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Original Article

Protective effect of Chinese sumac (Rhus typhina L.) fruit extract on angiotensin II-induced hypertension

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Abstract: This study aims to evaluate the effects of Chinese sumac fruit (CSF) extract on angiotensin-induced hypertension (HTN) in rats. The angiotensin II administration was performed in rats to establish the model of HTN. Rats in CSF or HTN group received CSF granule or normal saline gavage after angiotensin II treatment, respectively. Rats in control group were treated with normal saline only. The blood pressure of each rat was measured by intubation of carotid artery. The thickness of renal artery vessel was measured by hematoxylin and eosin (HE) staining. In addition, the mRNA and protein levels of total nitric oxide synthase (tNOS), endothelial nitric oxide synthase (eNOS), superoxide dismutase (SOD) and catalase (CAT) were detected, followed by the expression levels of nitric oxide (NO) and methane dicarboxylic aldehyde (MDA) in serum were measured. In CSF group, the blood pressure, thickness of renal arteries, expression levels of collagen I and collagen IV were decreased compared with HTN group. Furthermore, the mRNA and protein levels of SOD, CAT, tNOS, eNOS and expression levels of NO in CSF group were obviously elevated compared with HTN group ($P < 0.01$). MDA was remarkably decreased under the action of CSF ($P < 0.01$). In summary, CSF extract has properties to protect HTN. Decrease of oxidative stress and increase of NO are probably involved in the mechanism of CSF extract in treating HTN.

Keywords: Angiotensin, hypertension, oxidative stress

Introduction

Hypertension (HTN) is a chronic condition in which the blood pressure is elevated in arteries. It remains a major global public health burden, affecting 20-50% of adults in developed countries [1, 2]. HTN is a common leading cause of mortality worldwide with 7.5 million deaths annually [2]. In addition, HTN could bring about many life-threatening diseases, such as hypertensive heart disease and coronary artery disease [3]. Reduction of the blood pressure in patients suffering from HTN is in favor of a decreasing occurrence of stroke and heart disease [4-8]. Several drugs have been used for the treatment of HTN, including treprostinil [9], riociguat [10], propranolol and vasoactive intestinal peptide [11]. However, side effects are raised remarkably, including lack of energy, dizziness, and Raynaud's phenomenon. Therefore, it is urgent to search for new therapeutic methods.

Chinese sumac (Rhus typhina L.) is indigenous to the eastern area of North America and now extensively cultivated in China [12]. Chinese sumac fruit (CSF) is considered to be a traditional medicine, which has pharmacological functions, such as blood purifier and diuretic [13, 14]. It has been demonstrated that the extraction of CSF can be used as antioxidant [15, 16], and has the ability to enhance the antioxidant activities in patients to resist HTN [17-19]. Therefore, CSF extract may have an effect on reducing HTN. However, few studies have been reported to focus on the effect of CSF extraction HTN treatment.

In this paper, to study the effect of CSF extraction angiotensin II-induced HTN, we constructed a rat HTN model. The blood pressure, thickness of renal blood vessels and levels of perivascular collagen were measured after CSF treatment. Moreover, the mRNA and protein levels of enzymes innitric oxide (NO) system

[total nitric oxide synthase (tNOS), endothelial nitric oxide synthase (eNOS)] and antioxidase system [superoxide dismutase (SOD) and catalase (CAT)] were detected. In addition, the expression levels of NO and methane dicarboxylic aldehyde (MDA) were also measured. We are committed to reveal the effect and underlying mechanism of CSF extract on HTN therapy.

Materials and methods

Experimental animals

A total of 60male Sprague-Dawley (SD) rats, weighing 180-200 g (6-week-old), were provided by the Experimental Animal Division of Taishan Medical University Health Sciences Center (Taian, Shangdong, China). These rats were fed with normal fodder and tap water in breeding rooms under the controlled conditions (temperature 22 ± 3°C; humidity 55 ± 5%; artificial light 12 h/d). The current study was conducted under the protocol approved by Chinese Ministry of Health and Taishan Medical University Health Science Center.

