Unlocking Neurogenesis: PRGF Regenerative Therapy and the role of Tau Protein in Neurodegenerative Disease Models

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(2025)

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A thesis submitted to the National University of Sciences and Technology, Islamabad,

in partial fulfillment of the requirements for the degree of

Master of Science in

Biomedical Sciences

Supervisor: Dr. Saima Zafar

School of Mechanical and Manufacturing Engineering

National University of Sciences & Technology (NUST)

Islamabad, Pakistan

(2025)

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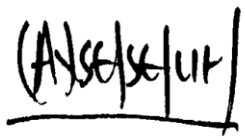
Student Signature: 

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Dedicated to my Parents, friends, and mentors, whose unwavering support, encouragement, and guidance have been invaluable throughout this journey. This achievement is as much yours as it is mine.

ACKNOWLEDGEMENTS

I am grateful to Almighty Allah for giving me the strength, patience, and knowledge that guided me during this study effort. My sincere thanks go out to my dear parents, (Syed Salih Muhammad and Abida Parveen), for their steadfast love, encouragement, and spiritual support. Their faith in me gave steady drive throughout difficult moments, and their prayers have been a source of strength throughout my journey.

I'd want to express my heartfelt gratitude to Dr. Saima Zafar, my supervisor, for her essential assistance, patience, and understanding. Her knowledge, insightful input, and encouragement were critical in completing my project, and I am thankful for the opportunity she gave me to improve both academically and personally.

I'd also want to convey my heartfelt gratitude to Zainab Naeem, whose help and encouragement were critical to finishing my thesis. Without Mary, I would have been unable to navigate the hurdles I experienced during my MS journey. I am also grateful to Amna Mansoor, Attia Abid, and Javaria Tahir for their unwavering support, friendship, and positivism throughout the most difficult stages of this study.

I also like to thank Wijeya, Eman and Fareen from the ASAB animal house and the entire team for their assistance with animal handling and research. Special thanks to my classmates, Asif, Durée Shahwar, Basma Jadon, Sahar Riaz whose companionship and camaraderie kept me focused and motivated.

Finally, I want to acknowledge all those who have contributed to this research, either directly or indirectly, including my college friends Aqsa Shams, Kainat Bashir, Bareera, and Aqsa Salih. Your encouragement and support have been a source of strength, and I am forever grateful for your presence in this journey.

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

Abbreviation	Abbreviation Full Form
Aβ	Amyloid beta
AD	Alzheimer's Disease
Ach	Acetylcholine
AChE	Acetylcholinesterase
$AlCl_3$	Aluminum Chloride
ANOVA	Analysis of Variance
APP	Amyloid Precursor Protein
AP	Amyloid Plaque
Apo A & E	Apolipoprotein A and E
BBB	Blood Brain Barrier
CNS	Central Nervous System
H & E	Hematoxylin And Eosin
IL-1B	Interleukin-1B
MWM	Morris Water Maze
NF-kß	Nuclear Factor-kß
NFT	Neurofibrillary Tangles
NOR	Novel Object Recognition
NMDA	N-methyl-D-aspartate
PA(%)	Percentage Alteration
PFA	Paraformaldehyde
PRGF	Platelet Rich Growth Factors
PRP	Platelet Rich Plasma
PSEN1	Presenilin 1

ABSTRACT

Alzheimer's disease (AD) is a neurological condition characterized by persistent cognitive impairment with few treatment options available. Platelet-rich plasma (PRP) and platelet-rich growth factors (PRGF), produced from human blood, have shown promise in treating a variety of neurological disorders. However, their use in Alzheimer's disease is underexplored, particularly in chemically produced models. This study looks at the therapeutic potential of PRGF and PRP in a chemically induced Alzheimer's mouse model using aluminum chloride (AlCl3), with a particular emphasis on the novel use of intranasal PRGF administration. This study to explore and compare the effects of PRGF and PRP in an Alzheimer's disease chemical model. Behavioral assessments were performed to evaluate cognitive deficits, memory retention, and spatial learning. The Y-maze and Elevated Plus Maze (EPM) tests demonstrated significant cognitive improvements in the PRGF-treated mice. PRGF administration resulted in enhanced memory performance and cognitive flexibility, with higher number of alterations and entries to novel arm. Additionally, PRGF-treated mice exhibited reduced anxiety-like behavior in the EPM. These findings suggest that PRGF treatment significantly improves cognitive abilities and memory retention however do not fully restore and cannot be used confidently in treatment. Histological analysis of the frontal cortex, cerebral cortex, hippocampus, dentate gyrus (DG) region, and liver was conducted following behavioral assessments to There were no significant improvements in cellular integrity observed in either the PRGF or PRP treated groups, indicating less to no neuroprotection and potential reversal of AlCl3-induced damage.

Keywords: Alzheimer's disease, Cognitive Function, Histopathology, Intranasal Administration, Neuroprotection, PRGF, PRP.

CHAPTER 1 : INTRODUCTION

Alzheimer disease (AD) is a chronic neurodegenerative disease and the most common type of dementia, which affects 50-70% of the elderly population(Ahmad et al., 2024). Originally described by Dr. Alois Alzheimer in 1906, the disease has gradually evolved into a worldwide problem owing to its increasing incidence and drastic consequences for patients, families, and health care organizations. Alzheimer's disease is characterized by dementia, memory loss, and is one of the most common causes of disability in the elderly(A. Singh et al., 2020).

The prevalence of Alzheimer worldwide is extremely high. Currently, 416 million people are affected by Alzheimer disease, and this is expected to double every two decades due to the increasing populations' average age. The increasing rate at which prevalence is rising among the elderly population presents immense pressures to healthcare and financial systems globally(Selkoe, 2012).

At a molecular level, Alzheimer disease is defined by several neuropathological abnormalities, such as extracellular amyloid-beta (Aβ) deposits and intracellular neurofibrillary tangles (NFTs) consisting of hyperphosphorylated tau protein(Braak et al., 1994). Aβ peptides aggregation results in the formation of plaques while the phosphorylation of tau results in the formation of neurofibrillary tangles(Blennow & Zetterberg, 2018). These pathological changes affect the intercellular communication and neuronal function and consequently lead to the brain atrophy. Alzheimer disease predominantly has late onset and is sporadic in nature, however less than 1% of cases are early onset familial Alzheimer disease due to mutations in the APP or PSEN 1/2 genes(Deture & Dickson, 2019).

It is important to know that clinical course of Alzheimer's disease takes many years. Some of the initial signs are short-term memory problems, impaired judgment, problem solving and communication challenges(Serrano-Pozo et al., 2011). This is true because as the disease progresses the symptoms worsen and may include aphasia, sleep disturbances, anxiety, hallucinations, and behavioral changes.

In the later stages, patients exhibit severe disability in mobility, speech, and swallowing, and require care all the time(Cloak & Al Khalili, 2022).

The societal and personal burden of Alzheimer disease is high as patients lose their ability to make decisions and care givers are stressed emotionally and financially(Licher et al., 2019). Today, there is no cure for the disease and treatment mainly involves managing the signs and symptoms. This underlines the necessity of the development of new therapeutic approaches, designed at molecular level(Serrano-Pozo et al., 2011).

Through this research, novel approaches are explored to address these challenges, including targeting neurodegeneration and improving drug delivery systems. Understanding and mitigating Alzheimer's disease's progression are crucial to alleviating its impact on individuals and society.

1.1 Neuropathological features in Alzheimer's Disease

AD has several neuropathological characteristics, and NFTs are considered a major component of the disease. These intracellular filamentous deposits discovered by Dr. Alois Alzheimer are central to the disease(Braak et al., 1994). The current methods of diagnosis of early AD are based mainly on histological characteristics, especially those that show changes in the cytoskeleton of neurons.

The tau protein, a microtubule-associated protein, is the major structural protein of NFTs(Serrano-Pozo et al., 2011). Normally, tau protein contributes to strengthening of microtubules in axons of neurons and acts to maintain cell structure. But in AD, the tau protein is hyperphosphorylated at different sites and is removed from microtubules and forms tangles. This accumulation interferes with normal neuronal functions resulting in neuronal death, apoptosis and decreased cell viability(Zhang et al., 2019).

Mature NFTs are present in neurons with bent dendrites and axons and the tau aggregates tend to move to the periphery of the soma. When these neurons die, the ghost NFTs linger due to the dissolution of dendritic and axonal

architecture(Rajmohan & Reddy, 2017). These tangles are mainly in the temporal region and disseminate to the isocortex and other parts of the brain and cause neurodegeneration and aberration of nerve connections. The other protein that has been associated with AD is amyloid precursor protein (APP) that when processed generates amyloid-β (Aβ) peptides. These peptides deposit in the brain in an abnormal way and form what is known as plaques which is characteristic of AD(Sehar et al., 2022). Aβ is involved in a number of critical cellular processes within the human body such as maintaining stability of neurons and intracellular transport effective for the nervous system, but when piled up in an abnormal manner, it forms plaques(Soeda & Takashima, 2020)(Moloney et al., 2021)

Aβ plaques are composed mainly of 40 or 42 amino acids, of which Aβ42 is most predominant. Aβ is generated because of a proteolytic cleavage of APP by secreting enzymes such as β-secretase (BACE1) and γ-secretase, which participate in normal APP processing(Kang et al., 1987). However, in AD the activity of the secretases is uneven; the toxic Aβ is produced instead of being degraded which leads to formation of the toxic protein aggregates, which are detrimental to the neurons(Blennow & Zetterberg, 2018).

The development of Aβ deposition is not random, but displays a temporal-spatial sequence: the limbic regions, the neocortex, the brainstem, and the cerebellum(Sciaccaluga et al., 2021). When the Aβ plaques develop, they impinge on some of the important parts of the brain, including the hippocampus that is responsible for memory storage(Hardy & Selkoe, 2002). Damage occurring in these areas cause disruption of the synapses and hence discontinuity of communication between different neurons; this accounts for the observed disability in patient's suffering from AD(Cras et al., 1991).

Besides the Aβ plaques, inflammation of neurons and impaired function of mitochondria are characteristic of AD. These pathophysiological events initiate a series of neurodegenerative processes such as oxidative stress and neuronal

apoptosis that are well correlated with dysfunction of synapses and memory repression(Blennow & Zetterberg, 2018).

Oxidative stress in AD results from relative decreased rate of ATP production and relative increased rate of ROS production within AD cells(Oron et al., 2007; Tao et al., 2019). This stress triggers different signaling cascade like; NFκB and PI3k/Akt that leads to neuronal damage and tau aggregation. Studies reveal that such substances as resveratrol and donepezil may decrease oxidative stress and stimulate the dephosphorylation of tau that may promote neuronal survival in AD (Hardy & Selkoe, 2002).

However, as pointed out by the findings, AD is still an untreatable ailment today. Despite the advancements in medical science numerous treatments which have been designed to cure the disease have not been found, though useful treatment in reducing the suffering of the affected individuals has been developed. Yet, present day research activities are aimed at the identification of better treatment modalities that directly address the pathological processes in AD such as tau and amyloid abnormalities, oxidative stress, and inflammation(Dhapola et al., 2024).

1.2. Current treatments

Cognitive intervention for Alzheimer's disease (AD) involves the use of drugs and other non-drug approaches. Drugs like cholinesterase inhibitors and NMDA receptor antagonists work on the relevant neurological channel to help in controlling the deterioration of brain and progression of the disease. These medications are useful in treating memory and other symptoms commonly seen in Alzheimer's disease. Besides pharmacological treatments, non-pharmacological treatments also form a major component in improving the QoL for patients. Cognitive training, changes in diet, exercise, and other creative approaches to cognitive and emotional treatment include music and art. With medical management, the objective is to give an integrative treatment with the purpose to reduce the symptoms and enhance the life span of patients with AD (Guzman-Martinez et al., 2021).

