Accepted Manuscript

Title: Rg3-enriched Korean Red Ginseng enhances blood pressure stability in spontaneously hypertensive rats

Author: Harsha Nagar Sujeong Choi Jung Saet-byel Byeong Hwa Jeon Kim Cuk-Seong

PII: S2213-4220(16)30047-6
DOI: http://dx.doi.org/doi:10.1016/j.imr.2016.05.006
Reference: IMR 206

To appear in:

Received date: 2-5-2016
Revised date: 23-5-2016
Accepted date: 30-5-2016

Please cite this article as: Harsha NagarSujeong ChoiJung Saet-byelByeong Hwa JeonKim Cuk-Seong Rg3-enriched Korean Red Ginseng enhances blood pressure stability in spontaneously hypertensive rats (2016), http://dx.doi.org/10.1016/j.imr.2016.05.006

Rg3-enriched Korean Red Ginseng enhances blood pressure stability in spontaneously hypertensive rats

Harsha Nagar[a1], Sujeong Choi[a1], Saet-byel Jung[b], Byeong Hwa Jeon[a], Cuk-Seong Kim[a*]

[a]Department of Physiology, School of Medicine, Chungnam National University, Daejeon 301-131, Republic of Korea

[b]Department of Endocrinology, Chungnam National University hospital, Daejeon 301-721, Republic of Korea

[1]These authors contributed equally to this work

Running Head : the effect of REKRG on high blood pressure

[*]Corresponding author:

Cuk-Seong Kim (cskim@cnu.ac.kr),

Department of physiology, School of Medicine, Chungnam National University, Daejeon 301-131, Republic of Korea

Tel) 82-42-580-8219, Fax) 82-42-585-8440

Abstract

Background: Korean Red Ginseng (*Panax ginseng*) has been shown to exert antihypertensive effects. In particular, ginsenoside Rg3 is thought to be a potent modulator of vascular function. The present study was performed to examine the antihypertensive efficacy of Korean Red Ginseng (KRG) extract and Rg3-enriched KRG (REKRG) extract.

Methods: Spontaneously hypertensive rats (SHR) and Wistar–Kyoto rats (WKY) were divided into six groups (WKY control, WKY-KRG, WKY-REKRG, SHR control, SHR-KRG, and SHR-REKRG), and systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured at the carotid artery, followed by injection of 3 mg/kg KRG or REKRG.

Results: REKRG treatment significantly decreased systolic blood pressure (SBP) and diastolic blood pressure (DBP) 3 hours post-treatment in SHR compared with SHR controls. However, SBP and DBP were not significantly different in KRG-treated SHR compared with SHR controls. Interestingly, REKRG treatment did not significantly alter SBP or DBP 3 hours post-treatment in WKY compared with WKY controls. Similarly, there were no differences in SBP or DBP with KRG treatment in WKY and WKY controls. Both KRG and REKRG increased endothelial nitric oxide synthase (eNOS) phosphorylation levels in the aorta, and the increases in eNOS phosphorylation levels by REKRG treatment were higher than those with KRG treatment. Similarly, nitric oxide production in plasma from WKY and SHR was also increased by both KRG and REKRG.

Conclusion: Taken together, these results suggest that REKRG has a more beneficial effect on blood pressure control than KRG in SHR.

Key words: Rg3-enriched Korean Red Ginseng, eNOS, SHR, nitric oxide

1. Introduction

Korean Red Ginseng (*Panax ginseng*) is a traditional Korean tonic medicine known for its efficacy in promoting physical strength and immunity, which may ameliorate certain chronic disease states such as vascular disease and hypertension[1]. Ginsenosides as a biologically effective extract of *P. ginseng* are a mixture of triterpene glycosides. The major fractions of ginsenosides consist of two groups according to chemical structure—the panaxadiol group is represented by Rb1, Rb2, Rb3, Rc, Rd, Rg3, Rh2, and Rs1, while the panaxatriol group consists of Re, Rf, Rg1, Rg2, and Rh1. Ginsenosides are well known to have hypotensive effects in experimental animals and hypertensive patients[2-4]. Moreover, they enhance cardiovascular functions associated with vasorelaxation and stimulation of nitric oxide (NO) production from endothelial NO synthase (eNOS)[3, 5]. Recently, individual ginsenosides have been shown to have different effects via various mechanisms in many tissues. In addition, studies of vascular disease are focusing on purified individual ginsenoside fractions from ginseng to identify the key components instead of using mixed ginsenosides. Among the ginsenosides of the panaxadiol and panaxatriol groups, ginsenoside Rg3 is a potent vasodilator that has been shown to provide vascular protective effects, such as antivascular contraction and antihypertensive effects as well as enhancement of NO production and eNOS activity[2, 6, 7]. Previously, we also showed that Rg3 improved vascular function through eNOS activation[2].

NO is a major endothelium-dependent relaxing factor, and its production by vascular endothelial cells plays a critical role in the regulation of vascular motor tone and stability of blood flow as well as blood pressure[8, 9]. NO is synthesized by the vascular endothelial cells using L-arginine as a substrate in a process catalyzed by NOS[10] and induces vascular smooth muscle relaxation by activation of guanylate cyclase[11]. Because it is an isoform of NOS that produces NO, eNOS also plays an important role in regulating systemic blood pressure[12]. Previous studies showed that a decrease in NO production can lead to hypertension[13], and eNOS mutation leads to impaired endothelium-dependent

vasorelaxation and may have hypertensive effects[14]. In addition, blood pressure was enhanced in rats in which eNOS was inhibited with Nω-nitro-L-arginine methyl ester (L-NAME)[15]. Recent studies suggested that production of NO is reduced and endothelium-dependent vasorelaxation is blunted in patients with essential hypertension[16].

