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Full Length Research Paper

Effects of fistular onion stalk extract on the level of NO and expression of endothelial NO synthase (eNOS) in human umbilical vein endothelium cells

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The stalk of *Allium schoenoprasum* L (fistular onion stalk) has been used in traditional Chinese medicine. In this study, we investigated the effects of fistular onion stalk extracts on nitric oxide (NO) production and endothelial NO synthase (eNOS) expression in cultured human umbilical vein endothelium cells (HUVECs). We found that the extracts of fistular onion stalk significantly increased the level of NO and the activity of eNOS. Our findings demonstrated that fistular onion stalk extracts could be a good candidate for new drugs to treat cardiovascular diseases by enhancing endothelial production of NO.

Key word: *Allium schoenoprasum* L, endothelium cells, nitric oxide, nitric oxide synthase.

INTRODUCTION

The stalk of *Allium schoenoprasum* L (fistular onion stalk), being one of the dietetic food, is the white portion of onion. It also has been used as a traditional Chinese medicine to treat a variety of diseases, including heart diseases (Rietz et al., 1993) due to its ability to lower blood pressure or inhibit platelet aggregation and thromboxane formation (Banerjee et al., 2002; Zhang et al., 2001; Briggs et al., 2001; Li et al., 2000; Boyle et al., 2000). Previous studies showed that the protective effects are attributed to the presence of steroid saponin and flavonoids in onion (Knekt et al., 1996). Recently, Jia-Huey had reported the effect of the green portion of Welsh onion on human platelet function *in vitro* (Chen et al., 2000). It is known that the blood vessel endothelial cells lie between blood flow and smooth muscle cells. Its damage and functional change play an important role in the start link of atherosclerosis working up (Hay et al., 2007; Cottone et al., 2007; Arbel et al., 2007). The obstacle of endothelial cell function is mainly demonstrated by a reduction in the blood vessel dilation factors or a decrease in the biological availability and an increase in the blood vessel constriction factors. Nitric oxide (NO) is produced from arginine by nitric oxide synthase (NOS). An increased NOS activity or NO concentration might help in protecting the endothelial cells and relaxing the blood vessel.

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Abbreviations: NO, Nitric oxide; **NOS,** nitric oxide synthase; **HUVECs,** human umbilical vein endothelium cells; **eNOS,** endothelial NO synthase.

#These authors contributed equally to this work.

MATERIALS AND METHODS

Preparation of extracts

Fistular onion stalk extracts were prepared as described previously (Chen et al., 2000). Briefly, the white-sheath part were separated and homogenized. After centrifugation, the supernatant was filtered, frozen at -80°C, and then lyophilized for 24 h. This dehydrated powder was dissolved in DMEM solution to obtain various concen-

trations.

Purification of extracts

Fistular onion stalk extracts were purified and screened for the presence of β-sitosterol, by using the methods previously described by Jayaprakasha (Arbel et al., 2007). The concentrated extract (80 g) was impregnated with 55 g of silica gel and chromatographed on silica column (100 cm × 35 mm). The column was washed with 2 L of dichloromethane (DCM), mixtures of DCM and acetone with increasing polarity and further concentrated under vacuum. The purity of the compounds was analyzed by high performance liquid chromatography (HPLC) using Varian Prsotar 240/330 (Varian, USA) equipped with a quaternary HPLC pump.

Preliminary phytochemical analysis

The β-sitosterol reference substance (HPLC content >98.0%) was purchased from Shanghai Tauto Biotech Co., Ltd., China. A Waters Prevail C18 analytical column (25 cm, 4.6 mm id, 5 lm particle size; Agilent, Zorbax, USA) was used. An isocratic mobile phase of 40% acetonitrile in water for 30 min, with a flow rate of 1.0 ml/min, was employed for elution. Prostar 330 wavelength detector at 210 nm was used to detect the compounds. The compounds were quantified using Star 6.0.

