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The Use of *Mus Musculus* Model to Evaluate Pharmacological Mechanisms of Curcumin in Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disease that is the primary cause of dementia in late adulthood. With the increasing global population and life expectancy, the incidence of AD is also rapidly rising. Currently, available drugs for AD cannot prevent disease progression but can only treat its symptoms, accompanied by severe side effects and high costs. Therefore, curcumin is expected to be an alternative treatment for AD. The aim of this study was to investigate the pharmacological pathway of curcumin's mechanisms of action on AD using mice as animal models. A search was conducted on four databases using the keywords "curcumin", "*Mus musculus*", and "Alzheimer's disease". Curcumin possesses multiple mechanisms of action, namely: (1) as an antioxidant agent by increasing antioxidant enzyme activity and decreasing the number of reactive oxygen species (ROS); (2) an anti-inflammatory agent characterized by a decrease in neuroinflammatory cytokines and inhibits endoplasmic reticulum stress; (3) inhibition of amyloid beta and tau hyperphosphorylation, resulting in a decrease in Aβ deposit and tau expression, as well as increased expression of beta-site APP cleaving enzyme-1 (BACE-1) and amyloid beta degrading enzyme; (4) Reducing the activated microglial cell population and decreasing ionized calcium binding adaptor-1 molecule (Iba-1) and glial fibrillary acidic protein (GFAP); (5) regulating important molecules in insulin signaling pathways and glucose metabolism; (6) Inhibition of activity of acetylcholinesterase (AChE) enzyme..

KEYWORDS: acetylcholinesterase, amyloid beta, microglia, neuroinflammation, tau hyperphosphorylation.

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I. INTRODUCTION

Alzheimer's disease (AD) is the most common types of dementia, a term referring to a decline in cognitive ability that interferes with daily activities (Kumar et al., 2023). As population and life expectancy continue to increase, the incidence of AD is rising globally. It is estimated that AD will increase to 65.7 million in 2030 and 115.4 million in 2050. The prevalence of AD increases with age from 1% per year before age 65 to 6% per year after age 85. The incidence of AD is slightly higher in women, especially those over 85 years old (Schachter and Davis, 2000).

Clinically, AD is characterized by brain abnormalities, such as extracellular aggregation of neuritic plaques and neurofibrillary tangles (NFT) in the brain. Neuritic plaque is an insoluble deposit of amyloid beta (Aβ) peptide derived from amyloid precursor protein (APP). The plaque first develops in brain areas associated with cognitive function and spreads to other cortical areas as the disease progresses. Meanwhile, NFT is an intracellular accumulation of tau protein that undergoes hyperphosphorylation. The formation of NFT is related to the severity of the disease. The more advanced the disease stage, the more NFTs are formed in the brain (Schachter and Davis, 2000).

The number of drugs available for AD is limited and most of them can only treat its symptoms. These treatments cannot prevent the further progression of the disease or reverse the effects that have already occurred. Drugs used for mild to moderate cases are classified as cholinesterase inhibitors, which prevent the breakdown of acetylcholine, which is necessary for memory and learning (Lee et al.,

2020). Drugs for mild to moderate cases include donepezil, rivastigmine, and galantamine. Meanwhile, for moderate to severe cases, memantine is generally given in combination with N-methyl D-aspartate (NMDA) antagonists. Treatments used in Alzheimer's cases have limited efficiency and significant side effects such as nausea, vomiting, diarrhea, headache, dizziness, fatigue, decreased consciousness, muscle spasms, and insomnia, as well as poor patient compliance (Lee et al., 2020). In addition, the cost of treatments is also a burden on patients. The significant increase in cases and limited drugs highlight the importance of developing alternative management strategies for AD patients (Lee et al., 2020).

Plants have long history of uses as medicine for various health problems. *Curcuma longa* (turmeric), which is commonly used as a spice, has long been known traditionally for the treatment of various diseases, such as cardiovascular disease, liver disease, obesity, cancer, inflammation, and neurodegenerative diseases. Main metabolites found in *C. longa* consist of a mixture of curcumin, diferuloylmethane (75-80%), demethoxycurcumin (15-20%), and bisdemethoxycurcumin (3-5%) (Mukhopadhyay, Ruidas, & Chaudhury, 2017). Pharmacological studies have researched the effects of curcumin on memory function, insulin receptors (IR), acetylcholinesterase (AChE) activity, and oxidative stress in dementia mice. Study results suggest that curcumin may be an important factor in protecting mice from AD. The aim of this study is to investigate the mechanism of action of curcumin as an antioxidant and anti-inflammatory agent against AD in mice.

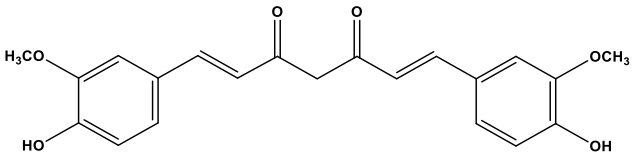

Figure 1 Curcumin.