1.2.1 Non-Pharmacological Therapies

Non-pharmacological treatments of Alzheimer's disease are aimed at increasing the patient's ability, alleviating the symptoms and optimizing the patient's quality of life. These are non-specific techniques that are considered to support pharmacotherapy to help to alleviate the worst symptoms of AD, such as the deterioration of cognition, memory loss, and other related symptoms(Olazarán et al., 2010; Yue et al., 2022).

Cognitive learning and rehabilitation therapies have provided the fundamental non-pharmacological strategies that help slow the progression of Alzheimer's Disease. These therapies are intended to promote cognitive function through purposeful stimulation of the brain that involves practice of tasks that demand memory, attention and executive control. Moreover, the use of an individualized approach to rehabilitation can bring a lot of benefits in everyday functioning in patients with AD(Bleibel et al., 2023). For example CST entails encouraging the patients to participate in highly structured group discussions, which enhance cognitive function and quality of life in patents with medium to moderately severe Alzheimer disease (Riemersma-van Der Lek et al., 2008). This is how cognitive rehabilitation is effective – patients are encouraged to form new neural connections that can partially compensate for the deterioration caused by amyloid plaques and tau tangles(Aboelwafa et al., 2020).

The role of physical activity in human health has been known for a long time, and recently, it was pointed out that it is especially significant in Alzheimer's Disease. The present study also revealed that exercise has many positive effects on AD patients, which include the following: increased brain functioning, decreased depression and increased quality of life. Aerobic exercises, such as walking, cycling and swimming are helpful in enhancing the cardiovascular system and blood flow whereas medication can only do so much with regard to enhancing cerebral metabolism and circulation in patients with AD(Guo et al., 2019). Also, resistance training and strength activity helps in building muscle mass and mobility and

decrease the chances of falling – a common problem in Alzheimer's disease that contributes to physical deterioration. Baker and colleagues mentioned that physical activity improves neuroplasticity, and the brain has the ability to cope with the pathological implication of AD, including amyloid plaques and tau tangles(S. Singh et al., 2016).

Rehabilitation can affect the cognitive and emotional state of the patient, even in the terminal stage of AD. The therapy has been shown to assist in the lessening of cognitive loss, but also the general well-being of the patients. Rehabilitation does not cure the disease but helps in making Alzheimer's patients more independent (H. et al., 2016) (Valenzuela et al., 2020).

In addition, exercise has been found to improve sleep, reduce anxiety and agitation, which are some of the behavioral symptoms of AD. Physical activity increases the production of endorphins and decreasing depressive symptoms, which are common in clients with Alzheimer's. Such benefits explain why caregivers should encourage physical exercise as part of the daily activities in patients suffering from AD as a way of improving cognitive as well as physical well-being of the patients(Blackman et al., 2021)(Olazarán et al., 2010).

Alzheimer's Disease (AD) requires the use of non-pharmacological approaches in its management, of which social support and cognitive stimulation are among the most effective. Social interaction is a critical determinant of the cognitive health and the psychological well-being of the person with AD. This is because social activities relieve feelings of loneliness and depression. Contact with relatives and friends, as well as with other caregivers, offers comfort and allows them to maintain a sense of normalcy and independence(Olazarán et al., 2010).Cognitive stimulation is a more structured approach that involves the use of games, puzzles and discussions with the patient. Proposing design frameworks that combine social interaction and cognitive exercise as components to enhance the quality of life of the patient since research shows that AD related patients only experience dementia when they have cognitive impairment and low mood. Group therapy such as

patients' memory training and discussion can also increase cognitive performance, social skills and decrease social isolation (Riemersma-van Der Lek et al., 2008).

Besides, the other organized learning activity, family and caregiver related interactions influence the emotional and psychological status of the AD patients significantly. Social interactions encourage self-care and a positive outlook above depressing feelings and thus make people's lives happier as they fight depression and anxiety. This approach helps in creating a supporting environment within which the AD patients would feel motivated (Valenzuela et al., 2020). Acupuncture and other forms of complementary and alternative medicine (CAM) have emerged as likely non-drug interventions for Alzheimer's Disease. According to TCM, Acupuncture aims at correct flow of energy or Qi by inserting thin needles at points on the body. Although the way acupuncture is helpful to AD patients has not been clarified, it has been reported in several studies that this treatment decreases the symptoms like agitation, anxiety, and depression. Some other complementary therapies like aromatherapy, music therapy, art therapy are also thought to work for patients with AD. For instance, aromatherapeutic uses of essential oils help in bringing down stress and lifting the mood; on the same way, music therapy also works in boosting cognitive function. Art therapy, however, enables a patient to use artistic products to communicate and may reduce patient's agitation and promote self-control. At the same time, improvements in the concept of AD treatment were marked by the inclusion of additional practices that potentially could complement traditional pharmacological interventions, strengthen the quality of life in such patients, and encourage further research on the effectiveness of these non-pharmacological forms of therapy (Öhman et al., 2016; Valenzuela et al., 2020).

The new treatment method that has been extensively used and has demonstrated some effectiveness in Alzheimer's Disease treatment is Hyperbaric oxygen therapy (HBOT). This therapy utilizes the breathing in of oxygen in a controlled chamber, thus raising the level of oxygen that reaches body parts, including the brain. HBOT increases oxygen levels in the brain, which encourages growth of new neurons and can rebuild injured neurons. It has been found that HBO

can enhance learning and memory and decrease amyloid deposition in AD experimental animals. Acceleration of oxygen supply enhances the process of cellular repair, reduces inflammation and awakens the neurons for regeneration. Besides the cognitive effects, HBOT seems to have positive antidepressant effects on AD patients who complain of depressed mood. Although the current literature in support of HBOT as a treatment for AD is still limited, current studies are still being conducted to establish the possibility of using the treatment to make positive changes in the cognitive and overall brain health of patients (Chang et al., 2018)(Zhang et al., 2019).

Alzheimer's Disease cannot be treated, but dietary interventions and nutrition are very important in controlling the disease. There is no food or supplement that has been found to prevent or treat AD, but a diet that is full of antioxidants, healthy fats, and essential nutrients can help slow down the progress of the disease and protect the brain. Certain vitamins and minerals including omega 3 fatty acids, which can be obtained from fish and certain nuts help to support brain activity and minimize inflammation. Other nutrients that have drawn interest in regard to AD prevention are antioxidants, such as vitamin E. Of all the vitamins, vitamin E has been found to decrease oxidative stress, which is believed to contribute to the development of AD. Other diets including the Mediterranean diet have also been linked with improved cognitive function in older people. The food choices in the Mediterranean diet are based on whole grain, fruits, vegetables and healthy fats which are thought to have an impact on inflammation and brain health. Besides guidelines on diet that may be beneficial in the management of AD, more recent research has looked into the possibility of using silymarin, which is sourced from Silybum marianum. Silymarin has shown neuroprotective properties mainly by modulating oxidative stress, inflammation and aggregation of amyloid-beta. These compounds may provide novel targets for dietary approaches towards Alzheimer's (Bleibel et al., 2023)(Aboelwafa et al., 2020)(Guo et al., 2019)

Another non-drug intervention that is receiving interest in the context of AD is phototherapy. This method employs light to activate the brain and provide relief

from signs of neurological disorders. Low-level light therapy (LLLT) also called photo biomodulation (PBM) delivers red or near infrared light that improves mitochondrial function, reduces oxidative stress and is neuroprotective. It was observed that the light therapy can make positive changes in cognitive function in AD patients, as well as decrease the formation of amyloid plaques because it aids in the increase of blood flow, and a higher level of brain metabolism (Anders et al., 2019; Hamblin, 2019). Also, red light therapy (RLT) has been established to reach the tissues and foster healing and revitalization of cells, ATP generation which is essential in the brain (Anitua et al., 2006).

1.2.2. Pharmacological therapies

Lecanemab is a newly approved drug for treating AD that works on amyloid-beta plaques. Thus, through the reduction and promotion of clearance of these toxic aggregates, Lecanemab has potential of slowing cognitive loss in AD patients. This drug has been approved by the FDA for clinical use for the treatment of AD. Nevertheless, the extent of its application and the clarity of the mechanisms of action and effectiveness of long-term pharmacological effects is still to be discovered(Cummings et al., 2023).

The other category of drugs used in the treatment of AD is acetylcholinesterase inhibitors including donepezil, rivastigmine and galantamine. These drugs act by raising the levels of a neurotransmitter called acetylcholine in the brain, which is normally depleted in AD. The drugs affect the functioning of acetylcholine, which is responsible for memory and thinking, they slow down the breakdown of this substance and thus help patients maintain cognitive abilities (Ribeiro-dos-Santos et al., 2023). While these drugs offer relief for the symptoms, they are associated with side effects such as gastrointestinal problems, dizziness, and nausea, which at times restrict the usage of the drugs (Caldieraro et al., 2021).

The other major drug used in managing of AD is memantine which the NMDA receptor antagonist. Memantine works on glutamate pathways and prevents the neuron from excitotoxicity which is a highly damaging phenomena in

neurodegenerative diseases. This one can enhance neuropsychological functioning and activities of daily living primarily in patients with moderate to severe AD but does not alter the pathological course of the disease (Tang et al., 2023). It is like many other drugs in that it is useful in controlling symptoms but does not slow the progression of the disease (McShane et al., 2019).

Another innovative anti-AD drug belongs to the monoclonal antibodies: aducanumab, which has been approved in the USA by the FDA. The main action of this drug involves the amelioration of amyloid-beta plaque production in the patient's brain. Another advantage of aducanumab is that it can enter the central nervous system (CNS), a factor that is important to address AD since most drugs never get into the CNS. It works against amyloid plaques believed to slow down the disease progression, but its working and effectiveness are not fully understood (Haddad et al., 2022). Aducanumab is a perfect example of the trend to create drugs that can penetrate through the BBB and act on the main pathological processes in AD.

Another therapeutic approach that shows potential in treating AD is gene therapy using CRISPR/Cas9 system. This approach focuses on attacking genes that are known to be mutated in the disease and include PSEN1, APOE and APP genes. Although gene therapy holds great potential, challenges remain in ensuring the accuracy of gene editing and preventing unintended genetic alterations. There are possibilities of using CRISPR/Cas9 to correct the gene basis of AD, but the technical and ethical challenges remain a topic of future research (Serrano-Pozo et al., 2011) (Ahmad et al., 2024).

One of the most recent approaches to the AD treatment is through Platelet RICH Plasma. Platelet-rich growth factors (PRGF) have emerged as a potential therapy strategy for tackling the complex issues of Alzheimer's disease (AD). PRGF, taken from human blood, is a concentrated supply of bioactive proteins, morphogens, and growth factors that help with tissue repair, regeneration, and neuroprotection. Its ability to promote cellular repair mechanisms and regulate inflammation makes it a

promising option for combating the neurodegenerative processes that underlying AD. Importantly, PRGF's therapeutic potential is enhanced by its suitability for intranasal administration, a non-invasive delivery technique that goes through the blood-brain barrier (BBB). This novel technique allows for direct access to the central nervous system, which is a major challenge in providing therapies for neurodegenerative conditions. The utilization of PRGF in the case of AD has two benefits: it treats the disease's structural and metabolic deficits. Amyloid-beta (Aβ) plaque buildup and tau hyperphosphorylation are examples of biochemical changes that impair synaptic integrity and neural transmission, resulting in gradual cognitive impairment. The pathogenesis of the illness is made worse by structural alterations, such as glial activation and extensive neuronal loss. By providing a large reservoir of endogenous growth stimulants and proteins that support growth of neurons, synaptic repair, and glial activity regulation, PRGF treatment has demonstrated the ability to reverse these alterations(Anitua et al., 2010).

