A systematic study of molecular targets of cannabidiol in Alzheimer's disease

Nil Patil[1,2], Khushalika Patil[2], Mukul Jain[1,2,3] (iD), Arifullah Mohammed[4] (iD), Alpa Yadav[5], Parmdeep Singh Dhanda[6], Chittaranjan Kole[7], Kirtan Dave[8], Prashant Kaushik[9], Mohammad Khairul Azhar Abdul Razab[10], Zulhazman Hamzah[3] (iD) and Norazlina Mat Nawi[11] (iD)

Abstract

Background: Alzheimer's disease (AD) is a prevalent, incurable, and chronic neurodegenerative condition characterized by the accumulation of amyloid-β protein (Aβ), disrupting various bodily systems. Despite the lack of a cure, phenolic compounds like cannabidiol (CBD), a non-psychoactive component of cannabis, have emerged as potential therapeutic agents for AD.

Objective: This systematic review explores the impact of different types of cannabidiol on AD, unveiling their neuroprotective mechanisms.

Methods: The research used PubMed, Scopus, and Web of Science databases with keywords like "Alzheimer's disease" and "Cannabidiol." Studies were evaluated based on title, abstract, and relevance to treating AD with CBD. No restrictions on research type or publication year. Excluded were hypothesis papers, reviews, books, unavailable articles, etc.

Results: Microsoft Excel identified 551 articles, with 92 included in the study, but only 22 were thoroughly evaluated. In-vivo and in-silico studies indicate that CBD may disrupt $A\beta_{42}$, reduce pro-inflammatory molecule release, prevent reactive oxygen species formation, inhibit lipid oxidation, and counteract Aβ-induced increases in intracellular calcium, thereby protecting neurons from apoptosis.

Conclusions: In summary, the study indicates that CBD and its analogs reduce the production of $A\beta_{42}$. Overall, these findings support the potential of CBD in alleviating the underlying pathology and symptoms associated with AD, underscoring the crucial need for further rigorous scientific investigation to elucidate the therapeutic applications and mechanisms of CBD in AD.

[1]Cell & Developmental Biology Lab, Research & Development Cell, Parul University, Vadodara, Gujarat, India
[2]Department of Life Sciences, Parul Institute of Applied Sciences, Parul University, Vadodara, Gujarat, India
[3]Faculty of Earth Science, Universiti Malaysia Kelantan, Jeli, Kelantan, Malaysia
[4]Department of Agriculture Science, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli, Kelantan, Malaysia
[5]Department of Botany, Indra Gandhi University, Meerpur, Rewari, India
[6]Department of Biochemistry, Punjab Agriculture University, Ludhiana, Punjab, India
[7]The Neotia University, Sarisha Jhinga, West Bengal, India
[8]Bioinformatics Laboratory, Research & Development Cell, Parul University, Vadodara, Gujarat, India
[9]Chaudhary Charan Singh Haryana Agricultural University, Hisar, India
[10]Medical Radiation Programme, School of Health Sciences, Universiti Sains Malaysia, Health Campus, Kubang Kerian, Kelantan, Malaysia
[11]Department of Nuclear Medicine, Radiotherapy & Oncology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, Kubang Kerian, Kelantan, Malaysia

Corresponding author:
Norazlina Mat Nawi, Department of Nuclear Medicine, Radiotherapy & Oncology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia.
Email: norazlina@usm.my

Introduction

Alzheimer's disease (AD), the leading source of dependency and disability among individuals aged 65 and older, stands as the predominant cause of dementia in the elderly. Although there is a lower age range that is afflicted, it affects women more often than males and adults over 65 more frequently. The World Alzheimer's Report of 2018 estimates that 50 million people worldwide are living with dementia, with AD affecting two-thirds of those individuals.[1,2] According to estimates, the number of people with AD will reach 152 million by the year 2050 as life expectancy rises.[3] AD can be treated with cannabidiol (CBD), which has been shown to slow down the disease progression.[4] According to the World Health Organization, 60–80% of the estimated 43.8 million dementia sufferers worldwide are thought to have AD, an age-related neurodegenerative disorder.[5] *Cannabis sativa* plants, also known as Hemp, naturally contain cannabinoids called phytocannabinoids which contain more than 400 different chemicals, the most well-known of which are delta-9-tetrahydrocannabinol (9-THC) and CBD.[6] The US FDA has approved the use of Sativex, a new formulation of THC and CBD, to treat multiple sclerosis and the injection of Sativex decreased the amounts of amyloid-β (Aβ) oligomers, phosphorylated tau, and GSK3 expression in a mouse model with human tau.[7] Consequently, the elements of the endocannabinoid system are thought to

be a possible target for phytocannabinoids as a defense against AD pathogenesis its capacity to play a crucial part in preserving the body's homeostasis, which includes the brain, endocrine, and immunological systems so, it is far more challenging to comprehend how phytocannabinoids affect endocannabinoid system regulation. However, CBD has no psychotropic side effects and is well tolerated.[8] It performs several neuroprotective actions by modulating different receptors (Figure 1).[9]

As a result, the medicinal potential of CBD, particularly for AD, has drawn growing attention to its neuroprotective and non-psychoactive qualities, because the majority of preclinical research has shown that CBD has largely beneficial neurological effects. As far back as the late 1800s, medical professionals in the UK and the United States noted that extracts from cannabis were associated with a decrease in the occurrence of epileptic seizures.[10] In comparison to conventional antiepileptic medications, CBD exhibits greater effectiveness as an anticonvulsant, offering increased specificity and fewer neurotoxic side effects.[11] Additionally, CBD has great selectivity, no excitability in the central nervous system, and can decrease the duration and amplitude of the post-discharge cAMP response element-binding protein (CREB). The intracellular protein CREB controls the expression of genes that are crucial for dopaminergic neurons. Through G protein-coupled receptors, dopamine modulates CREB's phosphorylation.[12] The creation of
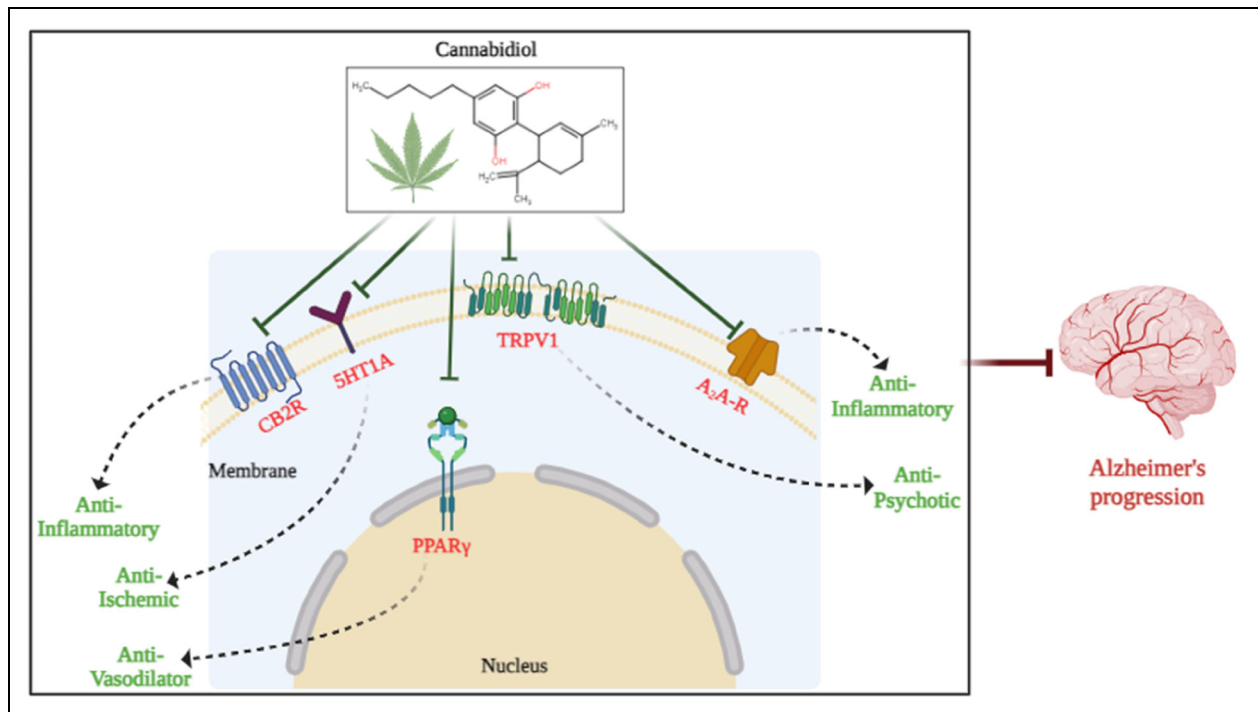
Figure 1. Potential biomarkers for CBD therapy of AD. Several potential biological targets of CBD involved in treating AD have been proposed, including CB2, 5-HT1A, TRPV1, PPARγ, and Adenosine receptors. These proposals based on the properties of each potential receptor target of CBD and well-established mechanism studies of CBD-mediated therapeutic functions.

efficient treatments for AD continues to be of utmost importance given its high prevalence and significant morbidity and mortality, since the identification of the neurofibrillary tangles and cerebral Aβ plaques, which are the pathological manifestations of AD and contain aggregates of hyperphosphorylated tau protein. On PC12 neuronal cells, research-employing CBD *in vitro* was carried out which findings demonstrated that CBD stimulated the Wnt/β-catenin pathway to activate the peroxisome proliferator-activated receptor (PPARγ) by exhibiting neuroprotection.[13] As a result, lowered reactive oxygen species (ROS) generation, lipid peroxidation, and proteins such as tau hyperphosphorylation, and suppressed acetylcholinesterase (AChE) while safeguarding PC12 cells from neurotoxicity and oxidative stress.[14] According to a study done on SH-SY5Y^{APP+} cells, it decreased the amounts of amyloid protein precursor (APP) full-length protein and Aβ synthesis.[15–17] AD is a neurodegenerative disorder that causes permanent memory loss and cognitive decline because of a little disruption in the cerebral cortex and hippocampus functioning.[18] In PC12 cells exposed to A, CBD reduced the formation of Aβ plaques, stopped the creation of ROS and the oxidation of lipids, restrained the amount of neuronal apoptosis caused by caspase 3 decrease, and counteracted increases in intracellular Ca^{+2} caused by Aβ. CBD was anti-inflammatory in an *in vivo* model.[19] CBD's act as an anti-inflammatory compound by reducing inducible nitric oxide synthase (iNOS) and interleukin (IL)-1β protein expression. Additionally, it prevented the hyperphosphorylation of tau protein in PC12 neurons triggered by Aβ. The mode of action of CBD was later demonstrated to be dosage-dependently antagonistic of pro-inflammatory nitric oxide (NO), tumor necrosis factor-alpha (TNF-α), and IL-1β via PPARγ.[20] By specifically inhibiting PPARγ-related NF-κB, GW9662 (a PPARγ antagonist) reduced reactive gliosis and prevented that impact. Following exposure to Aβ, neurogenesis was increased by activity of anandamide and CBD. Furthermore, recent preclinical studies have demonstrated various beneficial effects of CBD, such as its anticonvulsant, anti-nausea, and analgesic properties.

Moreover, these studies have shown that CBD provides superior protection for neurons against free radicals compared to ascorbate or tocopherol.[21] THC is equally cytotoxic as it is cytoprotective, protecting neurons and healthy cells but not many cancer cells. Various conditions, including multiple sclerosis, Parkinson's disease, AD, epilepsy, and other systemic disorders resulting from nervous system degeneration or anomalies, can be effectively treated with CBD, thanks to its neuroprotective advantages, as supported by both preclinical and clinical research.[22] CBD is a frequent subject of research in the context of neurodegenerative diseases and their associated patho-mechanisms.[23]

Materials and methods

Study protocol

The present systematic review was performed according to the Preferred Reporting Items for a Systematic Review and Meta-analysis (PRISMA) guidelines.[24,25]

Literature search strategy

Data were retrieved from PubMed, Scopus, and Web of Science (WoS) databases[26] from 7 May 2023 using the following keywords "Cannabidiol" and "Alzheimer's disease". There were only English-language and freely available (open-access) literatures were chosen.

Eligibility criteria

We evaluated each study's title and abstract before including, all eligible studies, which included studies that corresponded to our objectives for the treatment of AD with CBD, studies with sufficient and comprehensive data, studies without restrictions from *in vitro*, *in vivo*, or *in silico* research, and studies without limitations on publication year. Hypothesis, communication, systematic reviews, books and documents, meta-analyses, randomized controlled trials, posters, conferences, current opinions, letters to the editor, and unavailable articles were all omitted from the exclusion criteria.[27]

Collection of data

Two reviewers (N.P., K.P.) independently screened the titles and abstracts of papers using the aforementioned keywords after the duplicate articles had been removed.[28] After that, depending on the inclusion and exclusion criteria, relevant full-text articles were independently assessed.[29] Reviewer M.J. decided to exclude the publications that did not adhere to the study's objective.

Data synthesis and statistical analysis

Data were pooled using a random effects model when there was variability among the papers to give a more conservative assessment of the range of cannabinoids impact on AD. Heterogeneity was assessed using the I^2 index of 50% and the chi-squared test with a *p-value* of 0.05, both of which are used to determine a substantial level of heterogeneity.[30] Heterogeneity is the variance between distinct study findings that has not happened by mistake.[31] Reviewers M.J. & N.P. used Microsoft Excel (Windows XPC) for the statistical analysis.

