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Effect of catechins and high-temperature-processed green tea extract on scavenging reactive oxygen species and preventing Aβ_{1–42} fibrils’ formation in brain microvascular endothelium

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The present study investigated the effect of high-temperature-processed green tea extract (HTP_GTE) and its bioactive components on the reduction of reactive oxygen species (ROS) and amyloid-beta (Aβ) protein in human microvascular endothelial cells. Compared to Aβ_{1–42}-only treatment, pretreatment of HTP_GTE was revealed to effectively inhibit ROS generation (P<0.05). HTP_GTE and catechins not only inhibit Aβ_{1–42} fibril formation but also destabilize preformed Aβ_{1–42} fibrils. The presence of HTP_GTE, Aβ_{1–42} fibril formation was significantly inhibited in a dose-dependent manner at 12.5–100 μg/ml of HTP_GTE, showing 86–56%, respectively. Treatment of various concentrations of HTP_GTE and catechins steadily destabilized the preformed Aβ_{1–42} fibrils for 24 h in a dose-dependent manner. It was observed that the gallated groups such as epigallocatechin gallate, epicatechin gallate, gallocatechin gallate, and catechin gallate more effectively disturbed Aβ_{1–42} fibril formation and destabilized the preformed Aβ_{1–42} fibrils than the non-gallated group. Taken together, these findings supported that sterilized green tea could be promising natural anti-amyloidogenic agents associated with therapeutic approaches in Alzheimer’s disease by scavenging ROS generation and Aβ fibril in the brain tissue.

Keywords: High-temperature-processed green tea extract, Catechins, Reactive oxygen species, Aβ_{1–42} fibrils, Blood-brain barrier

Introduction
Alzheimer’s disease (AD) is an advanced neurodegenerative disease relating to most types of dementia.^{1} By 2050, the number of AD patients is predictable to more than 106 million worldwide, and it is assessed that 1 in 85 persons will be existing with the AD.^{2} Formation of amyloid-beta (Aβ) proteins as neuritic senile plaques’ constituent is a key risk factor and it plays a central pathogenic role in the commencement and progression of AD.^{3} The major species in Aβ proteins are the Aβ_{1–40} and Aβ_{1–42} peptides, with Aβ_{1–42} being a hallmark of AD. Aβ_{1–42} is considered more toxic than Aβ_{1–40} as it could aggregate much more quickly than Aβ_{1–40}, leading to provide further Aβ-accumulation-related potential neurodegenerative disorders.^{4,5}

A blood-brain barrier (BBB) located between the circulating blood and the brain not only forms an interface with tight junction but also retains various carrier-mediated transport systems.^{6,7} The BBB also regulates transport of compounds by efflux transporters such as P-glycoprotein (P-gp) and multidrug-resistance-associated proteins in order to support and protect central nervous system.^{8} Previous studies reported that Aβ induced numerous cytotoxic effects on neuronal cells and caused attenuation of the BBB through generation of reactive oxygen species (ROS).^{9,10} ROS make functional changes of the BBB, leading to an increase in its permeability.^{11,12}

It is known to be a key component of the Aβ formation
for neurodegeneration in AD.13,14 Ujiie et al. (2003) also reported that structural changes to the BBB induced by increased Aβ could take a major role in AD progression.15 Furthermore, Aβ accumulation in the synapse might be a damage to neuronal processes, which suggested that it could be a crucial factor that can affect the cognitive dysfunction of aged people and AD patients.16,17

The potential preventive action of phytochemicals has been recently focused on the neuronal death associated with neurodegenerative disorders. Several studies have demonstrated that polyphenols such as curcumin, resveratrol, and oligonol could inhibit Aβ-mediated damages.18–20 Among them, green tea (Camellia sinensis), widely consumed around the world as well as contained catechins, was extensively studied due to its strong antioxidant activity.21,22 For instance, it was well established that (-)‐epigallocatechin gallate (EGCG) provided potential functions to protect neuronal cells from toxicity caused by Aβ.23 Antioxidant compounds (‐)‐epicatechin (EC) and (‐)‐catechin (C) also effectively attenuated Aβ generation.24 Although other catechins presented in green tea have been known to provide free radical scavenging activity, it is limited to the anti‐amyloidogenic activity of EGCG. Therefore, the present study aimed to evaluate the inhibitory activity against ROS induced by Aβ1‐42 and anti‐amyloidogenic activity of catechins (epicatechins/non‐epicatechins) and sterilized green tea extracts by using an in vitro BBB model system.