As the main modulators of synaptic transmission and neuroinflammation, astrocytes are essential to the development of AD. It has been demonstrated that the degree of brain shrinkage and cognitive deterioration seen in AD patients is correlated with extensive and chronic astroglial activation (Cagnin et al., 2001; Parachikova & Cotman, 2007).Additionally, AD brains exhibit markedly lower levels of important neurotrophic factors, including brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF-I), and nerve growth factor (NGF)(Houeland et al., 2010; Moloney et al., 2021; Nagahara et al., 2009; Steen et al., 2005). These deficiencies lead to poor cognitive function, neuronal death, and synaptic malfunction.

By offering a treatment platform tailored to each patient and enhanced with physiologically balanced mediators, PRGF expands on this framework. Standardised PRGF-based formulations, such PRGF-Endoret, that are safe, simple to make, and therapeutically useful have been made possible by developments in regenerative medicine. The European Community and the U.S. Food and Drug Administration have approved this autologous treatment, confirming its potential for clinical use

(Anitua et al., 2010; Leslie, 2010). PRGF-Endoret provides a mix of neuroprotective and regenerative components that may promote neuronal repair and halt the course of illness by using the body's own healing processes.

The effects of intranasally delivered PRGF-Endoret in APP/PS1 mice, a recombinant model of AD, were examined in another research. Over the course of four weeks, this medication showed notable neuroprotective and restorative benefits, such as decreased tau hyperphosphorylation and Aβ buildup, reduced astroglial activation, increased synaptic plasticity, and enhanced cognitive function. These results imply that PRGF-Endoret is a unique therapy approach for AD that targets the disease's underlying processes in addition to symptom relief(Capsoni et al., 2002; Carro et al., 2006; Nagahara et al., 2009; Spuch et al., 2010). PRGF offers a whole strategy to controlling AD pathology by addressing the drawbacks of current medications, underscoring its promise as a game-changing therapy in the domain of neurological medicine.(Anitua et al., 2010)

The neuroprotective benefits of PRGF are further supported by recent research. Intraperitoneal delivery of conditioned media (CM) either by itself or in conjunction with PRP, improved hippocampal volume, improved bias ratios, and raised more neural density in key areas like the Dentate Gyrus (DG) and Cornu Ammonis 1 (CA1) in a streptozotocin-induced AD model(Cagnin et al., 2001; Parachikova & Cotman, 2007). To optimise treatment results, it is crucial to combine several regeneration modalities, as PRP alone showed limited efficiency. This is consistent with previous research showing that PRGF plays a part in reducing astrocytic activation, a defining feature of AD pathogenesis that is linked to brain shrinkage and cognitive decline (Cagnin et al., 2001; Parachikova & Cotman, 2007).

In a recent study, senescent mice between the ages - 16 and 18 months were used to examine the behavioral and mental effects of intravenous allogeneic PRP treatment. The experimental group got the PRP, which was extracted from age-matched donor mice, whereas the control group was given physiological saline. To examine locomotion, anxiety, depression-like behaviors, learning, and memory, the mice

were put through a various test. The findings showed that PRP therapy greatly enhanced learning and memory function in older rats and increased locomotor activity. Interestingly, the treated mice showed no signs of despair or anxiety, suggesting that PRP treatment maintained behavioral stability. The promise of PRP as a self-sourced, less intrusive treatment for age-related cognitive loss is highlighted by this study(Demir & Karagoz, 2020)

1.4. Need For Novel Therapeutic Approach

Alzheimer's disease remains a worldwide problem, and modern medicine has few approaches to treat this condition. The available medications mainly focus on alleviating the symptoms rather than addressing the causes of the disease. AD is characterized by amyloid plaques, tau tangles, and neuroinflammation, which requires new interventions. Current drugs including acetylcholinesterase inhibitors and glutamate antagonists are not very effective and are associated with side effects. Non-pharmacological treatments, as much as they are helpful are not capable of undoing the neural loss that the disease brings about. Thus, demands for new approaches that would address the pathophysiology of AD and offer disease modifying effects are high(Passeri et al., 2022).

One research finding that comes close to this is the application of Platelet-Rich Growth Factors (PRGF). Delivery of PRGF intranasally or intravenously has been explored and there is evidence of neurogenesis and improved brain reparative capabilities. PRGF should be targeted to deliver growth factors that promote neuronal regeneration and reduce the effects of AD to the BBB. This novel therapy could be a better and safer approach to the conventional treatments of the disease and give a new hope for reversing the process of dementia in AD patients. More studies are required to ascertain the value of PRGF as a disease modifying treatment for AD in the long term and its safety.

1.5. Intranasal Treatment with PRGF in Chemically Induced Mice Models

The literature search showed that intranasal delivery is a novel and potential therapeutic strategy for Alzheimer's disease. Although there are numerous publications on different pharmacological and non-pharmacological treatments, the intranasal route and, especially, chemical-induced models are not well investigated. This offers a favorable advantage of crossing the blood brain barrier, making it thus easier to deliver therapeutic agents to the CNS. Using this technique, our study aims to evaluate the efficacy of Platelet-Rich Growth Factors (PRGF) on neuronal regeneration and cognitive improvement in response to the neurotoxicity of Aluminum Chloride (AlCl3) induced Alzheimer's disease. This novel methodology is an innovative step toward the improvement of AD treatments.

1.6. Hypothesis

PRGF administered intranasally offers better neuroprotective and regenerative effects than intravenous administration in a chemically induced Alzheimer's disease model, because it crosses blood-brain barrier and directly acts on the CNS.

1.7. Objectives

The objectives of this research are multifaceted and aim to advance our understanding and treatment of AD:

1. PRGF was used to investigate the effectiveness of intranasal and intravenous administration in chemically induced rodent models of Alzheimer's disease.
2. Studying the difference in the impact of both intranasal and intravenous PRGF delivery on cognitive abilities, histopathological alterations, and behavior in AlCl3- induced AD models.

CHAPTER 2 : MATERIALS AND METHODOLOGY

2.1 Ethical Approval

Before starting the in vivo study, the National University of Science and Technology, Islamabad's NUST-IRB committee granted ethical permission (IRB Number 2024-IRB-A-40/40).

2.2 Animals

Male Balb/c albino mice that were between 6 and 8 weeks old were used in this study. The mice were kept in plastic cages with unlimited access to food and water for rodents until the day of dissection. The mice were given time to get used to the lab environment before the experiment started. Four groups of mice totaling twenty-four were created: n=6 for the Control group, n=6 for the AlCl3 group, n=6 for the AlCl3 group treated with PRGF administered intranasally (IN), and n=6 for the AlCl3 group treated with PRGF administered intravenously (IV).

2.3 Experimental Design

In this study, twenty-four healthy male Balb/c mice aged 6 to 8 weeks were used. The animals were first adapted to the animal facility for a week prior to the experiment. During the course of trial, the mice were housed in plastic cages with ad libitum access to food and water. Following the acclimation phase, the Alzheimer's disease was induced by administering AlCl3 to the diseased groups for 42 days. The treatment groups received platelet-rich growth factors (PRGF) through intravenous injection (IV) and intranasal delivery (IN). The IN treatment was administered for three times per week for four weeks, while the IV treatment was administered for once per week for three weeks. Behavioral assessments were performed after the treatment phase, spanning days 51 to 79, followed

by tissue sample collection on day 90. Histopathological analysis, including slide preparation, Hematoxylin and Eosin (H&E) staining, and microscopic examination,

was conducted from day 91 to 96. Figure 2.1shows the timeline and experimental design of the whole project.

2.4 Chemical Dosage

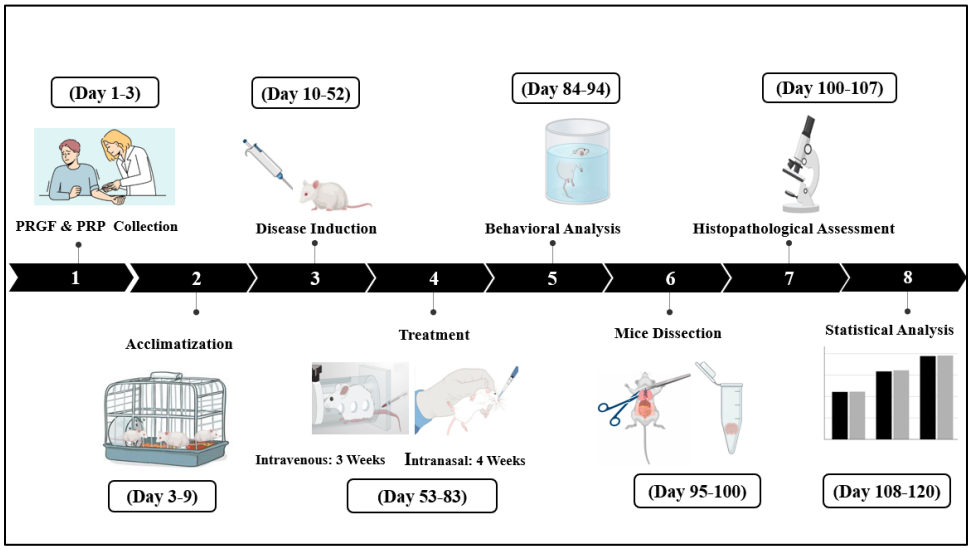

Figure 2.1(a): Timeline and experimental design of the study. The figure outlines the sequence of experimental stages, including animal acclimatization, treatment administration, behavioral assessments, and endpoint analyses. Key time points are indicated to provide an overview of the project's workflow and methodology.

2.4.1 Development of AD like Rodents

Alzheimer's disease-like models were induced in mice by administering AlCl3 orally at a dose of 20 mg/kg in distilled water over a period of 42 days. To prepare the stock solution, 20 mg of AlCl3 was dissolved in 10 ml of distilled water. Using a mouth gauge, 0.16 ml of the stock solution was administered to each mouse once daily (Wang et al., 2014; Yang et al., 2021).

2.4.2 Quality Assessment of PRGF for Treatment

The isolated blood from donors were analysed and checked for complete blood count CBC we checked platelet count of donor to be 323 unit 10^9 /L with reference values 150-450, serum IGF-1 = 328.23ng/ml with reference values 115.4-498.2, growth hormone 4.2 ng/ml with refence values <6.0.

2.4.3 Preparation for PRGF for Treatment

Blood was collected from healthy young male donors, after obtaining informed consent, through antecubital vein puncture at Saeed International Hospital, Islamabad. The blood was then placed in 9 mL tubes containing 3.8% sodium citrate (wt/vol). The samples were centrifuged at 580g for 8 minutes at room temperature to separate the plasma. The plasma fraction, which contained platelets but excluded the buffy coat and erythrocytes, was isolated. This plasma was then incubated with 5% calcium chloride (activator) for 1 hour at 37°C in glass tubes. Following incubation, the plasma was centrifuged at 1000g for 20 minutes at 4°C to collect the supernatant. The final platelet-enriched plasma fractions were aliquoted and stored at the Biochemistry Lab, National University of Sciences and Technology (NUST), until further use.

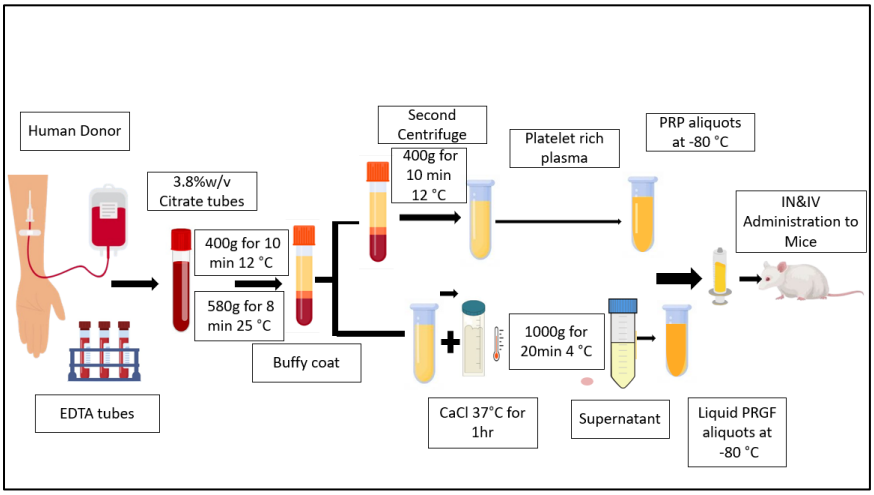

Figure 2.1(b) Figure shows the preparation protocol for PRP and PRGF

2.4.4 Dosage of Administration

PRGF was administered intranasally 3 times per week for 4 weeks, following a modified procedure based on previously described methods (Capsoni et al., 2002). Mice were carefully handled while awake, and a total volume of 48 µL of PRGF was delivered, 3 µL at a time, alternating between the nostrils with a 2-minute

interval between each administration, for a total of 16 applications. The control group were not given any dosage.

