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Effect on the immunological competence of *Physalis pubescens* L. in mice

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Effect of different parts of the *Physalis pubescens* L. (Pp) on the immunocompetence were investigated in Kunming mice inbred lines. After 35 consecutive days of feeding, the mice were sacrificed to evaluate the immunologic function through the relative weights of spleen and thymus, the delayed type hypersensitivity (DTH), carbon particle clearance test and serum agglutination test. The results are summarised as follows: the pulp of Pp had statistically significant effect on the mice's non-specific immunity, but not on the weight of the mice's thymus. The Pp's pulp, peel and seeds had significant effect on the mice's cellular immunity, respectively, they had regulating effect on the mice's T-lymphocytes. Any part of Pp didn't have statistically significant effect on the mice's humoral immunity.

Keywords: mice; immunoassays; *Physalis pubescens* L; DTH

Introduction

Physalis pubescens L. (Pp) is a plant belonging to *Physalis alkekengi* L. var. *franchetii* (Mast.) Makino. herbaceous perennid which is cultivated mainly in Hulunbeier League in Inner Mongolia and Greater Hignnan mountains in Heilongjiang province, China. It has been utilised both as eutrophy cordial and medicine. Pp is yellow, tastes sweet and sour, and contains plenty of Vc, carotene, calcium, iron and many other mineral substances. The calcium content is 73.3 times larger than that in tomato, 13.8 times in carrot. Pp contains 18 kinds of amino aids which are essential to human health, and account for 31% of pan-amino acid (Zhang, 2007). The E/T of amino acid is higher than most kinds of fruits we take in daily life. In traditional medicine, it was introduced as having various therapeutic effects including treatment of lung meridian, sore throat, parotitis, inflammation in urinary tract, difficulty in micturition and bloody urine (Wang, Zhang, & Li, 2007). It also helps to clear away heat, detoxicate, promote urination and stop bleeding. Most researchers are focusing on immunocompetence, the *Physalis francheti* Mast. var. *bulnyard* (Han, 2005). In this study, we investigated the immunological function of different parts of Pp. We used relative weights of spleen and thymus, the delayed type hypersensitivity (DTH), carbon particle clearance test and serum agglutination test to evaluate the

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immunocompetence of Pp. To our knowledge, this is the first study of the immunocompetence of Pp.

Materials

The whole fruit of Pp. were purchased in Dafa Market, Harbin, Heilongjiang province, P.R. China in June 2008 and properly identified by a well-known taxonomist, Prof. Wang Ping. A voucher sample was preserved in refrigerator under -13°C . The stuff to feed the mice were prepared with different parts of Pp such as the pulp, the sarcotesta, the seeds and the whole fruit by the ratio 1:1 in weight, with the forage and dried around 50°C .

Reagents

Indian ink was purchased from Beijing Xizhong Reagent Company and sheep red blood cell (SRBC) was purchased from Borun Bio-Tech Ltd. All other reagents, such as Absolute ethyl alcohol; NaCl, Na_2CO_3 , NaHCO_3 were AR.

Instruments

722s visible light spectrophotometer (Shanghai Precision Instrument Company Limited); JA2003 electronic balance (Shanghai Liangping Instrument and Meter Company Limited); Microsyringe (Shanghai Anting Microsyringe Factory); Pneumatic drier (Shanghai Yiting Scientific Instrument Company Limited); FW100 top speed omnipotence disintegrator (Tianjin Sitaite Instrument Company Limited); TDA-8002 Electric-heated thermostatic water bath (Tianjin Sitaite Instrument Company Limited); TDL-5 Bench centrifuge (Shanghai Anting Microsyringe Factory).

Experimental animals

Sixty (4- to 5-week-old) healthy Kunming mice inbred lines (20~25 g) were purchased from the Department of Animal Sciences, Heilongjiang Medical University and maintained on a 12 h light/12 h dark cycle from 5:00 AM to 17:00 PM under a regulated environment ($23 \pm 1^{\circ}\text{C}$) (Krzych, Thurman, Goldstein, Bressler, & Strausser, 1979). Animals were housed in plastic cages with free access to food and water. All procedures followed the guidelines for humane treatment of animals set by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Northeast Forestry University. Mice were divided into five groups randomly in the ratio of the mice female to male is 1:1 of weight: the pulp, the sarcotesta, the seeds; the whole fruit and the normal control.

