Immunization of mice with concentrated liquor from male zooid of *Antheraea pernyi*

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INTRODUCTION

The concentrated liquor from male zooid of *Antheraea pernyi* is a pure preparation of traditional Chinese medicine, which possesses many health-care functions. According to The Great Dictionary of Traditional Chinese Medicine, *A. pernyi* is the matured insect of silkworm. The major components of male zooid are proteins and more than 20 kinds of free amino acids, cytochrome C\(^{[1]}\), with the actions of tonifying the liver and invigorating the kidney, strengthening Yang Qi and astringing essence\(^{[2]}\). So male zooid is mainly employed to treat impotence, seminal emission, and stranguria with hematuria. This agent is made from unmated male zooid of *A. pernyi*. The effective components are extracted from its combined lixivium of edible level, then isolated, purified, and concentrated with advanced cryogenic techniques. The qualitative and quantitative analyses are tested by thin-layer chromatography. We undertook this animal experiment to study the effects of the concentrated liquor on cellular, humoral, and monocyte-phagocytic immune functions in mice.

MATERIALS AND METHODS

**Experimental materials**

Raw materials from male zooid of *A. pernyi* were provided by the Silkworm Research Institute of Shandong Agriculture Science Academy, purified and concentrated in our laboratory\(^{[3]}\).

The whole blood of Guinea pig was sampled and centrifuged. The concentrated RBCs of mice were added to 5 mL of extracted Guinea pig serum and stored at 4 °C for 30 min before use, then centrifuged at 1 500 r/min for 15 min. Preclusion of unspecific hemolysis caused by complement was eliminated by extraction of supernatant. The processed serum diluted in normal saline at 1:10 served as the experimental complement.

**Effects of concentrated liquor from male zooid of *Antheraea pernyi* on cellular immune function of mice**

Forty Kunming mice (6-8 wk) weighing 18-20 g were randomized into three tested groups (high-, medium-, and low-dosage group), and treated with 16.53, 2.62, and 0.564 mg/kg of concentrated liquor from male zooid of *A. pernyi* respectively. During the 15-d process of continuous oral filling, a dosage of 2% 0.2 mL (1×10\(^{6}\)/mL) (V/V) SRBC was injected into the abdominal cavity of each mouse on the 10\(^{th}\) d. Four days later, sizes of the left plantars of the immunized mice were measured with slide gaud, then another dosage of 20 μL (1×10\(^{6}\)/mL) 20 mol/L (V/V)
SRBC was injected subcutaneously at the measured site of each mouse. Twenty-four and forty-eight hours after injection, thickness of the left plantar of each treated mouse was measured respectively. Each site was measured thrice, and the average value was used to calculate the FSR. FSR = thickness of the left plantar before injection-thickness of the left plantar after injection (mm).

**Effects of concentrated liquor from male zooid of Antheraea pernyi on humoral immune function of mice**

Kunming mice (6-8 wk) weighing 18-22 g were randomized into three testing groups and fed with concentrated liquor from male zooid of *A. pernyi* at the dosages of 16.53, 2.62, and 0.564 mg/kg, respectively. During the 15-d process of continuous oral filling, a dosage of 0.2 mL (1×10⁶/mL) 20 mol/L (V/V) SRBC was injected into the abdominal cavity of each mouse on the 10th d. Five days later, whole blood was sampled from each mouse by eye extraction, and then the serum was prepared. Hemolysin in serum was determined as follows: the prepared serum from each mouse was diluted 1:50, 1 mL of the diluted serum was added into a 10-mL test tube, and then 0.5 and 1 mL complement of 10% SRBC was added into the tube. A control tube was added with normal saline instead of serum. After incubation at 37 °C for 30 min, the reaction was stopped in ice bath, centrifuged at 2 000 r/min for 10 min, the supernatant was extracted and 3 mL of Du’s reagent was added. At the same time, 0.25 mL 10% SRBC was added into Du’s reagent to a final volume of 4 mL and placed at room temperature. The optical densities of all preparations were tested by type-722 spectrophotometer in 1-cm color matching cups, the optical density value of each sample tube/the optical density value of SRBC at semi-hemolysis×dilution multiples.