Materials and methods
Standards and chemical reagents
(‐)‐Epigallocatechin (EGC), (‐)‐EC, (‐)‐EGCG, (‐)‐epicatechin gallate (ECG), (‐)‐gallocatechin (GC), (‐)‐catechin (C), (‐)‐gallocatechin gallate (GCG), and (‐)‐catechin gallate (CG) standards were purchased from Wako (Osaka, Japan). High‐temperature‐processed green tea extract (HTP_GTE) was kindly provided by AmorePacific R&D Center (Gyeonggi‐do, South Korea). Dulbecco’s phosphate saline buffer (DPBS), Dulbecco’s modified Eagle’s medium (DMEM), and 0.25% trypsin EDTA and penicillin/streptomycin were purchased from Corning Costar (Lake Forest, CA). Aβ1‐42 was purchased from Abcam (Cambridge, UK). The acetic acid solution was purchased from Sigma‐Aldrich (St. Louis, MO, USA). Water and acetonitrile (HPLC grade) were purchased from J.T. Baker (Phillipsburg, NJ, USA).

Sample preparation
The stock of synthetic Aβ1‐42 was dissolved in 0.1% ammonia solution to yield a final concentration of 250 μM, separated into aliquots, and then stored at −80°C until use. For (3‐(4,5‐dimethylthiazol‐2‐yl)‐2,5‐diphenyltetrazolium bromide (MTT) assay and 2', 7'-dichlorodihydrofluorescein diacetate (2'7'-DCFH‐DA) assay, 25 μM of Aβ1‐42 was pre‐incubated in DMEM without FBS for 24 h in order to form fibrils. Then, Aβ1‐42 was further diluted in a cell medium to obtain appropriate concentration. For the Thioflavin T (ThT) fluorescence assay, a stock solution of Aβ1‐42 was diluted to a concentration of 25 μM in phosphate buffer (50 mM, pH 7.5), containing 100 mM NaCl. Twenty five micrometer of Aβ1‐42 was used for further studies. Each catechin standards stock solutions was prepared in deionized distilled water. The stock solutions were stored at −80°C and diluted to the desired concentrations immediately before use.

Preparation of HTP_GTE
The camellia sinensis (CS) leaf used in this study was obtained from the Jeju‐do in South Korea. The dried CS leaf (1000 g) was extracted two times with 50% aqueous ethanol (41 × 3 times) using a heating bath at 60°C for 3 h to give 50% aqueous ethanol extract (255 g). The 50% aqueous ethanol extract was dissolved with 11 water and then subjected to a sterilization condition (at 121°C) for 90 minutes. The sterilized extract was concentrated with a rotary evaporator (Buchi R200) in vacuo (250 g).

Human brain microvascular endothelial cells cell culture
Human brain microvascular endothelial cells (HBMEC) were obtained from the Division of Pediatric Infectious Diseases, Johns Hopkins University School of Medicine (MD, USA). The cells were grown in DMEM supplement with 10% FBS and 1% penicillin/streptomycin. The cells were sustained in an incubator at 37°C with 5% CO2 of atmosphere and 95% air. The growth medium was changed every other day.

