AD cases worldwide, but the number of new cases is increasing by 7.7 million annually (Wang & Zhang, 2018). By 2050, there are projected to be 144 million AD patients worldwide (Gomez-Ramirez and Wu, 2014). One in three elderly people dies from Alzheimer's disease or a related dementia. According to the Alzheimer's Association (2013), AD is currently the sixth most common cause of death among all illnesses. According to the Alzheimer's and Related Disorders Society of India (2010), 50–75% of the 3.7 million Indians who have dementia have severe AD, and by 2030, this number is predicted to quadruple. The most prevalent type of age-related dementia, AD, is typified by a progressive loss of memory and deteriorating cognitive function, which eventually results in a decline in bodily functions and death. The neuropathological features of AD include senile plaques and protein clusters termed neurofibrillary tangles that are created in the brain. The intracellular bundles of paired helical filaments known as neurofibrillary tangles are mainly composed of tau, a microtubule-associated protein, which is abnormally phosphorylated (Ibrahim & Gabr, 2019).

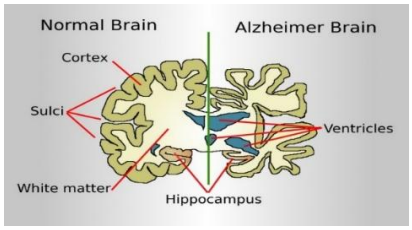

Figure 1: Alzheimer Disease

Dementia is not a huge health concern because of the aging population. Multiple cognitive deficiencies that are severe enough to interfere with day-to-day activities describe this clinical condition. The deficits include impairment of memory and any other cognitive domain disorder like agnosia, apraxia, aphasia, and so on. 60 to 70 percent of all cases of dementia in the elderly are caused by Alzheimer's disease (AD). A combination of several factors has made this a relevant issue in science and public health. The need to identify prophylactics that

can reduce risk or delay the onset of this is critical from the standpoint of choices of lifestyle. Here is an increase in a body of evidence to support the premise that intake of coffee/caffeine can bring down the risk of AD or delay its onset. This began with an epidemiologic human study and was supported by a controlled study in AD transgenic mice (Ramesh & Govindaraju, 2022). These studies gave an insight into the mechanisms where coffee/caffeine protects against AD even in the case of Mild Cognitive Impairment (MCI). The studies of Epidemiology have supported coffee/caffeine as being protective against such impairment and AD these studies though insightful will not be providing direct evidence to the prophylactic effect of coffee/caffeine against AD as this is all largely based on the recall which will not be able to isolate unequivocally the intake of coffee/caffeine from that of the other factors which may affect cognition over an entire lifetime (if they are not fully controlled). Luckily, creating the AD transgenic mice has permitted a highly controlled study that was performed to delve into the details of AD pathogenesis as well as its therapeutic development. The loss of both the structure and function of neurons is generally referred to as neurodegeneration. Although neuronal degradation is the primary cause of all diseases categorized as neurodegenerative disorders (NDDs), each disease affects a particular subset of neurons either physically or functionally. In addition, each neurodegenerative disease is thought to have unique pathogenic processes or causes of illness that culminate in neuronal death.

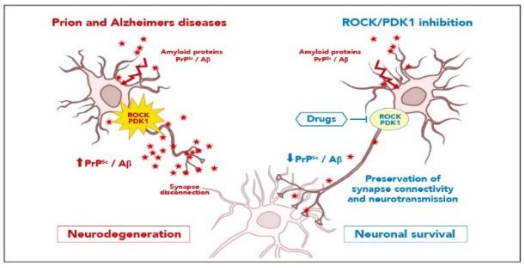

Figure 2: Prion Alzheimer Disease

However, certain diseases exhibit common symptoms due to overlap of the affected regions, either as a result of the disease's course or because of pathological commonality. Too far, over a hundred neurodegenerative diseases have been identified. However, for the above stated reasons, their classification is

still difficult. Typically, diseases are categorized based on the pathological hallmarks, clinical presentation, or the area of the brain that is afflicted.

