INVESTIGATION OF IMMUNOMODULATORY ACTIVITY OF HERICIUM ERINACEUS ON CYCLOPHOSPHAMIDE-INDUCED IMMUNOSUPPRESSION IN MICE

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The study was conducted to assess the immunostimulatory effect of Hericium Erinaceus on cyclophosphamide-induced immunodeficient mice. Animals were administered Hericium Erinaceus orally at 0.72 g/kg b.w and 1.44 g/kg b.w for seven consecutive days. On the 4th day, CP (200 mg/kg) was intraperitoneally injected into mice. The results illustrated that Hericium Erinaceus at 0.72 g/kg b.w only posed an immunostimulating potency in the cyclophosphamide-induced immunosuppression model. Besides, Hericium Erinaceus at 1.44 g/kg b.w ameliorated the effects of CP on delayed-type hypersensitivity (DTH) response, relative spleen weight, serum IL-2, TNF-α concentration, and micro-histological images of spleen and thymus substantially. This suggested the potential of Hericium Erinaceus to treat immunosuppression diseases in clinical practice.

Keywords: ***Hericium Erinaceus, immunostimulatory effect, mice, cyclophosphamide*.**

I. INTRODUCTION

The immune system protects the body from infection by pathogens, environmental toxins, allergens, and cellular damage. The immune system comprises two parts: innate immunity and adaptive immunity. Innate immunity represents the first line of defense against an intruding pathogen. Adaptive immunity is antigen-dependent and antigen-specific; therefore, it involves a lag time between exposure to the antigen and maximal response.[1] There are two types of adaptive responses: antibody-mediated immunity and cell-mediated immunity. Antibody-mediated immunity or humoral immunity is mediated by B-cells. When B-cells recognize the pathogen, they are proliferated and differentiated into traditional antibody-secreting plasma cells. T-cells carry out cell-mediated immunity or cellular immunity. After the T-cells are activated, they stimulate the cytokine (such as IL-2, TNF-α) production that further mediates the effective immune response.[2]

Immunosuppression is a state of immunity responding to pathogens below the required level. Immunostimulators used with chemotherapy drugs activate or induce the mediators or components of the immune system. Therefore, the study of new immunostimulatory agents has great promise concerning the prevention and treatment of immunosuppressive diseases.[1] Most of the prescription immunostimulants (levamisole, isoprinosine,...) are synthetic substances with

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severe side effects. There is a growing interest of using natural immunomodulators to modulate the complex human immune system. [3]

Hericium erinaceus is a type of mushroom belonging to the family *Hericiaceae,* a well-known edible and medicinal plant. For a long history, it has had many functional benefits, such as antitumor effects, immunomodulatory activities, antioxidant properties, cytotoxic effects, and neuron growth-promoting effects.[4,5] So far, the effect of *Hericium erinaceus* on As such, we conducted this study to validate the immunostimulatory potency of *Hericium Erinaceus* against the immunosuppression induced by cyclophosphamide.

II. MATERIALS AND METHODS

1. Preparation of *Hericium Erinaceus*

Hericium Erinaceus was produced by FUSI Pharma Company and formulated in capsule form. Each capsule contains 500 mg of *Hericium Erinaceus*. The recommended dose for humans is six capsules daily for oral use.

Hericium Erinaceus capsules were dissolved in distilled water before being given to mice.

Drugs and chemicals

Cyclophosphamide (Endoxan 200 mg, Baxter Oncology GmbH, Germany) was a standard immunosuppressive agent. Levamisole was obtained from Sigma (Aldrich) and used as a positive control in this experiment. Sheep red blood cells (SRBC) and OA solution (ovalbumin + $Al(OH)_3$) were used as the antigenic materials.

2. Animals

Swiss albino mice of either sex, weighing 20 ± 2 g, were purchased from the National Institute of Hygiene and Epidemiology. The mice were kept in cages (10 animals per cage) and water and standard food was provided ad libitum. Before the experiment, mice were adapted to the laboratory condition for seven days. This experiment was conducted at the Department of Pharmacology, Hanoi Medical University.

Experimental design

The animals were divided randomly into five groups of ten mice per cage. Group I (control) was given an i.p. injection of physiological saline. Group II (model) was injected with CP in a single dose (200 mg/kg) on the 4th day of the experiment. Group III (levamisole 10 mg/kg + CP), group IV (*Hericium Erinaceus* at 0.72 g/kg b.w (equivalent to the human recommended dose, conversion ratio 12) + CP)) and group V (*Hericium Erinaceus* at 1.44 g/kg b.w (2 times as high as the human recommended dose, conversion ratio 12) + CP)). Mice were administered orally levamisole and *Hericium Erinaceus* for seven consecutive days with a single injection of CP on the 4th day. On the 8th day, mice were sacrificed to collect blood samples, and the spleen and thymus were collected to evaluate immune parameters.