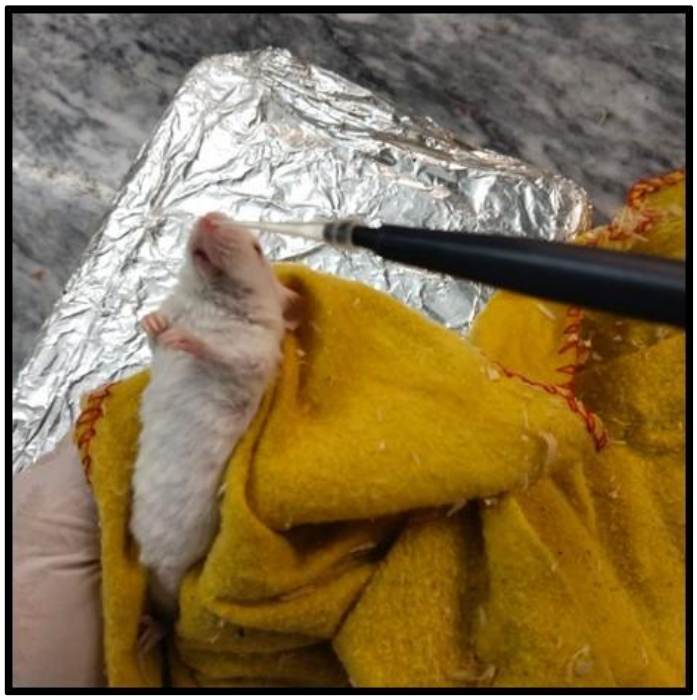

Figure 2.3: Intranasal administration of PRGF to mice. using a 10 µL pipette. The figure illustrates the method of delivering PRGF directly into the nasal passages of mice, highlighting the precise dosing technique employed during the experiment.

2.4.5 Preparation of PRP for Intra Venous Treatment

Blood was collected from healthy young male donors through antecubital vein puncture after informed consent. The blood samples were drawn into 9 mL tubes containing 3.8% (wt/vol) sodium citrate. The samples were then centrifuged at 400g for 10 minutes at 12 °C, again second centrifuge under same condition using a standard centrifuge. After centrifugation, the plasma fraction, which contained platelets but excluded the buffy coat and erythrocytes, was separated. The platelet-rich plasma (PRP) was then aliquoted into sterile containers and stored at -80°C at the Biochemistry Lab, National University of Sciences and Technology (NUST), until use

2.4.6 Dose Administration

For the intravenous treatment, PRP was administered at a dose of 100 µL per mouse once a week for 3 weeks. The treatment was carried out by tail vein injection without any activation, ensuring the PRP remained in its natural state.

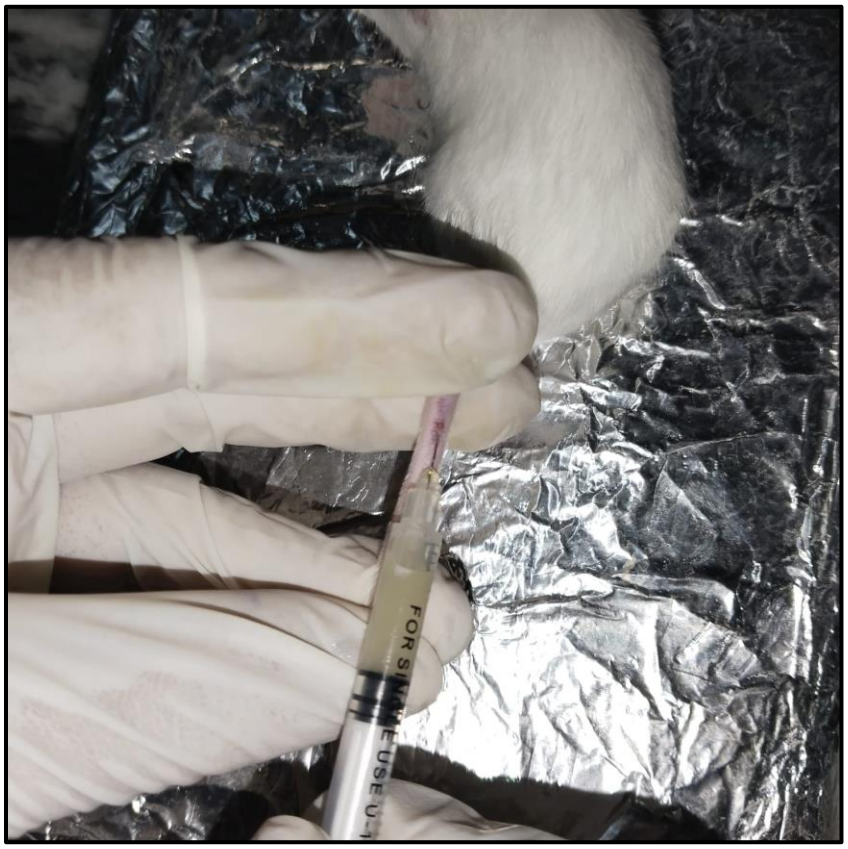

Figure 2.4: Intravenous administration of PRGF to mice using a 30-gauge needle. The figure demonstrates the technique for administering PRGF via the tail vein, emphasizing the precision required for successful intravenous delivery.

2.5 Behavioral Testing

After the AD-like symptoms have been elicited, behavioral tests were carried out to determine the presence of Alzheimer's disease indicators including impaired memory, learning and spatial navigation deficits.

2.5.1 Y-MAZE TEST

The animal was put in a Y-maze with three arms: the familiar arm, the start arm, and the novel arm. The arms were washed with 70% ethanol before each trial as required by the experimental procedure. After this, the mice were allowed to move and to explore the arms; the time spent in each arm together with the number of entries were measured. After the training phase, the mice were permitted to move around in all three arms for five minutes and the whole session was recorded on video for assessing cognitive abilities. Mice with Alzheimer-like disease perform poorly in this test especially in terms of memory since they are unable to switch between the arms effectively. A percentage alteration, indicative of cognitive decline, was calculated using the formula: $\{Spontaneous\ alternation/(Total\ number\ of\ arm\ entries - 2)\} \times 100$.

Pathological conditions of mice depict a percentage alternation of less than 22%, thus implying that mice with such diseases have learning and memory deficits as depicted in Figure 2.5 (Kraeuter et al., 2019).

Figure 2.5: Y-maze apparatus used for behavioral testing. The figure depicts the Y-shaped maze designed to assess spatial memory and exploratory behavior in mice. The three identical arms are arranged at 120° angles, allowing for the evaluation of spontaneous alternation performance.

2.5. Elevated Plus Maze

The Elevated Plus Maze (EPM) test is used to assess anxiolytic behavior in mice. Mice, naturally averse to elevated and open spaces, tend to avoid these areas, and this test evaluates their willingness to explore them despite their aversion. The apparatus consists of four arms: two enclosed passageways and two open passageways. It is constructed from an opaque iron alloy and elevated 75.5 cm above the ground. Each arm measures 30 cm in length and 5 cm in width. During the test, each mouse underwent a single five-minute trial, starting at the maze's center, facing away from the experimenter and oriented opposite to the closed arms, as shown in Figure 2.7. Behavioral analysis was conducted using video recordings to determine the total number of entries into the open arms and the amount of time spent in these open passageways. The results were interpreted by noting the values

for time spent on the open areas of arm, and on the enclosed arm, also number of entries to open and close arm were noted(Kruk et al., 2011)

Figure 2.6: Elevated Plus Maze (EPM) set up. for anxiety-like behavior assessment. The figure depicts the plus-shaped apparatus elevated above the ground, consisting of two open arms and two enclosed arms. The setup is used to evaluate exploratory behavior and anxiety levels in mice, based on their preference for open versus enclosed arms.

2.6 Dissection and Brain Tissue Preparation

Following the completion of behavioural testing, all animals were anesthetized using chloroform via inhalation to ensure deep sedation. A trapezoidal incision was performed on the thoracic region to expose the heart. A small incision was made in the upper left atrium to allow for the insertion of a 23-gauge perfusion catheter into the right ventricle. Perfusion was initiated with normal saline for five minutes, followed by a two- to three-minute infusion of 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS).

After perfusion, the brain was carefully removed and immediately placed in 4% PFA on ice. The skull was dissected with precision and divided along the mid-

sagittal plane using fine scissors, starting from the cerebellum and ending near the olfactory bulbs. The brain was split into left and right hemispheres, and the cortex was rapidly separated on ice. All samples were stored in 4% PFA to preserve them for histological analysis.

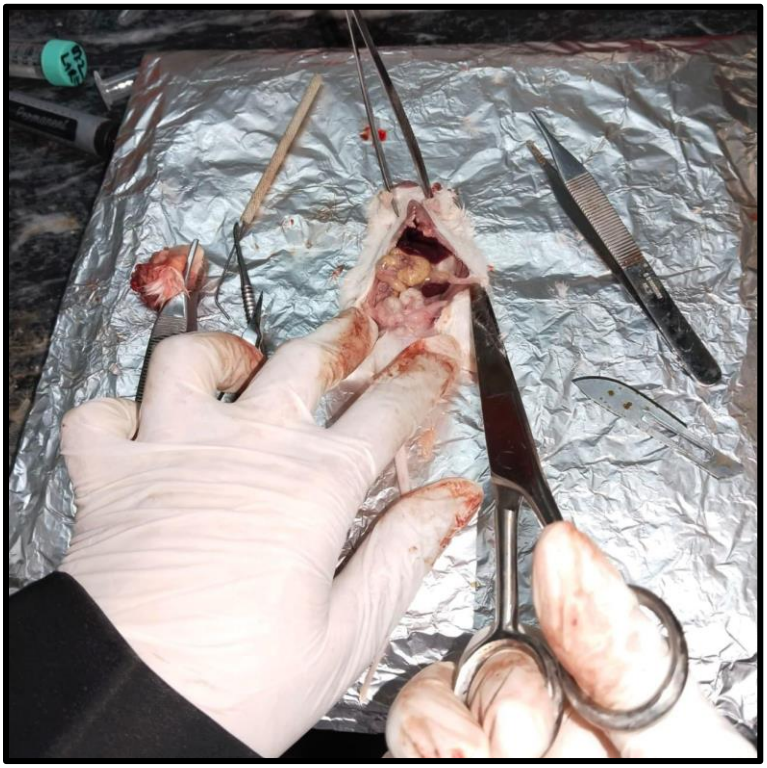

Figure 2.7: Dissection of mice for tissue collection. The figure illustrates the process of dissection, highlighting the precise extraction of tissues such as the brain, liver, or other organs for downstream analyses. The setup emphasizes sterile techniques and proper anatomical identification during the procedure.

2.7 Histological Analysis

To guarantee adequate fixing and retention of the tissues for histological testing, the brain was meticulously divided and submerged right away in 10% formalin. To attain total fixation, the brains were preserved in 10% formalin for 24 to 48 hours. To see certain structures, cells, tissues, or components, several staining procedures were used after fixation. The main technique used was hematoxylin and eosin (H&E) staining, which highlights the cytoplasm in pink and the nucleus in blue to clearly see cellular morphology. Furthermore, Nissl bodies, which are noticeable in

the bodies of neurons and suggest protein synthesis activity, were stained blue in order to selectively identify neurons using Nissl labelling. Collagen fibers, muscle, and cytoplasm were all distinguished using Masson's Trichrome Staining on liver tissue.

