J Food Sci Nutr
Vol 15, p 189～195 (2010)
DOI: 10.3746/jfn.2010.15.3.189

J Food Science and Nutrition

Vasorelaxant Activities of Aqueous Extracts from Twenty Medicinal Plants Used in Oriental Medicines in Isolated Rat Aorta

Eun-Young Kim[1], Kyung-Ok Lee[2], Dong-Il Kim[3], and Mee-Ra Rhyu[1†]

[1]*Functional Food Technology Research Group, Korea Food Research Institute, Gyeonggi 463-746, Korea*
[2]*Quality Control Lab, Hyundai Green Food Co., Ltd., Gyeonggi 448-503, Korea*
[3]*Department of Obstetrics & Gynecology, Dongguk University Ilsan Oriental Hospital, Gyeonggi 410-773, Korea*

Abstract

Water extracts from 20 medicinal plants, traditionally used for postmenopausal symptoms in Korea, were examined for their vasorelaxant activity in isolated rat thoracic aorta rings precontracted with norepinephrine (NE). Among the 20 medicinal plants, *Cornus officinalis* (CoEx, 0.3 mg/mL), *Schisandra chinensis* (ScEx, 0.3 mg/mL), *Erythrina variegate* (EvEx, 0.3 mg/mL), and *Epimedium koreanum* (EkEx, 0.3 mg/mL) showed rapid relaxation of endothelium-intact aorta (69±4%, 40±3%, 25±2%, and 23±3% of active tone induced by NE, respectively). In contrast, the extracts of *Erythrina variegata* (EvEx), *Angelica gigas* (AgEx), *Pueraria thunbergiana* (PtEx), and EkEx lead to gradual (i.e., long-term) relaxation to baseline in endothelium-intact vessels. The time to complete relaxation was 20～40 min. These 6 plant extracts were selected for the investigation of possible underlying mechanisms. The CoEx-, ScEx-, or EkEx-induced rapid relaxations were virtually abolished by endothelium denudation, and were significantly inhibited by pretreatment with nitric oxide (NO) synthase inhibitor N^G-nitro-L-arginine (L-NNA, 10 μM), indicating that increased formation of NO might contribute to the endothelium-mediated relaxation. In long-term responses, the endothelium denudation did not affect PtEx-induced relaxation, whereas it delayed responses by EvEx and AgEx, and significantly inhibited the effect of EkEx. Among EvEx, AgEx, and PtEx, EvEx attenuated the $CaCl_2$-induced vasoconstriction in high-potassium depolarized medium, implying that EvEx is involved in inhibition of the extracellular calcium influx to smooth muscle through voltage dependent calcium channels. These results provide the scientific rationale for the interrelationships between the use of 20 medicinal plants and their effects on cardiovascular health in estrogen deficient conditions.

Key words: medicinal plants, rapid relaxation, long-term vasorelaxation

INTRODUCTION

Medicinal plants have been used as traditional remedies in Asian countries for hundreds of years. Current research efforts are narrowing the knowledge gap between the traditional and scientific use of plants in treating various health conditions. Asian clinical prescriptions are usually composed of a combination of medicinal plants to provide synergistic pharmacological effects for greater efficacy than a single plant used alone. The following 20 plants: *Acanthopanax sessiliflorus*, *Aconitum carmichaeli*, *Angelica gigas*, *Astragalus membranaceus*, *Cnidium monnieri*, *Cornus officinalis*, *Cuscuta chinensis*, *Dioscorea japonica*, *Dipsacus asper*, *Epimedium koreanum*, *Erythrina variegata* var. *orientalis*, *Glycyrrhiza uralensis*, *Panax ginseng*, *Panax notoginseng*, *Polygonatum odoratum* var. *pluriflorum*, *Polygonatum stenophyllum*, *Polygonum multiflorum*, *Psoralea corylifolia*, *Pueraria thunbergiana*, *Schisandra chinensis*, have an extensive history of medicinal use as ingredients in various prescriptions for managing menopause-related symptoms in Korea (1,2). Despite their clinical use for alleviating menopausal symptoms, there is limited information available on their mechanism of action.

