Original Article

Anti-hypertensive effects of laiju extract in two different rat models

Suhong Chen MD[1], Guiyuan Lv Tech[2], Xiaodong Zhang MSc[3], Xiaoyu Liu MSc[2], Han Zhang MD[2,4], Yunwei Zhu MSc[2], Yin Wu MSc[2], Saiyue Liu MSc[2] and Zhunan Ni MSc[2]

[1]*Academy of Traditional Chinese Medicine, Wenzhou Medical College, Wenzhou, China*
[2]*Institute of Materia Medica, Zhejiang Chinese Medical University, Hangzhou, China*
[3]*Center for Drug Evaluation, State of Food and Drug Administration, Beijing, China*
[4]*Shanghai University of Traditional Chinese Medicine, Shanghai, China*

The aim of the study was to evaluate whether laiju extract (LJE) from *Semen Raphani* and *Flos Chrysanthemi* has an anti-hypertensive effect in renal hypertensive rat (RHR) and spontaneous hypertensive rat (SHR). LJE was prepared by extracting dried *Semen Raphani* and *Flos Chrysanthemi* with 70% ethanol. RHR and SHR models were prepared by standard methods. Forty RHRs and 40 SHRs were randomly divided into high LJE (300 mg/kg), moderate LJE (200 mg/kg), low LJE (100 mg/kg) and saline control four groups (n=10), respectively. Compared with saline control, blood pressure was significantly lowered at 6 and 5 hours in high and moderate LJE respectively in both RHR and SHR groups. However, blood pressure was significantly lowered at 2 and 3 hours in low LJE in both RHR and SHR groups, respectively. Compared with saline control, blood pressure remained significantly lower in SHR in all dosage groups with a single daily dose for 28 days of study. LJE has potential in the prevention management of hypertension. Further studies are needed to identify the active chemical constituents and mechanisms of action of LJE.

Key Words: food aid, expert system, disaster relief, monitoring, evaluation

Introduction

Hypertensive disease is a major public health problem. There are about 600 million patients suffering from hypertension world-wide. Reports from WHO show that the incidence of hypertension exceeds more than 10% of the worldwide population.[1] Self-awareness, treatment and control rates of hypertensive diseases were only 25%, 12.5% and 3%, respectively.[2] Hypertension is associated with many chronic conditions such as insulin resistance, obesity, carbohydrate tolerance, concomitance, haemaglutinin, hyperuricacidemia, atherosclerosis and cardiovascular diseases.[3]

Anti-hypertensive drugs can be divided into six catalogues, including diuretic, beta-blocking agent, calcium antagonist, angiotensin-converting enzyme inhibitors, angiotensin Ⅱ receptor antagonist and alpha-receptor blocking agent.[4-6] However, the efficacy of these drugs are only 40%-60%, and usually two or more anti-hypertensive drugs from different categories need be combined to achieve optimal results, however side effects from these medications are an important concern.[7] To find a safe and effective way to manage hypertension has challenged medical researchers for centuries. Traditional Chinese medicine extracted from the natural plants may be one of the most important directions in the future.

Laiju extract (LJE) was extracted from *Semen Raphani* and *Flos Chrysanthemi*, officinal and edible plants. *Semen Raphani* was recorded early in *Shijing* (600 B.C.). *Flos Chrysanthemi* was recorded as "long-term taking could promote Blood-Qi, lighten body, delay senility" in *Shennongbencaojing* (100 B.C.). They have been planted in China for over 2000 years.

Pharmacopeia of People's Republic of China records that the effects of *Semen Raphani* include promoting digestion, relieving flatulence, lowering adverse-rising energy and dissipating phlegm, which is suitable for chest distress, dyspeptic retention, sputum, cough with asthma and so on. *Flos Chrysanthemi* is applied in headache and vertigo with its effects on dispelling wind-evil, removing heat-evil, calming liver and improving eyesight.[8-9]

Clinical evidences and animal experiments suggested that *Semen Raphani* and *Flos Chrysanthemi* were potential materials as anti-hypertensives due to their LJE content. In the present study, renal hypertensive rat (RHR) and spontaneous hypertensive rat (SHR) were used to observe the effects of LJE on blood pressure (BP) and heart rate (HR).

