Vasodilatory effects and underlying mechanisms of the ethyl acetate extracts from Gastrodia elata Blume

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Vasodilatory effects and underlying mechanisms of the ethyl acetate extracts from *Gastrodia elata* Blume

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Abstract: The objective of this study was to assess the ethyl acetate extracts of *Gastrodia elata* Blume (GEB) on vascular tone and the mechanisms involved. GEB was extracted with 95% EtOH followed by a further extraction with ethyl acetate. The effects of GEB and its ingredients on the isometric tensions of the aortic rings from rats were measured. The ethyl acetate extract of GEB induced a vasodilator effect on rat aorta which was partially dependent on endothelium. Four chemical compounds isolated from GEB were identified as 3, 4-dihydroxybenzaldehyde (DB), 4-hydroxy benzaldehyde (HB), 4-methoxybenzyl alcohol (MA) and 4, 4'-dihydroxydiphenyl methane (DM), respectively. All of these compounds induced vasodilatations which were dependent on the endothelium to different degrees. After pretreated with *N*-nitro-L-arginine methyl ester, indomethacin or methylene blue, the vasodilatations induced by DB, HB and MA were significantly decreased. In addition, the contractions of the rat aortic rings due to *Ca*^{2+} influx and intracellular *Ca*^{2+} release were also inhibited by DM. Furthermore, the administration of DB significantly enhanced the productions of nitric oxide (NO) and the activities of the endothelial NO synthase in aorta and in endothelial cells. Thus, GEB may play an important role in the amelioration of hypertension by modulating vascular tones.

Key words: *Gastrodia elata* Blume, vasodilatation, endothelial cell, hypertension.
Introduction

As a saprophytic perennial herb of the Orchidaceae family, *Gastrodia elata Blume* (GEB, also known as *Tianma* in Chinese) was first recognized as one medicinal plant in Chinese Materia Medica (*Shennon bencaojing*), the oldest Chinese book on pharmacology (a compilation of oral traditions written between about 300 BC and 200 AD). It has been used since ancient times in China for treating a variety of disorders, such as vertigo, general paralysis, and epilepsy (Ahn et al. 2007; Yang et al. 2007). Meanwhile, GEB is also used for the treatment of cardiovascular diseases. For example, *Tianma Gouteng* Decoction, which uses GEB as the principal ingredient, is widely used to treat hypertension-related symptoms in clinical practice in East Asia (Wang et al. 2013; Zhang et al. 2012a). Up till now, several major physiological substances have been identified from GEB by our and other research groups (Duan et al. 2013; Lee et al. 2012; Yang et al. 2007). Among these active substances acidic polysaccharides purified from GEB have been demonstrated to decrease the systolic blood pressure in spontaneously hypertensive rats (Lee et al. 2012). Meanwhile, investigations also suggest GEB ameliorates the functions of vascular endothelial cells (ECs) (Hwang et al. 2009; Lee et al. 2009) and smooth muscle cells (Zhu et al. 2012) which play pivotal roles in the development of hypertension (Lacolley et al. 2012; Tang and Vanhoutte 2010).

The vascular ECs, the thin layer of cells that lines the inter surface of blood vessels, is currently considered as a central player for cardiovascular homeostasis (Tang and Vanhoutte 2010). The ECs can be activated by various chemical and physical stimuli to generate endothelium-derived autacoids to modulate the vascular tones (Tang and...
Vanhouette 2010). Since the changes of vascular tones play important roles in the regulation of blood pressure (Tang and Vanhouette 2010), the vasoactive effects of GEB and its active ingredients have also been investigated. Although the effects of GEB on vascular functions seem to be largely dependent on the endothelium (Kho et al. 2014), gastrodin, an active ingredient purified from GEB, has also been demonstrated to induce an endothelium-independent vasodilatation in rat thoracic aorta (Zhang et al. 2012b). These contradictory findings suggest that complicated mechanisms might be involved the GEB-induced vasodilatations. In addition, the vasodilatory effects of other active ingredients of GEB have hitherto not been investigated yet. Therefore, in the present study, we sought to investigate the vasodilatory effects and underlying mechanisms of the ethyl acetate extracts from GEB in rat aorta.

