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STUDIES ON RESIN PURIFICATION PROCESS OPTIMIZATION OF *EUCOMMIA ULMOIDES* OLIVER AND ITS ANTIHYPERTENSIVE EFFECT MECHANISM

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Abstract

Background: Through research it is found that the barks, stems, fruits and flowers of *Eucommia ulmoides* contain various compositions, such as lignin, cycloalkane and phenylpropanoids. The modern pharmacological research shows that *Eucommia ulmoides* has many functions, such as anti-aging, antitumor and bone cell proliferation. This paper studied the resin purification process optimization conditions and antihypertensive effect of *Eucommia ulmoides* Oliver.

Materials and Methods: The orthogonal experiment method was adopted to determine the purification effect of various macro-porous resins for eucommia ulmoides Oliver, and the SHR model was used to determine antihypertensive level of eucommia ulmoides Oliver.

Results: The optimal process of resin extraction and purification of *Eucommia ulmoides* Oliver was verified through the loading liquid concentration at 6 mg/ml, 20% ethanol used as the eluent; the flow rate control was 3.0, column volume per hour and the diameter-height ratio of adsorption column was 1:6; the extracts of *Eucommia ulmoides* Oliver can reduce SHR blood pressure, its antihypertensive effect is relatively stable and has no significant impact on HR, but the antihypertensive effect is lower than that of control medicine captopril. Also, the extracts of eucommia ulmoides Oliver can significantly increase the content of serum NO and reduce the content of ET.

Conclusion: The *Eucommia ulmoides* Oliver can relax blood vessels, reduce the peripheral resistance, reduce the returned blood volume, and eventually achieve the antihypertensive effect.

Key Words: *Eucommia ulmoides* Oliver; AB-8 Type Macroporous Resin; SHR

Introduction

The *Eucommia ulmoides* Oliver belongs to the dry bark of *Eucommia ulmoides* of Eucommiaceae plants, and was classified as a top grade medicine in the Compendium of Materia Medica. It has the effects of invigorating spleen-stomach and replenishing qi, strengthening bones and muscles, enhancing memory ability, prevents miscarriage, and ensures a refreshing and enduring hard work, after a long-term usage (Jiangsu, 1986). In recent years, scholars within and overseas, have conducted several researches on the chemical constituents of *Eucommia ulmoides*. Through research it is found that the barks, Oliver, stems, fruits and flowers of *Eucommia ulmoides* contain various chemical components, such as lignin, cycloalkane, phenylpropanoids, polysaccharide, flavonoids, amino acids and gutta-percha (Gonda et al., 1990; TomodoM et al., 1990; Cheng et al., 2002; Nakkamura et al., 1997). The modern pharmacological research shows that *Eucommia ulmoides* has many functions, such as anti-aging, anti-tumor, bone cell proliferation, and regulation of cardiovascular and immune systems (Wan et al., 2000; Xue et al., 1998; Cao et al., 2001; Luo et al., 2006). This experiment used different types of macro-porous resin to ensure an optimal extraction process of *Eucommia ulmoides* Oliver, at the same time, the anti-hypertensive effect of eucommia ulmoides Oliver extracts and its mechanism were studied, so as to lay the foundation for the in-depth development of *Eucommia ulmoides* Oliver.

Material and Methods

Experimental Animals

Male spontaneously hypertensive rats (SHR), purchased from Shanghai Institute of Hypertension, Shaghai.

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Reagent and Instrument

Captopril tablets manufactured by: Sino-American Bristol-Myers Squibb Pharmaceuticals Co., Ltd.; self-produced extracts of eucommia ulmoides Oliver, of which the concentration can be compounded according to the test requirements; Unitc-2100 ultraviolet visible-spectrophotometer manufactured by: Unico (Shanghai), Instrument Co., Ltd.; RE-52AA rotary evaporator manufactured by: Shanghai Yarong Biochemistry Instrument Factory; all reagents are analytically pure.

Resin Pre-treatment

The commercially available resin has been immersed with 95% ethanol for 18 hrs. After full swelling, it was packed into a column through wet process. 95% ethanol was washed until the effluent has no white turbidity by the addition of water, until the effluent and 95% ethanol were absorbed closely by ultraviolet scanning, then distilled water was used to wash until there was no ethanol flavor, for backup.

Preparation of Extracting Solution of Eucommia Ulmoides Oliver

Take 3 kg eucommia ulmoides Oliver shattered and dried. Take the powder of eucommia ulmoides Oliver extracted through 60% ethanol reflux extraction for three times, 2 hrs each, and the extraction temperature of 85°C. The extracts solutions are merged to recover the ethanol, and obtain the concentrated liquid. In application, appropriate water was used to dissolve the solution to the needed concentration.

