TARGETING TREM2: IN-SILICO STUDY OF INTERACTIONS WITH GUT MICROBIOTA BASED SUPPLEMENTS FOR ALZHEIMER'S DISEASE

A Thesis Submitted

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by

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CANDIDATE'S DECLARATION

I, Suprati Singh, 2K22/MSCBIO/50 student of M.Sc. Biotechnology, hereby certify that the thesis entitled **"Targeting TREM2: In-Silico Study of Interactions with Gut Microbiota Based Supplements for Alzheimer's Disease"** in partial fulfilment of the requirement for the award of the Degree of Masters of Sciences submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from May 2023 to May 2024 under the supervision of Prof. Pravir Kumar.

The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other institute.

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CERTIFICATE BY THE SUPERVISOR

Certified that **Ms. Suprati Singh**, 2K22/MSCBIO/50 has carried out their search work presented in this thesis entitled **"Targeting TREM2: In-Silico Study of Interactions with Gut Microbiota Based Supplements for Alzheimer's Disease"** from Department of Biotechnology, Delhi Technological University, Delhi under my supervision. The thesis embodies result of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

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ABSTRACT

Aim: To study the interaction between the receptor TREM2 and the supplements of bacterial strain *A. muciniphila* to target neuroinflammation in AD.

Methodology: Material and methodology used for whole study includes the variant determination, docking analysis of the test ligands, protein-protein interaction analysis or String analysis and ADME analysis to validate the results and finding.

Result: Herein we found that out of the considered supplements of the microbe of interest *A. muciniphila* mannitol, maltitol metformin, maltitol showed the maximum efficacy or binding affinity towards the receptor TREM2 but after conducting the ADME analysis we found that maltitol does not cross the Blood Brain Barrier (BBB), Lipophilicity in the optimum range, it is highly polar and flexible hence not suitable for oral administration, water soluble Pharmacokinetic analysis shows that is has lower absorption for GI and does not cross the BBB, also the ligand of interest does not respond to the inhibitors and it is not skin permeable.

Conclusion: The study concludes that the bacterial strain and its prebiotic supplements are not suitable for targeting neuroinflammation as it does not satisfy the required criteria for docking as well as drug development.

Keywords— Alzheimer's disease, gut microbiota, TREM2, molecular docking, STRING, neuroinflammation.

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LIST OF ABBREVIATIONS

ND	Neurodegenerative Disease
AD	Alzheimer's Disease
PD	Parkinsons Disease
ALS	Amyotrophic Lateral Sclerosis
MS	Multiple Sclerosis
MND	Motor Neuron Disease
HD	Huntington's Disease
SMA	Spinal Muscular Dystrophy
SCA	Spinocerebellar Ataxia
BBB	Blood Brain Barrier
SCFA	Short Chain Fatty Acid
GIT	Gastro Intestinal Tract
ADME	Absorption Distribution Metabolism Excretion
GI	Gastro Intestinal
SCFA	Short Chain Fatty Acid
CNS	Central Nervous System
GBA	Gut-Brain Axis
ENS	Enteric Nervous System
TLR	Toll Like Receptor
TREM2	Triggering Receptor Expressed on Myeloid Cells
FODMAP	Fermentable Oligo-, Di-, and Monosaccharides and Polyols
PPI	Protein-Protein Interaction

CHAPTER 1
INTRODUCTION

Neurodegenerative disorders are (NDD) are caused due to progressive neuronal loss which leads to synaptic dysfunction and memory decline. Some of the major NDDs are Multiple sclerosis (MS), Parkinson's disease (PD), Alzheimer's disease (AD), Amyotrophic lateral sclerosis (ALS) Huntington's disease (HD)[1]. Among these we have taken AD into account for our study which is one of the most prevalent diseases among the NDD. Here we are dealing with the concern of neuroinflammation in AD. Several investigations have demonstrated that GM has a major function in maintenance of body homeostasis which also involves the brain. Communication pipeline which connects the gut and brain is known as GBA [2]. Human gut constitutes a wide variety of microbial population which help to maintain the normal body functioning. In this study we are going to deal with a bacterial species *Akkermansia muciniphila* [3]. *A. muciniphila* also has been studied in other diseases also [4], and is responsible for lowering the inflammatory responses in Nasu Hakola disease [5].

In AD also neuroinflammation is a major problem in the progression of the disease to deal with this problem we are conducting this study. We have taken some supple ments of *A. muciniphila* and testing the binding of the supplements with the TREM2 receptor which is a key receptor in microglia for neuroinflammation. Neuroinflammation stands for the effects occurring due to pathological damage occurs in the peripheral or central nervous system, the CNS becomes sensitive, progressing towards secretion of tumour necrosis factor, pro-inflammatory cytokines chemokines, complement cytokines, and some small molecular messengers, such as (ROS) reactive oxygen species, prostaglandins and (NO) nitric oxide [6].

This study includes the interaction between the receptor TREM2 and the supplement molecules of *A. muciniphila* to test the blocking of hyper neuroinflammation also it includes the protein-protein interactions via String analysis and ADME analysis to validate the docking results and findings.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Neurodegenerative Diseases

NDD are collective phrase which denotes the disorders caused due to gradual declination of neurons. Neurons are the intricate building blocks or we can say basic structural component of our nervous system, function like miniature communication network. They receive, analyse, and communicate through electrical signals, enabling us to feel, move, think, and perceive our surroundings [7], [8]. However, in neurodegenerative conditions, these critical pathways are under threat. Neurodegeneration is linked to the dysfunction of the synaptic connections and deposition of physiochemically modified variants of peptides in brain Despite significant progress in modern medicine, some medical conditions remain highly challenging to treat effectively. The most common NDD includes AD, PD, MND, HD, Prions Disease, SMA, SCA [9] . As the brain governs numerous facets of the functions of the body, NDD have a comprehensive influence on human operations, constraining capacity to carry out basic such as movement, speech, balance, stability and convoluted tasks. Maximum NDDs enroot without exemption, nevertheless in a few instances, regimen aspire to ameliorate indications, lighten pain, or bring back the movement and coordination [10].There are mainly three kinds of neurological disorders, the first one is the congenital abnormalities which involves birth malformations in the structural makeup of the brain or spinal cord that arise in the uterus, or during the foetus development during pregnancy, second is the acquired neurological disorders that are the cognitive decline acquired by the patients during its lifespan, Third is the idiopathic disorder that is neuropathy occurs when nerve injury clashes with the operation of the PNS and the reason cannot be identified. The patients

suffering from ND are treated with several kinds of treatments to improve the symptomatic experiences as there is no particular drug or curative treatment for NDD Such as therapeutic approach, drugs like memantine, galantamine, donepezil, aducanumab, rivastigmine are used for AD, levodopa, carbidopa, apomorphine, pergolide, pramipexol for PD, riluzole and edaravone for ALS. Acupuncture oriental medicines for conjunctive therapies, yoga and exercises also plays important role in treatment of NDD. Other technology driven treatment methods are also present such as brain-computer interface, brain mapping to picturize the functional lobes and centres and that data is then analysed by the gamma knifes and cyber knifes that are radiation therapy and robotic radiosurgery system respectively, deep brain stimulation for tremor treatment [11], [12], [13]

2.2 Gut Microbiota

The human GI tract (250-400 m^2) is a major contact amidst the host and stimuli from environment and the irritant in the body. In an average life time, over 60 tonnes of food move through the human GI tract, biformed with a multitude of microbes from the ecosystem, which pose an immense danger to gut integrity [14] Collective term used for the mixed population of bacteria, algae, fungi, protozoa and archaea is the gut microbiota which are having symbiotic relationship inside the human gut [15] A healthy human gut possesses a huge population of microbes such as *Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, Verrucomicrobia, Bifidobacterium, Bacillus spp. Enterococcus*. During the condition of NDD the micro flora gets disturbed such as the level of *Firmicutes, Bacteroidetes, Escherichia/shigella* increases whereas level of *Verrucomicrobia, Lactobacillus spp*. decreases [16], [17] GM derived metabolites have momentous role in facilitating transmission betwixt the gut microbiome and the host, particularly the brain. Metabolites are classified into several classes, including:

- (SCFAs), includes propionate, burtyrate and acetate. These are thought to regulate immunological function, metabolism, and cognitive health.