This review aimed to study the mechanisms of curcumin action against AD using mice as a model. The study first describes curcumin and Mus musculus as an animal model to study curcumin efficacy for AD. It was then followed by description of mechanisms of actions of curcumin on AD, including anti-inflammatory, antioxidant, amyloid-β inhibition, tau inhibition, modulation of microglia, and modification of insulin signalling pathway.

II. METHOD

Screening of articles was conducted using PRISMA method and databases including Google Scholar, Pubmed, Elsevier, and ScienceDirect were used to find and collect relevant journals. The following keywords were used in literature search i.e. "curcumin," "Mus musculus," and "Alzheimer's disease". Inclusion criteria were set for the past 10 years and prioritized research articles over reviews. A total of 387 articles were identified. After review, 75 journals met the inclusion criteria. However, 40 journals were not used because they did not discuss the mechanism of curcumin action against AD in animal models or the outcomes of the studies were not relevant. After reviewing and screening the journals, 35 journals were found to meet the inclusion criteria and the study's goals.

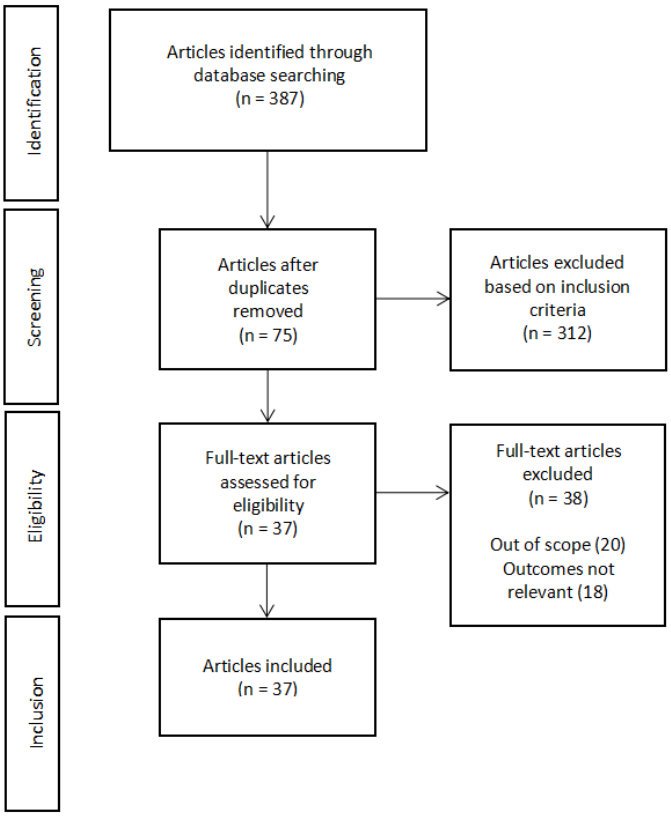

Figure 2. The Flowchart of article screening by PRISMA method for the study of in vivo investigations on the effect of curcumin on AD.

III. RESULTS

A. *Curcumin*

Curcumin (Figure 1), a hydrophobic polyphenol isolated from Curcuma longa, has been reported to have therapeutic effects on various diseases, including neurological, respiratory, cardiovascular, reproductive, digestive, musculoskeletal, endocrine, and autoimmune diseases (Voulgaropoulou et al., 2019). Several clinical trials have reported the bioactivity and pharmacology of curcumin, including anti-inflammatory, antioxidant, neuroprotective, and chemoprotective effects. Some studies have shown that curcumin has significant therapeutic effects on neurological diseases, especially AD (Huang et al., 2017). However, the main weakness of curcumin lies in its low bioavailability due to poor solubility, low absorption, rapid metabolism, and rapid excretion. Many efforts have been made to overcome this problem, and many curcumin formulations have been developed, including liposome encapsulation, nanoparticles, powder formulations, micelles, emulsions, co-

administration with other substances, or separate administration of its constituents.

B. *Mus musculus* types

Research using animal models is one of the most effective ways to investigate the pathogenic process of a disease. One commonly used animal model is Mus musculus or laboratory mouse. As a mammalian model system, mice have the advantages of a short lifespan and rapid reproduction, facilitating timely completion of experimental protocols. Furthermore, 99% of disease-related genes have homology with humans, which exceeds other animal models such as Danio rerio, Caenorhabditis elegans, and Drosophila melanogaster. The combination of these factors has made the laboratory mouse one of the most common animal models for Alzheimer's disease (AD).Two types of mice were used to study AD, i.e. wild mice and transgenic mice. In wild-type mice, there is no evidence of neurofibrillary tangles, which is one of the main characteristics of Alzheimer's disease (AD).

In contrast to wild-type mice, transgenic mice models can exhibit neurofibrillary tangles (NFTs), neurodegeneration, and memory loss as they age (Mckean, Handley, & Snell, 2021). Examples of transgenic mice include APP/PS1, 5xFAD, ApoE4, Tg2576, N2a/APPswe, and 3xTg types. The use of transgenic mice can be one way to manifest human AD pathology (Claeysen, Giannoni, & Ismeurt, 2020). This may be due to differences in tau sequence between mice and humans, with an estimated 88% sequence homology (Drummond and Wisniewski, 2017).

