Investigating the effect of Indigenous probiotic *Limosilactobacillus fermentum* Y55 and Quercetin on $AlCl_3$ induced Alzheimer's disease rat model.

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ISLAMABAD, PAKISTAN.

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A thesis submitted in partial fulfillment of the requirement for the degree of MS

In

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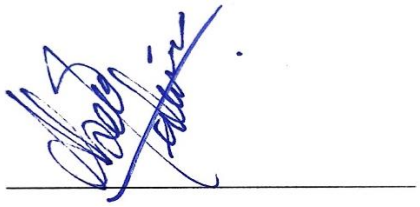

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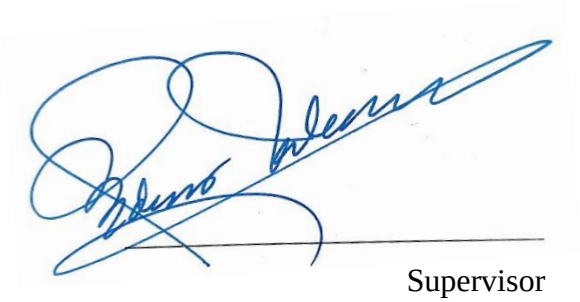

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List of Abbreviations

(CTF)-α	C-terminal fragment alpha
8-OHdG	8-Hydroxy-deoxyguanosine
AChe	Acetylcholinesterase
ACTH	Adrenocorticotropic hormone
AD	Alzheimer's disease
amyloid-β	Amyloid-beta
ANS	Autonomic nervous system
APP	Amyloid precursor protein
BACE-1	beta-secretase-1
BBB	Blood brain barrier
BDNF	Brain derived neurotrophic factor
cdk-5	cyclin-dependent kinase-5
cdk-5	cyclin-dependent kinase-5
CFU	Colony forming unit
ChAT	Choline acetyltransferase
CNS	Central nervous system
CoQ	Coenzyme Q
CREB	cAMP-response element binding protein
CRF	Corticotropin-releasing hormone
DC	Diseased control group
DPN	Diabetic peripheral neuropathy

ENS	Enteric nervous system
EPM	Elevated plus maze
ETC	electron transport chain
FA	Ferulic acid
FAO	Food and Agriculture Organization of the United Nations.
GABA	Gamma-aminobutyric acid
GBA	Gut brain axis
GI	Gastrointestinal
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
GSK-3b	Glycogen synthase kinase-3b
GSK-3b	Glycogen synthase kinase-3b
H&E	Hematoxylin and eosin
HPA	Hypothalamic-pituitary-adrenal axis
LAB	Lactic acid bacteria
MAPK	Mitogen-activated protein kinase
MAPT	Microtubule-associated protein tau
MGBA	Microbiota gut brain axis
MRS	De Man, Rogosa and Sharpe
MWM	Morris water maze
NC	Negative control group

NDs	Neurodegenerative diseases
NFT	neurofibrillary tangles
NGF	Nerve growth factor
NOR	Novel object recognition
Nrf2	Nuclear factor erythroid 2-related factor 2
NTS	Nucleus tractus solitarius
PC	Positive control group
PKC	Protein kinase C
ROS	Reactive oxygen species
sAPPα	Soluble ectodomain of amyloid precursor protein
SCFAs	Short chain fatty acid
SOD	superoxide dimutase
TP	Probiotic treatment group
TPQ	Probiotic and quercetin treatment group
TQ	Quercetin treatment group
Trkb	Tyrosine protein kinase
VN	Vagus nerve
WHO	World Health Organization

ABSTRACT

Alzheimer's disease (AD), the most common progressive neurodegenerative illness in older individuals, is characterized by declining cognitive ability. Cognitive decline and memory loss are two of the first pathological signs of Alzheimer's disease by many factors including oxidative brain damage. Several studies have reported improvement in memory and cognition impairment by the consumption of high doses of *Limosilactobacillus* strains and phytochemicals like Quercetin. Through promoting the growth of the HPA axis and the manufacture of neuromodulators such antioxidant enzymes, GABA, SCFAs, serotonin, and BDNF, the gut microbiota influences the gut-brain axis. The current study's objective is to investigate the anti-Alzheimer's effect of potential *Limosilactobacillus fermentum* Y55 ($1.5x10^9$ CFU) and Quercetin (25mg/kg) in combination on $AlCl_3$- induced AD rat models. Rats were assigned to six distinct groups at random (n = 6). Using a variety of behavioral tests, including the Elevated Plus Maze (EPM), New Object Recognition (NOR), Y-maze, and Morris Water Maze, researchers investigated the impact of this combination on anxiety and memory (MWM) to determine whether recovery plays a part in reducing Al's neurotoxic effects. Male rats were exposed to aluminum along with *Limosilactobacillus fermentum* Y55 and Quercetin mixed in distilled water and after completion of treatment their learning and memory was tested using Novel object recognition test, y maze test and Morris water test paradigms and collated with the controls. To assess the anxiety, an elevate plus maze test was performed. The animals from treated and their controls were euthanized and dissected after the behavioral tests and the brains were removed to extract hippocampus for further histological analysis. The results unfolded those rats treated with probiotic and polyphenol combination had exhibited significantly improved working, reference memory, recognition and spatial memory with reduced anxious behaviors. Histologically, this

combination has improved the normal architecture of the brain hippocampus in TPQ group. H &E staining showed that TPQ group had normal morphological features with proper round and intact cell bodies as compared to DC group. To cap it all, this study demonstrates that animals exposed to *Limosilactobacillus fermentum* Y55 and quercetin together show marked improvement in their cognitive functions and memory and improves the morphology of the main targeted parts of brain that is hippocampus.

CHAPTER 1

INTRODUCTION

Alzheimer's disease (AD), the most prevalent cause of dementia, is distinguished by two distinct types of lesions: amyloid β- (Aβ) plaques and intracellular neurofibrillary tangles (NFT) (Selkoe, 2001). AD pathology includes increased oxidative stress, synapse loss, localized cell death, and brain atrophy(Ashe & Zahs, 2010). There are several fiercely debated theories that attempt to explain the fundamental factor that triggers the onset of AD brain illness. In the past, work has been done to link several aberrations together under a single, basic pathogenic mechanism. (Hardy & Selkoe, 2002); (Markesbery, 1997). The optimal methods for treating and preventing AD are still unknown since the science lacks consensus on its etiology and pathology after decades of intensive research(M. de la Monte, 2012).

Alzheimer's disease (AD) primarily affects elderly persons aged 65 years or older and accounts for 60–80% of all dementia cases (DeTure & Dickson, 2019). 4.6 million new cases of AD are thought to be recorded annually, affecting around 35.6 million individuals worldwide. Age is a factor in the prevalence of AD; starting at 60 years old, the rate doubles every five years (Mayeux & Stern, 2012). Amyloid-(Aβ) aggregate buildup and tau protein hyperphosphorylation, which result in neurofibrillary tangles (NFTs) and synaptic dysfunction, are commonly linked to the pathophysiology of AD (Kommaddi et al., 2018).

Two significant systemic factors that exacerbate neurodegeneration are oxidative damage and inflammation, both of which are fueled by the usual physiological decline associated with

ageing. Reactive oxidative species (ROS) are formed when 0.4–4% of the electrons moving through the electron transport chain (ETC) escape and combine with an oxygen molecule to generate superoxide radicals, which are the principal source of ROS in the body (Murphy, 2009). Ordinarily, the cells' anti-oxidant defense mechanisms convert these free radicals into harmless species, but as people age, their cellular defenses gradually deteriorate, accumulating genetic, cellular, and membrane damage until death of cell occurs (Mitsuma et al., 2008).

ROS slowly builds up in neurons, which triggers microglial activation, cytokine release and neuroinflammation as a result. The pathophysiology of oxidative damage and inflammation leads to inflamm-aging, which is described as a persistent low-grade systemic proinflammatory state with raised cytokines and inflammatory mediators without any triggered cause. Aging via inflammation is the aggregate name for this vicious cycle." (Franceschi & Campisi, 2014).

In order to maintain oxidative homeostasis, complex regulatory systems that can mount self-limiting effector reactions in response to various forms of oxidative stress are needed. The redox-sensitive transcription factor NF-E2-related factor 2 (Nrf2) is well acknowledged as a crucial regulator of antioxidant processes(Raghunath et al., 2018). The multitude of antioxidant, cytoprotective, and immunomodulatory enzymes that this gene set encodes help regulate the cellular response to various stresses (Nguyen et al., 2009); (Tonelli et al., 2018).

The enteric nervous system and CNS maintain a communication pathway between them called gut brain axis (GBA). The GBA links the brains functions of cognition and emotion to the peripheral functions of intestine. The gut microbiota is an important variable of GBA (Dinan&Cryan, 2017). The gut microbiota, the dynamic endocrine organ, contains trillions of symbiotic bacteria that have an impact on the host's health. (Cho & Blaser, 2012). Lifestyle-related changes in the bacterial community's interindividual diversity affect human biochemistry

and disease resistance, including the susceptibility to brain illnesses, as well as a number of other alterations in human physiology. (Aziz et al., 2013). Through a variety of endocrine, neurological, and biochemical processes, the gut microbiota is able to communicate with the brain in a communication system that is known as the gut-brain axis. This system is bidirectional. (O'Mahony et al., 2015). The gut microbiota is in a very delicate equilibrium when it is healthy. Disorders or diseases can result from changes that disrupt this microecological balance owing to internal or external sources (Sochocka et al., 2019). *Bifidobacterium, Eubacterium rectale,* and *Dialister* are a few examples of the beneficial bacterial taxa that are less common in patients with AD's gut microbiota imbalance, while *Escherichia coli, Bacteroides,* and *Ruminococcus* are examples of the potentially pathogenic microbes that are more prevalent (Vogt et al., 2017); Cattaneo et al., 2017). It has been shown that the variety of the gut microbiota declines with advancing age, which may play an important part in the development of neurodegenerative diseases (Dinan & Cryan, 2017); Köhler et al., 2016). There is evidence that the gut microbiota can affect the course of neurological diseases and potentially cause illness development, particularly when it is in a dysbiotic state (Catanzaro et al., 2015). It is important to note that the relative balance of the microbiota population, and consequently its metabolic and genomic expression, are altered in response to environmental stresses, particularly oxidative stress. This results in extensive physiological alterations in metabolism and endocrine signaling in the human host (Ley et al., 2008).

There is no one therapy strategy for AD due to its baffling pathogenesis. For the symptomatic management of AD, a combination of medications with antioxidant, anti-inflammatory, and cholinergic characteristics is employed (Hager et al., 2001);(Herholz et al., 2004). Currently donepezil, rivastigmine, and other acetylcholinesterases are used to halt AD. They cure

symptoms but do not stop the spread of the disease. Also, they are linked to adverse effects that range in intensity from mild, like vomiting and weight loss, to severe, including bradycardia and sleeplessness (Joe & Ringman, 2019).

Hence the evidence of microbiota in gut health and modulation of brain function indicates that modulating diversity of gut microbiota and oxidative stress may serve as a potential route for therapy. A relatively new and alternate strategy which is being devised is the use of probiotics along with polyphenols together.

According to the definition of WHO, “probiotics are live micro-organisms when administrated inadequate amount confer health benefits”(Rigobelo, 2012). Lactic acid bacteria are usually probiotics and have been declared as safe by FAO and WHO (Anandharaj et al., 2015). Probiotics have been shown to reverse the dysbiosis of the gut microbiota, slow the onset of AD, especially in cases where inflammation and oxidative stress are present, and lessen cognitive loss (Evrensel & Ünsalver, 2018). In AD patients, probiotic use has been shown to reduce some oxidative stress and inflammatory indicators, providing evidence of a gut-brain connection throughout illness (Akbari et al., 2016). As a result of the numerous bioactive metabolites they produce, probiotics are also referred to as "the cell factory" and play crucial functions in maintaining human health (Lekchand Dasriya et al., 2022). Lactic acid, SCFA and acetic acid, antibiotic substances, and neurotransmitters are among those metabolites that probiotics create. Probiotics create short-chain fatty acids, which prevent amyloid from aggregating and regulate a number of signaling pathways. As a result, there is a decrease in neuro-inflammation, the production of amyloid plaques, and a positive regulation of repressed receptors (Seo & Holtzman, 2020).

Many vascular plants contain phenolic compounds called flavonoids, which are extracted from them. The capacity of flavonoids to reduce free radical production and scavenge free radicals is what gives them their antioxidant properties. An antioxidant flavonoid called quercetin, which is also present in apples and onions, boosts glutathione levels and affects the functioning of enzymes that fight free radicals (Ansari et al., 2009). There are numerous therapeutic plants that contain the flavonoid molecule quercetin (3, 5, 7, 3′, 4′-pentahydroxyflavone). Apparently acting as an antioxidant, quercetin scavenges reactive oxygen species (ROS) (Ossola et al., 2009). Moreover, it has antiviral, anti-inflammatory, and anticancer effects. Several studies have shown quercetin to have anti-amyloidogenic and neuroprotective benefits against neurological disorders (Elreedy et al., 2023).

Several research projects are being carried out in Pakistan that focus mainly on the health and industrial benefits of probiotics and polyphenols. The current study demonstrates how a combination of probiotic and a polyphenol plant extract might simultaneously alter numerous GBA signaling elements in order to halt the start of AD and delay its progression, possibly through antioxidant mechanisms. Although though both the probiotic formulation and quercetin can independently trigger effects on some of the risk variables of AD, the formulation is the only one that can consistently reduce all the studied risk factors, making the combinatorial therapy more effective than any of its ingredients.

OBJECTIVES

1. To analyze the prophylactic effects of *Limosilactobacillus fermentum* Y55 & Quercetin on anxiety and cognitive deficits of learning and memory via behavioral tests in $AlCl_3$ induced AD rat model.
2. To compare the effect of *Limosilactobacillus fermentum* Y55 and Quercetin with Donepezil on AD rat model.

CHAPTER 2

LITERATURE REVIEW

2.1 Alzheimer's Disease

A gradual age-related neurological disorder, Alzheimer's disease that was first identified as Dementia Praecox by the German neuropathologist Alois Alzheimer in 1907. Alzheimer presented his research at the 37th Congress of German Psychiatrists, noting that "in the center of an almost normal cell there stands out one or several fibers due to their unique thickness and peculiar impregnability. The uppermost layers contain many tiny foci. They are determined by the storage of specific substances in the cerebral cortex. Overall, we are dealing with a strange disease process" (Hippius & Neundörfer, 2003). It is now known that the impenetrable fibers and the millary foci, which were later known as neurofibrillary tangles and amyloid-based neuritic plaques, respectively, are the defining characteristics of AD (Figure 2.1). As there was little reaction to Alzheimer's discovery, senility and dementia were still seen as natural effects of ageing. Dr. Emil Kraepelin first referred to AD as a particularly severe type of senile dementia in 1910 (Graeber et al., 1997).

In the US, AD is the sixth leading cause of death. It is believed that the incidence of AD ranges from 1–3%, while its prevalence ranges from 10–30% of the population over the age of 65. This is due to the fact that AD typically lasts for 10 years (Sun et al., 2020). Alzheimer's disease is the most common kind of dementia that people suffer from. It is a neurodegenerative illness that worsens over time and is frequently recognized (H. J. Park et al., 2020). The disease has a very high death rate and is tightly linked to both hereditary predisposition and advancing age. The prevalent disease AD is a major contributor to dementia, which begins with an unchangeable

impairment in episodic memory and develops to a more general reduction in overall cognitive function in the elderly. Dementia begins with Alzheimer's disease (AD) symptoms and ends with dementia symptoms (Hafez et al., 2017; Osborn et al., 2016). Disorientation and a slow decline in memory and intelligence are hallmarks of AD (Garabadu & Verma, 2019).

Clinically, the disease manifests as a slow-moving cognitive and behavioral deterioration. The most prevalent form of the disease, dementia, affects about 50 million individuals worldwide, and by the year 2050, it's predicted that there will be 152 million instances worldwide (Yiannopoulou & Papageorgiou, 2020); Livingston et al., 2020). Primary carers for AD patients have negative emotional and physical effects as well. According to a study, 37% of nursing personnel who care for AD patients self-reported high emotional strain and 32% self-reported high physical stress (Albers et al., 2014).

2.2 Pathophysiology of AD:

The buildup of two proteins in the brain is what leads to the onset of Alzheimer's disease. These proteins, known as A-beta and tau, respectively produce external neuritic plaques and intracellular neurofibrillary tangles. Inflammatory reactions as well as neuronal malfunction and death follow. Instead of α-secretase and γ-secretase, β-amyloid-converting enzyme and γ-secretase break the amyloid precursor protein (APP), releasing A peptides that lead to the buildup of plaques and tangles (De-Paula et al., 2012). Amyloid plaque buildup, hyperphosphorylation of tau protein, mitochondrial dysfunction, neuroinflammation, and oxidative stress are all signs of AD pathogenesis (Choi, 1995).

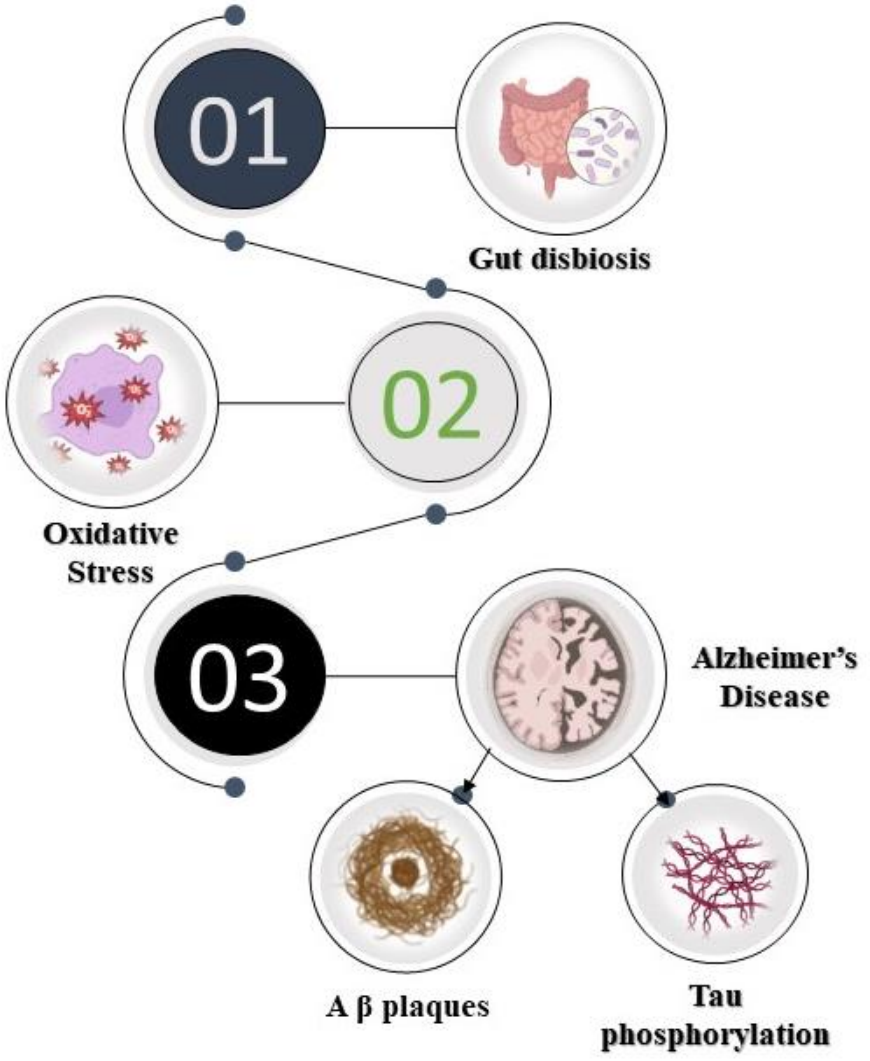

Figure 1: Pathophysiology of Alzheimer's disease

2.3 Hypothesis regarding AD

It is noteworthy that there are some established hypotheses connected to Alzheimer's disease (AD), such as the amyloid-beta cascade hypothesis, the tau protein hypo phosphorylation hypothesis, and the oxidative hypothesis, which may assist reveal the pathogenic processes that underlie the disease. The etiology of neurodegenerative diseases such Parkinson's disease, AD,

Huntington's disease, and multiple sclerosis all share the oxidative stress as a common factor (Tarafdar & Pula, 2018).

2.3.1 Amyloid beta Cascade Hypotheses:

This hypotheses was originally put forth in 1991 (Hardy & Allsop, 1991). When Paul Blocq and George Mannesco saw "circular buildup in the brains of aged patients" in 1892, they first hypothesized the existence of A-beta plaques. Glenner extracted "beta-amyloid" from the meningeal arteries of Alzheimer patients after nearly a century of study and found the peptide sequence in part (Glenner & Wong, 1984).

The first non-amyloidogenic pathological route involves the engagement of α- and γ-secretases in the creation of molecules that are neurotrophic and neuroprotective for nerve cells when conditions are normal. This pathway is followed when conditions are abnormal. These products include the soluble ectodomain of APP- (sAPPα), the C-terminal fragment (CTF)-α, and various smaller fragments. The first pathogenic pathway that is not amyloidogenic results in the production of these compounds. The amyloidogenic pathological route is the second step, in which APP is broken down by γ-secretase to CTF-β and then by γ-secretase to various lengths of Aβ peptides, including Aβ42. Aβ42 is more prone to aggregation and form plaque than Aβ40 is, and it also has a higher level of neurotoxicity (Carlo et al., 2012).

A considerable decline in spatial working memory, an increase in cholinergic dysfunction, a drop in ChAT and AChE activity, and an increase in AChE activity in prefrontal cortex, hippocampus, and amygdala are additional consequences of Aβ. Selected mouse brain regions also exhibit a significantly higher level of apoptosis. Moreover, in all brain regions of rat, A substantially decreased mitochondrial function, integrity, and bioenergetics (Semwal & Garabadu, 2020).

2.3.2 Tau Hypothesis

The production of tau, a microtubule-associated protein, is dependent on a particular type of alternative splicing that occurs in the MAPT gene. When Claude Wischik isolated tau from plaques that were found in the brains of Alzheimer's disease patients in 1988, he demonstrated for the first time that tau protein may be the primary cause of dementia (Wischik et al., 1988). Tau and microtubules are found in the axons of neurons in brain (Fan et al., 2020).

