

DECIPHERINGTHEMOLECULARLINKBETWEENALZHEIMER'S DISEASE AND PERIODONTITIS

Submitted in partial fulfillment of the requirements for the award of

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BY

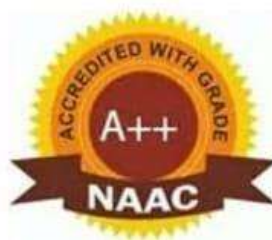
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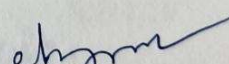
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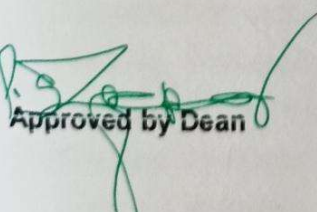
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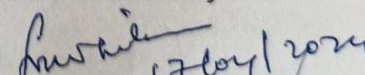
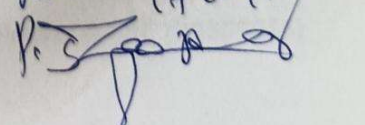
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DECLARATION

We hereby declare that the work presented in the thesis entitled "**DECIPHERING THE MOLECULAR LINK BETWEEN ALZHEIMER'S DISEASE AND PERIODONTITIS**" submitted by us to the Sathyabama Institute of Science and Technology (Deemed to be University), Chennai - 600119, for the award of Bachelor of Pharmacy (2020-2024) by research is an original record of research work carried out by us under the supervision of "**Dr. M. ArunSundar, M.Pharm., Ph.D., Associate Professor, School of Pharmacy**". The contents of this dissertation, in full or in parts, have not been submitted to any other institute or university for the award of any degree, diploma, membership, fellowship, associate-ship etc. In keeping with the general practicing reporting scientific observation, due acknowledgement has been made whenever the work described is based on the findings of other investigators.

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ABSTRACT

OBJECTIVE : Alzheimer's disease (AD) is a fatal neurodegenerative disease associated with elderly age group. *Porphyromonas gingivalis*, a central periodontal bacterium, is associated for the development of chronic inflammation state and neurodegenerative in AD. This can contribute to the accumulation of amyloid beta plaques and neurofibrillary tangles, increasing the involvement of pro-inflammatory cytokines like TNF α and markers such as NF- κ B in periodontitis exacerbate the inflammatory response in the brain. This chronic immune activation can lead to cognitive impairment in AD patients. Ongoing research aims to explore potential therapeutic interventions like the use of anti-inflammatory and antioxidant therapeutics to mitigate cognitive decline in AD.

METHODS: The study involved U251 cells cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. Cells were treated with low dose 10 nM and high dose 30 nM CBDV (Cannabidiol) and the effects were monitored. Cell viability was assessed by seeding cells into 96-well plates and treating them with A β 1-42 and LPS from *Porphyromonas gingivalis*. Protein concentrations were measured using commercial ELISA kits and Western blotting was performed to study the involved proteins. Statistical analysis was conducted using SPSS 16.0 software, and all graphs were produced using GraphPad Prism 7.0.

RESULTS : After analyzing the results *in vitro*, it was discovered that both low and high doses of CBDV effectively maintained normal antioxidant levels in SOD and CAT, despite the changes induced by A β +LPS. Additionally, the treatment successfully restored normal levels SIRT1 levels, while mitigating the abnormal increase in protein expression of NF- κ B caused by the A β +LPS challenge. The study ultimately concluded that high-dose CBDV treatment was more effective in replenishing antioxidant levels and counteracting the inflammatory insult caused by exposure to the A β +LPS cocktail.

CONCLUSION: The high dose of CBDV effectively reduced neuroinflammation caused by *Porphyromonas gingivalis* bacteria in Alzheimer's disease (AD) model. *In vitro* experiments conducted on cells exposed to a neurotoxic cocktail of A β +LPS showed that high-dose CBDV treatment could effectively reduce increased levels of pro-inflammatory cytokines TNF- α and IL-6 via suppression of NF- κ B /miRNA146a pathway mechanism. These findings highlight the potential therapeutic benefits of CBDV in targeting neuroinflammation caused by periodontal infection associated with AD pathology.

Keywords : Alzheimer's disease (AD), Periodontitis, *Porphyromonas gingivalis*, miRNA146a, Neuroinflammation, Cannabidiol (CBDV).

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LIST OF ABBREVIATIONS

1. AD	-	Alzheimer's disease
2. A β P	-	Amyloid β -peptide
3. A β	-	Amyloid beta
4. NFT	-	Neurofibrillary tangles
5. WHO	—	WORLD HEALTH ORGANIZATION
6. ISF	-	Interstitial fluid
7. CSF	-	Cerebrospinal fluid
8. pTau	-	Phosphorylated Tau
9. TBI	-	Traumatic brain injury
10. BBB	-	Blood Brain Barrier
11. APOE	-	Apolipoprotein
12. APP	-	Amyloid Precursor Protein
13. CD33	-	Cluster of differentiation 33
14. CRI	-	Cognitive Reserve Index
15. PSEN1	-	Presenilin 1
16. PSEN2	-	Presenilin 2
17. TREM2	-	Triggering Receptor Expressed on Myeloid Cells
18. MAPT	-	Microtubule Associated Protein
19. Kgp	-	Lysine Gingipain
20. LPS	-	Lipopolysaccharides
21. TLR4	-	Toll-like receptor 4

22. IL-1	-	Interleukin1
23. IL-6	-	Interleukin6
24. TNF- α	-	TumorNecrosisFactorAlpha
25. NF- κ B	-	Nuclear Factor kappa B
26. TLR	-	Toll-likereceptor
27. CPR	-	C-Reactive Protein (CRP)
28. CNS	-	Central nervous system
29. mRNA	-	Messenger ribonucleic acid
30. MAPK	-	Mitogen-activatedproteinkinase
31. LncRNAs	-	Long non-coding RNAs
32. AChE	-	Acetylcholinesterase
33. NMDA	-	N-methyl-D-aspartate
34. CAL	-	Clinical attachment loss
35. APL	-	Allostericpotentiatingligand
36. miRNAs	-	MicroRNAs
37. DOMINO	-	DonepezilandMemantine
38. 7,8-DHF	-	Dihydroxyflavone
39. CHF	-	Complement factor H
40. EA	-	Electroacupuncture
41. PG-LPS	-	<i>P.gingivalis</i> Lipopolysaccharide
42. CBD	-	Cannabidiol
43. 3'UTR	-	Threeprimeuntranslatedregion
44. CBG	-	Cannabigerol
45. CBC	-	CannabichromeneReactive
46. ROS	-	oxygen species Δ 9-
47. Δ 9-THCV	-	tetrahydrocannabivarin

48.CBDV	-	Cannabidivarin
49. Δ 9 -THCB	-	Δ 9-tetrahydro-cannabitol
50.RANKL	-	Receptor activator of nuclear factor κ B ligand Δ 9-
51. Δ 9 -THCVA	-	tetrahydrocannabivarinic acid
52.MMP	-	Metalloproteinase
53. Δ 9 -THCA	-	Δ 9-tetrahydrocannabinolic acid
54.THC	-	Tetrahydrocannabinol
55.ELISA	-	Enzyme-linked immunosorbent assay
56.NOS	-	Reactive nitrogen species

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CHAPTER 1

INTRODUCTION

ALZHEIMER'S DISEASE:

Alzheimer's disease (AD) is a fatal neurodegenerative disease figure 1.1 associated with elderly age group and a major health problem in the geriatric subjects worldwide. The incidence of AD significantly increases with age, reaching almost 50% in subjects aged 85 years (1). Alois Alzheimer, a German Physician, reported the first case of Alzheimer's disease in 1907 (2). The worldwide prevalence of dementia is estimated to be over 45 million people. Alzheimer's disease (AD) is the most common cause of dementia, responsible for 60-80% of cases (3). AD is characterized by the formation of extracellular amyloid β -peptide (A β P) plaques and neurofibrillary tangles (NFTs) of hyper-phosphorylated tau protein, leading to gradual loss of neuronal synapses and ultimately neuronal degeneration with domination of essential neurotransmitters (4).

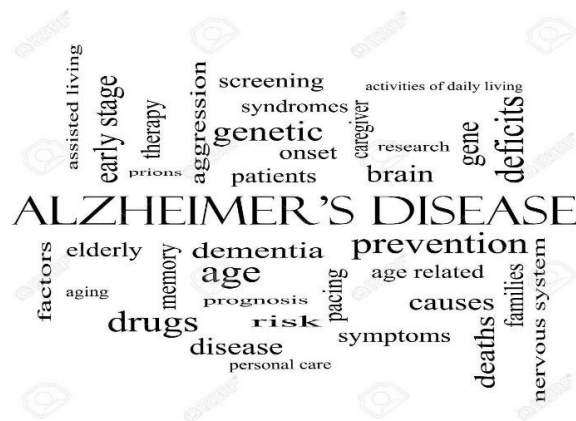


Fig1.1. Alzheimer's Disease –wordcloud

Vitamin D deficiency has been epidemiologically linked to Alzheimer's disease and other dementias, but no interventional studies have proved causality. Moreover, supplementation of vitamin D led to increased A β deposition and exacerbated AD figure 1.1. Collectively, these animal model and human cohort studies caution against prolonged use of vitamin D by AD patients (5). Approximately 70% of

the risk of developing AD can be attributed to genetics. However, acquired factors such as cerebrovascular diseases, diabetes, hypertension, obesity, and dyslipidemia increase the risk of AD development(6).

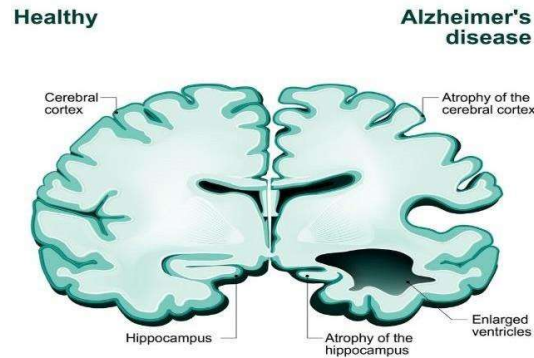


Fig1.2. Distinct types of AD

The global prevalence of dementia has been estimated to be as high as 24 million, and is predicted to double every 20 years at least 2040. In this review the prevalence and incidence rates, the established environmental risk factors, and the protective factors, and briefly review genetic variants predisposing to disease(7). As the disease progresses into AD dementia, symptoms of cognitive decline become more obvious, disrupt activities of daily living more frequently, and may prompt patients to seek medical attention(8).

Alzheimer's disease has been increasing steadily, and with today's aging population. WHO estimates that more than 55 million people (8.1 % of women and 5.4% of men over 65 years) are living with dementia. This number is estimated to rise to 78 million by 2030 and to 139 million by 2050. Aggregation of amyloid- β (A β) peptides into fibrils or other self-assembled states is central to Alzheimer's disease (AD) pathogenesis (9). Alois Alzheimer noticed a presence of amyloid plaques and a massive loss of neurons while examining the brain of his first patient that suffered from memory loss and change of personality before dying and described the condition as a serious disease of the cerebral cortex. Emil Kraepelin named this medical condition Alzheimer's disease for the first time (10) cardinal symptoms of Alzheimer's disease (AD) in the early stages are memory loss and modest changes in behavior (11).

AMYLOID- β :

Amyloid- β (A β) protein was proposed as the trigger for a cascade of events in the brain that lead to Alzheimer's disease (10). Amyloid- β (A β) deposit in the parenchyma is a major characteristic in Alzheimer's disease (AD), and the impaired glymphatic clearance contributes to the A β accumulation (11). Aggregation of amyloid- β (A β) peptides into fibrils or other self-assembled states is central to Alzheimer's disease (AD) pathogenesis (12). Beta-amyloid (A β) is present in the brain's interstitial fluid (ISF) and is considered a metabolic "waste product" (13). Essentially all neurodegenerative diseases are associated with misaccumulation of cellular waste products. Of these, misfolded or hyperphosphorylated proteins are among the most difficult for the brain to dispose. For example, tau and β -amyloid can accumulate as stable aggregates that are neurotoxic in conditions such as Alzheimer's disease (14). A widely accepted assumption is that AD begins with abnormal processing of amyloid precursor protein (APP), which then leads to excess production or reduced clearance of β -amyloid (A β) in the cortex (15). There are several A β species in the brain and plasma, such as A β 1-40 and A β 1-42, consisting of 40 and 42 amino acids, respectively. A β 1-42 is more toxic and aggregates more easily than A β 1-40 (16).

