Therapeutic Potential of Rutin-Bound Glucose Carbon Dots for Alzheimer's Disease

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Table of Contents

List of Figures

Abbreviations

Aβ	Amyloid Beta
AD	Alzheimer's Disease
ADAM10	A Disintegrin and Metalloproteinase Domain-Containing Protein 10
ADAS-Cog	AD Assessment Scale
AFM	Atomic Force Microscopy
$AlCl_3$	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
-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
GQDs	Graphene Quantum Dots
HIV	Human Immunodeficiency Virus
LOAD	Late-Onset AD
M1	Primary Motor
MMSE	Mini-Mental State Examination
MRI	Magnetic Resonance Imaging

MS4A4A	Membrane Spanning 4-Domains A4A
MWM	Morris Water Maze
NCDs	Nitrogen-Doped Carbon Dots
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
TJs	Tight Junctions
TREM2	Triggering Receptor Expressed on Myeloid Cells 2
WHO	World Health Organization
Y-CDs	Yellow-Emissive CDs

Abstract

World Health Organization (WHO) states that approximately 55 million people are suffering from dementia globally making it a major public health concern. Alzheimer's Disease (AD) is the most common cause of dementia constituting 60-80% of cases worldwide. Currently, the available drugs can only provide symptomatic relief. The blood-brain barrier is one of the obstacles in the path of the drugs that limits them from reaching the target area in the brain. Carbon Dots (CDs) 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. With the aim of improving its reach and effectiveness in the brain, in this study, nitrogen-doped carbon dots (NCDs) and Rutin were utilized to synthesize NCD-Rutin, which is a nanomaterial with high-performance capabilities. NCDs exhibited the ability to effectively suppress the hyperphosphorylation of tau protein. FTIR and UV-Vis Spectrum results confirm the doping of NCDs with Rutin. Additionally, utilizing AD-like rat model, it was observed that the NCD-Rutin was able to penetrate the blood-brain barrier. Aluminum chloride injection (150 mg/kg/day) with D-galactose (300 mg/kg/day) was administered intraperitoneally for 15 days in order to induce AD-like condition in 1-year-old male albino rats. A single injection of NCD-Rutin (10 mg/kg) was administered intraperitoneally to experimental animals. Significant improvement in memory impairment was observed in AD-like rat models a week after the injection of NCD-Rutin, which demonstrates its potential as a promising therapeutic agent for AD treatment.

Key Words: *Carbon dots, Rutin, Alzheimer's disease, Nanotechnology*

CHAPTER 1
INTRODUCTION

Alzheimer's disease (AD) has emerged as a considerably major challenge to public health, due to the augmented longevity of life of the general populace and a more comprehensive comprehension of the socio-economic implications of the ailment. Alois Alzheimer delineated AD in the year 1906, utilizing criteria of disorientation, progressive cognitive impairment, and pathological markers like senile plaques and neurofibrillary tangles (NFTs) (Schachter & Davis, 2000).

The early manifestation of AD can be attributed to the deposition of Amyloid beta (Aβ) peptide in the form of amyloid plaques in the brain, leading to neurodegeneration that results in cognitive and functional impairment (Selkoe & Hardy, 2016). The development of the disease is dependent on studies of rare genetic variations that either promote or reduce Aβ deposition, thereby providing evidence that amyloid plaques play a vital role (Fleisher et al., 2015). Moreover, the occurrence of amyloid plaques in the initial stages of the disease increases the likelihood of dementia progressing from simple cognitive impairment (Doraiswamy et al., 2012). The administration of treatments aimed at eliminating amyloid plaques may decelerate the clinical progression of AD. Alongside amyloid plaques, intracellular NFTs including hyperphosphorylated tau protein are another neuropathological marker of AD. Current disease models suggest that tau pathology is triggered by Aβ, and at a later stage, a complex and synergistic relationship between Aβ and tau emerges, hastening the course of AD (Busche & Hyman, 2020).

1.1. Epidemiology

In 2018, it was estimated by Alzheimer's Disease International that approximately 50 million individuals globally are affected by dementia, with a projected tripling of this figure by 2050, with two-thirds of cases being reported in under-developed countries (Scheltens et al., 2021). It is anticipated that the prevalence of incapacitating and financially disastrous ailments will increase in the middle of the century, impacting more than 131 million people by 2050 due to the ageing population (Prince et al., 2016).

The process of ageing is the key determinant of the primary risk factor for AD, resulting in a staggering increase in dementia incidence every 6.3 years, rising from a mere 3.9 per 1000 for individuals of the age group 60-90, to an alarming 104.8 per 1000 for those who have surpassed the age threshold of 90 (Prince et al., 2015). The frequency of dementia is higher in the older age group, with an estimated rate of 10% for those over the age of 65 and 40% for those over the age of 80 (DeTure & Dickson, 2019). Effective pre-clinical diagnosis and treatments are crucial in preventing disease progression before the onset of symptoms, given the mounting personal and financial burden.

There exist mainly two distinct classifications of AD: familial and sporadic. Furthermore, there are two distinct stages of the disease, (a) early-onset AD (EOAD), which ensues in individuals of age 65 and under, and (b) late-onset AD (LOAD), which occurs after the age of 65. EOAD cases are found to be more prevalent than LOAD but still constitute less than 5% of all AD cases with a pathology diagnosis. EOAD is often characterized by an aggressive course and presentation (Mendez, 2017). AD is believed to have an impact on 5.5% to 9% of the general populace every six months (Gao et al., 1998). Over the course of a decade, the disease's prevalence doubled. At present, almost half of those who are aged 85 or older suffer from AD. Mild cognitive impairment may be present in people with cognitive impairment but are generally not included under clinical criteria for AD, and still manifest a regression in cognitive performance and encounter difficulties with new learning. Ongoing studies indicate that 40% of this group will acquire AD within three years (Schachter & Davis, 2000).

1.2. Etiology

Amyloid precursor protein (APP), Presenilin 1 (PSEN1), and Presenilin 2 (PSEN2) genetic mutations have the potential to lead to dominantly genetic Familial AD (FAD). Appearing in under 1% of AD patients, FAD generally manifests at the age of 46.2 years, although it may occur as early as 20 years old (Ryman et al., 2014). The majority of patients exhibit dementia by the age of 65. Even though genetic risk factors, particularly the apolipoprotein E gene (APOE), have been identified as LOAD, which is more prevalent, and is still believed to be sporadic. The highest risks of developing AD include age, family history in a first-degree relative, and APOE4 genotype. Furthermore, it has been observed that the APOE4 allele significantly heightens the susceptibility to traumatic brain injuries, Down's syndrome, Lewy body dementia, and vascular dementia

(Verghese et al., 2011). The identification of approximately 30 genes through genome-wide association studies, such as Triggering Receptor Expressed on Myeloid Cells 2 (TREM2), Phospholipase D3 (PLD3), and A Disintegrin and metalloproteinase domain-containing protein 10 (ADAM10), has been related to an increased risk of LOAD. These genes not only have a direct impact on APP and tau, but also have a role in the up- or down-regulation of cholesterol metabolism, immune response, and endocytosis, with known functions (Karch & Goate, 2015). With a better comprehension of the functionality of the current and novel risk factors, insight into the pathophysiological mechanisms underlying AD can be gained. The major neuropathological characteristics of AD include senile plaques and NFTs. With the disease progression, the plaques gradually build up in brain regions linked to cognition before spreading to nearby cortical areas. These plaques embody insoluble Aβ deposits, which originated from APP. The Aβ peptide arises due to two events involving the proteolytic activity of β-secretase and γ-secretase. Two types of Aβ have been identified: $A\beta_{40}$, which is shorter, and $A\beta_{42}$, which is longer. The initial deposition of $A\beta_{42}$ may function in activating the mechanisms which eventually lead to the deposition of amyloid. Despite the mounting evidence that APP metabolic dysfunction, resulting in an increase in Aβ deposits, is a cause of AD, it remains unclear whether senile plaques are the causative agents of the disease or a by-product. Aβ is found to be toxic to neurons, either directly or indirectly by causing inflammation or an increase in free radical generation. Another distinguishing feature of AD is the collection of NFTs in the brain. Majority of these NFTs are composed of chemically reformed (irregularly folded and phosphorylated) Tau protein, which functions in the microtubules formation in neurons. The quantity of tau tangles in the cerebral cortex increases in proportion to the severity of the disease; as the disease progresses, so does the number of tangles. Recent research affirms the notion that changes in tau occur subsequent to Aβ build-up in AD patients. (Näslund et al., 2000).

1.3. Diagnosis

AD is characterized by cognitive and non-cognitive changes. The former includes disruptions in memory, language, executive function, and visuospatial orientation. The latter includes personality alterations, impaired judgment, mood disturbances, wandering, psychosis, sleep abnormalities and agitation. A reliable informant is required to provide a general medical, neurological, neuropsychiatric, and family history for suspected AD patients. Physical and

neurological examinations, routine laboratory examinations, optional laboratory examinations, and neuroimaging are included in the diagnostic evaluation. Complete blood count, thyroid function tests, sequential multiple analysis, vitamin B12, rapid plasma reagin, and folate are examples of routine laboratory tests, while erythrocyte sedimentation rate, serology for Lyme's disease, human immunodeficiency virus (HIV) serology, lumbar puncture, urinalysis, urine drug screen, and electroencephalography may be optional laboratory tests. Computed imaging or Magnetic Resonance Imaging (MRI) is used for neuroimaging. Utilizing standardized criteria such as the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM IV) criteria and the National Institute of Neurological and Communicative Diseases and Stroke—Alzheimer's Disease and Relatives, neuropathological examination (which involves identifying NFTs and plaques) from autopsy analysis has demonstrated to be 90% accurate in clinically detecting AD (Chao & Manita, 2013; McKhann et al., 1984).

AD gradually progresses, with an annual loss of 3 to 4 points on widely used diagnostic tools like the Mini-Mental State Examination (MMSE). While there are several deficiency patterns that may be observed, a common symptom is a gradual onset characterized by memory loss, ensued by the occurrence of aphasia, agnosia, and apraxia over time. In the early stages of AD, individuals may exhibit personality problems and irritability. Later on, patients typically experience gait and motor issues before becoming silent and bedridden. Despite the fact that the disease can persist for up to two decades, AD patients typically survive for 8 to 10 years post-diagnosis (Small et al., 1997).

Jack and his colleagues have undertaken the task of classifying biomarkers into three distinct categories, namely A (amyloid), T (phosphorylated tau), and N (neurodegeneration, assessed by the entirety of tau), constituting the ATN framework. This classification has been necessitated by the ever-evolving field of biomarkers, and the pressing need to render them efficacious in a diagnostic setting. According to their research model, the diagnosis of AD hinges on the presence of both Aβ and phosphorylated tau. It is imperative to note that AD diagnosis is solely established on biomarker data, and the manifestation of amyloid signifies an alteration in AD's pathology. It is noteworthy that AD is a protracted continuum that extends over several years, and the clinical phases may extend from cognitively mild to moderate cognitive deficiency and eventually dementia. The framework known as the ATN posits that Aβ and tau are the defining

features of AD, and thus biomarkers alone may be utilized to diagnose the condition. Furthermore, it unequivocally distinguishes between AD and dementia (Jack et al., 2018).

Despite receiving criticism for the omission of other significant causes of dementia, specifically vascular disease, the authors of the ATN framework maintain that the condition is underpinned by numerous underlying pathologies, with AD being one such pathology (Sweeney et al., 2019). It should be noted, however, that the diagnostic criteria for AD require the presence of Aβ and tau, although it is acknowledged that other pathologies may also be exhibited in affected patients (Jack et al., 2019). At present, the ATN approach is not considered to be suitable for clinical application due to the extensive amount of ATN categories. Additionally, the other disorders are not accounted for within the framework (Altomare et al., 2019). Furthermore, there exist pragmatic limitations regarding the definition of A, T, and N positive or negative, including the absence of established cutoff values for certain biomarkers and the grouping of diverse biomarkers into a solitary category. Despite the ATN approach being the basis for current research on disease-altering therapies, the clinical diagnosis of AD continues to depend on the standards established by the National Institute on Aging in 2011 (Cummings et al., 2019).

The ATN framework facilitates the possibility of personalized risk profiling for individuals suffering from mild forms of cognitive deficiency (Amsterdam et al., 2019). This advancement is significant as it allows for diagnosis prior to the onset of AD-associated dementia. Regardless of this development, a clinical research study examining doctor-patient interaction in clinics dealing with memory found that doctors tend to be hesitant in providing patients with mild cognitive impairment with specific prognostic information (Visser et al., 2020). The diagnosis of cognitive decline in predementia is considerably more challenging. ATN biomarkers have the ability to accurately predict the occurrence of dementia in those who experience subjective cognitive decline at a group level (Ebenau et al., 2020; Maurik et al., 2019). However, the process of personalized risk modelling remains challenging. According to a Delphi survey, both patients and their caregivers have expressed a preference for clear and specific information, even if it does not guarantee absolute assurance, during the diagnostic process (Fruijtier et al., 2019). The development of tools such as ADappt is urgently necessary to enhance decision-making and communication concerning a diagnosis of AD (Maurik et al., 2019).