Angiotensin II-induced HTN

All the rats were divided into 3 groups averagely, including control group, HTN group and CSF group. Rats in HTN group and CSF group received 200 ng/kg/min angiotensin II (Sigma chemical, St. Louis, MO, USA) administered for 3 weeks via subcutaneously implanted osmoticmini-pump 2002 (ALZA Corporation, Palo Alto, California, USA) for the establishment of HTN model. Briefly, rats were fasted for 12 hand mini-pump was inserted into the subcutaneous pocket on the back of the rats while the animals were anaesthetized with 1% pentobarbital sodium. When the mini-pump was fixed, the subcutaneous tissue was sutured meticulously layer by layer and skin was closed by continuous suture. Rats then were moved into breeding rooms once they were recovered spontaneously. Rats in the control group remained untreated as it had been shown previously that the implantation of empty osmotic pumps had no effect on the activity of enzymes involved in xenobiotic biotransformation [20].

Blood pressure determination

After the last feeding, blood pressure of each rat was assayed with intubation of carotid artery. After anesthetized and shaved, each rat received skin incision (1 cm) at the middle of the throat. Common carotid artery was separated from the fascia and intermuscular space by blunt dissection and was ligated at the distal end. Furthermore, the proximal end was clamped by bulldog clamp. A V-shaped incision was made at the arterial wall using ophthalmic scissors. Then, heparinized catheter was inserted into artery 1.5 cm deep oriented to the heart, and the bulldog clamp was removed quickly at the same time. At last, the catheter was ligated and fixated, and the waveform was recorded for 30 min when the arterial blood pressure was stable by rat arteria caudalis blood pressure determinator (RBP-1, China-Japan Friendship Institute of Clinical Medical Science, Beijing, China).

CSF administration

Fourteen days after angiotensin II administration, rats in HTN group and control group were given by gavage with normal saline (0.2 mL/d) once a day for a week. Rats in CSF group received CSF granule by gavage (1.35 g/kg/d) once a day for a week, too.

Hematoxylin and eosin (HE) staining

All rats were sacrificed 1 h after the last gavage with obtaining kidney and tail vein blood. The blood samples were collected in a centrifuge tube, added heparin, and centrifuged for 10 min (2500 r/min, 4°C). Then the rum was carefully removed and the plasma was obtained.

After the rats were sacrificed, about 1/4 kidney was fixed with 10% formalin and the remained kidney was fixed in 4% paraformaldehyde solution for 24 h and rehydrated gradually. Then the tissues were paraffin embedded and sliced in 4-μm sections. Sections were deparaffinized and stained with hematoxylin (Sigma Chemical Co., St. Louis, MO, USA) for 5 min and immersed into 1% HCl-ethanol solution to remove excessive hematoxylin. Sections were then counterstained with eosin for 3 min. After staining, all sections were dehydrated with ethanol, deparaffinized in xylene, and finally mounted with neutral balsam.

Western blot analysis

The fresh tissue around blood vessel was cracked by ultrasonic cell disruptor (JY92-IID, Ningbo Scientz Biotechnology Ltd. Co., Ningbo, China) in lysate buffer and centrifuged at 12,000 r/min for 30 min to obtain the supernate. The protein concentration was measured using bicinchoninic acid Protein Assay Kit

Table 1. The primer sequences used in quantitative real-time PCR

Primer	Sequence	Fragment size (bp)
tNOS	Sense 5'-ACCGCCTACATACCTCGC-3'	214
	Antisense 5'-CCGACAGGACTATAAAGA-3'	
eNOS	Sense 5'-ACACCCGGCTGGAAGAGCT-3'	180
	Antisense 5'-GTTTGGGGCTGAAGATGTC-3'	
SOD	Sense 5'-CACCTGAACCACAGCCTC-3'	400
	Antisense 5'-TTCCAGGGCCGTCAGCGCT-3'	
CAT	Sense 5'-GGTACCTATCTAGAAGCTCA-3'	461
	Antisense 5'-CTCACACCTAACCTGTCTC-3'	
β-actin	Sense 5'-CCTGATGCCATCCTGCGTCTG-3'	184
	Antisense 5'-TTCCTCGAAGTCTAGGGCAACATAG-3'	