TAU PROTEIN:

The 2 proteins that, in their aggregated state, histopathologically define AD are β -amyloid (A β) and tau (17). Tau is hyperphosphorylated, accumulating in the form of intracellular neurofibrillary tangles. This compromises its microtubule binding ability and promotes neurodegeneration, and leads to accumulation of microtubule-transported APP, further contributing to neurodegeneration (18). A number of studies have shown increased concentrations of microtubule-associated tau protein in cerebrospinal fluid (CSF) of individuals clinically diagnosed as having probable. Fig 1.4 Tau protein and neurofibrillary tangles Alzheimer's disease (AD), suggesting that CSF tau measurements might be useful in the diagnosis of AD and in the evaluation of treatment strategies (19). The normal tau protein is a human brain phosphoprotein with six isoforms ranging in size from 352-441 amino acids (20). Tau protein transference via the extracellular space, the compartment that is cleared by the glymphatic pathway. Neurofibrillary tangles (NFTs) are one of the key pathological

hallmarks of Alzheimer’s disease. NFTs primarily consist of phosphorylated tau (pTau). Clearance of extracellular tau from the brain is much slower than that of Ab, and specific transporters for tau exit across the blood-brain barrier have not been identified (21).

NEUROINFLAMMATION

In Alzheimer’s disease (AD), the initiation of neuroinflammation is primarily carried out by the brain’s microglia and astrocytes, which are responsible for maintaining the balance of synaptic transmission and its remodeling (22). Unfortunately, in the context of AD, these cells contribute to the accumulation of toxic tau and amyloid-β (Aβ) proteins, thereby leading to the sustained deterioration of the central nervous system (23). Through genomic studies, potential risk factors for neuroinflammation table 1.1, such as CD33, TREM2, or CR1, have been identified. Furthermore, proteomic analyses have uncovered connections between these genetic factors and dysfunctional signaling pathways that promote proinflammatory mechanisms (22), thus affecting crucial aspects like synaptic function, cell metabolism, and proliferation. Understanding the role of neuroinflammation in AD is of utmost importance in improving the diagnosis and treatment of the disease, as it is highly intertwined with the manifestation of symptoms in clinical settings.

Table 1.1 RISK FACTORS FOR AD

S.No	Risk Factors	Cause
1.	Age	Age is the strongest risk factor for AD. The risk of AD doubles every 5 years after the age of 65 and there is a 50% chance of having AD after the age of 85 (24).
2.	Gender	Age-adjusted rates for women were significantly higher than for men. This female excess is confirmed by 13 population surveys that report overall rates for AD by sex (25).
3.	Traumatic brain injury	the increased frequency of traumatic brain injury (TBI) in the previous history of AD patients. TBI could cause damage to the BBB resulting in leakage of plasma proteins and sensitization of the immune system to brain antigens

		normally isolated from them. Subsequently, a number of studies reported a connection between head injury and AD (26).
4.	Social class and education	Social class is a useful variable with which to begin an epidemiological examination of AD, because it could point to differential exposure or vulnerability. But the inquiry is hampered by the likelihood of biased case-finding, since many of the instruments used to detect dementia are highly sensitive to education, which is closely related to social class (27).
5.	Lifestyle	Alcohol drinking has been proposed as a possible risk factor for cognitive impairment and dementia, though understanding the association has proved difficult. Some studies have found no association between alcohol drinking and cognitive impairment or Alzheimer's disease, whereas others have found an association between heavy drinking and increased risk of dementia (28). smoking was associated with an increased risk of AD (pooled relative risk, 1.79; 95 % CI, 1.43-2.23). Thus, in contrast to the initial hypothesis of a possible protective effect, cigarette smoking actually increases the risk of AD (29).

NEUROGENETICS:

As a polygenic disorder, the etiopathogenesis of AD is based on several genetic and epigenetic (genetic-environmental interaction) factors. A gamut of additional genes including apolipoprotein E (APOE) E4 allele has been found to be potential risk factors for AD. The genetic basis of early onset of Alzheimer's disease is found to be based on three main genes which include amyloid precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2). According to AlzForum, additionally, two genes are linked with AD by means of genetics or the neuropathology of the encoded proteins (TREM2-Triggering receptor expressed on myeloid cells 2; MAPT-microtubule-

associated protein). A recent study showed that, of 53 mutation carriers identified in the cerebrospinal fluid (CSF) biomarkers, forty-six (87%) depicted aberrant changes in all the three CSF biomarkers including total tau protein (Tau), phospho-tau protein (P-Tau), and amyloid β ($A\beta$) figure 1.3.

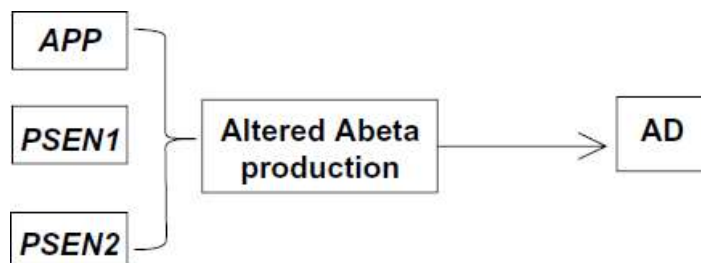


Fig 1.3 Neurogenetics

The amyloid precursor protein (APP) gene, presenilin (PSEN1 and PSEN2) genes involved in early onset Alzheimer's disease (AD); Abeta - Amyloid beta.

PERIODONTITIS:

Chronic inflammation associated with periodontitis promoted by bacterial infection leads to the release of pro-inflammatory molecules, cytokines, and chemokines into the bloodstream. They may pave the trail to neuroinflammation, which is well-known as a mechanism of Alzheimer's disease (30). Indeed, *Porphyromonas gingivalis*, a central periodontitis bacterium, has been detected in the brains of individuals who have been posthumously diagnosed with Alzheimer's disease, indicating a possible relationship among oral pathogens, systemic circulation, and neurodegenerative disease advancement (31). Additionally, the bacteria connected with dysbiosis in the pathogenesis of periodontitis produce toxins and enzymes that damage the infected gingival tissue figure 1.4. Persecuted by the formation of plaques and exudates on the gingiva and inflammation. Dysbiosis induces a low-grade systemic inflammatory response through the activation of the host immune response due to the disproportionate production of pro-inflammatory cytokines and matrix metalloproteinases (32). Periodontitis is a type of chronic and aggressive periodontitis that develops due to various factors of polymicrobial infection and is triggered by the presence of bacteria in the gingival crevice. Furthermore, the metabolic byproducts of these bacteria, which include acids, noxious gases, and virulence factors such as

enzymes, fimbriae, hemolysin, hemagglutinin, capsules, outer membrane vesicles, and lipopolysaccharides, as well as toxins, can directly harm the tissue surrounding the oral cavity (33).

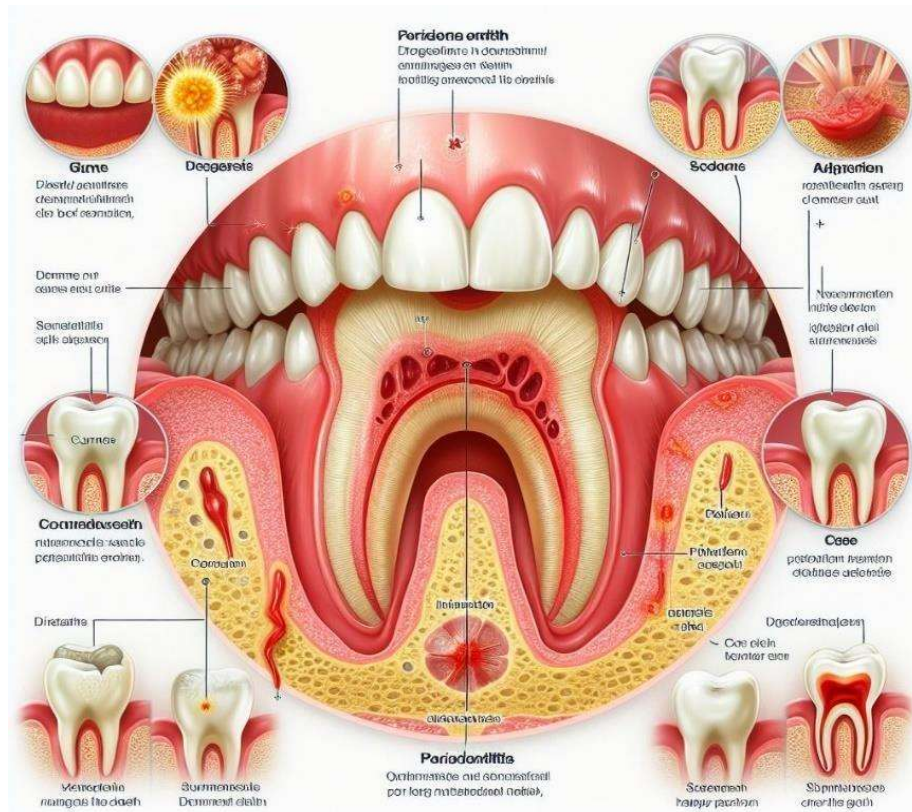


Fig1.4periodontitisdisease

For instance, *Porphyromonas gingivalis* is known to produce proteases that breakdown proteins in the periodontal tissues, contributing to the deterioration of the gum's structural integrity. Additionally, dysbiotic bacteria frequently develop biofilms on tooth surfaces and in periodontal pockets (33).The bacterium *Porphyromonas gingivalis*, a significant contributor to dysbiosis, generates gingipains enzymes that exert destructive effects on the periodontalligament figure1.4, the connective tissue between teeth and the surrounding bone. Additionally, gingipains may degrade host proteins, including those involved in immune responses, thereby contributing to immune evasion (34).According to Liu et al. (2017), Gingipains are proteolytic enzymesproducedby*Porphyromonasgingivalis*,aprimarypathogenlinkedtochronic periodontitis.Theseenzymesplayacrucialrolein*P.gingivalis*'virulencebyfacilitating thedegradationofhosttissuesandmodulatingthehostimmuneresponse.*P.*

gingivalis produce two main types of gingipains. Arginine Gingipains (RgpA and RgpB) are enzymes that hydrolyze peptide bonds containing arginine residues.

These large, multi-domain enzymes are instrumental in *P. gingivalis*'s capacity to infiltrate host tissues. Additionally *P. gingivalis* possesses Lysine Gingipain (Kgp), which targets peptide bonds with lysine residues. Kgp also contributes significantly to the degradation of host proteins, aiding *P. gingivalis* in evading the host immune response (36). Gingipains play a role in numerous pathogenic mechanisms linked to periodontitis. Specifically, they contribute to tissue destruction by breaking down proteins in the extracellular matrix and connective tissues, consequently leading to the deterioration of periodontal tissues figure 1.4. Moreover, gingipains aid in immune evasion by cleaving components of the host immune system.

GINGIPAINS:

Gingipains, proteolytic enzymes produced by the bacterium *Porphyromonas gingivalis*, are key virulence factors strongly associated with periodontal disease. They consist of arginine-specific gingipains (RgpA and RgpB) and lysine-specific gingipain (Kgp), which degrade peptides from the C-terminus involving arginine or lysine residues, respectively (37). These enzymes facilitate tissue invasion, modulate the host immune response, and cause destruction of periodontal tissues. Gingipains degrade components of the extracellular matrix, leading to periodontal ligament breakdown and bone loss. Additionally, they can cleave host immune system components, aiding *P. gingivalis* in evading host defenses and establishing chronic infection (34). Recent studies suggest a potential link between gingipains and neurodegenerative disorders like Alzheimer's disease, as they may degrade proteins associated with such conditions, including tau protein, a key component of neurofibrillary tangles. This degradation could contribute to Alzheimer's pathology by promoting neuroinflammation and the accumulation of pathological proteins in the brain (37). However, further research is needed to fully understand the mechanisms underlying this association. Overall, gingipains play a crucial role in the pathogenesis of periodontal disease and may also contribute to neurodegenerative processes, highlighting their significance in both oral and systemic health (38).