Corresponding Author: Professor Guiyuan Lv, Institute of Materia Medica, Zhejiang Chinese Medical University, 548 Binwen Road, Hangzhou, Zhejiang Province, China 310053
Tel: 86 571-86613601 ; Fax: 86 571-86613601
Email: lv.gy@263.net

Materials and methods

Animals

Sprage-Dawley rats, initially weighing 160-200 g, were purchased from experimental animal center, Academy of Medical Science of Zhejiang, China. Spontaneous hypertensive rats, weighing 180-220 g, were purchased from medical experimental animal center of Shanghai, Academia Sinica, China. Animals were kept at constant room temperature (20-25℃) and relative humidity (55%-75%), under a 12-hour light-dark cycle, and free access to food and water at all times. All animals were allowed to acclimatize to their holding cages for 3-4 days before any behavioral or surgical procedures. The study was performed after prior approval from the local ethical committee for animal experimentation in Zhejiang Chinese Medical University, China.

Preparation of the herb extract

Dried *Semen Raphani* and *Flos Chrysanthemi* was extracted with 2 x 70% ethanol, solvent was removed by vacuous. The LJE extract contained 1.5% of flavonids. LJE solution was prepared by dissolving the LJE extract into three different concentrations (30 mg/mL, 20 mg/mL, 10 mg/mL) with 0.9% saline.

Replication of renal hypertensive rat model

SD rats were anesthetized with 3% Pentobarbital sodium (30 mg/kg) by intraperitoneal injection. A ventral median line incision was performed, then a ring-shaped silver clip with 0.2 mm internal diameter was placed around the left renal artery. After six weeks, systolic pressure was measured by an indirect tail-cuff sphygmomanometer in preheated (37℃, 15 min) conscious rats. SD rats were considered to be renal hypertensive rat when systolic pressure was above 18.7 Kpa (140 mmHg).

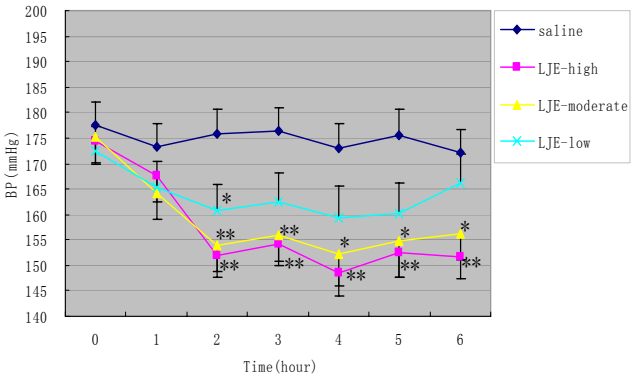

Figure 1. Effects of different concentrations of LJE or saline on BP for 6 hours in RHR. n=10. *p<0.05, **p<0.01.

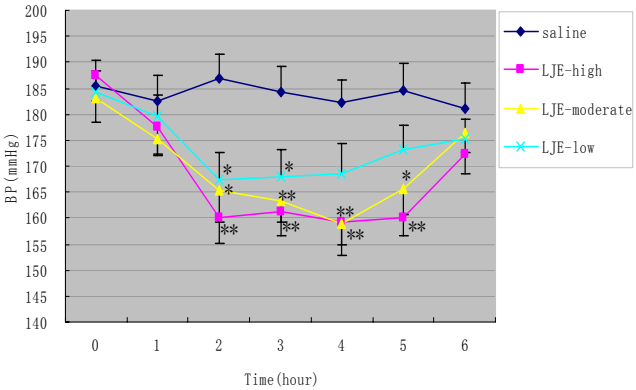

Figure 2. Effects of different concentrations of LJE or saline on BP for 6 hours in SHR. n=10. *p<0.05, **p<0.01.