Table 2. Blood pressure determination in rats after 7 days following angiotensin II administration and treatment with CSF

	Control group	HTN group	CSF group
SBP (mmHg)	119.4 ± 8.9	171.2 ± 5.1[a]	139.4 ± 8.3[a,b]
DBP (mmHg)	100.1 ± 5.4	132.6 ± 9.2[a]	111.5 ± 4.8[a,b]
PP (mmHg)	30.5 ± 0.09	43.5 ± 3.1[a]	35.2 ± 3.6[a,c]
MAP (mmHg)	109.3 ± 3.3	157.8 ± 6.3[a]	135.4 ± 5.9[a,c]

SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure; MAP: mean arterial pressure. [a]$P < 0.01$ vs. control group; [b]$P < 0.01$ vs. HTN group; [c]$P < 0.05$ vs. HTN group.

(Pierce Chemical Co., Rockford, IL, USA). Proteins were separated in 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membrane. The membrane was blocked with 5% nonfat milk and incubated with rabbit anti-rat polyclonal primary antibody of collagen I and collagen IV (1:1000, Abcam, Cambridge, UK) at 4°C overnight. Then, the membrane was incubated with Horse radish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody (1:50, Kangchen Bio-tech, Beijing, China) at room temperature for 90 min. After washing, immunoblots were visualized by Alpha Imager 2200 (Alpha Innotech, Santa Clara, USA) and the relative intensity of the immunoreactive bands was measured. β-actin was used as an internal reference.

Quantitative real-time polymerase chain reaction (RT-PCR) analysis

Total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA, USA), and cDNA were synthesized using First Strand cDNA Synthesis Kit (Promega, Madison, WI, USA) for quantitative RT-PCR. The primers (**Table 1**) of tNOS, eNOS, SOD and CAT were synthesized by Shanghai Sangon Biotech Co., Ltd (Shanghai, China). RT-PCR amplification mixtures contained: 2× PCR buffer (25 μL), 1.2 μL primers (25 μmol/L), 2× SYBR Green I (0.3 μL), 1 μL DNA template and 22.5 μL dH_2O. The cycling condition was polymerase activation at 94°C for 4 min; 35 cycles at 94°C for 20 s, 60°C for 30 s and 72°C 30 s. The mRNA levels of tNOS, eNOS, SOD and CAT were normalized by the β-actin level and calculated using the $2^{-\Delta\Delta Ct}$ method.

Oxidative stress markers detection

The expression levels of oxidative stress markers, including NO, tNOS, eNOS, SOD, MDA and CAT were measured with corresponding kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Samples were incubated at room temperature for 10 min and the absorbance of each was measured at 540 nm, 550 nm, 530 nm, 450 nm, 532 nm and 405 nm, respectively.

Statistical analysis

All data were represented as mean ± standard deviation (SD). Comparisons among groups were carried out using a Student's t-test. All statistical analyses were performed using SPSS 16.0 software (SPSS, Chicago, IL). $P < 0.05$ was considered to be statistically significant.

Results

Blood pressure determination

After blood pressure determination, the levels of systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP) and mean arterial pressure (MAP) in CSF group (139.4 ± 8.3 mmHg, 111.5 ± 4.8 mmHg, 35.2 ± 3.6 mmHg and 135.4 ± 5.9 mmHg, respectively) were obviously higher than those in control group ($P < 0.01$), and lower than those in HTN group (SBP and DBP: $P < 0.01$, PP and MAP: $P < 0.05$). In addition, the levels of SBP, DBP, PP