Previous studies have shown the effects of *P. ginseng* on vascular regulation and blood pressure control, and effects of total ginsenosides and Rg3 on eNOS activation and NO production. However, there is no previous study for comparison between KRG and REKRG. Therefore, in this study, the efficacy of total ginsenoside (KRG) and Rg3-enriched ginsenosides (REKRG) was compared in WKY and SHR. We examined the enhancement of blood pressure stability, eNOS phosphorylation and NO production by REKRG in SHR compared with WKY.

2. Methods

2.1 Preparation of Rg3-enriched KRG

Dried KRG (*P. ginseng*) root was purchased from Gumsan Nonghyup (Gumsan, Korea). Korean ginseng was extracted twice with 10 volumes of ethanol at 50°C for 7 hours (1st 50%, 2nd 85%), and then concentrated under vacuum at 50°C as described previously[2]. Briefly, the crude extract was dissolved in water, and enzyme-acid hydrolysis was performed to maximize ginsenoside Rg3 (raw ginsenoside was hydrolyzed to Rg3) under acidic (pH 2.5–3.5) and thermophilic (65°C–80°C) conditions. The enzyme, which has β-glycosidase activity including cellulose, hemicellulose, and glucosidase activities, was produced by *Aspergillus niger*. To remove the acid solution and concentrate Rg3, the reactant was passed through a column packed with DIAION HP20 resin (Mitsubishi Chemical Industries, Tokyo, Japan). The ginsenoside Rg3, kindly provided by BTGin Corporation (Occheon, Korea), was concentrated to powder under vacuum.

2.2 Animals and blood pressure measurements

Male 10- to 12-week-old spontaneously hypertensive rats (SHR) and Wistar–Kyoto rats (WKY) weighing 250 to 320 g were purchased from Central Lab. Animal Inc. (Seoul, Korea). SHR and WKY were randomly divided into six groups {WKY control, WKY-KRG (Korean Red Ginseng), WKY-REKRG (Rg3-enriched Korean Red Ginseng), SHR control, SHR-KRG (Korean Red Ginseng), SHR-REKRG (Rg3-enriched Korean Red Ginseng)}, and systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured at the carotid artery for 180 minutes, followed by jugular vein injection of 3 mg/kg KRG or REKRG as described previously[17]. Animals were raised under conditions of controlled lighting (06:00–18:00 daily) and temperature (24°C ± 1°C). Rats were anesthetized with urethane (1200 mg/kg), and the left common carotid artery was cannulated with a

cannula prefilled with heparinized normal saline (0.5 IU/mL) to measure arterial blood pressure. The arterial pressure was determined using a student physiography/data system (ADInstruments, Dunedin, New Zealand) and analyzed using Chart Pro software (ADInstruments). The jugular vein was cannulated for administration of saline as well as REKRG or KRG.

2.3 Western blotting analysis

Anti-phospho-eNOS antibody was purchased from Cell Signaling (Beverly, MA, USA). Anti-NOS3 antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Western blotting analysis was performed by adding 30 μg of tissue homogenate (obtained from rat aorta) to SDS–PAGE gel loading buffer followed by boiling and separation by electrophoresis and transfer onto nitrocellulose membranes. After incubation with appropriate primary and peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology), the chemiluminescent signal was developed using Super Signal West Pico or Femto Substrate from Thermo Fisher Scientific (Pierce, Rockford, IL). Blots were imaged and band densities quantified with a Gel Doc 2000 Chemi Doc system using Quantity One software (Bio-Rad, Hercules, CA). Values were normalized relative to β-actin loading control.

2.4 Nitrite and nitrate measurements

NO metabolites nitrite (NO_2^-) and nitrate (NO_3^-), the stable breakdown products of NO, were quantified using a commercially available kit (Nitrate/nitrite Fluorometric Assay Kit; Cayman Chemicals, Lexington, KY) according to the manufacturer's instructions. Plasma obtained from rat blood was deproteinized using a 10-kDa cutoff filter (Microcon YM10; Millipore, Bedford, MA). After subtraction of background fluorescence, values were normalized to determine the total protein level.

2.5 Statistical analysis

All experiments were performed at least three times. Statistical analysis was performed using SPSS version 13.0 (SPSS Inc., Chicago, IL). Data are presented as the mean ± standard deviation. Statistical significance was determined by analysis of variance (ANOVA) followed by the multiple comparison test with Bonferroni adjustment. In all analyses, $p < 0.05$ was taken to indicate statistical significance.

3. Results

3.1 REKRG reduces blood pressure of SHR

In our previous study, we showed that the concentration of ginsenoside Rg3 in REKRG is approximately 300-fold higher than that in KRG and that REKRG improves impaired endothelium-dependent vasorelaxation in the aorta in SHR[2]. In this study, we examined the effects of REKRG or KRG treatment on SBP and DBP in SHR and WKY. For this, REKRG and KRG were injected into the jugular vein of SHR and WKY at a dose of 3 mg/kg and the blood pressure was recorded for 180 minutes. Measurements were taken at baseline and then at 30, 60, 90, 120, 150, and 180 minutes. Many previous studies have shown that ginsenosides reduce blood pressure via increases in the production of endothelial NO[7] and that Rg3 is the most potent ginsenoside that activates eNOS in the rat aorta. As shown in Figure 1, administration of both KRG and REKRG to WKY rats caused no changes in either SBP (Figure 1A) or DBP (Figure 1B) recorded from 0 to 180 minutes following treatment. In contrast, administration of REKRG caused significant decreases in both SBP and DBP in SHR (Figure 2A and 2B) starting as early as 30 minutes post-treatment of REKRG. However, both SBP and DBP were unaffected by administration of KRG in SHR.