Cells culture

Aseptic neonate's umbilical cord was fetched and rinsed with aseptic phosphate buffered saline (PBS) before 12 to 15 ml 10.1% collagenase I was poured into the umbilical vein and hatched 15 min at 37°C. The collagenase solution containing a large number of endothelial cells was then collected using an aseptic syringe. After centrifugation for 10 min at 800 rpm/min, the supernatant was abandoned and DMEM solution was added into the tube and vaccinated in the culture flask. The endothelial cells were appraised by relevant antigen of VIII factor.

MTT assay for cell viability

The cytotoxicity of fistular onion stalk extracts was evaluated using MTT assay. In brief, 2×10^4 cells per well were plated into 96-well plates, cells/well to a final volume of 200 µl and then incubated at 37°C for 24 h before given a fresh change of medium. The cells were treated with various concentrations of fistular onion stalk extracts dissolved in DMEM. The control atorvastatin (HPLC content >98.0%, from Changzhou Comwin Fine Chemicals Co., Ltd, China) (100 µg/L) wells received the same amount of DMEM solution. After 24 h of incubation at 37°C, 20 µl of MTT (5 mg/ml) was added to each well and 4 h later, the cells were lysed with 150 µl of dimethy sulfoxide (DMSO). The plate was shaken for 10 and 30 min, later the optical densities for various concentrations of fistular onion stalk extracts were compared with the OD of the control or atorvastatin-stimulated wells to assess the cytotoxicity.

Nitric oxide assay

5×10^5 cells/well were seeded in 24-well plates, to a final volume of 500 µl followed by incubation at 37°C in 5% CO_2 for 24 h. The cells were further cultured for 24 h when treated with various concentrations of fistular onion stalk extracts dissolved in DMEM solution (final concentration from 100 to 12.5 µg/ml). The β-sitosterol and atorvastatin wells received the same amount of DMEM solution.

The supernatants were then collected and assayed for NO content by measuring the amount of nitrite using manufacturer's protocol of NO assay kit (R&D Systems, Minneapolis, MN). In this method, the nitrate present in the sample was reduced to nitrite by reduced nicotinamide adenine dinucleotide phosphate (GADPD) in the presence of the enzyme nitrate reductase. The nitrite formed reacted with sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride to give a red-violet diazo dye. Based on its absorbance within the visible range, the diazo dye was measured at 550 nm.

Western blotting

A cell lysates extract of the cells was resolved on SDS-acryl amide gel by electrophoresis and transferred to polyvinylidene difluoride membranes. The membranes were incubated with anti-NOS antibody (1:2000) at room temperature for 16 h followed by incubation with horse-radish peroxidase-conjugated anti-rabbit IgG antibody (1:2500) at room temperature for 3 h. Then, the blots were treated with ECL reagents and exposed to x-ray films.

Quantification of endothelial NO synthase (eNOS) mRNA by real-time PCR

The mRNA levels for eNOS was determined by real-time RT-PCR using QuantiTect SYBR Green RT-PCR Kit (Qiagen Ltd) on a LightCycler (Roche Diagnostics Ltd., Lewes, UK). Total RNA was isolated from cells of groups with the trizol reagent (Gibco BRL) according to the manufacturer's instruction. Primers for eNOS are (forward: TCG TGT GAA GAA CTG GGA GGTG and reverse: GGC CCT GTA GTT TCC GTG GA), human β-actin (CATGTACGTT GCTATCCAGGC and CTCCTTAATGTCACGCACGAT). RT-PCRs were performed in 20 µl reactions using 10 pmol of primers. Reverse transcription was carried out at 50°C for 15 min and cDNA was amplified in 32 cycles: 94°C for 10 s, 57°C for 5 s and 72°C for 5 s. Negative controls with no template were performed for each reaction. The relative quantity of gene expression was calculated according to the manufacturer's recommendations. Humam β-actin was used as an internal control to calculate the relative abundance of this gene.

Statistical analysis

The various concentrations of fistular onion stalk extracts and the relationship between different experimental groupings were compared and assessed by analysis of variance (ANOVA). Numerical values for each measurement are shown as mean±SD. Minimal statistical significance was judged at <0.05.

