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Original Article

The pharmacological activity of epigallocatechin-3-gallate (EGCG) on Alzheimer's disease animal model: A systematic review

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ABSTRACT

Background: Alzheimer's disease (AD) is currently incurable and there is an urgent need to develop new AD drugs. Many studies have revealed the potential neuroprotective effect of Epigallocatechin-3-O-gallate (EGCG), the main antioxidant in green tea, on animal models of AD. However, a systematic review of these reports is lacking.

Purpose: To assess the effectiveness of EGCG for AD treatment using systematic review and meta-analysis of preclinical trials.

Methods: We conducted a systematic search of all available randomized controlled trials (RCTs) performed up to November 2019 in the following electronic databases: ScienceDirect, Web of Science, and PubMed. 17 preclinical studies assessing the effect of EGCG on animal AD models have been identified. Meta-analysis and subgroup analysis was performed to evaluate cognition improvement of various types of AD models. The study quality was assessed using the CAMARADES checklist and the criteria of published studies.

Results: Our analysis shows that the methodological quality ranges from 3 to 5, with a median score of 4. According to meta-analysis of random-effects method, EGCG showed a positive effect in AD with shorter escape latency (SMD= -9.24, 95%CI= -12.05 to -6.42) and decreased $A\beta_{42}$ level (SD= -25.74,95%CI= -42.36 to -9.11). Regulation of α-, β-, γ-secretase activity, inhibition of tau phosphorylation, anti-oxidation, anti-inflammation, anti-apoptosis, and inhibition of AchE activity are reported as the main neuroprotective mechanisms. Though more than 100 clinical trials have been registered on the ClinicalTrials.gov, only one clinical trial has been conducted to test the therapeutic effects of EGCG on the AD progression and cognitive performance.

Conclusion: Here, we conducted this review to systematically describe the therapeutic potential of EGCG in animal models of AD and hope to provide a more comprehensive assessment of the effects in order to design future clinical trials. Besides, the safety, blood-brain barrier (BBB) penetration and bioavailability issues in conducting clinical trials were also discussed.

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease featured by cognitive impairment, memory loss and unpredictable behaviors. The disease caused a huge burden to the social healthcare system. However, the current medication for AD is disappointing (Alzheimer's, 2015, 2016; Hardy and Selkoe, 2002) and huge efforts have been spent on drug development, though most of the attempts failed. Amyloid plaques and neurofibrillary tangles composed of hyper-phosphorylated tau are the major pathological hallmarks of AD. Recent preclinical studies concluded that Aβ involved in the memory consolidation process by selectively impairing the cAMP/PKA/CREB pathway. The transcription factor CREB, which regulates the expression of cAMP response element (CRE)-containing genes, plays an essential role in learning and memory processes. Studies verified that pathologic (nanomolar) concentrations of $A\beta_{42}$ caused a sustained inhibition of CREB phosphorylation in hippocampal neuronal cultures and impaired learning and memory processes (Ettcheto et al., 2018;

Abbreviations: Aβ, β,-amyloid peptide; ALT, alanine aminotransferase; Akt, protein kinase B; AST, aspartate transaminase; ASAP, atypical small acinar proliferation; CHOP, binding protein homologous protein; CIN1, cervical intraepithelial neoplasia; CREB, cAMP response element binding; EGCG, Epigallocatechin-3-*O*-gallate; ERK, extracellular signal-regulated kinase; FAS, fatty acid synthase; GFAP, glial fibrillary acidic protein; GRP78, binding immunoglobulin protein; GSH-Px, glutathione peroxidase; GTE, green tea extract; HGF, hepatocyte growth factor; HGPIN, high-grade prostatic intraepithelial neoplasia; HPV, human papilloma virus; ICAM-1, intercellular adhesion molecule-1; LFTs, liver function tests

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Tong et al., 2001). Therefore, the Aβ and hyper-phosphorylated tau are still the most favored targets for development of AD drugs.

Historically, green tea (*Camellia sinensis*) originated in China and widely spread from Asia to western countries (Zaveri, 2006). Because of the broad spectrum of pharmacological activities, EGCG displayed therapeutic potential on various neurodegenerative diseases models. For example, oral administration of EGCG obviously alleviated dopamine neuron loss in the substantia *nigra* and tyrosine hydroxylase protein level depletion in PD model (Levites et al., 2001). EGCG also showed an anticonvulsive effect by reducing the neuroinflammatory process and seizure threshold (Cano et al., 2018). The neuroprotective effects of EGCG against Aβ-induced neuronal loss and tau toxicity in AD models were also reported in several studies (Buetler et al., 2002; Kaur et al., 2008). This systematic review conducted a quality assessment and a meta-analysis of the studies performed on the animal AD models in order to provide a comprehensive assessment of the effects. The underlying mechanisms and current obstacles were also thoroughly discussed.

Methods

Overview of retrieval methods

We performed an open-ended, language-restricted (English) search to screen for the studies on the efficacy of EGCG on animal AD models from databases including ScienceDirect, Web of Science, and PubMed according to previously reported method (Zhu et al., 2019). Identical keywords were preset for different search engines. Five keywords ("Epigallocatechin gallate" OR "Epigallocatechin-3-*O*-gallate" OR "Green tea extract" OR "Tea Catechin" OR "EGCG") of the primary domain were paired with the following subdomain words ("Alzheimer's disease" OR "Alzheimer disease" OR "β-amyloid peptide" OR "Tau protein" OR "amyloid plaques" OR "memory impairment" OR "cognition impairment" OR "AD"). The search was restricted to preclinical laboratory reports in animal models. Reference lists of all included articles were also hand-searched. Finally, the search results were reported compliance with the guidelines of the PRISMA statement (Kapoor et al., 2017; Moher et al., 2015).

Study selection procedure

The pre-established inclusion and exclusion criteria are listed below: Table 1. Inclusion criteria and exclusion criteria for screening literature.

Data extraction

Two investigators separately assessed the quality of articles and performed data collection procedures based on the inclusion criteria

Table 1
Inclusion criteria and exclusion criteria for screening literature.

Inclusion criteria:
Studies of EGCG administration on animal AD models;
EGCG is not tested in combination with other pharmacological interventions;
Free EGCG forms, not nanoparticles or EGCG analogs;
Laboratory animal is limited to mice/rats only and there is no restrictions on age, gender, or strain;
Depicting a group of control animals;
Raw data is independent of other studies and there is no restriction to methods of outcome assessment.
Exclusion criteria:
No original data;
Not the original study results;
Case reports, reviews, editorials, comments, abstracts or letters et al.;
No access to the databases.

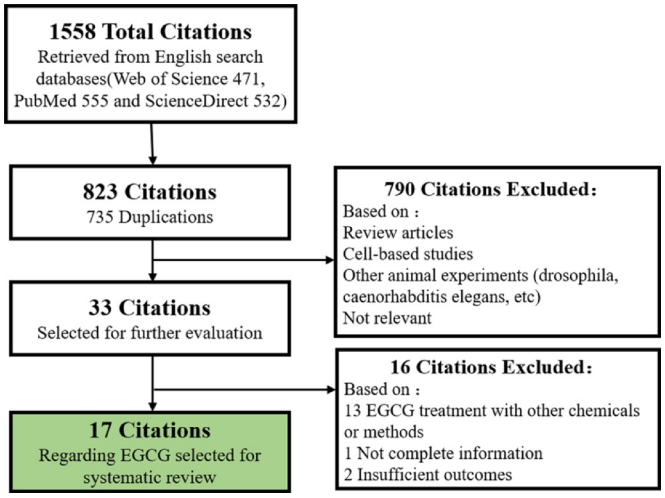

Fig. 1. Study selection flowchart.