Delayed type hypersensitivity (DTH) response

On the 2nd day of the model, animals were given an i.p. injection of sheep red blood cells, and OA 0.1 mL was injected into the spinal cords. DTH reaction was elicited five days later by injecting OA 50 µL into the right hind paw and physiological saline into the left after measuring the footpad's thickness. After 24 hours, the paw volume was measured again to assess the swelling degree of the footpad.

Counting of leukocyte quantity

Blood samples were collected on the day of sacrifice to determine the total WBC, lymphocytes, neutrophils, and monocytes.

Relative organ weight

The related weight of the spleen and thymus

was calculated using the following formula:

Relative organ weight = Organ weight (mg) / Body weight (g)

Histopathological study of spleen and thymus

The micro-histological examination was conducted at the Center for Research and Early Detection of Cancer (CREDCA). Assoc. Prof. Le Dinh Roanh, Director of CREDCA, gave results of pathological image analysis.

Assay for serum IL-2, TNF-α and IgM

On the 8th day of the experiment, blood samples were assayed to measure the concentration of serum IL-2, TNF-α, and IgM using an ELISA kit (Cloud-Clone Corp., USA).

3. Statistical analysis

The data were expressed as the Mean ± SD, and statistical analysis was carried out employing student's t-test.

III. RESULTS

1. Effect of *Hericium Erinaceus* on delayed-type hypersensitivity (DTH) response

Administration of CP (200 mg/kg, i.p) showed a significant decrease in the DTH response as compared with the control group (group I) ($p < 0.001$). In groups treated with *Hericium Erinaceus* at both doses of 0.72 g/kg/day (group IV) and 1.44 g/kg/day (group V), there was an upward trend in the DTH response compared with the model group. Still, no significant difference was observed ($p > 0.05$)(Figure 1).

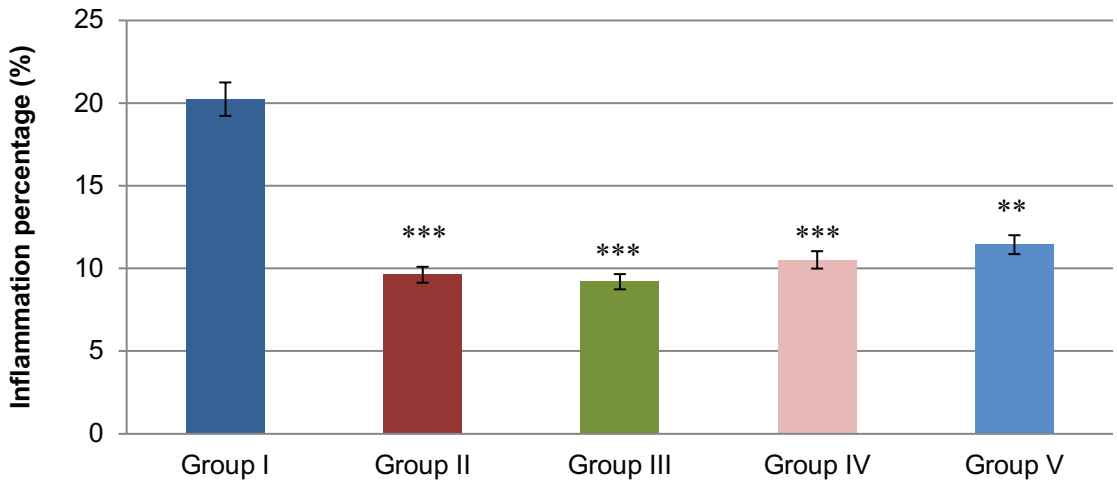


Figure 1. Effect of *Hericium Erinaceus* on delayed-type hypersensitivity (DTH) response

* $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$ compared with group I (control group)

$^{\Delta}$ $p < 0.05$, $^{\Delta\Delta}$ $p < 0.01$, $^{\Delta\Delta\Delta}$ $p < 0.001$ compared with group II (model group)

2. Effect of *Hericium Erinaceus* on leukocyte quantity

Mice treated with CP showed a significant reduction in leukocyte quantity compared to the control group. In groups treated *Hericium Erinaceus* at 0.72 g/kg/day and 1.44 g/kg/day, no significant difference was observed in total WBC count, lymphocytes, neutrophils, and monocytes count as compared with the model group ($p > 0.05$)(Table 1).

