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Calcium Channel-blocking Activity of Chinese Balloon Flower (*Platycodon grandiflorum*) for Producing Blood Pressure-lowering Functional Foods

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Abstract This study was conducted to evaluate the hypotensive properties of the extract of Chinese balloon flower (*Platycodon grandiflorum*)'s root. In the studies for calcium channel-blocking using *Xenopus* oocytes, the ethanol-extract (26.2±5.2%) showed higher activity than water-extract. Twenty female rats were fed 25, 35, and 45 mg/kg BW/day of the ethanol-extract for 14 days to observe the changes in blood pressures and heart pulses. Ethanol-extract decreased the systolic, diastolic, and mean blood pressures of the rats. Especially, the rats fed with 45 mg/kg BW/day of the ethanol-extract showed significant decreases in the blood pressures. These results suggested that a decrease in blood pressures was due to the extension of a blood vessel with calcium channel-blocking by ethanol-extract of Chinese balloon flower. Forty %-ethanol showed the highest efficiency for ethanol-extraction of Chinese balloon flower.

Keywords: Chinese balloon flower, calcium channel-blocking, blood pressure, heart pulse, hypotensive property

Introduction

Calcium ion can be demonstrated as the important signal intracellular messenger, participating in excitation and contraction of cardiac and vascular smooth muscle. It has been proved by many researches that calcium in the human body is involved in various physiological and biochemical processes, and related to some disease states (1,2). Calcium ion can control the normal physiological action of some visceral organs, such as heart, liver, kidney, or blood vessel, and can participate in a variety of physiological actions and life activities, such as excitation of neural cell, contraction of muscle, coagulation of blood, cell division, phagocytosis and immunity of cell, and so on (3-6). The metabolism of calcium in a human body is regulated by many factors to maintain the relative balance. If the equilibrium of our organisms was damaged, there would emerge some abnormal physiological or disease states. For example, coronary and other diseases in cardiac are due to immoderate influx of Ca^{2+} into cytoplasm (7-9). The diseases might be prevented or cured, if Ca^{2+} channels are blocked by medications or other substances (10,11), and thus, many calcium channel medications, such as Nifedipine, Verapamil, Prenylamine, Niludinine, Nisoldipine, and Felodipine, usually chemical compounds, have been used in treatment for coronary and other diseases (7,12). Besides these, the calcium blockers are effective for hypertension, renal failure, renal colic, stroke, arrhythmia, cerebral infarct, stenocardia, and angiospasm. Especially, many calcium blockers are used to block off influx of calcium ions, protect from contraction of cardiac and vascular smooth muscle, and then, lower blood pressure. However, overdose of the chemical calcium blocker may cause some harmful side effects (13,14). Therefore, the studies on natural substances have been actively progressed to solve this harmful side effect. Actually, various kinds of herbs including ginseng have become attractive as health-beneficial plants and as source materials for the development of medicines (15-20).

Another possible candidate for calcium blocker is Chinese balloon flower (*Platycodon grandiflorum*). The root of Chinese balloon flower has been used as a traditional oriental medicine for the symptoms of cough and sputum, and a remedy for bronchitis, tonsilitis, laryngitis, pulmonary pain, and inflammatory diseases in oriental countries (21,22). The extracts of Chinese balloon flower' root were reported to prevent from hyperlipidemia, hypercholesterolemia, obesity, liver damage, and diabetes (23,24). Therefore, the abilities of water and ethanol extracts of Chinese balloon flower for calcium channel-blocking, and thus, blood pressure depression were observed in this study, as a preliminary study for the further purpose of development of a new blood pressure-lowering beverage as a functional food without severe side effects.

Materials and Methods

Materials All natural Chinese balloon flower used in this work were purchased locally on an as-needed basis, freeze-dried, and extracted with various solvents, such as hexane, chloroform, ethyl acetate, butanol, water, methanol, and ethanol using accelerated solvent extractor (ASE 200; Dionex, Sunnyvale, CA, USA).

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Preparation of extracts from Chinese balloon flower One-hundred g of Chinese balloon flower was extracted with 300 mL of each solvent for 5 min at the temperature of 40°C under pressure of 1,500 psi. The extract was, then, filtrated with filter paper, evaporated under a reduced pressure, and freeze-dried. These extracts prepared with the various solvents, were to be tested for their calcium channel-blocking activities using *Xenopus* oocytes. The ethanol-extract was fed to rat to observe blood pressure changes.

