Investigating the Protective Effects of Phototherapy and various drugs in Alzheimer Disease

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(2024)

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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

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School of Mechanical and Manufacturing Engineering

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Islamabad, Pakistan

(2024)

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"*Dedicated to my family, friends and mentors, whose unwavering support, encouragement and guidance have been invaluable throughout this journey. To my dearest fiance, your consistent support has been my strength and inspiration. This achievement is as much yours as it is mine"*

ACKNOWLEDGEMENT

I am profoundly grateful to Almighty ALLAH (SWT) for bestowing the willpower and divine direction that guided me through the intricacies of this research project. My heartfelt appreciation goes to my beloved parents (Yameen Akhter and Tanveer Iqbal,) my mother in law (Gulshan Ara Kulsoom) and Ahmed Mujtaba, whose constant positivity and support got me through the most trying times in the lab. All it took to get this done was for him to listen to my problems and keep me motivated when things got tough and who have been unending pillars of support and prayers. Their moral and emotional encouragement helped me surmount disappointments, and their constant presence provided solace through thick and thin. This journey was made lighter by their belief in me and their enduring companionship.

I would like to thank my supervisor, Dr Saima Zafar for her help, understanding, and perseverance during my research and the work on this thesis. This work wouldn't have been complete without their knowledge and advices, and for that, I am genuinely thankful for the chances she gave me to develop in academics and as a person.

I also want to express my gratitude to the Dr Aneeqa Noor, without her it might have been a real nightmare to cope with all the difficulties I met in the process of conducting this research.

I would like to give a special thanks to Nadia Khan, Alveena Muti who encouraged me and provided helpful advice, and moral support during some of the hardest time. It was inspiring to hear about the wonderful friends you had throughout your journey.

I will also like to thank Hassan, Nida, Nabeel, Saria, Malaika, Uswa for the support, encouragement and love bestowed on me throughout the entire process. To my parents, siblings I would like to say that your unrelenting support on my part throughout this journey has never relented I am also indebted to all individuals whose contributions made this completion of this thesis possible.

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LIST OF 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
PBM	Photobiomodulation
Gal	Galantamine
Mem	Memantine
PSEN1	Presenilin 1

ABSTRACT

Alzheimer's disease (AD) is a prevailing public health concern as it causes neuronal degeneration resulting in cognitive impairment and memory loss with epidemiology of two-fold increase in every five years, which is predicted to double by 2060. There is no effective treatment exists currently which highlights the importance of development of a novel therapeutic mechanism to treat AD. In addition to it, various studies show that the medicines and therapies have been in practice, including phototherapy, which shows symptomatic improvement only. Therefore, this study evaluated the effectiveness of a combination of drugs and phototherapy on $AlCl_3$ induced male Balb/c mice models of AD. A phototherapy device prototype, featuring variable wavelengths ranging from 400nm-750nm by using RGB LEDs and the ESP32 microcontroller is designed in this study. The software design is created with VERO and Platform IO, featuring an intuitive interface that can be accessed from any device on the local network. A 625nm wavelength light, targeting the mice brain, held 2-3cm above head was used for the phototherapy. Galantamine and Memantine drugs were also administered through intra-peritoneal route at the same time to investigate the combined effect of phototherapy and drugs. Behavioral assessments were performed to validate the enhancement in cognitive deficits and spatial learning and memory. Memantine and phototherapy combination treatment group analysis displayed increased locomotor activity, spatial memory and cognitive improvements thus indicating enhanced cognitive abilities and memory retention in object recognition, elevated plus maze, y-maze, morris water maze and open field assessments while comparing to the diseased and alone drugs group. Behaviour analysis were followed by the dissection of mice for histological analysis, including H&E staining of cerebral and hippocampal tissues to verify the presence of healthy neuronal cells. Histology revealed improved cell morphology in the combination treatment groups (phototherapy-drug) compared to administering drugs alone, signifying the neuroprotective role of phototherapy in combination, as a novel therapeutic approach. The findings of this study suggests the use of combination therapies in the treatment of AD along with other neurodegenerative diseases

Keywords: Phototherapy, Combination treatment, Alzheimer’s disease, Galantamine, Memantine, Phototherapy device prototype, RGB LEDS, VERO, Wavelength

CHAPTER 1: INTRODUCTION

Alzheimer's disease is a global issue causing severe neurodegeneration and cognitive loss. Dr. Alois Alzheimer initially identified the condition in 1906 (Shukla & Singh, 2022). Alzheimer's is a highly prevalent disease nowadays and its diagnosis is through neuropathological confirmation of neurofibrillary tangles (NFTs) of protein tau and amyloid-β (Aβ) plaques (Blennow et al., 2006). When Aβ peptides accumulate abnormally in the extracellular space, they form Aβ plaques whereas excessively phosphorylated tau protein forms intraneuronal fibrils also called NFTs (Braak et al., 2006). Globally, neurodegenerative condirions including AD and abnormally transformed proteins are responsible for 50–70% cases of aged dementia. These conditions dramatically impair neuronal function and intercellular communication. (Ahmad et al., 2018; Blennow & Zetterberg, 2018). Dementia (AD) causes progressive loss of memory and difficulties with functioning and normal daily living tasks(Licher et al., 2019).

Global estimates of dementia patients are about 416 million in AD continum, and every 20 years, this number is expected to increase (Gustavsson et al., 2023). The incidence of AD is rapidly increasing in geriatric populations (Li et al., 2024). In today's society, the rising rate of illness is presenting significant health and financial challenges.

The hallmark signs of degenerative AD develops over a longer time (typically involves years), include a progressive decline in cognition accompanied by memory loss and related conditions like mobility and speech impairment (Albert, 2011). It also include aphasia, sleep disorders, early dementia-related illnesses, feeding difficulties, performance concerns and constitute a serious threat to public health. Patients with AD may also develop hallucinations, apathy, aggressiveness, and anxiety(Cloak & Al Khalili, 2019). As two main neuropathologic abnormalities that are seen in AD patients are NFTs and amyloid plaques, it leads to early symptoms like short-term memory loss which manifests difficulties in recalling names, events from the past, conversations, disorientation, communication issues, and behavioral changes that eventually result in trouble speaking, walking, and swallowing could all get worse with it (Serrano-Pozo et al., 2011). Other cases also involve genetic mutations involving amyloid-protein precursor (APP) and presenillin 1 and /presenillin 2 but cases are lesser than 1% (DeTure & Dickson, 2019).

1.1. Pathological Features of Alzheimer Disease

Alois Alzheimer initially identified the NFTs as cytoplasmic intraneuronal filamentous deposits in neuronal cells (Serrano-Pozo et al., 2011). Early identification of AD is made possible by histological evidence accompanied by cytoskeletal abnormalities (Braak et al., 1994). Tau is made up of protein which is linked to microtubules, is the main component of NFTs. This protein is helical in shape and it results from abnormal misfolding and hyperphosphorylation (Hur, 2022). Tau proteins aid in the formation and stability of microtubule (MT) proteins and are mostly found in the axonal regions of neurons. Due to decreased protein kinase and phosphatase activity, tau protein becomes hyperphosphorylated and detaches from microtubules and accumulate as NFTs in neuronal cell bodies. This accumulation leads to neuronal loss, apoptosis, and impairment of physiological functions (Al Mamun et al., 2020; Rajmohan & Reddy, 2017; Zhang et al., 2021).

Mature NFT containing neurons have twisted dendrites and axons as well. The nucleus of filamentous tau aggregates in the cytoplasm and is moved to the edge of the neuron's soma. Ghost NFTs are non-nucleated neurons that remain after the NFT-bearing neuron dies due to the collapse of its dendrites and axons. NFTs are dispersed across the temporal area that advances to the isocortex, causes neurodegeneration, and destroys the neural pathway. The entorhinal cortex and hippocampus regions make up the allocortex of the medial temporal lobe. The presence of NFTs has been considered as a cause of higher degree of dementia in AD patients because they impede the movement of nutrients and other growth-promoting substances that are important to the proper function and longevity of neurons. (Soeda & Takashima, 2020) (Moloney et al., 2021).

An important glycoprotein known as the amyloid precursor protein (APP) is broken down enzymatically into Aβ peptides, which results in Aβ accumulation in AD. The stability of neurons, development, signaling, and intracellular transport are all critically dependent on the APP. Nevertheless, an imbalance in the control of secretase homeostasis leads to the accumulation of insoluble and neurotoxic Aβ and the formation of plaques in the brain. But insoluble and neurotoxic Aβ tends to accumulate and form plaques in the brain due to unregulated secretase homeostasis (Sehar et al., 2022; Zhou et al., 2011).

Aβ containing forty or forty two amino acids is abnormally deposited and accumulates in AD. Because of its intractable nature and increased fibrillization. Aβ42 is more prevalent

than the other in plaques while linking to AD (Hur, 2022). The larger precursor molecule, APP, is produced in large quantities by brain neurons, blood cells (including platelets), and astrocytes, which also produce Aβ. Aβ is generated through two additional proteolytic cleavages of APP by γ-secretase at intramembranous sites and β-secretase at the ectodomain (β-APP-cleaving enzyme-1 (BACE1) (Blennow et al., 2006). The main constituents of neocortical neuritic plaques, a pathogenic feature of AD and an indication of brain aging, have been found to be tau NFTs and dense Aβ aggregates. AD is caused by these aggregations of tau and APP (Kang et al., 1987). Emerging quantitative neuroimaging analyses have revealed a spatial-temporal progression of brain Aβ buildup. This accumulation starts in cerebral regions where high number of neurons are present and have high rates of metabolic activity. Following that, it proceeds via the brainstem, neocortex, allocortex, and cerebellum (Cras et al., 1991).

In AD as there is abnormal extracellular aggregation and amyloid-β (Aβ) peptide deposition, which sets off a series of neurodegenerative events (Hardy & Selkoe, 2002), it first affects the brain's hippocampus, which is essential for memory consolidation and formation. (Goedert & Spillantini, 2006). When synaptic integrity in hippocampal neurons is compromised, aberrant communication between adjacent cells occurs (Sciaccaluga et al., 2021). Reduced cytochrome c oxidase (CCO) activity, neuronal inflammation and mitochondrial dysfunction are brought on by an imbalance between the fusion and fission proteins(Hatefi, 1985). Neural inflammation leads to changes in the activity of microglial and astrocyte, which leads to production of heat shock protein (HSP), activation of the cyclo-oxygenase pathway (COX) and the development of neuritis triggered by NFκB. Oxidative stress brought on by elevated reduced synthesis of adenosine triphosphate and levels of ROS, and intracellular neurofibrillary tangle expression. Thus, memory loss, or AD-related dementia, is one of the common symptoms of the disease. (Chang et al., 2018; Grillo et al., 2013; Oron et al., 2007; Tao et al., 2021; Wang et al., 2014).

As oxidative stress causes apoptosis, which is brought on by changes in the Nrf2 and ERK1/2 pathways, which are then followed by a reduction in PP2A activity and an enhanced GSK-3β expression. By interacting with multiple signalling pathways, including PP2A, CREB/ERK, NFκB, Nrf2, RCAN1 and NFκB, PI3K/Akt, oxidative stress promotes disease conditions. Researches indicated that TNF-α plays a part in the promotion of oxidative stress, which can be controlled by medications such as etanercept, which raise the levels of antioxidants. Resveratrol drug and Donepezil have been studied which shows low levels of

oxidative stress, stimulate the AMPK pathway, and activate PP2A, all of which help to promote tau dephosphorylation and the survival of neurons (Dhapola et al., 2024). Currently no perfect cure or very successful treatment for AD, despite multiple attempts to create medications and therapeutic agents. Although certain treatments have helped patients with AD live better lives, the illness is still largely incurable (Ghosh et al., 2016).

1.2. Current treatment strategies

Managing AD requires a multifaceted approach that combines both pharmacological and non-pharmacological treatments. Pharmacological treatments, including antagonists of NMDA receptors and cholinesterase inhibitors, are designed to alleviate the signs and slow disease progression by targeting specific neurological pathways. However, these medications are often complemented by other therapies nonpharmacological therapies such as cognitive training, dietary interventions, physical exercise and alternative therapies like music and art therapy. Both pharmacologic and non-pharmacologic therapies are applied to enhance patients' quality of life and mitigate the symptoms of AD (Guzman-Martinez et al., 2021).

1.2.1. Treatment approaches other than pharmacological

The goal of non-pharmacological treatment for AD patients is to improve their everyday functioning by improving cognitive function through memory training or art therapy to reduce depressed symptoms and address anomalies in sleeping patterns. Exercise, social support, cognition, acupuncture and rehabilitation training are among the first-mentioned therapies (Olazarán et al., 2010; Yue et al., 2022). Word games for memory along with music therapy and other activities have been shown in certain research to be beneficial for training cognitive function. Thus, in addition to other treatment modalities, non-drug therapy can be used to treat people with clinical AD. Related studies on food, genetics, or environmental factors will also yield more useful information for AD prevention strategies. It has been demonstrated that antioxidant mediators, such as vitamin E, are helpful in slowing the onset of AD (Bleibel et al., 2023). Silybum marianum seed extract is known as silymarin, utilizing these substances in AD models has an anti-amyloid neuroprotective effect. Silymarin targets oxidative stress in the cerebral cortex, which influences Aβ aggregation and other mechanisms including cellular apoptotic machinery, estrogenic receptor mediation, and inflammatory mechanisms. It improves learning memory, lowers resistance to insulin, boosts brain immunity, and increases antioxidative (enzymes) (Aboelwafa et al., 2020; Guo et al., 2019). Furthermore Aβ deposition

that result in cellular damage and free radical production are decreased by using in vitro nanoliquid crystals which are silymarin-encapsulated. (Singh et al., 2020).

Hypoxia may impact on a number of processes related to the pathophysiology of AD, including autophagy, mitochondrial function, cellular oxidative stress, and neurological inflammation. By applying hyperbaric oxygen treatment, hypoxic conditions are created and tissue supply of oxygen is increased in AD models. Patients with AD and MCI have indicated that this enhances brain glucose metabolism and improves cognitive functioning(Chen et al., 2020; Zhang et al., 2018). Several studies have discovered that cognitive stimulation, cognitive rehabilitation, and cognitive training enhanced the standard of living for people and their families suffering AD (Riemersma-van Der Lek et al., 2008). Treatment by utilizing exposure to light therapies in AD models decreased the depressed symptoms, functional limitations, and cognitive deterioration. Other non-pharmacological therapies, such as acupuncture, music therapy, aromatherapy, and regular, sustained exercise, may also help AD patients perform better both cognitively and non-cognitively (Öhman et al., 2016; Valenzuela et al., 2020).

Low light laser therapy (LLLT) is effective in treating disorders involving the neurological system. Several studies also show that LLLT have been proven to improve neurological processes that promote regeneration of nerves and synaptic development in the animal's spinal cord in a rodent model of AD (Byrnes, 2005; De Taboada et al., 2011). Planar arrays of numerous light-emitting diodes were suited for therapeutic applications due to their capacity to irradiate huge areas of tissue in a single hands-off session. These arrays provide therapeutically useful penetration depths and intensities along with wavelength selectivity that is nearly laser-like. It is found that three primary wavelengths have been noticed for LED-based systems, which are being utilized effectively in an expanding number of fields including near-infrared (about 830 nm), red (roughly 633 nm), and blue (nearly 415 nm). These wavelengths are clinically useful and have a strong biological foundation. Each of them has a distinct biological action spectrum, response, and cell target. The word "photobiomodulation therapy" (PBMT) describes the use of specific light wavelengths to treat a variety of diseases and ailments. These wavelengths are also applied in mice models to promote hair growth and healing of wounds. (Anders et al., 2019; Calderhead, 2007).

1.2.1.1. Phototherapy

Phototherapy has gained a lot of interest in the last several years as a therapeutic option for treating a range of conditions by non-invasive method called regenerative medicine to encourage wound healing. Photo-biomodulation (PBM) comprises as a treatment method that does not include medication and makes use of red or near-infrared light with varying wavelengths, and provided very promising results for treating AD (Caldieraro et al., 2021; Pan et al., 2023; Saltmarche et al., 2017). Red or near-infrared light can be used therapeutically help encourage recovery, lessen discomfort and inflammation, and prevent tissue death.

PBM was formerly known as LLLT, however, this term was changed to reflect the true nature as some processes were beneficially inhibited, lasers were not always necessary, and the term "low" was not well-defined (Anders et al., 2015; Mester & Mester, 2017). Later on public, physio therapists, and the medical community have all come to accept PBM more and more. LEDs that emit light in the red and near-infrared ranges and significant power densities (up to 100 mW/cm2 across quite large areas) are becoming more widely available, which contributes to their rising adoption, being non-invasive therapy. When it comes to performance, the LEDs are considered more convenient being on par with lasers with comparable power densities and wavelengths (Anders et al., 2019). Also LEDs are more cost-effective, safer, and more suited for residential use (Hamblin, 2019).

PBM has been considered safe by the U.S. Food and Drug Administration (FDA) because it is non-intrusive and has minimal significant side effects. Numerous studies indicate that PBM mechanisms are responsible for neuroprotection, increased brain tissue metabolism and microcirculation, decreased oxidative stress, and decreased inflammation (Enengl et al., 2020; Yang et al., 2021). Additionally non-intrusive transcranial photobiomodulation (tPBM) works by delivering near-infrared or low-level laser light into the brain through the transcranial region of the skull (Chang et al., 2018). It eliminates the required precursor protein (APP) and inhibits amyloid plaque formation (Lane, 2006). PBM restores mitochondrial homeostasis by cytochrome c oxidase activation and downregulation of nitric oxide (Manczak et al., 2011). Because it alters the COX pathway, suppresses the production of heat shock protein and cytokines that are pro-inflammatory, and controls ROS release as an antioxidant, PBMT has shown promise as a remedy for inflammation (Amaroli et al., 2021).

1.2.1.2. Mechanism of Photobiomodulation

Numerous studies demonstrated that CCO functions as a biological signal transmitter and cellular photoacceptor that are activated by photons in the red light and near-infrared light regions of the wavelength (Karu, 2010; Wong-Riley et al., 2005). In the catalytic center of CCO, photons given in PBM seem to promote a boost in electron availability for a decrease of molecular oxygen. It increases the potential of the membrane of the mitochondria and enhances the concentrations of ATP, cyclic AMP (cAMP), and reactive oxygen species—all of which are markers of enhanced mitochondrial activity and have the ability to start signaling pathways inside cells (De Freitas & Hamblin, 2016).

On the other hand, the CCO theory has been contested lately when two different cell types were genetically altered to be devoid of active CCO it was discovered that these altered cells responded to 660 nm light exposure in the same way that wild type cells did not. The study raises the possibility that CCO's involvement in cellular reactions to red light may not be as important as previously believed and highlights the need for more research into potential alternate mechanisms or routes (Lima et al., 2019).

CCO is still thought to be one of the main photoacceptor, even though other components of the succinate dehydrogenase and complexes I–IV in their electron transport chain exhibit enhanced activity in PBM. This idea is strengthened by the observation that elevated oxygen consumption is induced by low-intensity light exposure, such PBM, because most of the oxygen consumption occurs at complex IV, and the effects are countered by adding sodium azide, which is a CCO inhibitory substances, which eliminates the consequences of PBM (Núñez-Álvarez et al., 2017; Spitler et al., 2015).

When a certain molecular chromophore absorbs a photon can it have a biological impact. The chromophores that are thought to be helpful in PBM absorb light at many wavelengths in the electromagnetic bandwidth, which encompasses NIR, green, red, and blue (Hamblin, 2019). The final enzyme reffered as unit IV in the electron transport chain which is CCO, is present on the outer membrane of mitochondria. The electron transport chain (ETC) makes it easier for electrons to move across the mitochondria's inner membrane by facilitating a sequence of redox processes. Overall, these electron transport mechanisms produce a gradient of proton concentration throughout the membrane of the mitochondria, which encourages ATP synthase, also known as unit V, to produce high-energy adenosine triphosphate (ATP) from

ADP. CCO is a mediator in the transfer of electrons from cytochrome C to molecular oxygen. The assembly of the protein CCO has 2 copper and 2 heme centers and is composed of thirteen different polypeptide subunits. These copper and heme centres can be reduced or oxidised, resulting in sixteen distinct oxidation states. Each of these state of oxidation has an absorption spectrum that varies somewhat, but the near-infrared absorption of CCO is nearly unheard of in biological molecules. Moreover, Britton Chance calculated that this one enzyme may serve as a chromophore for about 50% of the near-infrared radiation is absorbed by tissue (Cooper et al., 1999).

