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A REVIEW ON THE EFFECTS OF *Cannabis sativa* L. ON HUMAN COGNITION

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ABSTRACT. Natural resources have been an important starting point in the medical and pharmaceutical sciences for the discovery of drug candidates. *Cannabis sativa*, a plant of the Cannabidaceae family, is regarded as the first psychoactive substance to be consumed. The use of cannabis has been shown to have positive effects on a variety of chronic conditions, including cancer, AIDS, and Alzheimer's. Consequently, legalizing cannabis use for both therapeutic and recreational purposes is currently the subject of heated controversy in numerous nations. *C. sativa* contains about 540 natural compounds known as phytocannabinoids. Among these compounds, cannabinol-type cannabidiol (CBD) and trans-Δ^{9}-tetrahidrocannabinol (THC) are more researched than other components. According to literature studies, it is known that the dominant psychotropic component in the species is trans-Δ^{9}-tetrahidrokanabinol, while the main non-psychoactive component is cannabidiol. In addition to its many traditional medical applications, cannabis has been shown to be effective against Alzheimer's disease-related cognitive dysfunction in numerous *in vitro* and *in vivo* investigations. Future research on cannabis extracts and components should, according to both in vitro and in vivo studies, focus on the potential advantages of cannabis extracts for both animal and human brain-cognitive health. In order to fully demonstrate the effects of cannabis and its various preparations on human cognition, longer-term clinical studies with larger numbers of subjects are needed.

Keywords: *Cannabis, Cannabinoid, Alzheimer's, Marijuana, Memory*

Cannabis sativa L.'NİN İNSAN BİLİŞİ ÜZERİNDEKİ ETKİLERİ ÜZERİNE BİR DEĞERLENDİRME

ÖZET. Doğal kaynaklar, ilaç adaylarının keşfedilmesinde tıp ve eczacılık bilimlerinde önemli bir başlangıç noktası olmuştur. Cannabidaceae familyasının bir üyesi olan *Cannabis sativa* eski çağlardan beri kullanılan en eski psikotrop ilaç olarak bilinmektedir. Literatürde geçen çok sayıda çalışma; *Cannabis* kullanımının kanser, AIDS ve Alzheimer hastalığı gibi çeşitli kronik rahatsızlıklar için yararlı etkilerini bildirmektedir. Bu nedenle, şu anda birçok ülkede tıbbi ve eğlence amaçlı *C. sativa* kullanımının yasallaştırılmasına ilişkin tartışmalar devam etmektedir. *C. sativa*, fitokanabinoidler olarak tanımlanan yaklaşık 540 doğal bileşik içermektedir. Bunlardan kannabinol tipi kannabidiol (CBD) ve trans-Δ^{9}-tetrahidrokanabinol (THC), diğer

bileşenlere kıyasla çok daha fazla araştırılan bileşiklerdir. Literatür çalışmalarına göre türde baskın psikotrop bileşen trans-Δ^9 tetrahidrokanabinol iken, psikoaktif olmayan ana bileşenin ise kannabidiol olduğu bilinmektedir. *Cannabis* türünün halk hekimliğinde çeşitli kullanımlarının yanısıra literatürde Alzheimer hastalığına bağlı bilişsel işlev bozukluğuna karşı olumlu etkisine atıfta bulunan birçok *in vivo* ve *in vitro* çalışma mevcuttur. Hem *in vivo* hem de *in vitro* çalışmalar kenevir ekstrelerinin ve bileşenlerinin gelecekteki çalışmaların hayvan ve insan biliş ve beyin sağlığı üzerindeki potansiyel faydalarını incelemesi gerektiğini göstermektedir. Cannabis ve çeşitli preperatlarının insan bilişi üzerindeki etkilerinin sağlıklı olarak ortaya konulabilmesi için özellike daha fazla sayıda denek ile daha uzun vadeli klinik çalışmalara ihtiyaç olduğu görülmektedir.

Keywords: *Cannabis, Cannabinoid, Alzheimer, Marijuana, Hafıza*

INTRODUCTION

Cannabis sativa L., a plant that belongs to the Cannabidaceae family and is native to Central Asia, is grown for both fiber and oil production as well as for therapeutic applications. This plant is recognized as the first psychoactive substance ever utilized. Chinese people have been using cannabis since 4000 BC, according to archeological discoveries [1]. It is a bipartite annual plant with flowers that has palm leaves with a distinctive cannabis pattern (Fig. 1). The Cannabis plant has three subspecies that are recognized: *C. sativa* ssp. *sativa* (L.), *C. sativa* ssp. *ruderalis* (Janisch), and *C. sativa* ssp. *indica* (Lam.) [2]. The most popular type produced in many climates is *C. sativa* [3].

Fig. 1. *Cannabis sativa* L.

In 2737 BC, the Chinese Emperor Shen Nung first provided a detailed description of the cannabis plant's characteristics and medicinal applications. Later, use of this herb expanded from China to India [4, 5]. In 1839, William O'Shaughnessy wrote about this plant's appetizing, analgesic, muscle-relaxing, anticonvulsant, and antiemetic properties in India. As a result, this herb started to be widely used for therapeutic purposes. Cannabis was made freely available in pharmacies in Western nations in 1854, when it was added to the list of dispensaries in the United States. Many studies in the literature discuss the benefits of using this plant for treating many chronic illnesses, including cancer, AIDS, and Alzheimer's. As a result, the abolition of cannabis prohibition for both medical and recreational purposes is currently being discussed in numerous nations [3].

