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Original Article

Effects and mechanism of total phenols of *Magnolia officinalis* combined with Maijunan Tablets on blood pressure of spontaneous hypertensive rats

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ABSTRACT

Objective: Maijunan (MJA) Tablets is a protected variety of traditional Chinese medicine (TCM) consisted of *Pueraria lobata*, hydrochlorothiazide (HTCZ), *Uncaria rhynchophylla* (366:1:980) and excipient. In the present work, MJA was consisted of the total flavones of *P. lobata*, HCTZ and total alkaloids of *U. rhynchophylla* (40:11:75). The combination of MJA and the total phenols of *Magnolia officinalis* (M-MJA) was consisted of the total flavones of *P. lobata*, the total phenols of *M. officinalis*, HCTZ and the total alkaloids of *U. rhynchophylla* (40:40:11:75). The aim of this work was to examine the effect and mechanism of M-MJA on the blood pressure of spontaneous hypertensive rats (SHRs).

Methods: Adult male SHRs were randomly divided into control group, MJA group (180 mg/kg·d), and the M-MJA group (218 mg/kg·d) ($n = 5$). SHRs were orally administered with M-MJA and MJA respectively once a day for 8 weeks, the blood pressure of SHRs was measured every two weeks, and the biochemical indicators related to blood pressures were detected at the last dosing.

Results: After oral administration of M-MJA to SHRs once a day for 8 weeks, the systolic and diastolic blood pressures of SHRs were deceased significantly. M-MJA affected renin-angiotensin-aldosterone system by decreasing the levels of Ren, Ang II and ALD, affected the endothelial function by decreasing the levels of ET-1 and 20-HETE, and increasing the level of eNOS, affected the oxidative stress by increasing the protein expression of Nrf2 and the activities of HO-1 and GSH-Px, and decreasing the protein expression of CYP2E1 and CYP4A, as well as the content of MDA.

Conclusion: These results indicated that M-MJA could regulate the renin-angiotensin-aldosterone system, improve endothelial function, and inhibit CYP4A activity to reduce the production of 20-HETE, alleviate the oxidative stress disorder of the visceral organs, and eventually exert antihypertensive effect. Additionally, the anti-oxidant ability, regulating the renin-angiotensin-aldosterone system and improving endothelial function of M-MJA are more powerful than that of MJA, suggesting that M-MJA may have a better anti-hypertensive effect than MJA.

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1. Introduction

As the major risk factor for stroke, hypertension seriously endangers human physical and mental health (Chobanian et al., 2003). Currently, western medicine is mainly used in the clinical treatment of hypertension in China, such as captopril, losartan, nifedipine, hydrochlorothiazide (HCTZ), propranolol and so on. There are also some important traditional Chinese medicine (TCM) used in the clinical treatment of hypertension, such as Decoction of *Gastrodia* and *Uncaria*, Maijunan (MJA) Tablets, Liuwei Dihuang Pills, Compound Qishao Antihypertensive Tablets, *Eucommia ulmoides* Antihypertensive Tablets, etc. Among them, thin film-coated MJA Tablets is a protected variety of TCM consisted of *Pueraria lobata*, hydrochlorothiazide (HTCZ), *Uncaria rhynchophylla* (366:1:980), and excipient. MJA Tablet has been applied to the patients with hypertension of chronic type I- and II-period for many years (Chinese Pharmacopoeia Commission, 1997). The long-term use of MJA Tablets can reduce blood viscosity and effectively prevent the hypertension-related complications. However, the anti-hypertensive resistance is a common phenomenon following the long-term use of MJA Tablets. It is well known that the activation of renin-angiotensin-aldosterone system (RAAS) and the

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hydroxylation of arachidonic acid is closely related to anti-hypertensive resistance. CYP4A is the main metabolic enzyme that catalyzes arachidonic acid to produce 20-hydroxyeicosatetraenoic acid (20-HETE) in rats (Zoccali, Mallamaci, & Grassi, 2015). 20-HETE is a potent vasoconstrictor, the up-regulation of which contributes to oxidative stress and increases blood pressure. Oxidative stress is closely related to the development of hypertension, and magnolol and honokiol are the major active components of the total phenols extracted from *Magnolia officinalis* (Qin et al., 2015), which have strong anti-oxidant effect. In addition, honokiol can inhibit the activity of cytochrome P450 4A (CYP4A) in rat liver (Wang, Zhai, & Chen, 2018). Therefore, it is speculated that the combination of MJA and the total phenols of *M. officinalis* could attenuate the oxidative stress injury of the primary organs such as heart, liver and kidney, and relieve anti-hypertensive resistance.