Identification of bioactive components in HTP_GTE by UPLC‐PDA‐ESI‐MS/MS
Bioactive components in HTP_GTE were determined by ultra‐high performance liquid chromatography (UPLC) with a photodiode array (PDA) detector and electrospray ionization (ESI) mass spectrometry (MS). HTP_GTE were analyzed through a column chromatography with reversed phased‐column (Shiseido, 250x4.6 mm, 5 μm, C18) with mobile phases of solvents A and B (A: 0.1% acetic acid in water, B: acetonitrile). The mobile phase gradient elution was accomplished by varying the ratio of solvents A and B with a 1 ml/min of flow rate. The injection volume was 20 μl. The initial phase of the gradient was 10% solvent B. Then, the gradient increased linearly to 15% of solvent B to 30 minutes, continuously...
increased to 20% for the next 12 minutes, and held at 20% for 2 minutes. The gradient deceased to 10% of solvent B for 44.1 minutes and held at 10% to 50 minutes until analysis of the next sample. The UV wavelength was set at 280 nm. Mass spectra were carried out by using an LCQ fleet with ESI-MS. MS was achieved in a negative ion mode ([M-H]−). ESI-MS conditions as follows: capillary voltage (−35.00 V), capillary temperature (270.00°C), source heater temperature (350.00°C), sheath gas flow (75.00), aux gas flow (40.00), tube lens (−70.00 V), source voltage (3.2 kV), and scan range (50–500 [m/z]).

Measurement of cell cytotoxicity
In accordance with the method reported in a previous study,25,26 MTT assay was conducted to determine the inhibition of cell growth by 50% (IC50). Briefly, HBMEC was seeded in the 96-well microplate at a density of 5×10³ cells/well. After the HBMEC was attached to a 96-well microplate for 24 hours, the medium was removed, and then each well was washed by using DPBS. To determine the IC50 value of Aβ1-42, HBMEC was treated by various concentrations (1 nM, 10 nM, 100 nM, 1 μM, 10 μM, and 25 μM) of Aβ1-42 for 24 hours. Non-cytotoxic concentration ranges of HTP_GTE, treatments of HTP_GTE (12.5, 25, 50, 100, 200, 400, and 800 μg/ml) were added into cells and maintained for 24 hours. Concentrations of each treatment above 90% of cell viability were used for further study.

Following treatment, the medium was removed and added to 100 μl of the MTT solution. Subsequently, 96-well microplate was incubated for 4 hours at 37°C under 5% of the CO2 atmosphere. Cells were incubated after replacing the MTT solution with dimethyl sulfoxide. And then, using a microplate reader (Varioskan Flash, Thermo Scientific, San Jose, CA), optical density (O.D.) was measured at 570 nm. This operation was repeated in three times, and the average means were used in formula as follows:

\[
\text{Percent of cell viability (\%)} = \frac{[\text{Average of test (O.D.)} - \text{Average of blank (O.D.)}]}{[\text{Average of control (O.D.)} - \text{Average of blank (O.D.)}]} \times 100
\]

Measurement of intracellular ROS
In accordance with the method reported in previous studies,25,27 2′,7′-DCFH-DA assay was carried out to measure intracellular ROS generation induced by Aβ1-42. Briefly, HBMEC was seeded in the 96-well black microplate at a density of 5x10³ cells per well. After the cells were attached to a 96-well black microplate for 24 hours, the medium was removed, and then each well was washed by using DPBS. HBMEC was treated by various concentrations (1 nM, 10 nM, 100 nM, 1 μM, 10 μM, and 25 μM) of Aβ1-42. To determine ROS scavenging capacity of HTP_GTE, HBMEC was treated by various

\[\text{Figure 1} \quad \text{Chemical structures of epicatechins (A) and non-epicatechins (B).} \quad * \text{G: gallate}\]
concentrations (12.5, 25, 50, and 100 µg/ml) of HTP_GTE for 2 hours and then, cells were treated with an IC_{50} value of Aβ_{1-42} for 24 hours. After treatment, the medium was removed and 27°C-DCFH-DA solution was added into each well followed by treating 0.25% trypsin EDTA. After reaction for 30 minutes at 37°C under 5% of the CO₂ atmosphere, the cell fluorescence was observed by using a microplate reader (Varioskan Flash, Thermo Scientific, San Jose, CA) at 488 nm for excitation and 525 nm for emission, respectively. This operation was repeated in three times, and the average means were used in formula as follows:

\[
\text{Percent of ROS generation (\%)} = \left( \frac{\text{Average of test}}{\text{Average of control}} \right) \times 100
\]