Age of Onset of AD

While early-onset AD usually appears at age 60 or younger, sporadic AD frequently appears at age 65. In order to evaluate changes in amyloid neta biomarkers, APOE4 carriers with a parental history of sporadic AD may find it helpful to be close to parental symptoms, per a recent study (Villeneuve S. et al, 2018).

Behavioural Symptoms of AD

Memory loss is the most typical sign of AD. New information, significant dates and occasions, individuals, languages, and the use of specific drugs can all be forgotten by patients. Disorientation in time and space is the second most prevalent symptom. Patients occasionally struggle to carry out duties in a familiar location on their own and frequently forget frequently used routes and locations. Planning and problem-solving are often difficult for AD patients. There have been a few documented instances of AD patients with visual impairments, such as trouble reading, determining an object's distance from them, distinguishing colors, and comprehending contrast.

Each AD patient has psychotic symptoms at a different frequency and with varying degrees of aggression or intensity. According to Rosenberg et al. (2015), agitation, apathy, depression, and psychosis (delusions) are the most prevalent neuropsychotic symptoms of AD. Twenty percent of all MCI patients in one population-based study experienced depression, twenty percent displayed significant apathetic symptoms, and fifteen percent displayed extreme agitation. 30 percent of dementia patients displayed indicators of agitation and violence, 32 percent of patients experienced depression, and 36 percent of patients displayed indifference.

2. PATHOLOGY OF ALZHEIMER'S DISEASE

The brain can compensate for neural dysfunctions because of a certain amount of redundancy. The symptoms begin to appear when the damage reaches a point

where the brain can no longer repair itself. The hallmark alterations or pathological characteristics linked to Alzheimer's disease include gliosis, amyloid plaques, neurofibrillary tangles, and extensive neuronal degeneration.

Presenilins

Intramembranous aspartyl proteases include presenilins. Nine transmembrane helix domains are present in both PSI and PSII. Transmembrane domains six and seven contain the aspartyl residues that exhibit catalytic activity (one on each domain). Between transmembrane six and seven, PSI and PSII go through proteolytic processing to create N-terminal and C-terminal fragments that stay connected to one another during complex formation. The motif that distinguishes PSI from PSII enables PSII to interact with AP-1 complexes and, consequently, endosome-lysosome complexes in a phosphorylation-dependent way. PSI has a larger spectrum of target proteins since it lacks this motif.

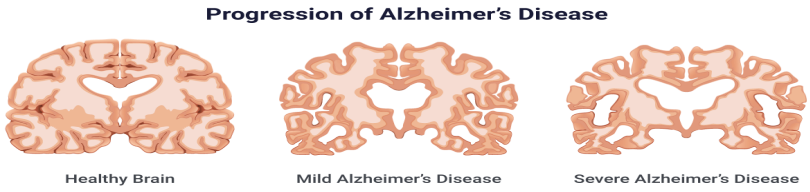

Figure 3: Progression of AD

Nicastrin

A type 1 integral membrane protein is called nicastrin. Its three structural domains are the transmembrane helical domain, the huge N-terminal extracellular ectodomain, and the C-terminal cytoplasmic domain. A conserved DYIGS motif found in the N-terminal domain aids in substrate selection. Only when substrates are cleaved by alpha or beta secretases is their N-terminal revealed. Nicastrin forms a scaffolding molecule with APH-1, which subsequently attaches to PEN-2 and PS1 or PS2. According to recent research, nicastrin is not required for gamma secretase action, despite its involvement in substrate recognition.

Anterior Pharynx-defective 1

The cytoplasmic C-terminal domain, the transmembrane segment, and the extracellular Nterminal area are the three domains of anterior pharynx-defective 1

(APH-1). It is believed that APH-1's interactions with other gamma secretase complex subunits are influenced by the conserved GXXXG motif present in the N-terminal region. Therefore, APH-1A and APH-1B are the two forms of APH-1 found in humans. The three paralogous genes that encode APH-1 in mice produce the three protein products known as APH-1A, APH-1B, and APH-1C. It's unclear what kind of APH-1 attaches to the PS1 or PS2 complex subunits.