Determination of Chlorogenic Acid Content

Take the extracts of *Eucommia ulmoides* Oliver according to Zhang Fengyun (Zhang et al., 1996), experimental method, and adopt the chromatographic separation UV-spectrophotometric method to determine the chlorogenic acid content.

Selection of Resin Type

The dynamic adsorption method was used to study the enrichment of different polarity resin on the extracts of *Eucommia ulmoides* Oliver. According to the physical and chemical properties of *Eucommia ulmoides* Oliver as well as adsorptive property of macro-porous resin, six kinds of resin such as X-5, D-101, HP-20, AB-8, ASD-7 and S-8 were selected for study. Take various types of resin about 10g, which are packed into a column through wet process after precision weighing. The extract solution of *Eucommia ulmoides* Oliver was added slowly into different resin columns. The control volume flow rate was 2.0 mL/min, used to determine the chlorogenic acid content of the extracts of *Eucommia ulmoides* Oliver in the ethanol elution parts, and to confirm the dynamic adsorptive property of various types of resin. The dynamic adsorptive results show that the adsorption quantity of AB-8 type macro-porous resin reached 36.73 mg/ml; for chlorogenic acid in the eucommia ulmoides Oliver, the adsorption rate and the elution rate reached 98.36% and 2.79% respectively. Compared with other five kinds of resin, AB-8 type macro-porous resin has good sorption-desorption capability, so it was finally selected for experiment.

Table 1: Comparison of Sorption-Desorption Capability of Resin for *Eucomia ulmoides* Oliver

Resin Type	Concentration of the Extracts of *Eucommia ulmoides* Oliver (mg/ml)	Adsorption Capacity of Chlorogenic Acid (mg/ml)	Adsorption Rate (%)	Analysis Rate(%)
X-5	3.25	3.49	10.76	1.86
D-101	3.25	4.58	12.14	1.73
HP-20	3.25	13.20	35.77	1.67
AB-8	3.25	36.73	98.36	2.79
ASD-7	3.25	26.84	72.69	2.34
S-8	3.25	28.39	74.58	1.24

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The experiment was carried out according to the L_9 (3^4), orthogonal design table, with the adsorption rate of chlorogenic acid as evaluation index, and the factor level design and test results are shown in Table 2, 3 and 4.

The intuitive analysis results show that the influencing sequence of factors was A > C > B > D. The results of variance analysis show that factor A was the main influencing factor, having significant difference, followed by factors C, B and D, having no significant difference. In combination with the practical situation of production, in line with the principle of energy saving, environmental protection, efficient extraction and purification, ultimately the optimal process of resin extraction and purification of eucommia ulmoides Oliver was selected as: A3B1C3D2, namely, the loading liquid concentration at 6 mg/ml, 20% ethanol was used as the eluent; the flow rate control was 3.0 column volume per hour and the diameter-height ratio of adsorption column 1:6.

Table 2: Factors Level Table of Orthogonal Experiment

Level	A Liquid Concentration (mg/ml)	B Ethanol Concentration (%)	C Flow Velocity (Bv/h)	Diameter-height Ratio of Adsorption Column
1	2	20	1.0	1：3
2	4	40	2.0	1：6
3	6	60	3.0	1：9

Table 3: Orthogonal Experimental Results of Resin Purification Process of Eucommia Ulmoides Oliver

Experiment No.	A	B	C	D	Adsorption Rate of Chlorogenic Acid (%)
1	1	1	1	1	57.84
2	1	2	2	2	59.21
3	1	3	3	3	68.43
4	2	1	2	3	62.55
5	2	2	3	1	67.98
6	2	3	1	2	58.12
7	3	1	3	2	74.68
8	3	2	1	3	68.91
9	3	3	2	1	73.56
K_1	61.827	65.023	61.623	65.073	
K_2	61.497	63.980	65.107	64.003	
K_3	72.383	66.703	68.977	66.630	
R	10.886	2.723	7.354	2.627	

Table 4: Variance Analysis Table

Factors	Sum of Deviation Squares	Degrees of Freedom	F Ratio	F Critical Value	Significance
Solution Concentration	230.072	2	21.981	19.000	*
Ethanol Concentration	11.327	2	1.082	19.000	
Flow Velocity	81.182	2	7.756	19.000	
Diameter-Height Ratio	10.467	2	1.000	19.000	
Error	10.47	2			

Notes: $F_{0.05}(2, 2) = 19.0$; $F_{0.01}(2, 2) = 99.0$.

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Effect of the Extracts of *Eucommia ulmoides* Oliver on SHR Blood Pressure

Experimental Animals and Reagent

50 Male clean-grade SHRs (spontaneous hypertension), of 12 weeks old, and (220±10g), body mass was used for the experiment after a week adaptive feeding. The self-produced Extracts of *Eucommia ulmoides* Oliver was compounded into 5, 10, and 15 mg/g according to the experimental requirements for backup.