3.2 REKRG stimulates the phosphorylation of eNOS

eNOS serves an important basal regulatory function in the vasculature. When subjected to stimuli, such as shear stress or acetylcholine, eNOS constitutively expressed in endothelial cells oxidizes L-arginine to generate L-citrulline and NO. Rg3 is one of the major biologically active components of ginseng and is regarded as the main compound responsible for its many pharmacological actions, including enhancement of eNOS phosphorylation and NO production. We examined eNOS phosphorylation by western blotting analysis of tissue homogenate from the aortas of both WKY and

SHR after administration of KRG or REKRG. As shown in Figure 3A, administration of both KRG and REKRG stimulated Ser-1177 phosphorylation of eNOS in WKY. Similarly, Ser-1177 phosphorylation of eNOS was also increased in SHR after administration of KRG and REKRG (Figure 3B), but the overall level of phosphorylation was higher in SHR than in WKY. In addition, the efficiency of REKRG to stimulate eNOS phosphorylation was much higher than that of KRG in both groups.

3.3 REKRG stimulates the production of NO

NO is an endothelium-derived relaxing factor that plays an important role in the control of vascular tone and vascular functions. The synthesis of NO by the vascular endothelium is responsible for vasodilator tone, which is essential for the regulation of blood pressure. High blood pressure is characterized by deficiency of eNOS and decreased NO production, especially in the endothelium. Therefore, we measured the plasma levels of NO in WKY and SHR after administration of KRG or REKRG. As expected, administration of both KRG and REKRG stimulated NO production in WKY and SHR (Figure 4A and B). The efficiency of REKRG to stimulate NO production was much higher than that of KRG in both groups. These results suggest that REKRG stimulates the eNOS signaling pathway leading to its phosphorylation, which in turn leads to an increase in NO production in SHR.

4. Discussion

High blood pressure is one of the major risk factors for the development of vascular diseases such as atherosclerosis, coronary heart disease, and stroke,[18] and several studies have reported cardiovascular risks associated with elevated blood pressure[19, 20]. High blood pressure is related to morphological changes and impaired function of vascular smooth muscle and endothelial cells. The beneficial effects of ginsenosides have been widely studied and shown to have new antihypertensive effects[3] and prevent or ameliorate various diseases, including atherosclerosis, cancer, and thrombosis[21-23]. In this study, we demonstrated that REKRG increases eNOS phosphorylation and NO production and decreases blood pressure in SHR. These results indicated that REKRG administration has the potential to stabilize blood pressure and prevent cardiovascular disease.

Several recent studies regarding cardiovascular disease focused on purified individual ginsenoside components of ginseng to show specific mechanisms instead of using whole ginseng extracts. Various components of whole ginseng extracts (i.e., ginsenosides Rg3, Rb1, and Re) have been shown to display antihypertensive and cardiovascular protective effects[7, 24]. Therefore, they are considered potential components for vascular protection. In particular, ginsenoside Rg3 has been studied extensively because it is the most potent vasodilator among the ginsenosides characterized to date. For example, Rg3 induces endothelium-independent vasorelaxation through inhibition of vascular smooth muscle tone by prevention of Ca^{2+} influx and stimulation of K^{+} efflux[7]. Moreover, it stimulates vascular endothelium-derived NO and induces vasodilation in the rat aorta[23,24]. Another important factor is that it can be easily extracted from red ginseng by a steaming and drying process. Processed ginseng, which contains high levels of Rg3, has emerged as a health-supporting agent in some East Asian countries, including Korea and China. In this study, we further investigated the vasoactive efficacy of the selected ginsenosides on vascular function and compared the effects of ginsenosides to two major isolated fractions (KRG and REKRG). In our previous study, we analyzed 11 ginsenosides

(Rg1, Re, Rf, Rh1, Rg2, Rb1, Rc, Rb2, Rg3, Rk1, and Rg5) by high-performance liquid chromatography[2]. The REKRG fraction was shown to contain Rg1, Re, Rf, Rh1, Rg2, Rb1, Rc, Rb2, Rg3, Rk1, and Rg5 at levels of 0.6, 1.9, 12.3, 5.0, 4.2, 3.8, 1.2, 1.0, 100.0, 12.0, and 21.0 mg/g, respectively, while the levels in KRG were 2.9, 4.2, 0.3, 0.1, 0.2, 5.9, 2.2, 2.1, 0.3, 0.05, and 0.12 mg/g, respectively. These results indicate that the concentration of ginsenoside Rg3 in REKRG is approximately 300-fold greater than that in KRG. We showed that REKRG improved endothelium-dependent vasorelaxation in the WKY rat and SHRs compared with controls, also REKRG treatment for 6 weeks reduced the mean aortic intima-media thickness compared with control. The results of the present study also indicate that REKRG stimulates endothelium-dependent vasorelaxation through not only eNOS phosphorylation but also NO production. Interestingly, although REKRG did not significantly decrease the blood pressure in WKY, it markedly decreased the blood pressure in SHR. In addition, REKRG has a greater antihypertensive effect than KRG at the same dose even though KRG has been well known to have an effect on high blood pressure. Therefore, these data suggest that Rg3 is the principal pharmacologically active component of KRG and that it could be an excellent candidate for use in antihypertensive therapy.

One of the limitations of our study was that we measured the blood pressure of rats under anesthesia. It has been shown previously that anesthesia itself can have an influence on cardiovascular function[25]. Anesthetic drug administration has an effect on the autonomic nervous system, thus limiting the evaluation of changes in blood pressure. Therefore, in this study, administration of KRG or REKRG to the rats under anesthesia may have caused changes in parameters, such as heart rate, resulting in tachycardia or bradycardia in anesthetized rats as a compensation against hypotension by the decrease of peripheral resistance[3]. Similarly, KRG or REKRG administration may also cause a decrease in heart contractility directly in anesthetized rats because rats under anesthesia may have reduced nervous reflex control and synaptic transmission in the autonomic nervous system.

NO is a radical generated from L-arginine by NOS, which plays a critical role as a second messenger in cell signaling[10]. Low levels of NO produced and released by the endothelial cells play critical roles in the maintenance of basal vascular tone. Some biochemical stimuli, such as thrombin, adenosine diphosphate (ADP), serotonin, acetylcholine, and bradykinin, as well as mechanical stimuli including shear stress and cyclic strain, result in increased synthesis of endothelial NO. Previous studies have shown a relationship between NO and hypertension. Inhibition of NO synthesis in the vasculature may lead to hypertension or ischemic stroke, likely through its effects on vascular tone. NO produced by vascular endothelial cells has been implicated in many of the effects of ginsenosides, including those on vessel relaxation, protection of the cardiovascular system, and antithrombotic and antiplatelet effects[23, 26].