Presenilin Enhancer 2

Three domains make up the 12K-Da protein called presenilin enhancer 2 (PEN-2): the transmembrane domain, the cytoplasmic domain at the C-terminus, and the extracellular domain at the N-terminus. There are three helical configurations in the transmembrane domain. PEN-2 primarily participates in the maturation and stabilization of the complex by binding to the fourth transmembrane region of presenilin-1.

Neurofibrillary Tangles

Tau is an axonal protein that stabilizes microtubules by binding to them via the MTBD. It speeds up tubulin polymerization, which facilitates microtubule assembly. Tau binding and detachment from microtubules are controlled by the phosphorylation and dephosphorylation cycles, respectively. The dynamic balance between tau phosphorylation and dephosphorylation is upset by mutations in APP or PS1 in AD, leading to abnormal phoshorylation and a decrease in tau's affinity for microtubules. The aberrantly phosphorylated tau begins to self-assemble into dimers, trimers, higher order oligomers, and protomers when it undergoes a conformational shift.

Gliosis

The process by which glial cells become activated in reaction to a stressor or challenge in the central nervous system is known as gliosis. Because post-mortem AD brains had higher amounts of proinflammatory cytokines and more activated microglia surrounding plaques, neuroinflammation is thought to be a major factor in AD pathogenesis. Glial cells (oligodendrocytes, microglia, and astrocytes) and cytokines that control inflammation are among the biological elements that are essential to the neuroimmune system. The development of AD is closely linked to

immune system dysfunction. The interaction between the chemokine fractalkine (CX3CL1) and its receptor (CX3CL1R) decreases microglia's capacity for phagocytic activity, indicating a reduction in Aβ clearance. Reduced Aβ deposition was the outcome of microglia having insufficient amounts of the NLRP3 inflammasome. It has been demonstrated that increased IL-10 levels result in neuronal dysfunction and 35 losses. While IL-4 and TGF-β inhibit the proinflammatory response, IL-6 and TNF-α mitigate the pathophysiology of AD. Aβ clearance is linked to microglia. Increased aggregation is the outcome of decreased Aβ clearance from the brain caused by compromised microglial function and insufficient phagocytic activity. Additionally, astrocytes can exacerbate immunological reactions. But through the Aβ-degrading enzyme neprilysin, they can also help with Aβ clearance.

Gross Neuronal Loss

Neuron loss is one of the pathogenic characteristics of AD. It is well recognized that AD causes changes in hippocampal volume. AD patients' post-mortem brains exhibit enlarged ventricles and brain atrophy. This is mostly caused by neuronal death and degeneration.

Mild Cognitive Impairment

Cognitive tests are used by the doctor to make the diagnosis (Roberts and Knopman, 2013). In comparison to someone of his own age and educational level, the person exhibits diminished cognitive capacities. The most frequent alterations are in executive processes such as memory and attention. Distinguishing MCI caused by AD from MCI caused by other illnesses is challenging.

3. ALZHEIMER'S DISEASE DEMENTIA

When a person's cognitive abilities steadily decline to the point where they can no longer operate independently, they are frequently diagnosed with dementia. Delirium or other mental disorders are not the source of this cognitive impairment. Health history, family history, and cognitive tests like the MMSE or MoCA are used to diagnose dementia. A person must have at least two of the following deficits, according to the diagnostic criteria, in order to be diagnosed with dementia: altered behaviour or personality, diminished reasoning, decreased language function, impaired visuospatial abilities, or impaired memory for new

knowledge. The diagnosis is dependent on biomarkers, the severity of symptoms, and the rate of advancement, and it can be difficult to differentiate AD from dementia. Biomarkers are measurable components that can be used to determine whether or not a disease is present. Additionally, it can be utilized to ascertain the disease's stage. AD can be identified for a long time since neurological alterations start early in the disease. Better disease management will result from early intervention. Direct and indirect biomarkers are the two categories into which AD biomarkers fall. The pathophysiology of disease is represented via direct biomarkers. For instance, tau and Aβ in AD. Neuronal damage in general, rather than a particular disorder, is represented by indirect biomarkers. Aβ is the most widely used direct biomarker for AD. The CSF of AD patients has lower amounts of Aβ than those of healthy people. Atrophy in the medial, basal, and lateral temporal lobes and decreased absorption of 18-fluorodeoxyglucose (FDG) in the temporo-parietal cortex are other signs of neuronal degeneration.