- Bile acids. Gut bacteria can change bile acids, resulting in compounds that affect host metabolism and inflammation [18]
- Tryptophan metabolites, include indole and its derivatives. These can affect the immune system and have neuroprotective properties.
- Trimethylamine N-oxide (TMAO). This metabolite, formed from choline and carnitine, has been linked to cardiovascular and metabolic problems. Branched chain amino acids. These amino acid metabolites levels vary and are linked to metabolic disorders [19]

2.3 Gut-Brain Axis

(GBA) associate the (CNS) and GI tract, enabling bidirectional transmission amid both the systems. Through spinal and vagal visceral afferent pathways, ENS, circulatory system and neuroendocrine system the gut communicates with brain and receives input from the sympathetic and parasympathetic nervous systems, as shown by gastrointestinal behaviour. GBA monitors and integrates intestinal function. In addition, the bowel's enteric nervous system, which is made up of motor neuron, interneurons, primary afferent neurons, perceives the enteric environment and regulates the precise patterns of intestinal motility and secretion [20].

Different modes of communication of GM and brain are as follows:

- Through production of metabolites, short-chain fatty acids, and neurotransmitters.
- Cortisol secretion by HPA in the condition of stress which affects production of mucus, intestinal motility and integrity. CNS can get affected (stress hormone modulation) by this alteration.
- By releasing chemokines and proinflammatory cytokines.

Immunity also plays a crucial role. Peptidoglycans and TLR specifically contribute as sensors of microbial biomolecules to intercede the immune resistance to microorganisms. Also, neuroinflammation as well as activation of microglia are the primary indicator for ND. Different routes can connect a local immunological response

to immune activation in the brain. There has been links between this low-grade immune activation and the pathophysiology of various types of depression and neurodegenerative diseases like AD [21]

2.4 Alzheimer's Disease

The most frequent explanation of dementia is AD, affects approximately 50,000,000 individuals worldwide and is a serious global health concern [22][23]. Alzheimer's is among the remarkably frequent category of dementia. Dementia is a generic term for intelligible deterioration. The hippocampus (which is responsible for memory and thoughts) is likely to be altered in case of AD. The general noticeable symptoms of AD include severe memory loss, spatial disorientation, mood changes, physical imbalance, and speech problems. A number of theories on the pathogenesis and progression of AD have been developed, the exact causes of the disease initiation and progression are still unresolved. In this context, Aβ polypeptide aggregation and hyperphosphorylated tau interlaces are likely evident tissue or organ level indicators for the hallmarks of (AD) are significant memory decline, cognition, and motor abilities. Pathophysiology of AD emphasize energy metabolism, calcium balance dysfunction, and neuroinflammation [6].

A region of the 4kDa amyloid precursor protein (APP) is referred to as amyloid-β that is largely produced by brain neurons, blood cells and vascular cells. Two fragments are formed as a result of consequent proteolytic cleavage of APP, βsecretase BACE-1 cleaves at the ectodomain and γ-secretase cleaves at the intramembranous site generating Aβ. With the help of neuroimaging studies, it has been investigated that spatiotemporal evolution of Aβ accumulation in the brain which initiates in cerebral region with high metabolic bioenergetic rate of activity for the population of neurons extends to the:

AD can be classified as:

1) *Early Onset AD (EOAD)*

EOAD is especially brought on by genetically driven inadequate regulation of amyloidogenic pathway with subsequent Amyloid β excessive production. EOAD is responsible for only 1% of the total AD cases mostly caused due to mutation in the *APP, PSEN1* and *PSEN2* genes. As APP genes are present on chromosome 21, the patient with down syndrome resulted from mutation on the locus exhibits cognitive decline corresponding to molecular characteristic of AD [22].

2) *Late Onset AD (LOAD)*

(LOAD) is much more prevalent and complicated as compared to early-onset Alzheimer's disease (EOAD), potentially involving multiple genes as well as interactions between genes and the environment [24].

APOE was considered as the only susceptible marker for LOAD before 2009. Since 2009, meta-analysis and GWAS studies have identified that there are approximately 25 additional loci: TREM2, ABCA7, CD33, HLA-DRB1/HLA-DRB5, CLU, CR1, MS4A4, EPHA1, INPP5D, SLC24A4/RIN3, CASS4, FERMT2, BIN1, CD2AP, NME8, PTK2B, SORL1 CELF1, PICALM [24].

2.5 Myeloid Cell

Myeloid cells are a diverse group of blood cells derived from myeloid progenitor cells from HSC in the bone marrow. They include a variety of adult blood cells:

- Granulocytes include neutrophils, eosinophils, and basophils.
- Monocytes turns into macrophages in tissues.
- Erythrocytes (red blood cells)

- Megakaryocytes generate platelets.

Myeloid cells perform a diverse role in neuroinflammation, contributing to both protective and harmful activities of the central nervous system (CNS) [25].

Defensive functions:

1. Debris clearing and tissue repair: Myeloid like macrophages and microglia, play a key part to maintain CNS equilibrium by eliminating pathogens, debris, and injured cells. This prevents the propagation of infection and promotes tissue healing.
2. Homeostasis Maintenance: Microglia, a type of myeloid cell found in the brain, help maintain CNS homeostasis during normal conditions.
3. Rejuvenation: In response to contamination or tissue injury, myeloid cells such as monocytes or granulocytes are often drawn from the circulation to initially induce inflammation and then contribute in healing and regrowth procedures [26].

Detrimental functions:

1 Proinflammatory response: Myeloid cells can generate pro-inflammatory cytokines and chemokines during pathological circumstances such as stroke or multiple sclerosis, leading to tissue damage and persistent inflammation in the central nervous system (CNS).

2 Amplified inflammation: Neutrophils can enhance autoimmune infiltrates in the CNS by maturing antigen-presenting cells, leading to neuroinflammation.

3 Pathological communication: The interaction amongst neurons and myeloid cells is highlighted to be vital for proper brain function, showing that abnormal interactions between myeloid cells and neurons might cause neuroinflammatory reactions.

2.5.1 Microglia

Microglia is one of the three types of glial cells; it is the smallest among all three. In normal condition microglia performs several functions such as removing

inappropriately communicating neurons, removes the protein aggregate or plagues and protein clumps and immunological functions. Microglia are not a uniform population. They reflect a variety of activation states based on the signals they get from their surroundings.
Resting State: This is the state in which microglia continually extend and retract processes to assess their surroundings.

Activated States: When microglia meet dangers, they can become activated, taking on diverse morphologies and functional profiles. These activated states can be broadly characterized as pro-inflammatory (M1-like) releasing cytokines and chemoattractant such as IL-1β TNFα, IL-6 or anti-inflammatory (M2-like) comparable to macrophages, releases cytokines and chemokines such as IL-4, TGFα, IL-13, IL-10 comparable to macrophages [27]

Microglia can be recognized as distinct from other set of myeloid cells by their unique gene expression delineation. Microglia require the molecules IRF8 (interferon regulatory factor 8), DAP12 (DNAX-activation protein 12), CSF1R (colony-stimulating factor 1 receptor), and transcription factor PU.1 to develop and exhibit their function [28].

2.6 Neuroinflammation

When pathological damage occurs in the peripheral or central nervous system, the CNS becomes sensitive, leading to the secretion of cytokines that are pro-inflammatory and tumour necrosis factor, chemokines, complement cytokines, and certain small molecular messengers, such as (ROS) reactive oxygen species, prostaglandins and (NO) nitric oxide [6].