RESULTS AND DISCUSSION

The objective of this paper is to investigate the effects of different concentrations of fistular onion stalk extracts on NO production in HUVECs. The effects of fistular onion stalk on the NO metabolism were also studied. Our experimental evidence showed for the first time that fistular onion stalk contains a high concentration of β-sitosterol which has antihypertensive and antioxidative properties. Therefore, β-sitosterol may be an important ingredient in onion that contributes to its medicinal function. In this paper, we indentified the presence of β-sitosterol in fistular onion stalk extracts (Figure 1). It was

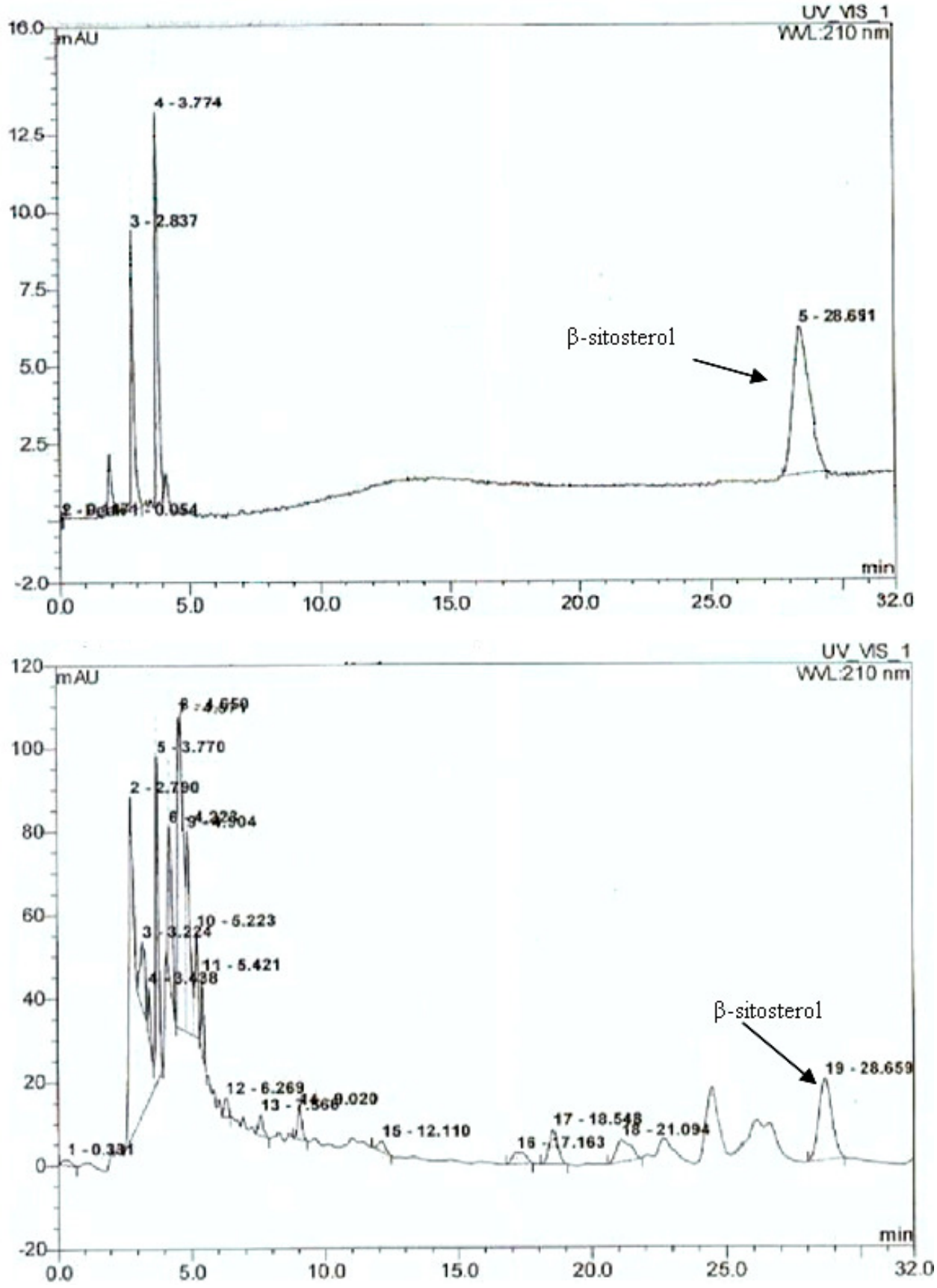

Figure 1. HPLC chromatogram of β-sitosterol. (A) Reference standards, (B) Fistular onion stalk extracts samples.