Phytoestrogens, a large family of plant-derived compounds, are similar in structure and function to estrogen (3). Phytoestrogens are believed to be important for the prevention of cardiovascular disease like estrogen (4), presumably due to their vasoprotective effect (5). *In vitro* experiments on vascular tissues have revealed that phytoestrogens produce endothelium-dependent or-independent relaxation, or both (6-8). Each of the 20 plants selected may provide phytoestrogens, which have a vasoprotective effect, based on its traditional uses in Korea. However, there has been no scientific data yet to show that they act on vascular tissues as phytoestrogens. The aim of this study was to examine the vasorelaxant effects in isolated aortic rings and possible mechanisms of action of 20 medicinal plants applied in various herbal pre-

†Corresponding author. E-mail: mrrhyu@kfri.re.kr
Phone: +82-31-780-9268, Fax: +82-31-709-9876

scriptions for managing menopause-related symptoms in Korean women.

MATERIALS AND METHODS

Preparation of plant extracts

All of the dried medicinal plants (Table 1) were purchased from a local market (Kyungdong Mart), and authenticated by Professor Yong Ki Park, Department of Oriental Medicine, College of Oriental Medicine, Dongguk University (Gyeongju, Korea). The 20 medicinal plants (100 g) were extracted consecutively under reflux with water (1 L) for 1 hr, evaporated under reduced pressure at low temperature (37～40°C) and lyophilized. The solid was stored at -20°C until use. A solution was prepared with distilled water at a concentration of 100～300 mg/mL on the day of the experiment. Each voucher specimen was deposited at the Korea Food Research Institute, Gyeonggi-Do, Korea (Table 1).

Preparation of rat aortic rings

Male Sprague-Dawley rats (200～250 g) were sacrificed by stunning and exsanguinations. The thoracic aorta was dissected free from the surrounding connective tissues and cut into rings 2～3 mm in length. The rings were then transferred to 4-mL horizontal-type muscle chambers, and bathed in physiological salt solution (PSS) containing (mM/L) NaCl (136.9), KCl (5.4), $CaCl_2$ (1.5), $MgCl_2$ (1.0), $NaHCO_3$ (23.8), glucose (5.5), and EDTA (0.01) at 37°C, in an atmosphere of 95% O_2 and 5% CO_2. The rings were mounted on stainless-steel hooks connected to a force-displacement transducer (FT 03; Grass, West Warwick, RI), connected to a polygraph system (RPS 212; Grass), and a computer analyzer (Power Laboratory 400, MacLab; AD Instruments, Castle Hill, Australia). A basal tension of 1 g was applied. Some segments were stripped of endothelium by gentle rubbing with a moistened swab. The functional state of the endothelium was verified by previous challenge of precontracted arteries with carbachol (1 μM) (9). Each experiment was performed on rings prepared from different rats. All studies were performed according to the Guiding Principles for the Care and Use of Laboratory Animals of the Ethics Committee of the Korea Food Research Institute.

Measurement of vascular reactivity of aortic rings

All rings were equilibrated for 60 min under a resting tension of 1 g and then exposed repeatedly to 72 mM/L KCl PSS until the responses stabilized. A control contraction was then produced by addition of 300 nM norepinephrine (NE). After sustained tension (60% of the maximal contraction in response to 72 mM KCl PSS in endothelium-intact rings) was obtained, test solutions

Table 1. Botanical names, family, plant parts, voucher specimen number, and ethnomedical uses of twenty medicinal plants