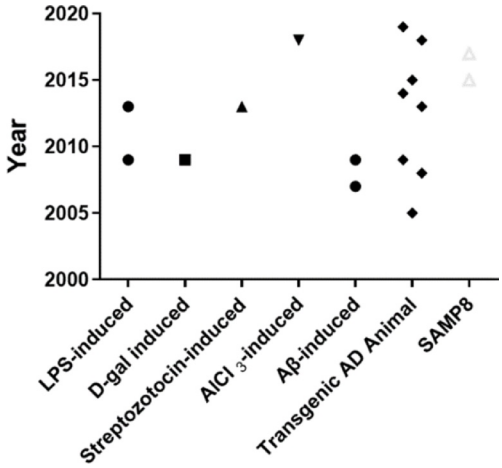

Fig. 2. Different AD Models.

(Table 1). If there is a discrepancy, the third party (Qi Zhu) will arbitrate through targeted discussion to resolve specific issues. The extraction of detailed information of included studies mainly involves three aspects: (1) Background information including the first author name, and year of publication; (2) Animal species, AD model types, and modeling methods; (3) Drug dosage, duration time, route of EGCG administration, and methods of outcome assessment.

Quality of evidence

Assessment criteria were established according to the CAMARADES study quality checklist (Moher et al., 2015) and the standards of published criteria (Macleod et al., 2009b) and modified according to the characteristics of experimental AD models. Then the methodological quality of studies was assessed using the following criteria: (1) sample size calculation; (2) random allocation of treatment/control group; (3) blinded assessment of outcome; (4) appropriate animal species and AD models; (5) publication in a peer-reviewed journal; (6) compliance with animal welfare regulations and (7) statement of potential conflicts of interest. Each study was given a quality score out of a maximum total of seven points, and the group median was calculated.

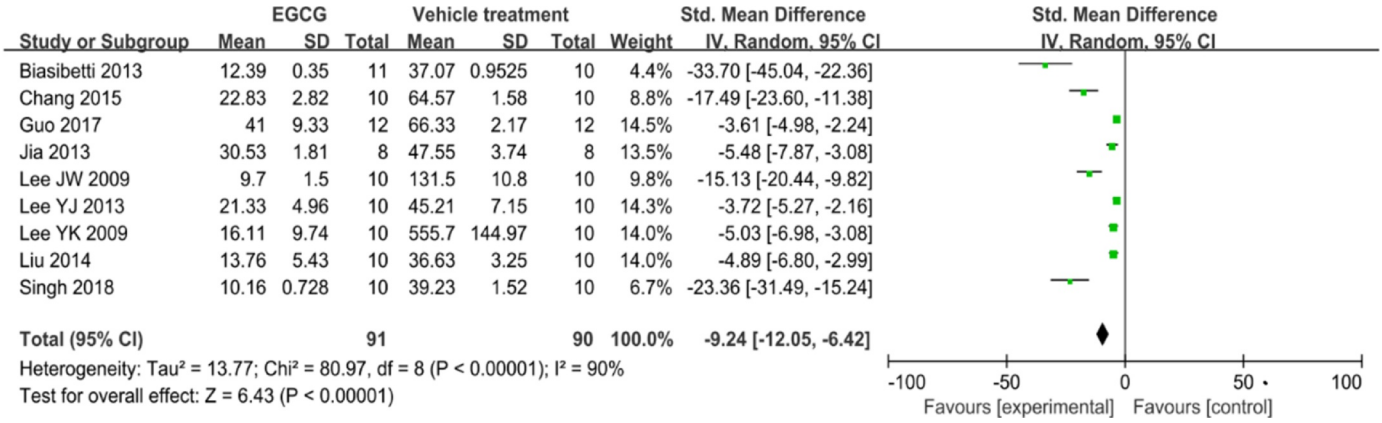

Fig. 3. Forest plot for comparison: EGCG versus vehicle treatment. Outcome: Escape latency (*sec*) in the Morris water maze test.

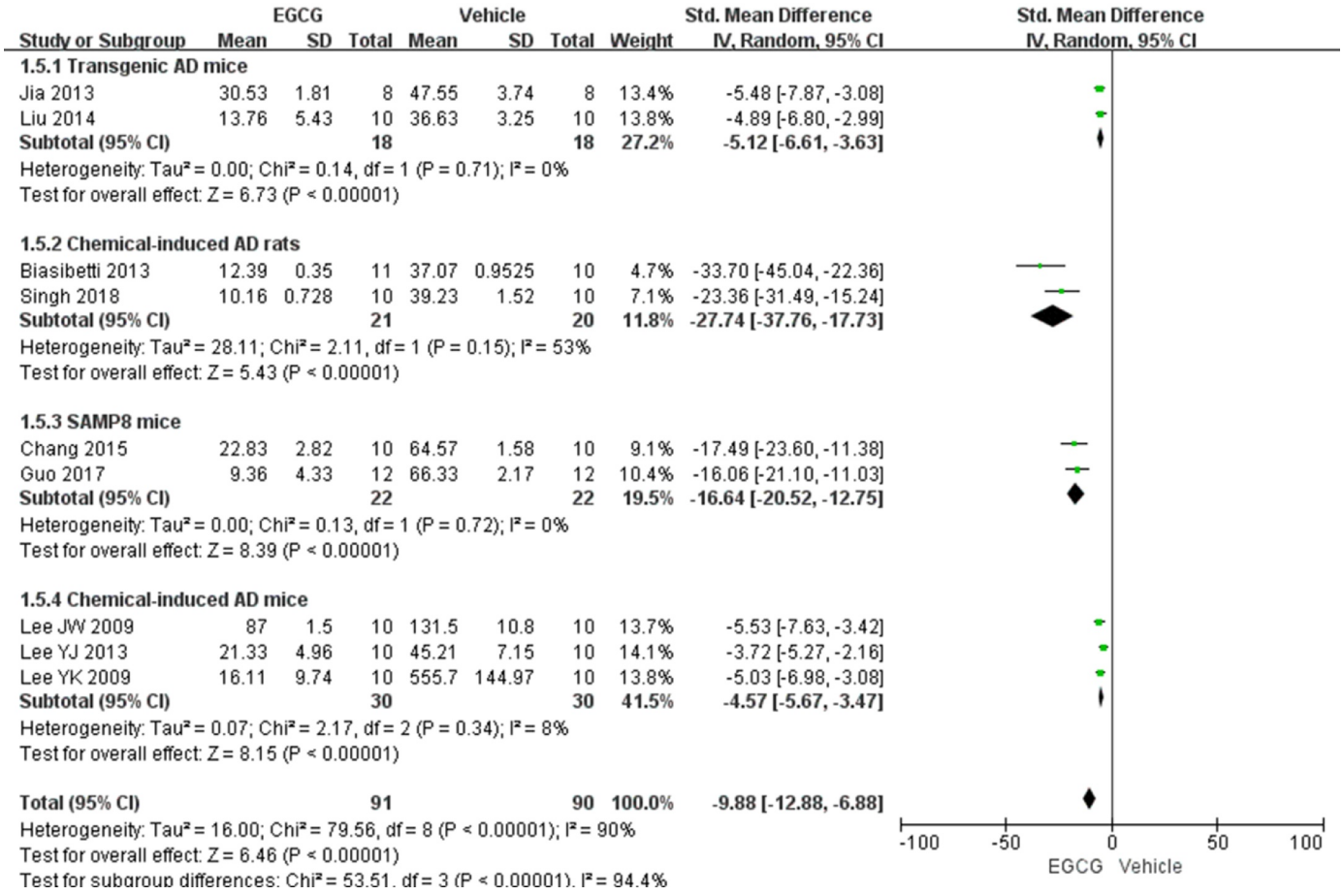

Fig. 4. Subgroup comparisons of different types of AD animal models after EGCG treatment (Morris water maze).