1.2.1.3. Red light therapy

LLLT sometimes known as red light treatment (RLT), is a kind of light therapy in which the body is exposed to very low levels of red or near-infrared light.Infrared (IR) and red light improve cellular activity, but ultraviolet (UV) light can cause damage to skin cells. Treatments for wounds, skin ailments, and neurodegenerative diseases like AD have all showed promise with this non-invasive therapy. The 600–1000 nm range, which has different characteristics, is the most often researched wavelength range for neurological effects (Gonzalez-Lima et al., 2014; Yu et al., 2015). The 630–660 nm wavelength range is useful for stimulating superficial tissues, improving wound healing, and lowering inflammation because it only penetrates a few millimetres into the tissue (Mirshafa et al., 2020).

RLT stimulates biological activities by entering the skin and underlying tissues. It is thought that RLT improves mitochondrial function by increasing the activity of mitochondria which are the cells powerhouses. It also improves ATP synthesis which is essential for cell survival and repair. RLT has the ability to remove amyloid plaques which is a common in AD, and it reduces neuroinflammation by modifying inflammatory pathways,

As the wavelength of the red spectrum at which traffic lights operate is 630 nm, the human eye is highly reactive to this particular red light (Wood et al., 2013). Retinol is very necessary for visual perception is transferred to the retina by formaldehyde degrading enzyme FDH (also known as ADH3) (Graymore et al., 1974). FDH is present throughout the retina. Red light (RL) at 630 nm may have an impact on hippocampal neurons' FDH activity (Gonzalez-Duarte & Albalat, 2005; Zhang et al., 2019). In vitro study, low-power laser phototherapy has the ability to cause amyloid-beta (Aβ) protein secondary structure disruption (Son et al., 2018). LED-RL lighting has been demonstrated to increase FDH activity,

decreasing the Aβ toxicity, endocannabinoid system (ECS) inhibition is also linked and mediated by Aβ. Furthermore, antibodies like p-tau181, PP2A and GSK-3β decreases the amount of CD45-positive reactive microglia and GFAP-positive reactive astrocytes which in result raises immunoreactivity of microtubule-associated protein (MAP)-2, and blocks the molecular pathways connected to phosphorylated tau. More significantly, it was discovered that there was a high rate of penetration of LED-RL at 630 nm into the human cortex. (Yue et al., 2019). Increased levels of AD biomarkers is linked to sleep disruption, suggesting a reciprocal association between sleep and AD. In AD patients, "sunset syndrome" refers to a complex of emotional and cognitive changes that occur when the light reduces (dusk, for example). Emotional issues, anxiety, restlessness, and a lack of concentration are some of the changes that can persist for several hours or the entire night. Surprisingly, recent studies suggest that LT may be able to lessen some of these effects by recovering emotional regulation, cognitive ability, and circadian rhythm (Bubu et al., 2020).

1.2.2. Pharmacologic therapies

Lecanemab is a medication used to treat AD. It may work by neutralising and eliminating potentially harmful amyloid-beta clumps that are present in the AD patients (Cummings et al., 2023).The FDA has officially authorized the use of lecanemab to treat AD. Memantine which is antagonist for N-methyl-D-aspartate receptor, and AChEs (donepezil, rivastigmine and galantamine) are among the authorized pharmaceutical therapies for AD. The most researched medications are GV-971, aducanumab, melatonin, and ginkgo biloba in addition to the ones already listed. The pharmacological toolkit for treating dementia is constantly growing as research into AD progresses. Drugs for dementia are still developing slowly, nevertheless, in comparison to those for other illnesses. (Chen et al., 2024).

Numerous substances have been discovered to lessen Aβ aggregation or neurotoxicity in vitro, as well as to slow the course or symptoms of AD. Along with memory loss, acetylcholinesterase activity rises in AD brains, indicating a role for the transmission of cholinergic neurons in processing memories and cognitive function (Ribeiro-dos-Santos et al., 2023). Acetylcholine is a primary neurotransmitter of the autonomic nervous system (ANS). It interacts with muscarinic and nicotinic receptors to exert its effects on the nervous system's core, the neuromuscular junction and peripheral nervous system. ACh is made up of its receptors, the enzymes that catalyze its creation and degradation in the cholinergic transmission system. The choline acetyltransferase enzyme converts choline and acetyl-coenzyme A into

Ach. ACh is hydrolyzed in choline and acetic acid by the enzymes acetylcholinesterase and butyrylcholinesterase, which degrades ACh. Since the this system is connected to attention and memory, transmission of cholinergic pathways plays an important role in cognition. ACh levels in the synaptic cleft are reduced in AD (Kaur et al., 2019). Ach inhibitors like donepezil are currently being used to treat AD patients. ACh is a neurotransmitter that is involved in memory. Increasing the amounts of ACh at cholinergic synapses may stabilize or enhance cognitive performance as they function by inhibiting the activity of the enzyme AChE. as well as have an impact on behaviour along with day-to-day functioning (Ribeiro-dos-Santos et al., 2023).

Increased glutamatergic transmission results in oxidative stress and deficits in the neurotransmitter ACh are also linked to AD. The major enzyme in cholinergic synapses' family of serine hydrolases that is vital to memory and cognition is acetylcholinesterase (AChE) (Kelly et al., 1997; Khoury et al., 2017). In addition to AD, Ach inhibition has been identified as a treatment approach for additional conditions, including dementia, glaucoma, Parkinson's disease , and myasthenia gravis (Craig et al., 2011).

At the moment, a lot of Ach inhibitors are utilized to treat AD symptoms, such as donepezil, rivastigmine, and Galantamine. Galantamine is a common alkaloid from the Amaryllidaceae family that is used to treat AD. Numerous clinical investigations have confirmed its efficacy since it was approved for therapeutic usage in 2001. Since an increase in life expectancy is a risk factor for neurodegenerative disorders, there has been a notable and growing global increase in instances of AD. This is expected to drive up demand for galantamine. Because of this aggravating element, scientists are searching for feasible substitutes for galantamine's larger-scale commercialization (Santos et al., 2020). The FDA approved galantamine hydrobromide which is sold as Razadyne, as a AD medication in 2001, following fifty years of research, to treat mild to moderate dementia (AD)-related disorientation. Galantamine helped people with AD function better cognitively and behaviorally. However, these medications has adverse effects, which include hepatotoxicity, hypotension, gastrointestinal issues, dizziness, diarrhoea, vomiting, and nausea, compromise their efficacy (Silva et al., 2023).

Memantine is another potent voltage-dependent antagonist of the N-methyl-D-aspartate receptor with modest affinity. Since 1989, it has been utilized to treat AD. It rose to the position of the second most widely used medication worldwide in 2018 to treatment of dementia. Memantine can only treat the symptoms of AD; it cannot reverse the disease's irreversibility.

The study and clinical use of memantine have advanced significantly throughout the previous 50 years (Tang et al., 2023). When it comes to treating mild AD, the efficacy of memantine in treating mild to severe AD is very different. It reduces symptoms like as memory loss and cognitive impairment, enables people with mild to severe AD symptoms carry out daily activities more readily, and has a positive safety and tolerability profile, this results in reduced progression of disease. A combination of medications may be the most successful line of action because a single medication is ineffective in treating AD due to its clinical heterogeneity. (Roberts et al., 2019).

With the exception of aducanumab, no new drug has received FDA approval for commercialization in over forty years. The only drug that the FDA has authorized for either preventing brain accumulation of amyloid or eliminating pre-existing amyloid deposits is aducanumab. Aducanumab has an FDA authorization, however its exact pharmacological mechanism is yet unclear. All other innovative drugs' clinical trials have failed (Mullane & Williams, 2019). Aducanumab is a medication that easily cross the blood-brain barrier (BBB). It functions like a single-chain antibody that attaches itself to amyloid aggregates in order to perhaps repair brain damage in AD patients by lowering β-amyloid plaques (Haddad et al., 2022). After passing across the blood-brain barrier, audanumab has the ability to adhere to and constrict amyloid plaques in the brain. Consequently, the plaques shrink in size and become tighter. In the brain, it also attaches to insoluble fibrils and aggregated soluble oligomers, which ultimately aids in controlling conformational changes (Leinenga et al., 2021; Watt et al., 2021).

Gene therapy appears to be able to lessen a number of degenerative disorders, its current application is constrained by pragmatic issues. The majority of AD cases are sporadic and driven by a number of unknown reasons, making the application of CRISPR technology in AD problematic. Many gene types have been linked to AD, including PSEN 1, APOE, APP and BACE1 (Albert, 2011; Kerwin et al., 2022). As obtaining optimally recognized beforehand diagnosis in the context of symptomatic disorders is also a difficult task. Late-onset AD advances rapidly and very earlier than the clinical symptoms, creating an unclear window for the application of preventative measures. CRISPR-Cas9 needs to have a high level of sensitivity and specificity in order to be a useful therapeutic tool. One of the biggest barriers that can prevent altered cells from functioning is off-target mutations; therefore, gene delivery to cell target areas might be ineffective. Random or off-targeting events can exacerbate germline mutations by having a deleterious, untreatable effect on healthy tissue. Trials are

needed to optimize guided RNA delivery into brain cells in order to meet the format efficacy, specificity, and sensitivity requirements (Ahmad et al., 2024).

A great prospective gene-editing tool with a variety of AD therapeutic options, CRISPR/Cas9 continues to be despite these obstacles. Since genome editing is an inevitable process, more research is required to verify the safety of CRISPR/Cas9 therapy (Karimian et al., 2019). Studies are being conducted to investigate the long-term consequences of CRISPR therapy. Ethical considerations must be strictly adhered to when conducting extensive research on the application of CRISPR/Cas9 in people (Barman et al., 2020; Karimian et al., 2019; Mianné et al., 2022)

1.3. Limitations

AD is rapidly worsening globally as a public health issue. For several decades, numerous therapeutic approaches have been investigated; yet, no cure has been found, hence prevention continues to be the most important approach. Preventive measures are now the best course of action before the disease reaches its advanced stages and can considerably slow the evolution of AD. In fact, the effects of current pharmacological therapies are only symptomatic, and disease-modifying drugs are currently unavailable. Drug transport to the central nervous system is complicated, which makes developing therapeutic and preventative strategies difficult. Researchers are investigating new strategies to improve the bioavailability of drugs to the brain. (Passeri et al., 2022).

1.4. The need for novel therapeutic approach

The lack of established etiological therapies highlights how important prevention is, in order to bridge this knowledge gap specialists evaluate the prospective benefits of utilizing animal models, as human conditions like AD need significant and costly study. This shows how different therapies may be beneficial in AD animal models to understand the different phases of AD. (Qin et al., 2020).

Numerous experimental and clinical therapeutic strategies, including tau-associated therapies, Aβ-targeted therapies, anti-inflammatory drugs, stem cell therapy, etc., have been established for treating AD. This, in turn, offers brief symptomatic relief but does not effectively change the progression of the condition. For this reason, gene therapies and

modifications are showing promise as innovative methods for treating a variety of neurological conditions, including AD (Pardo-Moreno et al., 2022; Silva et al., 2023).

1.5. Combining Light Therapy with Pharmacological Treatment

After a thorough analysis of the literature, we have determined that phototherapy, particularly the use of red light therapy in conjunction with medications such as galantamine and memantine, is a novel approach for both therapeutic and preventive strategies aimed at lowering the risk of AD and resolving issues with brain bioavailability. Researches indicates that phototherapy and medications hold great potential, as light with a wavelength range of 400 nm to 660 nm, along with cholinesterase inhibitors and NMDA receptor antagonists, can enter the brain and specifically target damaged neurons. The drug administration and therapeutic options for neurological condition like AD will possibly imply great effect.

According to previous study, SH-SY5Y neuronal cells before being exposed to Aβ1-42, were treated for 24 hours with LLLT (660 nm, 5mW/cm2, 3J/cm2) and donepezil (1μM). The two treatments continued for a further seven days. Viability rises significantly with donepezil therapy, LLLT treatment, and combined donepezil and LLLT treatment; 111%, 104%, and 129% viability, respectively. In response to 1μM Aβ1-42, donepezil and LLLT therapy both have a protective effect, boosting viability by 51% and 60%, respectively. This demonstrate that in the absence of any therapies, the donepezil and LLLT combination treatment of SH-SY5Y cells resulted in the highest increase in viability when compared to Aβ1-42 alone. Furthermore, compared to cells in the untreated control group, LLLT treatment resulted in a 161% increase in cell viability (Thammasart et al., 2019).

1.6. Hypothesis

Phototherapy, when combined with drugs like galantamine and memantine, may develop a more potent therapeutic effect to reverse the neuronal damage and death and improve cognitive decline in AD.

1.7. Objectives of the Study

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

1. Developing a customized RGB LED phototherapy device prototype intended for therapeutic use
2. Administering a combination of drugs and phototherapy on rodent models resembling AD.
3. Studying the combined effects of phototherapy and drugs on cognitive function by analyzing histopathology and conducting behavior tests

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: 03-2024-ASAB-01/01)

2.2. RGB based phototherapy device

2.2.1. Mechanical Design

The components utilized to construct the prototype of the Variable Wavelength RGB LED Controller comprised RGB light-emitting diodes (RGB LEDs) featuring integrated resistors, functioning within a wavelength spectrum of 400-750 nm, alongside a logic level converter (3.3V to 5V) to guarantee appropriate voltage compatibility between the LEDs and the ESP32 (ESP WROOM-32) microcontroller. Supplementary materials comprised a 5V power module, connecting wires, a breadboard for circuit assembly, resistors, and a USB power source. The prototype has the following essential components: the 3D-printed enclosure for accommodating the LED array, microcontroller based RGB LEDs with a 12V Power supply adapter and the VERO circuit board for regulating the LED functionality. The enclosure was fabricated by 3D printing with polylactic acid (PLA) filament, engineered to enhance thermal dissipation and safeguard interior components. The interior of the casing was black to prevent undesired reflections or interference from external light sources. The design of device is shown in figure 2.1.

2.2.2 Circuit Design

The circuit incorporates a logic level converter utilizing BSS138 MOSFETs (Q1, Q2, Q3, Q4) to facilitate voltage shifting between the ESP32 and additional components. The converter is facilitated by 10 kΩ resistors (R8–R14) for adequate signal conditioning. The ESP32 development board (ESP1) serves as the controller, interfacing via multiple GPIO pins, specifically D19, D18, D17, D16, D22, and D23, functioning at 3.3V. The circuit comprises six RGB LEDs (D1–D6), designated RGBLED-CA, which are of the common anode variation. Each LED is coupled with a 10Ω resistor (R1–R6) to control current flow. A voltage regulation circuit is supplied by a 12V, 2A power source (V1), which is reduced to 5V via a 7805 voltage

regulator (U1) to energize the LEDs and additional components. The configuration facilitates the regulation of RGB LEDs while overseeing various voltage levels using the level-shifting circuit shown in figure 2.2. The circuit was constructed using Proteus software, while the mechanical design of the device was constructed on VERO board and casing 3D model was created on Fusion 360 software.

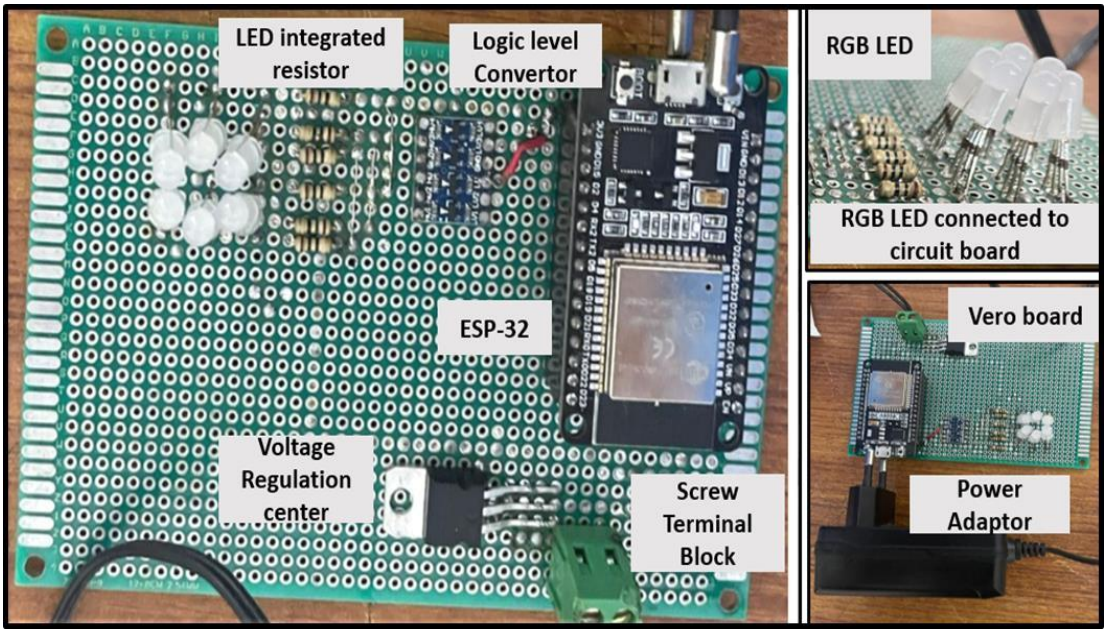

Figure 2.1 Hardware Device Design. The VERO circuit board is used in this light-based therapy device, utilizing RGB LEDs for power regulation. The circuit board manages light intensity and wavelength cycles, ensuring stable power and easy adjustment of light.

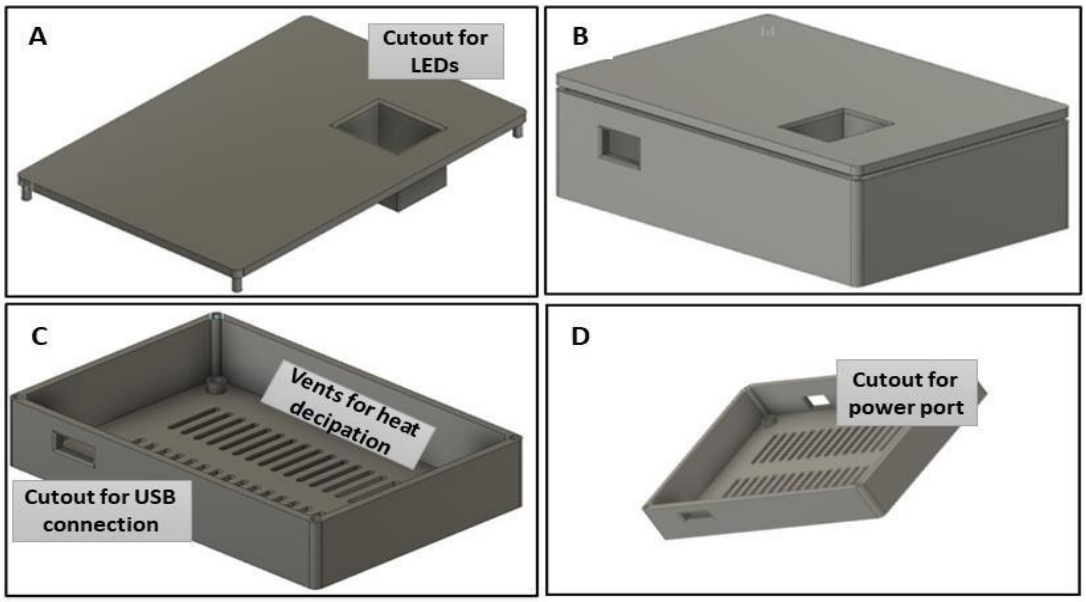

Figure 2.2 3D model of a custom-designed casing for the Variable Wavelength RGB LED Controller. (A) LEDs visibility cutouts, **(B)** Lid mounted on box**(C)** heat dissipation vents, USB connector cutouts and **(D)** An angled perspective showing power apertures.

