Systematic Review

Green Tea Catechins as Neuroprotective Agents: Systematic Review of the Literature in Animal Pre-Clinical Trials

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ABSTRACT

Alzheimer’s Disease (AD) is a neurodegenerative disorder and the most common form of dementia, with symptoms and manifestations that progressively get worse with increasing age. Therefore, with the ageing of the population worldwide, the prevalence of AD is increasing. There is no current cure for AD and, as a result, there has been a recent rise in interest in plant bioactive compounds that may prevent or improve symptoms of the disease. Currently, the nootropic potential of plant derived compounds that can combat damage posed by free radicals is being investigated. Antioxidants, in particular, the Green Tea Catechins (GTC), have been shown significant interest due to their exceptionally strong antioxidant and anti-inflammatory properties. The aim of this paper was to perform a systematic review based on the PRISMA guidelines in order to evaluate the effectiveness of GTC as a potential treatment to suppress or delay the onset of AD in pre-clinical animal trials. The paper reports on three animal pre-clinical trials in which rat or mice models of AD were used to test the effects of GTC or the pure form of the predominant GTC, Epigallocatechin gallate (EGCG), administered orally or by injection. The reviewed papers show that GTC extracts or pure EGCG had preventative effects on AD in the various animal models used, including the enhancement of learning and memory, possibly through the reduction in oxidative stress, β-amyloid plaque build up and Tau protein phosphorylation. Therefore, GTC extracts or EGCG in its pure form may serve as nootropic options in the prevention or treatment of neurodegeneration-associated diseases such as AD.

KEYWORDS: Green Tea Catechins; EGCG; Oxidative stress; Neuroprotection; Pre-clinical animal trials; Alzheimer’s disease; Beta amyloids; Tau protein.

ABBREVIATIONS: AD: Alzheimer’s Disease; GTC: Green Tea Catechins; LPO: Lipid peroxides; ROS: Reactive Oxygen Species; EGCG: Epigallocatechin gallate; MPTP: N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; EGC: Epigallocatechin; ECG: Epicatechin gallate; TBARS: Thiobarbituric acid-reactive substances; SOD: Superoxide dismutase; IP: Intraperitoneal; RAWM: Radial Arm Water Maze; FRAP: Ferric Reducing Ability of Plasma; APP: Amyloid Precursor Protein.

INTRODUCTION

Mental disease represents one of the world’s leading disabilities affecting over 25% of people at some period during their lifetime. In 2004, it represented 13% of the global burden of disease and it is proposed to increase to 15% by the year 2020, which will place it as the second leading cause of disability in the world. Alzheimer’s Disease (AD) and cognitive im-
There are several different factors which have been postulated to directly or indirectly play important roles in the initiation of these events: oxidative damage,9,26 genetic polymorphisms,21,22 gene mutations23 and abnormal levels of β-amyloid and tau proteins.24 Of these, the deposition of β-amyloid protein is seen as the primary event in the pathogenesis of AD. The other changes such as neurofibrillary tangles, synaptic degeneration and neuronal cell death are proposed to arise only as a consequence of the deposition of this protein in the brain.25

In addition, neurodegeneration is a common feature of AD with the main reasons described to be the combination of multi-factorial events such as neuro-inflammation, glutamate rich excitotoxicity and depletion of antioxidants.26 Hence, in recent years there has been developing evidence proposing that dietary polyphenols can potentially counteract and suppress the neuronal injury.26-29

Polyphenols are ubiquitous compounds classified as wide and complex group of plant secondary metabolites and to date there have been over 8000 compounds identified. Additionally, these compounds are exceptionally diverse in their structures, ranging from very simple molecules (pheno-lic acids) to more complex and highly polymerized structures (proanthocyanidins).30-36 The polyphenols are generally divided into two groups based on their molecular weight, low (500-3000 Da) and high molecular weight (>3000 Da).37 In addition, these compounds can also be classified into different groups based on the number of phenolic rings that they contain and on the basis of the structural elements by which these rings are connected to one another. They include such compounds as phenolic acids, flavonoids, stilbenes and lignans (Figure 1).