Measurement of BP and HR in RHR with LJE

RHRs were randomly divided into four groups, each receiving one of the following treatments: (i) LJE-high dose (30 mg/mL, Institute of Materia Medica, Zhejiang Chinese Medical University) (n=10), (ii) LJE-moderate dose (20 mg/mL) (n=10), (iii) LJE-low dose (10 mg/mL) (n=10), (iv) saline control (0.9% saline) (n=10). BP and HR were measured by RBP-1 blood pressure measurement of rats (Institute of Clinical Medicine, China-Japan Friendship Hospital, Beijing) at 0 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h after one intragastric administration (10 mL/kg).

Measurement of BP and HR in SHR with LJE

SHRs were the same as the RHRs with respect to dosage, route of administrations and method of measurements. This experiment included two parts, (i) After given one dose, BP and HR were measured at 0 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h. (ii) After multiple dosage administrations, BP and HR were measured once daily at 2 hours after the once daily intragastric administration for 7 days in the first stage, BP and HR were then measured once weekly at 2 hours after the once daily intragastric administration for 4 weeks in the second stage.

Statistical analysis

Data was analyzed using Microsoft Excel XP. Difference between the treatment and control at each point was analyzed by student *T*-test. Data were expressed as mean in all figures.

Results

Effects of LJE on BP and HR of RHR with one intragastric administration

Compared with saline control, BP of RHR was significantly decreased from 2-6 hours in both high and moderate LJE groups (p<0.01 or p<0.05; Fig1). However, BP of RHR was only significantly lowed at 2 h in low LJE group (p<0.05) (Fig 1). There were no significant changes in HR in either group (data not shown).

Effects of LJE on BP and HR of SHR with one intragastric administration

Compared to saline control group, three different concentrations of LJE could lower BP of SHR to different degrees with one intragastric administration (p<0.01 or p<0.05; Fig 2). In high LJE group, BP was significantly lowered at 2 h, 3 h, 4 h and 5 h compared with saline group (p<0.01) (Fig 2). In moderate LJE group, there were significant differences at 3 h and 4 h (p<0.01) and at 2 h and 5 h (p<0.05) when compared with saline group (Fig 2). In low LJE group, significant differences were found at 2 h and 3 h (p<0.05) (Fig 2). However, there were no significant changes in HR in either group (data not shown).

Effects of LJE on BP and HR of SHR with multiple intragastric administrations

With multiple administrations for 28 days, three doses of LJE had an effect on lowering BP of SHR at 2 h after one administration once daily (p<0.01 or p<0.05; Fig 3), their

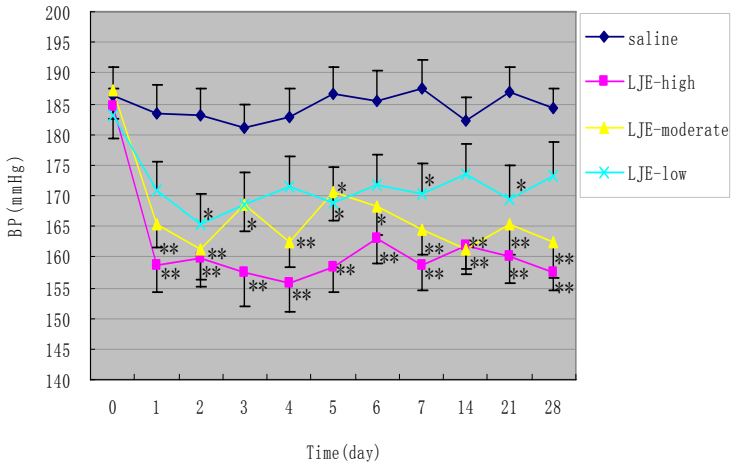

Figure 3. Effects of different concentrations of LJE or saline on BP once daily for 28 days in SHR. n=10. *p<0.05, **p<0.01.

action was stable and showed little fluctuation with time. In high LJE group, there were significant differences within 28 days with p<0.01, compared with saline group (Fig 3). In moderate LJE group, there were significant differences at 1 d, 2 d, 4 d, 7 d, 14 d, 21 d and 28 d with p<0.01, and at 3 d, 5 d and 6 d with p<0.05 when compared with saline group (Fig 3). In low LJE group, significant differences were found at 2 d, 5 d, 7 d and 21 d (p<0.05) (Fig 3), but there were no significant differences at other days while on descending trend. Meanwhile, HR in four groups showed no significant changes during the 28 days (data not shown).