**Materials and methods**

**Chemicals and drugs**

$\textit{N}^\omega$-nitro-L-arginine methyl ester (L-NAME), methylene blue (MB), indomethacin (INDO), acetylcholine (ACh), phenylephrine (PE), and ethylene glycol-bis(2-aminoethylether)-$N\text{,}N\text{,}N\text{,}N\text{'}$-tetraacetic acid (EGTA) were purchased from Sigma Aldrich Inc. (St. Louis, MO, USA). The other reagents were of analytical purity.

**Preparation of the extract of GEB**

Dried GEB used in the present study was purchased from Tianma Research Institute in Zhaotong (Zhaotong, Yunnan Province, P.R. China). The plant was identified at the Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, China, where a voucher specimen, MZYG0001, was deposited.
Five hundred grams of pulverized GEB was extracted three times with 95% EtOH at room temperature, and the organic solvent was evaporated under reduced pressure. Then the extracts were suspended in deionized water (1500 ml), followed by an extraction with ethyl acetate for 6 times. The extracts were filtered and concentrated under reduced pressure using rotary evaporator at 40°C until extraction solvent was completely dried. The extracts were stored in the refrigerator at 4°C for further use. They were dissolved in a vehicle containing ethyl acetate or deionized water before studies were carried out.

**Chromatographic analysis**

The high performance liquid chromatography (HPLC, UltiMate3000, Dionex, USA) equipped with Ultimate™ XB-C18 column (4.6 mm i.d.×250 mm, 5 µm particle size, Welch, Shanghai, China) and a diode-array detector (DAD, Agilent G 1315B) was used for analysis of ethyl acetate extract of the fruiting bodies of GEB. Acetonitrile (solvent A) and 1% acetic acid (solvent B) were used as mobile phase. Samples were eluted in gradient mode. The gradient elution program is shown in Table 1. The detection wavelength was set at 279 nm. The flow rate was 0.9 ml/min. The sample injection volume was 10 µL and the column temperature was maintained at 30°C. The solvents used for analysis were purchased from Fisher Scientific (Pittsburgh, PA, USA).

**Animals and tissue preparation**

Male Sprague-Dawley rats (250-300 g) were supplied from the Laboratory Animal Unit of Kunming Medical University (Kunming, China). All experiments performed in this study were approved by the Committee on the Use of Live Animals in Teaching and Research of Yunnan Minzu University. After anesthetized with pentobarbitone sodium (50
mg/kg, i.p.), the rats were killed by stunning and cervical dislocation and the thoracic aorta was dissected out. The aorta was cleaned of adhering fat and connective tissue and cut into 3 mm wide rings. Care was taken to avoid abrading the intimal surface in order to maintain the integrity of the endothelial layer. In certain experiments, endothelium was removed by gently rubbing the intimal space with a cotton swab.

**Isometric tension measurement**

The aortic rings were immediately immersed in Krebs solution (mM: 118 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 25 NaHCO₃, and 11.1 glucose, at 37°C), aerated with 5% CO₂/95% O₂ and connected to a force transducer (HV-4, TME Technology Co, Ltd., Chengdu, China). The rings were stretched progressively to their optimal resting tensions (2.0 g; determined in preliminary experiments) and allowed to equilibrate for 60 min. All changes in tension were expressed as a percentage decreased in the contraction to PE (1 µM). The absence of relaxation responses to ACh was taken as evidence that vessel segments were functionally denuded of endothelium.

**Protocol 1: vasodilatory effects of GEB on rat aorta**

PE was used to induce steady contraction in aortic rings followed by cumulative addition of 95% EtOH extract (3, 6, 9, 12, 18 mg/ml) and ethyl acetate extract (0.75, 1.5, 3, 6, 9 mg/ml) of GEB, respectively. After the ingredients were extracted from GEB, aortic rings were administrated with cumulative additions of the ingredients.

**Protocol 2: mechanisms underlying the vasodilatory effects of the ingredients from GEB.**

In order to study whether or not nitric oxide (NO), 3',5'-cyclic guanosine monophosphate (cGMP), and prostacyclin (PGI₂) were involved in the
endothelium-dependent vasodilatory effect of the ingredients from GEB, endothelium-intact rings were incubated with L-NAME (a endothelial NO synthase inhibitor; 100 $\mu$M), MB (a soluble guanylyl cyclase inhibitor; 10 $\mu$M), or Indomethacin (INDO; a nonselective inhibitor of cyclooxygenase, 10 $\mu$M) for 60 min prior to the application of PE, respectively. Ingredients extracted from GEB were then cumulatively added to the tissue.