Although acute neuroinflammation occurs as a defence mechanism for any damage that occurs in the nervous system, prolonged inflammation becomes chronic by the time and results in damaging neurons [29]. As the microglial are the major producers of inflammatory cytokines they are considered as the main source of

neuroinflammation [27]. Endogenous bioactive lipid are the molecules that helps to regulates the molecular processes and chronic inflammation. Some of the bioactive are eicosanoids, specialized pro-catabolic lipid mediators, Lys phospholipids, and endocannabinoid. Furthermore, tau protein phosphorylation, memory impairment, and synaptic loss are all strongly associated with microglia, making these biological processes the centres for the investigation of AD neuroinflammation [30][31].

2.6.1 Neuroinflammation Contemplated by Microglia

Betwixt the numerous exercises, microglia-mediated neuroinflammation has garnered interest due to its intricate and dynamic role in well-being and illness. Initial neuroinflammation is beneficial because it encourages the tissue healing, cellular remains elimination as well as invader eradication [29]. Nevertheless, microglia lose their homeostatic dactylogram and develop gradually stimulated with growing age or all along with pathological circumstances, developing into peculiar disease-allied phenotypic characters with persistent production of pro-inflammatory cytokines and chemokines that are pro-inflammatory [32], [33].

2.7 TREM 2 Receptors

In this league, microglial cells which are neuronal cells that produces inflammation response to any neuronal damage consist of a cell surface receptor named TREM2 [34], [35].

In this context, TREM2 receptors on the microglia has been proven as a potent biomarker for AD progression as it contributes to neuroinflammation due to the overexpression of TREM2 receptor and expression of certain variants of TREM2 receptor as well [33], [36].

Furthermore, deactivating TREM2 mutations have been implicated in dementia in pathological circumstances including the exceptional form of passed on, recessive, dementia that are early-onset recognized as Nasu Hakola Disease.[37] It's interesting to note that the enzyme required for Aβ peptides production is also responsible for accelerating the cleavage of TREM2: γ-secretase. Also, (PSEN1), the primary benefactor to the γ-secretase complex, interacts with TREM2 [38] [39].

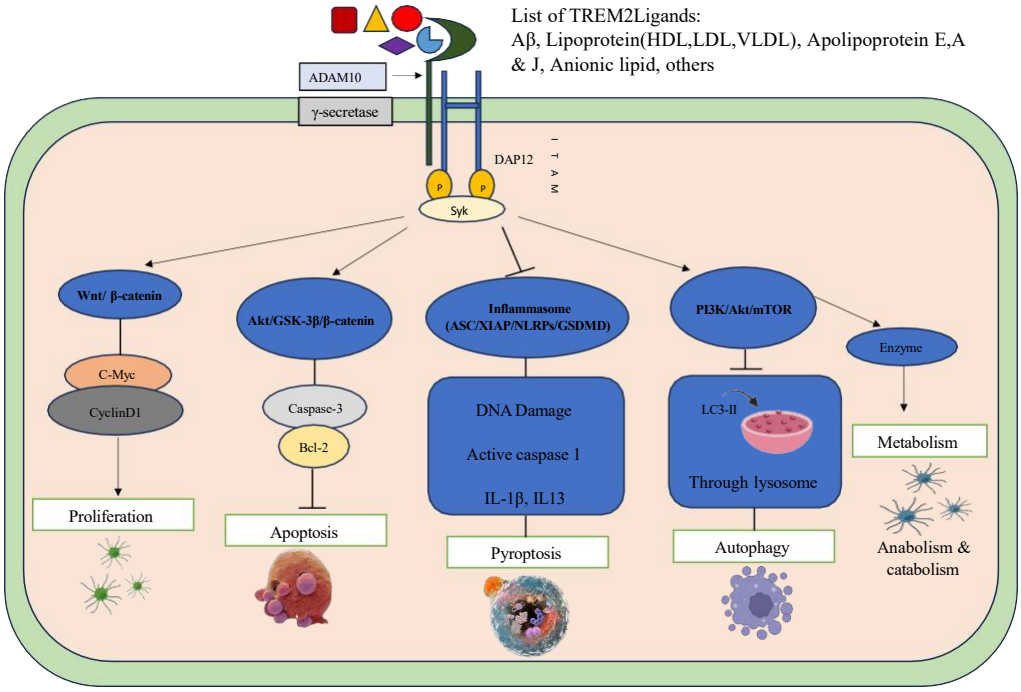


Fig. 2.1 TREM2 signalling pathway

2.8 *Akkermansia muciniphila*

A.*muciniphila* is an anaerobe, and a gram-negative bacterium which belongs to the Phylum: Verrucomicrobiae, Family: Akkermansiaceae, Species: *A. muciniphila*, Genus: Akkermansia[40] *A. muciniphila* has shown to accept modest levels of oxygen and so reclassified as an air-tolerant anaerobe. It improves the anti-incendiary properties of macrophages. This inquiry examined the anti-incendiary impact of A. muciniphila on THP-1 macrophages which are gliadin-accelerated [41]. A. muciniphilastrength to degrade mucin glycoproteins in gut using enzymes like proteases glycosyl hydrolases, sulphatases, and sialidase and utilize them as on

source of nitrogen and carbon; this whole process results in synthesis of (SCFAs) sulfate, acetate, 1.2-propanediol and propionate [42], [43]. Because of this breakdown mechanism, the bacteria improve mucin yield and thickness, strengthening the gut impediment reducing gut accessiblity to byproducts from microbes [2].

Recent research has found a relationship between A. muciniphila and a variety of ND, including autism spectrum disorder (ASD), psychosocial issues linked with IBD, refractory epilepsy, AD, PD and ALS disease.

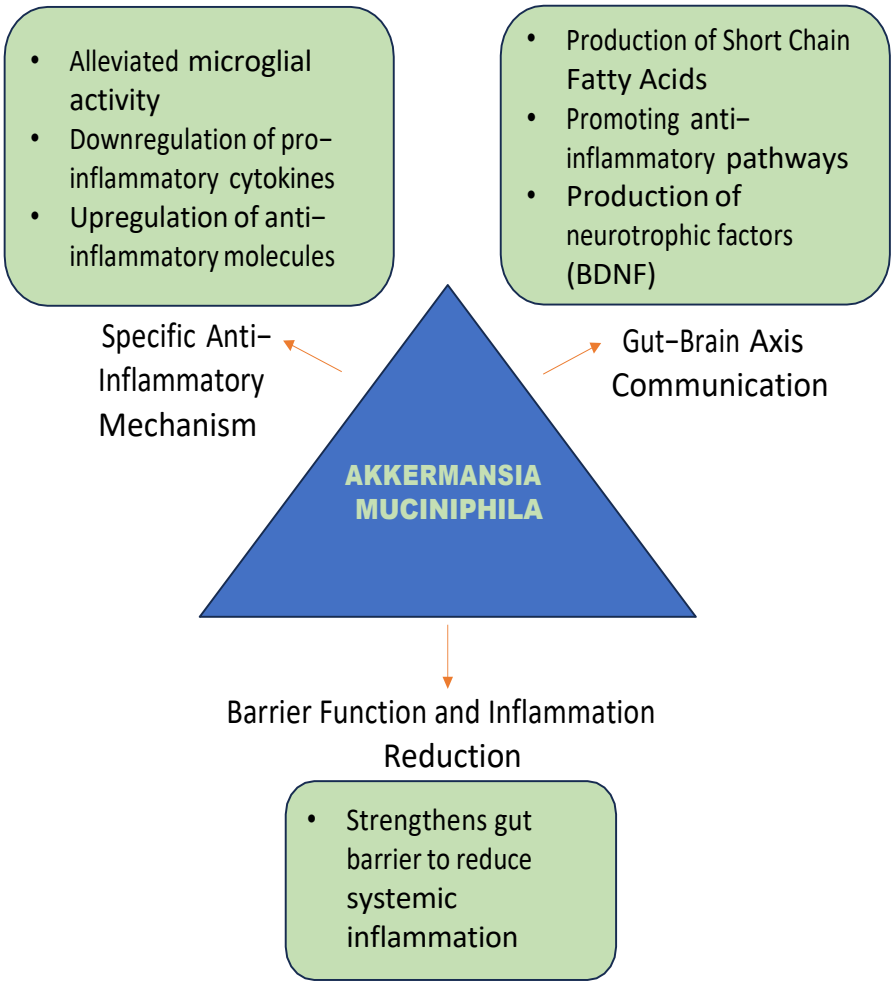


Fig. 2.2 Functions of *Akkermansia muciniphila*

A.muciniphila works in different ways to maintain homeostasis by downregulating proinflammatory cytokines, upregulating anti-inflammatory molecule, alleviates microglial activity, production of neurotrophic factors (BDNF), production of SCFA,

promoting anti-inflammatory pathways, strengthens gut barrier to reduce systemic inflammation [44].

