# Relaxant and vasoprotective effects of ginger extracts on porcine coronary arteries

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**Abstract.** Ginger (*Zingiber officinale* Roscoe) is a popular Chinese herbal medicine, which is considered to warm the stomach and dispel cold in traditional Chinese medicine. Ginger is widely used to treat stomach disorders, and it has been reported to exhibit antithrombotic activity via the inhibition of platelet aggregation and thromboxane $B_2$ production *in vitro*. Cardiovascular disease is associated with the aberrant functioning of the heart and circulatory system; the relatively narrow vessels of the circulation are commonly affected and blocked by atherosclerosis, which may result in angina or heart attack. Numerous drugs and medicines are used to treat myocardial infarction; however, they are often associated with numerous side effects. Therefore, it is important to identify substitutive drugs with no unbearable side effects. In the present study, the relaxant effects of ginger crude extract (GCE) were determined on porcine coronary arteries. The DPPH radical scavenging assay, lucigenin-enhanced chemiluminescence assay and western blot analysis were used to individually detect antioxidant assay of ginger extraction or superoxide anion produced by endothelial cells and molecular signaling. The results indicated that GCE induced relaxation of porcine coronary arteries in an endothelium-dependent manner. GCE increased vasoprotection via the suppression of nitric oxide synthase and cyclooxygenase. In addition, GCE possessed antioxidant ability, as determined using 1,1-diphenyl-2-picrylhydrazyl and lucigenin-enhanced chemiluminescence assays. Taken together, the present study demonstrated that GCE exerts marked vasoprotective effects and free radical-scavenging activities in porcine coronary arteries.

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## Introduction

Cardiovascular disease is the second leading cause of death among the ten leading chronic diseases in Taiwan according to the 2017 annual report of the Ministry of Health and Welfare, Taiwan, R.O.C. (1). A total of 20,812 people died, and the death rate was 88.5 per 100,000 population, increased by 8.1% from 2015 to 2016 (1). Cardiovascular disease includes coronary heart disease (CHD), peripheral arterial disease, aortic disease and stroke, and many risk factors are associated with the lesions (2,3). The drug treatments can greatly improve cardiovascular disease (4). Importantly, traditional Chinese medicine, dietary foods and supplements may prevent or help in fighting heart disease (5,6).

Ginger (*Zingiber officinale* Roscoe) is a natural herb that is widely used for medicinal and culinary purposes (7,8). Ginger exerts many health benefits and may be used to treat ailments, including cramps, arthritis and disorders of the gastrointestinal tract, such as constipation, dyspepsia, diarrhea, nausea and vomiting (8). In addition, ginger is recommended by traditional healers to treat cardiomyopathy, high blood pressure and palpitations (7,9,10). The main bioactive constituents of ginger are gingerol, shogaol, zingerone and paradol (11,12). Furthermore, the main aromatic components of ginger are zingiberol, gingediol, monoacyldigalactosyl-glycerol, iarylheptanoids and phytosterols (13). 6-Gingerol has numerous biological activi-

ties, including antioxidant, antitumor and anti-inflammatory effects (14-16). The pharmacological effects of 6-gingerol ameliorate hyperlipidemia by decreasing serum cholesterol and serum triglyceride levels (17). 6-shogaol is a dehydrated form of 6-gingerol, which is isolated from the dried or cooked rhizomes of ginger (18,19). In a previous study, ginger crude extract (GCE) was reported to exhibit hypotensive, endothelium-independent vasodilatory and cardiosuppressive properties, via its specific inhibitory action at voltage-dependent calcium channels (20). The present study aimed to investigate the relaxant effects of GCE on porcine coronary arteries *in vivo*.

## Materials and methods

*Reagents and chemicals*. DL-homocysteine (Hcy), 1H-[1,2,4] oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), bradykinin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), dimethyl sulfoxide, propranolol, *n*-butanol and other chemicals were high-grade products purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). $N^G$-nitro-$_L$-arginine ($_L$-NNA) and glibenclamide (Glib) were obtained from MP Biomedicals, LLC (Santa Ana, CA, USA). KH solution was composed of 70.2 mM NaCl, 4.2 mM KCl, 2.8 mM $CaCl_2$, 2.7 mM $MgSO_4$, 21.0 mM $NaHCO_3$, 0.2 mM $KH_2PO_4$ and 9.8 mM glucose, and the pH was adjusted to 7.4.

*Ginger extraction*. A total of 600 g fresh ginger rhizome was soaked in 2.5 l ethanol. The extracts were refluxed at 78˚C for 2 h; this was repeated three times. Subsequently, the filtrate was concentrated in a rotary evaporator. The weight of extracts was ~34.2 g (yield, 5.7%). The residue was then suspended in 50 ml water and extracted with 50 ml chloroform twice, after which the chloroform partition was evaporated to obtain 8.4 g residue (GCE). The aqueous phase was partitioned with *n*-butanol. The *n*-butanol partition was evaporated to obtain 6.3 g residue (ginger *n*-butanol extract, GNE). The water extract underwent reverse osmosis to obtain 19.5 g residue (ginger water extract, GWE); this process is summarized in Fig. 1. The stock solution of ginger extraction was prepared by dimethyl sulfoxide to dilute for further experiments.

*Coronary artery ring preparation*. Porcine hearts were freshly obtained from the local abattoir, immersed in cold 0.9% NaCl at 4˚C and were transported to the research laboratory. Excess connective tissue was removed and the arteries were cut into 5-mm rings. Endothelium-intact and -denuded porcine coronary artery rings were prepared, and the rings were then mounted with two stainless steel hooks in 10 ml KH solution-filled organ baths. KH solution was kept in oxygenated conditions (95% $O_2$ and 5% $CO_2$) at 37˚C and was replaced every 15 min to maintain continuous equilibration. The rings were perfused with 30 mM KCl in the organ bath until tonic phase contraction was achieved, as previously described (21) before pretreatment with 100 $\mu$M L-NNA, 10 $\mu$M ODQ, 10 $\mu$g/ml indomethacin, 20 $\mu$M propranolol, 1 $\mu$M Glib, 100 $\mu$M Hcy, 30 mM bradykinin and 77.5 mM $H_2O_2$, respectively, for indicated period of time.