Various factors can inhibit NO synthesis, one of which is excessive production of reactive oxygen species (ROS) by endothelial cells. ROS refers to oxygen radicals such as superoxide anion, hydroxyl radical, hydrogen peroxide, and peroxynitrite[27]. Studies over the past decade have indicated that ROS generated by endothelial cells play key roles in the signaling mechanisms and affect vascular homeostasis. Endothelial cells are not only major sources but also targets of ROS. Among the various ROS species, the superoxide anion destroys most of the NO[28] and further increases oxidative stress[29]. The superoxide anion level has also been shown to be increased in endothelial cells in SHR[30], which scavenges NO as soon as it is produced. Similarly, concentrations of superoxide anions produced by the aortic rings from SHR were greater than those produced by these structures from WKY[31]. Our results were consistent with this observation and showed that the level of NO production under basal conditions in WKY was higher than that in SHR because increased ROS in SHR immediately destroyed endothelium-derived NO. Some previous reports have demonstrated the anti-hypertensive effect of Rg3 enriched Korean red ginseng in human randomized control trials[32, 33]. Our results demonstrate anti-hypertensive effect of REKRG in comparison to KRG in rat model and we provide

the mechanistic pathway for its anti-hypertensive effect. We show that the vasodilatory effect of REKRG is endothelium dependent and eNOS phosphorylation followed by NO production is the major contributory factor to the observed vascular dilatation which has not been shown in human trials.

In conclusion, the present results indicate that REKRG has a significant antihypertensive effect in SHR, which may be due to the activation of eNOS and stimulation of NO production, and that REKRG intake may be beneficial for individuals with high risks of hypertension and cardiovascular diseases.

Conflict of Interest

No competing financial interests exist.

Acknowledgments

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2014R1A6A1029617 and NRF-2015R1D1A1A01061516). This study was supported by research fund of Chungnam National University in 2015. This study was supported by research fund of Chungnam National University Hospital.

The English in this document has been checked by at least two professional editors, both native speakers of English. For a certificate, please see: http://www.textcheck.com/certificate/C4x3Dm

References

1. Kim JH. Cardiovascular Diseases and Panax ginseng: A Review on Molecular Mechanis ms and Medical Applications. *J Ginseng Res* 2012;36:16-26.

2. Park JB, Kwon SK, Nagar H, Jung SB, Jeon BH, Kim CS, et al. Rg3-enriched Korean Red Ginseng improves vascular function in spontaneously hypertensive rats. *J Ginseng Res* 2014;38:244-50.

3. Jeon BH, Kim CS, Park KS, Lee JW, Park JB, Kim KJ, et al. Effect of Korea red ginseng on the blood pressure in conscious hypertensive rats. *Gen Pharmacol* 2000;35:135-41.

4. Sung J, Han KH, Zo JH, Park HJ, Kim CH, Oh BH, et al. Effects of red ginseng upon va scular endothelial function in patients with essential hypertension. *Am J Chin Med* 200 0;28:205-16.

5. Jeon BH, Kim CS, Kim HS, Park JB, Nam KY, Chang SJ, et al. Effect of Korean red ginse ng on blood pressure and nitric oxide production. *Acta Pharmacol Sin* 2000;21:1095-10 0.

6. Vuksan V, Sievenpipper J, Jovanovski E, Jenkins AL. Current Clinical Evidence for Korean Red Ginseng in Management of Diabetes and Vascular Disease: A Toronto's Ginseng Clinical Testing Program. *Journal of Ginseng Research* 2010;34:264-73.

7. Kim ND, Kang SY, Kim MJ, Park JH, Schini-Kerth VB. The ginsenoside Rg3 evokes endo thelium-independent relaxation in rat aortic rings: role of K+ channels. *Eur J Pharmaco l* 1999;367:51-7.

8. Rees DD, Palmer RM, Moncada S. Role of endothelium-derived nitric oxide in the regu lation of blood pressure. *Proc Natl Acad Sci U S A* 1989;86:3375-8.

9. Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on periphe ral arteriolar tone in man. *Lancet* 1989;2:997-1000.

10. Palmer RM, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide fr om L-arginine. *Nature* 1988;333:664-6.

11. Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological act ivity of endothelium-derived relaxing factor. *Nature* 1987;327:524-6.

12. Shesely EG, Maeda N, Kim HS, Desai KM, Krege JH, Laubach VE, et al. Elevated blood pressures in mice lacking endothelial nitric oxide synthase. *Proc Natl Acad Sci U S A* 1 996;93:13176-81.

13. Watson T, Goon PK, Lip GY. Endothelial progenitor cells, endothelial dysfunction, inflam mation, and oxidative stress in hypertension. *Antioxid Redox Signal* 2008;10:1079-88.

14. Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, Bevan JA, et al. Hypertensi on in mice lacking the gene for endothelial nitric oxide synthase. *Nature* 1995;377:23 9-42.

15. Bobadilla NA, Gamba G, Tapia E, García-Torres R, Bolio A, López-Zetina P, et al. Role of NO in cyclosporin nephrotoxicity: effects of chronic NO inhibition and NO synthases gene expression. *Am J Physiol* 1998;274:F791-8.

16. Panza JA, Casino PR, Kilcoyne CM, Quyyumi AA. Role of endothelium-derived nitric oxi de in the abnormal endothelium-dependent vascular relaxation of patients with essenti al hypertension. *Circulation* 1993;87:1468-74.

17. Parasuraman S, Raveendran R. Measurement of invasive blood pressure in rats. *J Phar macol Pharmacother* 2012;3:172-7.