2.8.1 *A. muciniphila* and AD

Deficiency of the respective bacteria was identified in faecal specimen from APP transgenics vulnerable to develop AD when in comparison to normal-type mice. This was inversely linked with the measure of antigenic Aβ-42 in brain**.** Further studies showed the administration of *A. muciniphila* in the mouse model of AD promoted the reduction of Aβ40-42 level in cerebral cortex, and boosted brain functions in AD mouse models [2].

2.8.2 *A. muciniphila* and PD

Colonic inflammation and higher intestinal permeability have been linked to PD [45] The functionally relevant explanation is, the bacteria which participates in mucus yield, may enhance gut accessibility in the individuals by producing hydrogen sulphide, changing the firmness of the intestinal protective layer and promoting the intestinal uptake of bacterial toxins [2].

2.8.3 *A. muciniphila* and ALS

A. muciniphila is lower in prevalence in ALS-prone mice having superoxide dismutase, and its supplement in transgenics, improves disease conditions, extends the animal's lifetime, mitigates atrophy of brain, and boosts spinal cord cell density. *A. muciniphila* functions via enhancing mitochondrial function, removing superoxide

radicals, and maintaining NAM adenine dinucleotide (NAD) equilibrium. These functions are known to be compromised [2], [46].

2.8.4 *A. muciniphila* and ASD

There was a substantial reduction in the pervasiveness of the bacteria in ASD suffers and their folks in comparison to controls in mouse model. Increased *A. muciniphila* levels were linked to lower levels of TNFα and Iba1 (microglial activation markers) and higher levels of MUC2. This suggests that A. muciniphila may improve disease progression by reestablishing integrity of the intestinal barrier through mucin production and release [2].

2.8.5 *A. muciniphila* and MS

In one observational investigation examining the GM makeup of 34 pairs of monozygotic twins ambiguous for MS, a higher concentration of bacteria was observed in the non-treated ones suffering from MS in contrast to either the normal twins or mouse suffering from MS that underwent any therapy; FMT from identical geminates having MS driven a substantially greater prevalence in transgenic mouse representation of disease of haphazard autoimmunity of brain, with IL-10 intricated in lowering disease progress [2].

2.9 *Akkermansia muciniphila* and its Supplements

A.muciniphila has various supplement, probiotic as well as prebiotic. Both the types are listed below:

2.9.1 Probiotic Supplements

There are no such evident studies done on TREM2, only the mice interventions studies have been reported [44].

Oral administration of the bacteria 2 × 10^8 CFU for 4 weeks inscreased the predominance of *A. muciniphila* in fecal product of high-fat-fed DIO [44]. Oral dispensation of a consolidation of *L. rhamnosus* LMG S-28148 and *B. animalis*, *subspp*. Lactis LMG P-28149 for a period of 14 weeks (5 days week_^{1}, 5 × 10^8 CFU for PBS strain) elevated *A. muciniphila* richness in the faecal product of the high-fat-fed DIO mice increased roughly 100 times [44].

2.9.2 Prebiotic Supplements

As mentioned in the section 2.9.1 there are no human studies reported, only the animal studies are the basis of research for now. In this context there are several studies done to find the prebiotic supplements of *A. muciniphila.* In this league, there are some prebiotics listed below, which showed positive results for promoting the growth of the species of interest.

2.9.2.1 FOS (fructo-oligosaccharides)

It a popular prebiotic, stimulated the appearance of *A. muciniphila* ob/ob mice, Sprague-Dawley rat models and in the intestinal tracts of DIO. It increased the prevalence of the strain by approximately 1000 folds. Another investigation discovered that five weeks of FOS administration by oral means at the similar dose raised the population of the respective bacteria in ob/ob mice by more than 80 times, accompanied along with bounty of the *E. rectale/C. coccoides* and *Bifidobacterium spp.* group [44]. In vitro culture research demonstrated that, amidst

16 different sources of carbon counting dietary fibres and prebiotics and, Feeding FOS to the media substantially boosted *A. muciniphila* growth, whereas other fibers/prebiotics did not, indicating that FOS may be a preferential nutrient for *A. muciniphila* [44], [47].

2.9.2.2 FODMAP

FODMAP[48] stands for fermentative oligo-, mono- and disaccharides and lower calorie sweetener: polyols, that comprise fructose, polyols such as mannitol maltitol, oligosaccharides, lactose and sugar alcohols[44]. Consumption of FODMAPs might elevate rapidly fermentable carbohydrates, resulting in functional gastrointestinal discomfort [49][50]. Several human investigations indicated that FODMAP level in the diet may decisively impact A. muciniphila prevalence[51]. The two human investigations demonstrated a favourable correlation between FODMAP in diets and A. muciniphila population in various patients/health individuals [51], [52].

2.9.2.3 Metformin

Metformin is typically used preliminarily for treating type 2 diabetes [53]. Remarkably, recent human and animal research demonstrated that metformin had the capacity to alter the GM, and this impact was connected with its anti-intemperate, anti-obesity, and therapeutic efficacy on metabolism of glucose [54].

CHAPTER 3

MATERIAL AND METHODOLOGY

3.1 Searching for the variants of TREM2 receptor having risk of AD

Search has been done through the literature review from PubMed and other sources.

3.2 String Analysis of TREM2:

String analysis of TREM2 in AD, PD, ALS, MS and HD has been performed to study the protein-protein interactions of TREM2 String Analysis has been performed. (https://stringdb.org/cgi/network?taskId=bzhkkTIh2C0u&sessionId=blMsgDZiKlYj)
String analysis provides the insight that TREM2 is involved in the neuroinflammation, amyloid β plague formation and regulation and well as it has been found to involve in various Signalling pathways and disease-gene association. The specific finding has been mentioned in result section.

3.3 Molecular Docking Study

3.3.1 Preparation of Receptor TREM2 for Docking

Macromolecule or the receptor TREM 2 is prepared for docking using BIOVIA software by removing the preexisting ligand and adding polar hydrogens.

3.3.2 Preparation of Ligand for Docking

Supplements of *A. muciniphila* are used as the Ligands. Mannitol, maltitol, metformin was prepared as ligands using Open babel software.

3.3.3 Choosing Reference Ligand

We have chosen two reference ligand molecules that are carpipramine and clocapramine as they have approximately same binding affinity with receptor TREM2. These are FDA approved drugs for neuroinflammation[55].

3.3.4 Docking of Receptor TREM2 and *A. muciniphila* Supplements

Metformin, mannitol and maltitol the supplements of *A.muciniphila* docking carried out with the TREM 2 receptor using the Autodock vina software [44].

3.3.5 Visualization of Docked Ligand and Receptor

3D and 2D visualisation of TREM 2 receptor docked with the highest efficacy ligand maltitol (supplement).

3.4 ADME Analysis

ADME analysis has been done using a tool named SWISSADME. The website permits you to evaluate physicochemical descriptors and forecast ADME metrics, pharmacokinetic attributes, druglike nature, and pharmaceutical friendliness of one or more small compounds to aid in drug discovery.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 List of variants of TREM2 receptor

Variants of receptor TREM2 are listed below in Table 1.

