



Original Article

Modified Saengmaeksan, an herbal formula containing six herbs, improves hypertension through RhoA/Rho kinase-mediated vasorelaxation



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ABSTRACT

Background: Saengmaeksan is a traditional herbal formula used in Korean medicine. We composed a modified formula based on Saengmaeksan (mSMS) with Puerariae Radix, Platycodonis Radix, Liriodopsis seu Ophiopogonis Tuber, Schisandrae Fructus, Coicis Semen, and Dioscoreae Rhizoma to investigate its in vivo and ex vivo antihypertensive effects and underlying mechanisms.

Methods: The antihypertensive efficacy of mSMS was assessed using L-NAME-induced hypertensive mice and spontaneously hypertensive rats (SHR). The animals were treated with mSMS, and blood pressure was monitored weekly. Vascular effects were evaluated through organ bath studies and aortic histology, while oxidative stress was assessed using DCF-DA staining. Renal protection was examined via H&E and immunofluorescence staining for AT1R expression. The molecular mechanisms were explored through Western blot analysis and GTP-RhoA assays.

Results: mSMS significantly reduced systolic and diastolic blood pressure in both hypertensive models, with the ethanol extract demonstrating superior efficacy. mSMS inhibited the RhoA/Rho kinase pathway, leading to reduced phosphorylation of MYPT1 and CPI17, and improved vascular relaxation. Additionally, mSMS attenuated aortic wall thickening, oxidative stress, and renal damage, with a marked decrease in AT1R expression in the kidneys.

Conclusions: mSMS exhibits potent antihypertensive effects through the inhibition of the RhoA/Rho-kinase pathway, reduction of vascular remodeling, oxidative stress, and renal protection. These findings support the potential of mSMS as a novel therapeutic agent for hypertension, offering a multi-targeted approach with fewer side effects compared to conventional therapies. Further clinical studies are required to confirm its clinical efficacy.

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1. Introduction

Hypertension, also known as high blood pressure, affects over 1 billion people worldwide. Hypertension is a major risk factor for cardiovascular diseases, stroke, and kidney failure, contributing significantly to morbidity and mortality rates globally.¹ In the United States, nearly half of adults are affected or treated for hypertension.² Current antihypertensive medications include diuretics, beta-blockers, ACE inhibitors, Angiotensin II receptor blockers (ARBs) and calcium channel inhibitors (CCIs). However, most of these drugs are associated with various side effects and limitations.^{3,4} Therefore, herbal medicines are gaining attention for their potential antihypertensive properties, due to the fewer side effects and multi-targeted nature.⁵ Emerging research has shown that certain herbal compounds can effectively reduce blood pressure and improve overall cardiovascular health, making them a promising adjunct or alternative to conventional antihypertensive drugs.^{6–9}

The small guanosine triphosphatase (GTP) binding protein Ras homolog family member A (RhoA) and its downstream effector Rho-kinase play an important role in smooth muscle contraction by agonist-induced Ca^{2+} -sensitization.¹⁰ Activated RhoA increases the Ca^{2+} sensitivity of both MLC phosphorylation and contraction in smooth muscle.¹¹ Activation of Rho-kinase decreases the activity of myosin light-chain phosphatase (MLCP) through phosphorylation of myosin phosphatase targeting subunit 1 (MYPT1) at Thr855 and protein kinase C (PKC)-potentiated inhibitory protein for heterotrimeric MLCP of 17 kDa (CPI17) at Thr38, enhancing vascular contractility.¹² Because of this key role in vascular smooth muscle contraction,¹³ RhoA/Rho-kinase pathway is a promising target for novel antihypertensive strategies.

This study focuses on the antihypertensive potential of a modified version of an herbal medicine decoction named Saengmaeksan (mSMS). We conducted a series of experiments including organ bath studies, mouse studies using the NG nitro L-arginine methyl ester (L-NAME) model,¹⁴ and studies on spontaneously hypertensive rats (SHR).¹⁵ Also, organ bath studies were designed to investigate the direct effects of mSMS on vascular smooth muscle tone. We also compared the effect of mSMS with currently prescribed hypertension drugs such as amlodipine and losartan. Our results provide evidence of the efficacy and potential mechanisms of mSMS as a novel antihypertensive agent.

2. Methods

2.1. Reagents

L-NAME, 11,9 epoxymethano-prostaglandin F2 α (U46619), KCl, phorbol 12,13-dibutyrate (PDBu), Ro31–8220, losartan, and amlodipine were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2. Preparation of mSMS

The herbal combination to prepare mSMS extracts were as follows: Puerariae Radix 40 g, Platycodonis Radix 40 g, Liriodopsis seu Ophiopogonis Tuber 40 g, Schisandrae Fructus 20 g, Coicis Semen 20 g, Dioscoreae Rhizoma 20 g. The herbs were mixed and extracted by 1.8 L of 100 °C D.W. for 2 h or by storing in 1.8 L of 70 % ethanol in water for 3 days at 20 °C. After being filtered, the extracts were concentrated by rotary evaporation (Eyela, Tokyo, Japan) then freeze-dried. The vouchers for each herb are stored in the Department of Pharmacology, College of Korean Medicine, Kyung Hee University.

2.3. High-performance liquid chromatography (HPLC)

mSMS was standardized based on the metabolites from its components using HPLC. Specifically, puerarin, daidzin, schizandrin, and gomisin A were chosen as marker compounds for standardization. The analytical HPLC system was equipped with a Waters Alliance e2695 HPLC system with 996 PDA detector (Waters Co., Manchester, UK) and

a RStech HECTOR-M C₁₈ (4.6 mm $\times$ 250 mm i.d.; 5 μ m). A HECTOR-M C18 column (4.6 $\times$ 250 mm, 5 μ m, 100 Å, RStech) was used with the column temperature maintained at 40 °C. The injection volume was 10 μ L, and samples were prepared at a concentration of 10 mg/mL in 100 % methanol. The mobile phase consisted of solvent A (water containing 0.1 % formic acid, w/w) and solvent B (acetonitrile containing 0.1 % formic acid, w/w). The flow rate was maintained at 1.0 mL/min from 0 to 24.0 min, increased to 1.2 mL/min at 24.1 min, and maintained until 33.0 min. The gradient elution program was as follows: starting at 85 % A and 15 % B at 0 min, changing to 80 % A and 20 % B at 9 min, then to 40 % A and 60 % B at 14 min, followed by 1 % A and 99 % B at 19 min, which was maintained until 24 min. At 24.1 min, the gradient was returned to the initial condition of 85 % A and 15 % B, and held until 33 min for re-equilibration.

2.4. Ex vivo studies

Ex vivo studies were approved by the Institutional Animal Care and Use Committee of National Institute for Korean Medicine Development (NIKOM-2022–14) and carried out in accordance with the Guide for the Care and Use of Laboratory Animals, 8th edition. Vascular tension was evaluated in thoracic aortic rings collected from Sprague-Dawley rats, weighing 320–350 g. A vasoconstriction study was performed as described previously.¹⁶ Briefly, the thoracic aorta was excised and cut into four ring segments of 3.5 mm. Endothelium denudation was done when needed, by gently rubbing the internal surface with the edge of a forceps. Each aortic ring was suspended in a water-jacketed organ bath (6 mL) maintained at 37 °C and 95 % O₂–5 % CO₂, connected to an isometric force transducer (Danish Myo Technology, Aarhus N, Denmark), and stretched to an optimal resting tension of 2.0 g, which was maintained throughout the experiment. Each ring was equilibrated in the organ bath solution for 60 min before the experiment measuring the contractile response after the addition of KCl. To determine the effect of mSMS on vascular tension in an endothelium-intact or endothelium-denuded rat aortic ring, vascular contractions were induced by thromboxane A₂ agonist U46619 (10 nM) or KCl (50 mM), or PKC activator PDBu (0.1 μ M). When each contraction reached a plateau, mSMS was added cumulatively (0.1–1.0 mg/ml) to elicit vascular relaxation. The effect of mSMS on vascular relaxation were determined after pretreatment with Ro31–8220 (1.0 μ M) or vehicle (0.1 % DMSO) for 30 min.

2.5. Western blot

After functional study, muscle strips were quickly immersed in acetone containing 10 % trichloroacetic acid (TCA) and 10 mM dithiothreitol (DTT) precooled to –80 °C. The aortic rings were washed in acetone containing 5 mM DTT to remove TCA, air-dried and stored at –80 °C until use. Western blot assays were performed as previously described.¹⁷ In brief, stored muscle samples were homogenized in RIPA buffer with protease and phosphatase inhibitors. Protein-matched samples (Bradford assay) were electrophoresed in SDS-PAGE gels, transferred to nitrocellulose membranes, and subjected to primary antibodies. Anti-mouse, anti-goat and anti-rabbit antibodies conjugated with horseradish peroxidase were used as secondary antibodies.

