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Original Research Article

Anti-hypertensive effect of Gastrodia elata Bl leaf extract in rats

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Abstract

Purpose: *To investigate the probable antihypertensive effects of Gastrodia elata Bl. extract (GEBE) in renovascular hypertensive rats as well as the mechanism involved in blood pressure reduction.*
Methods: *The two-kidney one-clip (2K1C) Goldblatt model of renovascular hypertension was used in Wistar rats. The 2K1C group rats were treated with captopril (40 mg/kg/day), low-dose GEBE (150 mg/kg/day) and high-dose of GEBE (300 mg/kg/day) for 6 weeks by intragastric administration. Systolic (SBP) and diastolic blood pressure (DBP) were measured by the tail-cuff method. Urine creatinine and urea levels of the rats were measured by enzyme-linked immunosorbent assay (ELISA). Superoxide dismutase (SOD) and malondialdehyde (MDA) levels were also evaluated.*
Results: *In the captopril- and GEBE-treated groups, blood pressure decreased progressively over the course of the 6-week treatment period compared with that of the untreated (control) rats ($p < 0.01$). High-dose GEBE also significantly increased plasma SOD activity but decreased plasma MDA concentration ($p < 0.05$). Renal function improved following captopril and GEBE (300 mg/kg/day) treatment ($p < 0.01$).*
Conclusion: *The results suggest that GEBE probably exerts an antihypertensive effect by inhibiting endothelin (ET)-converting enzyme and via its antioxidant activity.*

Keywords: *Antihypertensive, Gastrodia elata, Goldblatt renovascular hypertension, Endothelin-1, Hypertrophy*

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INTRODUCTION

Hypertension is the most common risk factor for myocardial infarction, stroke, heart failure, arterial fibrillation, aortic dissection and peripheral arterial diseases. It is among the most common chronic illnesses in the world [1,2] and remains the leading cause of death worldwide and one of the world's greatest public health problems. Although many new antihypertensive drugs with improved efficacy have been introduced to the market, they still possess serious side effects. On the one hand, nutrition and physical exercises are gaining more importance in the treatment of hypertension. On the other hand, attention has recently been focused on herbal and mineral preparations which are traditionally used as potential therapeutic agents in the prevention and management of cardiovascular diseases.

Of the various experimental or genetic models of hypertension, the Goldblatt chronic two-kidney, one-cliphypertension (2K1C) is a classical model of renovascular angiotensin-II-dependent hypertension. Experimental model of renal

(Goldblatt) hypertension is one of the widely used models for the study of pathophysiology of hypertension and antihypertensive drugs [3,4]. The fact that the rennin-angiotensin system (RAS) contributes critically to the pathophysiology of 2K1C Goldblatt hypertension is well established [5]. The 2K1C model, which exhibits a transient increase in the activity of RAS and a sustained rise in blood pressure, has been described as very close to human mature hypertension [6,7]. Thus, hypertension in this model is primarily the result of an augmented total peripheral resistance and, in mild cases of renal artery stenosis, bilateral reduction in renal-clearance function [8]. These physiological abnormalities are principally the result of a considerable increase in tissue and circulating levels, and direct actions of Ang-II [9]. Evidence shows that as the condition advances, the role of Ang-II in maintaining hypertension subsides, and other mediators become more effective in determining the levelof blood pressure [10].

To the best of our knowledge, there is no report on the anti-hypertensive effect of GEBE and the information regarding to the mechanism of GEBE against hypertension is very scarce. For these reasons, we describe, in the present paper, the anti-hypertensive effects of GEBE, together with changes in biological parameters such as SOD activity and MDA level, following the administration of GEBE to the renal hypertensive rat model.

EXPERIMENTAL

Collection and extraction of plant material

The herbal material, *Gastrodia elata Bl.* was collected from Guilin City, Guangxi Province in China in May 2016. Taxonomic identification of the plant was performed by a taxonomist, Professor Ping Wang of Department of Pharmacy of Guizhou University, Guiyang, China. A voucher specimen (no. GEBE 201605002) was deposited in the herbarium of College of Pharmacy, Guizhou University, China for future reference.

The leaves of *Gastrodia elata Bl.* were dried by an oven and decocted in water at 60 ℃ for three times, each time for 1 h. Then the extract was first dried in an oven at 80 ^{o}C and then freeze-dried to obtain GEBE.