Tau protein stabilizes microtubule assembly by interacting with tubulin through its isoforms as well as through phosphorylation of its own protein. Six isoforms of the tau protein family, with amino acid ranges of 352-441, make up this family the CNS isoform with the longest length, R1, R2, R3, and R4, includes a total of 441 amino acids distributed among four repeats and two inserts, whereas the isoform with the shortest length, R1, R3, and R4, has only three repeats and no inserts (352 amino acids total). All six isoforms of tau are found within the paired helical filaments that are characteristic of Alzheimer's disease, and many of them are in a hyperphosphorylated state (Mohandas et al., 2009).

Cyclin-dependent kinase-5 (cdk-5) and glycogen synthase kinase-3b (GSK-3b) are two of the several kinases that are implicated in the pathological process of tau hyperphosphorylation. They can target a number of tau phosphorylation sites (Gong et al., 2005). The cytoskeleton becomes unstable as a result of the hyperphosphorylation of the tau protein, and nerve cells deteriorate. It is vital to note that tau is critical for the stability of cytoskeletal microtubules in this sentence (Garcia & Cleveland, 2001).

2.3.3 Significance of Oxidative Stress in AD

Unbalanced levels of antioxidants and free radicals are referred to as oxidative stress. Another early sign of AD is oxidative stress (Qu et al., 2020). Reactive oxygen species (ROS or free

radicals), of which 95–98% originate from byproducts of the mitochondria's electron transport chain (ETC), may be a mediator of oxidative cell damage and cell death (Lum & Roebuck, 2001). An organ with a high oxygen demand is the brain for it to function effectively, making it vulnerable to the effects of ROS. Moreover, the brain has a low concentration of enzymes and other antioxidant molecules and has a high amount of polyunsaturated fatty acids that are susceptible to oxidation. Moreover, it has a significant amount of iron, a potent ROS catalyst (X. Wang et al., 2014). The ROS are typically reactive molecules that contain oxygen. ROS that are unstable, transient, and highly reactive are either free radicals or compounds that readily produce free radicals, ranging in reactivity from low to high (Madreiter-Sokolowski et al., 2020).

Overproduction of free radicals can contribute to the buildup of the tau and beta-amyloid (Aβ) proteins, two hallmarks of Alzheimer's disease (AD) (Abramov et al., 2020). At physiologically normal quantities, free radicals are crucial for synaptic plasticity, which in turn affects memory and learning. Nevertheless, when oxidative stress is elevated in neurodegenerative illnesses like AD, synaptic plasticity and memory are compromised (Massaad & Klann, 2011). As a therapeutic target, oxidative stress is a topic of extensive research for treating the learning and memory deficits in AD. 8-Hydroxy-deoxyguanosine (8-OHdG), a biomarker of DNA oxidative damage, is seen higher in the nuclear and mitochondria of Alzheimer's patients and animal models (Santos et al., 2012).

In the case of Alzheimer's disease, ETC is inhibited, resulting in a buildup of electrons in complex I and coenzyme Q (CoQ). It is possible for the electrons that have gathered there to be given directly to molecular oxygen to produce the nitric oxide, superoxide radical (O^{2-}) and the superoxide radical (O_2) can combine to generate the peroxynitrate radical (OONO-). The H_2O_2 is changed into the hazardous hydroxyl (OH) radical in the presence of transition metals. As a

result, AD is linked to ETC malfunction and the generation of free radicals. A lack of antioxidant capability is another feature of AD. Glutathione levels are low and the activity of the enzymes Cu/Zn SOD (superoxide dismutase) and (GSH) are diminished. As a result, antioxidants are powerless to stop the free radicals from causing cellular harm (Aliev et al., 2002).

2.4 Gut-brain Axis

It was theorized that "nervous weakness" may result in defective digestion, or on the other hand, that impaired digestion may permit accumulation of toxins from the intestines, including bacteria, that disrupt the action of the nervous system. The concept of connectivity between the stomach and the brain dates back as far as the eighteenth century. At the time, physicians reported on such illnesses as "neurasthenia gastrica" and "autointoxication" (Lillestøl, 2018).

The gut-brain axis, which interacts in both healthy and sick states, is an anatomical connection between the gut and the central nervous system (CNS) (Mayer, 2011). Understanding the crosstalk between the gut and the brain has revealed a complicated communication system that ensures the proper maintenance of gastrointestinal homeostasis. Moreover, several effects on mood, motivation, and higher cognitive abilities are anticipated from this system. "Gut-brain axis" (GBA) is a phrase that describes the intricate nature of these relationships (Rhee et al., 2009). Its job is to keep an eye on and integrate gut processes while also connecting the brain's affective and cognitive regions to peripheral intestinal processes and mechanisms include intestinal permeability, entero-endocrine signaling, enteric reflex, and immune activation. The mechanisms underlying GBA communications include the involvement of neuro-immuno-endocrine mediators (Tsigos & Chrousos, 2002).

It has been speculated for a long time that the gut may be home to as many as 10^{14} bacterial cells, which would explain for the reputed 10:1 ratio of bacterial cells to human cells that is frequently mentioned in the literature. Yet, according to recent studies, there are about $3.8\times\ 10^{13}$ bacterial cells overall in the gastrointestinal (GI) tract, making the above ratio 3:1 (Sender et al., 2016).

When a newborn is exposed to a variety of environmental stimuli, the gut microbiota grows in size and diversity. By the time an infant is one to two years old, an adult-like microbiome is visible. Because of its dynamic nature, the microbiome of the gut can be affected by a wide range of factors, including the method of birth delivery, the transfer of microbes from the mother to the child, genetics, food, infection, drugs (such as antibiotics), age, and stress (Penders et al., 2006); Yatsunenko et al., 2012). These factors have the potential to not only temporarily but also permanently alter the composition of the gut. Other phyla, such as Proteobacteria, *Actinobacteria, Fusobacteria,* and *Verrucomicrobia*, are found among the microbial diversity of gut, although in substantially lesser levels. Firmicutes and Bacteroidetes are the two bacterial phylotypes that predominate in the gut microbiome. Despite the fact that each person's gut microbiota is unique, people have been classified into three "enterotypes", each of which is dominated by a different species of bacteria: *Prevotella, Ruminococcus,* and *Bacteroides* (Arumugam et al., 2011).

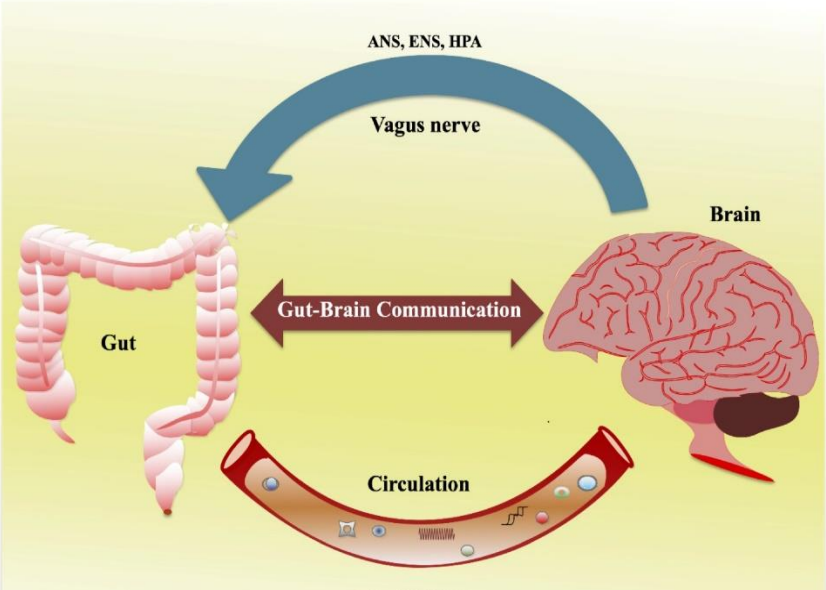

Figure 2: Bi-directional pathway of Gut Microbiota-Brain axis (Suganya & Koo, 2020)..

2.5 Bi-directional communication routes of Gut brain axis

The gut-brain axis is considered to be bidirectional due to the fact that the microbiota in the gut has an impact on the functioning of the central nervous system (bottoms-up approach), as well as the CNS has an effect on the composition of the microbiota in the gut. In other words, the microbiota in the gut has an effect on the CNS (top-down approach). The brain is able to control the function of the gastrointestinal tract by way of the hypothalamic-pituitary-adrenal axis as well as the autonomic nervous system. For instance, the brain secretes norepinephrine in response to stress, and this hormone has been shown to stimulate the proliferation of gut pathogens (Mart'yanov et al., 2021). On the other hand, the gut is able to influence the functions of the CNS by utilizing a wide range of metabolites and products derived from the microbiota, along with neuroactive chemicals and gut hormones that travel through the enteric nervous system. (Long-Smith et al., 2020).

2.5.1 Vagus nerve

In the vagus nerve (VN), also called cranial nerve X, there are 20% motor efferent fibers and 80% sensory afferent fibers (Fülling et al., 2019). The vagus nerve, the 10th cranial nerve, travels from the brain to muscular and mucosal layers of the gut. It plays several roles including regulation of peristalsis in gut, satiety and GI secretion (Breit et al., 2018)

It is the primary component of the parasympathetic nervous system. By the vagal circuit, it links the brain and microbiota. The microbiota and other gut metabolites stimulate afferent vagal neurons, causing them to fire action potentials and release glutamate, a neurotransmitter that is primarily excitatory, in the nucleus tractus solitarius (NTS). (Giridharan et al., 2022).

2.5.2 HPA axis

The HPA axis, which is a component of the neuroendocrine system, reacts to both psychological and physical stimuli and controls a variety of biological processes. Research shows that the HPA axis and the gut microbiome are directly connected. The HPA axis action alters the gut microbiota's makeup and raises gut permeability, making it easier for bacterial chemicals to enter the bloodstream and cause persistent low-grade inflammation (Doifode et al., 2021). Healthy human volunteers who consumed the B. longum1714 strain saw a reduction in cortisol levels. This translational psychobiotic simultaneously enhanced visuospatial memory performance, which is hippocampus dependent. The levels of plasma adrenocorticotropic hormone (ACTH), pro-inflammatory cytokines, corticosterone, hypothalamic corticotropin-releasing hormone (CRF) and intestinal permeability all increased in an animal model of partial restrict stress. Combining different probiotics caused reduction in inflammation, permeability of intestine, and the stress response of HPA axis (Giridharan et al., 2022).

There has been evidence of bacterial translocation in neuropsychiatric disorders associated with stress, such as depression. *Limosilactobacillus farciminis*, a potential probiotic, has the potential to regulate barrier leakiness brought on by stress-related HPA response (Ait-Belgnaoui et al., 2006). A human-derived microbiota cocktail containing five distinct *Enterococcus* and *Limosilactobacillus* strains, respectively, reduced gut dysbiosis, leaky gut, and cognitive deterioration in ageing mice fed high-fat diets (Ahmadi et al., 2020).

2.5.3 Neurotransmitters and Neuromodulators

Major contributors to the MGBA include metabolites and products generated from microorganisms, which mainly affect host tissues or cells through interactions with receptors. A byproduct of the microbially driven breakdown of carbohydrates, SCFAs have been proposed to support lymphocyte function, glucose balance, mucosal serotonin release, and learning and memory development through maintaining brain integrity (Clarke et al., 2013).

Another group of microbiota-related compounds, known as neuroactive molecules, also affects the MGBA, most likely via the ENS. Neuroactive chemicals like dopamine, noradrenaline, acetylcholine, melatonin, histamine and GABA that modulate the CNS have been shown to be modulated by gut microorganisms, if not directly synthesized (Hsieh et al., 2020).

Consuming *Limosilactobacillus plantarum* and Macleaya cordata extract also helped preserve the intestinal mucosal barrier and minimize intestinal oxidative stress in goats (Chen et al., 2020). Therefore, it is possible to draw the conclusion that the ingestion of probiotic bacteria may function as a biological barrier to the creation of reactive oxygen species (ROS) by stimulating antioxidant enzymes, increasing the production of antioxidant metabolites, and modulating antioxidant signaling pathways and gut microbiota (Amaretti et al., 2013; Y. Wang et al., 2017). Polyphenols also play a role in secreting antioxidant enzymes. In an earlier study, the

researchers utilized streptozocin to produce an AD model. After that, they utilized quercetin to reduce the amount of damage done to the myelin and axons in a rat model of diabetic peripheral neuropathy (DPN). In addition to this, they reduced the level of ROS production and increased the diversity of gut bacteria in all of the groups (Xie et al., 2020).

2.6 Alzheimer's and Gut-brain axis

Alzheimer's is a side effect of peripheral inflammation in addition to limited brain inflammation (Le Page et al., 2018). The fact that gut dysbiosis causes inflammation that worsens with age, disrupts the BBB, activates the immune system, and then causes neurodegeneration is evidence for this. On the other hand, a healthy, well-balanced stomach helps to lessen the negative consequences of ROS (Luca et al., 2019). Similarly, the 5xFAD mouse model which mimics Alzheimer's disease demonstrated shifts in the population of microbiota towards proinflammatory species in conjunction with changes in amino acid catabolism. On the other hand, therapy with antibiotics was able to reverse the effects, suggesting a probable link between the severity of the disease and an altered gut population (X. Wang et al., 2019).

A Chinese cohort used the 16s ribosomal RNA MiSeq sequencing technology to determine the changes in the gut microbial colonies of AD patients. According to the study, the gut microbiota of AD patients had dramatically different ratios of three important phylas. *Firmicutes* had a much lower relative abundance ($p = 0.008$) than proteobacteria, which had a significantly higher relative abundance ($p = 0.024$). Moreover, less *bacterioidetes* were found in AD patients. Important SCFA-producing bacteria are firmicutes. SCFAs are crucial for maintaining mucosal barrier permeability and safeguarding the BBB. A decrease in the amount of circulating SCFAs causes leaky gut and BBB degradation. Proteobacteria are proinflammatory microorganisms that release cytokines and contribute to the buildup of LPS. Less of the *clostridiaceae* and

ruminococcaceae were found in AD patients. Insulin resistance has been connected to their reduced abundance. For AD, insulin resistance is a significant risk factor (P. Liu et al., 2019). This data suggests that altering the diversity and composition of the microbiome through a variety of mechanisms could be used as a potential therapeutic approach to slow the onset and development of disease.

2.7 Conventional treatment for Alzheimer's disease

Since the pathophysiology of AD involves numerous pathways and is a complicated phenomenon, finding a single therapy approach is highly challenging. AD symptoms are treated with antioxidant, anti-inflammatory, and cholinergic medications. Some medications have negative side effects and do not halt the course of illness or neurodegeneration (Herholz et al., 2004). The most preferred method for treating AD is to block acetylcholinesterase (ACE), which recycles synaptic acetylcholine in grey matter. Decline in cholinergic neuron density is a preliminary feature of AD. Acetylcholine activity is prolonged when acetylcholinesterase is inhibited (Weller & Budson, 2018). Galantamine, rivastigmine, and donepezil are three of the more popular ACE inhibitors. These medications are taken orally and have a variety of side effects, such as heart block, bradycardia, sleeplessness, dizziness, allergic dermatitis, muscle cramps, and loss of appetite followed by weight loss. This list of negative effects is in no way complete; there may be more (Joe & Ringman, 2019) . Therefore, it is necessary to research innovative therapies that slow disease progression and have fewer negative consequences.

2.8 Alternative therapy for AD

The significance of the microbiome in relation to neurodegenerative illnesses has lately been investigated (Westfall et al., 2017). In addition, the axis between the gut and the brain is now

known to be an important contributor to the development of neurodegenerative illnesses. Hence, among the unique substances that might be used in AD prevention are polyphenols and probiotics, which are essential for the preservation of a healthy microbiome (Bistoletti et al., 2020).

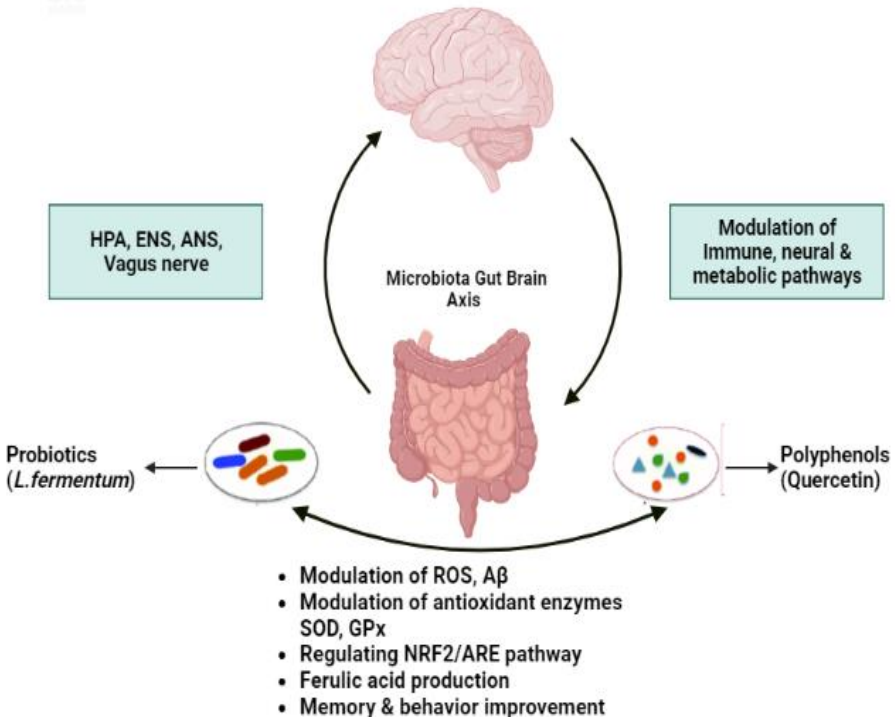

Figure 3: Mechanism of action of probiotics and polyphenols

2.8.1 Probiotics

The Food and Agricultural Organization of the World Health Organization provided the definition currently in use, which defines probiotics as "live bacteria that, when delivered in suitable proportions, impart a health benefit on the host." The definition can be changed in connection to food by highlighting the fact that microbes "when taken in suitable proportions as part of food" have the positive impact (*Joint FAO/WHO Expert Consultation On*, 2001). For health benefits, the Italian Ministry of Health (IMH) recommends consuming at least 1x10^9

CFUs of viable probiotic cells per day (Kechagia et al., 2013). Probiotics are a commonly used, reasonably priced, and well-tolerated therapeutic (Den et al., 2020). Although the precise process by which probiotics exert their effects is still not completely understood, they have a variety of mechanisms of action. These include anything from the creation of short chain fatty acids and bacteriocin to nutritional competition, the lowering of gut pH, and the activation of mucosal barrier function (Kechagia et al., 2013).

Probiotics have been shown to slow the progression of AD, notably the inflammatory response and oxidative stress, and to improve cognitive decline. The balance of the gut flora can also be brought back to its normal state with the help of probiotics (Evrensel & Ünsalver, 2018). In addition to acting as antibiotics, they regulate the pH level within the body, ensure the health of the intestinal lining, and stimulate the production of brain-derived neurotrophic factor (Larroya-García et al., 2019). The proteins, known as BDNF, aid in the survival and development of neurons in the brain. As a result, it is essential for neurological development. If these components of the brain are absent, common problems including learning deficits and memory impairments might develop (Naomi et al., 2022).

By altering the makeup of helpful bacteria in the gut microbiota and enhancing CNS processes, probiotics have favorable impacts on the central nervous system (CNS). Probiotics, in addition to brain neurotrophic factor, have an excellent prognosis for treating memory loss and mental problems by directly altering brain biochemical substances like serotonin, -aminobutyric acid (GABA), and dopamine (Ale & Binetti, 2021). The two genera of probiotic bacteria that are most frequently utilized are *Limosilactobacillus* and *Bifidobacterium*. As they don't contain lipopolysaccharides, eating them does not result in any kind of inflammation (Markowiak & Śliżewska, 2017). Probiotics' effects on spatial learning, memory, and other factors have been

studied in rats with AD. In comparison to the AD group, rats given probiotics (*B. bifidum, B. longum and L. acidophilus*) for four weeks demonstrated a significant increase in paired-pulse facilitation ratios, long-term potentiation, lipid profiles, and spatial learning and memory. (Rezaeiasl et al., 2019).

2.*8*.1.1 Anti-Alzheimer potential of *Limosilactobacillus fermentum*

Damage is caused to macromolecules such as proteins and nucleic acids when oxidative stress causes an increase in the quantity of oxygen radicals within the cell. ROS are dealt with by enzymes found within organisms, which neutralizes them and puts a stop to the damage they do. The enzyme SOD, GPx, GR and non-enzymatic antioxidants are the principal defense systems that protect the organism from the damaging effects of oxidative stress (Pizzino et al., 2017). As supporting antioxidants, the usage of antioxidants derived from biological sources is growing in popularity (Y. Wang et al., 2017).

The production of ROS is stimulated by redox-active metals like cobalt (Co), copper (Cu), and iron (Fe), which cause redox reactions (Georgiadou et al., 2018). The use of probiotics may confer several additional antioxidant effects. One of these approaches is the fact that certain lactic acid bacteria have been demonstrated to be capable of chelating metal ions, despite the fact that the precise mechanism of action remains a mystery (N. K. Lee et al., 2005).

In addition to encouraging the host to make antioxidant enzymes, lactic acid bacteria may manufacture their own antioxidant enzymes. For instance, it has been demonstrated that *Limosilactobacillus fermentum* contains SOD. (Kullisaar et al., 2002). Bacterial SODs have shown promise in the treatment of Crohn's disease in studies that have been conducted using mouse models (LeBlanc et al., 2011).

Moreover, *Limosilactobacillus fermentum* are known to activate a number of pathways, including MAPK, PKC, and Nrf2-Keap1-ARE, which play role in host as a responsive mechanism against oxidative stress (Y. Wang et al., 2017). In addition to the Nrf2 pathway, this lactic acid bacteria also stimulate the activity of Nrf2-related antioxidant enzymes like catalase, heme oxygenase 1, and SOD (Feng & Wang, 2020).