Over 100 of the 540 natural substances with a similar molecular skeleton structure found in *C. sativa* have been classified as phytocannabinoids [6]. Alkyl resorcinol and monoterpenes are found in the molecules of phytocannabinoids, which are cannabinoids with a lipid skeleton structure [7] (Fig. 2). After being biosynthesized as cannabinoid acids, cannabinoids are next decarboxylated to produce their neutral state.Phytocannabinoids are divided into several subclasses, such as Δ^9-tetrahydrocannabivarin type, cannabidiol type, tetrahydrocannabinol type, and cannabinol type [8]. The most researched of them are trans- Δ^9-tetrahydrocannabinol (THC) and cannabinol-type cannabidiol (CBD) (Fig. 2). Significant therapeutic promise exists for cannabinol-type cannabidiol in pathophysiological states or disorders. Studies of the literature revealed that trans-Δ^9-tetrahydrocannabinol was the predominant psychotropic component in the species, whereas cannabidiol was the primary non-psychoactive component [9].

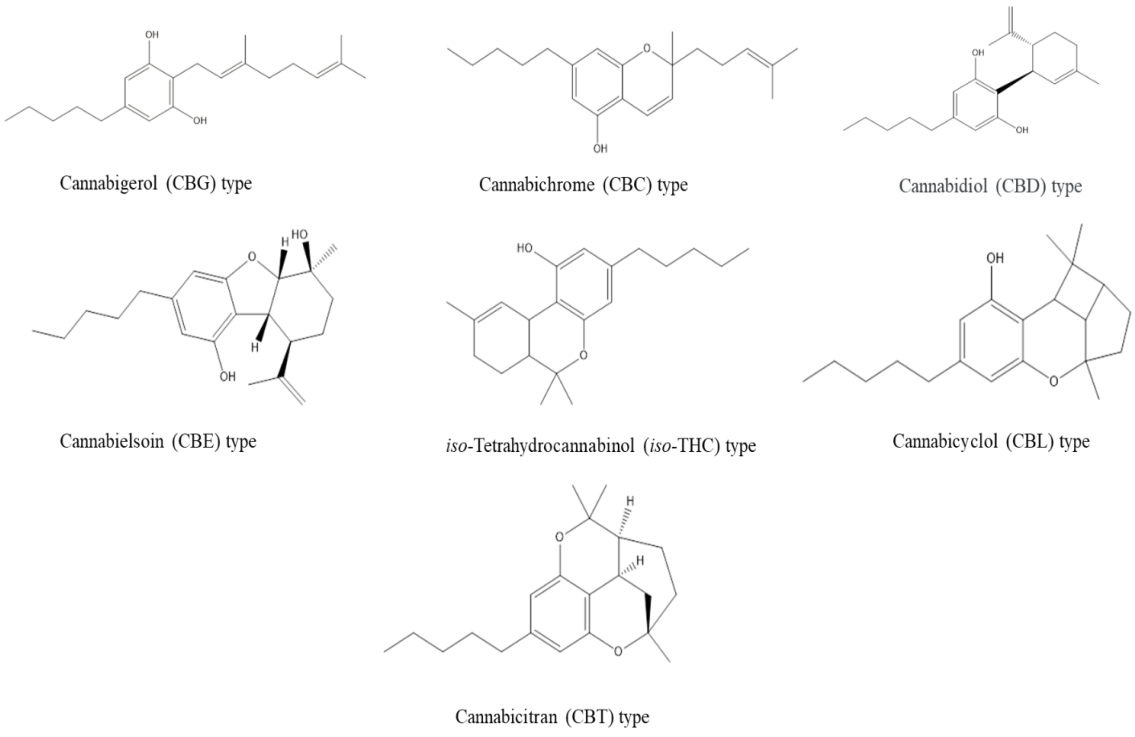

Fig.2. *Major active components in C. sativa*

The effects of *C. sativa* species on cognitive functions in humans were examined in this study, and more particularly, studies that had been published in the literature on Alzheimer's disease were evaluated and reported. These literatures were searched in databases such as Web of Science (WoS), Embase, Medline, Cochrane Library, Pubmed, Scopus, and Google Scholar with the keywords "*Cannabis*, *Cannabis sativa*, Cannabinoids, Anticholinesterase, Antialzheimier". In addition, binary keywords such as Alzheimer's disease and *Cannabis*, *Cannabis* and memory, *Cannabis* and cognitive dysfunction, *Cannabis* and Anticholinesterase, Cannabinoid and Alzheimer's, Marijuana and Alzheimer's were searched. Based on the in vitro and in vivo information retrieved from these databases, the effects of *Cannabis* species on human cognition are examined in this review, with a focus on the therapeutic effect related to Alzheimer's.

Studies of *Cannabis* and Its Many Preparations *In Vivo* and *In Vitro Studies*

In a study, the impact of *C. sativa* aerial parts on the enzyme activities of cholinesterases and beta-secretase, which are two potential causes of Alzheimer's disease, was investigated [10]. *Cannabis* extracts were made in this investigation using hexane, dichloromethane, dichloromethane: methanol (1:1), and water.