To verify whether or not extracellular Ca$^{2+}$ influx was involved in the endothelium-independent vasodilatations induced by ingredients from GEB, rat aortic rings with endothelium denuded were washed with Ca$^{2+}$-free Krebs solution four to five times before PE (1 $\mu$M) was applied. Then, Ca$^{2+}$ was added cumulatively to obtain a concentration-response curve (0.01-3 mM). Certain ingredients from GEB were added 10 min before the addition of Ca$^{2+}$.

To study whether or not the vasodilatations induced by ingredients from GEB were related to the inhibition of intracellular Ca$^{2+}$ release, the aortic rings with endothelium removed were exposed to Ca$^{2+}$-free solution with 50 $\mu$M EGTA for 15 min before the application of PE (1 $\mu$M) to induce the first transient contraction (con1). The rings were then washed three times and incubated with normal Krebs solution for at least 40 min to refill the intracellular Ca$^{2+}$ stores. Subsequently, the normal Krebs solution was rapidly replaced with Ca$^{2+}$-free solution and the rings were incubated for another 15 min. The second contraction (con2) was then induced by PE (1 $\mu$M) in the absence or presence of 100 mg/L SC or 1000 mg/L, which was added 10 min before PE application. The ratio of the second contraction to the first contraction (con2/con1) was calculated.
Culture of rat aortic arterial endothelial cells (RAECs)

RAECs were isolated from the aorta of SD rats, using the previous protocol with slight modification (Yang et al. 2006). Cells were incubated at 37°C (5% CO₂/95% air) in RPMI 1640 containing 10% fetal calf serum (Gibco, Invitrogen, California, USA), 100U/ml G-penicillin (Gibco) and 100 µg/ml streptomycin (Gibco). The identity of RAECs was confirmed by immunostaining using the goat anti-rat Factor VIII (an endothelial cell marker), goat anti-rat α-actin antibody (a vascular smooth muscle cell marker).

Measurements of the release of NO and the activity of eNOS in aorta and RAECs

For measuring the release of NO from aorta, the concentration of NO was measured in an organ bath system using carbon microsensors with a NO permeable membrane (ISONOPF100, World Precision Instruments Inc., USA). After the equilibration and rinse of the aortic rings, DB (0.1-10 µM) was administrated to the organ chamber. Then the concentration of NO in the buffer was measured 10 min after the administration of each dose of DB. For measuring the release of NO from RAECs, after the culture mediums were changed cells were incubated with different concentrations of DB (0.1-10 µM) for 10 min. Then concentration of NO in the culture medium was detected with NO kit (Jiancheng Bio-engineering Institute, Nanjing, China) according to the instruction manual. For detecting the effects of DB on the activities of eNOS in aorta and RAECs, aortic rings were incubated with DB (10 µM) in organ chambers, and RAECs were treated with DB (10 µM) for 10 min. Then, the activity of eNOS was determined as described previously (Wang and Abdel-Rahman 2002) by using a commercial kit (Jiancheng).

Statistical analysis
The changes in isometric tension were expressed as the percentage decreased in the contraction to PE. All data were expressed as means±S.E.M. Statistical analyses were performed using SPSS 22.0 (SPSS Inc., Chicago, IL). Comparison between two groups was analyzed using Student’s $t$-test. Comparison among three or more groups was analyzed using one-way ANOVA, $P<0.05$ was considered statistically significant.

**Results**

**The HPLC chromatogram of GEB**

The HPLC chromatogram of the ethyl acetate extract of GEB at 279 nm is presented in Fig. 1A. Four chemical compounds were isolated and identified as: (1) 3, 4-Dihydroxybenzaldehyde (DB); (2) 4-Hydroxy benzaldehyde (HB); (3) 4-Methoxybenzyl alcohol (MA); (4) 4, 4’-Dihydroxydiphenyl methane (DM) by using the reference standards (Fig. 1B). The Ultraviolet spectrum peaks of the above four compounds from GEB are shown in Fig. 1C-F [(C) MA; (D) HB; (E) DM; (F) DB]. Fig. 2A-D are the chemical structural formulas of the above four compounds.

