Neuroprotective Effect of Chlorogenic Acids against Alzheimer's Disease

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Abstract

With increasing trend of aging population, neurodegenerative diseases, including Alzheimer's Disease (AD) have become more prevalent in today's society. Coffee is a popular beverage that has been linked to health benefits, including the potential to mediate AD. Coffee contains different concentrations and isomers of chlorogenic acids (CGAs), which represent a group of phenolic compounds abundant in human diet that have garnered interest due to their antioxidant activity in relation to neurological benefits. Evidence supporting the neuroprotective effects of CGAs has been observed in epidemiological studies, as well as in vitro and in vivo studies. CGAs are capable of mediating oxidative stress and attenuating cell apoptosis due to different oxidative stressors and free radicals by modulating the accumulation of reactive oxygen species and by regulating the expression of key proteins and enzymes involved in cell apoptosis. As CGAs are highly bioavailable in humans compared to other bioactive polyphenols, these compounds have the potential to exert positive effects in alleviating AD.

Keywords: Alzheimer's Disease; Chlorogenic Acids; Coffee; Antioxidant Activity.

Introduction

As population aging becomes a trend in many regions of the world, age-related neurodegenerative diseases, such as Alzheimer's Disease (AD), have become more prevalent in today's society. AD is the 5th leading cause of death among elderly population age 65 or older in the United States with projected number of 13.8 million cases by 2050 [1]. Contributing factors to the development of AD are believed to be the accumulation of abnormally folded amyloid (Aβ) plaques, consisting mainly of 40- and 42-mer Aβ protein, and the accumulation of tau protein tangles [2, 3]. These accumulations lead to synaptic degeneration and neuronal damage, which are attributed to cognitive and memory impairments in AD patients. AD has also been associated with an increase in pro-inflammatory cytokines in the blood and cerebrospinal fluid, which is an inflammatory response likely as a repair mechanism that is triggered by neuronal damage [2].

Epidemiological studies have shown that coffee consumption is linked to risk reduction of brain diseases, including AD. Although caffeine content in coffee is only 1%, many studies focus on evaluating the effect of caffeine on AD prevention, which is likely due to psychoactive stimulating properties of caffeine and its ability to cross blood-brain barrier [4]. Compared to caffeine, coffee contains much higher amount of chlorogenic acids (CGAs), which is about 7-9% dry weight basis. Although some studies mention possible synergistic effect of caffeine, CGAs, and other bioactive phytochemicals in coffee to mitigate AG, the importance of CGAs may be overlooked. Moreover, studies on CGAs are necessary to further understand the neurological benefits of caffeinated coffee and decaffeinated coffee.

CGAs are a group of polyphenols abundant in human diet, which can be obtained by consuming fruits, vegetables, medicinal herbs, and coffee. These polyphenolic acids are esters of quinic and hydroxycinnamic acids, such as caffeic, ferulic, and p-coumaric acids. CGAs are the major phenolics in coffee, which are present in the forms of caffeoylquinic acids (CQA), feruloylquinic acids (FQA), di-caffeoylquinic acids (diCQA) and caffeoylquinic acid lactones (CQAL) isomers [5] (Figure 1).

CGAs have been the subject of scientific research due to their antioxidant capacity to scavenge free radicals, which is attributed to double bonds and hydroxyl groups in their structure. Accumulating evidence suggests that CGAs play a role in reducing the risk of neurodegenerative diseases, such as AD, by modulating the accumulation of reactive oxygen species and by regulating the expression of key proteins and enzymes involved in cell apoptosis. CGAs are highly bioavailable in humans compared to other bioactive polyphenols, these compounds have the potential to exert positive effects in alleviating AD.

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CGAs Composition in Coffee and the Effect of Processing on Total CGAs Content

CGAs content in green coffee beans is the highest compared to other food sources, comprising of up to 14% in dry weight basis (dwb) with 45 identified CGA compounds [6]. In green coffee, CGAs are present in the form of CQA, diCQA, FQA, p-coumaroylquinic acids (p-CoQA) and caffeoylferuoylquinic acids (CFQA). Other compounds, such as diferuoylquinic acids (diFQA), di-p-coumaroylquinic acids (di-p-CoQA), and dimethoxycinnamoylquinic acids, are also present although they only constitute less than 1% of total CGAs.