C. *Anti-inflammatory activity of curcumin in AD*

Chronic inflammation in neurons is one of important pathogenesis in AD. Several studies reported that deposition of Aβ was accompanied by pro-inflammatory compounds and microgliosis (Mishra and Palanivelu, 2008). Inflammation drugs work by targeting receptors and signaling pathways, modulating responses of target tissue towards inflammation, recovering inflammation effect on the target tissues, and inducing anti-inflammatory factors. Non-steroidal anti-inflammatory drugs have been shown to reduce AD symptoms. However, long term use of NSAID causes adverse side effects, such as toxicity to kidney, liver, and gastrointestinal system. Reports below shows mechanisms of action of curcumin in AD in modulating inflammatory pathways and inhibiting inflammatory mediators.

Table 1 Anti-inflammatory mechanisms of curcumin in AD.

Drug preparation, dosage, route of administration	Type of mice	Mechanisms and effects	References
Curcumin (4 g/kg) oral for 12 weeks	p25Tg	Reduced MIP-1, TNF-α, and IL-1β	(Sundaram et al., 2017)
Curcumin (40 mg/kg) via intraperitoneal for 3 weeks	ApoE4	Inhibition of ER stress	(Kou et al., 2021)
Low dose curcumin	APP/PS1	Decrease in IL-1β, miR-146A, and iNOS	(Gong and Sun, 2022)
Curcumin (100 mg/kg/day) oral from the age of 4 months	APP/PS1	Decrease in GFAP and COX2 expression	(Han et al., 2021)
Curcumin analog (TML-6) combined with anti-Aβ antibody NP106 for 17 weeks	APP/PS1	Decrease in TNFα and IL-6	(Su et al., 2022)
Curcumin analog (TML-6) 150 mg/kg oral	3xTg	Suppression of Iba-1 expression	(Su et al., 2020)
Tetrahydrocurcumin (400 mg/kg BW) intragastric	APP/PS1	Decrease in TNF-α and increase in TGF-β1 and Bag1	(Xiao et al., 2021)
Curcumin loaded lipid-core nanocapsules (LNC) 1 mg/kg intracerebroventricular for 14 days	Mice injected with amyloid β	Decrease in TNF-α and L-1β	(Giacomeli et al., 2019)
Co-administration of curcumin loaded and meloxicam (10 mg/kg BW) oral for 12 days	Swiss male mice injected with amyloid β	Downregulation of COX-2	(Gutierrez et al., 2021)
Curcumin and ambrosin (10 mg/kg) intraperitoneal for 7 days	Albino mice with LPS	Inhibition of TNF-α, IL-1β, COX-2, and iNOS expression	(Khalil et al., 2019)

Sundaram et al (2017) reported that treatment with Curcumin for 12 weeks on in p25Tg mice reduced proinflammatory cytokines. The study found that curcumin effectively reduced microglial activation and the production of proinflammatory cytokines MIP-1 (macrophage inflammatory protein-1), TNF-alpha (tumor necrosis factor-alpha), and IL-1β (Interleukin-1) (Sundaram et al., 2017).

However, TGF-β (transforming growth factor-β) did not change with curcumin treatment in p25Tg mice. The suppression of these proinflammatory cytokines also inhibited the progression of amyloid pathology and improved cognitive function in p25Tg mice. The cognitive function of mice was measured using a radial arm maze test.

Apolipoprotein E (ApoE) is a protein involved in the transportation of cholesterol and other lipids in the brain and throughout the body. ApoE promotes the aggregation of Aβ plaque. Thus targeting curcumin therapy to the ApoE4 protein could be an approach strategy in AD. Kou et al (2021) found that treatment by curcumin injection for three weeks to ApoE4 transgenic mice reduced high endoplasmic reticulum (ER) stress protein expression. Cognitive function was increased as seen based on the Morris water maze test (Kou et al., 2021).

In addition to Aβ, miR-146a is a useful biomarker for AD. Gong et al (2022) found that low-dose curcumin reduced the levels of miR-146a in the brain tissue (Gong and Sun, 2022). The treatment also decreased the levels of the inflammatory proteins IL-1β and iNOS, and increased complement factor H (CFH). CHF is associated with Aβ clearance (Gong and Sun, 2022).

In another study, Han et al (2021) found that treament on AD mouse model of APP/PS1 with curcumin (100 mg/kg/day) reduced the expression of GFAP (a biomarker as an astrocyte activation marker) and COX2 (an inflammation biomarker). Curcumin could modulate the HMGB1/NF-kB signaling pathway in the hippocampus of transgenic mice by decreasing the expression of HMGB1, RAGE, TLR4, and NF-κB p65 proteins (Han et al., 2021).