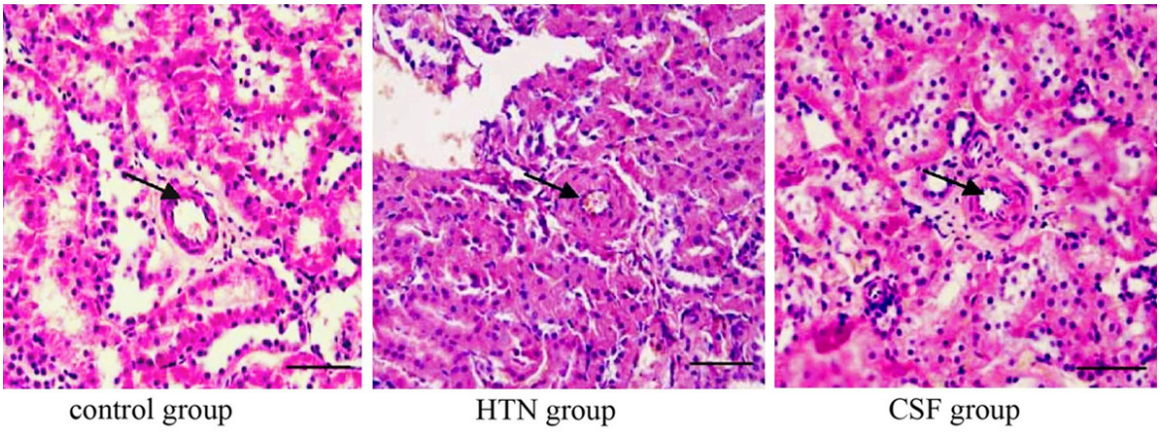

Figure 1. HE staining (× 400) for renal artery vessel of rats following angiotensin II administration and treated with Chinese sumac fruit (CSF).

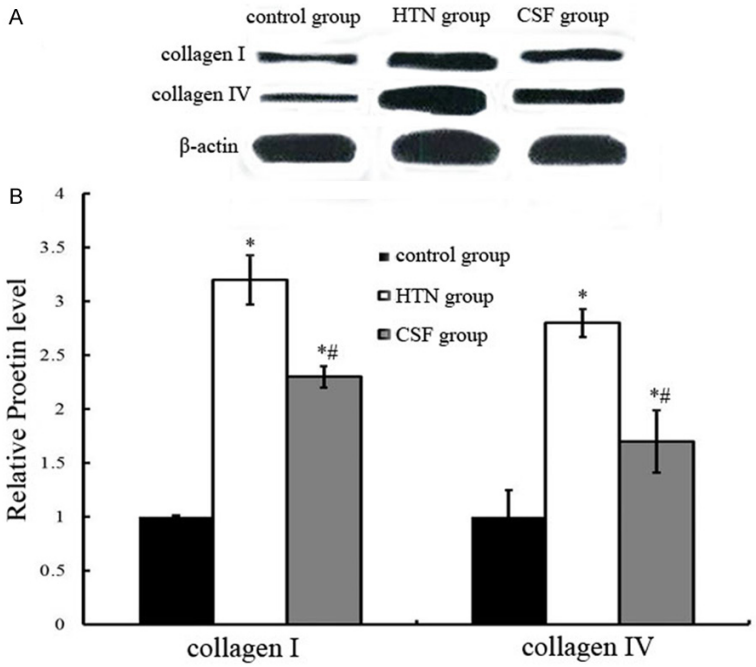

Figure 2. The protein expression (A) and relative expression levels (B) of collagen I and collagen IV in different groups. $^{*}P < 0.01$ *vs.*control group; $^{\#}P < 0.01$ *vs.* HTN group.

and MAP in HTN group (1.71 ± 5.1 mmHg, 132.6 ± 9.2 mmHg, 43.5 ± 3.1 mmHg, and 157.8 ± 6.3 mmHg, respectively) were higher than those in control group (119.4 ± 8.9 mmHg, 100.1 ± 5.4 mmHg, 30.5 ± 0.09 mmHg and 109.3 ± 3.3 mmHg, $P < 0.01$, **Table 2**).

The thickness of renal artery vessel analysis

Compared with the HTN group, the thickness of renal artery vessel was lower in CSF group (**Figure 1**), and the wall/lumen ratio of renal artery vessel in CSF group (40.2 ± 6.1%) was lower than that in HTN group (49.8 ± 3.1%, $P < 0.05$). In addition, compared with control group, the thickness of renal artery vessel in HTN group was increased, and the wall/lumen ratio of renal artery vessel in HTN group (49.8 ± 3.1%) was higher than that in the control group (38.4 ± 0.95%, $P < 0.05$).