2.6. GTP RhoA assay

Muscle strips were quick frozen by liquid nitrogen, and kept at –80 °C. G-LISA RhoA Activation Assay Biochem Kit (Catalog no. BK124, Cytoskeleton Inc., Denver, CO, USA) was used to perform GTP RhoA assays, according to the manufacturer's instructions as previously described.¹⁸ Briefly, muscle strip samples were homogenized in a lysis buffer and were centrifuged at 13,200 rpm for 15 min at 4 °C. The supernatant, containing 37 g of proteins, was transferred into a plate, and equal volumes of ice-cold binding buffer mixtures were added into each well. The plate was shaken on a cold orbital microplate shaker

(300 rpm) for 30 min at 4 °C, flicked out of the solution from wells, and incubated with diluted anti-RhoA primary antibodies followed by secondary antibodies on a microplate shaker (300 rpm) at room temperature for 45 min each. The plate was incubated with a horse radish peroxidase detection reagent for 15 min at 37 °C, and, after addition of a horseradish peroxidase stop buffer, the absorbance was immediately recorded at 490 nm. In total, four independent biological replicates were performed, using eight animals (2 vessels per replicate × 4 replicates). All contraction assays were completed prior to freezing, ensuring that the RhoA activation status reflected the contractile state induced by drug treatment.

2.7. Animal study

To evaluate the in vivo efficacy of mSMS, the L-NAME-induced hypertension mouse model and spontaneously hypertensive rat (SHR) model were used. All in vivo studies were approved by the Institutional Animal Care and Use Committee of Kyung Hee University (KHSASP-23-341) and carried out in accordance with the Guide for the Care and Use of Laboratory Animals, 8th edition. Doses of mSMS were selected based on the clinically used oral dose of 8000 mg/day in adult humans, with using converted doses of 500 mg/kg in mice and up to 800 mg/kg in rats.

For the L-NAME-induced hypertension study, 8-week-old female ICR mice and 8-week-old male C57BL/6 mice were used. The mice were first acclimated for one week in a controlled environment with a temperature of 25 ± 5 °C, humidity of 60 ± 20 %, and a 12-h light/dark cycle. During the acclimation period, the mice were provided with standard laboratory chow and water ad libitum. Following acclimation, hypertension was induced by administering L-NAME, an inhibitor of nitric oxide (NO) synthase.¹⁹ L-NAME was dissolved in drinking water at a concentration of 1 mg/mL and was provided to the mice ad libitum for a duration of three weeks. The water containing L-NAME was freshly prepared and replaced daily to ensure consistent dosing. Starting on the same day, mice were divided into two groups: the control group received PBS, while the treatment group received mSMS (500 mg/kg/day) via oral gavage for 3 weeks. For the SHR study, 8-week-old male SHRs, along with age-matched Wistar-Kyoto (WKY) rats as normotensive controls, were housed under standard laboratory conditions with a controlled temperature of 25 ± 5 °C, relative humidity of 60 ± 20 %, and a 12-h light/dark cycle. The animals were allowed free access to standard chow and water throughout the study period. mSMS, prepared in 100 μ L PBS at the indicated doses, was administered orally at consistent times. Losartan (20 mg/kg/day, per oral) and amlodipine (10 mg/kg/day, per oral) were used as positive controls.

Blood pressure was measured weekly using the CODA non-invasive blood pressure system (Kent Scientific, Torrington, CT, USA) as previously described.²⁰ Following 1-week acclimation to the instrument, SBP and DBP were measured weekly at the tail using volume pressure recording (VPR) sensor technology after a 10 min acclimation on a warming pad. Each session comprised 2 sets of 15 measurement cycles. Values retrieved from “Regular Cycle = True” and “Accepted = True” by the system’s automated quality assessment were considered valid. For each animal, values from the most stable window within a ± 10 mmHg range were averaged, and only datasets with at least four valid cycles per time point were included. After the final week of treatment, the mice were euthanized and blood and tissues were harvested for further analysis. Tissues were either processed for histological studies or frozen in liquid nitrogen and stored at -80 °C until further use.

2.8. Hematoxylin & eosin (H&E) staining

Aorta and kidney tissues were harvested and fixed in 10 % neutral-buffered formalin for 24 h. The fixed tissues were then embedded in paraffin and sectioned at a thickness of 5 μ m. The sections were deparaffinized in xylene and rehydrated through graded alcohols. H&E staining

was performed to evaluate the structural changes. Briefly, sections were stained with hematoxylin for 5 min, washed in running tap water, and then counterstained with eosin for 1–2 min. After dehydration through graded alcohols and clearing in xylene, the sections were mounted using a synthetic resin. The stained sections were observed under a light microscope, and the thickness of the aortic wall was measured using image analysis software.

2.9. Immunofluorescence (IF) staining

Oxidative stress in aortic tissues was assessed using 2',7'-dichlorofluorescein diacetate (DCF-DA) staining. Freshly isolated aorta segments were incubated with 10 μ M DCF-DA in a Krebs-Henseleit solution at 37 °C for 30 min in the dark. After incubation, the aorta segments were washed with PBS to remove excess DCF-DA. The fluorescence intensity, indicative of reactive oxygen species (ROS) levels, was captured using a fluorescence microscope with an excitation wavelength of 488 nm and an emission wavelength of 525 nm. The fluorescence images were analyzed using ImageJ software, and the mean fluorescence intensity was calculated as a measure of oxidative stress.

To assess the expression of angiotensin II type 1 receptor (AT1R) in kidney tissues, immunofluorescence (IF) staining was performed. Kidney sections were deparaffinized in xylene, rehydrated in graded alcohols, and subjected to antigen retrieval by heating in a citrate buffer (pH 6.0) for 20 min. The sections were then blocked with 5 % bovine serum albumin (BSA) in PBS for 1 h at room temperature to prevent nonspecific binding. After blocking, the sections were incubated with a primary antibody against AT1R (diluted 1:200 in PBS) overnight at 4 °C. Following primary antibody incubation, the sections were washed in PBS and incubated with a fluorescence-conjugated secondary antibody (Alexa Fluor 488, diluted 1:500 in PBS) for 1 h at room temperature in the dark. Nuclei were counterstained with DAPI for 5 min. The sections were mounted with antifade mounting medium and observed under a fluorescence microscope. The fluorescence intensity of AT1R was quantified using ImageJ software.

3. Results

3.1. HPCL analysis of mSMS

First, we analyzed the constituents of mSMS. HPLC analysis identified puerarin, daidzin, schisandrin, and gomisin A, the four known compounds of the herbal components of mSMS (Supplement 1).

3.2. mSMS shows vasorelaxation effects in endothelium-intact or endothelium-denuded aortic rings

We investigated the vasorelaxation response in aortic rings. U46619 caused contraction, and EtOH extract or water extract of mSMS was added cumulatively to elicit relaxation (Fig. 1a). EtOH extract of mSMS showed significant relaxation starting at 0.4 mg/mL. Water extract of mSMS at high concentrations also caused slight vasoconstriction in endothelium-intact aorta (Fig. 1b). In endothelium-denuded aortic rings (Fig. 1c), water extract of mSMS showed better relaxation effect than in endothelium-intact aorta but the effect of EtOH extract was decreased at 0.8 and 1.0 mg/mL concentrations ($p < 0.05$). Overall, EtOH extract of mSMS showed higher vasorelaxation effects in both endothelium-intact and endothelium-denuded aorta. Next, the tension of endothelium-denuded aortic rings induced by U46619 was measured after pretreatment with mSMS. The tension is expressed as percentage to initial contractions in response to 50 mM KCl. The contractile response to U46619 was prevented by both extracts of mSMS, with EtOH extract showing better effect (Fig. 1d, e).