Curcumin analogs have been synthesized and tested to improve the stability and metabolism of curcumin. Studies have demonstrated therapeutic properties of some curcumin analogs in AD treatment. Su et al. (2022) conducted a study comparing the therapeutic effects of combined treatment with anti-Aβ antibody NP106 and curcumin analog (TML-6) on APP/PS1 mice.(Su et al., 2022) The results showed that the combination treatment of NP106 and TML-6 was more effective in reducing brain Aβ and increasing microglial Aβ phagocytosis compared to monotherapy with NP106 or TML-6 alone. The level of IL-1β was slightly decreased by TML-6 and NP106 monotherapy, the level of TNFα was decreased by both monotherapies, while the level of IL-6 was significantly reduced by TML-6 monotherapy in the hippocampus but not in the cortex (Su et al., 2022).

Treatment of 3xTg mice with TML-6 succeeded in suppressing Iba-1 expression, which is a biomarker of microglial activation. Whereas treatment with curcumin did not cause a significant change in Iba-1 expression (Su et al., 2020).

Administration of 1,7-bis(4-hydroxy-3-methoxyphenyl) heptane-3,5-dione (tetrahydrocurcumin /THC), a curcumin derivative, can reduce Aβ burden in the hippocampus of APP/PS1 transgenic mice (Xiao et al., 2021). Xiao et al (2021) found that treatment with THC reduced Aβ42/Aβ40 ratio. The western blot analysis showed the reduction of abnormal Gab2, K-Ras, caspase-3, and TNF-α expression, while increasing TGF-β1 and Bag1 expression. In BV-2 cells, Aβ induced down-regulation of Gab2, K-Ras, TGF-β1, and excessive expression of caspase-3, PARP1, cleaved-PARP1, and TNF-α, which were restored by THC (Xiao et al., 2021).

Giacomelli et al. (2019) reported that curcumin loaded lipid-core nano capsules (LNC) can provide protection against neuroinflammation in female mouse models administered Aβ1-42 (Giacomeli et al., 2019). The mouse models were given LNC with intracerebroventricular injections at a dose of 1 mg/kg for 14 days. Treatment with LNC resulted in a decrease in TNF-α and L-1β in the mouse PFC (Giacomeli et al., 2019).

Combined treatment of curcumin and other substances were also reported to treat AD. Gutierrez et al. (2021) studied the combination effect of co-nano encapsulated curcumin and meloxicam, a commonly used drug to alleviate joint inflammation, in lipid core nano capsules (LCN) on cognitive impairment (Gutierrez et al., 2021). This study used male Swiss mice that were injected with amyloid beta peptide. The mice were injected with amyloid beta peptide aggregate (fragment 25-35, 3 nmol/3 μL) and then treated with curcumin-loaded LCN (10 mg/kg) or meloxicam-loaded LCN (5 mg/kg) or a combination of meloxicam and curcumin-co-loaded LCN (5 and 10 mg/kg, respectively). Treatment was given every other day for 12 days, with 6 doses given orally every 48 hours. The results reported an anti-inflammatory mechanism through downregulation of cortical COX-2 by the combination of curcumin and meloxicam (Gutierrez et al., 2021).

Khalil et al. (2019) reported that the use of curcumin with ambrosin in mice can inhibit the production of inflammatory mediators, NF-κβp65 transcript, and protein expression.(Khalil et al., 2019) Memory impairment in mice was induced by intraperitoneal injection of LPS (0.4 mg/kg) and treated with curcumin (100 mg/kg). Memory function in mice was measured using the Morris water maze and object recognition test. Treatment with curcumin resulted in the inhibition of proinflammatory and nitrosative mediators, namely TNF-α, IL-1β, COX-2, and iNOS. BACE1 was also inhibited, leading to a reduction in amyloid beta plaque deposition (Khalil et al., 2019).

D. Antioxidant activity of curcumin in AD

Aβ aggregates triggers oxidative stress due to the presence of reactive oxygen species (ROS) in the brain of AD sufferers. The oxidative neurotoxicity causes memory loss and cognitive deficit. Curcumin is able to inhibit activator protein-1 (AP-1) activity, which is a transcription factor involved in the expression of Aβ (Huang et al., 2017). Curcumin was reported to reduce oxidation of low density lipoprotein and protect brain mitochondria from oxidative stress damage, not only in AD, but also in other

neurodegenerative diseases such as Huntington's dan Parkinson's diseases. Descriptions below explains the most recent reports on the antioxidant activity of curcumin in AD.

An *in vitro* test using HT22 mouse hippocampal cells showed that curcumin was able to reduce oxidative stress. The cells were treated with 25 μM acrolein and followed by treatment with curcumin (5μg/mL) for 30 minutes. Results showed that curcumin decreased oxidative stress caused by acrolein, as evidenced by increased activity of the GSH and SOD enzymes and decreased MDA levels (Shi et al., 2018).