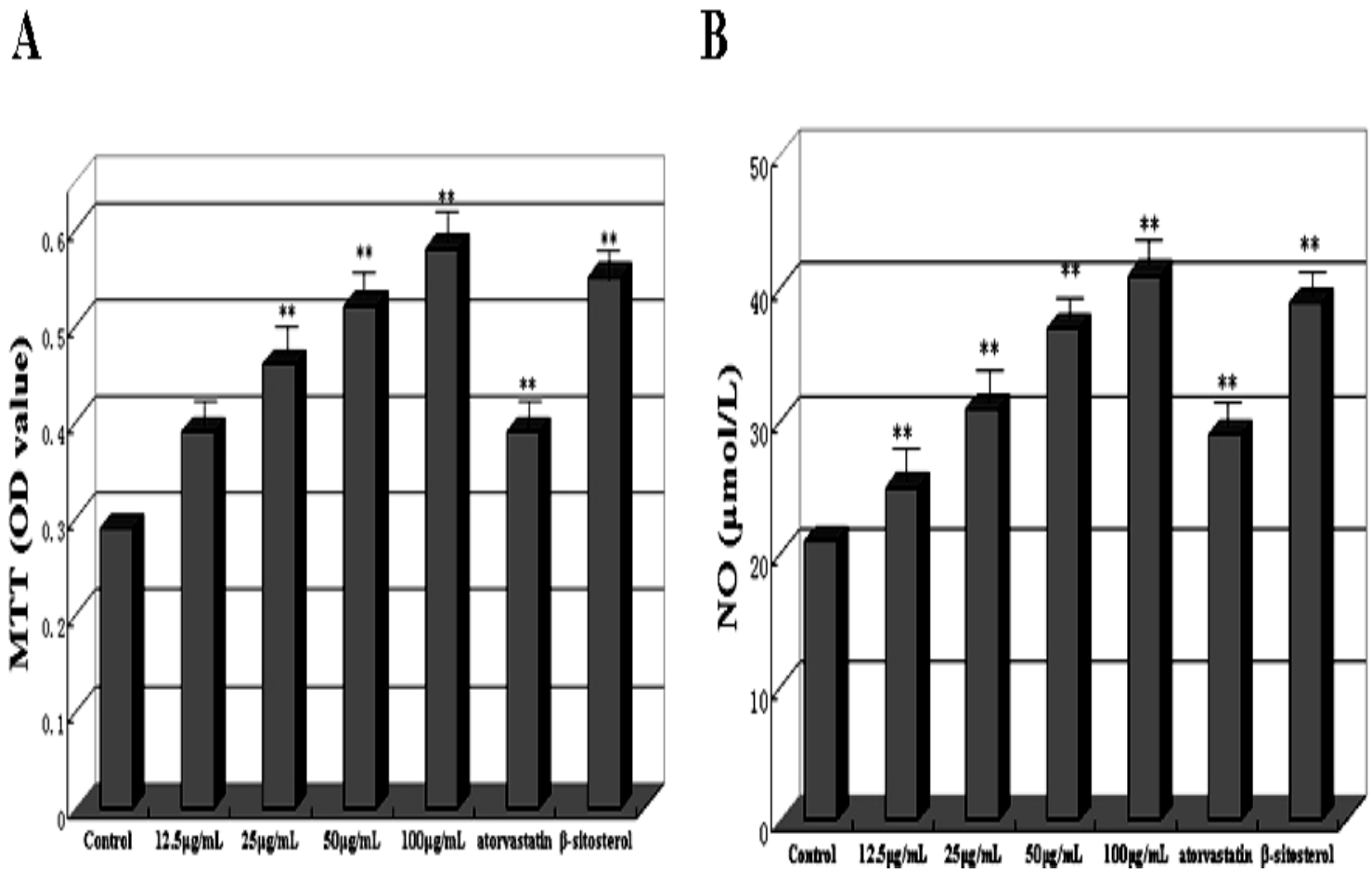

Figure 2. Effects of fistular onion stalk extracts on cell viability and NO production in HUVECs. It was demonstrated that fistular onion stalk extracts and β-sitosterol could reinforce the cell viability and increase NO production significantly in a dose-dependent manner in the HUVEC. Atorvastatin is the positive control. All data were obtained from three independent experiments. Error bars represent means+SD. Significantly different from the corresponding control (**P < 0.05, vs control group).