2.3. 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. Six groups of mice totalling twenty-four were created: n=4 for the Control group, n=4 for the $AlCl_3$ group, n=4 for the $AlCl_3$ group treated with galantamine, n=4 for the $AlCl_3$ group treated with memantine, n=4 for the $AlCl_3$ group treated with phototherapy and memantine, and four for the $AlCl_3$ group treated with phototherapy and galantamine.

2.4. Experimental Design

A total of twenty-four male Balb/c mice, 6-8 weeks, were acclimated in the animal house for a week as part of the experimental protocol with four mice per group. The mice in their cages had unrestricted access to food and water during this time. The $AlCl_3$-induced disease continued for 42 days starting on the 7th day. The treatment group received memantine and galantamine for a total of 7 and 14 days, respectively. Both phototherapy with memantine and phototherapy with galantamine were given to the treatment group simultaneously. Different behavioural assessments were conducted from the day 65 to 71 and tissue samples were collected on day 72 of the experiment. The process of histopathology was carried out from day 73 to day 78, which included the preparation of slides, staining with Hematoxylin and Eosin stain (H & E), and slide examination. Figure 2.3 shows the timeline and experimental design of the whole project.

2.5. Chemical Dosage

2.5.1. Development of AD like Rodents

Models of AD-like mice were developed by oral administration of 20 mg/kg of $AlCl_3$ in distilled water for 42 days. 20 mg of $AlCl_3$ in 10 ml of distilled water was needed to make the stock solution. Using a mouth gauge, 0.16 ml of the stock volume was used for administering $AlCl_3$ orally to each mouse once a day (Xing et al., 2018; Yang et al., 2013).

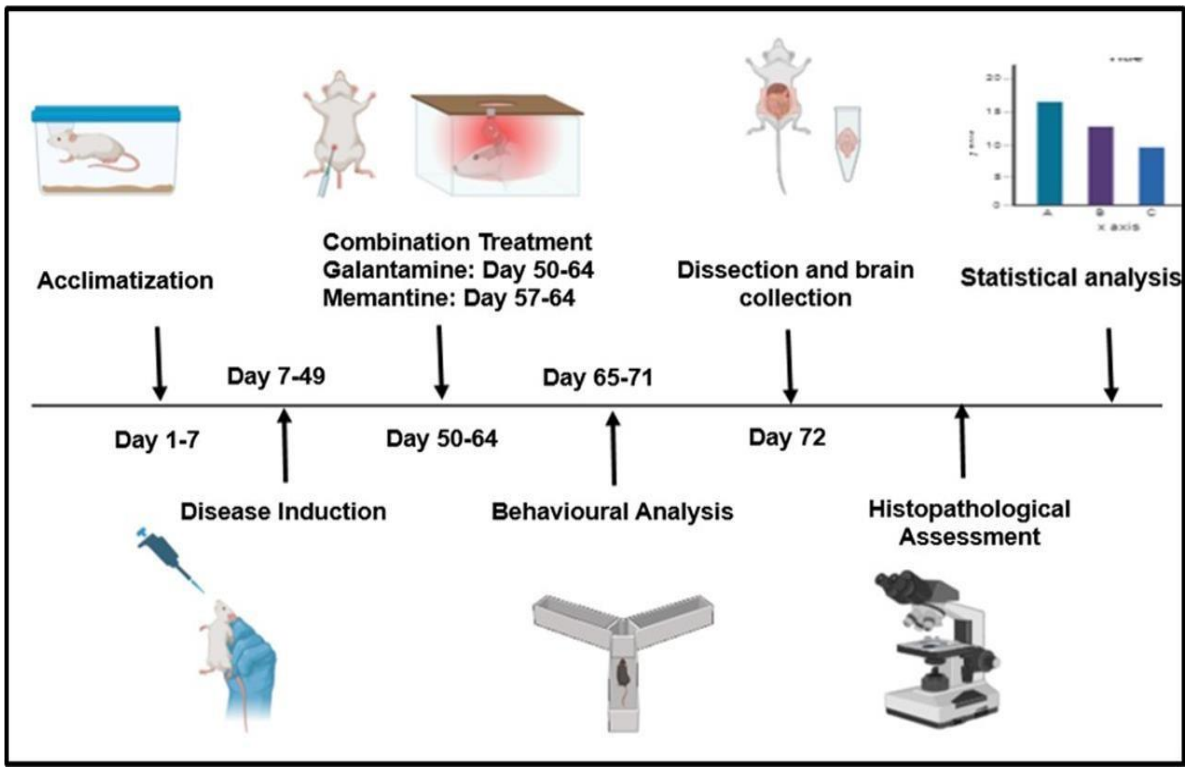

Figure 2.3 Timeline and Experimental Design. A meticulous timeline encompassed acclimatization, treatment administration, behavioral assessments, and comprehensive histopathological and molecular analyses.

2.5.2. Treatment of AD like Rodents

Galantamine was administered at 1.25mg/kg/day dissolved in 10ml/kg volume of saline by i.p route for 14 days (Van Dam et al., 2005). Memantine was administered at 3mg/kg/day for 7 days dissolved in 10ml/kg saline volume by i.p route (Bagewadi et al., 2015). Phototherapy was used as combination treatment of phototherapy and drugs administered using a RGB light device with 625nm wavelength of light applied over the top of head for 10mins daily for 7 days shown in figure 2.4A (Blivet et al., 2018).

2.6. Behavioral Testing

After developing AD-like mice model, behavioural testing was done on the mice to see if they had any symptoms or indicators of AD include memory loss, slower learning, and impaired spatial learning.

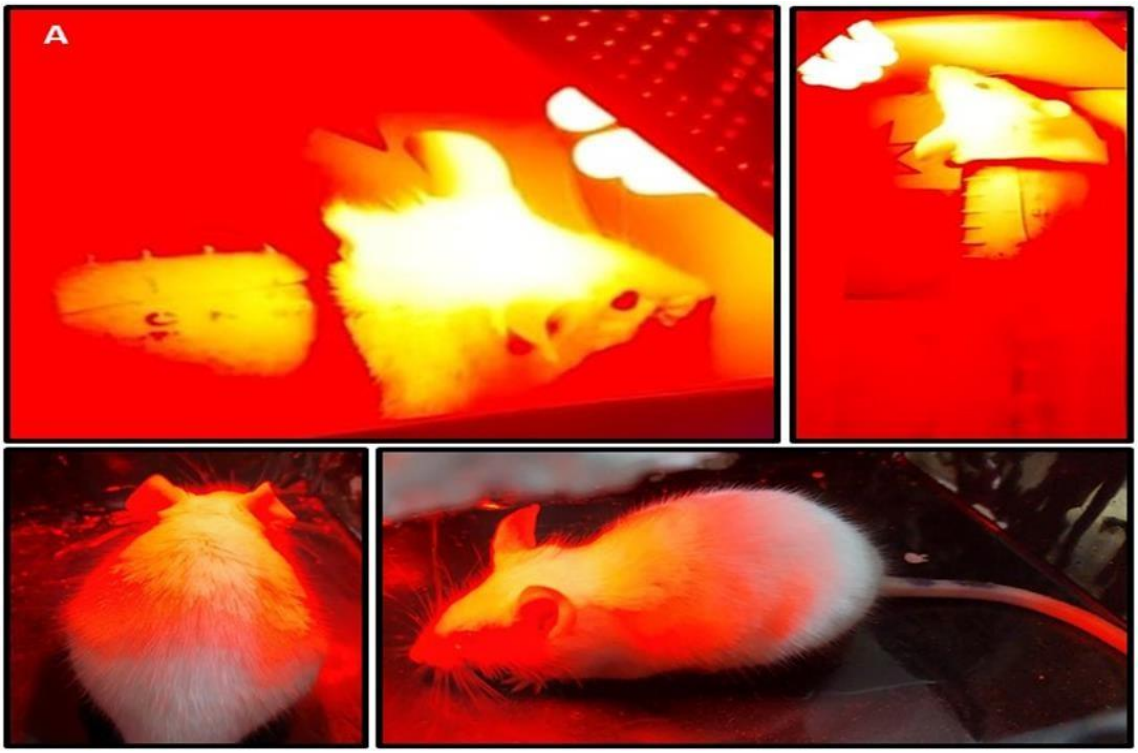

Figure 2.4 (A) Application of Phototherapy on a Mouse in Experimental Setup.
Investigating the therapeutic effects of phototherapy on cognitive function of Alzheimer's disease mice model.

2.6.1. Y-MAZE Test

The animal was put in a Y-maze with three arms: the start arm, the novel arm, and the familiar arm. Each arm was cleaned with 70% ethanol before re-entering a new animal. The ability to move between each arm and the time was recorded for each arm for each mice. After completing its training phase, it was permitted to explore all three arms for five minutes, recording the results with a video camera in order to compute the metrics indicating cognitive deterioration as shown in figure 2.5. When pathological conditions are introduced to mice, they lose their ability to remember the prior experiment and are unable to switch between the three arms and percentage alteration values are below 22%. A percentage alteration was calculated.by applying the subsequent formula as stated in literature (Kraeuter et al., 2019). $\{Spontaneous\ alternation/(Total\ number\ of\ arm\ entries\ -\ 2)\}\ \times\ 100.$

2.6.2. Novel Object Recognition Test (NORT)

The purpose of NORT was to determine whether the animals could identify the prior interacted object and spend more time with it by exposing it to both novel and familiar objects. This also aids in assessing the mice cognition, especially in terms of their recognition memory, as they frequently linger longer with objects they have not investigated previously (Lueptow,

2017). The apparatus consist of a box with objects placed diagonally to each other, every time a new mice is introduced into the apparatus, the apparatus was cleaned with 70% ethanol to get the accurate results. The test procedure comprises habituation, familiarization and testing phase and each mice was given 5 minutes to explore around. A video was recorded during the testing period to analyze the time spent with the novel object and familiar object. Figure 2.6 shows the NORT apparatus with a mice in testing phase.

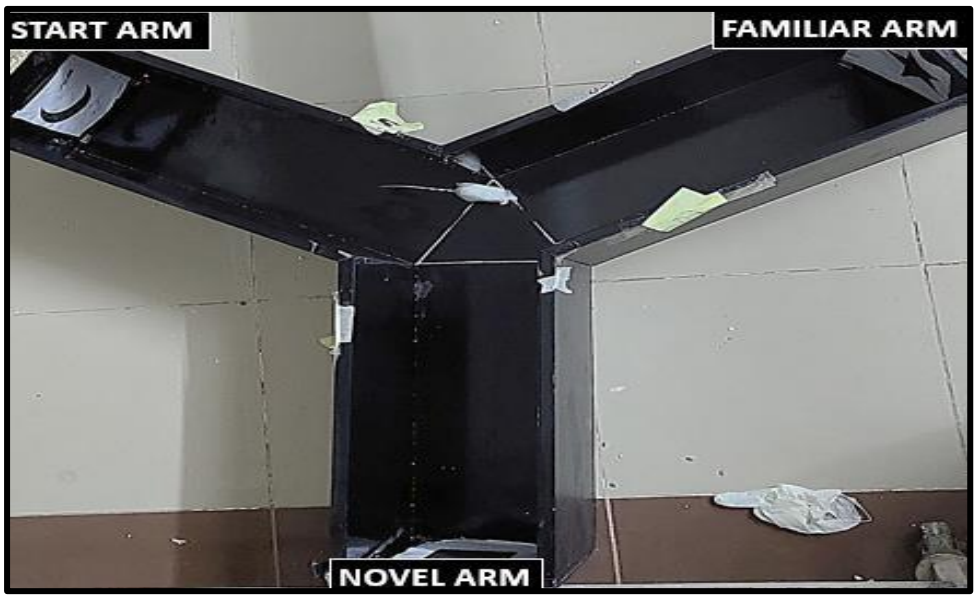

Figure 2.5 Y–Maze Test for spatial memory. This figure represents a mice moving from the initial arm to either a familiar arm or a novel arm in the Y-Maze Test. The percentage of alteration in the arms provide valuable insights into its cognitive abilities.

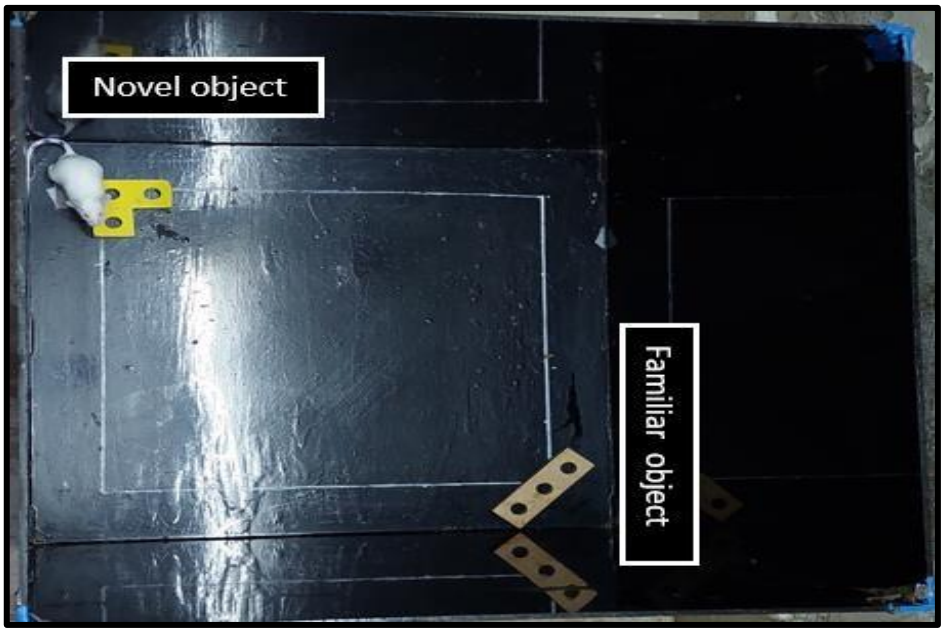

Figure 2.6 Novel Object Recognition Test for cognitive processes. This figure shows the movement of the mice between the two objects, one familiar and one novel object to study cognitive processes such as memory and attention in rodent models.

2.6.3. Morris Water Maze Test (MWM)

The apparatus consist of a circular stainless steel tank with markings indicating the directions to the north, south, east, and west. The tank measured around 2 meters in diameter and had walls that rose to a height of 60 cm and half filled with warm water (28 °C ± 3). 1-2 cm below the surface, a platform with a diameter of 10–12 cm was immersed in the tank's northeastern quarter. Mice were trained for the first five days to find the five-day underwater platform's escape latencies from various orientations were recorded. The platform was removed down on the sixth day, and the number of crossings in the target quadrant, the number of times the platform is crossed, and the amount of time is spent within the designated area were noticed (Singh et al., 2016). Figure 2.7 shows the MWM apparatus with a mice under test.

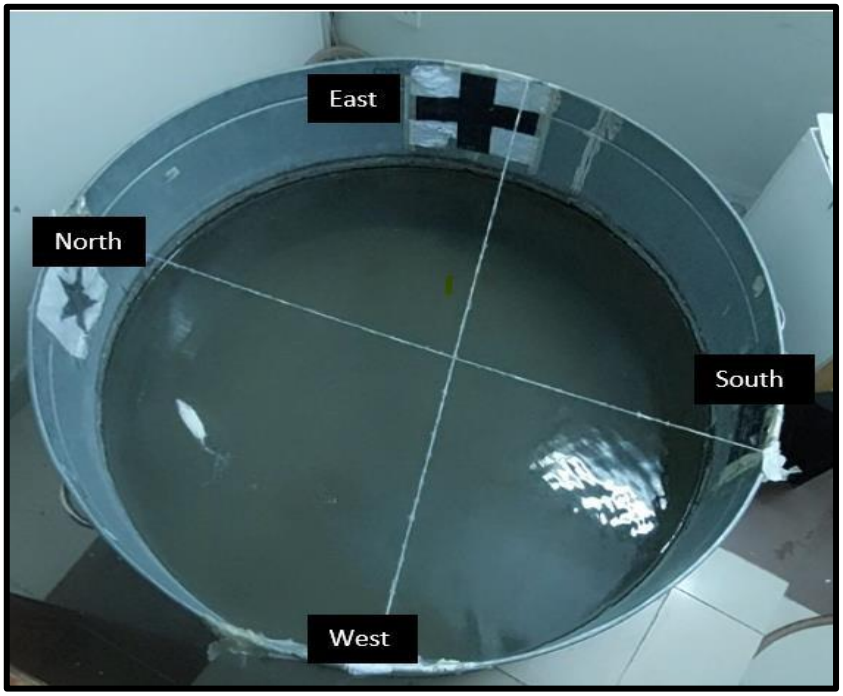

Figure 2.7 MWM Test apparatus. The figure shows a circular apparatus with tagged all four sides directions with a mice ongoing the escape latency experiment.

2.6.4. Open Field Test

The OF test is crucial in behavioral analysis especially in mouse models of $AlCl_3$-induced AD. This test measures mice's motor function, activity, exploratory behavior, and anxiety, which may be affected by AD. In mice, changes in mobility or movement patterns indicate disease severity and impact on physical ability. Behavior of mice in open spaces, such as prolonged presence near corners or edges (thigmotaxis), may suggest increased anxiety levels,

possibly from AD. The OF Test focuses on motor functions and anxiety-related behaviors. The OF equipment was built with a 60 cm x 60 cm wood cage and 60 cm high walls. The entire setup was black. The floor was divided into 16 uniform 15 cm by 15 cm squares shown in figure 2.8. Animal movements were recorded for five minutes using a video camera located far from the arena during the trial (Zimcikova et al., 2017).

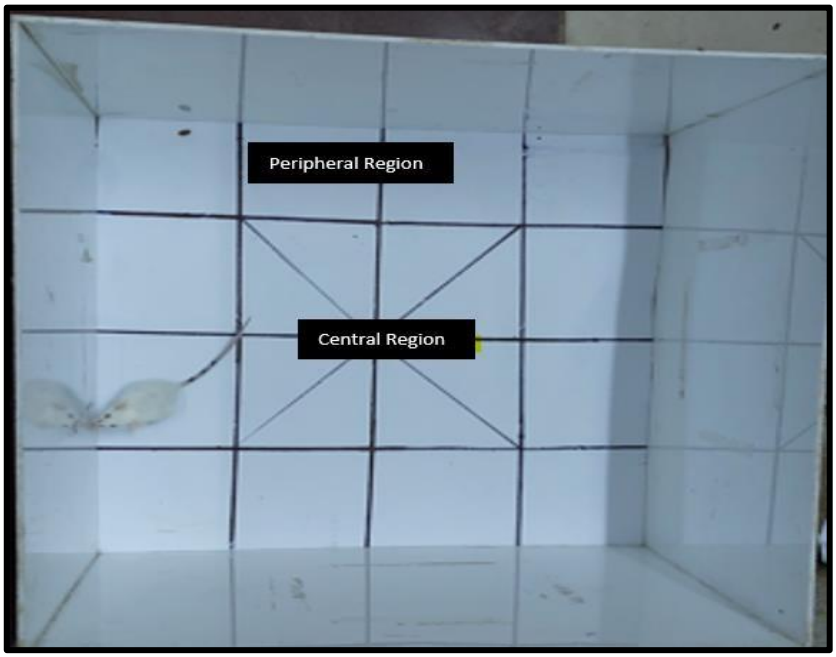

Figure 2.8 Open Field Test for exploratory behavior and anxiety level. The time spent in the central region and time spent in peripheral region is calculated. These measurements are crucial as they can provide insights into the mice exploratory behavior and anxiety levels.