Table 1. Effect of *Hericium Erinaceus* on leukocyte quantity

Group	Treatments	Leukocyte quantity (Means ± SD)			
		WBC (G/L)	LYM (G/L)	NEU (G/L)	MONO (G/L)
I	Control	6.11 ± 1.84	67.66 ± 8.42	8.63 ± 2.45	23.71 ± 8.03
II	Model	1.63 ± 0.58 ***	63.83 ± 14.21	10.87 ± 3.08	25.30 ± 12.83
III	Levamisole 10 mg/kg + CP	1.41 ± 0.26 ***	68.42 ± 9.89	15.38 ± 6.62 **	16.20 ± 9.50
IV	*Hericium Erinaceus* 0.72 g/kg + CP	1.77 ± 0.73 ***	54.83 ± 10.48 **	14.47 ± 4.93 **	30.70 ± 8.61
V	*Hericium Erinaceus* 1.44 g/kg + CP	1.47 ± 0.53 ***	63.82 ± 11.93	13.74 ± 3.42 **	22.44 ± 9.97

* $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$ compared with group I (control group)

$^{\Delta}$ $p < 0.05$, $^{\Delta\Delta}p < 0.01$, $^{\Delta\Delta\Delta}$ $p < 0.001$ compared with group II (model group)

3. Effect of *Hericium Erinaceus* on relative organ weight

Figure 2 shows that the relative spleen weight of the animals treated with *Hericium Erinaceus* at 0.72 g/kg increased slightly compared to group II. Still, no significant action was observed ($p > 0.05$). Besides, relative spleen weight in mice treated with *Hericium Erinaceus* at 1.44 g/kg dose increased markedly compared to group II ($p < 0.05$). There was no substantial difference between groups treated with *Hericium Erinaceus* and group II regarding the relative thymus weight ($p > 0.05$).

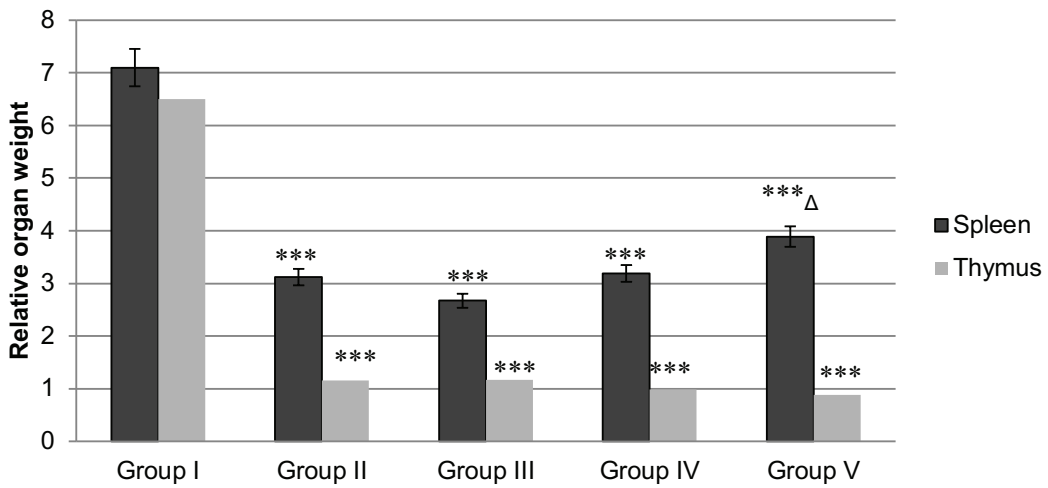


Figure 2. Effect of *Hericium Erinaceus* on relative organ weight

* $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$ compared with group I (control group)

$^{\Delta}$ $p < 0.05$, $^{\Delta\Delta}p < 0.01$, $^{\Delta\Delta\Delta}$ $p < 0.001$ compared with group II (model group)

4. Effect of *Hericium Erinaceus* on serum IL-2, TNF-α, and antibody IgM concentration

The table below (Table 2) illustrated a significant increase in serum IL-2 and TNF-α concentrations and a slight growth in serum IgM concentration in group treated levamisole compared to group II. *Hericium Erinaceus* at 0.72 g/kg showed an upward trend in serum IL-2 concentration and a significant increase in TNF-α concentration compared to group II. In the group treated with *Hericium Erinaceus* at 1.44 g/kg, serum IL-2, and TNF-α concentrations increased substantially compared to group II (with $p < 0.01$ and $p < 0.05$, respectively). There was no significant difference between groups treated with *Hericium Erinaceus* and group II regarding IgM concentration ($p > 0.05$).