Induction of renovascular hypertension (two-kidney, one-clip model)

Goldblatt 2K1C model of hypertension was induced according to the procedure described by Umar *et al* [11]. Briefly, the rats were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally). The left renal artery was exposed by retroperitoneal flank incision and dissected free of the renal vein and connective tissue. A silver clip with a lumen of 0.22 mm was placed around the artery for partial occlusion; in sham operations, the artery was not clipped. After 6 weeks the systolic blood pressure (SBP) was measured using the tail-cuff method in conscious rats. Only hypertensive rats (SBP above 150 mm Hg) were used in the experiments.

Animal groups

The mice were randomly divided into four groups as follows: (1) sham-operated control group (served as negative control) (n = 8); (2) an untreated model group (n = 8); (3) rats treated with captopril (40 mg/kg/day) group (n = 8); (4) rats treated with low-dose of GEBE (GEBE-L,150 mg/kg/day) (n = 8), and rats treated with high-dose of GEBE (GEBE-H, 300 mg/kg/day). Rats were treated for 6 weeks with daily oral administration of the products or the same volume of vehicle (0.9 % saline) (groups 1 and 2). Doses of GEBE were chosen with reference to doses commonly used in human and doses used in previous experiments. All rats were weighed and their blood pressure measured once weekly for 4 weeks. Drugs were dissolved in water, and administered using a 5-ml syringe with a 4-cm long gavage needle through the mouth to the mouth once daily for 3 weeks. The animal experiment was approved by the Animal Care and Use Committee of Guizhou University (approval ref no. 20100405) and was carried out in compliance with the Directive 2010/63/EU on the handling of animals used for scientific purposes [12].

Measurement of blood pressure

Systolic (SBP) and diastolic blood pressure (DBP) were measured by the tail-cuff method (BP-6 noninvasive Electro-Sphygmomanometer, Chengdu Taimeng Science and Technology, Chengdu, PR China) in awaken rats. The mean of three consecutive readings was taken. The arterial systolic and diastolic blood pressure was measured at the weekend and continuous measurement was carried out three times as the average blood pressure for weeks.

Assessment of renal function

At the last week of the experiment, the animals were placed in individual metabolic cages and acclimatized for two consecutive days before a

24-h urine collection. Creatinine and urea were measured with a commercial enzyme-linked immunosorbent assay (Nanjing Institute of biological engineering, China) as described by the manufacturer.

Determination of serum SOD activity and lipid peroxide level (MDA)

Serum superoxide dismutase (SOD) activity and the malondialdehyde (MDA) level were determined according to the instructions in the kit (Jiancheng Institute of Biotechnology, Nanjing, China).

Statistical analysis

Data are presented as mean ± standard deviation (SD), and were analyzed by one-way ANOVA followed by Tukey's multiple comparison using SPSS 16.0 software for Windows. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Effect of GEBE on blood pressure of 2K1C hypertensive rats

Untreated (model) renovascular hypertensive rats had markedly higher SBP and DBP than the sham-operated rats ($p < 0.01$). This hypertension was stable over 6 weeks of the experiment. In the captopril- and GEBE treated groups, the blood pressure decreased progressively over the course of the 6 weeks treatment compared with that of the untreated (model) rats ($p < 0.01$, Table 1).

Table 1: Effect of GEBE on the blood pressure of 2K1C hypertensive rats

Group	Dosage (mg/kg)	SBP (mmHg)	DBP(mmHg)
Normal	-	142.3±6.2**	76.7±2.5**
Control	-	213.5±8.1	110.2±3.4
Captopril	40	148.4±5.6**	80.5±3.2**
GEBE-L	150	172.1±6.4*	98.3±6.1
GEBE-H	300	153.2±4.3**	82.2±4.3**

**P < 0.05 and **p < 0.01 versus control group; GEBE-L: low dose of GEBE (150 mg/kg/day); GEBE-H: high dose of GEBE (150 mg/kg/day)*

Effect of GEBE on renal dysfunction in 2K1C hypertensive rats

The levels of blood urea nitrogen (BUN) and serum creatinine (Scr) were significantly higher in the untreated model group compared to the other group ($p < 0.05$). Both GEBE-L and GEBE-H were able to decrease the serum BUN and Scr levels ($p < 0.01$), respectively (Table 2).