Botanical names	Family	Plant parts	Voucher specimen No.	Ethnomedical use
Acanthopanax sessiliflorus (RUPR et MAX.) SEEM	Araliaceae	Cortex	AsEx-001	Antirheumatics or drugs for relieving rheumatic conditions
Aconitum carmichaeli DEBX	Ranunculaceae	Radix	AcEx-001	Drugs for dispelling internal cold
Angelica gigas NAKAI	Umbelliferaceae	Radix	AgEx-001	Tonics for blood
Astragalus membranaceus BUNGE	Leguminosae	Radix	AmEx-001	Tonics for Qi
Cnidium monnieri (L.) CUSSON	Umbelliferae	Fruit	CmEx-001	Tonics for Yang-energy
Cornus officinalis SIEB. et. ZUCC	Cornaceae	Fruit	CoEx-001	Astringents
Cuscuta chinensis LAM	Convolvulaceae	Semen	CcEx-001	Tonics for Yang-energy
Dioscorea japonica THUNB	Dioscoreaceae	Rhizoma	DjEx-001	Tonics for Qi
Dipsacus asper WALL	Dipsacaceae	Radix	DaEx-001	Tonics for Yang-energy
Epimedium koreanum NAKAI	Berberidaceae	Herb	EkEx-001	Tonics for Yang-energy
Erythrina variegata var. *orientalis* (L.) MERR	Leguminosae	Corex	EvEx-001	Drugs for relieving rheumatic conditions and articular pain
Glycyrrhiza uralensis FISCH	Leguminosae	Radix	GuEx-001	Tonics for Qi
Panax ginseng C.A.MEY	Araliaceae	Radix	PgEx-001	Tonics for Qi
Panax notoginseng BURK	Araliaceae	Radix	PnEx-001	Drugs for blood stagnation and bleeding
Polygonatum odoratum var. pluriflorum (Miq.) OHWI	Liliaceae	Rhizoma	PoEx-001	Tonics for Yin-energy
Polygonatum stenophyllum MAXIM	Liliaceae	Rhizoma	PsEx-001	Tonics for Yin-energy
Polygonum multiflorum THUNB	Polygonaceae	Radix	PmEx-001	Tonics for blood
Psoralea corylifolia LINNE	Leguminosae	Fruit	PcEx-001	Tonics for Yang-energy
Pueraria thunbergiana (SIEB. et ZUCC) BENTH	Fabaceae	Radix	PtEx-001	Drugs for relieving spasmodic
Schisandra chinensis (TURCZ.) BAILL	Schisandraceae	Fruit	ScEx-001	Astringents

were added to the bath solution. The high-potassium solution was prepared by replacing the NaCl in PSS with an equimolar amount of KCl (10).

In experiments using nitric oxide synthase inhibitor, inhibitors were added 20 min before precontraction. The inhibitor of extract-induced endothelium-dependent relaxation tested was N^G-nitro-L-arginine (L-NNA, 10 μM/L). To examine the effects of the extracts on calcium influx, the endothelium-denuded arteries were washed three times at 10 min intervals with calcium-free medium containing EGTA (1 mM). Then, the arteries were stimulated with calcium-free 72 mM KCl medium (with or without plant extracts) and increasing concentrations of $CaCl_2$ (0.3～10 mM) were added. For comparison, the L-type calcium channel blocker nicardipine (5 and 10 nM), instead of plants extracts, was evaluated in a separate series of experiments (11).

Reagents

Carbachol, L-NNA, nicardipine, and NE were purchased form Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals used for the measurement of vascular reactivity were all reagent grades, and were dissolved in PSS.

Data analysis

All results are expressed as means±SEM. The number of rings obtained from different rats is represented by *n*. Relaxation is expressed in terms of the percentage decrease in maximal contraction caused by NE (300 nM). One-way ANOVA and the Student Newman Keul test were used for statistical analyses of differences between groups and p values<0.05 were regarded as statistically significant.

RESULTS AND DISCUSSION

Vasorelaxant effect of water extracts of 20 plants in endothelium-intact rat aortic rings

The water extracts of 20 plants were assessed for effects on two phases relaxation in endothelium-intact rat aorta precontracted with NE (300 nM). A rapid relaxation, which occurred within seconds following administration of the plant extracts, was seen in CoEx (69±4%), ScEx (40±3%), EvEx (25±2%), and EkEx (23±3%) at 0.3 mg/mL; the other extracts were under 20% (Table 2). CoEx, ScEx, and EkEx caused concentration dependent responses (0.1～3.0 mg/mL; IC_{50} value: 0.18, 0.79, and 2.06 mg/mL, respectively) (Fig. 1), while EvEx showed no concentration dependency (0.3, 1.0, and 3.0 mg/mL showed 25±2, 42±3, and 25±3% relaxation, respectively).

