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Inhibitory Effects of Fermented *Gastrodia elata* on High Glucose-induced NO and IL-8 Production in Human Umbilical Vein Endothelial Cells

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Abstract – Hyperglycemia or high glucose (HG), is the hallmark of diabetes, known to induce oxidative stress, release of chemokines, and cytokines, which confer endothelial cell damage. On the other hand, microbial transformation of organic materials often leads to certain changes in their product structures which could enhance their biological activities. The aim of this study was to investigate the beneficial effects of fermented *Gastrodia elata* (FGE) in HG induced human umbilical vein endothelial cells (HUVECs) dysfunction. GE, fermented by *Saccharomyces cerevisiae*, which has an extensive history of safe use, exhibited higher phenolic compounds content than those of *Gastrodia elata* (GE). The HG-induced production of nitric oxide (NO) and interleukin-8 (IL-8) were significantly attenuated by FGE pretreatment to the cells, in a concentration dependent manner. In addition, FGE showed marked activity in free radical scavenging. These results suggest that FGE possesses beneficial effects in protecting against the oxidative stress, and inflammatory conditions in endothelial cells, caused by HG.
Keywords – *Gastrodia elata*, Fermentation, Human umbilical vein endothelial cells, High glucose, Nitric oxide, Interleukin-8

Introduction

In comparison to the general population, diabetic patients show increased risk for cardiovascular death. The global burden of diabetes is estimated to be 346 million cases, and the death-rates are expected to double by 2030 (WHO, 2011), indicating that new therapeutic tools to prevent and treat the disease is crucial. Hyperglycemia, generation of free radicals, glycation and oxidization of lipoproteins, abnormality in lipoprotein structure, and hypercoagulability are regarded as the major complications of diabetes. Among them, hyperglycemia or high glucose (HG) is the hallmark of diabetes, known to induce oxidative stress, release of chemokines, and cytokines, which confer endothelial cell damage (Betsy, 2008).

Several studies have demonstrated that HG induces the formation of nitric oxide (NO) (Liu *et al*., 2011), and chemo-attractant cytokine interleukin (IL)-8 (Temaru *et al*., 1997), in human umbilical venous endothelial cells (HUVECs). NO is a well recognized cytotoxic mediator of inflammatory responses (Xu *et al*., 2007). NO also have been known to react with hyperglycemia-induced reactive oxygen species (ROS) to yield peroxynitrite, a strong oxidant, which, in turn, contributes to endothelial dysfunction (Yamagishi and Matsui, 2011). Furthermore, it is reported that hyperglycemia might contribute the overproduction of ROS, which lead to vascular complications (Kuroki *et al*., 2003). ROS can change endothelial function, such as membrane lipids peroxidation, NF-κB activation, and reducing the availability of nitric oxide (NO) (Madamanchi *et al*., 2005). Therefore management of ROS production have recently regarded as a promising therapy to prevent endothelial dysfunction.

IL-8, a member of CXC chemokine family, also is found to be associated with atheroscleresosis and coronary heart disease (Moreau *et al*., 1999; Romuk *et al*., 2002). One study reported that serum levels of IL-8 were higher in diabetic patients as compared with the non-diabetic

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patients (Esposito *et al*., 2003). Moreover, IL-8 receptor knockout mice showed reduced susceptibility to atherosclerosis than in wild-type mice (Boisvert *et al*., 1998). IL-8 has been shown to promote migration of neurophils and T cells into the sub-endothelial layer, and trigger adhesion of monocytes to the endothelium (Gerszten *et al*., 1999).

Gastrodia elata Blume (GE), the tuber of orchid, has been used as a traditional medicine showing anticonvulsant, antiangiogenic, and analgesic activities in Korea, China, and Japan (Zeng *et al*., 2006). GE contains several phenolic compounds such as gastrodin, p-hydroxybenzyl alcohol (HBA), p-hydroxybenzaldehyde (HBZ) and p-hydroxy-3-methoxybenzaldehyde (Hwang *et al*., 2009). It has been reported that GE reduces lipid peroxide levels, and has free radical scavenging activities in rats (Hiesh *et al*., 2001). It also suppressed tumor necrosis factor (TNF)-α-induced vascular inflammatory processes via inhibition of oxidative stress and NF-κB activation, in HUVECs (Ha *et al*., 2001). These inhibitory activities have been similarly revealed by phenolic compounds which are present in GE. Gastrodin has a neuroprotective action against hypoxia via reduction in the glutamate levels in cortical neurons (Xiang *et al*., 2007). HBA has been shown to have protective role against oxidative damage-related diseases such as ischemic brain injury and coronary heart disease (Yu *et al*., 2005). Moreover, HBA isolated from GE exhibits anti-asthmatic activities by inhibiting the intraluminal accumulation of inflammatory cells, particularly eosinophils and neutrophils (Jang *et al*., 2010).

Several studies have shown that deglycated compounds are highly effective in *in vivo* physiological actions. To achieve this, various transformation methods have been utilized, including mild acid hydrolysis (Han *et al*., 1982), enzymatic conversion (Ko *et al*., 2003), and microbial conversion (Bae *et al*., 2002). However, the chemical methods of transformation show limitations associated with side reactions such as epimerization, hydration, and hydroxylation, and are also known to cause environmental pollution (Park *et al*., 2010).