Results

Results of retrieved literature

A total of 1558 articles were identified initially (471 from Web of Science, 555 from PubMed and 532 from ScienceDirect). 33 articles remained after screening by the titles and abstracts, of which 13 articles were removed for combination with other pharmacological interventions. Another 3 articles were excluded for insufficient outcomes or no full text. Finally, 17 whole animal studies were feasible for the systematic review (Biasibetti et al., 2013; Cano et al., 2019; Chang et al., 2015; Du et al., 2018; Guo et al., 2017; He et al., 2009; Jia et al., 2013; Lee et al., 2009a, 2013, 2009b; Lin et al., 2009; Liu et al., 2014; Rasoolijazi et al., 2007; Rezai-Zadeh et al., 2008, 2005; Singh et al., 2018; Walker et al., 2015). The study selection process is illustrated in a flowchart (Fig. 1).

Of the selected 17 articles, two species were included. 3 studies chose rats (total animal number 54) and 14 studies chose mice (total animal number 287). And these studies are divided into seven strains: Wistar rats (n = 3), SAMP8 and SAMR1 Mice (n = 2), B6/SJL mice (n = 3), C57BL/6 J mice (n = 5), ICR mice (n = 3), Kunming mice (n = 1). There were 6 articles using male animals, 3 articles using female animals, 3 studies using both males and females, and the other 5 studies not mentioning the sex of the animals used.

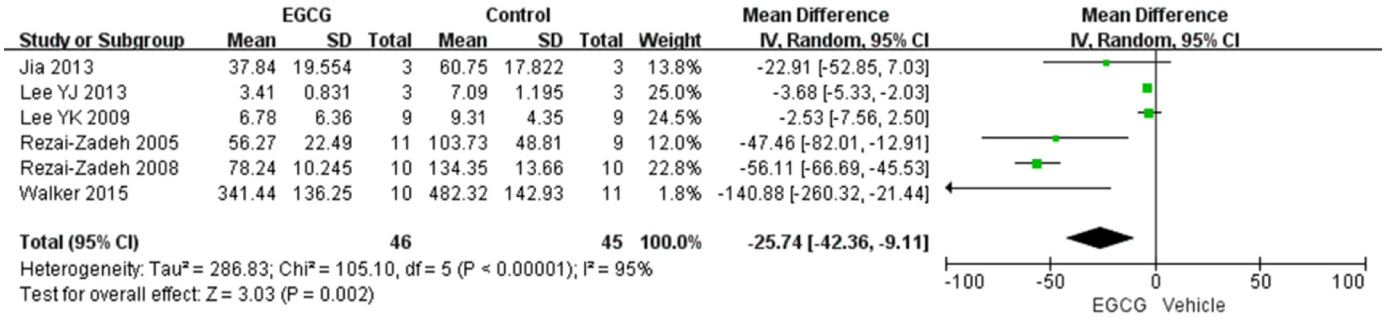

Fig. 5. Forest plot for comparison: EGCG versus vehicle treatment. Outcome: $A\beta_{1-42}$ pg/mg in the hippocampus measured by ELISA.

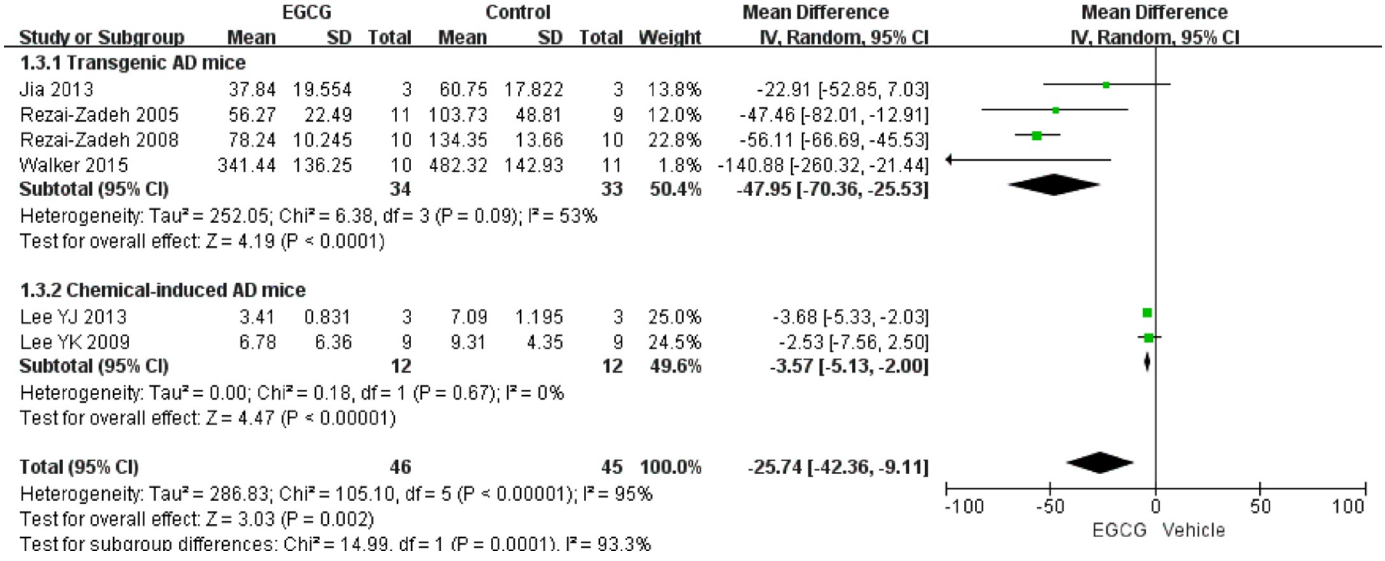

Fig. 6. Subgroup comparisons of transgenic or non-transgenic AD animals after EGCG treatment (insoluble Aβ).

Study characteristics

AD animal models

Animal models are commonly used for brain aging studies. Among these retrieved publications, five kinds of chemical-induced AD models were involved. They were Aβ-induced, LPS-induced, D-gal-induced, streptozotocin-induced and aluminum chloride ($AlCl_3$)-induced AD models respectively (Fig. 2). To mimic brain aging and cognitive deficits, Homa Rasoolijazi (Rasoolijazi et al., 2007) injected 2 nmol/μl $A\beta_{1-40}$ (4 μl) into the hippocampal fissure of male Wistar rats and Jae Woong Lee (Lee et al., 2009a) injected 0.5 μg $A\beta_{1-42}$ into the ventricles of male ICR mice. In addition, the LPS-induced AD model was used in two selected studies. Lee, Y. K. (Lee et al., 2009b) injected LPS directly into the third ventricle of the mice (1 μg/mouse) and Lee, Y. J. (Lee et al., 2013) injected 250 μg/kg LPS into mice intraperitoneally for 7 days to stimulate mice with neuronal degeneration and spatial memory impairment. Besides, subcutaneous injection of D-gal; lateral ventricle injection of streptozotocin; oral administration of $AlCl_3$ was also used to simulate cognitive impairment AD models. The common feature of these chemical and lesion-induced models is their ability to replicate the cognitive impairment symptoms as well as the neurodegeneration and neuroinflammation phenotypes of AD.