1.4. Genetics

The comprehension of the prospective mechanisms behind the four identified genes that lead to familial forms of AD that provides the most compelling evidence for the significant role of Aβ. Though these genetic mutations (APP on chromosome 21, PSEN1 on chromosome 14, and PSEN2 on chromosome 1) are exceedingly rare, accounting for less than 1% of cases, patients with familial early-onset autosomal dominant AD have three of these distinct mutations. All of these genes appear to elevate the cleavage of APP β- and γ-secretase, which subsequently seems to increase the cellular synthesis of $A\beta_{42}$. The APOE ε4 allele, while not entirely necessary or sufficient in causing AD, is a notable risk factor for its development in both genders across a broad range of ethnic and racial groups between the ages of 40 and 90, as evidenced by a meta-analysis that included over 14,000 AD patients and controls. Estimates suggest that hereditary factors, including APOε-4, account for between 45% and 60% of AD risk. Rather than increasing Aβ production, it would seem that APOE ε4 operates by enhancing Aβ aggregation or reducing its clearance. Furthermore, Lipoprotein, a newly discovered potential risk factor, appears to have a dual role in AD risk, protecting against late-onset AD in non-carriers while simultaneously increasing the risk of late-onset AD in carriers (Mooser et al., 2000). As part of the EURODEM (European Investigations of Dementia) initiatives, a series of retrospective inquiries have divulged that women exhibit an escalated risk for AD while simultaneously exhibiting an equivalent risk for vascular dementia. This heightened susceptibility to AD among women may be partly attributed to their extended lifespans. In light of the fact that women with AD tend to outlive their male counterparts, there exists a twofold increase in the number of women suffering from this ailment in the general population. Furthermore, these inquiries have evidenced that a history of dementia in the family and a history of head trauma do not significantly elevate the risk of AD (Andersen et al., 1999; Launer et al., 2014). At present, the only known risk factors for AD are ageing and APOE ε4. In spite of the existing knowledge, conducting genotyping in asymptomatic individuals is not recommended, regardless of their AD history, due to the indeterminate predictive value, the absence of a remedy for the ailment, and the potential for discriminatory practices (Lapham et al., 1996; Mehlman et al., 1996).

Twin studies demonstrate that hereditary factors impact a significant percentage of the vulnerability to AD, ranging from 60 to 80 per cent (Gatz et al., 2006). Although the prevalence of the APOE ε4 genotype partially accounts for the heritability of this disease, it does not fully

explain it (Ridge et al., 2013; Bellenguez et al., 2017). The latest large-scale genome-wide association study, which included over 150,000 individuals with AD and matched controls, as well as over 300,000 individuals with a proxy phenotype of AD (i.e., those with a family history of the disease) and controls without such history, has increased the number of risk alleles for AD to more than 40. Various studies on the genome-wide association have demonstrated that the highly prevalent APOE ε4 risk allele is linked to a 3-4 times higher risk of AD. However, other alleles that pose a risk for the disease have lesser contributions to the overall risk of the disease (Jansen et al., 2019).

1.5. Comorbidity

Additionally, AD can coexist with other forms of dementia such as vascular dementia or Lewy-body dementia. However, there is a lack of clinical research on treating individuals with comorbidity of this type. Furthermore, AD-affected individuals often exhibit high levels of medical comorbidity such as heart disease, malignancies, and diabetes (Schachter & Davis, 2022).

1.6. Pathophysiology

The initial stage of AD at the cellular level is commonly referred to as the preclinical phase by researchers in the field. Prior to any observable cognitive impairment, the disease progresses surreptitiously as a result of alterations in neurons, microglia, and astroglia (Strooper & Karran, 2016). Within this cellular environment, there exists a confluence of factors such as neuroinflammation, vascular changes, ageing, and failure of the glymphatic system that precede or occur concurrently with amyloid accumulation (Lu et al., 2014; Venegas et al., 2017; Plog & Nedergaard, 2018; Sweeney et al., 2018; Mesquita et al., 2018). The mechanism by which amyloid induces the propagation of tau pathology, leading to the emergence of necroptosis markers present in the neurons exhibiting granulovacuolar degeneration, remains unknown (Long & Holtzman, 2019; Koper et al., 2020).

Utilizing single-cell transcriptome analysis, the response of microglia has been elucidated (Keren-Shaul et al., 2017). The two primary genes associated with the risk of AD, APOE and TREM2, play a substantial role in this reaction (Keren-Shaul et al., 2017; Parhizkar et al., 2019; Frigerio et al., 2019). The genetic variations of TREM2 i.e., Arg47His, Arg62His, and Asp87Asn, which are linked to AD, diminish TREM2's ability to bind to amyloid plaques, which in turn bind

to APOE (Yeh et al., 2016). In addition to these, seventy other proteins such as Protein-tyrosine kinase 2-beta (PTKB2), Cortactin-CD2-associated protein (CD2AP), Ras and Rab interactor 3 (RIN3), and Bridging Integrator-1 (BIN1), which have been correlated with a genetic risk of developing AD, appear to function downstream of TREM2 and APOE signalling to regulate endocytosis, phagocytosis, and motility, in microglia. It is evident that the pathways of microglial response play a critical role in pathophysiology, given the congregation of so many risk genes for AD (Leyns et al., 2017, 2019).

The incongruous outcomes of the microglial response partially demonstrate the constraints of mouse models with tau-overexpression for the investigation of AD. The strong transgenic tau overexpression, which is present in some models, may lead to a synthetically elevated neuroinflammatory response that is absent in models with a lower expression level (Leyns et al., 2017, 2019). These conflicting observations can be clarified by employing mouse-human chimeric mice, animal models not overexpressing tau, or novel *in vitro* models originating from human-induced pluripotent stem cells (Park et al., 2018; Hasselmann et al., 2019; Mancuso et al., 2019).

Although the focus of research on AD has been on cellular pathology, there has also been a significant advancement in comprehending the biochemical phase of the illness, specifically the existence of amyloid according to ATN terminology. Cryo-electron microscopy has contributed to a better understanding of tau and Aβ fibrils (Gremer et al., 2017). Furthermore, through cryo-electron microscopy, Presenilins, which are the catalytic subunits of γ-secretases, and their interactions with Notch substrates and APP have been comprehensively understood (Yang et al., 2018; Zhou et al., 2019). It is an established fact that alterations in Presenilins disrupt the connections between γ-secretase and APP, steering to the untimely release of extended, aggregation-prone amyloid peptides. This understanding has been supported by practical studies on isolated γ-secretase complexes (Szaruga et al., 2017). These findings provide motivation for the development of innovative therapeutic advances to combat Aβ in AD.

1.7. Treatment

The primary goals of treatment entail enhancements in cognitive functioning and mitigations in behavioural disturbances, including depression, psychosis, agitation, and sleeplessness.

1.7.1. Pharmacotherapy

Cognitive enhancers for the amelioration of cognitive deficits in AD are presently accessible, alongside mood stabilizers, antipsychotic agents, antidepressant medications, and hypnotic agents for the alleviation of behavioural disturbances.

1.7.1.1. Cholinesterase Inhibitors

The cholinergic deficiency observed in individuals with AD serves as the foundation for employing cholinesterase inhibitors. Exclusively, cholinesterase inhibitors have exhibited significant outcomes for AD-affected individuals. These compounds prevent the degradation of acetylcholine by the enzymes responsible for its breakdown (acetylcholinesterase), thus elevating the amount of acetylcholine available for synaptic transmission. Over the course of the disease, particularly in the intermediate stage, these medications seem to be favourable (Schachter & Davis, 2022).

Currently, donepezil, tacrine, galantamine, and rivastigmine are the cholinesterase inhibitors that are globally available for clinical utilization (Knapp et al., 1994; Bores et al., 1996; Rogers et al., 1998; Rösler et al., 1999). While doctors and families may not immediately perceive improved symptoms, patients taking these drugs appear to experience less cognitive decline than control patients. In order for a medication to be approved for treating AD in the US, it must surpass a placebo in a randomized clinical trial that employs both global clinical measurements and psychometric testing. The study must also last for a minimum of three months. Two commonly employed scales are the Clinician Interview-Based Impression Scale (CIBIS) and the AD Assessment Scale (ADAS-cog). The latter assesses several cognitive functions, including language, orientation, recall, recognition, naming, command-following ability, and constructional and ideational praxis. The ADAS-cog yields a potential score ranging between 0 to 70. High scores suggest increased impairment.

The degree of AD appears to elicit varied responses to cholinesterase inhibition, with individuals in the middle phase of AD (as defined by MMSE scores of 11 to 17) demonstrating greater responsiveness than those with mild AD (MMSE scores of 18 to 26). These observations support the thought that cholinergic dysfunction becomes statistically larger at this juncture of AD (Davis et al., 1999). Moreover, cholinesterase inhibitors may be employed in treating behavioural disorders in AD patients, with clinical trials indicating improvements in psychosis, agitation, and mood disturbances through this class of drugs (Raskind, 1998; Nordberg & Svensson, 1998).

Unfortunately, there exists a paucity of research comparing the tolerance and safety of cholinesterase inhibitors. Conclusively determining which cholinesterase inhibitor to take based on head-to-head research is not feasible.

1.7.1.2. Anti-Inflammatory Agents

Retrospective epidemiologic investigations have yielded evidence supporting the notion that anti-inflammatory medication may impede the progression of AD (Breitner et al., 1994; Stewart et al., 1997). Prospective clinical trials employing non-steroidal anti-inflammatory medications (NSAIDs) in AD are exceedingly rare. Non-randomized studies utilizing NSAIDs (indomethacin, naproxen, ibuprofen, diclofenac), steroids (low-dose prednisone), and other anti-inflammatory drugs (colchicine, and hydroxychloroquine) have demonstrated hopeful outcomes in regulation of the course of the disease (Rogers et al., 1993; Aisen et al., 1996; Scharf et al., 1999). Unfortunately, these investigations have employed limited sample sizes. However, more recently conducted studies have not confirmed the earlier encouraging findings. During a 16-month, placebo-controlled study, 138 AD-affected patients were administered Prednisone. The results of the research revealed no discernible difference in the rate of cognitive deterioration in comparison to placebo (Aisen et al., 2000).

1.7.2. Lifestyle Alterations

The inaugural guidelines from the WHO regarding the mitigation of the risk of cognitive deficiency and dementia were issued in the year 2019 (World Health Organization, 2019). These guidelines acknowledge the possibility of offering recommendations, albeit with varying degrees of precision, for specific factors such as physical exertion, dietary choices, body mass index, hypertension, smoking and alcohol consumption, and diabetes. Limitations of the existing corpus of research include a dearth of long-term, randomized, controlled studies, standardization issues, and the absence of evidence from low- and middle-income nations where dementia incidence is rapidly mounting.

The SPRINT-MIND trial has shown that thorough control of blood pressure (with a target of <120 mm Hg) is highly effective against cognitive impairment in comparison to simple blood pressure control (with a goal of <140 mm Hg). This supports the concept that measures benefiting cardiovascular health are also beneficial for brain health. Nevertheless, determining the best possible therapeutic aim remains an unresolved issue, especially in those over 70 years of age (Williamson et al., 2019). The inadequacies of individual interventions in the past have

emphasized the key requirement for a multimodal preventative method that has demonstrated efficacy in the fields of diabetes and cardiovascular prevention (Kivipelto et al., 2018).

The initial extensive, randomized controlled trial, which evidenced that a lifestyle-based intervention with multiple domains could mitigate the risk of cognitive decline in at-risk individuals, was the Finnish FINGER study (Ngandu et al., 2015; Solomon et al., 2018). FINGER incorporated a well-balanced, healthy diet, social activities, physical exercise, and management of vascular as well as metabolic risk factors with mental training. Even in individuals with a genetic predisposition to AD, the trial's affirmative effects on cognition were perceptible. The Dutch PreDIVA trial focused on the management of metabolic and vascular risk factors, while the French MAPT trial explored the correlation between lifestyle mediation and omega-3 fatty acid supplementation (Charante et al., 2016; Andrieu et al., 2017). Despite the identification of cognitive benefits in certain participant subgroups at heightened risk of dementia, the aforementioned trials were unable to attain their primary goals. In a particular study of the amyloid-PET utilizing MAPT trial, a lifestyle intervention either on its own or when paired with omega-3 fatty acids was correlated with an improvement in primary cognitive ability in individuals exhibiting positive amyloid status (Delrieu et al., 2019). This investigation serves to demonstrate that lifestyle aspects are capable of contributing positively towards the outcomes of individuals with AD, even when they do not explicitly influence the pathology of the condition.

1.8. Role of Nanotechnology

The perplexing question of how medicinal drugs can traverse the blood-brain barrier (BBB) to advance into the central nervous system (CNS) and exert their therapeutic effects has long confounded the field of modern medicine. The BBB, characterized by its predominantly semipermeable nature, poses a significant obstacle to the delivery of treatment medicines for brain disorders (Sarrazin et al., 2012; Burgess & Hynynen, 2014). However, alternative modalities such as photodynamic and photothermal therapy have demonstrated promise in circumventing the BBB and are routinely implemented for the management of various CNS ailments. Nonetheless, the utilization of photosensitization techniques raises additional challenges, notably adverse effects such as damage to skin tissues (Dhas et al., 2021).

The key advantage of utilizing smart nanoparticles (NPs) is their ability to respond to both internal and external stimuli in predictable and specific ways, which enhances the control of the

medication delivery process. Additionally, research has demonstrated that the covalent coupling of NPs and various ligands can significantly improve the efficacy of drug delivery (Zhou, et al., 2018). Niosome and glutathione peptide are two such ligands that can enhance the stability and solubility of Nanoparticle-based drug delivery systems (DDS). Moreover, the exploitation of specific ligands, for example, transferrin, has the potential to aid in directed treatment and the traversal of biological obstacles such as cell membranes and the BBB (Gharbavi et al., 2018; Nosrati et al., 2019).

Carbon, the profusely abundant element on earth, exists in many allotropes, such as diamond, fullerene, and graphite. Carbon nanomaterials, a fresh classification of carbon-based substances, consisting of nanodiamonds, fullerenes, graphene quantum dots (GQDs), carbon dots, and carbon nanofibers, have garnered significant attention in the realm of nanotechnology. The most recent addition to this family, with a magnitude less than 10 nm, is the Carbon Dots (CD). These nanomaterials are enriched with exceptional features, such as remarkable quantum yield, amplified biocompatibility, satisfactory water solubility, low cytotoxicity, and augmented cell permeability, which render them advantageous for employment in the fields of biology (Jaleel & Pramod, 2018; Jhonsi et al., 2018). CDs can be synthesized using a diverse array of chemical and natural precursors, employing top-down and bottom-up approaches. Nonetheless, a significant proportion of researchers tend to resort to chemical precursors to produce CDs. Consequently, it is imperative to develop sustainable methods and resources for the synthesis of CDs, in order to mitigate the deleterious impacts associated with their production (Sharma et al., 2017; Thakur et al., 2019). In this regard, some researchers have turned to organic precursors for the production of CDs, owing to their high renewability, superior biocompatibility, and cost-effectiveness. CDs have been synthesized from an assortment of sources, including plants, beverages, fruits, and vegetables. Among these precursors, plant-derived CDs are currently receiving greater attention from researchers (Tejwan et al., 2022).