*Isometric tension of porcine coronary arteries*. The porcine coronary arteries were harvested, cut into numerous 5-mm rings, and were maintained in 5 ml organ baths containing 95% $O_2$ and 5% $CO_2$ at 37˚C. Ginger extracts were individually added to the 5-mm rings for 30 min and relaxation was observed. Alterations in tension were recorded using a Grass Force displacement transducer (model FT03; Grass; Natus Medical Incorporated, Pleasanton, CA, USA).

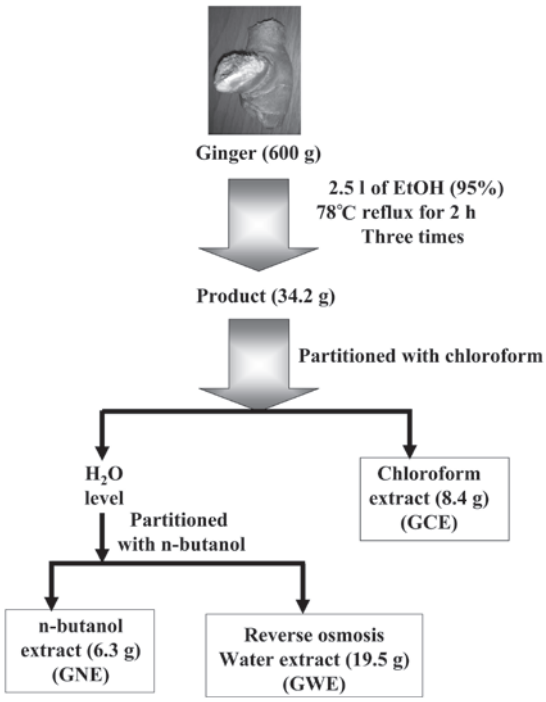


Figure 1. Flowchart depicting the preparation of three varieties of ginger extract (GCE, GNE and GWE). GCE, ginger crude extract; GNE, ginger *n*-butanol extract; GWE, ginger water extract.

*DPPH radical scavenging assay*. The DPPH radical scavenging assay was performed according to the method described by Sakanashi *et al* (21). Briefly, in each well of a 96-well plate, 50 $\mu$l sample extract was added to 150 $\mu$l 0.25 mM DPPH methanolic solution. After mixing thoroughly, the reactants were incubated in the dark for 30 min at room temperature. The control was prepared by mixing 50 $\mu$l methanol with 150 $\mu$l DPPH. The absorbance was detected at 517 nm using a spectrophotometer. Samples were measured in triplicate.

*Lucigenin-enhanced chemiluminescence assay*. The levels of superoxide anion produced by endothelial cells of the porcine arteries were detected using the lucigenin-enhanced chemiluminescence method, as previously described by Sun *et al* (22). Briefly, the samples of GCE, GNE and GWE were mixed with 5 $\mu$M lucigenin for 6 min. Time-based reading was recorded in a 5 min period using a luminometer. The area of each vessel segment was measured using a caliper and was used to normalize the data for each sample.

*Protein preparation*. Following treatment with or without GCE, GWE and GNE, porcine coronary artery endothelial cells were collected as previously described (23) and mixed with protein lysis buffer [50 mM Tris-HCl (pH 7.4), 1 mM NaF, 150 mM NaCl, 1 mM EGTA, 1 mM phenylmethane-sulfonyl fluoride, 1% NP-40 and 10 $\mu$g/ml leupeptin] on ice. The samples

were homogenized for 20 sec, incubated for 20 min on ice and centrifuged at 15,000 x g for 30 min at room temperature. The supernatants were then transferred into new tubes for protein quantification, as previously described (24,25).

*Western blot analysis*. A total of 50 $\mu$g protein was loaded and separated by 10% SDS-PAGE. The samples in the gels were then transferred onto polyvinylidene difluoride membranes. The membranes were incubated with 0.1% PBS-Tween containing 5% non-fat milk for 30 min at room temperature, and were then hybridized with cyclooxygenase-2 (COX-2; cat.no.GTX100656;1:1,000dilution;GenTex,Hsinchu,Taiwan), inducible nitric oxide synthase (iNOS; cat. no. GTX130246; 1:1,000 dilution; GenTex), endothelial nitric oxide synthase (eNOS; cat. no. 3GTX129843; 1:1,000 dilution; GenTex) and β-actin (cat. no. GTX109639; 1:5,000 dilution; GenTex) primary antibodies. Subsequently, membranes were incubated with horseradish peroxidase-conjugated rabbit IgG antibody (cat. no. GTX213110-01; 1:10,000 dilution; GenTex) at room temperature for 1 h and were then visualized using Immobilon Western HRP substrate kit (EMD Millipore, Billerica, MA, USA). Densitometric analysis of each band was performed utilizing National Institutes of Health (NIH) ImageJ 1.47 software (NIH, Bethesda, MD, USA).

*Statistical analysis*. Data are presented as the means ± standard deviation from at least three separate experiments. Statistical data were analyzed using one-way ANOVA with post hoc Dunnett's test for comparing groups to the control by SPSS version 13.0 for Windows (SPSS Inc., Chicago, IL, USA). P<0.05 was considered to indicate a statistically significantly difference.

## Results

*Ginger extracts (GCE, GNE and GWE) preparation*. The three varieties of ginger extract (GCE, GNE and GWE) were prepared according to the diagram presented in Fig. 1. These three ginger extracts were used in the present study to explore their effects on the vasorelaxation of porcine coronary artery rings.