Table I. Variants of receptor TREM2 having risk of AD

Variants (with reference)	Effect on TREM function	Signalling response	Expression	Phagocytosis of lipoproteins	References
R47H	Impaired	Decreased	Usual	Weakened	[56]
D87N	Impaired	Increased	Usual	Weakened	[38]
T96K	Impaired	Decreased	Increased	Weakened	[38]
R62H	Impaired	Decreased	Modest defect	Weakened	[38]
Y38C	-	-	Decreased	Weakened	[38]

Table I. shows that variant listed shows retarded and manipulated behavioural response, expression, signalling response and altered effects on TREM2 function.

4.2 String Analysis of Receptor TREM2

Study of PPI has shown that the number of edges and nodes in AD, PD, ALS, MS and HD are as follows:

Edges: 36, 134, 206, 271, 9 respectively.

Nodes: 11, 25, 29, 26, 6 respectively. Rest of the information such as biological functions, KEGG pathway, and disease-gene association is listed below in Table 2.

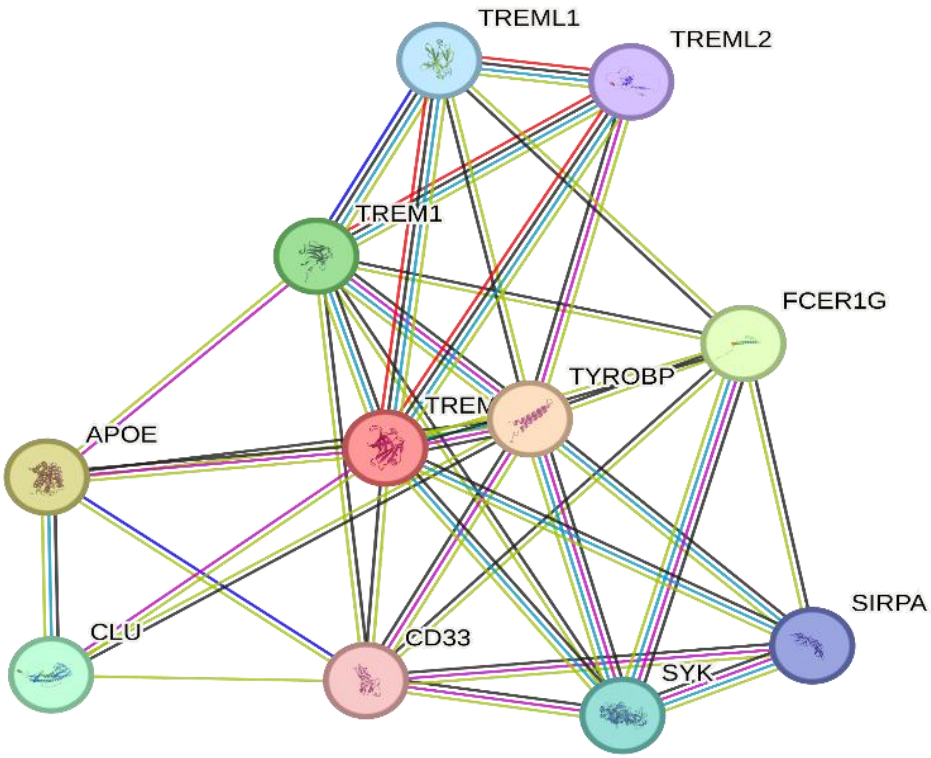


Fig. 4.1 String Network of TREM2 for AD with other proteins

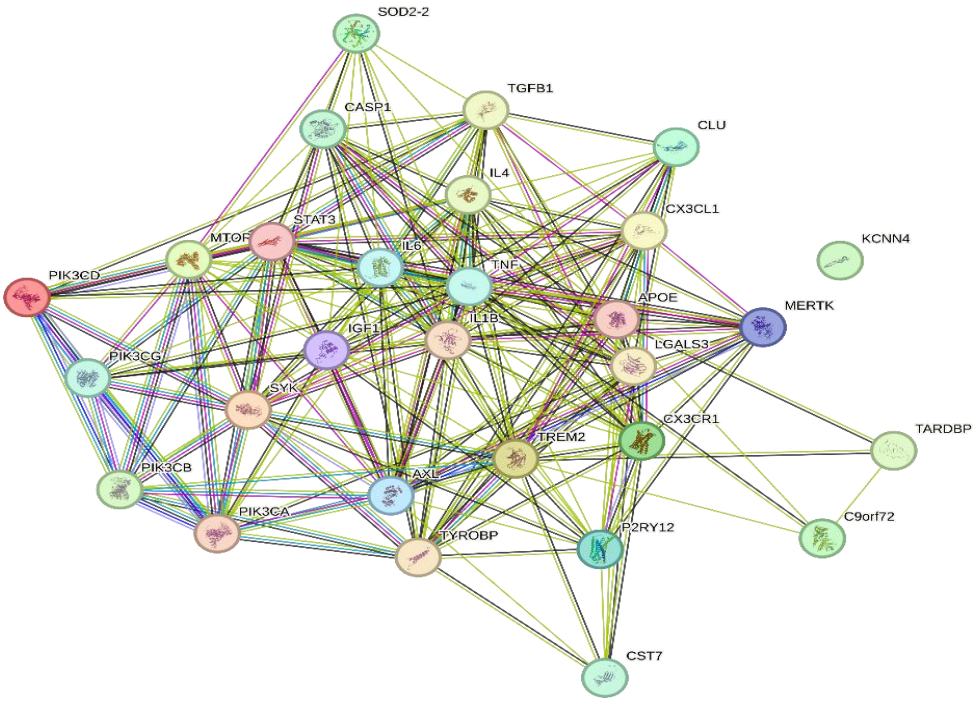


Fig. 4.2: String network of TREM2 with other proteins in PD

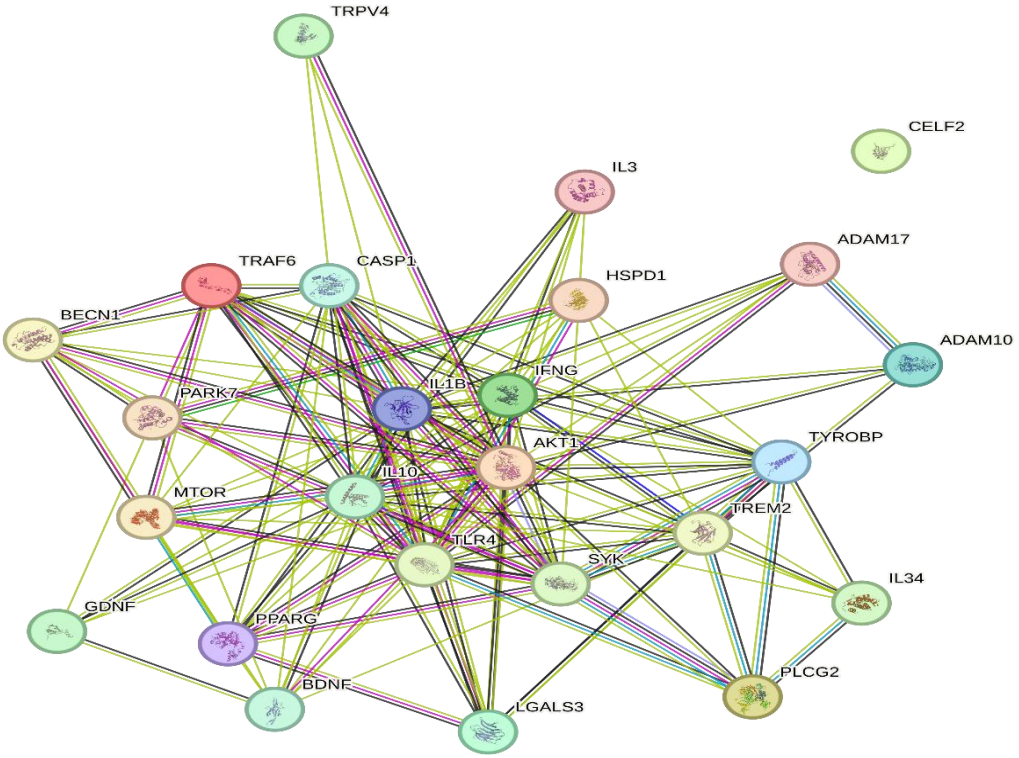


Fig. 4.3 network of TREM2 with other proteins in ALS Disease

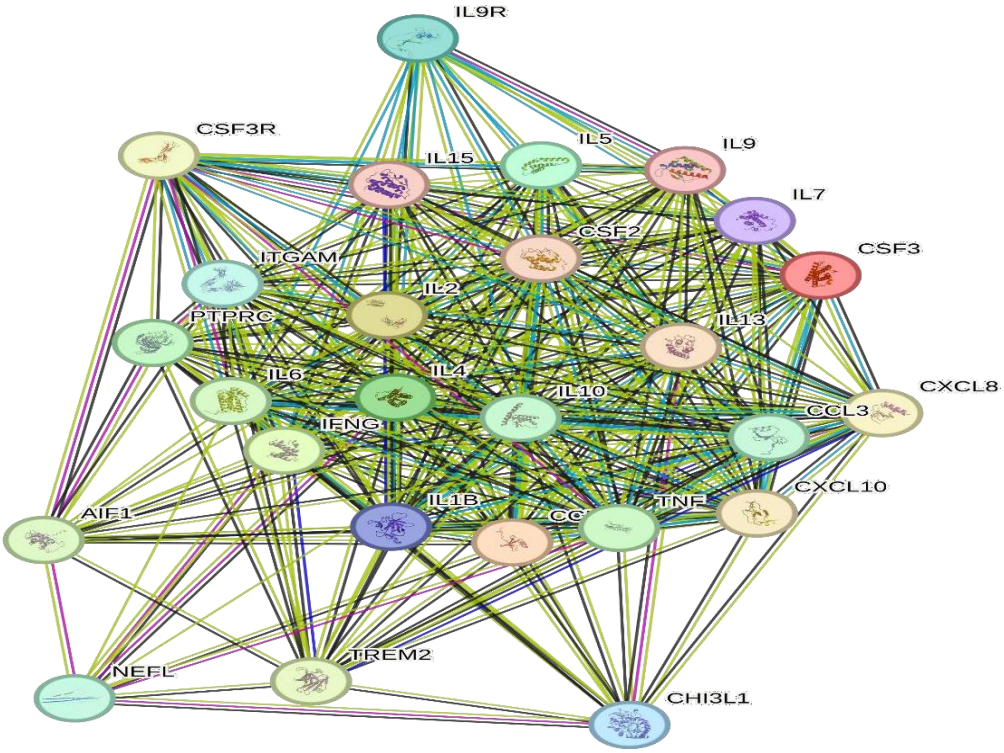


Fig. 4.4 String network of TREM2 with other proteins in MS

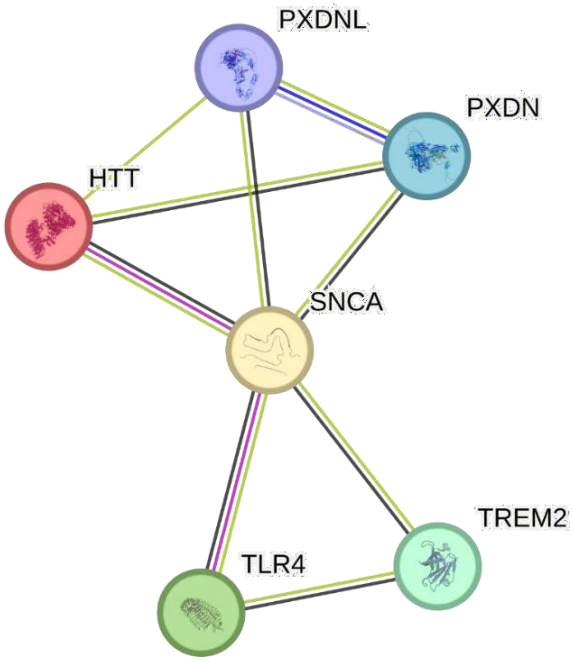


Fig. 4.5 String network of TREM2 with other proteins in HD

Table II. Data extracted from string analysis of receptor TREM2 in different ND

S. No.	Disease	Biological functions	Disease-gene association	KEGG Pathway
1.	AD	1. Positive regulation of neurofibrillary tangle assembly 2. Positive regulation of amyloid fibril formation 3. Regulation of hippocampal neuron apoptotic process 4. Microglial activation involved in immune response	1. Nasu- Hakola Disease 2. Lattice cornea dystrophy 3. ITM2B-related cerebral amyloid angiopathy 1 4. ITM2B-related cerebral amyloid angiopathy 2	1. Osteoclast differentiation 2. Natural killer cell mediated cytotoxicity