LIPOPOLYSACCHARIDES(LPS):

Lipopolysaccharides(LPS), key constituents of the outer membrane of gram-negative bacteria, serve as virulence factors in periodontitis and have been implicated in the pathogenesis of Alzheimer's disease (33). Mechanistically, LPS interacts with host cell receptors, notably Toll-Like receptor 4 (TLR4) expressed on immune cells like macrophages and microglia within the brain. Upon LPS binding, TLR4 initiates downstream signaling cascades, triggering the release of pro-inflammatory cytokines, including interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α). While this response is aimed at eliminating bacterial threats, persistent or excessive activation can lead to chronic inflammation (39), implicated in tissue damage and disease progression. In Alzheimer's disease, chronic neuroinflammation is considered contributory to hallmark pathologies, such as beta-amyloid plaque accumulation and neurofibrillary tangle (NFT) formation (40). Moreover, LPS-induced inflammation may compromise the blood-brain barrier, facilitating the entry of peripheral immune cells and neurotoxic molecules into the brain parenchyma, exacerbating neurodegeneration (41). Additionally, microglial activation in response to LPS may release reactive oxygen species, further damaging neurons. Thus, while LPS primarily underpins periodontal disease virulence, its capacity to induce inflammation and immune dysregulation may significantly impact neuroinflammatory processes in Alzheimer's disease, warranting further investigation for therapeutic insights (30).

PERIODONTAL INFLAMMATION:

Periodontitis triggers an immune response, leading to the release of inflammatory molecules called cytokines. These cytokines can enter the bloodstream and potentially reach the brain, where they can contribute to neuroinflammation. The neuroinflammation is characterized by the activation of immune cells in the brain, leading to the release of additional inflammatory molecules. This chronic inflammatory state can contribute to the accumulation of amyloid beta plaques and neurofibrillary tangles, increasing the involvement of pro-inflammatory cytokines like TNF α and markers such as CRP in periodontitis exacerbate the inflammatory response in the brain. This chronic immune activation can lead to neuronal damage, synaptic dysfunction, and ultimately cognitive impairment in AD patients. Additionally, the

association with *P. gingivalis*, a key pathogen in periodontitis, and the production of A β 1-42 implicated in amyloid plaques further highlights the potential role of oral health in the pathogenesis of AD. Moreover, the brains of AD patients consist of larger quantities of LPS- the primary component of the cell membrane of gram-negative micro bacteria, than those from normal subjects (42). Ongoing research aims to unravel the complex mechanisms underlying this relationship and explore potential therapeutic interventions, including the use of anti-inflammatory drugs like NSAIDs, to mitigate cognitive decline in AD.

Table 1.2 RISK FACTORS FOR PD

S.No	Risk Factors	Cause
1.	Oral dysbiosis	Periodontitis is the major risk factor. It release toxins and enzymes that damage the gingival tissue and contribute to the inflammatory response (43).
2.	Malocclusion	Misaligned teeth or a faulty bite can create areas that are difficult to clean, increasing the risk of plaque accumulation and periodontal disease (44).
3.	Poor oral hygiene	Inadequate brushing, flossing, and regular dental check-ups can lead to the accumulation of plaque and tartar, contributing to periodontal disease development.
4.	Microbial Factors	Besides the bacterial composition of dental plaque, factors such as bacterial virulence and antibiotic resistance can also impact the development and severity of periodontal disease (33).
5.	Genetics	Inherited factors can influence an individual's susceptibility to periodontal disease, including variations in immune function and inflammatory response genes (45).

PERIODONTAL GENETICS:

Periodontitis genetics is a multifaceted interplay of genetic factors that influences disease susceptibility and progression. Key players include microRNA-146a(miR-146a), Tumor Necrosis Factor- α (TNF- α), Nuclear Factor κ B(NF- κ B), and Interleukin-6 (IL-6), each contributing distinct roles in the inflammatory cascade underlying periodontal tissue destruction(40). miR-146a, a microRNA, acts as a negative regulator of innate immunity by targeting genes within the Toll-like receptor (TLR) signaling pathway, thereby influencing the expression of proinflammatory cytokines and chemokines in periodontal tissues(33). TNF- α , a potent proinflammatory cytokine primarily produced by macrophages, plays a central role in orchestrating immune responses and inflammation figure 1.5. In periodontitis, TNF- α promotes the recruitment and activation of immune cells, contributing to tissue destruction and bone resorption(39). Genetic variations, including TNF- α gene polymorphisms, have been studied for their associations with periodontitis susceptibility across different populations (41).

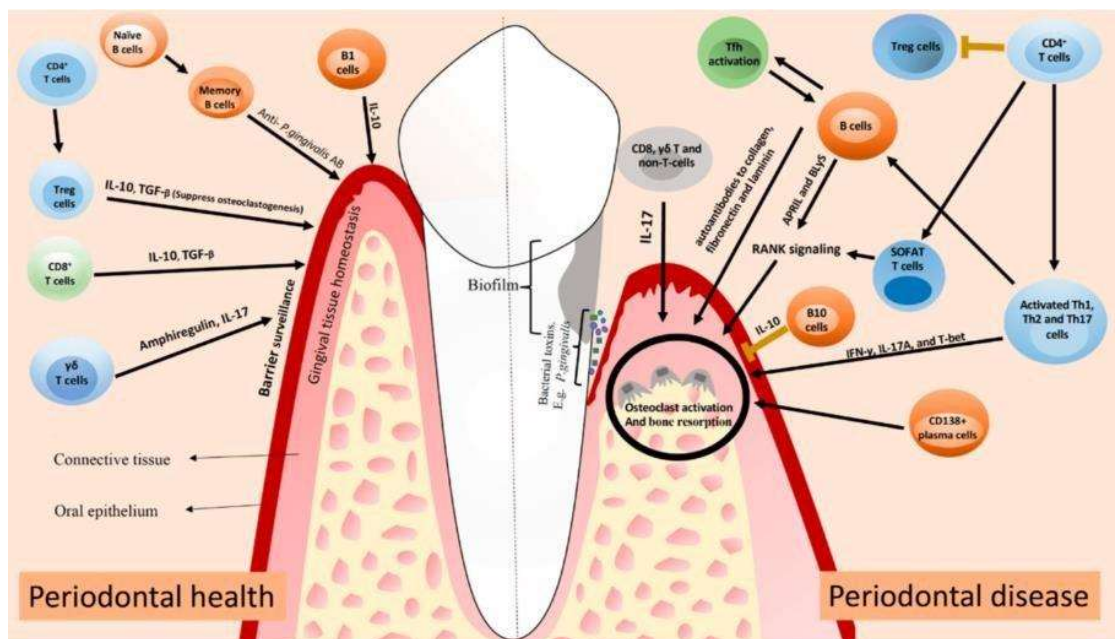


Fig1.5A Schematic View of periodontal inflammation

NF- κ B is a transcription factor pivotal in regulating the expression of genes involved in immune responses, inflammation, and cell survival. Activation of the NF- κ B signaling pathway is a hallmark event in the pathogenesis of periodontitis triggered by various stimuli, including bacterial pathogens and proinflammatory cytokines. This activation leads to the production of additional proinflammatory mediators and perpetuates the inflammatory response in periodontal tissues (46). IL-6, a pleiotropic cytokine produced by various cell types, including T cells and macrophages, plays diverse roles in immune regulation and tissue homeostasis. In periodontitis, IL-6 regulates osteoclast activity, bone resorption, and inflammatory responses in periodontal tissues. Genetic variations in the IL-6 gene have been investigated for their association with periodontitis susceptibility, although the findings have varied across different populations (47). The elucidation of genetic factors in periodontitis underscores the complex interplay between genetics, immune responses, and inflammation in disease pathogenesis. Understanding these molecular mechanisms offers insights into individual susceptibility to periodontitis and holds promise for developing personalized therapeutic approaches tailored to patients' genetic profiles (48).

Role of microRNA-146a:

the human oral microbiome and the brain. This pathogenic oral-brain circuit involves several key factors that work together to cause harm to the brain (48). These factors include lipopolysaccharide (LPS) (83) from the oral cavity-residing enterotoxigenic Gram-negative bacteria *P. gingivalis*, the transport of LPS across the oral cavity barrier and into the systemic circulation (49), and the transport of highly pro-inflammatory systemic PG-LPS across the blood-brain barrier into the brain-parenchyma and neuronal-cytoplasm (192). Furthermore, the activation and signaling via the pro-inflammatory NF- κ B (p50/p65) transcription-factor complex (82) figure 1.6, the coupling of NF- κ B with the significant up-regulation of the inducible pro-inflammatory microRNA-146a (miRNA-146a) (48), and the subsequent down-regulation of miRNA-146a regulated mRNA targets such as the encoding complement factor H (CFH) also play a critical role in this pathway (50). This complex pathway is responsible for regulating the innate-immune response, and its dysregulation can lead to neuro-

degeneration and inflammatory signaling. It is important to note that CFH is a key repressor of the innate-immune response. Down-regulation of CFH expression activates the complement system (48), which is the major non-cellular component of the innate-immune system, while propagating neuro-inflammation. Our findings suggest that other oral cavity microbes and their highly complex pro-inflammatory exudates may also contribute to this pathogenic oral cavity-brain pathway by miRNA146a.

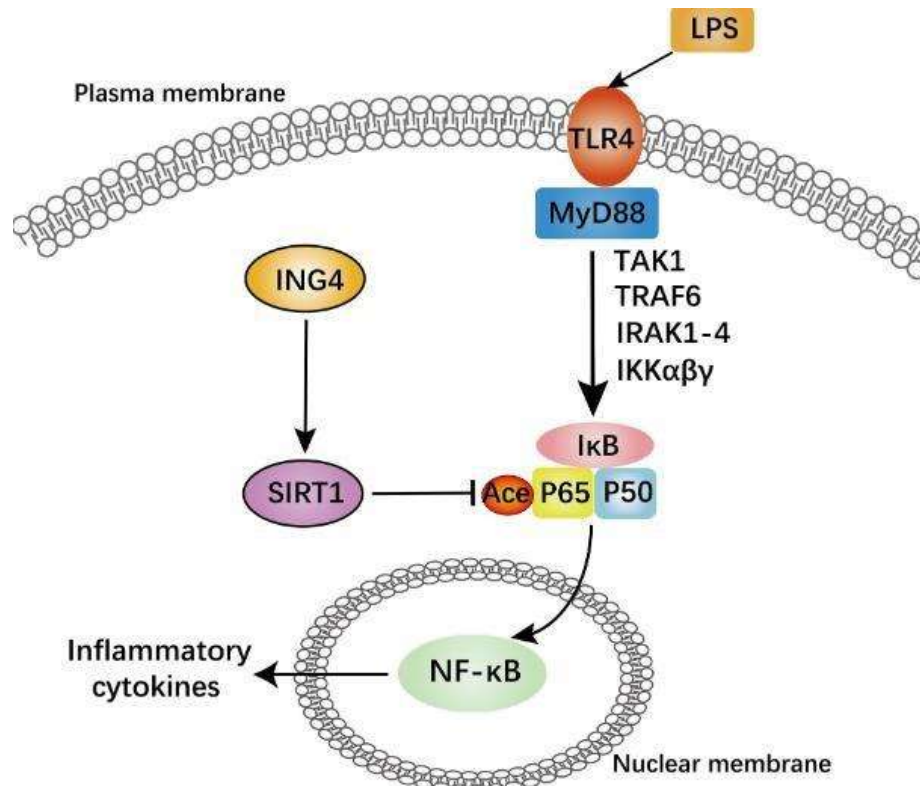


Fig1.6 Role of miRNA-146a

Moreover, it is noteworthy that *P. gingivalis*, which is the first Gram-negative anaerobic bacterial species that researchers studied as a potential contributor to the onset of Alzheimer's disease, appears to utilize damaged or leaky physiological barriers and an activated NF-κB (p50-p65) - pro-inflammatory miRNA-146a signaling circuit to convey microbiome-derived pathogenic signals into the brain (figure 1.6). The intricate nature of this pathway and the associated signaling mechanisms must be considered in the ongoing research into the relationship between oral health and brain function.

miR-146a and Alzheimer's disease:

Alzheimer's disease is a progressive neurodegenerative disorder that affects cognitive function, memory, and behavior. It is caused by the death of neurons, decreased number of synapses, and accumulation of amyloid beta protein isoforms (amyloid plaques) and phosphorylated tau protein in the neurons (1). These symptoms are associated with cerebral inflammation, which is the primary cause of Alzheimer's disease (126). The A β protein processing defect leads to the accumulation of A β sediments, which can cause oxidative stress, inflammation, and cellular death. Recent studies have shown that miR-146a is a biomarker that plays a significant role in the pathogenesis of Alzheimer's disease (49, 76). It can be used as a powerful tool for diagnosis and evaluation of the incidence risk of this disorder. The level of miR-146a has been found to be significantly downregulated in the serum, plasma, and CSF of patients and animals with Alzheimer's disease (75, 76). However, its remarkable upregulation has been evident in their brain tissues, especially in the neurons, and is positively associated with the density of plaques and synaptic pathology. Inflammatory signaling is also up-regulated, which is partly due to the overactivation of a transcription factor called NF- κ B (48). This inflammation supports the development and progression of AD. MicroRNAs (miRNAs) are small RNA molecules that can regulate gene expression by binding to specific messenger RNA targets. They can negatively regulate gene expression by decreasing the stability or translational efficiency of target mRNAs (51). The up-regulation of a brain-enriched miRNA-146a that is regulated by NF- κ B down-regulates a specific mRNA target that encodes complement factor H (CFH) (51), a repressor protein that is known to contribute to the regulation of the immune and inflammatory response of the brain.

