Effect of arctiin on hemorheology of experimental rats with blood stasis ayndrone

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ABSTRACT: OBJECTIVE To study influence of arctiin from seeds of *Arctium lappa* on hemorheology of experimental rats with the blood stasis syndrone. **METHODS** The blood hemorheology parameters, Fib, aPTT and PT of experimental rats with the blood stasis syndrone were evaluated using semi-automatic biochemical analysis. **RESULTS** Arctiin obviously decreased their high shear, middle shear, low shear, the blood viscosity, red blood cell aggregation index, red blood cell rigidity index and reductive viscosity. It also significantly prolonged the time of aPTT and PT and lowed the Fib concentration. **CONCLUSION** Arctiin apparently ameliorated the blood rheology abnormality and enhanced anti-coagulation effect on experimental rats with the blood stasis.

KEY WORDS: *Arctium lappa* L; arctiin; hemorheology; anti-coagulation

牛蒡子苷对血瘀大鼠血液流变学的影响

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摘要:目的 研究牛蒡子苷对血瘀大鼠血液流变学和血栓凝血因子的影响。方法 测定口服不同剂量的牛蒡子苷后对血瘀证大鼠流变学、Fib、aPTT和 PT的影响。结果 牛蒡子苷可以明显降低急性血瘀大鼠全血黏度,红细胞聚集指数,红细胞刚性指数和还原黏度,明显延长血瘀大鼠 PT和 aPTT时间,降低 Fib浓度。结论 牛蒡子苷能显著改善血瘀证大鼠血液流变学和抗凝血作用。

关键词:牛蒡;牛蒡子苷;血液流变学;抗凝作用
中图分类号:R285.5 文献标识码:A 文章编号:1007-7693(2006)06-0443-04

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Arctium lappa L., a biennal herb plant in Compositae, distributes all over China[1]. Its seeds has been used as chinese traditional medicine for treatment of the cold, mumps, measles, and throat sore[1]. Arctiin is one of the most important lignans isolated from seeds of *Arctium lappa*. Biological activity of arctiin on the treatment of the acute progress nephritis, chronic glomerulonephritis and nephritis was reported[2]. Arctiin also has the function of the enhancement of immunological activity[3], PAF antagonist[4-5], anti-inflammatory[3], Ca^{2+} antagonist and anti-hypertension[6]. In the present investigation, we reported effect of arctiin on the main hemorheological indexes of experimental rat with the blood stasis syndrone and the invigoration of blood circulation and anti-coagulation.

1 Materials and methods

1.1 Instrumentation

UV spectra were obtained in MeOH. IR spectra and FAB mass spectra were respectively acquired on Shimadzu FTIR-8700 using KBr as matrix and Zabspece mass spectrometer using glycerol as matrix. NMR spectra were recorded in CD_3OD using Varian Unity INVOA 600MHz NMR spectrometer. HPLC separation was performed in Delta 600 prep-HPLC system with 2690-photo-Diode-Array detector. Shimadzu C_{18} preparative HPLC column (10μm particle size, 250mm × 50mm) was used. S500+ BASIC Semi-automatic biochemical analysis (SECOMAM Company, France). FASCO-3010B Automatic Blood Rheometer (Chongqing University Veiduo Institute of Bioengineering). CD2001 Double Channel Blood coagulation analysis (Sichuan Maysun Industrial Company).

1.2 Chemicals

The standard arctiin compound was purchased from National Institute for the Control of Pharmaceutical and Biological Products of China, No. 0819-200203. HPLC grade acetonitril was obtained from Fisher Scientific (Fair Lawn, NJ, USA). Water for the HPLC mobile phase was purified in a mili-Q system (Milipore, Bedfold, MA). Adrenalin hydrochloride was obtained from Southwest pharmaceuticals manufactory Co. Ltd. (No. 030404). PT (Prothrombin time, No. 090303A), aPTT (activated partial thromboplatin time, No. 070302A) were purchased from BIOLABO Company in France.

1.3 Plant material

Seeds of *A. lappa* L. were purchased from an authentic Chongqing Company of Chinese Traditional Medica. A specimen has been deposited in the herbarium of Pharmacognosy department, Chongqing Academy of Chinese Traditional Medica.