Table 2 Antioxidant mechanisms of curcumin in AD

Drug preparation, dosage, route of administration	Type of mice	Mechanisms and effects	References
Curcumin (5μg/mL) by inhalation	Acrolein-induced HT22 mouse hippocampal cells	Increased SOD, GSH, decreased MDA levels	(Shi et al., 2018)
3% curcumin powder orally administered	Gasoline-exposed CD1 mice	Decreased AOPP, MDA and thiol compounds, increased SOD and catalase	(Elsayed et al., 2016)
Curcumin (200 mg/kg/day) and berberine (100 mg/kg/day) orally administered	B6C3-Tg	Reduced ROS, TBARS, APP and BACE1 levels, increased SOD	(Lin et al., 2020)
Curcumin in micelle formulation (APLB/CUR micelles) for 2 months	APP/PS1	Reduced R OS	(Lu et al., 2019)
Curcumin nanocapsules (10 mg/kg) orally administered	Swiss male mice with intracerebroventricular Aβ aggregate injection	Reduced ROS, increased SOD and catalase	(Fidelis et al., 2019)
PLGA nanoparticles + Aβ inhibitor S1 + curcumin	AD mice	Reduced ROS, increased SOD	(Huang et al., 2017)

Animal studies demonstrated the ability of curcumin to protect animal brain tissue from oxidative stress caused by gasoline exposure. In a study by Elsayed et al. (2016), a 3% powder was given orally to CD1 mice for 1 week before gasoline exposure and during the study. Mice were exposed to gasoline at a concentration of 9375 ppm for 2 hours/day for 3 weeks. An increase in advanced oxidation protein products (AOPP) due to gasoline inhalation was observed. However, AOPP was decreased after curcumin powder administration (Elsayed et al., 2016). In addition, MDA level was decreased, but the activity of the superoxide dismutase (SOD) and catalase enzymes was increased (Elsayed et al., 2016).

A synergistic effect of a combination of berberine (100 mg/kg/day) and curcumin (200 mg/kg/day) was studied using B6C3-Tg (APPswePSEN1dE9)/Nju mice. The combined treatment resulted in a decrease in oxidative stress in the cortex and hippocampus of AD mice, and improvement in cognitive function as indicated by a decrease in thiobarbituric acid reactive substances (TBARS) and increased SOD activity (Lin et al., 2020). The study observed a shorter duration in the Morris water maze (MWM) cognitive test (Lin et al., 2020). Additionally, the combination treatment also lowered APP and BACE1 levels and increased AMPK alpha phosphorylation and autophagy (Lin et al., 2020).

The use of micelle formulation can be a strategy to enhance curcumin bioavailability. Lu et al (2019) developed curcumin in micelles (PBL/Cur) and treated to APP/PS1 mice for three months. Treatment showed better ability to reduce ROS than regular curcumin, decreased neuroinflammatory cytokines, and Aβ plaques (Lu et al., 2019).

Fidelis et al. (2019) reported that curcumin-loaded nanocapsules (NLC C) can reduce oxidative stress and Increase the bioavailability and biodistribution of curcumin (Fidelis et al., 2019). This study used Swiss male mice as the animal model with intracerebroventricular Aβ aggregates (3 nmol/3 μL, i.c.v.) injection. NLC C was orally administered at a dose of 10 mg/kg every other day for 12 days. The results showed that NLC C was more effective in reducing oxidative stress due to Aβ in the prefrontal cortex than regular curcumin. This was evidenced by the decrease in ROS levels and the increase in SOD and catalase activity (Fidelis et al., 2019).

Huang et al (2017) developed curcumin with PLGA nanoparticles. Its combination with Aβ inhibitor S1(PQVGHL peptide) was treated to transgenic AD mice. Results showed reduced reactive oxygen species (ROS) and increased SOD activity. In addition, treatment decreased the production of cytokines IL-6 and TNF-α in the AD mouse brain (Huang et al., 2017). Cognitive function was evaluated using the Y-maze and object recognition tests.

E. Amyloid Beta (Aβ) Inhibition

Amyloid beta (Aβ) plaque is one of the hallmark symptoms of AD. Curcumin has been found to have many benefits for AD treatment, particularly in reducing Aβ

deposition. In a typical experiment, Aβ level can be measured using ELISA kit. Aβ plaques can also be assessed using immunolabeling with Aβ-specific antibody and cresyl violet staining, especially in prefrontal cortex (PFC), entorhinal cortex (EC), CA1, CA3, and subicular complex (SC).