The effects of extracts on acetylcholinesterase, butyrylcholinesterase, and beta-secretase activities were determined. Additionally, pre-adipocyte and normal vero cell lines underwent cytotoxicity testing. Chemical content analyses were performed using HPLC. In addition, the heavy metal content of *C. sativa* extract was determined by an atomic absorption spectrophotometer. The chemical content analysis showed that the cannabidiol ratio of the extracts was higher in hexane (166.2 µg/mg crude extract) and dichloromethane (5.7 µg/mg crude extract) than in methanol and dichloromethane: methanol (1:1) extracts. According to heavy metal content analysis, no heavy metals were detected in the aerial parts of *C. sativa*. AChE enzyme inhibition activity was determined as IC_{50}: 0.79 µg/ml for physostigmine used as control and IC_{50}:1.18 µg/ml for CBD. BChE enzyme inhibition activity was determined as IC_{50}: 0.76, 1.06, 1.81, and 1.95 ± 0.97 µg/ml for physostigmine, CBD, hexane, and DCM extracts, respectively. The beta-secretase enzyme inhibition activities of the extracts were determined to be 74%, 68%, 52%, 54%, 62%, and 50% µg/mL, respectively. However, CBD and THC showed weak β-secretase enzyme inhibition (below 15%). In addition, it was observed that all the extracts did not show any cytotoxic effects. In the study's results, it was reported that *C. sativa* extracts could be used to treat Alzheimer's disease [10].

Another study used modified human neural cells (SY-SH5Y) to examine the antioxidant properties of THC and CBD compounds [11]. In this work, DMSO solutions of Cannabis extracts were made at various concentrations. To assess antioxidant activity, standards of CBD:THC (10:90, 25:75, 50:50, 75:25, and 90:10 (w/w)) produced at various rates were also utilized. The neurotoxicity of cannabis extracts against engineeredhuman neuronal cells (SH-SY5Y) was assessed using the MTT test. The results of the study demonstrated that THC (IC_{50}: 0.4 µg/mL) had a greater capacity than CBD (IC50: 42.7 g/mL) to inhibit ROS produced by H_2O_2 in these cells. Additionally, the MTT test showed that CBD (1 µg/mL) and CBN (5 µg/mL) did not demonstrate any neurotoxicity

while THC at a dosage of 0.6 g/mL reduced cell viability by 50%. Furthermore, it was demonstrated that none of the THC:CBD samples produced at different ratios were harmful to cells at a concentration under 1 µg/mL and that the 50:50 ratio of THC:CBD had the highest IC_{50} value. Phase contrast microscopy was also used to examine how cannabinoids affected the shape of transformed neuronal cells.

DMSO was used as the negative control, and there were slender cells with elongated neurites in this group. The images showed that the CBD compound caused the death of these cells at concentrations of 10 µg/mL and above. Study results showed that *Cannabis* extracts and phytocannabinoids had promising potential for developing new drugs for the treatment of oxidative stress due to their antioxidant effects [11]. In the study, the *in vitro* anti-aggregative and neuroprotective features of the flavonoid, canflavin A, isolated from cannabis, against A $beta_{1-42}$ were evaluated [12] (Table 1). In addition, in order to broadly determine the bioactive properties of these flavonoids, a comparison was made with two geranyl-linked flavonoids, mimulon and diplacone. In the study, neuronal viability was determined on PC12 cells by MTT test under the effect of each flavonoid (1-200 µM) for 48 hours. Then, cells were exposed to each flavonoid at subtoxic quantities either alone or in combination with amyloid beta ($A\beta_{1–42}$; 0-2 µM) or the lipid peroxidant tert-butyl hydroperoxide. cellular morphology of PC12 was determined by Fluorescent staining for indication of $A\beta_{1–42}$ effects. Aggregation and A beta fibril formation caused by these compounds were evaluated by transmission electron microscopy (TEM) and Thioflavin T fluorometric kinetic assay. According to the results of the study, it was determined that canflavin A increased cell viability by 40% between 1-10 µM concentrations and exhibited intrinsic hormetic effect, but showed neurotoxicity at concentrations higher than 10-100 µM. Contrary to this biphasic effect observed in Canflavin A, concentration-dependent neurotoxicity of mimulon and diplacone was determined. Canflavin A, however, boosts cell survival by up to 40% at lower doses (< 10 µM), whereas 10 µM canflavin A reduces the neurotoxicity induced by $A\beta_{1–42}$-(0–2 µM), and it was also discovered that it reduced Aβ aggregate adherence to PC-12 cells and related neurite loss. As a result, it has been demonstrated that canflavin A has a neuroprotective and hormetic effect depending on concentration against neurotoxicity caused by $A\beta_{1–42}$ associated with the inhibition of Aβ fibrillation. In addition, it was determined that geranyl group-linked flavonoids exhibit relatively potent neurotoxicity which was not generally observed in many conventional flavonoids *in vitro* [12].

