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Original Research Article

Evaluation of protective effect of cyclodextrin glucanotransferase-treated *Gastrodia elata* Blume extract on ultraviolet B-induced premature skin aging

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Abstract

Purpose: *To investigate the protective effect of Gastrodia elata Blume (G. elata, GE) and cyclodextrin glucanotransferase (CGTase) enzyme-treated G. elata extract (EGE) against premature skin aging using ultraviolet B (UVB)-exposed normal human dermal fibroblasts (NHDFs).*

Methods: *The extract was characterized by liquid chromatography with tandem mass spectrometry (LC-MS/MS), ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC–QToF–MS) and nuclear magnetic resonance spectroscopy (NMR). The expression of matrix metalloproteinases (MMP-1,3), interleukin-6 (IL-6), transforming growth factor (TGF-β1) and procollagen type I was assayed using ELISA kits. Safety evaluation of EGE's dietary administration and topical application was performed by in vivo acute oral toxicity and local lymph node tests.*

Results: *Lower MMP-1 and IL-6 and higher procollagen type I and TGF-β1 levels were observed after treatment with EGE than with GE, indicating that EGE was more effective than GE in treating UVB-induced photoaging. With respect to phenolic composition, EGE had lower 4-hydroxybenzaldehyde (4-HBA) level and higher α-gastrodin level than GE. In UVB-irradiated NHDFs, α-gastrodin exhibited higher anti-aging activity than 4-HBA and β-gastrodin based on the expression of MMP-1, MMP-3, and procollagen type I. The in vivo data indicate that EGE was safe at concentrations of up to 2000 mg/kg for dietary administration and 0.1 % for topical application.*

Conclusion: *EGE protects UVB-induced photoaged human skin better than GE owing to its higher α-gastrodin content. Thus, EGE may be potentially useful agent in anti-aging cosmetic products.*

Keywords: *Gastrodia elata, α-Gastrodin, Anti-aging, CGTase, Ultraviolet B (UVB) irradiation, Matrix metalloproteinase, Procollagen, Normal human dermal fibroblasts*

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INTRODUCTION

Gastrodia elata Blume (Tianma) is a perennial herb in the *Orchidaceae* family used since a long time in traditional Chinese medicine to treat headaches, dizziness, paralysis and epilepsy [1]. Tianma exerts anti-angiogenic, anti-inflammatory, anticancer, antidiabetic,

antihypertensive, and neuroprotective effects [2-6]. These activities of *G. elata* were reported to be influenced by its high content of phenols [7].

Extrinsic skin aging was recently reported to be a result of exposure to UVB (280-320 nm). UVB can reduce skin collagens, which are the main components of extracellular matrix (ECM). Matrix metalloproteinases (MMPs) are increased in aged human skin to cleave collagen and other ECM proteins [8]. Additionally, transforming growth factor-β (TGF-β) has an important role in ECM biosynthesis [9]. Pro-inflammatory factors, such as interleukins (ILs), are also elevated and the oxidant-antioxidant cellular homeostasis is disturbed upon UVB exposure [10]. Thus, regulation of the expression of these factors is crucial to control extrinsic skin aging.

In the present study, it was hypothesized that the enzyme-treated ethanolic *G. elata* Blume extract (EGE) would enhance the synthesis of procollagen type I in exposed normal human dermal fibroblasts (NHDFs). Although phenolic compounds possess low toxicity, some plant extracts can irritate the skin by causing skin allergies. Based on this hypothesis, we investigated the anti-aging effect of EGE *in vitro* and its oral and topical safety *in vivo*.

EXPERIMENTAL

Plant material and extraction methods

The dried rhizomes of *G. elata* Blume (GE) were purchased from The Best Herb Company (Seoul, Republic of Korea). The dried extracts were prepared with five different concentrations of solvent (95 % EtOH, 75 % EtOH, 50 % EtOH, 25 % EtOH, and 100 % water) and extracted for 1, 3 or 6 h.