2. Materials and methods

2.1. Reagents and antibodies

The total alkaloids were extracted from *Uncaria rhynchophylla* (Miq.) Miq. ex Havil. (batch number 20 170 319, content 56.4%, Wuhan Warrickson Technology Co., Ltd., China); The total phenols were isolated from *Magnolia officinalis* Rehd. et Wils. (batch number, YS-HP2-20170210, content 95.02%, Shanghai Yuanye Biotechnology Co., Ltd., China); The total flavones were extracted from *Pueraria lobata* (Willd.) Ohwi (Batch number YS-GG2-20170217, content 94.85%, Xi'an Yuansen Biotechnology Co., Ltd., China); HCTZ (batch number 20160913, content 98%); Arachidonic acid (AA, batch number 0507786-1, Cayman Chemical Co., USA); 20-HETE-d6 (batch number 0417724-71, Cayman Chemical Co., USA); 20-HETE (batch number 10164, MedChem Express, USA); NADPH (BioFroxx, Germany); Detection kits including malondialdehyde (MDA), total superoxide dismutase (T-SOD), and glutathione peroxidase (GSH-Px) (Nanjing Jiancheng Bio., China); ELISA kits including renin (Ren), angiotensin II (Ang II), aldosterone (ALD) and endothelin-1 (ET-1), endothelial nitric oxide synthase (eNOS), and heme oxygenase 1 (HO-1) (Elabscience Corporation, China); Primary antibodies including polyclonal rabbit anti-mouse CYP2E1 antibody (diluted 1:500, Wuhan Boster Bio., China), Nrf2 antibody (diluted 1:500, Shenyang Wanlei, China), β-actin antibody (diluted 1:1000, Santa, USA), and monoclonal rabbit anti-mouse CYP4A antibody (diluted 1:1000, Abcam, USA); Secondary antibodies for Nrf2, CYP2E1, and CYP4A are horseradish peroxidase labeled goat anti-rabbit IgG ($H+L$), and for β-actin is horseradish peroxidase labeled goat anti-mouse IgG ($H+L$) (diluted 1:8000, KPL, USA); Rever Tra Ace® qPCR RT kit (Toyobo, Japan).

2.2. Sample preparation of MJA and M-MJA

The content ratio of the puerarin, HCTZ, and the total alkaloids of *U. rhynchophylla* in thin film-coated MJA Tablets is 40:11:75 (Zhang, Han, Du, & Chen, 2009). In the present work, M-MJA contains the same content of the total flavones of *P. lobata* and the total phenols of *M. officinalis*. Therefore, MJA and M-MJA were prepared by mixing the total flavones of *P. lobata*, HCTZ and the total alkaloids of *U. rhynchophylla* in the content ratio of 40:11:75, and by mixing the total flavones of *P. lobata*, the total phenols of *M. officinalis*, HCTZ and the total alkaloids of *U. rhynchophylla* in the content ratio of 40:40:11:75, respectively. The typical chromatograms of the total alkaloids extracted from *U. rhynchophylla*, the total phenols extracted from *M. officinalis* and the total flavones extracted from *P. lobata* were shown in Fig. 1 under the chromatographic conditions of Table 1.

2.3. Animal treatments

Male SHRs [15-week old, weight (283 ± 8.9) g, Certificate No. SCXK2016-0011], purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd., were acclimatized for at least one week and divided into the following three groups ($n=5$ per group): MJA group (dosed with 180 mg/kg·d of MJA) (Zhang, Han, Du, & Chen, 2009), M-MJA group (dosed with 218 mg/kg·d of M-MJA), and control group (dosed with the equal amount volume of 0.5% sodium carboxymethyl cellulose). M-MJA and MJA were prepared in 0.5% sodium carboxymethyl cellulose as the suspension liquid, and administrated on 9:00–10:00 a.m, once a day for 8 weeks. At the end of drug treatment, SHRs were fasted overnight but given free access to water, then anesthetized by pentobarbital (45 mg/kg, ip). The blood was collected from the rat abdominal aorta and the plasma and serum were separated by centrifugation. The tissues of liver, kidney, and heart were isolated and stored in liquid nitrogen.

2.4. Measurement of blood pressure

In the awakened state, the systolic (SBP) and diastolic (DBP) blood pressures of SHRs were measured once two weeks by tail-cuff method using a hemopiezometer (CODA Monitor, USA) after administration. Before the measurement of the blood pressure, rats were placed in muff for 10–15 min to acclimatize, then the blood pressure was measured continuously for at least five times and averaged.

2.5. Determination of biochemical indicators by detection kits

The frozen liver, kidney, and heart were homogenized in saline (1:9), respectively. The supernatant was collected by centrifuging

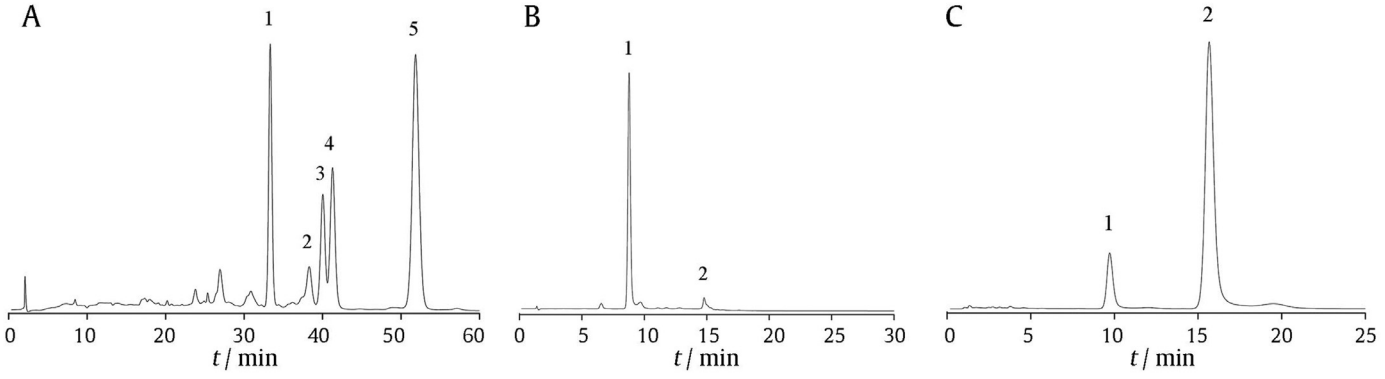

Fig. 1. HPLC chromatograms of total alkaloids of *U. rhynchophylla* (A) (1: isorhynchophylline, 2: isospeciofoline, 3: rhynchophylline, 4: corynoxine, 5: corynoxine), total flavones of *P. lobata* (B) (1: puerarin and 2: daidzein), and total phenols of *M. officinalis* (C) (1: honokiol and 2: magnolol).