Due to their distinctive properties, encompassing exceptional photoluminescence (PL), high biocompatibility, abundance of surface functional groups, non-toxicity, and nanoscale dimensions, CDs are among the most auspicious candidates for BBB penetration (Li et al., 2016; Seven et al., 2019; Zhou et al., 2019). In addition, their capacity to traverse the BBB can be substantially influenced by precursor materials, synthetic techniques, and post-synthesis procedures. According to numerous studies, CDs can traverse the BBB even with cargo molecules.

Essentially, CDs have demonstrated considerable capability as adaptable drug nanocarriers that can penetrate the BBB and provide treatment for CNS ailments (Kappe, 2004; Medeiros et al., 2019; Tajik et al., 2020).

1.9. Synthesizing Carbon Dots

Synthesis of CDs is carried out using top-down and bottom-up approaches, with a several forms of reaction and precursors employed (Tejwan et al., 2020). Microwave irradiation stands out as the most widely adopted technique for CD preparation, owing to its rapid response time and uniform heating (Kappe, 2004). The creation of CDs from polyethylene glycol (PEG) and sucrose using this method was first accomplished by Zhu et al. (2009). Subsequently, Wang et al. (2011) employed the same approach to generate CDs from a range of other carbohydrates. The production of CDs is predominantly accomplished through microwave-mediated processes, with hydrothermal/solvothermal procedures following closely behind. An illustration of this is demonstrated by Zhao et al., (2017) who utilized benzenetetramine to generate CDs with emission wavelengths exceeding 600 nm. The synthesis of CDs through hydrothermal or solvothermal means has been conducted using a variety of precursors, such as proteins, polysaccharides, and various organic acids including citric acids and amino acids (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). Conversely, ultrasonication is a more subtle approach in contrast to microwave irradiation or hydrothermal/solvothermal methods, albeit it is not as frequently applied (Tejwan et al., 2021). Zhou et al. (2018) reported that O-Phenylenediamine (OPD) and Citric acid were utilized as foundations to create a distinctive variety of excitation-independent yellow-emissive CDs (Y-CDs). In addition, Ajmal et al. suggested that probe sonication could be employed to create CDs from glucose (Ajmal et al., 2019).

CDs possess optical characteristics that are largely similar, with only minor differences. They are characterized by excitation-dependent emission, which was previously restricted to the blue-green range of light but has now also expanded to encompass the orange-red and even near-IR (NIR) range (Mintz et al., 2019; Shi et al., 2019). Remarkably, the orange-red and NIR PLs of CDs exhibit considerably lower excitation dependence than their blue-green counterparts. On the other hand, GQDs exhibit much more clearly defined structural characteristics than CDs, resulting in less variation in their PL properties (Zheng & Wu, 2017). However, the inclusion of heteroatoms

and the size of the sp^2 carbon complexes in the particle can still modify their emissions, often causing them to shift from blue to orange (Zhu et al., 2015; Shen et al., 2020; Wang et al., 2020). CDs and generic nitrogen-doped CDs (NCDs) exhibit similar characteristics (Zhou et al., 2013; Liyanage et al., 2019).

The primary differentiation among various CDs is found specifically in the core structure. Typically, the carbon nanodot centres are amorphous (Mintz et al., 2019). While some research proposes a hybrid sp^2/sp^3 structure for CDs, others demonstrate a more carbon-based/polymer-like structure (Xia et al., 2019). GQDs, by definition, consist of a few assembled graphene sheets (Tajik et al., 2020). In their cores, s-triazine or other carbon nitride structures are frequently found in CNDs. Although the fundamental structure of each class of CDs differs, it is believed that the surfaces of these molecules are composed of simple functional groups or small organic molecules.

Since their inception, CDs have been extensively scrutinized for a plethora of applications in the realm of nanomedicine, as previously alluded to (Tejwan et al., 2020). In 2007, Sun and colleagues initially detected CDs in human breast cancer cells (Cao et al., 2007). Various concentrations of CD solutions were administered to healthy or cancerous cells to assess their cytotoxicity and biocompatibility. Cell viability was gauged following exposure to CD solutions, and the findings evinced that all CDs had minimal cytotoxicity (Lu et al., 2016; Zhou et al., 2019). CDs' stability was assessed by examining their sizes, surface charges, and architectures subsequent to cell incubation under varied pH conditions. Comparable zeta potentials and sizes revealed that the physiological states and surface of CDs remained stable even after cellular internalization. Moreover, atomic force microscopy images demonstrated the stability of CDs for a duration exceeding seven days under various pH levels (Wang et al., 2017; Liu et al., 2018; Niu et al., 2020). The biocompatibility of CDs was first assessed in mice in 2009, wherein three distinct groups of mice were injected with CD aqueous solutions, and all survived the experimentation period of four weeks without displaying any clinical symptoms or aggressive behaviour. Furthermore, serum biochemistry assays affirmed the normal functioning of hepatic and renal systems (Yang, et al., 2009a). In the same year, Yang and colleagues provided the first instance of bioimaging using CDs in animal models, which initially received limited attention until the middle of the decade when interest in the subject surged dramatically (Yang et al., 2009b).

Both *in vitro* and *in vivo* models have been established to assess the proficiency of CDs and CD-conjugated derivatives to traverse the BBB. In their study, Lu et al. employed a one-pot

hydrothermal process to fabricate NCDs and evaluated their BBB-penetration potential employing an *in vitro* model comprising rat microvascular endothelial cells and astrocytes (Lu et al., 2016). The strong blue photoluminescence of NCDs under UV-Vis Spectroscopy served as evidence of their transport through the BBB in a concentration-dependent manner. Notably, the transwell model utilized in the NCDs investigation constitutes an *in vitro* approach. Nevertheless, this biomimetic model suffers from a primary limitation, namely its inability to accurately replicate the BBB, despite the existence of tunable parameters and minimal variations. Zhou et al. (2020) have identified a CD class that resembles gels and is capable of penetrating the BBB using a zebrafish model. It is noteworthy that the zebrafish CNS physiology shares several similarities with humans, including key nervous system constituents such as transmitters, hormones, and receptors (Panula et al., 2006). Moreover, zebrafish exhibit a higher rate of progeny generation compared to mice, thereby facilitating reproducibility of experiments (Zhou, et al., 2018). Furthermore, zebrafish progeny is cost-effective and can be efficiently raised in a limited area (Panula et al., 2006).

The creation of novel CD conjugates that can cross the BBB for drug delivery relies on a more comprehensive mechanism by which CDs traverse the BBB. The intercellular tight junctions (TJs) between endothelial cells uphold a distance of 4 nm to 6 nm, enabling CDs smaller than 4 nm to cross the gap through passive means (Cai et al., 2016). Furthermore, electric charge forms an additional obstacle to the gap. TJs feature anionic regions that attract supplementary CDs and cationic molecules (Bilensoy, 2010). For example, the NCDs fabricated from PEG, as mentioned above, exhibit a positively charged surface (Lu et al., 2016). The 2.6 nm particle size and positively charged surface of NCDs enable them to diffuse passively across the BBB. The BBB's internal lipid barrier is inherently hydrophobic. Nevertheless, the amphiphilic Y-CDs successfully surmounted the lipid wall and migrated through the cerebrospinal fluid (CSF) (Zhou et al., 2019). However, once the BBB is crossed, molecules must be hydrophilic to permeate through the CSF in the CNS. Passive diffusion is generally regarded as the most prevalent explanation for BBB penetration owing to the numerous intrinsic characteristics of CDs.

Furthermore, receptor-mediated endocytosis is a well-known technique for facilitating the crossing of CDs across the BBB. Li et al. (2016) demonstrated that the BBB cannot be penetrated by CDs alone but can be achieved through receptor-mediated endocytosis when combined with human transferrin. Another method involves the creation of self-targeting CDs using substances that are ligands for specific transporters. The resulting CDs will share similar functional groups

with their predecessors, thereby eliminating the need for further surface modification processes. Seven et al. showed that the BBB can be crossed via GLUT1-mediated transport by CDs made from glucose (GluCDs), a finding supported by two *in vivo* models: zebrafish and rats. Additionally, GluCDs were found to traverse the BBB with fluorescein (Seven et al., 2019). Furthermore, Mintz et al. utilized tryptophan as a precursor in the creation of CDs. The CDs synthesized with a tryptophan moiety on the surface were able to be identified and thus were able to successfully traverse the blood-brain barrier via LAT1-mediated transport. This demonstrates that CDs are efficacious delivery systems for medicinal treatment in central nervous system conditions (Mintz et al., 2019).

1.10. Application Of Carbon Dots in Treating CNS Diseases

CDs have exhibited their ability to traverse the BBB. Furthermore, the surfaces of CDs are endowed with abundant amine ($-NH_2$) and carboxyl (-COOH) groups, which can be leveraged to couple with diverse CNS pharmaceuticals. Additionally, CDs are biocompatible, rendering them suitable for biological therapeutics. For the treatment of AD and brain tumours, CDs serve as exceptional nanocarriers for delivering drugs into the CNS. One of the most widely used treatments for AD involves lowering the deposition of Aβ in the CNS.

Notably, Y-shaped carbon dots have exhibited the capacity to impede the generation of APP and Aβ in cells, rendering them suitable for employment as medicinal nanocarriers and nanomedicine in the context of AD (Zhou et al., 2019). In a research done by Liu et al. (2018), it was observed GQDs are capable of preventing Aβ aggregation. The potency of GQDs as an inhibitor of Aβ aggregation is further amplified upon their linkage with tramiprosate. Gong et al. (2016) found that functionalized CDs loaded with glycine-proline-glutamate effectively prevent Aβ aggregation and the reduction of proinflammatory cytokines. Similarly, the OPD-derived CDs developed by Chung et al. (2019) possess cationic surfaces and have been reported to be effective in preventing Aβ aggregation by coordinating with copper ions.

1.11. Natural Flavonoids as Treatment

Traditional medicinal practices have embraced a multitude of herbal remedies in treating various ailments since ancient times. These remedies, apart from serving as a source of nutrition, have proven to be effective in curing a range of disorders (Ang-Lee et al., 2001). In comparison to pharmaceuticals, certain plant extracts have demonstrated superior performance while being less

toxic and having fewer side effects (Awaad et al., 2011). The therapeutic characterestics of plants can be attributed to the presence of flavonoids, glycosides, tannins, alkaloids, terpenoids, and other secondary metabolites. The choice of solvent used during extraction plays a significant role in extracting physiologically active chemicals from plants. Out of the various solvents commonly used for extraction, such as chloroform, alcohols, ethyl acetate, and ether, water is by far the safest. While the antioxidant properties of flavonoids are widely recognized, their ability to modulate intracellular reactions, chiefly via the alteration of protein kinase signalling pathways, is indisputable (Rates, 2001). Furthermore, a considerable and growing body of evidence suggests that flavonoids can impede the pathways associated with AD. Nevertheless, there remains a substantial amount of ambiguity regarding their absorption, metabolism, and fundamental pharmacokinetics, which has hindered the progress of flavonoids as therapeutic interventions for AD.

The potential for Rutin (3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside), a hydrophobic natural flavonoid, to be delivered through CDs was explored in this study (Negahdari et al., 2021). Rutin is a significant component of our daily diet and provides considerable nutritional benefits. It can be found in substantial quantities in various fruits and vegetables such as asparagus, apples, buckwheat, figs, plums, grapes, and oranges, as well as in several beverages including green tea, elderflower tea, black tea (Hassan et al., 2018). Rutin demonstrates a diverse range of pharmacological effects and can be employed to treat a broad spectrum of ailments such as diabetes, hypertension, cancer, and hypercholesterolemia. Moreover, it is renowned for its cytoprotective, antibacterial, antioxidant, anticarcinogenic, vasoprotective, neuroprotective, and cardioprotective properties (Nafees et al., 2018). The limited water solubility and cell permeability of Rutin greatly impede its potential to induce pharmacological effects (Gullón et al., 2017). To overcome these restrictions, CDs were utilized as a drug transporting vehicle to enhance the pharmacological activities of the medication.

1.12. Protective Effects of Rutin

1.12.1. Central Nervous system

The efficacy of Rutin as a neuroprotective agent against brain ischemia has been established. Rutin administration has been observed to mitigate ischemic neural apoptosis through lipid peroxidation, reduction of p53 expression, and the upregulation of endogenous antioxidant

defence enzymes (Khan et al., 2009). Additionally, Pu et al., (2007) have reported its effectiveness in treating oxidative, glutamate, and hypoxia stress. Rutin administration has also been shown to reduce neuroinflammation in a sporadic AD-like rat model and to confer neuroprotective effects in mice treated with dexamethasone (Tongjaroenbuangam et al., 2011; Javed et al., 2012). Rutin also possesses anticonvulsant properties, as suggested by research. Furthermore, individuals with epilepsy may safely consume it as it does not demonstrate any adverse effects or interfere with the administration of antiepileptic drugs, according to Nieoczym et al.'s study conducted in 2014.

Aside from the neuroprotective effects, the neural crest, a progenitor with both mesenchymal and neural potentials, experienced enhanced trunk neural crest cell survival through Rutin treatment, while differentiation and proliferation remained unaffected. This phenomenon may be linked to the modification of extracellular signal-regulated kinase (ERK2) and phosphoinositide 3-kinases (PI3K) signalling, as suggested by (Nones et al., 2012). The treatment of schizophrenia poses a significant challenge due to the emergence of "tardive dyskinesia," a motor condition affecting the orofacial region, brought on by prolonged use of neuroleptic medications. This condition represents a major clinical problem. In a research study by Bishnoi et al., (2007), Rutin treatment was found to be effective in correcting behavioural alterations like stereotypic rearing, orofacial dyskinetic movements, locomotor activity, and percentage retention. Furthermore, the study revealed that Rutin treatment also led to the restoration of metabolic and neurochemical parameters in orofacial dyskinesia caused by haloperidol. Therefore, it appears that Rutin represents a key drug in the fight against hyperkinetic movement disorder.

