· 药理实验与临床观察 ·

Effect of protein and anthraquinone glucosides from *Semen Cassiae* on hemorrheology of hyperlipidemic rats

LI Xu-e, YANG Shui-yun*, ZHAO Wen-ming*, GUO Bao-jiang*

(Institute of Biological Technology of South China Normal University, Guangzhou Guangdong, 510631, China)

Abstract **Object** To study the effect of protein and anthraquinone glucosides from *Semen Cassiae* on hemorrheology of hyperlipidemic rats. **Methods** Rat hyperlipidemic model was prepared by ig lipid emulsion. After oral administration of the protein 0.25 or 1 mg/kg, the anthraquinone glucosides 5 or 20 mg/kg, and the protein 0.25 mg/kg plus the anthraquinone glucosides 5 mg/kg, the blood viscosity of high shear rate (ηH), blood viscosity of low shear rate (ηL), reduced viscosity (RV), plasma viscosity (ηP), platelet adhesion ratio (PAR), hematocrit (HCT), and erythrocyte sedimentation rate (ESR) of the hyperlipidemic rats were determined to assess the effect of the said drugs on these hemorrheologic parameters. **Results** The protein 1 mg/kg and the anthraquinone glucosides 20 mg/kg, as well as the protein 0.25 mg/kg plus the anthraquinone glucosides 5 mg/kg, could reduce the elevated ηH, ηL, RV, ηP, and PAR of hyperlipidemic rats ($P < 0.05$). **Conclusion** Protein and anthraquinone glucosides from *Semen Cassiae* could decrease ηH, ηL, RV, ηP, and PAR of hyperlipidemic rats.

Key words *Semen Cassiae*; protein; anthraquinone glucosides; hemorrheology

决明子蛋白质和蒽醌苷对高脂血症大鼠血液流变学的影响

李续娥,杨水云*,赵文明*,郭宝江

(华南师范大学生物技术研究所,广东 广州 510631)

摘 要:目的 探讨决明子蛋白质和蒽醌苷对高脂血症大鼠血液流变学的影响。方法 将大鼠制成高脂血症模型。测定决明子蛋白质和蒽醌苷的小、大剂量,以及两者小剂量合用后对高脂血症大鼠的全血粘度(高切变率下的ηH,低切变率下的ηL),全血还原粘度(RV),血浆粘度(ηP),血小板粘附率(PAR),红细胞压积(HCT)及血沉(ESR)的影响。结果 决明子蛋白质大剂量(1 mg/kg),蒽醌苷大剂量(20 mg/kg)以及两者的小剂量(蛋白质 0.25 mg/kg,蒽醌苷 5 mg/kg)合用,均可使高脂血症大鼠的ηH,ηL,RV,ηP和 PAR显著降低($P < 0.05$)。结论 决明子蛋白质和蒽醌苷皆可降低高脂血症大鼠的ηH,ηL,RV,ηP和 PAR

关键词:决明子;蛋白质;蒽醌苷;血液流变学

中图分类号:R286.2 文献标识码:A 文章编号:0253-2670(2002)05-0429-03

Semen Cassiae from *Cassia obtusifolia* L. or *C. tora* L. was known to be of therapeutic value in reducing fever, improving eyesight, nourishing intestine and relaxing bowel. Over recent years, it has been found that this drug is also effective to decrease blood pressure and serum lipid[1~2], and therefore attracted much public interest. In our previous study, protein and anthraquinone glucosides from *Semen Cassiae* could lower the elevated serum total cholesterol, triglyceride, and low den-

* **Received Date** 2001-10-06

Fund Item Project supported by Natural Science Foundation of Shanxi Province (No. 99SM74).

Resume of author LI Xu-e, female, associate Professor, Ph. D. studied at Xi'an University of Medical Science and Xi'an Jiaotong University respectively; worked at Tianjin Institute of Pharmaceutical Research, Xingping City Institute of Medicines and Chemical Reagents Inspection, and School of Chemical Engineering of Xi'an Jiaotong University respectively. Being a post-doctor student at Institute of Biological Technology of South China Normal University at present. Tel: 020-85211420

* Xi'an Jiaotong University

sity lipoprotein of hyperlipidemic rat ($P < 0.05$, $P < 0.01$)[3]. Hyperlipidemia could result in hemorrheologic abnormality which acted as a promoting factor, cause and worsen the pathological change and clinical symptoms of hyperlipidemia[4]. Therefore, the aim of this study was to investigate the effect of protein and anthraquinone glucosides from *Semen Cassiae* on hemorrheology of hyperlipidemic rat.