In this study, we performed the fermentation of GE (FGE) by *Saccharomyces cerevisiae,* which is classified as Generally Regarded As Safe (GRAS) by the U.S Food and Drug Administration (Nevoigt, 2008), and investigated the bioactivity of FGE against HG-induced oxidative stress and inflammatory response in HUVECs.

Experimental

Materials – Human umbilical vein endothelial cells (HUVECs) were purchased from the ATCC Global Bioresource Center (Manassas, VA). *Saccharomyces cerevisiae* (KCCM 50583) was obtained from Korea Culture Center of Microorganisms (KCCM, Seoul, Korea). Yeast extract (YE), dextrose, peptone, phenylmethylsolfonyl fluoride, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), sulfanilic acid, phosphoric acid and N-(1-naphthyl)-ethylenediamine were from Sigma-Aldrich (St. Louis, MO). Gastrodin, HBA, and HBZ, were kindly gifted by Muju Health Foods Co. Ltd. (Muju, Korea).

Fermentation of GE – GE was obtained from MJ Health foods co., Ltd. (Muju, Korea). It was washed and stored at −20 °C until further use. For fermentation, the fermentation medium (1 L) containing 1% yeast extract, 2% dextrose, 2% peptone, and distilled water, was adjusted to pH 5.0, and autoclaved at 121 °C for 15 min. Then, *Saccharomyces cerevisiae* were inoculated, and incubated at 30 °C on an orbital shaker at 120 rpm (revolution per minute) for 72 h. To prepare FGE, 500 g of crushed GE, 100 g of brown sugar, and distilled water, were adjusted to 1 L and autoclaved at 121 °C for 15 min. Fermentation medium was inoculated with 1% (v/v) cultures of *Saccharomyces cerevisiae* and incubated at 30 °C on an orbital shaker at 120 rpm for 3 weeks. The fermentation mixture was filtered and freeze-dried. During the process, pH and total sugar contents were measured every 3 days, using a digital pH-meter (Orion 410A) and Glucose Tester (HB-32b, PROEM, Korea), respectively. The GE without *Saccharomyces cerevisiae* was used as control.

Ultra performance liquid chromatography (UPLC) analysis – UPLC analysis was performed using an ACQUITY UPLC® BEH C_{18} (2.1 × 50 mm, 1.7 m, Waters Corp., Milford, USA) consisting of photodiode array detector. The detection wavelength was set at 220 nm. The column temperature was 30 °C, the flow rate was 0.6 mL/min, and injection volume was 2 L. To prepare the injection samples, 10 mg of GE, or FGE water extracts were dissolved in 1 ml of distilled water. The mobile phase consisted of 0.1% H_3PO_4 (A) and acetonitrile (B), and the solvent gradient changed according to the following condition: 0 min: A (7%); 0.5 min: A (7 - 15%); 2.5 min: A (15 - 90%); 3 min: A (90 - 7%); 3.5 min: A (7 - 93%).

MTT assay – The HUVECs were cultured in Endothelial cell basal medium-2 (EBM-2) supplemented with 2% fetal bovine serum. To investigate the effect of GE or FGE extracts on HUVECs viability, MTT assay was performed. Approximately, 4×10^4 cells/well were seeded into 24 well plates and added YE or varying concentrations

of GE or FGE for 48 h. And 50 L of the MTT working solution (2 mg/mL in PBS) was exposed to each well and incubated for 4 h at 37 ºC. The formazan crystals were dissolved in 1 mL of DMSO and the absorbance was measured spectrophotometrically at 540 nm.

HUVECs were plated and pretreated with YE or varying concentrations of GE or FGE for 2 h. After incubation, the cells were exposed with glucose (5.5 mM for basal control or 25 mM for diabetic condition) for another 24 h. Then the MTT solution was added to each well and incubated for 4 h at 37 ºC. The formazan crystals were solubilized in 1 mL of dimethyl sulphoxide (DMSO) and the absorbance at 540 nm was recorded.

Assay of NO production – HUVECs were seeded into 24 well plates at a density of 4×10^4 cells/well and pretreated with YE or various concentrations of GE or FGE in humidified atmosphere containing 5% CO_2 at 37 ºC for 1 h. After incubation, the cells were exposed to glucose (5.5 mM or 25 mM) for another 48 h. Nitrite in the medium was quantified spectrophotometrically using the Griess reagent. In brief, 0.5 mL sample was mixed with 0.25 mL of 1% sulfanilic acid in 5% phosphoric acid, and 0.25 mL of 0.1% N-(1-naphthyl)-ethylenediamine, and incubated for 30 min at room temperature. The absorbance was measured at 540 nm and compared with the control group. Similar method for the evaluation of NO inhibition was applied by phenolic compounds of FGE in HG treated HUVECs.