Preparation of EGE

Industrial grade cyclodextrin glucanotransferase (CGTase, EC 2.4.1.19) from *Bacillus stearothermophilus* and glucoamylase from *Rhizopus nevius* were purchased from Bision Corporation (Sungnam, Gyeonggi, Korea). A biotransformation mixture containing 100 mL of GE extract, a range of CGTase concentrations (1125 U, 2250 U, and 3375 U), 10 mM of $CaCl_2$, and 0.1 M of phosphate buffer (pH 6.0) was incubated for 24 or 48 h at 50 °C. The reaction was stopped by boiling in water for 10 min [11]. Finally, reaction products were continued using glucoamylase (40, 80, and 120 U) at pH 5.0 and 50 °C for 8 h.

Measurement of CGTase and glucoamylase activities

CGTase (or glucoamylase) and 0.3% soluble starch were dissolved at 40°C in a 20 mM acetate buffer (pH 5.5) containing 1 mM $CaC1_2$ for 10 min [11]. One unit of enzyme activity was defined as the amount of enzyme catalyzing 10% decrease in absorbance per minute under these conditions [12].

Standardized spectroscopic characterization of α-gastrodin

LC-MS/MS was performed using the Dionex Chromeleon™ Chromatography Data System (Thermo Fisher Scientific Korea Ltd., Seoul, Korea) with P580 and UVD100 detectors. For UPLC (Acquity UPLC I CLASS, Waters), the Luna Omega Polar column (150 × 2.1 mm, 3 µm particle size) was used. For QToF–MS (Vion IMS QToF, Waters), the mass spectrometer was operated in the ESI negative ion mode. NMR spectra were recorded at 25 °C on a Varian Unity Inova AS - 400 FT - NMR spectrometer (1H: 400 MHz, 13C: 100 MHz).

Cell culture, UVB irradiation and sample treatment

NHDFs were purchased from ScienCell Research Laboratories, Inc. (Carlsbad, CA, USA). Cells were seeded at 2 × 10^4 cells/cm^2 density for 24 h and then exposed to UVB (144 mJ/cm^2) radiation as described previously [13]. After UVB irradiation, cells were immediately incubated in serum-free medium with or without sample treatment. Normal cells were cultured in the same incubator conditions without UVB exposure or sample treatment.

MTT assay

To measure cell viability, 1 mg/mL MTT was added into cell culture dishes after 72 h of treatment with UVB and samples. After 2 h incubation with MTT, cell supernatants were discarded and 800 µL of DMSO (98% purity) was added to dissolve the formazan crystals. After gentle shaking for 10 min, the absorbance was measured at 570 nm h on a microplate reader (Molecular Devices FilterMax5, USA).

Measurement of secreted proteins

The concentrations of MMP-1, MMP-3, procollagen type I, IL-6, and TGF-β1 were determined in the cell culture supernatants after 72 h of sample treatment using ELISA kits according to the kit-specific instructions.

In vivo acute oral toxicity assay

The assay was conducted according to the OECD Guideline TG 423 (OECD, 2001). Female Sprague-Dawley (SD) rats (7-weeks-old; 56-62 g; n = 12) were purchased from Samtako Bio Korea Co. Ltd. (Gyeonggi-do, Korea). For toxicity analysis of EGE, SD rats were randomly divided into 4 groups with 3 rats each: 1) EGE5, 2) EGE50, 3) EGE300, and 4) EGE2000, which were provided a diet containing 5, 50, 300, and 2000 mg/kg of EGE. No experiment was carried out during adaptation (first week). The experimental protocol was approved by the Institutional Ethical Committee on Animal and Use Committee of Kyung Hee University (no. KHU ASP (SU)-12-09) and conducting according to Good Laboratory Practice (GLP) and conducted from 2017 July 30th to September 18th at ChemOn Inc. (Gyeonggi-do, Korea) [14].