demonstrated that fistular onion stalk extracts and β-sitosterol could reinforce the cell viability (Figure 2A). It was also found that, in comparison with the normal control group, fistular onion stalk extracts increased NO production significantly in a dose-dependent manner in the HUVEC (Figure 2B). In order to show whether the reinforced effect of fistular onion stalk extracts and β-sitosterol on NO production was due to its influence on eNOS synthesis, the expression of eNOS gene was examined. The quantitative eNOS mRNA analysis showed that, compared with the control group, eNOS mRNA abundance of cells significantly increased when the cells were treated by β-sitosterol and fistular onion stalk extracts of different concentrations (P < 0.05) (Figure 3A). As indicated by western blot analysis, the fistular onion stalk extracts and β-sitosterol can also increase eNOS protein expression (Figure 3B). Western blot analysis and real-time RT-PCR showed that in each group cell, a sustained increase in expression of eNOS was observed after treatment with β-sitosterol and different concentrations of fistular onion stalk extracts, indicating that fistular onion stalk extracts could up-regulate eNOS expression.

From a therapeutic standpoint, elevation of NO production could provide an efficacious strategy for inhibiting the blood vessel constriction and protecting the endothelial cells function. In the present study, fistular onion stalk extracts was discovered to increase NO production in the HUVEC and could be considered as a candidate for the development of new drugs to treat cardiovascular system diseases accompanied by the overproduction of NO.

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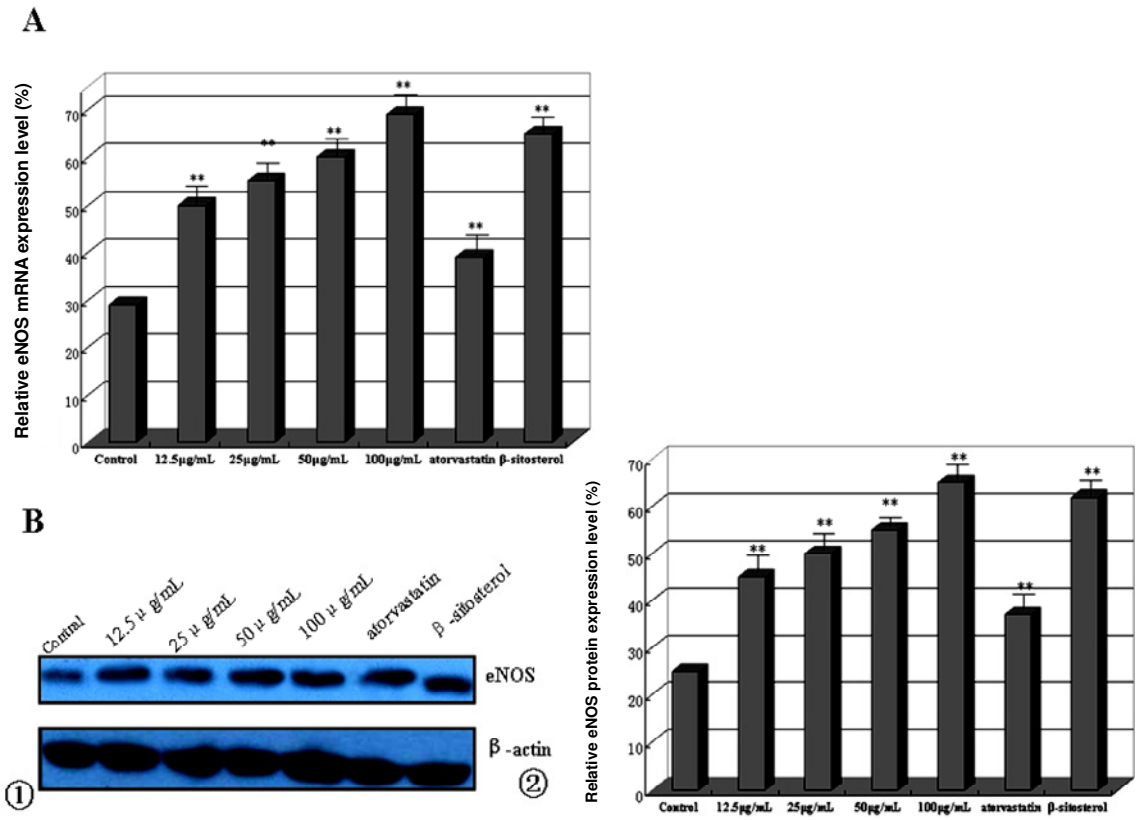