A second, slowly developing (i.e., long-term) relaxation to baseline, was seen in AgEx, EkEx, EvEx, and PtEx. Among these, EvEx showed the shortest time to developing relaxation to baseline (18 min), followed by AgEx, PtEx, and EkEx (Fig. 2A). In general, long-term relaxation was not affected by the endothelium denudation, because it was mediated by smooth muscle direct mechanisms. However, the vasorelaxations evoked by natural products were mediated by multiple mechanisms: through endothelium-dependent, endothelium-independent, and membrane channels (12). The relaxation caused by

Table 2. Two phases relaxant effects of twenty medicinal plants

Botanical names	Water extracts (Abbreviated name)	Vasorelaxant effect (%)	
		Rapid	Long-term
Acanthopanax sessiliflorus (RUPR et MAX.) SEEM	AsEx	19±5	26±13
Aconitum carmichaeli DEBX	AcEx	14±4	−
Angelica gigas NAKAI	AgEx	17±3	100±1
Astragalus membranaceus BUNGE	AmEx	−	−
Cnidium monnieri (L.) CUSSON	CmEx	−	−
Cornus officinalis SIEB. et. ZUCC	CoEx	69±4	−
Cuscuta chinensis LAM	CcEx	−	−
Dioscorea japonica THUNB	DjEx	10±5	−
Dipsacus asper WALL	DaEx	−	−
Epimedium koreanum NAKAI	EkEx	23±3	100±1
Erythrina variegata var. *orientalis* (L.) MERR	EvEx	25±2	100±1
Glycyrrhiza uralensis FISCH	GuEx	17±3	30±19
Panax ginseng C.A.MEY	PgEx	−	−
Panax notoginseng BURK	PnEx	−	45±20
Polygonatum odoratum var. pluriflorum (Miq.) OHWI	PoEx	−	−
Polygonatum stenophyllum MAXIM	PsEx	−	−
Polygonum multiflorum THUNB	PmEx	−	−
Psoralea corylifolia LINNE	PcEx	7±1	50±10
Pueraria thunbergiana (SIEB. et ZUCC) BENTH	PtEx	−	100±1
Schisandra chinensis (TURCZ.) BAILL	ScEx	40±3	−

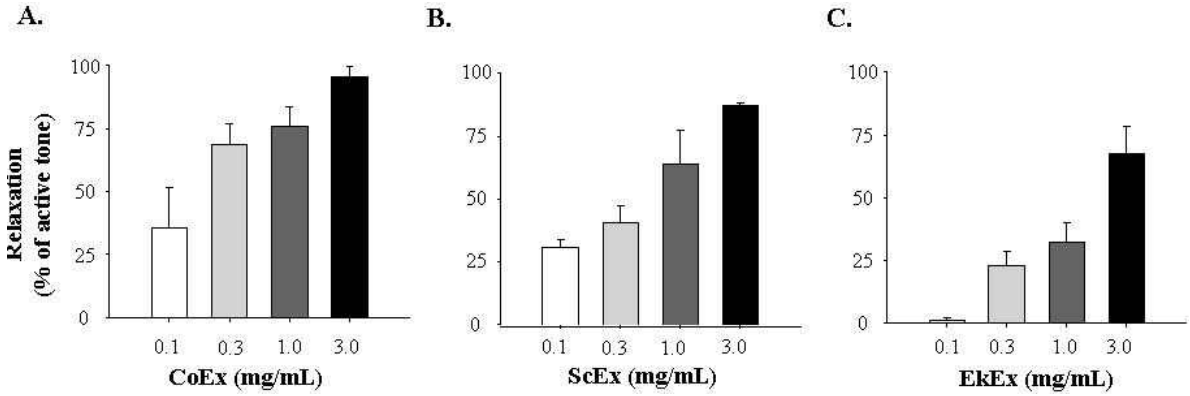

Fig. 1. The concentration dependency (0.1～3.0 mg/mL) of the extracts of *Cornus officinalis* (A, CoEx), *Schisandra chinensis* (B, ScEx), and *Epimedium koreanum* (C, EkEx). The ring contraction was induced with 300 nM norepinephrine (NE) and the each extracts were added to the muscle. The relaxation responses are expressed as the percentage of NE-induced tone.