Positron emission tomography (PET) and magnetic resonance imaging (MRI) are the two main imaging modalities used to investigate or diagnose AD. MRI is used to investigate changes in connectivity and gross anatomy. The distribution of tau and Aβ deposits is investigated using PET. The most conclusive diagnostic approach currently in use combines CSF markers with imaging methods. Long before AD symptoms appear, MRI has revealed structural and functional abnormalities in the brain. It has been demonstrated that cortical thinning is a predictor of cognitive decline. Another MRI-based marker for AD diagnosis is hippocampal volume. Diffusion anisotropy-based magnetic resonance imaging (MRI) has revealed structural alterations in white matter, particularly in the corpus callosum, cingulum bundle, uncinate fasciculus, and frontal and temporal lobes. According to functional MRI research, AD patients' brain activity is altered during the resting state as compared to normal people. According to functional MRI research, AD patients' brain activity is altered during the resting state as compared to normal people.

3XTG

Three genes—APP, PS1, and MAPT—have mutations in the 3XTG model. The mutations include PSEN1-M146V, MAPT-P301L, and APP-KM670/671NL

(Swedish). This model's genetic background is a hybrid between 129X/SvJ and 129S1/Sv. These mice exhibit neurofibrillary tangles in addition to plaques. While the tangled pathology is apparent by 12 months of life, the plaques begin to show as early as 6 months. By the time the child is 7 months old, there are more GFAP-positive cells, a sign of gliosis. By six months of age, synaptic dysfunctions including LTP impairment are evident. As early as four months of age, cognitive impairment manifests as retention-retrieval deficiencies.

APP23

The APP23 model, sometimes referred to as the Swedish model, is characterized by a single mutation in the APP gene (KM670/671NL). This model's genetic background is C57/BL6. The expression of APP is seven times higher in these mice. At six months of age, plaques are initially observed. In this model, neurofibrillary tangles are not present. By nine months of age, gliosis is evident. By 14 months of age, the hippocampal CA1 area has lost neurons. Beginning at three months of age, impaired spatial memory develops quickly. These models have not shown any changes in LTP/LTD till they are 12 months old.

APP/PS1

The Swedish mutation and the L166P mutation in PSEN1 are the two mutations found in APP/PS1 mice. The genetic background of this model is C57/BL6. The alterations are expressed at the Thy1 promoter, and APP expression is three times more. Around three months of life, plaque deposition begins in the hippocampal region, and by five months, it has extended to the cortex. At 4 weeks, there is a loss of spine, and at 17 months, there is a loss of neurons. In water maze trials, cognitive impairment is observed at 7 months of age. There are no tangles.

APPSwe/PSEN1dE9

Two mutations are present in these mice: a PSEN1 mutation (dE9) and a Swedish mutation. A C3:3 mouse with a Swedish mutation and hAPP expression was crossed with an S-9 mouse with a pSEN1 mutation to create the model. Around seven to eight months of age is when plaque deposition begins. Tangles and gliosis were not noticed until the child was 14 months old.

APP NL-F Knock-in

The APP NL-F Knock-in model was created to distinguish between the effects of excessive APP expression and those of excessive Aβ species production. Its two mutations are the Iberian mutation (APP I716" F") and the Swedish mutation (NL). These two mutations are incorporated into the humanized Aβ region and expressed under the mouse APP promoter. The Iberian mutation raises the ratio of Aβ42 to Aβ40, while the Swedish mutation causes overexpression of Aβ. This model's genetic background is C57/BL6. Deposition of plaque begins at six months of age. The investigations have not yet reported on tangles or 41 neuronal losses. At six months of age, activated GFAP-positive cells are visible around the plaques. Together with two other mouse strains, APP NL Knock-in and APP NL-G-F Knock-in, this model was created. There is only a Swedish mutation in APP NL Knock-in and no plaque pathology. Three mutations—Swedish, Iberian, and Arctic—are present in APP NL-G-F Knock-in, which has a more aggressive pathogenesis (APP E693G). As early as two months of age, gliosis and plaque accumulation can be noticed. There aren't any tangles and no evidence of neuronal loss yet. At six months of age, Y-maze tasks reveal memory impairment.

