Effectivity of Rutin-bound Carbon dots in preventing the aggregation of Amyloid beta

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DEDICATION

Dedicated to every kind soul I came across during this journey and to my brother who dispatched me millions of documents so I could win a scholarship to support this degree!

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LIST OF SYMBOLS, ABBREVIATIONS and acronyms

Aβ	Amyloid Beta
AD	Alzheimer's Disease
ADAM1	A Disintegrin and Metalloproteinase Domain-Containing Protein 10
ADAS	AD Assessment Scale
$AlCl_3$	Aluminum Chloride
ANOVA	Analysis of Variance
APOE	Apolipoprotein E Gene
APP	Amyloid Precursor Protein
BBB	Blood-Brain Barrier
BIN1	Bridging Integrator-1
CD	Carbon Dots
CD2AP	Cortactin-CD2-Associated Protein
CIBIS	Clinician Interview-Based Impression Scale
CNS	Central Nervous System
CHD	Coronary Heart Disease
-COOH	Carboxyl Group
CSF	Cerebrospinal Fluid
DDS	Drug Delivery Systems
DSM IV	Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition
EOAD	Early-Onset AD
ERK2	Extracellular Signal-Regulated Kinase
FAD	Familial AD
FTIR	Fourier Transform Infrared Spectroscopy
GluCDs	Glucose Carbon Dots
GCDs	Glucose Carbon Dots
GQDs	Graphene Quantum Dots
HIV	Human Immunodeficiency Virus
LOAD	Late-Onset AD
M1	Primary Motor
MS4A4A	Membrane Spanning 4-Domains A4A
MWM	Morris Water Maze
NFT	Neurofibrillary Tangles
$-NH_2$	Amine Group
NIR	Near-IR

NOR	Novel Object Recognition
OPD	O-Phenylenediamine
PA (%)	Percentage Alterations
PBS	Phosphate-Buffered Saline
PEG	Polyethylene Glycol
PI3K	Phosphoinositide 3-Kinases
PL	Photoluminescence
PLD3	Phospholipase D3
PSEN1	Presenilin 1
PSEN2	Presenilin 2
PTKB2	Protein-Tyrosine Kinase 2-Beta
RBCs	Red Blood Cells
RIN3	Ras and Rab Interactor 3
S1	Somatosensory
SEM	Standard Error of the Mean
SEM	Scanning Electron Microscope
TJs	Tight Junctions
TREM2	Triggering Receptor Expressed on Myeloid Cells 2
WHO	World Health Organization
Y-CDs	Yellow-Emissive CDs

ABSTRACT

According to the World Health Organization up to 55 million people have dementia resulting in concerning socio-economic implications. About 60-80% cases of dementia are associated with Alzheimer's disease. With all the advances in medicine, there still is no cure. Blood-brain barrier monitors the entry and exit of nutrients and molecules which makes it difficult for various drugs and therapeutic molecules to cross it. Carbon dots are made from glucose to overcome the BBB. Rutin is a naturally existing flavonoid extracted from plants and has therapeutic effects in neuroprotection. This study aims to improve the effectiveness of rutin combined with targeted delivery of carbon dots. Carbon dots were loaded with Rutin to make CD-Rutin, a nano-sized combination with a targeted drug. FTIR, UV-IR, and SEM analysis have produced positive results regarding the doping of CDs with Rutin. Administration of CD-Rutin was done in AD-like rat models at eight to twelve months of age. Alzheimer's was induced in rats with Aluminum Chloride and D-galactose through IP administration for two weeks. Behavioral tests were performed to check the progression of the disease. *In silico* analysis was also done to check ligand-protein interaction to check variation in the binding of Aβ isoforms with Rutin. Afterward, a single injection of CD-Rutin (10 mg/kg) was given intraperitoneally as well. Behavioral testing was done after the administration of the drug. Characterization techniques revealed the successful formation of CDs and subsequent loading of Rutin onto the CDs resulting in a CD-Rutin combination. *In silico* analysis provided strong binding affinities of Rutin with Aβ isoforms affirming Rutin as a favorable treatment to target amyloid aggregates. 3D configuration showed binding of Rutin with hydrophobic domains of protein oligomers Behavioral testing provided significant difference in the treated group with better memory retention and performance in activities involved in behavioral testing. After behavioral testing, rats were dissected for molecular analysis including H&E to assess cell degeneration and Thioflavin T staining to assess amyloid aggregates in the brain tissues. Results provided positive data in terms of cell count in the cortex. Overall results suggest that memory impairment and cognitive abilities were significantly improved after injections. The results demonstrate the positive therapeutic potential of CD-Rutin in Alzheimer's treatment.

Key Words: Carbon dots, Rutin, Alzheimer's disease, Nanotechnology, Amyloid beta.

CHAPTER 1: INTRODUCTION

Alzheimer's disease (AD) has persisted to be a chronic medical condition and, is a challenge to public health. It hinders longevity of life and has many socio-economic implications. Alois Alzheimer is the scientist who categorized the disease including disorientation, cognitive impairment, and distinct biomarkers i.e., senile plaques and tangles. (Schachter & Davis, 2000). Early Alzheimer's can be analyzed by the amount of Amyloid beta (Aβ) peptides in the form of plaques in the brain. These plaques affect the cognitive abilities of the individual and cause functional impairment (Selkoe & Hardy, 2016).

The disease is studied through genetic variations that either speed up or slow down the Aβ deposition so these Aβ plaques have a direct effect on the occurrence of AD (Fleisher et al., 2015). The existence of Amyloid beta plaques can be the cause of dementia that stems from cognitive impairment (Doraiswamy et al., 2012). The treatment resulting in the elimination of Amyloid plaques can slow down the progression of AD. Another causative agent responsible alongside Amyloid plaques are hyperphosphorylated Tau proteins tangles. Tau proteins are also an important biomarker of AD. The current disease research concludes that Tau pathology is the result of Aβ plaques deposition and in later stages, both factors play an equal role in the occurrence of disease. (Busche & Hyman, 2020).

1.1 Epidemiology

Alzheimer's disease affects 50 million individuals globally according to a study done by Alzheimer's disease International in 2018. This figure is predicted to increase three times by 2050. Two-thirds of the cases are reported in underdeveloped countries (Scheltens et al., 2021). It is estimated that the occurrence and more disastrous implications of this disease will prevail in 2050, affecting more than 131 million people because of the currently aging population (Prince et al., 2016). The occurrence of dementia is higher in the old age group. 10% for people who are more than the age of 65 and 40% for people who are older than 80 (DeTure & Dickson, 2019). Aging is an important factor governing the cause of dementia and chances increase every 6.3 years. Increasing from 3.9 per 1000 for people falling into the 60-90 age group to an alarming 104.8 per 1000 for people with age more than 90 (Prince et al., 2015). Diagnosis at an early stage and effective treatment are important for preventing the damaging outcomes of the disease including the personal and financial expenses inflicted on the families.

Alzheimer's is characterized by two stages: early-onset (EOAD) and late-onset (LOAD). Early onset occurs in people with age less than 65 and late onset ensues in people who have crossed the age threshold of 90 years. LOAD has more extensive implications and presentation (Mendez, 2017). AD is believed to influence 5.5% to 9% population every six months (Gao et al., 1998). During the previous decade, disease occurrence has doubled in the age group 85 and older. Mild Cognitive Impairment although not characterized under AD specifically, can still result in repressed cognitive performance and affect individuals. Current research suggests that this can lead to AD within three years (Schachter & Davis, 2000).

1.2 Etiology

Alzheimer's disease can also be differentiated into sporadic and familial AD. Genetic mutations of Amyloid Precursor Protein (APP), Presenilin 1 (PSEN1), and Presenilin 2 (PSEN2) can lead to dominantly genetic Familial AD (FAD). The occurrence of FAD generally happens at the age of 46.2 years; however, it can happen as early as 20 years (Ryman et al., 2014). Sporadic Alzheimer's can result from genetic factors, specifically the Apolipoprotein E gene (APOE). AD occurrence through this mutation comes under LOAD. It is more prevalent but is characterized by sporadic AD. Highest LOAD occurrences include age, family history, and APOE4 genotype. APOE4 allele increases the chances of brain injuries, Down's syndrome, Lewy body, and vascular dementia (Verghese et al., 2011). LOAD occurrence has been related to a genome-wide study of approximately 30 genes in the brain.

Not only do these genes have a direct impact on Aβ and Tau mechanisms but are also related to other disease conditions like up and down regulating metabolism, affecting cholesterol levels, endocytosis and immune response. (Karch & Goate, 2015). With a better understanding of the risk factors both current and novel, we can gain new insights into the mechanism of the disease itself. AD is characterized by senile plaques and NFTs. As the disease progresses the plaques accumulate even more and mount up to grow and increase in the cortical areas after spreading in the brain regions linked to cognition. The Aβ plagues include insoluble oligomers originating from APP. These peptides arise from abnormal proteolytic activity of b-secretase and gamma-secretase.

Two kinds of Aβ are identified, $A\beta_{40}$ which is shorter, and $A\beta_{42}$ which is longer. Initially, $A\beta_{42}$ activates the mechanism leading to the deposition of Amyloid. Deposition of Aβ results from APP metabolic dysfunction is a clear causative agent of Alzheimer's but it remains unclear whether senile plaques cause the disease or are the by-products. Free Aβ oligomers are toxic to neurons and cause inflammation by increasing free radical generation.

One clearer identification of Alzheimer's is the accumulation of NFTs (which is the abbreviation of neurofibrillary tangles) in the brain. NFTs are made of irregularly shaped and phosphorylated Tau proteins. These Tau proteins are responsible for making microtubules in neurons. The amount of Tau protein accumulated in the cortex increases with the progression of the disease. Current research studies also indicated a direct relation between Tau protein tangles and Aβ build-up in AD patients (Naslund et al., 2000).

1.3 Diagnosis

AD includes cognitive and non-cognitive changes in patients. Cognitive changes are disruption in memory, language, and executing motor functions along with visuospatial orientation. Non-cognitive changes include personality alterations, impairment in judgment, mood swings, sleep abnormalities, and agitation. While inspecting for the disease one has to go over the general medical history, neuropsychiatric, and family history for suspected Alzheimer's patients. Physical alongside neurological examinations, routine and optional examinations with neuroimaging are a part of diagnostic evaluation. Tests including total blood count, thyroid function tests, sequential multiple analysis, plasma reagin, folate, and vitamin B12 are some of the routine lab tests performed for diagnosis.

Erythrocyte sedimentation, serology of Lyme disease, human immunodeficiency virus (HIV), Lumbar puncture, urinalysis, urine drug screen, and electroencephalography are included in optional laboratory tests. MRI and computed imaging are also included for neuroimaging purposes. Using standardized criteria like the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM IV) criteria and National Institute of Neurological and Communicative Diseases, and Stroke-Alzheimer's Disease and Relatives, neuropathological examination (this test includes identification of NFTs and Plaques) while doing an autopsy analysis has resulted in 90% accuracy in clinically detecting AD (Chao & Manita, 2013; McKhann et al., 1984).

Using tools like Mini-Mental State Examination (MMSE) AD is characterized by a loss of 3-4 points annually. Other patterns that can be observed are gradual memory loss, occurrence of aphasia, agnosia, and apraxia over time. Early signs of AD can be behavioral changes like personality problems and irritability. With time, the affected individuals experience gait and issues performing motor functions eventually leading to them being silent and bedridden. AD itself persists for up to two decades but patients typically survive up to eight or may be ten years after the diagnosis of the disease (Small et al., 1997).

Biomarkers for Alzheimer’s are specified into categories, A for Amyloid, T for phosphorylated Tau, and N for neurodegeneration. These three biomarkers constitute the ATN

framework. This classification was necessary because of the ever-evolving need for biomarkers. According to the research of the group, they deemed it very important for the presence of Aβ and phosphorylated Tau for Alzheimer's diagnosis. Alzheimer's diagnosis is solely based on biomarkers data and utilization of Amyloid provides a significant alteration in AD's pathology. Alzheimer's disease is a condition that follows a gradual continuum. It extends over several years from cognitively mild to moderate Impairment which eventually leads to dementia.

The ATN framework depicts that Aβ and Tau are what define this disease and thus are very effective biomarkers. These also help in differentiating between AD and dementia (Jack et al., 2018). The research however omits significant causes of dementia like vascular disease, the scientists present their idea that dementia is the result of many underlying issues like Alzheimer's itself (Sweeney et al., 2019). The main criteria for characterization of AD is the occurrence of Aβ and Tau but other abnormalities in biomarkers are there as well in affected patients (Jack et al., 2019). Currently, ATN is too extensive for clinical application and it doesn't include other disorders (Altomare et al., 2019). ATN is the basic standard for research on Alzheimer's but due to the limitations of this approach, clinically the diagnosis still depends on the standards of the National Institute of Aging in 2011 (Cummings et al., 2019).

ATN profiling and analysis results in the possibility of risk profiling on a more personalized basis for people who suffer from mild cognitive impairment (Amsterdam et al., 2019). This research is significant as it provides diagnostics for the early onset of AD-related dementia. However, in a clinical setup, doctors are found to be reluctant to provide the stated prognosis to patients who have mild cognitive impairment (Visser et al., 2020). Diagnosing the early development in case of predementia is more challenging. With ATN biomarkers, it is possible to detect this condition characterized by mild cognitive impairment at a group level (Ebenau et al., 2030; Maurik et al., 2019). Personalized risk modeling remains unattainable because of its limitations. A survey done recently suggests that doctors and patients preferred clear communication even if not an absolute surety (Fruitier et al., 2019).

1.4 Genetics

The understanding of prospective mechanisms that lie behind identified genetic variation depends on APP on chromosome 21 and presenilin Proteins (PSEN1 on chromosome 14, and PSEN2 on chromosome 1). The studies including these genes show the compelling fact that Aβ plays a significant role. 1% of cases depicting familial early-onset autosomal dominant AD show three of the above-mentioned mutations. These mutations increase the

chances of cleavage of APP by the action of B- and G- Secretase. This cleavage increases the cellular synthesis of $A\beta_{42}$. The APOE ε4 allele is a noticeable factor and a risk of developing AD in a broad range of ethnic and racial groups that lie between the ages of 40 and 90 years. Although it is not entirely responsible for causing AD in these groups alone but holds a substantial importance.