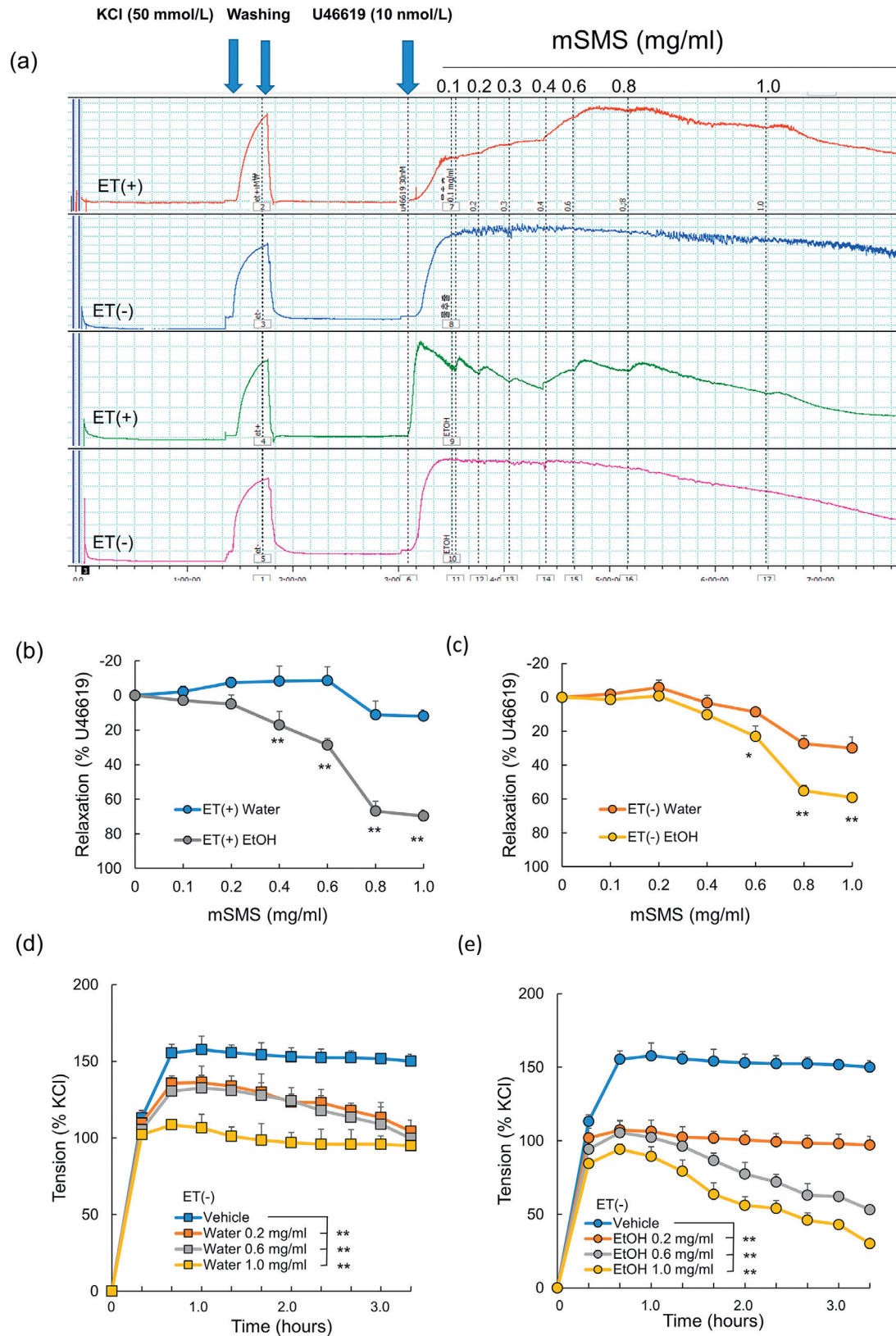


Fig. 1. Effect of mSMS water and EtOH extracts on vascular contraction. Isolated rat aorta rings were pre-contracted with U46619 and then treated with mSMS water extract and ethanol extract. The contractile response was measured using the organ bath method. (a-c) The relaxation responses were expressed as the percentage to maximal contractions in response to U46619 (10 nmol/L). mSMS was added cumulatively (0.1–1.0 mg/ml) to elicit relaxation in aortic rings precontracted with U46619. (d, e) Pretreatment effect of mSMS on U46619-induced contraction was determined. Developed tension is expressed as a percentage of the contraction to KCl (50 mmol/L). Data are expressed as means of 5 experiments with vertical bars showing SEM. * $P < 0.05$ vs. vehicle.

3.3. mSMS exerts vasorelaxation effects through inhibition of RhoA/Rho-kinase signaling

After pretreatment with mSMS, we added U46619 to induce contraction until plateau, and the amount of GTP-RhoA was determined. U46619 increased GTP-RhoA (1.98 ± 0.11 -fold vs. control), while EtOH extract of mSMS suppressed GTP-Rho levels (1.53 ± 0.10 -fold by 0.6 mg/mL and 1.45 ± 0.09 -fold by 1.0 mg/mL). Water extract showed insignificant changes (Fig. 2a). Next, we determined the level of the phosphorylation of Rho kinase substrates MLC20, MYPT1, and CPI17. U46619-stimulated MYPT1Thr⁸⁵⁵ phosphorylation increased significantly (2.10 ± 0.12 -fold) compared with the control. As shown in Fig. 2b and c, the phosphorylation level of MYPT1Thr⁸⁵⁵ was significantly decreased by mSMS (1.0 mg/ml mSMS_W, 1.59 ± 0.09 -fold; 0.2, 0.6, or 1.0 mg/ml mSMS_E, 1.65 ± 0.10 , 1.51 ± 0.11 and 1.40 ± 0.07 -fold). CPI17 is phosphorylated at Thr38. We also determined whether mSMS decreased the phosphorylation of CPI17Thr³⁸ induced by U46619. U46619 increased the phosphorylation level of CPI17Thr³⁸ as compared with the control, which was significantly decreased by pretreatment with mSMS (1.0 mg/ml mSMS_W, 0.76 ± 0.05 -fold; 0.2, 0.6, or 1.0 mg/ml mSMS_E, 0.62 ± 0.12 , 0.59 ± 0.07 and 0.58 ± 0.08 -fold).

After pretreatment with Ro31-8220, mSMS was treated, then aortic rings were challenged with PDBu. Pretreatment with Ro31-8220 inhibited the vascular contractility and phosphorylation of CPI17Thr³⁸ in response to PDBu. However, both mSMS_W and mSMS_E did not affect PDBu-induced vascular contraction (Fig. 2d) and CPI17 phosphorylation (Fig. 2e).

3.4. mSMS lowers blood pressure in L-NAME-induced hypertension mouse models

Next, we conducted animal studies to demonstrate the effect of mSMS *in vivo*. In male C57BL6 mice (Fig. 3a) and female ICR mice (Fig. 3b), EtOH extract of mSMS significantly suppressed systolic and diastolic blood pressures. Water extract was less effective. mSMS is a multi-herbal decoction, consisted of 6 herbs. When each herb extract was subjected to blood pressure tests *in vivo*, we could see that single herb extracts were not as effective as the decoction (Supplement 2). Next, we prepared six 5-herb-decoctions by excluding an herb one by one. It was found out that mSMS, containing all 6 herbs, showed far by the best effect compared to any of the 5 herb-combinations (Supplement 3).

3.5. mSMS lowers blood pressure in the SHR hypertension rat model

Next, we introduced the SHR rat model to evaluate the efficacy of mSMS. We also compared the antihypertensive effect of mSMS with the two most widely prescribed drugs for hypertension: losartan (an ARB) and amlodipine (a CCI). As seen in Fig. 4, while SBP rose to 210.7 ± 5.4 mmHg in SHR rats at week 4 (vs. 128.7 ± 2.7 mmHg in WKY rats), treatment with mSMS 400 mg/kg (186.0 ± 2.1 mmHg) and 800 mg/kg (180.2 ± 4.1 mmHg) significantly suppressed SBP. Losartan-treated SHR rats showed SBP of 156.5 ± 2.3 mmHg and amlodipine group showed 161.7 ± 2.6 mmHg. DBP results were similar, showing that mSMS effectively lowers DBP in SHR rats at a comparable level to positive controls.

3.6. mSMS reduces thickness and oxidative stress in the aorta of SHR rats

We measured the change in aorta thickness of SHR rats, since increased aorta thickness is a typical pathological sign in hypertension patients.²¹ As shown in Fig. 5a, a significant increase was observed in SHR rats compared to WKY control rats (141.44 ± 8.63 μ m vs. 263.13 ± 8.78 μ m, $p < 0.001$). mSMS treatment reduced the thickness of aorta at higher doses. Positive controls losartan and amlodipine were

effective as well. The vascular DCF-DA staining revealed a significant increase in ROS levels in SHR rats compared to normotensive WKY controls (Fig. 5b). Specifically, the hypertensive group exhibited a 7.2-fold increase ($p < 0.01$), indicating heightened oxidative stress. mSMS treatment significantly suppressed the level of DCF-DA intensity. Positive controls losartan and amlodipine both showed decrease of ROS.

3.7. mSMS reduces injury and AT1R expression in the kidney of SHR rats

Histological analysis using H&E staining was performed to assess the extent of renal injury in SHR rats (Fig. 6a). The untreated SHR rats exhibited significant renal pathology, characterized by glomerular hypertrophy, increased mesangial matrix, and tubulointerstitial damage, including tubular dilation and inflammatory cell infiltration. In contrast, SHR rats treated with mSMS displayed marked improvements in renal histology. H&E staining showed a significant reduction in glomerular hypertrophy and mesangial expansion compared to the untreated group. Additionally, the tubulointerstitial damage was notably attenuated, with fewer dilated tubules and reduced inflammatory infiltration observed in the treated rats. Moreover, the expression of AT1R, a key mediator in the pathogenesis of hypertensive renal injury, was assessed in the kidney tissue (Fig. 6b). Vehicle-treated SHR rats showed elevated AT1R expression compared to WKY rats, consistent with the hypertensive and renal damage observed. mSMS treatment showed a tendency to reduce AT1R expression in the kidney, however without statistical difference. Yet, at 400 and 800 mg/kg doses, the reduction rate was around 20 %. The ARB losartan also decreased the expression of AT1R as expected.

4. Discussion

4.1. Summary of key findings

In this study, we developed a modified herbal formulation, mSMS, based on Saengmaeksan to treat age-related hypertension. In both L-NAME-induced hypertensive mice and SHR rats, mSMS significantly reduced systolic blood pressure in a dose-dependent manner, with efficacy comparable to losartan and amlodipine. Mechanistically, mSMS attenuated vascular remodeling and reduced reactive oxygen species in the aorta, preserved renal structure, and downregulated AT1R expression. At the molecular level, mSMS inhibited RhoA activation and downstream phosphorylation of MYPT1 and CPI17, implicating suppression of the RhoA signaling axis as a central mechanism. These findings collectively position mSMS as a promising multi-targeted antihypertensive agent that exerts vasorelaxant and end-organ protective effects through suppression of vascular oxidative stress and contractile signaling.