Assay of IL-8 production – To examine the levels of IL-8 produced in HUVECs, 2×10^5 cells/well were seeded into a 24 well plate and pretreated with YE or varying concentrations of GE or FGE for 1 h. After incubation, the cells were exposed to glucose (5.5 mM or 25 mM) for another 48 h. Finally, the amount of IL-8 in the medium was analyzed by enzyme linked immunosorbent assay (ELISA) using a commercial kit (R&D Systems Ltd, Abingdon, UK), according to the manufacturer's instructions. Similar method was applied for the evaluation of IL-8 inhibition by phenolic compounds of FGE in HG treated HUVECs.

Antioxidant activity assay – The antioxidant activity was determined using the DPPH free radical scavenging assay. Briefly, 0.1 mM DPPH solution in methanol was prepared, and 1 mL of the solution was added to 0.5 mL of GE, or FGE at varying concentrations. The mixture was shaken vigorously and left for 30 min at room temperature and in dark. The absorbance was taken at 517 nm and compared with the control group. Percent scavenging of the DPPH free radical was measured using the following equation:

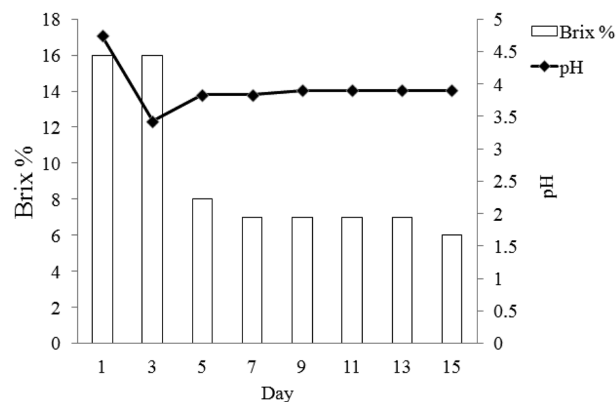

Fig. 1. pH and Brix during a process of fermentation.

$$\% \text{ DPPH radical scavenging } (\%) = [1 - (As / Ac)] \times 100$$

where, Ac=absorbance of control, As=absorbance of sample solution

Statistical analysis – Experimental results are represented as the mean ± SD. The statistical significance of the experiments was analyzed by the one-way ANOVA test and Student's *t*-test. $p < 0.05$ was considered statistically significant.

Results and Discussion

In the present study we utilized GRAS yeast *S. cerevisiae* (Nevoigt, 2008) to ferment GE, and the biological activity including antioxidative, cytotoxic, and anti-inflammatory effects of GE and FGE were examined in HG-induced HUVECs.

Fermentation is a process of decomposition of organic matters in an anaerobic condition. It can be carried out by lactic acid bacteria, such as *Bifidobacterium* sp., and some molds such as *Sachharomyces* sp. These microorganisms convert carbohydrates to alcohol and organic acids, which can enhance the bioactivity of the product (Jung *et al*., 2011). For instance, lactic acid bacteria fermented ginseng possesses enhanced cytotoxic effect against cancer cells (Bae *et al*., 2008; Wakabayashi *et al*., 1998). Moreover, *Lactobacillus* sp. fermented Hwangryunhaedok-tang showed certain components on it to have potent antioxidative and neuroprotective activity in mouse hippocampal cell line (Yang *et al*., 2011). During fermentation, physicochemical properties of FGE were evaluated every 3 days (Fig. 1). The initial pH of FGE was 4.74, which dropped to 3.43 after 3 days, and slowly rose to 3.98 at the end of the fermentation. On the other hand, total sugar contents of FGE was about 16% at the start, dropped sharply to 11% after 3 days, and maintained to about 12% until the end of

Table 1. The content of phenolic compounds in GE and FGE

Phenolic compounds	GE (ug/g)	FGE (ug/g)
Gastrodin	1.9 ± 0.06	6.5 ± 0.10
HBA	44.3 ± 0.67	111.0 ± 0.21
HBZ	ND	1.0 ± 0.00

Each value represents mean ± S.D. by triplicate independent experiments.
ND: not detected

the fermentation.

Furthermore, we performed UPLC analysis on the microbial transformation of GE by *Saccharomyces cerevisiae* indicated an enhanced presence of gastrodin, HBA, and HBZ. As illustrated in Table 1, there was about 3 fold increase in the content of gastrodin and HBA in FGE, in comparison to GE extract. Moreover, HBZ, which was not detectable in GE extract, was present in FGE. UPLC analysis showed enhanced presence of gastrodin, HBA, and HBZ, in FGE. It has been found that gastrodin inhibits the expression of inducible NO synthase, cyclooxygenase-2 (COX-2), and proinflammatory cytokines, in cultured LPS-stimulated microglia cells (Dai *et al*., 2011). Furthermore, gastrodin's metabolite, HBA has been shown to inhibit the activities of COX-1 and 2 (Lee *et al*., 2006). Moreover, HBA is known to inhibit oxidative damage, contributing to enhanced neuronal survival (Kim *et al*., 2007), and has also been shown to ameliorate ischemic injury induced by transient focal cerebral ischemia (Yu *et al*., 2010). HBZ, an analogue of HBA, has been shown to have antioxidative as well as positive GABAergic neuromodulation effect, suggesting its antiepileptic and anticonvulsive use (Ha *et al*., 2000). Then, the cytotoxicity of GE or FGE extracts on HUVECs was measured by MTT assay. GE or FGE extracts did not affect viability of HUVECs (data now shown). When HUVECs were treated with 25 mM glucose, there was about 3 fold decrease in cell viability compared to the cells treated with 5.5 mM glucose. Interestingly, when the cells were pretreated with 100 ug/ml FGE, cell viability increased significantly to 82.77%, showing 20% more viability than GE treated cells (Fig. 2).