Results

Screening and searching of literature

A thorough scan of the electronic databases up to May 2023 turned up 551 items. 371 publications were checked for titles and abstracts after duplicates were eliminated, and 180 of them were excluded. 220 articles met the requirements for inclusion and exclusion, allowing for a more thorough evaluation of their entire texts. The systematic review could comprise 91 papers in total. Figure 2 depicts the procedure of the literature search and screening process. Out of 91 papers, 21 articles were carried further for molecular mechanism elucidation in AD with CBD or its analogs. These 21 articles were mainly focused on *in silico*, *in vivo*, and *in vitro* approaches.

Data extraction

Authors NP & KP extracted the data from each article, which they subsequently compiled in Table 1, and author MJ checked it under the following headings: author information, route of administration (*in vivo, in vitro*, and *in silico*), molecular mechanism, treatment duration/agent dosage, and the effects of co-administering CBD and its equivalents on AD.

Discussion

Effects of CBD on AD pathology

The activation of microglia-induced neuroinflammation arises from multiple molecular pathways. In a 2020 study by dos-Santos-Pereira et al., CBD (1–10 μM) was found to inhibit the release of TNF-α, IL-1, and glutamate in LPS-induced microglial cell cultures. This effect was attributed to CBD's anti-inflammatory and antioxidant properties, which were evident through the downregulation of IkappaB (IκB) kinase-2 and NADPH oxidase apocynin. Notably, the anti-inflammatory impact of IB kinase-2 was synergized by its antioxidant capacity, as confirmed by the subsequent reduction in NF-κB-mediated signaling events.[53] Numerous studies have shown that the NF-κB pathway is essential for CBD's ability to inhibit neuroinflammation in TLR4-mediated pathways. When stimulated by extracellular inducers such as LPS, IRAK-1, and IκB undergo ubiquitination, leading to NF-κB activation and inflammation. CBD (1, 5, or 10 μM) partially inhibits IRAK-1 degradation, preserves IκB, and reduces NF-κBp65 phosphorylation, thus contributing to the suppression of inflammatory gene expression in LPS-activated BV-2 microglia via the IκB-NF-κB pathway reversal.[54] CBD's anti-inflammatory effects are mediated through the IFN-STAT pathway, which activates genes for CXCL10, CCL5, and CCL2. STAT family members express pro- and anti-inflammatory genes, with STAT 1 homodimer having pro-inflammatory effects. Increased IL-10 production or binding to STAT3- causes anti-inflammatory effects in different IL-10-inducible genes' consensus sequences. CBD dose-dependently decreased proinflammatory STAT1 activation and enhanced STAT3 activation, resulting in anti-inflammatory effects and inhibition of inflammation, linked to STAT1 and STAT3 expression control (Figure 1).[55] Additionally, CBD may reduce fatty acid amide hydrolase (FAAHs) enzymatic activity and boost the activity of anandamide and 2-AG concentrations, which would lower AD pathology, and excessive glutamatergic transmission.[23,56] The G protein-coupled receptor 55 (GPR55), which does not share any amino acid sequences with the conventional CB1 and CB2 receptors, is regarded as a third cannabinoid receptor.[57,58] In contrast to the Gi-coupled CB1 and CB2 receptors, the G-coupled receptor GPR55 is unique. Intracellular calcium and neuronal excitability are elevated when GPR55 is activated, which in turn affects cellular migration and proliferation and glutamate release in the hippocampus formation, which may be related to inflammation and cognitive functions.[59,60] CBD's ability to interact with diverse receptors, encompassing serotonin, vanilloid, adenosine, peroxisome proliferator-activated, opioid, and dopamine receptors.[51,61,62] Therefore, in addition to the presumed effects of cannabinoid receptors, CBD offers intriguing medicinal potential. Due to its antidepressant or anxiolytic effects[61] and its function as a neurotransmitter in the brain, it has been demonstrated that the serotonin 1A receptor (5-HT1A) receptors are primarily responsible for TRPV1 and are a crucial biological target for CBD treatment in numerous forms of pain and inflammation.[62] However, in the context of inflammation, all of these receptors may collaborate rather than function separately. For instance, a recent study revealed that blocking A2A receptors might enhance CB2 receptor-mediated signaling in the context of an A2A-CB2 receptor heteromer. Numerous target genes and signaling cascades are regulated by PPARγs, which also start distinct physiological reactions such as cell division, proliferation, and anti-inflammatory activities.[63,64] Fatty acid-binding proteins can transport CBD to the nucleus, where it subsequently binds to PPARγ directly, activating it and causing a variety of PPARγ-mediated alterations.[65] PPARγ has been frequently found to be a crucial regulator of the neuroprotective impact of CBD against AD progression.[66] Furthermore, CBD has been referred to as an allosteric opioid receptor modulator.[67] There were three types of CBD studies possible in AD *in silico*, *in vitro*, and *in vivo*.

In silico, in vitro, and in vivo study of CBD in AD

The Achilles server's *in silico* study tools were used to analyze the interaction between CBD and tau protein through best-fit docking. Donepezil, rivastigmine, and galantamine with binding affinities of −8.77, −7.0, and
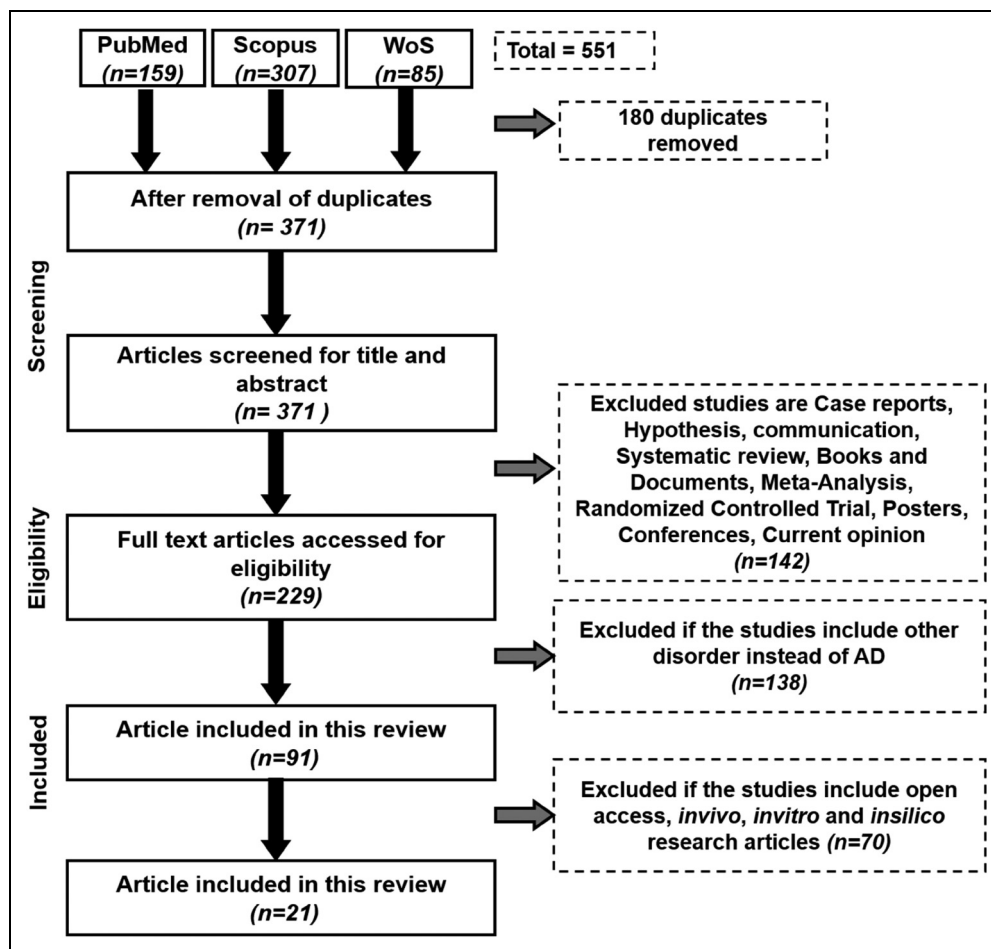
Figure 2. Schematic representation of the workflow.

−7.97 kcal/mol were employed as reference AChE receptor analogs for comparison.[68,69] Using memantine as a reference with a binding affinity of approximately 6.83 kcal/mol, THCV, Cannabinol C2, and Cannabidiorcol from cannabis demonstrated superior inhibition of AChE and butyrylcholinesterase (BuChE). Computational analysis suggests that THCV, Cannabinol C2, and Cannabidiorcol are the most effective compounds for inhibiting these enzymes associated with AD.[70,71] Through both biochemical and in silico methods, it was shown that CBD interacts with tau protein, inhibiting its amyloid fibril development via hydrogen bonds and hydrophobic forces. CBD also promotes the transformation of tau protein from a random coil shape to a more rigid structure, reducing the formation of tau clumps.[72]

Concerning *in vitro* studies, CBD has several properties that are directly related to its therapeutic effectiveness for AD[73] found that CBD reduced glycogen synthase kinase-3 (GSK-3; the enzyme that causes tau hyperphosphorylation). Importantly, CBD increased PPARγ-induced APP ubiquitination in APP-transfected human neuroblastoma cells, which resulted in decreased APP expression[74] and decreased Aβ peptide expression. In addition, CBD decreased the expression of the genes that code for the secretases involved in the enzymatic production of Aβ, such as BACE1, PS1, and PS2. CBD has also been found to weakly, but fascinatingly; inhibit direct secretase.[75] In addition, hemp seed oil (80% CBD) protected MC65 cells, an AD neuron-like cell model, from amyloid toxicity.[76] CBD also exhibits the potential to mitigate mitochondrial dysfunction, a significant aspect of AD. Moreover, CBD reduces oxidative stress by lowering ROS and NOX isoforms, including NOX, known for ROS production.[77] *In vitro* data suggest that CBD can rescue the Wnt/-catenin pathway, linked to tau hyperphosphorylation, and reduce iNOS protein synthesis and NO release.[78] Additionally, CBD's ability to reduce reactive gliosis may be associated with its inverse agonist role at the cannabinoid receptor 2 (CB2), a factor implicated in the condition.[79] Male 5xFAD transgenic mice aged 9 to 12 months were employed. Mice in the treatment group received eight intraperitoneal injections of CBD isolate at a level of 10 mg/kg every other day for two weeks. Mice receiving a placebo were given it on the same schedule as receiving CBD. With reduced amyloid levels in brain tissue and enhanced TREM2 expression, CBD may help

Table 1. The features of the listed studies.

Sr.	Authors	Route of Administration (in vivo /in vitro/ in silico)	Molecular mechanism	Treatment time/ Dosage of agent	Outcome
1	Khodadadi et al., 2021[32]	In vivo (5xFAD mice)	Reduction of Aβ, decreasing IL-6 expression in peripheral leukocytes	10 mg/kg Cannabidiol, intraperitoneal every other day for 8 doses over 2 weeks	Ameliorated the symptoms of AD, and retarded cognitive decline
2	De Paula Faria et al., 2022[33]	In vivo (Wistar male mice)	CBD protects the brain from STZ effects, which promotes dysregulation in glucose metabolism. The absence of alterations in glucose metabolism could be important for maintaining cognitive ability	20 mg/kg- STZ-Cannabidiol	Protect dysregulation in the glucose metabolism
3	Frandsen et al., 2022[34]	In vivo (C. elegans)	The glyoxalase pathway detoxifies methylglyoxal, a highly reactive metabolic byproduct that can accumulate in the brain and contribute to the severity of AD	100 µM- Cannabidiol	Cannabidiol can prevent methylglyoxal-mediated cellular damage through enhancement of the neural glyoxalase pathway and extend the lifespan
4	Chesworth et al., 2022[35]	In vivo (APPxPS1 mice)	Increased cognitive movement	20 mg/kg CBD for 8 months	CBD had limited effects on the expression of fear memory. These results indicate preventive CBD treatment can have a moderate spatial learning-enhancing effect in a female amyloid-β-based AD mouse model
5	Alali et al., 2021[36]	In vitro (Thioflavin T Assay)	—	CBD 20 µM	CBD inhibits the tau aggregations effectively
6	Hughes et al., 2019[37]	In vivo (C57Bl/6 mice)	CBD rescues Aβ-mediated deficits in LTP (long-term potentiation) through a mechanism independent of the 5HT1A, Adenosine A2A, or the CB1 receptor	CBD 10 µM	Presence of the PPARγ antagonist GW9662 the neuroprotective effect of CBD was prevented
7	Amini et al., 2022[38]	In vivo (Wistar male mice)	—	CBD nano chitosan (not specific)	Reducing Aβ plaques, increasing brain CB1 and level CB2, and improving learning and memory in Alz rats
8	Libro et al., 2016[39]	In vivo (Human gingiva-derived mesenchymal stem cells)	Inhibited the transcription of the kinases involved in tau phosphorylation (GSK3β and CDK5)	CBD (5 µM)	CBD led to the downregulation of the genes coding for kinases involved in tau protein phosphorylation, like GSK3β, CDK5, DYRK1A, CAMK2A, and the MAPKs indicating that CBD prevents tau hyperphosphorylation
9	Landucci et al., 2022[40]	Male and female Wistar rat pups	The neuroprotective effects of Cannabidiol were blocked by TRPV1, TRPV2, 5-HT1A, and PPARγ antagonists	CBD (not specific)	CBD was neuroprotective and its effect depended on TRPV2 and 5-HT1A receptors
10	Rathod et al., 2023[41]	In vitro analysis	CBR1 or CBR2 modulations result in reduced inflammatory cytokines such as IL-2 and IL-6, as well as a reduction in microglial activation, which	CBD (not specific)	CBD inhibits the NLRP3 inflammasome complex, which may offer significant neuroprotection

(continued)

Table I. Continued.