4.2. Comparison with previous studies

The original formulation of Saengmaeksan, consisting of Ginseng radix, Ophiopogonis radix, and Schisandrae fructus, was traditionally used to restore qi and revive the pulse. Historically, this formula was widely used to alleviate symptoms such as thirst, fatigue, and excessive sweating caused by summer heat.²² Notably, historical records indicate that it was highly valued by the Joseon royal court, where it was consumed as a functional herbal tea during the summer.²³ Saengmaeksan is shown to exert positive effects related to blood lipid, arteriosclerosis index, and relaxation of contract artery.²⁴ Saengmaeksan also protects liver damage induced by alcohol in rats²⁵ or improve physical stamina and reduces oxidative stress, thereby enhancing athletic performance and energy metabolism²⁶. Other studies demonstrated that Saengmaeksan has anti-inflammatory effects²⁷ and anti-hyperuricemic effects.²⁸ mSMS used in this study was developed with the specific goal of improving circulatory dysfunction, particularly in elderly individuals with a distinct hypertensive profile, which is characterized by elevated systolic blood pressure but relatively low diastolic pressure. To

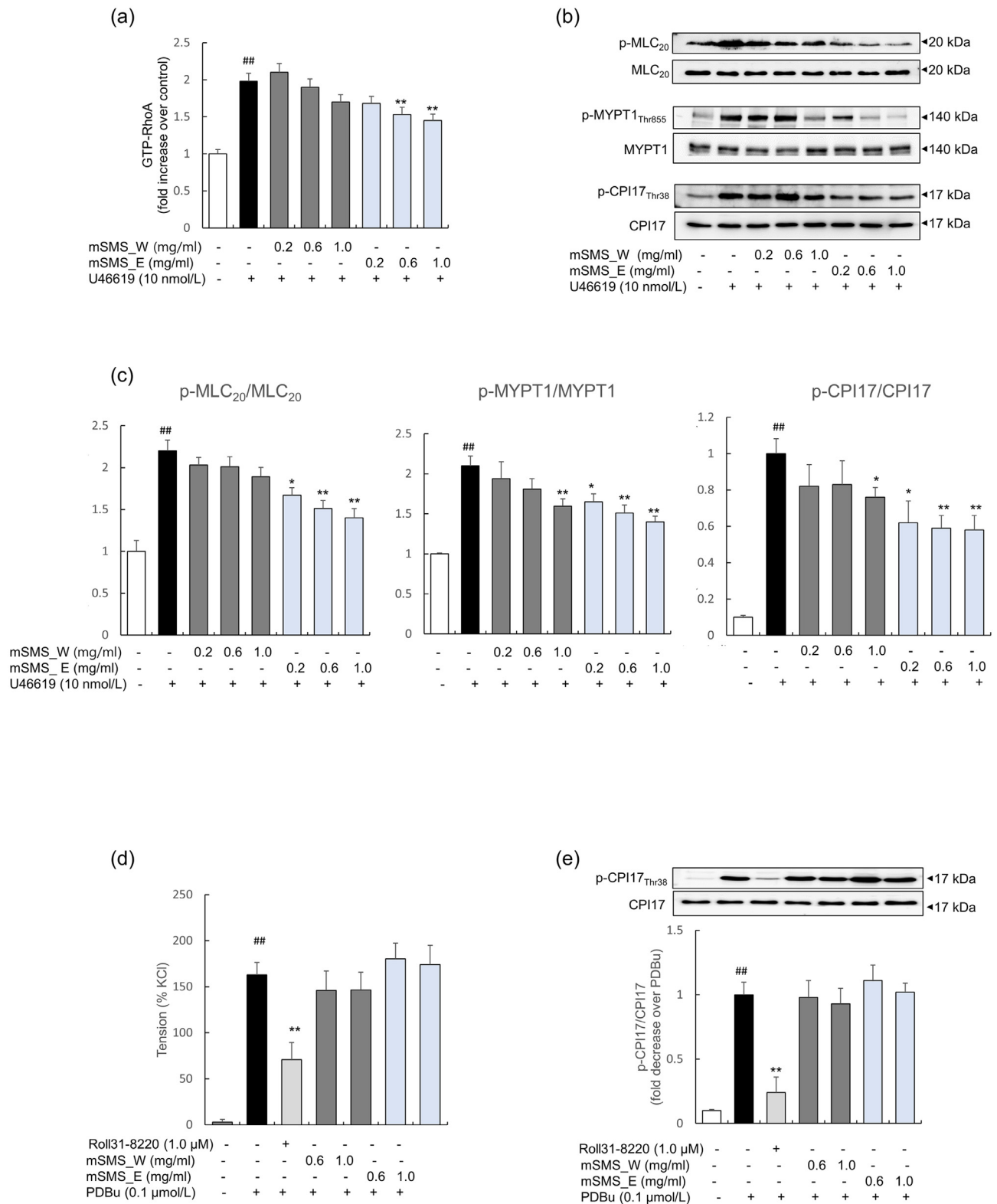


Fig. 2. Effect of mSMS water and EtOH extracts on Rho/Rho kinase pathway. (a) RhoA activation was assessed by G-LISA after treatment with U46619 (10 nmol/L). Modified Saengmaeksan had been pretreated for 30 min. Absorbance at the control (OD of around 0.4) was expressed as 1 arbitrary unit. (b) Phosphorylation levels of MLC20, MYPT1, and CPI17 were measured by western blot. (c) Relative ratios of phosphorylation against the total protein forms of MLC20, MYPT1, and CPI17 were measured. Data are expressed as means of 4 experiments with vertical bars showing SEM. (d) Effect of mSMS on PDBu-induced contraction in PKC-inhibited conditions. PDBu (0.1 μmol/L) was added to elicit tension in the presence or absence of Ro31-8220, mSMS (0.6 or 1.0 mg/ml) for 30 min in the aortic rings with endothelium denuded. Developed tension was expressed as a percentage of the maximum contraction to KCl (50 mmol/L). (e) CPI17 phosphorylation at Thr38 was assessed by Western blots after PDBu treatment. ## $P < 0.01$ vs. vehicle; * $P < 0.05$, ** $P < 0.01$ vs. U46619 alone (a-c) or PDBu alone (d, e).

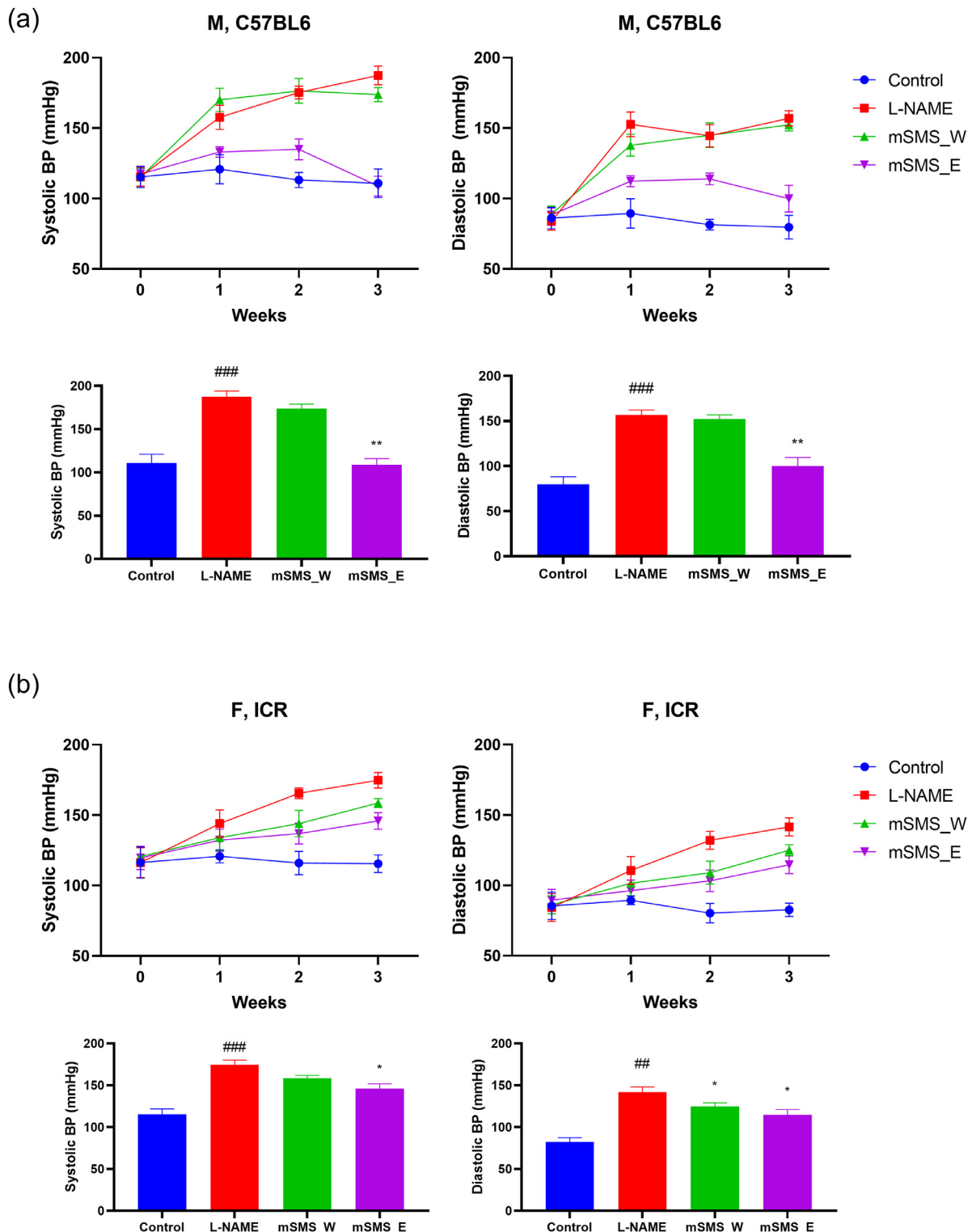


Fig. 3. Effect of mSMS extracts on blood pressure in L-NAME-induced hypertensive mice. (a) Male C57BL6 mice or (b) female ICR mice were administered L-NAME for 3 weeks to induce hypertension. mSMS water extract or ethanol extract (500 mg/kg/day) was orally administrated simultaneously. Blood pressure (SBP and DBP) was measured once a week using tail-cuff method. Bar graph show the blood pressure at Week 3 as mean $\pm$ SEM. $##P < 0.01$, $###P < 0.001$ vs. control group; $*P < 0.05$, $**P < 0.01$ vs. L-NAME hypertension group.

