

丝瓜子蛋白的提取分离及其对小鼠的抗早孕作用

张 颂 张宗禹 苏庆东¹ 刘晓松¹ 李雪峰¹

(生理学教研室)

丝瓜 *Luffa cylindrica* (L.) Roem. 与天花粉同科, 均系葫芦科植物。鉴于天花粉蛋白和四季豆蛋白等有抗生育作用^[1-3], 我们选用丝瓜子蛋白对小鼠进行了抗早孕作用研究。

方法和结果

一、丝瓜子蛋白的提取

丝瓜子购自南京蔬菜种子公司, 去壳后乙醚浸泡去油脂, 凉干, 磨碎, 过28目筛。取100 g, 加5倍量蒸馏水, 搅拌10 min, 置冰箱过夜, 并再搅拌2~3次。次日离心, 上清液以1 mol/L HCl调pH至4, 过滤, 滤液加4倍量-10℃的丙酮, 搅拌, 放置10 min, 离心(2800 r/min, 20 min)。沉淀用丙酮洗2次后真空干燥, 得粗蛋白8 g。

二、丝瓜子蛋白的分离和纯化

用羟基磷灰石柱层析法进行分离和纯化。羟基磷灰石按A Tiselius方法制备^[4]。装柱, 以0.001 mol/L, pH 6.8 磷酸缓冲液平衡过夜。取丝瓜子粗蛋白2 g, 溶于100 ml蒸馏水, 过滤进样, 流速每小时46 ml; 用pH 6.8 磷酸缓冲液0.001, 0.05, 0.1和0.2 mol/L依次洗脱。洗脱液在280 nm处测定吸收值, 可得I、II、III、IV 4个洗脱峰(图1)。合并各部分收集液, 分别用蒸馏水透析后冷冻干燥。

将4个洗脱峰的蛋白进行琼脂平板电泳鉴定。琼脂平板用离子强度0.05, pH 8.8的巴比妥缓冲液配成1%的琼脂溶液(内含0.3%羧甲基纤维素)。铺板, 用滴管在板的中间吸5个小孔。将所需电泳的蛋白溶液分别置于孔内。在200 V电压下, 电泳5 h, 经2%醋酸

固定后用氨基黑10 B染色, 结果见图2。

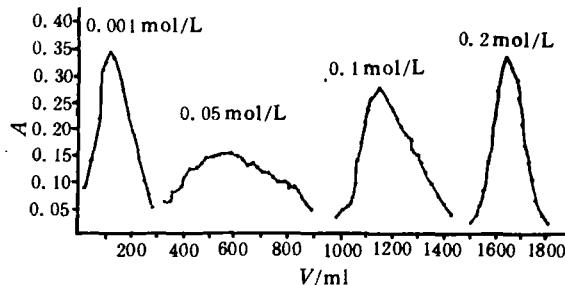


Fig 1. Elution profile of the protein of the seeds of *Luffa cylindrica* (L.) Roem. on hydroxylapatite column in stepwise elution with the pH 6.8 phosphate buffer, fractions were assayed for absorbance at 280 nm

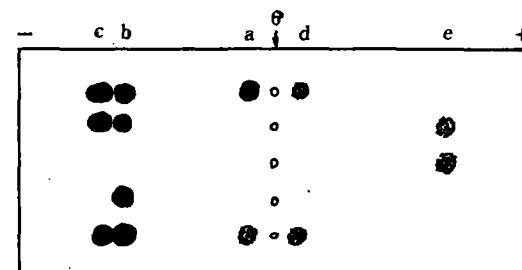


Fig 2. An agar gel electrophoresis of the protein of the seeds of *Luffa cylindrica* (L.) Roem. with 0.05 mol/L barbiturate buffer pH 8.8 in 1% agar gels

1. Crude protein.
2. Peak I, eluted with 0.001 mol/L pH 6.8 phosphate buffer.
3. Peak II, with 0.05 mol/L of same buffer.
4. Peak III, with 0.1 mol/L of same buffer.
5. Peak IV, with 0.2 mol/L of same buffer.
6. Sample origins.