When it comes to neurodegeneration, ferulic acid has an immediate effect on the neurons. Both in vivo and in vitro studies have shown that it can stimulate the proliferation of brain stem cells. In the former case, the number of neurons in the dentate gyrus increased as a result of FA of mice receiving corticosterone treatment, demonstrating its strong capacity to induce neurogenesis in vivo. For influencing communication between the commensal microbiota and the brain more recently, ferulic acid has been a major target. In addition to dietary sources, some gut microbiota have the ability to synthesis FA quickly and in large quantities through ferulic acid esterase gene. The most effective of these is *L. fermentum NCIMB 5221* (Westfall et al., 2017).

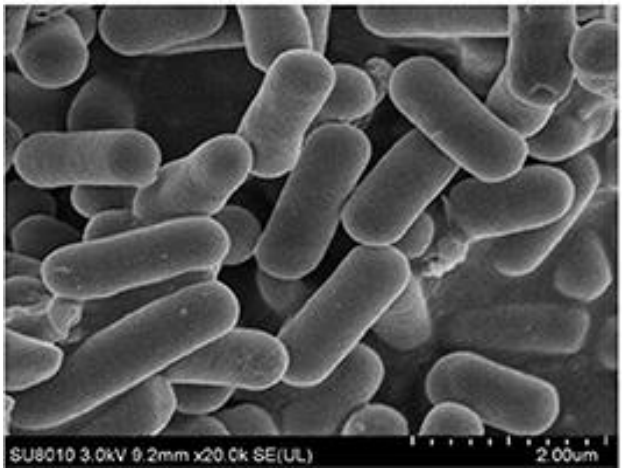

Figure 4: SEM image of *Limosilactobacillus fermentum* (Long et al., 2022)

2.8.2 Polyphenols

Phytochemicals are primarily secondary plant metabolites that can be found in a wide range of foods and beverages, including fruit, vegetables, cereals, nuts, cocoa/chocolate, juice, tea, and wine. Dietary intake of phytochemicals typically exceeds 1 g per day (Ovaskainen et al., 2008). The most varied class of phytochemicals, flavonoids are found in large quantities in higher plants and have exceptional medicinal potential (Nabavi et al., 2018). On the basis of their chemical makeup, flavonoids are further split into six classes: flavonols, isoflavonoids, anthocyanidins, flavanols and flavones (H. Khan et al., 2021).

The antioxidant and anti-inflammatory properties of flavonoids have received a lot of attention because they play key roles in the pathophysiology of AD (Devore et al., 2012). Research has shown that flavonoids may penetrate BBB. Hence, they could be used to prevent neurodegenerative disorders. Nevertheless, the BBB-crossing capacities of various flavonoid subclasses vary (Ullah & Khan, 2018). In the case of AD, its effectiveness is explained by a decrease in oxidative stress and the toxicity of Aβ (Deng et al., 2017).

2.*8*.2.1 Quercetin

The flavanols are a subgroup of flavonoids, and Quercetin is a flavanol. One of these compounds that people consume the most in their diets is Quercetin, with an average daily consumption of between 5 and 40 mg (Costa et al., 2016). Five hydroxyl groups and three ring configurations make up the chemical makeup of Quercetin. It has the ability to pass BBB, which, in the context of neurodegenerative disease, is critical. (Calfio et al., 2020).

Many qualities of Quercetin, such as its anti-inflammatory and antioxidant capacities, are advantageous to human health. This final point is especially important to keep in mind when

discussing neurodegenerative diseases because the brain is an organ that is particularly susceptible to oxidative stress. This is because the brain has a high concentration of unsaturated fatty acids, a high rate of oxygen consumption, and a limited capacity for antioxidants (Chopra et al., 2022). It is also possible to obtain it in free form by isolating it from the surfaces of leaf, fruit, or bud extracts. Passiflorae, Compositae, Solanaceae and Rhamnaceae, are plant groups that are high in quercetin (Alok et al., 2014). Quercetin is present in relatively high percentages in apples, mangoes, red leaf lettuce, onions, tomatoes, asparagus, capers, buck weed, tea, citrus, plums and berries (Kim et al., 2019) (Costa et al., 2016). Due to its ability to operate as a substrate for gut microorganisms to produce SCFAs while remaining undigested until it reaches the colonic section, the flavonoid can also function as a prebiotic (Chavarro et al., 2020); (Rice et al., 2019).

In order to check the ability of quercetin on SCFA a study was conducted on rmTBI mice. In faecal samples from rmTBI mice, we saw decreased levels of acetate, propionate, and butyrate. These levels recovered in the animals that had been given quercetin (Balasubramanian et al., 2022).

Quercetin has demonstrated therapeutic potential, enhancing cognitive abilities such as memory and learning in AD. In patients suffering from Alzheimer's , there is evidence that quercetin can improve learning, memory, and other cognitive skills (Anand David et al., 2016). In vitro models were used to draw the conclusion that quercetin treatment inhibited the AChE and secretase enzymes, inhibiting the breakdown of acetylcholine and reducing the synthesis of Aβ, respectively (M. T. H. Khan et al., 2009); (Shimmyo et al., 2008). In vitro and computer simulation studies have also shown quercetin can decrease beta-secretase-1 (BACE-1) enzyme

activity by forming hydrogen bonds. The C-3 OH group has a crucial role in the suppression of BACE-1 (Shimmyo et al., 2008).

Tauopathy frequently starts in the hippocampus, disrupting cognitive functions that depend on it, before spreading to other parts of the brain. It has been proven that quercetin can lower the phosphorylation of tau proteins and can limit the generation of NFTs in mice that have been genetically engineered to imitate age triple transgenic Alzheimer's disease (Sabogal-Guáqueta et al., 2015). The ability of quercetin to scavenge free radicals, chelate metals, and shield neurons from metal toxicity account for the majority of its antioxidant activities It is possible for quercetin to have an effect on enzyme systems, such as the nitric oxide synthase, as well as transcriptional factors, such as Nrf-2, which are responsible for activating genes that code for detoxifying and antioxidant proteins (Costa et al., 2016 ; Maccioni et al., 2022)

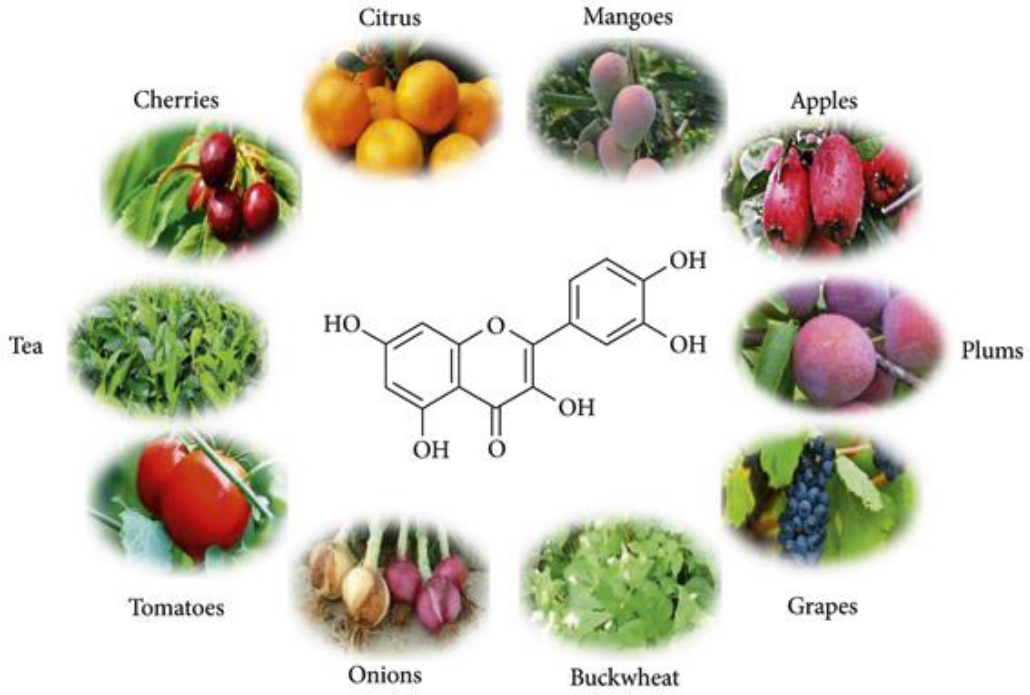

Figure 5: Sources of Quercetin (Costa et al., 2016)

CHAPTER 3

MATERIALS AND METHODS

3.1 Chemicals

Aluminum chloride hexahydrate ($AlCl_3.6H_2O$) was purchased from Scharlau (Product catalogue no. AL0770). Donepezil under the brand name Donecept® was purchased from local pharmacies in Islamabad, Pakistan. MRS Broth and MRS Agar were got from MERK. Chemicals for gram staining like Safranine, Gram's Iodine, Crystal Violet and Decolorizer were bought from Diachem. Quercetin in powder form was obtained from Dr. Imran at COMSAT university.

3.1.1 Probiotic strain selection (*Limosilactobacillus Fermentum* Y55)

Probiotic Isolate *Limosilactobacillus Fermentum* Y55 was selected that was previously isolated by lab fellow Noor, (2019). This strain is further characterized exhibiting antioxidant potential by in-silico analysis done by another lab fellow Ayesha, (2022).

3.2 Morphology assessment of *L. fermentum* Y55

3.2.1 Gram staining

Single colony was selected from MRS agar plate and dispensed on the droplet of distilled present on the slide to make smear which was then fixed by heat. The smear was then treated for 1 minute with crystal violet and removed with distilled water. It is then treated for 40 seconds with an iodine solution, washed with ethanol for 5 seconds before being stained with safranin for 40 seconds, and finally washed with distilled water. After drying by air, it was examined with

immersion oil under 100X objective lens microscope. Then catalase test was conducted. A single colony of probiotic strain was picked with sterile loop and transferred to dry clean slide. 3% H_2O_2 was added on the colony and mixed well.

3.3 Animals

Institutional Review Board (IRB) of ASAB, NUST under ethical code "07-2022-ASAB-01/02" approved the present study. Wister rats were bred and housed in the animal housed in the animal house of Atta ur Rahman School of Applied Biosciences (ASAB), National University of Sciences and Technology (NUST). Rats were housed in cages with a natural light-dark cycle and a constant temperature of 25°C (12-12 hours). Animals were provided with unlimited access to distilled water and a standard meal of crude protein (30%), crude fat (9%), crude fibre (4%), and moisture (10.4%). Experiments were conducted using male rats (n=36), weighing 150–200 g, and between 1.5 and 2 months old.

3.4 Study design

Before the start of animal trial probiotics were revived from the stocks and undergone gram staining and catalase test. Probiotic dosage of 1.5×10^9 CFUs/mL/day/rat was given to two groups i.e TP group and TPQ group. Probiotic strain of *Limosilactobacillus fermentum* Y55 was grown for 24 hrs. in MRS broth and then centrifuged for 10 minutes at 6000 rpm at 4˚ temperature. Cell pellet was washed with PBS two to three times. They have since been combined with distilled water, and their turbidity has been compared to the 0.5 McFarland standard. Optical density of dosage was matched with 0.5 McFarland standard i.e., 0.081-0.1. Fresh dosage was prepared on the same day of administration.

A 2.5-month long plan was formulated to evaluate the effect of *L. fermentum* Y55 and Quercetin in $AlCl_3$ induced Alzheimer's model of Rat and then compare the effect of probiotics and Quercetin given to rat both separately and collectively with Alzheimer's model and Positive control model i.e., Donepezil model. Afterwards behavioral tests were conducted to check the memory impairment and anxiety followed by decapitation of animals for histopathological studies.

Details of all the groups are provided in the table.

Table 1: Groups used in this study.

Sr No.	**Experimental Groups**	**(n)**	Treatment	
			30 Days	30 Days
1.	Negative Control group	6	Distilled H2O	Distilled H2O
2.	Diseased Control group	6	120 mg/kg $AlCl_3$	Distilled H2O
3.	Probiotic Treated group	6	120 mg/kg $AlCl_3$ + Probiotic 1.5×10^9 CFUs	Probiotic 1.5×10^9 CFUs
4.	Quercetin Treated group	6	120 mg/kg $AlCl_3$ + 25mg/kg Quercetin	25mg/kg Quercetin
5.	Probiotic & Quercetin Treated group	6	120 mg/kg $AlCl_3$ + Probiotic 1.5×10^9 CFUs + 25mg/kg Quercetin	Probiotic 1.5×10^9 CFUs + 25mg/kg Quercetin
6.	Positive Control group	6	120 mg/kg $AlCl_3$	3mg/kg Donepezil

3.6 Behavior studies

Behavior tests were done during the light cycle of rats, from 9 a.m. to 5 p.m., so that the rat's circadian rhythms would not cause any changes. Before beginning the behavior experiment, the rats were habituated for 30 minutes in the appropriate behavior room. The temperature was kept at 22 ± 2°C, and the room was sufficiently illuminated. The level of human intervention and disturbance was kept to a minimum. For video recordings of behavior tests, a video camera was connected to a tripod stand (S. Lee et al., 2012)**.**

3.6.1 Elevated plus maze test (EPM)

The EPM is used all over the world to examine the psychological and neurochemical causes of anxiety as well as to test rodent genotypes and anxiety-modulating drugs. Both conventional anxiety indices and ethologically derived "risk assessment" behavior are tested using the elevated plus-maze. There are many different regions involved, but the hippocampus and amygdala are the primary ones (Reddy & Kulkarni, 1998).

Apparatus

The elevated plus maze had four arms, two of which were closed alleys and two of which were open alleys. The apparatus had 30 × 5 cm-long arms and was composed of an opaque iron alloy. It was raised 75.5 cm off the ground.

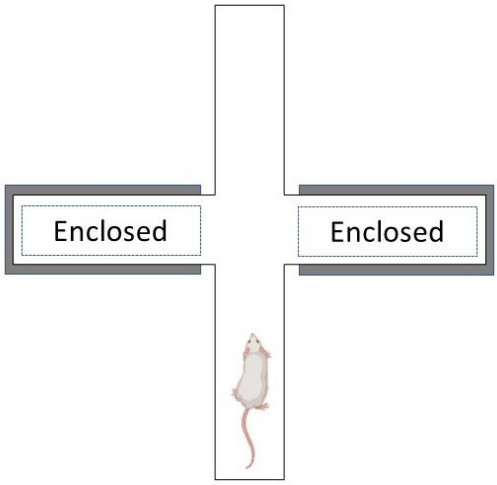

Figure 6: Elevated plus maze test apparatus.

Procedure

EPM was done in accordance with the established protocol by (Arendash et al., 2004). To start the experiment, rats were placed in the plus, facing the closed arm, and they were observed for 5 minutes. A spectator sat still 1.5 meters from the maze's center. A video recorder was set to record the behavior of rats in the above-mentioned time. Following parameters were analyzed through the video:

1. Time spent in open arm.
2. Number of entries in open arm.

3.6.2 Y-maze test

A well-established test of spontaneous exploration behavior in rodents is the Y-maze. It has also been referred to as a working memory test, an active or spontaneous working memory test, and a test of spontaneous alternation performance. Animals' natural behavior to explore undiscovered areas serves as its driving force.(Lalonde, n.d.). A mouse with an intact working memory, and consequently an intact prefrontal cortex, will recall the arms that have already been visited and

will have a propensity to enter a less often visited arm. During trial period or probe testing, one arm is blocked to test the animal's recollection of the arm that it has never visited before. (Kraeuter et al., 2019).

Apparatus

Three light-colored, opaque arms that are 120° apart from one another and each measure 16 cm high, 10 cm broad, and 40 cm long make up the Y-maze. The first arm is the starting arm, the second is the other arm, and the third is the novel arm, which is kept closed during the habituation phase and opened during the trial phase. The starting arm is where the animal is placed (Jung et al., 2020).

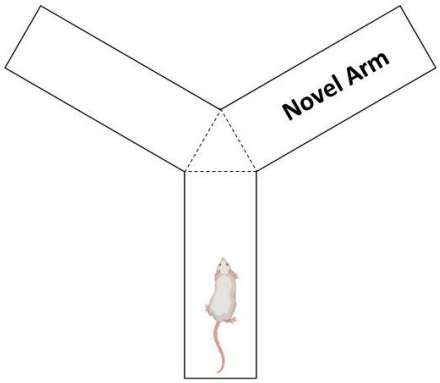

Figure 7: Y-maze test apparatus

Procedure

Animal was put inside a Y-maze during the habituation period, with the novel arm remaining closed off and only the start and other arms being open the animal was left free to explore its surroundings for 15 minutes while it was positioned in the start arm of the y-maze, facing the wall of the start arm. The animal was removed from the maze when the habituation period was over and placed back in its cage in order to create a 15-minute intertrial period between the

habituation and probe trials (Kraeuter et al., 2019). Animals were then free to roam the maze for around five minutes during the probing phase. A camera positioned above the maze captured the trial. Between habituation, the probing trial, and the following animal session, the maze was thoroughly cleansed and wiped with 70% ethanol to eliminate any odor cues for the animal. The following parameters will be evaluated using recorded videos:

1. Time spent in each arm
2. Spontaneous alternation performance (%)

   % Spontaneous alternation performance = (No. of Spontaneous alternation / total number of entries – 2) × 100

3.6.3 Novel object recognition test (NOR)

This behavior test was created in 1987 by Ennaceur and Delacour (Ennaceur & Delacour, 1987) based on the instinctive need of animals to investigate novelty. It focuses on the notion that a mouse will spend more time researching and examining an object that it has never seen before (a novel object), as opposed to spending time researching and exploring an object that it has seen before (familiar object).

Apparatus

The apparatus is made up of a box that is open on all sides and measures 80 centimeters in width, 80 centimeters in length, and 50 centimeters in height. The items that needed to be distinguished were made of physiologically inert substances like glass, plastic, or metal. The objects under the study should be weight to prevent the animals from moving them.

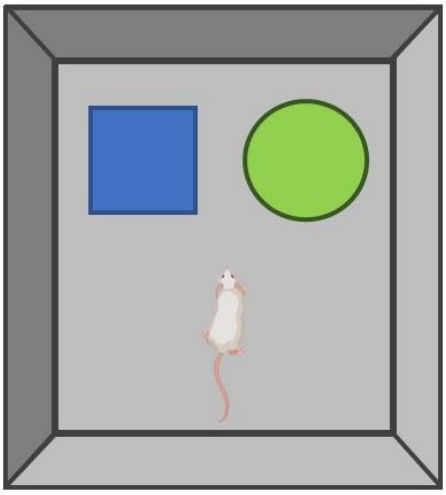

Figure 8: NOR test apparatus

Procedure

It comprises of two 10-minute test trials that are separated by 20-minute interludes. Five minutes were given for the participants to adjust to the square wooden box. Two comparable objects were put into the box being used to conduct the NOR test during the familiarization session, and the mouse was given free will to interact with the objects. One of the two familiar objects was taken away after the familiarization session. A new object was put in the spot of the one that was removed. This new item was referred to as a novel object. The rat was free to investigate and engage with both objects after the inter-trial break. Rat physically touching or sniffing the object is regarded as exploration.

- Time spent with novel and familiar object.
- The discrimination index.

3.6.4 Morris water maze test (MWM)

Few years ago, Morris, 1981 introduced a device to examine the spatial learning and reference memory in rat. With repeated training exercises, animals discover the platform and discover how

to get out of the pool. It has been used in some of the most complicated studies of the neurobiology and neuropharmacology of spatial learning and memory, despite the very simple core technique. (D'Hooge & De Deyn, 2001).

Apparatus

The tool was a circular steel pool with a height of 60 cm and a diameter of 120 cm. The pool was presumably divided into four quadrants and contained opaque water that was 34 cm deep. For the animals to exit the water, a submerged platform of 13 cm in diameter and 32 cm in height was positioned in the northwestern quadrant The animals might use spatial cues on the pool's walls to find a hidden platform by navigating from the release site around the pool's circumference.

Figure 9: MWM test apparatus

Procedure

With a few minor alterations, the test protocol was the same as that previously described (Bromley-Brits et al., 2011). It features a five-day acquisition phase and a one-day probe trial after that. A total of five trials were conducted for the learning assessment each day. Throughout the whole test, the platform's location was unaltered. It was planned for the starting direction to change for every trial, every day (Table:2). The trial's cutoff time was 90 seconds, and there was

a 10-minute break between trials. If the rat discovered the platform before the 90-second time limit, it was permitted to linger there for 5 seconds before being put back in its cage after drying. After 90 seconds, if the rat had not found the platform, it was placed there and given 20 seconds to remain there before being put back in its cage. The average of the five trials for each day was determined after the escape latency was recorded. On the sixth test day, one trial was conducted without a platform zone and with the release direction remaining unchanged. By monitoring the amount of time, the rat spent in the previously learned platform quadrant throughout the probing trial, reference memory was checked. Three parameters were calculated:

- Escape Latency over 5 days.
- Number of crossings over the platform on 6^{th} day.
- Time spent in the target quadrant on 6^{th} day.

Table 2 : Direction of release of rat, for Morris Water Maze Test

No. of Days	**DIRECTION OF RELEASE**				
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**
Day 1	West	South	North	East	South
Day 2	North	West	East	West	South
Day 3	North	East	West	South	North
Day 4	East	South	West	East	North
Day 5	West	South	North	East	South
Day 6	SINGLE TRAIL WITHOUT A PLATFORM RELEASE DIRECTION **WEST***				

3.7 Histopathological analysis of brain tissues

3.7.1 Perfusion fixation

As previously mentioned by (Gage et al., 2012), heart perfusion was carried out. Fixative solution was administered through the vascular system to fix tissue quickly and uniformly. The test mouse was put on the surgical table with its back facing down after being given anesthesia. Needles were driven through the appendages to hold them in place, securing the animal. By slicing through the connective tissues at the base of the diaphragm, the rib cage was made accessible. After that, the ribs were sliced just to the left of the midline of the rib cage. With the help of forceps, the heart was held steadily in a fixed position while a needle was introduced 5 mm deep into the left ventricular protrusion. The release of the perfusion drip valve allowed a slow, steady flow of 0.9% normal saline. To enable unrestricted solution flow through the vascular system, a cut was created in the right atrium with the use of sharp scissors. After passing saline, the body was free of blood. Instead of the saline, 4% paraformaldehyde (PFA) solution was used then. The liver's coloration lightened after PFA injection, and the animal's tail stiffened. Perfusion was terminated at this moment, and the brain was carefully extracted. The isolated brain was treated for dehydration and embedding after being maintained in formaldehyde.