Measurement of Aβ_{1-42} fibril formation and fibril destabilizing

Determination of fibril formation disrupts effect and fibril destabilizing effect was monitored by using a ThT binding assay. ThT specifically binds to the hydrophobic area on the surface of the β-sheet of Aβ_{1-42}, and thus the ThT fluorescence has been used as a quantitative indicator of amyloid fibrils. and as a result, the dye exhibits red-shifted and enhanced fluorescence emission. To measure the inhibition effect of HTP_GTE and catechins on Aβ_{1-42} fibril formation, 9 µl of 25 µM Aβ_{1-42} was added to the 96-well black microplate, then incubated with 1 µl of each sample for 24 hours. Plates were covered to minimize evaporation without agitation. After incubation, 200 µl of ThT solution containing 50 mM glycine, NaOH buffer, pH 8.5, at a final concentration of 10 µM was added to each well. To measure the effect of fibril destabilizing by HTP_GTE and catechins, Aβ_{1-42} was pre-incubated at 37°C for 24 hours to obtain Aβ_{1-42} fibrils. After pre-incubation, 9 µl of 25 µM Aβ_{1-42} was added to the black wells with 1 µl of each sample and incubated at various time intervals. Subsequently, 200 µl of ThT solution was added to each well and measured using a microplate reader (Varioskan Flash, Thermo Scientific, San Jose, CA) by fluorescence at 446 nm for excitation and 490 nm for emission, respectively. This operation was repeated in three times, and the average means were used in formula as follows:

\[
\text{Relative fluorescence intensity(\%)} = \frac{[\text{Average of test (Fluorescence intensity)} - \text{Average of each sample blank (Fluorescence intensity)}]}{[\text{Average of control (Fluorescence intensity)} - \text{Average of each sample blank (Fluorescence intensity)}]} \times 100
\]

Statistical analysis

Values were reported as the mean±standard deviation (SD) from at least three different experiments. Analysis of variance and Tukey’s post hoc test were accomplished to determine significant differences among groups at the significance level of 0.05 by using Graphpad Prism 3.0 software (Graphpad, San Diego, CA).

Results

Identification of bioactive components in HTP_GTE by UPLC-PDA-ESI-MS/MS

As illustrated in Fig. 2A, eight major peaks from HTP_GTE were detected at 4.67, 7.18, 9.39, 15.97, 16.91, 19.92, 31.51, and 32.77 minutes of retention time, respectively by using UPLC-PDA. Structural identification of eight major bioactive components in HTP_GTE was detected in the negative ion mode with mass fragment patterns obtained by mass spectrometry (MS^1 and MS^2 spectra). Since epicatechins and non-epicatehins are structural isomers, EGC, EC, EGCG, and ECG showed same fragment patterns with their epimer, GC, C, GCG, and CG, respectively (Fig. 2B and 2C). In the mass spectra for the peak 1 and 2 ([M-H]^-) ion at m/z 305.06 was obtained from MS^1 and further fragmented to mainly m/z 261.08, 218.98, and 179.01 in MS^2. For the peak 3 and 4, ([M-H]^-) ion at m/z 289.06 produced ion at m/z 245.03, 205.09, and 179.06 on MS^2. In case of the peak 5 and 6, ([M-H]^-) ion at m/z 456.95 produced ion at m/z 331.02, 305.09, and 168.91 on MS^2. Peak 7 and 8 ([M-H]^-) ion at m/z 441.01 produced ion at m/z 331.00, 289.03, and 169.02 on MS^2. Epicatechins and non-epicatechins were determined by matching ions mass fragment patterns in both MS^1 and MS^2 spectra by comparing mass fragment patterns achieved from own standard. Hence, eight major components (including epicatechins and non-epicatechins) were used for further studies as individual bioactive components in HTP_GTE.

Cell cytotoxicity and ROS scavenging capacity of HTP_GTE induced by Aβ_{1-42} in HBMEC