One of the foods rich in bioactive compounds, particularly flavonoids, is green tea (Camellia sinensis). In recent years, there has been a significant upward trend in the consumption of green tea and taking into consideration that overall tea (black and green) is one of the most consumed beverages in the world, it is not surprising that this trend was closely followed by academic research interest.

The most abundant polyphenolic compounds in green tea are the catechins, which account for nearly 30% of the dry tea leaf weight.38 In the recent literature, the Green Tea Catechins (GTC) have been related to a variety of different beneficial health effects particularly with respect to their potential for preventing and treating different cancers.39,40 cardiovascular diseases,41-43 inflammatory diseases44-47 and some of the neurodegenerative diseases45,48,49 in humans. Accounting for at least half of the total GTC, 38 the most predominant catechin found in green tea is Epigallocatechin gallate (EGCG), which has been ascribed numerous beneficial properties including antioxidant,50 anti-inflammatory,51,52 anti-microbial53,54 and anticancer effects.55-57

Although current trend in the scientific writing of systematic reviews is chiefly reserved for trials with human subjects...
from various cohorts, the use of well-designed pre-clinical animal trials is gaining value. This is primarily due to the increased number of well-developed gene-knockout animal models that can be used to mimic certain diseases commonly occurring in humans. Additionally, some of the illnesses such as AD can only be successfully diagnosed post-mortem. Therefore, keeping in mind that findings from animal models are not necessarily directly transferrable to humans, these studies still provide valuable insight into the potential mechanisms of action that may be applied to clinical research in humans. Therefore, the aim of this systematic review is to look into what is known about the relationship between GTC and the leading biomarkers associated with the development of AD.

**METHODS**

**Animals**

The animals used as models in the pre-clinical animal studies covered in this systematic review were rodents in particular rats (JCL: Wistar) and mice (C57/BL; Tg2576; APPsw; non-transgenic).

**Search Strategy**

The standardized criteria for conducting and reporting systematic reviews of observational studies based on the PRISMA 2009 guidelines were used. The search strategies were applied for articles published since the year 2000 using the following electronic databases: PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), the Cochrane Library (http://www.thecochranelibrary.com/view/0/index.html), and Scopus (http://www.elsevier.com/online-tools/scopus). Titles and abstracts were scanned for relevance and appropriate articles were selected.

The search used terms relating to the topic of this systematic review; “Green tea polyphenols” and “Alzheimer’s Disease” were used in the primary search followed by “Beta Amyloid”, “Neuroprotection” and “Catechins” in the secondary search. In addition, reference list checks of selected articles with relevance to the topic were also performed.

**Selection Criteria**

For the purpose of this systematic review, articles were selected only if they were entirely published in English, in peer-reviewed journals and were identified as pre-clinical animal trials. They were included if GTC were delivered as a part of a supplement, injection, oral consumption in tea, water, food or similar delivery methods. Furthermore, studies selected for review had sample sizes of over 5 animals (at the end of the study) of any gender and had identified looking at whether or not GTC affected one or all of the biomarkers in relation to the development and onset of AD.

**Primary Outcomes**

The primary outcomes were cognitive learning and abnormal levels of β-amyloid and tau proteins in brain homogenates.

**Secondary Outcomes**

The secondary outcomes were oxidative stress identification by the presence of Lipid peroxide (LPO) by-products and generation of Reactive Oxygen Species (ROS).
Materials

Studies used green tea extracts, GTC or pure EGCG in the intervention and the control groups were always administered with water containing no additional compounds or in a case of injections, animals were injected Intraperitoneally (IP) with sterile saline or not injected at all.

RESULTS

Characteristics of the Studies

Preliminary searches of the selected databases identified 4635 records with the terms “Green tea polyphenols” and “Alzheimer’s disease” alone (Figure 2) but the secondary search reduced this to 500 articles. These 500 abstracts were screened, of which only 3 studies (1 in rats and 2 in mice) were included in the review because they fitted all the eligibility criteria. These 3 articles were published after the year 2000; included animals in the pre-clinical setting treated orally or injected with a GTC supplement or an individual purified catechin and also included control groups.