2.6.5. Elevated Plus Maze Test

The EPM test measures mice anxiolytic behaviour. Since mice have aversions of elevations and open areas, this test measures their tendency to explore these areas in spite of their aversion. The apparatus included 4 arms, two of which were enclosed passageways and two of which were open passageways. The device was composed of opaque iron alloy and raised 75.5 cm from the ground. Each arm measuring 30 by 5 cm. Every mouse received a single, five-minute trial by positioning oneself near the maze's junction, appearing away from the experimenter and in the opposite direction of any of the two closed arms shown in figure 2.9. A video for behavior analysis was recorded to calculate the total number of entries and total amount of time spent in open passageways. The apparatus is shown in figure below:

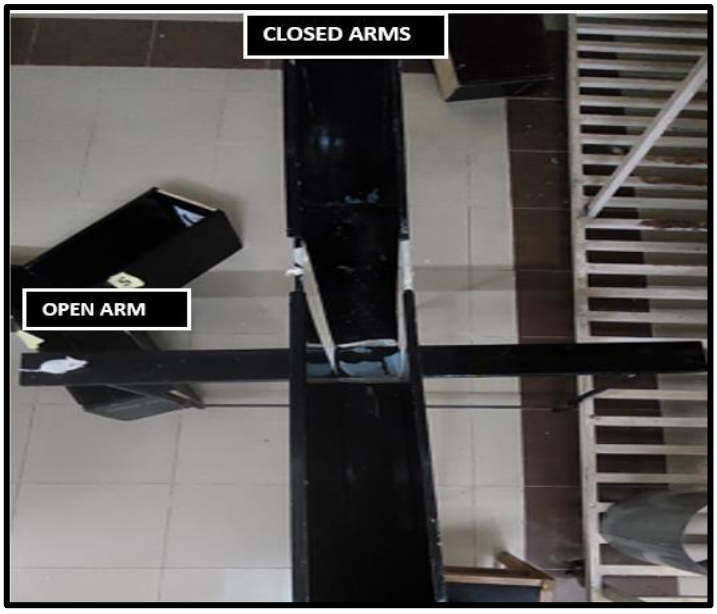

Figure 2.9 The Elevated Plus Maze (EPM) test. It evaluates anxiety-like behavior in AD mice. The maze consists of open and enclosed arms, with increased time in enclosed arms indicating heightened anxiety. The EPM test assesses therapeutic interventions on anxiety-related behaviors in AD.

2.7. Dissection and Brain Tissue Preparation

All animals were administered profound anesthesia via inhalation of chloroform following behavioral testing. A thoracic incision was performed utilizing a trapezoidal incision technique. A small incision was made in the upper left atrium to facilitate the implantation of a 23-gauge perfusion catheter into the right ventricle. Normal saline was administered for five minutes, succeeded by a two- to three-minute infusion of 4% paraformaldehyde (PFA) in PBS. The brain was removed and promptly stored in a 4% PFA and placed over ice. The skull was meticulously dissected and bisected in the mid-sagittal plane using fine scissors, commencing at the cerebellum and concluding at the bony region around the olfactory bulbs. The brain was bisected sagittally into the left and right hemispheres. The cortex was rapidly dissected on ice, and stored in 4% PFA for histological studies.

2.8. Histological Analysis

The brain was dissected and 4% PFA was passed through left ventricle after saline perfusion to prepare the brain tissue for histological investigation. Complete fixation was achieved by immersing it in 4% PFA for a suitable amount of time, usually between 24 and 48 hours, to enable complete fixation. Different staining techniques are employed to detect

specific structures, cells, tissues, or even metal components as the H&E staining is the most widely used method; it shows the nucleus in blue and the cytoplasm of cells in pink.

2.8.1. H&E staining

Fixed tissues from one brain of each group i.e., control, diseased and treated were sliced into approximately 4 µm thin slices and microscopic slides were prepared. These slides were deparaffinized by incubating them at 63°C for almost 30 minutes. Additionally, during incubation, slides were submerged in xylol for two minutes before being cleaned with different ethanol concentrations (100%, 90%, 80%, and 70%). Hematoxylin was used for three minutes, followed by a one-minute water wash, a one-minute differentiator with mild acid, another one-minute water wash, bluing, another one-minute water wash, ethanol, eosin, ninety-five percent ethanol, one-minute 100% ethanol, and finally, two minutes of xylene. At the end, slides prepared were covered with a coverslip.

2.9. Microscopy

A Binocular Light Microscope (S37242, Labo America Inc. USA) for cell count with a 4X–100X magnification was used to view H&E slides. Slides were displayed with a 40X magnification. Cells were counted and viewed in the cortex tissues using Image J software, and the differences were compared.

2.10. Statistical Analysis

Statistical analyses on the data obtained were carried out using GraphPad Prism (10.01, CA, USA). For the data sets with more than two groups, involving two factors, a two-way analysis of variance (ANOVA) was conducted. While performing the statistical analyses, it was assumed that the data were independent, approximately normally distributed, and showed equal variance within each group to be compared. The differences with a p-value of < 0.05 were regarded as statistically significant.

CHAPTER 3: RESULTS

3.1. Behavior Assessment Results

3.1.1. Spatial Memory and Exploratory Tendencies in Y-Maze Test

The Y-maze test highlights the importance of spatial working memory and cognitive ability in assessing memory and spatial learning in mice and it was used to analyse the short-term memory in mice of various groups. The control group results of Y-Maze test showed that the mice with intact working memory and prefrontal cortical functions showed better spatial learning and exploratory activity while diseased group showed decreased cognitive ability. The treatment group Memantine and Galantamine showed improved memory while comparing to disease group. Memantine group showed more number of entries and time spent in novel arm as compared to disease group. Similarly Galantamine showed improved exploratory tendency comparing to disease group. The drug Memantine showed better results of spatial memory in AD as compared to Galantamine.

Also our proposed combination therapy groups i.e Phototherapy and Galantamine combination group results in number of entries in novel arm are not very significant as compared to Galantamine alone but Phototherapy and Memantine combination showed much improved results as more number of entries in the novel arm were observed in it with more time spent in novel arm was noticed in these combinational therapy groups predicting the significant improvement in the spatial memory and exploration. Spontaneous alteration percentage in Phototherapy and Memantine group was also better. However, $AlCl_3$-treated mice showed a significant decrease in memory retention and route alterations. While comparing the results of combinational therapies with each other the Phototherapy and Memantine combination group showed much better results as compared to Phototherapy and Galantamine combination group in all the parameters of Y-Maze test predicting that the phototherapy combination with Memantine could work more efficiently in AD as shown in figure 3.1 and figure 3.2.

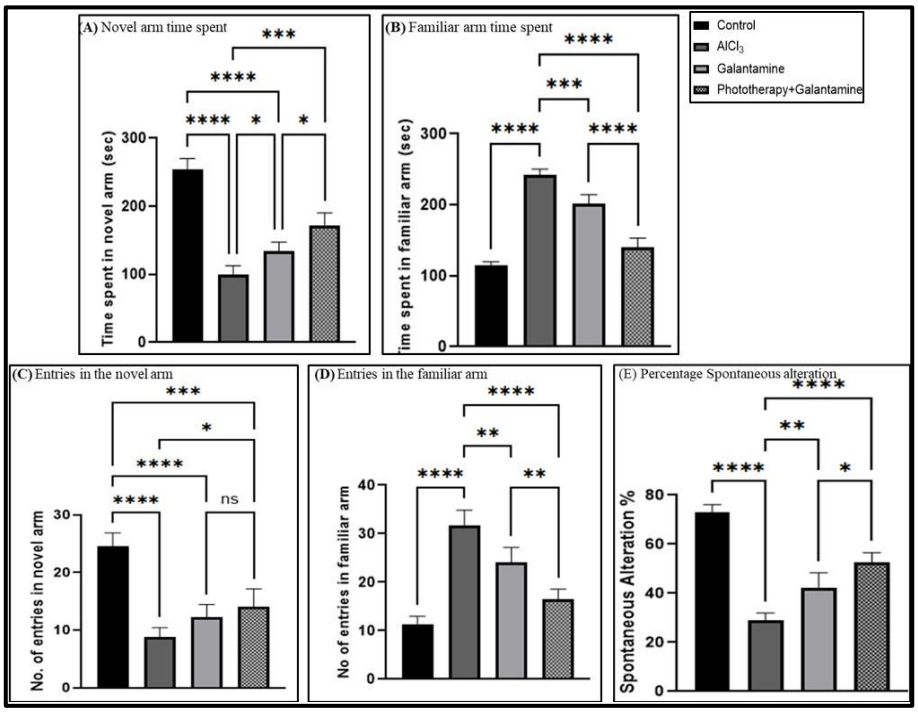

Figure 3.2 Graphs depict the performance in the Y-Maze test. There is high significance in the Phototherapy and GAL combination group comparing to disease and Galantamine alone. Statistical analysis, employing one-way ANOVA and Tukey's multiple comparison test, supported these findings, significance denoted by *$p \leq 0.05$, ***$p \leq 0.001$, ****$p \leq 0.0001$. Error bars representing the standard error of the mean (± SEM).

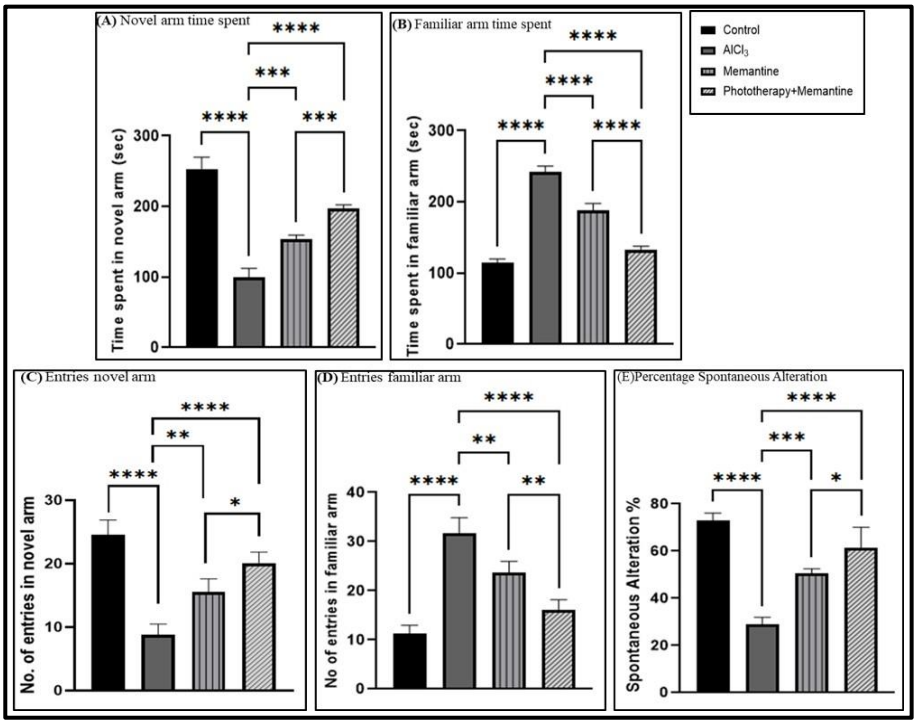

Figure 3.1 Graphs depict the performance in the Y-Maze test. There is high significance in the Phototherapy and MEM combination group comparing to disease and Galantamine. Statistical analysis, employing one-way ANOVA and Tukey's multiple comparison test, supported these findings, significance denoted by *$p \leq 0.05$, ***$p \leq 0.001$, ****$p \leq 0.0001$. Error bars representing the standard error of the mean (± SEM).

3.1.2. Memory Retention and Object Recognition Performance in NOR Test

Mice's memory retention and object recognition was analyzed using the NORT test's aspects, such as the time spent with novel object and time spent with familiar object. In figures 3.3A and 3.4A, the time spent with familiar object is presented. The time spent with familiar object suggests the impared memory as mice naturally, are exploratory in nature. Spending more time with familiar would indicate that the mice is unable to differenciate between the objects and suffering from cognitive impairment. The control group mice of both drug groups spent less time with familiar than any other group indication an intact memory. The disease group result predicted the severe memory impairment as they spent with more time with familiar object than other groups. The groups that were induced with Memantine and Galantamine had object recognition ability compared to the disease group. The results from the Memantine group shows the improved cognitive and recognition ability than the Galantamine group. This suggests that the drug can help AD patients maintaining the recognition memory. The results from the combination therapy groups revealed that Memantine and phototherapy in combination could be helpful in memory impairment and recognition, as there was less time spent with the familiar object than when phototherapy and Galantamine were used together.

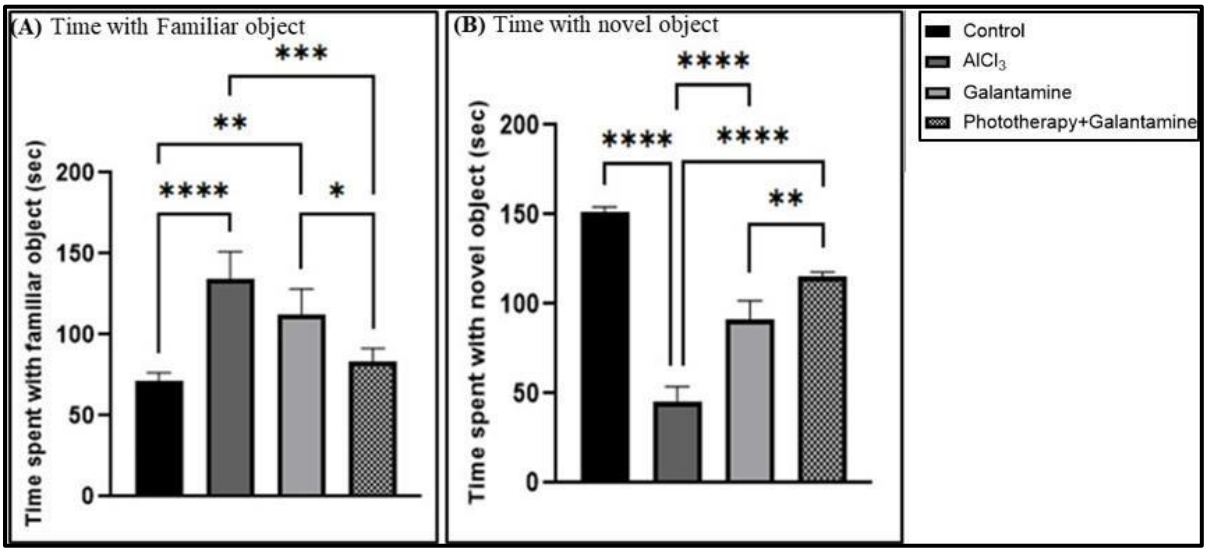

Figure 3.3 Graphs shows the Novel object recognition (NOR) test. Various comparisons among the groups are showing Galantamine and Phototherapy and GAL combination with control and disease group. Statistical analysis, employing one-way ANOVA and Tukey's multiple comparison test, supported these findings significance denoted by *$p \leq 0.05$, **$p \leq 0.01$, *** $p \leq 0.001$ and **** $p<0.0001$. Error bars representing the standard error of the mean (± SEM).

In the same way, Figures 3.3B and 3.4B shows time spent with novel object. Time spent with novel object reflects intact recognition memory and cognitive function, and the disease group spent less time with the novel object. The Memantine and Galantamine groups showed

more time spent with novel object comparing to disease group. The combination treatment groups, on the other hand, showed the prolonged time than the disease. It was also noticed that the group phototherapy and memantine combination spent the ample amount of time with novel object as compared to the phototherapy and Galantamine combination group. This suggests that the combination therapy has the improved results.

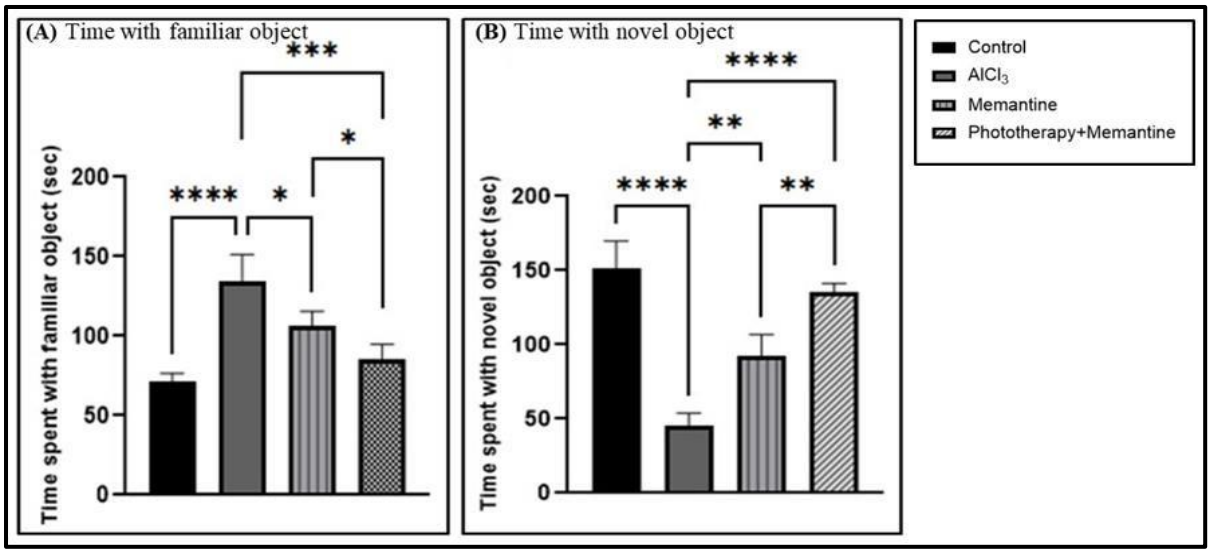

Figure 3.4 Graphs shows the Novel object recognition (NOR) test. Demonstrating the control group showed the highest exploration time with novel object, indicating normal cognitive function. Using one-way ANOVA and Tukey's multiple comparison test, statistical analysis showed that these results were significant (*$p \leq 0.05$, **$p \leq 0.01$, *** $p \leq 0.001$, and **** $p<0.0001$). The error bars show the standard error of the mean (± SEM).

3.1.3. Evaluation of Learning and Memory Acquisition

In this test, navigation, learning and memory acquisition skills of mice were assessed by evaluation of spatial retention memory using the MWM test. It was a 6 days test with training in the first 5 days and final test conduction day at 6th day. The video recorded during the testing was analyzed to calculate the number of entries and time in the target quadrant and crossing over the platform. The obtained results showed that the control group learned the most about the area and retained the memory of hidden platform with the most crossings and longest time spent in the target quadrant, while the diseased group displayed less entries and times in the target quadrant.

The treatment group Memantine and Galantamine showed improved memory while comparing to disease group. Also there was a significant difference in our proposed combination therapy group i.e Phototherapy and Galantamine combination, and Phototherapy and Memantine combination, as more number of entries in the target quadrant and more

crossing over the platform was noticed in these combinational therapy groups, predicting the significant improvement in the memory and learning. While comparing the results of combinational therapies with each other, the Phototherapy and Memantine combination group showed much better results as compared to Phototherapy and Galantamine combination group predicting that the phototherapy combination with Memantine could work more efficiently in AD as shown in figure 3.5 and figure 3.6.

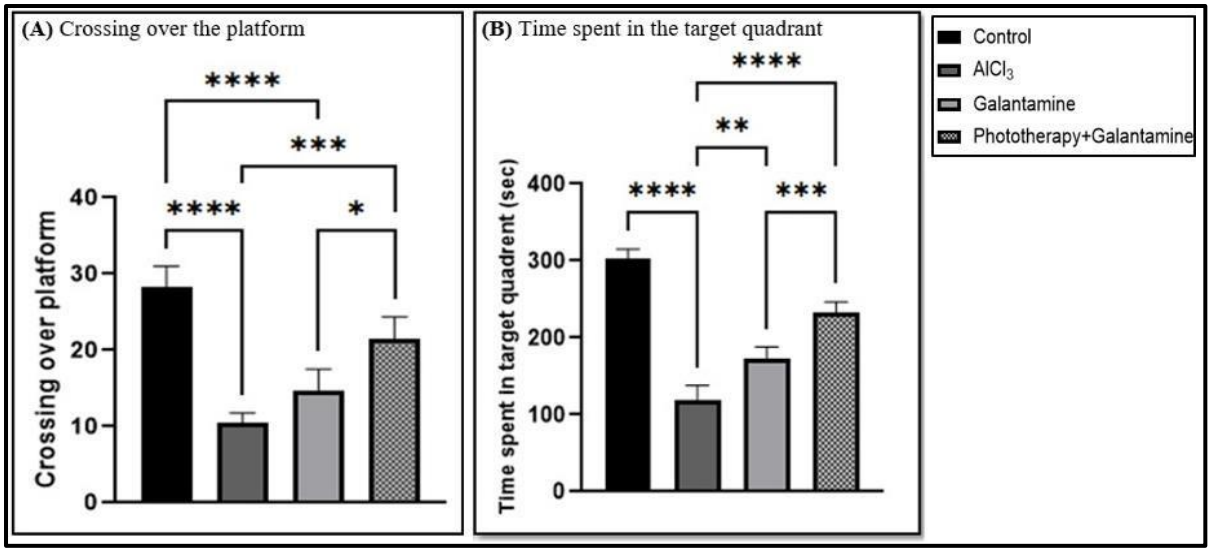

Figure 3.5 Graphs illustrate the performance in the MWM (MWM). The graphs predicts significant memory acquisition in phototherapy and Galantamine combination group as per time spent in the target quadrant is higher. Statistical analysis, employing one-way ANOVA and Tukey's multiple comparison test, supported these findings, significance denoted by *$p \leq 0.05$,**$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$. Error bars representing the standard error of the mean (± SEM).