Table 1
Chromatographic conditions of total alkaloids of *U. rhynchophylla*, total phenols of *M. officinalis* and total flavones of *P. lobata*.

Names	Wave length/nm	Mobile phase	Programs	Flow rate /(mL·min^{-1})
Total alkaloids of *U. rhynchophylla*	254	Methanol (B),10 mmol triethylamine in water (A)	0 – 20 min: 20%*B* – 60%B, 20–60 min:60%B	0.2
Total phenols of *M. officinalis*	294	Methanol (B), 0.1% formic acid in water (A)	0 – 25min:B:*A* = 65:35	0.3
Total flavones of *P. lobata*	250	Methanol (B), 0.1% formic acid in water (A)	0 – 5min:15%*B* – 25%, 5 – 12 min:25%B, 12–30 min: 85%B	0.3

at 3500 g for 15 min. The levels of MDA, T-SOD, GSH-Px, and HO-1 were measured by commercially available detection kits according to the manufacturer's instructions using a microplate reader (BIO-RAD, USA).

The levels of Ren, Ang II, ALD, and ET-1 in plasma, as well as ET-1 and eNOS in tissues and eNOS in serum, were measured by radioimmunoassay kits according to the manufacturer's instructions using a microplate reader (Multiskan MK3, USA).

2.6. Determination of 20-HETE by LC-MS/MS method

The microsomes of liver and kidney were obtained by differential centrifugation method (Fan et al., 2015). Briefly, the tissues of liver and kidney (0.5 g) were homogenized in 3 mL of Tris-HCl buffer (pH = 7.4) and centrifuged at 11 000 g for 20 min to collect the supernatant. The supernatant was further centrifuged at 100 000 g for 1 h to obtain the microsomal fraction. The pellets were suspended in 1.5 mL PBS buffer (0.1 mol/L) containing glycerol (PBS: glycerol = 1:4) and stored at −80 °C until assayed. The concentration of microsomal protein was measured using BCA kit. AA (40 μmol/L) used as the probe substrate of CYP4A was incubated with 0.3 mg/mL liver microsomes, or 0.4 mg/mL renal microsomes at 37 °C for 30 min in 200 μL PBS buffer containing 25 mmol/L $MgCl_2$ and 1 mmol/L NADPH. The reactions were terminated by adding 1 mL ice-cold ethyl acetate. Using 20-HETE-d6 (final concentration 200 ng/mL) as the internal standard (Williams et al., 2012), the content of metabolite 20-HETE was determined using an established LC-MS/MS system consisted of two LC-20 pumps, DGU-20A3R degasser, SIL-20AHT autosampler, CTO-20A column oven, Lab Solution data processing system (Shimadzu Corporation, Japan)

2.7. Western blotting

A total of 0.1 g tissues (liver and kidney) were homogenized in 500 μL RIPA lysate containing 1% PMSF and Cooktail (Shanghai Beyotime, China). Protein quantification was performed by using BCA kit (Thermo, USA). The denatured protein was separated by SDS-PAGE (5% stacking gel and 12% separating gel) and then transferred onto PVDF membranes (Millipore, USA). The membranes were blocked with nonfat milk for 2 h at room temperature, and then incubated with the primary antibodies (Nrf2, CYP2E1, CYP4A, β-actin) overnight at 4 °C and the secondary antibody for 1 h at room temperature in turn. The signal of the Western blotting was detected by ECL system (Shanghai Beyotime, China) and exposure to X-ray film in dark room. The density of Western blotting bands was quantified by gray scale scanning using Image J (NIH, Broken Symmetry Software).

2.8. Real-time quantitative PCR

Total RNA was extracted from the heart tissues by Trizol reagent (Invitrogen, USA), then transcripted into cDNA with Rever Tra Ace® qPCR RT kit (Toyobo, Japan). The cDNA was amplified by using SYBR Green I fluorescent quantitative PCR kit (Bio-Rad, USA) and CFX connect™ real-time system (Bio-Rad, USA). The RT-qPCR was performed for 40 cycles at the following conditions: pre-degeneration at 95 °C for 5 min, degeneration at 95 °C for 30 s, annealing at 59.4 °C for 30 s, and extended at 72 °C for 30 s. The primer sequences used in the present work were listed in Table 2. GAPDH was used as the reference gene to normalize the gene expression, and the relative quantification of mRNA level was determined by using the $2^{-\Delta\Delta Ct}$ method.

Table 2
Primers used in real-time PCR.

Genes	Forward primers	Reverse primers
CYP2E1	CCTACATGGATGCTGTGGTG	CTGGAAACTCATGGCTGTCA
CYP4A1	TTGAGCTACTGCCAGATCCCAC	CCCATTTTTGGACTTCAGCACA
CYP4A2	CTCGCCATAGCCATGCTTATC	CCTTCAGCTCATTCATGGCAATT
CYP4A3	CTCGCCATAGCCATGCTTATC	CCTTCAGCTCATTCATGGCAATC
CYP4A8	ATCCAGAGGTGTTTGACCCTTAT	AATGAGATGTGAGCAGATGGAGT
Nrf2	GCTATTTTCCATTCCCGAGTTAC	ATTGCTGTCCATCTCTGTCAG
GAPDH	AGGGCTGCCTTCTCTTGTGAC	TGGGTAGAATCATACTGGAACATGTAG

2.9. Statistical analysis

PSS 17.0 software was used for statistical analysis. Data are presented as means ± standard deviation (SD), and were analyzed by one-way analysis of variance. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of M-MJA on systolic and diastolic blood pressures of SHRs

Compared with control group, the systolic (SBP) and diastolic (DBP) blood pressures of MJA and M-MJA groups were significantly reduced during the 2 – 8 weeks of the drug administration ($P < 0.01$ or $P < 0.05$). However, there was no significant difference between MJA and M-MJA groups ($P > 0.05$) (Fig. 2).