The miRNA-146a is transcribed from a pre-miRNA-146a precursor gene, and the 5' regulatory region of that gene has been sequenced revealing three upstream NF- κ B-DNA binding sites (49). The regulation of miRNA-146a abundance is thought to be under the regulatory control of NF- κ B-DNA binding (49). Using gel shift assay, it was found that NF- κ B abundance was more abundant in AD neocortex and hippocampus than in controls (52). CFH protein abundance was found to be down-regulated in AD neocortex and hippocampus (78). One strong predicted mRNA target

formiRNA-146aistheCFHmRNA3'-UTR(53,79).Theup-regulationofmiRNA-146a coupledwiththedown-regulationofCFHwasobservedinstressedhumanneural(HN) cells exposed to interleukin-6, Amyloid beta42/40, and/or oxidative stress. The researchers conducted a transfection of HN cells with an NF- κ B containing pre-miRNA-146apromoter-luciferasereporterconstruct(51),whichshowedsignificantup-regulation of luciferase activity that paralleled decreases in CFH gene expression. In conclusion, Alzheimer's disease is a complex disorder that involves the upregulation of a particular NF- κ B-sensitive miRNA-146a in AD brains (49) figure 1.7.

This miRNA-146a targets complement factor H (CFH), an essential repressor of theinflammatory responseofthebrain.Thestudy shedslight on therole of miRNAs in the pathogenesis of Alzheimer's disease and their potential use as biomarkersfor diagnosisandevaluationofincidencerisk andalsotheuseofdirected anti-miRNAstrategiesto represstheeffectsofspecificallyup-regulatedmiRNAs may be an effective therapeutic approach against inflammation and related pathogenic signaling in stressed brain cells (51).

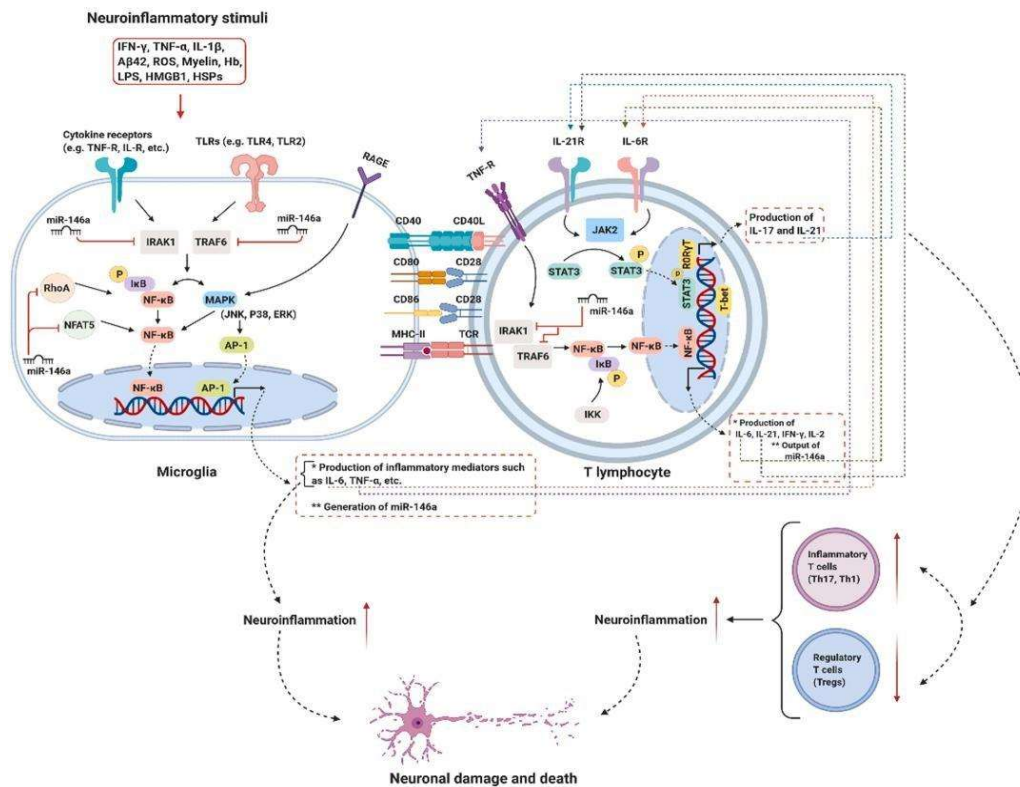


Fig1.7mechanismofneuronaldamageanddeathbymiRNA-146a

miR-146a and Periodontal disease:

Periodontitis is a chronic inflammatory disorder that affects the tissues surrounding the teeth. It is characterized by pocket formation and the destruction of alveolar bone around teeth, ultimately leading to tooth loss (54). Chronic periodontitis is the most common form of destructive periodontal disease, which is initiated by polybacterial infections (54). The bacterial components, such as lipopolysaccharide (LPS), (81) stimulate various host cells, including leukocytes, fibroblasts, and epithelial cells, through Toll-like receptors (TLRs) (55). This process leads to the induction of proinflammatory cytokines, such as tumor necrosis factor α (TNF- α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6) (80), and other inflammatory mediators (56,57). These local inflammatory mediators induce a number of events leading to the damages that occur during periodontitis (58,59,60). These events include upregulation of adhesion molecules on the endothelium, as well as production of chemokines that lead to leukocyte migration into the site of infection (58,59,60). To destroy bacteria, leukocytes produce reactive oxygen species (ROS), reactive nitrogen species (NOS), and matrix metalloproteinases (MMPs) that contribute to the damage of connective tissues. It has been shown that ROS and NOS, which are reactive molecules deriving from molecular oxygen and nitrogen, participate in the pathogenesis of periodontal disease (61,62). MMPs are endopeptidase that is known to be involved in the tissue remodeling. It is thought that MMPs are associated with the tissue destruction in periodontal disease (63,64).

In addition, proinflammatory cytokines disrupt the balances between bone resorption and formation leading to progressive alveolar bone loss in periodontitis (65,66,67). It has been shown that TNF- α and IL-1 β either alone or under synergistic interactions induce bone resorption which is mediated by regulating osteoclastogenic (65,66,67). The osteoclastogenic effect of TNF- α and IL-1 β was related to enhancing expression of receptor activator of nuclear factor kappa B ligand (RANK-L) (66,67). RANK-L has an important role in osteoclast differentiation and activation, which leads to bone resorption (68). Proinflammatory cytokines also inhibit bone formation via inhibiting osteoblast differentiation (69). MicroRNAs (miRNAs) are

a group of small noncoding ribonucleic acids (RNA) that play an important role in controlling several biological processes including cell division, differentiation, apoptosis, and embryogenesis (70).

They are involved in the regulation of gene expression by targeting messenger ribonucleic acid (mRNA), leading to mRNA degradation or translation inhibition (71). MiR-146a is a member of the miR-146 family that is involved in the control of innate immune responses and inflammation. (48) Upon stimulation of THP-1 cells with bacterial components such as LPS and proinflammatory cytokines, miR-146a was induced via a nuclear factor-kappa B (NF- κ B)-dependent pathway resulting in downregulation of adaptor proteins that are involved in inflammatory responses (48). Thus, miR-146a acts as a negative regulator of inflammation, and its levels have been shown to be associated with the pathogenesis of several inflammatory diseases (72,73).

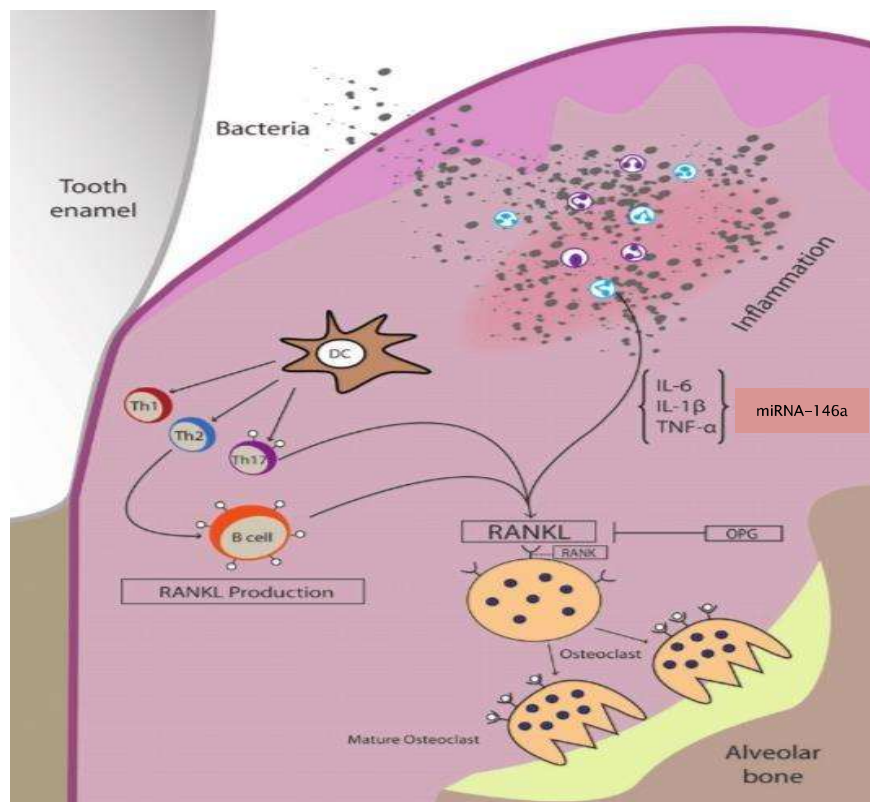


Fig 1.8 Role of miRNA-146a in periodontitis

In this study, we evaluated the expression levels of miR-146a and its downstream targets, such as TNF- α , IL-1 β , and IL-6 (74), in human patients with chronic periodontitis. We also assessed the association between the levels of miR-146a and its targets with clinical parameters, including probing depth (PD) and clinical attachment loss (CAL). The results of this study can provide insights into the role of miR-146a in the pathogenesis of chronic periodontitis and its potential as a therapeutic target for treating this disease. The crucial role of miR-146a as a negative feedback regulator of inflammation and its connection to the pathogenesis of inflammatory disorders in humans have been well-established. Recently, researchers have investigated the contribution of miR-146a to the pathogenesis of periodontal disease (73). In the study, protein level of miR-146a in gingival tissues of patients with chronic periodontitis was analyzed in *invitro* method figure 1.8. The results showed a significant 32.6-fold increase in miR-146a expression in cell line model compared to the control group (p-value < 0.001). Intriguingly, the level of miR-146a was positively correlated with scores of clinical parameters including PD and CAL in patients (correlation coefficients of 0.67 and 0.55 for PD and CAL figure 1.9, respectively, which were statistically significant, p-value < 0.05. Furthermore, the study evaluated the functional status of miR-146a in chronic periodontitis by assessing the levels of proinflammatory cytokines as the major targets of miR-146a (48). The level of TNF- α , IL-1 β , and IL-6 was assessed by a real-time PCR assay in gingival tissues of cell model (74). The results indicated a significant reduction in the expression level of TNF- α and IL-6 in cell line compared to the control group of cell line (p-value < 0.001) (Figure 2). Consistent with a decrease in the levels of TNF- α and IL-6 in patients, a reduction in IL-1 β was also observed (p-value = 0.096) (74). However, there was no correlation between the levels of proinflammatory cytokines and clinical parameters of cell line model.

