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# Immunoregulatory role of *Pleurotus eryngii* superfine powder through intercellular communication of cytokines

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*Pleurotus eryngii*, also known as thistle mushroom, is rich in proteins, dietary fibre and polysaccharides. It has been developed as a functional food due to its antioxidant, anti-fatigue, anti-viral and anti-tumour functions. The objective of this study was to investigate the mechanisms of the immunoregulatory and antioxidant effects of *P. eryngii* superfine powder (PESP) *in vivo*. Test mice were fed with a diet containing 6.67% PESP to explore its effect on mouse delayed-type hypersensitivity, phagocytic index, serum hemolysin, serum and liver glutathione peroxidase (GSH-$P_X$) activities, serum superoxide dismutase (SOD) activity and malondialdehyde (MDA) content in liver homogenate. For the human study, volunteers received PESP at doses of 5, 10 or 15 g. Serum levels of 39 cytokines were determined before and after ingestion using a Millipore Human Kit and a liquid chip scanner. Our results showed that, in mice, dietary *P. eryngii* supplementation significantly increased the weight difference of ears ($P < 0.01$), phagocytic index ($P < 0.05$), serum hemolysin ($P < 0.01$), serum and liver GSH-PX activities ($P < 0.01$) and serum SOD activity ($P < 0.01$), but decreased MDA content ($P < 0.01$) in liver homogenate. In human trials, 5 g of PESP significantly decreased serum epidermal growth factor, while 15 g of PESP significantly decreased serum granulocyte–macrophage colony stimulating factor, macrophage-derived chemokine and tumour necrosis factor-$\alpha$. No change was detected in serum cytokines after the administration of 10 g of PESP. *P. eryngii* can regulate innate and acquired immune functions in mice to execute antioxidant and anti-aging functions, and can alter human serum cytokines. The dual immunoregulatory effects of *P. eryngii* are consistent with the theory of traditional Chinese medicine.

**Keywords:** *Pleurotus eryngii*; immunoregulatory; cytokine; cell–cell communication network

## 1. Introduction

*Pleurotus eryngii*, commonly known as thistle mushroom, belongs to the genus of *Pleurotus* in the family of Pleurotaceae (Yao & Lan, 2004). Thistle mushroom is rich in protein, dietary fibre and polysaccharides (Rong, Li, Ye, Li, & Li, 2007), and exhibits antioxidant (Mi, Catherine, & Sang, 2012; Zhang & Wang, 2003), anti-anaphylaxis (Han et al., 2011), osteoclastogenesis inhibitory (Yokoyama, Bang, Shimizu, & Kondo, 2012),

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antiatherosclerotic (Mori, Kobayashi, Tomita, Inatomi, & Ikeda, 2008), anti-dermatitis (Choi et al., 2013), anti-fatigue (Zheng, Guo, & Fan, 2010), antiviral and anti-tumour functions (Chi, Xu, Wu, & Xing, 2006; Jeong, Jeong, Gu, Islam, & Song, 2010). While most studies of *P. eryngii* have focused on bioactive properties *in vitro*, its physiological activity is less clearly understood. Due to the inhibited communication between immune cells, *in vitro* trials cannot provide insight of the immunoregulatory effects of *P. eryngii* on humans and animals (Xiao & Pang, 2012). Therefore, the immunoregulatory functions of *P. eryngii* superfine powder (PESP) in animals and humans were investigated in our study.

In this study, mice were fed a diet supplemented with PESP. Physiological and biochemical parameters of mouse cells and immune function, as well as antioxidant activity, were then tested to evaluate the immunoregulatory and antioxidant functions of the mushroom. To explore signal pathways of immunoregulatory functions in healthy humans, the effect of PESP on 39 cytokines in serum was investigated using a liquid chip scanner. Intercellular cytokine communication networks representing immune regulation by PESP were proposed. The immunoregulatory function of PESP in humans was summarised.

## 2. Materials and methods

### *2.1. Materials and reagents*

*P. eryngii* was purchased from Jiangsu Tianfeng Biotechnology Co., Ltd. The purchased material was dried and passed through 300 mesh screen. Analytically pure dinitrofluorobenzene (DNFB) and chemically pure barium sulphate were purchased from Shanghai Chemical Reagent Corporation. Analytically pure acetone was obtained from Guanghua Chemical Factory Co., Ltd. Jilong Mountain Brand Pure sesame oil was purchased from Anhui Huaan Food Co., Ltd. Sheep red blood cells (SRBC) were prepared from sheep blood. Micro-scale blood coagulation test plates were obtained from Nantong Haimen Boyang Experimental Instrument Factory. Glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), malondialdehyde (MDA) and Coomassie bright blue protein test kits were purchased from Nanjing Jiancheng Bioengineering Institute. Interleukin-6 radioimmunoassay kit was purchased from Beijing Purevalley Biotechnology Co., Ltd. Human kit 96-well microplates were purchased from Millipore Corporation, USA and double distilled water was prepared using a Millipore-Q purification system.

