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• *BRIEF REPORTS* •

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

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Abstract

AIM: To study the effects of concentrated liquor from male zooid of *Antheraea pernyi* on immunological mice.

METHODS: For each experiment, 40 mice were randomly divided into normal saline group (control group) and three tested groups that were administered different dosages of concentrated liquor from male zooid of *A. pernyi* and food for 15 d. The typical FSR and HC_{50} value, monocyte-phagocytic exponent *K* and emendated monocyte-phagocytic exponent α were determined and calculated respectively.

RESULTS: After 24 and 48 h, the FSR values of the three tested groups improved significantly in comparison to the control group by variance analysis. The HC_{50} values showed a significant difference between the high dosage group and the control group, as well as between the high dosage group and other two tested groups. The monocyte-phagocytic exponent *K* and emendated exponent α showed rising tendencies, but no significant differences were found by variance analysis.

CONCLUSION: The concentrated liquor from male zooid of *A. pernyi* can significantly enhance cellular and humoral immune function in mice, but has no distinct influence on the monocyte-phagocytic system in mice.

Key words: *Antheraea pernyi*; Male zooid; Concentrated liquor; Mice; Immune function

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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 ℃ 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 (1×10^6/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 at 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 ℃ 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 of the control test tube was defined as zero value. The semi-hemolytic value (HC_{50}) was calculated as: HC_{50} = 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* for 15 d at the same dosages as the above. After the last dosage was given, charcoal particle clearance test was performed. The detailed procedure was as follows: Each mouse was injected with diluted Chinese ink through its tail vein, 20 μL whole blood was sampled from the medial canthus of each mouse at the 2nd and 10th min respectively, distilled water was added to a final volume of 2 mL, and the *A* value at 600 nm was determined. Then, the mice were killed by dearticulation, the liver, and spleen were removed and weighed. The monocyte-phagocytic exponent *K* was calculated as $K = (lgA2-lgA10)/(t2-t1)$, and the emendated monocyte-phagocytic exponent α as α = body weight/(liver weight+spleen weight)$\times K^{1/3}$. The A2 and A10 were the values at the 2nd and 10th min respectively, t1 and t2 represented the sampling time (both representing the ability of monocyte-phagocytic system to clear colloid charcoal particles in mice).

Statistical analysis

Variance analysis was performed with the statistical software SPSS (version 10.0). The data were expressed as mean±SD, $P<0.05$ was considered statistically significant.

RESULTS

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

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 cellular immune function of mice (mean±SD)

Group	Dosage (mg/kg)	Animal (*n*)	FSR (mm)	
			24 h	48 h
Control	dH_2O	10	0.66±0.25[b]	0.20±0.16[d]
Low-dosage	0.564	10	1.22±0.28	0.61±0.26
			<0.01	<0.01
Medium-dosage	2.62	10	1.24±0.23	0.61±0.19
			<0.01	<0.01
High-dosage	16.53	9	1.45±0.25	0.66±0.26
			<0.01	<0.01

[b]$P<0.01$, FSR (mm) of control group *vs* that of high-, medium- and low-dosage groups at 24 h respectively. [d]$P<0.01$, 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 HC_{50} 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*)	HC_{50}
Control	dH_2O	7	42.2±18.2[b]
Low-dosage	0.564	8	46.4±23.01[b]
Medium-dosage	2.62	8	40.13±15.16[b]
High-dosage	16.53	8	91.16±37.6
			<0.01[b,d]

[b]$P<0.01$, high-dosage group *vs* control group. [d]$P<0.01$, 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

The monocyte-phagocytic exponent *K* and emendated

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*	α
Control	dH_2O	10	0.0514±0.0122[a]	6.249±0.772[c]
Low-dosage	0.56	10	0.0527±0.0157	6.304±0.967
			P>0.05	*P*>0.05
Medium-dosage	2.62	10	0.0517±0.0104	6.354±0.761
			P>0.05	*P*>0.05
High-dosage	16.53	10	0.0570±0.0111	6.438±0.690
			P>0.05	*P*>0.05

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

monocyte-phagocytic exponent α (both representing the ability of monocyte-phagocytic system to clear colloid charcoal particles in mice) are shown in Table 3. Both exponent *K* and emendated exponent α displayed a rising tendency, but no significant differences were observed among the groups by variance analysis (*P*>0.05).

DISCUSSION

The male zooid is an animal material medicine in China, whose functions are well documented in Compendium of Materia Medica as follows[4]: invigorating essential Qi, strengthening vagina, untiring 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 and allergic conjunctivitis, and frostbite, heat-induced sore. Thus, male zooid can tonify the liver and invigorate the kidney, consolidate essence and strengthen Yang, check bleeding 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[5-10].

Hu *et al*.[11], 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 life-span of the male significantly increased when HB or SB was added to the culture medium. When the male silkworm moth ingredient was removed from HB or SB, the life-span prolongation effect of HB and SB drastically diminished, suggesting that the male silkworm moth is a key ingredient in combination with other components for specific prolongation of the life-span 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[12]. Zhang *et al*.[13], reported that the cecropins from Chinese oak silkworm *A. pernyi* possess effective anti-tumor activity with no cytotoxicity against normal eukaryotic cells, and impede the neoplastic process in murine large intestines.

T cells play a role in immune response, killing tumor cells and suppressing tumor growth. Due to the actions of estrogen, paracrine of tumor, negative nitrogen balance, the immune functions of patients can be suppressed and lead to immune escape, proliferation, and metastasis of tumor cells[14]. Th1 and Th2 are two subgroups of CD4+Th cells. Most tumor tissues can secret functional cytokine of Th2, causing the shift from Th1 to Th2 and immune suppression. Thus, promoting shift from Th2 to Th1 may be a method of tumor immunotherapy. The androgen-like action of male zooid can antagonize the immuno-suppression of high-level E2, promote high-expression of IL-18, induce production of IFN-γ, IL-2, IL-12, IL-18, and GM-CSF by monocytes, enhance cytotoxicity of NK and Th1 cells, accelerate proliferation of T cells and induce differentiation of Th1 cells[15]. Amino acids in male zooid can adjust negative nitrogen balance and restore immune functions. Peptides in male zooid can also improve immune functions, enhance T cell activity by killing tumor cells[16].

In general, the concentrated liquor from male zooid of *A. pernyi* can enhance non-specific immunity and CTL-mediated specific immunity, and has therapeutic functions in tumor therapy and adjuvant therapy.

Our study showed that the concentrated liquor from male zooid of *A. pernyi* could suppress tumor growth and improve immune function in mice. For immune modulation, it was observed in animal experiments that it could enhance cellular immunity significantly in all the three tested groups. In the high dosage group, the humoral immunity also obviously improved. A rising tendency was shown without significant difference.

In conclusion, the concentrated liquor from male zooid of *A. pernyi* is a potential anti-tumor agent by strengthening anti-pathogenic Qi. Further researches should be performed on its immuno-modulating effects on pancreatic and liver cancer.

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 Zhongyiyao 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 Yiyao* 2003; **24**: 248-249
9 **Mo ZQ,** Zhou SY, Sang T, Wen SK. The pharmocological investigation and application of Antheraea Pernyi. *Zhongyiyao* 1995; **18**: 101-103
10 **Cao Cai,** Wei HY. Pharmocological 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. *Eur 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 Yixue* 2002; **24**: 335-337

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