1 Materials and Methods

1.1 Preparations of the protein and the anthraquinone glucosides: *Semen Cassiae*, the dried and mature seed of *C. obtusifolia* L. was collected from Anhui Province, PRC in autumn. Its identity was authenticated by Prof. LI Yang-li, Department of Pharmacy, Xi'an Jiaotong University. The protein was extracted with buffer (KH_2PO_4 / NaOH, pH 8.0), then the extract was separated with ammonium sulfate fractionation. The protein, precipitated at 40% ~ 50% saturation of ammonium sulfate, was collected by centrifuge and dialyzed with double distilled water for 48 hours. The dialyzed protein was again centrifuged, and the centrifugate was purified on Sephadex G-75 column in double distilled water. The eluate that had an absorption peak at 280 nm was collected and lyophilized to obtain the purified protein with a content of 88.7%. Anthraquinone glucosides and anthraquinones were extracted from *Semen Cassiae* with 90% ethanol, and the anthraquinones were separated with chloroform. Anthraquinone glucosides were precipitated with Pb^{2+}, and then Pb^{2+} was removed by hydrogen sulfide and finally recrystallized. The content of anthraquinone glucosides was 94.8%.

1.2 Reagents: Duoxikang (mixture of eicosapentaenoic acid and docosahexaenoic acid) was produced by Zhejiang Hailisheng Pharmaceutical Factory (No. 980417). Tiamazole was a product of Guangzhou Shiqiao Pharmaceutical Factory (No. 980120).

1.3 Experimental rat model and treatment: SD rats, 200~ 240 g, were provided by Animal Experimental Center, Xi'an Jiaotong University (Grade Ⅱ, Certificate No. SX-05). Rats were divided into eight groups (Table 1). Every morning, rats in group Ⅰ (control) were given solvent which consisted of 10% polyoxyethylene (20) sorbitan monooleate + 10% glycerine + 80% distilled water, and the rats in other seven groups were given lipid emulsion which consisted of 20% lard + 10% cholesterol + 2% sodium cholate + 0.1% thiamazole + 10% polyoxyethylene (20) sorbitan monooleate + 10% glycerine + 47.9% distilled water. Every afternoon rats in group Ⅲ to Ⅷ were given drugs at different dosages as shown in Table 1. This procedure lasted 15 days.

Table 1 Experimental groups and treatment

group	diet and drug
Ⅰ	control (ig solvent, ip normal saline)
Ⅱ	hyperlipidemic model (ig LE, ip normal saline)
Ⅲ	positive drug control (ig LE, ip duoxikang 5 mL/kg
Ⅳ	small dosage P (ig LE, ip P 0.25 mg/kg)
Ⅴ	large dosage P (ig LE, ip P 1 mg/kg)
Ⅵ	small dosage AG (ig LE, ip AG 5 mg/kg)
Ⅶ	large dosage AG (ig LE, ip AG 20 mg/kg)
Ⅷ	small dosage P + small dosage AG (ig LE, ip P 0.25 mg/kg + AG 5 mg/kg)

LE: lipid emulsion P: protein AG: anthraquinone glucosides

1.4 Experimental methods and statistical analysis: After the last treatment, the rats were fasted for 12 h, and anesthetized with pentobarbital sodium (30 mg/kg, ip), and then blood samples were collected from carotid artery by intubation. The ηH ($200\ s^{-1}$), ηL ($40\ s^{-1}$) and RV were measured with the LG-Ⅲ tube-shaped spin blood viscometer (Transducer Technology Development Co., Chinese Academy of Sciences). The PAR was determined with 9103-3 platelet adhesiometer (Transducer Technology Development Co., Chinese Academy of Sciences). The ηP was determined with a falling ball viscometer (Huaxing High Technology Co.), and the ESR and HCT were taken with ESR and HCT reading board (Huaxing High Technology Co.). Results were expressed as $\bar{x} \pm s$, and statistical analysis was carried out using t-test.

2 Result

The changes of ηH, ηL, RV, ηP, PAR, ESR, and HCT in the eight groups were shown in Table 2.

Table 2 Changes in hemorrheologic parameters ($\bar{x} \pm s$, n= 10)

group	ηH (mPa·s)	ηL (mPa·s)	RV (mPa·s)	ηP (mPa·s)	PAR (%)	HCT (%)	ESR (mm/h)
I	3.77± 0.09**	9.31± 1.97**	6.21± 2.23**	2.85± 0.60**	20.36± 6.50	45.00± 3.90	1.63± 1.54
II	6.00± 1.26	15.19± 3.07	11.76± 3.29	4.26± 0.90	26.76± 6.43	45.30± 3.89	2.60± 2.40
III	4.04± 1.08**	9.08± 2.22**	7.72± 2.29**	3.29± 0.75*	18.20± 8.19*	44.80± 5.40	1.60± 1.70
IV	4.89± 1.59	12.13± 3.59	8.74± 2.88	3.60± 1.06	19.21± 6.72*	44.21± 6.51	1.90± 1.80
V	4.48± 1.22*	11.20± 3.38*	7.33± 2.60*	3.32± 0.82*	17.62± 7.79*	46.19± 5.06	1.30± 0.90
VI	5.49± 1.23	12.95± 3.05	9.65± 2.47	4.00± 0.81	19.19± 7.20*	44.25± 6.67	1.99± 1.58
VII	4.44± 1.45*	11.34± 3.29*	7.93± 2.41*	3.36± 0.93*	20.26± 6.70*	43.85± 5.84	1.60± 1.10
VIII	4.46± 1.54*	10.83± 4.05*	8.02± 4.26*	3.35± 1.05*	17.48± 3.71**	45.10± 4.30	1.30± 1.00