Statistical analysis

Data were expressed as means $\pm$ SD. Statistical analysis of the data for multiple comparisons was performed by comparing means followed by Pared-sample *t*-test by SPSS 10.01 version. $P < 0.05$ indicated a statistically significant difference. $P < 0.01$ indicated a statistically very significant difference.

Experimental methods

Experimental animal feeding

The experimental animals were divided into five groups randomly: the pulp, the sarcotesta, the seeds, the whole fruit and the normal control. The mice were weighed twice a week in order to adjust the feeding dosage. The food was a mixture of different parts of the Pp and the mice's forage by the ratio of 1:1 of weight. Each group had access to food and water freely. The feeding course needs 35 d (Zheng, 1999).

Assaying of relative weights of spleen and thymus

The body weights of the mice in each group were measured twice a week throughout experimental period and sacrificed by cervical dislocation after blood collection. The liver, thymus and spleen were collected and weighed.

Assaying of cellular immunity's function

The cellular immunity's function was assayed through the thickness of the voix pedis. All mice were first measured the thickness of the voix pedis and then immunised by intravenous (i.v.) injection of 0.1 ml of SRBC suspension (1×10^8 cells/ml). After 4 d immunity, all mice were injected a volume of 20 μ L of 20% SRBC hypodermic fraction (1×10^8 cells/ml). The footpad swelling was evaluated by measuring the thickness with a microcaliper (Mitutoyo Mfg., Japan) displayed in 0.01 mm gradations at 24 h and 48 h after the injection. The average value was obtained by executing the measurement three times on one point. The *D*-value between fore and after the aggression of the voix pedis was taken as the manifestation of the DTH (Burleson & Levey, 2007).

Assaying of the mice's englobement function of the mononuclear macrophage

The mice's englobement function of the mononuclear macrophage was assayed through the carbon particle clearance test. The mice were weighed before the injection with Indian ink (0.1 ml per 10 g) through the partes terminalis venovenostomy, reckoned by time immediately. Blood was trapped 20 μ L from the eyeground veniplex 2/10 min after the injection of the Indian ink, respectively, then quickly added into 2 ml Na_2CO_3 solution, m.b., which was assayed the light absorption value $(\text{OD}_1)/(\text{OD}_2)$ by the 722s visible light spectrophotometer at 600 nm, the Na_2CO_3 solution (0.1% w/w) as the blank sample.

Assaying of each part of Physalis pubescens L. (Pp) on the mice's cellular immunity

After 35 d feeding, the mice were injected 0.2 ml of 2% SRBC to acquiring immunity. Four to five days later, executed ophthalmectomy, and trapped the blood in the centrifuge tube. The sample was stewed for 1 h, and centrifuged the blood at 3000 r/min for 10 min in order to get the blood-serum. The blood-serum was diluted by multiple proportions with the normal saline (Chen, Hau, & Lee, 1995).

Put the blood-serum at different dilution factors into the micro-dose blood coagulation testing panel, 100 μ L for each hole, and it was added by 100 μ L 0.5%

SRBC suspension, m.b. The whole panel was put into the wet plat pan and incubated 3 h in the incubator at 37°C in an atmosphere of 5% CO₂, finally the extent of the blood clotting was observed (Wray, Morris, & Sojka, 1975).

Result and analysis

The influence that each part of the Physalis pubescens L. (Pp) on the relative weights of spleen and thymus of the mice

$$\text{Relative weight} = \text{viscera weight} / \text{body weight} \times 100\%$$

$$\text{Viscera index} = \text{viscera weight} / \text{hboxbodyweight} \times 100$$

According to Figure 1, the mixture of different parts of Pp was provided to Kunming mice inbred lines daily for 35 d, and normal mice were just given forage. Then the mice were sacrificed to eviscerate the liver, the spleen and the thymus. Each value represents the mean $\pm$ SD of results obtained from six to 10 mice. Asterisks denote a significant difference the pulp of the Pp compared with the value in normal mice not fed with Pp (* $P < 0.05$). Meanwhile, neither part of the Pp has statistically significant effect on the relative weights of thymus and thymus index of the mice.