Determination of Experimental Methods and Indexes

The SHR were divided into 5 groups, of 10, rats in each group, as follows: blank control group, low-mid-high dosage group of *Eucommia ulmoides* Oliver (5, 10 and 15 mg/g), and captopril group (10 mg/kg). Each group was dosed once daily, at 20 mL/kg each time, and continued for 3 weeks. The rat tail arterial blood pressure was measured in the waking state every week; the spectrophotometry used to determine the NO in the blood, and the radio-immuno-assay method was used to determine the content of plasma ET and Ang□.

From the experimental results, when compared with the blank control group, revealed that all experimental groups can reduce the diastolic and systolic blood pressure of SHR to varying degrees after 3 weeks, with a significant difference. Among them, the high dose group of *Eucommia ulmoides* Oliver (15 mg/g), has diastolic and systolic blood pressure of 116.1 and 140.2 mmgHg respectively after the 3-week dosing, showing certain dose-effect relationship.

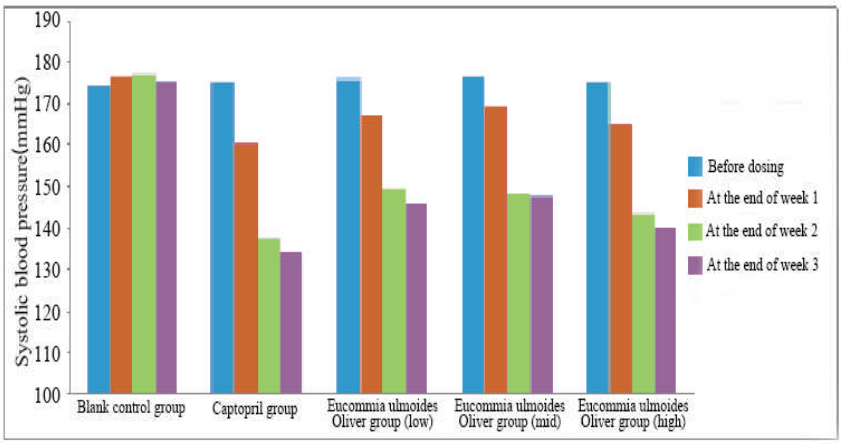

Figure 1: Graph for Changes of SHR Blood Pressure (Systolic Pressure) in Each Experimental Group

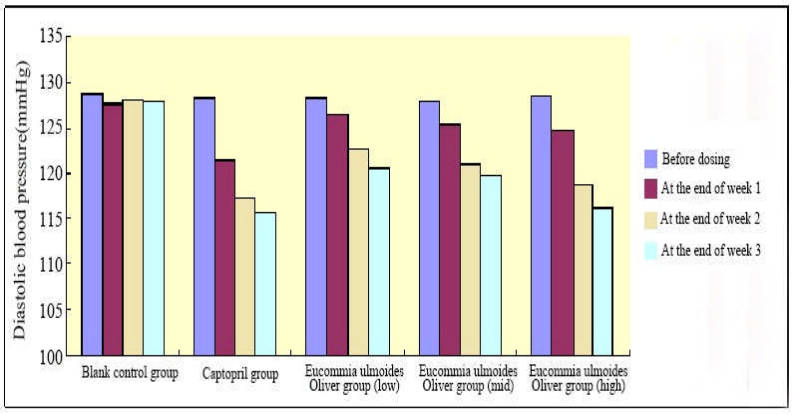

Figure 2: Graph for Changes of SHR Blood Pressure (Diastolic Pressure) in Each Experimental Group

Influence of *Eucommia ulmoides* Oliver on the contents of SHR serum ET and NO

Each dose group of *Eucommia ulmoides* Oliver can significantly increase the content of serum NO in rats, and reduce the content of serum ET, as shown in Fig. 3 and 4.