Sr.	Authors	Route of Administration (in vivo /in vitro/ in silico)	Molecular mechanism	Treatment time/ Dosage of agent	Outcome
			contributes to an inflammatory response in neurons		
11	Jiang et al., 2021[42]	In silico and In vivo (APP/PS1 transgenic mice)	Inhibits the Butyrylcholinesterase activity	Novel Cannabidiol-carbamate hybrids (not specific)	C16 (CBD carbamate derivative) significantly ameliorated the scopolamine-induced cognition impairment, and almost entirely recovered the Aβ1→42(iv)- impaired cognitive function to the normal level, showed better behavioral performance than donepezil and good anti-amyloidogenic effect
12	Wang et al., 2021[43]	In vivo (transgenic CL2355 strain of C. elegans and SHSY5Y cell line)	Rescued the activity of synaptic Ca^{+2}/calmodulin-dependent protein kinase II (CaMKII) from Aβ1→42 toxicity in C. elegans and in SHSY5Y cell line Aβ1→42 level monitoring	(not specific)	In C. elegans CBD treatment extends lifespan and improved health span and in the SHSY5Y cell line prevented neurite lesions induced by Aβ1→42 and increased the expression of fatty acid amide hydrolase (FAAH) and cannabinoid receptor 1 (CB1R)
13	Watt et al., 2020[44]	In vivo (4-month-old male TAU58/2 transgenic mice)	Motor impairment	CBD (50 mg/kg) starting 3 weeks before behavioral assessments	Motor deficits and changes in anxiety at this age do not impact on social domains
14	Watt et al., 2020[45]	In vivo (12-month-old AβPP$_{swe}$/PS1ΔE9 transgenic mice)	CBD tended to reduce insoluble Aβ40 levels in the hippocampus of AβPPxPS1 mice	50 m g/kg Cannabidiol	Improves cognition and moderately reduces Aβ40 levels
15	Yang et al., 2022[46]	In vivo (wild-type mice)	CBD increased the expression of microglial phagocytic receptors though TRPV2 activation and improved energy metabolism via mitochondrial functions in microglial cells in response to Aβ	CBD (5 μM)	TRPV2 expression was reduced in the cortex and hippocampus of AD model mice and Ad patients
16	Zhang et al., 2022[47]	In vivo (C. elegans CL4176)	CBD delayed the paralysis of CL4176 worms in a concentration-dependent manner in the range of 0–100 μM	CBD (0–100 μM)	The phenolic hydroxyl groups of CBD are critical for enhancing the oxidative stress resistance of worms, alleviating the aggregation of Aβ in vivo, and preventing the progression of AD
17	Fangyuan et al., 2023[48]	In vivo/In vitro (C. elegans CL4176)	Inhibit Aβ40 aggregation in vitro and found that β-asarone and CBD at a concentration of 2.5 μg/mL had a superior inhibitory effect	CBD (100 μM)	β-asarone and CBD shows inhibitory effects.
18	Kyudam et al., 2023[49]	In silico	Mutuation in APP and PSE1 can lead to early AD	Not specific	ENOS, MPO, APOE, APP, ADAM10, and PSEN1 with CBD from which APP and PSE1 complexes shows higher conformational stability and lower interaction energy
19	Juyong et al., 2023[50]	In vivo (Mice)	BDA and THCA have anti-AD effects and mitigate	CBDA (6 μM) and THCA (12 μM)	CBDA and THCA treated mice showed decreased

(continued)

Table I. Continued.

Sr.	Authors	Route of Administration (in vivo /in vitro/ in silico)	Molecular mechanism	Treatment time/ Dosage of agent	Outcome
			memory loss and resilience to increased hippocampal Ca^{+2}, Aβ, and p-tau levels		hippocampal Aβ and p-tau compared with Aβ1–42-treated mice
20	Long et al., 2023[51]	In vitro/In vivo (Mice C57/BL6)	Administration of CBD demonstrated a protective effect against Aβ toxicity both in vitro and in vivo, along with an amelioration of cognitive impairment in mice	CBD (0.25 μM-5 μM)	CBD's potential in ameliorating cognitive deficits in AD, primarily through synaptic restoration, neuronal protection, and the inhibition of glial activation and inflammatory factor release.
21	Nil et at., 2023[52]	In silico	THCV, Cannabinol C2, and Cannabidiorcol compounds are docked with AChE (Acetylcholinesterase), BuChE (Butyrylcholinesterase), γ-secretase, and BACE-1 enzymes.	Not specific	Enol and keto groups shows significant binding with AChE and BuChE

with amyloid clearance or prevent amyloid formation. Much like its enhancement of contextual memory in an AD mouse model, CBD increased the expression of IL-33 in astrocytes, a cytokine that stimulated microglial amyloid phagocytosis. Consequently, CBD could potentially serve as a safe and effective disease-modifying treatment to delay neurocognitive decline, partially by altering the function of glial cells.[32] Wistar male rats were administered 3 mg/kg of STZ, with [18F] FDG PET scans taken before and seven days after the injection. The animals were treated with either saline (STZ-saline) or 20 mg/kg of cannabidiol for a week. Novel object recognition assesses short- and long-term memory. The STZ-saline group exhibited significant reductions in [18F] FDG uptake in the whole brain, particularly in a hypometabolism cluster near the lateral ventricle. STZ-cannabidiol rats had a smaller cluster, and specific regions like the motor cortex, hippocampus, and thalamus showed less hypometabolism compared to STZ-saline animals. Memory performance was unchanged in the STZ-cannabidiol group. This suggests that cannabidiol may hold promise as an early treatment for the neurodegenerative processes observed in AD.[33]

Concerning in vivo studies to define the mechanism by which CBD reduces AD, the model organism *C. elegans* was selected. Transgenic *C. elegans* models have been created for several diseases, including AD. The advantages of using *C. elegans* over rat animal models include its short lifespan, low cost, ease of manipulation, and accessibility to a large number of multicolor reporter structures. The APP and tau genes share 38% of their genome sequences with *C. elegans*, according to Consortium and Elegans Sequencing (1998). Our understanding of the etiology and pathophysiology of AD as well as the molecular mechanisms behind treatment action has further advanced thanks to the discovery of transgenic *C. elegans* worms that express the human Aβ or tau protein. This study found that CBD prevented Aβ aggregation *in vivo*, which substantially reduced the course of AD. Importantly, it was found that CBD's phenolic hydroxyl groups are essential for slowing AD's progression. This study suggests CBD's anti-inflammatory qualities give it its anti-AD therapeutic effects.[34] Therapeutic benefits of CBD have been observed in APPxPS1 mice at different doses (ranging from 5 to 50 mg/kg and using CBD-enriched extract). Chronic CBD treatment reduces astrocytic response and the expression of the cell surface adhesion molecule CCL4 in the APPxPS1 microenvironment. However, its impact on Aβ levels in the hippocampus is only modest. Nevertheless, there is currently no evidence demonstrating a significant effect of therapeutic CBD treatment on other AD-related receptors and molecules in APPxPS1 mice, including brain-derived neurotrophic factor, PPARγ, ionized calcium-binding adaptor molecule 1 (IBA1), and various cytokines.[35]

CBD receptors present in the brain

CBD's lipophilic features led to the assumption that they cause various physiological functions via inhibiting cell membranes in an unstructured way.[80] Type 1 and type 2 cannabinoid receptors, respectively, make up the endocannabinoid system CB1 receptors, which are G protein-coupled receptors (GPCRs) linked to the Gi/o protein (G protein alpha subunits), inhibit intracellular adenylyl cyclase and N-type calcium channels when activated.[81] CB1 receptors have not been found in human microglia; however, they are found in the CNS, predominantly on neurons.[82] In addition, direct CB1 activation is said to be the most common GPCR in the mammalian brain and contains some adverse effects on the CNS which may be severe.[83] While inhibiting FAAH typically elevates anandamide levels and consequently boosts CB1 activation, it also leads to an increase in the levels of other endogenous FAAH substrates that activate various receptors. These receptors include the transient receptor potential cation channel subfamily V part 1 (TRPV1), the PPAR, the orphan GPCR 119 (GPR119), and the orphan GPCR 55 (GPR55).[84] Peripheral immune cells primarily contain the CB2 receptor.[85] CB2 is mostly present in microglia cells and only weakly expressed in the brain under normal circumstances.[86] Additionally, it was possible to distinguish a modest level of CB2 expression on neurons, where its activation controls neuronal excitability.[87] CB2 expression is elevated on microglia when there is an inflammatory response, as well as on T-cells, macrophages, or astrocytes. However, the inflammatory environment influences expression levels. Following an attempt with LPS or IFN-γ, microglia treated with CB2 agonists had a decreased pro-inflammatory response.[88–90] Recently, a study unveiled the structural changes of CB1R upon agonist binding, elucidating the conformational mechanism responsible for the diverse structures and signaling biases of CB1R agonists.[91] CBD is believed to act as a negative allosteric modulator (NAM) for both CB1R and CB2R. CB1R certainly serves as an NAM, regulating CB1 signaling when CBD are present. The most prevalent endogenous cannabinoid signaling neuronal model is CB1 NAMs, which are found in autaptic hippocampal neurons.[92] CBD's capacity to inhibit the CB2R active state (NAM activity) is a result of the pentyl chain's insertion into the hydrophobic pocket between TMs 2, 3, and 7. The activation of CB2R with various-length CBD analogs and in addition to the canonical long-form.[93,94] Two other CB1R isoforms with short N-termini that also result from alternative splicing have been discovered. Current investigations in non-human models found inconsistencies thus the biological[95] and pharmacological features of all the dual splice variants are still to be investigated. In comparison to the CB1R, the CB2R exhibits more species variations between humans and rats, with amino acid sequence similarity of just over 80% among humans and rodents. Two isoforms of CB2R are recognized in humans: one predominantly in the testis and minimally in brain reward regions, and another mainly in the spleen with limited brain presence. Their roles in endocannabinoid-mediated synaptic transmission are unclear.[96–98] CB1R is the most widely expressed GPCR in the brain, with highest levels in the olfactory bulb, cerebellum, basal ganglia, and hippocampus, and moderate expression in areas like the hypothalamus, amygdala, and cerebral cortex.[89] Mitochondrial CB1R is strongly linked to neuroprotection following cerebral ischemia/reperfusion.[99] CB2R, initially found in macrophages in the spleen, is prominent in immune cells and moderately expressed in peripheral tissues.[100,101] Both CB1R and CB2R are expressed in the CNS, with CB2R regulating brain activity via Ca^{+2}-activated channels.[102,103] The role of CB1 receptors (CB1R) in modulating physiological functions through the endocannabinoid system. CB1R, found in high numbers throughout the human body,[104] each with a range of physiological functions influenced by the CB1R and the endocannabinoid system. CB1R expression varies dramatically in a variety of clinical situations,[105] highlighting its vital function in a wide range of biological processes, influences neurotransmission by regulating the release of GABA and glutamate.[106,107] This receptor protects neurons from excitotoxicity, a harmful process in neurological conditions.[108,109] by reducing NO production, zinc mobilization, and enhancing brain-derived neurotrophic factor expression. It also inhibits calcium influx and glutamate release, highlighting its neuroprotective properties.[110,111]

In numerous neurodegenerative disorders, abnormal activation of the CB1R and other parts of the whole endocannabinoid system has been found.[112,113] Considering the foregoing, it is possible to speculate that Aβ deposition triggers the release of endocannabinoids in the neurons and glia, which stimulate CB1-mediated neuroprotective mechanisms and control the release of inflammatory mediators into microglia into CB2 receptors. Future research may explore the therapeutic potential of CB1/CB2 agonists and FAAH antagonists in AD. Recent pilot studies indicate that daily treatment with dronabinol for two weeks significantly improved overall Neuropsychiatric Inventory scores, including agitation, aberrant motor behavior[114] and nighttime behaviors in late-stage dementia patients (including those with AD and vascular dementia). Elevated CB1 receptor expression and endocannabinoid system activity suggest a possible pathway for compensating dopaminergic neuron degradation in the substantia nigra. Preclinical studies in AD animal models have also shown benefits from CB1R modulation.[115] The protective effects of Arachidonyl-2'-chloroethyl amide (ACEA) upon AE-induced memory impairment were eliminated when CB1 receptor blockade by AM251 was applied. Rats co-treated with AE + WIN + AM251 with AE + ACEA + AM251 did not display any memory-protecting effects.[116]

The CB2R plays a crucial role in immune function within the CNS and peripheral areas. Microglia, a persistent community of cells similar to macrophages, play a major role in this function. In pathological situations, microglia act as immune effector/accessory cells, moving and multiplying during damage and inflammation. They release cytokines like interleukin-1, IL-6, and tumor necrosis factor-α, which express MHC class I and II antigens and the CD11/CD18 complex complementing receptor.[117] Additionally, phagocytic, microglia can digest antigens and perform cytolytic actions when activated. Contrarily, these cells have been linked to nervous system disorders such as MD, AD, Parkinson's disease, and even acquired immune deficiency syndrome (AIDS) dementia in addition to their involvement in host defense and tissue regeneration in the CNS.[118]

Molecular mechanisms of CBD in AD (all the mechanisms)

Due to its anti-inflammatory and antioxidant effects, CBD's neuroprotective benefits are aimed at modulating receptors and channels implicated in neurodegenerative disorders.[117] G protein-coupled receptors, TRPV1, and PPARγ are just a few of the non-endocannabinoid signaling systems that CBD interacts with According to[113]; the GABAergic modulation of CBD may also contribute to its therapeutic effects Figure 3.