*GCE relaxes porcine coronary arteries*. Porcine coronary arteries were suspended in an organ bath. Various amounts of GCE (1, 3, 10, 30 and 100 $\mu$g/ml) were added to the porcine coronary arteries; water was used as a vehicle control. A dose-dependent increase in relaxation was observed in response to GCE (Fig. 2).

*GCE induces endothelium-dependent relaxation of porcine coronary arteries*. The results of the present study indicated that GCE induced endothelium-dependent vasorelaxation. Endothelium-intact and -denuded porcine coronary artery rings were incubated with various amounts of GCE (1, 3, 10, 30 and 100 $\mu$g/ml). GCE was able to reduce KCl-induced contraction and increase vasorelaxation from 27 to 99% in the endothelium-intact porcine coronary artery rings, whereas GCE exerted mild effects on the vasorelaxation of denuded porcine coronary artery rings (from 15 to 93%) (Fig. 3). Based on these data, it was suggested that endothelium-dependent

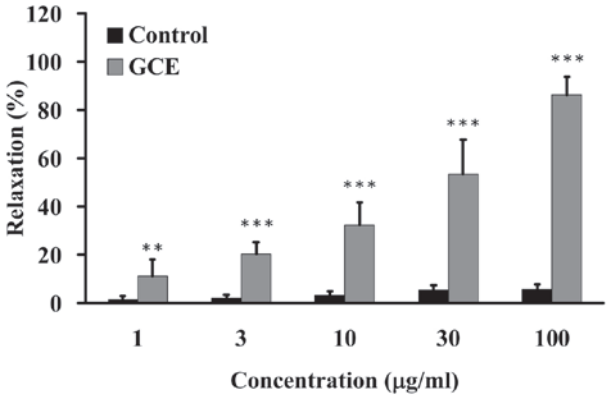


Figure 2. Vasodilatory effects of GCE on KCl-induced contraction. Various amounts of GCE were added to the porcine coronary arteries and KCl-induced contractions were evaluated. Data are presented as a percentage of 30 mM KCl-induced contraction based on tension changes. Values are expressed as the means ± standard deviation (n=6). $^{**}$P<0.01 and $^{***}$P<0.001 vs. the control group. GCE, ginger crude extract.

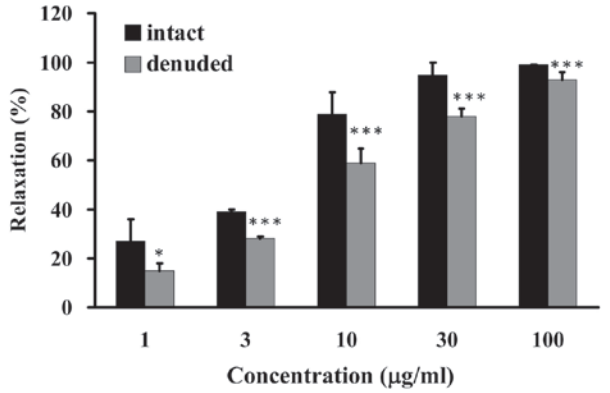


Figure 3. Effects of GCE on endothelium-dependent vasorelaxation. Endothelium-intact and -denuded porcine coronary artery rings were incubated with various amounts of GCE (1, 3, 10, 30 and 100 $\mu$g/ml). Tension was determined by isometric force transduction. Data are presented as a percentage of 30 mM KCl-induced contraction. Values are expressed as the means ± standard deviation (n=6). $^{*}$P<0.05 and $^{***}$P<0.001 vs. the intact group. GCE, ginger crude extract.

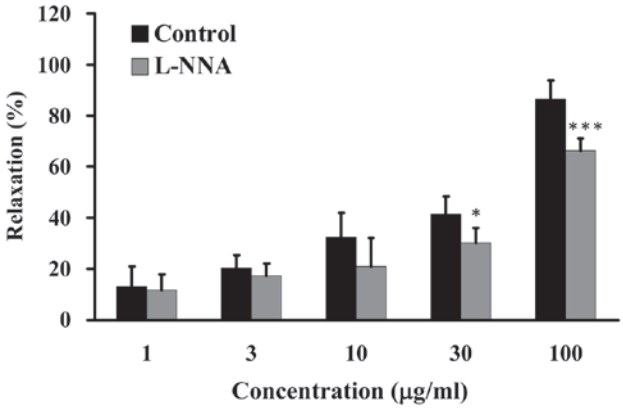


Figure 4. Effects of $_{L}$-NNA on GCE-induced vasorelaxation. Porcine coronary artery rings were incubated with various amounts of GCE (1, 3, 10, 30 and 100 $\mu$g/ml) in the absence (control) or presence of $_{L}$-NNA. Tension was determined by isometric force transduction. Data are presented as a percentage of 30 mM KCl-induced contraction. Values are expressed as the means ± standard deviation (n=6). $^{***}$P<0.001 vs. the control group. $_{L}$-NNA, $N^{G}$-nitro-$_{L}$-arginine; GCE, ginger crude extract.

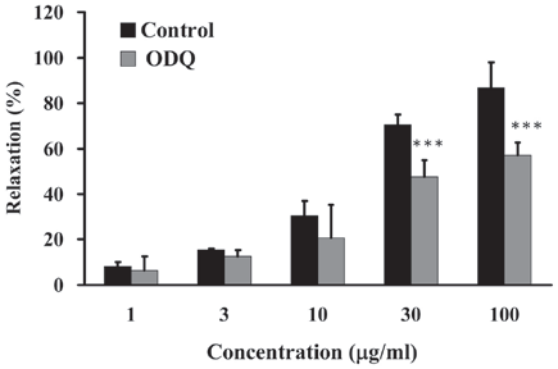


Figure 5. Effects of ODQ on GCE-induced vasorelaxation. Porcine coronary artery rings were incubated with various amounts of GCE (1, 3, 10, 30 and 100 $\mu$g/ml) in the absence (control) or presence of ODQ. Tension was determined by isometric force transduction. Data are presented as a percentage of 30 mM KCl-induced contraction. Values are expressed as the means ± standard deviation (n=6). $^{***}$P<0.001 vs. the control group. GCE, ginger crude extract; ODQ, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one.