A meta-analysis of over 14,000 AD patients showed that hereditary factors that include the gene i.e., APOE ε4, measure up to 45% to 60%. APOE ε4 doesn't relate to increasing Aβ production but rather operates to enhance the aggregation of Aβ and reduce clearance from the target site. Another causative agent is a newly identified Lipoprotein. Lipoprotein plays dual functionality, suppresses and protects non-carriers from the late onset of AD and increases the late onset of AD in carriers (Mooser et al., 2000). New research supports that women have a higher risk of AD with increased susceptibility to vascular dementia. This study is proposed by EURODEM (European Investigation of Dementia) through a series of retrospective studies. This increased susceptibility can also be related to women having longer lifespans.

Women suffering from AD live longer than their partners; this contributes to the existence of double the number of women with AD in the general population. Another contribution these studies have made is to help us understand that a history of dementia or head trauma does not increase the causes of AD (Andersen et al., 1999; Launer et al., 2014). The two known and confirmed risk factors are aging and APOE ε4. Conducting genotyping is not recommended in asymptomatic individuals, disregarding their AD history. The reason is indeterminate predictive values and the absence of a solid remedial solution for this condition (Lapham et al., 1996; Mehlman et al., 1996).

Hereditary factors contribute to 60% to 80% of vulnerability to AD (Gatz et al., 2006). APOE ε4 genotype does not fully explain this hereditary occurrence, although it does partially account for AD (Ridge et al., 2013; Bellenguez et al., 2017). Up-to-date research and increased the risk alleles for causing AD up to 40. This research includes a wide genomic study including 150,000 individual data and their comparison with controls. It also includes over 300,000 individuals with a proxy phenotype of AD (people who have AD running in family history) and a comparative analysis of their controls with family history. Individuals with prevalent APOE ε4 risk alleles have 3-4 times increased risk of AD. Other suspected alleles pose a risk factor too but contribute lesser to the occurrence and progression of the disease overall (Jansen et al., 2019).

1.5 Comorbidity

Alzheimer's can exist along with vascular dementia and Lewy body dementia. The study and treatment of individuals with this comorbidity is limited in clinical research. AD affectees exhibit other health conditions as well like heart diseases, malignancies, and diabetes (Schachter & Davis, 2022). Various lines of evidence provide the conclusion that type 2 diabetes is another risk factor for the occurrence of AD, Alzheimer's developing as an after-effect of diabetes is referred to as diabetes type 3 (Kandimalla et al., 2017; de la Monte, 2019). Lacunar strokes also called Silent Brain Infarcts lie under common ischemic strokes and are caused by occlusion of blood vessels that supply blood to the deep brain areas. Various researchers have linked the risk of cognitive impairment and AD with the occurrence of AD. It has been shown the presence of silent brain infarcts presence increased the risk of occurring dementia (Vermeer et al., 2003).

About gut diseases and cardiovascular complications, recent *in silico* studies suggest a link with occurrence of dementia. According to a study the genes linked to AD i.e., alpha 2 macroglobulin (*A2M)*, apolipoprotein E (APOE), and microtubule-associated protein 4 (*MAP4)*, found to be linked to the genes that were found to be associated with cardiovascular disease, this shows clear link between Alzheimer's disease and cardiovascular disorders (Ray et al., 2008). Crohn's disease which is a gastrointestinal disease is linked to AD. According to one study, AD and Crohn's disease were identified with a similar genetic identifier. A genetic variation alongside the IPMK gene, has been linked to Crohn's disease has increased the risk for AD (O'Donnell et al., 2019), (Yokoyama et al., 2016).

The association between dementia and coronary heart disease is studied with large research groups to draw solid conclusions. Another factor that lies in the acceleration of Alzheimer's is CHD. This linkage has been studied extensively and is supported by large epidemiological research. A study based on a large population provided strong evidence that unidentified myocardial infarction was associated with a high risk of dementia (Ikram et al., 2008). Another research involving 10 potential events of meta-analysis provided the understanding that CHD increased cognitive impairment and the occurrence of dementia (Deckers et al., 2017).

1.6 Pathophysiology

The preclinical phase of AD is described as the initial stage of AD which is at the cellular level. Before there is any visible cognitive impairment in the body and its functions, the disease spreads via alterations in neurons and its subcellular components (Strooper & Karran, 2016). These alterations within the neuronal environment include failure of the

glymphatic system along with neuroinflammation, aging, and vascular changes. These changes are happening simultaneously with Amyloid aggregation (Lu et al., 2014; Venegas et al., 2017; Plog & Nedergaard, 2018, Sweeney et al., 2018; Mesquita et al., 2018). But one mechanism remains unknown, i.e., the aggregation of Amyloid provides signals to Tau pathology which causes the emergence of biomarkers for necroptosis markers in the neurons which leads to granulovacuolar degeneration (Long & Holtzman, 2019; Koper et al., 2020).

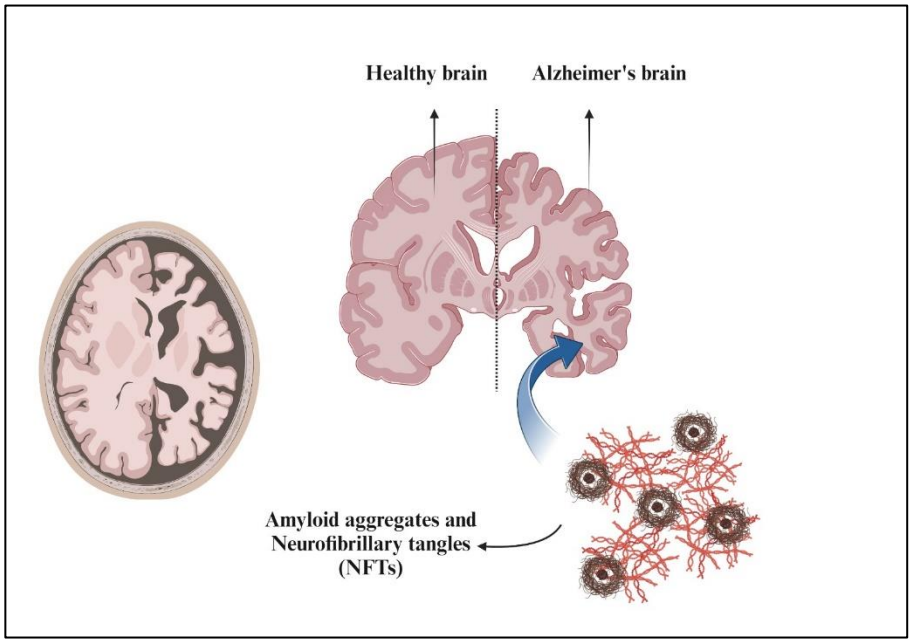

Figure 0.1 Comparison of healthy and AD patient's brain

Microglia, its structural changes, and response in AD have been thoroughly explained using single-cell transcriptome analysis (Keren-Shaul et al., 2017). Genetic variations that are widely studied and are primarily associated with AD are APOE, and TREM2, which play a significant role in above mentioned understanding (Keren-Shaul et al., 2017; Parhizkar et al., 2019; Frigerio et al., 2019). TREM2 has genetic variations i.e., Arg47His, Arg62His, and Asp87Asn, which decrease TREM2's efficiency in binding with Amyloid plaques. The unbound Amyloid-beta binds to APOE ε4 (Yeh et al., 2016). Other proteins like Protein-tyrosine kinase 2-beta (PTKB2), Ras and Rab interactor 3 (RIN3), Bridging Integrator-1 (BIN1), and Cortactin-CD2-associated protein (CD2AP) are being correlated to the development of AD. They play a downstream role compared to TREM2 and APOE. They help regulate cellular functions like endocytosis, phagocytosis, and motility in microglia.

Microglial pathways are important in the pathophysiology of AD because of the collective action of many risk genes for causing AD (Leyns et al., 2017, 2019). The constraints to study AD through mouse models with tau-overexpression for the investigation of AD results from the incongruous microglial response. Transgenic Tau-overexpression exists in

some mouse models which might lead to elevated neuroinflammatory responses that cannot predict the progression or occurrence of AD through this mechanism (Leyns et al., 2017,2019). To overcome these constraints, mouse-human chimeric mice, are animal models that don't overexpress Tau. Stem cells extracted by humans can also be used in *in vitro* settings (Park et al., 2018; Hasselmann et al., 2019; Mancuso et al., 2019).

The primary focus of AD research has been targeted cellular pathology, but the biochemical phase of illness has also been studied, targeting the existence of Amyloid in ATN terminology. Aβ and Tau fibrils have been studied and understood using cryo-electron microscopy (Gremer et al., 2017). Using the same technique researchers have studied Presenilines, catalytic subunits of gamma-secretase, they interact with APP and Notch substrates which is also studied using cryo-electron microscopy (Yang et al., 2018; Zhou et al., 2019). Preseniline alterations affect the gamma-secretase and APP connections. This leads towards releasing the Amyloid peptides that are susceptible to aggregation. This has been observed and studied on gamma-secretase complexes (Szaruga et al., 2017). This gives us the direction to work on finding innovative therapeutic solutions to target Aβ in AD.

1.7 Treatment

The goal of treating AD is to combat the negative effects that alter behavioral patterns like depression, psychosis, and agitation in addition to cognitive impairment and sleeplessness (Burke et al., 2019).

1.7.1 Pharmacotherapy

There are some pharmaceutical alternatives to enhance cognitive impairment that is the result of AD. To deal with irregular behavioral patterns there are mood stabilizers, antipsychotic agents, and antidepressants available for administration after consultation with the doctor.

1.7.2 Cholinesterase inhibitors

Cholinesterase inhibitors serve the purpose of combating cholinergic deficiency. These have had a positive action on AD-affected individuals. Acetylcholine which plays a part in synaptic transmission is broken down by acetylcholinesterase, these inhibitors stop the degradation thus making them available. These medications are useful in the intermediary stage throughout disease progression (Schachter & Davis, 2022). Available today in the market for cholinesterase inhibitors are drugs like donepezil, tacrine, galantamine, and rivastigmine (Knapp et al., 1994; Bores et al., 1996; Rogers et al., 1998; Rösler et al., 1999).

The results of these medications are not immediate but the patients have experienced better cognitive abilities after using them compared with controls.

The effectiveness of cholinesterase inhibitors is directly linked with the degree of AD progression. Patients with middle-phase suffering show great action while mild AD shows lesser effectiveness. The reason for this effectiveness has to do with the thought that cholinergic dysfunction is higher at this stage of AD (Davis et al., 1999). Cholinesterase inhibitors also improve behavioral disorders that have been observed in clinical trials (Raskind, 1998; Nordberg & Svenson, 1998). However, the constraints in administering cholinesterase inhibitors lie in their altering tolerance and safety. Determining head-to-head variance and research is not a feasible solution for its administration.

1.7.3 Anti-inflammatory Agents

Anti-inflammatory agents have been shown to reduce the progression of AD, this conclusion has been drawn by retrospective epidemiology (Breitner et al., 1994; Stewart et al., 1997). Non-steroidal anti-inflammatory Agents i.e., NSAIDs are rarely used in clinical trials. However, non-randomized studies done using these NSAIDS like ibuprofen, naproxen, Diclofenac, Steroids like low-dose Prednisone, and others have yielded positive results throughout AD progression (Rogers et al., 1993; Aisen et al., 1996; Scharf et al., 1999). However, the sample size for these results was limited. Recent findings don't encounter the previously supported results. Another experiment involving a 16-month administration of Prednisone in 138 patients suffering from AD compared with placebo-controls did not give any positive differential results in cognitive impairment (Aisen et al., 2000).

1.7.4 Lifestyle Alterations

Guidelines provided by the WHO for the mitigation of cognitive impairment risk factors and dementia were published in 2019 (World Health Organization, 2019). It offers guidelines and recommendations with degrees of variation for the precision of different factors like dietary choices, body mass index, physical exertion, alcohol consumption, and smoking. There is a limitation in the existence of research on long-term, controlled, and randomized studies relating to Alzheimer's including standardization issues and lack of evidence of research data in under developed and developing countries where dementia cases are piling up rapidly. A trial called SPRINT-MIND shows that controlling blood pressure with a target of less than 120 mm Hg is effective for slowing down cognitive degradation compared to normal control i.e., less than 140 mm Hg. This study confirms that having good cardiovascular health relates to good brain health.

However in individuals over the age of 70, determining possible therapy against AD is still unsolved (Williams et al., 2019). Requirements of a multimodal preventive method that shows effectiveness against diabetes and cardiovascular diseases along with other ailments is the key requirement (Kivipelto et al., 2018). The finish-FINGER study involved extensive, randomized controlled trials that showed a good lifestyle-based approach are useful in mitigation of cognitive decline risk in individuals at initial risk of AD (Ngandu et al., 2015; Soloman et al., 2018). The FINGER study consisted of a well-balanced and healthy diet with physical exercise and healthy social activities. It also entailed the management of vascular and metabolic risk factors along with mental training.

In cases of genetic predisposition to AD, this approach has had affirmative effects. French MAPT studies the correlation of lifestyle mediation with the supplementation of omega-3 fatty acids. The PreDIVA trial focuses on managing metabolic and vascular risk factors (Charante et al., 2016; Andrieu et al., 2017). There were cognitive benefits in some subgroups in the research with higher risk of dementia but they couldn't attain the needed results for primary focus. In another study involving amyloid-PET using MAPT trial, omega-3 fatty acids paired with lifestyle choices or a healthy lifestyle alone showed improved cognitive abilities in patients that showed a positive Amyloid status (Delrieu et al., 2019). These trials and research show that lifestyle choices can affect individuals positively suffering from AD even if they are not directly influencing the progression and pathology of AD.

1.8 Role of Nanotechnology

Modern medicine has faced the challenge of creating medicinal products that can cross the well-known blood-brain barrier (BBB) to cross the CNS and deploy their therapeutic effect on the target site. BBB is semi-permeable and can be a notable hindrance in the crossing of certain molecules (Sarrazin et al., 2012; Burgess & Hynyen, 2014). To overcome this obstacle there are some modalities like photothermal and photodynamic therapy. These are used in routine to manage CNS-related conditions. These technologies do however have side effects like damaging skin tissues from photosensitization (Dhas et al., 2021).

Nanoparticles (NPs) are smart and can respond to both internal and external stimuli. Their action is predictable and traceable, which increases their application in medicine and delivery of medicinal molecules. NPs can bind with various ligands and can enhance the process of drug delivery by many folds compared to traditional methods (Zhou et al., 2018). Glutathione and Niosome make nanoparticles more stable and a solution for a nanoparticle-based drug delivery system (DDS). Exploiting specific ligands like Transferrin can help

overcome biological obstacles like cell membranes and BBB and result in a more directed treatment (Gharbavi et al., 2018; Nosrati et al., 2019).