Rheostats for adenosine

ARs (A1R, A2AR, A2BR, and A3R) are endogenous adenosine-induced GPCRs that play a part in a variety of physiologic processes. Adenosine stimulation of A1R and A3R ends in the stimulation of repressive G (Gi/o) proteins,[119] which inhibits adenylate cyclase alongside reducing cyclic adenosine monophosphate (cAMP) within the cells. A2AR and A2BR, on another side, induce G protein activity and, as a result, an increase in cAMP. ARs stimulation includes the alteration of secondary messengers and supplementary signaling channels like that of phospholipase C, a Ca^{+2}-dependent protein kinase associated with cell interaction; phosphoinositide 3-kinases signaling convoluted during cell differentiation,[120] proliferation and growth; and ion channel initiation and Ca^{+2} modulation. Although CBD appears to work through a variety of signaling pathways,[121] it appears that A2AR is involved in its anti-inflammatory actions. CBD's anti-inflammatory impact is mediated directly through A2AR, whose activation causes the immune response to be regulated and pro-inflammatory cytokines to be reduced. In healthy conditions, the striatal neurons have a high level of A2AR expression compared to glial cells and neurons outside of the striatum. Additionally, this receptor subtype is found in the olfactory tubercle, caudate putamen, and nucleus accumbens. It is important to note that brain injuries cause microglia to express more

A2AR, which results in signal transductions that do not happen in cells that express the receptors normally,[122] such as promoting the release of cytokines. Strong support was offered for an A2AR-CB1R interaction taking place in the hippocampus by merging *in vivo* and complementing molecular approaches. The A2AR/CB1R oligomer appears to be important for how well CBD can counteract the cognitive impairment brought on by the psychoactive cannabis constituent 9-THC in a declarative and spatial memory exercise.[123] The pretreatment with the selectively pre- and postsynaptic A2AR antagonist SCH442416 as well as KW-6002, respectively, revealed for the first time that this CBD action was primarily reliant on presynaptic A2AR receptor activation.[124] Microglial activation results in an increase in CB2 and A2A receptor expression.[125] The goal was to identify microglial heteromer[126] and any variations in A2A-CB2Hets expression between dormant and active microglial cells. The outcomes from signal transduction assessments in resting microglia were consistent with those observed in co-transfected HEK-293 T cells. Through evaluations of cytosolic cAMP levels, ERK1/2 phosphorylation, and DMR, it was revealed that the A2AR and CB2R agonists generated a distinct and non-additive signal when applied together, resembling the signal achieved with CGS21680.[126] CBD tends to improve adenosine signaling,,[127] as a result, there is a rise in extracellular adenosine as well as a decrease in neuroinflammation. In cultured N13 cells and native microglial cells, CBD reduced ATP-activated intra-cellular Ca^{+2} rise. Over N13 microglial cells including basic rat microglia, the application of ZM241385. CB2 appears to be more crucial in controlling[128,129] the activation of microglia after stroke. Targeting CB1 is limited in therapeutic potential due to its widespread presence in the brain among both neurons and glia, the psychotropic effects of agonists like phytocannabinoids or synthetic CB1R agonists, and the adverse effects associated with the use of rimonabant, a selective CB1R ligand, in humans. Recently, paeoniflorin, a prominent constituent of Paeonia lactiflora, has been found to activate CB2R, which plays a role in regulating the polarization of microglia and macrophages in the CA1 area of the hippocampus in vascular dementia models.[130,131] *In vivo*, CBD with quite a dosage of 20 mg/kg was in a position to intercept amyloid-β-induced consequent disfigurement. CBD did lower the assertion of the gene-producing IL-6, a pro-inflammatory cytokine, following three weeks following intraperitoneal treatment. As a result of these findings, CBD that interacts with A2AR might be a promising treatment option.[132] In conclusion, CBD has anti-inflammatory effects in AD models via A2AR activation. In cultured N13 and primary microglial cells, CBD-suppressed ATP-induced intracellular Ca^{+2} rise, along with A2AR receptors can be implicated during this process. CBD inhibited the production associated with
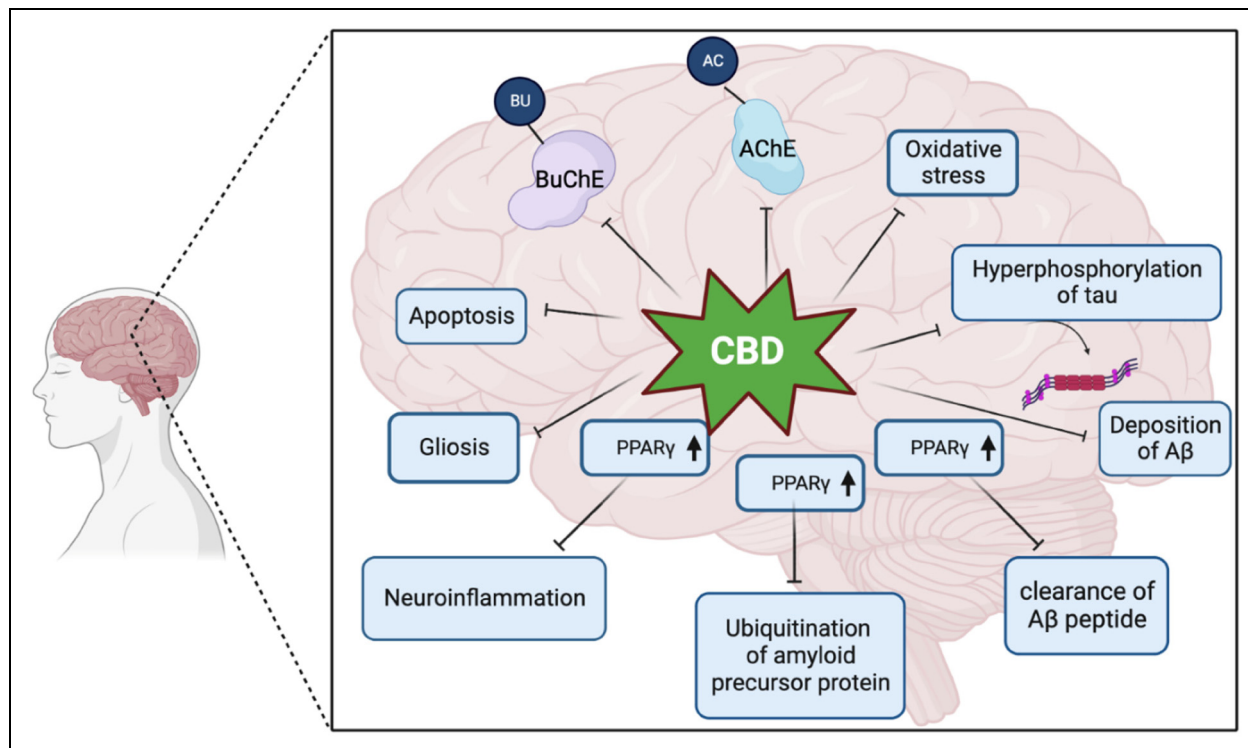
Figure 3. Different molecular mechanism that is affect by CBD in AD brain. BuChE, butyrylcholinesterase; AChE, acetylcholinesterase; PPARγ, peroxisome proliferator-activated receptor.

pro-inflammatory cytokine IL-6 *in vivo*,[133] which reversed the cognitive impairment caused by Aβ.

GPR3, GPR6, and GPR12

GPR3, GPR6, and GPR12 being known as forsaken receptors since their inland agonist is unknown. Before being cloned from human genomic libraries by numerous independent researchers,[134–137] GPR3 was first isolated from a mouse cDNA library in 1993.[138] It was also recently cloned from a library of porcine cDNA[139] GPR3 was located on chromosome 1p35–1p36.1 by Marchese et al.[130] This receptor's location was determined by Song et al.[134] and Iismaa et al.[138] to be 1p34.3-p36.1. Neurons across several brain areas, including the cortex, thalamus, hypothalamus, amygdala, hippocampus, pituitary, and cerebellum, express GPR3 mRNA widely.[136–140] Notably, postmortem brain tissue slices from people with AD show that the GPR3 protein is overexpressed in neurons.[140] Along with other tissues, the eye, lung, kidney, liver, testes, and ovary all express GPR3 mRNA.[136–140] In 1994, a rat cDNA library was used to clone GPR6, which was then given the name rCNL3.[141] Its cloning from human genomic DNA came next in 1995.[134] GPR6's chromosomal location was determined to be 6q21–22.1 by Heiber et al.[142] and Song et al.,[143] respectively. The brain's neurons,

notably those in the striatum (caudate, putamen, nucleus accumbens, and olfactory tubercle), are the primary sites of GPR6 mRNA expression. It can also be found, though to a lower extent, in the frontal cortex, retrosplenial cortex, hippocampus, amygdala, and hypothalamus.[140,141] GPR6 expression seems to be minimal in peripheral tissues. In a study by Song et al.,[142] GPR6 mRNA was not detected in various peripheral organs such as the heart, spleen, lung, liver, muscle, kidney, and testis in rats. GPR12, originally known as R334 in rats[144] and later as GPCR01 in mice,[145] was eventually cloned from human genomic DNA in 1995 and mapped to chromosomal region 13q12.[134] GPR12 mRNA was initially identified in the pituitary gland. In the brain, this transcript is present in neurons located in regions like the frontal cortex, piriform cortex, thalamus, hypothalamus, hippocampus, amygdala, and olfactory bulb. Peripherally, GPR12 mRNA can be found in the testis and oocytes.[140,144–146] However, the transmembrane domains of these types of forsaken receptors have 35% amino acid sequence similarity[147] with both CB1R and CB2R. It was only recently discovered that GPR3, GPR6, and GPR12 constitute new CBD markers. While CBD has numerous known markers, the latest finding in GPR3, GPR6, and GPR12[148] as additional CBD markers offer a fresh choice on the drug's therapeutic potential and benefits that calls for more research. In the β–arrestin 2 assignment experiment,[148] CBD was not the

powerful target for GPR3 (EC501 M) or GPR6 (EC50100 nM). It also is not a very potential target for GPR12-directed cAMP buildup (EC5010 M). CBD, on the other hand, still serves as a molecular framework for the development of new, possibly more powerful,[149] and efficient GPR3, GPR6, and GPR12 agonists. C1q, a protein that binds to Aβ plaques and assists in plaque clearing while simultaneously generating a harmful inflammatory response,[150] has been discovered in people with AD. GPR6 is elevated in AD animal models via a C1q-mediated pathway, in accordance to Benoit et al., 2013. In the mice model, it was discovered that GPR6 can require C1q-mediated neuroprotection with odds to Aβ -induced neurotoxicity. As a result, GPR6 may perform a powerful role in the neuroprotection of AD.[151]