上柱前的粗蛋白在琼脂平板电泳中主要含有a, b, c, d 4个区带, 未显示e区带。其中峰I除b, c区带外, 还含有向正极泳动的痕迹区带c; 峰II得单一向正极泳动的区带e; 峰III为单一的区带b; 峰IV除向负极泳动的3条区带a, b, c外, 还含有向正极泳动的区带d。

三、丝瓜子蛋白的抗早孕作用

抗早孕实验按以前报道的方法进行^[5]。结果见表1。峰Ⅱ和峰Ⅳ的抗早孕活性强,剂量3.3 mg/kg,腹腔注射1次的妊娠终止率为100%。

Tab 1. Abortifacient activity of the seeds of *Luffa cylindrica* (L.) Roem. in pregnant mice on day 7 of gestation

Group	Dose, mg/kg	Mice	Aborted mice (%)
Control		10	0 (0)
Crude protein	16.5	10	10 (100)
	6.6	10	6 (60)
Peak I	6.6	9	9 (100)
	3.3	10	3 (33)
Peak II	6.6	10	10 (100)
	3.3	10	10 (100)
Peak III	6.6	10	10 (100)
	3.3	10	6 (60)
Peak IV	6.6	10	10 (100)
	3.3	9	9 (100)

讨 论

1. 丝瓜子蛋白的分离纯化用二乙氨基纤维素、丙酮分级沉淀、硫酸铵分级盐析等方法未能获得理想结果,以羟基磷灰石柱层析方

法的效果较为满意。

2. 丝瓜子峰Ⅱ蛋白含量虽少,但活性较强。
3. 我们曾用两批丝瓜子分别进行过实验,经同样方法处理得到的蛋白,其抗早孕活性有较大差别,原因有待进一步研究。

关键词 丝瓜子; 蛋白质; 抗早孕

参 考 文 献

- 1 金善炜, 孙孝先, 汪绍福等. 天花粉蛋白的化学 I. 结晶天花粉蛋白的制备及其物理化学性质. 化学学报 1981; 39(9): 917-24
- 2 孙承琦, 王淑如, 胡卓逸等. 四季豆蛋白抗生育作用的研究. 药学学报 1983; 18(2): 81-5
- 3 Chan W Y, Tsim P P L, Yeung H W. The termination of early pregnancy in the mouse by β -momorcharin. Contraception 1984; 29(1): 91-100
- 4 Tisclius A, Hjerten S, Levin O. Protein chromatography on calcium phosphate columns. Arch Biochem Biophys 1956; 65: 132-55
- 5 张 领, 张宗禹, 刘 桦等. 大叶胡枝子根皮中的鞣质对小鼠的抗早孕作用. 中国药科大学学报 1990; 21(1): 57-8

Terminative Effect of the Seeds of *Luffa cylindrica* (L.) Ruem. on the Early Pregnant Mice

Zhang Song, Zhang Zonyu, Su Qingdong, Liu Xiaosong, Li Xueseng

Department of Physiology

The crude protein was extracted from the seeds of *Luffa cylindrica* (L.) Roem. It was shown that crude protein terminated early pregnancy in mice by intraperitoneal injection on day 7 of gestation. The crude protein was further purified on hydroxylapatite column (6×5 cm) equilibrated with 0.001 mol/L phosphate buffer pH 6.8, and elution was carried out with the same buffer (0.001, 0.05, 0.1 and 0.2 mol/L, respectively). The flow rate was 46 ml/h, and four fractions were obtained. One of these fractions, Peak II, eluted by 0.05 mol/L pH 6.8 phosphate buffer, showed a single band on agar gel electrophoresis. In early pregnant mice, terminating pregnancy rate by the main component (Peak II) was 100% at dose 3.3 mg/kg body weight by intraperitoneal injection.

Key words Seeds of *Luffa cylindrica* (L.) Roem.; Protein; Terminative early pregnancy