Figure 3 shows that HBMEC cell cytotoxicity by various concentrations of synthetic Aβ_{1-42} was observed in a dose-dependent manner, showing 91, 89, 83, 77, 54, and 33% of cell viability ranged from 1 nM to 25 µM of Aβ_{1-42}, respectively. Thus, the level of 10 µM Aβ_{1-42} concentration was used for...
further study as IC_{50} value. ROS generation caused by Aβ_{1-42} in HBMEC was also observed following exposure to 1 nM to 25 μM of Aβ_{1-42} for 24 hours (Fig. 4A). This result indicates that Aβ_{1-42} stimulated ROS generation resulting from cell apoptosis as reported in a previous study.²⁹ Hence, the protective effects of HTP_GTE on ROS generation induced by Aβ_{1-42} were measured at various concentrations of HTP_GTE, 12.5, 25, 50, and 100 μg/ml, which were determined not to cause cell cytotoxicity (data not shown) to verify scavenging capacity of HTP_GTE (Fig. 4B). When HTP_GTE was pretreated, ROS generation was significantly attenuated, ranging from 105 to 107% compared to Aβ_{1-42} only treatment (P<0.05). However, it was not shown in a dose-dependent manner. Particularly the most effective ROS scavenging ability was observed at 100 μg/ml concentration of HTP_GTE for protecting HBMEC against Aβ_{1-42} exposure. These results suggest that HTP_GTE could modulate ROS induced by Aβ_{1-42}, the source of oxidative stress in terms of the cellular integrity of BBB, resulting in brain protection.

**Inhibitory effects of HTP_GTE on Aβ_{1-42} fibril formation and Aβ_{1-42} fibril destabilization**

The present study investigated the effects of HTP_GTE on Aβ_{1-42} fibril formation using a ThT fluorescence assay. To measure the inhibitory
The effects of HTP_GTE on Aβ1-42 fibril formation, Aβ1-42 was incubated for 24 hours at 37°C with various concentrations of HTP_GTE (12.5, 25, 50, 100 μg/ml) and then Aβ1-42 fibril formation was expressed as relative fluorescence intensity (%). Figure 5A shows a fibril formation of Aβ1-42 in...
the presence or absence of HTP_GTE. The presence of HTP_GTE, Aβ1-42 fibril formation was significantly inhibited in a dose-dependent manner at 12.5–100 μg/ml of HTP_GTE, showing 86–56%, respectively. It indicates that 100 μg/ml of HTP_GTE could obviously inhibit Aβ1-42 fibril formation. However, there was no significant difference between 12.5 and 25 μg/ml of HTP_GTE (P > 0.05). One of the existing therapeutic approaches in the AD is to destabilize preformed fibrils in order to make Aβ clearance. Therefore, we further investigated the fibril destabilizing effects of HTP_GTE (Fig. 5B). ThT fluorescence was almost unchanged and showed the highest levels during the incubation of fresh Aβ1-42 at 37°C without HTP_GTE. After adding various concentrations of HTP_GTE, ThT fluorescence was considerably decreased between 0 and 1 hour of incubation time in all treatments and steadily decreased for 24 hours in a dose-dependent manner. Especially, the addition of 100 μg/ml HTP_GTE inhibition showed the highest activity to destabilize preformed fibrils during 24 hours of incubation. These results indicated that HTP_GTE could significantly inhibit and destabilize amyloid oligomer formation associated with AD due to catechins in HTP_GTE. Hence, we further studied the effects of bioactive components in HTP_GTE on an anti-Aβ1-42 activity in detail.

