Original Article

# The immune effects of edible fungus polysaccharides compounds in mice

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Most of the current researches on immune function of fungus polysaccharides were based on individual component. Only a few studies were involved in the mixture of proprietary compounds from different species of edible fungi. The main objective of this study was to evaluate mice's immune effects of the mixed polysaccharides (ratio 1∶ 0.5∶ 0.5) extracted from Lentinus edodes, Ganoderma lucidium and Grifola frondosa. Kunming mice weighted 20±2 g (10 mices/group) was treated for 30 consecutive days with polysaccharides compounds, which were added to basal diet at three concentrations: 0.17, 0.33 and 1.00 g/kg body weight. Then the NK cells' activities, ratio thymus/body and spleen/body, macrophage's activities, hemolysis, and delayed hypersensitivity (DTH) were determined by standard methods. Polysaccharides compounds at the concentrations of 0.33 and 1.00 g/kg body weight significantly increased the thymus and NK cells activities ($p$ <0.01), as well as the ability of macrophages to phagocyte latex particles and the activity of macrophages ($p$ <0.05). The hemolytic test and DTH of the tested groups had no remarkable difference compared to that of the control group ($p$ >0.05). The results indicated that the mixed polysaccharides compounds could enhance the cell immune of the mice.

**Key Words: mixed polysaccharides, immune, edible fungi**

## Introduction

The early studies of those anticancer substances from fungus came in the late 1960s reported by Ikewa *et al*., 1968, 1969[1,2] and Chihara *et al*., 1969,1970.[3,4] They demonstrated that extracts of several different mushroom species exhibited remarkable host-mediating antitumour activities against xenographs, e.g. sarcoma 180. In those studies the compounds were shown to be various types of polysaccharides.

Polysaccharides are now attracting greater attention within the field of medicine. This is because the Polysaccharides have a number of effects that include the ability to stimulate T-cell formation and potentiate the induction of different types of antitumor effector cells such as cytotoxic T-cells, NK cells and macrophages.[5] Many immunostimulating polysaccharides also possess anti-tumor properties.[6, 7]

The research is still going on and far from being closed because the roles and the mechanisms of polysaccharides on immune system are largely unknown. So far, most of previous studies have focused on immunological effects of known individual component. Little is known about the combined polysaccharides. Thus, here we explored the functions of edible fungus polysaccharides compounds in this field.

## Materials and methods

### *Extraction process of the polysaccharides compounds*

One kilogram edible fungus smashed was immersed in 30 L of 100 °C distilled water for 5 hours. After filtering, the extracted solution was concentrated. Alcohol was added to the concentration (alcohol: concentration is 6:1) and rested for 10 hours at 10 °C to sediment the polysaccharides compounds. The compounds were used in experiments after they were dried.

### *Effects of polysaccharides on thymus and spleen indexes*

The mice of each group were fed with samples once a day for 30 days. We sacrificed the mice and weighted their thymuses and spleens. The indexes were expressed as the thymus and spleen weight relative as body weight.

Spleen / thymus index = Spleen / thymus: body weight

### *Determination of NK cell's activity (LDH determination method)*

Forty healthy Kunming female mice were randomly divided into four groups (10 mice/group): three groups were fed respectively with high, middle, or low doses (0.17, 0.33 or 1.00 g/kg body weight) of the mixture of polysaccharides extracted from *Lentinus edodes*, *Ganoderma lucidium* and *Grifola frondosa*(ratio 1:0.5:0.5). Control group mice were fed with distill water. Each group of animals was allowed to feed on their dose once everyday successively during 30 days. On the last day of the feeding, take out each spleen and put it in a stable device of glass containing a Hanks disinfected solution.