In the mean time, the extractability of water and diluted spirits was also tested for development of a functional alcoholic beverage. Fifty g of sliced fresh Chinese balloon flower was soaked in a 300 mL of water, diluted spirits (ethanol content of 20, 40, 60, or 80%) for 30 days at room temperature. After filtration, the spirits soaked with Chinese balloon flower were evaporated under a reduced pressure to analyze soluble components.

Activity test of extracts for calcium channel-blocking using *Xenopus* oocytes cDNAs for the L (α1C, α2δ, and β3)-type Ca^{2+} channel subunits were provided from the University of Iowa, USA. *Xenopus* laevis were obtained from *Xenopus* I (Ann Arbor, MI, USA). Frogs were operated under anesthesia with an aerated solution of 3-amino benzoic acid ethyl ester to isolate oocytes. Oocytes were separated by treatment with collagenase and agitation for 2 hr in a Ca^{2+}-free medium containing 82.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, 2.5 mM sodium pyruvate, 100 units/mL penicillin and 100 μg/mL streptomycin. Stage V-VI oocytes were collected and stored in ND96 (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, and 5 mM HEPES, pH 7.5) supplemented with 0.5 mM theophylline and 50 g/mL gentamycin. This oocyte-containing solution was maintained at 18°C with continuous gentle shaking and changed every day. Electrophysiological experiments with the oocytes were performed within 5-6 days of their isolation. The drugs used in this study were bath-applied. One day after harvest, a 10 μL VWR microdispenser (VWR Scientific, San Francisco, CA, USA) fitted with a tapered glass pipette tip (15-20 μm in diameter) was used to inject 40 nL cRNAs (40 and 20 ng for α and other auxiliary subunits, respectively) into the animal or vegetal pole of each oocyte (25).

Recombinant plasmids containing calcium channel subunit cDNA inserts were linearized by digestion with appropriate restriction enzymes. cRNAs were obtained from the linearized templates with an *in vitro* transcription kit (mMessage mMachine, Ambion, Austin, TX, USA) using T7 RNA polymerase. The RNA was dissolved in RNase-free water at 1 μg/μL, divided into aliquots and stored at −70°C until used.

A custom-made Plexiglas net chamber was used for 2-electrode voltage-clamp recordings. The chamber was constructed by milling 2 concentric wells into the chamber bottom (diameter/height: upper well: 8/3 mm, lower well: 6/5 mm) and gluing plastic meshes (0.4-mm grid diameter) onto the bottom of the upper well. The perfusion inlet (1-mm diameter) was formed through the wall of the lower well, and a suction tube was placed on the edge of the upper well. The oocyte was then placed on the net separating upper and lower wells, the grids of the net serving as dimples keeping it in place during electro-physiological recording. The oocytes were impaled with 2 microelectrodes filled with 3 M KCl (0.2-0.7 MΩ). Recording solution consists of 10 Ba $(OH)_2$, 90 NaOH, 2 KOH, 5 HEPES (pH 7.0 adjusted with methanesulfonic acid) and 0.3 niflumic acid (mM). The electrophysiological experiments were performed at room temperature with an oocyte Clamp (OC-725C; Warner Instrument, Hamden, CT, USA), and stimulation and data acquisition were controlled with a pClamp 8 (Axon Instruments, Hamden, CT, USA). For most of the electrophysiological experiments on Ca^{2+} channel activity, the oocytes were clamped at a holding potential of −100 mV and depolarized to 10 mV for 500 msec, evoked every 10 sec. For current-voltage relationships, voltage steps were applied from −60 to +60 mV with 10-mV increments evoked every 10 sec for 500 msec. The values are presented as means±SEM. The differences between means of control and treatment data were analyzed using unpaired Student's *t*-tests.