Oxidative stress is a well established factor in the development and progression of diabetes and its complications (Baynes, 1991). The exposure of endothelial cells to HG increases the production of reactive oxygen species at the mitochondrial level, leading to increased cytotoxicity (Reusch, 2003). Antioxidant activity was expressed as amount of DPPH radical scavenged by GE or FGE. We examined the radical scavenging activity of GE, and FGE, each at 1, 10, and 100 ug/mL concentrations.

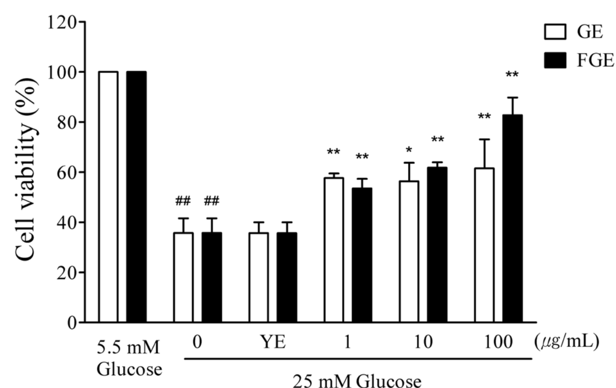

Fig. 2. Effects of GE extracts on cell viability in HG-treated HUVECs. 4×10^4 cells/well were seeded into 24 well plates and pretreated with YE or varying concentrations (1, 10, or 100 ug/mL) of extracts for 2 h. After incubation, the cells were treated with glucose (5.5 mM or 25 mM), and incubated for another 24 h. Each value represents mean ± S.D. by triplicate independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the HG (25 mM) and $^{\#\#}P < 0.01$ compared with the control.

Table 2. DPPH free radical scavenging activity of GE and FGE

Concentration (ug/mL)	Scavenging activity (%)	
	GE	FGE
1	0.56 ± 0.70	0
10	0.71 ± 1.23	1.55 ± 1.86
100	5.57 ± 4.76	44.08 ± 4.21**

Each value represents mean ± S.D. by triplicate independent experiments.
$^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control

Table 3. DPPH radical scavenging activity of phenolic compounds of FGE

Concentration (ug/mL)	Scavenging activity (%)		
	HBA	HBZ	Gastrodin
1	33.98 ± 1.40*	33.75 ± 1.12*	35.08 ± 1.74
10	34.47 ± 0.96**	32.90 ± 0.79**	33.59 ± 0.73**
100	46.49 ± 0.20**	32.85 ± 0.77**	33.39 ± 0.40**

Each value represents mean ± S.D. by triplicate independent experiments.
$^{*}P < 0.05$, $^{**}P < 0.01$ compared with control

We observed the dose dependent radical scavenging activity with our samples. As shown in Table 2, FGE was found to be a strong antioxidant as compared with GE. Particularly, at 100 ug/mL of concentration, FGE showed significantly potent antioxidant activity. As shown in Table 3, the antioxidative activities of HBA, HBZ, and gastrodin, were almost similar to each other at concentrations of 1 and 10 ug/mL. However, HBA at 100 ug/mL concentration showed the radical scavenging activity to be

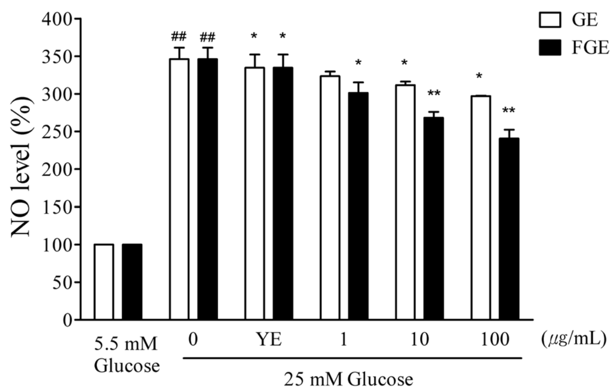

Fig. 3. Effect of FGE extracts on HG-induced NO production in HUVECs. HUVECs were seeded into 24 well plates at a density of 4×10^4 cells/well and pretreated with YE or various concentrations (1, 10, or 100 ug/mL) of extracts, in humidified atmosphere containing 5% CO_2 at 37 °C for 1 h. After incubation, the cells were treated with glucose (5.5 mM or 25 mM), and incubated for another 48 h. Each value represents mean ± S.D. by triplicate independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the HG (25 mM) and $^{\#\#}P < 0.01$ compared with the control.