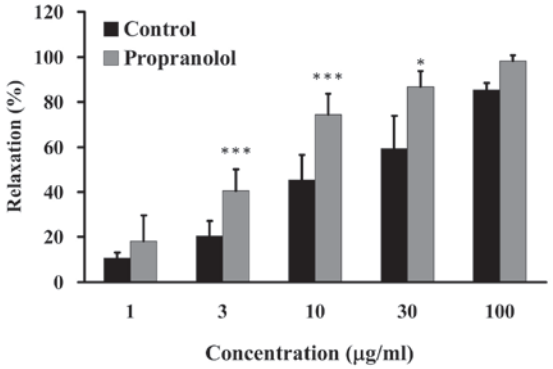


Figure 7. Effects of propranolol on GCE-induced vasorelaxation. Porcine coronary artery rings were incubated with various amounts of GCE (1, 3, 10, 30 and 100 $\mu$g/ml) in the absence (control) or presence of propranolol. Tension was determined by isometric force transduction. Data are presented as a percentage of 30 mM KCl-induced contraction. Values are expressed as the means ± standard deviation (n=6). $^{*}$P<0.05 and $^{***}$P<0.001 vs. the control group. GCE, ginger crude extract.

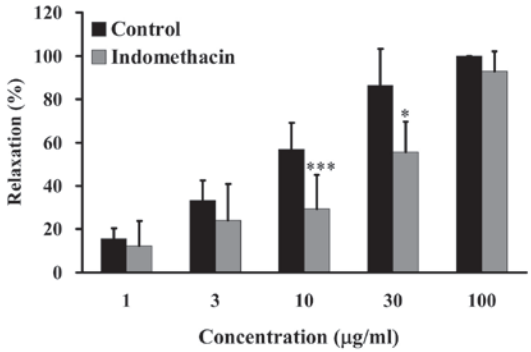


Figure 6. Effects of indomethacin on GCE-induced vasorelaxation Porcine coronary artery rings were incubated with various amounts of GCE (1, 3, 10, 30 and 100 $\mu$g/ml) in the absence (control) or presence of indomethacin. Tension was determined by isometric force transduction. Data are presented as a percentage of 30 mM KCl-induced contraction. Values are expressed as the means ± standard deviation (n=6). $^{*}$P<0.05 and $^{***}$P<0.001 vs. the control group. GCE, ginger crude extract.

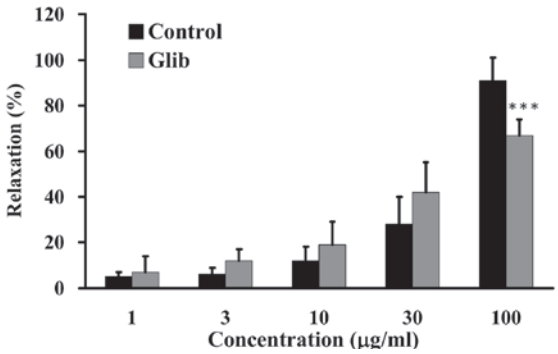


Figure 8. Effects of Glib on GCE-induced vasorelaxation. Porcine coronary artery rings were incubated with various amounts of GCE (1, 3, 10, 30 and 100 $\mu$g/ml) in the absence (control) or presence of Glib. Tension was determined by isometric force transduction. Data are presented as a percentage of 30 mM KCl-induced contraction. Values are expressed as the means ± standard deviation (n=6). $^{***}$P<0.001 vs. the control group. GCE, ginger crude extract; Glib, glibenclamide.

relaxation was increased in porcine coronary arteries following GCE exposure.

*The NOS signaling pathway is involved in GCE-induced relaxation*. Numerous *in vitro* and *in vivo* studies have reported that endothelium-dependent relaxation and vasodilatation persist in the presence of NOS inhibitors, including L-arginine analogues, such as $_{L}$-NNA (23,26). Porcine coronary artery rings were pretreated in the absence (control) or presence of 100 $\mu$M $_{L}$-NNA for 20 min, and were then incubated with 30 mM KCl to induce contraction until the tonic phase (27). Relaxation was examined in the presence of various concentrations of GCE (1, 3, 10, 30 and 100 $\mu$g/ml) in an organ bath. GCE induced relaxation of porcine coronary artery rings from 13 to 86% without $_{L}$-NNA pretreatment. Conversely, GCE (100 $\mu$g/ml) induced relaxation of porcine coronary artery rings to 66%, in the presence of $_{L}$-NNA (Fig. 4). These results revealed that GCE-induced relaxation of porcine coronary arteries may be mediated by the NOS signaling pathway.

*GCE improves relaxation via NO-activated soluble guanylate cyclase (sGC)*. The present study determined the effects of GCE on sGC-induced relaxation. Porcine coronary artery rings were pretreated in the absence (control) or presence of 10 $\mu$M ODQ for 20 min, and were then incubated with 30 mM KCl to induce contraction until the tonic phase. Relaxation was examined in the presence of various concentrations of GCE (1, 3, 10, 30 and 100 $\mu$g/ml) in an organ bath. GCE induced an increase in relaxation from 8 to 87% in porcine coronary artery rings following pretreatment without 10 $\mu$M ODQ. Conversely, relaxation of porcine coronary artery rings was significantly reduced following pretreatment with 10 $\mu$M ODQ and treatment with GCE at 30 and 100 $\mu$g/ml (Fig. 5). These results indicated that NO is a vital factor in GCE-induced relaxation of porcine coronary arteries.