Carbon exists abundantly in the earth's atmosphere in various forms diamond, graphite, and fullerene. Recently, carbon nanomaterials, a fresh classification of carbon-made substances have gained striding attention in Nanotechnology. Nanomaterials created from carbon exist in the form of nanodiamonds, carbon dots or quantum dots, fullerenes, and carbon nanofibers. The newest addition to this group is CDs with a magnitude less than 10 nm. These dots are wondrous in their properties of low cytotoxicity, greater biocompatibility, good solubility, and augmented cell permeability. These qualities make them exceptional to be used in the field of biology (Jaleel & Pramod, 2018; Jhonsi et al., 2018).

Another advantage of CDs is that they can be produced from various chemical and natural substances. Many researchers are producing CDs using chemical compounds. However, there is a need to mitigate deleterious by-products resulting from CD production using chemical compounds (Sharma et al., 2017; Thakur et al., 2019). Organic precursors are being preferred by some researchers to overcome the side effects associated with CD production due to their renewability, biocompatibility, and cost-effectiveness. Different organic sources like plants, fruits, beverages, and vegetables have been used for the production of CDs. Out of all these, plant-driven CDs are receiving more acclamation from scientists (Tejwan et al., 2022).

CD is the most suitable candidate to be used in drug delivery systems involving BBB because of have better biocompatibility, no toxicity, contain many functional groups, and have nano sizes (Li et al., 2016; Seven et al., 2019; Zhou et al., 2019). Their ability to cross the BBB can also be enhanced substantially and attaching various ligands, processes of production, and choice of starting material. CDs can also cross BBB along with cargo molecules which makes them excellent nanocarriers for transferring drugs to the target site that lies in areas crossing the Blood-brain barrier (Kappe, 2004; Medeiros et al., 2019; Tajik et al., 2020).

1.9 Synthesis of Carbon dots

Synthesizing CDs is done by different approaches including various processes with different reactions and starting materials. (Tejwan et al., 2020). Using the technique of microwave irradiation is the most common one for CD production due to the great response and uniformity in the heating process (Kappe, 2004). CD production with sucrose and polyethylene glycol (PEG) with microwave irradiation was done first by Zhu et al. (2009).

The same technique was used by Wang et al. (2011) to make CDs from other carbohydrates. After the microwave-mediated process, hydrothermal and solvothermal processes have been used.

Proteins, polysaccharides, and organic acids like citric acids and amino acids are used to synthesize CDs by hydrothermal and solvothermal processes (Zhou et al., 2015; Yang et al., 2017; Ghosh et al., 2017; Mintz et al., 2018; Seven et al., 2019; Xu et al., 2019; Sun et al., 2020). One other method that is not very frequently used is ultrasonication, which is more subtle than the processes aforementioned (Tejwan et al., 2021). Yellow-emissive CDs that are excitation-independent are produced by using O-phenylenediamine (OPD) and Citric acid. Probe sonication can also be used to create CDs from glucose (Ajmal et al., 2019). The optical characteristics of CDs are somewhat similar with slight differences. Their emission is excitation-dependent dependent previously restricted between the blue-green light range.

With new precursor material and processes of production, the wavelength range encompassed orange-red and near IR (NIR) range (Mintz et al., 2019; Shi et al., 2019). Orange-red wavelength and NIR CDs have low dependence on excitation compared to the blue-green forms. Glucose-based quantum dots have structural characteristics defined a lot better (Zheng & Wu, 2017). Carbon dots emissions can be modified by incorporating heteroatoms by which the size of sp^2 carbon complexes also changes and shifts the range of emission from blue to orange (Zhu et al., 2015; Shen et al., 2020; Wang et al., 2020). Carbon dots and nitrogen-doped carbon dots NCDs show similar excitation characteristics (Zhou et al., 2013; Liyanage et al., 2019). The core of different CDs and their structures result in various kinds of CDs.

A carbon nanodot has amorphous structures (Mintz et al., 2019). There is a hypothesis about a hybrid sp^2/sp^3 structure of CDs other researches show a polymer-like carbon-based structure (Xia et al., 2020). s-triazine and carbon nitride structures are also observed in Carbon nano-dots. Different CDs may differ in core structure, the surface however is made of small organic molecules and functional groups. Nanomedicine finds extensive applications for CDs (Tejwan et al., 2020). CDs were detected in cancer cells of human breasts by Sun in 2007 (Cao et al., 2007). Healthy and cancerous cells were targeted by Carbon dots to assess their biocompatibility and cellular toxicity.

Minimal cytotoxicity was determined by checking cell viability after exposure to CD solutions (Lu et al., 2016; Zhou et al., 2019). By accessing their size, surface charge, and architecture after cell incubation was observed to see CD's stability. CDs maintained their surface structure and physiological states after cellular internalization was assessed through zeta potentials and size. The stability of CDs for 7 days was assessed by atomic force

microscopy images under varying PH levels (Wang et al., 2017; Liu et al., 2018; Niu et al., 2020). Three different groups of Mice were injected with CDs aqueous solution for 4 weeks and they didn't display any aggravated behavior or clinical symptoms. The first bioimaging of CDs was done in animal models by Yang which rose to attention after CDs rose to attention in the medicinal field (Yang et al., 2009b).

1.9.1 Various derivatives of Carbon Dots and their production

CDs and their derivatives have been observed and assessed for their ability to cross BBB through *in vitro* as well as *in vivo* models. *In vitro* model consisted of rat microvascular system and astrocytes was used and CDs were made through a one-pot hydrothermal process. (Lu et al., 2016). Ability to cross the BBB for CDs was confirmed with strong blue photoluminescence of CDs using UV-Vis Spectroscopy. This experiment was an *in vitro* approach but it lacks the authenticity to replicate the BBB despite the existence of minimal variations and changeable parameters Zhou et al., (2020). One gel-like class of CDs has been identified and used to observe its ability to cross BBB in Zebrafish. Zebrafish nervous systems resemble humans in basic system constituents like transmitters, hormones, and receptors (Panula et al., 2006).

The progeny generation rate is also higher in Zebrafish than in mice which translates into reproducibility of experiments (Zhou et al., 2018). This option is cost-effective and the organism can be raised in a limited space (Panula et al., 2006). CD-conjugates structure and creation depend on the mechanism of how they move through the blood-brain barrier. Endothelial cells that align to form the membrane in the BBB are spaced together uploading gaps of 4nm to 6nm. This allows CDs smaller than 4nm to cross the barrier through the gaps through passive movement (Cai et al., 2016). The small size is one obstacle, another added concern is the electric charge on the gap. Yellow emissive CDs that are amphiphilic have successfully crossed the lipid wall and transverse through cerebrospinal fluid (CSF) (Zhou et al., 2019). Water solubility is another prerequisite for a molecule to cross the cerebrospinal fluid after they crossed the BBB. Passive diffusion is the most suited explanation for the penetration of BBB due because of the intrinsic properties of CDs.

Receptor-mediated endocytosis is another technique that helps CDs cross the BBB. Li et al. (2016) showed that CDs were not able to penetrate the barrier alone but can easily transverse through endocytosis mediated with a receptor i.e. Human Transferrin. Self-targeting CDs made from precursors as ligands that are transporters for specific substances can also be another direction to go towards. Another study shows that BBB can be transversed via GLUT1-mediated transport using glucose to make the CDs (GluCDs). This study was

supported by *in vivo* models of Zebrafish and rats. These glucose-based carbon dots were also found to transverse through the barrier with fluorescein (Seven et al., 2019). Tryptophan has also been used as a starting material to make CDs by Mintz et al. Using LAT1-mediated transport, tryptophan precursor CDs were successfully identified and we're able to cross BBB as well. This makes CDs an efficacious delivery system for medicinal applications involving nervous system conditions (Mintz et al., 2019).

1.10 Applications of Carbon dots in treating CNS Diseases

CDs contain surfaces that have abundant functional groups i.e., amine (-NH2) and carboxyl (-COOH) groups. These functional groups can be coupled with various CNS pharmaceuticals. CD's biocompatibility makes them excellent for biological therapy. CDs play an exceptional role as nanocarriers for delivering drugs in AD and brain tumors. The target treatment widely used for AD is lowering Aβ deposition in the Central Nervous System. Y-CDs (yellow-emissive CDs) have been observed to slow down the generation of APP and Aβ in cells making them exceptional candidates as nanocarriers and nanomedicine for AD (Zhou et al., 2019).

GQDs can halt Aβ aggregation. This ability of Glucose Quantum Dots has been amplified by the modification of the dots with tramiprosate. Research on CDs showed that modified dots with glycine-proline-glutamate prevented Aβ aggregation and reduced proinflammatory cytokines (Gong et al. 2016). Scientists made OPD-derived CDs that have cationic surfaces by coordinating them with copper ions. The research with these OPD-derived CDs has been effective against Aβ aggregation (Chung et al., 2019).

1.11 Natural Flavonoids as Treatment

Traditional medicine uses herbal extracts as remedies for treating different ailments. Herbal extracts have been effective against various disorders and also a source of nutrition (Ang-Lee et al., 2001). Herbal medicine when compared to synthetic pharmaceuticals has better performance is less toxic and has fewer side effects (Awaad et al., 2011). Plant extracts have flavonoids, glycosides, alkaloids, terpenoids, tannins, and many other beneficial metabolites. The extraction of physiologically active chemicals in herbal extracts is largely affected by the techniques and solvents used in the process. Different solvents used include chloroform, ethyl acetate, ether, and alcohol. Water is the safest choice.

Flavonoids have an exceptional property of modulating intracellular reactions by altering protein kinase signaling pathways. They also have antioxidant properties (Rates, 2001). They also impede pathways involved in the progression of AD. Ambiguity about absorption, metabolism, and fundamental pharmacokinetics related to flavonoids poses a hindrance in their applications for therapeutic interventions in AD. Rutin (3,3',4',5,7-pentahydroxyflavone-3-rhamnoglucoside) is a hydrophobic flavonoid that has been studied for its potential to be used with CDs (Negahdari et al., 2021). Rutin has incredible nutritional benefits and is an important component of our daily diet. It's abundantly present in fruits and vegetables like apples, asparagus, figs, buckwheat, oranges, and grapes.

Flavonoids are also present in some beverages like green tea, black tea, and elderflower tea (Hassan et al., 2018). Rutin is a wondrous flavonoid finding its therapeutic applications in many disorders like cancer, diabetes, hypertension, and hypercholesterolemia. Rutin has exceptional antibacterial, antioxidant, Vaso protective, neuroprotective, cytoprotective, and anticarcinogenic properties (Nafees et al., 2018). However, limitations caused by water solubility and cell permeability slow down its potential for pharmacological effects (Gullón et al., 2017). To tackle these limitations CDs have been used as drug delivery vehicles to make Rutin a better pharmacological candidate.

1.12 Protective effects of Rutin

1.12.1 Central Nervous System

Strong evidence has been found related to Rutin being a neuroprotective agent in cases of brain ischemia. Mitigation of neural ischemia through lipid peroxidation, upregulation of endogenous antioxidants, and reduction of p53 expression has been observed by the administration of Rutin (Khan et al., 2009). Rutin has also been researched for its effectiveness in treating glutamate, oxidative, and hypoxia stress. Reduction in neuroinflammation in a sporadic AD-like model of rats has been observed using Rutin. Rutin has also conferred neuroprotective effects in mice and treated them with dexamethasone (Tongjaroenbuangam et al., 2011; Javed et al., 2012). Anticonvulsant properties have also been associated with Rutin. Rutin is safe to be administered in patients with epilepsy because it does not affect the metabolism of antiepileptic drugs, according to studies done by Nieoczym et al., (2014).

Rutin is a progenitor having both mesenchymal and neural potentials. It elicits enhanced trunk neural crest cell survival with proliferation and differentiation unaffected. This effect of Rutin is linked with the modification of phosphoinositide 3-kinases (P13K) signaling, and extracellular signal-regulated kinase (ERK2) proposed by (Nones et al., 2012).

Schizophrenia, a serious neurodegenerative disease, causes "tardive dyskinesia". It affects our orofacial region, which is a result of neuroleptic medications. This is a major clinical obstacle. Rutin has provided amazing results in correcting orofacial dyskinetic movements, locomotor activity, stereotypic rearing, and percentage retention according to research done by Bishoni et al., (2007).

Rutin also leads to the restoration of neurochemical and metabolic parameters resulting from haloperidol in orofacial dyskinesia. So, Rutin has been related to fighting against hyperkinetic movement disorder. Stroke is a noticeable factor leading to adult mortality and disability (Lloyd-Jones et al., 2010). Inflammation and oxidative stress are the results of stroke (Deb et al., 2010). Rutin's protective effect has been studied on mice with localized cortical ischemia. This ischemia was induced by thermocoagulation somatosensory (S1) and primary motor (M1) cortical blood arteries. Sensorimotor loss has been recovered by Rutin's action as a result of reduced neurodegeneration (Ortolani et al., 1995). Rutin extracted from the plant *Schinus molle* was examined for its antidepressant role in mice through tail suspension and water maze swimming tests. Results suggest Rutin has an antidepressant-like outcome. This effect is attained by the enhancement of serotonin accessibility at the synaptic cleft (Machado et al., 2008).

1.12.2 Antiarthritic effects

Treatment of Rutin shows significant improvement in rheumatoid arthritis and Fanconi anemia by preventing oxygen radical overproduction (Ostrakhovitch & Afanas'ev, 2001). Rutin's protective effects have been found abundantly effective in chronic-stage inflammation. Rutin as a remedy for septic arthritis has been effective. This ability of Rutin is caused because of their anti-arthritic ability and antifungal (Han, 2009). Inhibiting catabolic and inflammatory markers was observed by Rutin in the case of osteoarthritic lesions (Horcajada et al., 2015).

1.12.3 Endocrine system

Hyperglycemia results from excessive glucose production in the body and lesser utilization (Chattopadhyay, 1993). An upregulation of insulin levels and a downregulation of glucose levels in plasma by continuous administration of Rutin has been found. Also, improved glycolytic levels and glycogen in diabetic rodents have been found by the action of Rutin. Pancreatic regeneration was observed in diabetic rats along with decreased fatty infiltration by the action Rutin (Srinivasan et al., 2005; Prince 2006). In Wistar rats grown with a high-cholesterol diet, hepatotoxicity was protected by Rutin and there was a reduction in amino-transferase, cholesterol, triglyceride, alanine transaminase, and LDL was the basis

of the aforementioned conclusion (Al-Rejaie et al., 2013). Better cardiac health has also been related to regular consumption of Rutin and other flavonoids (Kalgaonkar et al., 2010).