2.7.1 H&E staining

Fixed tissues from one brain in each group (control, diseased, and treated) were sectioned into approximately 4 µm thin slices, and microscopic slides were prepared. These slides were deparaffinized by incubating at 63°C for about 30 minutes. During this process, slides were immersed in xylene for two minutes and subsequently cleaned with graded ethanol concentrations (100%, 90%, 80%, and 70%). Hematoxylin staining was applied for three minutes, followed by sequential rinses: a one-minute water wash, one minute in mild acid for differentiation, another water wash, bluing, and a final water rinse. Afterward, the slides were treated with ethanol, stained with eosin, and processed through 95% ethanol, 100% ethanol, and a two-minute immersion in xylene. Finally, coverslips were mounted onto the slides to complete the preparation.

2.8 Microscopy

The H&E-stained slides were examined using a binocular light microscope (S37242, Labo America Inc., USA) with magnification settings ranging from 4X to 100X. The slides were examined at 40X magnification for analysis. Image J software was used to count the cells in the cortical tissues, and the results were compared to find any discrepancies.

2.9 Statistical Analysis

The data that was gathered was statistically analyzed using GraphPad Prism (version 10.41, CA, USA). 1-way analysis of variance (ANOVA) was used for data sets with two components and numerous. It was anticipated that the data were homogeneous of variance within each group, independent, and roughly

normally distributed. Any difference with a p-value < 0.05 considered to be statistically significant.

CHAPTER 3 : RESULTS

3.1. Behavior Assessment Results

3.1.1 Spatial Memory and Exploratory Tendencies in Y-Maze Test

In order to assess spatial memory and cognition in mice, the Y-maze test was employed; the impact of intranasal and intravenous PRP treatment was also assessed. The control group demonstrated enhanced cognitive function memory and exploratory activity. The diseased group, however, made significantly less accurate memory retention and exploration errors, which indicates the observed cognitive loss as a part of the disease model. When administered through both intranasal and intravenous administration, the PRP showed different levels of improvement relative to the diseased group. Treatment with PRP was found to bring improvements in spatial memory and exploratory behavior and therefore could be used to address the cognitive impairments in the diseased group. Intravenous treatment also enhanced cognitive function, but the results were not as significant as in the intranasal group, especially in the exploration and spatial learning. Taken together, these results indicate that intranasal delivery of PRP is a better strategy to enhance learning ability in the Alzheimer's disease model. However, both routes showed therapeutic potential and warrant further investigation to optimize their efficacy.

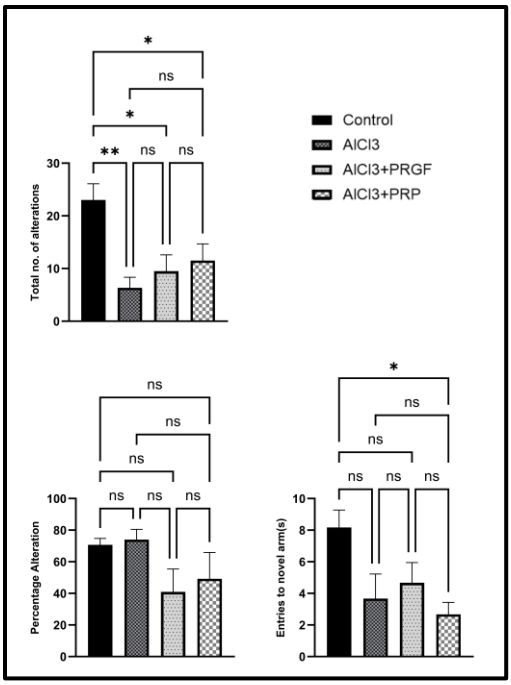

Figure 3.1: Graphs depict the performance in the Y-Maze test. There is high significance in the PRP group compared to the disease and PRGF groups, The PRP group showed significant improvement compared to both the PRGP and disease groups the percentage of alternations, entries to the novel arm (*p ≤ 0.05 when comparing experimental groups. Statistical analysis, employing one-way ANOVA and Tukey's multiple comparison test, supported these findings, with significance denoted by *p ≤ 0.05, p > 0.05 ns. Error bars represent the standard error of the mean (± SEM).

demonstrating improved spatial learning. Error bars represent the standard error of the mean (± SEM).

3.1.2 *Evaluation of Anxiety like behaviour*

The elevated plus maze test is commonly employed to measure anxiety like behaviors in rodents. Since fear and anxiety are but natural responses in animals, the height and openness of the high open arms triggered the anxiety reaction. Several parameters, including the time spent in the open and closed arms and the frequency of entering each arm, were analyzed in this test in order to evaluate anxiety-like responses of the animal groups. The parameters concerning open arms time, number of entries to the open arms are low while the parameters of closed arms time and number of entries to the closed arms are high, which reflects lower and higher

anxiety, respectively, in mice.The results showed that the control mice were less anxious as they spent more time in the open arms and made more entries into the open arms. Conversely, mice with disease had a high level of anxiety as they spent less time in the open arms and more time in the closed arms. PRGF and PRP treatment groups were less anxious than the diseased group as shown by increased time spent in the open arm of the maze and low entries into the closed arm. PRGF treatment group was more affected as they spent much time in the open arm and less time in the closed arm and made fewer entries in the closed arm than the PRP treatment group which suggests that the intranasal treatment has a more anxiolytic effect than the intravenous treatment. These results imply that both treatments could decrease the anxiety-like behaviors, and the intranasal treatment might be more efficacious in terms of anxiolytic effect. However, further studies are needed to validate these results and explore the relative effectiveness of the two treatment approaches.

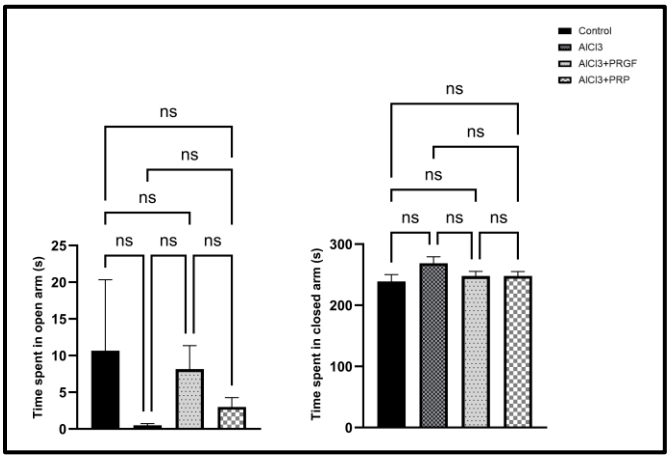

Figure 3.2: Graph depicts the performance in the Elevated Plus Maze (EPM) test. The PRGF-treated mice spent significantly more time in the open arms compared to the disease and PRP groups. Both the PRP and PRGF groups spent less time in the closed arms than the disease group. The PRGF group also made more entries into the open arms than the disease and PRP groups, and fewer entries into the closed arms compared to the disease and PRP groups, indicating reduced anxiety-like behavior. Statistical analysis, employing one-way ANOVA and Tukey's multiple comparison test, these findings were not significant with significance denoted by $p>0.05$. Error bars represent the standard error of the mean (± SEM).

3.2 Histological Evaluation of Neurodegenerative Changes in AD

Histopathological examinations are critical for studying Alzheimer's disease (AD) and related systemic changes because they give information about tissue shape and pathological abnormalities. This study uses hematoxylin and eosin (H&E) staining to determine total cell counts and structural integrity in important brain areas such as the cortex and hippocampus. The investigation compares control, illness, and treatment groups, with an emphasis on the outcomes of nasal PRGF and iv PRP. Both brain regions are examined to discover anatomical abnormalities associated with AD disease.
H&E-stained slices are examined using a light microscope at 40X resolution. Cellular alterations such as the loss of neurons, nuclear shrinkage, and malformed neurons are measured with ImageJ software. Figure 3.5 depicts the assessment of structural abnormalities in the cortex across several groups. While hippocampal modifications are examined to detect treatment-induced alterations to cellular architecture, as seen in Figure
Additionally, Masson's trichrome staining is used for liver tissue examination to assess fibrosis and collagen deposition. This staining gives an extensive view of hepatic structural alterations and supplements the overall histopathological examination, providing insights into systemic effects and treatment success. These combined analyses help to better understand the regenerative and systemic impacts of the suggested medicines.

3.2.1. structural and morphological analysis of frontal cortex

The histological study of H&E-stained frontal brain samples at 40x magnification indicated substantial differences between the control, Alzheimer's, and therapy groups. The control group's neuronal organization was unaffected, with usual cellular density and preserved well neuropil, and there was no neuroinflammation, necrosis, or aberrant aggregations. In contrast, the Alzheimer's group showed a significant decline in neuronal density, indicating neuronal death, as well as severe gliosis and neuroinflammation. Disorganized neuropil, vacuolation, cellular debris,

astrocytic hypertrophy, and microglial activation were among the structural changes, all of which indicated progressive neurodegeneration. In the PRGF-treated group, neuronal density and organization were partially restored, along with decreased gliosis and neuroinflammatory markers. Overall PRP and PRGF in terms of neuroprotective and regenerative properties did not performed well. Overall, our data show the therapeutic effects of PRGF and PRP in Alzheimer's pathology, with PRP having a stronger restorative impact.

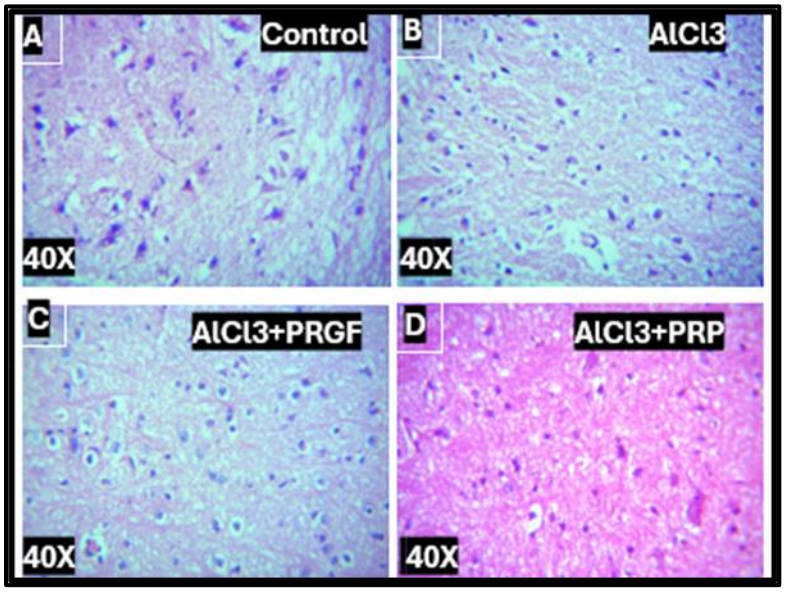

Figure 3.3: Microscopic examination of the frontal cortex in mice at 40x magnification. Figure illustrates the therapeutic effects of PRGF and PRP treatments compared to the control and diseased groups. Images were acquired using Optica Vision software.

3.2.1.1. cell counts in frontal cortex

Image J software was used to quantify cells in digital photomicrographs, and pictures were obtained using Optica Vision program. Figures 3.5 show that the control group had the greatest cell counts, whereas the AlCl3 group had the lowest, indicating considerable neuronal loss linked with the development of Alzheimer's disease (AD). These findings demonstrate the disease's pathogenic impact while also providing evidence for therapeutic efficacy. Both the PRGF- and PRP-treated groups demonstrated non-significant improvements in cortical cell counts as compared with the untreated group, with the control group acting as the standard

reference. The graphical analysis emphasizes the increased cellular preservation and possible therapeutic effects of PRGF and PRP therapies in preventing AD-related neuronal loss.