Besides the above-mentioned toxin-induced models, genetically modified mice provide a model that is closer to human AD pathology. The transgenic models such as APP/PS1, TgCRND8 and Tg2576 mice are based on the over-expression of mutant APP and/or PS1 protein these are associated with familial AD (Van Dam and De Deyn, 2011). A common feature of these three kinds of transgenic mice is that the amount of APP transgene expression is several times higher than the endogenous murine APP (Maia et al., 2013). Furthermore, all these transgenic mouse models are progressing with cortical and hippocampal β-amyloid peptide accumulation and caused profound cognitive deficits.

In addition to the AD models of toxin induction and genetic modification, two studies (Chang et al., 2015; Guo et al., 2017) utilized the senescence-accelerated prone mice (SAMP8) to mimic sporadic AD. Because more than 99% of clinical cases are sporadic late-onset AD (van der Flier et al., 2011), SAMP8 mice are considered a suitable model for exploring the pathogenesis and therapeutic treatments of AD.

Neuroprotective effects of EGCG on AD models

Cognitive function impairment is the major clinic symptom of AD thus the assessment of memory-related behavioral function is the most important assay to monitor the cognitive function in AD mice models. Morris Water Maze (MWM), the widely used behavioral tests, relies on recording the route of mice to navigate itself from start locations around the perimeter of an open swimming area to locate an underwater escape platform. According to the time it takes to find the platform (escape latency), time in the target quadrant, and a number of crossings over the platform position, the reference memory could be surmised. As the pathological hallmarks, Aβ accumulation and neurofibrillary tangles formation reflect the neuropathological changes in AD mice models. The data of MWM behavioral tests and Aβ content in the brain were extracted from the studies and the meta-analysis was conducted for a more objective appraisal of the research evidence.

Table 2
Main neuroprotective mechanisms and outcomes of EGCG towards AD.

Publication	Model	EGCG administration	Outcome measures	Neuroprotective mechanisms
Rezai-Zadeh, K et al.,2005	Tg APPsw line 2576	①EGCG (20 mg/kg) i.p. for 2 months ②EGCG (10 μg/mouse) intracerebroventricular injection for once.	Level of $A\beta_{1-40,\ 42}$ Product of nonamyloidogenic APP processing sAPP-α Activity assays of α-, β- and γ-secretase G8 Immunohistochemistry and thioflavin S Histochemistry test condition of Aβ deposition	Enhancing the nonamyloidogenic α-secretase proteolytic pathway
Homa Rasoolijazi,2007	Aβ-induced Alzheimer's Rat	EGCG (10 mg/kg) i.p. for three weeks	Psychomotor coordination (PMC) performance Spontaneous motor activity index	N/A
Rezai-Zadeh, K et al.,2008	Tg APPsw line 2576	①EGCG (50 mg/kg) orally administered for 6 months ②EGCG (20 mg/kg) i.p. for 2 months	Radial arm water maze (RAWM) Level of $A\beta_{1-40,\ 42}$ Product of nonamyloidogenic APP processing sAPP-α Levels of phospho-tau and total tau Immunohistochemistry and Congo red histochemistry test condition of Aβ deposition	N/A
Chih-Li Lin, et al.,2009	Tg APPsw line 2576	EGCG (20 mg/kg) orally administered for 4 months	Morris water maze Level of $A\beta_{1-40,\ 42}$ Changes of c-Abl-FE65 complex interaction and GSK3β-Tyr^{216} phosphorylation	Inhibiting APP proteolytic processing; Inhibiting cAbl/FE65 complex nuclear translocation and GSK3β activation
Lee, YK et al. 2009	LPS-induced mice	EGCG (1.5 or 3 mg/kg) orally administered for 3 weeks	Morris water maze test Passive avoidance performance Level of $A\beta_{1-42}$ Activity assays of β- and γ-secretase Levels of inflammatory factors- GFAP, C0X-2 and iNOS Level of apoptotic neurocytes	Anti-inflammation; Anti-apoptosis; Inhibiting beta- and gamma-secretase activity
He, Miao et al.,2009	D-gal induced mice	EGCG (2 or 6 mg/kg) intragastrical (i.g.) for 1month	Locomotor Activity Testing SMG-2 Water Maze Activities of T-SOD and GSH-Px (antioxidant enzymes) Contents of oxidative injury maker-MDA Level of apoptosis protein-cleaved caspase-3	Anti-inflammation; Anti-apoptosis
Lee, JW et al., 2009	Aβ-induced Alzheimer's mice	EGCG (1.5 or 3 mg/kg) orally administered for 3 weeks	Passive avoidance performance Water maze test Level of $A\beta_{1-42}$ Activity assays of α-, β- and γ-secretase Changes of JNK1, ERK2, and p38 MAP kinase Level of inflammatory factors -NF-κB Level of apoptotic neurocytes	Anti-inflammation; Anti-apoptosis; Modulating secretases activity; Inhibiting Aβ fibrillization; Inhibiting ERK/NF-kappaB pathway.
Lee, YJ et al., 2013	LPS-induced mice	EGCG (1.5 or 3 mg/kg) orally administered for 3 weeks	Morris water maze Probe test Passive avoidance performance Level of $A\beta_{1-42}$ Activity assays of β- and γ-secretase Levels of inflammatory factors- GFAP, COX-2 and iNOS(in addition to TNF-α, IL-1β, M-CSF, ICAM-1, IL-16)	Anti-neuroinflammation; Inhibiting cleavage secretase activities
Jia, N et al., 2013	APP/PS1 transgenic mice	EGCG (2 or 6 mg/kg/day) intragastrical for 1 month	Morris water maze test Level of $A\beta_{1-42}$ Immunohistochemistry test IRS-1pSer636 positive cells Level of inflammatory-related proteins-IRS-1, JNK, Akt and GSK3β	Remission insulin resistance by restoring IRS-1 signaling; Inhibiting TNF-α/JNK pathway; Reducing tau hyperphosphorylation
Biasibetti, R et al.,2013	Streptozotocin-induced rats	EGCG (10 mg/kg/day) i.g. for 1 month	Morris water maze Acetylcholinesterase activity Glial activation related proteins- GFAP and S100B Oxidative stress markers- NO, ROS Anti-oxidant enzyme-Glutathione peroxidase activity	Anti-neuroinflammation;Antioxidant action; Decreasing AchE activity
Mingyan Liu et al.,2014	APP/PS1 transgenic mice	EGCG (2 mg/kg) i.g. for 1 month	Morris Water Maze Test Passive Avoidance Test Locomotivity Test Level of $A\beta_{1-40}$ and APP Level of NGF and NGF-related TrkA/p75NTR signaling Level of apoptotic neurocytes	Increasing the level of NGF; Balancing TrkA/p75NTR signaling; Anti-apoptosis
Walker, JM et al.,2015	TgCRND8 mice		Barnes maze, Nest building	N/A

(*continued on next page*)

Table 2 (*continued*)