PPARγ

PPARγ is a nuclear receptor that is also a ligand-activated transcription factor that controls overall levels of expression for genes mediators of inflammation, trophic factor synthesis, redox balance, glucose, and lipid metabolism. A ubiquitin E3 ligase is also known as PPARγ. with many lysine residues, notably Lysine48 which is a type of ubiquitin chain that is linked by lysine 48 (Lys48) and serves as the most efficient trigger of protein degradation. It is synthesized by the ubiquitin-conjugating enzyme Ube2 K (also known as E2–25 K and Huntington Interacting Protein 2) that can form polyubiquitin chains. Indeed, PPARγ activation prevents the NF-κB signaling pathway by inhibiting their pro-inflammatory genes transcription,[152] such as TNF-α, IL-6, and IL-1β. It was demonstrated that the pro-neuroprotective effect was reliant on PPARγ receptor activation.[153] Significantly, CBD did not promote neuronal differentiation when a PPARγ antagonist GW9662 (2 μM) was also delivered, which hindered its neuroprotective effects.[154] It is known that GW9662 binds to PPARγ permanently and possesses an IC50 with in nM range.[155] Even if these data are insightful, they require further validation given the numerous protective effects of PPAR receptor activation alone.[149] Neurogenesis and hippocampal function are also essential for the symptomatology of diseases associated with anxiety, depression, and AD.[156] Since the endocannabinoid (eCB) system plays a crucial role in the regulation of neuronal production and lifespan, Kempermann's team first investigated whether a cannabinoid like CBD would increase the longevity of dentate gyrus (DG) newborn neurons in mice.[157,158] An innovative study using NeuN as a marker discovered that a diet rich in CBD significantly increased the co-localized immunoreactivity of 5-bromo-20-deoxyuridine (BrdU) and neuronal nuclei, suggesting pro-neurogenic effects. Notably, an intriguing counterargument was presented against THC's impact on this measure. Demirakca et al. (2010) later supported these pro-neurogenic effects of CBD in humans.[159] The endocannabinoid (ECB) system emerges as a crucial regulator of neonatal

neuron formation, functional integration, survival, and maturation in the adult hippocampus. Endocannabinoid signaling plays a role in the development of neural progenitor cell progeny, which possess a functional ECB system.[160] According to Andrés Mach et al.,[161] CB1 agonists cause neuronal growth and differentiation in the DG, in CB1 /- mice, which are also diminished.[162] Similar evidence has been presented for CB2 receptors, albeit in a more complicated way.[163,164] In other words, CB1 receptors help to maintain adult neurogenesis while CB2 receptors appear to aid the recovery from allos9otatic neurogenic conditions.[165] Most of the fundamental mechanisms behind memory loss are unclear. Epistolary memory, spatial identification, and visual and semantic orientation are all affected by early memory problems in AD.[166] The DG within the hippocampus serves as a neurogenic niche where neural stem cells differentiate into new neurons that become part of the granular cell layer.[167] These newly generated DG cells in the adult brain integrate into neural networks and contribute to hippocampal function.[168] The DG mediates the learning, extinction, and retrieval phases of contextual fear conditioning, with the latter two exhibiting distinct neural patterns.[169] In the erasure of memories, contextual discrimination, and hippocampal plasticity, immature neurons play an important role.[170,171] It has also been shown that immature neurons are drawn into networks for spatial memory.[172] Increasing neurogenesis in 5XFAD animals with Nestin-driven CreERT2 predicate Bax deletion restored the density of dendritic spines, increased the number of new neurons chosen into memory engrams, and changed the alterations made to both immature and mature neurons, eventually resulting in the recovery of contextual and spatial memory.[156,173] As a result, PPARγ agonists like CBD, by decreasing downstream gene transcription controlled at NF-κB,[174] may have a type of anti-inflammatory effect via the molecular process monitored by GSK3-β. Using SH-SY5YAPP + cells, as a model of AD[145] investigated this method of action. CBD (10^{-9}–10^{-6} M) was given to SH-SY5YAPP + cells during 24 h. CBD incorporation reduced the levels of expression and ubiquitination of those in the AβPP protein, resulting in a decrease in Aβ and neuronal death. To determine the specific role of PPARγ in mediating CBD's effects in SH-SY5YAPP + cells, CBD was manipulated using MK886 (3 μM) and GW9662 (9 nM), which are specific agonists of PPARα and PPARγ, respectively. Treatment with GW9662 (9 nM) led to a significant reduction in the effectiveness of CBD, indicating that the neuroprotective actions of CBD are mediated through PPARγ receptors. This suggests that CBD may have potential for AD treatment, partly due to its interaction with PPARγ. In an experiment using C57/black 6 mice, CBD was administered to hippocampus sections 30 min before soluble oligomer $Aβ_{1-42}$ injection. CBD treatment enhanced synaptic potentiation and transmission over time, safeguarding against cognitive deficits induced by $Aβ_{1-42}$. To better understand CBD's mechanisms, the perfusate was pre-treated with WAY 100635 (300 nM;

5-HT1A antagonist), ZM241385 (100 nM; A2AR antagonist), AM 251 (2 mM; CB1 inverse agonist), or GW9662 (2 mM; PPAR antagonist).[145] It was discovered that only GW9662 therapy reduced CBD's neuroprotective properties.[154] CBD is the cannabinoid with less affinity for PPARγ receptors from THCA, THC, CBGA, CBG, CBDA, and CBD[175] and this opens the door to new possible therapeutic options using acidic cannabinoids targeting PPARγ receptors at smaller dosages and in a more effective form.

Transient receptor potential channels (TRP)

TRP comprises a group of ion channels that are mostly found on cell membranes present in animal cells. CBD may bind with TRP,[176] hence reducing neuroinflammation. TRPV1, a member of the Vanilloid subfamily of TRP channels, acts as a robust antagonist of CBD. CBD stimulates TRPV1, which subsequently triggers the PI3 K/Akt signaling pathway, leading to the inhibition of glycogen synthase kinase 3. The inhibition of GSK-3 results in increased activity of the Wnt/β-catenin pathway,[177] which has a neuroprotective effect concerning neurotoxicity and oxidative stress that causes AD. CBD therapy inhibited hyperphosphorylation in tau protein-mediated to GSK-3β and β-catenin in Aβ-induced PC12 neurons, in keeping with this data for the *in vitro* model of AD. CBD also reduced Aβ-contents using SH-SY5Y transfected cells with amyloid progenitor (SH-SY5YAPP+),[145] as well and its treatment improved cognitive damage caused by AD in mice models. In a previous study, the research team investigated the role of TRPV2 in the biological mechanisms of CBD by comparing the behavior of human gingival mesenchymal stem cells treated with CBD (5 μM) and those that were not. CBD was found to suppress the expression of genes associated with AD, as revealed in a transcriptome analysis.[178]

CBD and its derivatives in clinical trials

Mechoulam et al. initially identified the structure of CBD in 1963.[179] In 1977, researchers identified two optical isomers and determined the crystal structure of CBD. These isomers vary in the stereochemistry of the linkage between the resorcinol molecule and the terpene core, with the aromatic and terpene ring nearly perpendicular to each other. Natural cannabis plants contain [–]-CBD, but [–]-CBD can only be synthesized through chemical methods. According to the most recent research, all CBD-type cannabinoids that are present in nature have a consistent structure. Of [–]-Trans-[1R, 6R], which is thought to correspond to a negative optical rotation. There are some derivatives of CBD, which are used in clinical trials with their structures and functions (Table 2).

Metabolism and toxic side effects of CBD in AD

Metabolism. CBD is primarily metabolized by the liver enzymes cytochrome P450 (CYP), particularly CYP2C19 and CYP3A4.[189] These enzymes break down CBD into various metabolites, some of which have therapeutic effects. The main metabolite of CBD is 7-hydroxycannabidiol (7-OH-CBD), which is known to be equally active as CBD.[190] AD is a neurodegenerative disorder characterized by the buildup of abnormal protein deposits in the brain. These deposits, called plaques and tangles, are thought to disrupt brain cell function and communication, leading to cognitive decline and memory loss. The potential interaction is the blood-brain barrier (BBB). The BBB is a highly selective membrane that protects the brain from harmful substances in the bloodstream.[191] CBD may have the ability to cross the BBB,[192] although the exact mechanisms are not fully understood. If impaired by AD, the BBB could allow for greater passage of CBD and its metabolites into the brain, potentially affecting its therapeutic effects.[193] AD may impair liver function, which could affect how CBD is metabolized.[194] A compromised liver might not be able to process CBD as efficiently, leading to higher levels of CBD and its metabolites in the body. This could potentially increase the risk of side effects.

Toxic side effects. CBD is generally well-tolerated, but it can cause side effects, especially at high doses.[195] While research on CBD for AD is ongoing, some potential side effects of CBD use to be aware of include:

- Liver problems: CBD can interact with the liver enzymes responsible for metabolizing drugs. This can lead to elevated liver enzymes in some people, especially those taking other medications.
- Diarrhea: CBD can cause loose stools, especially at higher doses.
- Fatigue: CBD may cause drowsiness or fatigue, especially when taken in high doses.
- Drug interactions: CBD can interact with certain medications, including blood thinners, antidepressants, and anti-seizure medications. It is important to talk to your doctor about all medications you're taking before starting CBD.

Limitations of CBD in Alzheimer's disease

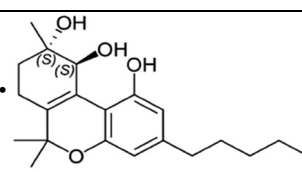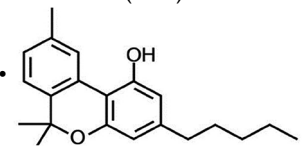Cannabis and cannabis-derived compounds have not been approved by the Food and Drug Administration (FDA) to treat AD, and very few trials to evaluate the use of THC (dronabinol and nabilone) or CBD have been done or are ongoing. For example, Nabilone, a synthesized cannabinoid currently approved for the treatment of chemotherapy-related nausea and vomiting, was found effective in reducing symptoms of

Table 2. Derivatives of CBD and their various function.

• Structure	• Functions	• Ref.
 [−]-Δ9-trans-Tetrahydrocannabinol (Δ9 − THC)	• THC-induced CB1 receptor activation, such as psycho activity, actually have more positive therapeutic effects than either Phytocannabinoid alone.	• 180
 [−]-Δ8- trans-Tetrahydrocannabinol (Δ8 − THC)	Potentiated the reversal of cocaine- and amphetamine-induced conditioned place-preference learning. A CB1 receptor blocker, indicating that additional neurochemical mechanisms may be at play, did not abolish this effect.	• 181
 Cannabigerol (CBG)	Nitro tyrosine, SOD1, and iNOS protein levels were all decreased by CBG pre-treatment, whereas Nrf-2 levels were increased.	• 182
 Cannabidiol (CBD)	• Reduce tau hyper-phosphorylation, control microglial cell migrations, lower A production by encouraging APP ubiquitination, stop hippocampal and cortical neurodegeneration, guard against A-mediated neurotoxicity and microglial-activated neurotoxicity, and enhance cell viability.	• 183
 Cannabielsoin (CBE)	CBE contains anti-inflammatory, antispasmodic, and analgesic properties that may be useful to people seeking a natural therapeutic option to conventional medicine. Due to its calming and relaxing effects, CBE can also aid in promoting overall well-being by easing stress and anxiety.	• 184
 Cannabichromene (CBC)	Antidepressant, anti-acne, neuroprotective, and anti-cancer benefits.	• 185
 Cannabicyclol (CBL)	• Anti-inflammatory, antibacterial, neurogenic, and antiemetic properties prevent or alleviate nausea and vomiting.	• 186

Table 2. Continued.

• Structure	• Functions	• Ref.
Cannabitriol (CBT)	• Reduce some of the THC's euphoric effects	• 187
Cannabinol (CBN)	• An agent with antibacterial, anti-inflammatory, painkilling, anti-insomnia, and anticonvulsant properties.	• 188

AD patients.[196] However, to patient safety, is become very important to closely monitor side effects such as sedation and may be cognitive decline. Considering cannabinoids as a therapeutic option, identifying an effective dosage and treatment time is challenging. It is known that molecular changes related to AD began several years before symptoms seen. As a result, neuroprotective and immunomodulatory effects of cannabinoids should be administered before AD is aggressive and prolonged in time. However, studies on the long-term effects of cannabinoids are not yet available. While studies on the long-term cognitive effects of heavy cannabis use suggest, cannabis negatively influences cognitive functions, such as episodic memory, attentional control, and motor inhibition.[197] for this reason, further studies to explore the short- and long-term effects of cannabinoids are urgently needed. Unfortunately, studies investigating cannabinoid drug-drug interactions are still limited. Several investigations would be fundamental to underpinning this critical point, considering patients with dementia take multiple medications, and cannabinoids could be included as an additional therapeutic strategy to tackling the symptoms of dementia.

Conclusion

In conclusion, the finding of this study indicates that cannabidiol/derivatives inhibit AD progression through various mechanisms and key hypotheses regarding AD pathology. Nave CBD can reduce Aβ, IL-6 expression in peripheral leukocytes, and retarded cognitive decline. Compare with other CBD derivatives, CBD carbamate derivatives notably reduced $A\beta_{1-42}$ levels, restored cognitive function to a normal state, and exhibited superior behavioral performance when compared to donepezil. CBD decreases Caspase 9, Caspase 3, and cleaved PARP1 protein levels and shows Antiapoptotic effects during cognitive decline. It also shows anti-cholinergic activity by inhibiting AChE and BuChE.

As a result, the expression of ChAT can be normalized. In terms of the neuroinflammatory process, the expression of proinflammatory miRNAs (miR-146a, miR-155, and miR-34a) associated with TLR and NF-κB signaling is reduce. Therefore, continued research efforts should focus on elucidating the precise mechanisms of action, exploring potential synergies with other AD medications, and optimizing CBD formulations and derivatization to maximize therapeutic benefits in AD patients. These observations underscore the significance of further research and exploration into the therapeutic applications of CBD in the context of AD.

ORCID iDs

Mukul Jain ⓘ https://orcid.org/ 0000-0003-3739-1247
Arifullah Mohammed ⓘ https://orcid.org/ 0000-0001-7793-8134
Zulhazman Hamzah ⓘ https://orcid.org/ 0000-0002-4837-4605
Norazlina MatNawi ⓘ https://orcid.org/0000-0002-0476-1398

Statements and declarations

Author contributions
Nil Patil (Conceptualization; Data curation); Khushalika Patil (Conceptualization; Data curation); Mukul Jain (Investigation; Supervision; Validation; Writing – original draft; Writing – review & editing); Arifullah Mohammed (Formal analysis); Prashant Kaushik (Project administration; Resources); Alpa Yadav (Formal analysis; Investigation); Parmdeep Singh Dhanda (Investigation; Methodology); Chittaranjan Kole (Project administration; Resources); Kirtan Dave (Resources; Software); Zulhazman Hamzah (Formal analysis; Investigation; Methodology; Project administration); Mohammad Khairul Azhar Abdul Razab

References

1. Koedam EL, Lauffer V, Van der Vlies AE, et al. Early-versus late-onset Alzheimer's disease: more than age alone. *J Alzheimers Dis* 2010; 19: 1401–1408.