this end, *Panax ginseng* was excluded due to its known hypertensive potential in certain populations,²⁹ and the formula was reconstituted with six herbal ingredients: Puerariae Radix, Platycodonis Radix, Liriodendron Ophiopogonis Tuber, Schisandrae Fructus, Coicis Semen, Dioscoreae Rhizoma. The rationale behind this modification was to preserve the

fluid-nourishing and pulse-reviving properties of the traditional Saeng-maeksan, while enhancing its therapeutic potential in vascular stiffness and pulse pressure imbalance. Here, we show that these six herbs synergistically exert an antihypertensive effect, yet each single herb do not have significant effects. Ingredients such as Puerariae radix³⁰ and Coicis

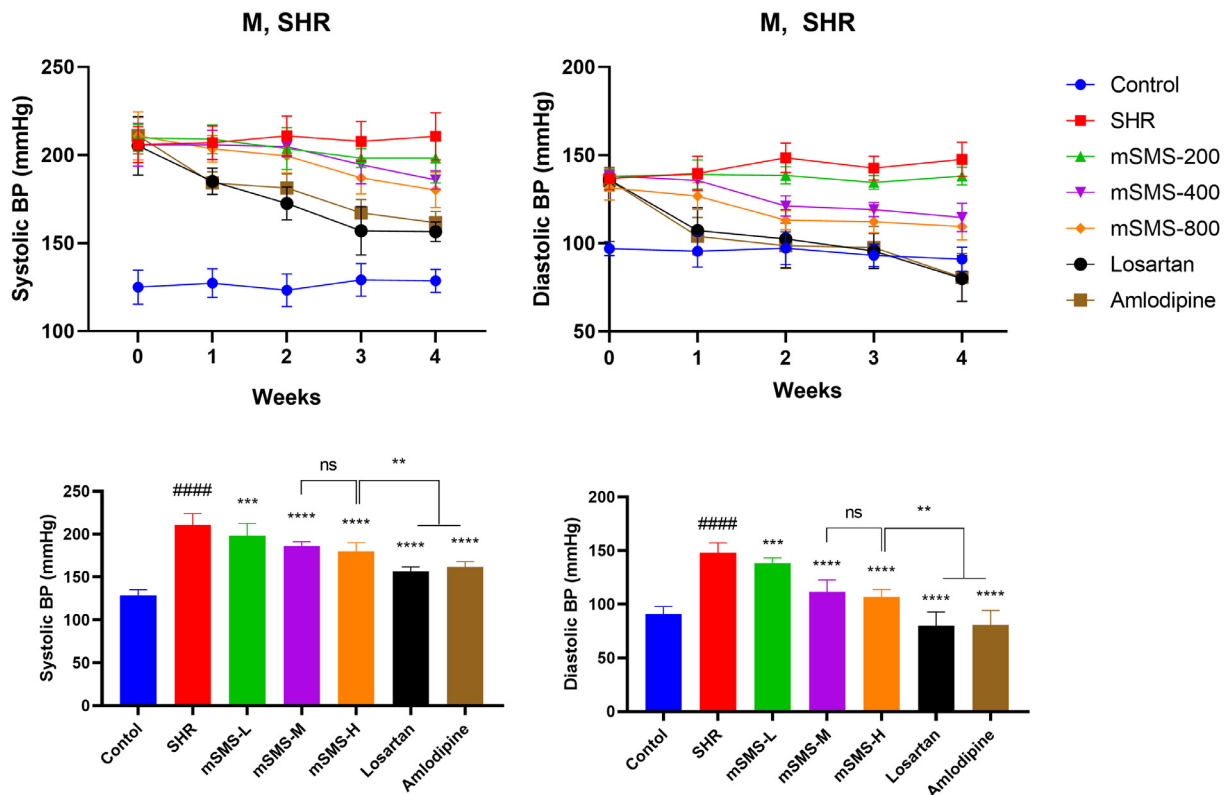


Fig. 4. Effect of mSMS extracts on blood pressure in SHR hypertensive rats. SHR rats were orally administrated with mSMS ethanol extract at 200, 400, and 800 mg/kg/day. Blood pressure (SBP and DBP) was measured once a week using tail-cuff method for 4 weeks. Losartan (20 mg/kg/day) and amlodipine (10 mg/kg/day) were used as positive controls. WKY rats were fed PBS as a control group. Bar graph show the blood pressure at Week 4 as mean $\pm$ SEM. #### $P < 0.0001$ vs. WKY control group; * $P < 0.05$, ** $P < 0.01$ vs. SHR hypertension group.

semen³¹ are known to improve peripheral circulation and reduce systolic overload, making the modified formulation more suitable for age-related vascular dysfunction. Platycodonis Radix improves vascular inflammation and oxidative stress,³² Schisandrae Fructus shows cardioprotective effects in ovariectomized and balloon-induced carotid artery injury rats,³³ and Dioscoreae Rhizomab exerts anti-hyperuricemic and nephroprotective effects in hypertensive mice.³⁴ Based on this experimental evidence, together with clinical aspects, suggest mSMS as a beneficial therapeutic for hypertension patients.

4.3. Clinical and scientific implications

In this study, we utilized two different *in vivo* models to determine the therapeutic effect of mSMS in hypertension: The L-NAME mouse model and SHR model. L-NAME, an inhibitor of NO synthase,¹⁹ induces hypertension in animals by reducing NO production, which is essential for vasodilation.³⁵ The SHR model is a genetically hypertensive rat model that mimics essential features of hypertension seen in human.¹⁵ mSMS effectively reduces blood pressure in the L-NAME-induced hypertension mouse model, both in males and females. Importantly, each of the 6 herbal components failed to exert antihypertensive effect *in vivo*, and all 5 herb-combinations showed less effect than mSMS. Using the SHR model, we confirmed the dose-dependent antihypertensive effect of mSMS. At doses of 400 mg/kg/day or higher, mSMS exerts a significant blood lowering effect in SHR rats. Its statistical difference to vehicle-treated SHR rats was comparable to an ARB losartan and a CCI amlodipine.

Aortic wall thickening is a common pathological change associated with hypertension, resulting from increased pressure load on the arterial walls, leading to vascular remodeling characterized by smooth muscle cell hypertrophy and increased extracellular matrix deposition.³⁶ This

structural alteration contributes to increased vascular stiffness, which further exacerbates hypertension and associated cardiovascular complications.³⁷ The reduction in aortic wall thickness not only reflects improved vascular structure but also implies a potential decrease in arterial stiffness, which could translate into better overall cardiovascular outcomes.³⁸ By preventing or reversing the pathological changes in the aorta, mSMS may help to alleviate the burden of hypertension on the cardiovascular system, reducing the risk of severe complications such as aortic aneurysm, dissection, and heart failure. Hypertension is closely associated with ROS, where excessive ROS production leads to oxidative stress therefore contributes to vascular dysfunction and increased blood pressure.³⁹ ROS can reduce the availability of NO, a key vasodilator, by forming peroxynitrite, which impairs vasodilation and increases vascular resistance. This oxidative stress also activates pathways that promote inflammation and vascular damage.⁴⁰ mSMS treatment resulted in decreased ROS levels in the aorta, indicating its antihypertension effects are related to oxidative stress and subsequent inflammation in the blood vessels.

Hypertension significantly affects kidney function through the overexpression of AT1R. Angiotensin II, a vital hormone in the renin-angiotensin-aldosterone system (RAAS), causes vasoconstriction, sodium retention, and aldosterone secretion via AT1R, raising blood pressure.⁴¹ In hypertension, the upregulation of AT1R in the kidneys intensifies these effects, worsening hypertension and causing renal damage. Higher kidney injury score in hypertensive patients often correlates with increased AT1R activity, indicating more severe kidney injury and poorer outcomes.⁴² ARBs like losartan and valsartan reduce these effects by inhibiting AT1R, lowering blood pressure, and protecting the kidneys. This intervention can decrease renal injury and improve overall cardiovascular and renal health.⁴³ Our results confirm that losartan reduced AT1R and histological damage in the kidney, corresponding to