GTC or EGCG Supplementation Doses

The 3 articles included in this review utilized either a GTC preparation or pure EGCG as the treatment method in animals. In the study with rats, the animals were orally treated with an aqueous GTC extract, referred to as Polyphenon E (Mi-tusi Norin CO. Ltd, Tokyo, Japan), which had an initial concentration of total catechins of 0.5% (w/v). The extract was diluted daily in the animals’ drinking water to provide the following concentrations of the individual catechins: EGCG (63%); EC (11%); EGC (6%) and ECG (6%). The control group received drinking water with no catechins in it.

One of the 2 studies in mice also used a GTC powdered extract (Plant extract GmbH & Co, KG, Vestenbergsgreuth, Germany) in which the catechin concentration was said to vary between 12 and 17% (w/w). In this study, some mice were IP injected with 0.5, 1 or 5 mg/kg of the GTC extract in saline (twice on the first day of the experiment with a 6 hr interval) followed by once for the next 4 days. Control mice were injected with saline. In the same paper, other mice received oral treatment with pure EGCG at two concentration levels (2 and 10 mg/kg/day) for 14 days.

In the other mice study, some of the animals (Tg2576 females) were orally administered with pure EGCG (50 mg/kg) in water or water only (control) on a daily basis for 6 months. Other mice (female APPsw) were treated by IP administration of EGCG (20 mg/kg) or vehicle only (control) daily for 60 days.

Cognitive Testing

Rats were tested for learning-related cognitive ability with the use of the eight-arm radial maze with a particular focus on the reference memory error and working memory error. The

![Flow chart of the publication selection process](image-url)
Radial Arm Water Maze (RAWM) was also used to assess the experimental mice for working memory.61

**Induction of Cognitive Deficits/Mimicking of Ad Symptoms in Animals**

One of the mice studies included “Swedish” mutant amyloid precursor protein overexpressing (APPsw, Tg) mice,61 while the other study injected the mice IP with N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) at levels of 24 mg/kg/day in order to replicate the clinical AD symptoms.60 However, in the Wistar rat study, the animals were infused with Aβ(1-40) (4.9-5.5 mmol/l) into the exposed left ventricle after the injection of AlCl3 (0.5 µg) was performed in the right ventricle.59

**Tissue Analysis/Testing**

In the mice studies, the brain homogenate tissues were analyzed using Western blots and immunohistochemistry techniques with a specific focus on soluble Aβ(1-40) and tyrosine hydroxylase.60 In the rat study,59 plasma and brain homogenates were tested for LPO concentrations using the Thiobarbituric acid-reactive substances (TBARS) assay while plasma was also analyzed for total antioxidant activity using the Ferric Reducing Ability of Plasma (FRAP) assay and for plasma triglycerides and total cholesterol using commercially available enzyme kits.

**Study Outcomes**

One of the studies by Rezai-Zadeh, et al.61 was done to determine if the oral administration of EGCG would cause a reduction in plaque build-up in the APPsw mice. It was found that the EGCG (50 mg/kg) treated mice had significantly reduced (p<0.05) plaque build-up compared to controls (Table 1). This was supported by the micrographs of the brain sections where it was identified that β-amyloid stained antibody sections were substantially reduced in the cingulate and entorhinal cortex, as well as in the hippocampus by 54%, 51% and 43%, respectively. This was further supported by analysis of the anterior quarter tissue/indicators, plasma and brain tissue (hippocampal and cerebral cortex) TBARS concentrations were unaffected (p>0.2) amongst the groups but the plasma triglycerides were not significantly different amongst the groups.

The hippocampal TBARS concentrations were significantly (p<0.0001) lower as were the ROS levels (p<0.0029) in the Aβ(1-40) infused rats pre-administered with Polyphenon E. The cerebral cortex TBARS concentrations were unaffected (p=0.2) amongst the groups but the Polyphenon E only group (no Aβ(1-40) infused) had significantly (p<0.05) lower concentrations of ROS compared to the other groups.

The subset analysis of the number of radial maze error scores also indicated that Polyphenon E has significant effects in both the vehicle only and Aβ(1-40) infused rats. The Polyphenon E pre-treated groups had significantly (p<0.0001) lower error scores than either the Aβ(1-40) infused or vehicle treated animals.