Figure 3. Effect of fistular onion stalk extraction on expression of eNOS gene. eNOS mRNA expression of HUVEC was detected by RT-PCR with different treatments (A).The eNOS protein expressions by Western blotting. β-Actin was used as an internal control (B-1). Protein expression of eNOS in HUVEC infected was quantified by densitometric analysis (B-2). Western blot analysis and real-time RT-PCR showed that in each group cell, a sustained increase in expression of eNOS was observed after treatment with β-sitosterol and different concentrations of fistular onion stalk extracts, indicating that fistular onion stalk extracts could up-regulate eNOS expression. Atorvastatin is the positive control. All data were obtained from three independent experiments. Error bars represent means+SD. Significantly different from the corresponding control (**P < 0.05, versus control group).

REFERENCES

Arbel Y, Dvir D, Feinberg MS (2007). The ASsociation between right coronary artery morphology and endothelial function. Int. J. Cardiol. 115: 19-23.

Banerjee SK, Dinda AK, Manchanda SC, Maulik SK (2002). Chronic garlic administration protects rat heart against oxidative stress induced by ischemic reperfusion injury. BMC Pharmacol. 16: 16-21.

Boyle SP, Dobson VL, Duthie SJ, Kyle JA, Collins AR (2000). Absorption and DNA protective effects of flavonoid glycosides from an onion meal Eur. J. Nutr. 39: 213-223.

Briggs WH, Folts JD, Osman HE, Goldman IL (2001). Administration of raw onion inhibits platelet-mediated thrombosis in dogs. J. Nutr. 131: 2619-2622.

Chen JH, Chen HI, Wang JS (2000). Effects of Welsh onion extracts on human platelet function *in vitro*. Life Sci. 66: 1571-1579.

Cottone S, Mule G, Nardi E (2007). Microalbuminuria and early endothelial activation in essential hypertension. J. Hum. Hypertens. 21: 167-172.

Hay A, Shi T, Juliet PA, Miyazaki A (2007). beta1 antagonist and beta2 agonist, celiprolol, restores the impaired endothelial dependent and independent responses and decreASed TNFalpha in rat with type II diabetes. Life Sci. 80: 592-599.

Knekt P, Hopia A, Jarvinen R, Reunanen A, Maatela J (1996). Flavonoid intake and coronary mortality in Finland: a cohort study. Br. Med. J. 312: 478-481.

Li G, Shi Z, Jia H, Ju J, Wang X, Xia Z, Qin L, Ge C, Xu Y, Cheng L, Chen P, Yuan G (2000). A clinical investigation on garlicin injectio for treatment of unstable angina pectoris and its actions on plasma endothelin and blood sugar levels. J. Tradit. Chin. Med. 20: 243-246.

Rietz B, Isensee H, Strobach H, Makdessi S, Jacob R (1993). Cardioprotective actions of wild garlic (allium ursinum) in ischemia and reperfusion. Mol. Cell Biochem. 119: 143-150.

Zhang WJ, Shi ZX, Wang BB, Cui YJ, Guo JZ, Li B (2001). *Allitridum mimics* effect of ischemic preconditioning by activation of protein kinase C. Acta Pharmacol. Sin. 22: 132-136.