The expression levels of collagen I and collagen IV

Protein expression levels of collagen I and collagen IV around vessels in both HTN and CSF groups were higher compared with control group (**Figure 2A**). For collagen I, the relative expression level in HTN group (3.2 ± 0.23) was higher than that in CSF group (2.3 ± 0.1, $P < 0.01$, **Figure 2B**). Similar with collagen I, the relative expression level of collagen IV in HTN group (2.8 ± 0.13) was also higher than that in CSF group (1.7 ± 0.29, $P < 0.01$). In addition, the expression levels of collagen I and collagen IV in both CSF group and HTN group were higher than those in control group (1 ± 0.01 and 1 ± 0.25, respectively).

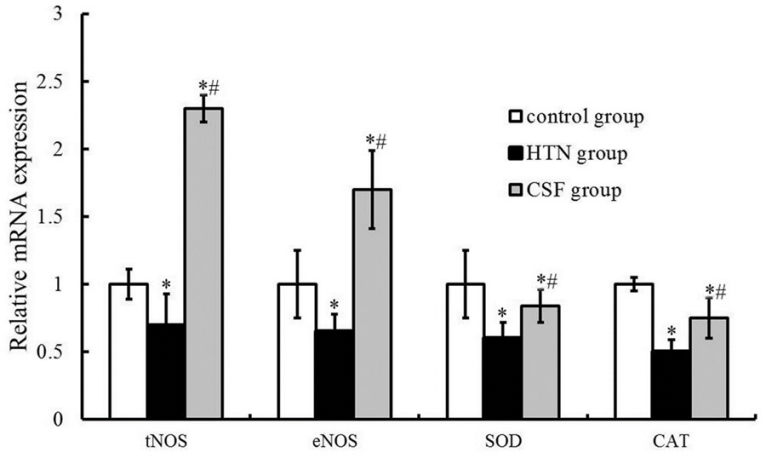

Figure 3. The mRNA expression of tNOS, eNOS, SOD and CAT in different groups. $^{*}P < 0.01$ *vs.* control group; $^{\#}P < 0.01$ *vs.* HTN group.

Table 3. The level of NO, tNOS, eNOS, SOD, CAT and MDA in rats following angiotensin II administration and treated with CSF

	Control group	HTN group	CSF group
NO (μmol/L)	22.4 ± 1.9	11.2 ± 4.3[a]	18.9 ± 2.3[a,b]
tNOS (U/mL)	30.1 ± 1.2	23.9 ± 2.1[a]	29.4 ± 0.8[a,b]
eNOS (U/mL)	12.8 ± 0.9	9.5 ± 1.3[a]	11.6 ± 1.6[a,b]
SOD (U/mL)	141.3 ± 12.3	90.8 ± 7.8[a]	117.4 ± 8.8[a,b]
MDA (μmol/L)	4.1 ± 0.7	6.3 ± 0.4[a]	3.5 ± 0.6[a,b]
CAT (U/mL)	7.3 ± 1.3	4.3 ± 1.2[a]	5.9 ± 0.4[a,b]

[a]$P < 0.01$ *vs.* control group; [b]$P < 0.01$ *vs.* HTN group.

mRNA expression of tNOS, eNOS, SOD and CAT

As shown in **Figure 3**, the relative mRNA levels of tNOS, eNOS, SOD and CAT in HTN group (0.7 ± 0.23, 0.65 ± 0.13, 0.6 ± 0.12, and 0.5 ± 0.09, respectively) were lower than those in CSF group (2.3 ± 0.1, 1.7 ± 0.29, 0.84 ± 0.12, and 0.75 ± 0.15, respectively, $P < 0.01$). Additionally, compared with control group, the mRNA level of tNOS in HTN group was decreased (1 ± 0.11 *vs.* 0.7 ± 0.23), and in CSF group was increased (1 ± 0.11 *vs.* 2.3 ± 0.21, $P < 0.01$), and the same as eNOS, SOD and CAT.