Blood pressure and heart pulse depression test using experimental animals The ordinary rats having average blood pressure and heart pulse were used, because the further purpose of this study was not to produce a hypertension-curing medicine, but to produce a hypertension-preventing beverage. A total of 20 female Sprague-Dawley rats (6-7 weeks old, weight 150-200 g) were obtained from animal distributor (Dae-Han Biolink, Chungbuk, Korea). They were kept in standard rat cage allowed to acclimate for 14 days with free access to feed and water. Lights were maintained on a reversed 12-hr light/dark cycle. Temperature and humidity were maintained at 23-24°C and 19%, respectively. Rats were divided into 4 groups. Each group was fed a standard feed plus 25, 35, or 45 mg/kg BW/day of the ethanol-extract, respectively. Control group was fed a standard feed only.

The rats were placed in plastic restrainers to measure the blood pressures and heart pulses. A cuff with a pneumatic pulse sensor was attached to the tail. Rats were allowed to habituate to this procedure for 3 days before experiments were performed. The experiment equipment which recorded blood pressure and heart pulse used a blood pressure monitor rat/Murochi model MK-2000 (Koreatech Inc., Daejeon, Korea). Blood pressure and heart pulse were averaged from 6 consecutive reading obtained from each rat (26).

Statistical analysis Statistical analysis was conducted using SPSS 10.0 package. All values were presented as means±SEM. The differences between means of control and treatment data were analyzed using Duncan's multiple range tests. A value of $p<0.05$ was considered statically significant (27).

Results and Discussion

Calcium channel-blocking abilities of Chinese balloon flower extracts The inward Ba^{2+} peak current (IBa) was recorded by the 2-electrode voltage clamp technique from oocytes injected with cRNAs encoding the L-type Ca^{2+} ion channel a and auxiliary subunits. The oocytes were held at −100 mV for L-type Ca^{2+} channels, and IBa were elicited

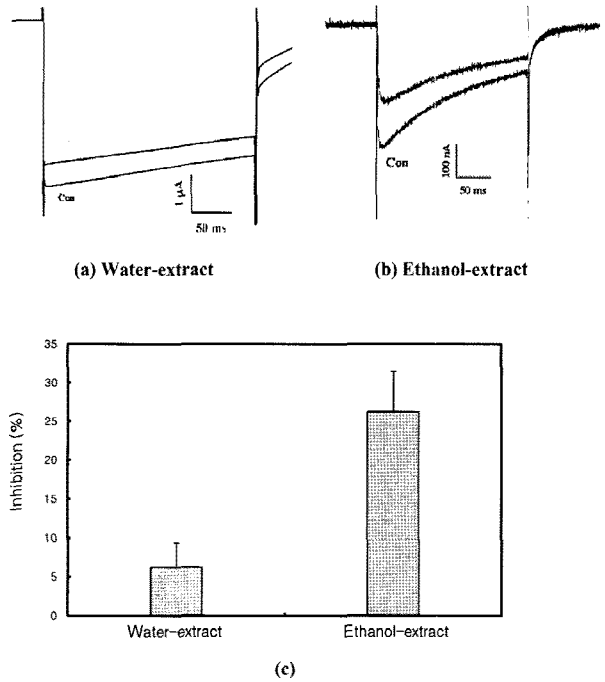

Fig. 1. Calcium channel trace of water-extract (a) and ethanol-extract (b) Chinese balloon flower, and the comparisons of the effects (c) on L-type calcium channel-blocking.

by depolarization to 10 mV, every 10 sec. As described in Fig. 1a and 1b, the depolarizing voltage steps induced large inward IBa in each type of Ca^{2+} channel, however, application of 100 mg/mL of the extracts reversibly inhibited IBa. The percent-inhibitions of IBa by Chinese balloon flower's extracts were 26.2±5.2% in ethanol-extract and 6.2±3.2% in water-extract (Fig. 1c).

The ethanol-extract showed the relatively strong inhibition to IBa, indicating the significant influence of these extracts to Ca^{2+} influx and efflux. L-Type Ca^{2+} channels are heteromultimeric structures that are minimally composed of a pore-forming a1C subunit and regulatory b and a2d subunits in vascular smooth muscle cells. The L-type Ca^{2+} channels are the primary pathways for voltage-gated Ca^{2+} influx that trigger excitation-contraction coupling in small resistance vessels (28). Notably, vascular smooth muscle cells of hypertensive rats showed an increased expression of L-type channel a1C subunits, which is