Thyroid iodide uptake is regulated by sodium-iodide symporter as a significant role in thyroid synthesis and detection of thyroid disorders. Thyroid cancer patients show reluctance in radioiodine therapy resulting in decreased iodine uptake capacity affecting chances of survival. It's a necessity for therapeutic technology to increase thyroid iodide absorption. Rutin administration has been linked to increased thyroid iodide absorption enhancing sodium-iodide symporter activity. This makes Rutin efficient in radioiodine therapy (Gonçalves et al., 2013).

1.12.4 Cardiovascular System

Protecting aortic endothelial cells from oxidative damage has been found by the use of Buckwheat. Buckwheat has Rutin in abundance and reduces nitrotyrosine and immunoreactivity. Detrimental effects of oxidative stress on arterial endothelial cells are observed to be diminished by using buckwheat germination extract (Dae et al., 2009). Administration of Rutin orally shows a decrease in vascular reactivity and baroreflex sensitivity. It was observed in hypersensitive rat models (Mendes-Junior et al., 2013). Rutin has been researched and observed to enhance endothelial activities in human endothelial cells, by generating increased amounts of nitric oxide (Ugusman et al., 2014). Platelet inhibition that is concentration-dependent was observed by Rutin in rabbit platelets. Rutin also increased intra-platelet-free calcium when administered as a dose-dependent therapy (Chen et al., 2002).

1.12.5 Gastrointestinal System

Ethanol is known to inflict damaging effects on stomach mucosa in both animals and humans. Stomach morphology, mucosal hyperemia, mucosal and submucosal hemorrhage, edema, and necrosis are the side effects of Ethanol when its concentration increases by 400ml/l. Lesions in the stomach are caused by oxygen producing free radicals (Pihan et al., 1987; Szelenyi & Brune, 1988). Before ethanol administration, Rutin treatment resulted in protection against necrosis. Oxidative stress and metabolic parameters were corrected in rats by Rutin. It was observed in an ulcer model induced by using indomethacin. Rutin also has protective benefits observed through histopathological studies (Abdel Raheem et al., 2010).

1.12.6. Respiratory System

To check the anti-asthmatic activity of Rutin, ovalbumin-sensitized guinea pigs were used to observe airway resistance. Airway opposition was observed in immediate-phase reaction and late-phase reaction after the exposure of aerosolized ovalbumin. Along with

airway resistance, phospholipase A2 reluctance, eosinophil peroxidase, and Histamine resistance were also checked. Rutin produced substantially positive results (Jung et al., 2007). Rutin can be an alternative treatment for whooping cough in place of Vitamin C and K. Rutin has also yielded positive results in idiopathic chylothorax in whippets and cats (Gould, 2004; Kopko, 2005; Schuller et al., 2011).

1.13 Aims and Objectives

This research aims to use CDs loaded with Rutin to improve cognitive impairment as a result of the pharmacological benefits of this hydrophobic drug. This hypothesis is based on research data associated with Rutin lowering neuroinflammation in rodents. They ameliorate dementia of Alzheimer's and provide a neuroprotective effect.

1) Design and synthesis of CDs
2) Characterization of CD-Rutin
3) Establishment of AD-like animal models
4) Evaluation of Rutin’s therapeutic potential through *In Silico* analysis.
5) Evaluation of effectivity of CD-Rutin using behavioral tests
6) Molecular Analysis of animal models

CHAPTER 2: MATERIALS AND METHODS

2.1 Ethical Approval

Before embarking on *in vivo* analysis ethical approval (IRB no. 05-2023-ASAb-02/02) was obtained from the NUST-IRB committee of the National University of Sciences and Technology, Islamabad.

2.2 Chemicals

Glucose (BCCF4025, Sigma Aldrich, Switzerland), Rutin hydrate (207671-50-9, Macklin, China), dialysis membrane (500 Dalton cut-off size, MD55, Scientific Research Special, China), and Ethylene alcohol (200-578-6, Sigma Aldrich Switzerland).

2.3 Preparation of CDs

Carbon dots were made by mixing Glucose with ethyl alcohol through the process of microwave irradiation. Glucose solution was made with deionized water as a solvent. 300 μm of Galactose solution was added to 5 ml. Microwave oven was used to heat the solution at (800 W) for 9 min. The brownish-red GNP solution was the result of heating and was allowed to cool down to 25 °C in a dark environment for over 2 hours. After cooling a filter membrane of 0.22 μm was used to filter the cooled solution. This resulting filtrate was purified in a dialysis bag with 500 Dalton pore size. This bag was placed inside a flask filled with deionized water for 48 hours. Afterwards, the GQCs were freeze dried or in an oven at a constant 75 °C for as long as needed to become solid and stored at 4 °C (or -20 °C). These could be stored for up to 6 months and used as needed.

2.4 Preparation of CD-Rutin

To prepare CD-Rutin loaded nanoparticles, Rutin hydrate was mixed in DMSO and was amalgamated in the aqueous extract of CDs from the dialysis bag. The ratio was 1:1 and for a whole night they were stirred constantly on a magnetic stirrer to allow Rutin to load efficiently. The resulting mixture was dialyzed for 2 hours with the dialysis membrane using a 500 Dalton pore size membrane to eliminate unbound impurities. To get CD-Rutin-bound molecules, the solution was subjected to lyophilization or oven drying.

2.5 Characterization of CDs

Structure of CDs was analyzed for their structural and chemical composition by different tests i.e., Ultraviolet-Visible (UV-Vis) Spectrophotometer, (FTIR), and Scanning Electron Microscopy (SEM) to confirm their size and molecular composition.

2.5.1 Fourier Transform Infrared Analysis

FTIR (Agilent Cary 630, Agilent Technologies, USA) was employed to analyze the functional groups present on CD surfaces. The main constituents of CDs are carbon, hydrogen, and oxygen. There are carboxyl or carboxylic acid groups along with hydroxyl groups on CDs' surface, these groups are widely studied to exist due to partial oxidation of carbon precursors. This partial oxidation occurs during the production of CDs. These bonds and functional groups were targeted to be observed in FTIR Spectroscopy using the range of 500-4000 cm^{-1} at 4 cm^{-1} resolution.

2.5.2 UV-Vis Spectroscopy

UV-VIS absorption Spectrophotometer (UV-1602, BMS, Germany) has a resolution of 1 nm ranging between 200 nm to 600 nm. CDs and Rutin binding were observed by measuring the wavelength spectrum of the mixture in the amount of 3 ml sample in a cuvette analyzed at room temperature.

2.5.3 SEM Analysis

Scanning electron microscopy (SEM) scans a sample using an electron beam and produces a focused image to analyze. It is used effectively in microanalysis. The microscope used in this study had a maximum of 0.2 µm magnification. It provided the size of the bound CD-Rutin combination and unbound Carbon dots. Microscope slides were cut into a size of 1 cm × cm. They were washed with ethyl alcohol and dried before adding the sample with the help of a pipette. sample solutions were applied onto the silicon glass slides after they were disinfected and dried. Slides were subsequently dried at 40°C in an oven, overnight.

2.6 *In Silico* Analysis

In Silico analysis was done by analyzing the PDB structure of Amyloid beta Proteins extracted from the AlphaFold Protein Structure Database. In addition, the structure of Amyloid Precursor Protein 1 was also used. The structure of Rutin was extracted from Drugbank. The docking of Rutin was observed with Aβ and APP1 separately**.** After finding out the binding

energies in Pyrx, the docking was observed using the Pymol setup. The Ligand-peptide interactions of different peptides of amyloid beta were also observed to check the binding affinity of Rutin with different sites on different lengths of amyloid beta. Results were analyzed and interpreted based on energy data with comparison to previously published studies that reported Autodock-based results (Morris et al., 1998).

2.7 Animal testing

Adult male albino rats (n = 12) were bought and kept in ASAB animal house, National University of Science and Technology (NUST), Islamabad. Control environmental conditions with temperature (25 ± 2 °C), natural light, and dark cycles ranging between 14 light and 10h dark. Feed and water ad libitum were provided to the rats regularly. There were four rats in three groups, diseased control, control and treated. Two groups were given doses of $AlCl_3$ (for two weeks) 3rd group was a control group with no drug administration. Group 2 was given doses of 10mg/kg of Rutin-bound CDs. PBS was the solvent used in the doses.

2.8 Behavioral testing

Behavioral tests were done on the rats after 2 weeks of $AlCl_3$ administration. The same tests were done one week following the administration of the CD-Rutin dose. Animals were moved to a separate room where behavioral tests were performed. These tests included a novel recognition test, an Open field test, Morris's water maze test, and a Y maze test.

2.8.1 Morris Water Maze (MWM) test

The Morris water maze (MWM) is used to analyze the cognitive abilities of rodents and it is done so based on the distal cues for navigating an open arena containing a partially inserted platform. Spatial learning is checked in rodents based on repeated trials. Memory changes are determined by noting the preference and inclination towards the platform area when it is removed. The rats were put in the water to swim around and locate the escape platform for 90 seconds. Rats were assisted to swim to the platform and were seated for 10 seconds for recognition in case they weren't able to reach the platform. This whole process was repeated for 4 consecutive days. The rats' memory was tested on the fifth day of the training without the platform. Their movement during the test was recorded by video (Takeuchi et al., 2011).

2.8.2 Y-Maze test

This system consists of three arms of varying lengths (8,30, and 15 cm) at a 120° angle. The whole experiment consists of two steps. The first of 2-trial tests consisted of letting the rats roam freely in 2 arms i.e., start arm and familiar arm for about 10 minutes. The third arm called the novel arm was closed. During the 2nd trial, the rats were free to roam in all three arms for 5 minutes. Total time that rats spent in novel arm was noted in a video. Rat's innate exploratory nature is shown by the time spent in the novel arm. The 3 arms in Y-Maze make the rats transition from the "familiar arm" to the "novel arm" and then ultimately towards the "start arm". However, when rats are suffering from neurodegenerative diseases, it affects their instincts. It makes them not remember the previous experience and so they do not alternate between the arms. Rats suffering from pathological conditions are less likely to explore the novel arm and hence provide us with the information we need (Takeuchi et al., 2011).

$$\frac{Number\ of\ alterations}{Total\ number\ of\ entries} x\ 100$$

2.8.3 Novel Object Recognition (NOR) Test

This test relies on rats' innate ability to interact with novel objects rather than familiar ones. Rats were free to move inside the box for the test for about 5 minutes, a day before the experiment. After placing two 2 identical objects in the box, rats were allowed to explore the objects freely for 5 minutes. Rats were removed and box and ethanol spray was used to rid of olfactory cues. After waiting for 24 hours, a different object was placed and one same object was kept and rats were recorded while interacting with the new object along with the old one (Zhang et al., 2012).

2.8.4 Open Field Test

The open field test is carried out in a hardwood floored box with W100cm X D100cm X H40cm divided into 16 squares (4X4) white lines. The rats were placed initially in one of the corners and their movement pattern was observed for 5 minutes. The number of squares the rats entered with both forelimbs counts as one. (a) Animals exploring 4 central squares and 12 external squares next to the wall regions were observed and noted separately. (b) The number of times a rat grooms itself i.e., licking its fur, cleaning its face, and scratching is also noted. (c) The times a rat sniffs, or scans the area while standing on its hindlimbs and also leaning against the wall with the forelegs was noted as well (Takeuchi et al., 2011).

2.9 Histopathology

2.9.1 Microscopy

H&E slides were observed under a Binocular Light Microscope (USA) with 4X — 100X magnification. Cell degeneration was observed through the cell count ratio between control, treated, and diseased tissues. The number of cells in each slide was counted using image J.

2.9.2 Hematoxylin and Eosin Staining (H&E)

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, this was achieved by incubating them at 63°C for almost 30 minutes. Also, in incubation, slides were immersed in xylol for 2 minutes of duration and then washed off with different amounts of ethanol (100%, 90%, 80%, 70%). Each washing of ethanol was for 2 minutes for each slide and then were rehydrated for 5 minutes with distilled water. H&E staining was done by using following reagents: hematoxylin for 3 minutes, water wash for 1 minute, then differentiator with mild acid for 1 minute, water washing for 1 minute, bluing for 1 minute, then again water washing for 1 minute, ethanol for 1 minute, eosin for 45 seconds, 95% ethanol for 1 minute, 100% ethanol for 1 minute and then at the end, xylene for 2 minutes. In the end, the slides prepared were covered with a coverslip and visualized using Binocular Light Microscope (USA) with 4X — 100X magnification (Feldman et al., 2014; Alturkistani et al., 2015).

2.9.3 Thioflavin T (ThT) Dye Staining

After deparaffinization and rehydration of the slides as previously described, a drop of a working solution of 0.5% ThT dye in 0.1 N HCl was placed on the slides, which were then kept in a humidity chamber for 15 minutes. The slides were rinsed in deionized water and then cover-slipped (Biancalana et al., 2010).

2.10 Statistical Analysis

Data was statistically assessed by using a t-test in the case of two samples (diseased and control) or analysis of variance (ANOVA) to compare three (diseased, control and treated groups) or more groups followed by Tuckey's multiple comparison test and a *p*-value of < 0.05 meant the data for groups analyzed was statistically significant.

CHAPTER 3: RESULTS

3.1 FTIR Analysis

FTIR analysis for CD-Rutin shows a peak at 3435 nm which corresponds with that of Rutin at 3342.48 nm. This shows the OH group in the CD-Rutin combination and Rutin. Other peaks of CD-Rutin at 1360 nm and 1425 nm show the C=C stretching along the structure and carboxyl bonds C=O in the combination molecules. Both these peaks resonated comparably with Rutin showing peaks at 1383 nm and 1500 nm which shows results of possible binding.