CGAs content in coffee is dependent on the source of coffee beans, as well as the degree of roasting and brewing. Total CGA content in Brazilian Coffea arabica green coffee samples range from 5.5-6.3 g/100 g dwb, while Brazilian Coffea canephora sample has 8.6 g total CGAs/100 g dwb [6]. Many studies have reported that C. canephora has higher CGAs content than C. arabica. In arabica samples from Brazil, Indonesia, and Ethiopia, total CGAs prior to roasting range from 5.1-6.4 g/100 g dwb [7]. Total CGA content in Robusta green coffee was reported to be 28% higher than Arabica variety [8]. Although CGA isomers composition varies depending on coffee variety and source, the main CGA class in all samples was CQA, with 5-CQA being the predominant form. 35% of total CGAs in coffee is in the form of 5-CQA. In total, CQA and diCQA isomers make up 92-95% of total CGAs in coffee [9].

In the roasting process, some CGAs are degraded into CGA lactones and other derivatives through pyrolysis. The main CGA lactones present in roasted coffee is cinnamoyl-1,5-γ-quinolactones. The extent of CGAs thermal degradation during roasting is dependent on roasting conditions (time-temperature combination). CGAs loss might reach up to 95% in dark roasted coffee, which is due to several mechanisms occurring during roasting process including isomerization, epimerization, lactonization and degradation [6].

At fast roasting time, the antioxidant capacity of C. arabica from Costa Rica was found to be higher than green beans. This is likely due to the generation of compounds with high antioxidant capacity during roasting [10]. Similarly, analyses comparing the antioxidant capacity of unheated and heated 5-CQA show that heated 5-CQA exhibits higher antioxidant capacity, which is attributed to the generation of 2-methoxy-4-vinylphenol, 4-vinylphenol, and pyrocatechol [11]. However, when roasting temperature reached 200°C, antioxidant capacity is significantly lowered as more CGAs are thermally degraded. Moon et al., [12] reported that 45-54% of CGAs are lost during light roast, while in higher roasts, up to 99% of CGAs can be lost. Aside from roasting, different brewing methods were also found to affect total CGAs content (Table 1).

Bioavailability of CGAs in Humans

Bioavailability is important in evaluating potential benefits of CGAs on human health. CGAs must be absorbed and be able to pass through blood-brain barrier and understanding whether intact CGAs or their metabolites pass through this barrier is crucial. Understanding the metabolic fate of CGA compounds could increase credibility to their proposed neurological benefits, especially determining specific compounds that impose the benefits.

Identification and quantification of CGA and their metabolites are prerequisites to assess whether these compounds are bioavailable in humans. Plasma sample analysis of human subjects after...
In urine sample analysis at baseline, phenolic compounds were identified in most subjects, including 5-CQA, sinapic, gallic, p-hydroxybenzoic, and dihydrocaffeic acids. Excluding CQA, these other compounds represented approximately 85% of total phenolics quantified from the urine sample. However, compounds identified in the urinary excretion of subjects also substantially varied. In the urine samples, 17 hydroxycinnamate derivatives were quantified, which was approximately 29% of total CGA intake [5]. These include CQAs, FQAs, caffeic, dihydrocaffeic, ferulic, dihydroferulic, dihydro (iso)ferulic, isoflavan, feruloylglycine along with sulfates and glucoronides of several compounds. No protocatechuic, dihydroferulic, benzoic, and hippuric acids was present although previous study had identified this in urinary excretion after CGA consumption [13]. The main metabolite detected in urine after CGAs ingestion is caffeic acid [15].

In a follow-up study done by Stalmach et al., [16], 59% of CQAs was recovered in ileal fluid of ileostomy subjects. They also found high recovery of several other compounds, including FQAs (77%), CQAL metabolites (56%), p-coumaroylquinic acids (40%) and diCQA (40%). In total, 71% of CGA and their metabolites were recovered in ileal fluid. However, only 8% CGAs and metabolites were recovered compared to 29% found in the previous study with healthy subjects.