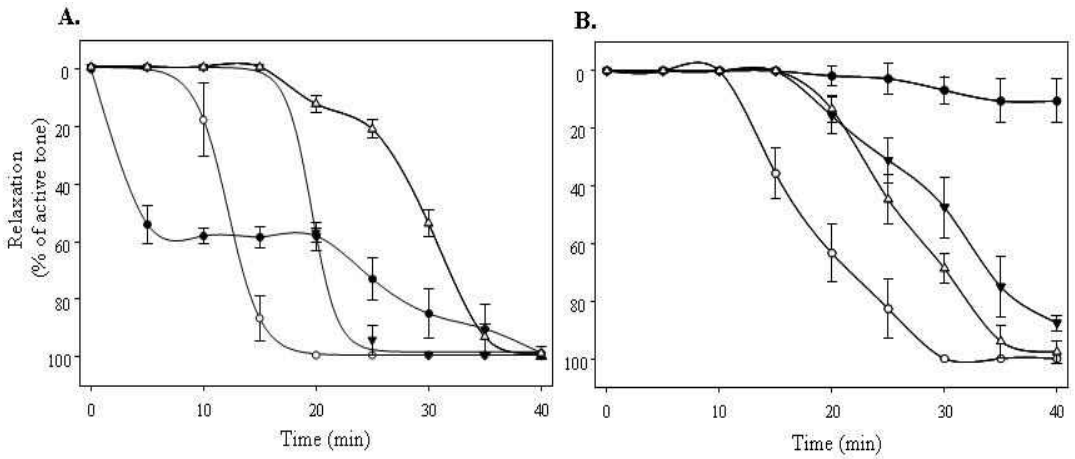

Fig. 2. The time-dependency of AgEx- (▼, 0.3 mg/mL), EkEx- (●, 0.3 mg/mL), EvEx- (○, 0.3 mg/mL), and PtEx- (△, 0.3 mg/mL) induced vascular relaxation in the endothelium-intact (A) and -denuded (B) rat aorta. The relaxation responses are expressed as the percentage relaxation of the NE-induced contraction.

PtEx was not affected by endothelium denudation, whereas the response of EkEx was significantly inhibited (Fig. 2B). This result suggested that the effect of EkEx in long-term relaxation was not mediated by the smooth muscle direct pathway, but by endothelium related function. Additionally, AgEx- or EvEx-induced long-term relaxations were slightly delayed in endothelium-denuded rings, implying that these effects were elicited by both endothelium-dependent and -independent mechanisms. Therefore, to further elucidate the underlying mechanisms, we selected CoEx, ScEx, and EkEx for the representative extracts which elicit endothelium-dependent relaxation, and chose AgEx, EvEx and PtEx as endothelium-independent relaxant extracts.

Mechanisms of selected medicinal plant extracts

In rapid relaxation, the endothelium participates as an important regulator of vascular tone by releasing endothelium-derived relaxing factor (EDRF) (13). Many EDRFs are secreted from endothelium, the most potent molecule is probably nitric oxide (NO) (14-16). As shown in Fig. 3, CoEx-, ScEx-, or EkEx-induced vasorelaxations were abolished by endothelium denudation, and these rapid relaxations were significantly inhibited by pretreatment with L-NNA (10 μM). Endothelial NO plays a major role in the control of vasomotor tone and structure (17). Under the pathological conditions of cardiovascular diseases, dysfunction exists in the vascular endothelium with a subsequent reduction in the release, bioavailability or action of NO. Thus, NO release and function is often decreased in cardiovascular diseases, such as hypertension (17), and atherosclerosis (18). We reported the vasorelaxant effect of *Schizandra chinensis* and *Angelica gigas* extracts (19,20), and quite recently, showed the synergistic vasorelaxant effect of *Ligusticum wallichii* and *Angelica gigas* by stimulation of NO release (21). Sohn et al. (22) reported that medicinal plant extracts containing *Cornus officinalis* and *Schisandra chinensis* reduced blood pressure and increased NO syn-