3.2. Effects of M-MJA on oxidative stress of SHRs

Compared with control group, the content of MDA in the kidney and heart of MJA and M-MJA groups was significantly decreased ($P < 0.01$ or $P < 0.05$), and no obvious change was observed in the liver. Compared with control group, the activities of GSH-Px in the liver, kidney, and heart of MJA and M-MJA were significantly increased ($P < 0.01$ or $P < 0.05$), while there was no significant difference observed for T-SOD activity in the tested tissues. Compared with control group, the activities of HO-1 in the liver, kidney, and heart of M-MJA group were significantly increased ($P < 0.01$), whereas only the hepatic HO-1 activity was significantly increased in MJA group ($P < 0.05$). Compared with MJA group, the content of MDA in M-MJA group in the heart was significantly reduced ($P < 0.05$), and the activities of HO-1 in the kidney and heart were increased significantly in M-MJA group ($P < 0.05$) (Fig. 3).

Compared with control group, the hepatic and renal CYP2E1 protein expression, as well as the cardiac CYP2E1 mRNA expression were inhibited significantly in MJA and M-MJA group ($P < 0.05$ *or* $P < 0.01$). Unlike the CYP2E1, a reverse effect was observed for Nrf2 protein expression following the drug administration ($P < 0.05$ or $P < 0.01$) (Fig. 4). Meanwhile, the hepatic and renal CYP4A protein

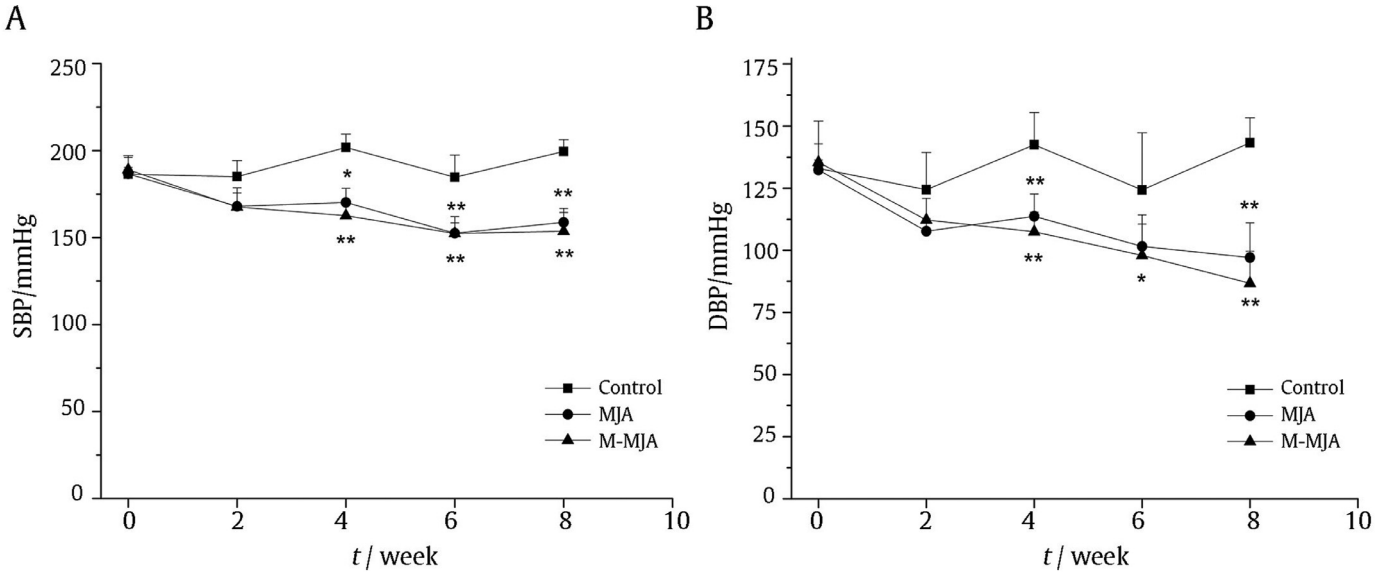

Fig. 2. Effects of MJA and M-MJA on systolic (A) and diastolic (B) blood pressures of SHRs during 2 – 8 weeks of drug administration ($n = 5$. $^{*}P < 0.05$, $^{*}P < 0.01$ *vs* control).

