

Influence of the mixture of *Epimedii Herba* and *Ginkgo Folium* extracts on the coronary flow of isolated hearts in rats

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ABSTRACT

Background: In clinical practice, *Epimedii Herba* and *Ginkgo Folium* preparations are widely used in treatment of diseases such as coronary heart disease (angina) in China. However, there are no studies on the two-drug combination. **Objective:** To explore the effect of the mixture of the *Epimedii Herba* extract (EE) and *Ginkgo Folium* extract (GE) on coronary flow of isolated hearts in rats. **Materials and Methods:** EE and GE were prepared by reflux in alcohol, and processed with HPD-100 macro-reticular resins; icariin from EE and total bilobalides from GE were determined by high performance liquid chromatography (HPLC). Fifty male Sprague-Dawley (SD) mice were subdivided into five groups (10 rats each): Normal control group (NC), EE – 10 mg group, GE – 10 mg group, EE – 5 mg + GE – 5 mg group, and EE – 10 mg + GE – 10 mg group. Isolated hearts uniform pressure perfusion was proceeded with Langendorff system. **Results:** The content of icariin in EE was 20.8%. The total content including four kinds of bilobalides (ginkolide A–C and bilobalide) in GE was 8.6%. The coronary flow in the NC group remained stable before and after treatment, and the coronaroy flow in the EE, GE, EE + GE groups was increased and the relative magnitude of heightening was 25.0–33.3%, and the coronary flow in EE + GE was significantly different from that in the single EE or GE group. **Conclusion:** EE or GE itself can heighten coronary flow of isolated hearts in rats. The activity of the mixture including EE and GE is better than that of single EE or GE, and the activity becomes larger when the dosage is doubled, and is related with dosage.

Key words: Bilobalide, Coronary flow, icariin, macro-reticular resins, traditional Chinese medicine

INTRODUCTION

Epimedium brevicornum Maxim. is a herb belonging to genus *Epimedium* of the family *Berberidaceae*. In China, there exist about five species belonging to the genus *Epimedium*, including *E. sagittatum* (Sieb. et Zucc.) Maxim., *E. pubescens* Maxim., *E. koreanum* Nakai, and *E. wushanense* T.S. Ying. In China, all of them have a history of thousands of years of combating different diseases, such as erectile dysfunction, fatigue, kidney disorders, joint pain, and are considered as hepatoprotective agent.^[1] These plants are known by their popular names as Yin Yang Huo.

Various well known and new chemical constituents were

isolated and characterized in *Epimedium* species, including flavonoids: cariin [Figure 1], epimedokoreanin A–D, chrysoeriol, quercetin, apigenin, luteolin, kaempferol, epimedin A–C, sagittasine A–C, ikarisoside A, icaricid II, yinyanghuo A–E, and baohuoside. Icariin was reported to be one of the main active components.^[2–4]

Icariin was found to increase the coronary blood flow of the isolated rat heart significantly, and attenuate cardiac contractility and effectively prevent increase in the whole blood cardiac contractility, plasma viscosity, packed erythrocyte volume, and fibrinogen.

Ginkgo biloba is an arbor belonging to Gymnospermae. In China, *Ginkgo biloba* leaves and fruits are used, especially, *Ginkgo Folium* is a frequently used drug in treating cerebrovascular disease. Terpene lactones include ginkgolide A–C [Figure 2] and bilobalid [Figure 3] in *Ginkgo Folium* has the functions of eliminating free radicals, anti-platelet-

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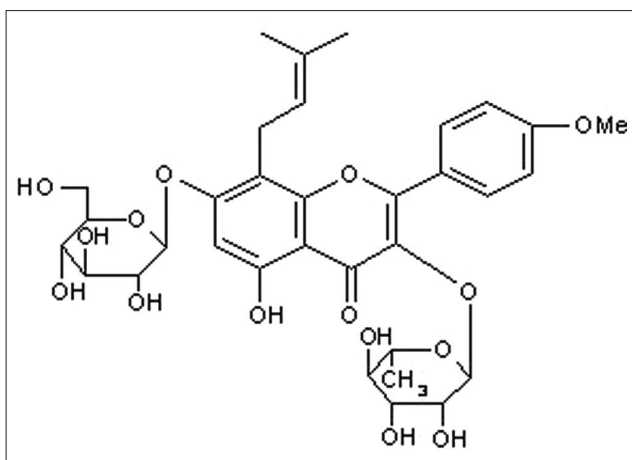