Table 3 Mechanisms of curcumin in the inhibition of amyloid β deposition

Drug preparation, dosage, route of administration	Type of mice	Mechanisms and effects	References
Curcumin at low dose (100 mg/kg/day), medium dose (200 mg/kg/day), and high dose (400 mg/kg/day)	APP/PS1	Decrease in Aβ formation and increase in β-amyloid-degrading enzymes	(Wang et al., 2014)
Curcumin at a dose of 120-300 mg/kg/day intragastrically for 60 days	5xFAD	Downregulation of BACE1 expression	(Zheng et al., 2017)
Curcumin at a dose of 83 mg	Tg2576	Reduction in Aβ40/Aβ42 levels in the frontal cortex and Aβ42 levels in the temporal lobe	(Sharman et al., 2019)
Curcumin (20 mg) encapsulated with selenium nanoparticles (Se-PLGA) administered intravenously	5xFAD	Binding of Aβ through hydrophobic binding of curcumin	(Huo et al., 2019)
FMeC1 (curcumin derivative) at a dose of 83 mg/kg for 6 months	APP/PS1	Reduction in formation and toxicity of Aβ deposits	(Yanagisawa et al., 2015)
TML-6 for 24 hours	N2a/APPswe	Reduction in insoluble forms of Aβ1–42 and Aβ1–40	(Su et al., 2020)
Combination of TML-6 and Anti-Aβ Antibody NP106 for 17 weeks	APP/PS1	Reduction in insoluble forms of Aβ1–42 and Aβ1–40	(Su et al., 2022)
Combination of curcumin and melatonin (50 mg/kg) orally	APP/PS1	Drastic reduction in Aβ plaques	(Gerenu et al., 2015)

Researchers have studied the effect of curcumin treatment on the formation and deposition of Aβ. Wang et al (2014) used APP/PS1 double transgenic AD mice, treated with 160 and 1000 ppm for six months. Curcumin reduced Aβ deposition by inhibiting the expression of Phosphatidylinositol 3-Kinase (PI3K), phosphorylated Akt, and rapamycin (mTOR).(Wang et al., 2014) Studies by Zheng et al (2017) used different dosages of curcumin at 150-300 mg/kg/day orally for 60 days. Analysis by ELISA showed decrease in Aβ in the cortex and hippocampus (Zheng et al., 2017). In addition, BACE1 was downregulated. Further, improvement of spatial learning and memory impairment in mice was observed by the Morris water maze (Zheng et al., 2017). Sharman et al (2019) used curcumin to treat male Tg2576 mice at a dose of 500 ppm or 83 mg/kg. By immunohistochemistry, the study observed a great reduction of Aβ plaques in cortical and hippocampal areas in the mouse brain, as well as a decrease in Aβ40/Aβ42 levels in the frontal cortex and Aβ42 levels in the temporal lobe (Sharman et al., 2019). However, the study found that a combination of curcumin with epigallocatechin-3-gallate (EGCG), docosahexaenoic acid (DHA), and α-lipoic acid (ALA) did not show a greater reduction in Aβ plaques (Sharman et al., 2019).

Curcumin nanoparticles have also been used by intravenous injection. Huo et al (2019) treated curcumin (20 mg) encapsulated with selenium nanoparticles (Se-PLGA) to 5XFAD mice. Curcumin and selenium have anti-inflammatory and antioxidant properties, so if combined together, they can result in a decrease in neuroinflammation and oxidative stress, which are the two main processes in AD pathogenesis. Curcumin molecules was visualized to bind Aβ oligomers in the nonpolar region of Aβ oligomers, thus can inhibit aggregation of Aβ oligomers (Huo et al., 2019).

Curcumin derivatives have been designed to increase curcumin bioavailability in the body and target specific signaling pathways in AD pathogenesis. A study by Yanagisawa et al. (2015) used curcumin derivative FMeC1, which was treated to APP/PS1 mice at a dose of 83 mg/kg for 6 months. Results showed that FMeC1 reduced insoluble Aβ deposits, decreased glial cell activity, and also improved cognitive deficits (Yanagisawa et al., 2015). Another curcumin derivative, TML-6, was tested on N2a/APPswe mouse models. A dose of 3.14 μg/mL was given for 24 hours. Results showed that treatment decreased Aβ40 and Aβ42 production by 86% and 80%. The treatment also upregulated Apo E suppressed NF-κB and mTOR expression, and enhanced Nrf2 antioxidant gene activity (Su et al., 2020).

Combined treatment of anti-Aβ Antibody NP106 and curcumin analog TML-6 has also been reported. Su et al

(2022) treated APP/PS1 for 17 weeks with a combination of NP106/TML-6. Staining with Amylo-Glo showed lower level of Aβ deposits in the cortex and hippocampus. insoluble forms of Aβ1–42 and Aβ1–40 in the brain were drastically reduced (Su et al., 2022). However, soluble forms of Aβ1–40 and Aβ1–42 were not found to have changed.

Gerenu et al. (2015) used a combination of curcumin and melatonin at a dose of 50 mg/kg to treat APP/PS1 mice. Drastic reduction of Aβ plaque levels was observed in the cerebral cortex and hippocampal area (Gerenu et al., 2015).

F. Tau Inhibition

Hyperphosphorylation of tau protein is one of the causes of the formation of neurofibrillary tangles, a hallmark of AD. Glycogen synthase kinase-3 (GSK-3), especially the β-isoform, involves in the phosphorylation of tau protein in multiple sites. GSK-3 is also the key kinase in the cleavage of APP into Aβ.