18. MacMahon S, Peto R, Cutler J, Collins R, Sorlie P, Neaton J, et al. Blood pressure, strok e, and coronary heart disease. Part 1, Prolonged differences in blood pressure: prospe ctive observational studies corrected for the regression dilution bias. *Lancet* 1990;335:7 65-74.

19. Stamler J, Stamler R, Neaton JD. Blood pressure, systolic and diastolic, and cardiovascul ar risks. US population data. *Arch Intern Med* 1993;153:598-615.

20. Kannel WB. Blood pressure as a cardiovascular risk factor: prevention and treatment. *J AMA* 1996;275:1571-6.

21. Zhang C, Liu L, Yu Y, Chen B, Tang C, Li X, et al. Antitumor effects of ginsenoside Rg3

on human hepatocellular carcinoma cells. *Mol Med Rep* 2012;5:1295-8.

22. Zhang YG, Zhang HG, Zhang GY, Fan JS, Li XH, Liu YH, et al. Panax notoginseng saponins attenuate atherosclerosis in rats by regulating the blood lipid profile and an anti-inflammatory action. *Clin Exp Pharmacol Physiol* 2008;35:1238-44.

23. Jin YR, Yu JY, Lee JJ, You SH, Chung JH, Noh JY, et al. Antithrombotic and antiplatelet activities of Korean red ginseng extract. *Basic Clin Pharmacol Toxicol* 2007;100:170-5.

24. Scott GI, Colligan PB, Ren BH, Ren J. Ginsenosides Rb1 and Re decrease cardiac contraction in adult rat ventricular myocytes: role of nitric oxide. *Br J Pharmacol* 2001;134:1159-65.

25. Carruba MO, Bondiolotti G, Picotti GB, Catteruccia N, Da Prada M. Effects of diethyl ether, halothane, ketamine and urethane on sympathetic activity in the rat. *Eur J Pharmacol* 1987;134:15-24.

26. Yu Z, Gao X, Yuan H, Liu T, Ma M, Chen X, et al. Simultaneous determination of safflor yellow A, puerarin, daidzein, ginsenosides (Rg(1), Rb(1), Rd), and notoginsenoside R(1) in rat plasma by liquid chromatography-mass spectrometry. *J Pharm Biomed Anal* 2007;45:327-36.

27. McIntyre M, Bohr DF, Dominiczak AF. Endothelial function in hypertension: the role of superoxide anion. *Hypertension* 1999;34:539-45.

28. Stuehr DJ. Mammalian nitric oxide synthases. *Biochim Biophys Acta* 1999;1411:217-30.

29. Suzuki H, DeLano FA, Parks DA, Jamshidi N, Granger DN, Ishii H, et al. Xanthine oxidase activity associated with arterial blood pressure in spontaneously hypertensive rats. *Proc Natl Acad Sci U S A* 1998;95:4754-9.

30. Grunfeld S, Hamilton CA, Mesaros S, McClain SW, Dominiczak AF, Bohr DF, et al. Role of superoxide in the depressed nitric oxide production by the endothelium of genetically hypertensive rats. *Hypertension* 1995;26:854-7.

31. Auch-Schwelk W, Katusic ZS, Vanhoutte PM. Contractions to oxygen-derived free radicals are augmented in aorta of the spontaneously hypertensive rat. *Hypertension* 1989;1

3:859-64.

32. Jovanovski E, Bateman EA, Bhardwaj J, Fairgrieve C, Mucalo I, Jenkins AL, et al. Effect of Rg3-enriched Korean red ginseng (Panax ginseng) on arterial stiffness and blood pressure in healthy individuals: a randomized controlled trial. *J Am Soc Hypertens* 2014;8:537-41.

33. Jovanovski E, Peeva V, Sievenpiper JL, Jenkins AL, Desouza L, Rahelic D, et al. Modulation of endothelial function by Korean red ginseng (Panax ginseng C.A. Meyer) and its components in healthy individuals: a randomized controlled trial. *Cardiovasc Ther* 2014; 32:163-9.

Figure legends

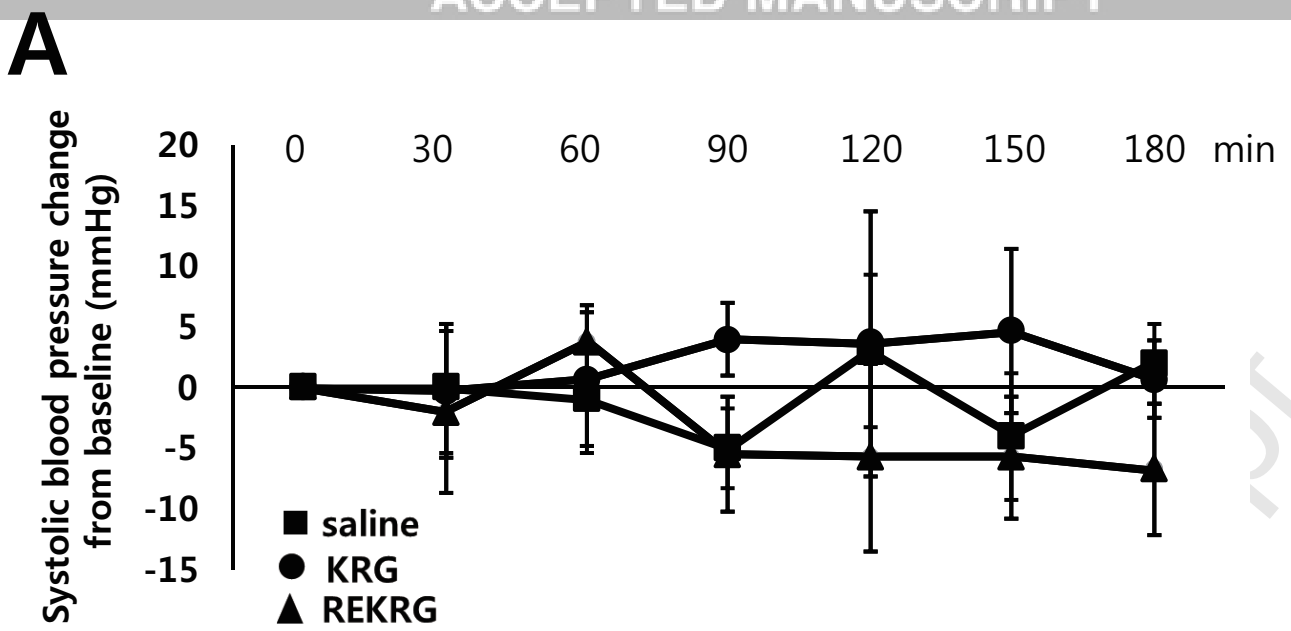
Figure 1. Acute effects of Rg3-enriched Korean Red Ginseng extract (REKRG) on changes in blood pressure in Wistar–Kyoto rats (WKY). Korean Red Ginseng extract (KRG) and REKRG were injected intravenously at 3 mg/kg, and both systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in WKY for 3 hours. (A, B) KRG and REKRG did not significantly alter SBP or DBP compared with controls. (n=6-7)