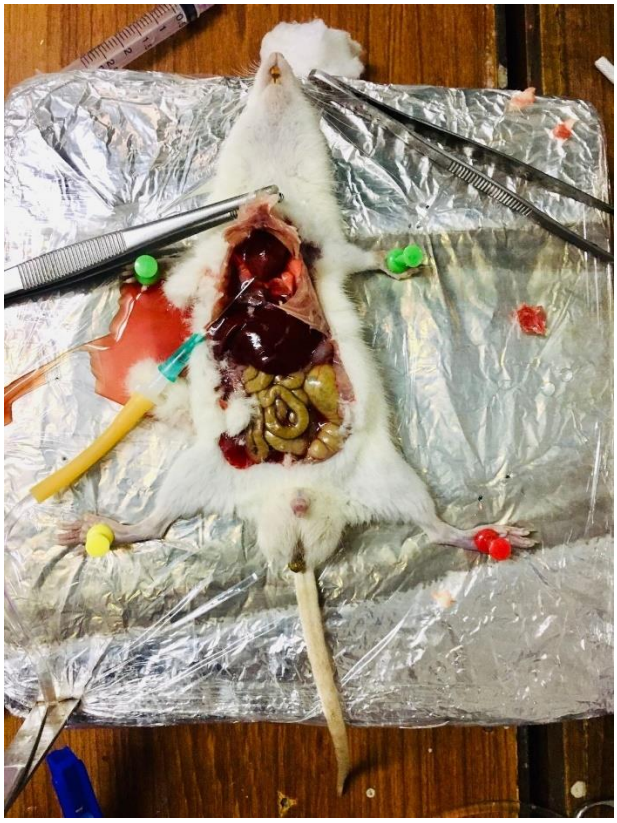

Figure 10: Perfusion fixation

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3.7.2 Paraffin embedding and block formation

The brain tissue was dehydrated over the course of 24 hours using a series of alcohols (isopropanol), 70% for 1 hour, 95% for 1 hour, and 100% for 1 hour, followed by paraffin infiltration. The brain tissues were next immersed in xylene for four hours. Paraffin embedding was then carried out by preserving the tissue in melted paraffin for 4 h at 60 °C, followed by four hours of cooling in a mould (block formation). After that, blocks were sectioned using a microtome at room temperature.

3.7.3 Hematoxylin and Eosin

On a 5µ tissue segment, conventional hematoxylin-eosin staining was carried out. The tissue was deparaffinized, incubated in Mayer's hematoxylin solution for 8 minutes, and then rinsed for 5 minutes in warm water. Eosin was used to counterstain the sections for a period of thirty seconds. Then soaking in 95% ethanol was done. Dry the slides and mount the cover slips. The images were seen under an inverted light microscope at magnifications of 4X, 10X, and 40X. OPTIKA Lite Software Version 2.11 was utilized for the acquisition and analysis of images.

3.8 Statistical analysis

Graphpad Prism version 8.0.1 was utilized in order to do the analysis on the results and to apply tests of One-way analysis of variance (ANOVA) or two-way ANOVA to dataset to determine whether statistical significance exists. Tukey's multiple comparisons test was also applied using graphpad prism 8.0.1 to determine group to group differences. Mean ± SEM was used to present error bars.

CHAPTER 4

RESULTS

4.1 Phenotypic Identification of *Limosilactobacillus fermentum* Y55

4.1.1 Colony Morphology

The purified bacterial isolates were cultured on MRS agar plates. These colonies were observed for their color and shape.

4.1.2 Gram staining

Gram staining was performed for the revived strain *Limosilactobacillus fermentum* Y55. The strain was gram positive.

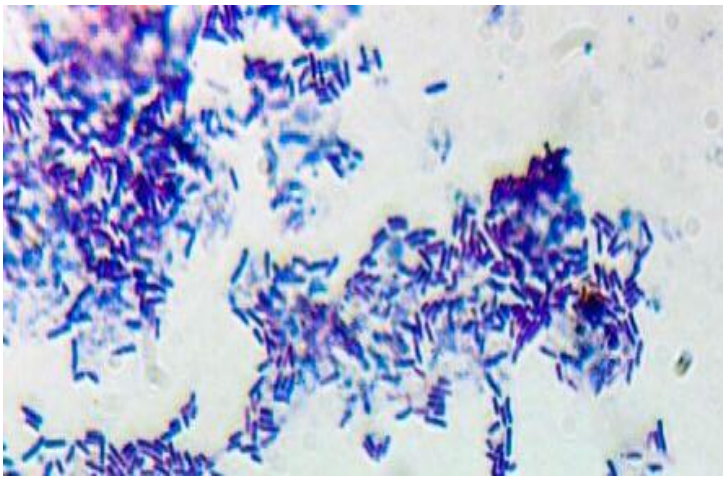

Figure 11: Different gram positive and rod shape morphologies of *L. fermentum* Y55 as revealed by gram staining observed under compound microscope at 100X resolution.

4.1.3 Catalase test:

No bubble formation was observed when *L. fermentum* Y55 was treated with 3% hydrogen per oxide. So, it was catalase negative.

4.2 Behavior Analysis

4.2.1 Effect of *Limosilactobacillus fermentum* Y55 and Quercetin on anxiety in EPM

Elevated plus maze is one of the most extensively used assays to detect anxiety like behaviors in rats. The assay leverages rodent's natural behaviors to provide a useful experimental analogue for anxiety. Animals having anxiety prefer to spend more time in closed arms on the other hand animals experiencing low levels of anxiety tend to explore open arm more.

Analysis of elevated plus maze revealed that number of entries in open arm by negative control group NC was more ($p < 0.001$) as compared to diseased control group. Similarly, Probiotic and quercetin treatment group TPQ showed significantly more ($p < 0.001$) entries into open arm than diseased control group. Likewise, Probiotic treatment group TP ($p < 0.01$) and Quercetin treatment group TQ ($p < 0.01$) showed improved entries into open arms in comparison to diseased control group DC. Positive control group PC showed less entries into open arm in contrast to NC ($p < 0.001$) and TPQ ($p < 0.001$) group (Fig 12):

In elevated plus maze more time spent in open arm is also an indicator for less anxious behavior. Rodents in NC ($p < 0.001$) and TPQ group ($p < 0.001$) spent more time in open arm as compared to diseased control group DC. Similarly, NC ($p < 0.01$) and TPQ ($p < 0.01$) group showed improved anxious behavior than PC group (Fig 13):

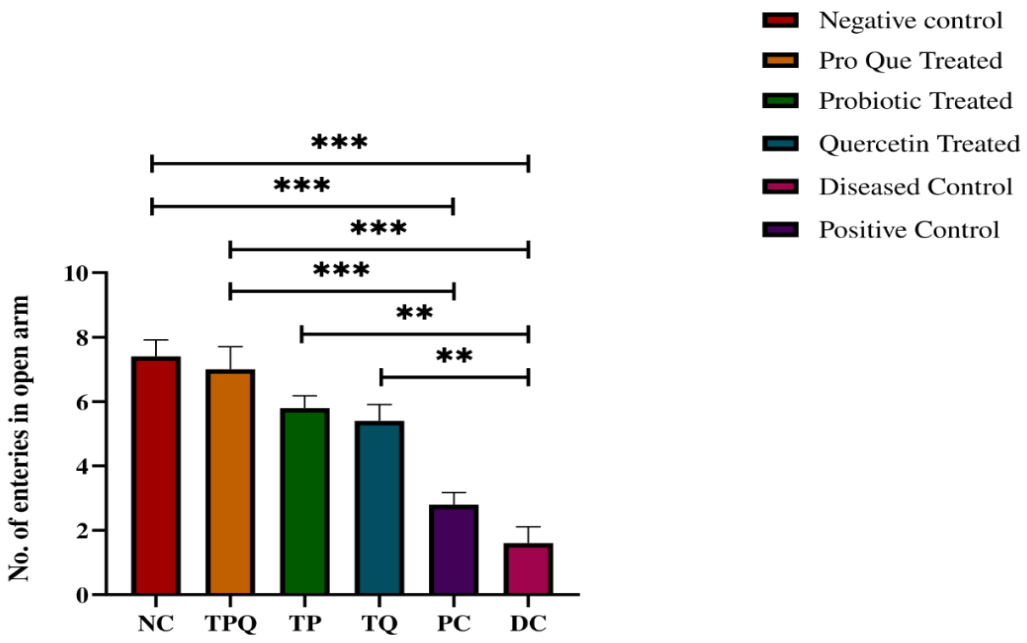

Figure 12: The effect of *L.fermentum* Y55 & quercetin on Number of entries in Open arm.

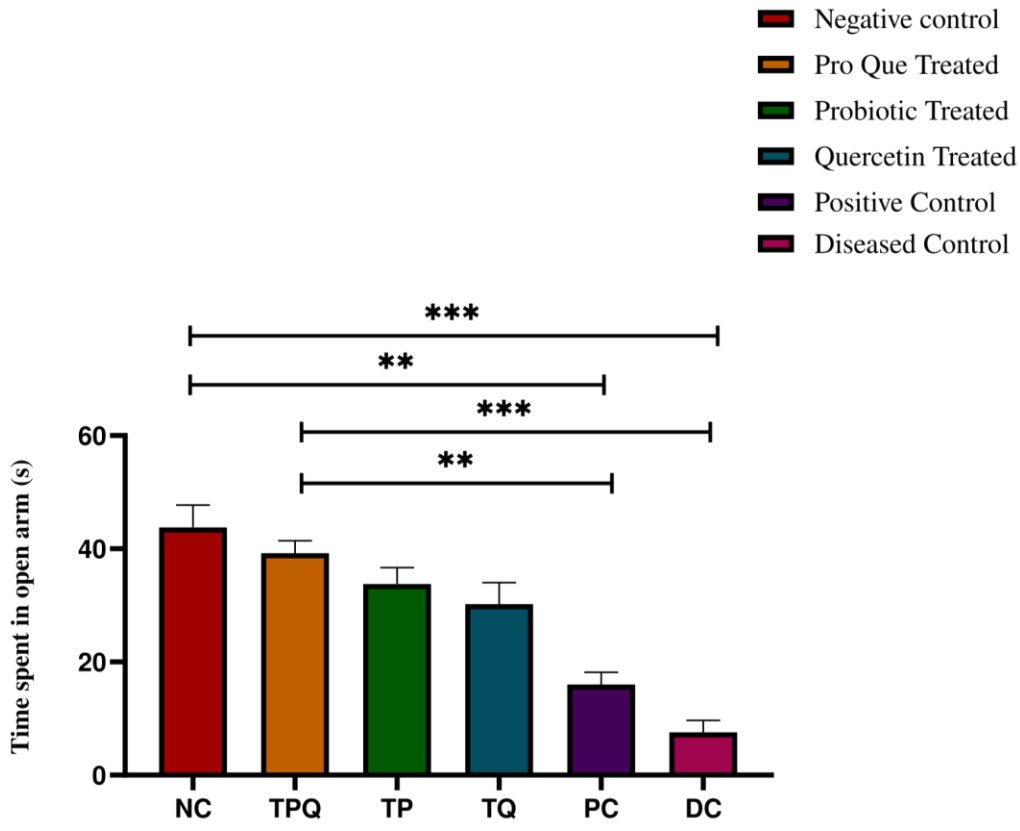

Figure 13:The effect of *L. fermentum* Y55 & quercetin on Time spent in Open arm.

4.2.2 Effect of *Limosilactobacillus fermentum* Y55 and Quercetin on spatial memory in Y-maze

Y-maze is a well-known behavioral test. Animals prefer to visit the novel arm of the maze rather than going to the arm that it has already visited. The time spent in each arm was recorded to look for the exploration time for the formerly blocked arm (Fig: 14). Rodents in negative control group spent more ($p<0.0001$) time in novel arm than disease control group similarly, TPQ group also tend to be spent more ($p<0.0001$) time in novel arm when compared to diseased control group which showed improved spatial memory. Likewise, TP ($p < 0.001$) and TQ ($p < 0.001$) group also showed significantly more time in novel arm than diseased control group. Positive control group has also showed improved ($p < 0.05$) spatial memory in contrast to diseased control group.

Spontaneous alternation was assessed as successive entry in all the three different arms on consecutive choices and is recorded when the rat enters in all the three different arms on consecutive choices. There was a significant increase of spontaneous alternation in NC ($p < 0.001$) and TPQ ($p < 0.01$) group as compared to DC group. Similarly, TP ($p < 0.05$) and TQ ($p < 0.05$) group showed significant improvement in spontaneous alternation in contrast to DC group. TPQ group ($p < 0.05$) and NC ($p < 0.05$) group had also shown significantly improved spatial memory than PC group (Fig: 15). The evidence suggests that the effect of oral consumption *Limosilactobacillus fermentum* Y55 and Quercetin in combination helps in improving spatial memory in AD rat models.

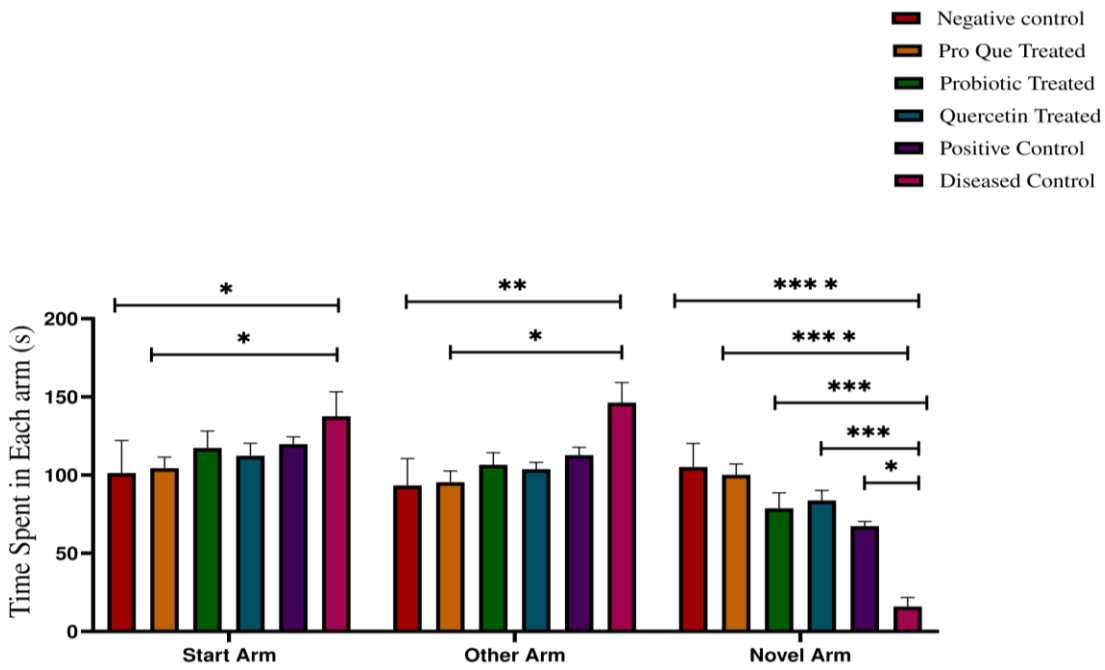

Figure 14: The effect of *L. fermentum* Y55 & Quercetin on Time spent in novel arm.

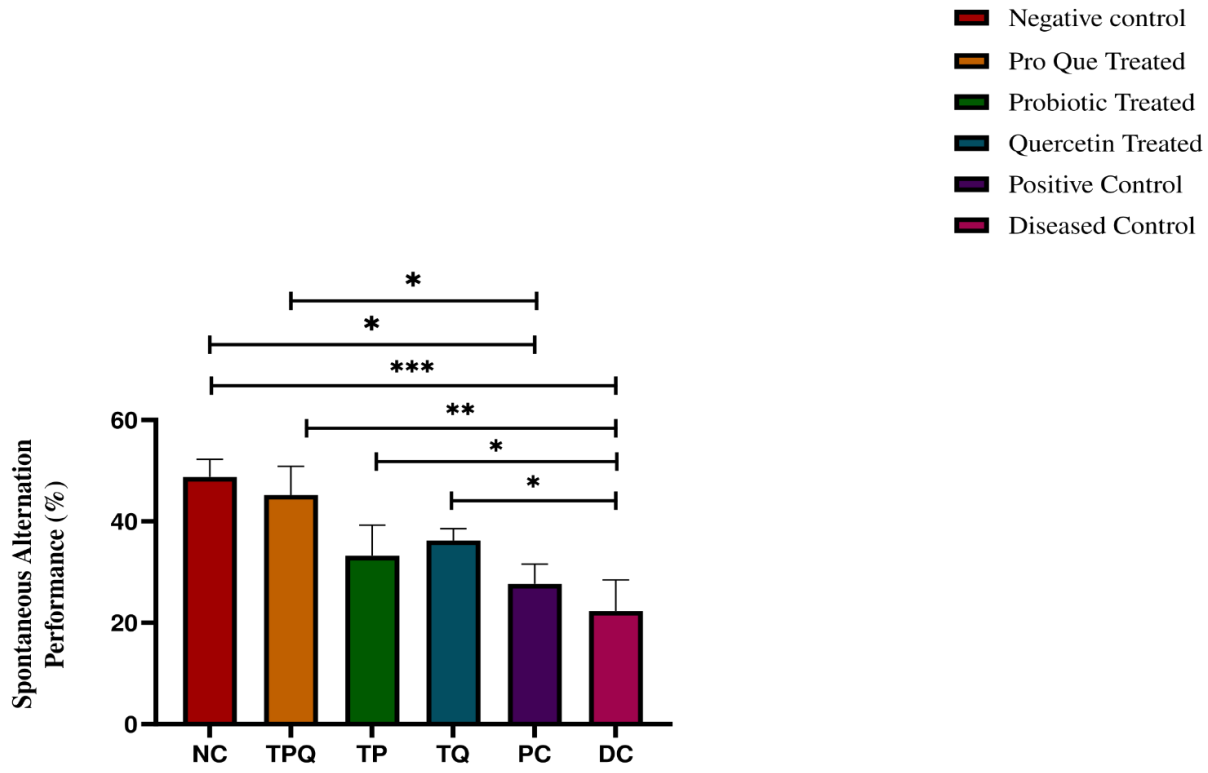

Figure 15: The effect of *L. fermentum* Y55 & Quercetin on (%) spontaneous alternation.

4.2.3 Effect of *Limosilactobacillus fermentum* Y55 and Quercetin on recognition memory in NOR

Recognition memory and exploratory behavior was determined using novel object recognition test, by exposing the test animal to different objects. Rodents tend to explore novel environments and objects rather than familiar ones. The recognition memory is measured in terms of DI.

Discrimination index of test session suggested that negative control spent significantly ($p < 0.01$) more time with novel object than familiar object. Similarly, TPQ group had also shown improved performance ($p < 0.01$) in spending more time with novel object. Recognition memory was also significantly improved in TP (0.05) and TQ ($p < 0.05$) group. The evidence shows that *Limosilactobacillus fermentum* Y55 and Quercetin treatment in combination was effective in restoring recognition memory of AD rat models (Fig: 16).

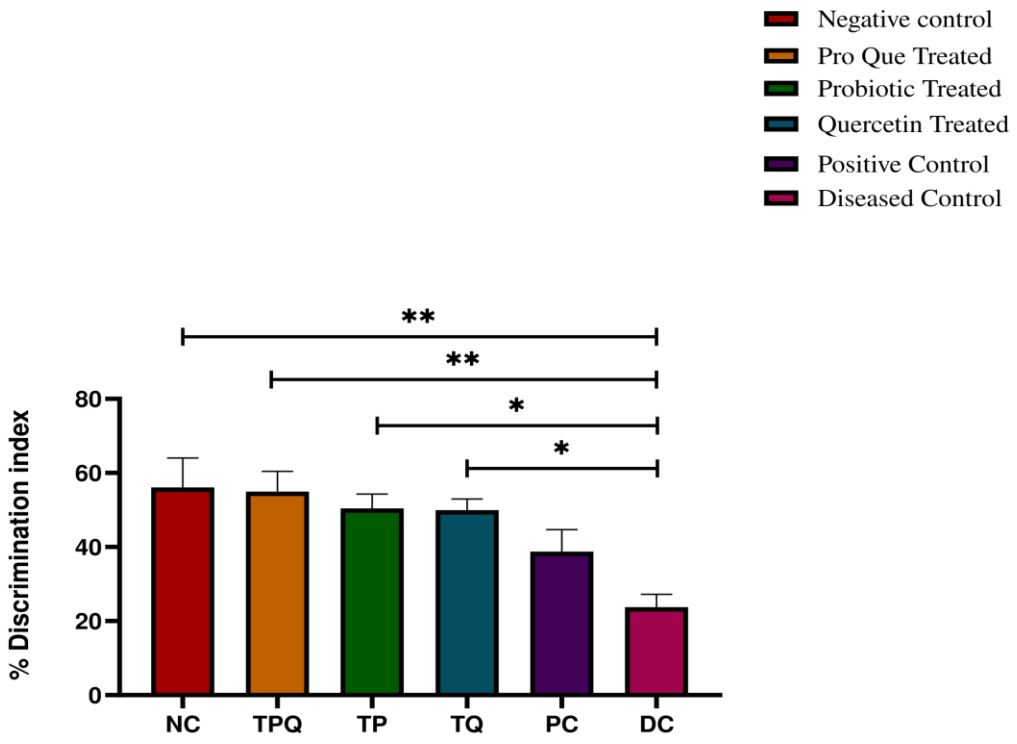

Figure 16: The effect of *L. fermentum* Y55 & Quercetin on recognition memory (% DI).

4.2.3 Effect of *L. fermentum* Y55 and Quercetin on spatial learning & memory in MWM

The MWM contains two phases: a training phase and a probe trial, and it lasts six days.The escape latency is used to test learning of spatial cues. The probe experiment was carried out on the final day for reference memory, and three parameters were counted: the number of platform crossings and the amount of time spent in target quadrant. On the training phases 5th day all groups displayed improvement in escape latency other than diseased control group. Diseased control group displayed significantly poor ($p < 0.0001$) improvement in escape latency on 5^{th} day as compared to negative control group. The TPQ group showed significant improvement ($p < 0.0001$) in escape latency as compared to DC group. The final escape latency was 4.04, 4.1, 6.76, 4.6, 5.44 and 25.76 on the 5th day for NC, TPQ, TP, TQ, PC and DC group respectively.