Local lymph node assay (LLNA)

The assay was conducted according to OECD Guideline 442B (OECD, 2010). Twenty-five female BALB/c mice (7-weeks-old; 17-20 g) were purchased from Central Lab. Animal Inc. (Seoul, Korea). No experiment was carried out during adaptation (first week). EGE was suspended in acetone: olive oil (4:1, v/v) and applied to the ear skin of the mice. As a positive control, 25% hexylcinnamaldehyde (HCA) in the same delivery vehicle was used. Concentrations of 0.05, 0.1, and 0.5% (v/v) of EGE extract were chosen for the 5 groups (n = 5) for the LLNA study.

Ears of each mouse were observed for erythema and scored using the OECD 442B criteria. On the 1st, 3rd, and 6th day, ear thickness was measured with an electronic digital caliper (Mitutoyo Korea Corporation). The experimental protocol was conducted according to GLP and conducted from 2017 July 20th to September 4th at ChemOn Inc. (Gyeonggi-do, Korea) [15].

Lymphocyte proliferation

A BrdU ELISA colorimetric kit (Roche, Germany) was used to evaluate lymphocyte proliferation. The stimulation index (SI) was calculated by dividing the mean BrdU labeling index/mouse in each test substance group and the positive control group by the mean BrdU labeling index for the solvent group. The BrdU labeling index is defined by calculating absorbance (ABS) values as: (ABS_{370} - $ABS_{blank370}$) - (ABS_{492} - $ABS_{blank492}$). The test result is considered positive when SI ≥ 1.6 [15].

Statistical analysis

Tukey's test was performed to compare the difference between treatments using one-way analysis of variance. Statistical significance was set at $p < 0.05$ for *in vitro* and *in vivo* data.

RESULTS

α–Gastrodin content of EGE

Among the 5 different concentrations of ethanol used and the 3 different duration of extraction, the 50% ethanolic extract processed after 3 h was identified as the most efficient based on its highest content of 4-HBA. The 4-HBA content of GE all extracts is shown in Table 1. Among 18 methods of enzyme modification, the 24-h treatment of CGTase (2250 U/100 mL) and glucoamylase (80 U/100 mL) gave the most active EGE, which contained 22.64 (mAU*min in peak area) of α-gastrodin, a targeted biotransformation product (Table 2, Figure 1). The biotransformed compound was identified as α-gastrodin by NMR analysis (Figure 2). Three batches were thereafter processed on a larger scale as shown in Figure 3. In 15 kg of crude GE, extraction yield was 0.920±0.030 g with 0.19% of 4-HBA. Finally, EGE containing 0.42% of α-gastrodin ($C_{13}H_{18}O_7$, 285.10636 MW) was selected for the skin anti-aging tests.

Effects of GE and EGE on MMP-1, procollagen type I, IL-6, and TGF-β1 secretion from UVB-irradiated NHDFs

No toxicity was observed in the control group as well as in the experiment group treated with GE and EGE. After UVB exposure, MMP-1 and IL-6 production was significantly higher while procollagen type I and TGF-β1 expression was lower than that in the normal group. However, treatment with GE and EGE reversed these responses by fibroblasts. EGE at 10 and 100 μg/mL significantly reduced MMP-1 production by 36.17 and 58.92 % as shown in Figure 4. GE treatment at 10 and 100 μg/mL reduced MMP-1 production by 25.07 and 35.60 %. In 100 μg/mL EGE-treated cells, procollagen type I and TGF-β1 levels increased by 197.80 and 216.82 %, respectively.

Effect of EGE's active components on MMP-1, MMP-3, and procollagen type I production

The 3 main components 4-HBA, α-gastrodin, and β-gastrodin were investigated. The compounds had no toxicity for NHDFs before or after UVB irradiation. At 50 μg/mL, α-gastrodin significantly suppressed MMP-1 and MMP-3 expression by

96.19 % and 74.14 %, respectively (Fig. 5). Procollagen type I level was higher following α-gastrodin treatment than after 4-HBA or β-gastrodin treatment.