Comparing the recovery of CGAs and their metabolites in plasma, urine, and ileal fluid samples, the recovery is high in plasma and ileal fluid and low in urine samples. Low urine recoveries indicate that it is not the preferred excretion route for CGA and their metabolites. Based on pharmacokinetic result of several studies, absorption of CGAs and their metabolites occur mainly in the colon. One third of CGA is potentially absorbed from the small intestine and stomach, while the rest reaches the large intestine to be metabolized by colonic microflora. The apparent bioavailability of CGAs from green coffee extract and CGA supplement are 33.3 ± 23% and 33 ± 17%, respectively [13, 17] (Table 2).

**Table 1. CGAs Content in Different Varieties of Green and Roasted Coffee Cultivars from Various Sources.**

| Cultivar            | Source                      | Roasting condition | Total CGAs content | Reference |
|---------------------|-----------------------------|--------------------|--------------------|-----------|
| C. arabica cv. Mundo Novo | Minas Gerais, Brazil        | Green              | 6267.4 ± 171       | [6]       |
|                     |                             | Medium²            | 2191.4 ± 27.4      |           |
|                     |                             | Dark²              | 237.9 ± 7.6        |           |
| C. arabica cv. Catuai Varmelho | Minas Gerais, Brazil      | Green              | 5520.9 ± 86.4      | [6]       |
|                     |                             | Medium²            | 2565.7 ± 35.3      |           |
|                     |                             | Dark²              | 305.6 ± 44.6       |           |
| C. canephora cv. Conillon | Minas Gerais, Brazil       | Green              | 8557.4 ± 119.7     | [6]       |
|                     |                             | Medium²            | 4201 ± 141.2       |           |
|                     |                             | Dark²              | 543.4 ± 11.6       |           |
| C. arabica cv. Bourbon | Minas Gerais, Brazil       | Green Regular      | 5087.6 ± 274.3     | [7]       |
|                     |                             | Green Decaf        | 6128.4 ± 233.2     |           |
| C. arabica cv. Sumatra | Sumatra, Indonesia         | Green Regular      | 5428.9 ± 326.3     | [7]       |
|                     |                             | Green Decaf        | 6442.7 ± 441.9     |           |
| C. arabica cv. Sumatra | Sulawesi, Indonesia       | Green Regular      | 6461.1 ± 370.4     | [7]       |
|                     |                             | Green Decaf        | 7266.8 ± 98.6      |           |
| C. arabica cv. Herloom | Harargue, Ethiopia        | Green Regular      | 5606.2 ± 73.9      | [7]       |
|                     |                             | Green Decaf        | 6405 ± 276.7       |           |
| C. arabica cv. Longberry | Ethiopia                  | Green              | 5699.2 ± 161.6     | [8]       |
|                     |                             | Dark²              | 540 ± 24.3         |           |
| C. canephora cv. Robusta | Uganda                  | Green              | 7577.3 ± 98.7      | [8]       |
|                     |                             | Dark²              | 732.8 ± 108.3      |           |

|              | **Total CGAs content is expressed as mg/100 g of dry coffee weight**  |
|--------------|-------------------------------------------------|
| **Cultivar** | **Source** |
| C. arabica cv. Mundo Novo | Minas Gerais, Brazil |
| C. arabica cv. Catuai Varmelho | Minas Gerais, Brazil |
| C. canephora cv. Conillon | Minas Gerais, Brazil |
| C. arabica cv. Bourbon | Minas Gerais, Brazil |
| C. arabica cv. Sumatra | Sumatra, Indonesia |
| C. arabica cv. Sumatra | Sulawesi, Indonesia |
| C. arabica cv. Herloom | Harargue, Ethiopia |
| C. arabica cv. Longberry | Ethiopia |
| C. canephora cv. Robusta | Uganda |

1 Total CGAs content is expressed as mg/100 g of dry coffee weight
2 Medium = 170˚C, 12 mins; Dark = 200˚C, 15 mins
3 Dark = 230˚C, 9 mins

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Proposed metabolic pathway of CGA and specific absorption sites for hydroxycinnamate derivatives in human digestive system