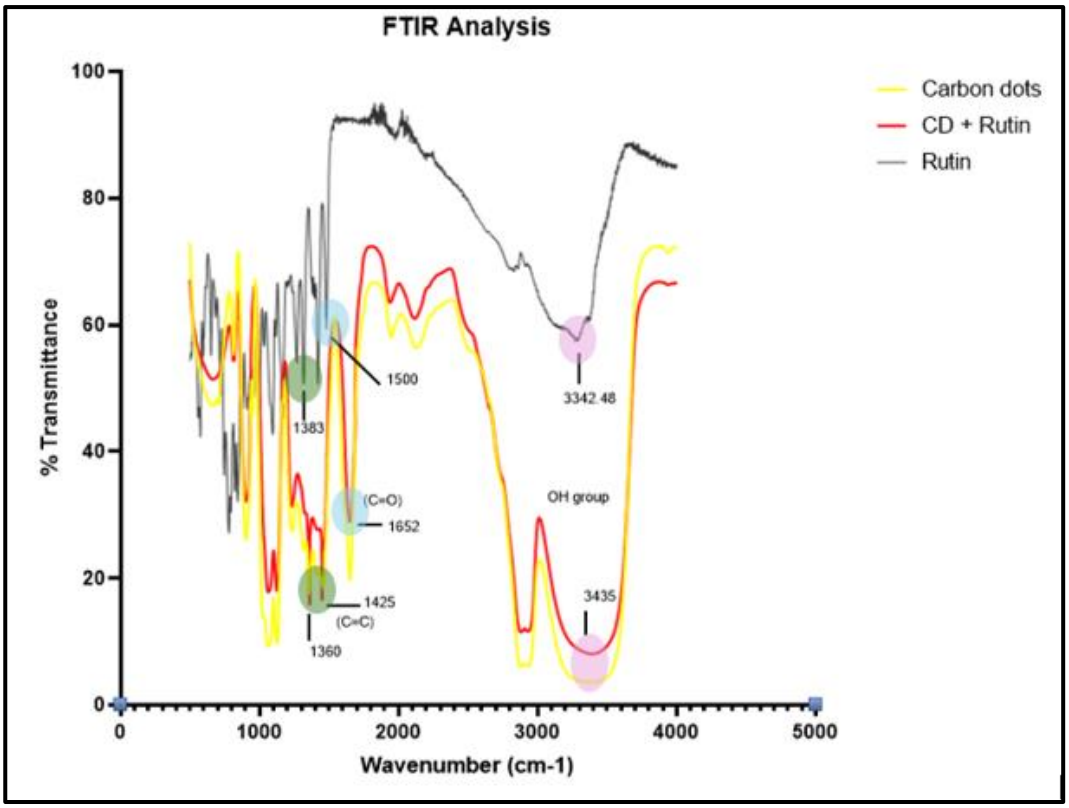

Figure 3.1 FTIR Analysis of CDs, CD-Rutin, and Rutin. The FTIR analysis shows peaks of Rutin (black) at 3342.48 nm, 1500 nm, and 1383. The graph for CDs (yellow) shows absorption peaks at 1425 nm and 3435 nm. Graph for CD-Rutin (Red) shows the peaks at 3435 nm, 1360 nm, and 1652 nm.

3.2 UV-Vis Spectrophotometry

The UV-Vis spectra of CDs provide us data with peaks at 240 nm and 345 nm. Rutin shows two peaks of absorption at 355 nm and 280 nm. CD-Rutin combination made a peak at 255 nm that lies in the absorption spectrum between rutin and CDs and another distinct peak at 358 nm. The peaks of carbon dots, CD-Rutin, and Rutin at 345 nm, 355 nm, and 358 nm align together to show uniformity in the resulting combination. The peak at 240 nm shows C=C stretching which is translated in CD-Rutin at 255 nm.

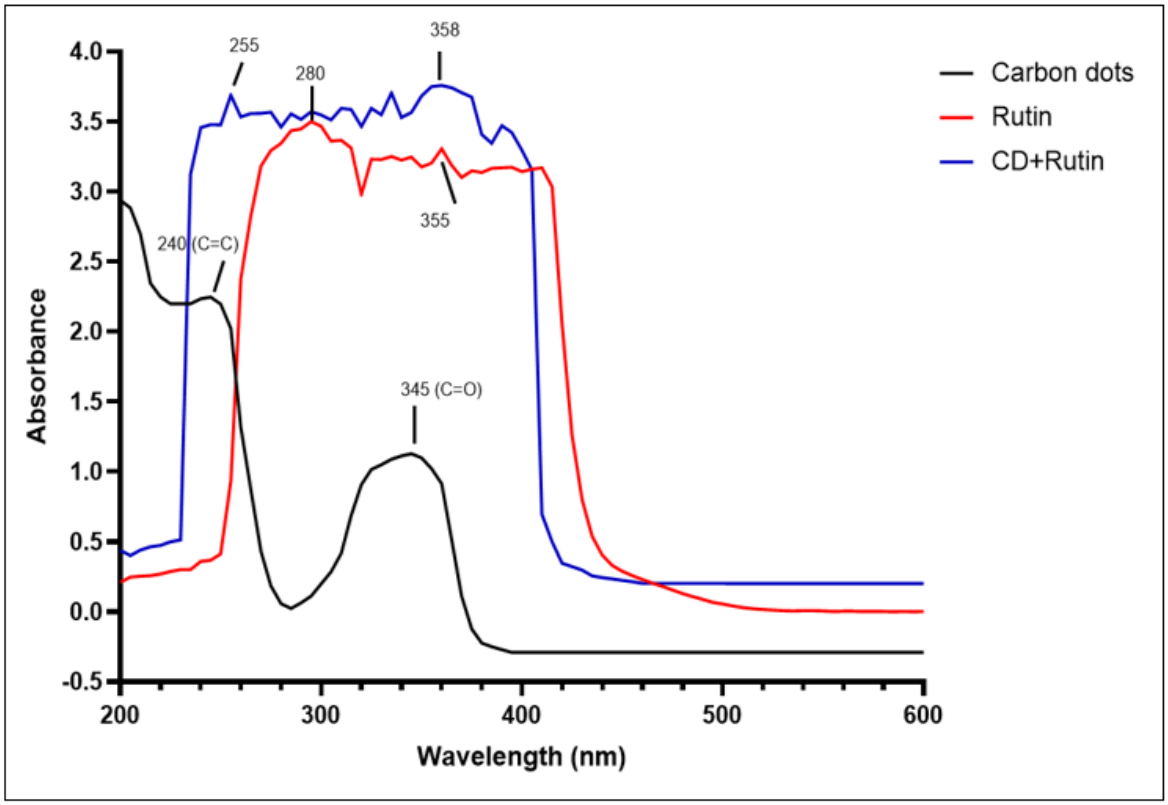

Figure 3.2 UV-Vis spectrum of CDs, Rutin and CD-Rutin. The UV spectra show peaks of CD (blue), 240 nm, and 345 nm. Rutin (red) shows peaks at 280 nm and 355 nm; and CD-Rutin (black) at 255 nm and 358 nm.

3.3 SEM Testing

Scanning electron microscopy was carried out with the magnification of 0.5 µm and 0.2 µm. The analysis shows two types of nanoparticles. The particles sized 14 ± 1 nm were unbound glucose CDs while particles with sizes more than 30 nm show the carbon dots loaded with drug i.e., Rutin. According to the literature, the size of Glucose CDs is ~10 nm. The mean of all the 30 nm plus nanoparticles is 34.05 nm. This gives the mean size of Glucose Carbon Dots loaded with Rutin as 34.05 ± 3 nm.

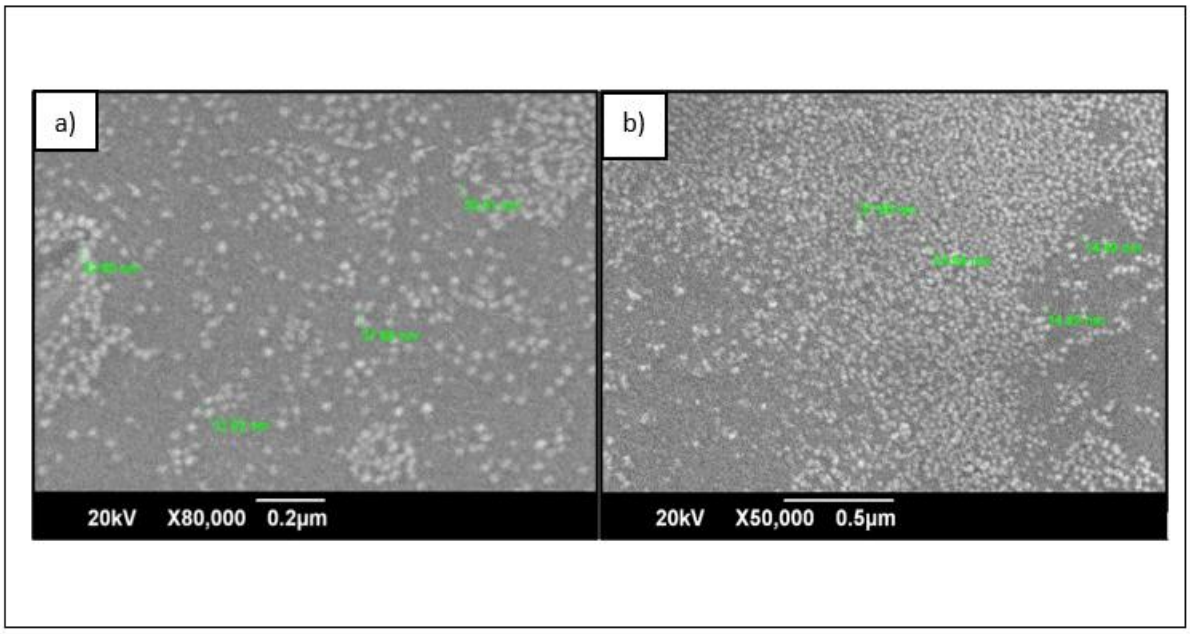

Figure 3.3 (a): Carbon Dots loaded with Rutin range around 30 nm with a mean calculated by taking into account all carbon dots pointed out is 34.05 ± 4 nm **(b)** Shows unbound carbon dots and Rutin bound carbon dots separately, given carbon dots size as 14 ± 1 nm.

3.4 Variation in binding affinities of Aβ Isoforms with Rutin

In Silico analysis performed in this study involved the analysis of binding energies and 3D configuration of different oligomers of Aβ and APP1. Negative binding energies in docking analysis of various oligomers with varying lengths of amino acids and APP1 show strong binding presenting Rutin as a favorable treatment option for Alzheimer's with added efficiency of targeted drug delivery.

3.5 Binding Energies

Binding energies show the affinity with which ligand (Rutin) binds with the target peptide. The conformations were observed, and each run was analyzed based on the binding energies. The lowest binding energy shows the strongest binding and the highest energy gives the weakest binding (Lin et al., 2011). The graph shows the strongest bonding with Amyloid beta Protein which shows that Rutin has the highest affinity to target the oligomers of Aβ. The lowest binding energy (strongest bonding) was found in the oligomers $A\beta_{1-42}$, and $A\beta_{1-40}$ i.e., -11.1 and -7.8 which shows that these oligomers are the ideal molecules to be targeted by Rutin which are abundant in Alzheimer's pathology in the brain**.**

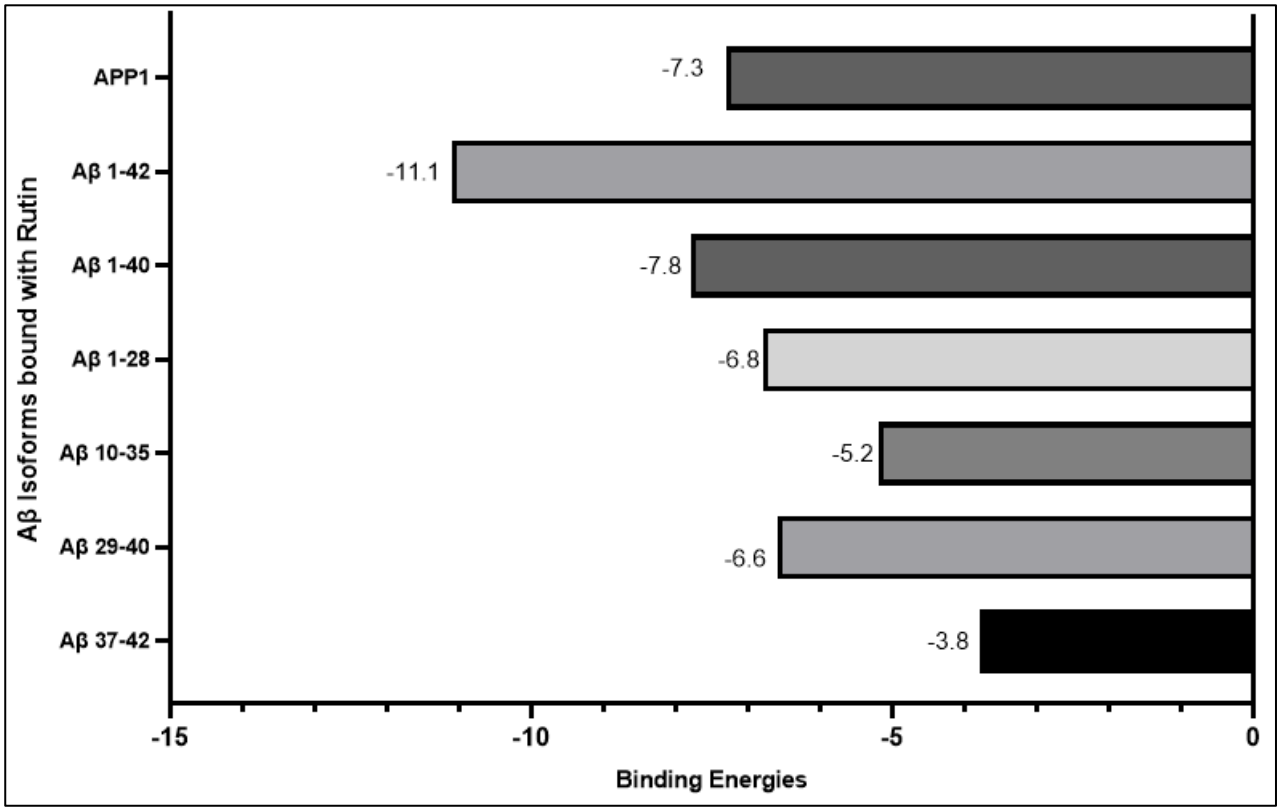

Figure 3.4 Binding affinities of various peptides of Aβ and APP1 protein. The lowest the binding energy, the strongest is the bonding between the ligand (Rutin) and the protein oligomers $A\beta_{1-42}$, and $A\beta_{1-40}$.

3.5.1 3-D structures visualization of Ligand-Peptide interaction

3D Structures of Ligand (Rutin) and various oligomers of Aβ were analyzed using PyRx to observe the binding sites for Rutin in each peptide. The structure of each oligomer was downloaded in a PDB format and uploaded along with that of Rutin as a ligand. Each analysis shows a distinct hydrophobic site for Rutin to bind in the Amyloid peptides.