Table 1: GE and its content of 4-HBA

Extraction condition	Extract yield (g)/10 g crude	4-HBA content (mg)
Type of solvent		
95% EtOH	0.412 ± 0.017	10.516 ± 0.870
75% EtOH	1.044 ± 0.038	12.248 ± 0.539
50% EtOH	1.233 ± 0.107	14.605 ± 1.204
25% EtOH	0.765 ± 0.017	6.231 ± 0.442
100% distilled water	0.408 ± 0.018	2.605 ± 0.061
Extraction time		
1 h	0.987 ± 0.017	1.157 ± 0.005
3 h	1.527 ± 0.057	1.858 ± 0.078
6 h	1.628 ± 0.072	1.956 ± 0.103

Table 2: Large-scale extraction of EGE and its content of α-gastrodin*

CGTase (2250 U/100 mL)	Glucoamylase (80 U/100 mL)	CG Tase treatment time	
		24 h	***48 h***
50%	50%	12.917 ± 0.021	15.550 ± 0.065
	100%	15.593 ± 0.038	14.542 ± 0.079
	150%	18.223 ± 0.028	15.645 ± 0.039
100%	50%	20.410 ± 0.022	23.389 ± 0.014
	100%	22.643 ± 0.041	20.672 ± 0.037
	150%	22.922 ± 0.026	20.123 ± 0.062
150%	50%	17.361 ± 0.052	19.702 ± 0.033
	100%	19.688 ± 0.083	19.821 ± 0.059
	150%	18.647 ± 0.063	19.075 ± 0.012

*The unit of peak area of α-gastrodin in EGE extracts was mAU*min

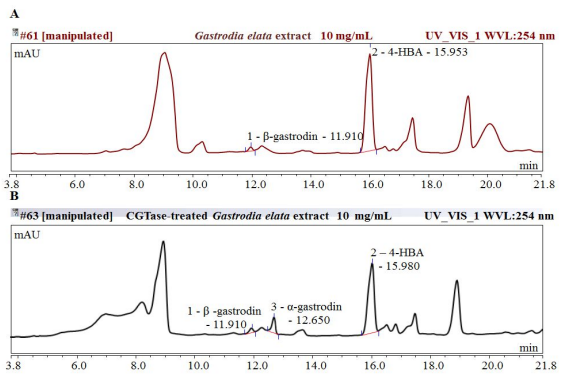

Figure 1: Analytical profile of the components of GE and EGE. A: *Gastrodia elata* extract - GE containing β-gastrodin and 4-HBA. B: Enzyme CTGase-treated *Gastrodia elata* extract - EGE containing β-gastrodin, α-gastrodin, and 4-HBA

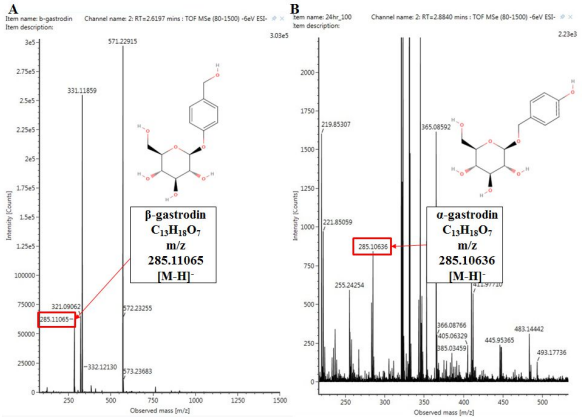

Figure 2: Characteristics of α-gastrodin (A) and β-gastrodin (B)