In order to treat Alzheimer's disease, 23 distinct multi-targeted molecules based on 2-aryl benzofuran were produced in a different investigation. Using the technique created by Ellman et al. [13], it was determined whether an inhibitor of these newly synthesized compounds affected the hydrolysis rate of acetylthiocholine or butyrylthiocholine as well as its ability to inhibit acetylcholinesterase-butyrylcholinesterase. Additionally, this study used MTT analysis to identify the neuroprotective effects of Aβ antiaggregant chemicals against $A\beta_{25-35}$ toxicity in human neuron cells (SHeSY5Y). Simultaneously, Congo Red staining and dihydroethidium analyses were used to determine if these compounds could bind $A\beta_{25-35}$-peptide to the cell membrane and prevent the generation of reactive oxygen species from $A\beta_{25-35}$-peptide. In the end, it was looked into how the novel chemicals affected the binding of human recombinant cannabinoid receptors (CB1 and CB2). The investigation found that, in accordance with their IC_{50} activities, increasing doses of these drugs lowered the inhibition of radioligand binding non-linearly.

The Cheng-Prusoff equation was used to calculate the Ki value when the IC50 value was less than 10 mM. Interestingly, the newly synthesized (2-{3-[7-(Benzylmethylamino) heptyloxy) phenyl}benzofuran-3-yl) phenyl methanone molecule displayed strong selectivity and moderate affinity for the CB1 receptor based on the results of the investigation. The findings of the study were claimed to have opened a fresh perspective in the field of research on Alzheimer's disease because it has long been known that cannabis ligands have neuroprotective qualities [14].

In a 2006 study, it was found that the active ingredient in cannabis, Δ^9-tetrahydrocannabinol, competitively inhibited the acetylcholinesterase (AChE) enzyme and prevented the formation of β-peptide(Aβ) amyloids, which is the main symptom of Alzheimer's disease [15]. THC was found to bind to the peripheral anionic region of AChE, which is a crucial site for the formation of amyloid, according to computational modeling of the THC&AChE interaction employed in the study. Compared to already licensed medications used to treat Alzheimer's disease, THC was discovered to be a good inhibitor of Aβ-aggregation, and their research showed a previously unknown molecular mechanism of cannabinoids that could directly alter the progression of this disease [15].

In a different study, the neuroprotective properties of cannabidiol were examined in relation to memory loss and oxidative stress brought on by amyloid beta-(Aβ), which is a significant contributor to cognitive decline and neuronal cell death [16]. Reactive oxygen species and lipid peroxide levels rose in the altered neuronal cell lines in the study (SH-SY5Y) after they had interacted with cannabidiol for 24 hours. This was discovered through analysis. In contrast to enzymatic antioxidants such superoxide dismutase, catalase, and glutathione reductase, it was discovered that Aβ (CP13-S202/205-PHF1-S396/404) therapy boosted both total tau expression and the phosphorylated forms of tau.

The expression of PSD-95 and Arc proteins, which are necessary for synaptic development and plasticity, was found to decrease with Aβ therapy. It was discovered that cannabidiol therapy in Aβ-treated SHSY5Y cells decreased the formation of lipid peroxide levels, controlled antioxidant activities, and enhanced the production of memory-related proteins. A mechanistic analysis of the therapeutic effects of cannabidiol on Aβ levels was also carried out in this study utilizing the SH-SY5Y cell line transfected with APP695 (SH-SY5Y-APP). According to the results of this study, it was found that cannabidiol did not show any change in APP-protein levels, α-secretase or gamma-secretase activity, on the contrary, it decreased the level of β-secretase and inhibited the activity of this enzyme, leading to a decrease in Aβ levels. Cannabidiol is a leading chemical in the development of medications for Alzheimer's disease since the results of the study showed that it boosted antioxidant activity and mitochondrial energy control while reducing Aβ levels [16]. It is well accepted that oxidative stress, specifically that caused by the actions of the Aβ-peptide aggregates on the membrane, is related to Alzheimer's disease. Cannabidiol, a key non-psychoactive component of the cannabis plant, was studied to determine how it affected beta-amyloid peptide-induced toxicity in cultured rat pheochromocytoma PC12 cells [17]. The results of the investigation showed that the viability of the cells significantly decreased after exposure to Aβ-peptide (1 μg/mL). It was discovered that cannabidiol had a combined neuroprotective, antioxidative, and antiapoptotic effect against beta-amyloid peptide toxicity, and that the signaling pathway for this neural protection was caused by inhibition of pro-caspase 3 by

cannabidiol, the dormant precursor of caspase 3. This study proved that cannabidiol could be effective in preventing neuronal cell death in Alzheimer's disease [17].

Another study looked into the impact of cannabidiol and other phytocannabinoids on microglial phagocytosis [18]. In this investigation, single-cell-Ca^{2+} imaging, immunoblotting, and immunocytochemistry were used to examine the effects of cannabidiol and other phytocannabinoids on the expression of mediator proteins. The amount of fluorescent latex bead phagocytosis that cannabidiol (10 μM) increased compared to the control was found to be 175±7% higher. Other phytocannabinoids such as endogenous and synthetic cannabinoids had no impact on phagocytosis. The results of the study indicated that pharmacological manipulation of TRPV-channel activity, alterations in microglial function, and the TRPV-induced phagocytosis-enhancing effect of cannabidiol may be a reasonable approach in the treatment of neuroinflammatory disorders, and that cannabidiol may serve as a potential starting point for the development of new therapeutics targeting the TRPV-receptor family in the future [18].