*GCE improves relaxation via COX*. The present study further examined the effects of GCE on relaxation following treatment with indomethacin, which is an inhibitor of COX. Porcine

coronary artery rings were pretreated in the absence (control) or presence of 1 $\mu$g/ml indomethacin for 20 min, and were then incubated with 30 mM KCl to induce contraction until the tonic phase. Relaxation was examined in the presence of various concentrations of GCE (1, 3, 10, 30 and 100 $\mu$g/ml) in an organ bath. GCE induced an increase in relaxation from 15 to 100% in porcine coronary artery rings without 1 $\mu$g/ml indomethacin treatment. Conversely, following pretreatment with 1 $\mu$g/ml indomethacin and treatment with low concentrations of GCE (3-30 $\mu$g/ml), relaxation of porcine coronary artery rings was significantly attenuated (Fig. 6). These results suggested that GCE attenuated relaxation induced by arachidonic acid. GCE-induced relaxation of porcine coronary arteries may be through COX pathway.

*GCE has no effect on relaxation induced by β1-adrenergic receptor blocker.* β-blockers have been widely used in the treatment of numerous cardiovascular diseases, particularly hypertension and atherosclerosis (28). Some β1-adrenergic receptor blockers cause vasodilation by increasing NO (29). The present study examined the effects of GCE on relaxation induced by propranolol, which is a β-blocker. Porcine coronary artery rings were pretreated in the absence (control) or presence of 20 $\mu$M propranolol for 20 min, and were then incubated with 30 mM KCl to induce contraction until the tonic phase. Relaxation was examined in the presence of various amounts of GCE (1, 3, 10, 30 and 100 $\mu$g/ml) in an organ bath. GCE at 3-30 $\mu$g/ml induced an increase in relaxation from 11 to 91% in porcine coronary artery rings without pretreatment with 20 $\mu$M propranolol (Fig. 7). These results indicated that GCE exhibited no apparent effect on propranolol-induced relaxation.

*ATP-sensitive potassium ($K_{ATP}$) channel blocker exerts no effects on GCE-induced relaxation.* $K_{ATP}$ channels are activated and opened by declining intracellular ATP levels and elevated cAMP concentration, which leads to hyperpolarization of endothelial cells and the promotion of NO formation *in vitro* (30,31). It has been suggested that endothelial cell hyperpolarization may contribute to vascular relaxation. $K_{ATP}$ channels are inhibited by sulfonylurea agents, including Glib (31,32). The present study examined the effects of Glib, a $K_{ATP}$ channel blocker, on GCE-induced relaxation. Porcine coronary artery rings were pretreated in the absence (control) or presence of 1 $\mu$M Glib for 60 min, and were then incubated with 30 mM KCl to induce contraction until the tonic phase. Relaxation was examined in the presence of various amounts of GCE (1, 3, 10, 30 and 100 $\mu$g/ml) in an organ bath. GCE induced an increase in relaxation from 15 to 76% in the porcine coronary artery rings without 1 $\mu$M Glib pretreatment (Fig. 8). These results suggested that Glib had no effect on GCE-induced relaxation.

*GCE prevents Hcy-induced endothelial vasomotor dysfunction.* The present study investigated the effects of GCE on Hcy-induced endothelial cell damage. Porcine coronary artery rings were incubated with 30 $\mu$g/ml GCE for 15 min, and were then treated with 100 $\mu$M Hcy for 30 min. Porcine coronary artery rings were placed in an organ bath containing 30 mM KCl to induce contraction until the tonic phase. Relaxation was examined following the addition of 30 mM bradykinin into the organ bath. Hcy reduced relaxation, whereas GCE significantly prevented Hcy-induced endothelial dysfunction (Fig. 9). These results indicated that GCE may improve Hcy-induced endothelial cell damage.

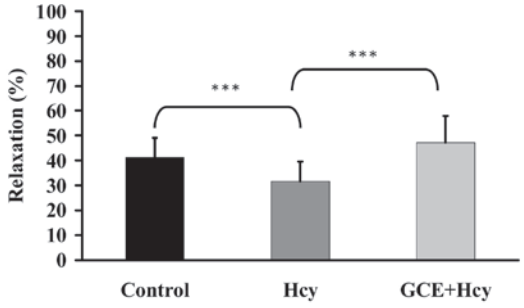


Figure 9. GCE improves Hcy-induced endothelial vasomotor dysfunction. Porcine coronary artery rings were incubated in the absence (control) or presence of 30 $\mu$g/ml GCE for 15 min, and were then treated with 100 $\mu$M Hcy for 30 min. Data are presented as a percentage of 30 mM KCl-induced contraction. Values are expressed as the means ± standard deviation (n=6). ***P<0.001. GCE, ginger crude extract; Hcy, homocysteine.

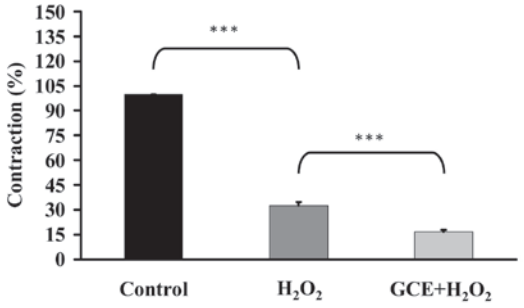


Figure 10. GCE improves $H_2O_2$-induced endothelial vasomotor dysfunction. Porcine coronary artery rings were incubated in the absence (control) or presence of 30 $\mu$g/ml GCE for 15 min and were then treated with 77.5 mM $H_2O_2$ for 30 min. Data are presented as a percentage of 30 mM KCl-induced contraction. ***P<0.001. GCE, ginger crude extract; $H_2O_2$, hydrogen peroxide.