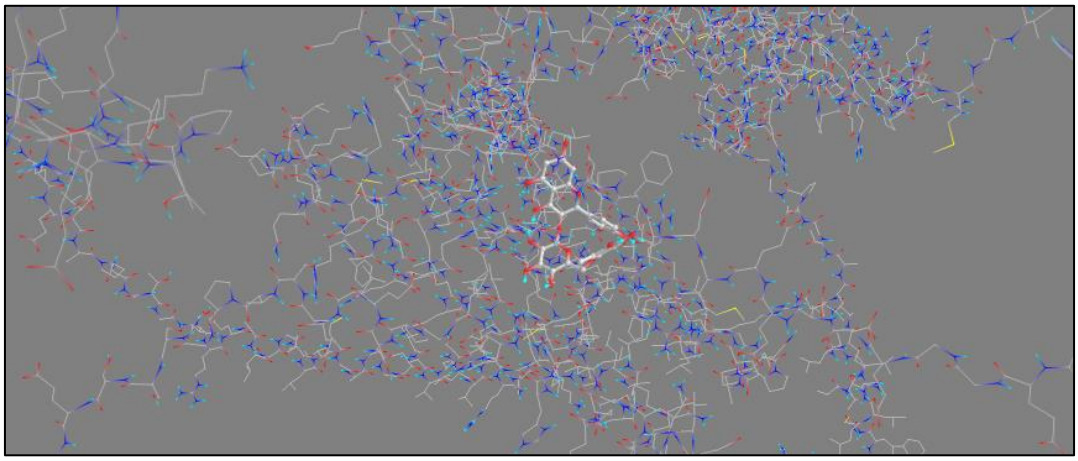

Figure 3.5 3D configuration of binding of Rutin and APP1 analyzed using PyRx. The average binding affinity for this ligand-protein interaction is -6.8.

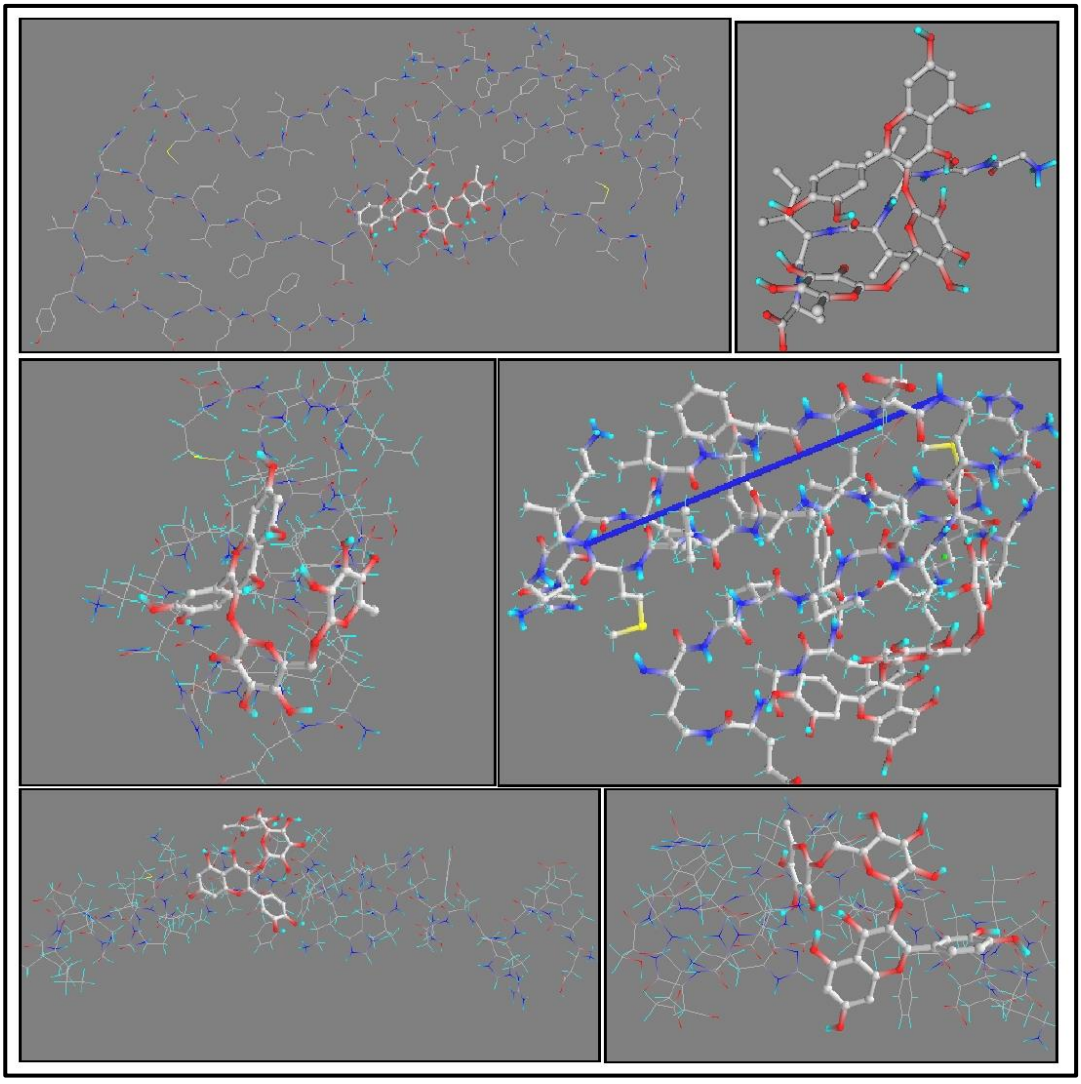

Figure 3.6 *In silico* analysis of various oligomers of Aβ. The binding energies show the binding affinities of Rutin and the ability to degrade the peptides accumulated in case of AD. The lowest binding energies were seen in $A\beta_{1-42}$ i.e., -11.1 and in $A\beta_{1-40}$ which was -7.8

3.6 Behavioral Tests after CD-Rutin Administration

Behavioral tests were performed to before the drug administration to confirm the induction of Alzheimer's and after confirmation, the drug was given and the same tests were repeated to check for the efficacy of the drug. The comparison of diseased, treated and control gives us the data about significant differences and how significant and non-significant were the results.

3.6.1 MWM Test

The escape latency experiment performed after drug and CD administration showed that the treated rats had a shorter latency in reaching the target platform when it was analyzed in comparison to the diseased rat group. It should be noticed that the treated rats showed significant results in terms of time that was in the quadrant where the platform was kept, in addition, the entry count in the target quadrant showed significant improvement. This gives positive results after the administration of CD-Rutin combination.

3.6.2 Y-Maze test

Percentage alterations i.e., increased no. of entry in both arms collectively were observed to be improved in treated rats. The number of entries in the familiar arm was also observed to be improved in the CD-Rutin administered group as compared to the diseased group. Percentage alterations, which is the collective number of switches between an arm during the time of the experiment. Improved PA% was clearly observed in the results.

3.6.3 NOR Test

There was no discernible change in the rats' capacity to recall item position or identification between the treated and sick groups, according to the findings of the test. Although the two groups exploratory activity patterns were identical, the treated rats showed a marginally higher propensity to leap onto the novel and familiar items.

3.6.1 Open Field Test

When investigating the open field, treated rats were seen to spend more time in the center than sick rats. Rats with illnesses, remained in the peripheral area the entire time.

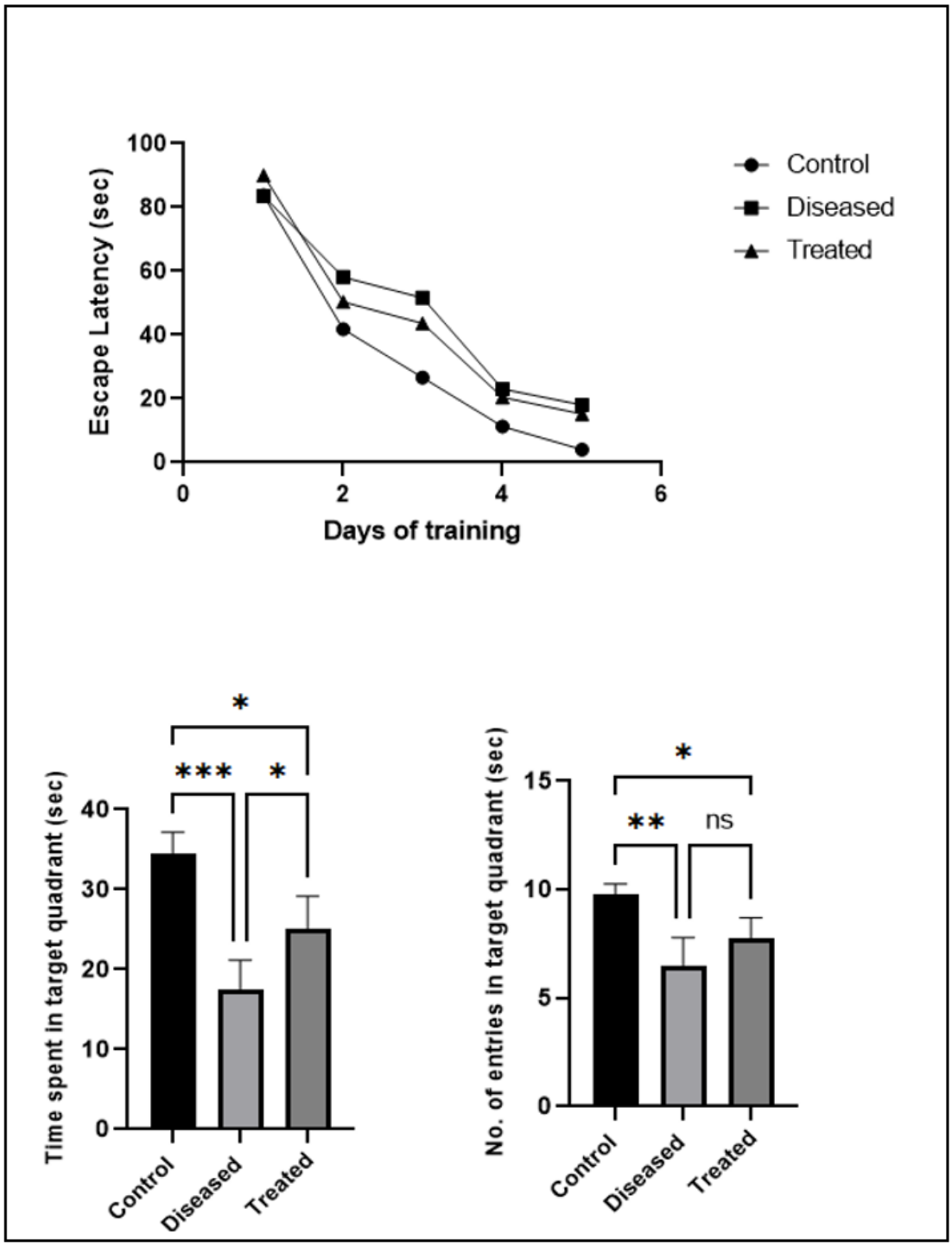

Figure 3.7 ***MWM test after CD-Rutin administration.*** (A) The escape latency graph showed decreased latency in the treatment group when it was compared to diseased rats. (B) And (C) The time and entries in the target quadrant are also increased in the treated rats. One-way ANOVA, followed by Tukey's multiple comparison test, was utilized for statistical analysis. Error bars present SEM (*p<0.05, **p<0.01, ***p<0.001).

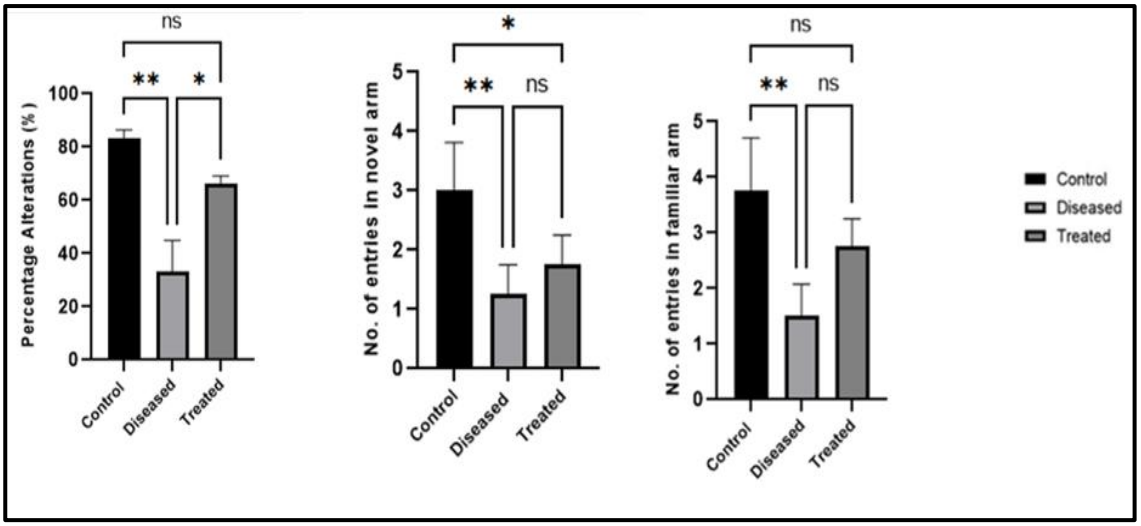

Figure 3.8 Y-Maze test after CD-Rutin administration. (A) Percentage alterations were observed to be increased in treated rats. (B) And (C) Exploring ability was also observed to have increased. One-way ANOVA, followed by Tukey's multiple comparison test, was used for statistical analysis. Error bars present SEM (*p<0.05, **p<0.01)..

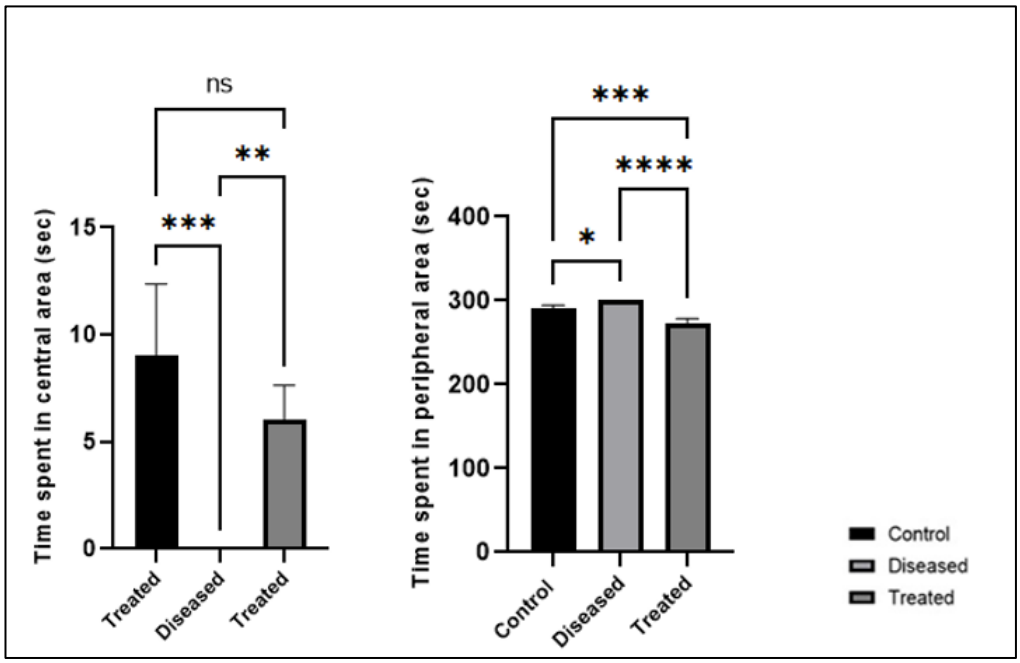

Figure 3.9 Open Field Test after CD-Rutin administration. (A) In treated rats, there was an increase in the amount of time spent in the central area, and vice versa. (B) i.e., time spent in the peripheral area. One-way ANOVA, followed by Tukey's multiple comparison test, was used for statistical analysis. Error bars present SEM (**p<0.01, ***p<0.001, and ****p<0.0001).