Aβ Related Treatment Approaches

The notion that Aβ species plays a significant role in the pathophysiology of AD is supported by several arguments: First off, the presence of amyloid plaques is one of the primary features of Alzheimer's disease. Second, synaptic toxicity caused by Aβ oligomers has been demonstrated. Thirdly, Aβ42 production is elevated in all the variants linked to early-onset AD. Fourth, amyloid plaque formations are associated with increased pro-inflammatory cytokines, activated microglia, and neuronal injury. Fifth, a higher Aβ load is linked to the risk allele APOE4. Researchers are now pursuing treatment approaches that target Aβ as a result of these findings.

Modulation of Aβ Production

Either α-secretase or β-secretase (BACE) can cleave the Aβ precursor molecule APP, and γ-secretase can then cleave it again. α-secretase cleavage is the non-amyloidogenic pathway since it produces Aβ40. The amyloidogenic pathway is

caused by BACE cleavage, which produces Aβ42, which has a greater tendency to aggregate.

Inhibition of Aß Aggregation

44 Oligomers can be formed by monomeric Aβ, namely Aβ42. Numerous investigations have demonstrated that these oligomers are neurotoxic. Oligomers develop into plaques. In addition to creating stress reactions around themselves, these aggregates are challenging to remove from the brain. Therefore, many groups have examined the strategy of preventing Aβ aggregation. The creation of compounds or peptides that can attach to monomeric fibrils and stop oligomerization has received a lot of attention. Successfully crossing the blood-brain barrier and getting the drug's concentration to approach ED50 in the brain are frequent issues that many of these molecule's encounter. Few inhibitors have advanced to clinical trials as a result of these crucial factors. One such chemical that shown effectiveness in Phase I and Phase II clinical studies as well as animal models was trimiprosate. The Phase III clinical trial was unsuccessful. Another such substance that did not pass a Phase II clinical trial was scyllo-inositol. Additionally, metal-protein attenuating substances—specifically, copper and zinc—are being investigated as possible inhibitors of Aβ aggregation. Clioquinol is one example of such a substance (Nguyen et al., 2021; Aswathy et al., 2019).

Enhancing Aß Clearance

Proteases that break down Aβ and facilitate its removal from the brain include plasmin, insulin-degrading enzyme, and neprilysin. It is challenging to specifically activate these proteases. Aβ42 levels were markedly reduced by PAI-1 inhibitors in animal models of AD. Targeting Aβ transporters like as LRP and RAGE is an additional strategy. Although several RAGE inhibitors are undergoing clinical studies, none of them have demonstrated any noteworthy impact.

Immunotherapy

According to the first set of research on immunotherapy for AD, passive immunization with Aβ42 significantly reduces AD pathology in animal models and produces a highly specific immune response to remove Aβ. As a result, mice developed antibodies against human Aβ42. When these antibodies were

administered to animal models, brain Aβ42 levels were significantly reduced, and cognitive scores improved. Table No. below lists the antibodies and agents that are undergoing clinical testing. The mechanism by which immunotherapeutic drugs work has been thoroughly examined. The agents appear to work primarily via activating microglia and phagocytosis.

Inflammation Related Approaches

Two facts serve as the primary foundation for the reasoning behind inflammation-related treatment approaches: first, recent genome-wide association studies have found a correlation between immune system-related genes, such as CR1, and AD; second, patients with arthritis who have been taking NSAIDs for a long time have a lower incidence of AD. Consequently, clinical trials were conducted to test a number of NSAIDs, including celecoxib, rofecoxib, and naproxen, however none of them demonstrated any therapeutic impact against AD.

4. EXISTING DRUGS FOR AD

46 Currently, symptomatic treatments are the medications given to treat AD. They delay the disease's course and alleviate behavioural and cognitive symptoms, but they cannot reverse the condition. Drug prescriptions differ depending on the patient's country of residency, related conditions, and the severity of the sickness. Drugs can be divided into two groups according to their modes of action (Godyń et al., 2016).