Lloyd-Jones et al., (2010) assert that stroke is a significant public health concern globally, owing to its major role in adult mortality and disability. Ischemic injury to the brain has been linked to inflammation and oxidative stress which are two pathogenic occurrences that are commonly observed (Deb et al., 2010). In mice induced with localized cortical ischemia triggered by thermocoagulation of the primary motor (M1) and somatosensory (S1) cortical blood arteries, Rutin's protective effects were investigated. The administration of Rutin significantly aided in the recovery of sensorimotor loss, as evidenced by a reduction in neurodegeneration in the cortical injury's periphery (Ortolani et al., 1995).

The present investigation scrutinized the properties of Rutin similar to anti-depressants isolated from plant *Schinus molle*, through the utilization of tail suspension and forced swimming tests in murine subjects. In the tail suspension test, a decrease in immobility time was observed

while the locomotor system activity remained unchanged. As per the findings, Rutin engenders an antidepressant-like outcome by enhancing the accessibility of noradrenaline and serotonin in the synaptic cleft (Machado et al., 2008).

1.12.2. Antiarthritic Effects

Rutin-treated animals demonstrated a substantial enhancement in rheumatoid arthritis and Fanconi anaemia due to the prevention of "oxygen radical overproduction" as noted by Ostrakhovitch & Afanas'ev, (2001). As per Guardia et al., (2001)'s study, Rutin exhibited greater efficacy during the chronic stage of inflammation. In the remedy for septic arthritis caused by *Candida albicans*, Rutin's antifungal and anti-arthritic properties proved to be effective according to Han, (2009). Moreover, in a separate study conducted on Hartley guinea pigs, Rutin was observed to inhibit the catabolic cartilage and inflammatory markers in osteoarthritic lesions (Horcajada et al., 2015).

1.12.3. Endocrine System

Hyperglycemia is primarily caused by excessive glucose synthesis and insufficient tissue utilization of glucose (Chattopadhyay, 1993). Researchers revealed that constant Rutin treatment effectively increased insulin levels decreased plasma glucose levels and improved the levels of glycolytic enzymes and glycogen in streptozotocin-induced diabetic rodents. Furthermore, Rutin-treated diabetic rats demonstrated significant pancreatic regeneration in addition to a decrease in fatty infiltration (Srinivasan et al., 2005; Prince, 2006).

In male Wistar rats fed a high-cholesterol diet, Rutin administration was found to protect against hepatotoxicity. This was evidenced by a reduction in plasma levels of aspartate aminotransferase, alanine transaminase, total cholesterol, triglyceride, and low-density lipoprotein, according to Al-Rejaie et al., (2013). Additionally, regular consumption of flavonoids, for example, Rutin was found to be beneficial for cardiac health, as described in a separate study by Kalgaonkar et al., (2010).

The phenomenon commonly referred to as "thyroid iodide uptake," which is under the regulatory control of the sodium-iodide symporter, plays a crucial part in the synthesis of thyroid hormones as well as in the detection and management of diverse thyroid disorders. Nonetheless, a considerable proportion of thyroid cancer patients exhibit reluctance towards radioiodine therapy, which leads to a significant decline in iodine uptake capacity and, consequently, diminishes the likelihood of survival. Hence, it becomes of utmost importance to explore the potential of organic

substances in facilitating thyroid iodide absorption. In an investigation, Rutin exhibited a slight reduction in serum levels of T4 and T3 but did not affect thyrotropin levels. This was accompanied by a decline in the activity of liver type-1 deiodinase and a substantial increase in type-2 deiodinase in the hypothalamus, brown adipose, and pituitary tissue. Rutin intake was observed to be correlated with an elevation in thyroid iodide uptake, possibly attributable to an augmentation in sodium-iodide symporter activity. Based on the findings of Gonçalves et al., (2013), Rutin demonstrates efficacy as an adjunct in radioiodine therapy.

1.12.4. Cardiovascular System

Buckwheat, a plant that is abundant in Rutin, has been found to decrease nitrotyrosine immunoreactivity and protect aortic endothelial cells from oxidative damage. This is due to the potent antioxidant properties of Rutin present in buckwheat. A study by Dae et al., (2009) has also shown that a buckwheat germination extract has antihypertensive effects and is capable of protecting arterial endothelial cells from the detrimental effects arising from oxidative stress. Furthermore, oral intake of Rutin has appeared to reduce oxidative stress, which is the key element in restoring decreased vascular reactivity and baroreflex sensitivity in rat models induced to be hypertensive, as reported by Mendes-Junior et al., (2013). Additionally, Ugusman et al., (2014) have exhibited that Rutin enhances endothelial activities by increasing the generation of nitric oxide in human endothelial cells.

In vitro, Rutin was observed to exhibit a concentration-dependent inhibition of platelet aggregation in rabbit platelets induced by a platelet-activating factor. Furthermore, in a dose-dependent manner, Rutin was also observed to inhibit the increase in intra-platelet-free calcium concentration caused by the platelet-activating factor. These findings were documented by Chen et al., (2002).

1.12.5. Gastrointestinal System

Ethanol, a noxious substance, has been shown by both animal and human studies to inflict damage on the stomach mucosa. According to Szabo & Goldberg, (2009), concentrations of ethanol exceeding 400 ml/l have an impact on the general morphology of the stomach and are linked to mucosal hyperemia, mucosal or submucosal haemorrhage, necrosis, and edoema. The production of free radicals by oxygen may be considered a crucial factor in the development of lesions, as suggested by Pihan et al., (1987) and Szelenyi & Brune, (1988). Prior treatment with Rutin, followed by ethanol administration, yielded considerable protection against necrosis. La

Casa et al., (2000) reported the restoration of glutathione peroxidase levels and an 'anti-lipoperoxidant effect'. Rutin treatment in rats led to the correction of oxidative stress and metabolic parameters in an ulcer model induced by indomethacin, likely due to the reduced oxidative stress generation, neutrophil infiltration, and replenishment of nitrate/nitrite levels. In addition, Abdel-Raheem, (2010) demonstrated the protective benefits of Rutin through histopathological studies.

1.12.6. Respiratory system

Airway resistance was evaluated in ovalbumin-sensitized conscious guinea pigs in order to investigate the anti-asthmatic activity of Rutin. Specifically, airway opposition during the immediate-phase reaction and the late-phase response were measured following exposure to aerosolized ovalbumin. Histamine resistance, phospholipase A2 reluctance, and eosinophil peroxidase were also examined. Results indicated that Rutin substantially reduced specific airway opposition and immediate-phase responsiveness, while also decreasing the recruitment of neutrophils and eosinophils into the lungs (Jung et al., 2007). Supplementarily to vitamins C and K, Rutin has been suggested for the treatment of whooping cough. Moreover, Rutin is efficacious in the treatment of idiopathic chylothorax in cats and whippets (Gould, 2004; Kopko, 2005; Schuller et al., 2011).

1.13. Aims and Objectives

This research hypothesizes that nanohybrids composed of NCDs and Rutin would demonstrate improvement in cognitive ability due to the pharmacological effects of the hydrophobic drug. This assertion is based on the fact that Rutin possesses the ability to reduce neuroinflammation in rodents of dementia of Alzheimer's type and to confer neuroprotective effects.

1) Design and synthesis of NCDs
2) Characterization of NCD-Rutin
3) Establishment of AD-like animal models
4) Evaluation of effectivity of NCD-Rutin using behavioural tests

CHAPTER 2
MATERIALS & METHODOLOGY

2.1. Ethical Approval

Before conducting *in vivo* study, ethical approval (IRB no. 05-2023-ASAB-02/02) was acquired from the NUST-IRB committee of the National University of Sciences and Technology, Islamabad.

2.2. Chemicals

Glucose (BCCF4025, Sigma Aldrich, Switzerland), Ammonia (10314944, Honeywell, Germany), Rutin hydrate (207671-50-9, Macklin, China), dialysis membrane (500 Da cut off, MD55, Scientific Research Special, China), Phosphate Buffered Saline (2810305, MP Biomedicals, France), Dimethyl sulfoxide (67-68-5, Sigma Aldrich, Switzerland) and Ultrapure Millipore water (7732-18-5, Sigma Aldrich, Switzerland). Sodium hydroxide (NaOH) was purchased from a local vendor.

2.3. Preparation of NCDs

Using the uniform heating technique, nitrogen-doped CDs were prepared by dissolving 5 g of glucose in 50 mL of water. Following vigorous swirling, 2.5 mL of ammonia was added to the aforementioned solution. The resulting translucent mixture was subjected to a temperature of 250 °C for a duration of 30 minutes in a laboratory oven. The colour transition of the sample from translucent to pale yellow is indicative of the presence of NCDs. To ensure the complete elimination of any remaining ammonia, the pale-yellow solution was rapidly agitated for an hour after being chilled. After that, pH 7 was preserved by adding NaOH/HCl solution dropwise using a basic pH meter. To eliminate residual unfused small and large molecules, the aqueous solution of NCDs underwent a dialysis process against Millipore water for a duration of 48 hours using a dialysis membrane prior to characterization.

2.4. Preparation of NCDs-Rutin

The dissolution of Rutin in DMSO was amalgamated with the aqueous CD solution using a mass ratio of 1:1. Stirring was performed at room temperature throughout the night while Rutin was being loaded. The resulting solution was subsequently dialyzed for 2 hours to remove any unbound molecules. To acquire NCDs-Rutin in the form of powder, the purified, drug-loaded NCDs were subjected to lyophilization.

2.5. Characterization Of NCDs-Rutin

The morphological structure and chemical composition of prepared, NCDs were characterized using different techniques, i.e., Ultraviolet-Visible (UV-Vis) Spectrophotometer, Atomic Force Microscopy (AFM), and Fourier transform infrared radiation (FTIR).

2.5.1. FTIR

FTIR (Agilent Cary 630, Agilent Technologies, USA) has been employed to detect the functional groups present on the surface of NCDs. The primary constituents of the NCDs under investigation are carbon, hydrogen, nitrogen, and oxygen. The prevalence of carboxyl or carboxylic acid groups and hydroxyl groups on the CDs' surface is a well-established fact, owing to the partial oxidation of a carbon precursor during the creation of CDs. The bonds and functional groups in the sample were analysed by FTIR spectroscopy in a range of 500-4000 cm^{-1} at 4 cm^{-1} resolution.

2.5.2. UV-Vis Spectrophotometer

UV-Vis absorption spectrophotometer (UV-1602, BMS, Germany) with a resolution of 1 nm between the range of 200 and 600 nm was used. The binding of NCDs with Rutin was monitored by measuring the spectrum of the reaction mixture in a UV-Visible spectrophotometer with the amount of suspension that is 3 ml of the sample was pipette in a cuvette and analysed at room temperature.

2.5.3. AFM

AFM investigations were conducted using a commercial silicon tip (RTESPA 300, Bruker, USA) with a resonance of 300 kHz and nominal elastic constant of 40 N m^{-1} on a Multimode 8 Bruker AFM microscope equipped with a Nanoscope V controller. The ScanAsyst^{TM} was utilized with a scan size of 3 mm. To prepare the samples, 6 mL of solutions were applied onto a silicon glass slide and subsequently dried at 40 ºC in an oven, overnight.

2.6. Haemolytic Assay

The haemolytic assay was implemented as a means of evaluating compatibility with blood. To isolate red blood cells (RBCs), 0.5 ml of whole blood sample was combined with 1 ml of phosphate-buffered saline (PBS). This centrifugation process was executed at 10,000 g for a duration of 5 minutes. The RBCs underwent five additional washes with 1 ml of PBS. 0.2 ml of diluted RBC suspension was added to (a) 0.8 ml of the nanoparticle PBS suspension at a concentration of percentages 0.1, 0.5, 1, 1.5, 2, 10, 20, and 30; (b) deionized water (positive group), and (c) PBS (negative group). Following an incubation period of 60 mins at 37ºC temperature and centrifugation for five minutes at 10000 g, 100 µl of the supernatant from all samples was transferred to a 96-well plate. Subsequently, utilizing a microplate reader (Thermo Scientific™ Multiskan™ Sky), the absorbance was measured at 577 nm. The rate of haemolysis was quantified using the haemolytic ratio, which is calculated as follows:

$$\text{Haemolysis rate (\%)} = \frac{(OD_{(\text{test})} - OD_{(\text{negative control})})}{(OD_{(\text{Positive control})} - OD_{(\text{negative control})})}\ x\ 100$$

2.7. Animal Testing

Adult male Albino rats (n = 12) were purchased and housed in the animal house of Atta-ur-Rahman School of Applied Biosciences (ASAB), National University of Sciences & Technology (NUST), Islamabad, under controlled environmental conditions (25 ± 2 °C). Natural light and dark cycles (14 h light and 10 h dark) were followed. Feed and water ad libitum were provided to the animals. They were divided into three groups of 4, designated as Groups 1-3. Group 3 was a control group and Groups 1 and 2 were $AlCl_3$-treated groups (for 15 days). Group 2 was then treated with a 10 mg/kg dose of Rutin-bound NCDs. PBS was used as a vehicle.

2.7.1. Behavioural Testing

Behaviour tests were performed on the 16^{th} day of the aluminium treatment and one week after administering the NCD-Rutin dose. Animals were moved to the behavioural testing area 30 minutes prior to the beginning of the behaviour test. The tests performed were novel object recognition test, Open field Test, Morris water maze test, and Y maze test.

2.7.1.1. Morris Water Maze (MWM) Test

The maze in this test consists of a pool containing opaque water and a platform submerged underwater. The rats were trained by allowing them to swim for 90 seconds and find the platform.

Each was allowed to remain on it for 10 seconds. Those unable to find the platform were steered to it. The training was given for 5 consecutive days. On the last day of training, the rats' memory was tested without the platform. A video of the performance of each was recorded (Takeuchi et al., 2011).