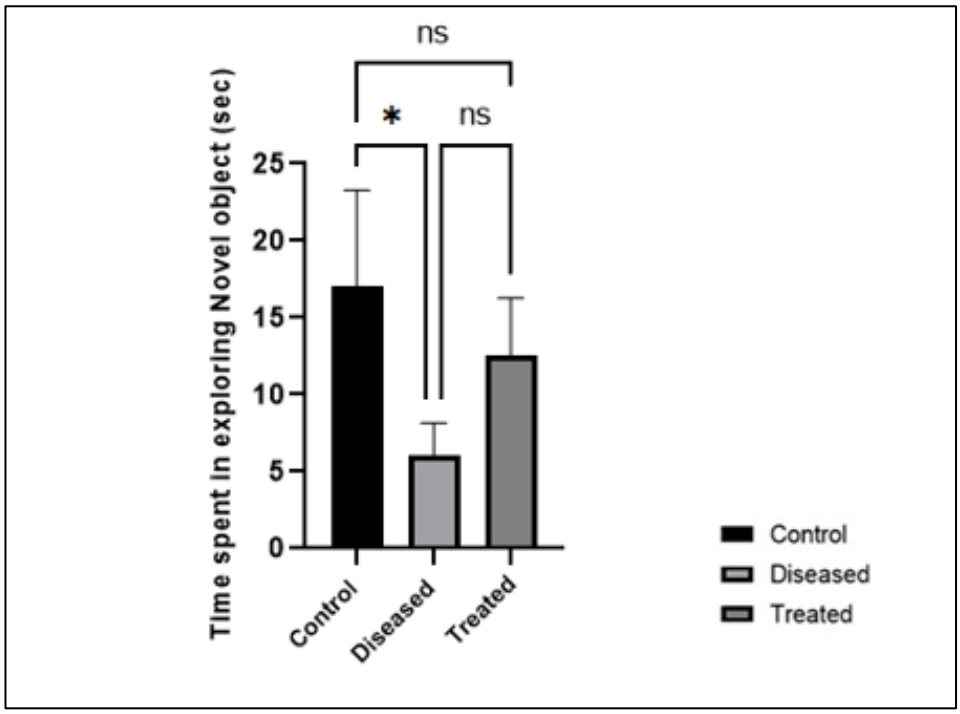

Figure 3.10 NOR Test after CD-Rutin administration. The graph represents the control, diseased group, and treated group, the rats explored a novel object, and the time was noted. Although no significant difference was found, the intrinsic ability to leap onto the novel object was increased. One-way ANOVA, followed by Tukey's multiple comparison tests, was used for statistical analysis. Error bars present SEM (*$p<0.05$) while ns shows non-significant differences.

3.7 Histopathology

Histopathology is the process of fixing brain tissue on a microscopic slide and staining it with relevant stains to observe and analyze various parts for the extent of the disease. Albino rats were subjected to PFA fixation which involves injecting PFA solution into the pumping heart of an unconscious subject that reaches the brain and fixes the tissues in their present condition with the stage of disease progression. The brain is then dissected and a thinly sliced sagittal portion is fixed onto a microscopic slide.

3.7.1 H&E staining

Histopathological tests were performed to check for the extent of cell degeneration in the cortex of diseased, healthy rats and rat groups after the administration of the Rutin-CD combination. Slides were visualized under the magnification power of 20X. With the help of Image J software, cells were observed and counted for the tissues of the cortex in all groups i.e., control, diseased, and treated, and differences were compared.

3.7.1.1. Diseased and Control Sample before Drug Administration

In Alzheimer's disease there is rapid call degeneration and E cells shrink with spaces around them. The overall number of cells is also decreased shows rapid degeneration when compared with healthy cells and shows multiple cells with smaller sizes. This observation affirms the induction of Alzheimer's disease in the diseased group.

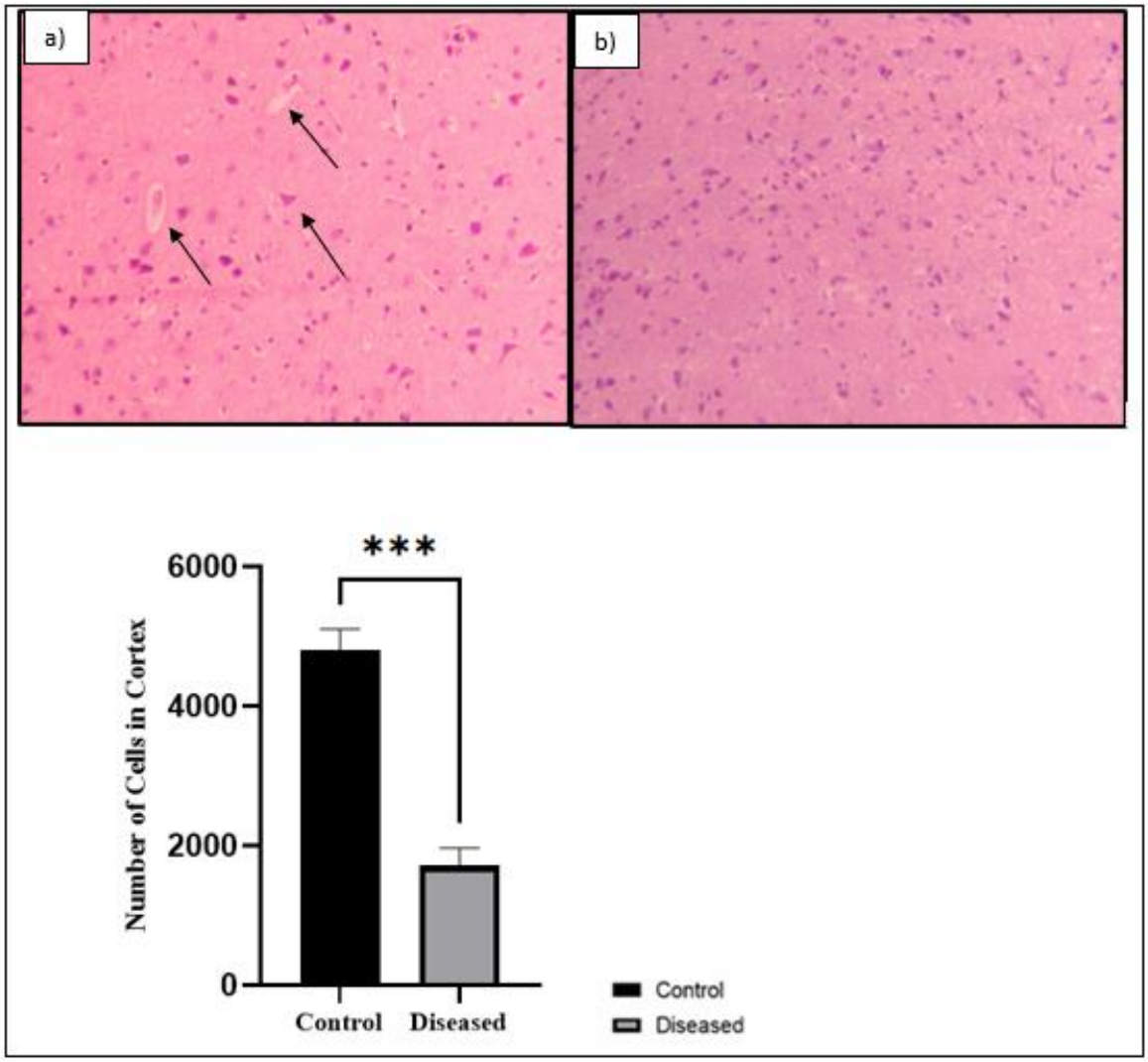

Figure 3.11: Diseased Samples: (A) Hematoxylin and Eosin (H&E) Staining results of Cortex show clear degradation and decreased number of cells-**(B)** Shows healthy cells with no degradation or amyloid deposition. One-way ANOVA, followed by Tukey's multiple comparison test, was used for statistical analysis. Error bars present SEM (***$p<0.001$).

3.7.1.2. CD-Rutin Administration

After the administration of CD plus Rutin, the results clearly show positive results, there is stopped degradation of cells in treated tissues with lesser shrunken E cells. This gives positive results in the case of Alzheimer's with the administration of CDs plus Rutin. The graphical representation gives the total cell count in all three samples.

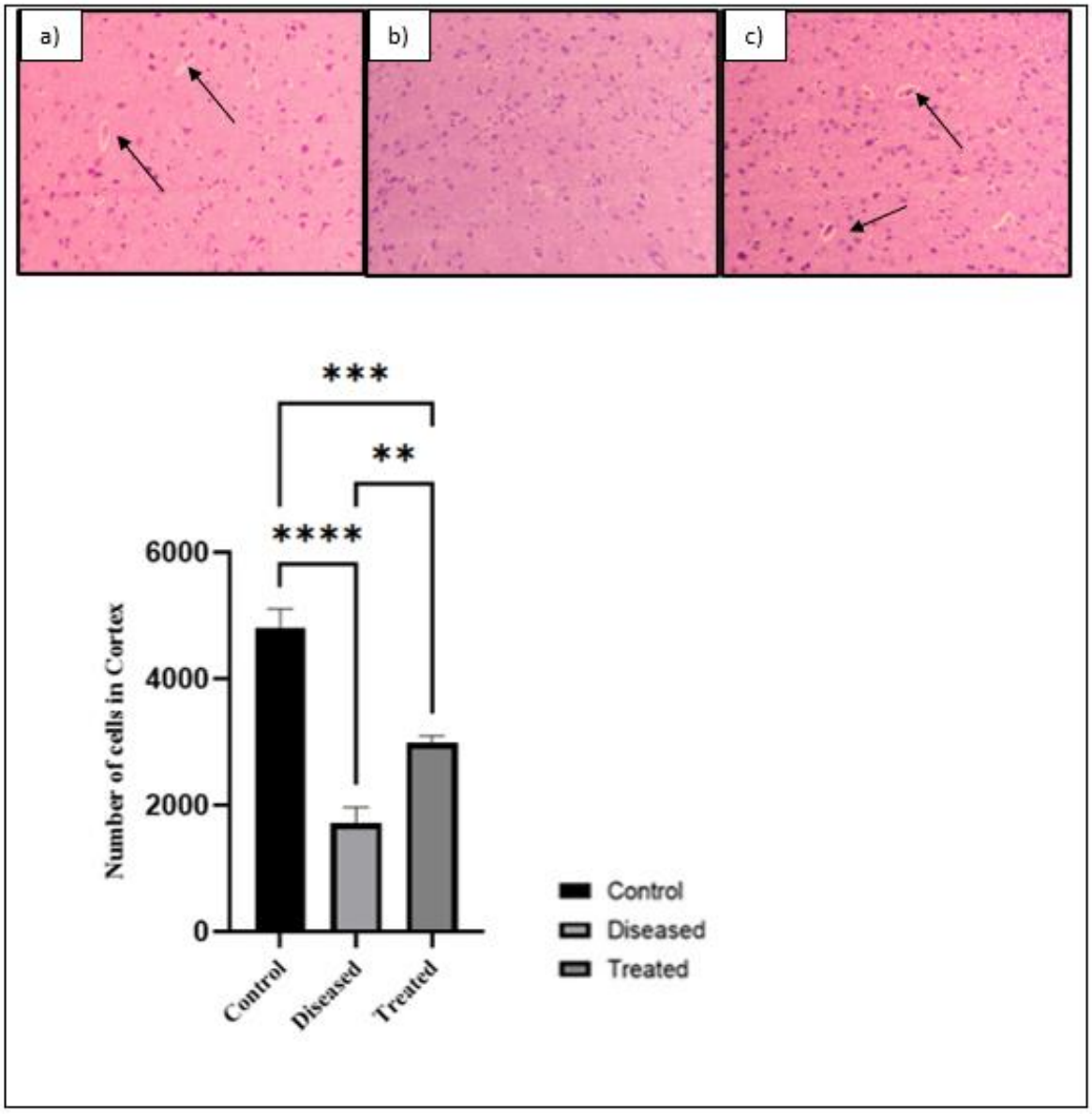

Figure 3.12: Administration of CD-Rutin: One-way ANOVA analysis shows positive results with P value <0.0001. There is a significant difference between Treated (A), Diseased (B), and Control (C) tissue samples in terms of number of cells in the Cortex. One-way ANOVA, followed by Tukey's multiple comparison test, was used for statistical analysis. Error bars present SEM (**p<0.01, ***p<0.001, and ****p<0.0001).

3.7.2. Thioflavin T staining

Thioflavin T (ThT) stained slides were analyzed to check for the amyloid aggregation as the stain molecules have the affinity to attach to the Aβ aggregates. The slides were viewed under a fluorescent microscope with 10X magnification. The green color shows fluorescence that indicates the presence of amyloid plaques in the brain tissues, specifically the cortex. ImageJ was used to count the number of cells.