Many compounds that were not originally present in the coffee samples were recovered from plasma, urine, and ileal fluids. This suggests that combination of CGA hydrolysis, as well as complex metabolism might be occurring in human digestive system. Also...
The time when C max is detected (T max) for certain compounds, in the small intestine and the liver [5]. Sulfated metabolites might be due to sulfation occurring in the small intestine. However, these compounds were found to be capable of producing esterase to break down CGAs in the colon [15]. More - over, the studies proposed that feruloylglycine and dihydroferulic acid lactone-O-sulfates (CQAL-O-sulfates), and three FQAs, the metabolites of CQA, FQA, and CQAL, sulfate and glucuronide metabolites of CQA, FQA, and CQAL, p-coumaroylquinic, caffeic, and ferulic acids were found to be capable of producing esterase to break down CGAs in the colon [15]. Moreover, the studies proposed that feruloylglycine and dihydroferulic acid conversion from ferulic acid and metabolism of caffeic acid occurred in the colon [16]. For 5-CQA, two caffeoylquinic acid lactone-O-sulfates (CQAL-O-sulfates), and three FQAs, the Tmax values were between 0.6 to 1.0 h, which suggest absorption in the small intestine. However, these compounds were found to have low C max values ranging from 2.2 to 27 nM, which is indicative of low-level absorption [5].

The table when C max is detected (T max) for certain compounds, such as free dihydroferulic acid and dihydrocaffeic acid and their sulfated conjugates, was between 4.7 – 5.2 h, indicating that absorption occurred in the large intestine. Strains of Escherichia coli, Bifidobacterium lactis, Lactobacillus gasseri were found to be capable of producing esterase to break down CGAs in the colon [15]. Moreover, the studies proposed that feruloylglycine and dihydroferulic acid conversion from ferulic acid and metabolism of caffeic acid occurred in the colon [16]. For 5-CQA, two caffeoylquinic acid lactone-O-sulfates (CQAL-O-sulfates), and three FQAs, the Tmax values were between 0.6 to 1.0 h, which suggest absorption in the small intestine. However, these compounds were found to have low C max values ranging from 2.2 to 27 nM, which is indicative of low-level absorption [5].

These and other findings suggest that colonic metabolites CGAs might play an important role in contributing to health benefits of CGAs because two-thirds of CGAs are metabolized in the colon. Low C max values of certain compounds, including CQAs, indicate that some CGAs are not well absorbed in their intact form. Substantial variability from different subjects might due to some genetic factors or microbial variation of different individuals affecting metabolism and absorption processes since no correlation was found between excretion with gender, age, weight, height, or body mass index [13].

Neuroprotective Mechanisms of CGAs on Key Components of Alzheimer's Disease

AD and other neurodegenerative diseases have been associated with oxidative neuronal death injury. Disease mechanism of AD has been linked to cellular damage due to oxidative stress in the brain, which is mediated by detrimental compounds called reactive oxygen species (ROS), including highly reactive hydroxyl radicals produced from hydrogen peroxide (H₂O₂) [4]. Antioxidant properties of CGAs are related to direct scavenging of hydroxyl radicals in a dose-dependent manner [18]. ROS promote apoptosis as well as oxidative damage to cellular protein, lipids, mitochondria, and DNA, subsequently leading to chronic diseases.

Studies have shown that brain of AD patients is under considerable oxidative stress since brain has relatively low amount of antioxidant enzymes, making it susceptible to oxidative damage [4, 19]. Aβ1-42 peptide contains methionine 35 residue that contributes to oxidative stress, which leads to neurotoxicity [20]. Mitochondrial dysfunction, which is commonly associated with apoptosis, resulted from ROS accumulation and expression of pro-apoptotic proteins, including Bax. ROS accumulation and pro-apoptotic proteins can increase mitochondrial permeability causing mitochondria to release cytochrome c. This leads to the activation of cysteine proteases called caspases, which consequently cause neuronal apoptosis [21]. Endogenous antioxidant enzymes, including glutathione peroxidase and catalase, are usually able to diminish adverse effects caused by ROS for tissue to maintain a healthy redox state [22].

Chemical assays, cell cultures, and animal models are useful investigation methods to elucidate the mechanisms underlying the beneficial effect of CGAs on cells and enzymes that are linked to AD.