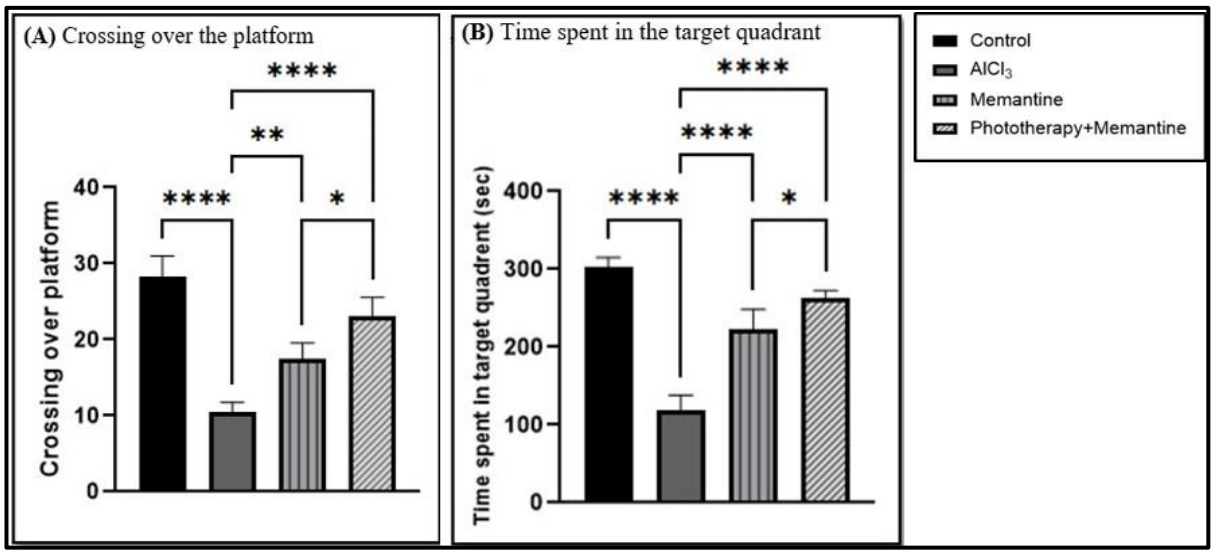

Figure 3.6 Graphs illustrate the performance in the MWM. The graphs predicts significant memory acquisition in Phototherapy and MEM combination group as per time spent in the target quadrant is higher. Mice learning improvement is predicted by crossing over the platform place when it was removed. Statistical analysis, employing one-way ANOVA and Tukey's multiple comparison test, supported these findings, significance denoted by *$p \leq 0.05$,**$p \leq 0.01$, , ****$p \leq 0.0001$. Error bars representing the standard error of the mean (± SEM).

3.1.4. Evaluation of Exploratory and Anxiety Response

The exploratory and anxiety response of mice were evaluated using OF test's various aspects like number of entries in central area and peripheral areas. In figure 3.7A and 3.8A represents the number of entries in central area. The central area entries represents the lower anxiety and greater exploratory behavior and control group mice of both drug groups entered the central region more often as compared to all other groups. The diseased mice were showing the high anxiety response and were moving in the peripheral region therefore the entries by disease group is in central area were very less. The treatment groups of Memantine and Galantamine represented improved anxiety like response while comparing to disease group. The Memantine group showed the better results than the Galantamine group indicating that the drug is capable of reducing the anxiety like responces in AD patients. The combination therapy groups were also better in results while comparing to drug alone. In OF test, number of entries in central region by Memantine and phototherapy combination was also high as compared to Phototherapy and Galantamine combination.

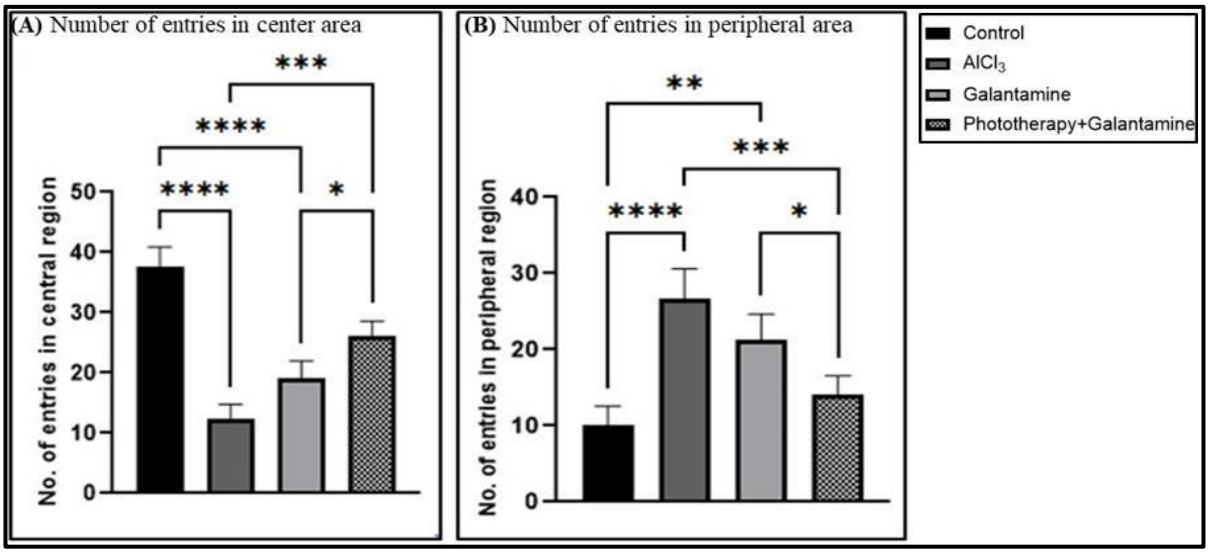

Figure 3.7 This graph shows the Open Field Test. The more entries by the Disease group in the peripheral area show how the mice reacted to anxiety, while the more entries by the treatment groups in the center area show how the mice responded to exploring. Using one-way ANOVA and Tukey's multiple comparison test, statistical analysis showed that these results were significant (*$p \leq 0.05$, **$p \leq 0.01$, *** $p \leq 0.001$, and **** $p<0.0001$). The error bars show the standard error of the mean (± SEM).

Similarly the Figure 3.7(B) and 3.8(B) represents the number of entries in peripheral region. Peripheral region entries indicates high anxiety responces and disease group exhibited the more number of entries in peripheral region. The Memantine and Galantamine alone groups displayed less number of entries comparing to disease. While the combination treatment groups also revealed less entries in comparison to alone drugs and disease. It is also noted that the

phototherapy Memantine combination group displayed less entries in the peripheral area predicting that this combination is more workable as compared to phototherapy and Galantamine.

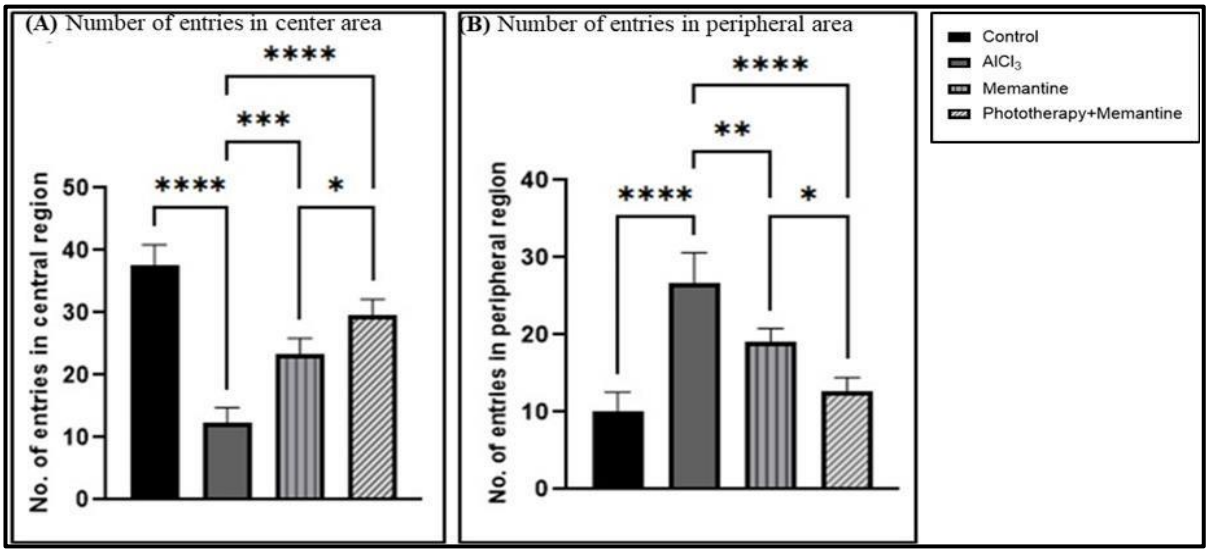

Figure 3.8 This graph shows the Open Field Test. The more entries by the Disease group in the peripheral area show how the mice reacted to anxiety, while the more entries by the treatment groups in the center area show how the mice responded to exploring. Using one-way ANOVA and Tukey's multiple comparison test, statistical analysis showed that these results were significant (*$p \leq 0.05$, **$p \leq 0.01$, *** $p \leq 0.001$, and **** $p<0.0001$). The error bars show the standard error of the mean (± SEM).

3.1.5. Anxiety like Behavior Assessment by Elevated Plus Maze Test

The animal's anxiety is triggered by being placed on an elevated open arm, which is primarily due to height and openness therefore elevated plus maze test apparatus is used to observe the anxiety like responces of mice. In this test different aspects were used to analyze the anxiety like behavior of the animal groups including time spent in open and close arms and number of entries in open and close arms.

The figure 3.9 A, 3.9 B and 3.10 A, 3.10 B represents the time spend in open arm reflects the reduced anxiety, and the disease group spent less time in open arm indicating higher anxiety. The Memantine and Galantamine groups showed more time spent in open arm comparing to disease group, which shows their exploratory behavior and indicate lower anxiety behavior. The combination treatment groups, on the other hand, showed the prolonged time spent in open arm than the disease. It is also observed that the phototherapy and memantine combination spent the maximum amount of time in open arm as compared to the phototherapy and Galantamine combination group. This suggests that the combination therapy has the improved results than the phototherapy combination with Galantamine and vice versa for the

time spent in close arm as the high anxiolytic response of disease group was noticed while calculating the amount of time spend in close arms and number of entries in close was analyzed.

The figure 3.9 C, 3.9 D and 3.10 C, 3.10 D represents the number of entries in open arm and close arm to evaluate the anxiety and feared response of mice. The open arm entries represents the lower anxiety and greater exploratory behavior and control group mice of both drug groups explored the open arm more often as compared to all other groups. The diseased mice were showing the high anxiety response and were moving in the close arms and spending more time there, therefore the entries by disease group is in open arm were very less. The treatment groups of Memantine and Galantamine represented improved anxiety like response while comparing to disease group. The Memantine group showed the better results than the Galantamine group indicating that the drug is capable of reducing the anxiety like responces in AD patients. The combination therapy groups were also better in results while comparing to drug alone. In this test, number of entries in open arm by Memantine and phototherapy combination was high as compared to Phototherapy and Galantamine combination so it indicates that our this treatment group showed the significant results.

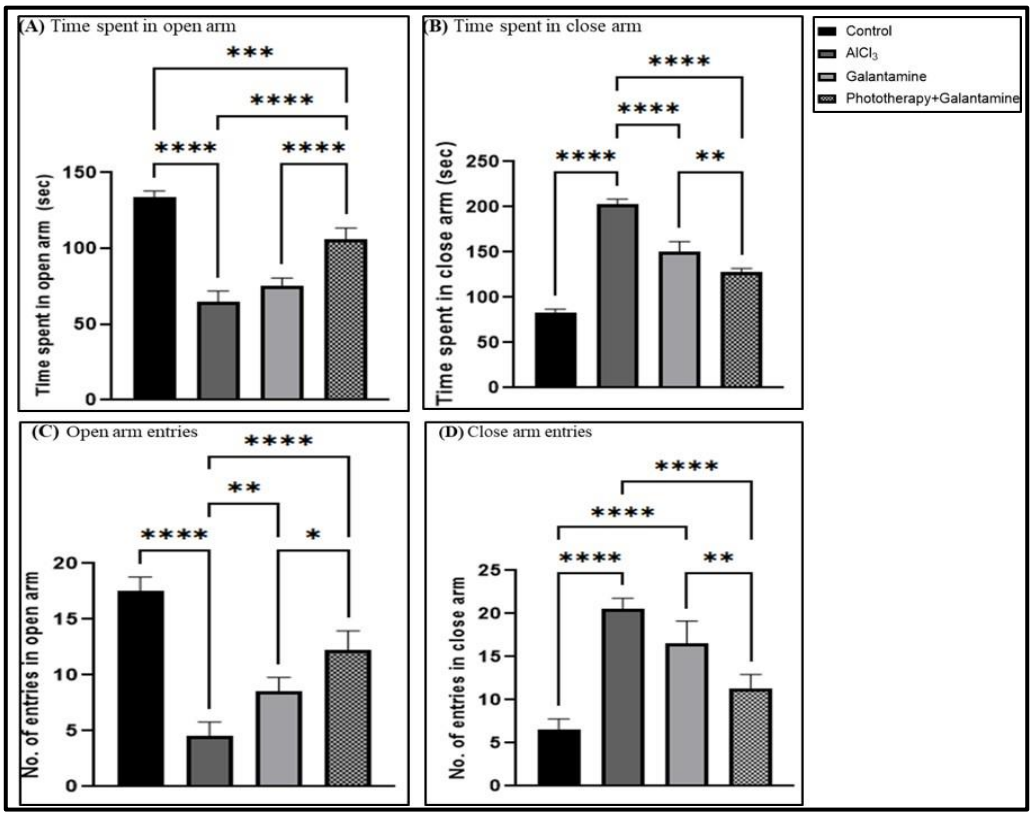

Figure 3.6 The graphs include Elevated Plus Maze test. The graph illustrates the comparison of anxiety response of Galantamine and Phototherapy and GAL combination group with control and disease among various parameters of EPMT. Statistical analysis, employing one-way ANOVA and Tukey's multiple comparison test, supported these findings, significance denoted by *p ≤ 0.05 ,**p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001. Error bars representing the standard error of the mean (± SEM).

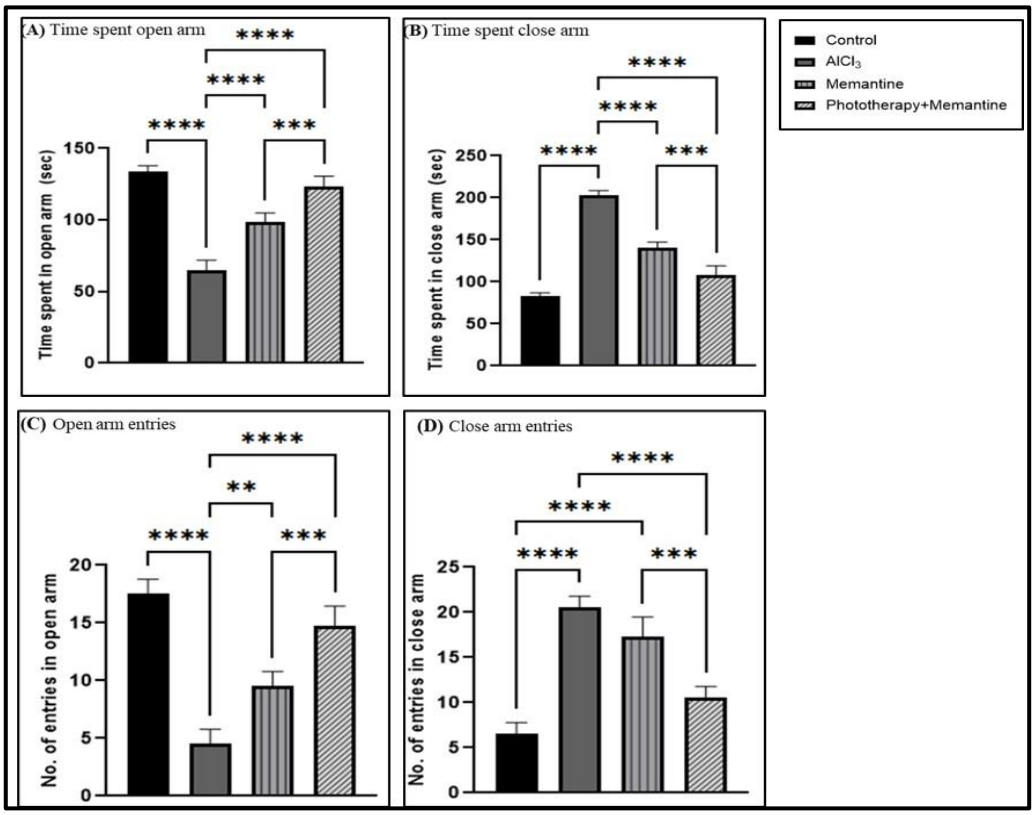

Figure 3.7 The graphs include Elevated Plus Maze test. The graph illustrates the comparison of anxiety response of Memantine and Phototherapy and MEM combination group with control and disease among various parameters of EPMT. Statistical analysis, employing one-way ANOVA and Tukey's multiple comparison test, supported these findings, significance denoted by *p ≤ 0.05 ,**p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001. Error bars representing the standard error of the mean (± SEM).

3.2. Histological Evaluation of Neurodegeneration Changes in AD

Histopathological analyses are crucial for investigating AD as they reveal changes in brain tissues. This research uses H&E staining to analyze total cell count and anatomical brain regions like the cortex and hippocampus. It compares histological outcomes between control, diseases, and treatment groups including Galantamine, Memantine, phototherapy and Galantamine combination and phototherapy and memantine combination. This analysis focuses on the brain hemispheres and examines structural alterations resulting from AD. The H&E-stained slides were visualized under a light microscope at 40X magnification. The cells under observation were analyzed using Image J software and damage to cell like neuronal loss, nucleus shrinkage, and deformed neurons were analyzed along with observation of alterations in the treatment groups. Different magnifications are used to observe the structural deformity in cortex among various groups as shown in figure 3.11. The hippocampus is also analyzed this study to observe the cell structure alteration in various groups as shown in figure 3.12.