The study by Haque, et al.59 in JCL: Wistar rats was focused on the effects of GTC on three measures: AD precursors/indicators, plasma and brain tissue (hippocampal and cerebral cortex) oxidative stress and outcomes of the eight arm radial maze test (working and reference memory errors) (Table 1). The plasma TBARS concentrations were significantly (p=0.02) higher in the control rats infused with the vehicle only and in rats infused with Aβ(1-40), whether they were pre-administered with Polyphenon E or not, compared to the animals infused with Polyphenon E only. However, the plasma FRAP was significantly (p<0.0001) higher in the Polyphenon E pre-administered groups, compared to controls, whether they were infused with Aβ(1-40) or not. Furthermore, the plasma cholesterol levels were significantly (p<0.05) lower in the polyphenon E treated Aβ(1-40) infused group compared to the rest of the experimental groups although the plasma triglycerides were not significantly (p>0.05) different amongst the groups.

The study by Levites, et al.60 investigated the neuroprotective properties of a GTC extract and pure EGCG in the MPTP mouse model of dopaminergic neurodegeneration similar to that found in sufferers of Parkinson’s disease (Table 1). In this study, it was identified that pre-treatment of the mice with either GTC at two doses (0.5 and 1 mg/kg) or with pure EGCG at two doses (2 and 10 mg/kg) prevented the neuronal losses caused by the MPTP treatment and concomitantly prevented the depletion of dopamine and tyrosine hydroxylase in the substantia nigra of the mice’s brains. It was identified that the GTC extract provided significant protection against the MPTP induced decrease in the melanin containing dopamine neurons at the GTC doses of 0.5 and 1 mg/kg although the higher dose of 5 mg/kg GTC was not effective. In addition, the pure EGCG at 2 and 10 mg/kg also considerably prevented the decrease in striatal dopamine neurons induced by the MPTP.

Furthermore, the activities of the SOD and catalase enzymes were assessed in the striatum of the mice treated with EGCG, MPTP or both of these compounds. It was observed that the MPTP increased the activity of both of these enzymes while...
The studies outlined in this review have exemplified containing structures are potential antioxidants and free-radical in the prevention of neurodegeneration has been associated with pressing the development of AD and Parkinson’s disease alike. act as vehicle mediated “backbones” for human trials on sup-

pre-treatment with EGCG prevented these effects at 2 mg/kg but not at 10 mg/kg.

**DISCUSSION**

The recent advances in drug development as well as more identifiable characteristics of neurodegeneration have set a path for the utilization of drugs that exhibit free radical scavenging and iron-chelating properties. These compounds can consequently serve as potential treatment of neurodegeneration and act as vehicle mediated “backbones” for human trials on suppressing the development of AD and Parkinson’s disease alike. The utilization of pure EGCG and more complex GTC mixtures in the prevention of neurodegeneration has been associated with the catechol-like structures as it is well established that catechol-containing structures are potential antioxidants and free-radical scavengers.62,63

The studies outlined in this review have exemplified some of the putative neuroprotective properties that GTC extracts and pure EGCG have on the suppression of neurodegeneration and the preservation of learning and behavioral patterns commonly affected by AD. The studies reviewed identified the benefits of GTC on either the suppression of development or the maintenance of symptoms associated with AD.

The study by Rezai-Zadeh, et al.61 identified that the oral administration of EGCG diminished amyloidosis in an AD-induced animal model, which is readily identified as one of the hallmarks in the development of AD; EGCG reduced the formation of β-amyloid plaque in the primary neuronal cells. Previous studies have predicted and observed that EGCG promotes the cleavage of Amyloid Precursor Protein (APP) into soluble-APP associated with an elevation of a-secretase cleavage, the main pathway for APP processing.61,64 Therefore, the findings by Rezai-Zadeh and colleagues61 indicate that EGCG may potentially reduce the formation of the toxic Sarkosyl soluble phospho-tau isofoms that are considered to be one of the features of