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Spleen was softly smashed using nippers and filtered with 200 meshes-sieves. The collected single impure cells were washed and calculated. Finally, adjust the solution of cells to $5\times10^6$ cell/mL with the RPMI 1640 medium. The YAC-1 (target cells) was transferred for culture before 24 hours. Before culturing, cells were washed three times with the Hanks solution and adjusted their concentration to $1\times10^5$cell/mL with the RPMI 1640 medium. Cultured target cells and impure cells of spleen (Effective cells), every 100 μL (Target rate 50:1), in round bottom 96-well plates. Naturally to set free target cells with feeding liquid to 100 μL. Take the biggest flask to dilute target cells with 100 μLof 1% $NP_4O$. Three repeated wells were used for each concentration. The cells were incubated in a 5% $CO_2$-air mixture at 37℃ for 4 hours and centrifuged. Then 100 μLof floating liquid of the mixture was taken out and placed on a 96-well micro plate with 100 μl of LDH solution. After 3 minutes, 30 μL of 1 mol/L of salty solution was added to each well. Measure the value of the absorbency and calculate the vitality of NK cells.

NK cell activity was calculated as by the following equation:

NK cell activity (%) =$[(T_0- (S-E))/T_0] \times100$

Where $T_0$ is optical density of the control target cell; S is that of test; E is that of effect cell.

***Experiment on the macrophages of the abdominal cavity of mice by phagocytosis the erythrocytes of chicken***

Make 4 sets of 10 mice with a blank group as control. Feed each set of animals with a precise quantity (0.17, 0.33, 1.00 g/kg) of combined polysaccharides, once every day successively during 30 days. An hour before sacrifice, mice were injected with 1 mL of 20% (V/V) chicken's red blood cells (CRBC) solution. After sacrifice, mice were injected with 2 mL of physiological saline solution to extract the fluid from the abdomen. The extracted cells were incubated for 30 min at 37 ℃. The numbers of CRBC ingested by 100 macrophages were counted in an optical microscope and expressed as phagocytosis index (PI). The percentages of macrophages that phagocytosed CRBC were determined and expressed as phagocytic efficiency index (PEI).

***Dinitrofluorobenzene introduced delayed type hypersensitivity test***

The mice in each group were fed with samples infusion once a day for 30 days. On the 25th day, 50 μL of dinitrofluorobenzene solution was introduced to the abdomen area of each mouse (abdomen area was pre-denuded 24 hours before). Five days later, 10 μL dinitrofluorobenzene solution was introduced to the right ear. Kill the mice after 24 hours, cut 8 mm diameter circular ear tissue from each ear, weight and calculate the difference between left ear and right ear.

**Mouse micronucleus test**

Fifty ICR mice (weight from 21 to 25 g) were randomly divided into five groups (10 mice/group, every group includes five males and five females). Polysaccharide complex was dissolved in distilled water and administered orally one times, 24 hours apart, at doses of 2.5, 5.0, 10.0 g/kg body weight every day. The animals were killed at 6 hours after the final doses. Their sternum marrow cells were analyzed following methanol fixing and Giemsa staining. The frequency of micronuclei (MN) was counted based on an examination of 1000 polychromatic erythrocytes (PCE) per mouse. Distilled water and cyclophosphamide (60mg/kg) were given to mice as negative and positive control, respectively.

**Results and discussion**

***Ratios of thymus/body and spleen/body***

The mixture of polysaccharides caused an increase in the thymus index compared with the control group (Table 1). The effect of the polysaccharides mixture at a dosage of 1.00 g/kg on ratio of thymus to body was the significant and strongest increase as compared with other dosages. On the other hand, the polysaccharides complex had no significant effect on the spleen/body ratio. Thymus and spleen are main immunization organs; some immunosuppressive agent can make them shrink while immunopotentiator can increase their weight. The bigger the immunity index is, the stronger the immune capability is. The increase of the thymus index indicated that the mixture of polysaccharides could enhance the cell-mediated immunity, and thus have the ability to stimulate T cell formation.

***Natural killer cells***

Natural Killer cells are antitumour effector cells. Patients with cancer typically have fewer NK cells in their blood.