### *2.2. Instruments and equipment*

FA1104 analytical balance was purchased from Shanghai Shunyu Hengping Scientific Instrument Co., Ltd. Puncher was purchased from Beijing Ji'an Delphi Technology Co. Ltd. TGL-16G type centrifuge was purchased from Shanghai Anting Scientific Instrument Factory. 3K15 centrifuge was purchased from Sigma Co., USA. Light biochemical incubator was purchased from Donglian Ltd. Co., Harbin. Millipore Liquid Chip Scanner was purchased from Millipore Corporation, USA.

### *2.3. Methods*

#### *2.3.1. Animal trials*

*2.3.1.1. Animal grouping.* Sixty specific pathogen free (SPF) female mice [18–22 g, Certification No. SCXK (Su) 2008-0010; Animal usage licence No. SYXK (Su) 2008-0003] were provided by Animal Laboratory Centre of Nantong University. Animal

experiments were conducted in three batches. Data collected from the first batch were related to cellular immune function, the ratios of thymus to body weight, spleen to body weight and liver to body weight; SOD in serum and liver; and concentrations of interleukin-6 and MDA in liver. In the second batch, the monocyte–macrophage function was measured. In the third batch, the humoral immune function was measured. The 20 mice in each batch were randomly divided into two groups: 10 mice representing the negative control group and 10 in the *P. eryngii* group. All experimental animals were allowed unrestricted access to food and clean drinking water. Cellular immune functions and humoral immune functions were measured after the mice had been continually fed with the experimental diets for four weeks, while the monocyte–macrophage function was measured after five weeks of continuous feeding (Huang, 2010). The dosage of *P. eryngii* used in this study was 1.0 g/kg·bw, which is 10-fold higher than the recommended dosage for adult humans (60 g/d or 1.0 g/kg·bw).

*2.3.1.2. Preparation of mouse diets.* The negative control group was provided with a full-nutrition complex diet (20% crude protein, 4% crude fat, 5% crude fibre, 8% crude ash, 10% water, 1.0–1.8% calcium and 0.6–1.2% phosphorus). Considering that the daily dietary intake for mice is approximately 15% of body weight, the supplemented *P. eryngii* was provided at a level of 6.67%. The diet supplemented with *P. eryngii* was prepared as described below. Briefly, 121.5 g PESP was added to 1700 g basal diet and mixed evenly. After adding boiling water and stirring, the mixture was then twisted into small balls and dried at 60°C for 6 h.

*2.3.1.3. Determination of physiological and biochemical parameters.* Delayed-type hypersensitivity (DTH) of mice induced by DNFB was tested by ear swelling method. The phagocytic function of mononuclear phagocytes in mice was measured by carbon clearance test. The serum hemolysin levels of mice were determined by blood clotting method. The activities of GSH-Px and SOD in serum and liver of mice were determined by test kits. Interleukin-6 levels in livers of mice were determined using a test kit.

*2.3.1.4. Data analysis.* All experimental data were analysed using SPSS17.0. All data were expressed as Mean ± SD. Significance tests were conducted using one-way analysis of variance (ANOVA). Cytokine data were analysed by a self-paired control *t*-test using SPSS11.5 at the 95% confidence interval. Significance and high significance were indicated at $P < 0.05$ and 0.01, respectively.

### *2.3.2. Human cytokine trials*

*2.3.2.1. Recruitment of volunteers.* Twelve volunteers (5 male and 7 female) were recruited from a group of healthy college students, and divided into three groups of four for dosage purposes. All volunteers signed an informed consent and liability agreement. During the entire trial period, all volunteers were required to study and rest according to a normal schedule, and to avoid violent exercise, smoking and drinking. Except for regular meals and drinking water, volunteers were not allowed to consume any other foods, drugs or beverages. Additionally, volunteers were required to maintain a calm mood during the trial period (Wu, Gao, Luo, & Gui, 2010).

The trial period lasted two days. On the first day, all volunteers received regular unbiased diets and maintained a normal daily schedule. On the second day, in addition to the regular diet, lunch food was supplemented with *P. eryngii*. Blood was collected from each subject at 2:30 pm on each day of the trial, incubated at 4°C, and centrifuged

at 4°C (3200 r/min for 10 min). The serum was harvested and stored at –70°C until analysis.

*2.3.2.2. Doses and treatments.* *P. eryngii* was administered at three dosage levels: 5, 10 and 15 g representing the low, middle and high dose, respectively. The dried powder was mixed with boiling water, stirred into a paste and steamed for 15 min before being administered by volunteers.

*2.3.2.3. Determination of cytokines.* The 39 cytokines evaluated using a liquid chip scanner and 96-well microplates included: epidermal growth factor (EGF), eotaxin, fibroblast growth factor (FGF-2), Flt-3, fractalkine, granulocyte colony stimulating factor (G-CSF), granulocyte–macrophage colony stimulating factor (GM-CSF), growth regulated oncogene (GRO), interferon (IFN)-$\alpha$2, IFN-$\gamma$, interleukin (IL)-1$\alpha$, IL-1$\beta$, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p40), IL-12(p70), IL-13, IL-15, IL-17, IFN-inducible protein (IP)-10, monocyte chemoattractant protein (MCP)-1, MCP-3, macrophage-derived chemokine (MDC), macrophage inflammatory protein (MIP)-1$\alpha$, MIP-1$\beta$, sCD40L, sIL-2Ra, transforming growth factor (TGF)-$\alpha$, tumour necrosis factor (TNF)-$\alpha$, TNF-$\beta$ and vascular endothelial growth factor (VEGF). A plot illustrating the concentration change rates of these cytokines was established. The change rates of these cytokines can be expressed by the following equation on the basis of the determined concentrations:

$$\text{Change rate}(\%) = (C_{Experiment} - C_{Control}) = C_{Control}.$$

*2.3.2.4. Construction of cytokine networks.* According to the previous studies (Hua, Pang, & Guo, 2010 and Li et al., 2012) and *Cytokines & Cells Online Pathfinder Encyclopedia Version 29.0* (http://www.copewithcytokines.de/cope.cgi), the data related to remarkably varied cytokines in response to *P. eryngii* at three doses have been summarised and a value representing the intensity was assigned to each cytokine to define positive stimulation and negative inhibition. In the source table of cytokines, the change rate of 0 represents no regulatory effect; while 1 indicates up-regulatory effect and –1 indicates down-regulatory effect.

Intracellular communication networks representing intercellular interactions in response to *P. eryngii* at three doses were mapped using Microsoft Visio 2007 in which the connection lines between both type of cells indicated mutual message transmission and exchange among cytokines. In intracellular communication, full lines represent inhibition, dashed lines represent stimulation and line thickness represents the intensity of inhibition or stimulation (Frankenstein, Alon, & Cohen, 2006).

## 3. Results

### *3.1. Effect of* P. eryngii *on immune and antioxidant functions of mice*

As seen in Table 1, the administration of *P. eryngii* at 10 g resulted in significant differences between treatment and control mice with respect to ear weight, phagocytic index, serum hemolysin, serum and liver GSH-$P_X$ activities, serum SOD activity and MDA content in liver homogenate. Mice were sensitised by epicutaneous application of DNFB on shaved abdominal skin and were challenged with DNFB on ear skin after sensitisation for 4–7 days. Ear swelling reached its maximum peak after the challenge

Table 1. Effect of *P. eryngii* on immune and antioxidant indexes in mice.

| Immune indexes | Weight difference between both ears (mg) | Phagocytic index | Serum hemolysin | Serum GSH-$P_X$ (U/Ml) | Liver homogenate (0.25%) GSH-$P_X$ (U/mg protein) | Serum SOD (U/mL) | Liver homogenate (1%) SOD (U/mg protein) | Liver homogenate (10%) IL-6 (pg/mL) | Liver homogenate (10%) MDA(nmol/mg protein) |
|---|---|---|---|---|---|---|---|---|---|
| *P. eryngii* | 12.5 ± 2.8** | 4.48 ± 0.67* | 84.6 ± 9.9** | 583.49 ± 156.52** | 331.56 ± 70.26* | 198.62 ±18.69** | 59.04 ± 10.12 | 278.73 ± 86.33 | 0.291 ± 0.100** |
| Negative control | 9.3 ± 2.8 | 3.96 ± 0.44 | 60.9 ± 8.7 | 281.77 ± 103.97 | 261.29 ± 88.37 | 169.10 ± 14.40 | 50.96 ± 6.30 | 235.47 ± 25.28 | 0.832 ± 0.187 |

*Means a significant difference compared with the negative control ($P < 0.05$).
**Means a highly significant difference compared with the negative control ($P < 0.01$).

between 24 and 48 h, and was detected during the evaluation of DTH. These results demonstrated an increase in weight difference between both ears, suggesting that *P. eryngii* enhanced cellular immune functions in mice. In addition, an increase was also observed for mouse phagocytic index, demonstrating that *P. eryngii* enhanced the phagocytic function of mononuclear phagocytes in mice. The measured SRBC agglomeration efficiency of anti-SRBC antibodies generated in immunised mice revealed an increase in serum hemolysin which indicated that *P. eryngii* also enhanced the humoral immune function in mice. Additionally, *P. eryngii* may produce a significant increase in GSH-$P_X$ activity in serum and liver, and increased SOD activity in serum as well as a decrease in MDA level in liver of mice, suggesting that *P. eryngii* may scavenge excessive free radicals and execute antioxidant and anti-ageing functions.

### *3.2. Effects of* P. eryngii *on human cytokines*

#### *3.2.1. Effects of low-dose* P. eryngii *on human cytokines*

Cytokine analysis (Figure 1) indicated that *P. eryngii* had little effect on 23 kinds of the cytokines evaluated, including eotaxin, Flt-3L, G-CSF, IL-1$\beta$, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p40), IL-13, IL-15, MCP-1, MCP-3, MDC, sCD40L, TNF-$\alpha$ and TNF-$\beta$ in serum. Lower levels of serum FGF-2, Fractalkine, GM-CSF, IFN-$\alpha$2, MIP-1$\alpha$, sIL-2Ra, TGF-$\alpha$ and VEGF were detected in PESP-treated group on the second day compared with the first day, indicating that the expression of these cytokines was inhibited. Conversely, the expression levels of EGF, GRO, IFN-$\gamma$, IL-1$\alpha$, IL-12 (p70), IL-17, IP-10 and MIP-1$\beta$ were up-regulated, while the Student's *t*-test