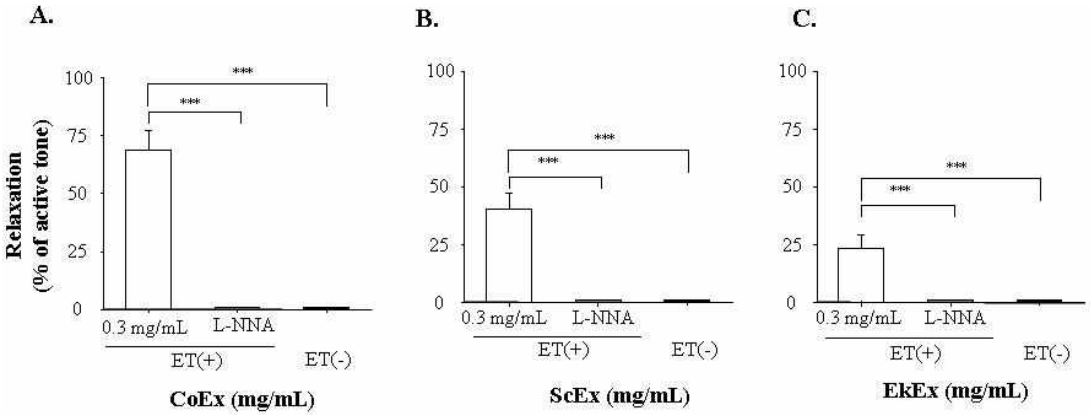

Fig. 3. Comparison of the responses to CoEx (A), ScEx (B), or EkEx (C) of endothelium-intact (□) and inhibitor treated rat aortas (■), and endothelium-denuded rat aortas (■). In endothelium intact rings, each strip was pretreated with L-NNA for 20 min in the quiescent preparation, and each extract was added to NE-contracted aorta. The relaxation responses are expressed as the percentage of NE-induced tension.

thase in spontaneously hypertensive rats. *Epimedium* extract was reported to exert partial endothelial NO-dependent vasorelaxation in rat aorta (23). Additionally, many studies have attempted to identify plant extracts with vasorelaxant effects, and they found that the vasorelaxant effects are related to stimulation of NO release from vascular tissues (24-26). Therefore, CoEx, ScEx, or EkEx elicited the vasorelaxation *via* endothelial NO production, and these endothelium-dependent vasorelaxations may contribute to the beneficial effects on the cardiovascular system.

The influx and bioavailability of calcium is an important mediator of excitation-contraction coupling in smooth muscle cells, and inhibition of calcium influx is one of the major mechanisms in smooth muscle direct vasorelaxation (27). The rise of intracellular calcium concentration can be due to either Ca^{2+} entry through membrane channels, receptor-mediated Ca^{2+} release from the sarcoplasmic reticulum or both (28,29). Transmembrane Ca^{2+} influx depends on the classical ionotropic function of ion channels, among which the L-type voltage-dependent calcium channels (VDCCs) are particularly relevant (30). We compared the effects of AgEx, EvEx, and PtEx on cumulative concentrations of $CaCl_2$ (0.3～10 mM) in a 72-mM KCl/L depolarized calcium-free medium containing EGTA with the L-type calcium channel inhibitor, nicardipine. In our results, EvEx (0.3 mg/mL) significantly shifted the $CaCl_2$-inducd contraction curves to the right and downward compared with a control to a similar level as nicardipine (5 nM/L), but AgEx and PtEx did not (Fig. 4), suggesting that EvEx elicited the vasorelaxation *via* inhibition of calcium influx, especially through VDCCs. Although AgEx or PtEx did not affect the calcium-induced contraction in our experimental conditions, it is possible that these extracts produced the vasorelaxant effect by calcium related mechanisms other than extracellular calcium, such as intracellular release of calcium. The extract of *Erythrina variegate* exhibits beneficial effects against osteoporosis induced by estrogen deficiency through suppression of bone calcium turnover (31) and helps maintain calcium homeostasis (32). Therefore, the vasorelaxant effect mediated through calcium homeostasis, also, may be an important mechanism of the effect of EvEx in the management of menopausal symptoms. In these results, the inhibition of calcium influx was a possible mechanism of EvEx-induced vasorelaxation, but the other mechanisms of the EvEx-mediated effect may also contribute to the AgEx-, and/or PtEx-induced va-

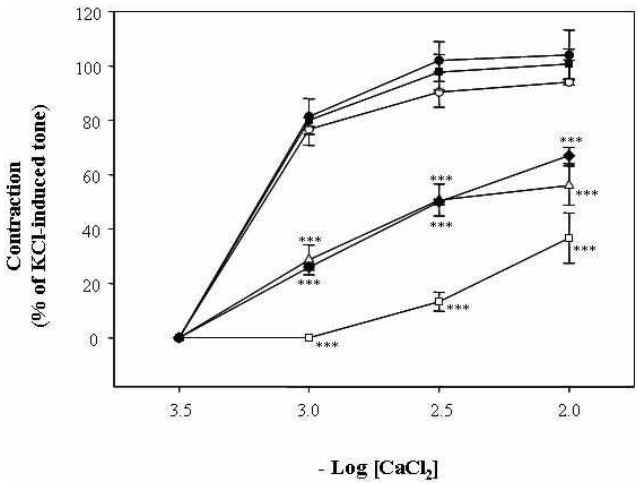

Fig. 4. The effects of AgEx (○, 0.3 mg/mL), EvEx (△, 0.3 mg/mL), and PtEx (■, 0.3 mg/mL) on calcium concentration-dependent contraction curves in rat thoracic aorta without endothelium compared to the effects of nicardipine (□, 5 and ◆, 10 nM/L). Data are expressed as a percentage of the maximum contraction induced by Ca^{2+} in controls (●, means± SEM). ***$p<0.001$ compared to the corresponding controls.

sorelaxation.