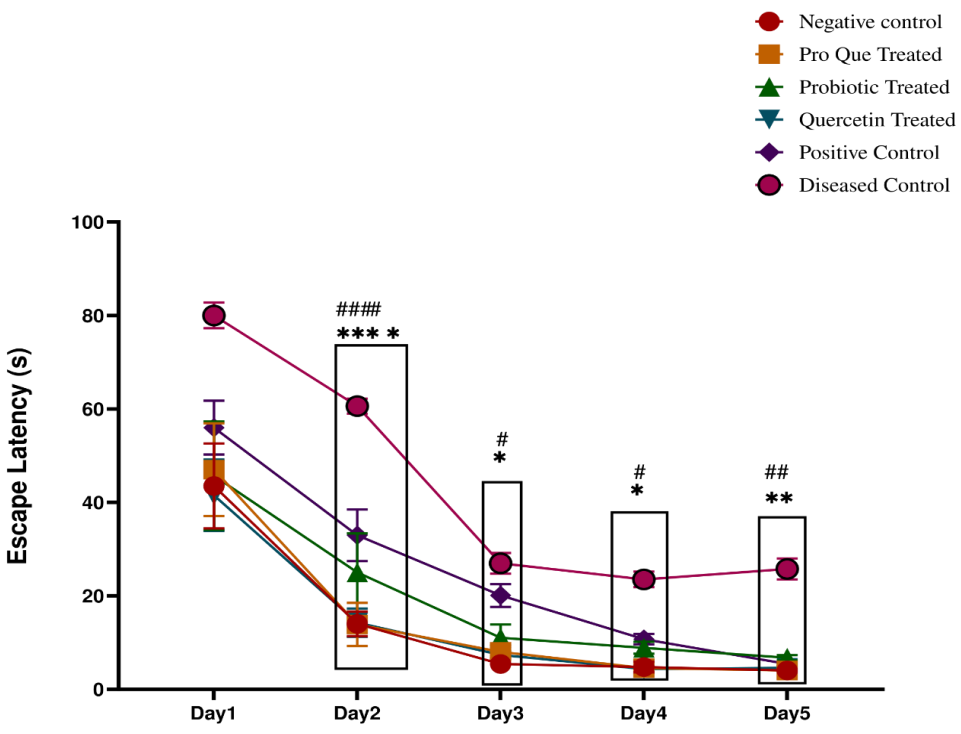

Figure 17: The effect of *L. fermentum* Y55 & quercetin on escape latency in MWM.

The symbols * , # represent significance between Negative control and Diseased control group, and Probiotic Quercetin treated group and Diseased control group respectively. The negative control NC group made significantly more crossings ($p < 0.001$) as compared to diseased control. The *L. fermentum* Y55 and quercetin treatment TPQ group had significantly higher performance ($p < 0.001$) as compared to diseased control DC group. Improvement in number of crossings across platform was also shown by *L. fermentum* Y55 treatment TP group ($p < 0.05$) and quercetin treatment TQ group ($p < 0.05$) in contrast to diseased control group DC (Fig:18).

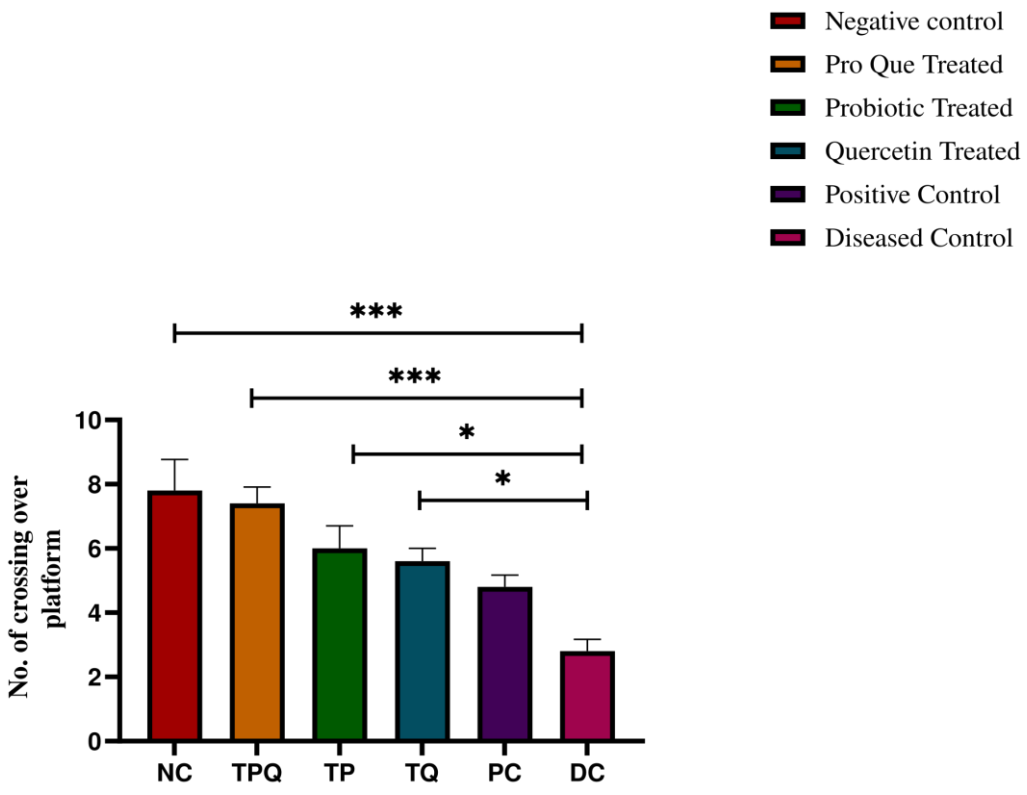

Figure 18: The effect of *L. fermentum* Y55 & Quercetin on spatial memory in MWM.

The time duration which rats had spent in target quadrant was also evaluated. The negative control NC group showed significantly more time ($p < 0.001$) in respective quadrant as compared to DC group. Similarly, the duration of rats in TPQ group is significantly ($p < 0.001$) more in target quadrant in comparison to diseased control DC group. Similar to what was seen with TP ($p < 0.01$), tremendous progress was made there and TQ ($p < 0.01$) group as compared to diseased control DC group. Positive control group PC group displayed significant ($p < 0.05$) improvement in time spent in target quadrant as compared to diseased control DC group (Fig: 19).

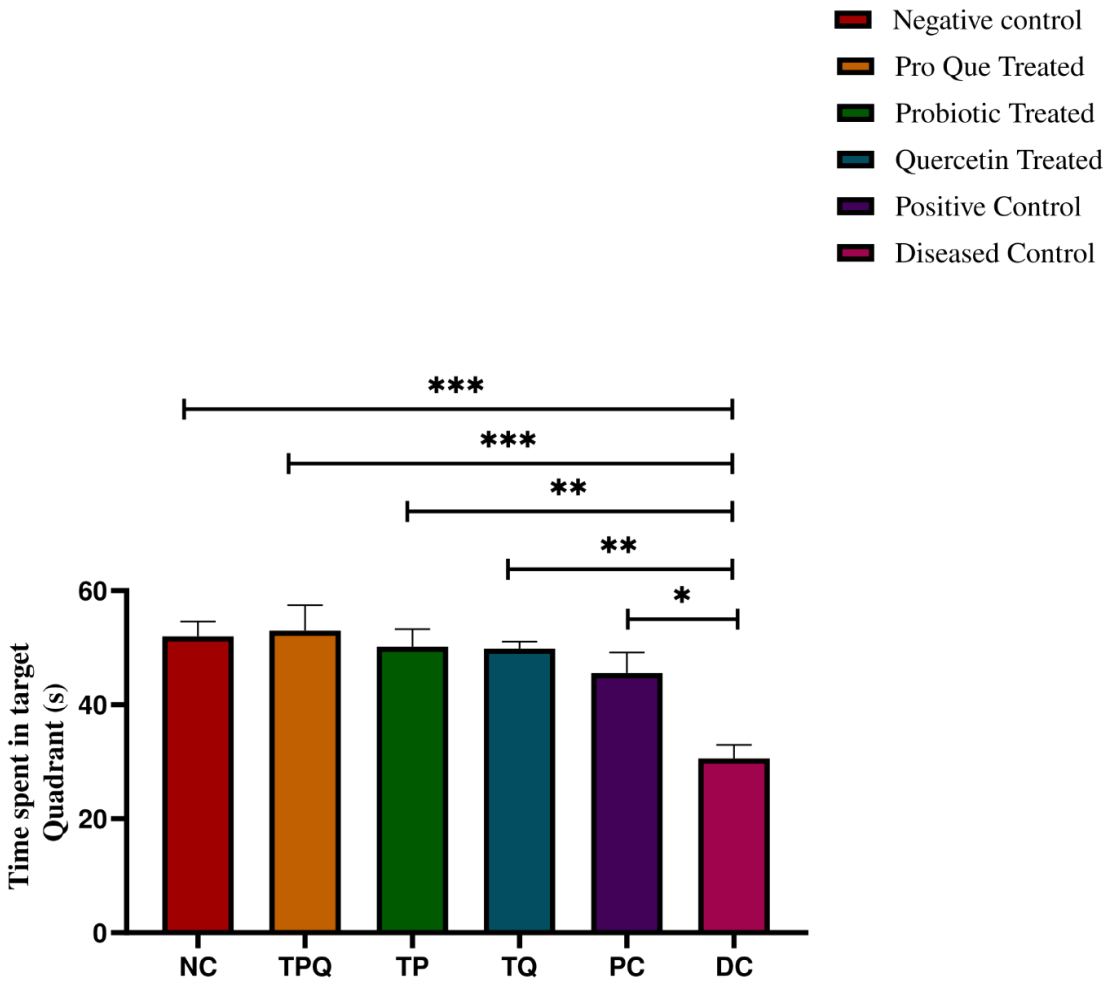

Figure 19: The effect of *L. fermentum* Y55 & Quercetin on time spent in target quadrant in MWM .

4.3 Histopathological assessment

The hippocampus of rat brain was stained with hematoxylin and eosin stain. Results of staining showed that neurodegeneration was observed in diseased control DC group in hippocampus as compared to the negative control. Combination treatment of *L. fermentum* Y55 and Quercetin has significantly restored neurodegeneration in $AlCL_3$-induced model showing neuroprotective effect of both. On the other hand, probiotic treatment TP group, quercetin treatment TQ group and positive control group PC also showed comparable better results than diseased control group.

The morphology of neuronal cell bodies was analyzed, and these findings constitute the qualitative outcomes of that analysis (Fig: 20,21,22). A marked difference was observed in cell bodies in diseased control DC ($p < 0.001$) as compared to negative control NC group. Number of observed cell bodies was also significantly improved in TPQ ($p < 0.01$) group. Probiotic treatment TP group ($p<0.05$) and quercetin treatment TQ ($p < 0.05$) also showed significant improvement. (Fig: 23)

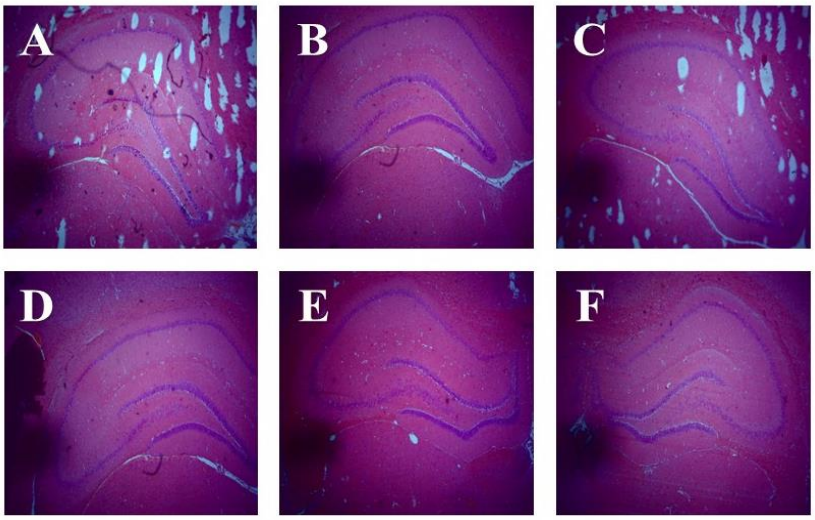

Figure 20: H&E-stained coronal sections of hippocampus 4X magnification. (A) Negative Control (B) *L. fermentum*Y55 & Quercetin treatment TPQ group (C) *L. fermentum* Y55 treated TP group (D) Quercetin treated TQ group l (E) Donepezil treated positive control PC group (F) $Alcl_3$ induced diseased control DC group.

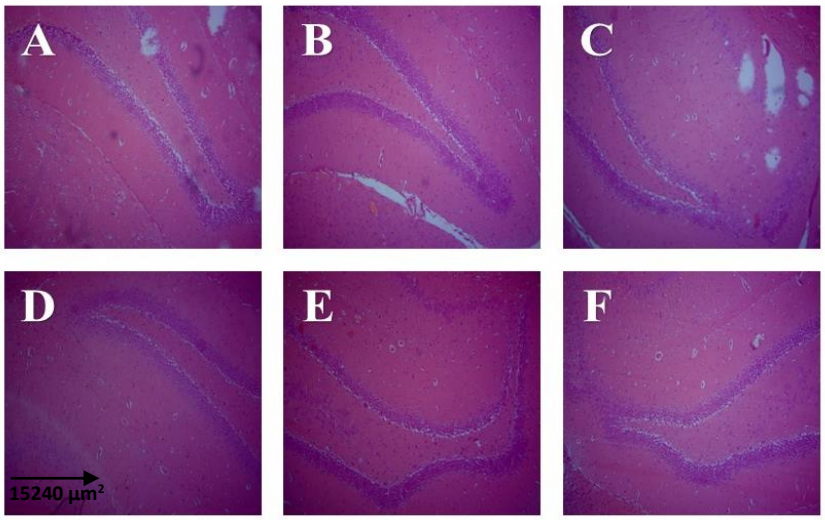

Figure 21: H&E stained coronal sections of hippocampus 10X magnification. (A) Negative Control (B) *L. fermentum*-Y55 & Quercetin treatment TPQ group (C) *L. fermentum* Y55 treated TP group (D) Quercetin treated TQ group l (E) Donepezil treated positive control PC group (F) $Alcl_3$ induced diseased control DC group

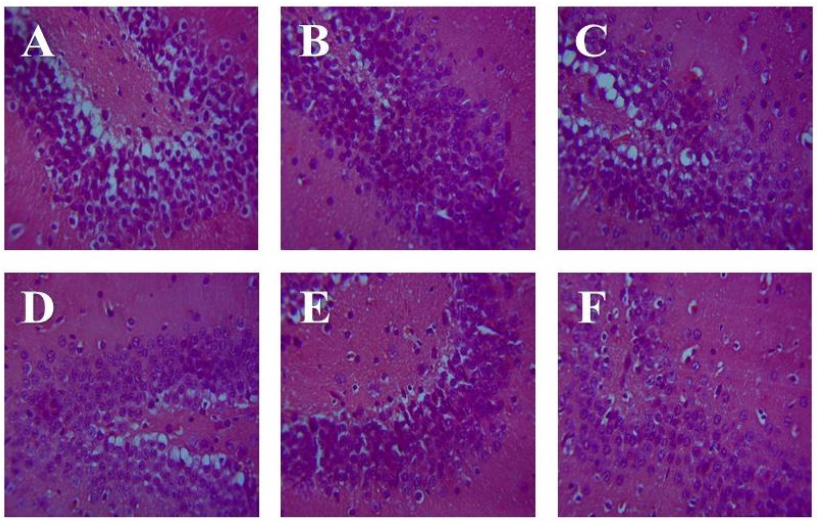

Figure 22: H&E stained coronal sections of hippocampus 40X magnification. (A) Negative Control (B) *L. fermentum*-Y55 & quercetin treatment TPQ group (C) *L. fermentum* Y55 treatment TP group (D) Quercetin treatment TQ group l (E) Donepezil treatment positive control PC group (F) $Alcl_3$ induced diseased control DC group

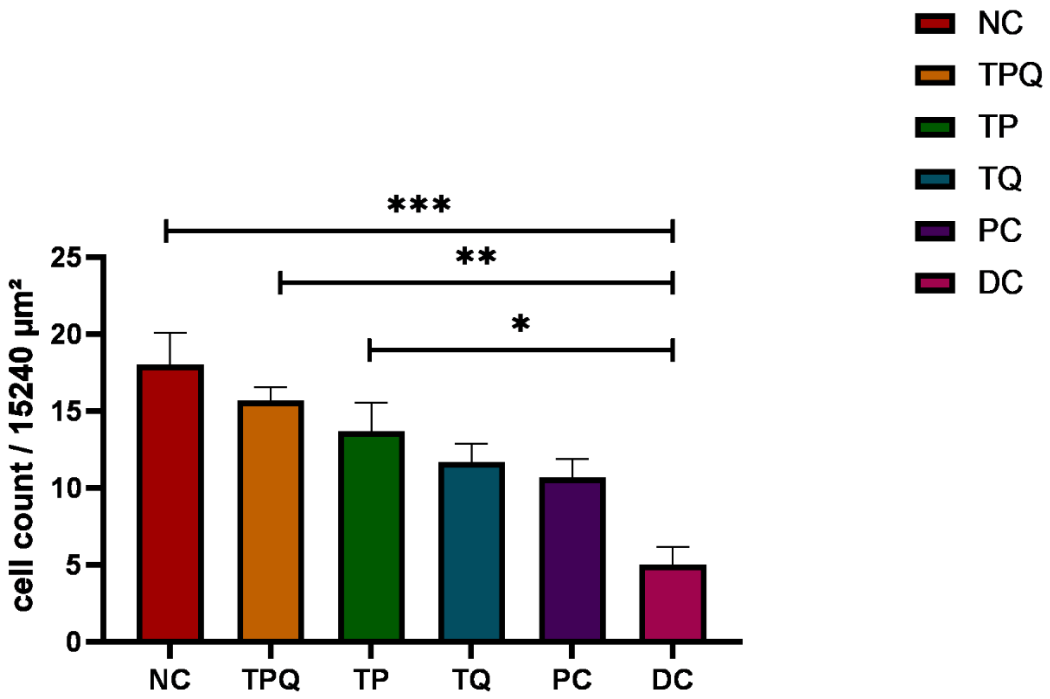

Figure 23: The effect of *L. fermentum* Y55 & Quercetin on cell count in dentate gyrus, hippocampus.

CHAPTER 5

DISCUSSION

Alzheimer’s is a complicated, age-related, and chronic condition, the pathophysiology of which cannot be attributed to a single cause. Alzheimer's disease affects the brain's ability to store and retrieve memories. There is no single pharmaceutical drug that can concurrently target all of these risk factors in order to effectively prevent the progression of disease because of the heterogeneous nature of these risk factors. This is because there is no single risk factor that can do so. There are a number of factors that can play a role in the progression of Alzheimer's disease. Some of these factors include neuroinflammation, metabolic instability, heightened oxidative stress, and an imbalance in neurochemical signaling. In a similar manner, the treatments that are currently available for Alzheimer's disease only address certain symptoms of the disease, such as the memory loss, abnormalities in acetylcholine levels, behavioral problems, or modifications in sleep patterns, as well as amyloid beta and neurofibrillary tangle accumulation (Broadstock et al., 2014).

In this study previously isolated strain *Limosilactobacillus fermentum* Y55 was selected based on their antioxidant potential as confirmed by Ayesha, 2022 in whole genome sequencing of this strain. Its safety and survival profile in GIT was already assessed by Noor, 2019. This probiotic strain due to its antioxidant potential serves as a protective strategy to overcome oxidative stress leading to AD. As oxidative stress is higher in Alzheimer’s causing the accumulation of amyloid beta plagues. *Limosilactobacillus fermentum* Y55 has different potential benefits like maintenance of gut microbiota, cholesterol reduction and liver diseases. *Limosilactobacillus fermentum* and other probiotics have been utilized as a therapy for AD. Moreover, to enhance the antioxidant activity a polyphenol named as Quercetin is also used which together constitutes a

symbiotic formulation. Quercetin also has anti-Alzheimer's and antioxidant activity and is a good approach to overcome prevailing cognitive impairment like Alzheimer's.

The current study evaluated that both pre and post supplementation of *L. fermentum* Y55 days at a dosage of $1.5x10^9$ CFU per day and Quercetin (25mg/kg) on AD rat models treated with $AlCl_3$ (120 mg/kg) has a neuroprotective effect against AD due to their antioxidative properties. The experiment was carried out for 60 days. For the first 30 days $AlCl_3$ was given to all groups except the negative control group. TP, TQ, TPQ were given probiotics, quercetin and mixture of probiotics and quercetin respectively prior to $AlCl_3$. After 30 days $AlCl_3$ administration was stopped in all groups, but Probiotic, Quercetin and administration of their combination was continued. In addition to this donepezil (3mg/kg) was given to PC group for 30 days. Anxiety and memory impairment was detected through various behavior tests. The results showed that $AlCl_3$ induced Alzheimer's was significantly attenuated by supplementing probiotics and quercetin together.

The hippocampal and amygdala regions of the brain are involved in EPM, which calculates depression and anxiety respectively (Iqbal et al., 2016). The elevated plus maze is one of the most popularly used tests for determining whether animals exhibit behaviors associated with exploration or anxiety. The variables in the elevated plus maze. are the most appropriate for assessing the anxiety and exploratory activity in the animals as soon as they are placed in the elevated maze. This is done by assessing the No. of times they enter the open arms and time they tend to spend in open arm, which ultimately determines how anxious the animal is when exposed to a certain height and how it responds to this novel and aversive environment (Ikram et al., 2021).

Limosilactobacillus strain has shown to exhibit non-anxious behavior in elevated plus maze test. A study on the novel probiotic *Limosilactobacillus fermentum* PS 150 against chronic mild stress was done to assess the psychotropic potential of probiotics against depressed behavior in rats. According to the findings, this particular strain performed better on the elevated plus maze test than the diseased control group did in terms of depression and anxious behavior. This is because PS 150 equalized the serotonin and IDO levels induced by CMS and stopped the degeneration of the rat brain (Y.-W. Liu et al., 2019). Rats treated with *Yersinia enterocolitica* strain 8081 displayed more nervous attitude in the elevated plus maze test than rats given *Limosilactobacillus fermentum* strain 9338 and diclofenac. Upregulated immunological, inflammatory, apoptotic, and antioxidant defense response proteins are to responsible for this (Ahlawat et al., 2021).

