Supplementary data

**Dietary supplementation of a high-temperature-processed green tea extract attenuates cognitive impairment in PS2 and Tg2576 mice**

Juewon Kim, Shinichiro Funayama, Naotaka Izuo, Takahiko Shimizu

Methods

Table S1

Figure S1 - S3

**Methods**

**1. HPLC method**

1. Control reagent

1) Standard reagent

GC : (-)-gallocatechin (Wako chem : 072-04761)

EGC : (-)-epigallocatechin (Wako chem : 056-06761)

C : (-)-catechin (Wako chem : 035-18461)

EC : (-)-epicatechin (Wako chem : 059-06751)

EGCG : (-)-epigallocatechin gallate (Wako chem : 056-08961)

GGC : (-)-gallocatechin gallate (Wako chem : 079-04771)

ECG : (-)-epicatechin gallate (Wako chem : 052-06741)

CG : (-)-catechin gallate (Wako chem : 035-18471)

2) Solvent

Trifluoroacetic acid (TFA): HPLC analysis

Acetic acid : HPLC analysis

Acetonitrile : HPLC grade

Methanol : HPLC grade

Water : HPLC grade

3. Standard reagent

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Standard | Standard volume (mg) | Purity (spec) (%) | Amount (mg) | Total volume (mL) | Concentration (g/mL) |
| GC | 10 | 98 | 1 | 20 | 50 |
| EGC | 10 | 98 | 5 | 250 |
| C | 10 | 98 | 1 | 50 |
| EGCG | 50 | 99 | 8 | 400 |
| EC | 10 | 98 | 2 | 100 |
| GCG | 10 | 98 | 5 | 250 |
| ECG | 10 | 98 | 2 | 100 |
| CG | 10 | 98 | 1 | 50 |
| Caffeine | 100 (g) | 98 | 8 | 400 |

4. Analysis

Column : Agilent Zorbax Eclipse XDB C18 HT (2.1 mm x 100 mm, 1.8 m)

Inject volume : 2 μL

Detector: PDA detector (UV 280 nm)

Temperature: 40℃

Move solvent: 0.05% (v/v) Trifluoroacetic acid solution (A), Methanol:acetonitrile (70:30) solution (B)– gradient elution

Table 2. Gradient elution condition

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Time (min) | Flow (mL/min.) | %A | %B | Curve |
| 0.0 | 0.4 | 90 | 10 | 6 |
| 2.0 | 0.4 | 85 | 15 | 6 |
| 3.0 | 0.4 | 85 | 15 | 6 |
| 6.5 | 0.4 | 70 | 30 | 6 |
| 7.0 | 0.4 | 0 | 100 | 6 |
| 8.50 | 0.4 | 0 | 100 | 6 |
| 9.00 | 0.4 | 90 | 10 | 6 |
| 10.0 | 0.4 | 90 | 10 | 6 |

2. HPLC-PDA analysis method

2.1 GC: (-)-gallocatechin

|  |  |  |  |
| --- | --- | --- | --- |
|  |  |  |  |
| Analytical method | Instrument |  | HPLC-PDA (Alliance 2695/2996 PDA, Waters) |
|  | Sample process |  | Liquid extraction |
|  | Calibration range |  | 10 ~ 200 ㎍/mL |
| Method validation | Specificity |  | No interference observed  Retention time : 6.0 min |
|  | Linearity | N = 3 | 10 ~ 200 ㎍/mL  R2 ≥ 0.999 |
|  | System Precision | N = 3, 5 | Intra-day (n = 5) 0.74 ~ 2.13 %  Inter-day (n = 3) 2.53 % |
|  | Repeatability(\*) | N = 3, 6 | Intra-day (n = 3, 6) 0.40 ~ 1.08 %  Inter-day (n = 3) 0.99 % |
|  | Accuracy (Recovery) | N = 3 | 91.7 ~ 98.1 % (CV 0.18 ~ 0.40 %) |
|  | LOD, LOQ | N = 5 | LOQ : 1.47 ㎍/mL  LOQ : 0.48 ㎍/mL |

\* Repeatability

2.2 EGC: (-)-epigallocatechin

|  |  |  |  |
| --- | --- | --- | --- |
|  |  |  |  |
| Analytical method | Instrument |  | HPLC-PDA (Alliance 2695/2996 PDA, Waters) |
|  | Sample process |  | Liquid extraction |
|  | Calibration range |  | 5 ~ 100 ㎍/mL |
| Method validation | Specificity |  | No interference observed  Retention time : 9.9 min |
|  | Linearity | N = 3 | 5 ~ 100 ㎍/mL  R2 ≥ 0.999 |
|  | System Precision | N = 3, 5 | Intra-day (n = 5) 0.62 ~ 1.22 %  Inter-day (n = 3) 1.27 % |
|  | Repeatability | N = 3, 6 | Intra-day (n = 3, 6) 0.29 ~ 1.08 %  Inter-day (n = 3) 1.16 % |