In a different study, the impact of cannabidiol on the hyper-phosphorylation of tau proteins in PC12 neuronal cells induced by Aβ, one of the most obvious symptoms of Alzheimer's disease, was evaluated [19]. 24 hours after adding $A\beta_{1–42}$ to PC12 cells, the effect of Aβ (1μg/ml) on the expression of the proteins -catenin and GSK-3 as well as its impact on the expression of the hyperphosphorylated tau proteins (68 kDa) were investigated. According to the concentration, p-GSK-3 β levels caused by $A\beta_{1–42}$ were dramatically decreased in PC12 cells by cannabidiol (10^{-7}-10^{-5} M). Additionally, PPP-tau expression was markedly sped up in PC12 cells treated with Aβ compared to untreated cells, which had only weakly detectable hyper-phosphorylated tau protein levels (PPP-tau). This study demonstrated the important role that cannabidiol played in the treatment of Alzheimer's disease [19] and provided new mechanistic insights into the neuroprotective effects of the compound, particularly in light of its low toxicity in humans.

Esposito et al. [20] looked at the potential inhibitory effect of cannabidiol on nitrite production and iNOS-protein expression in Aβ-induced nitrosative stress in neuronal cell lines. When compared to unstimulated cells, the synthesis of nitrite was significantly accelerated in the study when modified PC12 cells were stimulated with $A\beta_{1\text{-}42}$ (1 g/mL) for 36 hours. At various doses, both the non-selective iNOS inhibitor l-NAME (0.3-30M) and the more focused iNOS inhibitor SMT (0.3-30M) were found to inhibit the expression of the iNOS-protein. Additionally, it was discovered that the effects of A1-42 on nitrite production and iNOS-protein expression were both suppressed by cannabidiol (10^{-6}-10^{-4} M). Cannabidiol may have a neuroprotective function, and the results of this study stressed the significance of this substance in preventing β-amyloid-induced neurodegeneration given its low toxicity in humans [20].

In a different study, cannabidiol (CBD) pretreatment at 5 μM may have had an impact on the transcriptional profile of gingival-derived mesenchymal stem cells (GMSCs) [21]. The downregulation of genes linked to Alzheimer's disease, including those that encode the kinases responsible for phosphorylating tau, was discovered to be promoted by CBD. To do this, the expression profiles of GMSCs under control (CTR-GMSCs) and under CBD treatment (CBD-GMSCs) were compared. Additionally, CBD triggered the PI3K/Akt signal to limit the development of GSK3β, a protein that is essential in the

etiology of Alzheimer's disease, according to study on immunocytochemistry. In conclusion, this study discovered that pretreatment with CBD decreased the expression of proteins in GMSCs that may be related to tau phosphorylation and generation, and that GMSCs with a favorable molecular profile may be more effective for treating Alzheimer's disease [21].

In a study by Scuderi et al. [22], the impact of cannabidiol as a potential regulator of amyloid precursor protein-(APP) production in SHSY5Y$^{(APP+)}$ neurons was investigated. Additionally, the potential function of the proposed molecular site for the effects of CBD, the peroxisome proliferator-activated receptor γ (PPARγ), was investigated. The results of the study revealed that CBD made the SHSY5Y(APP+) APP protein ubiquitinated, which reduced the production of Aβ and significantly decreased the levels of APP full-length protein. CBD has also been shown to increase the lifespan of SHSY5Y(APP+) neurons by reducing the rate of long-term apoptosis. Additionally, it was claimed that all of the known effects of CBD were solely caused by PPARγ activation [22]. The cell lines employed and the findings from the in vitro literature research of cannabis and its various components are listed in Table 1.

Table 1: *Studies of Cannabis and its different formulations in vitro.*

Cell Types	Observed Effects	References
PC12 cells	It was determined that at low concentrations (<10 μM) cannflavin A increased cell viability by up to 40%, while 10 μM cannflavin A reduced Aβ aggregate adhesion to PC-12 cells and associated neurite loss by inhibiting the neurotoxicity elicited by $A\beta_{1-42}$ (0–2 μM).	**[12]**
SHeSY5Y neuronal cells	It was determined that the synthesized 1 and 18 complexes showed good selectivity and moderate affinity for the CB1 receptor.	**[14]**
--	Compared with currently approved drugs used for the treatment of Alzheimer's disease, THC was reported to be an effective inhibitor of Aβ aggregation.	**[15]**
SH-SY5Y neuronal cells	It was determined that cannabidiol treatment reduced lipid peroxide accumulation, regulated antioxidant activities and improved the expression of memory-related proteins in Aβ-treated SHSY5Y cells.	**[16]**
Rat pheochromocytoma PC12 cells	It was determined that cannabidiol had neuroprotective, anti-oxidative and anti-apoptotic effects against beta-amyloid peptide toxicity, and inactive precursor of caspase 3, pro-caspase 3, was inhibited by cannabidiol.	**[17]**
P12 cells	It was determined that cannabidiol (10^{-7}-10^{-5} M) significantly inhibited the levels of Aβ (1-42)-induced p-GSK-3β in PC12 cells depending on concentration.	**[19]**
P12 cells	It was reported that cannabidiol (10^{-6} -10^{-4} M) inhibited both nitrite production and iNOS protein expression induced by Aβ (1–42).	**[20]**
human mesenchymal stem cells	It was determined that pretreatment with CBD prevented the expression of proteins potentially related to tau phosphorylation and Aβ production in GMSCs.	**[21]**
SHSY5Y$^{(APP+)}$ neuron cells	CBD was shown to induce ubiquitination of the APP protein in SHSY5Y(APP+), resulting in a significant reduction in APP full-length protein levels.	**[22]**