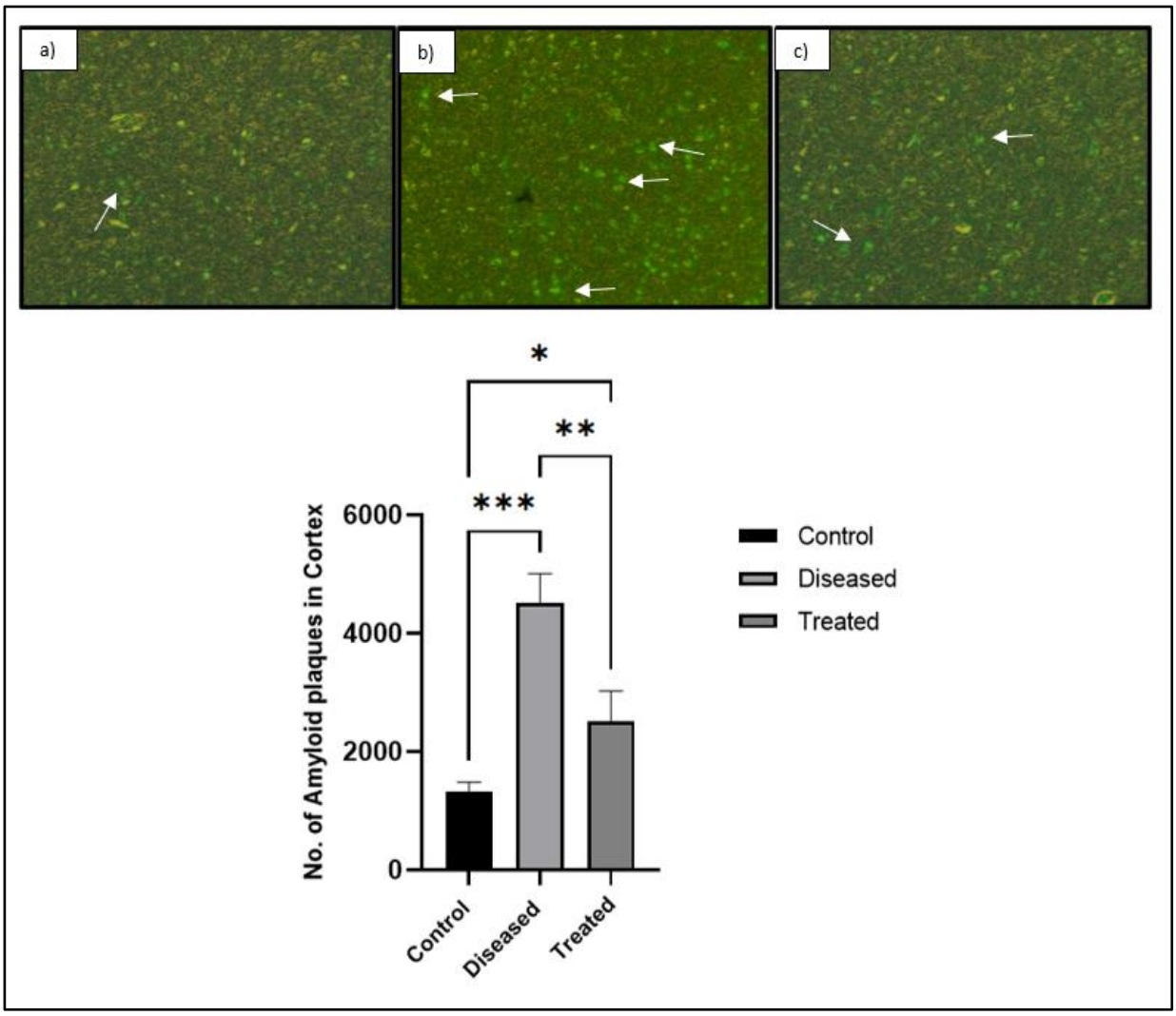

Figure 3.13: ThT staining of rat cortex indicates the presence of amyloid plaques in the regions that fluoresce bright green. The slides were photographed under a fluorescent microscope at 10X magnification. the arrows in the diseased cortex show aggregated Aβ deposits. a) shows the control group with very few aggregates b) shows the diseased cortex with multiple Aβ aggregates having bright green color c) indicates the treated group with lesser no. of aggregates as compared to diseased group. One-way ANOVA, followed by Tukey's multiple comparison test, was used for statistical analysis. Error bars present SEM (**p<0.01, ***p<0.001).

CHAPTER 4: DISCUSSION

A report submitted by WHO in 2016 says that neurological conditions and related abnormalities are accountable for almost 276 million disability-adjusted lives and caused 9 million deaths worldwide (Feigin et al., 2019). This worldwide prevalence presents an extreme need for effective treatments. Blood-brain barrier and its selective permeability halt the crossing of many macromolecules along with pharmaceuticals from entering the CNS and deploying their therapeutic effect. This poses an obstacle in circumventing neurology-related issues. Recent research in nanoparticle technology, specifically nanocarriers as drug delivery vehicles has made substantial progress over the years. Surface molecules of CDs have great potential to serve as drug delivery systems that can penetrate BBB to resolve neurological conundrums.

CDs can bind with useful ligands and remedy brain tumors, like glioblastoma that has been researched extensively by Leblanc and colleagues. Most research done to date lacks thorough analysis and extensive research to produce effective data about CDs and their neuroprotective effectiveness. It's imperative to understand the mechanism of this intricate system. Rutin activates the MAPK pathway and inhibits apoptosis, which is caused by Aβ peptides, which is the primary basis of its neuroprotective effects. In addition, it increases the survival of neural crest cells by causing the stimulation of ERK2 and P13K pathways (Wang et al., 2015). Rutin also causes amplification of many antioxidant enzymes like Catalase, Superoxide Dismutase, and Inhibitor of iNOS Activity (Yu et al., 2015). It restrains the activity of Amylin aggregation. Also, can diminish microglial activation started by lipopolysaccharides (Simonyi et al., 2015).

Presently the research focuses on Rutin-bound CDs and their properties to cross BBB and ability to treat AD. The CD bound with the Rutin complex prepared has hydroxyl, carboxy, and carbonyl groups which indicates their water dispersibility and exceptional stability (Guo et al., 2021). The UV-Vis spectra of carbon dots (Figure 3.2) showed two peaks at 240 nm and 345 nm which can be related to their π-π* transition of C=C and C=O bonds respectively (Marfa Egorova et al., 2023) . Rutin exhibits two important bands at 360 nm and 295 nm. These are consistent results when compared with studies done by (Panhwar & Memon, 2014). CD-Rutin showed corresponding peaks of 255 nm and 358 nm. These readings can be related to the π-π* transition of a system with a benzene ring conjugation.

Examining UV-Vis's spectra Rutin, CDs, and CDs-Rutin, it can be shown that Rutin has been successfully loaded onto the Carbon dots to make a stable combination. SEM analysis provides an estimation of the sizes of molecules produced with unbound carbon dots having a size of 14.25 nm and bound carbon dots with Rutin falling in the range of around 30 nm size. SEM analysis confirms the loading of carbon dots with that of Rutin successfully. *In silico* analysis provided us with the binding energies to assess the affinity of Rutin with target sites of Amyloid beta peptides with varying lengths. The negative energies show a strong affinity with favorable bonding and thus positive *in silico* results (Lin et al., 2011). The strongest bonding and lowest energy were found in the interaction of Rutin with $A\beta_{1-42}$ and $A\beta_{1-40}$ and was -11.1 and -7.8. These oligomers have been studied widely with their relation to Alzheimer's pathology so a strong affinity of Rutin with these oligomers presents it as a favorable treatment against amyloid aggregation leading to AD due to these aggregates (Festa et al., 2019).

Behavioral tests performed to check the Alzheimer's induction with $AlCl_3$ showed that subject rats had a gradual decline in cognitive abilities. In the MWM test, diseased rats stayed for decreased amount of time in the target quadrant in comparison to the control group. The control group that stayed for longer in the target quadrant and also showed increased entries in the quadrant. The escape latency graph also showed impairment in the rat's cognitive capability. Assessing the spatial memory shown by PA% in the Y-Maze test in the diseases group was lower significantly ($p > 0.05$) in comparison to the control group (difference between means control and diseased ± SEM = -20.0±5.675). However, the mean PA% value in the control group was 83.14% and was significantly more ($P > 0.05$) as were in the AD group. The graph shows alterations of rats in all three arms of Y-Maze, the movements and alterations in the control group were more than in the AD group. The diseased group explored the familiar arm more than the novel arm.

The open field test was done to observe alterations and movements in both control and diseased groups. As Figure 3.4.3 shows. Interactions of novel and familiar objects were observed in NOR tests between rats and objects. Rats with AD didn't explore the novel object as much as diseased rats. The diseased group showed more cognitive decline as compared to the control group. The same experiments were repeated after administration of the drug. Treated rats exhibited a lesser latency in reaching the platform when compared with diseased rats. They spent more time in target area also the number of entries was significantly more. The Y-Maze test showed significant improvement in percentage alterations in the treated group as compared to the diseased group.

The treated group had better alterations between the arms and entries in the familiar arm. In an open-field test, treated rats spent increased amount of time exploring the central area when compared with diseased rats who were more focused on the peripheral area. The NOR test produced a slight betterment in treated rats in exploring novel objects compared to the diseased rats, and their intrinsic inclination to jump to the objects was improved. The overall results suggest CDs function as a smart system for Rutin to be delivered. After behavioral analysis, the rat groups were dissected for histopathological analysis that provided significant differences between healthy, diseased, and treated groups. H&E-stained slides under a microscope with 20X magnification showed clear cell degeneration, the cell count was significantly different in all three groups suggesting positive results of the study.

Direct future applications still suffer some issues due to limited research and the viability of experimentation on human subjects. Enhancing the optical properties of CD-Rutin is needed. For this, a wide range of CDs with tunable compositions, sizes, forms, and crystallinity need to be produced. Extensive research and progress on the procedures for the production of CDs are time-consuming, unfavorable environmentally, and result in minimal quantities. Therefore, it is important to establish sustainable, effective, and manageable techniques to make CDs in larger quantities and better quality. PL properties of CDs in blue or green fluorescence have been studied extensively (Jiang et al., 2015). CDs exhibiting NIR fluorescence, phosphorescence, and electroluminescence with enhanced Raman scattering are rare in occurrence.

Above mentioned advantages make CDs better suited for bioimaging, light emission, and sensitive sensing. So, CDs with such enhanced optical properties would have greater potential. Also, it is absolutely necessary to describe the mechanisms that underlie the optical characteristics of CDs. Examination of PL mechanisms for CDs has gained considerable attention in association with their edge and surface states. Not to mention their quantum confinement effect. Nevertheless, there is insufficient data for recommending more widely accepted PL methods for CD production. Mechanisms involving the optical features of CDs remain rather unexplored (Changqin et al., 2014)

An approach effective to enhance the optical characteristics of CDs needs their binding with advantageous materials. So, it is important to study CDs-based hybrids for their surface functionalization and interfacial impacts. Theoretical calculations and contemporary characterization techniques are needed to understand the relationship between optical properties and their interfacial interactions. A thorough understanding of optical traits for CDs will result in many innovative applications. CDs are environmentally friendly and safe materials with their viable photoelectric applications.

CHAPTER 5: CONCLUSION AND FUTURE RECOMMENDATIONS

In this investigation, the CD-Rutin combination was prepared through microwave irradiation. Carbon dots were formed and subjected to a comprehensive characterization process to validate their formation. Structural characterization revealed the functional groups exposed on the surface and the size of molecules produced providing validation of CD-Rutin binding. This extensive molecular analysis provided the basis for their capability to cross the blood-brain barrier. Water dispersibility of CD-Rutin was confirmed using FTIR analysis which confirmed the existence of functional groups like hydroxyl and carboxyl groups in the produced molecules. SEM analysis indicated the size of unbound carbon dots was 14.25 nm which correlates with the size of carbon dots given in literature being ~10 nm. The size of bound carbon dots is 34.05 nm ± 3 nm. *In silico* analysis of Rutin and amyloid oligomer interactions proves it to be a favorable treatment option. *In vivo,* behavioral testing also demonstrated positive results. In adult male albino rat models, cognitive impairment before and after the administration of drug-bound carbon dots was analyzed and was significantly and noticeably improved in treated rats. Biocompatibility and no visible toxicity were found in the rats. Histopathology of brain tissues of subject groups also provided significant results of drug combination action. As a result of this progressive research, CD-Rutin might serve to be a novel and beneficial platform that shows potential in the domain of therapeutics to treat AD.

CHAPTER 6: SUMMARY

Cases of Alzheimer's are around 50 million worldwide and are piling up rapidly, these numbers are expected to triple by 2050. The most common cause of dementia is Alzheimer's. About 60-80% of dementia cases are associated with Alzheimer's disease. Considering the socio-economic consequences of the disease, there is a dire need for a sustainable and effective cure. With all the advances in medicine, there still are no medications. Currently, the available drugs can only manage symptomatic altercations. The objective of this study was to find a green solution for Alzheimer's disease that is highly targeted, efficient, non-toxic, and biocompatible. CDs have been found to have excellent properties such as biocompatibility, low cytotoxicity, and large surface area to volume ratio which makes them a potential candidate for drug delivery. Rutin, a naturally occurring flavonoid has many biological effects, one of which is the neuroprotective effect. Previously, this drug has been found to reduce Aβ oligomer levels and neuroinflammation in APP/PS1 mouse model of AD with improvement in spatial memory.

To improve its reach and effectiveness in the brain, in this study, a CD-Rutin combination was produced by making glucose-based CDs with microwave irradiation and loading the resulting nanoparticles with Rutin. The comprehensive characterization techniques such as FTIR, UV-Vis, and SEM analysis confirmed the formation of stable and water-dispersible CDs loaded with Rutin. The existence of various functional groups in the resulting molecules confirmed the formation of CD-Rutin. Administration of CD-Rutin was done in AD-like rat models at eight to twelve months of age. Alzheimer's was induced in rats with Aluminum Chloride (150 mg/kg/day) and D-galactose (300 mg/kg/day) through IP administration for two weeks. Behavioral tests were performed to check the progression of the disease afterwards, a single injection of CD-Rutin (10 mg/kg) was given intraperitoneally as well. Behavioral testing was done again after the administration of the drug. *In silico* analysis was also done to check ligand-protein interaction to check variation in the binding of Aβ isoforms with Rutin.

In silico analysis of Rutin and Aβ isoforms provided binding energies and 3D configuration. The analysis was based on the fact that low binding energies showed strong bonding and translated into high affinities. For Rutin to target the Amyloid aggregates in the brain that accumulate to cause AD low binding energies were required and were observed. The 3D configuration provided visualization of binding sites for Rutin onto the Aβ isoforms. CD-Rutin combination showed better positive therapeutic outcomes and targeted drug delivery. Better performance and reduced cognitive impairment in the

treatment group were confirmed by multiple behavioral tests before and after the administration of the drug including Morris's water maze test, open field test, novel object recognition test, and Y-Maze test. Histopathological analysis showed lesser Amyloid aggregates in treated groups as compared to diseased groups and with significantly positive results in terms of cell count and elongated E cells. The research provides a new direction where modified carbon dots can be utilized in other neurodegenerative diseases. An exhaustive and greater comprehension of CD-Rutin, its surface properties, and optical traits will facilitate the emergence of numerous innovative and therapeutic applications.

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