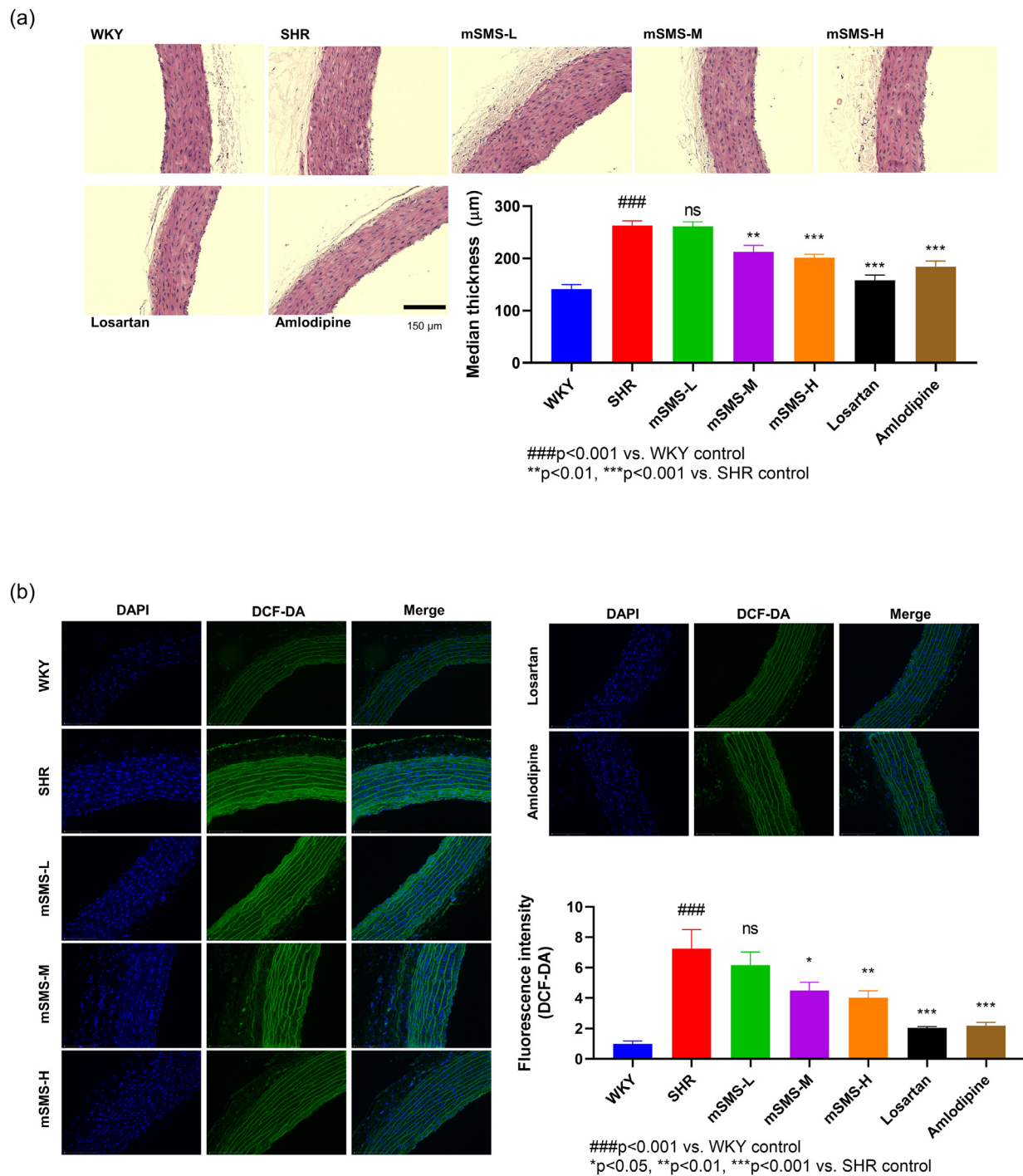


Fig. 5. Effect of mSMS extract on histological changes and oxidative stress in aorta of SHR rats. (a) Representative images of aorta sections stained with H&E, showing the structural integrity and morphology of the aortic wall. Bar graph represent the thickness of aorta. (b) Representative images of aorta sections stained with DCF-DA to assess ROS levels. Increased fluorescence indicates higher levels of oxidative stress in the aortic tissue. Bar graph represent the relative fluorescence intensities. ###p < 0.001 vs. WKY control group; *P < 0.05, **P < 0.01, ***P < 0.001 vs. SHR hypertension group.

previous knowledge. mSMS treatment, while improving renal injury, did not significantly reduce AT1R expression, suggesting its mechanism of action may not involve AT1R.

RhoA kinase is an enzyme that regulates vascular smooth muscle contraction and blood pressure. Overactivation of the RhoA/Rho kinase pathway significantly contributes to hypertension by increasing smooth muscle contractility and impairing endothelial function, leading to elevated vascular resistance.⁴⁴ This pathway is crucial for the signaling of vasoconstrictive agents like endothelin-1 and angiotensin II,

key players in hypertension.⁴⁵ Inhibition of Rho kinase has been shown to lower blood pressure, induce vasodilation, and improve endothelial function.⁴⁶ Our study demonstrated that U46619, a thromboxane A2 receptor agonist,⁴⁷ activated RhoA and increased phosphorylation of MLC20, inducing contraction. This activation was inhibited by mSMS, suggesting that mSMS may suppress GTP RhoA activation. Additionally, mSMS inhibited U46619-induced phosphorylation of MYPT1 at Thr855, a site targeted by Rho kinase,⁴⁸ indicating regulation of vasoconstriction through suppression of the Rho-kinase-MYPT1 pathway. CPI17, another

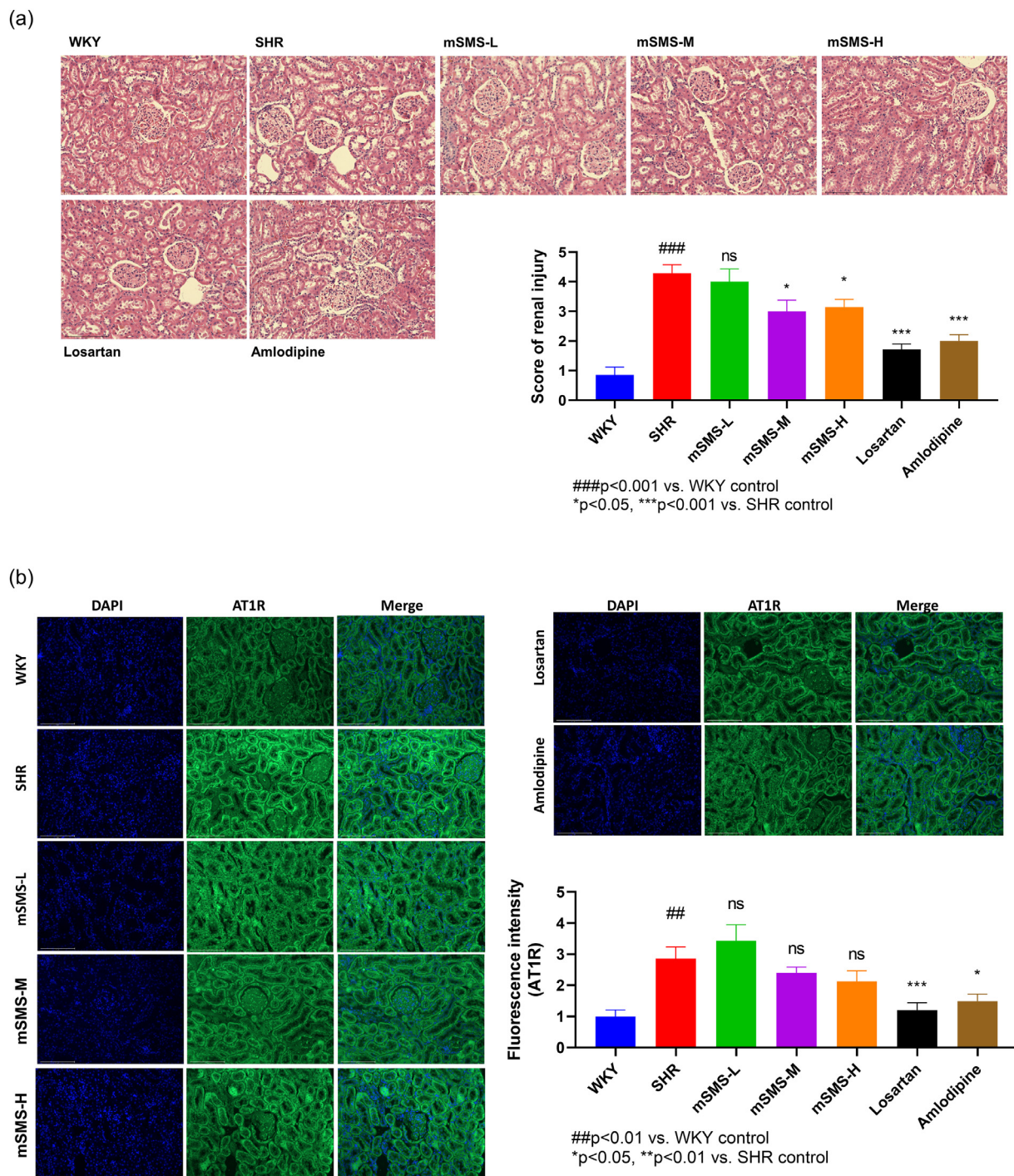


Fig. 6. Effect of mSMS extract on histological changes and AT1R expression in kidney of SHR rats. (a) Representative images of kidney sections stained with H&E. Bar graph quantifies the kidney injury score, highlighting the degree of renal damage. (b) IF staining of kidney sections for AT1R. Bar graph presents the quantification of AT1R fluorescence intensity. ###p < 0.01, ###p < 0.001 vs. WKY control group; *p < 0.05, ***p < 0.001 vs. SHR hypertension group.

downstream effector of Rho-kinase,⁴⁹ was also inhibited by mSMS, as shown by reduced CPI17Th³⁸ phosphorylation. However, mSMS did not affect the PKC pathway, indicating the importance of the RhoA/Rho kinase pathway in the mechanism of action of mSMS. Overall, mSMS reduced MLC20 phosphorylation and vasoconstriction, suggesting its potential as a therapeutic agent targeting the Rho kinase pathway, which is crucial in the pathophysiology of hypertension.