Table 2. Identification of CGAs and Metabolites Detected in Plasma, Urine, and Ileal Fluid Samples of Human Subjects after the Ingestion of Green Coffee Extract and 200 ml of Instant Coffee.

| Source            | Sample       | Compounds                                                                 | Reference |
|-------------------|--------------|---------------------------------------------------------------------------|-----------|
| Green coffee      | Plasma       | 3-CQA, 4-CQA, 5-CQA, 3,4-diCQA, 3,5-diCQA, 4,5-diCQA, caffeic, ferulic, isoferic, and p-coumaric acids. | [13]      |
|                   | Urine        | 4-CQA (trace), 5-CQA (trace), sinapic, gallic, p-hydroxybenzoic, vanillic, siringic, dihydrocaffeic acids. |           |
| Instant coffee    | Plasma       | 5-CQA, 3-FQA, 4-FQA, 5-FQA, dihydrocaffeic, dihydroferulic acids, sulfates and glucuronide metabolites of caffeic, CQAL, dihydrocaffeic, dihydroferulic, ferulic, and isoferic acids. | [5]       |
|                   | Urine        | 3-FQA, 4-FQA, 5-FQA, dihydrocaffeic and dihydroferulic acids, feruloylglycine, sulfates and glucuronide metabolites of caffeic, CQA, CQAL, dihydrocaffeic, dihydroferulic, dihydroisoferulic, ferulic, and isoferic acids. |           |
| Instant coffee    | Ileal fluid  | 3-CQA, 4-CQA, 5-CQA, 3-FQA, 4-FQA, 5-FQA, 3,4-diCQA, 3,5-diCQA, 4,5-diCQA, sulfate and glucuronide metabolites of CQA, FQA, and CQAL, p-coumaroylquinic, caffeic, and ferulic acids | [16]      |

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Antioxidant Capacity of CGAs (Chemical Assays)

1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), and oxygen radical absorbance capacity (ORAC) are common assays that are used to measure antioxidant capacity of phenolic compounds in vitro. CGAs have been shown to exhibit dose dependent antioxidant activity in scavenging DPPH radicals, ABTS radicals, hydroxyl radicals, peroxyl radicals, as well as superoxide anions.

In DPPH and malonaldehyde-gas chromatography (MA-GC) assays the activity of 1μg/mL and 20μg/mL of 5-CQA ranged from 5.18 to 50.69% for DPPH assay, and from 1.36 to 44.41% for MA-GC assay [11]. DPPH-radical scavenging activities of 3-CQA, 4-CQA, and 5-CQA were similar, suggesting that ester bond position in the structure does not have much effect on the activity. However, compared to FQAs, CQAs have better scavenging activity, indicating that hydroxyl groups at 3-and 4-positions in the benzene rings are important in determining antioxidant activity [23]. At 10μM, CQAs are also able to scavenge superoxide radicals by 48-51%, which can also be attributed to hydroxyl groups in the benzene ring structure [23].

As most of CGAs are extensively metabolized in the colon, it is necessary to determine the antioxidant activity of CGA metabolites. ABTS and ORAC analyses showed that caffeic, isoforulic and dihydroforulic acids have stronger antioxidant activity than CGA. Colonic metabolites tested, such as m-coumaric acid and 3-(hydroxyphenyl) propionic acid, also showed antioxidant activity, indicating that hydroxyl groups at 3-and 4-positions in the benzene rings are important in determining antioxidant activity [23].

Antioxidant Capacity of CGAs (Cell-Based Assays)

CGAs provide protective effects against cytotoxicity stimulated by various oxidative stressors including H2O2, methylglyoxal, and methylmercury. In a study using primary cortical neurons, H2O2 for 24 h showed strong inhibition of this phenomenon in neuronal cells [4]. The proposed mechanism is through the prevention of H2O2-induced down-regulation of anti-apoptotic proteins Bel-2 and Bel-XL, which promotes cell survival [4]. Moreover, cell viability is increased due to prevention of caspase-3 cleavage and subsequently poly (ADP-ribose) polymerase (PARP) cleavage by caspase-3 [4]. Pretreatment also resulted in upregulation of NADPH: quinine oxidoreductase 1 (NQO1) expression in neurons, which helps to protect neurons from H2O2-induced down-regulation of anti-apoptotic proteins Bel-2 and Bel-XL, which promotes cell survival [4]. Moreover, cell viability is increased due to prevention of caspase-3 cleavage and subsequently PARP cleavage. Mitochondrial membrane depolarization is one of the critical characteristics of ROS-induced neuronal cell death. Reduction of apoptotic cells, caspase-3 activity, and suppression of ROS formation were observed [26]. The proposed mechanism is related to the delay of cellular antioxidant consumption by glutathione peroxidase (GPx) because an increase in intracellular GSH values and activity of GPx were observed [26].