1.4 Isolation and purity

Ground seeds (4kg) of *A. lappa* were Soxhelt-extracted successively with hexane, then with EtOH. The EtOH extract was evaporated to dryness under reduce pressure. The residue was chromatographed by polyamide column with MeOH. The fraction containing arctiin was concentrated and refrigerated. A white participtate was obtained and further puried by use of Prep-HPLC. The mobile phase is acetonitrile and water (35∶65) at a flow rate of 10mL/min. The 10g of white powder was yielded and identified as arctiin (see Fig 1) compared with UV, IR, MS and NMR data of standard compound.

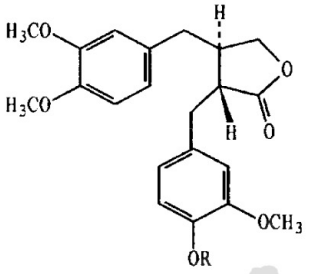

Fig 1 Structure of compound

图 1 化合物结构

R = Glucosyl

1.5 Animals

Wister rats [(330±5) g male, certificate No. SCXK (YU) 20020004] were supplied by Laboratory Animal Center of Chongqing Academy of Chinese Materia Medica. They were housed under standard animal laboratory condition. All experiments was conducted according to the NIH Guide for the Care and Use of Laboratory Animal (NIH, Publication No. 80-23, revised 1996). The experiment procedures were approved by the local committee on Animal Care and Use.

1.6 Experimental rat model and treatment

Seventy wister big rats were divided into 7 groups at the start of the experiments. The rats of the first group and second group were taken as control and model group respectively. Each rat of control group and model group was orally administered 2mL of distilled water for 7 days. The rats of other 5 groups were orally administered arctiin at doses of 15, 30, 60, 90 and 150mg/(kg·d) respectively. Rats of model group and treatment group were made into the blood stasis syndrone model on 8th day referring to pPharmacological experiments for Chinese Traditional Medicine[7-11]. After 24 h, the blood samples were collected from belly aorta. 3ml blood of them injected into heparinized tube rapidly and shaken gently for measuring blood viscosity, plasma viscosity, red blood cell aggregation index, red blood rigidity index, red blood ability out of shape and hematocrit etc. 2.0ml of the blood samples injected into tube with citric acid sodium (1∶9 V/V) and shaken gently for measuring PT and aPTT.

1.7 Data analysis

Results were expressed as $\bar{x} \pm s$, and statistical analysis was carried out using *t*-test.

2 Results

2.1 Effects of arctiin on hemorheology with blood stasis

As shown in table 1, arctiin at doses of 30mg/(kg·d), 60mg/(kg·d), 90mg/(kg·d) and 150mg/(kg·d) respectively had significant improvement in whole blood high shear, middle shear, low shear, plasma viscosity, reduced viscosity, red blood cell aggregation index and rigidity index compared with model group. The best doses arrange is 30 ~ 60mg/kg.

Red blood cell rigidity indexis very important parameter used to measure the red blood cell abnormality. It is one of the main reason to cause blood stasis in blood cycle-system, especially blood micro-cycle. In the present study, we found arctiin had the function to prevent red blood cell from abnormality. The results indicated arctiin had a great role in improving blood stasis.

Tab 1 Effect of arctiin on hemorheology of experimented rats with blood stasis($n=5, \bar{x}\pm s$)

表 1 牛蒡子苷对血瘀大鼠流变学影响($n=5, \bar{x}\pm s$)