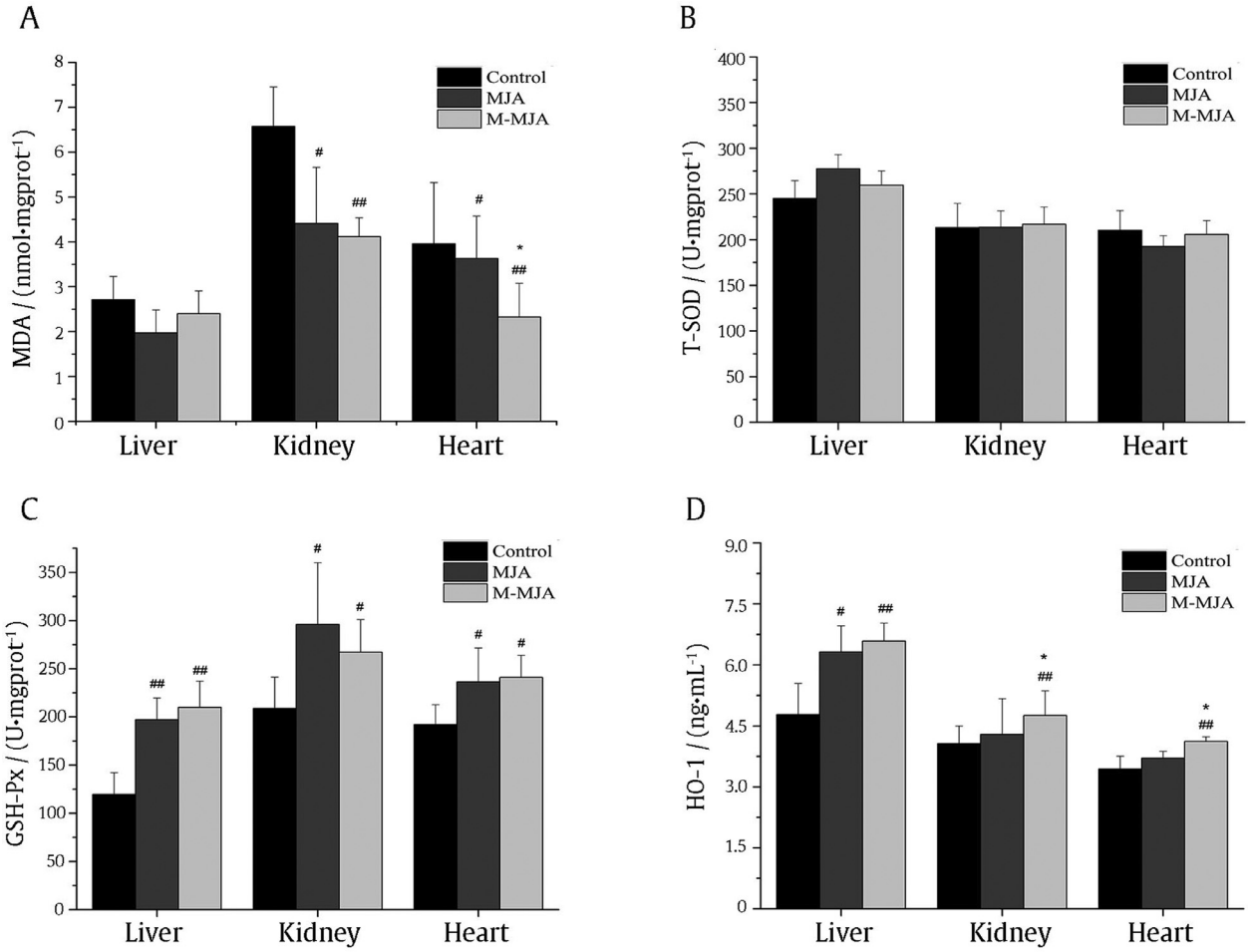

Fig. 3. Effects of MJA and M-MJA on levels of MDA (A), T-SOD (B), GSH-Px (C) and HO-1 (D) in liver, kidney and heart of SHRs ($n = 5$. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs* control. $^{*}P < 0.05$ M-MJA *vs* MJA).

levels in M-MJA group were decreased significantly ($P < 0.05$ or $P < 0.01$), and the same changes for the cardiac CYP4A mRNA expression were also observed in both drug administration groups ($P < 0.05$ or $P < 0.01$). Compared with MJA group, the protein levels of hepatic and renal CYP4A and CYP2E1, as well as the mRNA levels of cardiac CYP4A and CYP2E1, were significantly reduced after M-MJA treatment ($P < 0.05$ or $P < 0.01$), and an opposite effect was observed for Nrf2 expression following M-MJA treatment ($P < 0.05$ or $P < 0.01$) (Fig. 4).

3.3. Effects of M-MJA on RAAS of SHRs

Compared with the control group, the levels of plasma ALD, Ang II and Ren in M-MJA group were significantly decreased ($P < 0.05$); Although the levels of them in MJA group were lower than those in control group, there were no statistical significance between them. Additionally, the level of AngII in M-MJA group was significantly lower than that in MJA group ($P < 0.05$) (Fig. 5).

3.4. Effects of M-MJA on endothelial factor ET-1, eNOS, and 20-HETE of SHRs

The levels of plasma and cardiac ET-1 in M-MJA and MJA groups were significantly lower than those in control group ($P < 0.05$ or $P < 0.01$), and the hepatic and plasmatic ET-1 level in M-MJA group was significantly lower than those in control and MJA groups ($P < 0.05$ or $P < 0.01$). No statistical significance was found for the renal ET-1 level in the three groups. At the same time, the levels of