* $P < 0.05$, ** $P < 0.01$ *vs* group II

Compared with control group (I), ηH, ηL, RV, ηP, and PAR of hyperlipidemic model group (II) were significantly increased ($P < 0.05$, $P < 0.01$), group with duoxikang (III) could lower the above raised indexes ($P < 0.05$, $P < 0.01$). This manifested that the hyperlipidemic model of rat was successfully established by ig lipid emulsion for 15 d.

Results showed that the protein 1 mg/kg (V), the anthraquinone glucosides 20 mg/kg (VII), and the protein 0.25 mg/kg plus anthraquinone glucosides 5 mg/kg (VIII) could decrease ηH, ηL, RV and ηP of the hyperlipidemic rats ($P < 0.05$); but neither protein 0.25 mg/kg (IV), nor the anthraquinone glucosides 5 mg/kg (VI) showed any effect ($P > 0.05$). The protein 0.25 (IV) and 1 mg/kg (V), the anthraquinone glucosides (VI) 5 and 20 mg/kg (VII), and the protein 0.25 mg/kg plus the anthraquinone glucosides 5 mg/kg (VIII) could lower PAR of the hyperlipidemic rats ($P < 0.05$), but could not lower ESR and HCT of the hyperlipidemic rats ($P > 0.05$).

3 Discussion

Clinical study indicated that a kind of tea made from *Semen Cassiae* could lower serum lipid[1]. In our previous study, we found that protein and anthraquinone glucosides from *Semen Cassiae* could lower serum lipid of hyperlipidemic rats. The results of the present study showed that they could also lower the elevated ηH, ηL, RV, ηP, and PAR of hyperlipidemic rats. Therefore, protein and anthraquinone glucosides from *Semen Cassiae* are not only beneficial to hyperlipidemia, but also beneficial to hemorrheologic disorders.

It has been reported that *Semen Cassiae* has eyesight-improving function and can be used to cure many kinds of eye diseases[2]. The severity of diabetic retinopathy was positively correlated with total cholesterol and low density lipoprotein[5]. One of the main pathogenesis of retinal vein occlusion was due to abnormalities of hemorrheology[6], and the raised blood viscosity and the change of platelet activity played an important role as the chief culprit[7,8]. Protein and anthraquinone glucosides from *Semen Cassiae* could lower total cholesterol, low density lipoprotein, blood viscosity, and platelet adhesion ratio, thus they were useful for treating diabetic retinopathy and retinal vein occlusion. This might, in part at least, account for the improvement of eyesight by *Semen Cassiae*.

References

[1] Hu K C, Hu Y M. Treating hypertension and hyperlipidomia with tea made from *Semen Cassiae* [J]. Zhejiang J Tradit Chin Med, 1992, (9): 432.

[2] Hua H Q. Study and clinical application of *Semen Cassiae* [J]. Chin J Chin Mater Med, 1995, 20(9): 566-567.

[3] Li X E. Study on the effective lipid-lowering components and lipid-lowering protein structure of *Cassiae* seed [Thesis for the Doctorate]. Xi'an Institute of Biological Engineering of Xi'an Jiaotong University, 2000.

[4] Zhao C Q, Zhao Z W. Clinical Hemorrheology [M]. Beijing: The People's Medical Publishing House, 1997.

[5] Li C Z, Zhang S H, Wang X L, *et al*. Relationship between dyslipidemia and diabetic retinopathy in non-insulin-dependent diabetes mellitus [J]. Chin J Ocul Fundus Dis, 1998, 14(1): 21-23.

[6] Xi X H, Tan J Q, Nie A G, *et al*. The effect of combined treatment of xue-shuan-tong and isovaemic haemodilution on activities of fibrinolysis and hemorrheology in patients with retinal vein occlusion [J]. Chin J Ocul Fundus Dis, 1998, 14(1): 7-9.

[7] Ring C P, Pearson T C, Sanders M D. Viscosity and retinal vein thrombosis [J]. Br J Ophthalmol, 1976, 60: 397.

[8] Wang F, Zhang H R, Li F M. Observation of platelet function in patients with central retinal vein occlusion [J]. Ocul Fundus Dis, 1987, 3(4): 197.