Microglia activation promotes neurotoxicity due to the release of pro-inflammatory factors, such as nitric oxide (NO), superoxide, and tumor necrosis factor-α (TNF-α) [27]. By diminishing the activation of microglia, progression of neurodegenerative diseases could be impeded. CGAs were able to suppress lipopolysaccharide (LPS)-stimulated microglia activation by reducing NO production and release of TNF-α, subsequently increasing survival of dopaminergic (DA) neuron. CGAs prevented nuclear factor-kappaB (NF-αB) translocation, as well as decreased LPS-induced phosphorylation and inhibitory kappa B-alpha degradation [27].

In another study that investigated the effect of CGAs in human hepatoma cell line (HepG2), treating the cells with CGAs for 18 h without exposing the culture to pro-oxidant resulted in increased glutathione levels, while ROS formation was decreased. CGAs were also found to exert protective effect on mouse Neuro-2A neuroblastoma (Neuro-2A) cell subjected to methylglyoxal. High level of methylglyoxal in blood or tissue has been associated with diabetic models, which can eventually lead to diabetic neuropathy, such as AD [25]. Treatment with CGAs resulted in lower methylglyoxal-induced cell apoptosis in similar mechanisms with aforementioned studies. These mechanisms include reduction of mitochondrial disruption and caspase-3 activity, inhibition of Bax expressions, JNK and p38 MAPK activation, decreased cleavage of PARP, as well as increased Bel-2 expression [25].
treatment had little effect on cell viability as caspase cascade and expression levels of Bcl-XL, Bcl-2, and Bax proteins were not affected [28]. Beneficial effect of CGAs against oxidative damage is likely due to enhanced cellular defense system related to lower ROS formation and increase in antioxidant potential related to glutathione (GSH) formation [28].

These findings suggest that CGAs provide protective effects by reducing the accumulation of intracellular ROS and by regulating the MAPK signal pathways. CGAs are also able to up-regulate Bel-XL and down-regulate caspase-3 and caspase-9, which are key proteins and enzymes linked to apoptosis. Moreover, CGAs are able to inhibit microglia activation; hence they might inhibit the progression of AD.

In relation to Aβ, treating human neuroblastoma SH-SYSY cells with 3,5-diCQA was shown to inhibit Aβ-induced neurotoxicity. Increased mRNA expression of phosphoglycerate kinase 1 (PGK 1) glycolytic enzyme and intracellular adenosine triphosphate (ATP) level were observed [29]. 3,5-diCQA and 4,5-diCQA were also shown to possess anti-amyloidogenic property. In human neuroblastoma SH-SYSY cells, there is an observed dose-dependent inhibition of 42-mer amyloid beta-protein (Aβ42) aggregation. Aβ42 is important in the pathogenesis of AD due to its ability to aggregate in the form of beta sheet to induce neurotoxicity. 20 μM of 4,5-diCQA reduced Aβ42-induced neurotoxicity and restored cell viability by preventing beta sheet formation [3]. This property is related to caffeoyl group in CQA that is able to form covalent bond with Aβ42 residues, which prevents the formation of Aβ oligomers and destabilizes Aβ42 aggregates [3] (Table 3). Antioxidant Capacity of CGAs (Animal Studies)