Table 4: Mechanisms of curcumin in the tau inhibition

Drug preparation, dosage, route of administration	Type of mice	Mechanisms and effects	References
Low dose (0.16 g/kg) and high dose (1.0 g/kg) of curcumin were administered orally	APP/PS1	Decrease in tau expression (P-Tau(ser404)/Tau) and caveolin-1	(Sun et al., 2017)
Curcumin analog C1	5xFAD, P301S, and 3xTg-AD	Decreased APP, CTF-β/α, and tau aggregates	(Song et al., 2020)
Combination of ferulic acid (0.05%), phosphatidylserine (0.05%), and curcumin (0.01%) administered orally for 3 months	APP/PS1	Reduced the AT8/TAU-5 ratio and phosphorylation of tau protein	(Okuda, Fujita, & Sugimoto, 2019)
PLGA-PEG-B6/Cur	APP/PS1	Reduced hyperphosphorylation of tau	(Fan et al., 2018)

Curcumin treatment in APP/PS1 double transgenic mice could reduce the expression of tau (P-Tau (ser404)/Tau) (Sun et al 2017). This study also found reduced level of caveolin-1 (a membrane caveolae protein marker that plays a role in the formation of Aβ and cleavage of APP), but the expression of phosphorylated GSK-3 increased (Sun et al., 2017).

Treatment of curcumin analog C1 could also reduce tau aggregation. Study by Song et al (2020) proved that treatment of curcumin analog C1 to three models of AD mice (5xFAD, P301S, and 3xTg-AD mice) could effectively activate TFEB, leading to increased autophagy and lysosomal activity, and reduction in APP, APP C-terminal fragments (CTF-β/α), Aβ deposits, and tau aggregates (Song et al., 2020). This was followed by improvement in synaptic and cognitive function in transgenic mice.

Ferulic acid (FA) and phosphatidylserine (PS) have been extensively investigated for their potential in treating AD due to their neuroprotective effects. Okuda et al (2019) studied the effect of combination of ferulic acid (FA), phosphatidylserine (PS), and curcumin for AD. Treatment of APP/PS1 mice with 0.05% FA, 0.05% PS, and 0.01% Cur for three months proved to reduce tau phosphorylation in the mice brain (Okuda et al., 2019). Also observed were the reduction of insoluble Aβ1-42 and insoluble Aβ1-40 compared to the control group. The cognitive function of mice, evaluated using the Y-maze, was also improved (Okuda et al., 2019).

Application of curcumin for AD suffers from low bioavailability and absorption of the compound. To increase penetration through blood brain barrier, alternative formulation is required. Fan et al. (2018) designed a poly(lactide-co-glycolide)-block-poly(ethylene glycol) (PLGA-PEG) nanoparticle conjugated with B6 peptide and containing Cur (PLGA-PEG-B6/Cur) administered to APP/PS1 mice. The formulation could reduce the diameter of Cur, leading to increased cellular uptake and good compatibility (Fan et al., 2018). Morris Water Maze Results proved that the combination of PLGA-PEG-B6/Cur could improve spatial learning and memory of APP/PS1 mice. compared to regular curcumin (Fan et al., 2018).

G. Modulation of Microglia

Microglia, a type of glial cell, is an important inate immune surveillance in the central nervous system (CNS). In response to phatological brain condition such as in the accumulation of Aβ , microglia releases proinflamatory cytokines and free radicals. The activation of microglia is important in the phagocytosis Aβ. However long term activation causes chronic neuro inflammation and oxidative stress condition in CNS.

In a study using Tg2576 mice by Teter et al (2019), treatment of low dose of curcumin (160 ppm) for 10-16 months could stimulate microglial migration and Aβ plaque phagocytosis (Teter et al., 2019). Moreover, low-dose curcumin also reduced the level of miR-155, a micro-RNA associated with microglial neurodegenerative phenotype (Teter et al., 2019).

Curcumin treatment can decrease glial cell activity of APP/PS1 transgenic mice, by decreasing immunoreactivity

of GFAP and Iba-1 in the cerebral cortex and hippocampus of APP/PS1 mice (Yanagisawa et al., 2015). Xiao et al. (2021) showed that treatment with tetrahydrocurcumin (THC) 400 mg/kg can reduce TNF-α and increase TGF-β1 secretion in APP/PS1 mice (Xiao et al., 2021).

Table 5 Mechanisms of curcumin in the modulation of microglia in AD.