Table 1. Compositions of a standard feed for rats

Component	Content (%)
Crude protein	20.0
Crude fat	7.0
Crude fiber	7.0
Moisture	10.0
Ash	6.0
Calcium	0.5
Phosphorus	0.5
Corn starch	44.5
Mineral mixture	3.5
Vitamin mixture	1.0

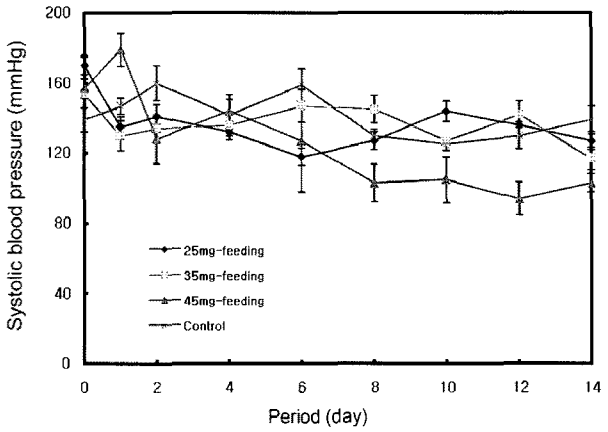

Fig. 2. Changes in systolic blood pressure of rats fed with 0, 25, 35, and 45 mg/kg BW/day of ethanol-extract of Chinese balloon flower for 14 days. Values reported as means±SD. *$p<0.05$ by Duncan's multiple range test.

associated with elevated Ca^{2+} influx and the development of abnormal arterial tone (29). Therefore, the extract of Chinese balloon flower, especially the ethanol-extract, was expected to decrease the blood pressure by calcium channel-blocking, and thus, decided to utilize in development of a beverage possessing blood pressure-lowering functionality.

Blood pressure and heart pulse-lowering abilities of the ethanol-extract Generally, the decrease in a blood pressure reduces cardiovascular diseases in hypertension patients (30). The ethanol-extract was tested *in vivo* to observe the changes in the blood pressure and heart pulse of rats, because of its strong inhibition to IBa.

Systolic and diastolic blood pressures, which constitute blood pressure, change with age. Ordinarily, systolic blood pressure of human increases with aging, while diastolic blood pressure increases until age of 50 and then decreases gradually (31). Diastolic blood pressure is related to risks of cardiovascular disease and hypertension in youth and systolic blood pressure is related to those in the old ages (32). The changes in systolic blood pressures of the rats fed with 25, 35, or 45 mg/kg BW/day of the ethanol-extract. The rats fed with 25 or 35 mg/kg BW/day of the ethanol-extract did not show significant blood depression. However, the systolic blood pressures of the rats fed with 45 mg/kg BW/day of the ethanol-extract showed a significant decrease during the first 2 days and gradual decreases after 2 days (Fig. 2).

All of the experimental rats, except control, showed the trends of decreases in diastolic blood pressures (Fig. 3). Even the rats fed with 25 mg/kg BW/day of the ethanol-extract showed a slightly lower diastolic blood pressure than control during the whole period of experiment. The diastolic blood pressure of the rats fed with 35 mg/kg BW/day of the ethanol-extract decreased drastically after 10 days of feeding (97±16.1 to 40±8.7 mmHg). The diastolic blood pressure of the rats fed with 45 mg/kg BW/day of the ethanol-extract decreased sharply after 2 days of feeding (109±6.3 to 45.4±17.5), but remained relatively unchanged after 6 days of feeding. Although there were severe differences in results according to the individual variety,

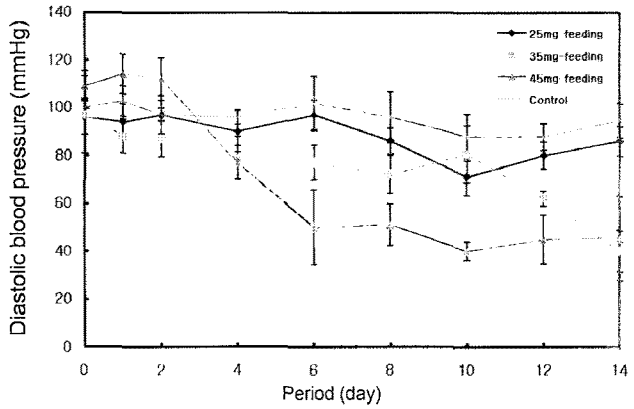

Fig. 3. Changes in diastolic blood pressure of rats fed with 0, 25, 35, and 45 mg/kg BW/day of ethanol-extract of Chinese balloon flower for 14 days. Values reported as means ± SD. $*p < 0.05$ by Duncan's multiple range test.