By acting as an antioxidant, flavanols like quercetin are said to be useful in treating AD and neurodegenerative illnesses linked to oxidative stress. This method has also demonstrated encouraging outcomes against anxiety, which can be demonstrated as being raised plus maze test (Pattanashetti et al., 2017). In order to examine the effects of this flavanol on the behavioral reactions of rats, Quercetin was administered to obese wistar rats that were receiving a high fat and fructose diet (HFCD). It was shown that rats given quercetin exhibited less nervous behavior in the elevated plus maze test because they spent more time in the open arm than the diseased control group (Mzhelskaya et al., 2020).

On the other hand, donepezil causes anxiety. Donepezil, as was to be expected, lowered the amount of acetylcholinesterase activity in the brain while simultaneously raising the levels of cortisol throughout the body. This provides additional evidence connecting acetylcholinesterase inhibition to anxiety-like behavioral and endocrine responses (Giacomini et al., 2020). This

demonstrates that AD patients with elevated cortisol levels did not experience a reduction in anxiety after therapy (Chang et al., 2018). On 15 young adults, a double-blind randomized control study was conducted. It was shown that given donepezil therapy, their assessments of vigour and anxiety were much higher than those of the controls (Pompeia et al., 2013).

This study corroborated the existing evidence that probiotics and Quercetin are helpful in attenuating anxiety-like behavior. Combination therapy of *Limosilactobacillus fermentum* Y-55 and Quercetin caused significant decrease in anxiety like behavior in TPQ group as compared to probiotic and Quercetin given individually when given along with $AlCl_3$. Reduced anxiolytics behavior was indicated by an increase entry and time spent in open arm. On the other hand, donepezil causes anxiety. So, it is found out that *L. Fermentum* Y55 and Quercetin together can show more promising results in overcoming anxious behavior associated with AD.

Several parameters of memory including spatial memory, reference memory and long-term spatial memory were studied by employing different behavioral tests to better determine the effectivity of probiotic and quercetin treatment on deficits memory and learning in AD as different procedures target distinct idiosyncrasies within a domain. Spatial reference and working memory are assessed through the Y-maze test. The animal's natural exploratory activity and spontaneous alternation can also be checked via this test. Total entries in each arm were counted in order to evaluate spatial and reference memory deficits along with time spent in each arm was evaluated and then % spontaneous alternation is determined. The impact of *Limosilactobacillus fermentum* JDFM216 on old mice was examined in a study to better understand how probiotic ingestion affects memory and behavior. It was concluded that JDFM216 has increased the spontaneous alternation as compared to aged mouse group resulting in improvement of spatial working memory (M. R. Park et al., 2020).

The usefulness of quercetin has been documented, including its neuroprotective effects on learning and spatial memory. It has been demonstrated that the bioflavonoid quercetin can slow the progression of Alzheimer's disease when coupled with the compound $AlCl_3$. Working memory was significantly enhanced in the group that received quercetin treatment as compared to the group that received $AlCl_3$ treatment. This is as a result of the fact that in the y-maze test, the group that was given quercetin treatment exhibited higher instances of spontaneous alternations (Elreedy et al., 2023). In another study 25-30 mg/kg Quercetin improved the spatial working memory in Y-maze test. As the spontaneous alternations are higher in Quercetin treated group as compared to Rotenone treated group. This is due to the fact that Quercetin lessens the neuroinflammation (Jain et al., 2022). Similarly, according to their performance in the Y-maze, donepezil treatment at 10 mg/kg and 3 mg/kg reversed memory impairment in hairless rats caused by scopolamine. Before receiving an intraperitoneal injection of scopolamine, rats pre-treated with donepezil showed a much higher percentage of spontaneous alternation (Shin et al., 2018). Consistent with the above-mentioned results, improved spontaneous alternations were observed for TPQ group i.e., *Limosilactobacillus fermentum* Y55 and Quercetin treated group as compared to diseased group.

In the task of novel object recognition, the cortex and hippocampus mediate learning and memory formation (Antunes & Biala, 2012). Mice and rats frequently interact with novel objects. Spending more time interacting with the novel object sets object recognition apart (Bevins & Besheer, 2006). Probiotics have the capacity to improve compromised detoxification mechanisms by lowering oxidative stress and reestablishing the gut's flora. A study demonstrates the neuroprotective and antioxidant effects of a probiotic combination made up of strains of

Limosilactobacillus and *Bifidobacteria*. As compared to rats fed aflatoxin, those fed this consortium for eight weeks demonstrated better memory and learning in NOR (Aytekin Sahin et al., 2022). In a similar vein, there are several pieces of data that support the antioxidant capabilities of quercetin. When the rodents were given Quercetin, they had an increase in SOD, which is an antioxidant enzyme, and were able to alter the activity of GPx. As a consequence of this, quercetin was able to ameliorate the memory impairment in mice brought on by rotenone, as demonstrated by an improvement in the discrimination index in novel object recognition (Madiha et al., 2021). Following the administration of donepezil, rats that had been previously exposed to ethanol exhibited significantly improved performance in the NOR and MWM tests. This was accomplished by donepezil's ability to reduce the oxidative stress and its ability to raise dopamine levels in the dorsal hippocampus (Arif et al., 2022). Our results also unraveled that *Limosilactobacillus fermentum* Y55 and Quercetin has improved the recognition memory in TPQ group which was much decreased in aluminum exposed group. Other groups showed improvement too but TPQ is better as polyphenols potentiates the effect of probiotics (Kadlec & Jakubec, 2014).

The Morris water maze test is ideal for determining one's ability to acquire and remember spatial relationships, as well as for determining one's reference memory (Vorhees & Williams, 2006). Escape latencies that are measured on a daily basis are a good indicator of long-term memory (Morris, 1981). Donepezil also possesses antioxidant qualities. Because it demonstrates the rise in GSH levels when compared to the GSH levels in the streptozotocin rat model of Alzheimer's disease. Resultantly, the rat performs well in MWM as seen by a decrease in latency time in trials that were repeated for five days, showing that learning and memory function were intact (Saxena et al., 2008). Combination therapy comprising of *Limosilactobacillus fermentum,*

Limosilactobacillus acidophilus and Bifidobacterium lactis was given to diabetic rats to assess the effect of this combination against oxidative stress and impaired memory in diabetic conditions. The results indicated that probiotics are beneficial in improving spatial learning and memory in MWM in addition to optimizing antioxidant defense system in rats (Saeideh et al., 2012). Mice that had been supplemented with LAB had a shorter escape latency and travelled a shorter distance, but they spent a much longer amount of time in the platform zone. This was linked to a reduction in the amount BACE1 mRNA in brains as well as a significant decrease in the amount of nitric oxide. LAB12 was also responsible for an appreciable increase in glutathione (Ahmad Alwi et al., 2022). According to research, quercetin can restore the cognitive loss brought on by long-term reserpine use detected through MWM (Naidu et al., 2004). Quercetin's ability to scavenge free radicals and oxidize metabolites, as well as the qualities of iron or copper that chelate them and limit lipid peroxidation, may be the primary or indirect processes behind this action. According to certain reports, quercetin stimulates glutathione peroxidase (Sriraksa et al., 2012). Regarding the effects of *Limosilactobacillus fermentum* Y-55 and quercetin given collectively, had shown that Long-term spatial memory and learning was improved as observed in escape latency in MWM. Moreover, rats in TPQ group had improved reference memory as observed in probe trial. Hitherto, our experiment had showed that pretreatment with combo of probiotics and quercetin improved the behavior of animals. Now our study further validated the results through histological evaluation. From the histological examination, our results demonstrated that H&E - stained rat brain hippocampus showed improved neuron structures along with pyknotic nuclei in TPQ groups as compared to aluminum exposed group. $AlCl_3$ causes neurodegeneration due to increased inflammation, oxidative stress and reactive glosis (Prakash et al., 2013). Cell counts achieved through treatment with probiotic

& quercetin combination, probiotics, quercetin and donepezil were comparable to those achieved through the negative control group. Donepezil is effective in both curing Alzheimer's disease and recovery from injuries to the brain caused by trauma. Donepezil stimulates the production of new neurons by controlling the activity of the cholinergic system and modulating signaling that is dependent on BDNF and TrkB. Expression of BDNF and levels of phosphorylated TrkB are both increased as a result (Zheng et al., 2018). Similarly, *Limosilactobacillus fermentum* inhibits the activity of acetylcholinesterase (Handajani et al., 2022) and improve BDNF production thus contributing to neurogenesis (Romo-Araiza & Ibarra, 2020). In a similar vein, the antioxidative quercetin possessed the capacity to influence the expression of genes involved in the regulation of neurogenesis such as BDNF, CREB, and NGF (Karimipour et al., 2019). Hence, pretreating with *Limosilactobacillus fermentum* and quercetin had proved to be a good option in maintaining the morphology of neurons in TPQ group as compared to exposure group treated with aluminum.

Conclusion and prospects

The current study showed that the strain *Limosilactobacillus fermentum* Y55 along with quercetin have the therapeutic potential to mitigate the effect of Alzheimer's disease. This combination is effective in improving cognitive decline and anxiety-like behavior associated with AD. As both probiotics and quercetin are cheap and well-tolerated in GIT, including them in diet would be beneficial in lessening down oxidative stress and Alzheimer's symptoms in aged individuals. To precisely understand why these probiotics and quercetin combination were particularly successful in treating AD, further research is required to fully understand the intricate connection that exists between the microbiota in the gut and the brain.

In future, 16s RNA profiling of fecal microbial diversity could be done for the purpose of analyzing the impact of probiotics and quercetin on microbial diversity in gut. Furthermore, the effect of this combination must be studied on antioxidant and neurogenesis markers via RT-PCR or ELISA kits to give further insight as to how gut microbiota attenuates the severity of disease symptoms.

CHAPTER: 06

References

Abramov, A. Y., Potapova, E. V., Dremin, V. V., & Dunaev, A. V. (2020). Interaction of Oxidative Stress and Misfolded Proteins in the Mechanism of Neurodegeneration. *Life*, *10*(7), Article 7. https://doi.org/10.3390/life10070101

Ahlawat, S., Shankar, A., Vandna, Mohan, H., & Sharma, K. K. (2021). Yersinia enterocolitica and Lactobacillus fermentum induces differential cellular and behavioral responses during diclofenac biotransformation in rat gut. *Toxicology and Applied Pharmacology*, *431*, 115741. https://doi.org/10.1016/j.taap.2021.115741

Ahmad Alwi, N. A., Lim, S. M., Mani, V., & Ramasamy, K. (2022). Lactobacillus Spp.-Enhanced Memory is Strain-Dependent and Associated, in Part, with Amyloidogenic and anti-Oxidant/Oxidative Stress Interplay in Amyloid Beta Precursor Protein Transgenic Mice. *Journal of Dietary Supplements*, *0*(0), 1–18. https://doi.org/10.1080/19390211.2022.2103608

Ahmadi, S., Wang, S., Nagpal, R., Wang, B., Jain, S., Razazan, A., Mishra, S. P., Zhu, X., Wang, Z., Kavanagh, K., & Yadav, H. (n.d.). A human-origin probiotic cocktail ameliorates aging-related leaky gut and inflammation via modulating the microbiota/taurine/tight junction axis. *JCI Insight*, *5*(9), e132055. https://doi.org/10.1172/jci.insight.132055

Ait-Belgnaoui, A., Han, W., Lamine, F., Eutamene, H., Fioramonti, J., Bueno, L., & Theodorou, V. (2006). Lactobacillus farciminis treatment suppresses stress induced visceral hypersensitivity: A possible action through interaction with epithelial cell cytoskeleton contraction. *Gut*, *55*(8), 1090–1094. https://doi.org/10.1136/gut.2005.084194

Akbari, E., Asemi, Z., Daneshvar Kakhaki, R., Bahmani, F., Kouchaki, E., Tamtaji, O. R., Hamidi, G. A., & Salami, M. (2016). Effect of Probiotic Supplementation on Cognitive Function and Metabolic Status in Alzheimer's Disease: A Randomized, Double-Blind and Controlled Trial. *Frontiers in Aging Neuroscience*, *8*. https://www.frontiersin.org/articles/10.3389/fnagi.2016.00256

Albers, G., Van den Block, L., & Vander Stichele, R. (2014). The burden of caring for people with dementia at the end of life in nursing homes: A postdeath study among nursing staff. *International Journal of Older People Nursing*, *9*(2), 106–117. https://doi.org/10.1111/opn.12050

Ale, E. C., & Binetti, A. G. (2021). Role of Probiotics, Prebiotics, and Synbiotics in the Elderly: Insights Into Their Applications. *Frontiers in Microbiology*, *12*. https://www.frontiersin.org/articles/10.3389/fmicb.2021.631254

Aliev, G., Smith, M. A., Seyidova, D., Neal, M. L., Lamb, B. T., Nunomura, A., Gasimov, E. K., Vinters, H. V., Perry, G., LaManna, J. C., & Friedland, R. P. (2002). The Role of Oxidative Stress in the Pathophysiology of Cerebrovascular Lesions in Alzheimer's Disease. *Brain Pathology*, *12*(1), 21–35. https://doi.org/10.1111/j.1750-3639.2002.tb00419.x

Alok, S., Jain, S. K., Verma, A., Kumar, M., Mahor, A., & Sabharwal, M. (2014). Herbal antioxidant in clinical practice: A review. *Asian Pacific Journal of Tropical Biomedicine*, *4*(1), 78–84. https://doi.org/10.1016/S2221-1691(14)60213-6

Amaretti, A., di Nunzio, M., Pompei, A., Raimondi, S., Rossi, M., & Bordoni, A. (2013). Antioxidant properties of potentially probiotic bacteria: In vitro and in vivo activities. *Applied Microbiology and Biotechnology*, *97*(2), 809–817. https://doi.org/10.1007/s00253-012-4241-7

Anand David, A. V., Arulmoli, R., & Parasuraman, S. (2016). Overviews of Biological Importance of Quercetin: A Bioactive Flavonoid. *Pharmacognosy Reviews*, *10*(20), 84–89. https://doi.org/10.4103/0973-7847.194044

Anandharaj, M., Sivasankari, B., Santhanakaruppu, R., Manimaran, M., Rani, R. P., & Sivakumar, S. (2015). Determining the probiotic potential of cholesterol-reducing Lactobacillus and Weissella strains isolated from gherkins (fermented cucumber) and south Indian fermented koozh. *Research in Microbiology*, *166*(5), 428–439. https://doi.org/10.1016/j.resmic.2015.03.002

Ansari, M. A., Abdul, H. M., Joshi, G., Opii, W. O., & Butterfield, D. A. (2009). Protective effect of quercetin in primary neurons against Aβ(1–42): Relevance to Alzheimer's disease. *The Journal of Nutritional Biochemistry*, *20*(4), 269–275. https://doi.org/10.1016/j.jnutbio.2008.03.002

Antunes, M., & Biala, G. (2012). The novel object recognition memory: Neurobiology, test procedure, and its modifications. *Cognitive Processing*, *13*(2), 93–110. https://doi.org/10.1007/s10339-011-0430-z

Arendash, G. W., Lewis, J., Leighty, R. E., McGowan, E., Cracchiolo, J. R., Hutton, M., & Garcia, M. F. (2004). Multi-metric behavioral comparison of APPsw and P301L models for Alzheimer's Disease: Linkage of poorer cognitive performance to tau pathology in forebrain. *Brain Research*, *1012*(1), 29–41. https://doi.org/10.1016/j.brainres.2004.02.081

Arif, M., Rauf, K., Rehman, N. U., Tokhi, A., Ikram, M., & Sewell, R. D. (2022). 6-Methoxyflavone and Donepezil Behavioral Plus Neurochemical Correlates in Reversing Chronic Ethanol and Withdrawal Induced Cognitive Impairment. *Drug Design, Development and Therapy*, *16*, 1573–1593. https://doi.org/10.2147/DDDT.S360677

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D. R., Fernandes, G. R., Tap, J., Bruls, T., Batto, J. M., Bertalan, M., Borruel, N., Casellas, F., Fernandez, L., Gautier, L., Hansen, T., Hattori, M., Hayashi, T., Kleerebezem, M., … Bork, P. (2011). Erratum: Enterotypes of the human gut microbiome (Nature (2011) 473 (174-180)). *Nature*, *474*(7353), 666. https://doi.org/10.1038/nature10187

Ashe, K. H., & Zahs, K. R. (2010). Probing the Biology of Alzheimer's Disease in Mice. *Neuron*, *66*(5), 631–645. https://doi.org/10.1016/j.neuron.2010.04.031

Aytekin Sahin, G., Karabulut, D., Unal, G., Sayan, M., & Sahin, H. (2022). Effects of probiotic supplementation on very low dose AFB1-induced neurotoxicity in adult male rats. *Life Sciences*, *306*, 120798. https://doi.org/10.1016/j.lfs.2022.120798

Aziz, Q., Doré, J., Emmanuel, A., Guarner, F., & Quigley, E. M. M. (2013). Gut microbiota and gastrointestinal health: Current concepts and future directions. *Neurogastroenterology and Motility: The Official Journal of the European Gastrointestinal Motility Society*, *25*(1), 4–15. https://doi.org/10.1111/nmo.12046

Balasubramanian, R., Bazaz, M. R., Pasam, T., Sharief, N., Velip, L., Samanthula, G., & Dandekar, M. P. (2022). Involvement of Microbiome Gut–Brain Axis in Neuroprotective Effect of Quercetin in Mouse Model of Repeated Mild Traumatic Brain Injury. *NeuroMolecular Medicine*. https://doi.org/10.1007/s12017-022-08732-z

Bevins, R. A., & Besheer, J. (2006). Object recognition in rats and mice: A one-trial non-matching-to-sample learning task to study "recognition memory." *Nature Protocols*, *1*(3), Article 3. https://doi.org/10.1038/nprot.2006.205

Bistoletti, M., Bosi, A., Banfi, D., Giaroni, C., & Baj, A. (2020). Chapter Two - The microbiota-gut-brain axis: Focus on the fundamental communication pathways. In L. J. Kasselman

(Ed.), *Progress in Molecular Biology and Translational Science* (Vol. 176, pp. 43–110). Academic Press. https://doi.org/10.1016/bs.pmbts.2020.08.012

Breit, S., Kupferberg, A., Rogler, G., & Hasler, G. (2018). Vagus Nerve as Modulator of the Brain–Gut Axis in Psychiatric and Inflammatory Disorders. *Frontiers in Psychiatry*, *9*. https://www.frontiersin.org/articles/10.3389/fpsyt.2018.00044

Broadstock, M., Ballard, C., & Corbett, A. (2014). Latest treatment options for Alzheimer's disease, Parkinson's disease dementia and dementia with Lewy bodies. *Expert Opinion on Pharmacotherapy*, *15*(13), 1797–1810. https://doi.org/10.1517/14656566.2014.936848

Bromley-Brits, K., Deng, Y., & Song, W. (2011). Morris water maze test for learning and memory deficits in Alzheimer's disease model mice. *Journal of Visualized Experiments: JoVE*, *53*, 2920. https://doi.org/10.3791/2920

Calfio, C., Gonzalez, A., Singh, S. K., Rojo, L. E., & Maccioni, R. B. (2020). The Emerging Role of Nutraceuticals and Phytochemicals in the Prevention and Treatment of Alzheimer's Disease. *Journal of Alzheimer's Disease*, *77*(1), 33–51. https://doi.org/10.3233/JAD-200443

Carlo, M. D., Giacomazza, D., & Biagio, P. L. S. (2012). Alzheimer's disease: Biological aspects, therapeutic perspectives and diagnostic tools. *Journal of Physics: Condensed Matter*, *24*(24), 244102. https://doi.org/10.1088/0953-8984/24/24/244102

Catanzaro, R., Anzalone, M., Calabrese, F., Milazzo, M., Capuana, M., Italia, A., Occhipinti, S., & Marotta, F. (2015). The gut microbiota and its correlations with the central nervous system disorders. *Panminerva Medica*, *57*(3), 127–143.

Cattaneo, A., Cattane, N., Galluzzi, S., Provasi, S., Lopizzo, N., Festari, C., Ferrari, C., Guerra, U. P., Paghera, B., Muscio, C., Bianchetti, A., Volta, G. D., Turla, M., Cotelli, M. S., Gennuso, M., Prelle, A., Zanetti, O., Lussignoli, G., Mirabile, D., … Frisoni, G. B. (2017).

Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers in cognitively impaired elderly. *Neurobiology of Aging*, *49*, 60–68. https://doi.org/10.1016/j.neurobiolaging.2016.08.019

Chang, Y. S., Wu, Y. H., Wang, C. J., Tang, S. H., & Chen, H. L. (2018). Higher levels of thyroxine may predict a favorable response to donepezil treatment in patients with Alzheimer disease: A prospective, case–control study. *BMC Neuroscience*, *19*(1), 36. https://doi.org/10.1186/s12868-018-0436-x

Chavarro, J. E., Martín-Calvo, N., Yuan, C., Arvizu, M., Rich-Edwards, J. W., Michels, K. B., & Sun, Q. (2020). Association of Birth by Cesarean Delivery With Obesity and Type 2 Diabetes Among Adult Women. *JAMA Network Open*, *3*(4), e202605. https://doi.org/10.1001/jamanetworkopen.2020.2605

Chen, K., Liu, Y., Cheng, Y., Yan, Q., Zhou, C., He, Z., Zeng, J., He, J., & Tan, Z. (2020). Supplementation of Lactobacillus plantarum or Macleaya cordata Extract Alleviates Oxidative Damage Induced by Weaning in the Lower Gut of Young Goats. *Animals*, *10*(4), Article 4. https://doi.org/10.3390/ani10040548

Cho, I., & Blaser, M. J. (2012). The human microbiome: At the interface of health and disease. *Nature Reviews. Genetics*, *13*(4), 260–270. https://doi.org/10.1038/nrg3182

Choi, B. H. (1995). Oxidative stress and Alzheimer's disease. *Neurobiology of Aging*, *16*(4), 675–678. https://doi.org/10.1016/0197-4580(95)00065-M

Chopra, A. S., Lordan, R., Horbańczuk, O. K., Atanasov, A. G., Chopra, I., Horbańczuk, J. O., Jóźwik, A., Huang, L., Pirgozliev, V., Banach, M., Battino, M., & Arkells, N. (2022). The current use and evolving landscape of nutraceuticals. *Pharmacological Research*, *175*, 106001. https://doi.org/10.1016/j.phrs.2021.106001

Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R. D., Shanahan, F., Dinan, T. G., & Cryan, J. F. (2013). The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Molecular Psychiatry*, *18*(6), Article 6. https://doi.org/10.1038/mp.2012.77

Costa, L. G., Garrick, J. M., Roquè, P. J., & Pellacani, C. (2016). Mechanisms of Neuroprotection by Quercetin: Counteracting Oxidative Stress and More. *Oxidative Medicine and Cellular Longevity*, *2016*, e2986796. https://doi.org/10.1155/2016/2986796

Den, H., Dong, X., Chen, M., & Zou, Z. (2020). Efficacy of probiotics on cognition, and biomarkers of inflammation and oxidative stress in adults with Alzheimer's disease or mild cognitive impairment—A meta-analysis of randomized controlled trials. *Aging (Albany NY)*, *12*(4), 4010. https://doi.org/10.18632/aging.102810

Deng, Y.-H., Wang, N.-N., Zou, Z.-X., Zhang, L., Xu, K.-P., Chen, A. F., Cao, D.-S., & Tan, G.-S. (2017). Multi-Target Screening and Experimental Validation of Natural Products from Selaginella Plants against Alzheimer's Disease. *Frontiers in Pharmacology*, *8*. https://www.frontiersin.org/articles/10.3389/fphar.2017.00539

De-Paula, V. J., Radanovic, M., Diniz, B. S., & Forlenza, O. V. (2012). Alzheimer's Disease. In J. R. Harris (Ed.), *Protein Aggregation and Fibrillogenesis in Cerebral and Systemic Amyloid Disease* (pp. 329–352). Springer Netherlands. https://doi.org/10.1007/978-94-007-5416-4_14

DeTure, M. A., & Dickson, D. W. (2019). The neuropathological diagnosis of Alzheimer's disease. *Molecular Neurodegeneration*, *14*(1), 32. https://doi.org/10.1186/s13024-019-0333-5

Devore, E. E., Kang, J. H., Breteler, M. M. B., & Grodstein, F. (2012). Dietary intakes of berries and flavonoids in relation to cognitive decline. *Annals of Neurology*, *72*(1), 135–143. https://doi.org/10.1002/ana.23594

D'Hooge, R., & De Deyn, P. P. (2001). Applications of the Morris water maze in the study of learning and memory. *Brain Research Reviews*, *36*(1), 60–90. https://doi.org/10.1016/S0165-0173(01)00067-4

Dinan, T. G., & Cryan, J. F. (2017). Gut instincts: Microbiota as a key regulator of brain development, ageing and neurodegeneration. *The Journal of Physiology*, *595*(2), 489–503. https://doi.org/10.1113/JP273106

Doifode, T., Giridharan, V. V., Generoso, J. S., Bhatti, G., Collodel, A., Schulz, P. E., Forlenza, O. V., & Barichello, T. (2021). The impact of the microbiota-gut-brain axis on Alzheimer's disease pathophysiology. *Pharmacological Research*, *164*, 105314. https://doi.org/10.1016/j.phrs.2020.105314

Elreedy, H. A., Elfiky, A. M., Mahmoud, A. A., Ibrahim, K. S., & Ghazy, M. A. (2023). Neuroprotective effect of quercetin through targeting key genes involved in aluminum chloride induced Alzheimer's disease in rats. *Egyptian Journal of Basic and Applied Sciences*, *10*(1), 174–184. https://doi.org/10.1080/2314808X.2022.2164136

Ennaceur, A., & Delacour, J. (1987). Effect of combined or separate administration of piracetam and choline on learning and memory in the rat. *Psychopharmacology*, *92*(1), 58–67. https://doi.org/10.1007/BF00215480

Evrensel, A., & Ünsalver, B. Ö. (2018). *Gut Microbiota: Brain Axis*. BoD – Books on Demand.

Fan, L., Mao, C., Hu, X., Zhang, S., Yang, Z., Hu, Z., Sun, H., Fan, Y., Dong, Y., Yang, J., Shi, C., & Xu, Y. (2020). New Insights Into the Pathogenesis of Alzheimer's Disease. *Frontiers in Neurology*, *10*. https://www.frontiersin.org/articles/10.3389/fneur.2019.01312

Feng, T., & Wang, J. (2020). Oxidative stress tolerance and antioxidant capacity of lactic acid bacteria as probiotic: A systematic review. *Gut Microbes, 12*(1), 1801944. https://doi.org/10.1080/19490976.2020.1801944

Franceschi, C., & Campisi, J. (2014). Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *69 Suppl 1*, S4-9. https://doi.org/10.1093/gerona/glu057

Fülling, C., Dinan, T. G., & Cryan, J. F. (2019). Gut Microbe to Brain Signaling: What Happens in Vagus…. *Neuron*, *101*(6), 998–1002. https://doi.org/10.1016/j.neuron.2019.02.008

Gage, G. J., Kipke, D. R., & Shain, W. (2012). Whole Animal Perfusion Fixation for Rodents. *Journal of Visualized Experiments : JoVE*, *65*, 3564. https://doi.org/10.3791/3564

Garabadu, D., & Verma, J. (2019). Exendin-4 attenuates brain mitochondrial toxicity through PI3K/Akt-dependent pathway in amyloid beta (1-42)-induced cognitive deficit rats. *Neurochemistry International*, *128*, 39–49. https://doi.org/10.1016/j.neuint.2019.04.006

Garcia, M. L., & Cleveland, D. W. (2001). Going new places using an old MAP: Tau, microtubules and human neurodegenerative disease. *Current Opinion in Cell Biology*, *13*(1), 41–48. https://doi.org/10.1016/S0955-0674(00)00172-1

Georgiadou, E. C., Kowalska, E., Patla, K., Kulbat, K., Smolińska, B., Leszczyńska, J., & Fotopoulos, V. (2018). Influence of Heavy Metals (Ni, Cu, and Zn) on Nitro-Oxidative Stress Responses, Proteome Regulation and Allergen Production in Basil (Ocimum basilicum L.) Plants. *Frontiers in Plant Science*, *9*. https://www.frontiersin.org/articles/10.3389/fpls.2018.00862

Giacomini, A. C., Bueno, B. W., Marcon, L., Scolari, N., Genario, R., Demin, K. A., Kolesnikova, T. O., Kalueff, A. V., & de Abreu, M. S. (2020). An acetylcholinesterase inhibitor, donepezil, increases anxiety and cortisol levels in adult zebrafish. *Journal of*

Psychopharmacology (Oxford, England), *34*(12), 1449–1456. https://doi.org/10.1177/0269881120944155

Giridharan, V. V., Barichello De Quevedo, C. E., & Petronilho, F. (2022). Microbiota-gut-brain axis in the Alzheimer's disease pathology—An overview. *Neuroscience Research*, *181*, 17–21. https://doi.org/10.1016/j.neures.2022.05.003

Glenner, G. G., & Wong, C. W. (1984). Alzheimer's disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochemical and Biophysical Research Communications*, *120*(3), 885–890. https://doi.org/10.1016/S0006-291X(84)80190-4

Gong, C.-X., Liu, F., Grundke-Iqbal, I., & Iqbal, K. (2005). Post-translational modifications of tau protein in Alzheimer's disease. *Journal of Neural Transmission*, *112*(6), 813–838. https://doi.org/10.1007/s00702-004-0221-0

Graeber, M. B., Kösel, S., Egensperger, R., Banati, R. B., Müller, U., Bise, K., Hoff, P., Möller, H. J., Fujisawa, K., & Mehraein, P. (1997). Rediscovery of the case described by Alois Alzheimer in 1911: Historical, histological and molecular genetic analysis. *Neurogenetics*, *1*(1), 73–80. https://doi.org/10.1007/s100480050011

Hafez, H. S., Ghareeb, D. A., Saleh, S. R., Abady, M. M., El Demellawy, M. A., Hussien, H., & Abdel-Monem, N. (2017). Neuroprotective effect of ipriflavone against scopolamine-induced memory impairment in rats. *Psychopharmacology*, *234*(20), 3037–3053. https://doi.org/10.1007/s00213-017-4690-x

Hager, K., Marahrens, A., Kenklies, M., Riederer, P., & Münch, G. (2001). Alpha-lipoic acid as a new treatment option for Azheimer type dementia. *Archives of Gerontology and Geriatrics*, *32*(3), 275–282. https://doi.org/10.1016/S0167-4943(01)00104-2

Handajani, Y. S., Turana, Y., Yogiara, Y., Sugiyono, S. P., Lamadong, V., Widjaja, N. T., Christianto, G. A. M., & Suwanto, A. (2022). Effects of Tempeh Probiotics on Elderly With Cognitive Impairment. *Frontiers in Aging Neuroscience*, *14*. https://www.frontiersin.org/articles/10.3389/fnagi.2022.891773

Hardy, J., & Allsop, D. (1991). Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends in Pharmacological Sciences*, *12*, 383–388. https://doi.org/10.1016/0165-6147(91)90609-V

Hardy, J., & Selkoe, D. J. (2002). The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science (New York, N.Y.)*, *297*(5580), 353–356. https://doi.org/10.1126/science.1072994

Herholz, K., Weisenbach, S., Zündorf, G., Lenz, O., Schröder, H., Bauer, B., Kalbe, E., & Heiss, W.-D. (2004). In vivo study of acetylcholine esterase in basal forebrain, amygdala, and cortex in mild to moderate Alzheimer disease. *NeuroImage*, *21*(1), 136–143. https://doi.org/10.1016/j.neuroimage.2003.09.042

Hippius, H., & Neundörfer, G. (2003). The discovery of Alzheimer's disease. *Dialogues in Clinical Neuroscience*, *5*(1), 101–108. https://doi.org/10.31887/DCNS.2003.5.1/hhippius

Hsieh, T.-H., Kuo, C.-W., Hsieh, K.-H., Shieh, M.-J., Peng, C.-W., Chen, Y.-C., Chang, Y.-L., Huang, Y.-Z., Chen, C.-C., Chang, P.-K., Chen, K.-Y., & Chen, H.-Y. (2020). Probiotics Alleviate the Progressive Deterioration of Motor Functions in a Mouse Model of Parkinson's Disease. *Brain Sciences*, *10*(4), Article 4. https://doi.org/10.3390/brainsci10040206

Ikram, M. F., Farhat, S. M., Mahboob, A., Baig, S., Yaqinuddin, A., & Ahmed, T. (2021). Expression of DnMTs and MBDs in AlCl3-Induced Neurotoxicity Mouse Model.

Biological Trace Element Research, *199*(9), 3433–3444. https://doi.org/10.1007/s12011-020-02474-4

Iqbal, G., Iqbal, A., Mahboob, A., M. Farhat, S., & Ahmed, T. (2016). Memory Enhancing Effect of Black Pepper in the AlCl3 Induced Neurotoxicity Mouse Model is Mediated Through Its Active Component Chavicine. *Current Pharmaceutical Biotechnology*, *17*(11), 962–973.

Jain, J., Hasan, W., Biswas, P., Yadav, R. S., & Jat, D. (2022). Neuroprotective effect of quercetin against rotenone-induced neuroinflammation and alterations in mice behavior. *Journal of Biochemical and Molecular Toxicology*, *36*(10), e23165. https://doi.org/10.1002/jbt.23165

Joe, E., & Ringman, J. M. (2019). Cognitive symptoms of Alzheimer's disease: Clinical management and prevention. *BMJ*, *367*, l6217. https://doi.org/10.1136/bmj.l6217

Joint FAO/WHO Expert Consultation on. (2001).

Jung, D. H., Ahn, S. M., Pak, M. E., Lee, H. J., Jung, Y. J., Kim, K. B., Shin, Y.-I., Shin, H. K., & Choi, B. T. (2020). Therapeutic effects of anodal transcranial direct current stimulation in a rat model of ADHD. *ELife*, *9*, e56359. https://doi.org/10.7554/eLife.56359

Kadlec, R., & Jakubec, M. (2014). The effect of prebiotics on adherence of probiotics. *Journal of Dairy Science*, *97*(4), 1983–1990. https://doi.org/10.3168/jds.2013-7448

Karimipour, M., Rahbarghazi, R., Tayefi, H., Shimia, M., Ghanadian, M., Mahmoudi, J., & Bagheri, H. S. (2019). Quercetin promotes learning and memory performance concomitantly with neural stem/progenitor cell proliferation and neurogenesis in the adult rat dentate gyrus. *International Journal of Developmental Neuroscience: The Official Journal of the International Society for Developmental Neuroscience*, *74*, 18–26. https://doi.org/10.1016/j.ijdevneu.2019.02.005

Kechagia, M., Basoulis, D., Konstantopoulou, S., Dimitriadi, D., Gyftopoulou, K., Skarmoutsou, N., & Fakiri, E. M. (2013). Health Benefits of Probiotics: A Review. *ISRN Nutrition*, *2013*. https://doi.org/10.5402/2013/481651

Khan, H., Ullah, H., Martorell, M., Valdes, S. E., Belwal, T., Tejada, S., Sureda, A., & Kamal, M. A. (2021). Flavonoids nanoparticles in cancer: Treatment, prevention and clinical prospects. *Seminars in Cancer Biology*, *69*, 200–211. https://doi.org/10.1016/j.semcancer.2019.07.023

Khan, M. T. H., Orhan, I., Şenol, F. S., Kartal, M., Şener, B., Dvorská, M., Šmejkal, K., & Šlapetová, T. (2009). Cholinesterase inhibitory activities of some flavonoid derivatives and chosen xanthone and their molecular docking studies. *Chemico-Biological Interactions*, *181*(3), 383–389. https://doi.org/10.1016/j.cbi.2009.06.024

Kim, D. H., Khan, H., Ullah, H., Hassan, S. T. S., Šmejkal, K., Efferth, T., Mahomoodally, M. F., Xu, S., Habtemariam, S., Filosa, R., Lagoa, R., & Rengasamy, K. R. (2019). MicroRNA targeting by quercetin in cancer treatment and chemoprotection. *Pharmacological Research*, *147*, 104346. https://doi.org/10.1016/j.phrs.2019.104346

Köhler, C. A., Maes, M., Slyepchenko, A., Berk, M., Solmi, M., Lanctôt, K. L., & Carvalho, A. F. (2016). The Gut-Brain Axis, Including the Microbiome, Leaky Gut and Bacterial Translocation: Mechanisms and Pathophysiological Role in Alzheimer's Disease. *Current Pharmaceutical Design*, *22*(40), 6152–6166. https://doi.org/10.2174/1381612822666160907093807

Kommaddi, R. P., Das, D., Karunakaran, S., Nanguneri, S., Bapat, D., Ray, A., Shaw, E., Bennett, D. A., Nair, D., & Ravindranath, V. (2018). Aβ mediates F-actin disassembly in dendritic spines leading to cognitive deficits in Alzheimer's disease. *The Journal of Neuroscience*, *38*(5), 1085–1099. https://doi.org/10.1523/JNEUROSCI.2127-17.2017

Kraeuter, A.-K., Guest, P. C., & Sarnyai, Z. (2019). The Y-Maze for Assessment of Spatial Working and Reference Memory in Mice. In P. C. Guest (Ed.), *Pre-Clinical Models* (Vol. 1916, pp. 105–111). Springer New York. https://doi.org/10.1007/978-1-4939-8994-2_10

Kullisaar, T., Zilmer, M., Mikelsaar, M., Vihalemm, T., Annuk, H., Kairane, C., & Kilk, A. (2002). Two antioxidative lactobacilli strains as promising probiotics. *International Journal of Food Microbiology*, *72*(3), 215–224. https://doi.org/10.1016/S0168-1605(01)00674-2

Larroya-García, A., Navas-Carrillo, D., & Orenes-Piñero, E. (2019). Impact of gut microbiota on neurological diseases: Diet composition and novel treatments. *Critical Reviews in Food Science and Nutrition*, *59*(19), 3102–3116. https://doi.org/10.1080/10408398.2018.1484340

Le Page, A., Dupuis, G., Frost, E. H., Larbi, A., Pawelec, G., Witkowski, J. M., & Fulop, T. (2018). Role of the peripheral innate immune system in the development of Alzheimer's disease. *Experimental Gerontology*, *107*, 59–66. https://doi.org/10.1016/j.exger.2017.12.019

LeBlanc, J. G., del Carmen, S., Miyoshi, A., Azevedo, V., Sesma, F., Langella, P., Bermúdez-Humarán, L. G., Watterlot, L., Perdigon, G., & de Moreno de LeBlanc, A. (2011). Use of superoxide dismutase and catalase producing lactic acid bacteria in TNBS induced Crohn's disease in mice. *Journal of Biotechnology*, *151*(3), 287–293. https://doi.org/10.1016/j.jbiotec.2010.11.008

Lee, N. K., Choi, Y. G., Baik, J. Y., Han, S. Y., Jeong, D., Bae, Y. S., Kim, N., & Lee, S. Y. (2005). A crucial role for reactive oxygen species in RANKL-induced osteoclast differentiation. *Blood*, *106*(3), 852–859. https://doi.org/10.1182/blood-2004-09-3662

Lee, S., Ahmed, T., Lee, S., Kim, H., Choi, S., Kim, D.-S., Kim, S. J., Cho, J., & Shin, H.-S. (2012). Bidirectional modulation of fear extinction by mediodorsal thalamic firing in mice. *Nature Neuroscience*, *15*(2), Article 2. https://doi.org/10.1038/nn.2999

Lekchand Dasriya, V., Samtiya, M., Dhewa, T., Puniya, M., Kumar, S., Ranveer, S., Chaudhary, V., Vij, S., Behare, P., Singh, N., Aluko, R. E., & Puniya, A. K. (2022). Etiology and management of Alzheimer's disease: Potential role of gut microbiota modulation with probiotics supplementation. *Journal of Food Biochemistry*, *46*(1), e14043. https://doi.org/10.1111/jfbc.14043

Ley, R. E., Hamady, M., Lozupone, C., Turnbaugh, P., Ramey, R. R., Bircher, J. S., Schlegel, Michael. L., Tucker, T. A., Schrenzel, M. D., Knight, R., & Gordon, J. I. (2008). Evolution of mammals and their gut microbes. *Science (New York, N.Y.)*, *320*(5883), 1647–1651. https://doi.org/10.1126/science.1155725

Lillestøl, K. (2018). 'Neurasthenia gastrica' revisited: Perceptions of nerve-gut interactions in nervous exhaustion, 1880–1920. *Microbial Ecology in Health and Disease*, *29*(2), 1553438. https://doi.org/10.1080/16512235.2018.1553438

Liu, P., Wu, L., Peng, G., Han, Y., Tang, R., Ge, J., Zhang, L., Jia, L., Yue, S., Zhou, K., Li, L., Luo, B., & Wang, B. (2019). Altered microbiomes distinguish Alzheimer's disease from amnestic mild cognitive impairment and health in a Chinese cohort. *Brain, Behavior, and Immunity*, *80*, 633–643. https://doi.org/10.1016/j.bbi.2019.05.008

Liu, Y.-W., Ong, J. S., Gan, C. Y., Khoo, B. Y., Yahaya, S., Choi, S. B., Low, W. Y., Tsai, Y.-C., & Liong, M. T. (2019). Lactobacillus fermentum PS150 showed psychotropic properties by altering serotonergic pathway during stress. *Journal of Functional Foods*, *59*, 352–361. https://doi.org/10.1016/j.jff.2019.05.043

Livingston, G., Huntley, J., Sommerlad, A., Ames, D., Ballard, C., Banerjee, S., Brayne, C., Burns, A., Cohen-Mansfield, J., Cooper, C., Costafreda, S. G., Dias, A., Fox, N., Gitlin, L. N., Howard, R., Kales, H. C., Kivimäki, M., Larson, E. B., Ogunniyi, A., … Mukadam, N. (2020). Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. *Lancet (London, England)*, *396*(10248), 413–446. https://doi.org/10.1016/S0140-6736(20)30367-6

Long, X., Wu, H., Zhou, Y., Wan, Y., Kan, X., Gong, J., & Zhao, X. (2022). Preventive Effect of Limosilactobacillus fermentum SCHY34 on Lead Acetate-Induced Neurological Damage in SD Rats. *Frontiers in Nutrition*, *9*. https://www.frontiersin.org/articles/10.3389/fnut.2022.852012

Long-Smith, C., O'Riordan, K. J., Clarke, G., Stanton, C., Dinan, T. G., & Cryan, J. F. (2020). Microbiota-Gut-Brain Axis: New Therapeutic Opportunities. *Annual Review of Pharmacology and Toxicology*, *60*(1), 477–502. https://doi.org/10.1146/annurev-pharmtox-010919-023628

Luca, M., Di Mauro, M., Di Mauro, M., & Luca, A. (2019). Gut Microbiota in Alzheimer's Disease, Depression, and Type 2 Diabetes Mellitus: The Role of Oxidative Stress. *Oxidative Medicine and Cellular Longevity*, *2019*, e4730539. https://doi.org/10.1155/2019/4730539

Lum, H., & Roebuck, K. A. (2001). Oxidant stress and endothelial cell dysfunction. *American Journal of Physiology-Cell Physiology*, *280*(4), C719–C741. https://doi.org/10.1152/ajpcell.2001.280.4.C719

M. de la Monte, S. (2012). Brain Insulin Resistance and Deficiency as Therapeutic Targets in Alzheimer's Disease. *Current Alzheimer Research*, *9*(1), 35–66. https://doi.org/10.2174/156720512799015037

Maccioni, R. B., Calfío, C., González, A., & Lüttges, V. (2022). Novel Nutraceutical Compounds in Alzheimer Prevention. *Biomolecules*, *12*(2), Article 2. https://doi.org/10.3390/biom12020249

Madiha, S., Batool, Z., Tabassum, S., Liaquat, L., Sadir, S., Shahzad, S., Naqvi, F., Saleem, S., Yousuf, S., Nawaz, A., Ahmad, S., Sajid, I., Afzal, A., & Haider, S. (2021). Quercetin exhibits potent antioxidant activity, restores motor and non-motor deficits induced by rotenone toxicity. *PLOS ONE*, *16*(11), e0258928. https://doi.org/10.1371/journal.pone.0258928