2.	PD	1. Positive regulation of calci 1-monooxygenase activity 2. positive activation of	1. Type 2 diabetes mellitus 2. Alzheimer's disease	1. Inflammatory Bowel disease 2. Rheumatoid arthritis

		complement activation 3. Positive regulation of neuroinflammatory response 4. Positive regulation of NLRP3 inflammasome complex assembly	3. Multiple sclerosis 4. Nasu- Hakola disease 5. Central nervous system disease	3. JAK-STAT signalling pathway 4. Glioma 5. TNF signalling pathway
3.	ALS disease	1. Negative regulation of primary miRNA processing 2. Positive regulation of calci 1-monooxygenase activity 3. Positive regulation of neurofibrillary tangle assembly 4. Microglial activation	1. Nasu- Hakola disease 2. Artery Disease 3. Diabetes mellitus 4. Hematopoietic stem cell disease	1. AGE-RAGE signalling pathway 2. EGFR tyrosine kinase inhibitor resistance 3. Salmonella infection 4. Pathways in cancer

		5. Positive regulation of miRNA- mediated gene silencing		5. PI3k-Akt signalling pathway
4.	Multiple Sclerosis	1. Regulation of chronic inflammatory response to antigenic stimulus 2. Positive regulation of calci 1-monooxygenase activity 3. Regulation of hippocampal neuron apoptotic process 4. Regulation of microglial activation 5. Regulation of amyloid-beta clearance	1. Intestinal Infection 2. Autoimmune disease of musculoskeletal system 3. Lower respiratory tract infection 4. Leukocyte disease 5. Allergic disease	1. Malaria 2. Chagas disease 3. Cytokine-cytokine receptor reaction 4. JAK-STAT signalling pathway 5. NF-kappa B signalling pathway

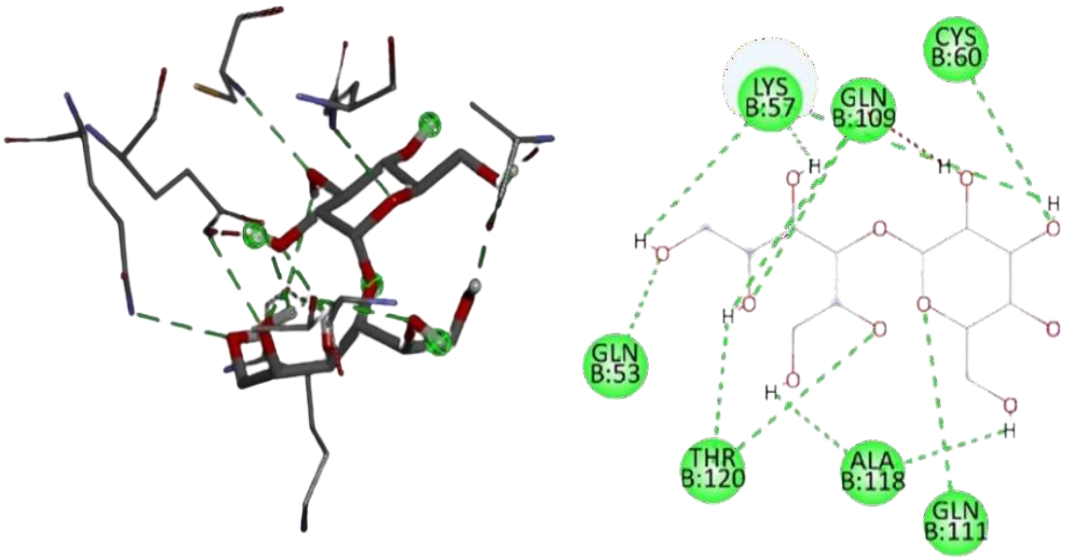
5.	HD	1. Regulation of CAMKK-AMPK signalling cascade 2. Regulation of cytokine production involved in inflammatory response 3. Cellular response to amyloid-beta 4. Response to oxidative stress 5. Positive regulation of mitochondrial organisation	1. Huntington's disease 2. Early-onset Parkinsons disease	