Lipoprotein Receptor Related Protein 1

Numerous proteinase inhibitors, which are typically present in plasma at high quantities, control blood protease activity. A proteinase and its inhibitor combine to produce a complex, which is subsequently removed from the bloodstream by the receptor system.

Withania Somnifera

Ashwagandha, or Withania somnifera in Sanskrit, is a plant in the Solanaceae family. Common names for it include Indian ginseng and winter cherry. The plant grows widely in drier regions of tropical and sub-tropical zones and can reach

heights of 0.5 to 2 meters. Most of Africa, North America, India, China, and Australia are known to cultivate it. Ashwagandha is primarily grown for its therapeutic properties in India. It falls within the category of "medhyarasayana" formulations, which are treatments for the central nervous system. Medicinal formulations are made using either the entire plant or specific sections of it, such as the leaves, stem, or root (Cao et al., 2018). Additionally, it can be utilized either by itself or in conjunction with other therapeutic plants.

5. NOVEL TREATMENT APPROACHES

We created a reporter assay technique based on luciferase to screen the fractions and sub-fractions in vitro. This approach was justified by the idea that the luciferase gene's expression beneath the LRP promoter would provide an indirect readout of the fractions' activity on the promoter region. A fraction's luminescence will increase with its enhancer activity. This method also allowed us to address the question of where the enhancer target sites are on the LRP gene and examine the different LRP promoter regions independently. Selecting a promoterless vector containing the luciferase gene was the initial step in answering the aforementioned issues (Folch et al., 2016). Promega Corporation in Wisconsin, USA, provides pGL3-Basic, which we used as the vector. Its 4.8 kbp size comprises a luciferase gene, an ori site, a multiple cloning site (MCS), and the antibiotic resistance gene ampicillin.

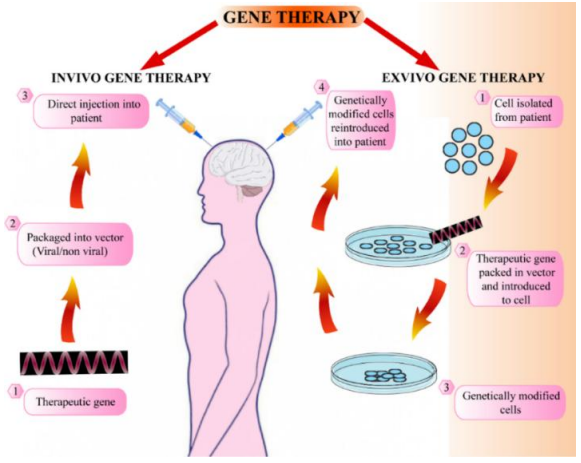

Figure 3: Gene Therapy

The first step in cloning a gene's promoter region into a vector is to separate the DNA that contains the gene. Creating forward and backward primers that will attach to the region of interest and aid in its amplification is the second stage. Following the creation of appropriate primers, the region of interest is amplified using the polymerase chain reaction (PCR). It is now necessary to ligate this amplified product into the vector. This is accomplished by digesting the vector and the amplified product using the same set of restriction enzymes, followed by ligation (Ju & Tam, 2022). To validate that the vector contains the appropriate promoter region, restriction digestion and PCR are utilized once more. The vector and insert are changed into competent bacterial cells after their identities have been verified. Following the inoculation of these cells into LB broth, the vector containing the insert is cloned. From these cells, the plasmid DNA is subsequently extracted. By using PCR or restriction digestion, a tiny quantity of plasmid can be utilized to verify the clone's identification once more. The next sections provide a detailed description of the fundamental idea, necessary supplies, and steps for each of the aforementioned processes.

The Qiagen DNeasy Tissue Kit's DNA extraction process is founded on the ideas of spin-column-based nucleic acid purification. A lysis reagent made up of chaotropic chemicals is used to lyse the cells open. The cells release their nucleic acids. The samples are then combined with a buffer solution that contains either isopropanol or ethanol. The binding solution is this. At the proper pH, it makes nucleic acid binding to the silica column easier. The bound nucleic acid is subsequently released with the help of elution buffer, allowing it to be collected in a vial.