Discussion

The present study suggests that LJE has an anti-hypertensive effect. We adopted two kinds of hypertensive animal models, RHR and SHR, to observe the influences of LJE on BP and HR with one or multiple intragastric administrations. Compared to saline group, the effects of LJE on decreasing BP can last 6 hours in RHR, and 5 hours in SHR with one administration. The decreasing effects on BP in SHR can last 28 days with once daily administration for 28 day study length. The effects of LJE on HR in two kinds of hypertensive rats showed no significant changes. LJE can lower BP abut has no significant effects on HR.

Traditional Chinese medicine is playing more and more important roles in health and nutrition fields. The main raw materials of health food for auxiliary anti-hypertension are from natural plants such as *Semen Cassiae*, *Prunella Vulgaris*, *Apocynum Venetum*, *Flos Sophorae*, *Gastrodia Elata*, *Eucommia Ulmoides*, *Puerariae Radix*, leaves of *Cyclocarya Paliurus (batal) iljinsk*, dry extract from *Ginkgo Bilob leaves*, and *Tea Polyphenols*. They have more or less anti-hypertensive effects.[10-11]

Studies from human and animal have indicated that *Semen Raphani* could be suitable for hypertension, hypertension with coronary diseases, endocrine disorders, and digestive or respiratory system diseases.[12] In the treatment of phase-Ⅱ hypertension, *Semen Raphani* lowered blood pressure and cholesterol, which could reduce the incidence of heart diseases, cerebral hemorrhage, coronary diseases and kidney harm caused by hypertension.[13] *Semen Raphani* decoction could reduce hyperlipemia and hypertension by diuresis, which is effective to improve primary hypertensive syndromes, especially for phase-Ⅰ and phase-Ⅱ hypertensive patients.[14] Animal experiments also proved that *Flos Chrysanthemi* decoction could decrease blood pressure, dilate coronary artery, increase coronary blood flow, lighten heart muscle ischemia symptom and restrain vasopermeability.[15]

Studies had shown that the bioactive constituents extracted from plants were mainly flavonoids, alkaloids, anthraquinone and so on.[16-19] *Eucommia Ulmoides* contained flavonoids such as *Quercetin*, *Rutin* and so on, with the possible anti-hypertensive mechanism of depressing the activity of angiotensin-converting enzyme.[20] The anti-hypertensive effects of Jueming extract are related to the suppression of the renin-angiotensin system, but not to aldosterone, endothelin and atrial natriuretic peptide.[21] *Semen Raphani* contained flavonoids, alkaloids and so on.[22-23] *Semen Raph*ani lowered BP by dilating vessel and decreasing vascular resistance.[8] The chemical constituents of *Flos Chrysanthemi* include flavonoids, volatile oil, amino acid, infinitesimal elements and so on.[24] Research of material bases on anti-hypertensive effects indicate that total flavonids of *Flos Chrysanthemi* (57mg/Kg, i.v.) could reduce BP of rats.[25] Laiju extract was from the prescription of *Semen Raphani* and *Flos Chrysanthemi*. The possible mechanism of action of laiju extract on lowering BP may be related to the renin-angiotensin system.

In conclusions, LJE can lower BP in RHR and SHR, thus it may be a potential natural product which can be used to manage hypertension. Because of abundant resources of *Semen Raphani* and *Flos Chrysanthemi* in China, it may be possible for these findings to contribute to the development of a health food or natural product to manage hypertension. As there are several limitations in this study, further research is needed to identify bioactive compound(s) and the anti-hypertensive mechanism(s) of action of laiju extract.

Acknowledgement

The authors would like to acknowledge all the participates. This project funded by Zhejiang Provincial Natural Science Foundation of China under Grant No. ZB0205.