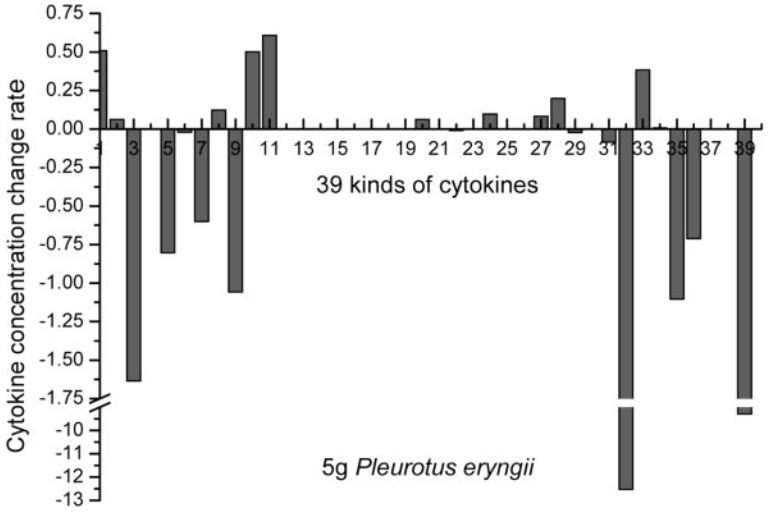


Figure 1. Effect of *P. eryngii* at the dosage of 5 g on 39 kinds of human cytokines.
Note: 1. EGF, 2. Eotaxin, 3. FGF-2, 4. Flt-3L, 5. Fractalkine, 6. G-CSF, 7. GM-CSF, 8. GRO, 9. IFN-$\alpha$2, 10. IFN-$\gamma$, 11. IL-1$\alpha$, 12. IL-1$\beta$, 13. IL-1ra, 14. IL-2, 15. IL-3, 16. IL-4, 17. IL-5, 18. IL-6, 19. IL-7, 20. IL-8, 21. IL-9, 22. IL-10, 23. IL-12(p40), 24. IL-12(p70), 25. IL-13, 26. IL-15, 27. IL-17, 28. IP-10, 29. MCP-1, 30. MCP-3, 31. MDC, 32. MIP-1$\alpha$, 33. MIP-1$\beta$, 343. sCD40L, 35. sIL-2Ra, 36. TGF-$\alpha$, 37. TNF-$\alpha$, 38. TNF-$\beta$ and 39. VEGF.

indicated that only serum EGF was significantly down-regulated by *P. eryngii* (Table 1). Thus, *P. eryngii* at a dosage of 5 g had a down-regulatory effect on EGF.

### *3.2.2. Effects of middle-dose P. eryngii on human cytokines*

*P. eryngii* at a dosage of 10 g had no effect on serum FGF-2, Flt-3L, fractalkine, GRO, IFN-$\gamma$, IL-1$\beta$, IL-1ra, IL-2n IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40), IL-13, IL-15, IL-17, IP-10, MCP-1, MCP-3, MDC, sIL-2Ra, TGF-$\alpha$, TNF-$\beta$ and VEGF (Figure 2). A decline in serum EGF, eotaxin, G-CSF, GM-CSF, IFN-$\alpha$2, IL-12(p70), MIP-1$\beta$, sCD40L and TNF-$\alpha$ was detected in the subjects supplemented with *P. eryngii* on the second day compared with the first day, which demonstrated that the expression of these nine cytokines was down-regulated by *P. eryngii*. Serum IL-1$\alpha$ and MIP-1$\alpha$, however, exhibited an up-regulation effect. No significant change was observed for any cytokine as demonstrated by both the *t*-test and the statistical significance analysis, therefore, *P. eryngii* at a dosage of 10 g had no significant effect on the expression of the 39 kinds of cytokines evaluated in the study.

### *3.2.3. Effects of high-dose* P. eryngii *on human cytokines*

*P. eryngii* at a dosage of 15 g had no significant effect on serum Flt-3L, GRO, IFN-$\gamma$, IL-1$\alpha$, IL-1$\beta$, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12(p40), IL-15, MCP-3, MIP-1$\alpha$, sIL-2Ra or TNF-$\beta$ (Figure 3). The concentrations of EGF, eotaxin, FGF-2, fractalkine, G-CSF, GM-CSF, IFN-$\alpha$2, IL-8, IL-12 (p70), IL-13, IL-17, IP-10, MCP-1, MDC, MIP-1$\beta$, sCD40L, TGF-$\alpha$, TNF-$\alpha$ and VEGF were lower on the second day than those on the first day, which suggested down-regulated expression. As seen in Table 2, both the *t*-test and statistical significance analysis indicated that GM-CSF, MDC