Madreiter-Sokolowski, C. T., Thomas, C., & Ristow, M. (2020). Interrelation between ROS and Ca2+ in aging and age-related diseases. *Redox Biology*, *36*, 101678. https://doi.org/10.1016/j.redox.2020.101678

Markesbery, W. R. (1997). Oxidative Stress Hypothesis in Alzheimer's Disease. *Free Radical Biology and Medicine*, *23*(1), 134–147. https://doi.org/10.1016/S0891-5849(96)00629-6

Markowiak, P., & Śliżewska, K. (2017). Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. *Nutrients*, *9*(9), Article 9. https://doi.org/10.3390/nu9091021

Mart'yanov, S. V., Botchkova, E. A., Plakunov, V. K., & Gannesen, A. V. (2021). The Impact of Norepinephrine on Mono-Species and Dual-Species Staphylococcal Biofilms. *Microorganisms*, *9*(4), Article 4. https://doi.org/10.3390/microorganisms9040820

Massaad, C. A., & Klann, E. (2011). Reactive Oxygen Species in the Regulation of Synaptic Plasticity and Memory. *Antioxidants & Redox Signaling*, *14*(10), 2013–2054. https://doi.org/10.1089/ars.2010.3208

Mayer, E. A. (2011). Gut feelings: The emerging biology of gut–brain communication. *Nature Reviews Neuroscience*, *12*(8), Article 8. https://doi.org/10.1038/nrn3071

Mayeux, R., & Stern, Y. (2012). Epidemiology of Alzheimer Disease. *Cold Spring Harbor Perspectives in Medicine*, *2*(8), a006239. https://doi.org/10.1101/cshperspect.a006239

Mitsuma, T., Odajima, H., Momiyama, Z., Watanabe, K., Masuguchi, M., Sekine, T., Shidara, S., & Hirano, S. (2008). Enhancement of gene expression by a peptide p(CHWPR) produced by Bifidobacterium lactis BB-12. *Microbiology and Immunology*, *52*(3), 144–155. https://doi.org/10.1111/j.1348-0421.2008.00022.x

Mohandas, E., Rajmohan, V., & Raghunath, B. (2009). Neurobiology of Alzheimer's disease. *Indian Journal of Psychiatry*, *51*(1), 55–61. https://doi.org/10.4103/0019-5545.44908

Morris, R. G. M. (1981). Spatial localization does not require the presence of local cues. *Learning and Motivation*, *12*(2), 239–260. https://doi.org/10.1016/0023-9690(81)90020-5

Murphy, M. P. (2009). How mitochondria produce reactive oxygen species. *The Biochemical Journal*, *417*(1), 1–13. https://doi.org/10.1042/BJ20081386

Mzhelskaya, K. V., Shipelin, V. A., Shumakova, A. A., Musaeva, A. D., Soto, J. S., Riger, N. A., Trusov, N. V., Kirbaeva, N. V., Apryatin, S. A., & Gmoshinski, I. V. (2020). Effects of quercetin on the neuromotor function and behavioral responses of Wistar and Zucker rats fed a high-fat and high-carbohydrate diet. *Behavioural Brain Research*, *378*, 112270. https://doi.org/10.1016/j.bbr.2019.112270

Nabavi, S. F., Atanasov, A. G., Khan, H., Barreca, D., Trombetta, D., Testai, L., Sureda, A., Tejada, S., Vacca, R. A., Pittalà, V., Gulei, D., Berindan-Neagoe, I., Shirooie, S., & Nabavi, S. M. (2018). Targeting ubiquitin-proteasome pathway by natural, in particular polyphenols, anticancer agents: Lessons learned from clinical trials. *Cancer Letters*, *434*, 101–113. https://doi.org/10.1016/j.canlet.2018.07.018

Naidu, P. S., Singh, A., & Kulkarni, S. K. (2004). Reversal of Reserpine-Induced Orofacial Dyskinesia and Cognitive Dysfunction by Quercetin. *Pharmacology*, *70*(2), 59–67. https://doi.org/10.1159/000074669

Naomi, R., Embong, H., Othman, F., Ghazi, H. F., Maruthey, N., & Bahari, H. (2022). Probiotics for Alzheimer's Disease: A Systematic Review. *Nutrients*, *14*(1), Article 1. https://doi.org/10.3390/nu14010020

Nguyen, T., Nioi, P., & Pickett, C. B. (2009). The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. *The Journal of Biological Chemistry*, *284*(20), 13291–13295. https://doi.org/10.1074/jbc.R900010200

O'Mahony, S. M., Clarke, G., Borre, Y. E., Dinan, T. G., & Cryan, J. F. (2015). Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behavioural Brain Research*, *277*, 32–48. https://doi.org/10.1016/j.bbr.2014.07.027

Osborn, L. M., Kamphuis, W., Wadman, W. J., & Hol, E. M. (2016). Astrogliosis: An integral player in the pathogenesis of Alzheimer's disease. *Progress in Neurobiology*, *144*, 121–141. https://doi.org/10.1016/j.pneurobio.2016.01.001

Ossola, B., Kääriäinen, T. M., & Männistö, P. T. (2009). The multiple faces of quercetin in neuroprotection. *Expert Opinion on Drug Safety*, *8*(4), 397–409. https://doi.org/10.1517/14740330903026944

Ovaskainen, M.-L., Törrönen, R., Koponen, J. M., Sinkko, H., Hellström, J., Reinivuo, H., & Mattila, P. (2008). Dietary Intake and Major Food Sources of Polyphenols in Finnish Adults. *The Journal of Nutrition*, *138*(3), 562–566. https://doi.org/10.1093/jn/138.3.562

Park, H. J., Kwon, H., Lee, J. H., Cho, E., Lee, Y. C., Moon, M., Jun, M., Kim, D. H., & Jung, J. W. (2020). β-Amyrin Ameliorates Alzheimer's Disease-Like Aberrant Synaptic Plasticity

in the Mouse Hippocampus. *Biomolecules & Therapeutics*, *28*(1), 74–82. https://doi.org/10.4062/biomolther.2019.024

Park, M. R., Shin, M., Mun, D., Jeong, S.-Y., Jeong, D.-Y., Song, M., Ko, G., Unno, T., Kim, Y., & Oh, S. (2020). Probiotic Lactobacillus fermentum strain JDFM216 improves cognitive behavior and modulates immune response with gut microbiota. *Scientific Reports*, *10*, 21701. https://doi.org/10.1038/s41598-020-77587-w

Pattanashetti, L. A., Taranalli, A. D., Parvatrao, V., Malabade, R. H., & Kumar, D. (2017). Evaluation of neuroprotective effect of quercetin with donepezil in scopolamine-induced amnesia in rats. *Indian Journal of Pharmacology*, *49*(1), 60–64. https://doi.org/10.4103/0253-7613.201016

Penders, J., Thijs, C., Vink, C., Stelma, F. F., Snijders, B., Kummeling, I., van den Brandt, P. A., & Stobberingh, E. E. (2006). Factors Influencing the Composition of the Intestinal Microbiota in Early Infancy. *Pediatrics*, *118*(2), 511–521. https://doi.org/10.1542/peds.2005-2824

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., & Bitto, A. (2017). Oxidative Stress: Harms and Benefits for Human Health. *Oxidative Medicine and Cellular Longevity*, *2017*, e8416763. https://doi.org/10.1155/2017/8416763

Pompeia, S., Gouveia, J. R., & Galduróz, J. C. F. (2013). Acute mood effect of donepezil in young, healthy volunteers. *Human Psychopharmacology: Clinical and Experimental*, *28*(3), 263–269. https://doi.org/10.1002/hup.2319

Prakash, D., Gopinath, K., & Sudhandiran, G. (2013). Fisetin Enhances Behavioral Performances and Attenuates Reactive Gliosis and Inflammation During Aluminum Chloride-Induced

Neurotoxicity. *NeuroMolecular Medicine*, *15*(1), 192–208. https://doi.org/10.1007/s12017-012-8210-1

Qu, Z., Sun, J., Zhang, W., Yu, J., & Zhuang, C. (2020). Transcription factor NRF2 as a promising therapeutic target for Alzheimer's disease. *Free Radical Biology and Medicine*, *159*, 87–102. https://doi.org/10.1016/j.freeradbiomed.2020.06.028

Raghunath, A., Sundarraj, K., Nagarajan, R., Arfuso, F., Bian, J., Kumar, A. P., Sethi, G., & Perumal, E. (2018). Antioxidant response elements: Discovery, classes, regulation and potential applications. *Redox Biology*, *17*, 297–314. https://doi.org/10.1016/j.redox.2018.05.002

Reddy, D. S., & Kulkarni, S. K. (1998). Inhibition of neuronal nitric oxide synthase (n-cNOS) reverses the corticotrophin-induced behavioral effects in rats. *Molecular and Cellular Biochemistry*, *183*(1), 25–38. https://doi.org/10.1023/A:1006815125689

Rezaeiasl, Z., Salami, M., & Sepehri, G. (2019). The Effects of Probiotic Lactobacillus and Bifidobacterium Strains on Memory and Learning Behavior, Long-Term Potentiation (LTP), and Some Biochemical Parameters in β-Amyloid-Induced Rat's Model of Alzheimer's Disease. *Preventive Nutrition and Food Science*, *24*(3), 265–273. https://doi.org/10.3746/pnf.2019.24.3.265

Rhee, S. H., Pothoulakis, C., & Mayer, E. A. (2009). Principles and clinical implications of the brain–gut–enteric microbiota axis. *Nature Reviews Gastroenterology & Hepatology*, *6*(5), Article 5. https://doi.org/10.1038/nrgastro.2009.35

Rice, M. W., Pandya, J. D., & Shear, D. A. (2019). Gut Microbiota as a Therapeutic Target to Ameliorate the Biochemical, Neuroanatomical, and Behavioral Effects of Traumatic Brain Injuries. *Frontiers in Neurology*, *10*. https://www.frontiersin.org/articles/10.3389/fneur.2019.00875

Rigobelo, E. (2012). *Probiotics*. BoD – Books on Demand.

Romo-Araiza, A., & Ibarra, A. (2020). Prebiotics and probiotics as potential therapy for cognitive impairment. *Medical Hypotheses*, *134*, 109410. https://doi.org/10.1016/j.mehy.2019.109410

Sabogal-Guáqueta, A. M., Muñoz-Manco, J. I., Ramírez-Pineda, J. R., Lamprea-Rodriguez, M., Osorio, E., & Cardona-Gómez, G. P. (2015). The flavonoid quercetin ameliorates Alzheimer's disease pathology and protects cognitive and emotional function in aged triple transgenic Alzheimer's disease model mice. *Neuropharmacology*, *93*, 134–145. https://doi.org/10.1016/j.neuropharm.2015.01.027

Saeideh, D., Sayyed Alireza, T., Mansoureh, S., Hojatollah, A., & Mahmoud, S. (2012). *[<The> effect of co-administration of lactobacillus probiotics and bifidobacterium on spatial memory and learning in diabetic rats]*. 531–539.

Santos, R. X., Correia, S. C., Zhu, X., Lee, H.-G., Petersen, R. B., Nunomura, A., Smith, M. A., Perry, G., & Moreira, P. I. (2012). Nuclear and mitochondrial DNA oxidation in Alzheimer's disease. *Free Radical Research*, *46*(4), 565–576. https://doi.org/10.3109/10715762.2011.648188

Saxena, G., Singh, S. P., Agrawal, R., & Nath, C. (2008). Effect of donepezil and tacrine on oxidative stress in intracerebral streptozotocin-induced model of dementia in mice. *European Journal of Pharmacology*, *581*(3), 283–289. https://doi.org/10.1016/j.ejphar.2007.12.009

Selkoe, D. J. (2001). Alzheimer's Disease: Genes, Proteins, and Therapy. *Physiological Reviews*, *81*(2), 741–766. https://doi.org/10.1152/physrev.2001.81.2.741

Semwal, B. C., & Garabadu, D. (2020). Amyloid beta (1–42) downregulates adenosine-2b receptors in addition to mitochondrial impairment and cholinergic dysfunction in memory-

sensitive mouse brain regions. *Journal of Receptors and Signal Transduction*, *40*(6), 531–540. https://doi.org/10.1080/10799893.2020.1767136

Sender, R., Fuchs, S., & Milo, R. (2016). Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLOS Biology*, *14*(8), e1002533. https://doi.org/10.1371/journal.pbio.1002533

Seo, D.-O., & Holtzman, D. M. (2020). Gut Microbiota: From the Forgotten Organ to a Potential Key Player in the Pathology of Alzheimer's Disease. *The Journals of Gerontology: Series A*, *75*(7), 1232–1241. https://doi.org/10.1093/gerona/glz262

Shimmyo, Y., Kihara, T., Akaike, A., Niidome, T., & Sugimoto, H. (2008). Flavonols and flavones as BACE-1 inhibitors: Structure–activity relationship in cell-free, cell-based and in silico studies reveal novel pharmacophore features. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *1780*(5), 819–825. https://doi.org/10.1016/j.bbagen.2008.01.017

Shin, C. Y., Kim, H.-S., Cha, K.-H., Won, D. H., Lee, J.-Y., Jang, S. W., & Sohn, U. D. (2018). The Effects of Donepezil, an Acetylcholinesterase Inhibitor, on Impaired Learning and Memory in Rodents. *Biomolecules & Therapeutics*, *26*(3), 274–281. https://doi.org/10.4062/biomolther.2017.189

Sochocka, M., Donskow-Łysoniewska, K., Diniz, B. S., Kurpas, D., Brzozowska, E., & Leszek, J. (2019). The Gut Microbiome Alterations and Inflammation-Driven Pathogenesis of Alzheimer's Disease—A Critical Review. *Molecular Neurobiology*, *56*(3), 1841–1851. https://doi.org/10.1007/s12035-018-1188-4

Sriraksa, N., Wattanathorn, J., Muchimapura, S., Tiamkao, S., Brown, K., & Chaisiwamongkol, K. (2012). Cognitive-Enhancing Effect of Quercetin in a Rat Model of Parkinson's

Disease Induced by 6-Hydroxydopamine. *Evidence-Based Complementary and Alternative Medicine : ECAM, 2012*, 823206. https://doi.org/10.1155/2012/823206

Suganya, K., & Koo, B.-S. (2020). Gut–Brain Axis: Role of Gut Microbiota on Neurological Disorders and How Probiotics/Prebiotics Beneficially Modulate Microbial and Immune Pathways to Improve Brain Functions. *International Journal of Molecular Sciences*, *21*(20), Article 20. https://doi.org/10.3390/ijms21207551

Sun, M., Ma, K., Wen, J., Wang, G., Zhang, C., Li, Q., Bao, X., & Wang, H. (2020). A Review of the Brain-Gut-Microbiome Axis and the Potential Role of Microbiota in Alzheimer's Disease. *Journal of Alzheimer's Disease: JAD*, *73*(3), 849–865. https://doi.org/10.3233/JAD-190872

Tarafdar, A., & Pula, G. (2018). The Role of NADPH Oxidases and Oxidative Stress in Neurodegenerative Disorders. *International Journal of Molecular Sciences*, *19*(12), Article 12. https://doi.org/10.3390/ijms19123824

Tonelli, C., Chio, I. I. C., & Tuveson, D. A. (2018). Transcriptional Regulation by Nrf2. *Antioxidants & Redox Signaling*, *29*(17), 1727–1745. https://doi.org/10.1089/ars.2017.7342

Tsigos, C., & Chrousos, G. P. (2002). Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress. *Journal of Psychosomatic Research*, *53*(4), 865–871. https://doi.org/10.1016/S0022-3999(02)00429-4

Ullah, H., & Khan, H. (2018). Anti-Parkinson Potential of Silymarin: Mechanistic Insight and Therapeutic Standing. *Frontiers in Pharmacology*, *9*. https://www.frontiersin.org/articles/10.3389/fphar.2018.00422

Vogt, N. M., Kerby, R. L., Dill-McFarland, K. A., Harding, S. J., Merluzzi, A. P., Johnson, S. C., Carlsson, C. M., Asthana, S., Zetterberg, H., Blennow, K., Bendlin, B. B., & Rey, F. E.

(2017). Gut microbiome alterations in Alzheimer's disease. *Scientific Reports*, *7*(1), Article 1. https://doi.org/10.1038/s41598-017-13601-y

Vorhees, C. V., & Williams, M. T. (2006). Morris water maze: Procedures for assessing spatial and related forms of learning and memory. *Nature Protocols*, *1*(2), Article 2. https://doi.org/10.1038/nprot.2006.116

Wang, X., Sun, G., Feng, T., Zhang, J., Huang, X., Wang, T., Xie, Z., Chu, X., Yang, J., Wang, H., Chang, S., Gong, Y., Ruan, L., Zhang, G., Yan, S., Lian, W., Du, C., Yang, D., Zhang, Q., … Geng, M. (2019). Sodium oligomannate therapeutically remodels gut microbiota and suppresses gut bacterial amino acids-shaped neuroinflammation to inhibit Alzheimer's disease progression. *Cell Research*, *29*(10), Article 10. https://doi.org/10.1038/s41422-019-0216-x

Wang, X., Wang, W., Li, L., Perry, G., Lee, H., & Zhu, X. (2014). Oxidative stress and mitochondrial dysfunction in Alzheimer's disease. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1842*(8), 1240–1247. https://doi.org/10.1016/j.bbadis.2013.10.015

Wang, Y., Wu, Y., Wang, Y., Xu, H., Mei, X., Yu, D., Wang, Y., & Li, W. (2017). Antioxidant Properties of Probiotic Bacteria. *Nutrients*, *9*(5), Article 5. https://doi.org/10.3390/nu9050521

Weller, J., & Budson, A. (2018). *Current understanding of Alzheimer's disease diagnosis and treatment* (7:1161). F1000Research. https://doi.org/10.12688/f1000research.14506.1

Westfall, S., Lomis, N., Kahouli, I., Dia, S. Y., Singh, S. P., & Prakash, S. (2017). Microbiome, probiotics and neurodegenerative diseases: Deciphering the gut brain axis. *Cellular and Molecular Life Sciences*, *74*(20), 3769–3787. https://doi.org/10.1007/s00018-017-2550-9

Wischik, C. M., Novak, M., Edwards, P. C., Klug, A., Tichelaar, W., & Crowther, R. A. (1988). Structural characterization of the core of the paired helical filament of Alzheimer disease. *Proceedings of the National Academy of Sciences*, *85*(13), 4884–4888. https://doi.org/10.1073/pnas.85.13.4884

Xie, J., Song, W., Liang, X., Zhang, Q., Shi, Y., Liu, W., & Shi, X. (2020). Protective effect of quercetin on streptozotocin-induced diabetic peripheral neuropathy rats through modulating gut microbiota and reactive oxygen species level. *Biomedicine & Pharmacotherapy*, *127*, 110147. https://doi.org/10.1016/j.biopha.2020.110147

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R. N., Anokhin, A. P., Heath, A. C., Warner, B., Reeder, J., Kuczynski, J., Caporaso, J. G., Lozupone, C. A., Lauber, C., Clemente, J. C., Knights, D., … Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. *Nature*, *486*(7402), Article 7402. https://doi.org/10.1038/nature11053

Yiannopoulou, K. G., & Papageorgiou, S. G. (2020). Current and Future Treatments in Alzheimer Disease: An Update. *Journal of Central Nervous System Disease*, *12*, 1179573520907397. https://doi.org/10.1177/1179573520907397

Zheng, H., Niu, S., Zhao, H., Li, S., & Jiao, J. (2018). Donepezil improves the cognitive impairment in a tree shrew model of Alzheimer's disease induced by amyloid-β1–40 via activating the BDNF/TrkB signal pathway. *Metabolic Brain Disease*, *33*(6), 1961–1974. https://doi.org/10.1007/s11011-018-0303-6

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1. Research Project Title: Investigating the effect of indigenous probiotic Lactobacillus spp. Strains and Quercetin on AlCl3 induced Alzheimer's disease rat model.

2	Name of PI:	Dr Abdul Rahman
3	Duration:	03-04 months
4	Name of Institution / Department	ASAB, NUST
5	IRB No.	07-2022-ASAB-01/02

6. The project proposal entitled above has been reviewed by the NUST Institutional Review Board Meeting held on July 01, 2022.

7. The Board approves project proposal on scale and criteria given below to be implemented before/during project execution.

- Safety Measures
- Workspace Requirements
- Protection from potential hazards & Risks
- Confidentiality Requirements (If Any)

Note: The Ethical Review Committee reserves the rights to re-review the project during the project execution to address the suggested guidelines.

Prof. Dr. Muhammad Tahir
Deptt of Plant Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST, Islamabad

Signature of the Head of Department/Head of Thematic Research

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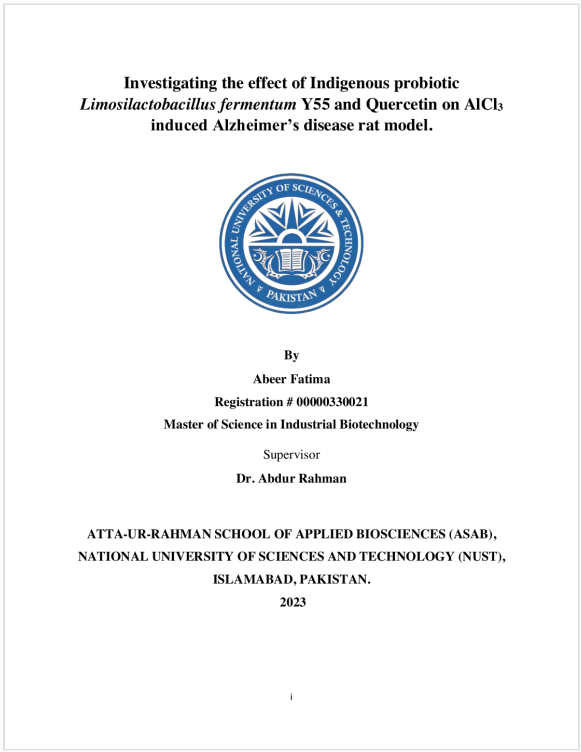

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By

Abeer Fatima

Registration # 00000330021

Master of Science in Industrial Biotechnology

Supervisor

Dr. Abdur Rahman

ATTA-UR-RAHMAN SCHOOL OF APPLIED BIOSCIENCES (ASAB), NATIONAL UNIVERSITY OF SCIENCES AND TECHNOLOGY (NUST), ISLAMABAD, PAKISTAN.

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