2.7.1.2. Y-Maze Test

This apparatus consists of three arms of different lengths (8, 30 and 15 cm) at a 120° angle. In the first of these 2-trial tests, the rats were permitted to roam freely in 2 of the arms which are the start arm and the familiar arm for 10 minutes. The third arm, which is the novel arm, was closed. In the second trial, the rats were allowed to roam freely in all three arms for 5 minutes. The time spent in the novel arm was documented in the form of a video. Rats exhibit exploratory behaviour characterized by frequent visits to novel locations. In the Y-maze, rats are expected to alternate between the three arms, transitioning from the "familiar arm" to the "novel arm" and ultimately to the "start arm," while bearing in mind the prior examination of the preceding arms. However, under pathological conditions, rats fail to alternate between the three arms, exhibiting an inability to recall the prior investigation of one arm. The percentage alteration is calculated using the following formula (Takeuchi et al., 2011).

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

2.7.1.3. Novel Object Recognition (NOR) Test

This test focuses on the ability of rats to interact with novel objects rather than familiar objects. Initially, they were allowed to freely explore a behavioural arena box for 5 minutes, a day before the test. They were then trained by placing them in a box having 2 identical objects at specific locations, for 5 minutes. The box was then wiped with ethanol to remove olfactory cues. After a retention time of 24 hours, the rats were tested by replacing the original objects with a novel object and allowed to explore for 5 minutes (Zhang et al., 2012).

2.7.1.4. Open Field Test

The flooring of the hardwood equipment, measuring W100 cm x D100 cm x H40 cm, was partitioned into 16 squares (4x4) white lines. The rats were situated in one of the corners, and the following conduct was observed for a duration of five minutes. (a) The number of squares entered

(including both forelimbs) which counts as one. The animal's examination of the central 4 squares and the external 12 squares next to the wall regions were tallied separately. (b) The act of grooming, which encompasses activities such as licking one's fur, cleaning one's face, and scratching, is also noted. (c) The number of instances of rearing, or the act of sniffing and scanning the area while standing on the hind limbs and intermittently leaning against a wall with the forelegs (Takeuchi et al., 2011).

2.8. Statistical Analysis

The data was statistically analysed by student's t-test or analysis of variance (ANOVA) followed by Tukey's multiple comparison test and a *p*-value of <0.05 was considered to be statistically significant. All data are expressed as mean ± standard error of the mean (SEM).

CHAPTER 3

RESULTS

3.1. FTIR Analysis

The presented Figure 3.1 indicates the presence of hydroxyl, carboxyl, and carbonyl groups on the surface edges of NCDs. They displayed an absorption peak at 1637.88 cm^{-1} (C=O) and 1425.82 cm^{-1} (C-H). The IR absorption peak for hydroxyl (O-H) was observed at 3278.78 cm^{-1}, and the vibration region C=N stretching was noted at 1630 cm^{-1}, as depicted in Figure 3.1.

The vibrational frequency of the important *ν*(C=O) bond in Rutin was discovered to be 1651.62 cm^{-1}. Notably, the benzene ring skeleton in Rutin exhibits *ν*(C=C) stretching bonds at 1595.63 cm^{-1}. The stretching vibrations of the OH/H_2O groups were observed as wide bands at 3342.48 cm^{-1}. NCD-Rutin depicted a peak at 3266.48 cm^{-1} and 1593.16 cm^{-1} corresponding to the original samples showing OH groups and C=C bonds.

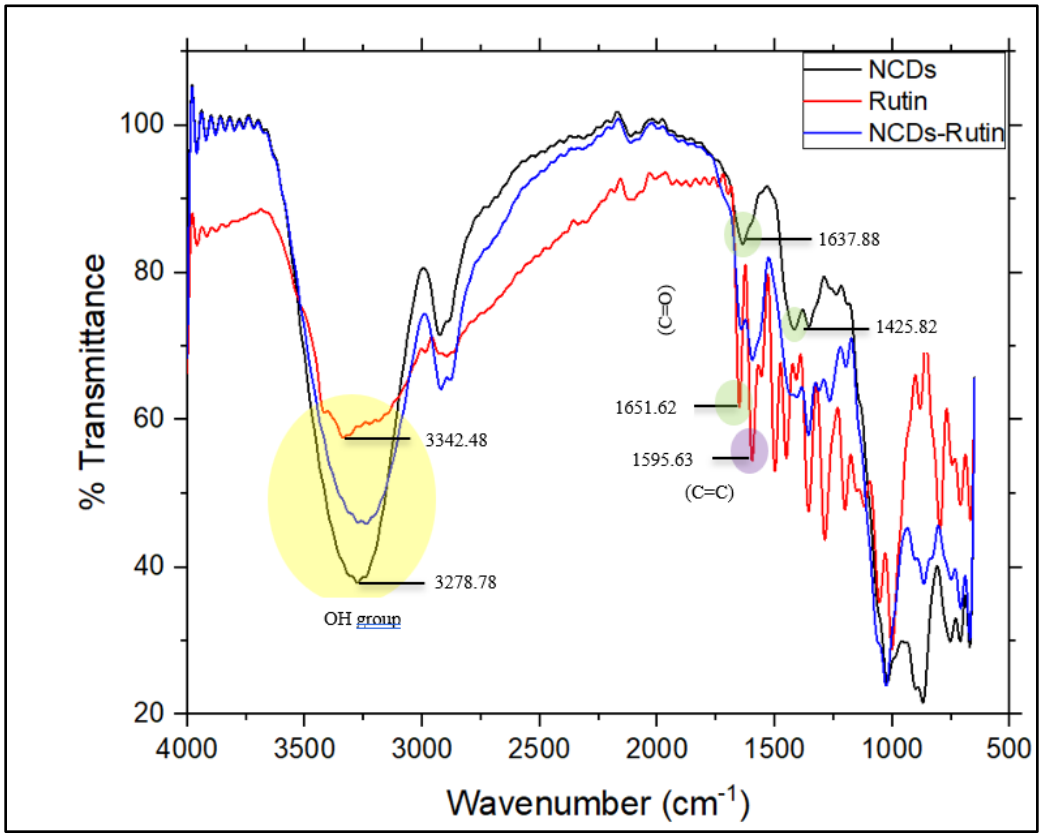

Figure 3.1: FTIR spectra of NCDs, Rutin and NCD-Rutin. The black line depicts the IR spectra of simple NCDs, red represents the Rutin, while blue shows Rutin-bound NCDs. The yellow

highlighted area depicts the presence of OH groups in the compounds. Green represents the C=O bond and purple shows the C=C bond.

3.2. UV-Vis Spectrophotometry

The UV-Vis spectra of NCDs (Figure 3.2) exhibited distinct absorption peaks at 215, 270 nm and 310 nm. Rutin exhibits two absorption bands, specifically at 360 nm (band I) and 295 nm (band II). NCD-Rutin showed similar peaks to Rutin at 295 nm and 355 nm.

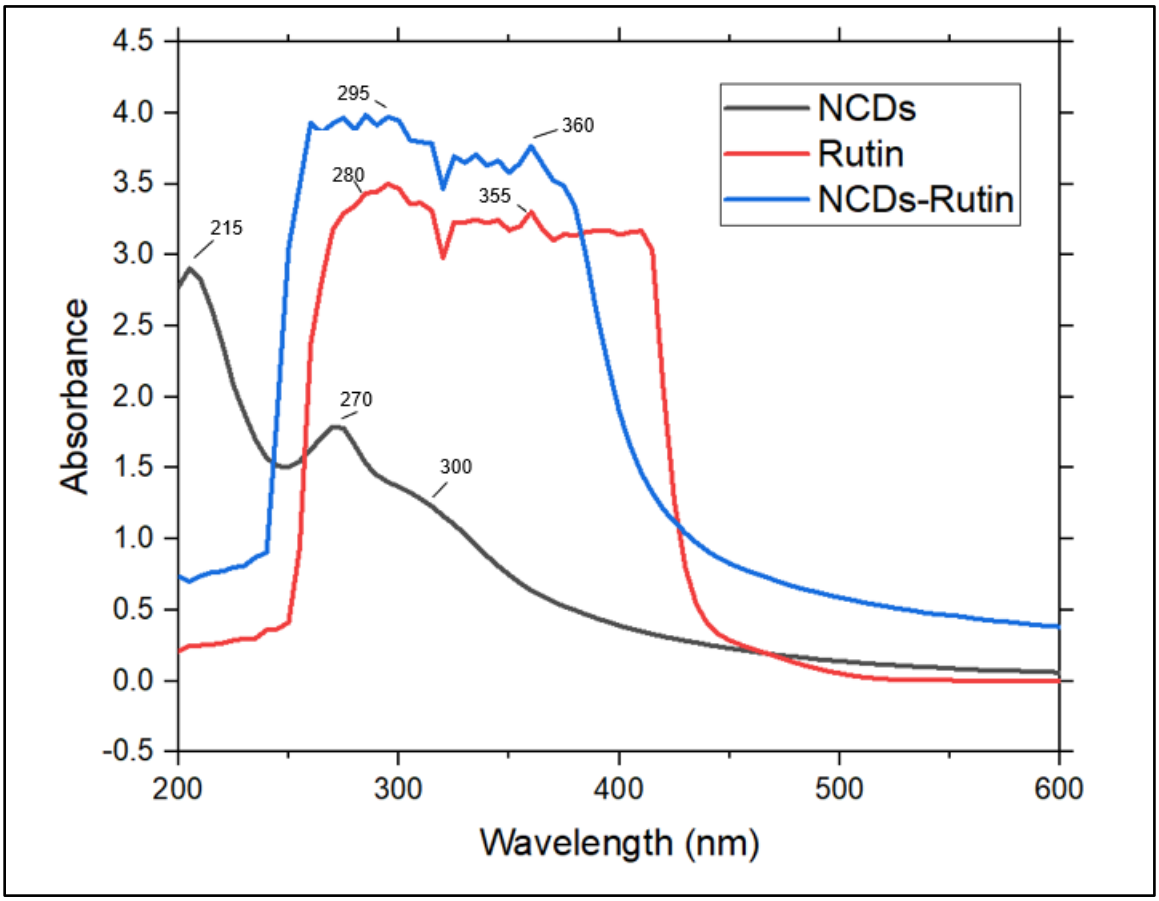

Figure 3.2: UV- Vis spectrum of NCDs, Rutin and NCD-Rutin. The UV spectra depict the peaks of NCDs (black) at 215 nm, 270 nm, and 300 nm, Rutin (red) at 280 nm and 355 nm; and NCD-Rutin (blue) at 295 nm and 360 nm.

3.3. AFM Analysis

The morphology of the synthesised NCDs and NCDs-Rutin was examined using AFM, as depicted in Figure 3.3. The results revealed the existence of <100 nm-sized minute aggregates and individual NCD-Rutin particles. Notably, the height observed of NCD-Rutin is 49 nm.

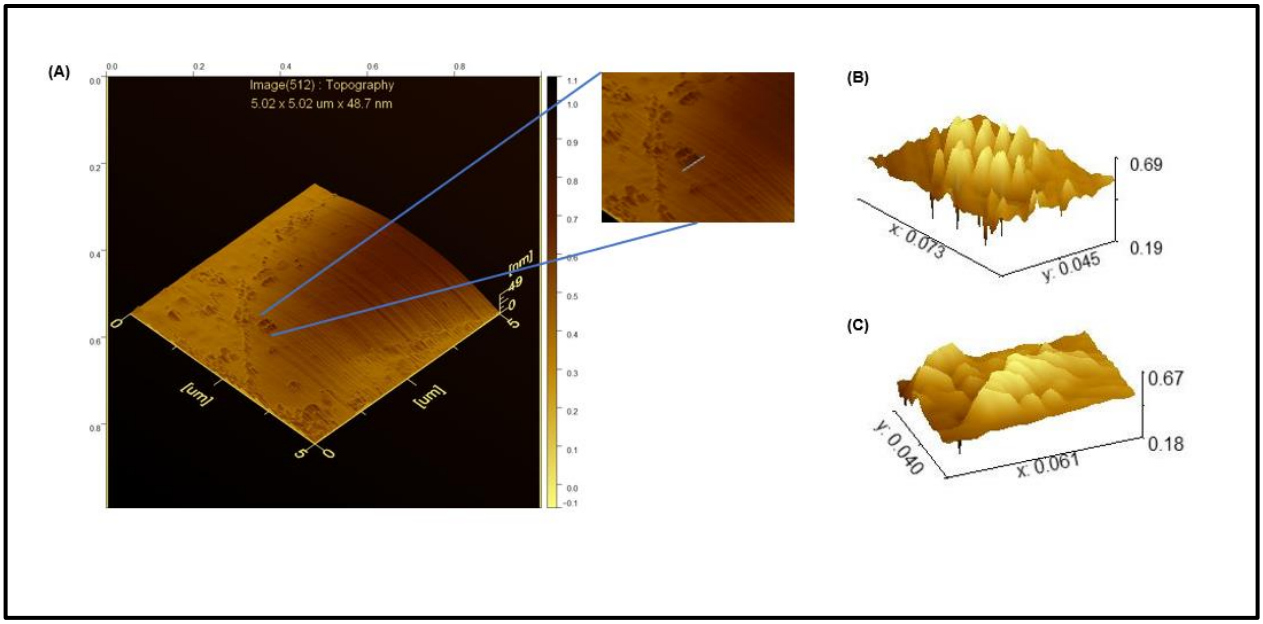

Figure 3.3: AFM results for NCD-Rutin, depicting topography. (A) shows the particle aggregates on a 1 cm glass slide, with an enlarged image of a specific area where particles can be observed. (B) and (C) exhibit 3D images of the enlarged area depicting the height and thickness of the NCD-Rutin particles

3.4. Haemolytic Test

Different concentrations of the NCD-Rutin were assessed and all of them did not induce significant haemolysis, as indicated in Figure 3.4. The haemolysis rates of NCD-Rutin were observed to be substantially lower than the universally recognized standard of 5%, across a concentration range of 0.1% to 30% (Choi et al., 2011). The haemolysis rate decreased from 10% to 1% and showed a slight increase in 1.5% and 2%.

3.5. Behavioural Tests

3.5.1. MWM Test

The first MWM test revealed that the cognitive processes pertaining to memory and learning were significantly disrupted in the rats induced with AD due to $AlCl_3$ exposure. The prolonged escape latency in AD animals was indicative of severe impairment in their learning ability. Furthermore, the $AlCl_3$ challenge exhibited the rats' ability to inhibit their memories and leave the target quadrant promptly.