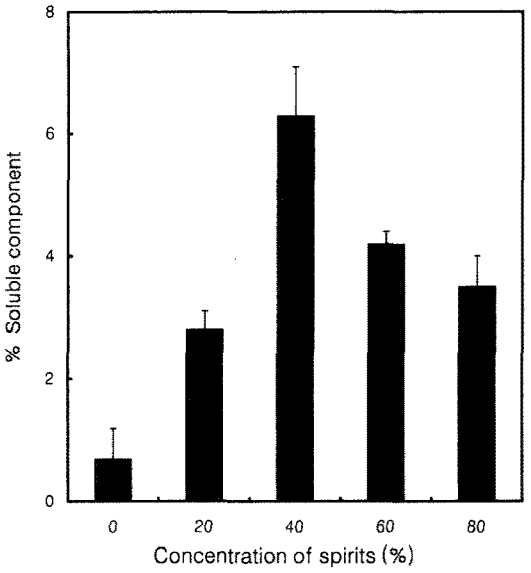

Fig. 4. Amounts of extracts with spirits of 0-80% ethanol concentration.

the significant effects of the ethanol-extracts on the both blood pressures. The changes in the mean blood pressure of the 3 groups of the rats were described in Table 2. Significant decreases in the mean blood pressure of the rats fed with 35 and 45 mg/kg BW/day of the ethanol-extract, were observed. Although an accurate mechanism was not verified yet, Lim *et al.* (33) also reported the inhibition of Platycodin D extracted from Chinese balloon flower to the contractile responses. The rates of decreases were in the proportion to the amount of the fed ethanol-extract. The mean blood pressure of each group decreased gradually during the experiment period (data not shown). This result was in accordance with the results of previous experiments for systolic and diastolic blood pressures.

Although the ethanol-extract decreased the blood pressures effectively, it did not significantly change the heart pulses (Table 3). However, the works of Fatehi *et al.* (34) dealing with the heart pulses affected by *Crocus sativus* petals showed the similar results. Consequently, the

Table 2. Changes in mean blood pressures of female Sprague-Dawley rats by feeding 0-45 mg/kg BW/day of ethanol-extract of Chinese balloon flower for 14 days

Group	Mean blood pressure[1] (mmHg)		Rate of decrease (%)
	Before feeding	After feeding for 14 days	
Control	116±4^{cd}	105±7.9^{ab}	8.48±2.9
25 mg/kg BW/day-feeding	120±3.9^{a}	100±7.5^{a}	15.69±3.4
35 mg/kg BW/day-feeding	99±17.3^{b}	66±5.3^{a}	30.01±6.2
45 mg/kg BW/day-feeding	126±3.5^{d}	69±13.2^{a}	42.23±5.9

[1]Values reported as means±SD; means with different letters in a column are significantly different by Duncan's multiple range test ($p<0.05$).

Table 3. Changes in heart pulses of rats fed with 0, 25, 35, and 45 mg/kg BW/day of ethanol-extract of Chinese balloon flower for 14 days

Intake period (day) \ Groups	Heart pulse[1] (beats/min)			
	25 mg/kg BW/day-feeding	35 mg/kg BW/day-feeding	45 mg/kg BW/day-feeding	Control
0	475±8^{d}	478±22^{c}	511±15^{d}	471±6^{e}
1	412±9^{a}	473±11^{c}	486±13^{bc}	456±8^{d}
2	441±8^{bc}	458±9^{bc}	423±34^{a}	439±2^{c}
4	459±10^{cd}	480±10^{c}	432±16^{a}	404±4^{a}
6	435±11^{b}	463±19^{bc}	431±29^{a}	433±3^{c}
8	414±7^{a}	451±14^{bc}	461±10^{ab}	422±3^{b}
10	397±21^{a}	438±23^{ab}	445±16^{a}	460±3^{d}
12	460±8^{cd}	415±23^{a}	448±16^{a}	419±12^{b}
14	451±9^{bc}	435±18^{ab}	444±17^{a}	399±6^{a}

[1]Values reported as means±SD; means with different letters in row are significantly different by Duncan's multiple range test ($p<0.05$).

effect of the ethanol-extract on peripheral resistance seems important.