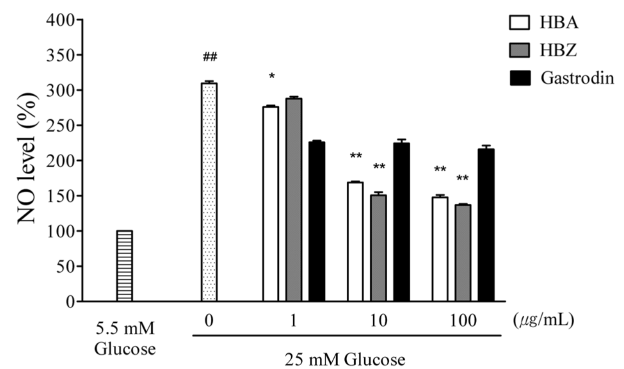

Fig. 4. Effect of the phenolic compounds of FGE on HG-induced NO production in HUVECs. 4×10^4 HUVECs/well were treated with various concentrations (1, 10, or 100 ug/mL) of phenolic compounds of FGE for 1 h, followed by HG (25 mM) for another 48 h. The NO production was measured by Griess reagent. The method was replicated for each phenolic compound, individually. Each value represents mean ± S.D. by triplicate independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the HG (25 mM) and $^{\#\#}P < 0.01$ compared with the control.

more potent than either of HBZ, or gastrodin. Importantly, our study showed that FGE exhibits enhanced free radical scavenging activity, suggesting that it may have protective role against high glucose induced oxidative damage to HUVECs.

Nitric oxide plays a key role in endothelial functions. In appropriate level, NO acts as a strong vasodilatating agent (Lirk, 2002). But when NO is generated in large amounts, it reacts quickly with superoxide anion to yield peroxynitrite, a powerful oxidant which, in turn, causes adverse impacts to DNA, and proteins, leading to inflammation and tissue damage (Maeda and Akaike, 1998). In this study, we focused on the pathways of inflammation and enhancing several chronic diseases. Thus, we examined the inhibitory effect of FGE on NO production in HG-induced HUVECs. As shown in Fig. 3, when compared to 5.5 mM glucose, the levels of NO production by HUVECs were significantly increased by 25 mM glucose treatment. YE pretreated HUVECs did not showed any changes on NO production. But as expected, in comparison to GE treated cells, FGE treatment inhibited the induction of NO production levels, in a concentration dependant manner, to 303.11 ± 14.10%, or 269.98 ± 7.83%, or 242.25 ± 11.77% at concentrations of 1, or 10, or 100 ug/mL of FGE, respectively. Although, gastrodin showed similar trend of inhibition with its varying concentrations, each of the other two compounds, HBA and HBZ, showed about 50% inhibition of HG induced NO production at all other concentrations except 1 ug/mL (Fig. 4). Our finding indicated that FGE pretreatment significantly inhibited HG-induced NO production in HUVECs, which strengthens its importance as anti-inflammatory agents.

IL-8 is one of the mediators of inflammation and has been known to play a key role in the formation of atherosclerotic lesions (Moreau *et al.*, 1999), which is a major complication of diabetes. IL-8 is a chemokine which mediate inflammatory responses, and is a potent chemo-attractant that is crucial for mediating monocyte-endothelium adhesion (Gerszten *et al.*, 1999). Furthermore, hyperglycemia is known to stimulate IL-8 production in HUVECs (Kinoshita *et al.*, 2008), and enhanced production of inflammatory mediators has been correlated with diabetic complications (Marigo *et al.*, 2011). Therefore, these data suggest that inhibition of IL-8 in HUVECs could prevent the cells adhesion leading to prevention of atherosclerosis particularly in diabetic patients. In our experiments, FGE showed marked inhibitory activity against HG-induced IL-8 production in HUVECs. Similarly, *Buddleja officinalis* has also been shown to inhibit expression of HG-induced IL-8 mRNA, and reactive oxygen species generation, in HUVECs (Lee *et al.*, 2008a; Lee *et al.*, 2008b). Moreover, GE has been found to inhibit TNF-α-induced vascular inflammatory processes via inhibition of oxidative stress, and NF-κB activation, in HUVECs (Lee *et al.*, 2006), suggesting its potential role in anti-inflammatory activities.

Therefore, to know whether FGE exhibits any effect against IL-8 production, HUVECs were pretreated with YE, or GE, or FGE, for 48 h, followed by 25 mM of

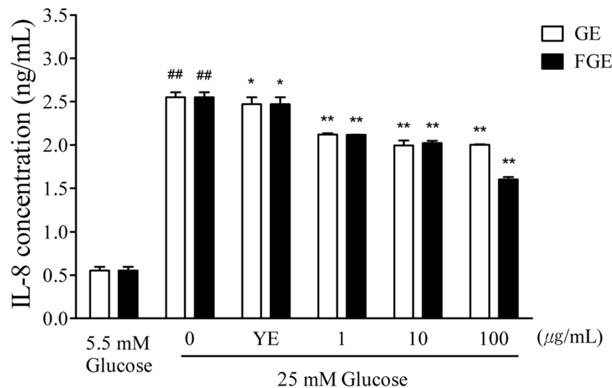

Fig. 5. Effect of FGE extracts on HG-induced IL-8 production in HUVECs. 2×10^5 HUVECs/well were seeded into a 24 well plate and pretreated with YE or varying concentrations of extracts for 1 h. After incubation, the cells were exposed to glucose (5.5 mM or 25 mM), and incubated for another 48 h, followed by centrifugation at 20,000 × g for 30 min. Finally, the amount of IL-8 in supernatant was analyzed by ELISA. Each value represents mean ± S.D. by triplicate independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the HG (25 mM) and $^{\#\#}P < 0.01$ compared with the control.