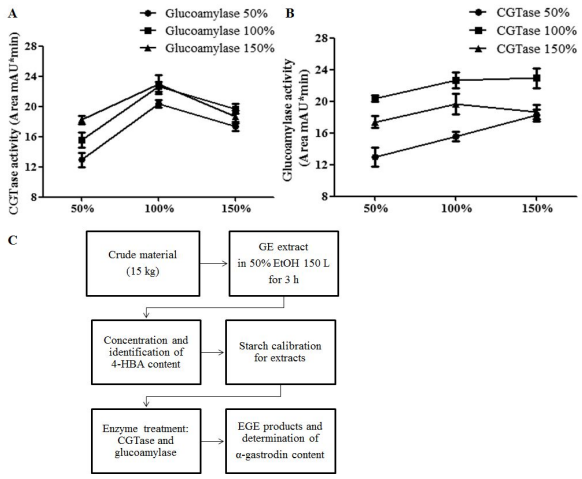

Figure 3: Biotransformation process of GE to EGE. A, B: Enzyme activity of CGTase and glucoamylase; C: Biotransformation process diagram of GE to EGE

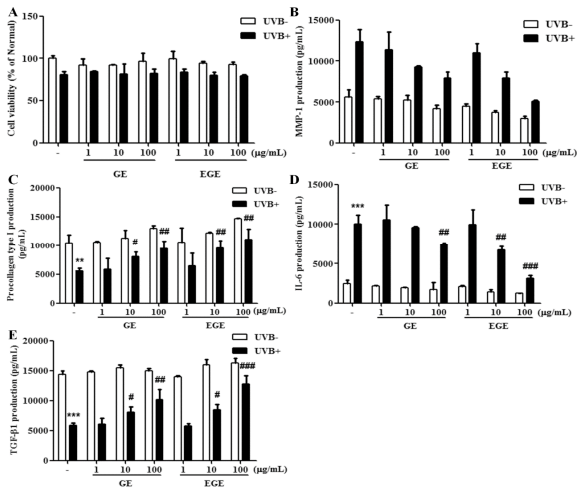

Figure 4: Effect of GE and EGE on UVB-irradiated NHDF viability (A), on MMP-1 (B), procollagen type I (C), IL-6 (D), and TGF-β1 (E) production

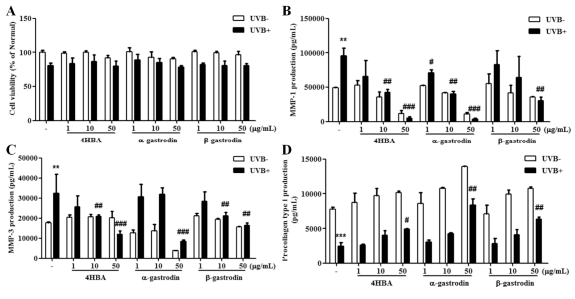

Figure 5: Effects of 4-HBA, α-gastrodin, and β-gastrodin on the viability of UVB-irradiated NHDFs (A), and levels of MMP-1 (B), MMP-3 (C), and procollagen type I (D) in these cells

Acute oral toxicity of EGE

Upon oral administration of EGE, SD rats did not exhibit any signs of toxicity in the skin, hair, eyes, mucous membranes, respiratory, circulatory, and autonomic, and central nervous systems, or abnormalities in behavior patterns. A negligible increase was observed after 15 days approximately 16 - 18 g/rat for all groups (Table 3). No death was observed in any of the groups (even at a dose of 2000 mg/kg); therefore, EGE may be considered to be safe for oral administration.

Table 3: Body weight of SD rats

D#	Group (mg/kg)			
	G1 (300)	*G2 (300)*	*G3 (2000)*	*G4 (2000)*
1	169.43 ± 12.12	173.85 ± 11.47	180.77 ± 13.58	187.59 ± 4.69
2	187.66 ± 12.34	193.22 ± 14.65	199.26 ± 17.90	206.52 ± 3.57
4	192.66 ± 15.83	200.80 ± 11.98	207.42 ± 16.10	215.84 ± 5.71
8	205.37 ± 18.39	210.70 ± 13.88	216.56 ± 16.51	227.33 ± 4.65
15	224.19 ± 21.95	223.77 ± 17.84	232.17 ± 19.44	238.01 ± 6.35
Gain*	54.76 ± 10.60	49.92 ± 6.47	51.40 ± 5.91	50.42 ± 8.92
N	3	3	3	3