Studies conducted in rodent models further corroborate the neuroprotective effect of CGAs shown to attenuate oxidative stress in cell cultures. Pathogenesis of AD has been linked to decrease in cholinergic neurons, leading to deposition of amyloid protein and hence progression of AD [19]. As acetylcholinesterase (AChE) and butyrylcholinesterase (BchE) are key enzymes linked to AD, inhibiting the activity of these enzymes is regarded as an effective strategy against AD by enhancing cholinergic neuronal function [19]. Dose-dependent inhibition of AChE and BchE activity was observed when rats were subjected to 0-12 μg/mL CGA and caffeic acid. However, caffeic acid was found to exhibit stronger activity in inhibiting AChE and BchE activity compared to CGA. AChE and BchE break down acetylcholine and butyrylcholine in the brain, which result in reduced communication between nerve cells using acetylcholine or butyrylcholine as a chemical messenger. Thus, the inhibition of these enzymes results in a temporary improvement of AD symptoms as inhibition results in slower acetylcholine and butyrylcholine breakdown in brain and prevention of oxidative neurodegeneration [19].

Table 3. Summary of Evidence from Cell-Based Assays that Evaluated the Neuroprotective Effects of CGAs.

| Model                                      | Compound | Treatment                  | Results                                                                 | Reference |
|--------------------------------------------|----------|---------------------------|-------------------------------------------------------------------------|-----------|
| Primary cortical neurons + H₂O₂            | CGA      | 12.5-100 μM for 1 h       | Inhibition of nuclear condensation, prevention of Bcl-2 and Bcl-XL down-regulation, reduced caspase-3 and PARP cleavage, increased NQO1 expression | [4]       |
| PC12 neuronal cell + H₂O₂                  | 5-CQA    | 1 and 5 μM                | Inhibition of nuclear condensation and DNA fragmentation, inhibition of JNK and p38 MAPK activation, reduced ROS accumulation | [21]      |
| PC12 neuronal cell + H₂O₂                  | 3-CQA, 4-CQA, 5-CQA | 10μM for 20 min         | Higher percentage of mitochondrial membrane polarization was maintained, decreased caspase-9 activation | [23]      |
| Mouse neuro-2A neuroblastoma + methylglyoxal | CGA      | 10-50μM for 72 h          | Reduced mitochondrial disruption, caspase-3 activity, and PARP cleavage, Bax expression, increased Bcl-2, inhibition of JNK and p38 MAPK activation | [25]      |
| PC12 neuronal cell + Methylmercury          | CGA      | 0.05-1.35μM               | Increased cell viability, reduced caspase-3 activity and ROS formation, increased intracellular GSH values and GPx activity | [26]      |
| Primary microglia + LPS                    | CGA      | 1-4 μM                    | Decreased NO production and TNF-a release, decreased phosphorylation and inhibitory kappa B-alpha degradation | [27]      |
| Human hepatoma cell                         | CGA      | 1-100μM for 18h           | Decreased ROS formation, increased intracellular GSH value | [28]      |
| Human neuroblastoma SH-SYSY cell + Aβ      | 3,5-diCQA | 20 μM for 72h             | Increased PGK1 and ATP level | [29]      |
| SH-SYSY cell + Aβ                          | 4,5-diCQA | 1-20μM for 48h            | Decreased β-sheet formation and Aβ42 aggregation | [3]       |
In *ex vivo* rat cortical slices treated with H$_2$O$_2$, CGA was found to prevent decline in intensity of 5-triphenyltetrazolium chloride (TTC) staining in a dose-dependent manner, and reduce H$_2$O$_2$-induced MDA and ROS increase in brain tissues [22]. CGA had protective effect at as low as 0.1 μM, although 10 μM was required to have similar staining level as control. While caffeic acid has similar neuroprotective effect as CGA, quinic acid did not show strong protective effect against H$_2$O$_2$-induced alteration in the brain [22].

Administering 3,5-diCQA to senescence accelerated-prone mice (SAMP8) resulted in reduced cognitive impairment as spatial learning and memory were improved. An observed increased in intracellular ATP is likely due to overexpression of a glycolytic enzyme, phosphoglycerate kinase-1 (PGK1), which plays a role in glycolysis pathway [29]. Similarly, in mice with scopolamine-induced amnesia, CGA was found to increase spontaneous alternation behavior in Y-maze test, which indicates improvement of short-term memory [30]. Moreover, CGA treatment improved long-term memory and reversed scopolamine-induced cognitive impairments by inhibiting AchE activity and reducing MDA levels in hippocampus and frontal cortex [30]. Similarly, decaffeinated coffee attenuated scopolamine-induced memory impairment in rats by reducing TNF-α release and suppressing NF-κB stimulation in their hippocampus [31]. On the contrary, treating rats with decaffeinated coffee without scopolamine was not found to improve learning and memory. Pretreating mice with 100 mg/kg CGA before injecting LPS was found to attenuate LPS-induced release of interleukin-1β and TNF-α in injection site, which was substantia nigra (SN). CGA attenuated microglia activation in SN and protected against LPS-induced neurotoxicity [27].