The previously mentioned results on the blood pressures and calcium channel blocking experiments, suggested that a decrease in blood pressure may be due, at least in part, to an extension of a cardiac vascular as a consequence of Ca^{2+} channel blockade induced by the ethanol-extract of Chinese balloon flower. Therefore, the effects of ethanol concentrations in spirits on extractability of the solutes in Chinese balloon flower were studied for efficient extraction of the ethanol-extract of Chinese balloon flower.

The spirit with ethanol concentration of 40% extracted the largest amount of yellowish and sticky solute among the tested concentration (0-80%) of ethanol (Fig. 4). The amount of extract increased with increasing concentration of ethanol, however, decreased with higher ethanol concentrations than 40%. Therefore, a 40% ethanol-extraction of Chinese balloon flower was strongly recommended to produce a beverage possessing blood pressure depressing functionality.

Acknowledgments

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References

1. Bers DM. Excitation-Contraction Coupling and Cardiac Contractile Force. Kluwer Academic, Dordrecht, Netherlands. pp. 26-27 (1991)
2. Katz AM. Physiology of Heart. 2nd ed. Raven Press, New York, NY, USA. pp. 35-38 (1992)
3. Bolton RB. Mechanism of action or transmitters and other substances on smooth muscle. Physiol. Rev. 59: 606-718 (1979)
4. Godfraind T. Alternative mechanism for the potentiation of the relaxation evoked by isoprenaline in aortae from young and aged rats. Eur. J. Pharmacol. 53: 273-279 (1979)
5. Furchgott RF. Sprial-cut strip of rabbit aorta for *in vitro* studies of response of arterial smooth muscle. Method Med. Res. 8: 177-186 (1976)
6. Reuter H, Beeler GW. Calcium current and activation of contraction in ventricular myocardial fibres. Science 162: 399-401 (1969)
7. Zhang YJ. Application and progress of calcium antagonist. Prog. Cardioangiol. 16: 43-51 (1995)
8. Fleckenstein A, Tritthart FB, Herbst A, Grun G. A new group of competitive Ca-antagonist (Iproveratril, D600, prenylamine) with highly potent inhitory effect on excitation-contraction coupling in mammalian myocardium. Pflueg. Arch. Eur. J. Phy. 307: R25-R32 (1969)
9. Godfraind T. Actions of Nifedipine on calcium fluxes and contraction in isolated ratarteries. J. Pharmacol. Exp. Ther. 224: 443-450 (1983)
10. Godfraind T, Dieu D. The inhibition by flunarizine of the norepinephine-evoked contraction and calcium influx in rat aorta and mesenteric arteies. J. Pharmacol. Exp. Ther. 217: 510-515 (1881)
11. Godfraind T, Miller RC. Specificity of actions of Ca entry blockers, a comparision of their actions in rat arteries and in human coronary. Circ. Res. 43: 251-256 (1983)
12. Godfraind T, Govoni S. Recent advances in the pharmacology of Ca^{++} and K^+ channels. J. Pharmacol. Sci. 16: 1-4 (1995)
13. Shimizu Y, Kataoka M, Seto H, Kido J, Nagata T. Nifedipine induces gingival epithelial hyperplasia in rats through inhibition of apoptosis. J. Periodontol. 73: 863-867 (2002)
14. Kanamasa K, Kimura A, Miyataka M. Incidence of cancer in post-myocardial infarction patients treated with short-acting nifedipine and diltiazem, secondary prevention group. Cancer 85: 1369-1374 (1996)
15. Kerekgyarto C, Virag L, Chihara G, Fachet J. Strain differences in the cytotoxic activity and TNF production of murine macrophages stimulated by lentinan. Int. Immunopharmacol. 18: 347-353 (1996)
16. Han S-B, Kim Y-H, Lee C-W, Park S-M, Lee H-Y, Ahn K-S. Characteristic imunostimulation by angelan isolated from *Angelica gigas* Nakai. Int. Immunopharmacol. 40: 39-48 (1998)