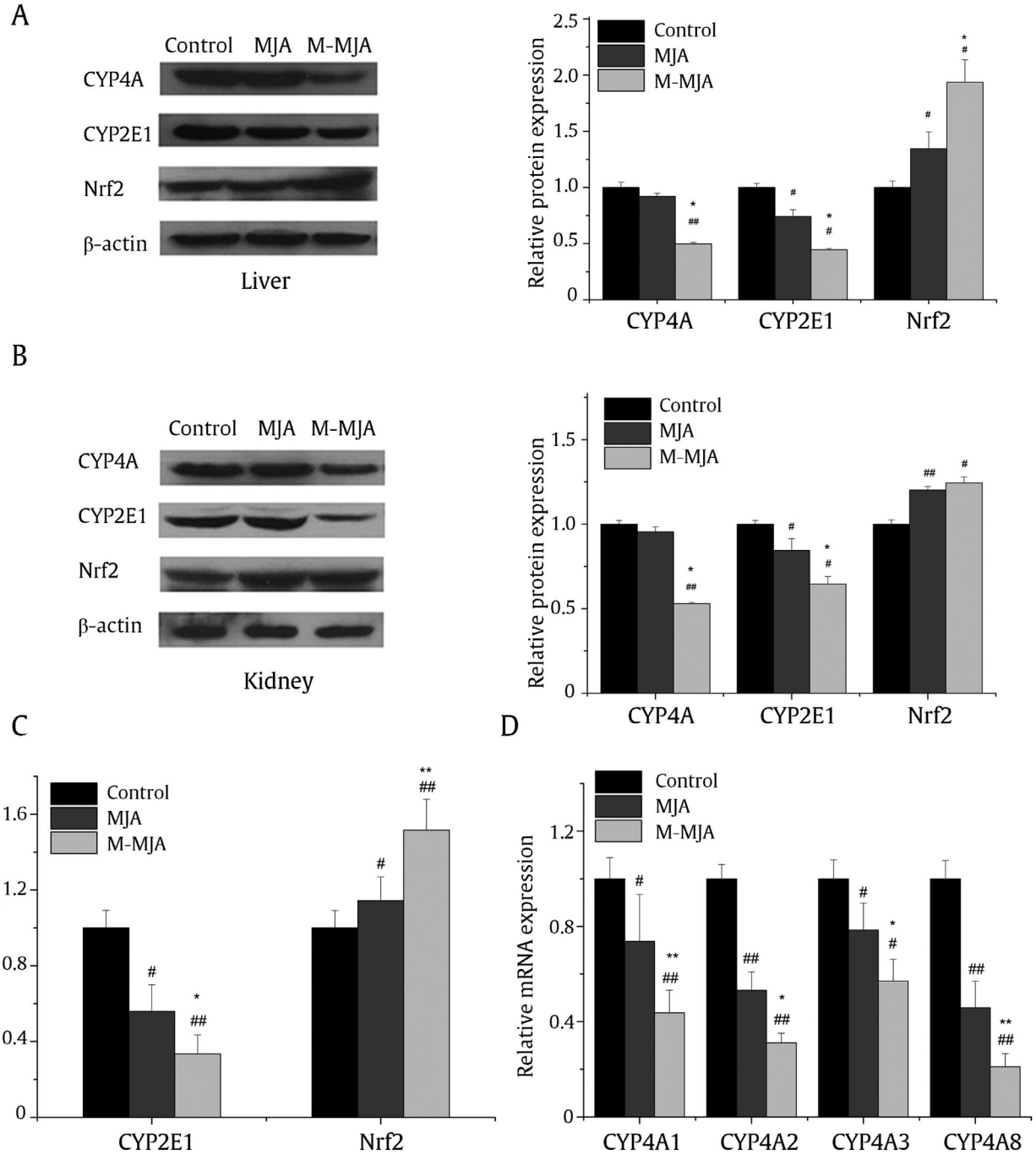

Fig. 4. Effects of MJA and M-MJA on expression levels of CYP4A, CYP2E1, and Nrf2 in liver(A), kidney(B) and heart (C, D) of SHRs (mean ± SEM, $n = 5$) ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs* control. $^{*}P < 0.05$ M-MJA *vs* MJA).

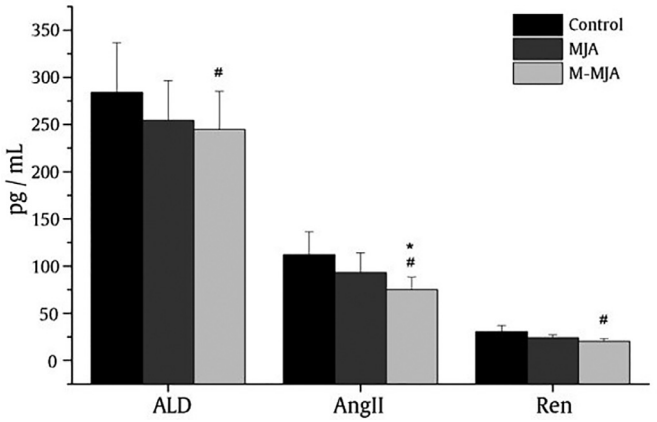

Fig. 5. Effects of MJA and M-MJA on levels of plasma ALD, Ang II and Ren in SHRs ($n = 5$. $^{\#}P < 0.05$ *vs* control. $^{*}P < 0.05$ M-MJA *vs* MJA).

eNOS in serum, liver and kidney of M-MJA and MJA groups were significantly higher than those in control group ($P < 0.05$ or $P < 0.01$), and the levels of eNOS in serum and heart of M-MJA group were significantly higher than those in MJA group ($P < 0.05$ or $P < 0.01$). Meanwhile, the content of 20-HETE in the liver and kidney of M-MJA and MJA groups was significantly lower than that in control group ($P < 0.05$ or $P < 0.01$), but there was no significant difference between the two groups (Fig. 6).