CONCLUSION

This study investigated the vasorelaxant effects and possible mechanisms of action of 20 medicinal plants, which have been used to manage postmenopausal symptoms in Korea. CoEx, ScEx, or EkEx elicited endothelial NO-dependent rapid vasorelaxation, and EvEx elicited long-term vasorelaxation *via* inhibition of calcium influx. Although the AgEx- and PtEx-induced vasorelaxation mechanisms were not identified in this study, it will be useful to further analyze those medicinal plants. These results may provide information on the cardio-protective effects of these herbs.

ACKNOWLEDGEMENTS

This study was supported by Technology Development Program for Agriculture and Forestry, Ministry for Agriculture, Forestry and Fisheries, Republic of Korea (GA0831) and the National Research Foundation of Korea Grant funded by the Ministry of Education, Science and Technology (KFR-2007-531-F00009).

REFERENCES

1. Donguibogam. 1999. Interlinear ed. Bubin Publishers Co., Seoul, Korea.
2. Kim DI, Woo DY, Seo JW, Lee TK. 2000. The management of climacteric syndrome clinic and the herbal medicine for that clinic. *Kor J Orient Gynecol* 13: 418-416.
3. Albertazzi P, Purdie DW. 2002. The nature and utility of the phytoestrogens: a review of the evidence. *Maturitas* 42: 173-185.
4. Lissin LW, Cooke JP. 2000. Phytoestrogens and cardiovascular health. *J Am Coll Cardiol* 35: 1403-1410.
5. Ishimi Y, Miyaura C, Ohmura M, Onoe Y, Sato T, Uchiyama Y, Ito M, Wang X, Suda T, Ikegami S. 1999. Selective effects of genistein, a soybean isoflavone, on B-lymphopoiesis and bone loss caused by estrogen deficiency. *Endocrinol* 140: 1893-1900.
6. Figtree GM, Griffiths H, Lu YQ, Webb CM, Macleod K, Collins P. 2000. Plant-derived estrogens relax coronary arteries in vitro by a calcium antagonistic mechanism. *J Am Coll Cardiol* 35: 1977-1985.
7. Mishra SK, Abbot SE, Choudhury Z, Cheng M, Khatab N, Maycock NJR, Zavery A, Aaronson PI. 2000. Endothelium-dependent relaxation of rat aorta and main pulmonary artery by the phytoestrogens genistein and daidzein. *Cardiovasc Res* 46: 539-546.
8. Lee MYK, Man RYK. 2003. The phytoestrogen genistein enhances endothelium-independent relaxation in the porcine coronary artery. *Eur J Pharmacol* 481: 227-232.
9. Sudjarwo SA, Hori M, Karaki H. 1992. Effect of endothelin-3 on cytosolic calcium level in vascular endothelium and on smooth muscle contraction. *Eur J Pharmacol* 229: 137-142.
10. Rhyu MR, Kim DK, Kim HY, Kim BK. 2000. Nitric oxide-mediated endothelium-dependent relaxation of rat thoracic aorta induced by aqueous extract of red rice fermented with *Monascus rubber*. *J Ethnopharmacol* 70: 29-34.
11. Salom JB, Burguete MC, Perez-Asenisio FJ, Centeno JM, Toregrosa G, Alborch E. 2002. Acute relaxant effects of 17-β-estradiol through non-genomic mechanisms in rabbit carotid artery. *Steroids* 67: 339-346.
12. McNeill JR, Jrugens TM. 2006. A systematic review of mechanisms by which natural products of plant origin evoke vasodilatation. *Can J Physiol Pharmacol* 84: 803-821.
13. Torok J. 2000. Histamine-induced relaxation in pulmonary artery of normotensive and hypertensive rats: relative contribution of prostanoids, nitric oxide and hyperpolarization. *Pharmacol Res* 49: 503-508.
14. Gryglewski RJ, Palmer RMJ, Moncada S. 1986. Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. *Nature* 320: 454-456.