In Vivo Studies

A nonselective cannabinoid receptor agonist WIN55,212-2 (WIN), the prototype for anticholinesterase, was thought to have the capability of altering the acute toxicity of paraoxon in an in vivo study [23]. In this investigation, the effects of WIN on locomotor activity in male 2-month-old rats were assessed. Paraoxon (0.4 or 0.6 mg/kg, sc) or vector (1.5 mg/kg, subcutaneously) was applied to rats and then given an acute dose of WIN to assess any potential impact on paraoxon toxicity. Indicators of cholinergic toxicity were monitored from 0 to 4 hours following paraoxon dosage at regular intervals. In contrast to the rats in the control group, who received peanut oil in a volume equivalent to paraoxon, peanut oil was used to dissolve paraoxon and delivered subcutaneously (0.4-0.6 mg/kg) in a volume of 1 mL/kg. A third party observer who was ignorant of the treatment groups rated the signs of cholinergic toxicity in the rats, such as tremors and SLUD (Lacrimation, Urination, Defecation, and Salivation) indications. According to study findings, acute and repeated cannabinoid agonist treatment could differentially affect acute cholinergic toxicity by modulating acetylcholine release and adapting cannabinoid signaling associated with repeated cannabis exposure [23].

In another work, cholinesterase activity and cholinergic indicators of AChE release in the tissues of 8-week-old cannabinoid receptor CB1 knockdown male mice were assessed in relation to the effects of chlorpyrifos (CPF), an organophosphorus (OP) insecticide [24]. CPF (300 mg/kg, 2 ml/kg in peanut oil, sc) was administered to mice of both genotypes for the study, and functional neurochemical alterations were assessed. The cortex, cerebellum, and heart of both genotypes showed comparable cholinergic signals and cholinesterase inhibition (82–95%) 48 hours after dose. In vitro hippocampal slices from mice of the wild type showed reduced depolarization-induced ACh-release when treated with WIN; however, hippocampus or striatal slices from live or dead mice of either genotype had no effect. CPF or CPB oxon-induced increases in ACh release were reported to be brain-regionally susceptible to regulation by CB1-mediated eCB signaling, yet, in mice, ablation of the CB1 receptor had little effect on the development of acute toxicity after CPF [24].

The purpose of a marijuana study was to ascertain whether brain perfusion in certain areas in marijuana users during functional neuroimaging, particularly those affected by Alzheimer's disease, was different from that of controls [25]. In this study, 92 patients served as the control group and 982 individuals with cannabis use disorder were compared using SPECT and perfusion neuroimaging techniques while at rest and during periods of concentration. The findings of the study showed that cannabis users had less cerebral perfusion ($p<0.05$). It was claimed that Alzheimer's disease primarily targets the hippocampus, the prominent brain area that distinguishes cannabis users from healthy individuals [25].

Another study looked into the viability of giving people with cannabis use disorder (CUD) galantamine, an acetylcholinesterase-inhibitor licensed for the treatment of Alzheimer's disease and other dementias, as well as the drug's effects on cognition [26]. In randomized, double-blind, parallel-group research, 30 CUD sufferers took part. Regarding demographic and baseline factors, there was no discernible difference between the galantamine and placebo groups, and there were no appreciable adverse effects from galantamine. *Cannabis* withdrawal subsided steadily over the course of the investigation. Over the course of the 10-day period, cognitive outcomes somewhat improved. For instance, it was shown that both groups showed a statistically significant rise in the measures of response inhibition and a trend toward improvement in the measures of

attention. Analysis, however, did not reveal a significant main impact for the treatment or for time-dependent treatment interactions. In brief, the results indicated the viability of giving galantamine to those with CUD. Galantamine may be able to help with the cognitive deficiencies linked to CUD, although further research into this claim requires adequately strong, randomized, placebo-controlled studies [26].

Another study evaluated older persons 65 and older with or without regular cannabis usage in terms of resting-state functional connectivity (rsFC) [27]. A higher relationship between sources in the hippocampus and parahippocampal cortex and targets in the anterior lobes of the cerebellum was discovered in older adult cannabis users compared to non-users at the conclusion of the study. Aged non-users aged 25 to 35 years were likewise shown to have the same enhanced connection between the hippocampus and cerebellar regions. According to these findings, future research should look at potential of the cannabinoids for harming older persons as well as for helping them think clearly and take care of their brains [27].

A different study by Chen et al. [28] sought to establish a connection between the synaptic and cognitive problems and the activation of cyclooxygenase-2 (COX-2) that ensue from ongoing exposure to Δ^9-THC in the brain. The study on CB1 knockout, C57BL/6, COX-2 knockout, Thy1-EGFP transgenic, and 5XFAD-APP transgenic mice with 10 mg/kg of Δ^9-THC injection found that genetic or pharmacological inhibition of COX-2, downregulation, and internalization of glutamate-receptor subunits blocked the changes in dendritic spine density of hippocampal neurons induced by repeated Δ^9-THC exposures. The findings demonstrated that the advantages of beta-amyloid plaques and the neurodegeneration caused by Δ^9-THC in rats with Alzheimer's disease were maintained in the context of COX-2 inhibition. The results of the study suggested that concurrent COX-2 inhibition could increase the efficacy of medicinal cannabis [28].