		EGCG (50 mg/kg/day) orally administered for 4 months	Open-field Level of soluble Aβ	
Xiang Chang et al.,2015	SAMP8 mice	EGCG (5 or15 mg/kg) i.g. for 2 months	Morris water maze test Level of $A\beta_{1-42}$ Expression of neprilysin Apoptosis proteins-Bcl-2, Bax	Enhancing neprilysin activity; Anti-apoptosis
Guo, Y. F. et al.,2017	SAMP8 mice	EGCG(5 or 15 mg/kg) orally administered for 4 months	Morris water maze test Locomotor activity test Y-maze test Level of $A\beta_{1-42}$ and phosphorylated-^{tauSer396} Level of β-secretase Synaptophysin(SYN) and postsynaptic density protein 95 (PSD95);	Increasing level of synaptic proteins; Inhibiting β-secretase activity
Du, K. et al.,2018	APP/PS1 transgenic mice	EGCG (2 mg/kg/day) i.g. for 1 month	Apoptosis protein-cleaved-caspase 3 ER stress-associated proteins- GRP78, CHOP and cleaved-caspase 12	Inhibiting ER stress-associated neuronal apoptosis
Singh, N. A. et al.,2018	$AlCl_3$-induced AD rats	EGCG or nanoEGCG (10 mg/kg) i.g. for 1 month	Morris Water Maze Open Field Novel Object Recognition Level of $A\beta_{1-42}$ and APP Acetylcholinesterase activity Oxidative stress markers-NO, ROS Changes of PDK1, GSK3β	Antioxidant; Decreasing AchE activity; Reducing tau hyperphosphorylation
Amanda Cano. et al.,2019	APP/PS1 transgenic mice	EGCG or nanoEGCG (40 mg/kg) orally administered for 3 months	Morris water maze test Novel Object Recognition test Soluable and insoluable Aβ	Inhibiting Aβ fibrillization

Morris water maze analysis

Nine studies on MWM tests were used to evaluate cognition and memory function in experimental AD models. A total number of 91 animal was included and treated with EGCG at different doses, with 90 vehicle-treated animal added as controls. Escape latency (*sec*) was used in the correlation because it represents the difference in memory function between EGCG and vehicle treatment groups. Univariate statistical analysis was performed using Revman 5.3 software. In preclinical AD models, EGCG showed strong efficacy in shortening escape latency ($p < 0.00001$) compared with vehicle-treated animals, SMD −9.24 (95% CI = −12.05 to −6.42, Fig 3). Due to detection of significant heterogeneity ($I^2 = = 90\%$), the random-effects model was used. Subgroup comparison was also conducted according to different types of AD models (Fig 4). The elevated heterogeneity may be explained by the use of different AD models (transgenic/chemicel-induced models) and animal strains (mouse/rat).

$A\beta_{1-42}$ detection analysis

Pathological determination of insoluble $A\beta_{1-42}$ in the hippocampus were reported in 6 of 16 total studies. In AD animals, $A\beta_{1-42}$ in the hippocampus was significantly reduced ($p = 0.002$) compared to the vehicle-treated group with an SD of −25.74 (95% CI = −42.36 to −9.11, Fig 5). Due to the high heterogeneity ($I^2 = 84\%$), we conducted a subgroup comparison (Fig 6). The elevated heterogeneity may be explained by the use of different models (transgenic AD model/chemicel-induced AD model).

Neuroprotective mechanisms of EGCG on AD models

The accumulated toxic Aβ peptide and hyperphosphorylated tau protein, increased oxidative stress, neuroinflammation, insulin resistance and aberrant metabolism have all been proposed to be involved in the etiology and pathogenesis of AD (Ansari and Emerald, 2019; Minter et al., 2016; Morris et al., 2011; Riek and Eisenberg, 2016). Table 2 summarized the main neuroprotective mechanisms and efficacy of EGCG on AD models in these retrieved publications. In the studies, EGCG has been revealed to exert anti-oxidation, anti-inflammation, anti-apoptosis, regulation of α-, β-, γ-secretase activity, inhibition of hyperphosphorylation of tau and reduction of AchE activity (Fig.7). In addition, two articles reported the effects of EGCG on levels of neprilysin and synaptic proteins in the brain.

Cognitive and memory impairment is the most direct phenotype associated with AD and mitigating this dysfunction is the most important index in assessing successful modeling and therapeutic efficacy of candidate drugs. Most of the publications listed here (87.5%) performed behavioral tests on AD animals. The main behavioral tests including the Morris water maze, locomotivity test, performance of passive avoidance and open field test. These tests are frequently used to evaluate spatial learning and memory functions.

EGCG decreased the accumulation of Aβ. Aβ peptide is produced through endoproteolytic processing from amyloid precursor protein (APP) by β- and γ-secretase-mediated cleavage. Alternatively, APP can be cleaved within the Aβ sequence by α-secretase which hinders the formation of Aβ (Selkoe, 2001). Fourteen publications have reported EGCG lowered soluble or insoluble Aβ levels and rescued cognitive impairment. Three studies have reported changes in secretase activity caused by EGCG. Kavon Rezai-Zadeh (Rezai-Zadeh et al., 2005) showed that EGCG acts as an α-secretase agonist which upregulated nonamyloidogenic APP proteolysis and reduced the deposition of β-amyloid plaques in Tg APPsw mice. They also detected reduced soluble and insoluble $A\beta_{1-40,42}$ levels with elevated levels of α-CTF and sAPP-α in brain tissue as supporting evidence. Besides, Jae Woong Lee et al. (Lee et al., 2009a) and Young Kyoung Lee et al. (Lee et al., 2009b) verified that EGCG treatment downregulated β- and γ-secretase activities and eventually decreased toxic Aβ levels in the cortex and hippocampus.

EGCG regulates GSK3β activity. Tau hyperphosphorylation contributes to the formation of the neurofibrillary tangle (NFT) and causes neuropathological lesions (Takashima, 2006). There are also three studies revealed the potential role of EGCG on the glycogen synthase kinase-3β (GSK-3β), a pivotal tau kinase in the brain promoting the production of paired helical filament in NFT. Chih-Li Lin et al. (Lin et al., 2009) reported that EGCG relieved Aβ-induced neurotoxicity by inhibiting GSK3β activation and c-Ab/FE65 complex