Figure 2. Acute effects of Rg3-enriched Korean Red Ginseng extract (REKRG) on changes in blood pressure in spontaneously hypertensive rats (SHR). Korean Red Ginseng extract (KRG) and REKRG were injected intravenously at 3 mg/kg, and both systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in SHR for 3 hours. (A, B) REKRG significantly decreased SBP and DBP compared with controls. However, KRG did not. *$p < 0.05$ compared with control. Data represent the mean ± SD ($n = 6–7$).

Figure 3. Effects of Rg3-enriched Korean Red Ginseng extract (REKRG) on phosphorylation of endothelial nitric oxide synthase (eNOS). The phosphorylation of eNOS was measured in the aorta 3 hours after intravenous injection of Korean Red Ginseng extract (KRG) and REKRG 3 mg/kg. (A, B) REKRG and KRG significantly increased eNOS phosphorylation compared with saline control in both WKY and SHR. The levels of eNOS phosphorylation were quantified by densitometric analysis (lower panels in A and B). All western blots shown are representative of three independent experiments. *$p < 0.05$ compared with control. Data represent the mean ± SD.(n=5-6)

Figure 4. Effects of Rg3-enriched Korean Red Ginseng extract (REKRG) on nitric oxide (NO) production. Metabolites of NO (nitrite and nitrate) were measured in the plasma of rats 3 hours after

intravenous injection of Korean Red Ginseng extract (KRG) and REKRG 3 mg/kg. (A, B) REKRG significantly increased NO production compared with saline control in both WKY and SHR. Data are representative of three independent experiments. *$p < 0.05$ compared with control. Data represent the mean ± SD (n = 5–6).



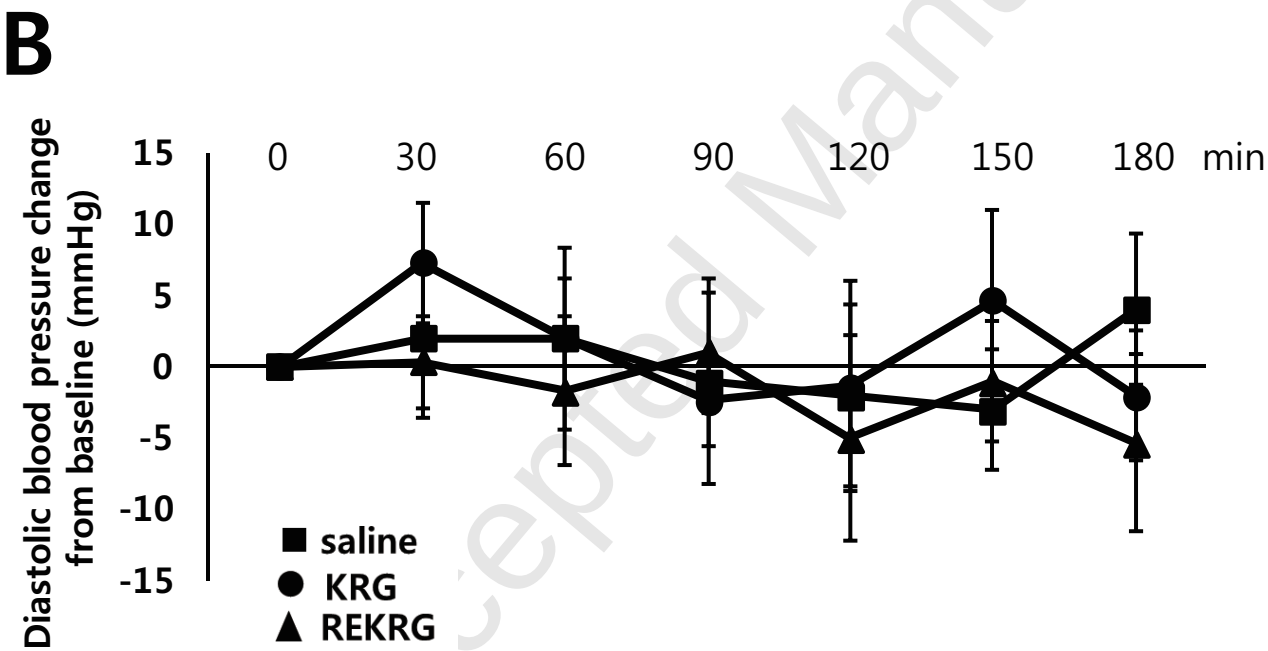
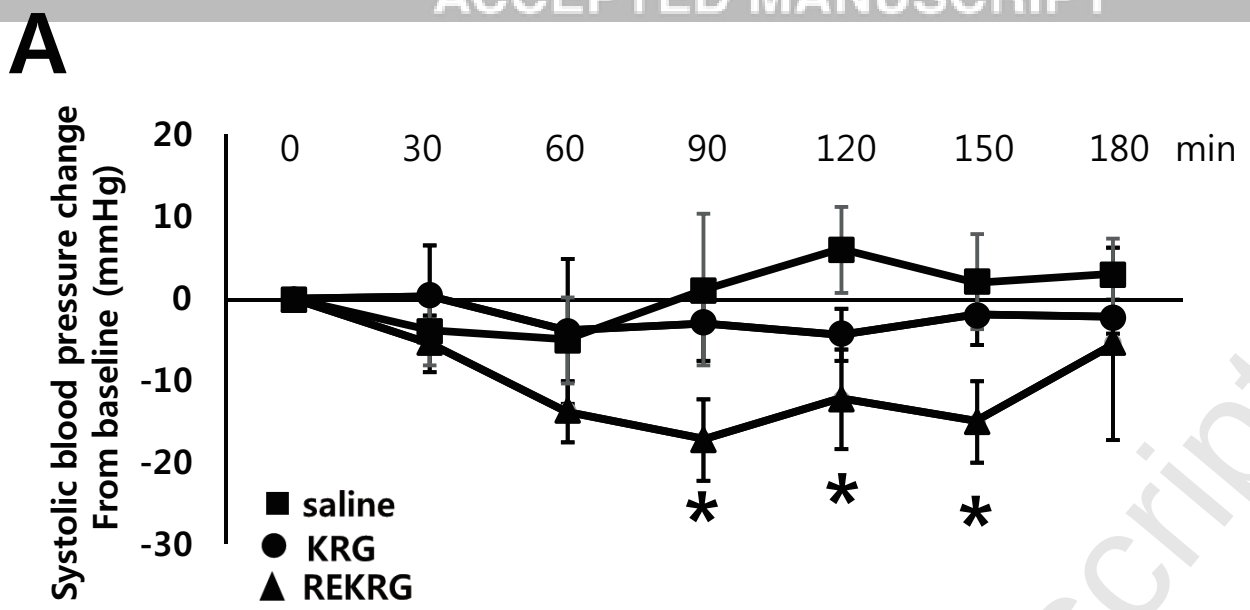