The ability of cannabidiol to reduce inflammation in vivo in the mouse brain were examined in a different study [29]. In the experimentation stage of the study, human $A\beta_{1-42}$ was intrahippocampally administered into C57BL/6J three to five month old mice. After that, the mice received intraperitoneal CBD dosages (2.5 or 10 mg/kg) for 7 days. Results show that CBD has a dose-dependent ability to decrease glial fibrillary acidic protein (GFAP) mRNA and protein expression. One of the primary characteristics of reactive gliosis is assumed to be the presence of GFAP, the most well-known marker of active astrocytes. These findings suggested that CBD could lessen reactive gliosis brought on by Aβ [29].

Given that PPAR-activated receptors are upregulated in Alzheimer's patients, a different investigation examined how PPAR-γ contributes to the therapeutic effects of cannabidiol [30]. In this investigation, the hippocampus of adult male rats was treated with cannabidiol (10 mg/kg) for 15 days in the presence or absence of a PPAR-γ or PPAR-α-receptor antagonist after being injected with human Aβ-42. In rat astrocytes, the expression of GFAP, iNOS, S100 calcium-binding protein B (S100B), and p56 and p50 antibodies was reduced by cannabidiol in a dose-dependent manner. It shows that cannabidiol has anti-inflammatory capabilities because iNOS and GFAP are important components of reactive gliosis. In addition, the decreased expression of p50 and p56 genes showed that cannabidiol had the ability to inhibit NF-kB, and that both PPAR-γ and NF-kB were effective in the emergence of cannabidiol's anti-inflammatory properties. In addition, as a result of this study, it was stated that the anti-inflammatory

effect of cannabidiol was mediated by the PPAR-γ receptor. Finally, it was reported that CBD can restore CA1-pyramidal neurons of rats to similar-integrity to control rats [30].

Effects of cannabidiol on cognition in pharmacological animal models of Alzheimer's disease were examined by Martin-Moreno et al. [31]. In this investigation, 2.5 μg of fibrillary Aβ was intraventricularly injected into three-month-old C57/Bl6 mice. These mice were then given injections of CBD at a dose of 20 mg/kg every day for the first week and three times a week for the subsequent two weeks. The MorrisWaterMaze was then used to assess the spatial learning of the mice. The findings demonstrated that cannabidiol therapy could restore the cognitive abnormalities in mice that had received an injection of Aβ. The therapeutic impact of cannabidiol on cognition, however, was found to occur via different mechanisms in this investigation because selective CB2 agonists failed to stop cognitive loss. The behavioral advantages of cannabidiol in mice may be attributable to the control of glial activation, as evidenced by the discovery that cannabidiol therapy inhibits Aβ-induced IL-6 gene expression [31].

In a different study, transgenic mice were used to examine the healing potential of continuous cannabidiol treatment for Alzheimer's disease. In this experiment, CBD-(20 mg/kg CBD, daily injections) was administered to 6-month-old male APPxPS1 mice for 3 weeks after the beginning of cognitive impairments and Alzheimer's disease pathology. The results of the study showed that cannabidiol therapy corrected cognitive deficits in mice in social and object recognition memory without changing the anxiety parameters [32].

In a study, cannabidiol administration in transgenic mice with Alzheimer's disease was examined for its ability to inhibit the development of chronic Alzheimer's disease [33]. Male APP-PS1 mice that were 2.5 months old were treated for 8 months utilizing a daily oral administration procedure with 20 mg/kg of cannabidiol or cannabidiol pellets. Before the start of Alzheimer's disease, this application was used to assess the long-term impact of cannabidiol. In summary, this study showed that long-term cannabidiol administration delayed the onset of memory problems in social recognition in transgenic mice with Alzheimer's disease while having no effect on the brain's anxiety regions [33].

In a distinct study, behavioral deficits associated with frontotemporal dementia (FTD) and Alzheimer's disease were caused by the P301S mutation in human tau carried by TAU58/2-transgenic mice [34]. Three weeks prior to the implementation of behavioral paradigms for FTD and Alzheimer's disease, 14-month-old female TAU58/2-transgenic and wild-type (WT) mice were given 100 mg/kg CBD. TAU58/2-females were discovered to have decreased motor function, lower body weight, and less anxious behavior than WT. Additionally, it was discovered that long-term CBD use improved the transgenic mice's impaired spatial reference memory. All mice treated with chronic CBD showed a decrease in the freezing and anxiety-like behaviors linked to contextual fear. According to the study, prolonged CBD treatment reduced various disease-related symptoms in 14-month-old TAU58/2-transgenic mice and could potentially be utilized to treat behavioral problems associated with tauopathy, such as cognitive deficits [34].