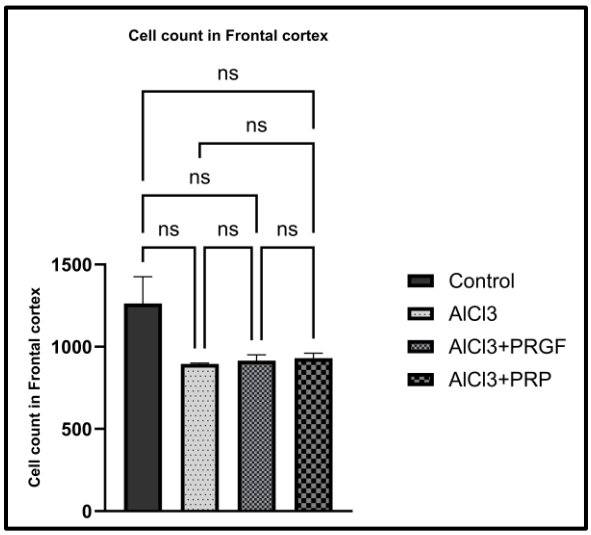

Figure 3.4: Cell counts in the frontal cortex. The figure illustrates the cell count across experimental groups in the frontal cortex. Statistical analysis using one-way ANOVA and Tukey's multiple comparison test demonstrated non-significant differences among the groups ($p > 0.05$). Error bars indicate the standard error of the mean (± SEM).

3.2.2. structural and morphological analysis of cerebral cortex

The H&E-stained pictures of the cerebral cortex at various resolutions (4x, 10x, and 40x) show clear disparities in the progression of the disease and treatment success.

At 4x magnification, the normal cortex has well-defined layers and typical neuronal density, with no evidence of disease or degeneration. In contrast, the untreated Alzheimer's cortex has altered cortical architecture, reduced neuronal density, and increased cell spacing, indicating neurodegeneration. PRGF-treated cortex exhibits a certain reconstruction of cell density and structure, but PRP therapy results in more substantial benefits, such as near-normal cortical organization and decreased neurodegeneration. At 10x magnification, the control group retains its homogenous structure and cellular organization. The Alzheimer's cortex has obvious diseases, including gliosis, hypercellularity, and spongiform degeneration. PRGF therapy decreases these abnormal indicators while improving cellular structure to a

considerable extent. PRP-treated samples show improved cortical preservation, with less gliosis and inflammation and compare more closely to the control.

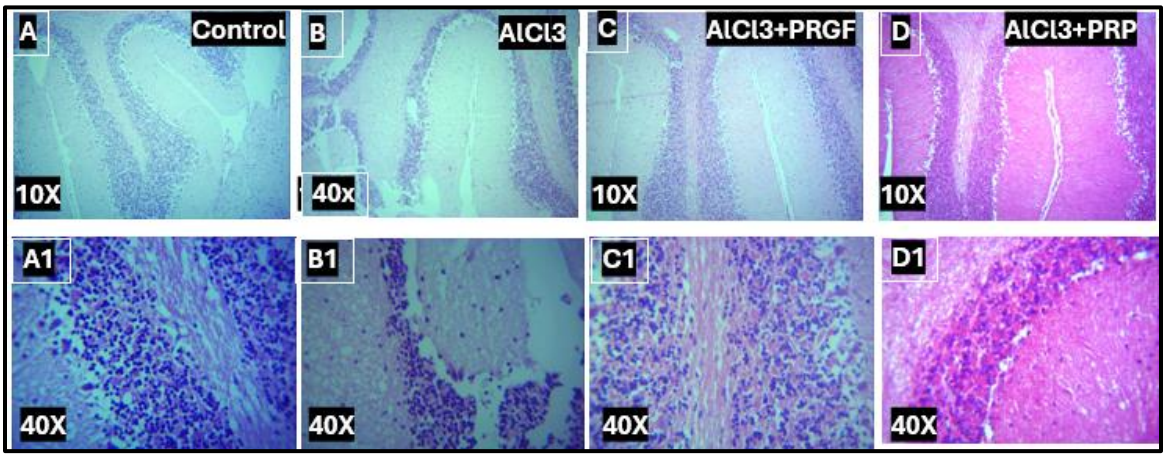

Figure 3.5: illustration of the cerebral cortex across different experimental groups. The figure shows histopathological images of the cerebral cortex from the PRP, PRGF, disease, and control groups at 10x and 40x magnification. Images were captured using Optica Vision software.

At 40x magnification, the normal cortex shows organized neuronal layers with neither inflammatory or degenerative alterations. The Alzheimer's cortex is severely disorganized, with diminished neuronal density and indications of inflammation. PRGF therapy improves cortical structure and lowers gliosis, but PRP treatment considerably improves neuronal organization and pathological characteristics, approaching control group settings. These findings imply that, while both PRGF and PRP therapies have therapeutic effects, PRGF is more effective in decreasing Alzheimer's-related neurodegeneration and encourages cortical regeneration. Quantitative measures of neural density and inflammatory markers are advised for a more detailed analysis.

3.2.2.1. Cell counts in cerebral cortex

The total count of cells on digital microscopy images was counted using Image J software and Optica Vision software. The results, presented in Figure 3.8, reveal that the control group had the greatest cell count, while the diseased group had the lowest cell density in the cortex, which is consistent with the course of

Alzheimer's. The afflicted group's lower cell count verifies AD's pathogenic effect. When compared to the treated groups, the PRGF-treated cortex had a much higher cell count than the diseased group, while it was still lower than the control group. In contrast, the PRP-treated group had a comparable cell count to the AlCl3 group, with no significant change, indicating that therapeutic effectiveness was restricted.

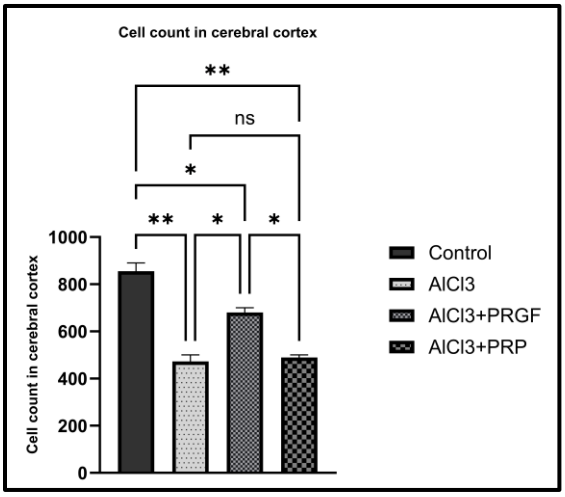

Figure 3.6: Graphical representation of cell count in the cerebral cortex at 40x magnification. The figure illustrates the cell count across experimental groups. Statistical analysis using one-way ANOVA and Tukey's multiple comparison test revealed a significant difference among the groups (*p ≤ 0.05, *p<0.01). Error bars represent the standard error of the mean (± SEM).

3.2.3 structural analysis of hippocampus

To investigate structural alterations in the brain hippocampus DG area, the number of cells, structural deformity, plaques, and cellular density were deemed the primary indicators of illness progression and alteration following therapy. The illness group contains fewer neurons with malformed nuclei, abnormal cell shape, shrinking of the nucleus, elongated cells, and changed sizes. The hippocampus study indicates significant variations in neural density and integrity of structure across groups. The control group had the maximum cell count, normal architecture, and no evidence of deterioration or inflammation. In contrast, the untreated Alzheimer's group shows substantial neuronal loss, gliosis, and morphological disruption, as well as the lowest cell count, showing that the disease has advanced. The PRGF-treated hippocampus has a larger cell count than both the untreated illness group and the

PRP group, but it stays less than that of the control group. This shows that PRGF therapy causes partial regeneration of neuronal density while preserving structural integrity. The PRP-treated hippocampus contains far fewer neurons than the PRGF group and substantially fewer than the control, reflecting not as effective recovery with regard to of cell density, despite a considerable reduction in inflammation.

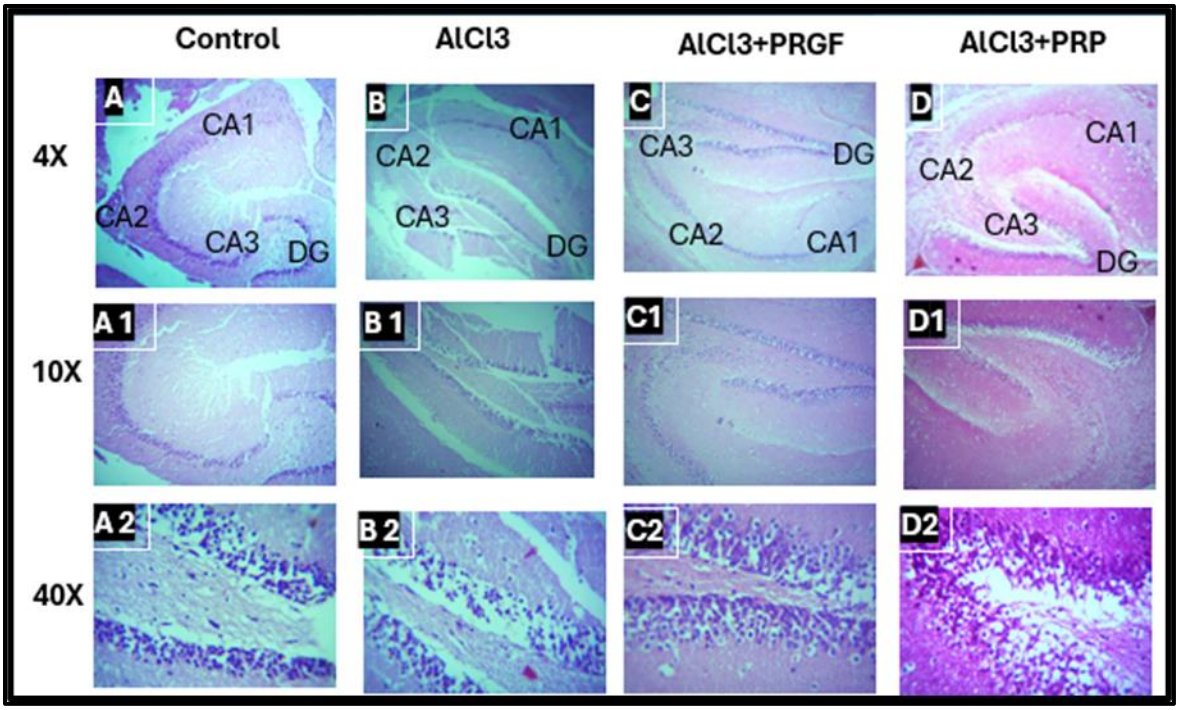

Figure 3.7: Histopathological analysis of the mice hippocampus at 4x, 10x, and 40x magnification. Figure Shows the hippocampus from the PRGF and PRP treatment groups in comparison to the control and disease groups. The images were captured using Optica Vision software.

These findings indicate that PRGF has a greater influence on cell counts and integrity of tissue than both the Alzheimer's and PRP, yet PRP therapy still has some neuroprotective effects, notably in terms of inflammation reduction and neuronal morphology preservation.

3.2.3.1. hippocampus DG region

A 40x magnification study of the dentate gyrus (DG) areas in the hippocampus revealed major variations across the AlCl3, control, PRGF-treated, and PRP-treated groups. In the disease control group, there was an increased cellular density

(hypercellularity), as well as disorganized neuronal layers and a lack of normal DG architecture, which are signs of Alzheimer's disease development. The PRGF therapy resulted in better cellular organization than the disease, control, with less hypercellularity and more delineated layers. Furthermore, nuclear morphology improved in PRGF-treated samples, with more regular nuclei and less evidence of apoptosis. The PRGF and PRP therapy showed the greatest improvement, with further decrease in hypercellularity and more normalized cell density.