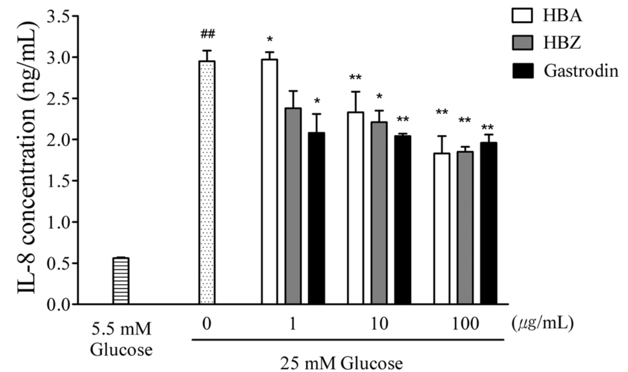

Fig. 6. Effect of the phenolic compounds of FGE on HG-induced IL-8 production in HUVECs. 2×10^5 HUVECs/well were pretreated with the phenolic compounds (HBA, or HBZ, or gastrodin) for 1 h, followed by glucose (25 mM) for another 48 h. IL-8 production was estimated by ELISA. The method was replicated for each phenolic compound, individually. Each value represents mean ± S.D. by triplicate independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the HG (25 mM) and $^{\#\#}P < 0.01$ compared with the control.

glucose treatment for 1 h. Thereafter, the cell-free supernatant was collected and subjected to ELISA. As shown in Fig. 5, YE did not affect IL-8 production on HUVECs. GE pretreated cells, although, at varying concentrations, inhibited the HG-induced IL-8 production to almost similar levels about 2 ng/mL. FGE treated cells also showed almost similar pattern, but in comparison to GE treated cells and other concentrations of FGE, 100 ug/mL of FGE showed marked inhibition of IL-8 production (1.60 ± 0.03 ng/mL). The IL-8 inhibition activities of HBA, HBZ, and gastrodin were also studied. We observed the concentration dependent IL-8 production inhibition by them. Only at higher concentrations (100 ug/mL) potent inhibitory activity against IL-8 production in HG-induced cells was observed with HBA and HBZ. However, in a similar condition, varying concentrations of gastrodin showed almost similar pattern of inhibition (Fig. 6). These results indicate that FGE could play an important preventive role in HG-induced inflammatory activities through its enhanced free radical scavenging activity, and by inhibition of HG-induced NO, and IL-8, productions.

Our research findings confirmed FGE as a strong antioxidative as well as anti-inflammatory agent. FGE significantly enhanced cells viability, leading to suppressed cytotoxicity in HG-induced HUVECs, in a concentration dependent manner. FGE pretreatment showed enhanced free radical scavenging activity. In addition, FGE significantly inhibited the HG-induced NO and IL-8 production, in a concentration dependent manner, in HUVECs. Taken together, the beneficial effects of GE were enhanced by fermentation, and HBA and HBZ played a major role in these beneficial effects. These findings raise the possibility that FGE might be useful for the treatment and prevention of endothelial dysfunction associated with diabetes

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References

World Health Organization. Diabetes 2011. Available at: http://www.who.int/mediacentre/factsheets/fs312/en/.

Bae, E.A., Choo, M.K., Park, E.K., Park, S.Y., Shin, H.Y., and Kim, D.H., Metabolism of ginsenoside Rc by human intestinal bacteria and its related antiallergic activity. *Biol. Pharm. Bull.* **25**, 743-747 (2002).

Bae, E.A., Park, S.Y., and Kim, D.H., Constitutive beta-glucosidases hydrolyzing ginsenoside Rb1 and Rb2 from human intestinal bacteria. *Biol. Pharm. Bull.* **23**, 1481-1485 (2008).

Baynes, J.W., Role of oxidative stress in development of complications in diabetes. *Diabetes* **40**, 405-412 (1991).

Betsy, B.D., The pathophysiology of cardiovascular diseases and diabetes: beyond blood pressure and lipids. *Diabetes Spectrum*, **21**, 160-165 (2008).

Boisvert, W.A., Santiago, R., Curtiss, L.K., and Terkeltaub, R.A., A leukocyte homologue of the IL-8 receptor CXCR-2 mediates the accumulation of macrophages in atherosclerotic lesions of LDL receptor-deficient mice. *J. Clin. Invest.*, **101**, 353-363 (1998).