References

1. Wang W. Brief introduction of the third international academic seminar on hypertension and the related diseases. Chin J hypertens 2002; 10: 63-93.
2. Su DF, Miao CY. Treatment of hypertension: old topic, new concept. Chin J hypertens 2001; 9: 4.
3. Zhu SJYL. Risk factors and hypertension complication. Japanese Medical Introduction 1997; 18: 200-202.
4. Zhou BY. The progress of clinical diagnosis and treatment in hypertension. AnHui Medical and Pharmaceutical Journal 2003; 7: 465.
5. Ke L. Review and prospect of medication of hypertension. The College of Sanitary and Management Staff of SiChuan Province 2004; 20: 138.
6. Wang Z. Progress on treatment of essential hypertension. Job and Health 2004; 20: 138.
7. Du YL, Chen SX. Combinative application of anti-hypertension drugs. World Clinical Drugs 2005; 26: 592-602.
8. Tan P, Jiang HY, Lv WH. Research survey of Semen Raphani. Journal of Practical Traditional Chinese Medicine 2005; 21: 254.
9. Zhang J, Li YB, Qian DW, Qian SH, Duan JA, Ding AW. Research survey on chemical composition and pharmacologic actions of Flos Chrysanthemi. Lishizhen Medicine and Materia Medica Research 2006; 17:1941-1942.
10. Fan QL, Zhao DJ, Liu FM. The advances in TCM research on hypertension. Research of Traditional Chinese Medicine 2002; 15: 47.
11. Lv GY, Chen SH, Lv HJ, Chen Y. Studies on anti-hypertension effect of Xueling. Journal of Zhejiang college of TCM 2003; 27: 47-49.
12. Ma Q, Ma J. Effect of Semen Raphani on anti-hypertension. Tradit Chin Med 1998; 39: 454.
13. Zhang YH, Chen SW, Zhou M, Deng YF. Experimental studies on anti-hypertension effect of Semen Raphani injection. JiLin Tradit Chin Med 1996; 41.
14. Zeng JF. Clinical study of Semen Raphani in the treatment of hyperlipidemia 38 cases in elder patients. Zhejiang Tradit Chin Med 1995; 30: 494.
15. Wang CX. Progress in research on chemical constituents of Flos Chrysanthemum. Chin Med Mater 2004; 27: 224-226.
16. Li XE, Guo BJ, Zeng Z. Experimental research of protein, oligosaccharide and anthraquinone glycoside of Semen Cassiae on anti-hypertension. Chinese Traditional and Herbal Drugs 2003; 34: 842-843.
17. Li W, Yang M, Wang Q, Fu P, Chen YL, Li GD. Experimental research of veratridine on anti-hypertension. Journal of Chinese Modern Traditional Chinese Medicine 2006; 4: 292-296.
18. Zheng H, Wang XJ. Research survey on pharmacological action of Pueraria Lobata. CJGMTCM 2006; 49-51.
19. Hou JJ, Han LW, Yang GE, Li QS. Research survey on chemical constituent and pharmacological activity of Folium Apocyni Veneti. Chinese Traditional and Herbal Drugs 2006; 37:7-9.
20. Luo LF, Wu WH, Ouyang DS. Anti-hypertensive component and mechanism of Eucommia Ulmoides. Chinese Traditional and Herbal Drugs 2006; 37: 150-152.
21. Chen SH, LV GY, Shi B, Li WL, Zhang LY. Studies on mechanism of anti-hypertension effect of extracts from Jueming compound. Chinese Traditional and Herbal Drugs 2004; 35: 180-182.
22. Liu LF, Wang YX, Zhang XY, Liu XF. Determination of sinapine in Semen Raphani. Chinese Traditional Patent Medicine 2002; 24: 52-54.
23. Xue M, Wang ZJ, San YJ, Zhou J. Microwave technique extraction and determination of total flavonoids and polysaccharides from Semen Raphani. Journal of Shihezi University (Natural Science) 2005; 23: 155-157.
24. Zhang J, Qian DW, Li YB, Yin ZQ. Research on chemical constituents from Flos Chrysanthemi. Nat Prod Res Dev 2006; 18: 71-73.
25. Dai M, Liu QY, Li DZ. Research of material bases on anti-febrile and hypertensive effects of Flos Chrysanthemi. Journal of Chinese Medicinal Materials 2001; 7: 505-506.