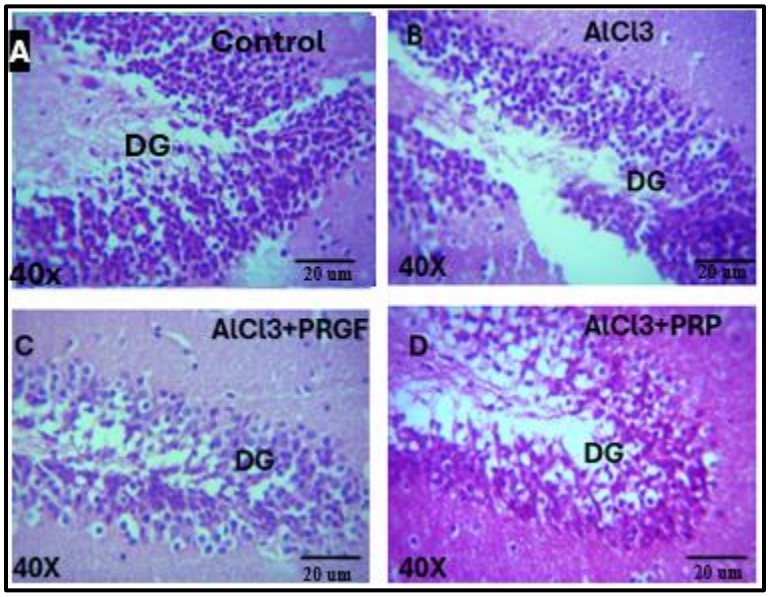

Figure 3.8: Histopathological analysis of the DG region of the hippocampus at 40x magnification. Figure Displays the DG region of the hippocampus from the PRGF and PRP-treated groups, compared to the control and disease groups. Images were captured using Optica Vision software.

The nuclear morphology in the PRP-treated samples was almost normal, with no indication of apoptotic or necrotic cells. Glial proliferation and gliosis were decreased in both treatment groups, but the PRP group had the smallest glial response, indicating stronger anti-inflammatory activity. The extracellular space, which had grown in the disease control owing to tissue deterioration, was decreased in both treated groups, with the PRP group having the most compact tissue, indicating greater preservation of tissue integrity. Overall, both PRGF and PRP-treated group preserved the most DG structure, including the curvature and granule cell layers, when compared to the disease indicating that PRGF and PRP have the

potential to be the most effective treatment for Alzheimer's-related neurodegeneration.

3.2.3.1.2. Cell counts in DG region

Cell counts in the hippocampus dentate gyrus (DG) area were quantified with ImageJ software and plotted with GraphPad Prism, and the findings are shown in Figures 3.11. The control group had the maximum cell density in the hippocampus DG area, which served as a baseline for comparisons. In contrast, the sick group, which is symptomatic of Alzheimer's disease (AD), had a considerable fall in cell count, emphasizing the neuronal loss associated with AD development. This decrease in cell density supports the pathogenic alterations associated with the disease. Both the PRGF and PRP therapy groups did not showed an increase in cell count and density compared to the sick group, indicating a non-therapeutic effect. These findings did not support the potential usefulness of PRGF and PRP in reducing cell loss related to Alzheimer's disease.

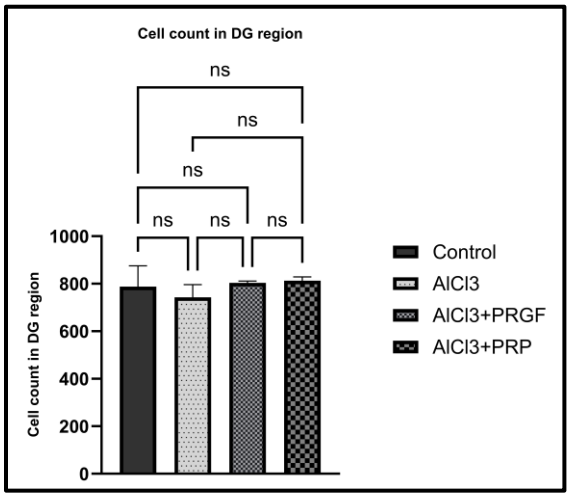

Figure 3.9: Graphical representation of cell count in the DG region of the hippocampus. The figure illustrates the cell count across experimental groups. Statistical analysis using one-way ANOVA and Tukey's multiple comparison test revealed a significant difference among the groups ($p > 0.05$). Error bars represent the standard error of the mean (± SEM).

3.2.4. histological analysis of Liver

The liver histology shows clear differences between the control, Alzheimer's, PRGF-treated, and PRP-treated groups. In the placebo group, the hepatic lobules have well-preserved architecture with little to no collagen deposition, and the hepatocytes are homogeneous in size and distribution, with identifiable nuclei. The sick group's hepatic architecture is significantly disrupted, with the disappearance of distinct lobular borders. Collagen deposition is significantly enhanced, especially in the periportal and perisinusoidal regions, accompanied with hepatocyte deterioration, necrosis, and cytoplasmic vacuolation.

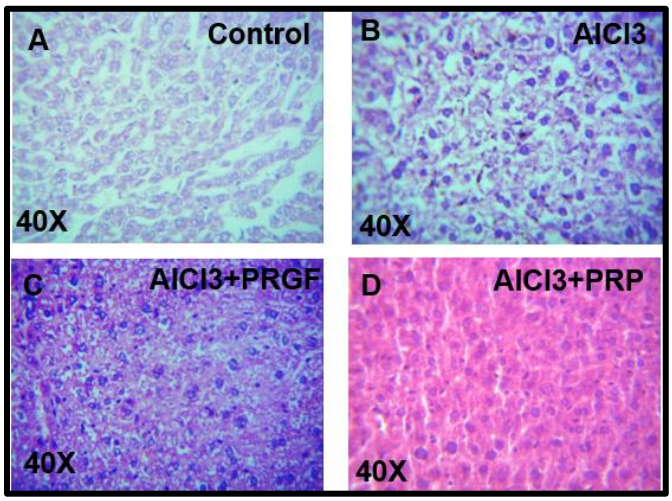

Figure 3.10: Histopathological images of the liver with H&E stain at 40x magnification. The figure displays the liver tissue stained with H&E and Masson's Trichrome, providing detailed insights into tissue structure and fibrosis. Images were captured using Optica Vision software.

In comparison to the sick group, the PRGF-treated group recovers somewhat and has less fibrotic tissue. The blue staining for collagen is less visible, and hepatocytes exhibit partial repair, with fewer necrotic regions and moderate cellular growth consistent with tissue repair. The PRP-treated group did not show any healing. Hepatocytes in this category have a near-damaged configuration, with conspicuous nuclei and low cytoplasmic damage, indicating successful healing and tissue regeneration. In summary, collagen deposition gradually declines from sick to control groups, with PRGF therapy demonstrating a little restoration of hepatocyte

integrity and hepatic architecture. PRGF therapy offers an intermediate level of improvement.

3.2.4.1. cell counts in Liver

Cell counting data, as analyzed with ImageJ software, indicated that the placebo group had the greatest number of cells. The AlCl3 group had a lowest cell count, suggesting considerable neuronal loss due to disease progression. In contrast, the PRGF-treated group had a bit higher cell count than the disease and PRP-treated groups, indicating a modest improvement in cell preservation. However, the PRGF-treated group still had a lower cell count than control group, demonstrating that, while PRGF therapy has some neuroprotective effects, it does not completely restore cell density to normal. The PRP-treated group had a lower cell count than the AlCl3 group, but the cell density remained lower than that of the control and PRGF-treated groups. These findings demonstrate the different levels of success among therapies, with PRGF demonstrating the most hopeful recovery when compared to the diseased group.

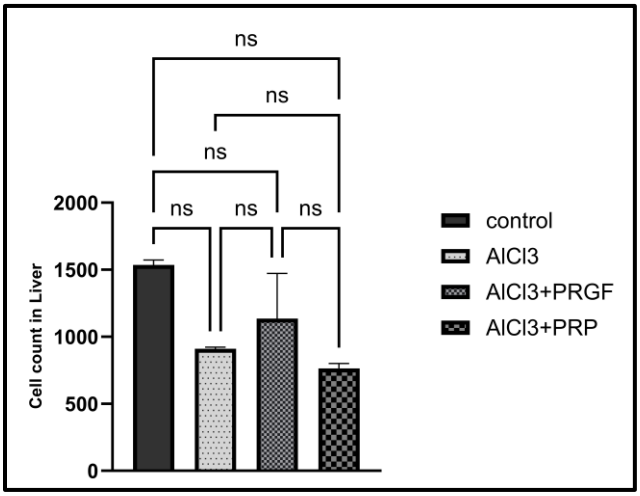

Figure 3.11: Graphical representation of cell count in the liver. The figure illustrates the cell count across experimental groups. Statistical analysis using one-way ANOVA and Tukey's multiple comparison test revealed a non-significant difference among the groups ($p > 0.05$). Error bars represent the standard error of the mean (± SEM).

CHAPTER 4 : DISCUSSION

Over the last several decades, a lot of research has been conducted on Alzheimer's disease (AD), a progressive neurodegenerative illness, with the amyloid hypothesis serving as a main theoretical structure(Kang et al., 1987). Many pharmaceutical strategies have been investigated, including as medications that target the manufacture and catabolism of amyloid-beta (Aβ); however, it is still unknown how well these strategies work in AD patients and animal models(M.L., 2003). Cholinesterase inhibitors, NMDA receptor antagonists, and alternative treatments including music and art therapy are the mainstays of current treatment approaches, which concentrate on symptom management(Blackman et al., 2021; Theleritis et al., 2017). Galantamine, rivastigmine, and donepezil are a few examples of cholinesterase inhibitors that are frequently used to treat the behavioral and cognitive signs of AD(S. Singh et al., 2016). Although galantamine's capacity to enhance cognitive performance is very well-known,

Side effects such hepatotoxicity, gastrointestinal issues, and light-headedness restrict its therapeutic usefulness (Ribeiro-dos-Santos et al., 2023).Although it does not stop the course of AD, memantine, a voltage-dependent receptor for NMDA antagonist, assists with decline in memory and cognitive impairment (Pardo-Moreno et al., 2022). In light of these constraints, new therapeutic modalities are attracting interest because of minimal negative effects and antioxidant qualities, such as combined therapies and non-invasive alternatives like phototherapy.
One of the novel therapies that has shown promise in addressing the complex pathophysiology of AD is platelet-rich growth regulators (PRGF), which are extracted from human blood and are a settled availability of transforming factors, morphogens, and bioactive proteins that foster regeneration of tissues and repair. PRGF provides double advantages for therapy by solving both structural stability and biochemical deficits in AD, such as tau hyperphosphorylation, amyloid-beta plaque accumulation, and synaptic dysfunction; its capacity to control inflammation and encourage cellular repair further highlights its potential to lessen

neurodegenerative processes; additionally, intranasal delivery of PRGF permits non-invasive treatment of the central nervous system by bridging the blood-brain barrier, increasing its clinical applicability.

Significant neuroprotective and restorative effects have been shown by this method in animal models, such as decreased tau hyperphosphorylation, decreased astroglial activation, and enhanced cognitive function (Cagnin et al., 2001; Houeland et al., 2010; Parachikova & Cotman, 2007) PRGF offers a revolutionary therapeutic strategy for AD with significant practical application potential by overcoming the shortcomings of existing therapies and utilizing the body's natural repair processes (Anitua et al., 2010; Leslie, 2010). In these investigational studies, we used two different delivery methods intravenous and intranasal to examine the impact of Platelet-Rich Growth Factors (PRGF) in chemically induced Alzheimer's disease mice. The Y Maze and Elevated Plus Maze were among the behavioral evaluations used to compare the effectiveness of these therapies. To assess the structural alterations linked to PRGF therapy, a histological examination of several brain areas was also carried out.