**The vasodilatation induced by GEB**

As shown in Fig. 3A, 95% EtOH extract of GEB induced an endothelium-independent vasodilatation. The EC50 values are 0.93 mg/ml. The ethyl acetate extract of GEB also induced a vasodilatatory effect on rat aorta with a slight lower EC50 value (0.72 mg/ml, Fig. 3B). However, in endothelium-denuded aorta, the vasodilatation induced by the ethyl acetate extract of GEB was significantly attenuated ($P<0.05$, compared with endothelium-intact group). Fig. 3C and D showed the endothelium-independent vasodilatations produced by sodium nitroprusside, a NO donor, and the
endothelium-dependent vasodilatations induced by ACh in rat thoracic aortas.

The vasodilatation induced by the ingredients from GEB

Among the four obtained chemicals extracted from GEB, MA, HB and DB induce vasodilatations which were largely dependent on the endothelium (Fig. 4A-C). The EC50 values are 1.51 mM (MA), 0.63 mM (HB) and 3.89 µM (DB), respectively. However, the vasodilatation induced by DM was not largely dependent on the endothelium, with an EC50 value at 0.47 mM (Fig. 4D).

Mechanisms underlying the vasodilatory effects of the ingredients from GEB

Fig. 5A-C shows that the vasodilatory effects of MA, HB and DB were significantly reduced by L-NAME, INDO and MB to a different extend. However, the vasodilatation induced by DM was markedly impaired by L-NAME but was not notably influenced by INDO or MB (Fig. 5D).

To study the effect of DM on Ca^{2+} channels, studies were performed in Ca^{2+}-free preparations. Priming with PE (1 µM) produced small increase in resting tone, and subsequent cumulative addition of CaCl_2 (10 µM to 3mM) caused stepwise constriction. Fig. 5E shows that the Ca^{2+}-dependent contraction was inhibited when the aortic rings were pre-incubated with DM (0.3 mM) for 30 min before PE was applied (P<0.05, compared with control group). The Ca^{2+}-dependent contraction was further attenuated when higher concentrations of DM (1 mM and 3 mM) were applied (1 mM: P<0.05; 3 mM: P<0.01).

To study the effect of DM on intracellular Ca^{2+} release, first contraction was induced by PE (1 µM) in Ca^{2+}-free buffer with EGTA (50 µM). The intracellular Ca^{2+} was then refilled by incubating the tissue in normal Krebs solution. The second contraction was
induced by PE in Ca\textsuperscript{2+}-free solution again. Fig. 5F shows that the ratio of second contraction to first contraction was significantly decreased when the tissue was pretreated with DM before second contraction (0.3 mM: \( P<0.05 \); 1 mM: \( P<0.01 \); 3 mM: \( P<0.01 \)).

In addition, the productions of NO from aorta and RAECs were concentration-dependently enhanced by the administration of DB (Fig. 6A and B). Similarly, the activities of eNOS in aorta and RAECs were also markedly elevated after treatment with DB (Fig. 6C and D).

**Discussion**

Traditionally, hypertension is diagnosed when an individual crosses an established blood pressure threshold, systolic blood pressure of 140 mmHg or higher, and a diastolic blood pressure of 90 mmHg or higher (Giles et al. 2005). However, the evolving definition of hypertension have put additional insight into early hypertension-related cardiovascular diseases (CVDs), such as the endothelial dysfunction, since these early CVDs may be established before identifiable blood pressure thresholds are crossed (Giles et al. 2005). The physiological substances purified from GEB have been demonstrated to decrease the systolic blood pressure in spontaneously hypertensive rats (Lee et al. 2012). Meanwhile, it has also been demonstrated that GEB enhanced ACh-induced vasodilatation in endothelium-intact thoracic aorta (Feng et al. 2012) and ameliorated the impaired endothelium-dependent vasodilatation in rat carotid arteries (Kho et al. 2014). These findings suggest that GEB and its ingredients may not only decrease the blood pressure but also ameliorate the early hypertension-related CVDs.