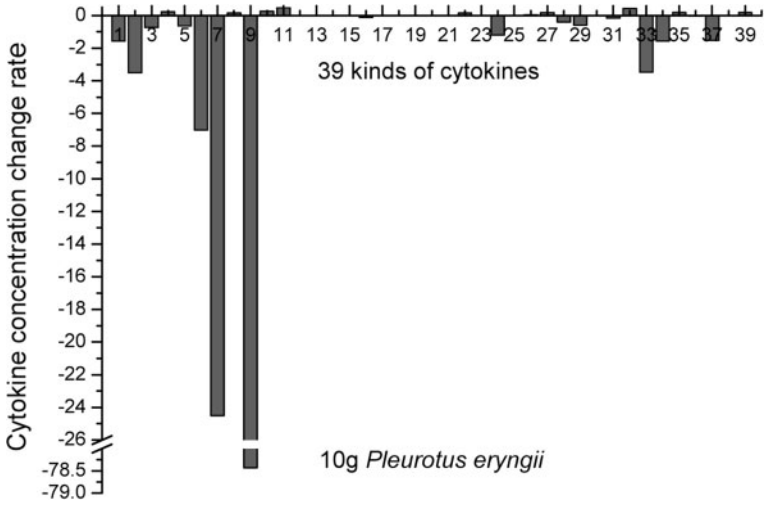


Figure 2. Effect of *P. eryngii* at the dosage of 10 g on 39 kinds of human cytokines.
Note: 1. EGF, 2. Eotaxin, 3. FGF-2, 4. Flt-3L, 5. Fractalkine, 6. G-CSF, 7. GM-CSF, 8. GRO, 9. IFN-$\alpha$2, 10. IFN-$\gamma$, 11. IL-1$\alpha$, 12. IL-1$\beta$, 13. IL-1ra, 14. IL-2, 15. IL-3, 16. IL-4, 17. IL-5, 18. IL-6, 19. IL-7, 20. IL-8, 21. IL-9, 22. IL-10, 23. IL-12(p40), 24. IL-12(p70), 25. IL-13, 26. IL-15, 27. IL-17, 28. IP-10, 29. MCP-1, 30. MCP-3, 31. MDC, 32. MIP-1$\alpha$, 33. MIP-1$\beta$, 343. sCD40L, 35. sIL-2Ra, 36. TGF-$\alpha$, 37. TNF-$\alpha$, 38. TNF-$\beta\beta$ and 39. VEGF.

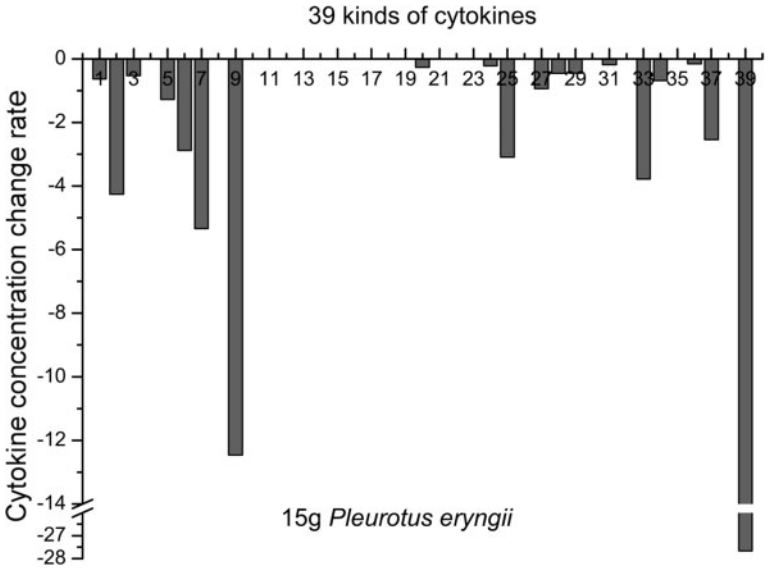


Figure 3. Effect of *P. eryngii* at the dosage of 15 g on 39 kinds of human cytokines.
Note: 1. EGF, 2. Eotaxin, 3. FGF-2, 4. Flt-3L, 5. Fractalkine, 6. G-CSF, 7. GM-CSF, 8. GRO, 9. IFN-$\alpha$2, 10. IFN-$\gamma$, 11. IL-1$\alpha$, 12. IL-1$\beta$, 13. IL-1ra, 14. IL-2, 15. IL-3, 16. IL-4, 17. IL-5, 18. IL-6, 19. IL-7, 20. IL-8, 21. IL-9, 22. IL-10, 23. IL-12(p40), 24. IL-12(p70), 25. IL-13, 26. IL-15, 27. IL-17, 28. IP-10, 29. MCP-1, 30. MCP-3, 31. MDC, 32. MIP-1$\alpha$, 33. MIP-1$\beta$, 343. sCD40L, 35. sIL-2Ra, 36. TGF-$\alpha$, 37. TNF-$\alpha$, 38. TNF-$\beta$ and 39. VEGF.

Table 2. Cytokines that changed significantly in response to ingestion of 15 g of *P. eryngii*.

| Cytokines | *P* value | Possibility | Rate of change |
|---|---|---|---|
| GM-CSF | 0.097 ↓ | * | 5.343 |
| MDC | 0.086 ↓ | * | 0.181 |
| TNF-$\alpha$ | 0.045 ↓ | ** | 2.540 |

*Means a significant difference compared with the negative control ($P < 0.05$).
**Means a highly significant difference compared with the negative control ($P < 0.01$).
↓Means concentration decreased.

and TNF-$\alpha$ exhibited significant reductions in the expression, indicating a down-regulatory effect at this dosage.