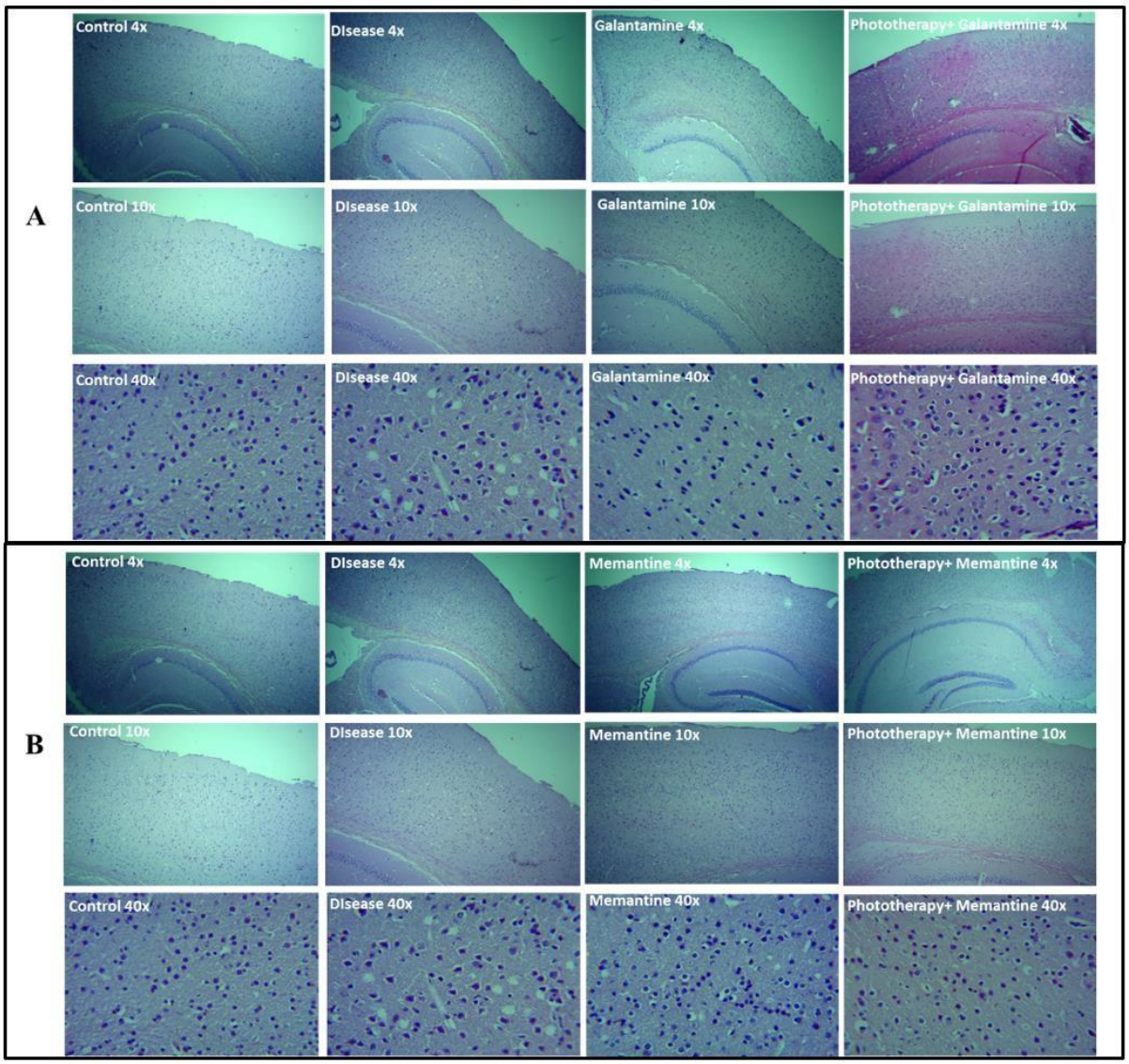

Figure 3.8 Histopathology of mice cortex at 4x, 10x and 40x magnification. (A) Represents the Galantamine and combination therapy group in comparison to control and disease group. **(B)** represents the Memantine and combination therapy group in comparison to control, disease group. Pictures are captures using Optica vision software.

3.2.1. Structural and morphometric analysis of Cortical Region

In AD, hematoxylin and eosin staining is performed to find neuronal death, which is a hallmark of the disease. It colors the cytoplasm pinkish purple and nucleus is stained bluish purple. The disease group in the figure 3.13 A 3.14 A has fewer neurons with disfigured nuclei, irregular cell shape, shrinkage of nucleus is observed, the cells are elongated and size is altered. The control groups illustrate healthy, round shaped cells with intact nucleus and no sign of deformity and cellular density is greator as compared to the disease group which is a sign of neurodegeneration. In contrast the Galantamine and memantine treatment group are showing here fewer clusters of neurons in the cortex but there is an observation of cell structure

improvement, number of cells is also increased as compared to disease group. The combination therapy treatment shows some recovered cells and some irregular shaped cells with very few disfigured neurons. Some structural deformity is observed but on the same side intact nucleus is also observed. The phototherapy and Memantine combination therapy is shown to improve the cellular structure of cortex cell more efficiently as compared to Phototherapy and Galantamine combination.

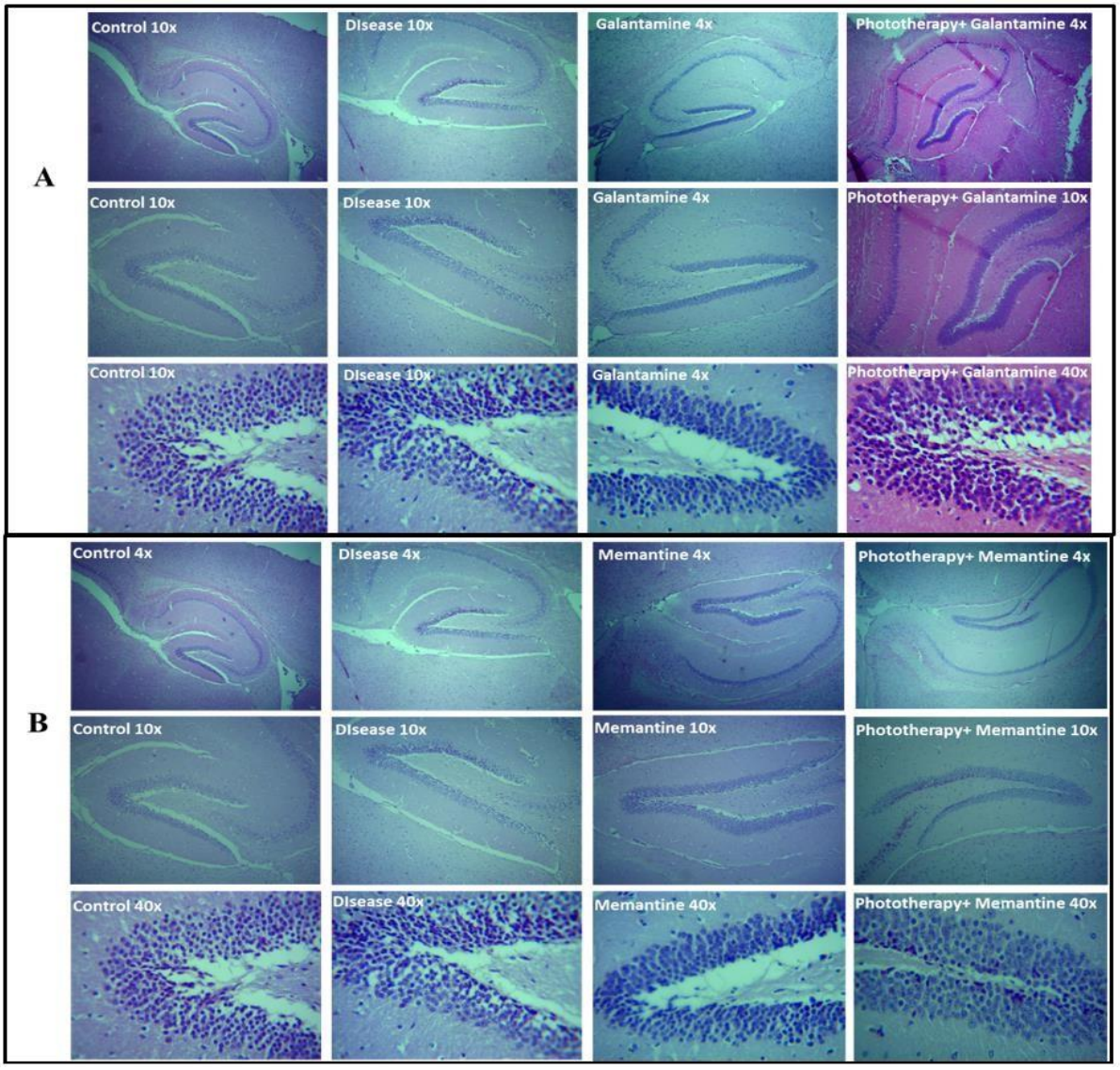

Figure 3.9 Histopathology of mice Hippocampus at 4x, 10x and 40x magnification. (A) Represents the hippocampus of Galantamine and combination therapy group in comparison to control and disease group. (B) Represents the Memantine and combination therapy group in comparison to control, disease group. Pictures are captures using Optica vision software.

3.2.1.1. Cell count in cortex

Image J software was used to count the number of cells on digital photomicrographs using Optica Vision software. The results are shown in Figure 3.13 B and 3.14 B. The control group has the highest number of cell count and disease has the lowest number of cells present in their cortex predicting the onset of AD. The number of cells confirms the pathology of disease and treatment efficacy of the treated group by comparing them all. In this graphical representation of number of cells the Galantamine and Memantine group cortex shows little improvement in cell count despite of having the AD. The combination therapy treatment groups have much improved number of cells in cortex as compared to disease and keeping the control as standard shown in figure 3.13 B and 3.14 B respectively.

While comparing the combination treatment efficacy among different drugs the number of cell count in cortex of phototherapy and memantine combination group is significantly higher as compared to disease group and there is not very significant difference in between both combinational treatment groups.

3.2.2. Structural and morphometric analysis of Hippocampus Region

To study the structural changes in brain hippocampus DG region was observed and the number of cell count, structural deformity, plaques and celluar density was considered main indication of disease progression and alteration after the treatment. The disease group has fewer neurons with disfigured nuclei, irregular cell shape, shrinkage of nucleus is observed, the cells are elongated and size is altered. The control groups illustrate healthy, round shaped cells with intact nucleus and no sign of deformity and cellular density is greator as compared to the disease group which is a sign of neurodegeneration. In contrast the Galantamine and memantine treatment group are showing here fewer clusters of neurons in the cortex but there is an observation of cell structure improvement, number of cells is also increased as compared to disease group. The combination therapy treatment shows some recovered cells and some irregular shaped cells with very few disfigured neurons. Some structural deformity is observed but on the same side intact nucleus is also observed. The phototherapy and Memantine combination therapy is shown to improve the cellular structure of cortex cell more efficiently as compared to Phototherapy and Galantamine combination. The yellow arrow shows round, healthy neurons that are well-formed and show no signs of degeneration. While red indicates dead neural cells that are not organized in space in the figure 3.15A and 3.15A.

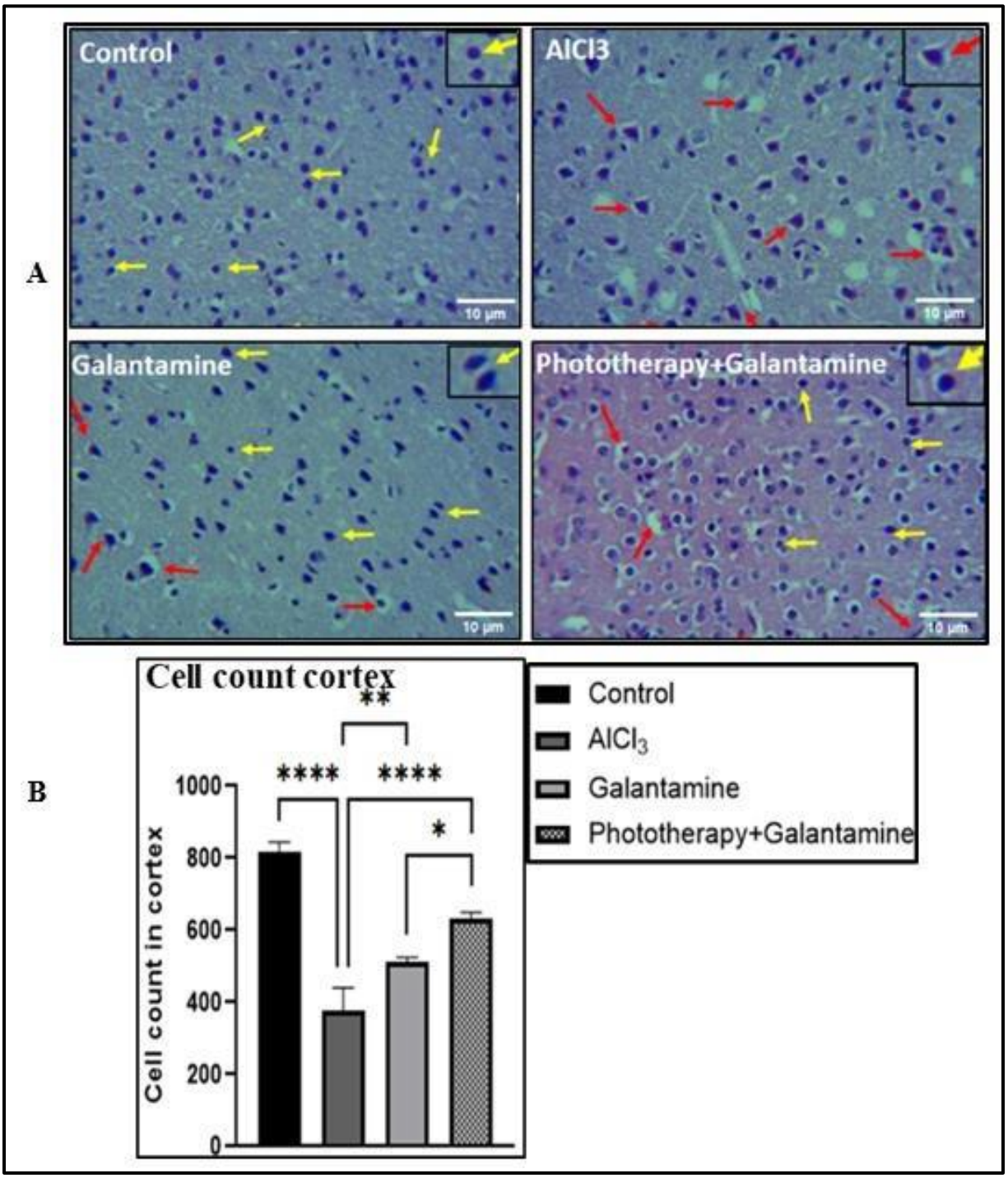

Figure 3.10 Histopathology of mice Cortex at 40x magnification. (A) Represents the hippocampus of Control, Disease, Galantamine and Phototherapy and GAL combination group. **(B)** Represents the graphical representation of cell count in the same group. Statistical analysis, employing one-way ANOVA and Tukey's multiple comparison test, supported these findings, significance denoted by *p ≤ 0.05, ***p ≤ 0.001, ****p ≤ 0.0001. Error bars representing the standard error of the mean (± SEM).

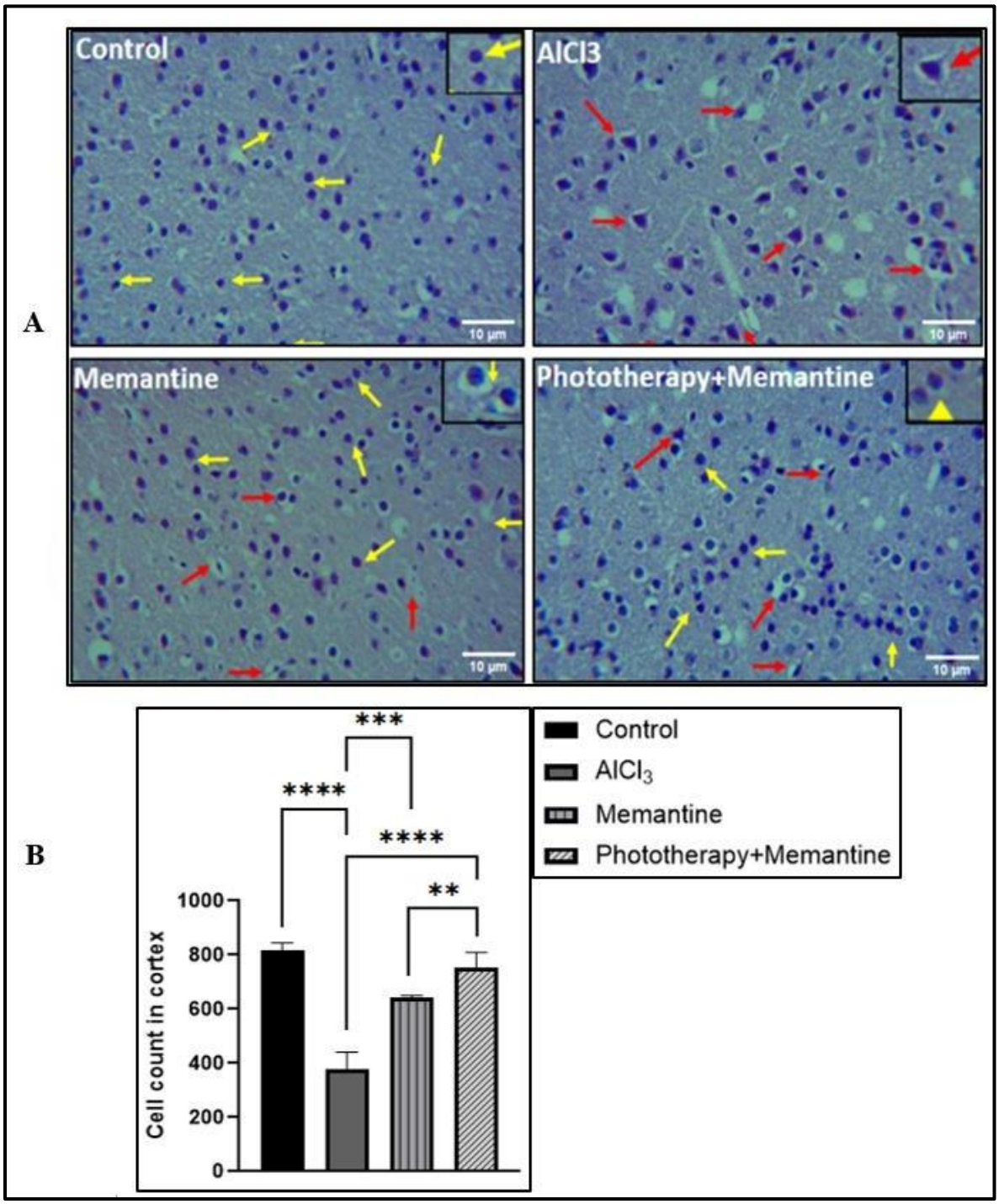

Figure 3.11 Histopathology of mice Cortex at 40x magnification. (A) Represents the hippocampus of Control, Disease, Memantine and Phototherapy+ Memantine group. **(B)** Represents the graphical representation of cell count in the same group. Statistical analysis, employing one-way ANOVA and Tukey's multiple comparison test, supported these findings, significance denoted by *$p \leq 0.05$, ***$p \leq 0.001$, ****$p \leq 0.0001$. Error bars representing the standard error of the mean (± SEM).

3.2.2.1. Cell count in DG region of Hippocampus

The cell count graph for hippocampus was plotted over Graphpad Prism by utilizing the imageJ software. The results are shown in Figure 3.15 B and 3.16 B.