As an early hypertension-related CVD, endothelial dysfunction is a common feature
of hypertension, and it results from the imbalanced release of endothelium-derived vasodilators, in particular NO and PGI₂, and endothelium-derived vasoconstrictors, such as angiotensin II an ET-1 (Tang and Vanhoutte 2010). The endothelium-dependent vasodilatation is achieved by combined effects of endothelium-derived vasodilators, NO, PGI₂, and endothelium-derived hyperpolarizing factor (EDHF) (Garland et al. 2011). EDHF plays a more significant contribution to the vasodilatations in smaller resistance arteries whereas NO and PGI₂ play greater roles in large-conduit arteries (Feletou and Vanhoutte 2004). NO and PGI₂ can be synthesized by ECs in response to physiological stimuli such as shear stress or chemical stimuli (Khazaei et al. 2008). Diffused from ECs, NO elicits vasodilatations by activating soluble guanylyl cyclase and increasing the production of cGMP, whereas PGI₂ stimulates adenylyl cyclase and potentiates the generation of cAMP in vascular smooth muscle to induce the vasodilatory effects (Bryan et al. 2005; Khazaei et al. 2008). In the present study, 95% EtOH extract of GEB induced an endothelium-independent vasodilatation but the ethyl acetate extract of GEB induced a stronger vasodilatatory effect on rat aorta with a quite lower EC50 value which was partially dependent on endothelium. This finding indicates that quite a few active ingredients extracted from GEB by ethyl acetate may induce vasodilatations which are dependent on endothelium. Although the EC50 values of these three active chemicals extracted from GEB by ethyl acetate are quite different, the mechanisms involved in their endothelium-dependent vasodilatations are almost the same. The activations of both NO-cGMP pathway and/or PGI₂ pathway greatly contribute to the endothelium-dependent vasodilatations induced by MA, HB and DB. These mechanisms were further confirmed
since DB also enhanced the activities of eNOS and the productions of NO in both ECs and aorta.

Although the activation of NO pathway may also be involved in DM-induced endothelium-dependent vasodilatation, the major vasodilatory effect of DM seems not dependent on endothelium. Our findings suggest the mechanisms involved in DM-induced endothelium-independent vasodilatation are closely associated with the inhibitions of Ca\(^{2+}\) influx and intracellular Ca\(^{2+}\) release. Ca\(^{2+}\) is a critical factor in excitation-contraction coupling in smooth muscle cells (Lohn et al. 2000; Wellman and Nelson 2003). There are two types of Ca\(^{2+}\) channels on the plasma membranes of vascular smooth muscle cells: receptor-operated Ca\(^{2+}\) channels (ROCCs) and voltage-dependent Ca\(^{2+}\) channels (VDCCs) (Xiong and Sperelakis 1995). Influx of Ca\(^{2+}\) through ROCCs and VDCCs and the release of Ca\(^{2+}\) from sarcoplasmic reticulum by activation of 1,4,5-triphosphate inositol and ryanodine receptors (Imtiaz et al. 2006; Thorneloe and Nelson 2005) result in the increased intracellular Ca\(^{2+}\), which causes vascular smooth muscle contraction. Based on our results, we believe that DM inhibits the Ca\(^{2+}\) influx through VDCC and attenuate the intracellular Ca\(^{2+}\) release in vascular smooth muscle cells, thereby leading to a fall in intracellular Ca\(^{2+}\) level and hence causing vasodilatation.

In conclusion, our study has demonstrated that the ethyl acetate extracts of GEB induce both endothelium-dependent and -independent vasodilatations in rat thoracic aorta. The endothelium-dependent vasodilatations induced by the ethyl acetate extracts of GEB are predominantly mediated by NO-cGMP-dependent pathway but the PGI\(_2\)-dependent mechanism may be partly involved. The endothelium-independent vasodilatation induced
by the ethyl acetate extracts of GEB may be related to the inhibitions of voltage-dependent
Ca\(^{2+}\) channels and intracellular Ca\(^{2+}\) release. Our findings may provide assistance to the
better understandings of the mechanisms underlying the therapeutical effect of GEB on
cardiovascular system. In addition, our findings may pave the ways to discover useful
herbal drugs for the treatment of hypertension as well as for the amelioration of the early
hypertension-related CVDs.

**Acknowledgements**

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Table 1

HPLC elution programme profile.

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Figure legends

**Fig. 1** The HPLC chromatogram and the ultraviolet spectrum of main peaks of the acetoacetate extract of GEB at 279 nm. (A) HPLC chromatogram of GEB at 279 nm; (B) HPLC chromatogram of reference substances. (C) The ultraviolet spectrum of the peak at RT 8.980 min; (D) The ultraviolet spectrum of the peak at RT 12.913 min; (E) The ultraviolet spectrum of the peak at RT 16.613 min; (F) The ultraviolet spectrum of the peak at RT 32.153 min.