### *3.3. Construction and analysis of intercellular communication networks*

Cytokines are highly effective, multi-functional small protein molecules that are secreted and synthesised by immune and non-immune cells. And they play multiple roles in regulating cellular functions (Manson & Annan, 1971). Due to these multiple functions and cross-interactions, it is difficult to analyse the specific functions of these cytokines. To systematically analyse the overall interactions among cytokines with a significant change in response to *P. eryngii* ingestion, we searched the *Cytokines & Cells Online Pathfinder Encyclopedia Version 29.0* (http://www.copewithcytokines.de/cope.cgi) for data on cells responsible for cytokine secretion and their target cells, and subsequently

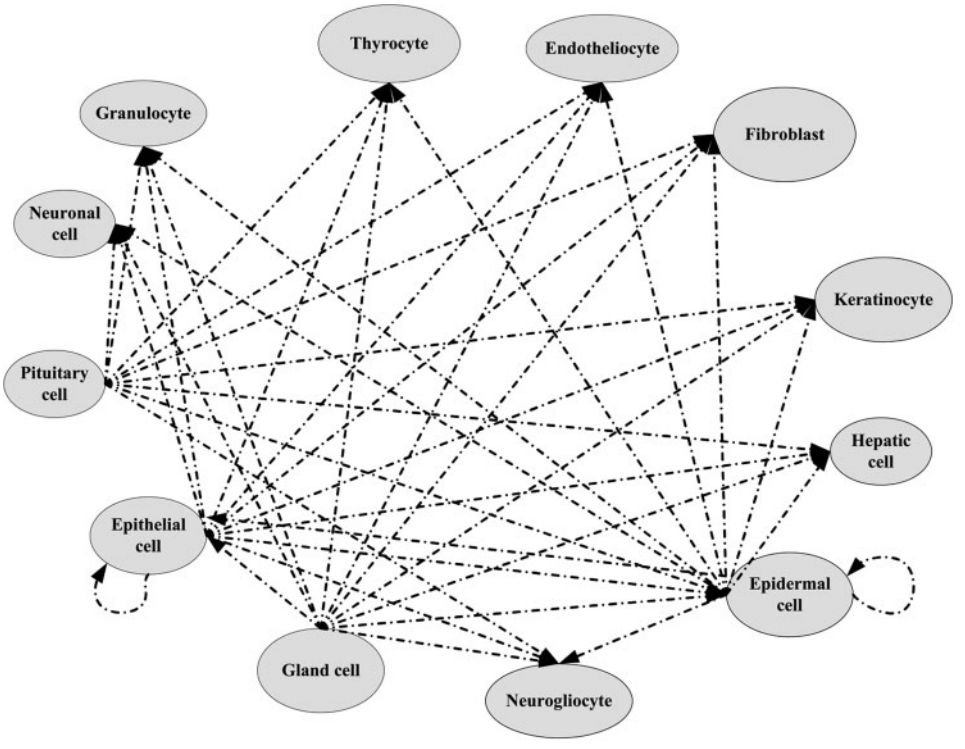


Figure 4. Intercellular communication network that represents the interactions of 12 types of cells in response to ingestion of 5 g of *P. eryngii*.

established intercellular communication networks representing their mutual activations or inhibitions (Pang, Chen, Hu, & Xie, 2010). Based on intercellular interaction analysis, further efforts will be needed to clarify the immunoregulatory effects and possible mechanisms of *P. eryngii* at different doses in humans.

### *3.3.1. Low-dose intercellular communication network*

An intercellular communication network representing the interactions among 12 types of cells in response to *P. eryngii* at a dosage of 5 g is presented in Figure 4. Among these, signalling cells include pituitary and glandular cells; receiving cells include epidermal and epithelial cells; and effector cells include neuronal cells, granulocytes, thyroid cells, endothelial cells, fibroblasts, keratinocytes, hepatocytes and neuroglial cells.

We speculated that the secretion of EGF by pituitary cells, glandular cells and epidermal cells was activated upon ingestion of 5 g *P. eryngii*. EGF performs a variety of physiological functions, such as promoting normal cellular differentiation, stimulating the proliferation of epidermal cells, fibroblasts and thyroid cells, inducing the formation of newly generated blood vessels, accelerating wound healing (Chen, Wu, Jiang, Huang, & Wang, 2005) and participating in uptake and absorption of nutrients and the proliferation, differentiation and maturation of gastrointestinal epithelial cells (Dvorak, 2010). In addition to affecting cell-cycle transitions in target cells and consequently accelerating their differentiation and proliferation, EGF plays an important role in tissue repair by