15. Lüscher TF. 1994. The endothelium in hypertension: bystander, target or mediator? *J Hypertens* 12: S105-S116.
16. Barton M, Cosentino F, Frandes RP, Moreau P, Shaw S, Lüscher TF. 1997. Anatomic heterogeneity of vascular aging: role of nitric oxide and endothelin. *Hypertension* 30: 817-824.
17. Lüscher TF, Vanhoutte PM. 1986. Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. *Hypertension* 8: 344-348.
18. Kanazawa K, Kawashima S, Mikami S, Miwa Y, Hirata K, Suematsu M, Hayashi Y, Itoh H, Yokoyama M. 1996. Endothelial constitutive nitric oxide synthase protein and mRNA increased in rabbit atherosclerotic aorta despite impaired endothelium-dependent vascular relaxation. *Am J Pathol* 148: 1949-1956.
19. Rhyu MR, Kim EY, Yoon BK, Lee YJ, Chen SN. 2006. Aqueous extract of *Schizandra chinensis* fruit causes endothelium-dependent and -independent relaxation of isolated rat thoracic aorta. *Phytomedicine* 13: 651-657.
20. Rhyu MR, Kim JH, Kim EY. 2005. Radix Angelica elicits both nitric oxide-dependent and calcium influx-mediated relaxation in rat aorta. *J Cardiovasc Pharmacol* 46: 99-104.
21. Kim EY, Rhyu MR. 2010. Synergistic vasorelaxant and antihypertensive effects of *Ligusticum wallichii* and *Angelica gigas*. *J Ethnopharmacol* 130: 545-551.
22. Sohn DW, Kim HY, Kim SD, Lee EJ, Kim HS, Kim JK, Hwang SY, Cho YH, Kim SW. 2008. Elevation of intracavernous pressure and NO-cGMP activity by a new herbal formula in penile tissues of spontaneous hypertensive male rats. *J Ethnopharmacol* 120: 176-180.
23. Wang GJ, Tsai TH, Lin LC. 2007. Prenylflavonol, acylated flavonol glycosides and related compounds from *Epimedium sagittatum*. *Phytochemistry* 68: 2455-2464.
24. Kuramochi T, Chu J, Suga T. 1994. Gou-teng (from *Uncaria rhynchophylla* MIQUEL)-induced endothelium-dependent and -independent relaxations in the isolated rat aorta. *Life Sci* 54: 2061-2069.
25. Chen ZY, Zhang ZS, Kwan KY, Zhu M, Ho WKK, Huang Y. 1998. Endothelium-dependent relaxation induced by hawthorn extract in rat mesenteric artery. *Life Sci* 63: 1983-1991.
26. Chiou WF, Chang PC, Chou CJ, Chen CF. 2000. Protein constituents to the hypotensive and vasorelaxant activities of *Cordyceps sinensis*. *Life Sci* 66: 1369-1376.

27. Meisheri KD, Hwang O, Breemen C. 1981. Evidence for two separate Ca^{2+} pathways in smooth muscle plasmalemma. *J Membrane Biol* 59: 19-25.
28. Somlyo AP, Somlyo AV. 1994. Signal transduction and regulation in smooth muscle. *Nature* 372: 231-236.
29. Bolton TB, Prestwich SA, Zholos AV, Gordienko DV. 1999. Excitation-contraction coupling in gastrointestinal and other smooth muscles. *Annu Rev Physiol* 61: 85-115.
30. Urena J, Valle-Rodrigues A, Lopez-Barneo J. 2007. Metabotropic Ca^{2+} channel-induced calcium release in vascular smooth muscle. *Cell Calcium* 42: 513-520.
31. Zhang Y, Li XL, Lai WP, Chen B, Cow HK, Wu CHF, Wang NL, Yao XS, Wong MS. 2007. Anti-osteoporotic effect of *Erythrina variegat*e L. in ovariectomized rats. *J Ethnopharmacol* 109: 165-159.
32. Zhang Y, Li XL, Yao XS, Wong MS. 2008. Osteogenic activities of genistein derivatives were influenced by the presence of prenyl group at ring A. *Arch Pharm Res* 31: 1534-1539.

(Received April 27, 2010; Accepted September 10, 2010)