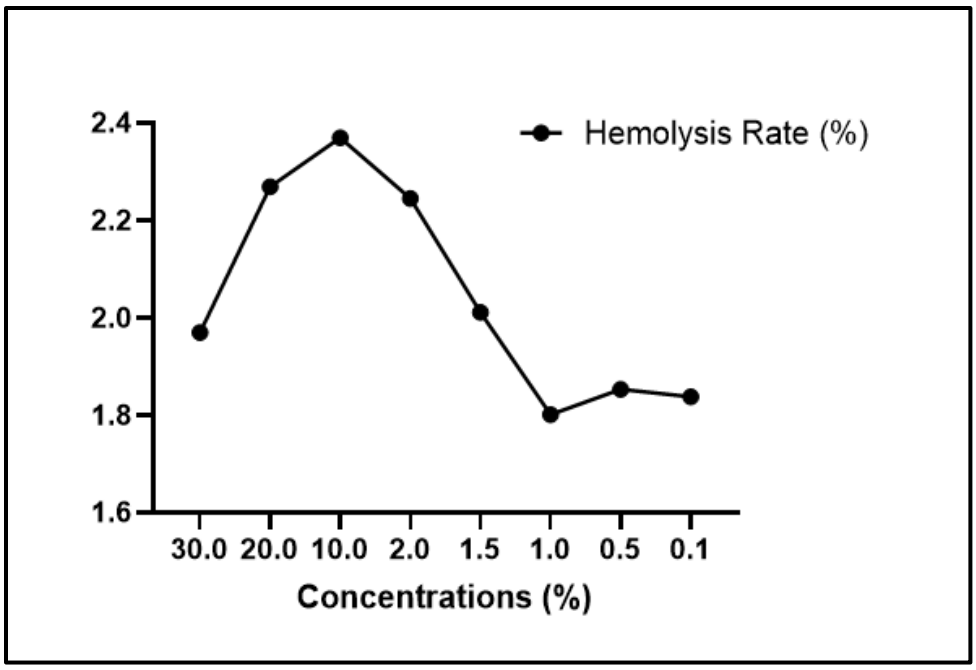

Figure 3.4: Haemolysis rate (%) of RBCs treated with different concentrations of NCD-Rutin. The OD was checked on 577 nm. Deionized water was used as positive control whereas PBS was used as negative control in this experiment. All the rates lie below 5%, which is as per universally recognized standards (Choi et al., 2011).

3.5.2. Y-Maze Test

Assessment of working memory showed that the percentage alterations (PA(%)), in the Y-maze test, of the AD group was 63% which was statistically significantly lower ($p<0.05$) than the corresponding mean value in the control group (-20.10 ± 5.675). In comparison, the mean value of PA% in the control group was 83.14% which was significantly higher ($p<0.05$) than that of the AD group.

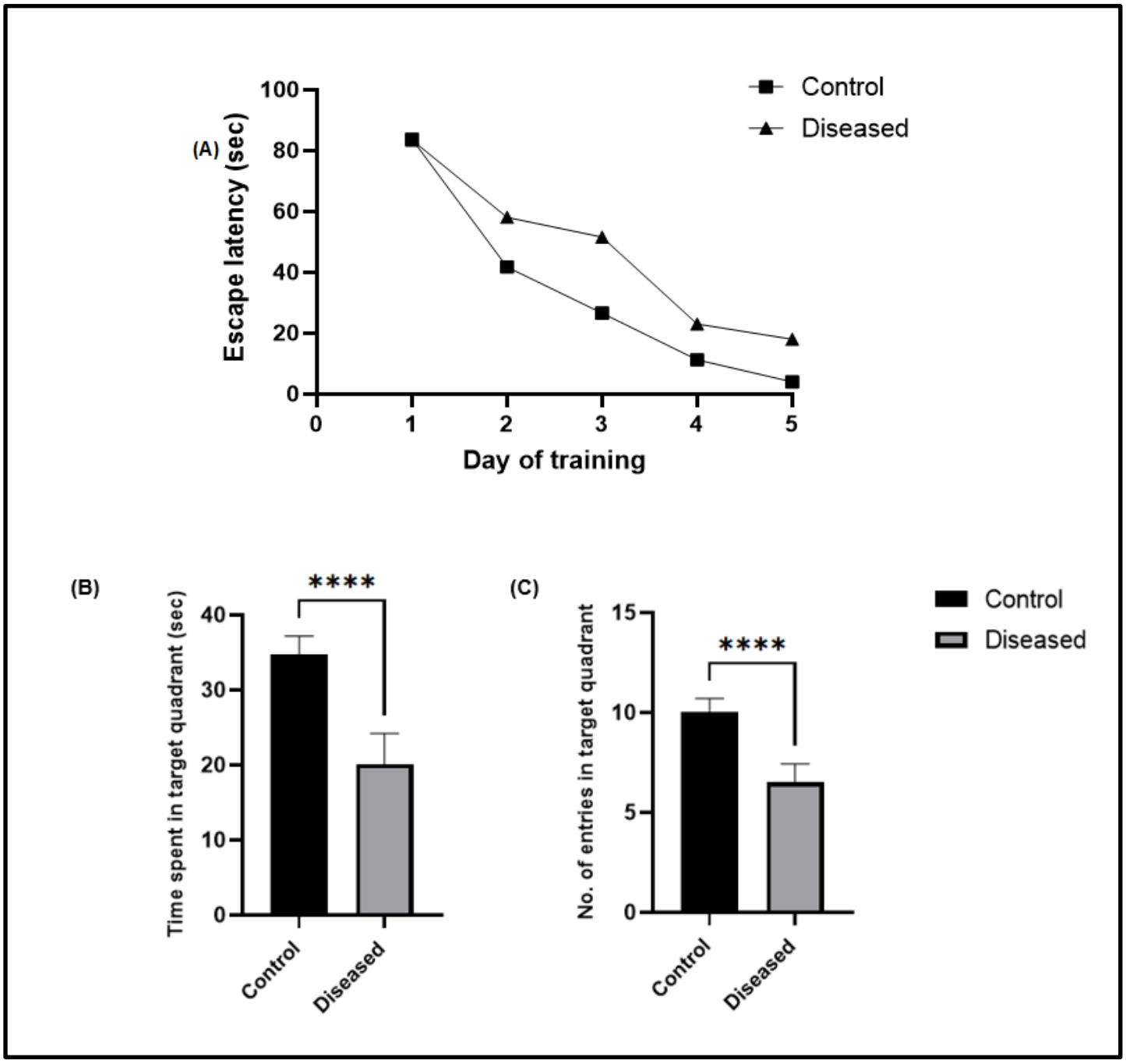

Figure 3.5.1: MWM test (A) Escape latency in 90 seconds measured for 5 days (B) Time spent in target quadrant on test day (C) No. of times rats enter the target quadrant on test day. The escape latency graph shows the declining memory of rats as observed from the seconds spent in searching for the platform submerged in the water. Graphs (B) and (C) depict the memory of the rats searching for the platform in the target quadrant. Significant differences can be observed in the control and diseased groups.

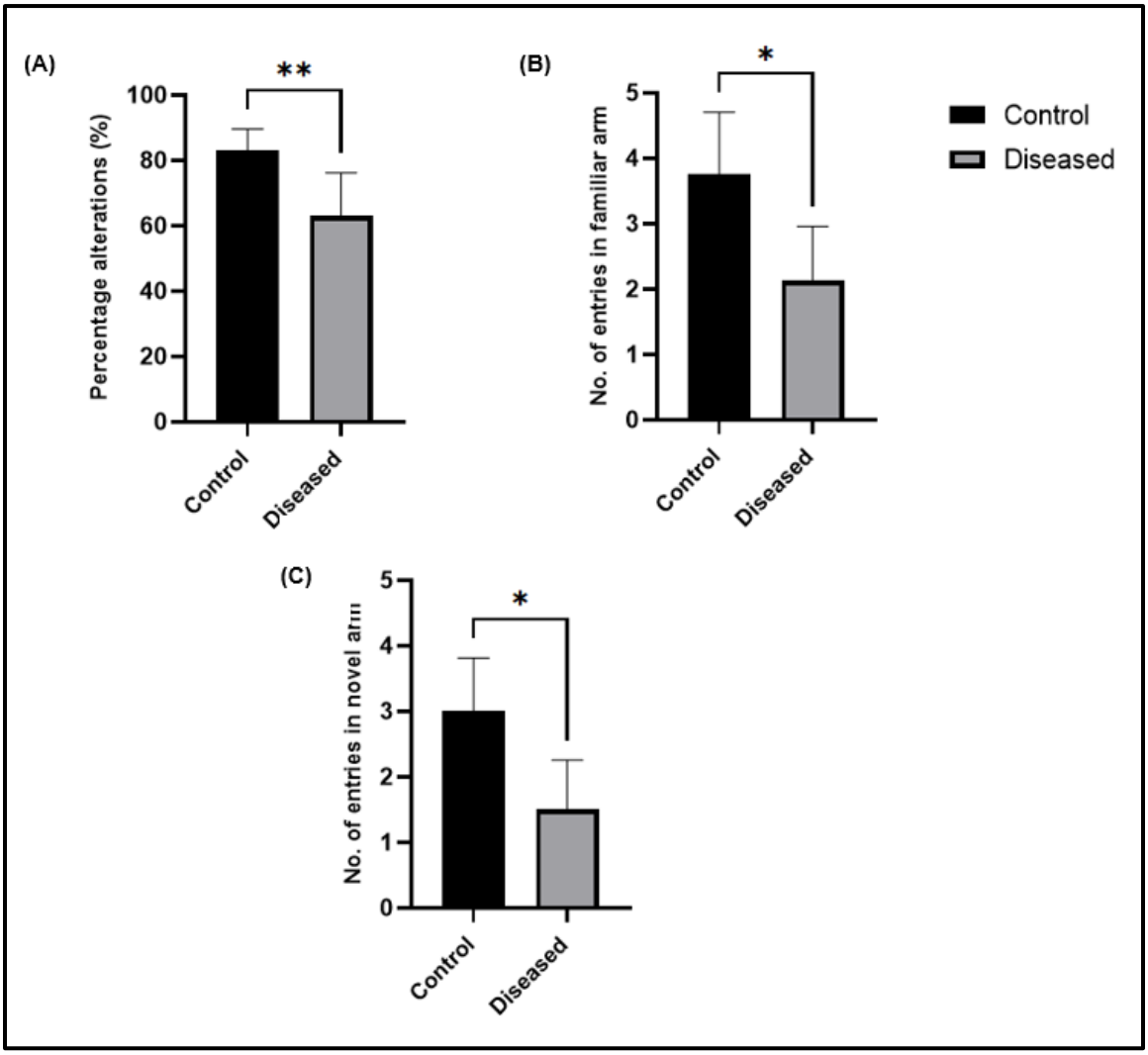

Figure 3.5.2: Y-maze test. (A) PA(%) of both the groups between the three arms of the Y maze (B) No. of entries in the novel arm, (C) No. of entries in the familiar arm. The percentage of alternations was significantly decreased in the diseased group, suggesting that cognitive ability was impaired in AD model rats. Controls (n = 4); AD model rats (n = 8). Evaluated with student t-test.

3.5.3. Open Field Test

Utilizing an open field test, alterations in behaviour among both control and treated animals were discerned and subsequently recorded, as depicted in Figure 3.5.3. Relative to control animals, those affected by $AlCl_3$-induced AD exhibited, a decrease in rearing frequency, and an increase in grooming frequency. Whereas the control group showed increased rearing frequency and decreased grooming frequency.

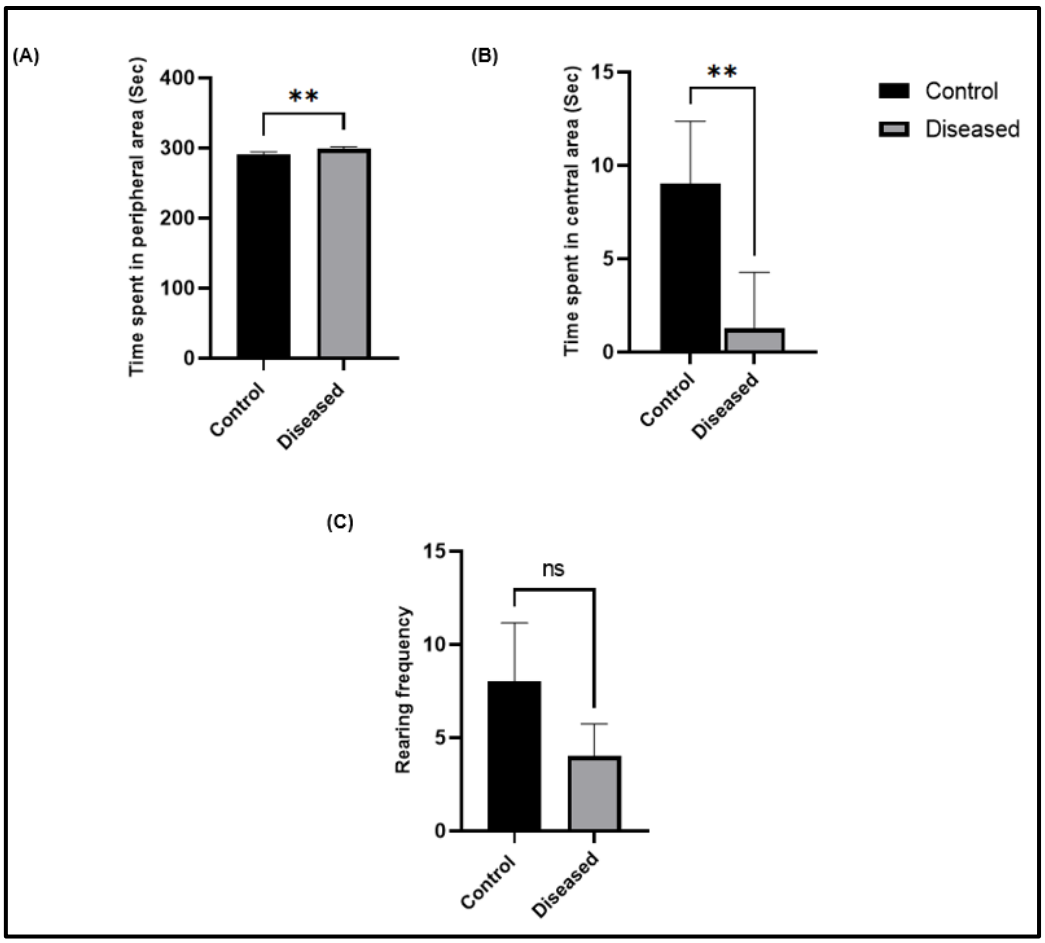

Figure 3.5.3: Open Field test. (A) Time spent in peripheral area, (B) Time spent in central area (C) Rearing frequency. Diseased rats spent more time in the peripheral corners of the field instead of exploring the field. AD-like models were also observed to be rearing on hind legs lesser as compared to the control group. The difference in rearing frequency failed to reach significance. Controls (n = 4); AD model rats (n = 8). Evaluated with student t-test.