According to Figure 2, effects of different parts of Pp on the DTH reaction in Kunming mice inbred lines was studied. The mixture of different parts of Pp was

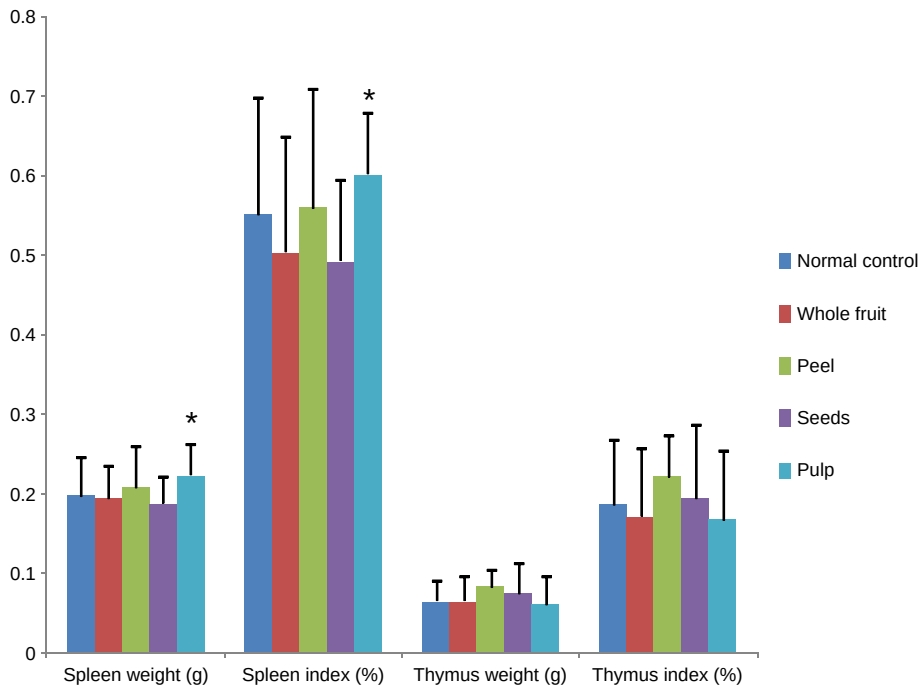


Figure 1. Effect of each part of the Pp on the relative weights of spleen and thymus of the mice ($\bar{X} \pm \text{SD}$).

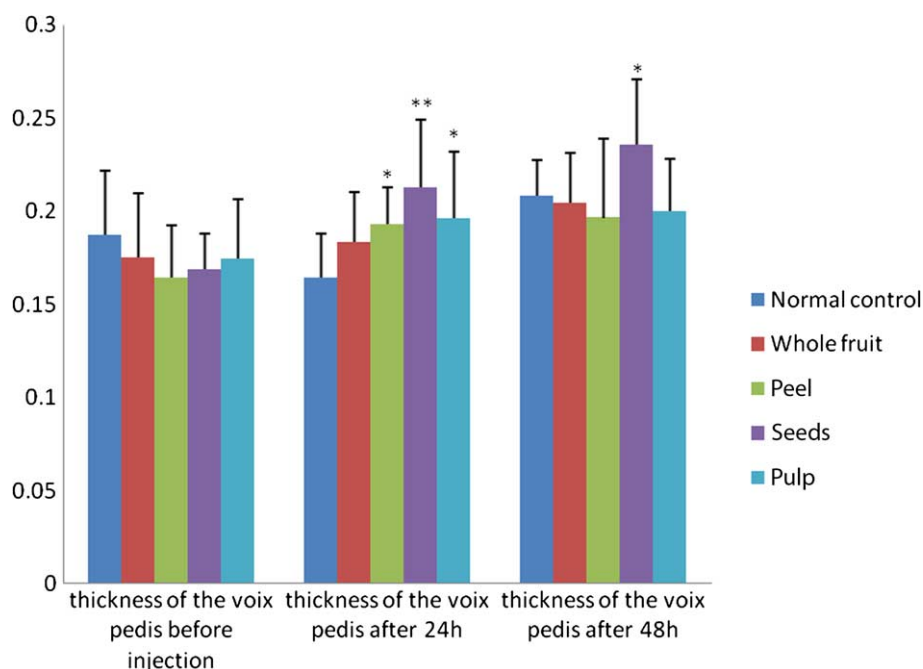


Figure 2. Effect of each part of Pp on the mice's DTH ($X \pm SD$) (cm).

provided to Kunming mice inbred lines daily for 35 d, and normal mice were just given forage. All mice were first measured the thickness of the voix pedis and then immunised by i.v. injection of 0.1 ml of SRBC suspension (1×10^8 cells/ml). After 4 d immunity, all mice were injected a volume of 20 μ L of 20% SRBC hypodermic fraction (1×10^8 cells/ml). The thickness of the left voix pedis was measured at 24 h and 48 h after the injection. Executed the measurement three times on one point, obtained the average value. The *D*-value between fore and after the aggression of the voix pedis was taken as the manifestation of the DTH (Burleson & Levey, 2007). Footpad swelling is shown as the difference between the thicknesses of the footpads challenged with SRBC, respectively. Each column represents the mean $\pm$ SD of results obtained from six to 10 mice. Asterisks denote a significant difference compared with the value in normal mice not fed Pp (* $P < 0.05$; ** $P < 0.01$). The peel of Pp has statistically significant effect on the DTH of the mice after 24 h, ($p < 0.05$). The seeds and the pulp of Pp have statistically significant effect on both the DTH of the mice after 24 h and 48 h ($p < 0.05$).