The first day of administration is designated as Day 1.All values are presented as mean ± SD (g); # Day; * Body weight gained after 15 days

In vivo skin sensitization potential of EGE

Ear thickness, erythema index, and individual animal observations indicated that only one mouse in the 0.5% EGE treatment condition had local dermal irritation. The BrdU labeling indexes of the normal and 0.05% EGE groups were not significantly different (Table 4, Table 5, Table 6, and Table 7). The SI value of the positive control was 2.54. Treatment with EGE resulted in a dose-dependent increased in lymphocyte proliferation. However, SI values of EGE-treated mice ears were all below 1.6. Based on these SI values, EGE was not considered as a dermal sensitizer in the LLNA.

DISCUSSION

It is increasingly accepted that the effects of UVB on the skin can be modeled *in vitro* using UVB-irradiated NHDFs. In the present study, EGE exhibited a greater anti-aging activity than GE in UVB-irradiated NHDFs. EGE regulated MMP-1 and procollagen type I production via IL-6 and TGF-β1 expression. The higher content of α-gastrodin contributed to the higher activity of EGE than that of GE. Both dietary and topical application of EGE was recognized as safe at concentrations of up to 2000 mg/kg and 0.1% (w/v), respectively.

Table 4: Ear and absolute auricular lymph node weights (N = 5)

Dose (%)	Ear (g)	Auricular lymph node (g)
0	0.0158±0.0005	0.0034±0.0012[a)]
0.05	0.0154±0.0009	0.0050±0.0009
0.1	0.0152±0.0008	0.0058±0.0012
0.5	0.0195±0.0017*	0.0176±0.0028**
HCA 25	0.0169±0.0006*	0.0088±0.0010**

Data are expressed as mean ±SD. (*p < 0.05; **p < 0.01)

Table 5: Ear thickness (mm, N = 5)

Dose (%)	Ear thickness					
	Day 1		*Day 3*		*Day 6*	
	Left	*Right*	*Left*	*Right*	*Left*	*Right*
0	0.169 ± 0.003	0.166 ± 0.009	0.167 ± 0.004	0.166 ± 0.005	0.160 ± 0.003	0.162 ± 0.005
0.05	0.168 ± 0.004	0.166 ± 0.008	0.168 ± 0.003	0.166 ± 0.007	0.160 ± 0.002	0.161 ± 0.005
0.1	0.167 ± 0.008	0.166 ± 0.004	0.167 ± 0.004	0.165 ± 0.005	0.161 ± 0.006	0.161 ± 0.002
0.5	0.168 ± 0.008	0.165 ± 0.005	0.167 ± 0.003	0.166 ± 0.005	0.160 ± 0.003	0.159 ± 0.005
HCA 25	0.163 ± 0.006	0.168 ± 0.005	0.169 ± 0.006	0.167 ± 0.004	0.164 ± 0.004	0.161 ± 0.003

Table 6: Erythema scores (N = 5)

Dose (%)	Erythema score	
	Left	*Right*
0	0.0±0.00	0.0±0.00
0.05	0.0±0.00	0.0±0.00
0.1	0.0±0.00	0.0±0.00
0.5	0.3±0.55	0.3±0.55
HCA 25	0.0±0.00	0.0±0.00

Data are expressed as mean ± SD

Table 7: Cell proliferation in auricular lymph node (N = 5)

Dose (%)	BrdU labeling index (mean)	SI	Evaluation [a)]
0	0.124 ± 0.001	1.00	-
0.05	0.127 ± 0.003	1.03	+
0.1	0.145 ± 0.001	1.17	+
0.5	0.179 ± 0.002	1.45	+
HCA 25	0.315 ± 0.004*	2.54	+

Test article: EGE; Vehicle: 4:1 acetone/olive oil. [a)] Only one lymph node in an animal was evaluated due to organ loss; Data are expressed as mean ± SD (*p < 0.05)