stimulating intracellular DNA, RNA and protein synthesis (Fu, Sun, & Wang, 1999). Studies have demonstrated that EGF activates a number of different cellular signalling pathways upon binding to receptors (Zhao & Liu, 2011), including: (1) the receptor tyrosine kinase-Ras-mitogen-activated protein kinase (RTK-Ras-MAPK) signalling pathway which promotes innate immune responses and activates acquired immune responses; (2) the phosphoinositide 3-kinase/protein kinase B (PI3K-PKB) signalling pathway which induces cellular growth through Bcl-2 family, blocks cell apoptosis and maintains cell survival through Bad and Caspase 9, and participates in vesicular transport, cell migration and other activities; and (3) the janus kinase/signal transducer and activator of transcription (JAK-SATA) signalling pathway which is involved in cell proliferation, differentiation, apoptosis, immunoregulation (He & Wu, 2009), and the regulation of gene transcription (Li, 2008). Data from this study indicated that *P. eryngii* at a dosage of 5 g enhanced the ability of liver to detoxify and remove toxins, promoted tissue repair and activated innate and acquired immune responses by driving EGF secretion. It also stimulated the proliferation of epidermal cells, fibroblasts and endothelial cells and activated corresponding signalling pathways (Figure 4).

### *3.3.2. High-dose intercellular communication network*

Figure 5 demonstrates an intercellular communication network representing the interactions among 19 types of cells in response to *P. eryngii* at a dosage of 15 g, which includes: osteoblasts, mast cells, B-cells, hepatocytes and smooth muscle cells as signalling cells, neutrophil cells, monocytes, fibroblasts, endothelial cells, monocyte-derived dendritic cells, macrophages, T-cells, synovial cells, astrocytes, microglia and natural killer (NK) cells as receiving cells; and eosinophils, megakaryocytes and osteoclasts as effector cells.

As seen in Table 2 and Figure 5, the high-dose of *P. eryngii* dramatically down-regulated GM-CSF and MDC, thus preventing an overexpression induced by inflammation, and increasing protection from excessive inflammation and chemotaxis. GM-CSF, which is important for chemotaxis and activation of inflammatory cells, is induced in T-cells, macrophages, endothelial cells and mast cells at inflammation sites (Middleton & Thatcher, 1998). It not only participates in the regulation of neutrophils and macrophages through an auto-secretion mechanism in combination with other cytokines, such as TNF-$\alpha$ and IL-6, but also directly effects the differentiation and proliferation of epithelial cells, fibroblasts and vascular endothelial cells (Bussolino et al., 1989; Hancock, Kaplan, & Cohn, 1988; Imokawa, Yada, Kimura, & Morisaki, 1996; Kawada et al., 1997). The overexpression of GM-CSF is reversely down-regulated by activated mature macrophages, thus relieving inflammation. TNF-$\alpha$ expression was also down-regulated following the ingestion of 15 g *P. eryngii*. TNF-$\alpha$ promotes the growth, differentiation and apoptosis of cells and induces inflammation by binding to specific cell membrane receptors (Qiu, Hou, & Huang, 2007). TNF-$\alpha$, in combination with IL-1, induces changes in endothelial cells, resists anti-coagulation action and promotes thrombosis. In addition, while normal immune action of TNF-$\alpha$ is essential for the body, overexpression indicates high risk for developing severe diseases. Inflammation and thrombosis can be inhibited by down-regulation of TNF-$\alpha$. *P. eryngii* in the 15 g dose group can promote the activity of NK cells and also play a role in inhibiting tumour (Jeong et al., 2010).

GM-CSF plays a positive role in the development of prostate cancer by activating the MAPK signalling pathway (Chen et al., 2006). It also regulates the expression of protease activated receptors (PARs) in mast cells and stimulates the secretion of IL-4 and IL-6 in