4.3 Docking Results

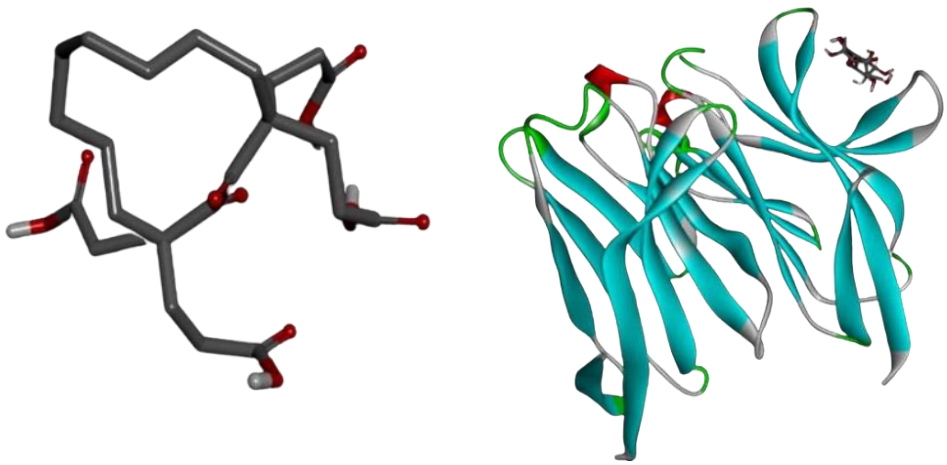
As a result of docking Maltitol has been visualized as the highest efficacy ligand to bind with the TREM 2 receptor as a supplement of bacterium *A. muciniphila.*

According to the docking results Maltitol can be considered as the supplement of *Akkermansia muciniphila* for prevention of inflammation during AD.

But at the same time, it does not stand out on comparing with our reference molecules which are carcapramine and calcapramine



(a) **(b)**



(c) **(d)**

Fig. 4.6: (a) 3D interaction of receptor and ligand (b) 2D interaction of receptor and ligand (c) Ligand (Maltitol) with highest efficacy with the TREM 2 receptor (d) 3D representation of receptor and ligand docking.

Table III. Binding affinity of ligands including reference ligands

Molecular Formula	ID	Name	Binding Affinity
C28H38N4O	ZINC597537	Carpipramine	-7.5
C28H37C1N4O	ZINC608266	Calcapramine	-7.5
C4H11N5	(PDB)4091	Metformin	-4.2
C6H14O6	(PDB)6251	Mannitol	-4.9
C12H24O11	(PDB)493591	Maltitol	-5.9

4.4 ADME Analysis

ADME analysis of the ligand maltitol has been done.

Analysis shows the following results. It cannot cross the BBB, it is not approved from CNS, figure 7 shows that the ligand is highly polar and flexible hence it is not suitable for oral administration.

i. Lipophilicity has the optimum range of 3-4.5 but the ligand does not satisfy the optimum range.
ii. The ligand is water soluble
iii. Pharmacokinetic analysis shows that is has lower absorption for GI and does not cross the BBB, also the ligand of interest does not respond to the inhibitors and it is not skin permeable.
iv. This ligand does not the satisfy the criteria for drug likeliness.

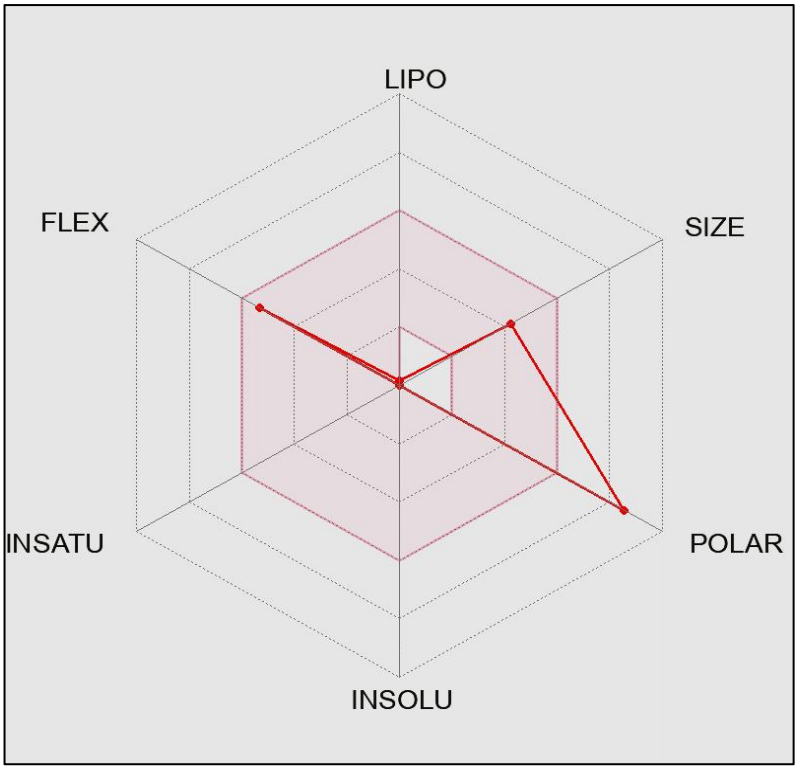


Fig. 4.7: ADME analysis of Maltitol

Physicochemical Properties	
Formula	C12H24O11
Molecular weight	344.31 g/mol
Num. heavy atoms	23
Num. arom. heavy atoms	0
Fraction Csp3	1.00
Num. rotatable bonds	8
Num. H-bond acceptors	11
Num. H-bond donors	9
Molar Refractivity	70.31
TPSA	200.53 Å²

(a)

Medicinal Chemistry	
PAINS	0 alert
Brenk	0 alert
Leadlikeness	No; 1 violation: Rotors>7
Synthetic accessibility	5.37

(b)

Lipophilicity	
Log $P_{o/w}$ (iLOGP)	0.39
Log $P_{o/w}$ (XLOGP3)	-5.21
Log $P_{o/w}$ (WLOGP)	-5.76
Log $P_{o/w}$ (MLOGP)	-4.77
Log $P_{o/w}$ (SILICOS-IT)	-3.59
Consensus Log $P_{o/w}$	-3.79

(c)

Water Solubility	
Log *S* (ESOL)	1.84
Solubility	2.36e+04 mg/ml ; 6.85e+01 mol/l
Class	Highly soluble
Log *S* (Ali)	1.64
Solubility	1.52e+04 mg/ml ; 4.41e+01 mol/l
Class	Highly soluble
Log *S* (SILICOS-IT)	4.20
Solubility	5.51e+06 mg/ml ; 1.60e+04 mol/l
Class	Soluble

(d)

Pharmacokinetics	
GI absorption	Low
BBB permeant	No
P-gp substrate	Yes
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-12.10 cm/s

(e)

Fig. 4.8: Different properties of a drug candidate obtained from ADME analysis through SWISSADME. (a) Physiochemical properties (b) Medicinal chemistry (c) Lipophilicity Water solubility (d) Water solubility (e) Pharmacokinetics.