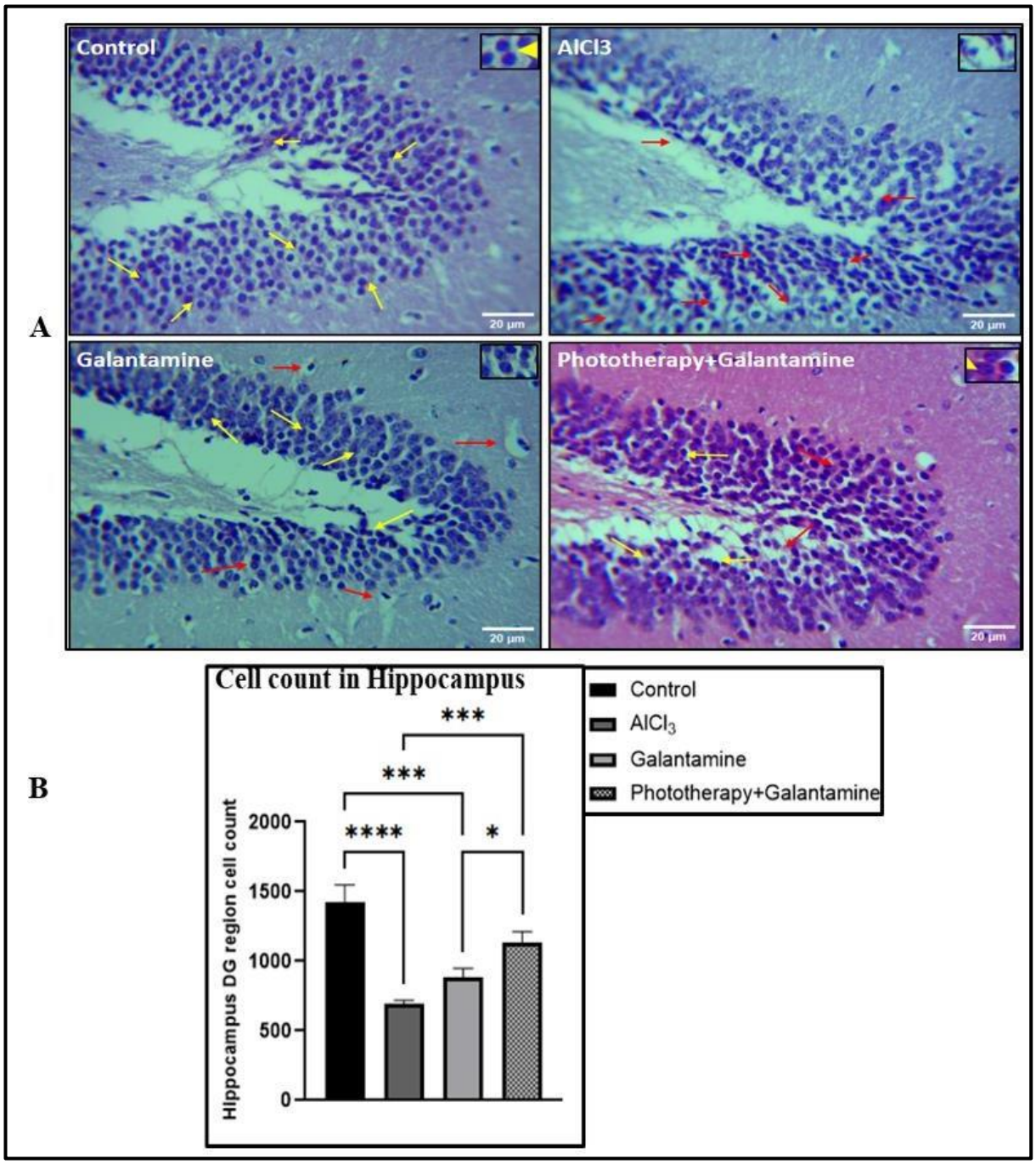

Figure 3.12 Histopathology of mice Hippocampus at 40x magnification. (A) Represents the hippocampus of Control, Disease, Galantamine and Phototherapy+ Galantamine group. (B) Represents the graphical representation of cell count in the same group. Statistical analysis, employing one-way ANOVA and Tukey's multiple comparison test, supported these findings, significance denoted by *$p \leq 0.05$, ***$p \leq 0.001$, ****$p \leq 0.0001$. Error bars representing the standard error of the mean (± SEM).

The control group has the highest number of cell count in the hippocampus DG region and disease has the lowest number of cells present in their DG region predicting the onset of AD. The number of cells confirms the pathology of disease and treatment efficacy of the treated group by comparing them all. In this graphical representation of number of cells the Galantamine and Memantine group shows little improvement in cell count and density despite of having the AD shown in fugure 3.15 and 3.16 respectively.

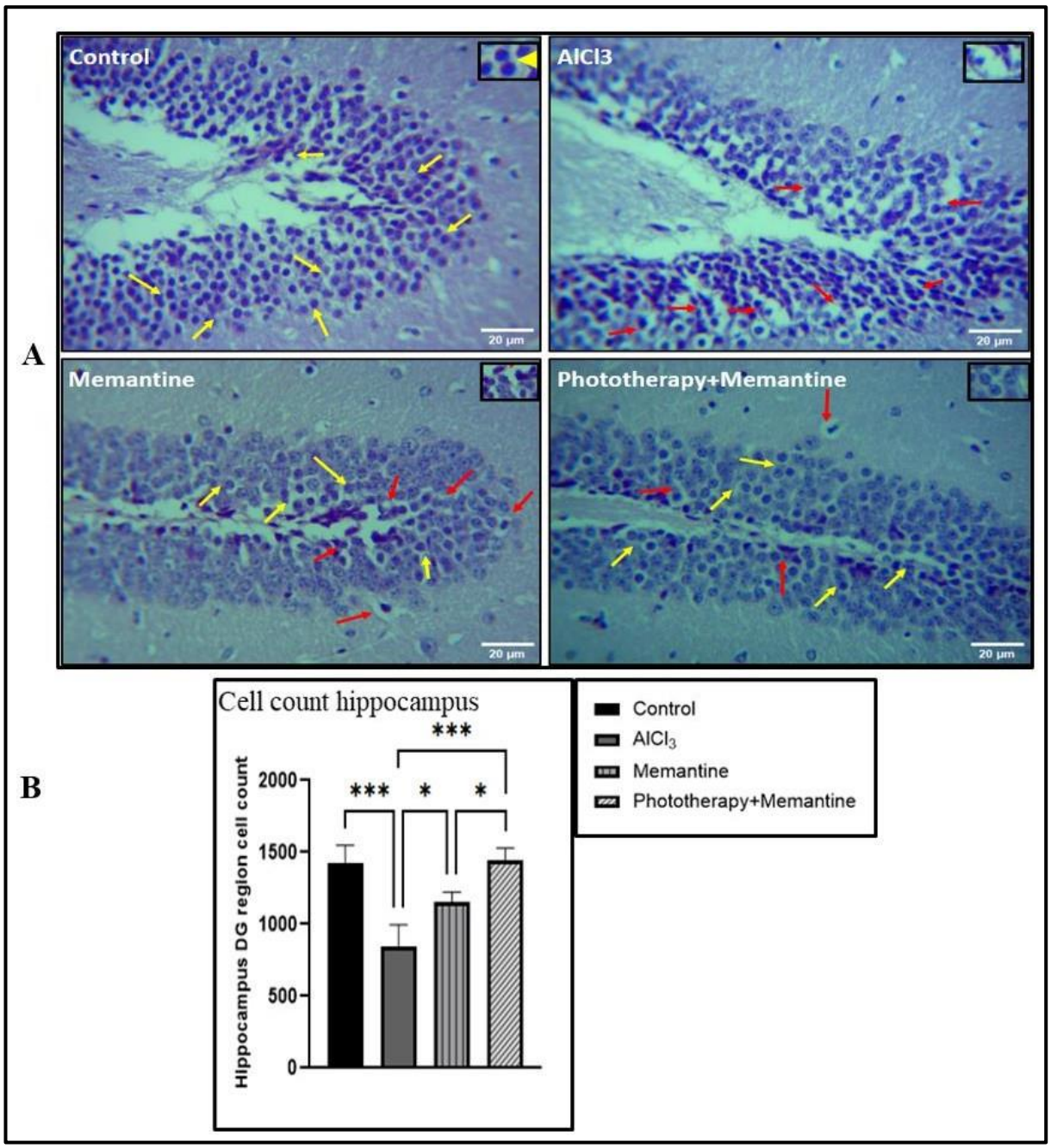

Figure 3.13 Histopathology of mice Hippocampus at 40x magnification. (A) Represents the hippocampus of Control, Disease, Memantine and Phototherapy+ Memantine group. **(B)** Represents the graphical representation of cell count in the same group. Statistical analysis, employing one-way ANOVA and Tukey's multiple comparison test, supported these findings, significance denoted by *p ≤ 0.05, ***p ≤ 0.001, ****p ≤ 0.0001. Error bars representing the standard error of the mean (± SEM).

The combination therapy treatment groups have much improved celluar density and number of cells in cortex as compared to disease and keeping the control as standard. While comparing the combination treatment efficacy among different drugs the number of cell count in hippocampus of phototherapy and memantine combination group is significantly higher as compared to disease group and there is not very significant difference in between both combination treatment groups. The visual differences in the cell count confirms the efficacy of Phototherapy and combination therapy.

CHAPTER 4: DISCUSSION

AD is progressive disorder causing neurodegeneration over the past few decades, significant efforts have been made to study the pathology of AD, the amyloid hypothesis being the predominant hypothesis (Masters et al., 2015). Some drugs have been designed to increase or decrease Aβ catabolism or synthesis, but their effectiveness in animal models or AD patients remains uncertain (Michaelis, 2003). Current treatment involves managing AD through a combination of pharmacological and non-pharmacological treatments, including NMDA receptor antagonists, cholinesterase inhibitors, and alternative therapies like music and art therapy are being investigated (Blackman et al., 2021; Theleritis et al., 2017). AChE inhibitors like donepezil, rivastigmine, and galantamine are used to manage AD symptoms(Singh et al., 2024). Galantamine is widely used for treating AD, with prolonged lifespan posing a risk to neurological diseases, there is a growing demand for drugs leading manufacturers to seek more suitable options for large-scale applications. It improves cognitive and behavioral functioning in individuals with Alzheimer's. However, side effects like hepatotoxicity, hypotension, gastrointestinal disturbances, dizziness, diarrhea, vomiting, and nausea can reduce its efficacy (Nozaki et al., 2024; Prvulovic et al., 2010; Santos et al., 2020).

Another drug, Memantine, a voltage-dependent NMDA antagonist, has been used to treat AD. Although it can't reverse its irreversibility but it can reduces symptoms like memory loss and cognitive impairment (Pardo-Moreno et al., 2022). Combining drugs may be the most effective treatment such as phototherapy, a non-invasive treatment for conditions like AD, is considered safe by the FDA due to its minimal side effects and antioxidant properties (Saeedi & Mehranfar, 2022). In this study we analyzed the combination therapy effects of drugs and phototherapy on AD. In the this study we investigated the impacts of Phototherapy as a combination treatment for drugs like Galantamine, Memantine and their synergistic effects on an animal model of AD, utilizing the MWM, NORT, Y-Maze , EPMT and OF as the assessment tool.

The Y-maze test reveals information pertaining to the working memory and the spatial recognition. This study examines the comparative behavioral performance of groups across five parameters: the amount of time spent in the novel arm, the amount of time spent in the familiar, the number of entries in the novel arm, the number entries in the familiar arm of the maze and the percentage of time it spent by calculating the spontaneous alternation between

the two arms. In this study the control group the cognitive functions remained healthy because no sign of neurodegeneration was observed. The control animals spent more of the time in the novel arm and had higher spontaneous alternation percentages are recorded while comparison control group performance with the $AlCl_3$ group ($p < 0.0001$). On the other hand, the $AlCl_3$ induced animals exhibited a significant impairment in learning and memory as well as cognitive flexibility. The disease group spent more time in the familiar arm of the maze and only made fewer entries into the novel arm of the maze which clearly indicates spatial memory dysfunction, as previously explained in the literature linking the Alzheimer's pathology to impaired hippocampal function. Literature reveals that AD pathology ($AlCl_3$ group) is linked with low performance in spontaneous alternation than the control group which help in the determination of the working memory (Rao et al., 2021).

Further similar effect was observed in APP/PS1 transgenic mice which is the genetic model of AD; using Y-maze test, there were spatial memory deficits, as indicated by reduced entries into the novel arm and a spontaneous alternation percentage. Accordingly, the deficits were found to be related to hippocampal atrophy and formation of the amyloid-beta plaque in the patients (Zhu et al., 2017). The comparison of $AlCl_3$ group and Galantamine administered group indicated as highly significant ($p<0.0001$). The time spent and entries into novel arm of Galantamine treated group and the spontaneous alteration percentage was also significantly higher ($p<0.0001$) than $AlCl_3$ group. This enhancements suggest a recovery of spatial memory and learning ability of Galantamine group. Literature also indicated the the administration of galantamine is liked with increased levels of Ach required for memory and learning, and had shown to be reduced the amyloid burden in models of AD(Wang et al., 2017). Phototherapy and Galantamine combination improved the performance when compared with treatments alone. The combination group spend more of its time in the novel arm $P < 0.0001$, and displays higher spontaneous alternation $P < 0.0001$ than $AlCl_3$ group. The combination treatment also showed reduced time spent in the familiar arm ($p < 0.0001$). Phototherapy is thought to reduce neuroinflammation and possibly contribute to increase the function of mitochondria in neurons. Such mechanisms may have played a role in enhancing the cognition and have a neuroprotective effect of combining pharmacological and non-pharmacological treatments in AD models (Rong et al., 2021; Wang et al., 2021).

After comparing the results for the Galantamine group and the combination therapy group there was evidence of significant differences. The combined treatment group was found

to spent slightly more time than the control group in the familiar arm $p < 0.05$) and had higher percentage of spontaneous alternation $p < 0.05$ indication some cognitive impairment. This indicates that the phototherapy has added value in combination with the Galantamine but still control was better in performance. But for some extent the number of entries into the novel arm, time spent in novel arm comparing to treatment alone showed overall improved effect of two treatments as different non significantly (ns), which points both to the efficacy of the treatments and to the potential of additional phototherapy effects to be more significant in the long-term cognitive functions compared to the immediate exploratory behavior. (Li et al., 2014). The differences between the control group and the $AlCl_3$ diseased group, the results obtained indicate that the disease affected cognitive losses in all the parameters that were measured. The time spent in the novel arm was considerably higher in the control group when compared to the rest of the groups ($p < 0.0001$) suggesting normal exploratory behavior and memory. Therefore, the $AlCl_3$ group exhibited a significant less time spent in the novel arm. Likewise, the time in the familiar arm was significantly higher in $AlCl_3$ group as compared to the control group ($p < 0.0001$) these results also suggest than the animals in the $AlCl_3$ group had an impaired cognitive ability. Various studied revealed the same results of $AlCl_3$ in comparison with memantine treatment approaches (Sadiq & Al-Zubaidy, 2024).Unlike the novel arm, the control group made significantly higher entries into the novel area and with this confirmed better cognitive capability as compared to the $AlCl_3$ group. Further, the rate of spontaneous alternation was significantly lower in diseased group than in control, pointing towards the working memory impairment.

In comparison with the $AlCl_3$ group, the Memantine group and the combination therapy with memantine, all the indicators were significantly better. In the novel arm, the Memantine group spend more time compared to the baseline ($p < 0.001$) while showing less time in the familiar arm compared to baseline ($p < 0.0001$) which suggest the group had some form of memory retrieve ability. The effect was more evident in the studying Memantine and Phototherapy group to demonstrate further increase in time with the novel arm and a significant reduction in time with the familiar arm. The number of entries into the novel and the familiar arm was higher for both treatment groups than in the $AlCl_3$ group. However the combined treatment had a tendency to promote better cognitive recovery. The percentage of spontaneous alternation also increased in both the treatment groups, but not to the level of the control group, the maximum recovery was seen in the Memantine Phototherapy combination group.

A comparison of the results in Memantine and combination of phototherapy demonstrated more significant positive trends in the cognitive functioning comparison to the $AlCl_3$ group in all three trials of the study, but higher efficacy of the combined therapy is noticed. The memantine showed similar number of entries into novel and familiar arms but the Memantine and phototherapy combination had a higher percentage of spontaneous alternation. These findings imply that there may be a synergistic effect when used together with phototherapy and Memantine; the effect on spatial and working memory functions is better than with Memantine. The impact of the drugs Galantamine and Memantine in the AD model is still debatable however, the results reveal that the drug enhanced cognitive functions to a large extent. In a study Low level light therapy (LLLT) on an AD rat model was performed and the overall cognitive performance of the rats was enhanced and the parameter of spontaneous alternation in the Y-maze was found significantly different. The authors also stated that LLLT lowered the deposition of amyloid-beta and enhanced the synaptic plasticity consequently the memory(Montazeri et al., 2021).

In the NOR test, the comparison of the performances of the control group and the $AlCl_3$ diseased group shows that the $AlCl_3$ diseased group has impaired cognitive ability as compared with the control group. The time activated with the novel object was significantly higher in the control group compared with the familiar object ($p < 0.0001$) demonstrating that the memory and cognitive function was intact. On the other hand, the $AlCl_3$ group has exhibited memory loss, taking more time with the familiar object and significantly less time with the novel object which points to their poor ability at recognising new objects, hence the deficit in recognition memory. This reduction in object recognition ability observed in the $AlCl_3$ group is in compliance with $AlCl_3$ induced AD like condition in which memory and learning related ability is impaired due to neurodegenerative alterations. The normal healthy animals will tend to spend more time exploring a novel object since they have proper and efficient intact memory and cognitive pliability while the animals with AD, or AD like manifestations such as animals which were injected with $AlCl_3$ tend to spend much more time exploring familiar objects since their memory is impaired. As highlighted in previous studies, AD models have been found to have cognitive deficits and less active exploratory preference for new objects(Stazi & Wirths, 2021)

In the course of the present study, it was observed that the efficacy of both treatments of Galantamine and phototherapy and Galantamine combination significantly enhanced the

cognitive changes compared to the $AlCl_3$ group. The time spent with the familiar object was reduced significantly with Galantamine treated animals and did not differ significantly with the control and more time was spent with the novel object ($P < 0.0001$) compared with $AlCl_3$ group indicating partial restoration of memory by Galantamine. This improvement could be attributed to the fact that Galantamine's action in a way facilitates the cholinergic neurotransmission which is very essential in learning and memory. The results of the phototherapy and Galantamine group were even better as compared to the Phototherapy group; thus, the rats of this group spent significantly more time exploring the novel object ($p < 0.0001$) and less time exploring the familiar object ($p < 0.05$) as compared to the Phototherapy group only it can be pointed out that the phototherapy and galantamine combination led on this regard, these studies suggest the possibility of enhancing memory performances in other AD models through combination therapies. Several existing experiments have shown that treatment by Galantamine enhances the performance in memory related tasks some of which are the NOR test where the treated animal spends more time exploring the novel object thus suggesting the restoration of the recognition memory. (Labban et al., 2021). Such enhancements are in concordance with the mode of action of Galantamine that helps overcome the cholinergic dysfunction that characterizes AD induced cognitive dysfunction. The findings indicated that the group receiving both Galantamine and phototherapy post-treatment has a better cognitive performance than the group receiving only Galantamine. The time spent by the mice of the combination therapy group with the novel object was significantly more compared to the Galantamine group but the time spent with the familiar object was significantly less compared to the Galantamine group. Thus the effects of the phototherapy and galantamine combination based interventions are better than those of Galantamine alone. This shows that there is the potential of enhancing phototherapy with pharmacological interventions for further improvement in the management of memory defect that is characteristic with AD.

In another treatment group as well, Memantine caused significant enhancement in the score of the test, as the animals in this group spent less time with the familiar object ($p < 0.001$) and more time with the novel object ($p < 0.01$), which suggest that Memantine partially retrieved the recognition memory. It was found that memantine's neuroprotective effect and its influence on the synaptic plasticity may have contributed to this improvement. Such improvement signifies the efficacy of phototherapy, and when combined with Memantine, this Phototherapy group recorded more time with the novel object than the $AlCl_3$ group whose treatment only involved the drug ($p < 0.0001$) and lesser time with the familiar object ($p < 0.$

001) proving that the therapy is made better with the addition of memantine for Alzheimer's patients. Earlier literatures have revealed that, Memantine treatment in AD models enhances the ability to perform on NOR tests in which the treated animals demonstrated the preference towards the novel object like untreated animals suggesting improved recognition memory (Ihalainen et al., 2011). As a result of memantine, the neuroprotection is noticeable in AD since excitement toxicity plays a role in neuron destruction and loss of functions which is in line with the results from the current study. Finally, Memantine and Memantine + Phototherapy, the use of both therapies was more beneficial in improving the cognitive abilities. We found that the time spent with the novel object was less with the familiar object in the Memantine and Phototherapy group than with the Memantine-only group ($p < 0.01$), suggesting that phototherapy enhances the recovery of memory by Memantine. This seems to imply that easing the pharmacological treatment with cognitive-behavioral therapy might be more effective in patients with AD since it seems to make the improvement brought by pharmacological treatment alone greater.

The MWM evaluates spatial learning and memory, both of which are compromised in AD. The research examines platform crossings and duration in the target quadrant to evaluate memory retention and spatial memory. In behavior analysis, the control group was significantly higher than other groups showing significantly more time spent in target quadrant in comparison to the $AlCl_3$ group giving the p value ($p < 0.0001$). The reductions in memory and learning abilities evidenced in the $AlCl_3$ group predicts the neurotoxic effects of $AlCl_3$ that have been documented in the literature. Results have established that $AlCl_3$ interferes with the hippocampus thus affecting spatial memory (Cao et al., 2017) as evidenced by fewer platform crossings and less time spent in the target quadrant in this set. The MWM test results for the control, $AlCl_3$, Memantine-treated, and phototherapy and memantine combinationgroups were also analyzed. In the control and disease comparison, the number of platform crossings and time spent in the target quadrant for the control group were significantly higher ($p < 0.0001$), showing that $AlCl_3$ causes severe cognitive impairment. These findings are consistent with prior research on $AlCl_3$, which induces amyloid-beta aggregation, oxidative stress, and synaptic disruption in mice, resulting in severe spatial learning impairment (Zhou et al., 2019).