4-HBA and β-gastrodin are the main phenolic constituents of GE [16]. They reduce oxidative stress by upregulating Nrf2 [17,18]. β-Gastrodin downregulated IL-6 and MAPKs [6]. Sun *et al.* [19] and Hwang *et al.* [20] indicated that an anti-aging candidate that promoted Nrf2/ARE signaling and reduced IL-6 and MAPKs could inhibit the expression of MMPs and increase procollagen type I. MMP-1 and procollagen type I are important markers in photoaging. Thus, we hypothesized that GE and EGE containing 4-HBA and β-gastrodin could have an effect on the secretion of procollagen type I. In this study, EGE was found to contain 4-HBA, β-gastrodin, and α-gastrodin. Both GE and EGE, as well as their individual compounds decreased the production of MMPs and increased the production of procollagen type I. Among the 3 main compounds studied, the anti-aging activity of *α*-gastrodin was demonstrated to be the best. EGE possibly possessed a greater anti-aging activity than GE because of its higher α-gastrodin content.

CGTase (EC 2.4.1.19) from *B. stearothermophilus* is an *α*-amylase which is generally recognized as safe (GRAS) and is widely used in the food, pharmaceutical, chemical and cosmetic industries [21]. α-Gastrodin-enriched EGE possessed high anti-aging and no significant toxic activities in UVB-irradiated NHDFs as well as in SD rats and BALB/c mice. The results also showed that EGE is safe for ingestion as well as topical use (at < 2000 mg/kg and <0.1 %w/v, respectively).

CONCLUSION

The results suggest that UV-induced premature skin aging may be regulated by treatment with GE and EGE. This study allows us to better appreciate biotransformed functional foods or cosmetics with tested safety. GE and EGE containing *α*-gastrodin, β-gastrodin, and 4-HBA show protective effects in photoaged fibroblasts *in vitro* as well as in rats and mice *in vivo*. Follow-up research is required to determine the anti-aging or anti-wrinkle activities of EGE in artificial human skin models and in clinical trials.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of Authors

We declare that this work was done by all authors mentioned in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. DrS Yi and Hwang conceived and designed the study. DrS Ngo, Yang and Park collected and analyzed the data. All authors wrote, read and approved the manuscript for publication must be specified.

REFERENCES

1. *Xiao F, Yan B, Chen L, Zhou D. Review of the use of botanicals for epilepsy in complementary medical systems Traditional Chinese Medicine. Epilepsy Behav 2015; 52: 281-289.*
2. *Ahn EK, Jeon HJ, Lim EJ, Jung HJ, Park EH. Anti-inflammatory and anti-angiogenic activities of Gastrodia elata Blume. J Ethnopharmacol 2007; 110: 476-482.*
3. *Heo JC, Woo SU, Son M, Par Y, Choi WS, Chang KT, Kim SU, Yoon EK, Kim YH, Shin HM, et al. Anti-tumor activity of Gastrodia elata Blume is closely associated with a GTP-Ras-dependent pathway. Oncol Rep 2007; 18: 849-853.*
4. *Yang HJ, Kim MJ, Kwon DY, Kim DS, Lee YH, Kim JE, Park S. Anti-diabetic activities of gastrodia elata blume water extracts are mediated mainly by potentiating glucose-stimulated insulin secretion and increasing β-cell mass in non-obese type 2 diabetic animals. Nutrients 2016; 8: 1-15.*