Figure 1: Structure of icariin

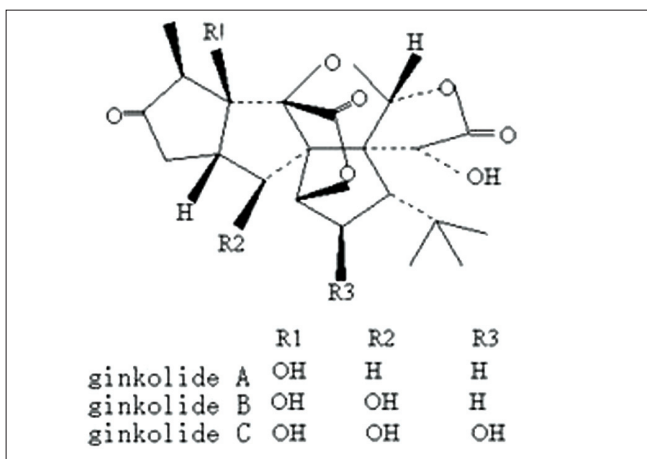


Figure 2: Structure of ginkgolide A–C

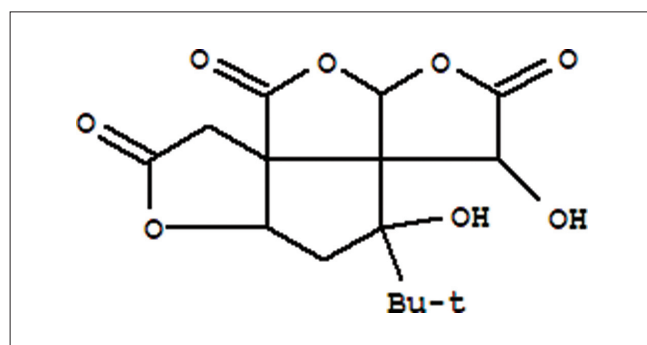


Figure 3: Structure of bilobalide

activating factor, controlling the activation of white cells, protecting vascular endothelial cells,^[5] relaxing blood vessel, anti-oxidation, improving learning and memory, and treating Alzheimer's disease.^[6]

In clinical practice, *Epimedii Herba* and *Ginkgo Folium* preparations are widely used in diseases such as coronary

heart disease (angina) in China. However, there is no studies on two-drug combination. In this paper, the mixture of EE and GE in improving coronary flow was designed to be evaluated and compared with EE or GE.

MATERIALS AND METHODS

Plant Materials

Both *Epimedii Herba* and *Ginkgo Folium* were collected from Shenyang North-East Drug Store Chain, Shenyang, China, and were identified as *Epimedium brevicornum* Maxim. and *Ginkgo biloba* L. by Prof. Changji Yuan.

Preparation of Epimedium herb extract (EE) and HPLC analyses

Epimedii Herba was ground into powder and refluxed in 70% alcohol (V/V) (100 g/800 mL, 1.5 h ×2). The filtrate was concentrated under reduced pressure, and processed with HPD-100 macro-reticular resins (purchased from Cangzhou Baoen Chemical Industry Company). The resulting powder was used. Content of icariin in the powder was determined by high performance liquid chromatography (HPLC), using Diamonsil C₁₈ (150 mm × 4.6 mm, 5 μm) column at 30 °C. The mobile phase was acetonitrile–water (V:V = 30:70) and the detection wavelength was set at 270 nm.

Preparation of Ginkgo Folium extract (EE) and HPLC analyses

Ginkgo Folium extract was ground to a powder and refluxed in 50% alcohol (V/V) (100 g/800 mL, 1.5 h ×2). The filtrate was concentrated under reduced pressure, and processed with HPD-100 macro-reticular resins (purchased from Cangzhou Baoen Chemical Industry Company). The resulting powder was used. The total bilobalides in the powder were determined by HPLC-ELSD, using Diamonsil C18 (150 mm × 4.6 mm, 5 μm) column at 30°C. The mobile phase was methanol-tetrahydrofuran–water (V:V:V = 25:8:67).