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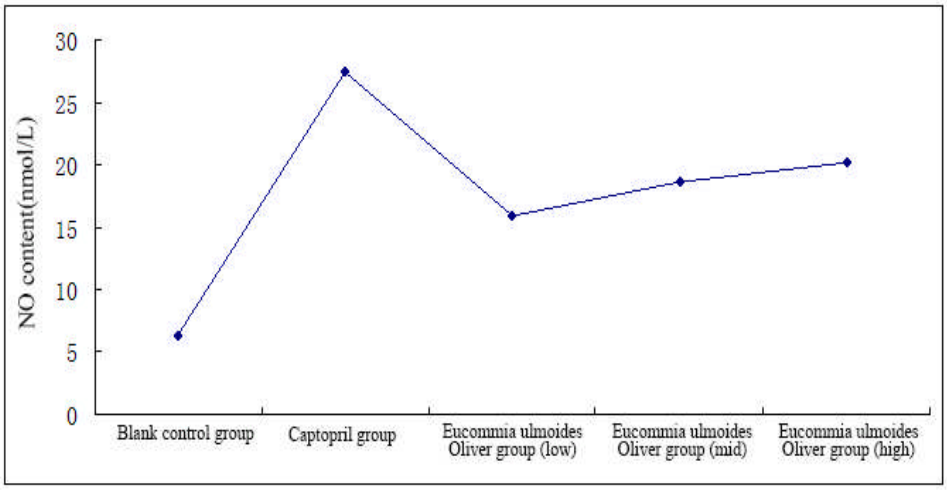

Figure 3: Graph for Changes of SHR serum NO in Each Experimental Group

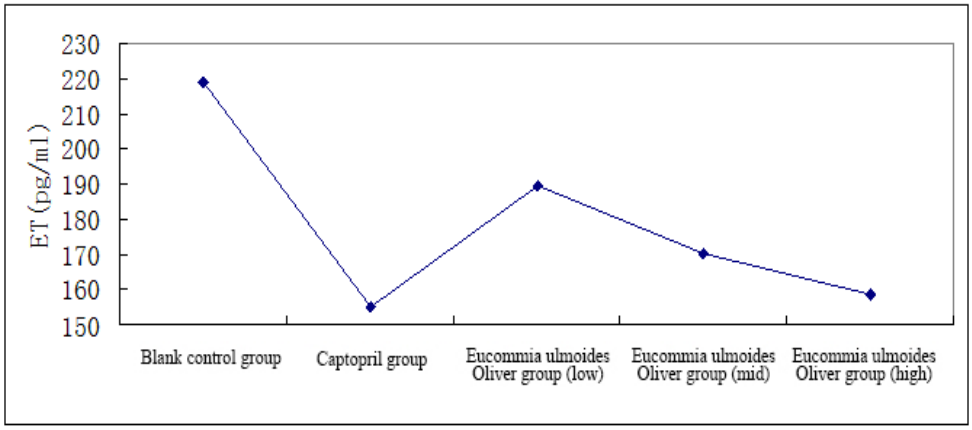

Figure 4: Graph for Changes of SHR serum ET in Each Experimental Group

Discussion

The etiology and pathogenesis of primary hypertension are unknown yet. At present, the Page Mosaic theory is relatively comprehensive, arguing the hypertension results from the interaction of many factors, which includes: 1. Genetic factors. Studies have shown that about 75% of patients have genetic qualities. There is a kind of hormone in their serum, inhibiting Na+/K+－ATP enzyme activity, reducing sodium potassium pump function, resulting increase of concentration of intracellular Na+ and Ca2+, strengthening SMC contraction on the arterial wall, increasing the density of adrenergic receptor, intensified vascular reactivity, and the elevation of blood pressure. 2. Dietary electrolyte. The diet of high salt and low potassium is susceptible to the hypertension 3. Socio-psychological stress. The social psychological stress can change the balance of hormones in the body, thus affecting all metabolic processes. 4. Kidney factor. The interstitial cells of kidney medulla secrete anti-hypertensive substances such as prostaglandin imbalance, and the sodium dysfunction may be associated with the pathogenesis of hypertension. 5. Neuro-endocrine factor. The increase of fiber excitability of sympathetic nerve of the arteriole remains an important neural factor for the pathogenesis of this disease (Cai et al., 2004).

However, Schiffrin (Schiffrin, 1995), research proves that the disequilibrium of NO and ET ratio is one of important pathogenesis of the hypertension, especially for primary hypertension. In 1980, Furchgatl found that the relaxing function of vascular smooth muscle of acetylcholine (Ach), depends on the integrity of endothelial cells, and proposed that vascular endothelial cell can produce a kind of factor prompting vasodilatation, EDRF (endothelium derived relaxing factor). It was proved that its main ingredient is nitric oxide (NO). ET is a kind of bioactive peptides secreted by vascular endothelial cells, one of the known strongest vaso-excitor materials. The NO and ET jointly maintain the balance of structure and functional state of vascular endothelial cells in the body.

The experiment proves that the extracts of *Eucommia ulmoides* Oliver can reduce SHR blood pressure, its anti-hypertensive effect was

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relatively stable and has no significant impact on HR, but the anti-hypertensive effect was lower than that of control medicine, captopril. At the same time, the extracts of eucommia ulmoides Oliver can significantly increase the content of serum NO and reduce the content of ET, so as to relax blood vessels, reduce the peripheral resistance, reduce the returned blood volume, and eventually achieve the anti-hypertensive effect.

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