3.5.4. NOR Test

The interaction between rats and objects is considerably influenced by a variety of item characteristics, encompassing size, shape, substance, presence of protrusions or incursions, surface roughness, and colour brightness. To ensure unbiased selection, we selected items for pre-screening that possessed a simple shape, and washable materials (such as odourless plastic or metal), which were equivalent in size to the rats, and had colours with similar brightness. Rats of the control group were observed to explore the novel object more in comparison to those in the diseased group. This depicted that the diseased group showed cognitive impairment by failing to recall that it had already explored the familiar object.

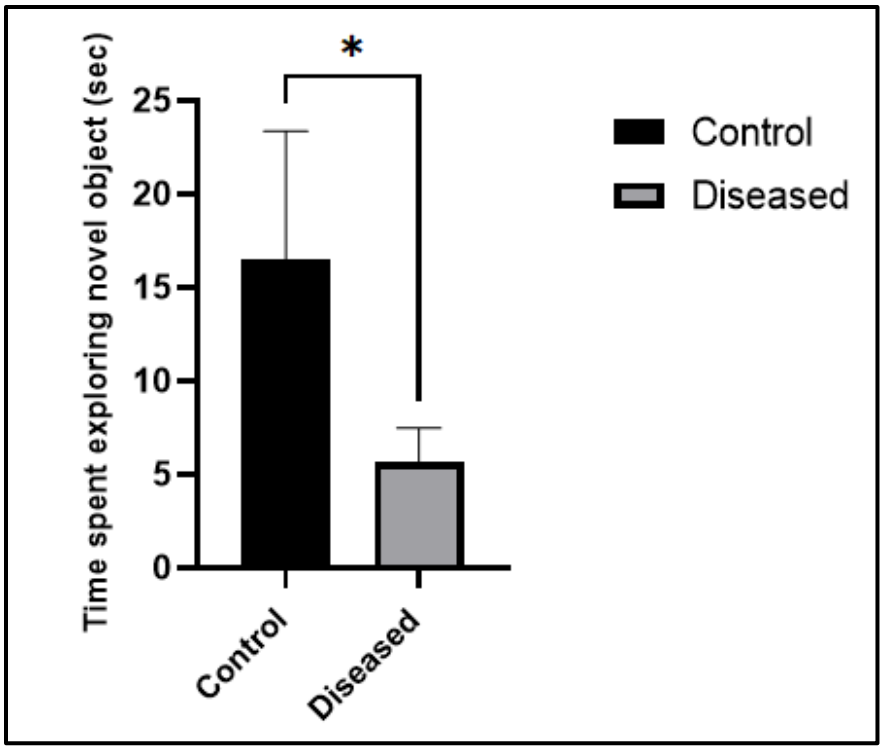

Figure 3.5.4: NOR test. The Graph represents the control group and diseased group, in which the rats explored a novel object and the time they spent exploring was observed. An unpaired t-test was used on the data.

3.6. Behavioral Tests After NCD-Rutin Administration

3.6.1. MWM Test

The results of the escape latency test indicated that the treated rats exhibited a slightly shorter latency in reaching the platform in comparison to the diseased group. However, the treated rats exhibited significant results in the duration spent in the target quadrant, in addition to the number of entries.

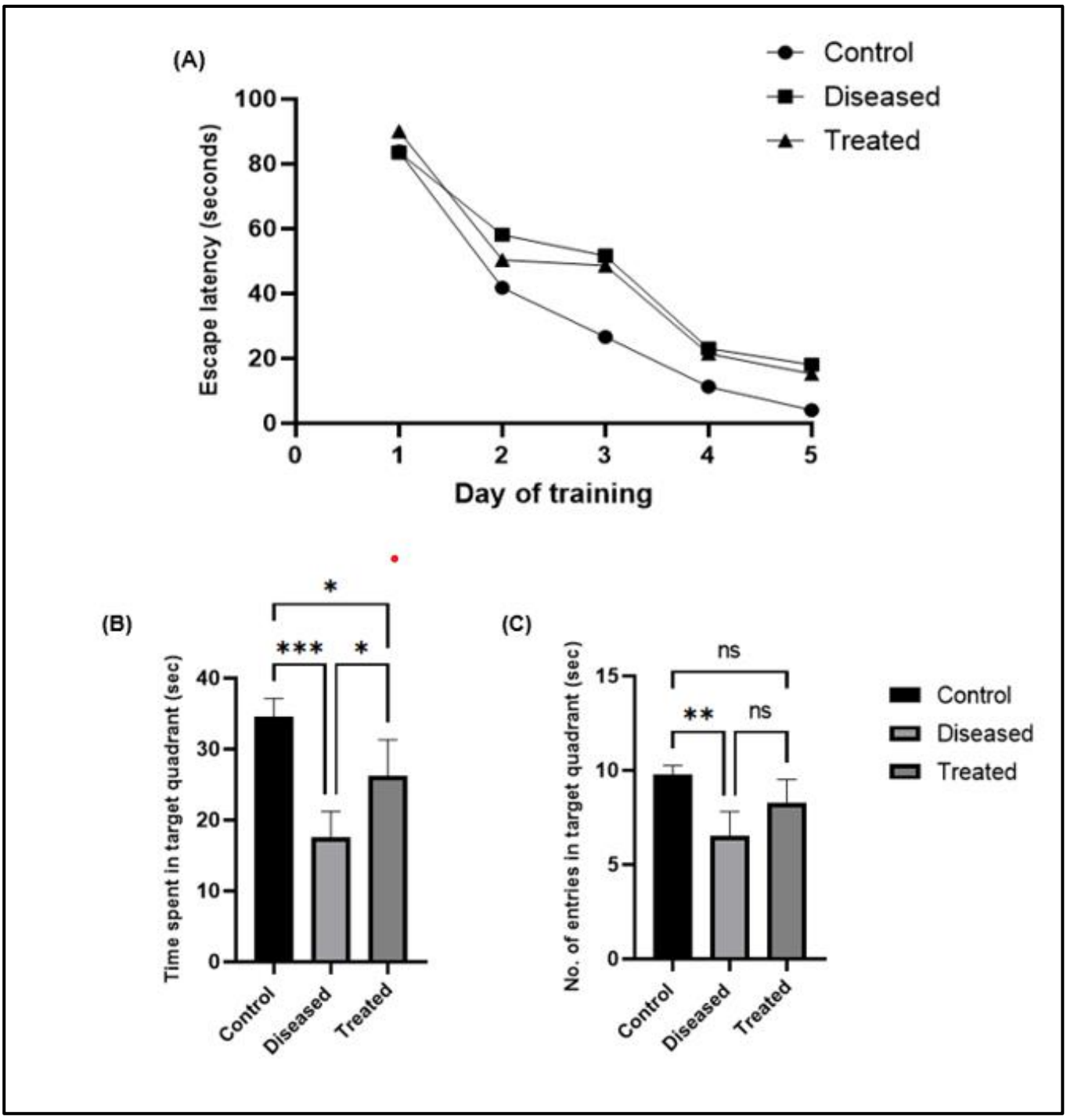

Figure 3.6.1: MWM test after NCD-Rutin administration. (A) The escape latency graph shows slightly decreased latency in treated rats when compared to diseased rats. (B) and (C) The time spent and number of entries in the target quadrant is observed to be increased in the treated rats. One-way ANOVA, followed by Tukey's multiple comparison test, was used for statistical analysis. Error bars present SEM (*p<0.05, **p<0.01, ***p<0.001).

3.6.2. Y-Maze test

Only the parameter of time spent in the novel arm showed significant results while the other two did not. It is noteworthy that the treated group did show recovery trends in alterations and entries in the familiar arms.

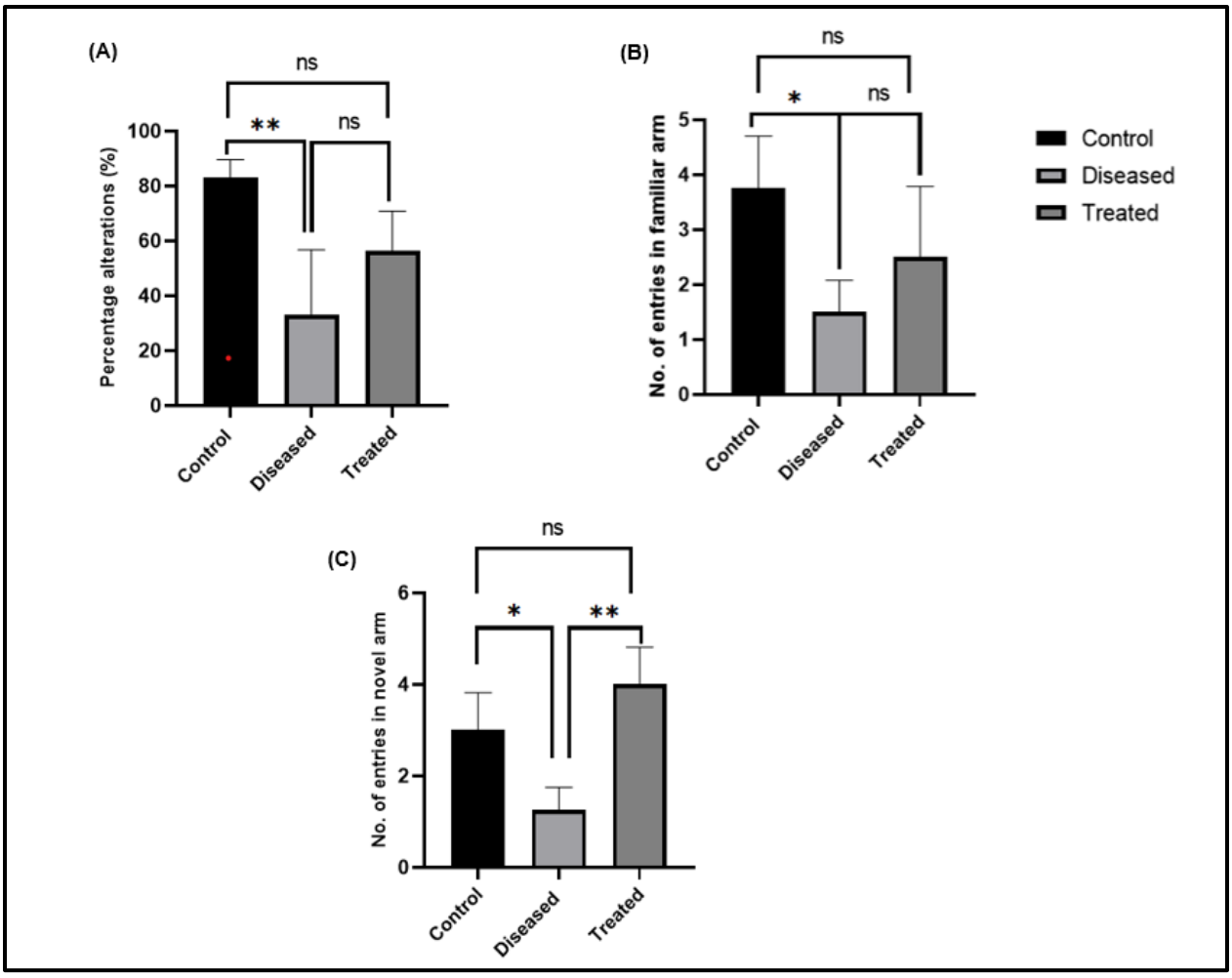

Figure 3.6.2: Y-Maze test after NCD-Rutin administration. (A) PA% was observed to be increased in treated rats. (B) and (C) Exploring ability in rats can also be observed to have increased. One-way ANOVA, followed by Tukey's multiple comparison test, was used for statistical analysis. Error bars present SEM (*p<0.05, **p<0.01).

3.6.3. Open Field Test

Treated rats were observed to spend more time in the central area than the diseased rats while exploring the field. Whereas diseased rats spent the whole time in the peripheral area.