Group	η_H	η_M	η_L	ηp	RAI	RV	HCT	RI7
control	4.10±0.08	5.12±0.51	10.21±0.89	1.07±0.11	9.77±1.39	8.98±1.59	0.40±0.08	7.95±1.23
model	11.31±1.24**	12.77±2.05**	18.04±2.13**	1.14±0.10	5.87±1.26**	20.86±1.26**	0.54±0.07	17.96±1.59**
arctiin (15mg/kg)	11.07±1.62	12.55±1.03	17.03±1.21	1.10±0.10	15.05±1.23	21.89±0.13	0.40±0.06	18.92±1.82
arctiin (30mg/kg)	6.40±1.98△△	8.11±1.22△△	13.11±1.32△△	1.01±0.10	11.98±1.43△△	13.17±1.53△△	0.41±0.07	11.87±1.49△△
arctiin (60mg/kg)	6.16±1.73△△	8.03±1.32△△	12.96±1.26△△	1.11±0.09	12.18±1.27△△	12.95±1.39△△	0.46±0.07	12.14±1.41△△
arctiin (90mg/kg)	7.54±1.89△	8.67±1.32△△	14.01±1.39△△	1.12±0.08	12.75±1.53△	12.54±1.19△△	0.43±0.06	11.82±1.54△△
arctiin (150mg/kg)	8.21±1.98△	9.48±1.63△	15.02±1.81△	1.10±0.09	13.22±1.54△	13.04±1.34△△	0.44±0.05	13.54±1.78△

* $P<0.05$, ** $P<0.01$ vs control group; △ $P<0.05$, △△ $P<0.01$ vs model group. η_H = high shear; η_M = middle shear; η_L = low shear; ηp = plasma viscosity HCT = hematocrit; RV = reduced viscosity; RAI = red blood cell aggregation index; RI = red blood cell rigidity index.

2.2 Anti-coagulation effect of arctiin

Two main parameters, aPTT and PT, which reflect the function of blood coagulation system, were determined to observe anti-coagulation effect of arctiin. The aPTT was mainly reflected the function of endogenic grumb system, which especially had strong relationship with coagulation factors such as Ⅱa、Ⅴ、Ⅶ and ⅩⅡ. The PT was mainly reflected the function of exogenic grume system. As shown in Table 2, when arctiin was orally administered at doses of 30mg/kg and 60mg/kg respectively, the index PT and aPTT were prolonged in comparison with model group($P<0.01$), and the content of Fib was significantly lowed. These results showed that arctiin invigorated blood circulation and improved high grume state of experimental rats with the blood stasis syndrone.

Tab 2 Effect of arctiin on coagulation factors of experimented rats with blood stasis($n=5, \bar{x}\pm s$)

表 2 牛蒡子苷对血瘀大鼠凝血因子的影响($n=5, \bar{x}\pm s$)

Group	PT(s)	aPTT(s)	Fib(g/L^{-1})
control	24.8±1.8	35.7±1.4	2.55±0.39
model	13.9±1.3**	22.1±2.0**	4.02±0.45**
arctiin(15mg/kg)	15.9±1.2	24.8±1.1	3.98±0.56
arctiin(30mg/kg)	25.1±0.11△△	46.5±2.2△△	2.49±0.41△△
arctiin(60mg/kg)	21.7±1.22△△	34.9±2.2△△	2.69±038△△
arctiin(90mg/kg)	18.6±1.73	24.6±1.4	3.49±0.56
arctiin(150mg/kg)	16.2±1.42	22.9±1.8	3.78±0.58

* $P<0.05$, ** $P<0.01$ vs control group; △ $P<0.05$, △△ $P<0.01$ vs model group

3 Discussion

Seeds of *Arctium lappa* has been used as Chinese traditional medicine for treatment of the cold, mumps, measles and throat sore[1]. Lignans isolated from seeds of *Arctium lappa* are the effective part. Arctiin isolated from the lignans is main effective component. In this present investigation, we found that arctiin had significant effect on whole blood high shear, middle shear, low shear, plasma viscosity, reduced viscosity, red blood cell aggregation index and rigidity index. Arctiin also obviously prolonged indexes of prothrombin and activated partial thromboplatin, and lowed the content of Fib. These results indicated that arctiin invigorate and improve blood circulation. Lwakami et al (1992) and Kazuo et al (1986) reported PAF antagonist, Ca^{2+} antagonist and anti-hypertension. Furthermore, the present studies pharmacologically shown that arctiin will play an important role in treating vascular dieases.

4 Acknowledgements

The project was supported by Visiting Scholar Foundation of Key Lab. In University. We thank associate Prof. Ran (Chongqing Academy of Chinese Materia Medica) for the valuable assistance.

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收稿日期: 2005-02-21