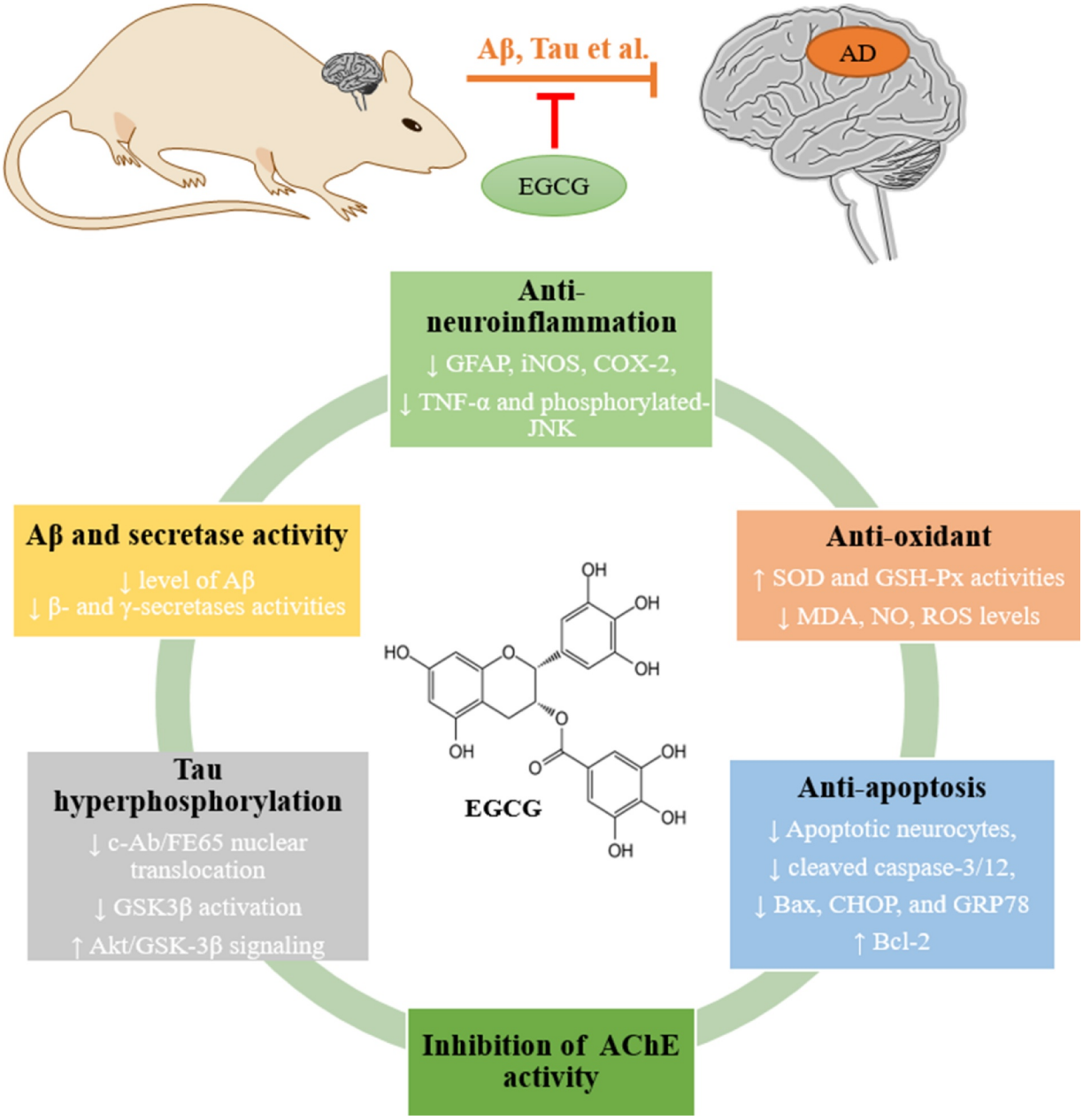

Fig. 7. Neuroprotection mechanisms of EGCG.

nuclear translocation in Tg2576 transgenic mice. They demonstrated that EGCG treatment decreased GSK3β activation by monitoring Tyr^{216} phosphorylation level. Ning Jia et al. (Jia et al., 2013) and Singh, N.A. et al. (Singh et al., 2018) also showed that EGCG was able to increase hippocampal p-GSK3βSer9 levels by restoring the downstream Akt/GSK-3β signaling and reduce the expression levels of GSK3β in APP/PS1 mice or $AlCl_3$-induced AD rats.

EGCG ameliorates oxidative stress. Oxidative stress is recognized, at least in part, to be an important motivation of AD (Bonda et al., 2010). Key antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) can protect brain tissue from cell damage caused by oxidative stress. Malondialdehyde (MDA) produced by lipid peroxidation is a marker for oxidative damage. Three studies have indicated that EGCG exerted antioxidant function in murine AD models. Miao He et al. (He et al., 2009) demonstrated that EGCG enhanced the activity of T-SOD and GSH-Px and reduced MDA content in the hippocampus. Regina Biasibetti et al. (Biasibetti et al., 2013) and Singh, N. A. et al. (Singh et al., 2018) reported that EGCG relieved hippocampal oxidation and nitrosative stress by reducing reactive oxygen species(ROS) level and NO production in streptozotocin-induced and $AlCl_3$-induced models respectively.

EGCG exerts anti-neuroinflammation action. Neuroinflammation promotes AD pathogenesis as much as Aβ plaques and neurofibrillary tangles rather than being activated by them (Zhang et al., 2013). Three studies showed the anti-inflammatory function of EGCG. Glial fibrillary acidic protein (GFAP), inducible nitric oxide synthetase (iNOS) and cyclooxygenase-2 (COX-2) are representative markers of neuroinflammation. Both Young Kyoung Lee et al. (Lee et al., 2009b) and Lee Young-Jung et al. Lee et al., 2013 found that EGCG prevented LPS-induced elevation of GFAP, iNOS, and COX-2. Lee Young-Jung et al. further demonstrated that EGCG prevented LPS-induced astrocytes activation and cytokines elevation including IL-1β, IL-16, TNF-α, soluble intercellular adhesion molecule-1 (sICAM-1) and

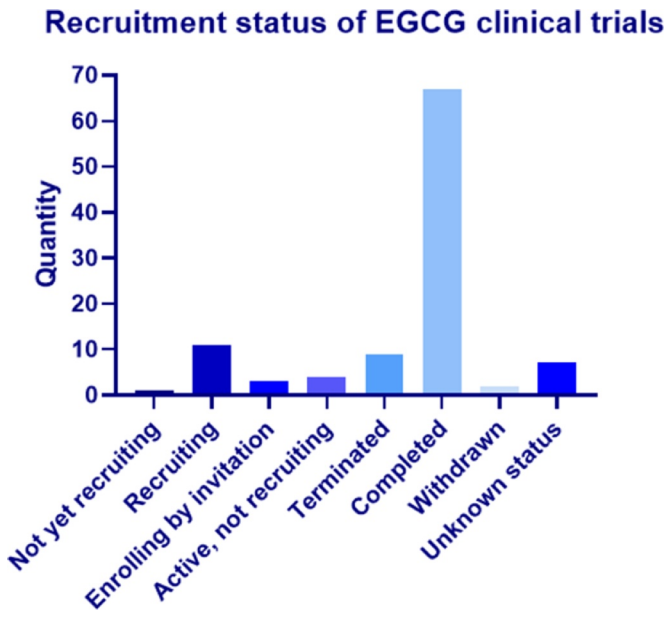

Fig. 8. Recruitment status of EGCG clinical trials.

macrophage colony-stimulating factor (M-CSF). Besides, Ning Jia et al. (Jia et al., 2013) demonstrated that EGCG exhibited the anti-inflammatory effect by reducing hippocampal TNF-α and phosphorylated-JNK levels.

EGCG exerts anti-apoptosis action. Five studies showed that EGCG administration could effectively reduce neuronal apoptosis and improve the functional performance of AD animals. Miao He et al. (He et al., 2009), Jae Woong Lee et al. (Lee et al., 2009a) and Mingyan Liu et al. (Liu et al., 2014) all demonstrated that apoptotic neurons in the hippocampus were decreased by EGCG intake in D-Galactose-induced AD model, $A\beta_{1-42}$-induced AD model and APP/PS1 transgenic mice, respectively. Caspase 3 cleavage is a typical mark of apoptosis, three publications (Miao HE et al. (He et al., 2009), Du, K. et al. (Du et al., 2018) and Mingyan Liu et al. (Liu et al., 2014)) reported that EGCG administration reduced the expression of cleaved caspase 3 and the level of apoptotic neurons in the hippocampus. Furthermore, Xiang Chang et al. (Chang et al., 2015) found that EGCG administration could up-regulate the anti-apoptotic protein Bcl-2 and amplify the Bcl-2/Bax ratio. Du, K. et al. (Du et al., 2018) revealed the role of EGCG in endoplasmic reticulum stress-mediated apoptosis. They found that EGCG treatment reduced the expression levels of CHOP, GRP78 and cleaved caspase 12 in the cerebral cortex of APP/PS1mice (Du et al., 2018).

EGCG inhibits AChE activity. As the main neurotransmitter, acetylcholine (ACh) maintains brain function such as cognition and learning ability and short-term memory. In addition, the elevated level of acetylcholinesterase (AChE) is associated with the loss of cholinergic neurons (Lombardo and Maskos, 2015). Three studies reported the efficacy of EGCG in preventing the turnover of the ACh to alleviate memory loss symptoms. Both Singh, N.A. et al. (Singh et al., 2018) and Regina Biasibetti (Biasibetti et al., 2013) reported that EGCG was capable to impair the AchE activity and prevent cholinergic neuron loss in the cortex and hippocampus.