Effect of each part of Physalis pubescens L. (Pp) on the mice's carbon particle clearance

The verification was taken, and the carbon particle clearance's capacity was expressed by englobement index. Calculate out the englobement index (englobement speed) *K*.

$$K = (1gOD_1 - 1gOD_2) / (t_2 - t_1)$$

$$a = K^{(1/3)} \times \text{weight} / (\text{spleen} + \text{liver})$$

k – englobement index

t_1 – the time of the first time trapped blood after ink injection, min

t_2 – the time of the second time trapped blood after ink injection, min

OD_1 – light absorption at t_1

OD_2 – light absorption at t_2

According to Table 1, the mixture of different parts of Pp was provided to Kunming mice inbred lines daily for 35 d, and normal mice were just given forage. The mice were weighed before the injection with Indian ink (0.1 ml per 10 g) through the partes terminalis venovenostomy, reckoned by time immediately. Blood was trapped 20 μ L from the eyeground veniplex 2 min/10 min after the injection of the Indian ink, respectively, then quickly added into 2 ml Na_2CO_3 solution, m.b., which was assayed the light absorption value (OD_1)/(OD_2) by the 722s visible light spectrophotometer at 600 nm, the Na_2CO_3 solution (0.1% w/w) as the blank sample. Each value represents the mean $\pm$ SD of results obtained from six to 10 mice. Asterisks denote a significant difference on the effect of mice's carbon particle clearance of the pulp of the Pp compared with the value in normal mice not fed with Pp (* $P < 0.05$).

Effect of each part of Physalis pubescens L. (Pp) on the mice's cellular immunity

Antibody was calculated progression according to the serum agglutination extents classification. We compared the difference of the antibody progression between the control group and the other groups. The bigger the antibody progression the more antibodies can be found in serum. The results were shown in Table 2. Serum agglutination extent was generally divided into five levels (0–IV). We could calculate the antibody progression, compared the difference of the antibody progression between the control group and the other groups.

$$\text{Antibody progression} = (S_1 + 2S_2 + 3S_3 + \dots nS_n)$$

In this formula, 1, 2, 3 $\dots n$ delegate the index of the multiple proportions dilution, S delegates the agglutination extent progression. The bigger the antibody progression the more antibodies can be found in serum:

- All the red blood cells went down to the bottom of the hole and formed a dot, the liquid around the dot was clear.
- Most of the red blood cells went down to the bottom of the hole and there were a few agglutinative red blood cells found around the dot.

Table 1. Effect of each part of Pp on the mice's carbon particle clearance ($X \pm SD$).

Groups	Englobement index (a index)
Normal control	0.3388 ± 0.7950
Whole fruit	0.1789 ± 1.4351
Peel	0.4393 ± 0.6016
Seeds	0.0631 ± 1.770
Pulp	$0.3853 \pm 0.8118^*$

Values are the mean $\pm$ SD for 10 mice. Compared to normal control. * $p < 0.05$.

Table 2. Effect of each part of Pp on the mice's humoral immunity ($X \pm SD$).

Groups	Antibody progression
Normal control	32 ± 4.3
Whole fruit	43 ± 8.5
Peel	23 ± 9.9
Seeds	36.5 ± 9.3
Pulp	29.3 ± 11.2

Values are the mean $\pm$ SD for 10 mice. Compared to normal control. * $p < 0.05$.

- The agglutinative red blood cells forms a lamella on the bottom of the hole and a obvious loose dot could be found in the centre of the bottom.
- The agglutinative red blood cells forms a uniform lamella on the bottom of the hole and a indistinct dot could be found in the centre of the bottom.
- The agglutinative red blood cells forms a uniform lamella on the bottom of the hole and the clot might be contortuplicate sometimes.