Table 1.3 clinical parameter

Group	PD (mm)	CAL (mm)	Age (year)
Chronic periodontitis	4.6 $\pm$ 1.3	5.9 $\pm$ 1.4	44 $\pm$ 8
Healthy individuals	2 $\pm$ 0.5	2.1 $\pm$ 0.6	32 $\pm$ 12

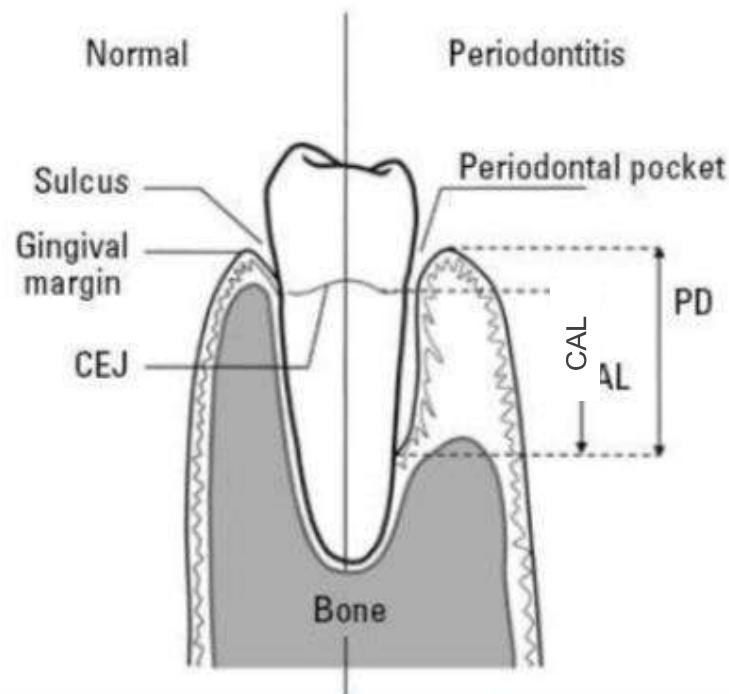


Fig1.9schematicrepresentationofPDandCAL

In summary, the findings of this study suggest that miR-146a may have a direct or indirect influence on the pathology of periodontal disease. These results lay the groundwork for further studies investigating the role of miR-146a in the development and/or progression of periodontal disease and its potential as a therapeutic target against the disease.

TREATMENT:

APPROVED DRUGS FOR ALZHEIMER DISEASE:

Current treatments for patients with Alzheimer's disease aim to improve behavioral, cognitive, and non-cognitive symptoms. The acetylcholinesterase (AChE) inhibitors, rivastigmine, donepezil, and galantamine, and the N-methyl-D-aspartate (NMDA) receptor antagonist, memantine, have been the main pharmacological treatments for Alzheimer's disease (84).

1. **Donepezil's** cost-effectiveness was demonstrated regardless of whether outcomes were measured in terms of improvements in cognitive impairment,

functional impairment or health-related quality of life, and whether costs were measured just for the health and social care system or for society as a whole (85).

2. **Rivastigmine** is a modern cholinesterase inhibitor with established therapeutic efficacy. Rivastigmine is particularly good in patients fulfilling the criteria of dementia with Lewy body (86).
3. **Galantamine** (Reminyl), an approved treatment for Alzheimer's disease (AD), is a potent allosteric potentiating ligand (APL) of human $\alpha 3\beta 4$, $\alpha 4\beta 2$, and $\alpha 6\beta 4$ nicotinic receptors (nAChRs). Galantamine acts as an nAChR inhibitor. The other approved AD drugs, donepezil and rivastigmine, are devoid of the nicotinic APL action; at micromolar concentrations they also block nAChR activity (87).
4. N-methyl-D-aspartate (NMDA) antagonist **memantine** appears to be helpful in patients with moderate-to-severe Alzheimer's disease. There is evidence that an increased plasma homocysteine level is an independent risk factor for the development of Alzheimer's disease (88).
5. The **Donepezil and Memantine** in Moderate to Severe Alzheimer's Disease (DOMINO) study was a multicenter, double-blind, placebo-controlled, clinical trial with a two-by-two factorial design. The outcomes were assessed for 52 weeks (89).
6. **Aducanumab**: The FDA recently approved aducanumab (Aduhelm) for people with mild symptoms of Alzheimer disease, such as individuals who are still independent in basic daily functioning. It reduced brain amyloid plaque (protein deposits), a main feature of Alzheimer disease. Plaque reduction, however, has not been a reliable marker for cognitive function in past trials. Not everyone with plaque has or will get Alzheimer disease, and other plaque-reducing medications have not shown meaningful patient benefit. Adverse effects were common. In the trials, 41% of patients experienced brain swelling or bleeding. While most cases were mild and managed with dose reduction, 1% to 2% of patients required hospitalization or had long-lasting impairment. A recent patient death linked to aducanumab is under investigation.
7. **Lecanemab** reduced markers of amyloid in early Alzheimer's disease and resulted in moderately less decline on measures of cognition and function than placebo at 18 months but was associated with adverse events. Longer trials are warranted to determine the efficacy and safety of lecanemab in early Alzheimer's disease.

DRUGS UNDER CLINICAL TRIAL:

In addition to the anti-amyloid and anti-tau targeted drugs, various other drugs are under clinical trials which include Nicotinic Receptors Agonist, Antioxidants, PPAR gamma agonist, Gamma secretase inhibitor, 5HT-6 Antagonist, Statins and Others.

Table 1.4 Drugs under clinical trials:

Class	Drugs
Nicotinic Receptors Agonist	4OH-GTS-21
Antioxidants	• Ginkgo biloba • Vitamin-E • Melatonin
PPAR gamma agonist	Pioglitazone
Gamma secretase inhibitor	Semagacestat
5HT-6 Antagonist	SB-271046
Statins	• Simvastatin • Pravastatin
Others	• heavy metal chelators • Estrogens • Anti-inflammatory drugs

PRODRUGS/ MIMETICS:

Many prodrugs and biomimetics are also used in various animal models for the treatment of AD. Examples; ramiprosate, a glycosaminoglycan mimetic; 7,8-dihydroxyflavone (7,8-DHF), a BDNF mimetic; Huperzine A (HupA), isolated from the Chinese herb *Huperzia serrata* (Thunb) selective AChE inhibitor; ALZ-801 is an oral, small-molecule inhibitor of beta-amyloid (A β) oligomer formation; Memogain has been recently described, this pro-drug of galantamine, etc.

ALTERNATIVE THERAPIES:

a) Acupuncture:

Acupuncture and electroacupuncture (EA) have been commonly used as a complementary therapy by TCM practitioners in China for the treatment of various neurodegenerative disorders (90).

b) Physical Exercise:

Exercise causes changes in the brain at the anatomic, cellular, and molecular levels by inducing a cascade of cellular and molecular processes that promote different physiologic phenomena, including angiogenesis, neurogenesis, synaptogenesis, and stimulation of neurotrophic factors that enhance learning, memory, and brain plasticity (91).

c) Ethnobotanicals:

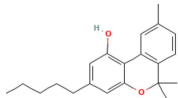
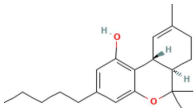
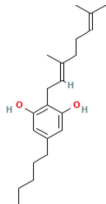
Many ethnobotanicals (plant extracts and plant derived active constituents) have been studied for the treatment of neurodegenerative diseases including AD. In the current study cannabis derived compounds (Cannabinoids) were assessed for their possible glymphatic clearance and neuroprotective effects.

1.4.6 CANNABIS SATIVA:

Cannabis is the botanical name of a genus within the Cannabaceae family. The genus includes three species, *C. sativa*, *C. ruderalis* and *C. indica*. The vernacular name of *Cannabis sativa*: Ganja-chedi in Tamil, Marijuana in English, jia in Bengali and Bhangganja in Assamese. The extracts of *C. sativa* have been demonstrated to have analgesic, antiemetic, anxiolytic, anticonvulsant activity and neuro-protective effect (92).

There are over 100 different Phyto cannabinoids, including cannabidiol (CBD), cannabigerol (CBG), cannabichromene (CBC), Δ^9 - tetrahydrocannabivarin (Δ^9 -THCV), cannabidivarin (CBDV), Δ^9 - tetrahydrocannabinol (Δ^9 -THC), Δ^9 -tetrahydrocannabiphorol (Δ^9 -THCP), Δ^9 - tetrahydro-cannabutol (Δ^9 -THCB), Δ^9 -tetrahydrocannabivarinic acid(Δ^9 -THCVA) and Δ^9 - tetrahydrocannabinolic acid (Δ^9 -THCA) etc., (93).

Table 1.5 Different types of Phytocannabinoids:

S.no	DRUG	CHEMICAL STRUCTURE	DESCRIPTION
1	Cannabinol		Cannabinol is a cannabinoid isolated from the plant <i>Cannabis</i> that is a metabolite of tetrahydrocannabinol (THC), with potential immunosuppressive and anti-inflammatory activities (94).
2	Δ^9 -tetrahydrocannabinol		Dronabinol is an isomer of tetrahydrocannabinol (THC) that is the main and most active isomer found in the <i>Cannabis sativa</i> L. plant, with potential anti-emetic, analgesic and appetite stimulating activities (95).
3	Cannabigerol		Cannabigerol is a member of the class of resorcinols that is resorcinol which is substituted by a (2E)-3,7-dimethylocta-2,6-dien-1-yl group at position 2 and by a pentyl group at position 5 (96).

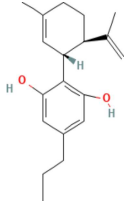
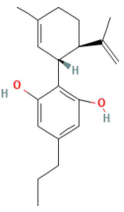
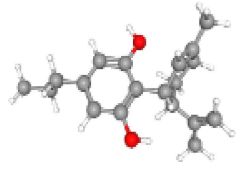
4	Cannabidivarin		Cannabidivarin, also known as cannabidivarol or CBDV, is a non-psychoactive cannabinoid and CBDV has demonstrated anticonvulsant activity in animal and human models (97).
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Table 1.6 Cannabidivarin 2D and 3D structures:

CANNABIDIVARIN:

2D Structure	3D Structure
	

Cannabidivarin, also known as cannabidivarol or CBDV, is a non-psychoactive cannabinoid found within [DB14009]. It is one of over 100 cannabinoids identified from the *Cannabis* plant that can modulate the physiological activity of *Cannabis*, or marijuana. Compared to its homolog, [DB09061], CBDV is shortened by two methyl (CH₂) groups on its side chain. Notably, both [DB09061] and CBDV have demonstrated anticonvulsant activity in animal and human models and are demonstrating promising clinical trial results (97).

CHAPTER 2

LITERATURE SURVEY

Liccardo et al (2020) demonstrated that oral pathogens and their toxic proteins infect the brain of AD patients. The aim is to keen interest in the identification and modification of the risk factors and novel molecular mechanisms associated with the development and progression of AD. They provided updates on the emerging (preclinical and clinical) evidence that supports the relationship between periodontitis and AD. Moreover, the data attest that periodontitis and AD share common risk factors and a similar hyper-inflammatory phenotype (98).

Poole S et al. (2013) reviewed evidence suggesting that oral infections, especially related to periodontitis, may contribute to neuroinflammation and neurodegeneration in Alzheimer's disease pathogenesis, emphasizing the need for further research into the oral-systemic link (99).

Singh Rao et al (2014) demonstrated that The long-term effect of inflammatory mediators, pathogens, and/or their virulence factors, reaching the brain systemically or otherwise would, over time, prime the brain's own microglia in individuals who have inherent susceptibility traits. The objective is the Periodontal pathogens *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* are implicated in the development of a number of inflammatory pathologies at remote organ sites, including Alzheimer's disease (AD). The initial inflammatory hypothesis proposed that AD hallmark proteins were the main contributors of central nervous system (CNS) inflammation (100).

Elgreu et al (2021) demonstrate that the periodontal bacteria and their virulence factors can trigger host immune defense and induce many systemic diseases cause inflammation and invasion. Analysis of some oral bacteria like *P. gingivalis* and *T. denticola*, and its related treatments and mediators by the specific methods called western blot and ELISA, which will inhibit inflammation and related disease that have potential to be further applied clinically (101).

Eslami et al (2023) demonstrate that there is a relationship between AD and periodontitis-involved bacteria as a risk factor. Alzheimer's disease (AD) is

pathologically characterized by senile plaques comprising beta-amyloid and neurofibrillary tangles (NFTs) formed by hyperphosphorylated tau as a microtubule-associated protein with a key role in the pathogenesis of AD (102).

Fu et al (2023) demonstrated that articles have shown that *P. gingivalis* infections increase the risk of PD and may indirectly lead to the development of AD. The aim of this review was to explore whether *P. gingivalis* periodontal infection is associated with AD and to provide possible mechanisms of association. PD is mainly by infection with *Porphyromonas gingivalis* (*P. gingivalis*) and by the synergistic action of various microorganisms. AD, the most common neurodegenerative disease, is characterized by poor memory and specific hallmark proteins (103).

Ishida et al. (2022) Researched on the 5-Caffeoylquinic Acid Ameliorates Cognitive Decline and Reduces A β Deposition by Modulating A β Clearance Pathways in APP/PS2 Transgenic Mice (3). Histochemical analysis revealed that 5-CQA substantially reduced A β plaque formation and neuronal loss in the hippocampi. And They showed the result that 5-CQA reduces A β deposition in the brain by modulating the A β clearance pathways and ameliorating cognitive decline and neuronal loss in APP/PS2 mice. Thus, 5-CQA may be effective in preventing cognitive dysfunction in AD (104).