*GCE prevents hydrogen peroxide ($H_2O_2$)-induced endothelial cell damage.* The present study clarified the effects of GCE on $H_2O_2$-induced endothelial cell damage. Porcine coronary artery rings were incubated with 30 $\mu$g/ml GCE for 15 min, and were then placed in an organ bath containing 30 mM KCl to induce contraction until the tonic phase. Rings were treated with 77.5 mM $H_2O_2$ for 15 min and contraction was examined. $H_2O_2$ induced endothelial contraction, whereas GCE significantly prevented $H_2O_2$-induced endothelial dysfunction (Fig. 10). These data revealed that GCE may attenuate $H_2O_2$-induced endothelial cell injury.

*Ginger extracts possess antioxidant abilities.* Reactive oxygen species (ROS) are produced under oxidative stress and adverse cellular environments (33). Vitamins E and C, β-carotene, flavonoids and polyphenols have previously been demonstrated to possess free radical-scavenging abilities (34). In the present study, the antioxidant properties of ginger extracts were individually determined according to DPPH and lucigenin-enhanced chemiluminescence assays. DPPH absorbance decreased

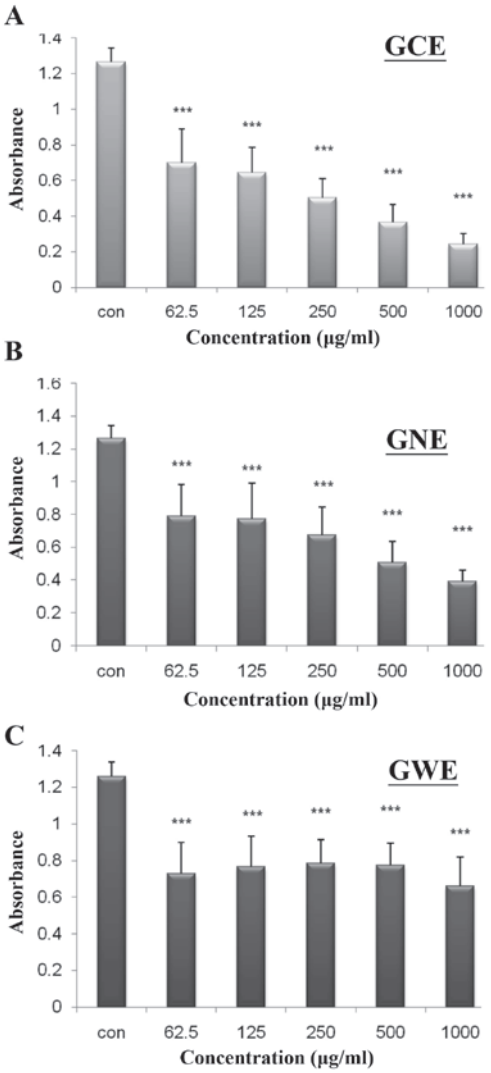


Figure 11. DPPH-scavenging activities of three varieties of ginger extract. DPPH was mixed with various amounts (62.5, 125, 250, 500 and 1,000 $\mu$g/ml) of (A) GCE, (B) GNE and (C) GWE. Data are presented as absorbance read at 517 nm. Values are expressed as the means ± standard deviation. ***P<0.001 vs. the control group (n=6). DPPH, 1,1-diphenyl-2-picrylhydrazyl; GCE, ginger crude extract; GNE, ginger *n*-butanol extract; GWE, ginger water extract.

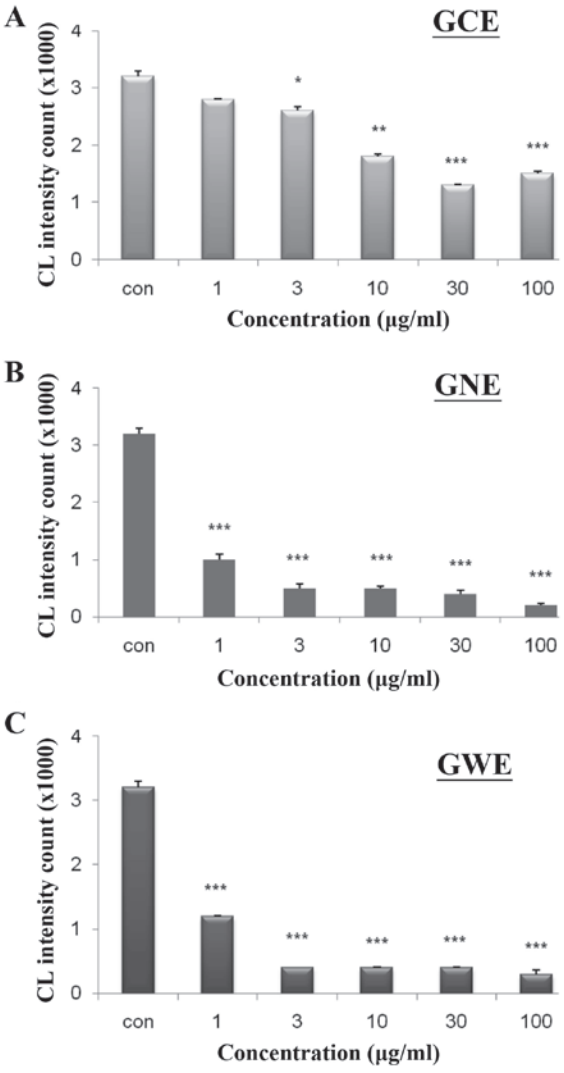


Figure 12. Antioxidant activities of three varieties of ginger extract were evaluated by lucigenin-enhanced chemiluminescence assay. Lucigenin was mixed with various amounts (1, 3, 10, 30 and 100 $\mu$g/ml) of (A) GCE, (B) GNE and (C) GWE. Data are presented as absorbance read at 517 nm. Values are expressed as the means ± standard deviation (n=6). *P<0.05, **P<0.01 and ***P<0.001 vs. the control group. GCE, ginger crude extract; GNE, ginger *n*-butanol extract; GWE, ginger water extract; CL, chemiluminescence.