Influence of EE and GE on coronary flow of isolated hearts in rats

Fifty male Sprague–Dawley (SD) mice were supplied by the Experimental Animal Centre, Liaoning University of Traditional Chinese Medicine (SYXK (Liao) 2010-0032). The animals were housed in an air-conditioned room at an ambient temperature of 24°C and 50–65% relative humidity with automatic 12-h light/12-h dark cycles. The mice were randomly subdivided into four groups (10 rats each): Normal control group (NC), EE – 10 mg group, GE – 10 mg group, EE – 5 mg + GE – 5 mg group, and EE – 10 mg + GE – 10 mg group. Isolated hearts uniform pressure perfusion was proceeded with Langendorff system. The changes of coronary flow were

recorded before and after treatment 10 min mean of coronary flow before administration (\bar{F}) and maximum of coronary flow after administration (F_{max}) was found, and then the maximum increase rate (%) [$(F_{max} - \bar{F})/\bar{F}$] was calculated. All results were expressed as mean \pm SE. Data were analyzed using one-way analysis of variance (ANOVA) followed by Student's *t*-test using SPSS computer software version 16.0. The level of significance was fixed at 0.05.

RESULTS

Content of icariin from Epimedii Herba extract (EE)

A linear relationship between icariin and the peak area was established in the range of 0.0987 μg –0.8883 μg ($r = 0.9997$), and the mean recovery was 99.8% (RSD = 0.9%). The content of icariin in EE was 20.8% [Figures 4 and 5].

Content of the total bilobalides from Ginkgo Folium extract (GE)

A linear relationship between ginkgolide A,B,C, bilobalide and logarithm of peak area was respectively established in the range of 0.7008 μg –4.9056 μg ($r = 0.9999$), 0.3976 μg –2.7832 μg ($r = 0.9997$), 0.4008 μg –2.8056 μg ($r = 0.9999$), 0.6048 μg –4.2336 μg ($r = 0.9999$), and the mean recovery was, respectively, 98.1% (RSD = 0.9%), 97.9% (RSD = 1.7%), 96.3% (RSD = 1.2%), and 98.7% (RSD = 2.0%). The total content of four kinds of bilobalides (ginkgolide A–C and bilobalide) in GE was 8.6%. [Figures 6 and 7].