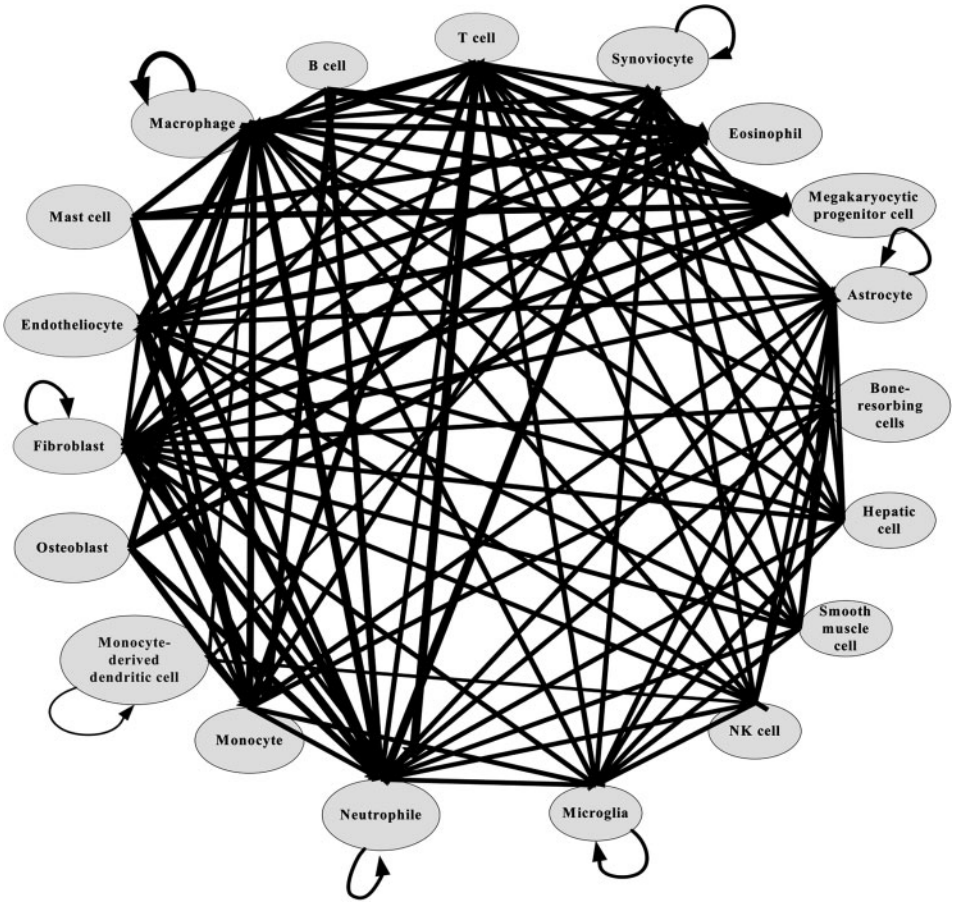


Figure 5. Intercellular communication network that represents the interactions of 19 types of cells in response to ingestion of 15 g of *P. eryngii*.

mast cells by activating PKB and extracellular signal regulated kinase (ERK) signalling pathways, which suggests that GM-CSF exerts condition-dependent regulatory effects on Th1 and Th2 cytokine secretion and plays a vital role in allergic inflammation (Zhang, 2008). TNF-$\alpha$ inhibits the growth of non-regulatory lymphocytes and thus reduces the possibility of developing cancer by activating the nuclear factor-κB (NF-κB) signal pathway (Jin & Liu, 2000). By activating the c-Jun N-terminal kinase (JNK) signalling pathway, TNF-$\alpha$ also phosphorylates insulin receptor substrate-1 and thus inactivates its activity. Serine phosphorylation of insulin receptor substrate-1 in lipocytes is induced while tyrosine phosphorylation is inhibited, resulting in insulin resistance (Wang & Du, 2009). The p38 MAPK signalling pathway plays a crucial role in TNF-$\alpha$-induced malfunction of vascular endothelial cells (Yuan, Wang, & Yang, 2009). Administration of *P. eryngii* at a dosage of 15 g down-regulated the expression of GM-CSF, MDC and TNF-$\alpha$ (Figure 5) by activating related signalling pathways and thereby inhibiting the proliferation of fibroblasts, endothelial cells and macrophages, and functioning to inhibit tumour growth, relieve allergic inflammation and resist diabetes.

## 4. Discussion

The effects of *P. eryngii* on human cytokine change varied by dosage. Low and high doses of *P. eryngii* resulted in different immune regulatory effects, that is, dual regulation, which is in agreement with the theory of traditional Chinese medicine. We speculated that the mechanism responsible for these effects was associated with concentration changes of different cytokines and complex intercellular communication networks in response to *P. eryngii* at various doses. However, further studies are needed to explore the molecular mechanisms. Dosage selection according to the constitution and physical condition of different populations would produce different health benefits. Low-dose *P. eryngii* ingestion could promote EGF secretion, stimulate the proliferation, differentiation and migration of epidermal cells and enhance immune responses and blood circulation, whereas high-dose ingestion could induce anti-inflammation, antioxidant and anti-ageing effects, prevent and resist cancers and restrain excessive immune responses.

Cytokines and intercellular communication networks are a self-stabilising and self-regulating system in healthy populations, which should be free from excessive intervention; otherwise metabolic and endocrine disorders may be induced. Therefore, regulation and intervention with foods, dietary supplements or medicines may be considered when the body largely deviates from its normal conditions, that is, "bias" in the theory of traditional Chinese medicine. *P. eryngii* is safe to eat, presumably because the mushroom at the middle dose of 10 g does not cause any significant cytokine change in the body. This study indicates that long-term, high-dose ingestion of *P. eryngii* may play a role in the adjuvant therapy for modern diseases that are induced by excessive nutrients or excessive immune response and which represent global epidemiological problems.

## 5. Conclusion

This study demonstrates that dietary supplementation with PESP regulated innate and acquired immune functions and produced antioxidant and anti-ageing effects in mice. Different regulatory effects on human cytokines were observed for *P. eryngii* ingestion at doses of 5 and 15 g. Ingestion of 5 g *P. eryngii* promoted EGF secretion which stimulated the proliferation of epidermal cells, fibroblasts and endothelial cells, activated the RTK-Ras-MAPK, PI3K-PKB, PKC and JAK-SATA signalling pathways, and enhanced the ability of the liver to filter out and remove toxins, promote tissue repair and activate innate and acquired immune functions. By contrast, *P. eryngii* ingestion at 15 g inhibited the proliferation of fibroblasts, endothelial cells and macrophages, activated MAPK, Akt, ERK, NF-κB, JNK and P38/MAPK signalling pathways and down-regulated the expression of GM-CSF, MDC and TNF-$\alpha$ which can prevent excessive immune responses, suppress tumorigenesis, relieve allergic inflammation and promote resistance to diabetes.

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