Figure 1



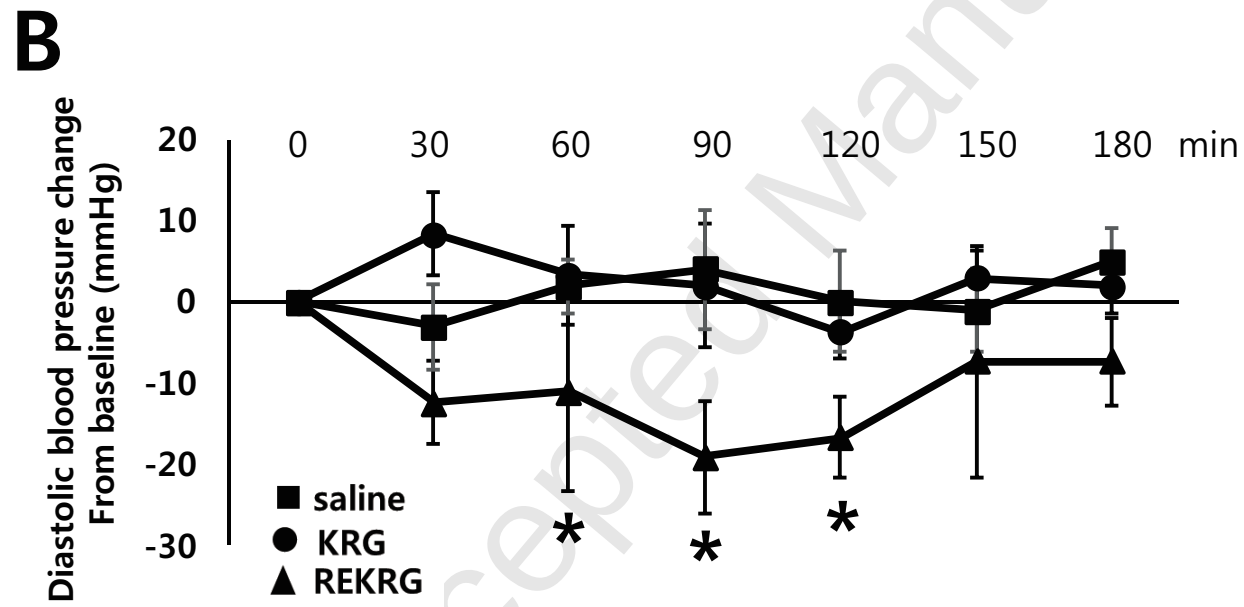


Figure 2