Table 2: Effect of GEBE on renal dysfunction in 2K1C hypertensive rats

Group	Dosage (mg/kg)	BUN (mmol/L)	SCr (μmol/L)
Normal	-	45.3±4.6**	31±4.1**
Control	-	83.4±6.2	63.6±5.8
Captopril	40	47±5.1**	45±7.6**
GEBE-L	150	69±7.6	53±3.7
GEBE-H	300	52±4.8**	37±3.5**

**P < 0.05 and **p < 0.01 versus control group. GEBE-L: low dose of GEBE (150 mg/kg/day); GEBE-H: high dose of GEBE (150 mg/kg/day)*

Effect of GEBE on antioxidant status in 2K1C hypertensive rats

The plasma SOD activity in the untreated control group was significantly lower than that in the sham-operated group. The SOD activity of the GEBE and captopril treated groups were significantly higher than that in the untreated control group ($p < 0.01$). The GEBE and captopril-treated groups showed significantly lower MDA level ($p < 0.05$) than the untreated model group (Table 3).

Table 3: Effect of GEBE on antioxidant status in 2K1C hypertensive rats

Group	Dosage (mg/kg)	SOD (pg/mL)	MDA (pg/mL)
Normal	-	124.5±8.5**	4.7±1.2**
Control	-	85.9±6.4	13.6±3.4
Captopril	40	117.6±6.7**	7.4±2.3*
GEBE-L	150	98.4±5.9*	6.5±2.7*
GEBE-H	300	118.7±6.6**	5.2±3.6*

**P < 0.05 and **p < 0.01 versus control group. GEBE-L: low dose of GEBE (150 mg/kg/day); GEBE-H: high dose of GEBE (150 mg/kg/day)*

DISCUSSION

The 2K1C renovascular hypertension is a classic animal model of renin-dependent hypertension, which is considered to be similar to human renal hypertension. The 2K1C hypertension is an angiotensin-II (Ang-II) dependent model of hypertension where increased plasma and intrarenal AngII concentrations [13,14], enhanced production and systemic delivery of Ang-II by the clipped kidney, form the basic endocrine disturbance [15].

The endothelin (ET) system and RAAS are two of the most potent vasopressor mechanisms identified to date and in conditions where both systems are activated, their interrelationships have been proposed to contribute to the

development of hypertension [16]. Previous reports showed that blood pressure regulation is dependent on the relationship between the ET system and RAAS [17]. The active component of the RAAS is Ang-II, which is a potent vasoconstrictor and the mechanism of RAAS-induced hypertension has generally been attributed to the direct vasoconstrictor effects of Ang-II and the mineralocorticoid effects of aldosterone. Ang-II can also elevate blood pressure by augmenting noradrenaline release from sympathetic nerve endings in the vasculature [18] by increasing secretion of potent vasoconstrictor ET-1.

A recent hypothesis pointed out a possible role of oxidative stress as a key player in the pathogenesis of insulin resistance, cell dysfunction, and hypertension [19] and many mechanisms have been implicated in processes underlying oxidative stress-mediated hypertension. The relationship between the development of hypertension and the increased bioavailability of ROS or decreased antioxidant capacity, or both, have been demonstrated in many experimental models of hypertension as well as in human hypertension [20]. These findings are based, in general, on increased levels of biomarkers of lipid peroxidation and oxidative stress [21].

Research data suggest that oxidative damage and proinflammatory processes may precede full-blown hypertension [22]. As malondialdehydes (MDA) are a class of terminal lipid peroxidation metabolites, determination of their content can directly reflect lipid peroxidation levels. On the other hand, SOD is a natural antioxidant enzyme which can remove superoxide anion radicals in vivo to maintain the production of free radicals in the body and clear the dynamic equilibrium of a metal enzyme. Therefore, protection of the endothelial function and reduction of free radical damage in the treatment and prevention of hypertensive target organ damage can have a far-reaching significance.

The results obtained suggest that GEBE may play a key role in scavenging OFR, thus causing anti-oxidation effects and can clear free radicals from the system, which in turn effectively improved the endothelial lipid oxidative damage and high blood pressure to the same extent as captopril, thus guaranteeing its therapeutic effects.

CONCLUSION

This study demonstrate the significant antihypertensive effect of GEBE which is achieved by inhibiting endothelin-converting enzyme and via its antioxidant activity. Thus, GEBE has the potential to be developed as a therapeutic agent for the treatment of hypertension.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Hua Fang and Wei-jing Zhang contributed equally to this work.

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