2. Zhang XX, Tian Y, Wang ZT, et al. The epidemiology of Alzheimer's disease modifiable risk factors and prevention. *J Alzheimers Dis* 2021; 8: 313–321.

3. Mura T, Dartigues JF and Berr C. How many dementia cases in France and Europe? Alternative projections and scenarios 2010–2050. *Eur J Neurol* 2010; 17: 252–259.

4. Watt G and Karl T. In vivo evidence for therapeutic properties of cannabidiol (CBD) for Alzheimer's disease. *Front Pharmacol* 2017; 8: 20.

5. Nichols E. *Estimating the global mortality from Alzheimer's disease and other dementias: a new method and results from the Global Burden of Disease Study 2019.* Doctoral Dissertation, University of Washington, 2019.

6. Aso E, Sánchez-Pla A, Vegas-Lozano E, et al. Cannabis-based medicine reduces multiple pathological processes in AbetaPP PS1 mice. *J Alzheimers Dis* 2015; 43: 977–991.

7. Casarejos MJ, Perucho J, Gomez A, et al. Natural cannabinoids improve dopamine neurotransmission and tau and amyloid pathology in a mouse model of tauopathy. *J Alzheimers Dis* 2013; 35: 525–539.

8. Pertwee RG. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. *Br J Pharmacol* 2008; 153: 199–215.

9. Dos Santos RG, Hallak JE and Crippa JA. Neuropharmacological effects of the main phytocannabinoids: a narrative review. *Adv Exp Med Biol* 2021; 1264: 29–45.

10. Friedman D and Sirven JI. Historical perspective on the medical use of cannabis for epilepsy: ancient times to the 1980s. *Epilepsy Behav* 2017; 70: 298–301.

11. Consroe P and Wolkin A. Cannabidiol–antiepileptic drug comparisons and interactions in experimentally induced seizures in rats. *J Pharmacol Exp Ther* 1977; 201: 26–32.

12. Turkanis SA, Smiley KA, Borys HK, et al. An electrophysiological analysis of the anticonvulsant action of cannabidiol on limbic seizures in conscious rats. *Epilepsia* 1979; 20: 351–363.

13. Vallée A and Lecarpentier Y. Alzheimer disease: crosstalk between the canonical Wnt/beta-catenin pathway and PPARs alpha and gamma. *Front Neurosci* 2016; 10: 459.

14. Rajesh M, Mukhopadhyay P, Bátkai S, et al. Cannabidiol attenuates cardiac dysfunction, oxidative stress, fibrosis, and inflammatory and cell death signaling pathways in diabetic cardiomyopathy. *J Am Coll Cardiol* 2010; 56: 2115–2125.

15. Scuderi C, Stecca C, Bronzuoli MR, et al. Sirtuin modulators control reactive gliosis in an in vitro model of Alzheimer's disease. *Front Pharmacol* 2014; 5: 89.

16. Scuderi C, Stecca C, Valenza M, et al. Palmitoylethanolamide controls reactive gliosis and exerts neuroprotective functions in a rat model of Alzheimer's disease. *Cell Death Dis* 2014; 5: e1419.

17. Cheng D, Low JK, Logge W, et al. Chronic cannabidiol treatment improves social and object recognition in double transgenic APPswe/PS1ΔE9 mice. *Psychopharmacology (Berl)* 2014; 231: 3009–3017.

18. Vallée A, Lecarpentier Y, Guillevin R, et al. Effects of cannabidiol interactions with Wnt/β-catenin pathway and PPARγ on oxidative stress and neuroinflammation in Alzheimer's disease. *Acta Biochim Biophys Sin (Shanghai)* 2017; 49: 853–866.

19. Iuvone T, De Filippis D, Esposito G, et al. The spice sage and its active ingredient rosmarinic acid protect PC12 cells from amyloid-β peptide-induced neurotoxicity. *J Pharmacol Exp Ther* 2006; 317: 1143–1149.

20. Navin N, Kendall J, Troge J, et al. Tumour evolution inferred by single-cell sequencing. *Nature* 2011; 472: 90.

21. Yang S, Du Y, Zhao X, et al. Cannabidiol enhances microglial beta-amyloid peptide phagocytosis and clearance via vanilloid family type 2 channel activation. *Int J Mol Sci* 2022; 23: 5367.

22. Singh K, Bhushan B, Chanchal DK, et al. Emerging therapeutic potential of cannabidiol (CBD) in neurological disorders: a comprehensive review. *Behav Neurol* 2023; 2023: 8825358.

23. Zhang Y, Li H, Jin S, et al. Cannabidiol protects against Alzheimer's disease in C. elegans via ROS scavenging activity of its phenolic hydroxyl groups. *Eur J Pharmacol* 2022; 919: 174829.

24. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Syst Rev* 2021; 10: 89.

25. Lunny C, Brennan SE, McDonald S, et al. Toward a comprehensive evidence map of overview of systematic review methods: paper 1—purpose, eligibility, search and data extraction. *Syst Rev* 2017; 6: 1–27.

26. Falagas ME, Pitsouni EI, Malietzis GA, et al. Comparison of PubMed, scopus, web of science, and google scholar: strengths and weaknesses. *FASEB J* 2008; 22: 338–342.

27. Garg R. Methodology for research I. *Indian J Anaesth* 2016; 60: 640–645.

28. Covidence. http://www.covidence.org (accessed 4 May 2023).

29. Patino CM and Ferreira JC. Inclusion and exclusion criteria in research studies: definitions and why they matter. *J Bras Pneumol* 2018; 44: 84.

30. Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. *BMJ* 2003; 327: 557–560.

31. Fletcher J. What is heterogeneity and is it important? *BMJ* 2007; 334: 94–96.

32. Khodadadi H, Salles ÉL, Jarrahi A, et al. Cannabidiol ameliorates cognitive function via regulation of IL-33 and TREM2 upregulation in a murine model of Alzheimer's disease. *J Alzheimers Dis* 2021; 80: 973–977.

33. de Paula Faria D, Estessi de Souza L, Duran FLS, et al. Cannabidiol treatment improves glucose metabolism and memory in streptozotocin-induced Alzheimer's disease rat model: a proof-of-concept study. *Int J Mol Sci* 2022; 23: 1076.

34. Frandsen J and Narayanasamy P. Effect of cannabidiol on the neural glyoxalase pathway function and longevity of several C. elegans strains including a C. elegans Alzheimer's disease model. *ACS Chem Neurosci* 2022; 13: 1165–1177.

35. Chesworth R, Cheng D, Staub C, et al. Effect of long-term cannabidiol on learning and anxiety in a female Alzheimer's disease mouse model. *Front Pharmacol* 2022; 13: 931384.

36. Alali S, Riazi G, Ashrafi-Kooshk MR, et al. Cannabidiol inhibits tau aggregation in vitro. *Cells* 2021; 10: 3521.

37. Hughes B and Herron CE. Cannabidiol reverses deficits in hippocampal LTP in a model of Alzheimer's disease. *Neurochem Res* 2019; 44: 703–713.

38. Amini M and Abdolmaleki Z. The effect of cannabidiol coated by nano-chitosan on learning and memory, hippocampal CB1 and CB2 levels, and amyloid plaques in an Alzheimer's disease rat model. *Neuropsychobiology* 2022; 81: 171–183.

39. Libro R, Diomede F, Scionti D, et al. Cannabidiol modulates the expression of Alzheimer's disease-related genes in mesenchymal stem cells. *Int J Mol Sci* 2016; 18: 26.

40. Landucci E, Mazzantini C, Lana D, et al. Cannabidiol inhibits microglia activation and mitigates neuronal damage induced by kainate in an in-vitro seizure model. *Neurobiol Dis* 2022; 174: 105895.

41. Rathod SS and Agrawal YO. Phytocannabinoids as potential multitargeting neuroprotectants in Alzheimer's disease. *Curr Drug Res Rev* 2024; 16: 94–110.

42. Jiang X, Zhang Z, Zuo J, et al. Novel cannabidiol-carbamate hybrids as selective BuChE inhibitors: docking-based fragment reassembly for the development of potential therapeutic agents against Alzheimer's disease. *Eur J Med Chem* 2021; 223: 113735.

43. Wang Z, Zheng P, Xie Y, et al. Cannabidiol regulates CB1-pSTAT3 signaling for neurite outgrowth, prolongs lifespan, and improves healthspan in Caenorhabditis elegans of Aβ pathology models. *FASEB J* 2021; 35: e21537.

44. Watt G, Shang K, Zieba J, et al. Chronic treatment with 50 mg/kg cannabidiol improves cognition and moderately reduces Aβ40 levels in 12-month-old male AβPPswe/PS1ΔE9 transgenic mice. *J Alzheimers Dis* 2020; 74: 937–950.

45. Watt G, Chesworth R, Przybyla M, et al. Chronic cannabidiol (CBD) treatment did not exhibit beneficial effects in 4-month-old male TAU58/2 transgenic mice. *Pharmacol Biochem Behav* 2020; 196: 172970.

46. De Petrocellis L, Vellani V, Schiano-Moriello A, et al. Plant-derived cannabinoids modulate the activity of transient receptor potential channels of ankyrin type-1 and melastatin type-8. *J Pharmacol Exp Ther* 2008; 325: 1007–1015.

47. Duan F, Ju T, Song C, et al. Synergetic effect of β-asarone and cannabidiol against Aβ aggregation in vitro and in vivo. *Comput Struct Biotechnol J* 2023; 21: 3875–3884.

48. Choi K, Lee Y, Kim C, et al. An in silico study for expanding the utility of cannabidiol in Alzheimer's disease therapeutic development. *Int J Mol Sci* 2023; 24: 16013.

49. Kim J, Choi P, Park YT, et al. The cannabinoids, CBDA and THCA, rescue memory deficits and reduce amyloid-beta and tau pathology in an Alzheimer's disease-like mouse model. *Int J Mol Sci* 2023; 24: 6827.

50. Chen L, Sun Y, Li J, et al. Assessing cannabidiol as a therapeutic agent for preventing and alleviating Alzheimer's disease neurodegeneration. *Cells* 2023; 12: 2672.

51. Patil N, Chandel V, Rana A, et al. Investigation of Cannabis sativa phytochemicals as anti-Alzheimer's agents: an in silico study. *Plants* 2023; 12: 510.

52. Dash R, Ali MC, Jahan I, et al. The emerging potential of cannabidiol in reversing proteinopathies. *Ageing Res Rev* 2021; 65: 101209.

53. Dos-Santos-Pereira M, Guimarães FS, Del-Bel E, et al. Cannabidiol prevents LPS-induced microglial inflammation by inhibiting ROS/NF-κB-dependent signaling and glucose consumption. *Glia* 2020; 68: 561–573.

54. Kozela E, Pietr M, Juknat A, et al. Cannabinoids Δ9-tetrahydrocannabinol and cannabidiol differentially inhibit the lipopolysaccharide-activated NF-κB and interferon-β/STAT proinflammatory pathways in BV-2 microglial cells. *J Biol Chem* 2010; 285: 1616–1626.

55. Lunn CA, Fine JS, Rojas-Triana A, et al. A novel cannabinoid peripheral cannabinoid receptor-selective inverse agonist blocks leukocyte recruitment in vivo. *J Pharmacol Exp Ther* 2006; 316: 780–788.

56. Li H, Liu Y, Tian D, et al. Overview of cannabidiol (CBD) and its analogs: structures, biological activities, and neuroprotective mechanisms in epilepsy and Alzheimer's disease. *Eur J Med Chem* 2020; 192: 112163.

57. Rock EM and Parker LA. Constituents of Cannabis sativa. *Adv Exp Med Biol* 2021; 1264: 1–13.

58. Ryberg E, Larsson N, Sjögren S, et al. The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol* 2007; 152: 1092–1101.

59. Lauckner JE, Jensen JB, Chen HY, et al. GPR55 Is a cannabinoid receptor that increases intracellular calcium and

inhibits M current. *Proc Natl Acad Sci U S A* 2008; 105: 2699–2704.

60. Marichal-Cancino BA, Fajardo-Valdez A, Ruiz-Contreras AE, et al. Advances in the physiology of GPR55 in the central nervous system. *Curr Neuropharmacol* 2017; 15: 771–778.

61. De Gregorio D, McLaughlin RJ, Posa L, et al. Cannabidiol modulates serotonergic transmission and reverses both allodynia and anxiety-like behavior in a model of neuropathic pain. *Pain* 2019; 160: 136.

62. Costa B, Giagnoni G, Franke C, et al. Vanilloid TRPV1 receptor mediates the antihyperalgesic effect of the nonpsychoactive cannabinoid, cannabidiol, in a rat model of acute inflammation. *Br J Pharmacol* 2004; 143: 247–250.

63. Franco R, Reyes-Resina I, Aguinaga D, et al. Potentiation of cannabinoid signaling in microglia by adenosine A2A receptor antagonists. *Glia* 2019; 67: 2410–2423.

