Original Article

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

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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 worldwide. 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 categories, including diuretic, beta-blocking agent, calcium antagonist, angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonist and alpha-receptor blocking agent.⁴ 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.⁵ 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.⁶⁷

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).
Materials and methods

Animals

Sprague-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°C) 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 vacuum. The LJE extract contained 1.5% of flavonoids. 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) with 0.9% saline. 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 pre-renal artery. After six weeks, systolic pressure was measured once weekly for 4 weeks in the second stage.

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; Fig 1). However, BP of RHR was only significantly lowered at 2 h in low LJE group (p<0.05) (Fig 2). 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
They have more or less anti-hypertensive effects.10-11

Studies from human and animal have indicated that 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 Raphani 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 flavonoids 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.

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References