4. Discussion

The main active component in the total flavones extracted from *P. lobata* is puerarin. The total flavonoids and puerarin cannot only induce the activity of anti-oxidant enzymes and improve the oxidative stress injury of liver and kidney (She, Liu, Li, & Hong, 2014), but also affect RAAS and vascular endothelial factors such as lowering ET-1 content and increasing NO content in blood of SHRs, which can result in the antihypertensive effect (Cai et al., 2011; Huang, Zhang, Cui, & Ding, 2012). The main antihypertensive components in the total alkaloids extracted from *U. rhynchophylla* are rhynchophylline and isorhynchophylline. The antihypertensive action of this total alkaloids is associated with the alleviated oxidative stress and vascular endothelial dysfunction in SHRs (Li et al., 2015). Magnolol and honokiol are the major active components of the total phenols extracted from *M. officinalis.* The antihypertensive action of honokiol is related to its anti-oxidation and improvement of vascular endothelial function (Zhang et al., 2010). HCTZ is a classic diuretic drug and is used to achieve diuretic effect by inhibiting the Na^+/Cl- cotransporter protein in renal distal convoluted tubules. The long-term antihypertensive effect of HCTZ is associated with the direct vasodilation by opening Ca^{2+}-activated K^+ channel on the vascular smooth muscle cell membrane, and also with the improvement of endothelial function (Kedziora-Kornatowska et al., 2006). However, the long-term use of HCTZ can activate RAAS and partially counteract its antihypertensive effect, and also can dose-dependently lead to some adverse reactions such as hypokalemia, hyponatremia, hyperuricemia, insulin resistance, and visceral fat accumulation (Messerli et al., 2011). In this paper, the regulatory mechanism of M-MJA and MJA on the blood pressure of SHRs has been studied comparatively, and the experimental results are discussed as follows.

RAAS plays an important role in regulating fluid balance and blood pressure. Ren is a protease which activates angiotensinogen to produce angiotensin I (Ang I). Ang I is catalyzed by Ang-converting enzyme (ACE) to form Ang II, which can promote vasoconstriction and the secretion of aldosterone (ALD) from adrenal cortex. ALD can inhibit the secretion of Na^+ in the kidney and cause peripheral vascular constriction (Lv et al., 2013). In the present work, we found that MJA had no significant effect on the levels of Ren, Ang II, and ALD in SHRs, while M-MJA significantly decreased the levels of Ren, Ang II, and ALD, indicating that M-MJA can achieve the antihypertensive efficacy by adjusting the RAAS of SHRs.

Vascular endothelial cells can secrete various active substances, including ET and NO. ET (ET-1, ET-2, ET-3, and ET-4) is the effective contractile vascular substance, and ET-1 is the predominant isoform among them (Lüscher & Barton, 2000). NO synthesized by the necessary enzyme eNOS (Jiang et al., 2015), is an endothelium-dependent vasodilation factor, the impairment of endothelial function is closely related to the decrease of endothelial dependent NO content (Jiang et al., 2015). NO and ET are checks and balances in the endothelial system. The ET-1 produced in the renal vascular endothelial cells and renal tubules leads to renal vasoconstriction and water-sodium retention. Too much ET-1 produced in the kidney can damage the synthetic ability of NO, resulting in increased blood pressure of glomerular capillary and glomerular cellulose necrosis (Schiffrin, 2001). Long-term suffering from high blood pressure can cause myocardial hypertrophy, the neural endocrine hormone secreted by heart, especially Ang II and ET-1 play a major role in causing myocardial hypertrophy (Luodonpaa, Vuolteenaho, Eskelinen, & Ruskoaho, 2001). In the liver, the increased ET-1 synthesis and decreased NO release can lead to the increase of hepatic vascular resistance (Hu, George, & Wang, 2013). The results of this paper showed that both M-MJA and MJA significantly reduced ET-1 content and increased eNOS activity in the blood and tissues of SHRs. By contrast, the effect

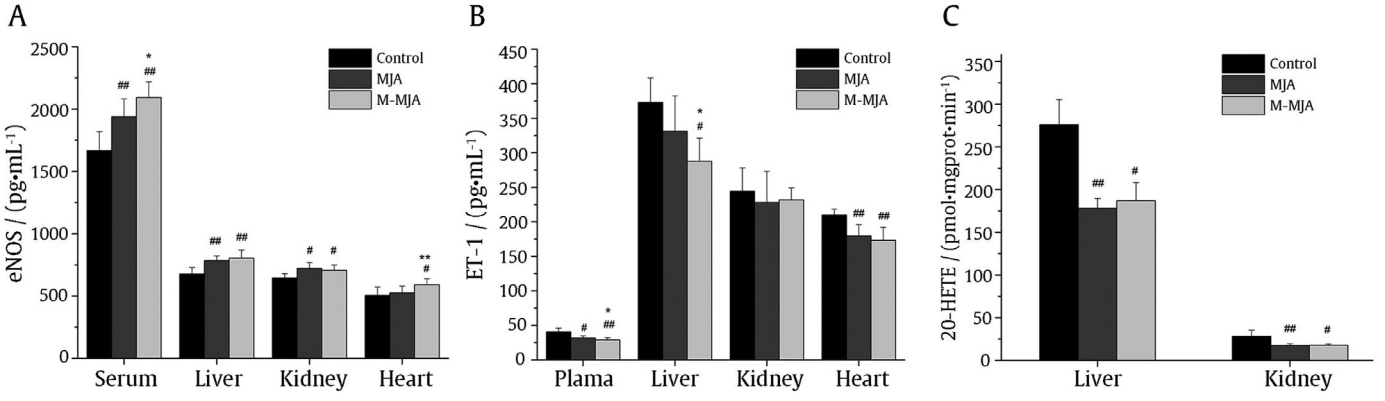

Fig. 6. Effects of MJA and M-MJA on levels of eNOS (A), ET-1(B) and 20-HETE(C) in tested tissues and blood of SHRs ($n = 5$). $^{\#}P < 0.05$, $^{\#}P < 0.01$ *vs* control. $^{*}P < 0.01$ M-MJA *vs* MJA.