The levels of oxidative stress markers

The expression levels of NO (18.9 ± 2.3 μmol/L), tNOS (29.4 ± 0.8 U/mL), eNOS (11.6 ± 1.6 U/mL), SOD (117.4 ± 128.8 U/mL), MDA (3.5 ± 0.6 μmol/L) and CAT (5.9 ± 0.4 U/mL) in CSF group were higher than those in HTN group (11.2 ± 1.3 μmol/L, 23.9 ± 2.1 U/mL, 9.5 ± 1.3 U/mL, 90.8 ± 7.8 U/mL, 6.3 ± 0.4 μmol/L and 4.3 ± 1.2 U/mL, respectively) and lower than those in control group (**Table 3**, $P < 0.01$).

Discussion

HTN is the leading cause of cardiovascular mortality worldwide according to the World Health Organization [21]. The prevention and treatment of HTN have drawn more attention in the medical field. In this study, we investigated the protective effect of CSF extract on angiotensin II-induced HTN.

Compared with HTN group, blood pressure, thickness of renal arteries vessel, and expression levels of collagen I and collagen IV were significantly reduced under the action of CSF extract. This may be due to the chemical composition stannin in the CSF extract. It has been reported that tannin can reduce the blood pressure and prolong the life span of the HTN rats [22, 23]. Tannin exhibits the hypotensive effects reducing the blood pressure significantly in the HTN rats induced by angiotensin I [24]. Furthermore, tannin is useful on ameliorating one or more symptom of HTN [23]. It can inhibit platelet aggregation, which is commonly associated with arterial HTN [25]. Thus, CSF extract may be effective in resisting the progress and development of HTN.

Accumulating evidences suggest that HTN is associated with oxidative stress [26, 27], which is an excessive production of reactive oxygen species (ROS) overwhelming antioxidant capacity [28]. Our results showed that the MDA level was significantly increased at the HTN rats and returned to normal under the action of CSF extract. Moreover, antioxidant activities (SOD, CAT) were elevated by treating with CSF extract, which suggested that oxidative stress was decreased in HTN rats. It has been proposed that enhancing antioxidant activities in patients can resist HTN [17-19]. A lifelong antioxidant-

rich diet has the ability to diminish the severity of HTN, ameliorate or reverse abnormalities expressions of antioxidant enzymes (including SOD and CAT) [29]. It has been verified that CSF extracts possess strong antimicrobial and antioxidant activities [16, 30]. Thus, CSF extracts may attenuate oxidative stress through inducing antioxidant enzymes activities so as to perform its protection in the process of HTN.

On the other hand, NO, a potent vasodilator produced by endothelial cells, plays an important role in the regulation of blood pressure and regional blood flow [31]. It is produced mainly by NOS, especially eNOS in vessels. In our study, we found that the levels of NO, tNOS and eNOS were suppressed in HTN rats. This was corresponded with previous research, which showed NO inactivation in HTN rats [32]. Moreover, the main reason for oxidative stress resulting in HTN was the inactivation of NO [33]. Whereas, the levels of NO, tNOS and eNOS were increased and returned to normal under the effect of CSF extract, which meant that the regulation of blood pressure had been restored. Previous evidence indicates that somatic delivery of the human eNOS gene induces a prolonged reduction of high blood pressure [34]. Furthermore, infusion of antioxidantim proves endothelial vasodilation by restoring NO activity [35]. Therefore, as an antioxidant, CSF extract may play its protective role in HTN through ameliorating abnormality level of NO.

The major limitations in the current study showed as follow: First, total NO synthesis includes not only tNOS and eNOS, but also inducible nitric oxide synthase (iNOS). However, we did not investigate the changes of iNOS level in this study, and further studies are still needed to cover this shortage. Second, the specific active ingredients of CSF extract were not studied.

In conclusion, CSF extract has properties to protect HTN induced by angiotensin II. The conceivable mechanism of CSF extract in protecting HTN may be associated with decreasing oxidative stress and increasing the level of NO. These findings indicate the feasibility of CSF extract for treating HTN.

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Disclosure of conflict of interest

None.

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