64. Peters JM, Shah YM and Gonzalez FJ. The role of peroxisome proliferator-activated receptors in carcinogenesis and chemoprevention. *Nat Rev Cancer* 2012; 12: 181–195.

65. Elmes MW, Kaczocha M, Berger WT, et al. Fatty acidbinding proteins (FABPs) are intracellular carriers for Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD). *J Biol Chem* 2015; 290: 8711–8721.

66. Silvestro S, Schepici G, Bramanti P, et al. Molecular targets of cannabidiol in experimental models of neurological disease. *Molecules* 2020; 25: 5186.

67. Kathmann M, Flau K, Redmer A, et al. Cannabidiol is an allosteric modulator at mu- and delta-opioid receptors. *Naunyn Schmiedebergs Arch Pharmacol* 2006; 372: 354–361.

68. Esposito G, De Filippis D, Maiuri MC, et al. Cannabidiol inhibits inducible nitric oxide synthase protein expression and nitric oxide production in β-amyloid stimulated PC12 neurons through p38 MAP kinase and NF-κB involvement. *Neurosci Lett* 2006; 399: 91–95.

69. Mooko T, Bala A, Tripathy S, et al. Cannabis sativa L. flower and bud extracts inhibited in vitro cholinesterases and β-secretase enzymes activities: possible mechanisms of cannabis use in Alzheimer disease. *Endocr Metab Immune Disord Drug Targets* 2022; 22: 297–309.

70. Raja A, Ahmadi S, de Costa F, et al. Attenuation of oxidative stress by cannabinoids and cannabis extracts in differentiated neuronal cells. *Pharmaceuticals* 2020; 13: 328.

71. Esposito G, De Filippis D, Carnuccio R, et al. The marijuana component cannabidiol inhibits β-amyloid-induced tau protein hyperphosphorylation through Wnt/β-catenin pathway rescue in PC12 cells. *J Mol Med* 2006; 84: 253–258.

72. Ashrafian H, Zadeh EH and Khan RH. Review on Alzheimer's disease: inhibition of amyloid beta and tau tangle formation. *Int J Biol Macromol* 2021; 167: 382–394.

73. Walter L and Stella N. Cannabinoids and neuroinflammation. *Br J Pharmacol* 2004; 141: 775–785.

74. Iuvone T, Esposito G, Esposito R, et al. Neuroprotective effect of cannabidiol, a non-psychoactive component from Cannabis sativa, on β-amyloid-induced toxicity in PC12 cells. *J Neurochem* 2004; 89: 134–141.

75. Hampson AJ, Grimaldi M, Axelrod J, et al. Cannabidiol and [−] Δ9-tetrahydrocannabinol are neuroprotective antioxidants. *Proc Natl Acad Sci U S A* 1998; 95: 8268–8273.

76. Beccano-Kelly D and Harvey J. Leptin: a novel therapeutic target in Alzheimer's disease? *Int J Alzheimers Dis* 2012; 2012: 594137.

77. Janefjord E, Mååg JL, Harvey BS, et al. Cannabinoid effects on β amyloid fibril and aggregate formation, neuronal and microglial-activated neurotoxicity in vitro. *Cell Mol Neurobiol* 2014; 34: 31–42.

78. Gaoni Y and Mechoulam R. Isolation, structure, and partial synthesis of an active constituent of hashish. *J Am Chem Soc* 1964; 86: 1646–1647.

79. Matsuda LA, Lolait SJ, Brownstein MJ, et al. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 1990; 346: 561–564.

80. Devane CL. Differential pharmacology of newer antidepressants. *J Clin Psychiatry* 1998; 59(Suppl 20): 4491.

81. Hernandez SS, Coelho FG, Gobbi S, et al. Effects of physical activity on cognitive functions, balance and risk of falls in elderly patients with Alzheimer's dementia. *Braz J Phys Ther* 2010; 14: 68–74.

82. Araque A and Navarrete M. Glial cells in neuronal network function. *Philos Trans R Soc Lond B Biol Sci* 2010; 365: 2375–2381.

83. Schmöle AC, Lundt R, Ternes S, et al. Cannabinoid receptor 2 deficiency results in reduced neuroinflammation in an Alzheimer's disease mouse model. *Neurobiol Aging* 2015; 36: 710–719.

84. Maccarrone M, Bab I, Bíró T, et al. Endocannabinoid signaling at the periphery: 50 years after THC. *Trends Pharmacol Sci* 2015; 36: 277–296.

85. Dengel LT, Van Zee KJ, King TA, et al. Axillary dissection can be avoided in the majority of clinically node-negative patients undergoing breast-conserving therapy. *Ann Surg Oncol* 2014; 21: 22–27.

86. Putnam NH, Butts T, Ferrier DE, et al. The amphioxus genome and the evolution of the chordate karyotype. *Nature* 2008; 453: 1064–1071.

87. Ehrhart J, Obregon D, Mori T, et al. Stimulation of cannabinoid receptor 2 (CB2) suppresses microglial activation. *J Neuroinflammation* 2005; 2: 29.

88. Ma X, Zhang Q, Zhu Q, et al. A robust CRISPR/Cas9 system for convenient, high-efficiency multiplex genome editing in monocot and dicot plants. *Molecular Plant* 2015; 8: 1274–1284.

89. Wang L, Guo L, Lu L, et al. Synaptosomal mitochondrial dysfunction in 5xFAD mouse model of Alzheimer's disease. *PLoS One* 2016; 11: e0150441.

90. Zhang J, Chen C, Hua S, et al. An updated meta-analysis of cohort studies: diabetes and risk of Alzheimer's disease. *Diabetes Res Clin Pract* 2017; 124: 41–47.

91. Chen WT, Lu A, Craessaerts K, et al. Spatial transcriptomics and in situ sequencing to study Alzheimer's disease. *Cell* 2020; 182: 976–991.

92. McPartland JM, Norris RW and Kilpatrick CW. Coevolution between cannabinoid receptors and endocannabinoid ligands. *Gene* 2007; 397: 126–135.

93. Atwood BK, Wager-Miller J, Haskins C, et al. Functional selectivity in CB2 cannabinoid receptor signaling and regulation: implications for the therapeutic potential of CB2 ligands. *Mol Pharmacol* 2012; 81: 250–263.

94. Li S, Tan HY, Wang N, et al. The role of oxidative stress and antioxidants in liver diseases. *Int J Mol Sci* 2015; 16: 26087–26124.

95. Dhopeshwarkar A and Mackie K. CB2 Cannabinoid receptors as a therapeutic target—what does the future hold? *Mol Pharmacol* 2014; 86: 430–437.

96. Mackie K. Distribution of cannabinoid receptors in the central and peripheral nervous system. *Handb Exp Pharmacol* 2005; 168: 299–325.

97. Munro S, Thomas KL and Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 1993; 365: 61–65.

98. Howlett AC. The cannabinoid receptors. *Prostaglandins Other Lipid Mediators* 2002; 68: 619–631.

99. Atwood BK and Mackie K. CB2: A cannabinoid receptor with an identity crisis. *Br J Pharmacol* 2010; 160: 467–479.

100. Naudé PJ, Nyakas C, Eiden LE, et al. Lipocalin 2: a novel component of proinflammatory signaling in Alzheimer's disease. *FASEB J* 2012; 26: 2811.

101. Console-Bram L, Brailoiu E, Brailoiu GC, et al. Activation of GPR 18 by cannabinoid compounds: a tale of biased agonism. *Br J Pharmacol* 2014; 171: 3908–3917.

102. McPartland JM, Duncan M, Di Marzo V, et al. Are cannabidiol and Δ9-tetrahydrocannabivarin negative modulators of the endocannabinoid system? A systematic review. *Br J Pharmacol* 2015; 172: 737–753.

103. Pacher P, Bátkai S and Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev* 2006; 58: 389–462.

104. Kano M, Ohno-Shosaku T, Hashimotodani Y, et al. Endocannabinoid-mediated control of synaptic transmission. *Physiol Rev* 2009; 89: 309–380.

105. Marsicano G, Goodenough S, Monory K, et al. CB1 Cannabinoid receptors and on-demand defense against excitotoxicity. *Science* 2003; 302: 84–88.

106. Chiarlone A, Bellocchio L, Blázquez C, et al. A restricted population of CB1 cannabinoid receptors with neuroprotective activity. *Proc Natl Acad Sci U S A* 2014; 111: 8257–8262.

107. Martinez-Martin P, Rodriguez-Blazquez C, Kurtis MM, et al. The impact of non-motor symptoms on health-related quality of life of patients with Parkinson's disease. *Mov Disord* 2011; 26: 399–406.

108. Kim D, Jeong S and Moon J. Synthesis of silver nanoparticles using the polyol process and the influence of precursor injection. *Nanotechnology* 2006; 17: 4019.

109. Brotchie JM. CB1 Cannabinoid receptor signaling in Parkinson's disease. *Curr Opin Pharmacol* 2003; 3: 54–61.

110. Waksman Y, Olson JM, Carlisle SJ, et al. The central cannabinoid receptor (CB1) mediates inhibition of nitric oxide production by rat microglial cells. *J Pharmacol Exp Ther* 1999; 288: 1357–1366.

111. Zou S and Kumar U. Cannabinoid receptors and the endocannabinoid system: signaling and function in the central nervous system. *Int J Mol Sci* 2018; 19: 833.

112. Sharma P, Mehta M, Dhanjal DS, et al. Emerging trends in the novel drug delivery approaches for the treatment of lung cancer. *Chem Biol Interact* 2019; 309: 108720.

113. Ahmad QR, Allen RC, Andersen TC, et al. Direct evidence for neutrino flavor transformation from neutral-current interactions in the Sudbury Neutrino Observatory. *Phys Rev Lett* 2002; 89: 011301.

114. Carlsson GE, Möller A, Blomstrand C, et al. European stroke initiative recommendations for stroke management–update 2003. *Cerebrovasc Dis* 2003; 16: 311–337.

115. Van der Stelt M, Mazzola C, Esposito G, et al. Endocannabinoids and β-amyloid-induced neurotoxicity in vivo: effect of pharmacological elevation of endocannabinoid levels. *Cell Mol Life Sci* 2006; 63: 1410–1424.

116. Cabral GA, Raborn ES, Griffin L, et al. CB2 Receptors in the brain: role in central immune function. *Br J Pharmacol* 2008; 153: 240–251.

117. Premoli M, Aria F, Bonini SA, et al. Cannabidiol: recent advances and new insights for neuropsychiatric disorders treatment. *Life Sci* 2019; 224: 120–127.

118. Bakas T, Van Nieuwenhuijzen PS, Devenish SO, et al. The direct actions of cannabidiol and 2-arachidonoyl glycerol at GABAA receptors. *Pharmacol Res* 2017; 119: 358–370.

119. White JA, McKinney BC, John MC, et al. Conditional forebrain deletion of the L-type calcium channel CaV1.2 disrupts remote spatial memories in mice. *Learn Mem* 2008; 15: 1–5.

120. Acton D, Ren X, Di Costanzo S, et al. Spinal neuropeptide Y1 receptor-expressing neurons form an essential excitatory pathway for mechanical itch. *Cell Rep* 2019; 28: 625–639.

121. Bettelli E, Carrier Y, Gao W, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 2006; 441: 235–238.

122. Illes P, Rubini P, Ulrich H, et al. Regulation of microglial functions by purinergic mechanisms in the healthy and diseased CNS. *Cells* 2020; 9: 1108.

123. Aso Y, Ray RP, Long X, et al. Nitric oxide acts as a cotransmitter in a subset of dopaminergic neurons to diversify memory dynamics. *Elife* 2019; 8: e49257.

124. Orru M, Bakešová J, Brugarolas M, et al. Striatal pre-and postsynaptic profile of adenosine A2A receptor antagonists. *PLoS One* 2011; 6: e16088.

125. Concannon RM, Okine BN, Finn DP, et al. Differential upregulation of the cannabinoid CB2 receptor in neurotoxic and inflammation-driven rat models of Parkinson's disease. *Exp Neurol* 2015; 269: 133–141.

126. Franco-Duarte R, Černáková L, Kadam S, et al. Advances in chemical and biological methods to identify microorganisms—from past to present. *Microorganisms* 2019; 7: 130.

127. Magen I, Avraham Y, Ackerman Z, et al. Cannabidiol ameliorates cognitive and motor impairments in mice with bile duct ligation. *J Hepatol* 2009; 51: 528–534.

128. Schmidt W, Schäfer F, Striggow V, et al. Cannabinoid receptor subtypes 1 and 2 mediate long-lasting neuroprotection and improve motor behavior deficits after transient focal cerebral ischemia. *Neuroscience* 2012; 227: 313–326.

129. Zarruk JG, Fernández-López D, García-Yébenes I, et al. Cannabinoid type 2 receptor activation downregulates stroke-induced classic and alternative brain macrophage/microglial activation concomitant to neuroprotection. *Stroke* 2012; 43: 211–219.

130. Sam AH, Salem V and Ghatei MA. Rimonabant: from RIO to ban. *J Obes* 2011; 2011: 432607.

131. Kong Y, Jiang B and Luo X. Gut microbiota influences Alzheimer's disease pathogenesis by regulating acetate in Drosophila model. *Future Microbiol* 2018; 13: 1117–1128.