**Effects of concentrated liquor from male zooid of Antheraea pernyi on monocyte-phagocytic function of mice**

Kunming mice (6-8 wk) weighing 18-22 g were randomized into three tested groups and fed with concentrated liquor from male zooid of *A. pernyi* at the dosages of 1.24±0.28 mg/kg, 0.61±0.26 mg/kg, and 0.20±0.16 mg/kg, respectively. During the 15-d process of continuous oral filling, a dosage of 0.2 mL (1×10⁶/mL) 20 mol/L (V/V) SRBC was injected into the abdominal cavity of each mouse on the 10th d. Twenty-four and forty-eight hours after immunization with SRBC, the FSR values of the three tested groups improved significantly compared to the control group by variance analysis (Table 1), indicating that the cellular immune function of mice could be improved obviously by concentrated liquor from male zooid of *A. pernyi*.

### Table 1 Effects of concentrated liquor from male zooid of *A. pernyi* on cell immunity function of mice (mean±SD)

| Group      | Dosage (mg/kg) | Animal (n) | 24 h FSR (mm) | 48 h FSR (mm) |
|------------|----------------|------------|---------------|---------------|
| Control    | dH₂O           | 10         | 0.66±0.25     | 0.20±0.16     |
| Low-dosage | 0.564          | 10         | 1.22±0.28     | 0.61±0.26     |
| Medium-dosage | 2.62      | 10         | 1.24±0.23     | 0.61±0.19     |
| High-dosage | 16.53         | 9          | 1.45±0.25     | 0.66±0.26     |

1 P<0.01, FSR (mm) of control group vs that of high-, medium- and low-dosage groups at 24 h respectively. 2 P<0.01, FSR (mm) of control group vs that of high-, medium- and low-dosage groups at 48 h respectively.

**Effects of concentrated liquor from male zooid of Antheraea pernyi on humoral immune function of mice**

As shown in Table 2, the HC₃₀ values representing the humoral immune function of mice showed a significant difference between the high dosage group and the control group by variance analysis (F= 7.965, P<0.01). The same results were also observed between the high-dosage group and the other two tested groups (P<0.01). The concentrated liquor from male zooid of *A. pernyi* had certain positive effect on the humoral immune function of mice.

### Table 2 Effects of concentrated liquor from male zooid of *A. pernyi* on humoral immune function of mice (mean±SD)

| Group      | Dosage (mg/kg) | Animal (n) | HC₃₀ |
|------------|----------------|------------|------|
| Control    | dH₂O           | 7          | 42.2±18.2 |
| Low-dosage | 0.564          | 8          | 46.4±23.0 |
| Medium-dosage | 2.62      | 8          | 40.1±15.6 |
| High-dosage | 16.53         | 8          | 91.6±37.6 |

1 P<0.01, high-dosage group vs control group. 2 P<0.01, high-dosage group vs low- and medium-dosage groups respectively.

**Effects of concentrated liquor from male zooid of Antheraea pernyi on monocyte-phagocytic function of mice**

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Table 3  Effects of concentrated liquor from male zooid of *A. pernyi* on monocyte-phagocytic function of mice (mean±SD)

| Group         | Dosage (mg/kg) | Animal (n) | K             | a               |
|---------------|----------------|------------|---------------|-----------------|
| Control       | dH2O           | 10         | 0.0514±0.0122 | 6.249±0.727     |
| Low-dosage    | 0.56           | 10         | 0.0527±0.0157 | 6.304±0.967     |
| Medium-dosage | 2.62           | 10         | 0.0517±0.0104 | 6.354±0.761     |
| High-dosage   | 16.53          | 10         | 0.0570±0.0111 | 6.438±0.690     |

*P<0.05, *K* value, high-, medium-, and low-dosage groups vs control group respectively. *P>0.05, *α* value, high-, medium-, and low-dosage groups vs control group respectively.

**DISCUSSION**

The male zooid is an animal material medicine in China, whose functions are well documented in *Compendium of Materia Medica* as follows: **invigorating essential Qi, strengthening vagina, uniting of sexual intercourse, and arresting essence.** In *Ri Hua Zi Ben Cao* (*Ri Hua Zi Materia Medica*), it is documented to have the following actions: strengthening sexual function, checking spermatorrhea and stranguria with hematuria, warming kidney, extinguishing sore and scar, indications: wound from metal instrument injury, acute catarrhal conjunctivitis, allergy, ascites, vaginal, untiring of sexual intercourse, and arresting essence. Thus, male zooid can tonify the liver and invigorate the kidney, and promote muscle growth. The male zooid of *A. pernyi* contains many active substances, such as brain hormone, pro-thymosin, hormone of *A. pernyi*, diuretics, which can adjust metabolism and restore immune functions.