17. Liu F, Fung MC. Analysis of immunomodulating cytokine mRNAs in the mouse induced by mushroom polysaccharides. Life Sci. 64: 39-48 (1999)
18. Mark SP, Woo M-N, Vuksan VM. Korean red ginseng lowers blood pressure in individuals with hypertension. Am. J. Hypertens. 17: 33-38 (2004)
19. Hah J-S, Kang B-S, Kang D-H. Effect of *Panax Ginseng* alcohol extract on cardiovascular system. Yonsei Med. J. 19: 2-9 (1978)
20. Seog H-M, Jung C-H, Kim Y-S, Park S-H. Phenolic acids and antioxidant activities of wild ginseng (*Panax ginseng* C.A. Meyer) leaves. Food Sci. Biotechnol. 14: 371-374 (2005)
21. Lee E-B. A comparison of experimental pharmacological effects of crude platycodin with clinical indication of *Platycodi radix*. J. Pharm. Soc. Jpn. 93: 1188-1194 (1973)
22. Kim K-S, Ezaki O, Ikemoto S, Hitakura M. Effects of *Platycodon grandiflorum* feeding on serum and liver lipid concentrations in rats with diet-induced hyperlipidemia. J. Nutr. Sci. 41: 485-491 (1995)
23. Kim K-S, Seo E-K, Lee Y-C, Lee T-Y, Cho Y-W, Ezaki O, Kim C-H. Effect of dietary *Platycodon grandiflorum* on the improvement of insulin resistance in obese Zucker rats. J. Nutr. Biochem. 11: 420-424 (2000)
24. Lee Y-H, Kim Y-J, Kim H-I, Cho H-Y, Yoon G-H. Potent HAT inhibitory effect of aqueous extract from bellflower (*Platycodon grandiflorum*) roots on androgen receptor-mediated transcriptional regulation. Food Sci. Biotechnol. 16: 457-462 (2007)
25. Jeong S-M, Lee J-H, Yoon I-S, Nah S-Y. Gene cloning, tissue distribution, and its characterization of Ca^{++}-activated Cl^- channel activated by ginsenosides in *Xenopus* laevis oocytes. J. Ginseng Res. 29: 167-175 (2005)
26. Kubota Y, Umegaki K, Kagota S, Tanaka N, Nakamura K, Kunitomo M, Shinozuka K. Evaluation of blood pressure measured by tail-cuff methods (without heating) in spontaneously hypertensive rats. Biol. Pharm. Bull. 29: 1756-1758 (2006)
27. Sung H-Y, Lim Y-J, Choi Y-S. Soy isoflavones do not alter the effects of fructooligosaccharide on the intestinal ecosystem of colon-cancer model rats. Food Sci. Biotechnol. 15: 931-936 (2006)
28. Sonkusare S, Palade PT, Marsh JD, Telemaque S, Pesic A, Rusch NJ. Vascular calcium channels and high blood pressure: Pathophysiology and therapeutic implications. Vasc. Pharmacol. 44: 131-142 (2006)
29. Harder DR, Brann L, Halpern W. Altered membrane electrical properties of smooth muscle cells from small cerebral arteries of hypertensive rats. Blood Vessels 20: 154-160 (1983)
30. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: A meta-analysis in individual data for one million adults in 61 prospective studies. Prospective studies collaboration. Lancet 360: 1903-1913 (2002)
31. Franklin SS, Gustin W, Wong ND, Larson MG, Weber MA, Kannel WB, Levy D. Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study. Circulation 96: 308-315 (1997)
32. Franklin SS, Gustin W, Wong ND, Larson MG, Weber MA, Kannel WB, Levy D. Does the relation of blood pressure to coronary heart disease risk change with aging? The Framingham Heart Study. Circulation 103: 1245-1249 (2001)
33. Lim D-Y, Kim B-C, Lee E-B. Comparison of vasodilator effect of Platycodin D and D3 in rats. Korean J. Physiol. Pharmacol. 7: 149-155 (2003)
34. Fatehi M, Rashidabady T, Zahra FH. Effects of *Crocus sativus* petals' extract on rat blood pressure and on responses induced by electrical field stimulation in the rat isolated vas deferens and guinea-pig ileum. J. Ethnopharmacol. 84: 199-203 (2003)