Inhibitory effects of epicatechins and non-epicatechins on Aβ1-42 fibril formation
To measure the inhibitory effects of epicatechins (EGC, EC, EGCG, and ECG) on Aβ1-42 fibril formation, Aβ1-42 was incubated for 24 hours at 37°C with various concentrations of epicatechins (10, 20, 40, and 80 μM) and Aβ1-42 fibril formation was expressed as relative fluorescence intensity (%). In all groups, relative fluorescence intensity as a surrogate of Aβ1-42 fibril formation was attenuated by epicatechins in a dose-dependent manner at all the concentrations. EGC inhibited relative fluorescence intensity at all the concentrations in a dose-dependent manner, ranged from 88 to 50% for 10–80 μM of concentrations, respectively (Fig. 6 A). Relative fluorescence intensity by pretreatment of EC ranged from 92 to 53%, indicating a pattern with EGC (Fig. 6B). However, in contrast to EGC, a dramatic decreasing was observed between 10 and 20 μM of EC, showing 92–71%, respectively. Although each relative fluorescence intensity decreased by 88 or 92% at 10 μM concentration of EGC or EC, there was no significant difference between Aβ1-42 treatment only and 10 μM of EGC or EC treatment (P > 0.05), respectively. It was also observed that the gallated groups such as EGCG and ECG disturbed Aβ1-42 fibril formation, indicating that relative fluorescence intensity was ranging from
77 to 41% and from 82 to 44% for 10–80 μM EGCG (Fig. 6C) and ECG (Fig. 6D), respectively. Both EGCG and ECG showed the linear decreasing pattern on Aβ1–42 fibril formation; especially EGCG showed a higher inhibition effect on Aβ1–42 fibril formation than that of ECG. Our results suggest that epicatechins could effectively disrupt Aβ1–42 fibril formation associated with the AD. Since non-epicatechins are other components in green tea, we performed further experiments for measuring the inhibitory effects of non-epicatechins on Aβ1–42 fibril formation (Fig. 7). In all groups, relative fluorescence intensity was attenuated by non-epicatechins in a dose-dependent manner at all the concentrations (10, 20, 40, and 80 μM), implying that epicatechins as well as non-epicatechins in green tea inhibited Aβ1–42 fibril formation. In detail, the relative fluorescence intensity in a GC and C treatment ranged from 96 to 78% and 93 to 73%, respectively (Fig. 7A and 7B). The gallated groups such as GCG and CG remarkably disturbed Aβ1–42 fibril formation, showing 89–40% and 90–45% of relative fluorescence intensity for GCG (Fig. 7C) and CG (Fig. 7D), respectively. Apart from other groups, all the values in the GCG group showed a significant difference when compared to Aβ1–42-only treatment (P<0.05). Compared to other non-epicatechins, the level of Aβ1–42 fibril formation treated with GCG showed the lowest at all concentrations, indicating that GCG provided relatively higher inhibition activity than others.

**Effects of epicatechins and non-epicatechins on Aβ1–42 fibril destabilization**

The destabilizing effect of catechins on Aβ1–42 fibril was evaluated during 24 hours incubation with the presence of 80 μM of each catechin at 37°C and it was expressed as % of aggregation (Fig. 8). In all groups, marked destabilizing was observed between 0 and 0.5 hours of incubation time. The efficiency of destabilizing Aβ1–42 fibril was in the order of EGCG > GCG > CG > ECG > GC > EGC > EC > C. Gallated groups such as EGCG, ECG, GCG, and CG showed a much higher destabilizing effect compared to non-gallated groups. Especially, it was shown that EGCG has the highest ability in disaggregation of Aβ1–42 fibril by showing 44% at 24 hours followed by GCG. In the case of non-gallated catechins, GC showed the highest destabilizing effect on Aβ1–42 fibril among non-gallated catechins, having 81% at 24 hours of incubation time. These results implied that green tea catechins could protect the brain against an AD by destabilizing effect on Aβ1–42 fibril.

**Discussion**

Here, we demonstrated that the HTP_GTE and its bioactive components, catechins (including...
epicatechins and non-epicatechins), exhibited ROS scavenging capacity in HBMEC induced by Aβ1–42 as well as anti-amyloidogenic effects such as fibril formation and fibril destabilization in vitro. Misonou et al. (2000) reported that oxidative stress induced by hydrogen peroxide (H2O2) increased the intracellular Aβ levels in human neuroblastoma SH-SY5Y cells.30 Other previous studies also found that oxidative stress induced by Aβ peptides directly initiate ROS formation associated with cellular dysfunction, mitochondrial dysfunction, and neuronal death in the pathogenesis of AD.31,32 Based upon results from the present study, Aβ could induce ROS generation in the human brain endothelial cell line (Fig. 4A). It could be considered that Aβ and ROS are in a close relationship with each other in neuronal disorder, leading to the AD. Thus, plant extracts containing the flavonoids have been extensively studied for modulation of oxidative status induced by Aβ. For instance, Bastianetto et al., 2000 investigated the antioxidant capacity of Ginkgo biloba extract obtained from green leaves against the intracellular and mitochondrial ROS in rat hippocampal cells induced by Aβ peptides.33 Like our results, intracellular and mitochondrial ROS levels were reduced 85, 65, 49%, and 88, 75, 64%, respectively, at 10, 50, and 100 μg/ml concentrations of Ginkgo biloba extract after exposure to cells. According to the previous study, Ginkgo biloba leaves contained 167 μg/g of EC, the most abundant compound among other flavonoids, followed by rutin (135 μg/g), apigenin (32.6 μg/g), quercetin (11.0 μg/g), and luteolin (10.4 μg/g).34 In addition, it was demonstrated that HTP_GTE treatment substantially reduced oxidative DNA damage by reducing the formation of 8-hydroxydeoxyguanosine, which has been implicated in neuronal cell death by Aβ25–35.35,36 Hence, these findings support our result that HTP_GTE could effectively contribute to prevention of AD by restraining of Aβ accumulation induced by oxidative stress through the antioxidant activity.