2.3 C: (-)-catechin

|  |  |  |  |
| --- | --- | --- | --- |
|  |  |  |  |
| Analytical method | Instrument |  | HPLC-PDA (Alliance 2695/2996 PDA, Waters) |
|  | Sample process |  | Liquid extraction |
|  | Calibration range |  | 5 ~ 100 ㎍/mL |
| Method validation | Specificity |  | No interference observed  Retention time : 13.5 min |
|  | Linearity | N = 3 | 5 ~ 100 ㎍/mL  R2 ≥ 0.999 |
|  | System Precision | N = 3, 5 | Intra-day (n = 5) 0.47 ~ 0.69 %  Inter-day (n = 3) 0.61 % |
|  | Repeatability | N = 3, 6 | Intra-day (n = 3, 6) 0.04 ~ 2.50 %  Inter-day (n = 3) 1.99 % |
|  | Accuracy (Recovery) | N = 3 | 99.0 ~ 102.9 % (CV 0.10 ~ 0.40 %) |
|  | LOD, LOQ | N = 5 | LOQ : 0.29 ㎍/mL  LOQ : 0.10 ㎍/mL |

2.4 EC: (-)-epicatechin

|  |  |  |  |
| --- | --- | --- | --- |
|  |  |  |  |
| Analytical method | Instrument |  | HPLC-PDA (Alliance 2695/2996 PDA, Waters) |
|  | Sample process |  | Liquid extraction |
|  | Calibration range |  | 5 ~ 100 ㎍/mL |
| Method validation | Specificity |  | No interference observed  Retention time : 22.4 min |
|  | Linearity | N = 3 | 5 ~ 100 ㎍/mL  R2 ≥ 0.999 |
|  | System Precision | N = 3, 5 | Intra-day (n = 5) 0.18 ~ 0.37 %  Inter-day (n = 3) 3.34 % |
|  | Repeatability | N = 3, 6 | Intra-day (n = 3, 6) 1.05 ~ 2.09 %  Inter-day (n = 3) 2.82 % |
|  | Accuracy (Recovery) | N = 3 | 100.1 ~ 103.2 % (CV 0.07 ~ 1.46 %) |
|  | LOD, LOQ | N = 5 | LOQ : 2.38 ㎍/mL  LOD : 0.79 ㎍/mL |

2.5 EGCG: (-)-epigallocatechin gallate

|  |  |  |  |
| --- | --- | --- | --- |
|  |  |  |  |
| Analytical method | Instrument |  | HPLC-PDA (Alliance 2695/2996 PDA, Waters) |
|  | Sample process |  | Liquid extraction |
|  | Calibration range |  | 10 ~ 200 ㎍/mL |
| Method validation | Specificity |  | No interference observed  Retention time : 23.4 min |
|  | Linearity | N = 3 | 10 ~ 200 ㎍/mL  R2 ≥ 0.999 |
|  | System Precision | N = 3, 5 | Intra-day (n = 5) 0.13~ 0.50 %  Inter-day (n = 3) 1.73 % |
|  | Repeatability | N = 3, 6 | Intra-day (n = 3, 6) 0.36 ~ 1.21 %  Inter-day (n = 3) 0.92 % |
|  | Accuracy (Recovery) | N = 3 | 101.9 ~ 108.8 % (CV 0.12 ~ 0.14 %) |
|  | LOD, LOQ | N = 5 | LOQ : 0.29 ㎍/mL  LOQ : 0.89 ㎍/mL |

2.6 GCG: (-)-gallocatechin gallate

|  |  |  |  |
| --- | --- | --- | --- |
|  |  |  |  |
| Analytical method | Instrument |  | HPLC-PDA (Alliance 2695/2996 PDA, Waters) |
|  | Sample process |  | Liquid extraction |
|  | Calibration range |  | 10 ~ 200 ㎍/mL |
| Method validation | Specificity |  | No interference observed  Retention time : 27.3 min |
|  | Linearity | N = 3 | 10 ~ 100 ㎍/mL  R2 ≥ 0.999 |
|  | System Precision | N = 3, 5 | Intra-day (n = 5) 0.22 ~ 0.41 %  Inter-day (n = 3) 1.16 % |
|  | Repeatability | N = 3, 6 | Intra-day (n = 3, 6) 0.44 ~ 1.67 %  Inter-day (n = 3) 1.25 % |
|  | Accuracy(Recovery) | N = 3 | 106.0 ~ 112.0 % (CV 0.08 ~ 0.12 %) |
|  | LOD, LOQ | N = 5 | LOQ : 0.26 ㎍/mL  LOQ : 0.09 ㎍/mL |