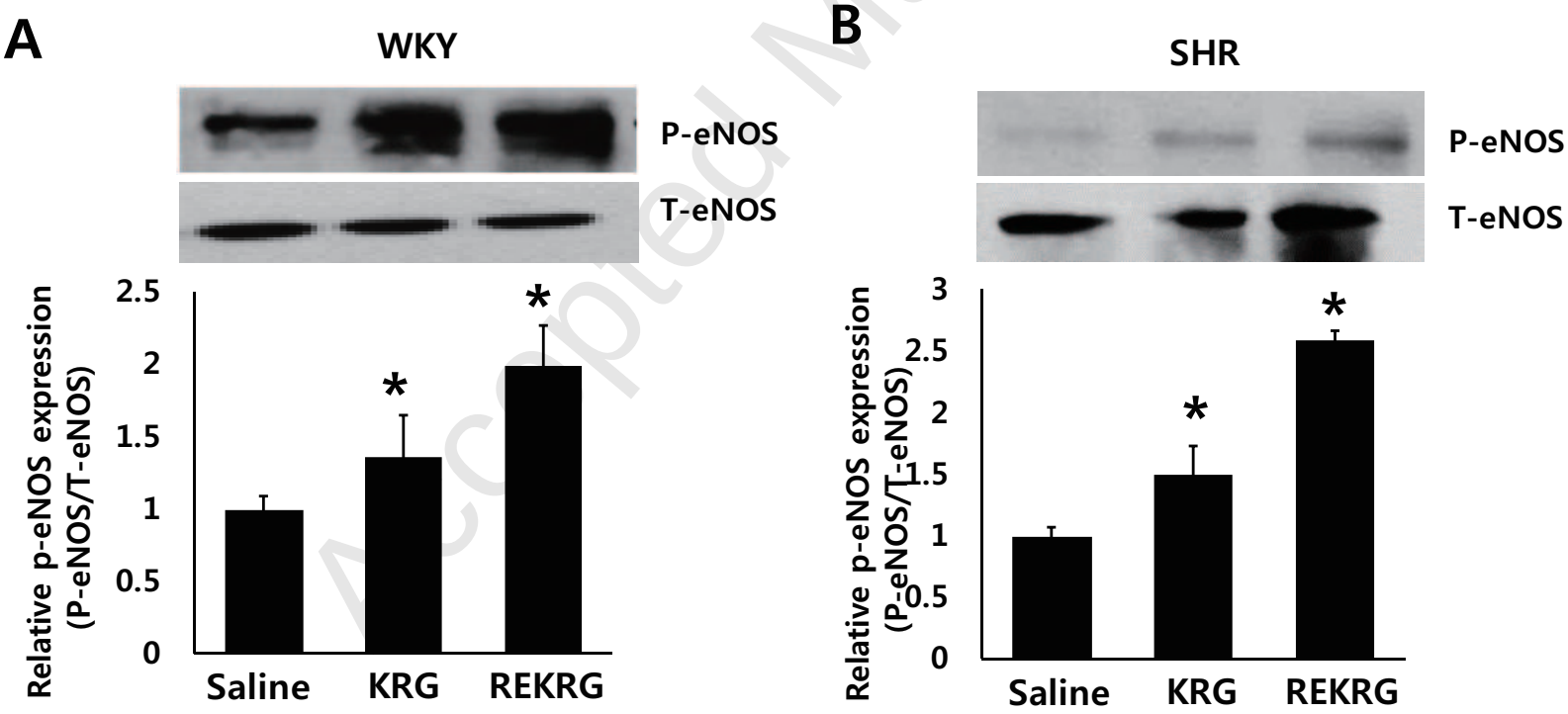


Figure 3

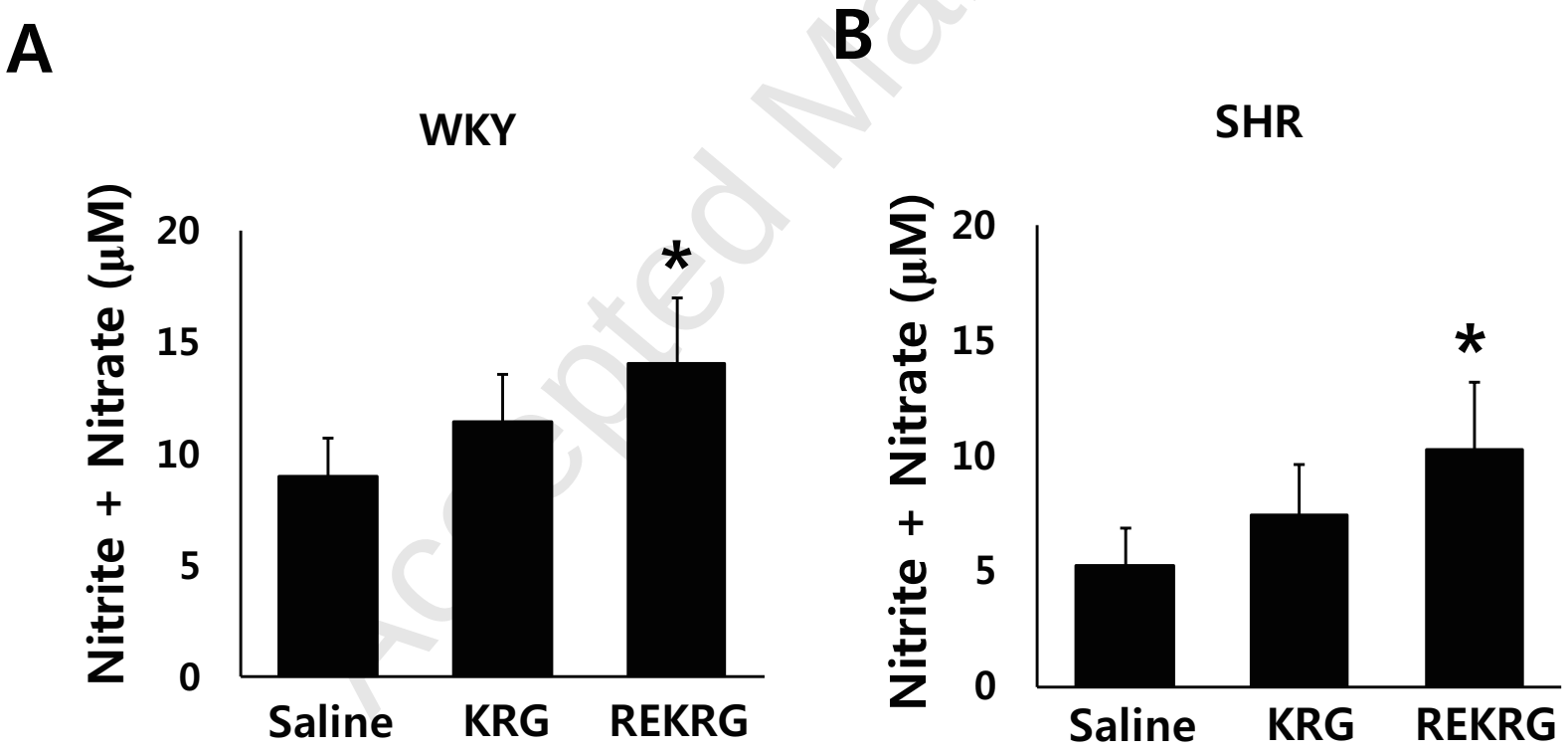


Figure 4