Brownlee, M., The pathobiology of diabetes complications: a unifying mechanism. *Diabetes*, **54**, 1615-1625 (2005).

Dai, J.N., Zong, Y., Zhong, L.M., Li, Y.M., Zhang, W., Bian, L.G., Ai, Q.L., Liu, Y.D., Sun, J., and Lu, D., Gastrodin inhibits expression of

inducible NO synthase, cyclooxygenase-2 and proinflammatory cytokines in cultured LPS-stimulated microglia via MAPK pathways. *PLoS One*, **6**, e21891 (2011).

Esposito, K., Nappo, F., Giugliano, F., Di Palo, C., Ciotola, M., Barbieri, M., Paolisso, G., and Giugliano, D., Cytokine milieu tends toward inflammation in type 2 diabetes. *Diabetes Care*, **26**, 1647 (2003).

Gerszten, R.E., Garcia-Zepeda, E.A., Lim, Y.C., Yoshida, M., Ding, H.A., Gimbrone, M.A Jr., Luster, A.D., Luscinskas, F.W., and Rosenzweig, A., MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. *Nature*, **398**, 718-723 (1999).

Ha, J.H., Shin, S.M., Lee, S.K., Kim, J.S., Shin, U.S., Huh, K, Kim, J.A., Yong, C.S., Lee, N.J., and Lee, D.U., In vitro effects of hydroxybenzaldehydes from *Gastrodia elata* and their analogues on GABAergic neurotransmission, and a structure-activity correlation. *Planta. Med.*, **67**, 877-880 (2001).

Hsieh, C.L., Chiang, S.Y., Cheng, K.S., Lin, Y.H., Tang, N.Y., Lee, C.J., Pon, C.Z., and Hsieh, C.T., Anticonvulsive and free radical scavenging activities of *Gastrodia elata* Bl. in kainic acid-treated rats. *Am. J. Chin. Med.*, **29**, 331-341 (2001).

Hwang, S.M., Lee, Y.J., Kang, D.G., and Lee, H.S., Anti-inflammatory effect of *Gastrodia elata* rhizome in human umbilical vein endothelial cells. *Am. J. Chin. Med.*, **37**, 395-406 (2009).

Ha, J.H., Lee, D.U., Lee, J.T., Kim, J.S., Yong, C.S., Kim, J.A., Ha, J.S., and Huh, K., 4-Hydroxybenzaldehyde from *Gastrodia elata* Bl. is active in the antioxidation and GABAergic neuromodulation of the rat brain. *J. Ethnopharmacol*., **73**, 329-333 (2000).

Han, B.H., Park, M.H., Han, Y.N., Woo, L.K., Sankawa, U, Yahara, S, and Tanaka, O., Degradation of ginseng saponins under mild acidic conditions. *Planta. Med.*, **44**, 146-149 (1982).

Jang, Y.W., Lee, J.Y., and Kim, C.J., Anti-asthmatic activity of phenolic compounds from the roots of *Gastrodia elata* Bl. *Int. Immunopharmacol*., **10**, 147-154 (2010).

Jung, Y.M., Lee, S.H., Lee, D.S., You, M.J., Chung, I.K., Cheon, W.H., Kwon, Y.S., Lee, Y.J., and Ku, S.K., Fermented garlic protects diabetic, obese mice when fed a high-fat diet by antioxidant effects. *Nutr. Res.*, **31**, 387-396 (2011).

Kim, H.J., Hwang, I.K., and Won, M.H., Vanillin, 4-hydroxybenzyl aldehyde and 4-hydroxybenzyl alcohol prevent hippocampal CA1 cell death following global ischemia. *Brain Res.*, **1181**, 130-141 (2007).

Kinoshita, K., Tanjoh, K., Noda, A., Sakurai, A., Yamaguchi, J., Azuhata, T., Utagawa, A., and Moriya, T., Interleukin-8 production from human umbilical vein endothelial cells during brief hyperglycemia: the effect of tumor necrotic factor-alpha. *J. Surg. Res.*, **144**, 127-131 (2008).

Ko, S.R., Choi, K.J., Uchida, K., and Suzuki, Y., Enzymatic preparation of ginsenosides Rg2, Rh1, and F1 from proto-panaxatriol-type ginseng saponin mixture. *Planta. Med.*, **69**, 285-286 (2003).

Kuroki, T., Isshiki, K., and King, G.L., Oxidative stress: the lead or supporting actor in the pathogenesis of diabetic complications. *J. Am. Soc. Nephrol*., **14**, S216-S220 (2003).

Lee, J.Y., Jang, Y.W., Kang, H.S., Moon, H., Sim, S.S., and Kim, C.J., Anti-inflammatory action of phenolic compounds from *Gastrodia elata* root. *Arch. Pharm. Res.*, **29**, 849-858 (2006).

Lee, Y.J., Kang, D.G., Kim, J.S., and Lee, H.S., *Buddleja officinalis* inhibits high glucose-induced matrix metalloproteinase activity in human umbilical vein endothelial cells. *Phytother. Res.*, **22**, 1655-1659 (2008a).