Ilu et al (2023) demonstrate that, the "double-edged sword" affecting the occurrence and development of periodontitis, as well as its function in periodontitis-related systemic disorders. High expression of PD-1/PD-L1 can inhibit the inflammatory response and reduce the destruction of periodontal supporting tissues so the PD-1/PD-L1 pathway could be a new avenue for periodontal and its related systemic disorders therapy (105).

Qiu et al. (2009) Epidemiology of Alzheimer's disease: occurrence, determinants, and strategies toward intervention (32). They brief about the long-term multidomain interventions toward the optimal control of multiple vascular risk factors and the maintenance of socially integrated lifestyles and mentally stimulating activities are expected to reduce the risk or postpone the clinical onset of dementia, including Alzheimer's disease (106).

Cogswell et al. research in 2008 focuses on how miRNA146a influences the innate immune response in Alzheimer's disease. The study highlights the importance of microRNAs in the development of neuroinflammatory disorders such as Alzheimer's, providing valuable information on potential treatment targets and the mechanisms involved in the disease's advancement (107).

Jiang et al. (2019) conducted a study to examine how miRNA146a could protect the brain in a rat model of Alzheimer's disease. Their results indicate that miRNA146a might have a crucial role in reducing neurodegeneration linked to Alzheimer's, presenting a potential treatment option for this condition. This research provides important information on the use of miRNA-based approaches for protecting the brain in Alzheimer's disease (108).

Xia et al. (2020) conduct research on the DNA methylation patterns of the miRNA146a gene promoter in Alzheimer's disease. Their study delves into epigenetic changes to explore possible regulatory mechanisms involved in the irregularity of miRNA146a in Alzheimer's development. This investigation enhances our knowledge of the epigenetic environment linked to Alzheimer's and provides valuable details on the involvement of miRNA146a in the disease progression (109).

Song et al. (2015) investigated how NF- κ B prompts the production of miRNA146a and how this in turn impacts cellular senescence by repressing USP2. By unraveling the control systems at play, the study highlights the complex interactions between miRNAs, NF- κ B signaling, and cellular senescence. These findings offer important knowledge about the molecular pathways involved in aging of cells and could have relevance for comprehending conditions related to age like Alzheimer's disease (110).

Vilardo et al. (2010) investigate the relationship between microRNA (miRNA)-146a and amyloid precursor protein (APP) mRNA in Alzheimer's disease, shedding light on how this interaction could affect disease progression. By uncovering this regulatory process, the study enhances our knowledge of how APP is controlled at the post-transcriptional level and its significance for Alzheimer's disease. These findings advance our comprehension of the molecular mechanisms involved in Alzheimer's development and present possibilities for exploring miRNA-based therapies (111).

Maes et al. (2016) examine the intricate relationship between miRNA146a and NF- κ B in Alzheimer's disease, with a focus on amyloid-beta metabolism pathways. Their findings reveal detailed molecular connections among miRNA146a, NF- κ B signaling, and amyloid-beta metabolism, offering potential therapeutic targets for Alzheimer's disease. This research provides valuable understanding of how epigenetics, miRNAs, and neurodegeneration interact in the progression of Alzheimer's disease (112).

Iliff et al. (2012) have researched on A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β (40). On the basis of in vivo two-photon imaging of small fluorescent tracers, they showed that CSF enters the parenchyma along paravascular spaces that surround penetrating arteries and that brain interstitial fluid is cleared along paravenous drainage pathways. At the last they brief about the Clearance through paravenous flow may also regulate extracellular levels of proteins involved with neurodegenerative conditions, its impairment perhaps contributing to the mis-accumulation of soluble proteins (113).

In 2021, Nevins et al. conducted a research study on Comparison of a Novel Sonic Toothbrush to Manual Brushing on Plaque Control and Gingival Inflammation: A Multicenter, Randomized, Controlled Clinical Trial, which was published in the International Journal of Periodontics and Restorative Dentistry. The main goal of the study was to assess how effective a new sonic toothbrush is compared to manual brushing in managing plaque and reducing gum inflammation. This was a controlled clinical trial conducted across multiple centers involving a variety of researchers and participants. The results of this research could offer valuable insights into the benefits of using the innovative sonic toothbrush for enhancing oral hygiene and periodontal health (114).

Yagnik et al. (2019) conducted research to examine the relationship between miRNA-146a, periodontal health, and cardiovascular conditions by analyzing its expression in subgingival plaque samples from chronic periodontitis patients with and without coronary heart disease. The study aimed to uncover possible links between periodontitis and cardiovascular diseases, providing valuable insights into the underlying mechanisms. The results may help advance our understanding of the

pathophysiology of these two conditions and could have implications for the development of new diagnostic or therapeutic strategies targeting miRNA-146a (115).

Monea et al. (2014) delve into the complex connections among oxidative stress, diabetes mellitus, and periodontal disease in their review. They emphasize how oxidative stress acts as a key factor bridging diabetes and periodontitis, shedding light on the possible mechanisms that drive the reciprocal interaction between these ailments. Recognizing the influence of oxidative stress on diabetes and oral health may pave the way for tailored treatments to address and forestall complications related to these conditions (116).

Ghotloo et al.'s study on Evaluation of microRNA-146a in generalized aggressive periodontitis and its relationship with disease severity, delves into the significance of microRNA-146a (miR-146a) in generalized aggressive periodontitis (GAgP) and its connection to the seriousness of the condition. The researchers are likely looking into the levels of miR-146a expression in GAgP patients and its potential as a marker for the severity of the disease. This investigation illuminates the molecular mechanisms involved in GAgP and could potentially lead to new ways of diagnosing and treating the condition (117).

Yoneda et al. (2019) conducted a case-control study to explore the link between serum microRNAs and chronic periodontitis. The goal was to evaluate microRNAs as potential biomarkers for diagnosing and treating chronic periodontitis, with the hope of offering new ways to manage this oral health issue (118).

Ma et al. (2011) delve into the complex connection between microRNAs and the NF- κ B signaling pathway. The study elucidates how microRNAs regulate NF- κ B activation and subsequent signaling, revealing the intricate relationship between these two key elements in inflammation and immune response. Unraveling the impact of microRNAs on NF- κ B signaling may provide valuable insights into the development of diseases marked by disrupted inflammation and immunity, opening up possibilities for targeted therapeutic approaches (119).

Kozela et al. (2010) researched on the Cannabinoids Delta(9)-tetrahydrocannabinol and cannabidiol differentially inhibit the lipopolysaccharide-activated NF- κ B and interferon-beta/STAT proinflammatory pathways in BV-2

microglial cells (79). In the summary, their observations show that CBD and THC vary in their effects on the anti-inflammatory pathways, including the NF-kappaB and IFNbeta-dependent pathways (120).

Götz et al. (2019) have done research on Structure-Effect Relationships of Novel Semi-Synthetic Cannabinoid Derivatives. They conclude with the CBD- and CBDV-derivatives studied are suitable for targeting CB-receptors. Some may be used as selective CB2 agonists. The length of the aliphatic rest at R² of CBD (pentyl) and CBDV (propyl) did not correlate with the binding affinity. Higher polarity at R¹ appeared to favor the agonistic activity at CB2-receptors (121).

CHAPTER3

AIM AND SCOPE OF THE WORK

Alzheimer's disease has been increasing steadily, and with today's aging population. Alzheimer's disease (AD) is the most frequent cause of dementia in the world. WHO estimates that more than 55 million people (8.1% of women and 5.4% of men over 65 years) are living with dementia. Currently more than 55 million people live with dementia worldwide, and there are nearly 10 million new cases every year. The assemblage of Amyloid beta ($A\beta$) and tau proteins in the hippocampus region of brain cause Alzheimer's disease (AD). Aggregation of amyloid- β ($A\beta$) peptides into fibrils or other self-assembled states is central to Alzheimer's disease (AD) pathogenesis (5).

Periodontitis is a condition that causes chronic inflammation in the gums, leading to the release of cytokines and chemokines into the bloodstream. This can result in neuroinflammation. Bacteria present in the gingival crevice can cause polymicrobial infection, leading to damage of the tissue around the oral cavity. Bacteria associated with dysbiosis produce toxins and enzymes that harm gingival tissue and cause a low-grade systemic inflammatory response. *Porphyromonas gingivalis*, a primary pathogen that causes chronic periodontitis, produces two types of gingipains, enzymes that play a vital role in its virulence by breaking down host tissues and modulating the host immune response. Gingipains are enzymes produced by *Porphyromonas gingivalis* that contribute to tissue damage by breaking down proteins in the extracellular matrix and connective tissues, leading to the deterioration of periodontal tissues. They also aid in immune evasion by cleaving components of the host immune system. Recent studies suggest that there might be a link between gingipains and neurodegenerative disorders like Alzheimer's disease, but more research is necessary to fully understand the mechanisms underlying this association. Lipopolysaccharides (LPS) found in the outer membrane of gram-negative bacteria play a role in both periodontitis and Alzheimer's disease.

LPS interacts with host cell receptors, leading to the release of pro-inflammatory cytokines that cause chronic inflammation and damage to tissues. In Alzheimer's disease, chronic neuroinflammation is associated with the accumulation of amyloid beta plaques and neurofibrillary tangles. Moreover, *P. gingivalis*, a pathogen in periodontitis, is linked to the production of A β 1-42, which is implicated in amyloid plaques.

MicroRNA-146a is a small RNA molecule that is involved in regulating immune responses and inflammation. Recent studies have revealed that miRNA-146a plays a significant role in the pathogenesis of Alzheimer's disease and periodontitis. In Alzheimer's disease, miRNA-146a is upregulated in response to chronic neuroinflammation, and it contributes to the regulation of the production and clearance of amyloid beta. On the other hand, in periodontitis, miRNA-146a is upregulated in response to bacterial infection and inflammatory cytokines, where it regulates the expression of genes that are involved in the immune response and tissue remodeling. Interestingly, miRNA-146a has also been found to modulate the inflammatory response to *Porphyromonas gingivalis*, a bacterial pathogen associated with periodontitis, by targeting the Toll-like receptor 4 (TLR4) signaling pathway. However, more research is required to fully comprehend the role of miRNA-146a in the pathogenesis of Alzheimer's disease and periodontitis, as well as its therapeutic potential in treating these conditions.

To date only six drugs/ combinations were approved by the FDA for the treatment of AD. These drugs encompass cholinesterase inhibitors: donepezil, rivastigmine, galantamine; NMDA receptor antagonist: memantine; combination of donepezil (cholinesterase inhibitor) plus memantine; anti A β monoclonal antibody: aducanumab and Lecanemab. The drugs used for Alzheimer's disease shows some side effects like head ache, dizziness, nausea and diarrhea. But aducanumab shows brain swells, small bleeds (Microhaemorrhage) and brain bleeding along the surface of the brain (superficial siderosis). They primarily target bacterial infections but also have anti-inflammatory effects. Doxycycline (e.g., Vibramycin, Doxy 100, Doryx) and minocycline (e.g., Ximino) fall into this category; Chlorhexidine: An antiseptic used to treat gingivitis and periodontitis. It helps reduce inflammation and bacterial load in the oral cavity.

Recent research has shed light on the pivotal role of miR-146a in both Alzheimer's disease (AD) and periodontal disease. In AD, miR-146a's dysregulation contributes to neuroinflammation, with its downregulation in serum, plasma, and cerebrospinal fluid of patients indicating its potential as a diagnostic marker. Conversely, its upregulation in brain tissues, particularly in neurons, correlates with plaque density and synaptic pathology, suggesting its involvement in disease progression. Additionally, miR-146a influences AD pathogenesis through pathways involving STAT1, MYC, and TLR2, NF- κ B, TNF- α impacting inflammatory responses and A β sediment clearance.

In periodontal disease, miR-146a plays a complex role, with conflicting findings regarding its effects on inflammation and cytokine regulation. However, its expression positively correlates with deeper probing depths, indicating its potential as a biomarker for disease severity. Moreover, miR-146a is implicated in inflammatory conditions of NF κ B, LPS, TNF- α target genes and involvement in oxidative stress responses. So these findings having the intricate interplay between miR-146a, inflammation, and disease pathogenesis in both AD and periodontal disease.

Overall, these findings underscore the intricate interplay between miR-146a, inflammation, and disease pathogenesis in both AD and periodontal disease.