The impact of *C. sativa* on acetylcholinesterase and butyrylcholinesterase activity was examined in a study as potential cholinergic biomarkers of neurotoxicity [35]. In the study, rats were administered cannabis resin (20, 10, or 5 mg/kg), tramadol (20, 10, and 5 mg/kg), and cannabis resin (20, 10, and 5 mg/kg) mixed with tramadol (10 mg/kg) subcutaneously daily for 6 weeks. Cannabis resin is comparable to the active component,

Δ^9-tetrahydrocannabinol. In the brain and serum of the experimental groups, butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) activity were assessed. Additionally, the study assessed the paraoxonase-1 (PON1) activity in the serum of rats given these medications. According to the study, AChE activity in the brains of experimental groups rose (16.3–36.5%) following the application of cannabis resin (10–20 mg/kg). AChE activity did not alter in the brains of rats given tramadol treatments ranging from 5 to 20 mg/kg, according to the research. The amount of brain AChE activity decreased by 14.1%, 12.9%, and 13.6% in rats treated with cannabis resin (20, 10, or 5 mg/kg) and tramadol (10 mg/kg), respectively. Based on these findings, the researchers hypothesized that cannabis resin may have distinct effects on BChE and AChE activities, which may be related to memory issues and a deterioration in cognitive function in long-term users [35]. In vivo literature studies of cannabis and doses of its different components and outcomes are summarized in Table 2.

Table 2: *Studies on Cannabis and its many formulations in vivo*

Test Organisms	Application and Dosage	Observed Effects	References
2 -month-old male rats	1 mL/kg subcutaneously (0.4-0.6 mg/kg)	It was determined that acute and repeated exposure to cannabinoid agonists could differentially alter acute cholinergic toxicity through modulation of acetylcholine release and adaptation in cannabinergic signaling associated with repeated cannabinoid exposure.	[23]
8-week-old male mice	Chlorpyrifos 300 mg/kg, 2 ml/kg in peanut oil	It was observed that WIN reduced depolarization-induced ACh release in hippocampal slices from wild-type mice *in vitro*, however, there was no effect on hippocampal slices or striatal slices of both genotypes in dead mice.	[24]
Human	The group of 982 subjects with CUD and the control group of 92 subjects were compared with SPECT and perfusion neuroimaging at resting and concentration.	Cannabis users were found to have, on average, lower cerebral perfusion ($p < 0.05$).	[25]
Human	30 individuals with CUD participated in a randomized, double-blind, parallel-group study for 10 days.	The feasibility of administering galantamine for individuals with CUD was supported.	[26]
Human	The resting-state functional connectivity (rsFC) was compared in older adults aged 65+ with or without regular use of Cannabis	It was found that older adult cannabis users had stronger connections than non-users between sources in the hippocampus and parahippocampal cortex and targets in the anterior lobes of the cerebellum.	[27]
C57BL/6, CB1knockout, Thy1-EGFP transgenic,COX-2 knockout and 5XFAD APP transgenic mice	10 mg/kg Δ^9-THC injection	The beneficial effects of beta-amyloid plaques and neurodegeneration reduced by Δ9-THC in animals with Alzheimer's disease were found to be maintained in the presence of COX-2 inhibition.	[28]
3–5-month-old C57BL/6J mice	2.5 or 10 mg kg-1 CBD administered for 7 days	It was shown that CBD was able to dose-dependently inhibit glial fibrillary acidic protein (GFAP) mRNA and protein expression.	[29]
Adult male Sprague-Dawley rats	10 mg/kg CBD administered for 15 days	It was determined that cannabidiol dose-dependently reduced the Aβ-induced expression of iNOS, GFAP, S100 calcium-binding protein B (S100B) and p50 and p56 antibodies in rat astrocytes.	[30]
C57/Bl6 mice, 3-month-old	Mice were treated with CBD injections of 20 mg/kg daily for the first 1 week and 3 times a week for the following 2 weeks.	It has been determined that cannabidiol treatment inhibits Aβ-induced IL-6 gene expression.	[31]
6-month-old APPxPS1 mice	20 mg/kg CBD administered daily for 3 weeks	It was determined that cannabidiol treatment reversed the cognitive deficits in object recognition and social recognition memory without affecting the anxiety parameters of the subjects.	[32]
2.5-month-old APP PS1 mice	20 mg/kg cannabidiol or cannabidiol pellets were administered for 8 months using the daily oral administration protocol.	It has been demonstrated by the study that long-term cannabidiol treatment prevents the development of memory deficits in social recognition without affecting anxiety areas of the brain in transgenic mice with Alzheimer's disease.	[33]
14-month-old female TAU58/2 transgenic mice	100 mg/kg CBD was administered for 3 weeks.	It was shown that CBD improved impaired spatial reference memory, reduced anxiety-like behaviors and freezing associated with contextual fear in the transgenic mice	[34]
130-140 g rats	Rats were treated with cannabis resin (5, 10, or 20 mg/kg) tramadol (5, 10, and 20 mg/kg), and cannabis resin (5, 10, and 20 mg/kg) combined with tramadol (10 mg/kg) subcutaneously daily for 6 weeks.	It was reported that cannabis resin might exert differential effects on AChE and BChE activities, which could contribute to memory problems and a decline in cognitive function in chronic users.	[35]

CONCLUSIONS

Looking at the *in vitro* data overall, it is clear that phytocannabinoids and cannabis extracts, particularly those found in research using the neuronal cell lines PC12 and SH-SY5Y, show promise in reducing -amyloid-induced neurodegeneration. Likewise, *in vivo* studies suggest that future studies of *Cannabis* extracts and components should examine the potential benefits of animal and human cognition and brain health.

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