Recent clinical trials involving multi-herbal decoctions have highlighted the potential of various herbal medicines to treat hypertension.⁵⁰⁻⁵⁴ In a clinical trial in 17 male college tennis players, Saengmaek-

san significantly reduced fatigue markers and enhanced immune function, suggesting improved fatigue resistance, liver function, and immune response with Saengmaeksan intake during high-intensity exercise.⁵⁵ An RCT examined the effect of Saengmaeksan on energy metabolism during exercise. Participants were evaluated for changes in energy expenditure and metabolic efficiency.²⁶ Another pilot RCT evaluated the efficacy and safety of a combined treatment of Ojeok-san plus Saengmaeksan for gastroesophageal reflux-induced chronic cough, indicating that the combination could be effective for chronic cough management.⁵⁶ After demonstration of the efficacy of mSMS in a single-arm, prospective,

observational study,⁵⁷ and the positive results of this study, our investigational new drug (IND) application to the Korean ministry of Food and Drug Safety has been recently approved based on. We are currently preparing for a phase I clinical trial to further evaluate the safety and pharmacokinetics of mSMS.

4.4. Strengths and limitations

This study offers several notable strengths. First, we developed a rationally designed multi-herb decoction (mSMS) based on the traditional herbal formula Saengmaeksan, particularly optimized for age-related vascular dysfunction. Importantly, this optimization reflects scientific evidence and clinical rigor, balancing traditional therapeutic concepts with modern biomedical understanding. Second, the antihypertensive efficacy of mSMS was validated across two established in vivo models of hypertension, L-NAME-induced mice and SHR rats. Moreover, we confirmed blood pressure-lowering effects in both male and female animals, strengthening the translational relevance. Another important finding is from our mechanistic studies, in which we elucidated multiple therapeutic pathways targeted by mSMS, including RhoA signaling, AT1R expression, and oxidative stress. Lastly, the comparative analysis of individual herbs, 5-herb combinations, and the full 6-herb formulation demonstrated a clear synergistic effect of mSMS in vivo, supporting the holistic principle of herbal synergy.

However, also certain limitations should be addressed through further investigation. First, while mechanistic studies were rigorously performed, the causal role of these pathways was not fully validated in vivo. Second, long-term safety and toxicity of chronic mSMS administration remain to be evaluated, although a single-dose toxicity study (data not shown) confirmed acceptable short-term safety. Also, additional translational studies are required to determine whether the observed antihypertensive effects and mechanistic insights can be replicated in human populations. Further investigations including pharmacokinetics, bioavailability, and potential herb-drug interactions will be necessary to support clinical application of mSMS.

4.5. Conclusion

In conclusion, our study demonstrates that the multi-herbal decoction, mSMS, exhibits significant antihypertensive effects both in vitro and in vivo. Through a series of experiments, we confirmed that mSMS exerts its vasorelaxation effects primarily by inhibiting the RhoA/Rho-kinase signaling pathway, which plays a crucial role in smooth muscle contraction and the development of hypertension. The ethanol extract of mSMS showed superior efficacy compared to the water extract, indicating that specific phytochemicals within mSMS, such as isoflavones and gomisins A, are key contributors to its therapeutic effects. In animal models, mSMS not only effectively reduced systolic and diastolic blood pressure but also mitigated vascular remodeling and oxidative stress, which are common pathological features of hypertension. The findings of this study are presented as a schematic diagram in Supplement 4. These findings suggest that mSMS has the potential to serve as a novel therapeutic agent for hypertension, offering a multi-targeted approach with fewer side effects compared to conventional antihypertensive drugs.

CRediT authorship contribution statement

Young Mi Seok: Conceptualization, Methodology, Investigation, Writing – original draft. **Hye-Lin Kim:** Investigation, Data curation, Formal analysis, Writing – original draft. **Hyo In Kim:** Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Hobin Moon:** Conceptualization, Methodology, Funding acquisition. **Yohan Han:** Data curation, Writing – review & editing. **Eun Sang Cho:** Methodology. **Hyun Hee Leem:** Writing – review & editing. **Hyo Jung Kim:** Investigation. **Sujin Shin:** Methodology. **Se-Jin Kim:** Data curation. **Jin Ki Jung:** Data curation. **Wei Huang:** Methodology, Data curation.

Kyung Oh Jung: Data curation. **Doo Suk Lee:** Data curation. **Sung Gyu Jin:** Data curation. **Hyunwoo Kim:** Investigation. **Changsoo Yang:** Conceptualization, Methodology, Data curation, Writing – review & editing, Funding acquisition. **Kyungjin Lee:** Methodology, Data curation, Writing – review & editing, Supervision. **Jinbong Park:** Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

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Ethics statement

Ex vivo studies were approved by the Institutional Animal Care and Use Committee of National Institute for Korean Medicine Development (NIKOM-2022-14). In vivo studies were approved by the Institutional Animal Care and Use Committee of Kyung Hee University (KHSASP-23-341). All studies involving animals were carried out in accordance with the Guide for the Care and Use of Laboratory Animals, 8th edition.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.imr.2025.101207](https://doi.org/10.1016/j.imr.2025.101207).

References

- Mills KT, Stefanescu A, He J. The global epidemiology of hypertension. *Nat Rev Nephrol.* 2020;16(4):223–237.
- Chobufo MD, Gayam V, Solunty J, Rahman EU, Enoru S, Foryoung JB, et al. Prevalence and control rates of hypertension in the USA: 2017–2018. *Int J Cardiol Hypertens.* 2020;6:100044.
- Khalil H, Zeltser R. *Antihypertensive Medications*. StatPearls Publishing; 2024 Available from <https://www.ncbi.nlm.nih.gov/books/NBK554579/> Accessed August 2024.
- Whelton PK, Carey RM, Aronow WS, Casey Jr DE, Collins KJ, Dennison Himmelfarb C, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American College of cardiology/American heart association task force on clinical practice guidelines. *Hypertension.* 2018;71(6):e13–e115.
- Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol.* 2014;4:177.
- Xinke Z, Yingdong L, Mingxia F, Kai L, Kaibing C, Yuqing L, et al. Chinese herbal medicine for the treatment of primary hypertension: a methodology overview of systematic reviews. *Syst Rev.* 2016;5(1):180.
- Ajebli M, Eddouks M. Phytotherapy of hypertension: an updated overview. *Endocr Metab Immune Disord Drug Targets.* 2020;20(6):812–839.
- Kamyab R, Namdar H, Torbati M, Ghojzadeh M, Araj-Khodaei M, Fazljou SMB. Medicinal plants in the treatment of hypertension: a review. *Adv Pharm Bull.* 2021;11(4):601–617.
- Xue Z, Li Y, Zhou M, Liu Z, Fan G, Wang X, et al. Traditional herbal medicine discovery for the treatment and prevention of pulmonary arterial hypertension. *Front Pharmacol.* 2021;12:720873.
- Somlyo AP, Somlyo AV. Ca²⁺ sensitivity of smooth muscle and nonmuscle myosin II: modulated by G proteins, kinases, and myosin phosphatase. *Physiol Rev.* 2003;83(4):1325–1358.
- Shimokawa H, Sunamura S, Satoh K. RhoA/rho-kinase in the cardiovascular system. *Circ Res.* 2016;118(2):352–366.
- Nunes KP, Webb RC. New insights into RhoA/rho-kinase signaling: a key regulator of vascular contraction. *Small GTPases.* 2021;12(5–6):458–469.