2.7 ECG: (-)-epicatechin gallate

|  |  |  |  |
| --- | --- | --- | --- |
|  |  |  |  |
| Analytical method | Instrument |  | HPLC-PDA (Alliance 2695/2996 PDA, Waters) |
|  | Sample process |  | Liquid extraction |
|  | Calibration range |  | 5 ~ 100 ㎍/mL |
| Method validation | Specificity |  | No interference observed  Retention time : 41.2 min |
|  | Linearity | N = 3 | 5 ~ 100 ㎍/mL  R2 ≥ 0.999 |
|  | System Precision | N = 3, 5 | Intra-day (n = 5) 0.37 ~ 0.68 %  Inter-day (n = 3) 0.79 % |
|  | Repeatability | N = 3, 6 | Intra-day (n = 3, 6) 0.29 ~ 1.49 %  Inter-day (n = 3) 1.22 % |
|  | Accuracy (Recovery) | N = 3 | 102.9 ~ 105.0 % (CV 0.29 ~ 0.35 %) |
|  | LOD, LOQ | N = 5 | LOQ : 0.58 ㎍/mL  LOQ : 0.19 ㎍/mL |

2.8 GC: (-)-catechin gallate

|  |  |  |  |
| --- | --- | --- | --- |
|  |  |  |  |
| Analytical method | Instrument |  | HPLC-PDA (Alliance 2695/2996 PDA, Waters) |
|  | Sample process |  | Liquid extraction |
|  | Calibration range |  | 5 ~ 100 ㎍/mL |
| Method validation | Specificity |  | No interference observed  Retention time : 43.7 min |
|  | Linearity | N=3 | 5 ~ 100 ㎍/mL  R2 ≥ 0.999 |
|  | System Precision | N = 3, 5 | Intra-day (n = 5) 0.15 ~ 0.78 %  Inter-day (n=3) 0.90 % |
|  | Repeatability | N = 3, 6 | Intra-day (n = 3, 6) 0.64 ~ 1.82 %  Inter-day (n = 3) 2.01 % |
|  | Accuracy (Recovery) | N = 3 | 98.1 ~ 101.9 % (CV 0.11 ~ 0.95 %) |
|  | LOD, LOQ | N = 5 | LOQ : 0.85 ㎍/mL  LOQ : 0.27 ㎍/mL |

Table S1. The amount of GCG after HTP-GTE administration

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Time (h)** | **Dose** | **Plasma (ng/mL)** | **Brain tissue(ng/g)** | **T/P ratio** |
| **0.5** | **1 g/kg** | **201.3 ± 190.1** | **5.80 ± 0.48** | **0.046 ± 0.027** |
| **1** | **1 g/kg** | **90.1 ± 17.0** | **5.70 ± 0.41** | **0.066 ± 0.015** |

The amount of GCG in plasma or brain tissue was evaluated on 0.5 and 1 h after oral treatment of HTP-GTE (1 g/kg) by HPLC analysis. We used ethyl acetate as extraction buffer because of solubility of catechin component. T/P ratio means tissue to plasma ratio of GCG. Data represent means ± S.D. The experiments performed three times independently.

a

**d:\Users\ap493069\Desktop\Figure S1A.tif**

b

d:\Users\ap493069\Desktop\Figure S1B.tif

Figure S1. The HPLC data of catechin component. (a) GTE and (b) HTP-GTE were depicted.

a

d:\Users\ap493069\Desktop\AD_GTE\Submission\Figure. S3b.tif

b

d:\Users\ap493069\Desktop\Fig.S2b.tif

Figure S2. The effects of HTP-GTE on anxiety-related behavior and locomotive activity. (a) Open arm ratio (time spent in open field/total) was observed. (b) Locomotive activity of mice was evaluated in 7 consecutive days. Data are represented as means with average of experimental values. n = 6.

a

b

Figure S3. The pharmacokinetics of 100 mg/kg EGCG (a) or GCG (b) was estimated for 4 h. The concentrations of EGCG or GCG in mice blood sample were evaluated. The AUC (area under the plasma level-time curve) was 113.4 ng⋅hr/ml for EGCG and 865.7 ng⋅hr/ml for GCG, respectively. C max (maximum plasma concentration) was 186.6 ng/ml for EGCG and 314.5 ng/ml for GCG, respectively. Data are depicted as means ± S.D.

a b

d:\Users\ap493069\Desktop\AD_GTE\Submission\Revision\GCG.tif d:\Users\ap493069\Desktop\AD_GTE\Submission\Revision\EGCG.tif

Figure S4. The chemical structure of GCG (a) and EGCG (b) was represented.