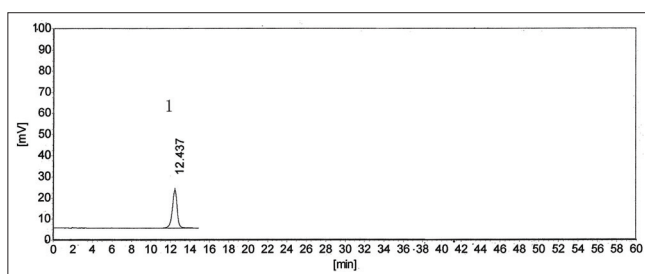


Figure 4: HPLC Chromatograph of icariin

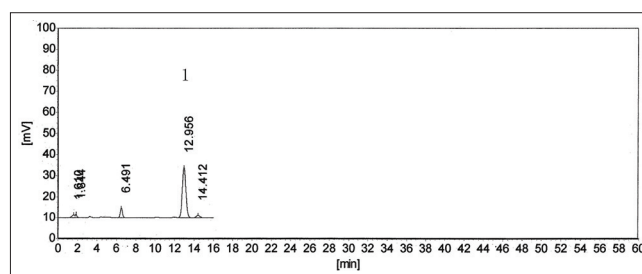


Figure 5: HPLC Chromatograph of *Epimedium Herb* extract

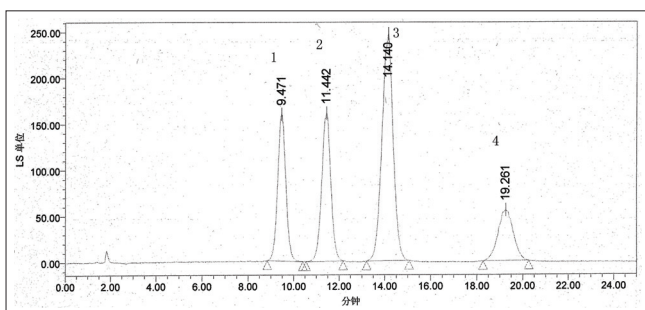


Figure 6: HPLC Chromatograph of ginkgolide A–C and bilobalide (1- ginkgolide C; 2- bilobalide; 3- ginkgolide A; 4- ginkgolide B)

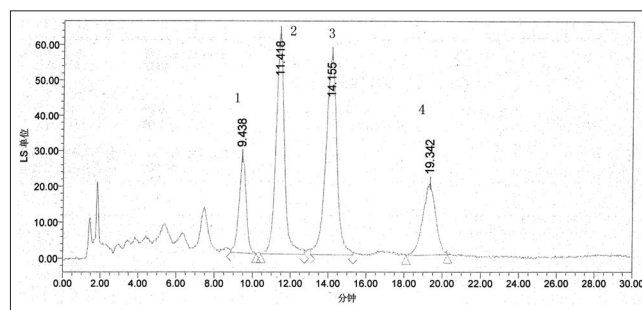


Figure 7: HPLC Chromatograph of *Ginkgo Folium* extract (1- ginkgolide C; 2- bilobalide; 3- ginkgolide A; 4- ginkgolide B)

Coronary flow of isolated hearts in rats

The coronary flow in NC group kept stabilization before and after treatment, that in EE, GE, EE + GE groups was increased, and the relative magnitude of heightening was 25.0–33.3%, and the coronary flow in EE + GE was significantly different from that in single EE or GE group ($P < 0.05$) [Table 1].

Conclusion and discussion

When *Epimedii Herba* extract (EE) or *Ginkgo Folium* extract (GE) was used alone, both can heighten coronary flow of isolated hearts in rats. The activity of the mixture of both EE and GE is better than that of just EE or GE alone, and the activity becomes larger when the dosage is doubled, and it is related with dosage.

Both of *Epimedii Herba* and *Ginkgo Folium* are have had a long history in treating diseases of circulatory system in China. *Xianlingpi Granule*, a preparation of *Epimedii Herba* in China, is a drug in treating coronary artery disease (CAD), hypertension, chest distress, and rheumatism.^[7] *Epimedii Herba* also is used in coronary artery disease (CAD) with the other traditional Chinese herb, for example, *Yanghuo Sanqi Pian*, a compound preparation with *notoginseng radix*, is used in coronary artery disease and angina pectoris.^[8] *Ginkgo Folium* has been processed in the form of tablet, soft capsule, and drop pill, and has also been developed into more compound drugs. The main indications are thoracic obstruction, apoplexy, hemiplegia, and so forth.^[9]

Table 1: Influence of EE and GE on coronary flow of isolated hearts in rats

Group	Dose (mg)	Before administration (ml/min)	After administration (ml/min)	Maximum increase rate (%)
NC	—	7.3±1.3	7.4±1.3	1.4±2.6
EE	10	7.1±1.0	8.9±1.9 ^a	25.3±20.2 ^a
GE	10	7.2±1.2	9.1±1.9 ^a	25.0±13.5 ^a
EE+GE	5+5	7.1±1.0	9.2±1.7 ^{abc}	29.6±15.0 ^{abc}
EE+GE	10+10	7.2±1.0	9.6±1.3 ^{abc}	33.3±10.4 ^{abc}

All values are expressed as mean ± SE. ^a*P* < 0.05, when compared with normal control rats. ^b*P* < 0.05, when compared with EE rats. ^c*P* < 0.05, when compared with GE rats.

Epimedii Herba has been used alone or as a compound in treatment of circulatory system diseases, but there is no other drug including Ginkgo Folium. In this study, the activity of the mixture including EE and GE is better than that of single EE or GE. It is significant that the mixture maybe be developed into new drug in treating circulatory system diseases.

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