Marion M. Bradford created the Bradford Assay in 1976. Protein concentration is determined using a quantitative spectroscopic technique. It is predicated on the idea that arginine, lysine, and histidine are basic amino acids that, when bound by Coomassie blue dye, form a blue-colored complex under basic circumstances. The intensity of color produced increases with protein concentration. The dye comes in three varieties: anionic (blue), neutral (green), and cationic (red).

Antigen-antibody interaction is the fundamental idea behind ELISA. An enzyme and a specific antibody are used to incubate the antigen, which is a material with

an unknown concentration. When a chromogenic substrate is present, this enzyme aids in the creation of color. The 88 pure or recombinant antigens used to generate the standards are whose concentration in the sample needs to be ascertained.

TRI reagent and bromochlorophenol (BCP) were used to isolate RNA. Phenol and guanidine isothiocyanate are present in a monophasic solution in the TRI reagent. Protein denaturation, RNase inactivation, and rRNA separation from ribosomes are all caused by guanidium, thiocyanate, and phenol. Additionally, phenol aids in the separation of DNA and RNA. Additionally, BCP denaturates proteins. By eliminating lipids and separating monophase into three different phases—the organic phase, interphase, and aqueous phase—it facilitates improved RNA extraction. Protein is found in the organic phase, whereas DNA is found in the interphase. After being extracted, RNA is placed in an aqueous phase that can be separated and subsequently precipitated with ethanol.

Single-stranded cDNA was prepared using Applied Biosystems' High-Capacity cDNA Reverse Transcription Kit. Single-stranded complementary DNA is produced from RNA by reverse transcriptase. The kit's instructions were followed in order to prepare the reaction mixture. The reagents utilized are as follows: 0.4 ul of 25X 100mM dNTP mix, 1 ul of 10X reverse transcription buffer, 1 ug of RNA, 0.5 ul of reverse transcriptase, and 1 ul of 10X random hexamer primers.

With real-time PCR, the PCR product may be precisely quantified at each cycle. Fluorescent reporter dyes added to the product are used to track the PCR reaction in real time throughout the exponential phase. DNA binding dyes, fluorescent primers, and fluorescent probes are examples of fluorescent reporters that are frequently utilized. When SYBR green attaches to double-stranded DNA (dsDNA), it fluoresces, and the quantity of dsDNA present determines how strong the fluorescent signal is. The number of amplicons produced during the exponential phase is directly correlated with the rise in the fluorescent signal. This makes it possible to monitor the PCR product accumulation. Fluorophores are linked to certain primers in primer-based fluorescent detectors; as the primers are integrated into the PCR product, the fluorescence rises, signifying an increase in the number of amplicons (Wolfe, 2002).

Proteins on polyacrylamide support migrate in an electric field with a net negative charge. Proteins can be quantified and described using a technique called sodium dodecyl sulphate-polyacrylamide gel electrophoresis, or SDS-PAGE.

Since the LRP promoter area has not been thoroughly investigated, little is known about the transcription factors and other regulatory components linked to LRP overexpression. Determining how well various promoter regions drive the production of LRP when treated with the extract is one method to answer this question (in the context of the current investigation). We could identify the transcription factors and co-activators linked to the overexpression of LRP by identifying the active promoter area. The next part provides a description of the experiment's findings. Additionally, we chose to use just the 400bp clone for all subsequent tests because the activity of fractions and WE was similar among the three clones.