In transgenic Drosophila model of AD, decaffeinated coffee suppressed the decrease in lifespan of the fly due to Aβ expression. Neuroprotective mechanism of decaffeinated coffee is related to the activation of cytoprotective transcription factor NF-E2-Related factor (Nrf2) [32]. However, other components present in decaffeinated coffee besides CGAs might contribute to its neuroprotective effect (Table 4).

### Antioxidant Capacity of CGAs (Human Studies)

Although CGAs and their metabolites are able to attenuate oxidative stress and provide neuroprotective effects *in vitro* and in animal models, current clinical trial is limited to studying the effect of coffee consumption on reducing oxidative damage. Consumption of CGAs enriched coffee shows changes in biomarkers of oxidative damage, including reduced lipid peroxidation measured by 8-Isoprostaglandin F2α in urine and 3-nitrotroxyamine in plasma, and reduced formation of oxidized purines and pyrimidines marked by lower DNA migration in lymphocytes [33]. Moreover, lower plasma level of oxidized LDLs and malondialdehyde (MDA) were observed, indicating increased total antioxidant capacity [33]. Increased expression of several genes, including NQO1, superoxide dismutase (SOD) and glutathione-S-transferases (GST), were observed after coffee consumption although the increase was not significant [33]. In another study by Mišiket al., [34], coffee consumption reduced DNA migration due to oxidized purines. However, there was no significant change in other biomarkers, including LDLs and MDA levels.

In addition to mitigating oxidative stress, decaffeinated coffee enriched with CGA positively affects cognition in healthy elderly population. High CGA content coffee reduced headache and mental fatigue, as well as increased alertness in subjects when compared to low CGA content coffee, indicating that CGA improves brain-related function [35].

### Conclusions

According to current bioavailability studies, CGAs are highly bioavailable in humans with approximately 33% absorption from the small intestine. Considering that CGAs undergo extensive metabolism in the colon, it is necessary to evaluate the role of colonic microflora and the effect of CGA metabolites in neuroprotection. CGA metabolites, including caffeic and dihydroferulic acids show high antioxidant capacity *in vitro*. Studies evaluating the antioxidant capacity of CGAs in modulating oxidative stress *in vitro* and *in vivo* show promising results in regards to the potential of

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**Table 4. Summary of Evidence from Animal Models that Evaluated the Neuroprotective Effects of CGAs.**

| Model                          | Compound                        | Treatment          | Results                                              | Reference |
|-------------------------------|---------------------------------|--------------------|------------------------------------------------------|-----------|
| Rat brain *in vitro*          | CGA                             | 0-12 μg/mL         | Inhibition of AchE and BchE activity, reduced MDA and ROS levels | [19]      |
| Rat cortical slices + H$_2$O$_2$ | CGA, caffeic, quinic acids     | 0.1 μM–100 μM for 1h | Decreased MDA and ROS levels                         | [22]      |
| Senescence-accelerated-prone mice 8 | 3,5-diCQA                    | 6.7 mg/kg × day for 1 month | Reduced cognitive impairment, improved spatial learning and memory | [29]      |
| Mice + scopolamine            | CGA                             | 0.9 mg/kg          | Improved short-term and long-term memory, reduced cognitive decline | [30]      |
| Rats + scopolamine            | Decaffeinated coffee            | 120 or 240 mg/kg   | Reduced memory impairment                            | [31]      |
| Mice + LPS                    | CGA                             | 100 mg/kg          | Reduced interleukin-1β and TNF-α release, decreased TNF-α release NF-kB stimulation | [27]      |
| Drosophila model of AD        | Decaffeinated coffee            | 18.4g/100ml extract | Increased lifespan                                   | [32]      |
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