According to Table 2, the mixture of different parts of Pp was provided to Kunming mice inbred lines daily for 35 d, and normal mice were just given forage. After 35 d feeding, the mice were injected 0.2 ml of 2% SRBC to acquiring immunity. Four to five days later, executed ophthalmectomy, and trapped the blood in the centrifuge tube. The sample was stewed for 1 h, and centrifuged the blood at 3000 r/min for 10 min in order to get the blood-serum. The blood-serum was diluted by multiple proportions with the normal saline (Chen et al., 1995). Put the blood-serum at different dilution factors into the micro-dose blood coagulation testing panel, 100 μ L for each hole, and it was added by 100 μ L 0.5% SRBC suspension, m.b. The whole panel was put into the wet plat pan and incubated 3 h in the incubator at 37°C in an atmosphere of 5% CO₂, finally the extent of the blood clotting was observed (Wray et al., 1975). Each value represents the mean $\pm$ SD of results obtained from six to 10 mice. Neither part of Pp has statically significant effect on the mice's humoral immunity.

Conclusion and discussion

Through the relative weight of spleen and thymus, the DTH, carbon particle clearance test and serum agglutination test, we could concluded that the pulp of Pp had statistically significant effect on the mice's non-specific immunity ($p < 0.05$), but not on the weight of the mices' thymus. The Pp's pulp, peel and seeds had significant effect on the mices' cellular immunity, respectively ($p < 0.05$, $p < 0.05$, $p < 0.01$), they had regulating effect on the mice's T-lymphocytes. Any part of Pp didn't have statistically significant effect on the mice's humoral immunity.

The mechanisms and paths of action that the Pp effect on the mice's T-lymphocytes might be as following (Battisto, Borek, & Bucsi, 1972):

1. Pp induced to produce some certain kinds of cell factor by stimulating the non-specificity immunological cells such as the macrophage to active the T-lymphocytes.
2. Some unknown competences in Pp might have the function the same as the para cytokine which can combine with the receptors on the surface of the cells to actuate the multiplication and the differentiation of the T-lymphocytes (Liu & Sun, 2006).
3. Some micro-molecular fat-soluble competences in the seeds of Pp can come directly into the T-lymphocytes, this may affect the constructive metabolism of the cells and secretion of the cell factor.

The action principles of the Pp's active ingredient for the specific immunity are deserved for further researches.

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References

- Battisto, J.R., Borek, F., & Bucsi, R.A. (1972). Studies on the isolation of lymphocytes active in cell-mediated immune responses: III. The pattern of recovery of immuno-competence by various lymphoid cell populations following cessation of chronic administration of antilymphocyte serum. *Cellular Immunology*, 4, 333–340.
- Burleson, R., & Levey, R.H. (2007, June). *Tumor cell lysate-specific DTH reaction correlates with longer survival and disease stability in vaccinated melanoma patients Salazar-Onfray F Conference Information*. 7th Annual Meeting of the Federation of Clinical Immunology Societies, Chile.
- Chen, W.C., Hau, D.M., & Lee, S.S. (1995). Effects of ganoderma-lucidum and krestin on cellular immunocompetence in gamma-ray-irradiated mice. *American Journal of Chinese Medicine*, 23, 71–80.
- Han, Y. (2005). *Preliminary study on the effective constituents of Physalis alkekengi L. var. francheti (Mast.) Makino*. Unpublished master's thesis, Xinjiang University, Xinjiang, China.
- Krzych, U., Thurman, G.B., Goldstein, A.L., Bressler, J.P., & Strausser, H.R. (1979). Sex-related immunocompetence of balb-C Mice 1. Study of immunological responsiveness of neonatal, weanling, and young-adult mice. *Journal of Immunology*, 123, 2568–2574.
- Liu, R., & Sun, F. (2006). Study on extraction and immune activity of polysaccharide from Korean pine seed. *Journal of Chinese Institute of Food Science and Technology*, 6, 290–293.
- Wang, F., Zhang, S.-W., & Li, P.-Y. (2007). Effects of alcohol extractive of the fruit of *Physalis pubescens* L. on the diuresis and anti-inflammation activities in rats. *Special Wild Economic Animal and Plant Research*, 4, 35–38.
- Wray, C., Morris, J.A., & Sojka, W.J. (1975). Comparison of indirect hemagglutination tests and serum agglutination tests for serological diagnosis of salmonella-dublin infection in cattle. *British Veterinary Journal*, 131, 727–737.
- Zhang, S. (2007). *Study on chemical constituents of the Physalis pubescens L. and its pharmacological activities*. Unpublished master's thesis, Jining University.
- Zheng, J. (1999). *Functional foods* (Vol. 3). China: China Light Industry Press.