**Fig. 2.** The chemical structural formulas of the four compounds isolated from GEB by ethyl acetate. (A) 4-Methoxybenzyl alcohol; (B) 4-Hydroxy benzaldehyde; (C) 4, 4’-Dihydroxydiphenyl methane; (D) 3, 4-Dihydroxybenzaldehyde.

**Fig. 3.** Vasodilatory effects of 95% EtOH and ethyl acetate extracts of GEB on rat aortic rings with or without endothelium. (A) Vasodilatations induced by the 95% EtOH extract of GEB (B) Vasodilatations induced by the ethyl acetate extract of GEB. Changes in tension were expressed as the percentage decreased in the contraction to PE (1 µM). Values are means±S.E.M. (n=6). *P<0.05 compared with endothelium-intact group; **P<0.01 compared with endothelium-intact group.

**Fig. 4.** Vasodilatory effects of the ethyl acetate extracts of GEB on rat aortic rings with or without endothelium. (A) Vasodilatations induced by 4-Methoxybenzyl alcohol (MA); (B) Vasodilatations induced by 4-Hydroxy benzaldehyde (HB); (C) Vasodilatations induced
by 3, 4-Dihydroxybenzaldehyde (DB); (D) Vasodilatations induced by 4, 4’-Dihydroxydiphenyl methane (DM). Changes in tension were expressed as the percentage decreased in the contraction to PE (1 μM). Values are means±S.E.M. (n=6). *P<0.05 compared with endothelium-intact group; **P<0.01 compared with endothelium-intact group.

Fig. 5. Mechanisms underlying the vasodilatory effects of ethyl acetate extracts of GEB. (A)-(D) Effects of different pharmacological inhibitors on the vasodilatations-induced by 4-Methoxybenzyl alcohol (MA), 4-Hydroxy benzaldehyde (HB), 3, 4-Dihydroxybenzaldehyde (DB) and 4, 4’-Dihydroxydiphenyl methane (DM). Vasodilatory effects of the above four chemicals extracted from GEB on rat aorta were measured in the absence (control) or presence of methylene blue (MB, 100 μM; a soluble guanylyl cyclase inhibitor), L-NAME (100 μM; an endothelial NO synthase inhibitor) or indomethacin (INDO, 30 μM; a cyclooxygenase inhibitor). (E) Effect of DM on Ca^{2+}-dependent contraction in rat aorta. Experiments were carried out in Ca^{2+}-free Krebs solution. After addition of PE (1 μM), Ca^{2+} (10 μM to 3 mM) was cumulatively added to the aorta in the absence (control) or presence of DM (0.3 mM, 1 mM or 3 mM). (F) Effect of DM on intracellular Ca^{2+} release. The first contraction was induced by PE (1 μM) in Ca^{2+}-free solution with EGTA (50 μM). After the refill of the intracellularCa^{2+} in normal Krebs solution, the second contraction was induced by PE in Ca^{2+}-free solution again. Changes in tension were expressed as the percentage decreased in the contraction to PE (1 μM). Values are means ± S.E.M. (n = 6). *P <0.05 compared to the control. **P <0.01 compared to the
control.

Fig. 6. Effect of 3, 4-dihydroxybenzaldehyde (DB) on the productions of NO and the activities of eNOS in aorta and rat aortic endothelial cells (RAECs). (A) Effect of DB on the production of NO in aorta. Ten minutes after the administration of each dose of DB (0.1-10 µM), concentration of NO was measured in an organ bath system using carbon microsensors with a NO permeable membrane; (B) Effect of DB on the production of NO in RAECs. After cells were incubated with different concentrations of DB (0.1-10 µM) for 10 min, concentrations of NO in the culture medium were detected with a NO kit; (C) Effect of DB on the activity of eNOS in aorta; (D) Effect of DB on the activity of eNOS in RAECs. Values are means±S.E.M. (n=6). *P<0.05, **P<0.01 compared with Control group.
Figure 1.

508x557mm (300 x 300 DPI)
Figure 2.

(A) 4-Methoxybenzyl alcohol

(B) 4-Hydroxy benzaldehyde

(C) 4,4’-Dihydroxydiphenylmethane

(D) 3,4-Dihydroxybenzaldehyde
Figure 3.

550x513mm (300 x 300 DPI)
Figure 4.

550x444mm (300 x 300 DPI)
Figure 5.

508x563mm (300 x 300 DPI)
Figure 6.

550x441mm (300 x 300 DPI)