CHAPTER-4

EXPERIMENTALWORK

INVITROSTUDY

Cellculture:

U251 cells were obtained from the BeNa Culture Collection (No. BNCC337874) (Beijing, China) and cultured in Dulbecco's modified Eagle's medium (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (Zhejiang Tianhang Biotechnology Co., Ltd., Huzhou, China). Cannabidiol (CBDV) was dissolved in 100% ethanol. At a concentration of 10 nM (low dose) and 30 nM CBDV was added to the cell culture. 0.01% of ethanol is added to control culture and kept in a humidified 5% CO₂ incubator at 37 °C.

Cellviabilityassay:

U251 cells were seeded into 96-well plates at a density of 8×10^3 cells per well. After 12 h of incubation, cells were pretreated with Cannabidiol (CBDV) for 3 h at doses of 10 nM (low dose) and 30 nM (high dose), and then co-incubated with or without 2.5 μ M and 1 μ g/ml of A β 1-42 and LPS at 37 °C for a further 24 h. Twenty microliters of 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (5 mg/mL) was added to each well, and the cells were further incubated for 4 h at 37 °C in darkness. After removing the supernatant, 150 μ L of dimethyl sulfoxide were added to dissolve crystalline formazan. The absorbance at 490 nm was measured using a microplate reader (Synergy 4, Omega Bio-tek, Inc., Norcross, GA, USA).

Reactiveoxygenspecies(ROS):

U251 cells were seeded into 6-well plates at a density of 2×10^5 cells per well, pretreated with 10 nM (low dose) and 30 nM (high dose) of CBDV for 3 h, and co-incubated with or without 2.5 μ M and 1 μ g/ml of A β 1-42 and LPS at 37 °C for 12 h. The intracellular concentrations of ROS were measured using an ROS assay kit (E004, Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

The intracellular concentrations of Ca^{2+} were measured using Fluo-4 AM dye (F14201, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The fluorescence intensity of cells under light excitation was observed using a fluorescent inverted microscope (CKX31, Olympus, Tokyo, Japan).

ELISA:

The collected cells were quantified using a Pierce™ BCA Assay Kit. The concentrations of the following proteins were analysed using the corresponding commercial ELISA kits (Jiangsu Kete Biological Technology Co., Ltd, Jiangsu, China): TNF- α , IL-6, IL-1- β . The absorbance at 450 nm was detected using a microplate reader (Synergy 4, Omega BioTek, Inc., Norcross, GA, USA).

Western blot:

U251 cells were seeded into 6-well plates at a density of 2×10^5 cells per well, pretreated with 10 nM (low dose) and 30 nM (high dose) of CBDV for 3 h, and then co-incubated with or without 2.5 μM and 1 $\mu\text{g/ml}$ of A β 1-42 and IA at 37 °C for 24 h. The brain tissues collected from experimental mice and treated cells were lysed using RIPA buffer containing PMSF, stored on ice, and quantified using the Pierce™ BCA Protein Assay Kit. As in our previous study, 40 μg of protein and 4 μL of marker (26616, Thermo scientific, Waltham, MA, USA) were separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis and then transferred to polyvinylidene fluoride membranes (0.45 μm) (GE Healthcare Life Science, Beijing, China). The loaded membranes were blocked in 5% bovine serum albumin at 4 °C for 5 h and then incubated separately with the following primary antibodies at 4 °C overnight: SIRT1 and NF- κB . After washing with tris-buffered saline containing 0.1% Tween 20, the membranes were incubated separately with the following secondary antibodies: goat anti-mouse IgG (H+L) (peroxidase/HRP conjugated) and goat anti-rabbit IgG (H+L) (peroxidase/HRP conjugated). The membranes were then exposed using an electrochemiluminescence kit (Merck, Darmstadt, Germany) to obtain blots, which were visualized using an automatic chemiluminescence image analysis system (Tanon 5200, Tanon Science and Technology Co., Ltd., Shanghai, China) and quantified using Image-pro Plus 6.0 analysis software (Media Cybernetics, Bethesda, MD, USA).

Statistical analysis:

Data were expressed as means $\pm$ standard errors of the mean (SEM). Statistical significance was determined using one-way analysis of variance with SPSS 16.0 software (IBM Corp., Armonk, NY, USA). Post hoc analysis was performed using multiple comparisons (Dunn's test). A p value < 0.05 was considered to indicate a statistically significant difference. All of the graphs were produced using GraphPad Prism 7.0.

CHAPTER 5

RESULTS AND DISCUSSION

INVITRO ANALYSIS:

Effect of CBDV (low and high doses) and A β +LPS on SOD and CAT protein levels on U251 cells.

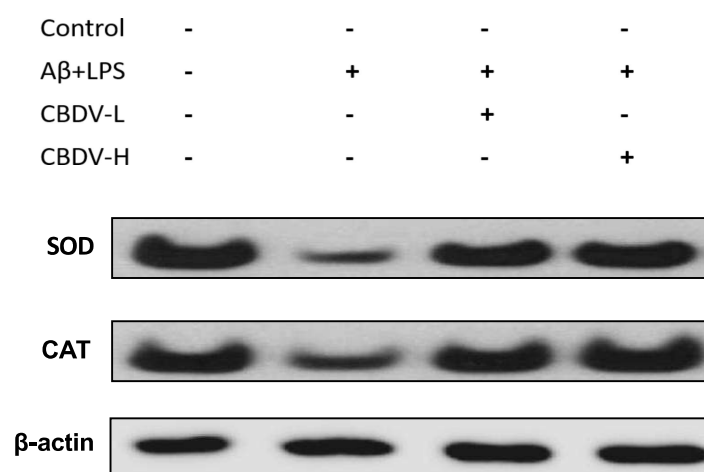
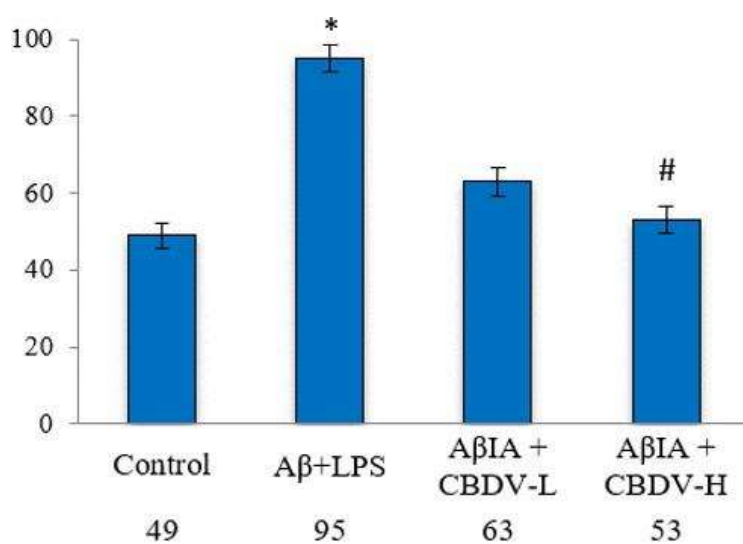


Fig5.1(A) Representative Western blots of SOD and CAT expressions



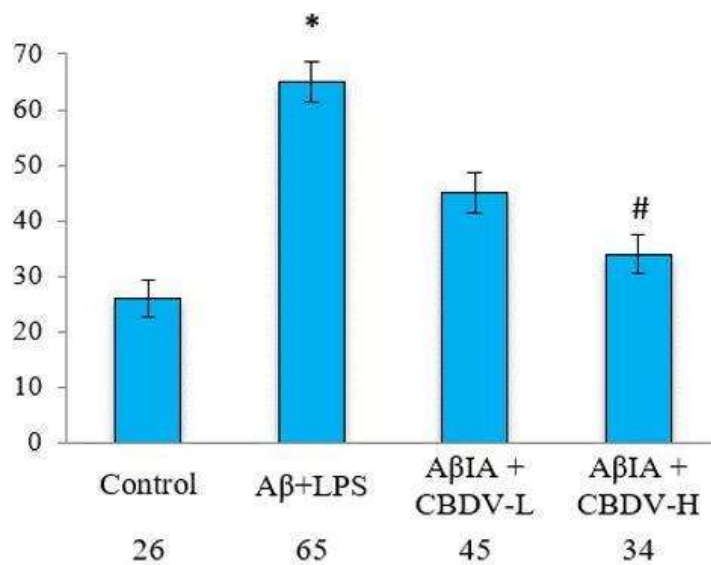


Fig 5.1(B) The relative SOD and CAT protein levels in U251 cells.

Cannabidiol (SOD and CAT) of U251 cells against Aβ+LPS

When we probed the U251 cells for the pivotal antioxidants SOD and CAT, we observed that SOD and CAT levels were significantly ($P < 0.05$) diminished and elevated by 65% and 95% respectively in the Aβ+LPS challenged cells, as compared to that of the controls. Nevertheless, CBDV-L (low dose) and CBDV-H (high dose) treated cells depicted near normal SOD and CAT levels in the cell lysates. Especially, high-dose CBDV (CBDV-High dose) treated was more effective in replenishing the antioxidant levels in the Aβ+LPS challenged cells.

Aβ1-40 with LPS-treated rats showed a significant increase in the mitochondrial membrane fluidity and a significant increase in the activity of SOD of the hippocampus, as well as a marked increase in the content of MDA of the hippocampus (122). The observation that blockade of catalase Compound II formation prevents Aβ inhibition of catalase suggests that Aβ may be involved in oxidizing the enzyme, a mechanism already proposed for Aβ inactivation of other enzymes, and this process appears to involve Aβ binding to catalase (123).

Effect of CBDV (low and high doses) and A β +LPS on SIRT1 levels in U251 cells.

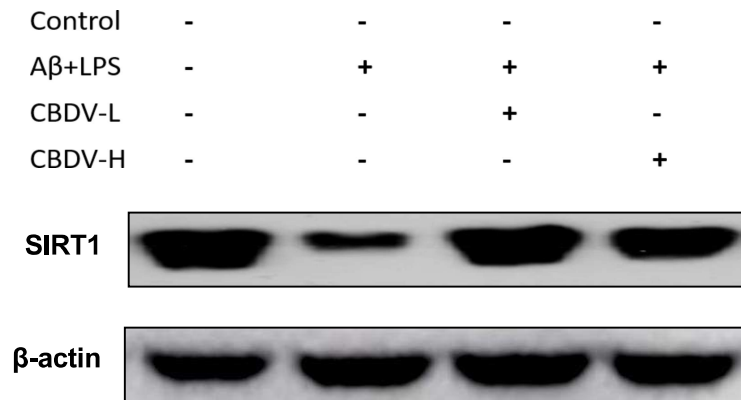


Fig 5.2 Representative Western blots of SIRT1 expressions

The SIRT1 is treated with A β +LPS-challenged cells.

Translocation of SIRT1 is increased in the cells due to the neurotoxic assault provoked by A β +LPS. We observed that treatment with both low-dose and high-dose of CBDV reinstated the normal levels of SIRT1 levels. Of note, CBDV-High dose treatment displayed better activity when compared to CBDV-Low dose treatment.

SIRT1 increased the A β 42- immunoreactive area in the cytoplasm and increase the cell surface where SIRT1 was colocalized with A β 42.

CBDV agonist treatment increased LPS-induced SIRT1 secretion but not influencing SIRT1 mRNA in peritoneal macrophages. CBDV could promote the intracellular degradation of SIRT1 via the autophagy-lysosome pathway in macrophage (124).

Effect of CBDV (low and high doses) and Aβ + LPS on the relative NF-κB on U251 cells.