Drug preparation, dosage, route of administration	Type of mice	Mechanisms and effects	References
Curcumin (160 ppm) for 10-16 months	Tg2576	Reduction of miR-155 levels	(Teter et al., 2019)
Tetrahydrocurcumin (400 mg/kg BW) intragastric	APP/PS1	Decreased TNF-α and increased secretion of TGF-β1	(Xiao et al., 2021)
Curcumin with micelles formulation (APLB/CUR micelles) for 2 months	APP/PS1	Reduction of activated microglia	(Lu et al., 2019)
Combination of curcumin analog (TML-6) and anti-Aβ antibody NP106	APP/PS1	Increased microglial phagocytosis with Iba-1 colocalization in Aβ plaques and changes in microglial morphology	(Su et al., 2022)
Curcumin + melatonin (50 mg/kg) orally	APP/PS1	Reduction of type IV glial cell population	(Gerenu et al., 2015)
FMeC1 (curcumin derivative) with a dose of 83 mg/kg for 6 months	APP/PS1	Reduction of GFAP- and Iba-1 immunoreactivity	(Yanagisawa et al., 2015)

Similar findings were reported by Lu et al (2018) which showed that treatment with curcumin formulated with micelles for two months could decrease activated microglia, followed by a decrease in the expression of neuroinflammatory markers, tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β), in the mouse brain, and a decrease in oxidative stress (Lu et al., 2019).

Combination treatment of curcumin analog (TML-6) and anti-Aβ antibody NP106 in APP/PS1 mice can enhance phagocytosis of Aβ by microglia. In the study by Su et al (2022), colocalization of Iba-1 (a marker of microglia) and Aβ was observed (Su et al., 2022). Changes in microglial morphology was also observed due to treatment. In another study, Gerenu et al (2015) found that oral treatment of combination of curcumin and melatonin for 12 weeks could decrease microglia activation, as seen by decrease in the most activated glial cell type, type IV (Gerenu et al., 2015).

H. Modification of the Insulin Signaling Pathway

Deficits in glucose, dysfunction in insulin signaling, and insulin resistance in the brain often occur in the pathogenesis of AD. Feng et al. (2016) reported that treatment with curcumin at a dose of 200 mg/kg/day for six months has the potential to regulate important molecules in the insulin signaling pathway in the brain.(Feng et al., 2016) The results showed an expression of insulin receptor (InR) and a decrease in insulin receptor substrate (IRS) in the CA1 area of APP/PS1 double transgenic mice hippocampus. Meanwhile, the expression of phosphatidylinositol-3 kinase (PI3K), phosphorylated PI3K (p-PI3K), serine-threonine kinase (AKT), and phosphorylated AKT (p-AKT) increased (Feng et al., 2016).

Wang et al. (2017) reported that curcumin can regulate insulin signaling and glucose metabolism (Wang et al., 2017). This study monitored glucose metabolism in the brains of APPswe/PS1dE9 mice using micro-positron emission tomography (PET) technique. The results showed an increase in cerebral glucose uptake in AD mice. The IHC results showed that curcumin could improve defective insulin signaling pathways by upregulating insulin-like growth factor (IGF)-1R, IRS-2, PI3K, p-PI3K, Akt, and p-Akt, while IR and IRS-1 experienced downregulation mechanisms (Wang et al., 2017). With increased glucose metabolism and improved insulin signaling, spatial learning and memory also improved, demonstrating that curcumin has the potential to treat insulin resistance in the brain.

Although curcumin has the potential to treat insulin resistance in AD, its low bioavailability limits the absorption of curcumin in the body and reaching the brain. This limitation causes limitations in its effectiveness in treating insulin resistance. Therefore, strategies to improve its bioavailability are needed to increase the circulation of curcumin to the brain.

I. Inhibition of Acetylcholinesterase

Acetylcholine is a neurotransmitter responsible for regulating cognitive function, the central cholinergic system, and plays an important role in memory and learning processes. Acetylcholine is degraded by cholinesterase, causing cholinergic deficiency in AD cases. Inhibition of cholinesterase enzyme is one of the therapeutic targets in AD. Hussain, et al. (2022) conducted research with six synthetic curcumin analogs and investigated their potential for in vivo cholinesterase inhibition using elevated plus maze (EPM), Y-maze, and novel object recognition (NOR) behavioral tests in mice (Hussain et al., 2022). The mice were given scopolamine (1 mg/kg) to induce AChE enzyme activity. The studied curcumin analogs showed a decrease in

time in the EPM and an increase in %SAP in the Y-maze in the NOR model mice (Hussain et al., 2022). Investigation into cholinesterase inhibition was conducted using MOE software, and the results showed inhibition of AChE enzyme activity.

IV. CONCLUSION

Curcumin has been shown to have therapeutic effects in AD. The mechanisms of action include: (1) as an antioxidant agent characterized by reducing MDA levels and the amount of ROS as well as increasing SOD, GSH, and catalase activity; (2) anti-inflammatory agent characterized by reducing neuroinflammatory cytokines, inhibiting or downregulation of COX, and inhibiting ER stress; (3) Inhibition of Aβ formation as seen by reduction of Aβ 40 and Aβ 42 deposition and increased activity of Aβ degrading enzymes; (4) reduced hyperphosphorylation of tau; (5) increased microglial phagocytocis and reduced activated microglia; (6) regulating important molecules in the insulin signaling pathway and glucose metabolism; (7) inhibition of acetylcholinesterase (AChE) activity.

CONFLICT OF INTEREST

None to declare

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