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<td>Anti-amyloidigenic effects of orally administered EGCG in APPsw mice.</td>
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<td>Haque, et al. (2008)</td>
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<td>Long-term administration of GTC and effect on oxidative stress and cognitive impairment in Aβ infused AD rat model.</td>
<td>Treatment group was administered GTC combination [EGCG (63%), EC (11%), EGC (6%) and ECG (8%)] in water for 26 wk.</td>
<td>Treatment prevented β-amyloid associated impairment in cognitive learning (p&lt;0.0001). Treated rats showed significant decreased number of reference and working memory errors (p&lt;0.05). Treated rats also had lower LPO (TBARS) in hippocampus and plasma and ROS in hippocampus and cortex and higher FRAP in plasma.</td>
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<td>Leviotes, et al. (2001)</td>
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<td>Mice were IP injected twice on the first day with GTC extract (0.5mg/kg), followed by injection of MPTP (24mg/kg) and GTC extract (0.5mg/kg) for the subsequent four days.</td>
<td>The injected GTC extract and oral EGCG showed a protective effect against MPTP-induced decreases in dopamine and tyrosine hydroxylase protein levels (p&lt;0.05).</td>
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| | The effect of EGCG on MPTP-induced neuro-degeneration | The injected GTC extract also prevented the MPTP-induced decrease in dopamine neurons in the brains. (p<0.02). |
| | The prevention of the MPTP-induced increase in the antioxidant enzymes SOD and Catalase by EGCG. | Oral EGCG at 2mg/kg/day, but not at 10mg/kg/day, prevented the MPTP-induced increase in SOD and Catalase. |

Table 1: Summary of GTC effects in animal pre-clinical trials.
The oral administration of EGCG for a six-month period to the AD transgenic mice also reduced β-amyloid deposition and its build up in important cognitive areas of the brain, improving working memory to near perfect results. Therefore, the findings of this study also supported the potential of EGCG to provide cognitive benefits in the transgenic mice models of AD as measured by RAWMS. Finally, these findings strengthen the possibility that the consumption of EGCG can be seen as a feasible therapeutic approach against AD and other similar cognitive impairments, which result from neuro-degeneration.

The study by Haque, et al. demonstrated that the long term pre-administration of PolyphenonE could prevent the development of β-amyloid-induced spatial cognitive learning difficulties in an AD rat model. In this model, the infusion of Aβ40 into the rat’s hippocampus induced a deficit in long-term potentiation and also in working memory. The β-amyloid infusion also increased hippocampal ROS concentrations, suggesting that ROS generation may lead to impaired learning cognitive functions.

The findings indicated that the neuroprotection and prevention of cognitive learning impairments seen with the GTC extract may have been due to its ability to decrease the β-amyloid induced oxidative stress. The pre-administration of PolyphenonE decreased the LPO (TBARS) and ROS concentrations, induced by β-amyloid infusion, in both the brain and plasma of the rats; the Polyphenon E also increased the FRAP in plasma. These results suggested that there was a significant and independent antioxidant effect of the GTC extract, which could potentially be harnessed to prevent cognitive damage in AD. This increase in antioxidant activity was proposed by the authors to be the main enhancing influence on the memory-related learning observed. These findings were also consistent with other observations that the oral consumption of GTC and other antioxidants is associated with the activation of antioxidative enzymes in various mouse models.

In one part of the study in mice by Levites, et al. they utilized MPTP as an initiator of neuro-degeneration caused by depletion of dopamine and tyrosine hydroxylase and showed that a GTC extract could prevent the MPTP-induced decreases in dopamine and tyrosine hydrolase. In a further study using a mice model of cerebral ischemia, pure EGCG injected immediately after ischemia, resulted in less memory impairment and reduced hippocampal neuron damage. Levites Y, et al. suggested that the EGCG’s neuroprotective properties may also include the regulation of antioxidant enzymes.

In conclusion, the papers reviewed in this systematic-review indicate that GTC and/or pure EGCG possess neuroprotective properties, which may be useful for preventing the development, or for the maintenance, of AD. Each of the papers reviewed also show effects on one or more of the current biomarkers linked to the development of AD. Although the findings of these articles are associated with pre-clinical animal models, they never the less provide a “stepping stone” for the utilization of GTC or EGCG as nootropic nutraceuticals for the potential suppression of neurodegenerative diseases such as AD.

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