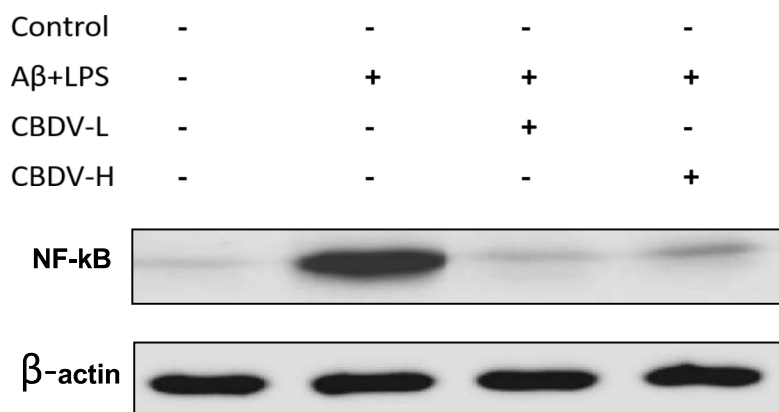


Fig5.3(A) Representative Western blots of NF- κ B expressions

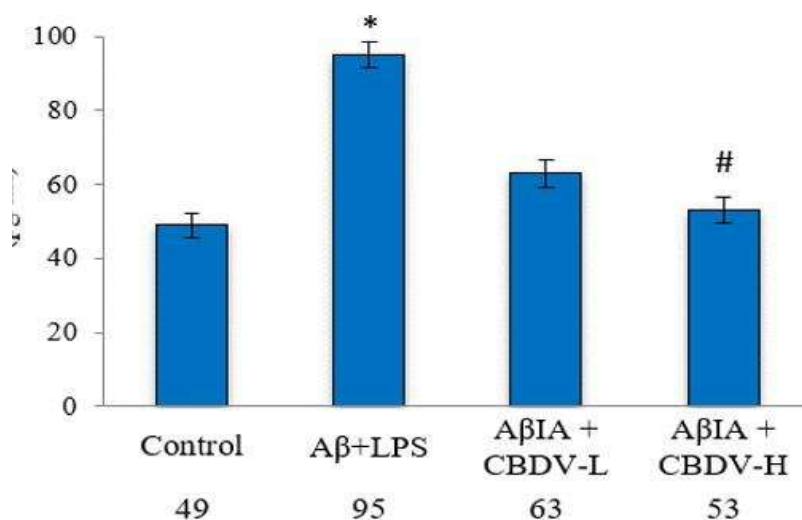


Fig5.3(B)NF- κ Blevelexpression

Cannabidiol inhibits NF- κ B in U251 cells against A β +LPS

In this study, about 5-fold increase in the protein expression of NF- κ B was observed in the A β +LPS challenged cells. However, treatment with the CBDV (both 0 50 100 150 200 55 CBDV-Low dose and CBDV-High dose) increase in the protein expression of NF- κ B. CBDV reduces the activity of the NF- κ B pathway, a primary pathway regulating the expression of proinflammatory genes. Moreover, CBDV up-

regulates the activation of the STAT3 transcription factor, an element of homeostatic mechanism inducing anti-inflammatory events (49). The transcription factors NFAT and NF- κ B in T-cells strongly indicate that the tested CBDV-agonists are more likely to inhibit the NFAT signalling pathway than the NF- κ B pathway (49).

Effect of CBDV (low and high doses) and A β +LPS on the relative miRNA-146a protein levels on U251 cells.

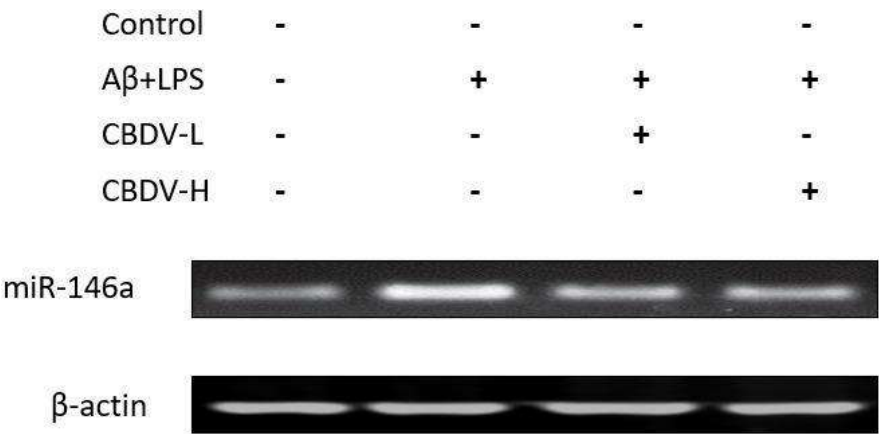


Fig5.4(A) Representative Western blots of miRNA146a expressions

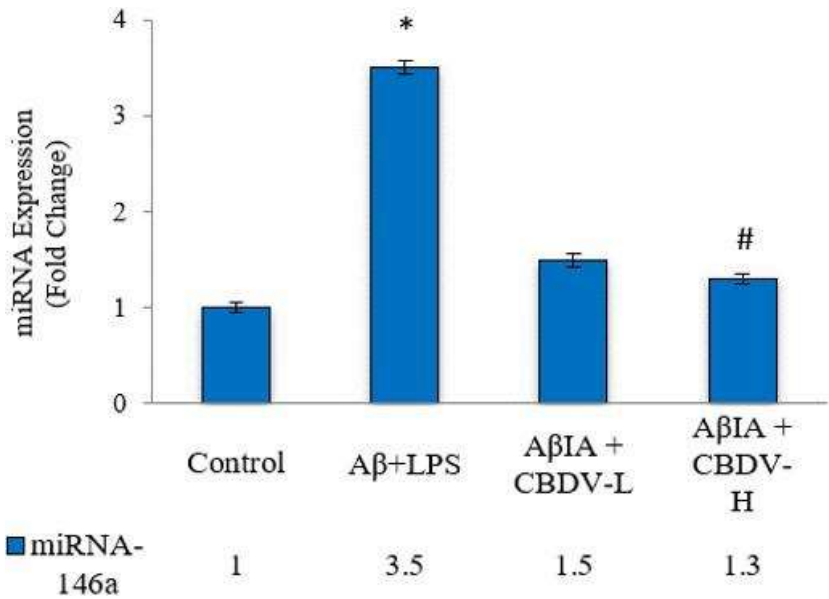
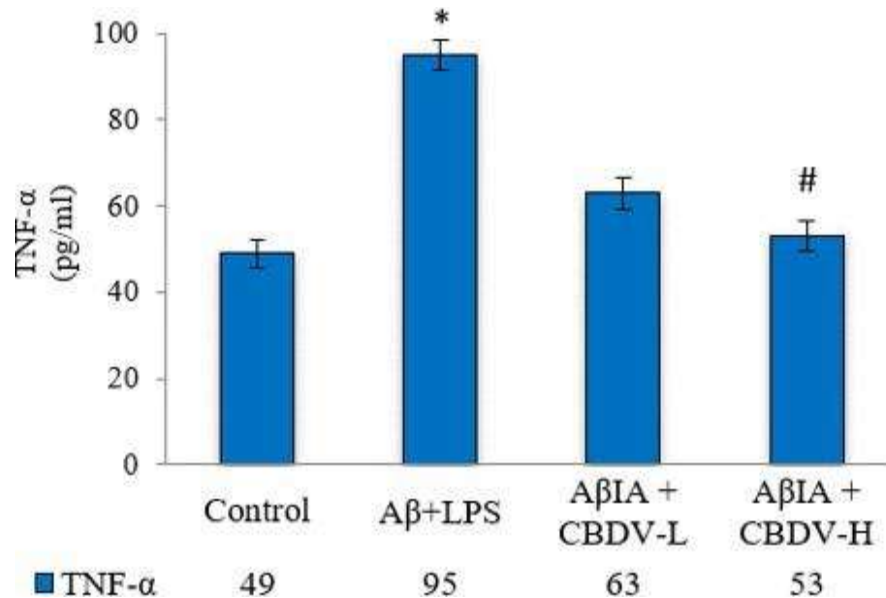


Fig5.5(B) Level of miRNA146a

In this study, about 3.5-fold increase in the protein expression of miRNA-146a was observed in the A β +LPS challenged cells, whereas, CBDV-Low dose and CBDV-High dose having 1.5 and 1.3 folds respectively. From the observation, high-dose CBDV (CBDV-High dose) is more effective in replenishing the miRNA146a levels in the A β +LPS challenged cells.

miRNA146a deficiency is directly involved in impaired A β brain clearance via NF- κ B/TNF- α . miRNA146a mediated might counteract blood-brain barrier abnormalities and age-related disease. (48,49,51).

Effect of CBDV (low and high doses) and A β +LPS on the relative TNF α and IL-1 β protein levels on U251 cells.



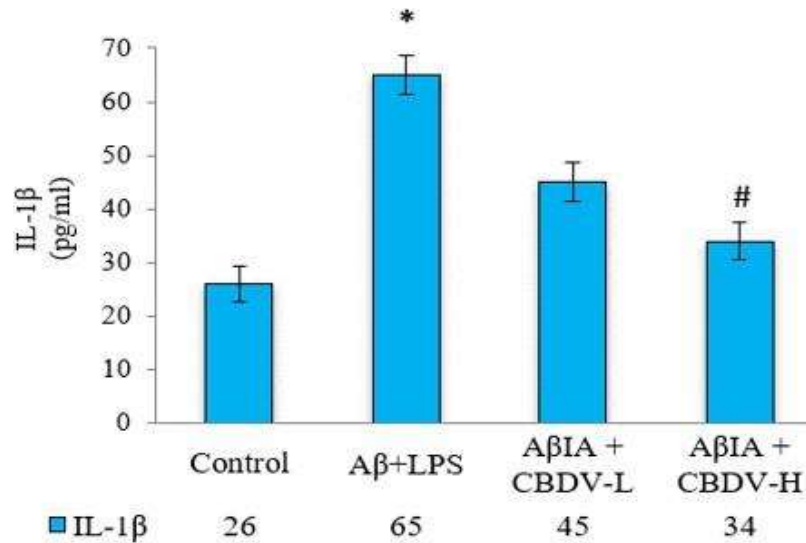


Fig5.6 Level of TNFα and IL-1β

Secretion of the pro-inflammatory cytokines including TNFα and IL-1β is an immediate effector function of the cells challenged with the neurotoxic cocktail of Aβ+LPS. We observed levels were diminished and elevated by 65% and 95% respectively in the Aβ+LPS challenged cells, as compared to that of the controls. Increased in the levels of TNFα when compared to IL-1β in the Aβ+LPS challenged cells, while high-dose CBDV treatment more effectively counteracted the inflammatory insult kicked-off by the exposure of cells to Aβ+LPS cocktail. However, treatment with low-dose CBDV (CBDV-Low dose) did not show any significant anti-inflammatory effect against the neurotoxins.

A pivotal molecule in inflammation is the pro-inflammatory cytokine TNF-α. Several lines of evidence using genetic and pharmacological manipulations indicate that TNF-α signaling exacerbates both Aβ and tau pathologies *in vivo* (125). The proinflammatory cytokine interleukin-1β (IL-1β) is upregulated in human AD and believed to promote amyloid plaque deposition (126).

Tumor necrosis factor alpha, interleukin IL-1β and interferon gamma were the most commonly studied pro-inflammatory cytokines and their levels were consistently reduced after treatment with CBDV.

SUMMARY

The neuroprotective effects of Cannabis sativa as well as its active constituents mainly cannabinoids, have been already reported. However, their role in miRNA AND periodontitis clearance pathway in AD has not been analyzed to assess their interaction between miRNA-146a clearance mechanism.

Based on the study we found that Cannabigerol has medium-high interaction with two molecular targets (beta-amyloid and tau). Cannabinol has medium-high interaction with three molecular targets (beta-amyloid, tau and interleukin-6. Cannabidiol (CBDV) has medium-high interaction with four molecular targets (tau, NF-kB, LPS, interleukin-6 and SIRT1).

Overall CBDV has been chosen for further analysis using *in-vitro* studies. For the *in-vitro* studies U251 cell line was used to study the effect of CBDV on various molecular targets including antioxidant's (SOD and CAT), inflammatory mediators (SIRT1, NF-KB, TNF- α and IL-6) and pivotal target in the miRNA.

We found that the expression of antioxidant's (SOD and CAT) were significantly diminished in the A β +LPS challenged cells (vs control). Nevertheless CBDV (especially CBDV-H) treated cells depicted near normal SOD and CAT levels in the cell lysate.

Translocation of SIRT1 from the nucleus to cytosol is increased in the cells due to the neurotoxic assault provoked by A β +LPS. We observed that treatment with high-dose of CBDV reinstated the normal levels of nuclear and cytosolic SIRT1 levels.

We also found that the expression of anti-inflammatory agents (NF-KB) were significantly increased in the A β +LPS challenged cells (vs control). The treatment with

CBDV (mainly CBDV-H) ameliorated the abnormal increase in the protein expression of NF- κ B.

The expression of anti-inflammatory agents (TNF- α and IL-6) were shown some significantly increase in the A β +LPS challenged cells (vs control). High-dose CBDV (CBDV-H) treatment was more effectively counteracted the inflammatory insult kicked off by the exposure of cells to A β +LPA cocktail.

CONCLUSION

To summarize precisely CBDV showed better interaction with miRNA TARGET system.

Further analysis using *in-vitro* studies clearly indicate the antioxidant, anti-inflammatory and miRNA clearance facilitating properties of CBDV.

Hence, CBDV might be a putative neurotherapeutic agent for the treatment of Alzheimer's Disease which was influenced by periodontitis and by miRNA146a. However, further studies are warranted to elucidate the comprehensive effect of CBDV on various upstream and downstream targets in the miRNA pathway. Besides the effects of CBDV on cognitive dysfunction in AD which was caused by periodontitis need to be studied.

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