A previous study established that Aβ1–42 peptides contain two stacked parallel β-sheet structure.37 The formation of ordered β-sheet structures is predominantly a consequence of the formation of highly directional inter-chain hydrogen bonds.38 Therefore, the structure activity relationships of polyphenols have been considered for a steric interference in β-sheet interaction, resulting in not only inhibition but also destabilization of Aβ. Several other known inhibitors supporting this fact, OH substitutors, blocked Aβ aggregation by hydrogen bonding with β-sheet of Aβ.39,40 The number of hydroxyl groups on each side of the molecule is related to the anti-amyloidogenic activity of polyphenol. The more OH groups in the molecular structure, the higher the anti-amyloidogenic activity was shown.41 Previous studies suggested that EGCG could act as anti-amyloidogenic agents by both binding to the Aβ structure and modifying the pathway to form non-toxic oligomers and stabilizes amyloidogenic proteins, preventing them from forming the amorphous aggregates associated with fibril formation.42,43 Since EGC, EC, ECG, and ECGG have 6, 5, 8, and 7 of OH groups as shown in Fig. 1, these aspects support our results that gallated groups showed higher anti-amyloidogenic activity than non-gallated groups on inhibition of Aβ1–42 fibril formation and fibril destabilizing of Aβ1–42 peptides (Figs. 6 and 8). GC, C, GCG, and CG have the same

Figure 8 Effects on Aβ1–42 fibril destabilization by epicatechins and non-epicatechins. Aβ1–42 was incubated at 37°C for 24 hours in 50 mM phosphate buffer prior to the experiment. The resulting Aβ1–42 fibrils were then further incubated at 37°C with 80 μM of each catechin. Values are mean±SD of triplicate samples (n=3).
number of OH groups with each EGC, EC, EGC, and ECG, respectively; thus non-epicatechins also showed similar patterns with the results of epicatechins (Figs. 7 and 8). Furthermore, the number of OH groups is associated with not only anti-amyloidogenic activity but antioxidant activity. It was established that the antioxidant activity related to the chemical structure was found to be reliable on the number of OH or NH₂ groups.⁴⁴ This result means that both antioxidant activity and anti-amyloidogenic activity are closely related. In addition, Ono et al. (2003) reported that chirality of catechins is another factor affecting the anti-amyloidogenic activity. Therefore, it may be reasonable to consider that polyphenols with antioxidant motifs could bind specifically to Aβ, inhibiting Aβ formation or destabilizing preformed Aβ. To our knowledge, there is limited information on the inhibitory effects of whole catechins, bioactive components in HTP_GTE on Aβ₁₋₄₂ as a hall mark of the AD.

In conclusion, the present study demonstrated that sterilized green tea was able to attenuate the generation of ROS in human brain endothelial cell line and be reliable on the number of OH or NH₂ groups.⁴⁴ This result means that both antioxidant activity and anti-amyloidogenic activity are closely related. In addition, Ono et al. (2003) reported that chirality of catechins is another factor affecting the anti-amyloidogenic activity. Therefore, it may be reasonable to consider that polyphenols with antioxidant motifs could bind specifically to Aβ, inhibiting Aβ formation or destabilizing preformed Aβ. To our knowledge, there is limited information on the inhibitory effects of whole catechins, bioactive components in HTP_GTE on Aβ₁₋₄₂ as a hall mark of the AD.

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