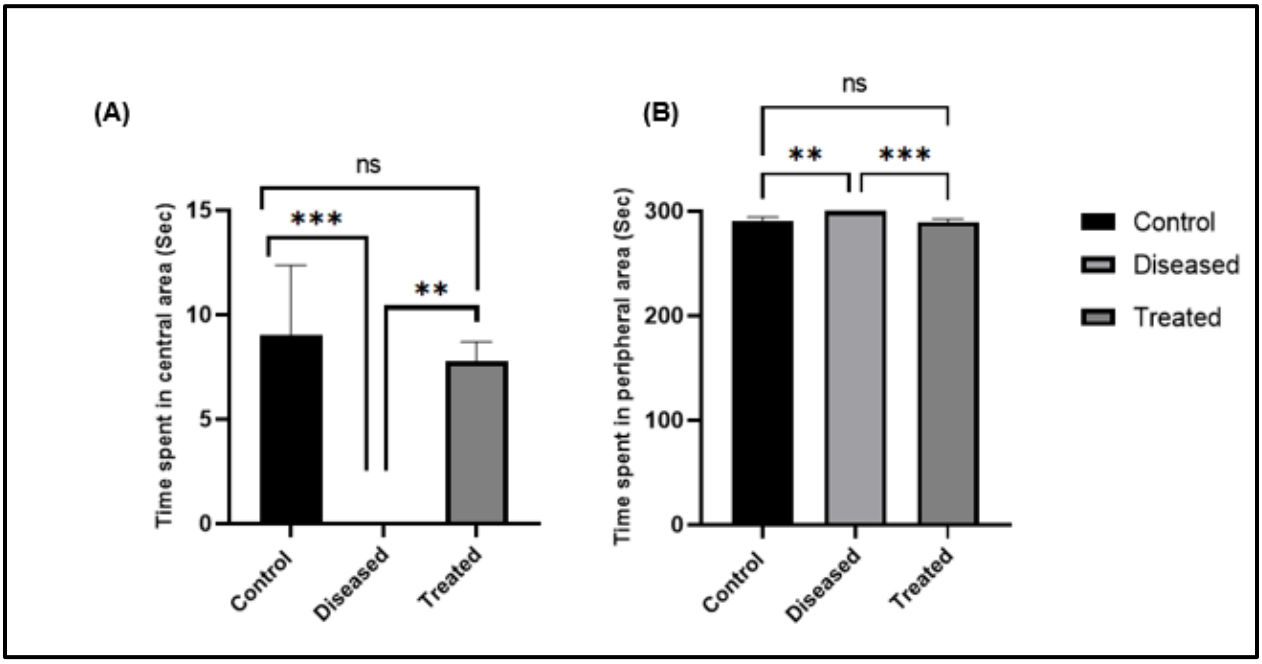

Figure 3.6.3: Open Field Test after NCD-Rutin administration. (A) Time spent in the central area was observed to be increased in treated rats and vice versa was observed in (B) i.e., time spent in the peripheral area. One-way ANOVA, followed by Tukey's multiple comparison test, was used for statistical analysis. Error bars present SEM (**p<0.01, ***p<0.001).

3.6.4. NOR Test

The results of the test conducted to assess the rats' ability to recall object position or identification, showed no significant difference between the treated and the diseased group. While both groups displayed similar patterns of exploratory behavior, the treated rats exhibited slightly more inclination to jump onto the objects.

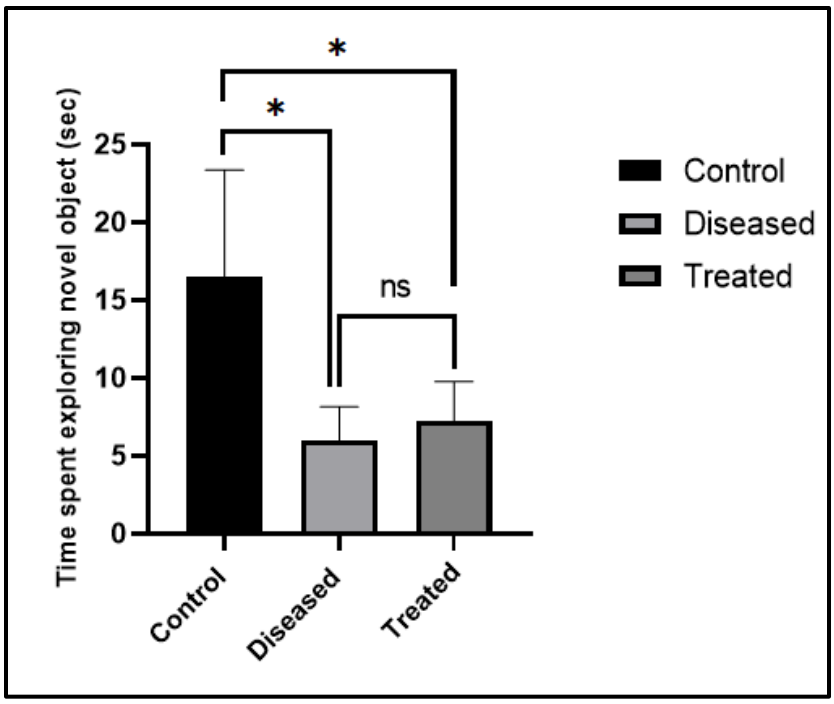

Figure 3.6.4: NOR Test after NCD-Rutin administration. The graph represents the control, diseased group and treated group, in which the rats explored a novel object and the time they spent exploring was observed. No significant difference was noted between the treated and the diseased group. One-way ANOVA, followed by Tukey's multiple comparison tests, was used for statistical analysis. Error bars present SEM (*$p<0.05$).

CHAPTER 4
DISCUSSION

In accordance with a 2016 report by the WHO, abnormal neurological conditions were accountable for over 276 million disability-adjusted life years and approximately 9 million fatalities worldwide (Feigin et al., 2019). The escalating prevalence of neurological illnesses warrants a pressing need for efficacious treatments. However, the blood-brain barrier's remarkably selective semi-permeability impedes the majority of macromolecules, including pharmaceuticals, from accessing the CNS, posing a significant obstacle to neurological illness treatment. Fortunately, recent advancements in nanoparticle technology, particularly in nanoparticle-mediated drug transport, have made substantial progress in overcoming the blood-brain barrier. Furthermore, the manifold surface molecules of CDs offer immense potential for conjugation with one or more ligands, serving as CD-based DDS capable of penetrating the BBB to remedy neurological conditions and brain tumours, such as glioblastoma, which has been extensively researched by Leblanc and his colleagues. Currently, most proposed processes lack thorough analysis. To achieve enhanced solutions for the persistent challenges associated with CNS medication delivery, comprehension of these intricate systems is imperative. As drug components, natural flavonoids such as Rutin can be utilized to bind to the CDs and can exhibit synergistic effects against AD.

Rutin's neuroprotective qualities primarily entail the activation of the MAPK pathway and the inhibition of apoptosis, which is induced by Aβ oligomers. Additionally, Rutin has the ability to bolster the survival of neural crest cells by stimulating the ERK2 and PI3K pathways (Wang et al., 2015). Rutin also amplifies the activity of several enzymes with anti-oxidant properties, such as Superoxidase Dismutase, Catalase, and Inhibitor of iNOS Activity (Yu et al., 2015). Furthermore, it significantly restrains the aggregation of Amylin. Rutin also possesses the capacity to substantially diminish the amount of microglial activation instigated by IFNγ and lipopolysaccharide (Simonyi et al., 2015).

The present study focuses on the production of Rutin-bound NCDs, wherein their capacity to penetrate the BBB is analyzed, and their efficacy in the treatment of AD was observed. The prepared NCD-Rutin complex was characterized using FTIR, UV-Vis Spectrophotometer, and AFM. The resulting spectra (figure 3.1) indicated the presence of hydroxyl, carboxyl, and carbonyl

groups, thereby confirming their exceptional stability and water dispersibility (Guo et al., 2021). They displayed an absorption peak at 1637.88 cm^{-1}, which can be attributed to the asymmetric stretching of carbonyl (C=O), and at 1425.82 cm^{-1}, which corresponds to the C-H symmetric stretching vibration (Dhiman et al., 2019). As reported by Zheng et al. (2016), the IR absorption peak for hydroxyl (O-H) asymmetry stretching was observed at 3278.78 cm^{-1}, and the vibration region C=N stretching was noted at 1630 cm^{-1}, as depicted in Figure 3.1. NCD-Rutin depicted a peak at 3266.48 cm^{-1} corresponding to the OH group in the complex while a peak at 1593.16 cm^{-1} matched the original peaks of C=C bonds.

The UV-Vis spectra (Figure 3.2) exhibit three distinct absorption peaks at 215, 270 nm and 310 nm, which can be accredited to the $\pi - \pi*$ transition of C=C, C=N, and C=O bond, respectively. Rutin exhibits two noteworthy absorption bands, specifically at 360 nm and 295 nm, within the ultraviolet/visible region. This observation is consistent with the results from the study conducted by Panhwar & Memon, (2014). NCD-Rutin showed similar peaks to Rutin at 295 nm and 355 nm. The aforementioned bands can be attributed to the $\pi - \pi*$ transition of a benzene ring-conjugated system. By examining the UV-Vis spectra differences between Rutin, NCDs, and NCDs-Rutin, it can be concluded that Rutin has been successfully anchored to the surface of GluCDs. AFM results presented the morphology of the synthesised NCDs and NCDs-Rutin, as depicted in Figure 3.3. The results revealed the existence of <100 nm-sized minute aggregates and individual NCD-Rutin particles. Notably, the height observed of NCD-Rutin is 49 nm and the average size is 25 ± 2 nm. These aggregates were formed due to the drying process undergone by the sample prior to examination. The AFM findings intimate that NCD-Rutin is more prone to aggregating into larger structures as compared to NCDs.

The haemolysis rates of NCD-Rutin were observed to be substantially lower than the universally recognized standard of 5%, across a concentration range of 0.1% to 30%. The rates decreased from 10% to 1% and showed a slight increase in 1.5% and 2% concentration. The behavioural test performed to confirm the induction of Alzheimer's disease using $AlCl_3$ presented results indicating that the diseased rats moderately lost their cognitive function. In the MWM test, diseased rats spent less time in the target quadrant as compared to the control group which not only spent more time but also exhibited an increased number of entries in the target quadrant. An escape latency graph was also deduced from the test, which confirmed that diseased rats' cognitive capability was impaired. Assessment of spatial working memory showed that PA%, in the Y-maze

test, in the AD group was 63% which was statistically significantly lower ($p<0.05$) than the corresponding mean value in the control group (-20.10 ± 5.675). On the other hand, the mean value of PA% in the control group was 83.14% which was significantly higher ($p<0.05$) than that of the AD group. In the graph depicting the number of alterations, the control group altered in all three arms of the Y-maze more than the diseased group, which tended to return to the same arm they had already explored. Even though the graph of the number of entries showed very little difference between both the groups, it does not invalidate the fact that the cognitive ability of the rats was impaired because entries into arms which was already explored were also considered.

Utilizing an open field test, alterations in behaviour among both control and treated animals were discerned and subsequently recorded, as depicted in Figure 3.5.3. The interaction between rats and objects was observed in the NOR test. Rats of the control group explored the novel object more in comparison to those in the diseased group. This depicted that the diseased group showed cognitive impairment by failing to recall that it had already explored the familiar object.

The behavioural tests were repeated after drug administration. The results of the escape latency test indicated that the treated rats exhibited a slightly shorter latency in reaching the platform in comparison to the diseased group. However, the treated rats exhibited significant results in the time spent in the target quadrant, in addition to the number of entries. In the Y-maze test, time spent in the novel arm showed significant results while the other two did not. It is noteworthy that the treated group did show recovery trends in alterations and entries in the familiar arms. Open field test showed that the treated rats spent more time in the central area than the diseased rats while exploring the field. Whereas diseased rats spent the whole time in the peripheral area. The results of the NOR test conducted to assess the rats' ability to recall object position or identification, showed no significant difference between the treated and the diseased group. While both groups displayed similar patterns of exploratory behaviour, the treated rats exhibited slightly more inclination to jump onto the objects.

The results indicate that NCDs function as a smart approach to administering drugs such as Rutin for AD, affording the ability to monitor progress and alter delivery. Despite having knowledge of the exceptional properties of NCD-Rutin, several issues need to be resolved to direct their future applications. In particular, heightened focus should be placed on enhancing the optical performance of NCD-Rutin. To modify their optical characteristics, it is imperative to first synthesize a diverse range of NCDs with tunable compositions, sizes, forms, crystallinity, and

electrical structures. Despite notable progress in the preparation of multifunctional CDs, the majority of documented procedures are time-consuming, environmentally unfavourable, and produce minimal quantities of product. As such, it is crucial to establish an effective, manageable, and sustainable synthetic technique to produce NCDs in significant quantities and of superior quality.

Secondly, it is noteworthy that in the majority of CD types, the PL properties, particularly those with blue or green fluorescence, have been extensively studied. Nevertheless, the observation of CDs that exhibit NIR fluorescence, phosphorescence, electrochemiluminescence, and surface-enhanced Raman scattering capabilities is a rare occurrence. These optical attributes of CDs render them more suitable for applications such as bioimaging, light emission, and highly sensitive sensing. Thus, the proficient preparation of distinctive NCDs with such unique optical characteristics is of great importance for potential applications.

Thirdly, it is imperative to clarify the mechanism underlying the optical characteristics of CDs. The examination of the PL mechanism of CDs has garnered considerable attention due to its association with the edge and surface states, as well as the quantum confinement effect. However, there exists an insufficiency of robust data to recommend a more widely accepted PL method for CDs. Most critically, the mechanics underlying the other optical features of CDs remain largely unexplored. To comprehensively comprehend the mechanism of CDs' optical qualities, additional theoretical and experimental research must be undertaken.

Finally, an efficacious approach to ameliorate the optical characteristics of CDs involves their hybridization with other advantageous materials. In this regard, it is imperative to regulate the surface functionalization and interfacial impact of CDs-based hybrids. Furthermore, a considerably greater focus is required to scrutinize the correlation between the optical properties and their interfacial interactions, including theoretical calculations and contemporary characterization techniques. An exhaustive comprehension of NCD-Rutin optical traits will facilitate the emergence of numerous innovative applications. Moreover, CDs are deemed as one of the environmentally friendly and safest materials, thereby rendering photoelectronic applications for CDs viable.

CHAPTER 5
CONCLUSION

In this particular investigation, NCD-Rutin was prepared through the carbonization process. The carbon dots that were formed during this procedure were also subjected to a comprehensive characterization process. Structural characterization revealing the size and surface functional groups validated the binding of NCDs and Rutin and that it has the capability of crossing the BBB. The water dispersibility of NCD-Rutin was confirmed through FTIR analysis that revealed the existence of hydroxyl and carboxyl functional groups. AFM indicated the average size of the particle to be 25 ± 2 nm. These NCD-Rutin particles exhibited exceptional biocompatibility as implied by the hemolysis test. The rates were observed to be substantially lower than the universally recognized standard of 5%, with the maximum rate falling under 2.4%. *In vivo* tests also demonstrate positive results. In adult male albino rat models, cognitive conditions before and after administration of NCD-Rutin were determined. Treated rats indicated improved results in comparison to diseased rats. No visible toxicity was observed in the rats. Consequently, these NCD-Rutin might serve as a novel platform, exhibiting potential in the domain of therapeutics for addressing AD.

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