The animal treated with galantamine demonstrated non-significant changes in crossing over the platform as compared to the $AlCl_3$ group while the time spend in the target quadrant is significantly more compared to the $AlCl_3$ group (**$p < 0.01$). Previous studies suggesting

that galantamine improves learning ability in AD models through the cholinergic system (Liu et al., 2018). The Memantine treatment resulted in increased platform crossings ($p < 0.0001$) and more time spent in the target quadrant compared to the disease group, indicating that it can alleviate cognitive deficiencies by attenuating glutamate signals and preventing excitotoxic effects. Memantine has been shown in clinical and preclinical studies to have neuroprotective benefits, as proven by decreased brain damage and enhanced synaptic plasticity (Stazi & Wirths, 2021).

The galantamine-treated group showed better performance than disease group but not effective as in the control group(Baakman et al., 2022). The use of phototherapy with galantamine showed enhanced effect on spatial memory than using galantamine alone; the results for the physiological observations were a significantly increased in platform crossings ($p<0.01$) and time spent in the target quadrant ($p < 0.001$). It has been seen from the literature that phototherapy can enhance the efficiency of mitochondria, enhance ATP generation, and decrease neuroinflammation which plays a major role in AD (Wang et al., 2017).

When comparing the results of the Memantine and Phototherapy combination group to both the disease group and the Memantine-alone group there was an additional improvement in cognitive function in terms of platform crossings and time in the target quadrant with ($p < 0.0001$) and ($p < 0.5$). This suggests that phototherapy improves the favorable effects of light in a cumulative manner, which could be attributable to its effects on mitochondrial activity, neurogenesis, and neuroinflammation. Also, there is evidence that phototherapy improves synaptic plasticity and decreases amyloid-beta levels in animal models (Han et al., 2018). Previous studies also support these observations with reports of improved prognosis with multimodal interventions that address both the neurotransmitter and the neurodegenerative aspects of AD compared to single agent treatments in animal models (Xu et al., 2013). The increase number of platform crossings and time in target quadrant shown by the phototherapy and Galantamine combination group compared with the control group revealed that the combined treatment was able to reduce the AD-like cognitive impairments to some extent. In this test while comparing both combination therapy groups results it is revealed that combination treatment overall provided better result but comparing them with each other shows that the phototherapy and Galantamine provided us the better results.

The results from the OF test describe some aspect of the anxiety-like behavior in the different groups. In comparing the control group with the $AlCl_3$ diseased group changes in the

anxiety like behaviour are visible. The control group recorded many entries into the central area compared to the peripheral area ($p < 0.0001$) revealing normal exploration and reduced anxiety. However, to the $AlCl_3$ group the level of anxiety was higher than in the control group as evidenced by the low number of entries into the central area and high number of entries in the peripheral zone. This implies that $AlCl_3$ caused the animals to develop anxiety like behavior, an indication that these animals preferred to stay in the periphery zone of the open field area which was relatively safe as compared to the central area. Prior animal research has shown that the AD associated brain changes including hippocampal changes give rise to increased anxiety and the deficits in attention to exploratory activities in animals. (Lazarova et al., 2021; Pentkowski et al., 2021).

When the results of the diseased group were compared with that of the two treatment groups; Galantamine and combination of phototherapy and Galantamine they showed a significant improvement in decreasing the anxiety like behavior in the mice. In addition to this, the Galantamine group showed a great increase in the number of entries to the central area which was statistically significant $p < 0.001$ and a decrease in the number of entries to the peripheral area $p < 0.01$ thus a suggestion that Galantamine had an anxiolytic effect and promoted exploratory activity. This suggest that there was a further enhancement when Galantamine was administered together with phototherapy, as the Galantamine and Phototherapy and Galantamine combination group made significantly more entries into the central area ($p < 0.001$), and significantly fewer entries into the peripheral area ($p < 0.001$), than the diseased group. This indicates that there is effectiveness of both the treatment methods but there could be even more advantage with the combined therapy treatment. Galantamine was observed to enhance cognitive ability due to increased level of AchE in the brain since the cholinergic function is impaired in the AD patient. The studies conducted earlier identified the fact that the Galantamine has memory enhancing effects and decrease the anxiety – like behavior in the AD models through cholinergic transmission. Galantamine improved the anxiety status as evidenced by increase in the number of entries into the central region and decrease in peripheral entries(Bhattacharya et al., 2014).

While comparing the result between two treatment groups, this study also found that the mice in combination therapy with galantamine group had a significantly lower anxiety score than that in the Galantamine only group. the Galantamine and Phototherapy group entered the central area more frequently ($p < 0.05$), and entered the peripheral area less frequently ($p <$ 0.

05). This suggests that the interactions between phototherapy and Galantamine increase the effectiveness of treatment, and therefore reduce the anxiety-like behavioral response and increase the exploratory activity as compared to the group treated only with Galantamine.Similarly when comparing the $AlCl_3$ group to other treatment groups; Memantine and Phototherapy and Memantine combination; both treatment significantly enhanced the mice performance in this test. The Memantine group demonstrated an increase in central area entries ($p < 0.001$) and a decrease in peripheral entries ($p < 0.001$) when compared with the $AlCl_3$ group implying less anxiety. The same was observed in the case of the Memantine and Phototherapy group combination where there was a highly significant increase in the central area entries ($p < 0.0001$) and a decrease in peripheral area entries ($p < 0.0001$) compared to the diseased group which further proved that the combination treatment reduced anxiety and restrained normal exploratory activity in a more effective manner. It has been reported that memantine with affinity to NMDA receptors has been used to prevent excitotoxicity along with enhancing synaptic plasticity in models of AD. It is famed to boost alertness and decrease anxiety related behavior because it shields neurons from toxic impact of glutamate (Calcagnini, 2020). When the two treatments were compared Phototherapy and memantine combination was more effective than was memantine alone. The combination therapy yielded higher place entry in the central area ($p < 0.05$) and lower place entry in the peripheral area. This emphasizes the integration of phototherapy with Memantine which was more effective in the treatment of anxiety like behavior and offered greater extent of restorative effect in the exploratory activity as compared to memantine alone treated animals.

In the elevated plus maze (EPM) test, as represented in the graphical representations, we can observe significant differences in anxiety-like behavior across the groups. Time spent in open arm, the time spent in open arm indicated that the control group spent significantly more time in open arm than $AlCl_3$ group ($p < 0.0001$) suggesting that $AlCl_3$ fond anxiety like behavior since open arms are considered to have low anxiety. The result shows that the Galantamine group had significantly less open arm time spent than the Control group ($p < 0.001$) but more than the $AlCl_3$ group ($p < 0.05$). Interestingly, when we compared Phototherapy and Galantamine combined, the result was significantly improved compared with the $AlCl_3$ and Galantamine alone ($p < 0.001$) and the time spent on the open arms was near to the control group's behavior which indicates that phototherapy has anxiolytic effect increasing the efficacy of galantamine. The time spent in closed Arm, is consistent with anxiety-related behavior; the $AlCl_3$ group again spended most time in the closed arms as compared to the

control group ($p < 0.0001$). Thus, this anxiety-like behaviour was alleviated on the part of galantamine administration since the time spent in the closed arms was compared to the $AlCl_3$ group ($p < 0.001$).

The combined treatment with Phototherapy and Galantamine lowered the time in closed arm even more, indicating that phototherapy can augment the anxiolytic effect of Galantamine. Similarly the number of entries into the open and closed arms were observed. The open arm entries in the control group was found to be significantly higher in comparison with the $AlCl_3$ group ($p < 0.0001$) that revealed the anxiogenic action of this compound. With reference to the open arm entries it was revealed that the Galantamine group was significant with the $AlCl_3$ group ($p < 0.001$) but not with the control group. The combined effect of both Phototherapy and Galantamine enhanced open arm entries as compared to the Galantamine alone group and $AlCl_3$ group indicating the positive effects of phototherapy in reducing anxiety. On the other hand, the closed arm entries emerged fewer in the $AlCl_3$ group in comparison to the control ($p < 0.0001$), the combination therapy statistically significantly reduced the closed arm entries than the $AlCl_3$ and Galantamine groups. These findings support previous studies showing that $AlCl_3$ causes neurological and anxiety-like behaviors because of the increased time spent in the closed arms and decrease entries in the open arms (Kruk et al., 2011; Ullah et al., 2021). Some of these effects have been addressed through the use of the galantamine that works as an AchE inhibitor that increases cognitive capacity and decreases anxiety (Bohra & Kale, 2018)The addition of phototherapy to the Galantamine treatment indicates a beneficial increase in anxiolytic effect, which can be attributed to the neuroprotective and anti-inflammatory mechanisms of photobiomodulation (N. Danappanvar et al., 2023). These findings suggest that phototherapy could be used as an additional approach to combating anxiety-associated behaviors in NDs, alongside pharmacological management.

The memantine group results shows that the control group recorded relatively long time in the open arms compared to $AlCl_3$ groups (< 0.0001) confirming that $AlCl_3$ has an anxiogenic effect as more time in the open arm is indicative of low anxiety. Compared to the $AlCl_3$ group, the Memantine group was found to spent more time in the open arms ($p < 0.0001$) which is consistent with an anxiolytic effect of Memantine. Specifically, the administration of both Phototherapy and Memantine enhanced the time spent in the open arms as compared to the Memantine only group; thus, pointing towards an interaction effect of phototherapy in minimizing anxiety like behavior. The comparable results for time spent in the closed arm

indicate that the anxiety-like effects of $AlCl_3$ are valid, as this group spent significantly more time to the closed arms than the control group ($p<0.0001$). The duration in the closed arms was markedly reduced in the Memantine-treated group compared to the $AlCl_3$ group ($p < 0.0001$), and the time spent in the closed arms was further diminished in the Phototherapy with Memantine group relative to the Memantine-only group. In accordance with these findings, we contend that phototherapy may enhance the effectiveness of memantine in diminishing anxiety-related behaviors. The frequency of entry into the open and closed arms mirrors the trends noted for the duration spent in these arms. The $AlCl_3$ group had a considerably reduced number of open arm entries compared to the control group ($p < 0.0001$), indicating the impact of $AlCl_3$ on heightened anxiety levels. In comparison to the $AlCl_3$ group, the Memantine group demonstrated a higher frequency of open arm entries ($p < 0.01$), although still lower than that of the control group. Phototherapy in conjunction with Memantine resulted in a greater number of open arm entries compared to the Memantine-only group ($p < 0.001$), reinforcing the hypothesis that phototherapy may augment the anxiolytic efficacy of Memantine. Conversely, the mean of closed arm entries was markedly lower in the $AlCl_3$ group, which further diminished significantly in the Memantine and combination therapy groups, with an insignificant p-value ($p < 0.001$) in the latter. These findings support prior research indicating that $AlCl_3$ elicits an anxiogenic response and is associated with neurochemical and metabolic changes that result in cognitive deficits and heightened anxiety-like behavior in experimental animals (Bagewadi et al., 2015; Minkeviciene et al., 2008) Memantine, an NMDA receptor antagonist, is recognized for its ability to improve cognitive function and reduce anxiety in neurological conditions. The enhancement noted with phototherapy may be attributed to its neuroprotective and anti-inflammatory characteristics, as discussed by Hamblin in the context of possible applications of photobiomodulation for cognitive and behavioral improvements (Hamblin, 2019). These findings suggest that the combined use of phototherapy and Memantine may serve as an effective treatment strategy for addressing anxiety-like behavior in neurotoxic or neurodegeneration.

The histopathological analysis of the cortex of control group represents the highest number of cells in the cortex compared to the $AlCl_3$ group ($p < 0.0001$). This drastic change in morphology emphasizes the neurotoxicity of $AlCl_3$, which has previously been linked to neuronal loss and decreased cell survival in the brain. Experimental reports have shown that $AlCl_3$ induce oxidation stress and affect the neuronal structure leading to reduced cell number. A high level of cell count improvement in the cortex was recorded in the group of animals

administered with Galantamine ($p < 0.0001$). Oxidative stress and decrease in AchE levels in the neuronal cells are reversed by Galantamine, a cholinesterase inhibitors which has neuroprotective effect to counteract the neuronal damage induced by $AlCl_3$. Yet, an average cell count of the Galantamine group was significantly higher than the disease group, but still the control group comparing with Galantamine has some neuroprotective effect, perhaps using it alone may not be effective enough to remedy much of the damage. Notably, the combination of Phototherapy plus Galantamine brought the cell count of the cortex even closer to the control group compared to the group treated with Galantamine alone '($p < 0.05$). It emerges that phototherapy triggers neurogenesis as well as reduces inflammation, which could further boost the impact of Galantamine in rehabilitating the compromised integrity of cortical cells (Hamblin, 2019). The combination therapies shows that phototherapy enhances the neuroprotective impacts of galantamine in the brain.

Similar changes are observed among the groups treated with Memantine. Cortical cells in the control group are observed to be more numerous compared to the others, especially the $AlCl_3$ group, a difference which is statistically significant, ($p < 0.0001$). Memantine treatment demonstrated a significant protective effect on cortical neuron survival versus the $AlCl_3$ group ($p < 0.0001$) but did not reach control group levels. But even higher results were received combining Phototherapy and Memantine, as the cell count is significantly higher than in case of Memantine only ($p < 0.01$), which evidences that Phototherapy enhances Memantine neuroprotective effect not only through the reduction of inflammation but also via stimulation of photoprotective processes in cells mitochondria (Hamblin, 2019). These findings were similar to what has been observed in previous studies revealing that phototherapy, administered together with neuroprotective drugs like donezepil, makes a great impact in promoting the survival of neurons in neurodegenerative disorders (Minkeviciene et al., 2008; Thammasart et al., 2019). The application of combination therapy provided the favourable effects of these may also constitute a promising strategy for preventing the neuronal loss associated with neurodegenerative disorders (Thammasart et al., 2019).

The histopathological changes were quantified through counting the total number of cells within the hippocampus, and particularly in the DG region, and illustrated the impact of neurodegeneration induced by $AlCl_3$ and the potential of combination therapy for the prevention of it. It is evident that the overall cell density of the control group is higher with the highest cell count was found in the hippocampal DG region, which goes in accordance with

the fact that the control animals are healthy and exhibit normal cognitive capabilities and neuroprotection and is in concordance with the previous studies(Penner et al., 2010). $AlCl_3$-treated group possessed significantly less neurons in the hippocampus (****, $p < 0.0001$) than that of the control group, suggesting that $AlCl_3$ triggers severe neurodegeneration in the hippocampus which is characterized the neuropathological changes of AD, including hippocampal atrophy and neurons loss(Serrano-Pozo et al., 2011). Treatment with Galantamine reverse the suppressed hippocampal cell density which is somewhat close to the control group (**, $p < 0.01$). The neuroprotective tendency of galantamine can therefore be linked to the factor that it promotes cholinergic neurotransmission and decrease in neuronal lost in models of AD(Geerts, 2005). Compared to $AlCl_3$ group, the combined use of Galantamine and phototherapy significantly elevated the cell count in the hippocampus ($p < 0.05$). Thus, phototherapy is synergistic with Galantamine in preventing hippocampal neurodegeneration. It was also noted in previous studies that Galantamine not only enhances the aspects of cognition but also has a neuroprotective effect whereby it helps prevent cell death in particular areas of the brain such as the hippocampus to support the current findings(Liu et al., 2010).

Also, Memantine group increased hippocampal cell compared to the $AlCl_3$ group (***, $p < 0.001$). Memantine, which is NMDA receptor antagonist, has beneficial effects of decreasing the excitotoxicity, which is a critical factor for neuronal layer damage in AD. Several studies have reported its neuro protective properties whereby it increases the survival of cells in hippocampus (Parsons et al., 2013). The synergy between Memantine and phototherapy on hippocampal cells revealed a statistically significant enhancement on Memantine treatment alone ($.p < 0.05$), which confirms that phototherapy enhances the neuroprotective benefits of Memantine. Phototherapy might promote neurogenesis and reverse the impaired neuronal survival, which in turn can result in the increased number of cells in the hippocampal DG region. It was observed that both Galantamine and Memantine treatments on their own increase the numbers of hippocampal cells in AD models; however, when phototherapy was applied together with either of the drugs, additional neuroprotective effects were observed, suggesting that combination treatments could be useful in preventing neurodegeneration. There is evidence from earlier studies that these drugs help safeguard against hippocampal loss and neurons in the AD models.

CHAPTER 5: SUMMARY OF RESEARCH WORK

AD is a progressive form of dementia characterized by the decline of cognitive and memory abilities, as well as the incapacity to perform daily activities individually. Current treatments such as galantamine and memantine are symptomatic and do not alter the disease's pathogenesis. There is a growing interest in utilizing phototherapy as an adjunct treatment to enhance the efficacy of pharmaceutical medicines. This study seeks to evaluate the impact of combining administration of these medications with phototherapy to improve therapeutic efficacy and augment neuroprotection in models of AD. This study examines the protective effects of phototherapy in combination with pharmacological treatments in AD models, specifically analyzing the two drugs: galantamine, a cholinesterase inhibitor, and memantine, an NMDA receptor antagonist. The objective of the trial is to see if phototherapy can enhance the effectiveness of these medications and offer additional neuroprotective advantages. The preventive effects of phototherapy in combination with pharmacological therapies in AD animals. A phototherapy device has been designed to provide light-based therapy with a changeable wavelength range of 400-750 nm, aimed at establishing an innovative approach. The results compared the outcomes because the treatments were given as either phototherapy in addition to single-drug therapy .Behavioral performance was assessed by several measures, while histological analyses of the brain and dentate gyrus (DG) region were conducted to evaluate neuroprotective activity. The findings indicated that memantine combined with phototherapy had improved results compared to other groups in terms of enhancing performance metrics and treating pathological indicators of neurodegeneration. The memantine-phototherapy group exhibited a considerable enhancement in working memory during the behavioral assessments. Histological research indicated that memantine plus phototherapy could reduce the extent of damage and preserve neuronal structural integrity in both the cortex and the dentate gyrus region. This suggests that the synergistic effect of phototherapy and memantine may yield a greater neuroprotective benefit than either treatment alone.

The study presents data for future research exploring the potential integration of phototherapy with pharmacological treatment for AD, particularly emphasizing the memantine-phototherapy regimen on molecular level. These results underscore the importance of researching strategies for treating AD across several modalities to engage the multiple and interrelated pathways by which the disease impacts the body.

CONCLUSION AND FUTURE RECOMMENDATIONS

This research investigated at how effectively phototherapy works when used along with medicines like galantamine and memantine in AD models in animals. A phototherapy device with an emission spectrum that changes from 400 to 750 nm was used, and the two treatment types were compared in terms of how well they worked. According to the study, the phototherapy and memantine combinationled to the most significant improvements in both behavioral analysis and tissue changes compared to other treatment groups. Based on the changes we noticed in the cortex and dentate gyrus (DG), we came to the conclusion that the combination therapy protects neurons better against damage and improves the structure of the tissue. Phototherapy and memantine seem to work better together to protect neurons in people with AD as compared to Pahototherapy and galantamine combination. Additional research should be done on the evaluated combination of phototherapy and memantine, to determine if they are safe and effective for treating AD. More research needs to be done on the transcriptional and translational levels of this study to confirm the effects of the neuroprotection found in this study. To make this intervention more useful, we need to learn more about how phototherapy affects mitochondrial activity, reduces oxidative stress, and changes neuroinflammation on molecular level. It could be broadened to include more drugs to find other options that might target tau proteins or amyloid-beta more effectively. Additional studies are needed to find out if the combined therapies work in the long run to keep the therapeutic benefits going and stop the disease from progression. Modifying phototherapy factors like wavelength, intensity, and frequency will also be necessary to make the treatment work better. This study lays the foundation for the development of combined therapy approaches that can help people with AD suffering from a variety of conditions

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