from 0.70 to 0.24, as GCE concentration increased from 62.5 to 1,000 $\mu$g/ml (Fig. 11A). The rate of inhibition was increased from 40 to 85%in a dose-dependent manner. DPPH absorbance decreased from 0.79 to 0.39, as GNE concentration increased from 62.5 to 1,000 $\mu$g/ml. The rate of inhibition was increased from 37 to 78% in a dose-dependent manner (Fig. 11B). DPPH absorbance decreased from 0.73 to 0.66, as GWE concentration increased from 62.5 to 1,000 $\mu$g/ml. The rate of inhibition was increased from 42 to 48% (Fig. 11C). These findings indicated that GCE possesses a stronger ability to reduce free radical levels. To determine whether ginger extracts possess $H_2O_2$-scavenging abilities, a lucigenin-enhanced chemiluminescence assay was conducted. Various concentrations of GCE, GNE and GWE were used to evaluate their ability to remove $H_2O_2$. The $H_2O_2$-scavenging ability was increased from 10 to 52% in response to GCE (Fig. 12A). The $H_2O_2$-scavenging ability was increased from 68 to 94% in response to GNE (Fig. 12B) and from 63 to 90% in response to GWE (Fig. 12C). These findings indicated that GCE may possess a stronger antioxidant ability to scavenge free radicals.

*GCE exerts strong vasoprotective effects*. The present study investigated the effects of ginger extracts on Hcy-induced endothelial cell damage by analyzing the protein expression levels of endothelial NOS (eNOS), iNOS and COX-2. Hcy increased eNOS, iNOS and COX-2 expression. In the absence of Hcy, GWE induced eNOS, maintained iNOS and reduced COX-2 expression (Fig. 13A). Conversely, low concentration (10 $\mu$g/ml) of GWE slightly reduced eNOS, slightly induced iNOS and reduced COX-2 expression in the presence of Hcy. A high concentration (30 $\mu$g/ml) of GWE markedly reduced the expression levels of eNOS, iNOS and COX-2 in the presence of Hcy. GCE markedly reduced eNOS, iNOS and COX-2 expression in the presence of Hcy, whereas GNE markedly induced eNOS, iNOS and COX-2 expression in the presence of Hcy. These findings indicated that GCE exerts

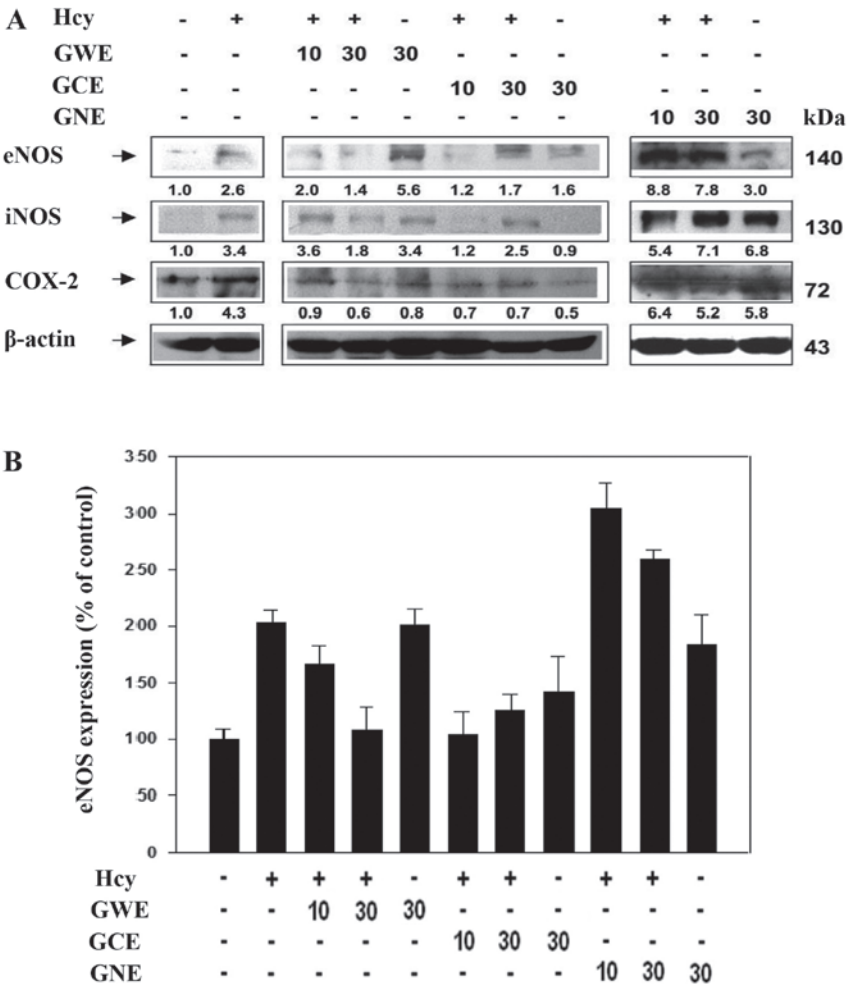


Figure 13. Vasoprotective effects of GCE were examined by western blot analysis. Porcine coronary artery rings were pretreated in the absence (control) or presence of 10 or 30 $\mu$g/ml GWE, GCE and GNE for 15 min, and were then incubated with 100 $\mu$M Hcy for 30 min. Cells were harvested and (A) eNOS, iNOS and COX-2 expression were analyzed by western blot analysis. (B) eNOS activity was also analyzed. Values are expressed as the means ± standard deviation of at least three independent experiments. COX-2, cyclooxygenase-2; eNOS, endothelial nitric oxide synthase; GWE, ginger water extract; GCE, ginger crude extract; GNE, ginger *n*-butanol extract; iNOS, inducible nitric oxide synthase.

stronger vasoprotective effects. In addition, eNOS expression was quantified from western blot analysis (Fig. 13B). These data suggested that GCE, not GWE or GNE, possesses a strong vasoprotective effect.