5. Kho MC, Lee YJ, Cha JD, Choi KM, Kang DG, Lee HS 2014. Gastrodia elata ameliorates high-fructose diet-induced lipid metabolism and endothelial dysfunction. Evid Based Complement Alternat Med 2014; 2014: 1-10.
6. Kim SY, Back HI, Park SH, Park EO, Oh MR, Kim MG, So BO, Chae SW, Lee GH, Kim DS, et al. Neuroprotective effect of Gastrodia elata Blume ethanol extracts (GEB) against beta-amyloid-induced BV2 cell death: Gastrodin and 4-hydroxybenzaldehyde (4-HBA) are the bio active candidates. FASEB J 2011; 25: 298-298.
7. Kim MJ, Yang HJ, Moon BR, Kim JE, Kim KS, Park S. Gastrodia elata Blume Rhizome Aqueous Extract Improves Arterial Thrombosis, Dyslipidemia, and Insulin Response in Testosterone-Deficient Rats. Evid Based Complement Alternat Med 2017; 2017: 1-11.
8. Quan T, Fisher GJ. Role of age-associated alterations of the dermal extracellular matrix microenvironment in human skin aging: a mini-review. Gerontology 2015; 61: 427-434.
9. Jabłonska-Trypuc A, Matejczyk M, Rosochacki S. Matrix metalloproteinases (MMPs), the main extracellular matrix (ECM) enzymes in collagen degradation, as a target for anticancer drugs. J Enzyme Inhib Med Chem 2016; 31: 177-183.
10. Nishimura N, Tohyama C, Satoh M, Nishimura H, Reeve VE. Defective immune response and severe skin damage following UVB irradiation in interleukin-6-deficient mice. Immunology 1999; 97: 77-83.
11. Suzuki Y, Suzuki K. Enzymatic formation of 4G-α-D-glucopyranosyl-rutin. Agric Biol Chem 1991; 55: 181-187.
12. Qi Q, She X, Endo T, Zimmermann W. Effect of the reaction temperature on the transglycosylation reactions catalyzed by the cyclodextrin glucanotransferase from Bacillus macerans for the synthesis of large-ring cyclodextrins. Tetrahedron 2004; 60: 799-806.
13. Hwang E, Park SY, Lee HJ, Lee TY, Sun ZW, Yi TH. Gallic acid regulates skin photoaging in UVB-exposed fibroblast and hairless mice. Phytother Res 2014; 28: 1778-1788.
14. OECD/OCDE TG 423 [Internet]. OECD GUIDELINE FOR TESTING OF CHEMICALS: Acute Oral Toxicity - Acute Toxic Class Method [cited 2001 December]. Available from: https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl423.pdf
15. OECD/OCDE 442B [Internet]. OECD GUIDELINE FOR TESTING OF CHEMICALS: Skin Sensitization: Local Lymph Node Assay: BrdU-ELISA [cited 2010 July]. Available from: https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd-tg442b.pdf
16. Huang WY, Zhang HC, Liu WX, Li CY. Survey of antioxidant capacity and phenolic composition of blueberry, blackberry, and strawberry in Nanjing. J Zhejiang Univ Sci B 2012; 13: 94-102.
17. Zhang Z, Zhou J, Song D, Sun Y, Liao C, Jiang X. Gastrodin protects against LPS-induced acute lung injury by activating Nrf2 signaling pathway. Oncotarget 2017; 8: 32147–32156.
18. Kam KY, Yu SJ, Jeong N, Hong JH, Jalin AMA, Lee S, Choi YW, Lee CK, Kang SG. p-Hydroxybenzyl alcohol prevents brain injury and behavioral impairment by activating Nrf2, PDI, and neurotrophic factor genes in a rat model of brain ischemia. Mol Cells 2011; 31: 209-215.
19. Sun Z, Park SY, Hwang E, Zhang M, Seo SA, Lin P, Yi TH. Thymus vulgaris alleviates UVB irradiation induced skin damage via inhibition of MAPK/AP-1 and activation of Nrf2-ARE antioxidant system. J Cell Mol Med 2017; 21: 336-348.
20. Hwang E, Ngo HT, Seo SA, Park B, Zhang M, Yi TH. Protective effect of dietary Alchemilla mollis on UVB-irradiated premature skin aging through regulation of transcription factor NFATc1 and Nrf2/ARE pathways. Phytomedicine 2018; 39: 125-136.
21. Leemhuis H, Kragh KM, Dijkstra BW, Dijkhuizen L. Engineering cyclodextrin glycosyltransferase into a starch hydrolase with a high exo-specificity. J Biotechnol 2003; 103: 203-212.