Lee, Y.J., Kang, D.G., Kim, J.S., and Lee, H.S., Effect of *Buddleja officinalis* on high-glucose-induced vascular inflammation in human umbilical vein endothelial cells. *Exp. Biol. Med. (Maywood)*, **233**, 694-700 (2008b).

Lirk, P., Hoffmann, G., and Rieder, J., Inducible nitric oxide synthase-time for reappraisal. *Curr. Drug Targets Inflamm. Allergy,* **1**, 89-108 (2002).

Liu, J., Wei, S., Tian, L., Yan, L., Guo, Q., and Ma, X., Effects of endomorphins on human umbilical vein endothelial cells under high glucose. *Peptides*, **32**, 86-92 (2011).

Madamanchi, N.R., Vendrov, A., and Runge, M.S., Oxidative stress and vascular disease. *Arterioscler. Thromb. Vasc. Biol.,* **25**, 29-38 (2005).

Maeda, H. and Akaike, T., Nitric oxide and oxygen radicals in infection, inflammation, and cancer. *Biochemistry (Mosc)*, **63**, 854-865 (1998).

Marigo, L., Cerreto, R., Giuliani, M., Somma, F., Lajolo, C., and Cordaro, M., Diabetes mellitus: biochemical, histological and microbiological aspects in periodontal disease. *Eur. Rev. Med. Pharmacol. Sci.*, **15**, 751-758 (2011).

Moreau, M., Brocheriou, I., Petit, L., Ninio, E., Chapman, M.J., and Rouis, M., Interleukin-8 mediates downregulation of tissue inhibitor of metalloproteinase-1 expression in cholesterol-loaded human macrophages: relevance to stability of atherosclerotic plaque. *Circulation*, **99**, 420-426 (1999).

Nevoigt, E., Progress in metabolic engineering of *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.*, **72**, 379-412 (2008).

Park, C.S., Yoo, M.H., Noh, K.H., and Oh, D.K., Biotransformation of ginsenosides by hydrolyzing the sugar moieties of ginsenosides using microbial glycosidases. *Appl. Microbiol. Biotechnol.*, **87**, 9-19 (2010).

Reusch, J.E., Diabetes, microvascular complications, and cardiovascular complications: what is about glucose? *J. Clin. Invest.*, **112**, 986-988 (2003).

Romuk, E., Skrzep-Poloczek, B., Wojciechowska, C., Tomasik, A., Birkner, E., Wodniecki, J., Gabrylewicz, B., Ochala, A., and Tendera, M., Selectin-P and interleukin-8 plasma levels in coronary heart disease patients. *Eur. J. Clin. Invest.*, **32**, 657-661 (2002).

Temaru, R., Urakaze, M., Satou, A., Yamazaki, K., Nakamura, N., and Kobayashi, M., High glucose enhances the gene expression of interleukin-8 in human endothelial cells, but not in smooth muscle cells: possible role of interleukin-8 in diabetic macroangiopathy. *Diabetologia*, **40**, 610-613 (1997).

Wakabayashi, C., Murakami, K., Hasegawa, H., Murata, J., and Saiki, I., An intestinal bacterial metabolite of ginseng protopanaxadiol saponins has the ability to induce apoptosis in tumor cells. *Biochem. Biophys. Res. Commun.*, **246**, 725-730 (1998).

Xiang, X., Yanying, L., and Xiaodong, B., Protective effects of gastrodin on hypoxia-induced toxicity in primary cultures of rat cortical neurons. *Planta. Med.*, **73**, 650-654 (2007).

Xu, X., Lu, Y., and Bie, X., Protective effects of gastrodin on hypoxia-induced toxicity in primary cultures of rat cortical neurons. *Planta. Med.*, **73**, 650-654 (2007).

Yamagishi, S. and Matsui, T., Nitric oxide, a janus-faced therapeutic target for diabetic microangiopathy-Friend or foe? *Pharmacol. Res.*, **64**, 187-194 (2011).

Yang, H.J., Weon, J.B., Lee, B., and Ma, C.J., The alteration of components in the fermented Hwangryunhaedok-tang and its neuroprotective activity. *Pharmacogn. Mag.*, **7**, 207-212 (2011).

Yu, S.J., Kim, J.R., Lee, C.K., Han, J.E., Lee, J.H., Kim, H.S., Hong, J.H., and Kang, S.G., *Gastrodia elata* Blume and an active component, *p*-hydroxybenzyl alcohol reduce focal ischemic brain injury through antioxidant related gene expressions. *Biol. Pharm. Bull.*, **28**, 1016-1020 (2005).

Yu, S.S., Zhao, J., Zheng, W.P., and Zhao, Y., Neuroprotective effect of 4-hydroxybenzyl alcohol against transient focal cerebral ischemia via anti-apoptosis in rats. *Brain Res.*, **1308**, 167-175 (2010).

Zeng, X., Zhang, S., Zhang, L., Zhang, K., and Zheng, X., A study of the neuroprotective effect of the phenolic glucoside gastrodin during cerebral ischemia *in vivo* and *in vitro*. *Planta. Med.*, **72**, 1359-1365 (2006).

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