**Table 1.** Effects of polysaccharides complex on thymus/body and spleen/body ratio

| Group | Thymus/body (Mean±SD) | Spleen/body (Mean±SD) |
|---|---|---|
| Control | 0.28±0.07 | 0.37±0.05 |
| 0.17 g/kg | 0.32±0.08 | 0.36±0.13 |
| 0.33 g/kg | 0.35±0.07 | 0.44±0.12 |
| 1.00 g/kg | 0.38±0.06* | 0.35±0.05 |

* $p$ <0.05, n=10

**Table 2.** NK cells' activities of the four groups mice

| Group | NK Cells' activities (%) |
|---|---|
| Blank | 15.0±5.6 |
| 0.17 g/kg | 17.3±3.8 |
| 0.33 g/kg | 21.7±6.1 |
| 1.00 g/kg | 63.0±15.0** |

** $p$ <0.01, n=10

**Table 3.** Results of the Macrophage phagocytosis assay

| Group | phagocytic efficiency index | phagocytosis index |
|---|---|---|
| Blank | 25.8±7.2 | 0.50±0.25 |
| 0.17 g/kg | 35.6±7.6 | 0.70±10.76 |
| 0.33 g/kg | 36.1±8.3* | 0.70±10.76 |
| 1.00 g/kg | 33.8±10.8 | 0.69±0.24 |

*$p$ <0.05, n=10

**Table 4.** Amount of antibody in the four groups mice by hemolysis test

| Group | Amount of antibody (Mean±SD) |
|---|---|
| Control | 213.0±32.2 |
| 0.17 g/kg | 207.8±19.7 |
| 0.33 g/kg | 209.9±19.2 |
| 1.00 g/kg | 216.5±31.8 |

n=10

Thus, it is essential to enhance natural killer activity to kill certain types of cancer cells. NK cells' activity in high dose groups was significantly higher than that in the control group ($p$<0.01, Table 2). Results showed that kumming female mice given 1g/kg of mixture of polysaccharides from *Lentinus edodes, Ganoderma Lucidium and Grifola frondosa* in Table 3, indicated that the treat group with the dosage of 1 g/kg polysaccharides complex had higher macrophage phagocytosis activity compared with control group ($p$<0.05). A significant increase in macrophage phagocytosis was induced in mice tested. The mixture of polysaccharides could markedly increase macrophage phagocytosis. Macrophages play a pivotal role in non-specific immunity because it stimulated macrophages and natural killer cells to destroy cancer cells. The results showed that the feeding of mixture of polysaccharides can augment both the ability of macrophages to phagocyte latex particles and the activity of the macrophages. The dosage of 1 g/kg had the strongest effect among three dosages.

***Macrophages***

The macrophages test was carried out in the Table 3. There is no remarkable difference between control group and experimental groups ($p$>0.05) except for the experimental group 0.33 g/kg ($p$<0.05). Protective immunity against tumor is composed of both humoral and cellular immunity. Mixture of polysaccharides to mice could increase the ability of macrophages to phagocyte latex particles and spreading activity of the macrophages. The concentration of 0.33 g/kg had the best effect.

The results showed that the mixture of polysaccharides doesn't accelerate B cell antibody formation, which indicates that it could not restore the humoral immunity against sheep red blood cells (SRBC).

***Hemolysis***

A delayed hypersensitivity test is an immune function test measuring the presence of activated T cells that recognize a certain substance. There is no remarkable difference between control group and experiment group in this test ($p$>0.05, Table 4).

The results indicated all the same the stimulatory effect of mixture of polysaccharides on delayed type hypersensitivity (DTH) cells and other immune cells involved in antigen processing. The results showed that the mixture of polysaccharides did not accelerate B cell antibody formation. It could not restore the humoral immunity against sheep red blood cells (SRBC).

**Table 5.** Delayed type hypersensitivity (DTH) of the four groups mice

| Group | Ear weight difference (mg) (Mean±SD) |
|---|---|
| Control | 9.4±3.8 |
| 0.17 g/kg | 9.1±3.8 |
| 0.33 g/kg | 11.4±3.8 |
| 1.00 g/kg | 11.8±4.5 |

n=10

***Dinitrofluorobenzene test***

The result of dinitrofluorobenzene test was demonstrated in Table 5. There was no remarkable difference between control group and treated groups (variance analysis, $p$>0.05). This indicated that all concentration of mixed polysaccharides had the same effects on delayed type hypersensitivity (DTH).

The present study demonstrated that the treatment in mice with fungus polysaccharides compounds at certain doses can improve some immune activities, and thus increase the tumor control rate. There was report that the highest control rate can reach 49.3% [8]. In the present study, the polysaccharide compounds not only increased the rate of tumor control, but also enhanced the activity of NK cells as well as the phagocytic function of mice macrophages. The thymus weight was also increased.

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