The Y-maze exam assesses working memory and spatial recognition ability. This study compares the behavioral performance of various groups using five key parameters: number of entries into the novel arm, percentage spontaneous alterations between the two arms and total alteration. The control group's cognitive performance was intact, with no evidence of neurodegeneration, indicating normal brain activity.

In our studies there were notable variations between the outcomes of the intravenous and intranasal therapy groups. The intranasal therapy group showed a higher proportion of voluntary alternation ($p < 0.05$) indicating a certain level of cognitive improvement in comparison to the AlCl3 group. The comparison of the $AlCl_3$-induced infected group with the control group showed that the condition caused cognitive deficits in every parameter that was assessed. Interestingly, the control group spent a much greater amount of time in the new arm than any other group

(*p<0.05), suggesting that their memory processes and exploration behavior were normal. Furthermore, contrasted to the $AlCl_3$ group, the control group entered the novel arm substantially more frequently, indicating even better cognitive function. Additionally, the diseased group's random alternation rate was noticeably lower than the control groups, indicating working memory problems. All cognitive measures were significantly superior in each of the treatment groups when compared to the $AlCl_3$ unit to the intravenous and PRGF treatment groups. A certain amount of memory retrieval was evident since the nasal group spent a longer period in the newly developed arm than in the baseline.

The intranasal PRGF group showed the most significant memory recovery, in the two groups receiving treatment showing an increase in unpredictable alternation, although not reaching those levels seen in the control group. There were also significant improvements in total alternation (*p<0.05), but the intranasal group showed the most substantial recovery.

In contrast to the $AlCl_3$-induced Alzheimer's disorder (AD) group, the effectiveness of the Platelet-Rich Plasma (PRP) given intravenously and PRGF intranasally during the current investigation markedly enhanced cognitive function.

The elevated plus maze (EPM) test offers useful information about anxiety-like behavior. $AlCl_3$'s anxiogenic effects were confirmed by the fact that the group treated with the substance spent considerably less time in the open arms than the control group, an indication of decreased anxiety. On the other hand, the groups that received PRGF showed better anxiety-related metrics. In particular, the intra-nasal (IN) PRGF group outperformed the $AlCl_3$ group in terms of time spent in the open arms, and the intra-venous (IV) PRGF group outperformed the $AlCl_3$ group as well. Remarkably, the IN PRGF group had an open-arm time that was more in line with the control groups, indicating that the nasal route had a stronger anxiolytic effect than the IV method. Despite IV group performed substantially better compared to the $AlCl_3$ group even though it spent a shorter span in the open arms than the IN group. This suggests that both delivery routes, though to varying degrees, reduce the

anxiety that is brought on by $AlCl_3$.Compared to the control group, the $AlCl_3$ group spent considerably more time in the enclosed arms, which is frequently linked to increased anxiety. In comparison to the $AlCl_3$ group, PRGF treatment, especially through the IN route, dramatically decreased the amount of time spend in the confined arms. This decrease demonstrates how well PRGF works to reduce anxiety-like behavior brought on by $AlCl_3$.Though not as successfully as the IN route, the IV group also showed a reduction in the duration spent within the closed arms in comparison to the $AlCl_3$ group. These results were further supported by open arm entries. The anxiogenic effect of $AlCl_3$ was highlighted by the control group's considerably higher number of entries into the open arms during comparison with the $AlCl_3$ group. Open arm entries were significantly higher in the IN and IV PRGF treatment groups than in the $AlCl_3$ group. Nevertheless, neither group achieved the open arm entries of the control group, to the IN group once more outperforming the IV group.

These results are consistent with other research showing that $AlCl_3$ increases time spent in the closed arms and decreases entry into the open arms, causing neurological disorders and anxiety-like behavior (Kruk et al., 2011). Normal behavior was somewhat restored in the PRGF-treated groups. Particularly, the IN group showed anxiety-related metrics that were more in line with the control groups, highlighting the possibility that nasal delivery could strengthen PRGF's anxiolytic effects.

Overall, the findings support $AlCl_3$'s anxiogenic effects, as shown by the much longer time and more entry in the enclosed arms but statistically results were not significant. PRGF treatment—particularly through the IN route—effectively offset these effects. With the IN route providing a more effective delivery strategy than the IV route, these results demonstrate a chance of the growth factor PRGF as an effective therapy for reducing anxiety-like behaviors linked to neurotoxic exposures.

The histological and quantitative examinations of brain areas such as the hippocampus, dentate gyrus (DG), frontal cortex, and cerebral cortex indicated non-

significant differences between the control, disease, PRGF, and PRP therapy groups, as validated by ANOVA and Brown-Forsythe tests. The hippocampus, an important site for memory and learning, showed significant neuronal loss in the disease control group. PRGF therapy preserved more neuronal density than PRP, although neither treatment completely restored cell counts to control levels. The ANOVA analysis showed a non-significant $p>0.05$, and the Brown-Forsythe test confirmed with a p-value, indicating not very strong variations in cellular density between the groups. These findings demonstrate the non-therapeutic of PRGF in preventing hippocampus degeneration associated with Alzheimer's disease (AD).

Decreases in cell density were found in the DG area of the hippocampus, which is critical for neurogenesis, when compared to the control group. PRGF and PRP therapy did not result in recovery and better neuronal preservation than PRP. The ANOVA analysis yielded a p-value of > 0.05, showing substantial variations in treatment effectiveness. The findings did not support PRGF's potential to preserve and partially rebuild neuronal morphology in the DG area. Although the Brown-Forsythe test was not applicable in this case, the statistical significance of the ANOVA indicates confidence in the observed patterns.

The frontal brain, which is liable for higher-order cognitive activities, also differed significantly between the groups. The control group had the greatest cell density, accompanied by the PRGF, PRP, and diseased groups. PRGF therapy preserved cellular structure better than PRP. The ANOVA findings revealed a non-significant $p > 0.05$, corroborated by a Brown-Forsythe, indicating non-significant differences between groups. These data imply that PRGF provides more protective effects on neurons in the frontal cortex than PRP, yet both did not show potential in reducing AD pathology.

In a similar way in the cerebral cortex, the placebo group had the maximum neuronal density, whereas the illness group had considerable decreases. PRGF therapy resulted in partial healing and had a better neuroprotective impact than PRP. The ANOVA analysis yielded a significant $*p<0.05$, while the Brown-Forsythe test supported the variation with a p-value of <0.05. These findings suggest that, while

PRGF and PRP are both effective in maintaining cortical architecture, PRGF has a stronger effect.

Overall, the ANOVA and Brown-Forsythe tests show statistically significant differences in only cerebral cortex locations studied. PRGF consistently outperformed PRP in terms of neuroprotective and regenerative benefits across all areas, showing its potential as a treatment for Alzheimer's disease. The statistical significance obtained in each investigation supports the robustness of these findings, highlighting PRGF's therapeutic promise for treating neurological damage and preserving tissue integrity in AD.

The statistical examination of the liver histology results reveals substantial findings that demonstrate the role of PRGF and PRP therapies in hepatic recovery in Alzheimer's disease. The ANOVA findings showed a p-value of 0.0033, showing a significant variation in collagen deposition and tissue architecture across each category (control, diseased, PRGF-treated, and PRP-treated). The Brown-Forsythe test verified heterogeneity in variance (p-value < 0.0001), indicating substantial variations in standard deviations groups.
The findings suggest that the AlCl3 group has substantial fibrosis and disturbed hepatic architecture, whereas PRGF therapy causes modest recovery with decreased collagen deposition and better hepatocyte integrity. PRP therapy, however, did not show any significant improvement, nearly approximating the diseased group with low fibrosis and restored tissue structures. These data support the curative effects of PRGF in reducing the damage to the liver associated with Alzheimer's disease pathology, and they imply that PRGF, while helpful, is less effective than PRP. The statistically significant results verify the therapies' effectiveness and emphasize their distinct effects on liver histology.

CHAPTER 5 : SUMMARY

Alzheimer's disease (AD) is a progressively neurodegenerative condition characterized by cognitive impairment, memory loss, and neuroinflammation. It is predominantly connected with the buildup of amyloid plaques and tau tangles, which causes neuronal death. Current treatment options are limited, with an emphasis on symptom management rather than halting or reversed disease progression. In recent years, platelet-rich plasma (PRP) and platelet-rich growth factors (PRGF) generated from human blood have received interest for their possible therapeutic benefits in a variety of disorders, including neurological diseases. However, the use of these medicines in Alzheimer's disease is yet underexplored, particularly in chemically produced AD models. The current study sought to examine the therapy potential of PRGF and PRP in a chemically induced mouse model of Alzheimer's disease, employing aluminum chloride (AlCl3) to cause cognitive impairments. PRGF was administered intranasally for the first time in a chemical model of Alzheimer's disease, as previous research had primarily focused on its effects in transgenic mice. Moreover, a comparative analysis of PRGF and PRP therapies was conducted, adding novel insights into their potential benefits for AD treatment. Behavioral tests such as the Y-maze and Elevated Plus Maze (EPM) were used to examine cognitive function, anxiety-like behavior, and memory performance. Significant changes in spontaneous alternation behavior were seen in the Y-maze, with PRGF-treated mice exhibiting better memory and cognitive flexibility than the disease and PRP groups In the Elevated Plus Maze, PRGF treatment led to reduced anxiety-like behavior, as evidenced by increased time spent in open arms and few entries into close arms. Histopathological analysis of the frontal cortex, cerebral cortex, hippocampus, dentate gyrus (DG) region, and liver revealed significant improvements in cellular integrity following PRGF treatment. The cellular count in the DG region and frontal cortex did not show promising results in both PRGF-treated groups, indicating no neuroprotection and potential reversal of damage induced by AlCl3. In the liver, PRGF also demonstrated somehow positive effects, highlighting its systemic therapeutic potential.

In conclusion, the study provides evidence that PRGF, particularly when administered intranasally, offers a promising therapeutic approach in some aspects like cerebral cortex for Alzheimer’s disease. It highlights that both PRGF and PRP cannot be confidently used as treatments for AD, with significant improvements in cognitive function and histopathological outcomes. Further research is required to elucidate the underlying mechanisms of PRGF’s action and its long-term effects in Alzheimer's treatment.

CHAPTER 6 : CONCLUSIONS AND FUTURE RECOMMENDATION

This study focused on the behavioral analysis of PRGF and PRP in the treatment of Alzheimer's disease (AD) in a chemically induced BALB/c mice model using aluminum chloride ($AlCl_3$). While the findings highlight the potential cognitive and neuroprotective effects of intranasal PRGF and PRP, further investigations are essential to fully elucidate their therapeutic mechanisms and applications.

Since this research is the first to employ intranasal PRGF administration in a chemically induced Alzheimer's model, future studies should delve deeper into the molecular and cellular pathways affected by these therapies. Specific focus should be given to the analysis of neuroinflammatory markers, oxidative stress parameters, and neuronal apoptosis to better understand how PRGF and PRP modulate these processes. Additionally, exploring their impact on amyloid-beta plaques and tau phosphorylation will provide crucial insights into their disease-modifying effects.

It is also imperative to expand the scope of the research to include proteomic and transcriptomic analyses of brain tissues, particularly in regions like the hippocampus, dentate gyrus, and frontal cortex. These studies could help identify specific proteins and genes influenced by PRGF and PRP, shedding light on their mechanisms of action.

Furthermore, long-term studies should be conducted to evaluate the sustained therapeutic benefits and potential side effects of PRGF and PRP. Investigating their effects in transgenic AD models and in combination with other therapeutic agents could also open new avenues for treatment optimization.

In summary, while this study provides a foundation for the behavioral and therapeutic potential of PRGF and PRP, advancing toward molecular-level research is essential to unlock their full clinical potential in Alzheimer's disease treatment.

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