132. Martín-Moreno AM, Reigada D, Ramírez BG, et al. Cannabidiol and other cannabinoids reduce microglial activation in vitro and in vivo: relevance to Alzheimer's disease. *Mol Pharmacol* 2011; 79: 964–973.

133. Tambe SM, Mali S, Amin PD, et al. Neuroprotective potential of cannabidiol: molecular mechanisms and clinical implications. *J Integr Med* 2023; 21: 236–244.

134. Song ZH, Modi W and Bonner TI. Molecular cloning and chromosomal localization of human genes encoding three closely related G protein-coupled receptors. *Genomics* 1995; 28: 347–349.

135. Marchese A, Docherty JM, Nguyen T, et al. Cloning of human genes encoding novel G protein-coupled receptors. *Genomics* 1994; 23: 609–618.

136. Iismaa TP, Kiefer J, Liu ML, et al. Isolation and chromosomal localization of a novel human G-protein-coupled receptor (GPR3) expressed predominantly in the central nervous system. *Genomics* 1994; 24: 391–394.

137. Eggerickx D, Denef JF, Labbe O, et al. Molecular cloning of an orphan G-protein-coupled receptor that constitutively activates adenylate cyclase. *Biochem J* 1995; 309: 837–843.

138. Saeki Y, Ueno S, Mizuno R, et al. Molecular cloning of a novel putative G protein-coupled receptor (GPCR21) which is expressed predominantly in mouse central nervous system. *FEBS Lett* 1993; 336: 317–322.

139. Zhang B, Ding J, Li Y, et al. The porcine Gpr3 gene: molecular cloning, characterization, and expression level in tissues and cumulus–oocyte complexes during in vitro maturation. *Mol Biol Rep* 2012; 39: 5831–5839.

140. Hinckley M, Vaccari S, Horner K, et al. The G-protein-coupled receptors GPR3 and GPR12 are involved in cAMP signaling and maintenance of meiotic arrest in rodent oocytes. *Dev Biol* 2005; 287: 249–261.

141. Thathiah A, Spittaels K, Hoffmann M, et al. The orphan G protein–coupled receptor 3 modulates amyloid-beta peptide generation in neurons. *Science* 2009; 323: 946–951.

142. Heiber M, Docherty JM, Shah G, et al. Isolation of three novel human genes encoding G protein-coupled receptors. *DNA Cell Biol* 1995; 14: 25–35.

143. Song ZH, Young WS III, Brownstein MJ, et al. Molecular cloning of a novel candidate G protein-coupled receptor from rat brain. *FEBS Lett* 1994; 351: 375–379.

144. Eidne KA, Zabavnik J, Peters T, et al. Cloning, sequencing, and tissue distribution of a candidate G protein-coupled receptor from rat pituitary gland. *FEBS Lett* 1991; 292: 243–248.

145. Ignatov A, Lintzel J, Hermans-Borgmeyer I, et al. Role of the G-protein-coupled receptor GPR12 as a high-affinity receptor for sphingosylphosphorylcholine and its expression and function in brain development. *J Neurosci* 2003; 23: 907–914.

146. Pratico D, Uryu K, Leight S, et al. Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer's amyloidosis. *J Neurol Sci* 2001; 21: 4183–4187.

147. Laun AS, Shrader SH, Brown KJ, et al. GPR3, GPR6, and GPR12 as novel molecular targets: their biological functions and interaction with cannabidiol. *Acta Pharmacol Sin* 2019; 40: 300–308.

148. Joo IL, Lai AY, Bazzigaluppi P, et al. Early neurovascular dysfunction in a transgenic rat model of Alzheimer's disease. *Sci Rep* 2017; 7: 46427.

149. Ye J, Chen K, Wu T, et al. Heterogeneous data fusion for Alzheimer's disease study. In: Proceedings of the 14th ACM SIGKDD international conference on knowledge discovery and data mining, 2008, pp.1025–1033.

150. Benoit ME, Hernandez MX, Dinh ML, et al. C1q-induced LRP1B and GPR6 proteins expressed early in Alzheimer's disease mouse models are essential for the C1q-mediated protection against amyloid-β neurotoxicity. *J Biol Chem* 2013; 288: 654–665.

151. Luo X, Hou L, Shi H, et al. CSF Levels of the neuronal injury biomarker visinin-like protein-1 in Alzheimer's disease and dementia with Lewy bodies. *J Neurochem* 2013; 127: 681–690.

152. Esposito G, Scuderi C, Valenza M, et al. Cannabidiol reduces aβ-induced neuroinflammation and promotes hippocampal neurogenesis through PPARγ involvement. *PLoS One* 2011; 6: e28668.

153. Ryan D, Drysdale AJ, Pertwee RG, et al. Differential effects of cannabis extracts and pure plant cannabinoids on hippocampal neurones and glia. *Neurosci Lett* 2006; 408: 236–241.

154. Lee DK, George SR, O'Dowd BF, et al. Orphan G protein-coupled receptors in the CNS. *Curr Opin Pharmacol* 2001; 1: 31–39.

155. Szabo MP, Mishra S, Knupp A, et al. The role of Alzheimer's disease risk genes in endolysosomal pathways. *Neurobiol Dis* 2022; 162: 105576.

156. Wolf SA, Bick-Sander A, Fabel K, et al. Cannabinoid receptor CB1 mediates baseline and activity-induced survival of new neurons in adult hippocampal neurogenesis. *Cell Commun Signal* 2010; 8: 1.

157. Aguado T, Monory K, Palazuelos J, et al. The endocannabinoid system drives neural progenitor proliferation. *FASEB J* 2005; 19: 1704–1706.

158. Demirakca T, Sartorius A, Ende G, et al. Diminished gray matter in the hippocampus of cannabis users: possible protective effects of cannabidiol. *Drug Alcohol Depend* 2011; 114: 242–245.

159. Prenderville JA, Kelly ÁM and Downer EJ. The role of cannabinoids in adult neurogenesis. *Br J Pharmacol* 2015; 172: 3950–3963.

160. Andres-Mach M, Zagaja M, Haratym-Maj A, et al. Long-term treatment with arachidonyl-2′-chloroethylamide combined with valproate increases neurogenesis in a mouse pilocarpine model of epilepsy. *Int J Mol Sci* 2017; 18: 900.

161. Zimmermann T, Ludewig S, Korte M, et al. Functional impact of the cannabinoid type 1 receptor in adult neurogenesis. *Eur Neuropsychopharmacol* 2016; 26: S186–S187.

162. Palazuelos J, Ortega Z, Díaz-Alonso J, et al. CB2 Cannabinoid receptors promote neural progenitor cell proliferation via mTORC1 signaling. *J Biol Chem* 2012; 287: 1198–1209.

163. Mensching L, Djogo N, Keller C, et al. Stable adult hippocampal neurogenesis in cannabinoid receptor CB2 deficient mice. *Int J Mol Sci* 2019; 20: 3759.

164. Oddi S, Scipioni L and Maccarrone M. Endocannabinoid system and adult neurogenesis: a focused review. *Curr Opin Pharmacol* 2020; 50: 25–32.

165. Lazarov O and Hollands C. Hippocampal neurogenesis: learning to remember. *Prog Neurobiol* 2016; 138–140: 1–18.

166. Kempermann G, Song H and Gage FH. Neurogenesis in the adult hippocampus. *Cold Spring Harb Perspect Biol* 2015; 7: a018812.

167. Gonçalves JT, Schafer ST and Gage FH. Adult neurogenesis in the hippocampus: from stem cells to behavior. *Cell* 2016; 167: 897–914.

168. Lacagnina AF, Brockway ET, Crovetti CR, et al. Distinct hippocampal engrams control extinction and relapse of fear memory. *Nat Neurosci* 2019; 22: 753–761.

169. Akers KG, Martinez-Canabal A, Restivo L, et al. Hippocampal neurogenesis regulates forgetting during adulthood and infancy. *Science* 2014; 344: 598–602.

170. Gage FH. Adult neurogenesis in mammals. *Science* 2019; 364: 827–828.

171. Kee N, Teixeira CM, Wang AH, et al. Preferential incorporation of adult-generated granule cells into spatial memory networks in the dentate gyrus. *Nat Neurosci* 2007; 10: 355–362.

172. Oakley H, Cole SL, Logan S, et al. Intraneuronal β-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. *J Neurosci* 2006; 26: 10129–10140.

173. O'Sullivan SE. An update on PPAR activation by cannabinoids. *Br J Pharmacol* 2016; 173: 1899–1910.

174. Nadal L, Coupé P, Helmer C, et al. Differential annualized rates of hippocampal subfields atrophy in aging and future Alzheimer's clinical syndrome. *Neurobiol Aging* 2020; 90: 75–83.

175. De Petrocellis L, Ligresti A, Moriello AS, et al. Effects of cannabinoids and cannabinoid-enriched Cannabis extracts on TRP channels and endocannabinoid metabolic enzymes. *Br J Pharmacol* 2011; 163: 1479–1494.

176. Cassano T, Villani R, Pace L, et al. From Cannabis sativa to cannabidiol: promising therapeutic candidate for the treatment of neurodegenerative diseases. *Front Pharmacol* 2020; 11: 124.

177. Scuderi C, Steardo L and Esposito G. Cannabidiol promotes amyloid precursor protein ubiquitination and reduction of beta amyloid expression in SHSY5YAPP+ cells through PPARγ involvement. *Phytother Res* 2014; 28: 1007–1013.

178. Mechoulam R and Shvo Y. Hashish—I: the structure of cannabidiol. *Tetrahedron* 1963; 19: 2073–2078.

179. Kumar A, Kindig K, Rao S, et al. Structural basis for cannabinoid-induced potentiation of alpha1-glycine receptors in lipid nanodiscs. *Nat Commun* 2022; 13: 4862.

180. Parker LA, Burton P, Sorge RE, et al. Effect of low doses of Δ9-tetrahydrocannabinol and cannabidiol on the extinction of cocaine-induced and amphetamine-induced conditioned place preference learning in rats. *Psychopharmacology* 2004; 175: 360–366.

181. Gugliandolo A, Pollastro F, Grassi G, et al. In vitro model of neuroinflammation: efficacy of cannabigerol, a non-psychoactive cannabinoid. *Int J Mol Sci* 2018; 19: 1992.

182. Batalla A, Bos J, Postma A, et al. The impact of cannabidiol on human brain function: a systematic review. *Front Pharmacol* 2021; 11: 618184.

183. Morales P, Hurst DP and and Reggio PH. Molecular targets of the phytocannabinoids: a complex picture. In: Kinghorn AD Falk H, Gibbons S, et al., (eds) *Phytocannabinoids: unraveling the complex chemistry and pharmacology of Cannabis sativa*. Cham: Springer International Publishing, 2017, pp.103–131.

184. Russo EB. Cannabis therapeutics and the future of neurology. *Front Integr Neurosci* 2018; 11: 51.

185. Ferraro JM and Umstead WJ. Chiral separation of cannabichromene, cannabicyclol, and their acidic analogs on polysaccharide chiral stationary phases. *Molecules* 2023; 28: 1164.

186. De Vita S, Finamore C, Chini MG, et al. Phytochemical analysis of the methanolic extract and essential oil from leaves of industrial hemp futura 75 cultivar: isolation of a new cannabinoid derivative and biological profile using computational approaches. *Plants* 2022; 11: 1671.

187. Worth T. Cannabis's chemical synergies. *Nature* 2019; 572: S12–S13.

188. Beers JL, Fu D and Jackson KD. Cytochrome P450-catalyzed metabolism of cannabidiol to the active metabolite 7-hydroxy-cannabidiol. *Drug Metab Dispos* 2021; 49: 882–891.

189. Zhang Q, Melchert PW and Markowitz JS. Pharmacokinetic variability of oral cannabidiol and its major metabolites after short-term high-dose exposure in healthy subjects. *Med Cannabinoids* 2024; 7: 1–9.

190. Daneman R and Prat A. The blood-brain barrier. *Cold Spring Harb Perspect Biol* 2015; 7: a020412.

191. Yau GTY, Tai W, Arnold JC, et al. Cannabidiol for the treatment of brain disorders: therapeutic potential and routes of administration. *Pharm Res* 2023; 40: 1087–1114.

192. Kolesarova M, Simko P, Urbanska N, et al. Exploring the potential of cannabinoid nanodelivery systems for CNS disorders. *Pharmaceutics* 2023; 15: 204.

193. Abbotts KSS, Ewell TR, Butterklee HM, et al. Cannabidiol and cannabidiol metabolites: pharmacokinetics, interaction with food, and influence on liver function. *Nutrients* 2022; 14: 2152.

194. Huestis MA, Solimini R, Pichini S, et al. Cannabidiol adverse effects and toxicity. *Curr Neuropharmacol* 2019; 17: 974–989.

195. Herrmann N, Ruthirakuhan M, Gallagher D, et al. Randomized placebo-controlled trial of nabilone for agitation in Alzheimer's disease. *Am J Geriatr Psychiatry* 2019; 27: 1161–1173.

196. Croxford JL. Therapeutic potential of cannabinoids in CNS disease. *CNS Drugs* 2003; 17: 179–202.

197. Kroon E, Kuhns L and Cousijn J. The short-term and long-term effects of cannabis on cognition: recent advances in the field. *Curr Opin Psychol* 2021; 38: 49–55.