Others. Other mechanisms involved in EGCG neuroprotection have also been proposed: regulation of neprilysin, synaptic proteins and nerve growth factor (NGF) levels. In detail, Xiang Chang (Chang et al., 2015) found that EGCG can up-regulate the expression of neprilysin, which helps to degrade the abnormally folded Aβ peptide in neural tissue. Guo Yufang (Guo et al., 2017) reported that EGCG can relief Aβ-induced synaptic dysfunction by rescuing the decreased levels of synaptic protein marker synaptophysin (SNY) and postsynaptic density protein 95 (PSD95). Besides, Mingyan Liu et al.(Liu et al., 2014) demonstrated that EGCG could ameliorate the NGF starvation in AD mice by increasing both NGF and proNGF levels and maintain the TrkA/p75NTR balance.

Analysis of EGCG clinical trials

Recruitment status of current EGCG clinical trials

105 clinical trials related to EGCG have been registered on the ClinicalTrials.gov (Fig. 8). Most of clinical trials have been completed (n = 67), and the other status of clinical trials are: not yet recruiting (n = 1); recruiting (n = 11); enrolling by invitation (n = 3); active, not recruiting (n = 4); terminated (n = 9); withdrawn (n = 2) and unknown status (n = 7).

Clinical trial on the cognitive improvements of EGCG in down syndrome

Due to triplication of the APP gene in trisomy of chromosome 21, 90% of individuals with Down syndrome (DS) showed neurodegenerative features of AD after age 60. Alzheimer's disease in DS often presents with cognitive decline, behavioral symptoms, myoclonus/seizures (Zis and Strydom, 2018). A double-blind, placebo-controlled, phase 2, single-center trial of EGCG for DS treatment has been completed. After the 12 months of EGCG treatment, higher preservation of memory and an improvement of executive functioning compared with the placebo have been observed, as measured with the ABAS-II functional academics score ($p < 0.002$). The efficacy of EGCG was suggested to depend, at least partly, on the ability of EGCG to inhibit DYRK1A kinase activity, a strong candidate gene for learning defects associated with DS (De la Torre et al., 2014).

Safety of EGCG reported by the completed clinical trials

67 clinical trials have completed the recruitment process. Among these trials, 12 studies have reported relevant results. The condition or disease of the clinical trials include prostate cancer (n = 3); breast cancer (n = 3); Down's syndrome (n = 1); HPV infection (n = 1); ulcerative colitis (n = 1); bladder cancer (n = 1); multiple sclerosis (n = 1) and lymphocytic leukemia (n = 1). The general characteristics of completed clinical trials are shown in Table 3.

In preclinical and phase I clinical trials, it has been shown that bioavailability of EGCG is increased when it is consumed on a fasting basis. However, the rate of hepatotoxicity is also increased (Crew et al., 2012; Wu et al., 2011). The FDA does not allow human studies of Polyphenon E on a fasting basis, as mortality of dog is high when 1200 mg Polyphenon E is used in the fasting state (Kapetanovic et al., 2009). The highest safe dose for more than a month of treatment allowed by FDA is 800 mg of EGCG daily with food.

Among these clinical trials, 6 (50%) studies selected 800 mg EGCG as a daily dose (duration from 6 weeks to 1 year) and EGCG was well tolerated with no grade 2 or higher toxicities except for one trial of multiple sclerosis showing a high risk of hepatotoxicity (Lovera et al., 2015). The grade of adverse events (AEs) compliances with the standardized definitions published by the National Cancer Institute to describe the severity of organ toxicity for patients (https://ctep.cancer.gov/). AEs including nausea, dizziness, stomach ache, heartburn, abdominal pain, headache, and muscle pain has been reported after EGCG treatment. However, most of the reported events were rated as mild events with no significant difference with placebo group (Bettuzzi et al., 2006; Campbell et al., 2010; Chow et al., 2001, 2003; de la Torre et al., 2016; Dryden et al., 2013; Garcia et al., 2014; Lovera et al., 2015; McLarty et al., 2009; Samavat et al., 2019). Recent studies using Polyphenon E to treat chronic lymphocytic leukemia have escalated the daily dose to 4000 mg with reasonable safety for six months treatment (16% discontinuation due to elevated liver function tests)

Table 3
Safety of EGCG reported by the completed clinical trials.

No.	Trials	Disease	Type of study	Study Phase	N (Case: Control)	Mean age (SD/ Range)	Follow-up time	Intervention	Main results	Safety and Tolerability
1	de la Torre R, 2016	Down's syndrome	Double-blind, randomised, placebo-controlled trial	Phase 2	84	22(4)	12 months	600 or 800 mg EGCG daily	Improvement of executive function and recognition memory.	Well tolerated; AEs were mainly mild with no differences between the study groups.
2	Jesus Lovera MD, 2015	Multiple Sclerosis	Randomized, placebo-controlled trial	Phase 2	13	48(33–59)	12 months	800 mg EGCG daily	Not futile at increasing brain NAA level.	A high risk of hepatotoxicity with elevation of liver enzymes (5/7 participants); common AEs were nausea and abdominal pain.
4	Nagi B. Kumar, 2016	Prostatic Hyperplasia	Randomized, placebo-controlled trial	Phase 2	97	63.02(7.9)	12 months	400 mg EGCG daily	EGCG accumulated in plasma ($p < 0.001$) in men with baseline HGPIN or ASAP.	Well tolerated; no liver or other dose limiting toxicities.
5	McLarty J, 2009	Prostate Cancer	Open-label, single-arm trial	Phase 2	33	58.6(7.1)	6 weeks	polyphenol E (containing 800 mg EGCG) daily	Levels of serum HGF, VEGF and PSA decreased in men with prostate cancer ($p < 0.03$).	No AEs on liver function (albumin, aspartate aminotransferase, alkaline phosphatase, and amylase significantly decreased).
6	Zhang Z, 2016	Prostate Cancer	Randomized, double-blind, placebo-controlled trial	Phase Not Applicable	89	63(6.3)	5 months	600 mg EGCG daily	No significant difference in FAS or Ki-67 changes.	No difference for AEs between treatment and placebo groups.
7	Samavat H, 2019	Breast Cancer	Randomized, double-blind placebo-controlled trial	Phase 2	1075	59.8(5.02)	12 months	GTE containing 843 mg EGCG daily	Increasing levels of blood estradiol ($p = 0.02$) and bioavailable estradiol ($p = 0.03$).	Common AEs were infections and gastrointestinal disorders (no significant difference); well tolerated; AEs > 98% were mild (grade 1 or 2);
8	Julie l. Campbell, 2010	Breast Cancer	Open-label, single-arm trial	Phase 2	32	51.8(7.7)	2–4 weeks	800 mg EGCG daily	No significant change in serum HGF level.	Well tolerated; no grade 2 or higher toxicities; no significant elevation of liver or pancreatic enzymes.
9	Katherine D. Crew,2012	Breast Cancer	Randomized, double-blinded, placebo-controlled trial	Phase 1	40	52 (36 to 64)	6 months	polyphenon E of 400, 600 or 800 mg twice daily	reduction estradiol level ($p = 0.05$) and SHBG level ($p = 0.03$);	Common AEs (mainly grade 1) are gastrointestinal disorder (nausea; indigestion; diarrhea; constipation, etc.).
10	Garcia FA, 2013	HPV infection	Randomized, double-blind, placebo-controlled trial	Phase 2	98	28.28 (8.39)	4 months	Polyphenon E (containing 800 mg EGCG) daily	No significant change in clearance of high-risk HPV and related CIN1.	Well tolerated; 97% were grade 1 or grade 2 AEs (nausea, vomiting, cervicitis, sinus infection, dizziness, back pain, stomach ache).
11	GW Dryden, 2013	Ulcerative Colitis	Randomized, double-blinded, placebo-controlled trial.	Phase 2	20	40(15)	2 months	400 mg or 800 mg EGCG daily	Polyphenon E showed therapeutic benefit ($p = 0.03$).	No serious AEs (grade 1 or 2); no significant differences of bilirubin, alkaline phosphatase, ALT, and AST levels.
12	Shanafelt TD, 2013	Lymphocytic Leukemia	Single group trial	Phase 2	42	60 (41 to 78)	6 months	2000 mg EGCG twice daily	Declination of absolute lymphocyte count (30%) and/or lymphadenopathy (70%).	Well tolerated; the most severe toxicity was hocyte co (nausea; abdominal pain; diarrhea; yperglycemia);

Table 4
Methodological score of included studies.