of M-MJA was more remarkable than that of MJA, suggesting that M-MJA may reduce hypertension-induced endothelial injury by increasing NO content and reducing ET-1 content in blood and tissues, and achieve the antihypertensive efficacy. In addition, 20-HETE is a metabolite of arachidonic acid via CYP hydroxylase, which plays an important role in regulating vascular tension, blood pressure and renal function (Joseph et al., 2017). In rats, the main hydroxylase for the production of 20-HETE is CYP4A, which is highly expressed in the liver and the kidney (Zoccali et al., 2015). 20-HETE can restrain Ca^{2+}-activated K^{+} channel opening and promote the depolarization of vascular smooth muscle, activate L-type Ca^{2+} channel and improve the intracellular Ca^{2+} concentration to produce excitation-contraction coupling and vasoconstriction (Zeng et al., 2010). Moreover, 20-HETE can regulates other endothelial factors such as NO and Ang II to produce vasoconstriction, for instance, the 20-HETE produced in the vessel wall can act on endothelial cells by EGFR-MAPK-IKK beta signaling pathways, which on the one hand, uncoupling eNOS to decrease the NO level and increase the O^{2-}concentration (Cheng et al., 2010), and thereby cause endothelial dysfunction; On the other hand, induce ACE expression and increase the content of Ang II in endothelial cells (Garcia, Shkolnik, Milhau, Falck, & Schwartzman, 2016). Compared with the model group, this paper found that M-MJA and MJA decreased CYP4A activity and 20-HETE content in the microsomes of liver and kidney, indicating that M-MJA and MJA can cause the antihypertensive efficacy by inhibiting CYP4A expression and reducing the production of 20-HETE, as well as regulating the levels of Ang II and NO in the liver and kidney of SHRs.

Oxidative stress is closely related to the development of hypertension, which may be one of the causes of hypertension, and may also be a complication of hypertension (Rodriguez-Iturbe, Zhan, Quiroz, Sindhu, & Vaziri, 2003). Nuclear factor erythroid 2-related factor 2 (Nrf2) is a key inflammatory regulator in the antioxidant signaling pathway and plays an important role in regulating oxidative stress (Vomund, Schäfer, Parnham, Brüne, & Knethen, 2017). Reactive oxygen species (ROS) can interact with NO, Ang II, and 20-HETE to aggravate the development of hypertension. ROS is an important cause of the lipid peroxidation of cell membranes, and its level is regulated by endogenous anti-oxidant system (T-SOD, GSH-Px, HO-1, etc.) and ROS generation system (Koek, Liedorp, & Bast, 2011; Tariq, Green, & Hodson, 2014). eNOS can catalyze *L*-arginine and four hydrogen biopterin (BH4) to generate NO, which combines with superoxide to form peroxynitrite. The excessive peroxynitrite will lead to BH4 oxidation, promote eNOS uncoupling and ROS generation, and finally result in increased oxidative stress injury (Kuzkaya, Weissmann, Harrison, & Dikalov, 2003). Ang II can further activate NADPH oxidase to generate ROS by activating protein kinase C, tyrosine kinases, and phosphatidylinositol-3-kinase (Medhora et al., 2008). ET-1 increases vascular superoxide via an endothelin(A)/ NADPH oxidase pathway (Li et al., 2003). Additionally, 20-HETE can also stimulate the formation of ROS by activating Rac1/2 and inducing the translocation and phosphorylation of NADPH oxidase subunit p47 (phox) (Medhora et al., 2008). The oxidative stress of the kidney caused renal inflammation and sodium retention. After rats were fed with the antioxidant-rich diet, the symptoms of hypertension were relieved, and the content of nitrotyrosine and MDA in the kidney was reduced (Rodriguez-Iturbe et al., 2003). The liver has an important effect on blood pressure (Duarte et al., 2001) due to highly expressed CYP2E1 and CYP4A, which can oxidize various small molecular substrates to form the superoxide anions (Koek et al., 2011). The effects of oxidative stress on cardiovascular damage and hypertension cannot be ignored, and the superoxide-NO balance is an important factor in maintaining cardiac function. The short-term exposure to ROS can increase blood pressure by stimulating heart rate and stress, and the long-term exposure to ROS can also lead to chronic hypertension likely by inducing hypertrophy of the heart muscle (Lassègue & Griendling, 2004). In this paper, we found that M-MJA and MJA up-regulated the protein and mRNA expression of Nrf2 in the tested tissues, and therefore inhibited the activity and expression of CYP2E1 and CYP4A, increased the activity of GSH-Px and HO-1. These results indicated that M-MJA and MJA can reduce the blood pressure to a certain extent by improving the oxidative stress disorder of the visceral organs of SHRs.

5. Conclusion

In conclusion, we firstly found that M-MJA could regulate the RAAS, improve endothelial function, inhibit CYP4A activity to reduce the production of 20-HETE, alleviate the oxidative stress disorder of the visceral organs, and eventually produce antihypertensive effect. The anti-oxidant ability, regulating the renin-angiotensin-aldosterone system and improving endothelial function of M-MJA are more powerful than that of MJA, suggesting that M-MJA may have a better anti-hypertensive effect than MJA. The results of this study are of great significance to elucidate the mechanism of M-MJA and MJA against hypertension.

Conflict of interest

The authors declare no conflicts of interest.

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