6. DISCUSSION

About 35 million people worldwide suffer with Alzheimer's disease (AD), a progressive neurological illness. There is currently no cure for AD, despite the fact that the genetic abnormalities linked to the disease were discovered more than 20 years ago. Only symptomatic treatment is offered by commercially accessible medications like donepezil and memantine. The incidence of AD patients is rising annually due to an increase in average life expectancy, making the development of more effective therapeutic drugs that can change the course of the illness essential. Traditional medical systems like Ayurveda and medicinal plants have received a lot of interest as a result of the desire to develop better therapeutic tactics and identify better therapeutic targets. More than 200 WS root formulations have been documented in traditional Indian medical systems; the most of these were used to treat anxiety, tension, and memory problems. According to earlier research from our lab, a semipurified extract of Withania somnifera root cures plaque pathology and behavioral abnormalities in nine-month-old APPSwe/PS1dE9 rats by upregulating hepatic LRP (Sehgal et al, 2012). Amyloid beta 42 (Aβ42) is sucked up by LRP1 in its soluble form (sLRP). Therefore, higher plasma levels of sLRP lead to higher plasma clearance of Aβ42. This causes Aβ42 monomers to flow from the brain into the periphery. The oligomers begin

breaking down into monomers as soon as the monomers are eliminated from the brain, and plaque destruction also starts (Barage & Sonawane, 2015; Graham et al., 2017; Pinheiro & Faustino, 2019; Nalivaeva & Turner, 2019; Gao et al., 2018).

LRP expression has also been linked to nuclear respiratory factor 1 (NRF1). The NRF1 element, also known as the NRF1 binding sequence, was located at -143 base pairs and contained intrinsic sites for the binding of interferon gamma (IFN gamma) and cyclic adenosine monophosphate (cAMP).

Circadian rhythms are essential to almost every type of life. The SCN in mammals synchronizes autonomous cellular clocks to produce a complex rhythm of energetics and metabolism that results in organism-level behaviour. Our goal was to determine how continuous light affected the pathophysiology of AD (Zhang et al., 2021; Derakhshankhah et al., 2020; Oualla et al., 2017; Perricone & Vander Heide, 2014; Jain & Jain, 2013).

However, in at-risk AD rats, circadian disturbance facilitated the aggregation of exogenously given Aβ42. While rifampicin simply stopped plaque from forming and had no effect on cognitive symptoms or oxidative brain damage, daily fluoxetine medication totally restores animals' circadian rhythms and rids them of AD-like pathology. Additionally, the levels of circadian rhythm markers Per2, PRX1, and PRX-SO2/3 in the SCN were disturbed after four months of continuous light. Apart from the memory impairment observed in animals exposed to continuous, long-term light, the neurobiochemical analyses verified that AD pathology is in progress, as demonstrated by a notable increase in endogenous Aβ42 levels and dysregulation of several genes related to AD, including Bace1, Sirt1, Prokr2, and Mgat3.

7. CONCLUSION

Alzheimer's disease (AD), a degenerative neurological condition, is characterized by cognitive and decision-making difficulties in addition to memory loss. Even though the first case of AD was reported more than a century ago, there is still no treatment for it. The commercially available medications, such as donepezil and memantine, only partially alleviate symptoms. They neither stop the disease's

progression nor treat it. Therefore, it is essential to develop novel therapeutic approaches and targets.

The results demonstrate that Wistar rats who are exposed to continuous light for an extended period of time develop behavioural abnormalities and brain oxidative damage. Additionally, prolonged exposure to light triggers a number of upsetting molecular processes that lead to a phenotype resembling AD. By restoring circadian regularity, fluoxetine therapy saved rats against an AD-like phenotype. We suggest that shift workers may be more susceptible to AD, and that symptoms may manifest earlier in individuals who are predisposed, making it more difficult to administer the right treatment on time.

Even though Aβ deposition form toxic aggregates is believed to be the hallmark of AD, dementia and cognitive impairment cannot be caused by this alone. The accumulation of p-tau, which is connected to neuronal dysfunction and death, is more strongly associated with the onset of cognitive decline. Neuroinflammation may have two functions: it can be advantageous when it promotes the removal of Aβ and protects against damage, and it can be harmful when it is continuously triggered, feeding a number of pro-inflammatory pathways linked to neurodegeneration. Relying exclusively on misfolded protein deposition may only be a partially successful strategy. Nonetheless, it is crucial to identify neurodegenerative alterations early on at a potentially curable stage of cognitive decline. One of the most significant issues with treating AD that researchers have faced in the last 20 years is determining the appropriate targets at the appropriate time. A multimodal approach that incorporates anti-amyloid and tau therapies with the management of neuroinflammatory reactions may work well in this field.

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