CHAPTER 5

CONCLUSION

AD is one a prevalent neurological disease which results in cognitive deterioration or loss pf memory and is characterized by a steady decline in neuronal cells and progresses by several factors such as neuroinflammation. It becomes very important to control and regulate the damages until we find the cure. Neuroinflammation is one of the various problems caused in the condition of AD. In this study we are targeting neuroinflammation. The primary causative agents bind to the receptor TREM2 in microglia which leads to proinflammatory and anti-inflammatory responses in case the proinflammatory responses exceeds the normal, it has to be controlled. In this context, gut microbe is found very useful to regulate the brain health through gut-brain axis. There are plenty of intestinal microbes which resides in our intestine and affect the homeostasis, one of those is the bacterium *Akkermansia muciniphila*. The study discusses the potential of *A. muciniphila* to reduce neuroinflammation and minimize the progression of AD and the supplements that can be considered for the patients of Alzheimer's disease such as FOS, FODMAP, metformin. Analysis of the supplements through docking has been done and the docking results conclude that we can consider the supplements for therapy but they don't show the required binding affinity as compared to the reference drug also through docking we can conclude among all the taken ligands maltitol showed maximum efficacy for receptor TREM2 to reduce the hyper proinflammatory responses during AD conditions. Hence, they are not a potent blocking agent for neuroinflammation. Also, ADME analysis has been done for maltitol which concluded that it is not a suitable candidate for drug development as it does not satisfy the required criteria for drug development.

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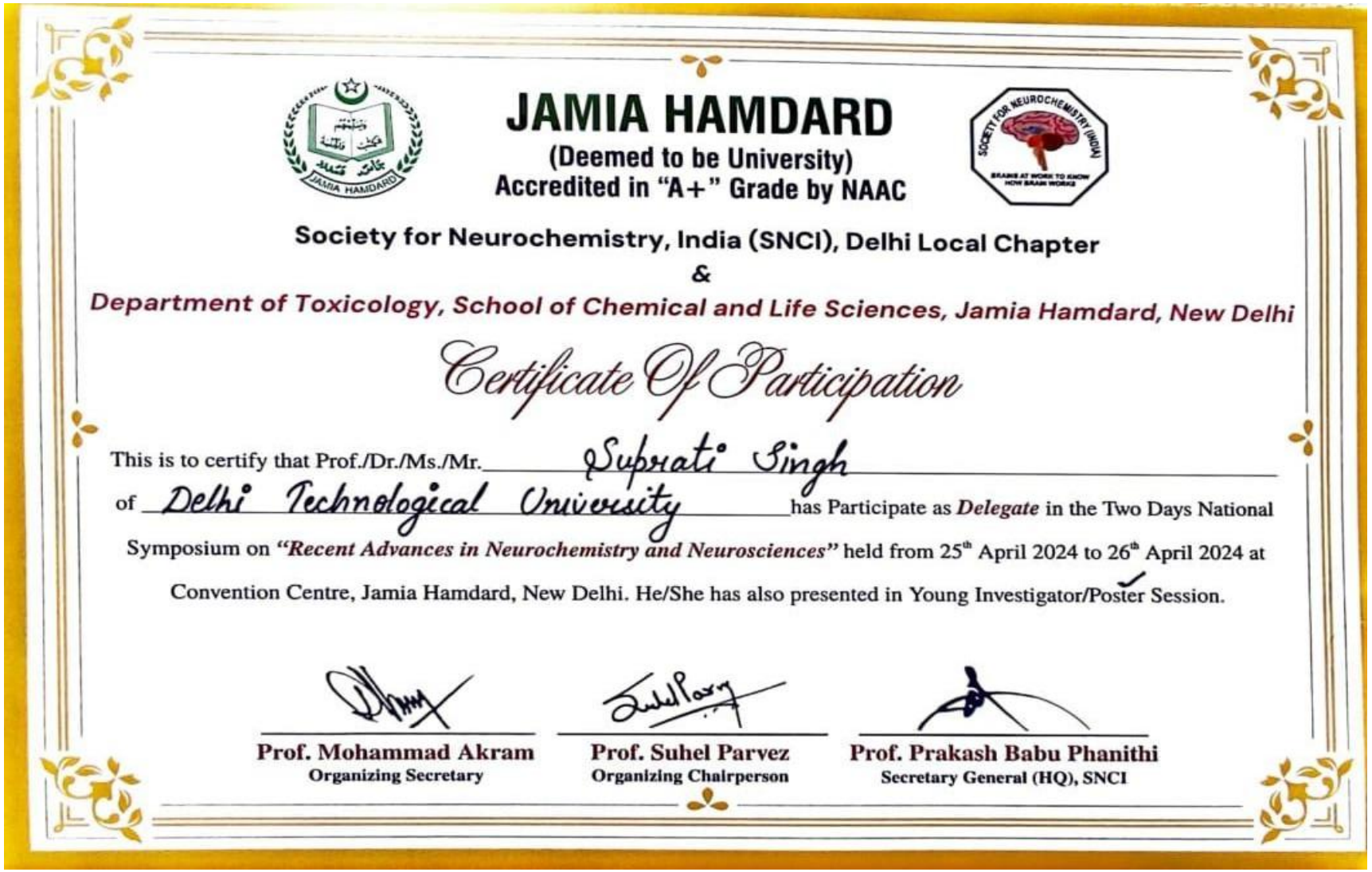

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This is to certify that Prof./Dr./Ms./Mr. Suprati Singh
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Title of the Thesis Targeting TREM2: In-Silico Study of Interactions with Gut Microbiota Based Supplements for Alzheimer's Disease

Total pages 38 Name of Scholar Suprati Singh

Supervisor (s)

(1) Prof. Pravir Kumar

Department Department of Biotechnology

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CURRICULUM VITAE

SUPRATI SINGH

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EDUCATION

Course	Year	Institution	CGPA/ Percentage
M.Sc. (Biotechnology)	2022-2024	Delhi Technological University, New Delhi	82.7%
B.Sc. (Biotechnology)	2019-2022	Chhatrapati Shahu ji Maharaj University, Kanpur	78.8%
CBSE (Class XII)	2019	Kendriya Vidyalaya, Azamgarh	78.9 %
CBSE (Class X)	2017	Kendriya Vidyalaya No. 1 AFS Chakeri Kanpur	9.2 CGPA

INTERNSHIPS

Subject Matter Expert Intern (Learn with Life)

- Developed and delivered comprehensive, engaging and well-structured NEET Foundation study material based on the exam pattern and latest trends.

CONFERENCES

S. No.	Conference Name	Organization
1.	Antimicrobial Resistance: Noval Drug Discovery and Vaccine Development: Challenges and Opportunities	SRM University, Delhi-NCR, Sonipat
2.	Translational Neuroscience: Bridging the gap between Bench to Beside	Jamia Hamdard University, New Delhi, SNCI and DST-STUTI

WORKSHOPS

I. Industrial Visit, National Sugar Institute, Kanpur

Attended one-day workshop at the National Sugar Institute for Bioethanol Production:

- Demonstration of bioethanol production at pilot scale
- Demonstration of brewery
- Soil Texture investigation demonstration

II. Society for Neurochemistry India (SNCI) and DST- STUTI

Attended one week workshop on "Translational Neuroscience: Bridging the gap between Bench to Beside"

POSTER PRESENTATIONS

S.No.	Poster Title	Organizing Institution
1.	*Lactiplantibacillus plantarum*: It's Prophylactic Effect and Potential Probiotic for Alzheimer's Disease	SRM University, Delhi NCR, Sonipat
2.	*Akkermansia muciniphila*: Interactions with TREM 2 Receptors: Probiotic for Alzheimer's Disease?	Jamia Hamdard University, New Delhi

PROJECTS

Currently working on Gut Microbiota Brain Axis, it's impact on Alzheimer's Disease for Dissertation.

TECHNICAL SKILLS

- MS Office
  (MS Word, MS Excel, MS PowerPoint)
- Molecular Docking and simulation

OTHER SKILLS

- Book chapter writing

- Medical Writing
- Subject matter expert (Lifesciences)
- Manuscript writing
- Literature review
- Scientific writing