## Discussion

Numerous phytochemicals used in traditional Chinese medicine have beneficial health effects on blood pressure and endothelial function (19,35). Ginger, which is a spice used to enhance the flavor of foods, has been used for centuries in the Taiwanese, Chinese, Indian, Arabic, Tibetan, Unani and Siddha systems of traditional medicine (7-9). It has previously been reported that ginger possesses various beneficial pharmacological effects, including hypoglycemic, insulinotropic and hypolipidemic activities, in humans and animals (13-16). Ginger, and its extracts, have also been reported to possess anticancer, analgesic and antioxidant pharmacological activities (11-13). The present study demonstrated that GCE exerts strong vasoprotective effects and exhibits free radical-scavenging abilities in porcine coronary arteries *in vivo*.

Ginger has been used to treat cardiovascular diseases for a long time, and it is known to exert diuretic and blood pressure-lowering functions (7,9,10). In the present study, GCE relaxed porcine coronary arteries in a dose-dependent manner (Fig. 2). In rats, ginger has been reported to exhibit hypotensive, endothelium-dependent and -independent vasodilatory effects (36). Distinct receptors on the surface of the aorta and coronary arteries result in varying responses to stimulants. For example, epinephrine induces vasoconstriction of the aorta, but vasodilation of the coronary arteries (37). The present results indicated that GCE may relax KCl-induced contraction of endothelium-intact porcine coronary artery rings, whereas GCE only exerted a mild effect on relaxation of endothelium-denuded porcine coronary artery rings (Fig. 3). These data suggested that GCE may induce endothelium-dependent relaxation of porcine coronary arteries. NO is a major mediator of endothelium-dependent arterial relaxation.

Vasodilators, including NO, prostaglandin $I_2$ and endothelium-derived hyperpolarizing factor, contribute to endothelium-dependent relaxation (38). The present results indicated that GCE-induced endothelium-dependent relaxation was markedly inhibited by $_L$-NNA, an endothelial NOS inhibitor (Fig. 4). NO activates sGC, which is responsible for the enzymatic conversion of GTP to cyclic GMP (cGMP). An

increase in cGMP has been reported to mediate relaxation of coronary arteries. ODQ, which is a potent inhibitor of NO-activated sGC, inhibits NO-stimulated activity (39). In the present study, GCE-induced relaxation was significantly attenuated in the porcine coronary artery rings in response to pretreatment with ODQ (Fig. 5). These results indicated that the NO signaling pathway may be involved in GCE-induced relaxation of porcine coronary arteries. Arachidonic acid causes endothelium-dependent relaxation of coronary arteries (40). COX converts arachidonic acid into prostaglandin G2 (41). The present results indicated that GCE -induced relaxation was significantly attenuated in porcine coronary artery rings in response to pretreatment with indomethacin (Fig. 6). These data suggested that COX may be involved in GCE-induced relaxation of porcine coronary arteries.

Elevated Hcy levels in the blood (hyperhomocysteinemia) induce endothelial cell injury and are correlated with the occurrence of blood clots, which in turn may lead to atherogenesis. Hcy is a possible risk factor for coronary artery disease (42). Ilkhanizadeh *et al* (43) demonstrated that ginger extract may significantly reduce cardiac structural abnormalities in diabetic rats, and these effects were associated with improvements in serum apolipoprotein, leptin, cathepsin G and Hcy levels. The present results suggested that Hcy reduced relaxation, whereas GCE significantly prevented Hcy-induced endothelial dysfunction.

ROS are well-known mediators of vascular damage. $H_2O_2$ induces contraction in isolated canine basilar arteries (44). The present study revealed that GCE improved $H_2O_2$-induced endothelial cell injury, and possessed a stronger antioxidant ability to scavenge free radicals, compared with GNE and GWE. Dugasani *et al* (14) demonstrated that [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol exhibited substantial scavenging activities with half maximal inhibitory concentration ($IC_{50}$) values of 26.3, 19.47, 10.47 and 8.05 $\mu$M against DPPH radical, $IC_{50}$ values of 4.05, 2.5, 1.68 and 0.85 $\mu$M against superoxide radical and $IC_{50}$ values of 4.62, 1.97, 1.35 and 0.72 $\mu$M against hydroxyl radical, respectively. 6-Shogaol exhibited the most potent antioxidant and anti-inflammatory properties. In addition, elevated Hcy levels in the blood are associated with atherogenesis. It has been reported that Hcy increases the mRNA expression levels of eNOS and upregulates iNOS expression, thus resulting in COX-2 production, which eventually leads to the inflammatory response (45,46). The present study examined the effects of ginger extracts on Hcy-induced endothelial cell damage and on the protein expression levels of eNOS, iNOS and COX-2. Hcy increased eNOS, iNOS and COX-2 expression, whereas GCE markedly reduced eNOS, iNOS and COX-2 expression in the presence of Hcy (Fig. 13). These results indicated that GCE may exert a strong vasoprotective effect.

In conclusion, the present study is the first, to the best of our knowledge, to demonstrate that GCE may induce relaxant and vasoprotective effects on porcine coronary arteries, and may possess free radical-scavenging activities. Therefore, GCE may be considered a potential cardioprotective factor in the context of human diseases.

## Competing interests

The authors declare that they have no competing interests.

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