Hu *et al.* using the fruit fly (*Drosophila melanogaster*) as a longevity model, examined the effect of hu-bao (HB) and seng-bao (SB), two marketed health products made from a mixture of natural ingredients, and found that the effect of HB and SB are specific for the male fly. The lifespan of the male significantly increased when HB or SB was added to the culture medium. When the male silk worm moth ingredient was removed from HB or SB, the lifespan prolongation effect of HB and SB drastically diminished, suggesting that the male silk worm moth is a key ingredient in combination with other components for specific prolongation of the lifespan of male flies. The immune system in the Chinese oak silk moth, *A. pernyi*, originated from a single ancestral gene with that of the Cecropia moth whose antibacterial activity has been tested against nine different bacterial species. Zhang *et al.* reported that the cecropins from Chinese oak silk worm *A. pernyi* possess effective anti-tumor activity with no cytotoxicity against normal eukaryotic cells, and impede the neoplastic process in murine large intestines.

**REFERENCES**

1. Piao HS, Ju Y, Zheng YH, Li YJ, Zheng CJ, Shen GH. Extraction separation and identification of amino acid components in the semen of male silk moths. *Yanbian Daxue Yixue Xuebao* 1997; 20: 147-148
2. Li QY, Hu PL. The study of antheraea pernyi and boxbyxmoril. *Shanghai Zhongyiyao Zazhi* 1996; 11: 45-47
3. Xu SY, Bian RL, Chen X. Experimental methodology of pharmacology. *Beijing: the People’s Health Press* 1991: 1233-1238
4. Ming D, Li SZ. Compendium of materia medica. 4TH edition. *Beijing: the People’s Health Press* 1981: 2247-2255
5. Tang JH. The effect of antheraea pernyi. *Huaxia Yixue* 1999; 12: 341
6. Liu TY, Li DJ. Chinese medicine: Antheraea Pernyi. *Beijing Zhongyi Daxue Xuebao* 1994; 17: 22-23

7. Mao G, Cui DJ, Xu QM. The effect of Antheraea Pernyi. *Liaoning Zhongyi Zazhi* 1994; 21: 231-232

8. Liu XM, Zhou LS. The anti-fatigue function of the capsule Wei Li Kang. *Guangdong Yiyoao* 2003; 24: 248-249

9. Mo QZ, Zhou SY, Sang T, Wen SK. The pharmacological investigation and application of Antheraea Pernyi. *Zhongyi Yao* 1995; 18: 101-103

10. Cao Cai, Wei HY. Pharmacological study of Antheraea Pernyi. *Zhongguo Zhongyi Zazhi* 1991; 16: 368-370

11. Hu K, Wang Q, Hu PQ. The male silkworm moth (Antheraea pernyi) is a key ingredient in hu-bao and sheng-bao for specific prolongation of the life-span of the male fruit fly (Drosophila melanogaster). *Am J Chin Med* 2002; 30: 263-270

12. Qu Z, Steiner H, Engstrom A, Bennich H, Boman HG. Insect immunity: isolation and structure of cecropins B and D from pupae of the Chinese oak silk moth, Antheraea pernyi. *Ear J Biochem* 1982; 127: 219-224

13. Zhang WM, Lai ZS, He MR, Xu G, Huang W, Zhou DY. Effects of the antibacterial peptide cecropins from Chinese oak silkworm, Antheraea pernyi on 1, 2-dimethylhydrazine-induced colon carcinogenesis in rats. *Diyi Junyi Daxue Xuebao* 2003; 23: 1066-1068

14. Vermeiren J, Ceuppens JL, Van Ghelue M, Witters P, Bullens D, Mages HW, Kroczek RA, Van Gool SW. Human T cell activation by costimulatory signal-deficient allogeneic cells induces inducible costimulator-expressing anergic T cells with regulatory cell activity. *J Immunol* 2004; 172: 5371-5378

15. Makar KW, Wilson CB. DNA methylation is a nonredundant repressor of the Th2 effector program. *J Immunol* 2004; 173: 4402-4406

16. Xie ZW. The effects of Chinese traditional medicine on the Th1/Th2 balance. *Guowai Yiyou* 2002; 24: 335-337