References	1	2	3	4	5	6	7	Total
Rezai-Zadeh, K(2005)				√	√	√		3
Homa Rasoolijazi(2007)		√		√	√			3
Rezai-Zadeh, K(2008)		√		√	√	√		4
Chih-Li Lin(2009)				√	√	√	√	4
Lee, YK(2009)				√	√	√		3
He, Miao(2009)		√		√	√	√		4
Lee, JW(2009)				√	√	√		3
Lee, YJ(2013)				√	√	√		3
Jia, N(2013)		√		√	√	√	√	5
Biasibetti, R(2013)				√	√	√		3
Mingyan Liu(2014)				√	√	√	√	4
Walker, JM(2015)				√	√	√		3
Xiang Chang(2015)		√		√	√	√		4
Guo, Y. F(2017)		√		√	√	√	√	5
Du, K(2018)		√		√	√	√	√	5
Singh, N. A(2018)		√	√	√	√	√		5
Natalie Hudson (2019)				√	√	√	√	4

These assessment criteria are: (1) a sample size calculation; (2) random allocation to treatment group; (3) blinded assessment of outcome; (4) appropriate animal species and AD models; (5) publication in a peer-reviewed journal; (6) compliance with animal welfare regulations and (7) statement of potential conflict of interests.

(Shanafelt et al., 2013). Besides, one trial has conducted the maximum tolerated dose test (25% dose-limiting toxicity), and 600 mg Poly E twice daily has been defined for breast cancer patients (Campbell et al., 2010). Therefore, the higher dose may be tolerable for cancer treatment but the dose of 800 mg daily is probably the dose that most patients would be able to consistently tolerate.

Methodological quality of included studies

The methodological quality scores of selected studies ranged from 3 to 5 out of a total 7 points and the median score is 4. All the papers were published in a peer-reviewed journal with proper animal models; compliance with animal welfare regulations was reported by 16 publications (94.1%); random allocation to the group was reported by 8 publications (50.0%); potential conflict of interest was mentioned by 6 publications (35.3%), and blinded assessment of outcome was only conducted in 1 study. In addition, sample size calculation was not conducted in publications identified in this systematic review (Macleod et al., 2009a). All the details are shown in Table 4.

Discussion

Up to now, the FDA approved drugs for AD treatment are limited to acetylcholinesterase inhibitors (donepezil, galantamine and rivastigmine) indicated for mild-to-moderate AD and *N*-methyl-D-aspartate receptor antagonist, memantine indicated for moderate-to-severe AD (Cummings et al., 2015). Although AchEIs have been shown to improve cognitive function, they can not stop AD progression and accompanied with multiple side effects. Therefore, it is necessary to develop more efficacious and less expensive disease-modifying drugs with a better safety and tolerability profile. The results of this review support the therapeutic effect of EGCG on AD animal models. However, some limitations of the present meta-analysis and AD animal models in this study deserve acknowledgement. First, it is recommended to estimate the required sample size for detecting a particular effect size. Because the number of animals in each group will affect the likelihood of detecting difference(s) between groups. Second, random allocation to the treatment or control group is a significant step to minimize the effects of subjective bias. Thus the method of allocation should be described in subsequent studies. Third, 17 retrieved studies involved transgenic animal models and chemical-induced AD models. The chemical-induced models replicate many of the clinical symptoms associated with AD such as memory and learning loss. However, due to a lack of typical senile plaques and NFTs, these models do not represent accurate modeling systems for AD. Despite such concerns, these studies confirmed that EGCG improved the spatial memory performance in the MWM test and decreased the brain A β burden and phosphorylation of Tau.

EGCG has shown well tolerance and safety in most clinical trials under a variety of diseases. The FDA does not allow human studies of Polyphenon E on a fasting basis and the highest safe dose is 800 mg of EGCG daily with food. In 11 clinical trials of phase 1–2, most of the reported AEs (stomach ache, nausea, vomiting, abdominal pain, diarrhea, dizziness, back pain, etc.) were rated as mild events and the incidence reported in the polyphenol-treated groups was not more than the placebo group. 800 mg as the most common dose is generally well-tolerated and a minimal number of severe gastrointestinal responses were seen in the current trial.

Three clinical trials using EGCG as a therapeutic tool aimed to reduce AD progression and only one trial showed improvement of cognitive performance in Down syndrome subjects. de la Torre R (NCT01699711) conducted the first randomized controlled clinical trial which combined pharmacological treatment (9 mg/kg EGCG daily) with cognitive function training. They planned to monitor $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides and their truncated forms as biomarkers of AD. Due to technical issues with the assay for plasma Aβ forms, these measurements were not reported. Finally, this study shows a significant improvement in memory, executive function, and facilitated adaptive behavior. Besides, 200 ~ 800 mg sunphenon EGCG was used to treat early stage of AD for 18 months and no results were reported so far (NCT00951834). Another trial planed to combine EGCG (260–520 mg EGCG daily) and multimodal intervention to the prevention of cognitive decline in ApoE4 carriers of AD (NCT03978052) while the status of this trial was not yet recruiting.

There are still some challenges on EGCG new drug development process in spite of the supporting evidence. Recent studies have demonstrated that EGCG undergos methylation, glucuronidation and sulfation in vivo which significantly reduce EGCG's half-life from 18 h down to < 4 h. Therefore, understanding of the absorption, metabolism, how can EGCG permeate BBB, molecular targets of EGCG and AD markers can be of importance to better utilize it as an anti-AD agent. The first challenge is about the stability of EGCG. Modifying nanolipidic EGCG particles upregulating the oral bioavailability *in vivo* and *in vitro* by several folds over free EGCG has been verified (Chen et al., 2012; Smith et al., 2010; Zhang et al., 2014). The second challenge is BBB penetration property of EGCG. Nanoparticles also have been proposed to facilitate or even open a specific transport route across the BBB. Neha Atulkumar Singh and AmandaCano find that brain uptake of nanoEGCG occurred through destabilized BBB endothelial junctions by diffusion and it was enhanced due to longer dwell time and greater stability in the blood (Cano et al., 2019; Singh et al., 2018). The third challenge is the structure-activity relationship of EGCG and AD biomarkers in biological molecular techniques, such as computer-aided docking, surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC). It may be helpful to identify and characterize the binding sites and find the biomarkers “hot spots” such as the key residues, pockets or PPI surface that could bind EGCG. Such perspectives and challenges can guide further preclinical tests and clinical trials. In the end, we envision that EGCG might be a promising candidate that is worth further experimental and clinical trials for AD.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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