13. Sawma T, Shaito A, Najm N, Sidani M, Orekhov A, El-Yazbi AF, et al. Role of RhoA and Rho-associated kinase in phenotypic switching of vascular smooth muscle cells: implications for vascular function. *Atherosclerosis*. 2022;358:12–28.
14. Leong XF, Ng CY, Jaarin K. Animal models in Cardiovascular research: hypertension and atherosclerosis. *Biomed Res Int*. 2015;2015:528757.
15. Pinto YM, Paul M, Ganten D. Lessons from rat models of hypertension: from Goldblatt to genetic engineering. *Cardiovasc Res*. 1998;39(1):77–88.
16. Seok YM, Azam MA, Okamoto Y, Sato A, Yoshioka K, Maeda M, et al. Enhanced Ca²⁺-dependent activation of phosphoinositide 3-kinase class I α isoform-rho axis in blood vessels of spontaneously hypertensive rats. *Hypertension*. 2010;56(5):934–941.
17. Kim HI, Park J, Gallo D, Shankar S, Konecna B, Han Y, et al. DANGER signals activate G-protein receptor kinases suppressing neutrophil function and predisposing to infection after tissue trauma. *Ann Surg*. 2023;278(6):e1277–88.
18. Seok YM, Baek I, Kim YH, Jeong YS, Lee IJ, Shin DH, et al. Isoflavone attenuates vascular contraction through inhibition of the RhoA/rho-kinase signaling pathway. *J Pharmacol Exp Ther*. 2008;326(3):991–998.
19. Scheller M, Blobner M, Von Loewenich C, Schneek H, Stadler J, Franke C, et al. The NO synthase inhibitors L-Name and L-NMMA, but not L-arginine, block the mammalian nicotinic acetylcholine receptor channel. *Toxicol Lett*. 1998;100–101:109–113.
20. Park J, Shin S, Bu Y, Choi HY, Lee K. Vasorelaxant and blood pressure-lowering effects of *Cnidium monnieri* fruit ethanol extract in Sprague Dawley and spontaneously hypertensive rats. *Int J Mol Sci*. 2024;25(8):4223.
21. Liu CY, Chen D, Bluemke DA, Wu CO, Teixido-Tura G, Chugh A, et al. Evolution of aortic wall thickness and stiffness with atherosclerosis: long-term follow up from the multi-ethnic study of atherosclerosis. *Hypertension*. 2015;65(5):1015–1019.
22. Ouyang Y, Tang L, Hu S, Tian G, Dong C, Lai H, et al. Shengmai san-derived compound prescriptions: a review on chemical constituents, pharmacokinetic studies, quality control, and pharmacological properties. *Phytomedicine*. 2022;107:154433.
23. Kim S-s. From Woohwang Cheongsimwon to Ginseng - the history of medicine use in the Joseon era. *Korean J Med Hist*. 2017;26(2):147–180.
24. Park SH, Kim WJ. Study on oriental prescription for medicinal food applications-effects and mechanism of Saengmaegsan on the relaxation of contracted artery and regional cerebral blood flow. *East Asian Soc Diet Life*. 2005;15(5):606–612.
25. Park KJ, Lee MJ, Kang H, Kim KS, Lee SH, Cho I, et al. Saeng-Maek-San, a medicinal herb complex, protects liver cell damage induced by alcohol. *Biol Pharm Bull*. 2002;25(11):1451–1455.
26. Baek S, Kim J, Nam MH, Park SM, Lee TS, Kang SY, et al. Saengmaeksan, a traditional polyherbal formulation containing Panax ginseng, improves energy metabolism during exercise. *PLoS One*. 2024;19(1):e0296487.
27. Jeong MY, Park DH, Kim MC, Park J, Kim DS, Jeon YD, et al. Saengmaeksan inhibits inflammatory mediators by suppressing RIP-2/caspase-1 activation. *Immunopharmacol Immunotoxicol*. 2013;35(2):241–250.
28. Sung YY, Yuk HJ, Kim DS. Saengmaeksan, a traditional herbal formulation consisting of Panax ginseng, ameliorates hyperuricemia by inhibiting xanthine oxidase activity and enhancing urate excretion in rats. *J Ginseng Res*. 2021;45(5):565–574.
29. Zaheri S, Marandi SM. The effect of ginseng supplement on heart rate, systolic and diastolic blood pressure to resistance training in trained males. *Artery Res*. 2016;15(1):6–11.
30. Cai RL, Li M, Xie SH, Song Y, Zou ZM, Zhu CY, et al. Antihypertensive effect of total flavone extracts from *Puerariae Radix*. *J Ethnopharmacol*. 2011;133(1):177–183.
31. Yeh WJ, Ko J, Cheng WY, Yang HY. Dehulled adlay consumption modulates blood pressure in spontaneously hypertensive rats and overweight and obese young adults. *Nutrients*. 2021;13(7):2305.
32. Chung MJ, Kim SH, Park JW, Lee YJ, Ham SS. Platycodon grandiflorum root attenuates vascular endothelial cell injury by oxidized low-density lipoprotein and prevents high-fat diet-induced dyslipidemia in mice by up-regulating antioxidant proteins. *Nutr Res*. 2012;32(5):365–373.
33. Kim EY, Baek IH, Rhyu MR. Cardioprotective effects of aqueous *Schizandra chinensis* fruit extract on ovariectomized and balloon-induced carotid artery injury rat models: effects on serum lipid profiles and blood pressure. *J Ethnopharmacol*. 2011;134(3):668–675.
34. Su J, Wei Y, Liu M, Liu T, Li J, Ji Y, et al. Anti-hyperuricemic and nephroprotective effects of *Rhizoma Dioscoreae septemlobae* extracts and its main component dioscin via regulation of mOAT1, mURAT1 and mOCT2 in hypertensive mice. *Arch Pharm Res*. 2014;37(10):1336–1344.
35. Tousoulis D, Kampoli AM, Tentolouris C, Papageorgiou N, Stefanadis C. The role of nitric oxide on endothelial function. *Curr Vasc Pharmacol*. 2012;10(1):4–18.
36. Brown IAM, Diederich L, Good ME, DeLalio LJ, Murphy SA, Cortese-Krott MM, et al. Vascular smooth muscle remodeling in conductive and resistance arteries in hypertension. *Arter Thromb Vasc Biol*. 2018;38(9):1969–1985.
37. Taghizadeh H, Taghizadehghalehjoughi A, Yildirim S, Ozkaraca M, Genc S, Yeni Y, et al. Deteriorated vascular homeostasis in hypertension: experimental evidence from aorta, brain, and pancreatic vasculature. *J Pers Med*. 2022;12(10):1602.
38. Kim HL. Arterial stiffness and hypertension. *Clin Hypertens*. 2023;29(1):31.
39. Rodrigo R, González J, Paoletto F. The role of oxidative stress in the pathophysiology of hypertension. *Hypertens Res*. 2011;34(4):431–440.
40. Chen Q, Wang Q, Zhu J, Xiao Q, Zhang L. Reactive oxygen species: key regulators in vascular health and diseases. *Br J Pharmacol*. 2018;175(8):1279–1292.
41. Su C, Xue J, Ye C, Chen A. Role of the central renin-angiotensin system in hypertension (Review). *Int J Mol Med*. 2021;47(6).
42. Nistala R, Wei Y, Sowers JR, Whaley-Connell A. Renin-angiotensin-aldosterone system-mediated redox effects in chronic kidney disease. *Transl Res*. 2009;153(3):102–113.
43. Schmieder RE, Ruilope LM, Barnett AH. Renal protection with angiotensin receptor blockers: where do we stand. *J Nephrol*. 2011;24(5):569–580.
44. Yao L, Romero MJ, Toque HA, Yang G, Caldwell RB, Caldwell RW. The role of RhoA/rho kinase pathway in endothelial dysfunction. *J Cardiovasc Dis Res*. 2010;1(4):165–170.
45. Gómez-Garre D, Ruiz-Ortega M, Ortego M, Largo R, López-Armada MJ, Plaza JJ, et al. Effects and interactions of endothelin-1 and angiotensin II on matrix protein expression and synthesis and mesangial cell growth. *Hypertension*. 1996;27(4):885–892.
46. Surma M, Wei L, Shi J. Rho kinase as a therapeutic target in cardiovascular disease. *Future Cardiol*. 2011;7(5):657–671.
47. Scornik FS, Toro L. U46619, a thromboxane A₂ agonist, inhibits KCa channel activity from pig coronary artery. *Am J Physiol*. 1992;262(3 Pt 1):C708–C713.
48. Baek I, Jeon SB, Song MJ, Yang E, Sohn UD, Kim IK. Flavone attenuates vascular contractions by inhibiting RhoA/Rho Kinase pathway. *Korean J Physiol Pharmacol*. 2009;13(3):201–207.
49. Kim JI, Urban M, Young GD, Eto M. Reciprocal regulation controlling the expression of CPI-17, a specific inhibitor protein for the myosin light chain phosphatase in vascular smooth muscle cells. *Am J Physiol Cell Physiol*. 2012;303(1):C58–C68.
50. Tong XL, Lian FM, Zhou Q, Xu LP, Ji HY, Xu GC, et al. Prospective multicenter clinical trial of Chinese herbal formula JZQG (Jiangzhuoqinggan) for hypertension. *Am J Chin Med*. 2013;41(1):33–42.
51. Zhong GW, Chen MJ, Luo YH, Xiang LL, Xie QY, Li YH, et al. Effect of Chinese herbal medicine for calming Gan (肝) and suppressing hyperactive yang on arterial elasticity function and circadian rhythm of blood pressure in patients with essential hypertension. *Chin J Integr Med*. 2011;17(6):414–420.
52. Chen Y, Fu DY, Chen Y, He YM, Fu XD, Xu YQ, et al. Effects of Chinese herbal medicine Yiqi Huaju Formula on hypertensive patients with metabolic syndrome: a randomized, placebo-controlled trial. *J Integr Med*. 2013;11(3):184–194.
53. Azushima K, Tamura K, Haku S, Wakui H, Kanaoka T, Ohsawa M, et al. Effects of the oriental herbal medicine Bofu-tsusho-san in obesity hypertension: a multicenter, randomized, parallel-group controlled trial (ATH-D-14-01021.R2). *Atherosclerosis*. 2015;240(1):297–304.
54. Yuwen Y, Liu YQ, Wang YP, Dai JG, Liu DS, Wang YX, et al. The add-on effect of a Chinese herbal formula for patients with resistant hypertension: study protocol for a pilot cohort study. *J Integr Med*. 2015;13(2):122–128.
55. Juan S, Lee JH, Won SJ, Oh S, Ha MS. Effect of Saengmaeksan on fatigue, liver function, and immunity combined with high-intensity training. *J Immunol Res*. 2023;2023:3269293.
56. Lyu YR, Kim KI, Yang C, Jung SY, Kwon OJ, Jung HJ, et al. Efficacy and safety of Ojeok-San plus Saengmaek-San for gastroesophageal reflux-induced chronic cough: a pilot, randomized, double-blind, placebo-controlled trial. *Front Pharmacol*. 2022;13:787860.
57. Cho N, Moon H, Shin KM, Kang BK, Leem J, Yang C. Safety and effectiveness of an herbal decoction (modified Saengmaeksan) in hypertensive patients: protocol for a real-world prospective observational study. *PLoS One*. 2025;20(1):e0316276.