Table 2. Effect of *Hericium Erinaceus* on serum IL-2, TNF-α, and antibody IgM concentration

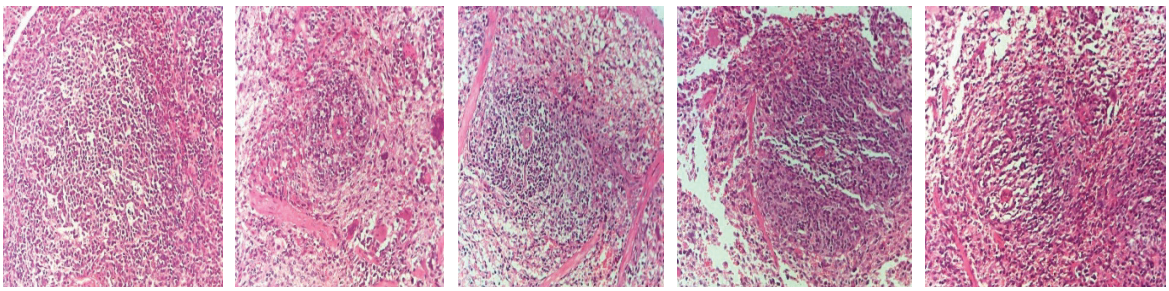
Group	IL-2 concentration Mean ± SD (pg/mL)	TNF-α concentration Mean ± SD (pg/mL)	IgM concentration Mean ± SD (µg/mL)
I	20.18 ± 4.30	19.11 ± 5.30	83.37 ± 18.04
II	18.34 ± 4.44	7.51 ± 2.38***	31.84 ± 9.09***
III	27.09 ± 9.18*$^{\Delta}$	18.61 ± 8.10$^{\Delta\Delta\Delta}$	37.37 ± 9.66***
IV	19.02 ± 5.90	13.53 ± 5.80*$^{\Delta\Delta}$	26.09 ± 7.04***
V	29.03 ± 10.71*$^{\Delta\Delta}$	10.21 ± 2.97***$^{\Delta}$	26.19 ± 8.55***

* $p < 0.05$, ** $p < 0.01$; *** $p < 0.001$ compared with group I (control group)

$^{\Delta}$ $p < 0.05$, $^{\Delta\Delta}$ $p < 0.01$, $^{\Delta\Delta\Delta}$ $p < 0.001$ compared with group II (model group)

5. Histopathological study of spleen and thymus

The micro-histological images (Figures 3 and 4) demonstrated that in the spleen and thymus of animals treated with CP, the number of lymphocytes decreased dramatically compared to the control mice. Levamisole and *Hericium Erinaceus* at both doses restored significantly the lymphocyte quantity in the spleen and thymus.



Group I Group I Group I Group I Group I

Figure 3. Micro-histopathological images of spleens (HE × 20)

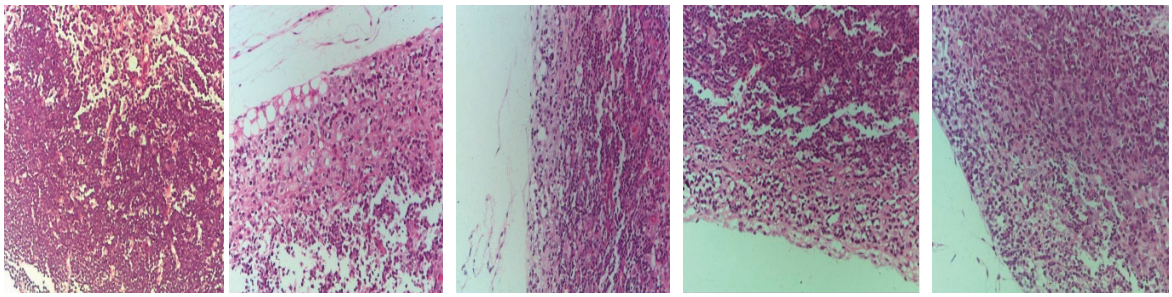


Figure 4. Micro-histopathological images of thymuses (HE × 20)

IV. DISCUSSION

Cyclophosphamide is one of the most commonly used alkylating agents, which produces toxic side effects, including immunotoxicity, hematotoxicity, and mutagenicity. It has a pronounced effect on lymphocytes and is usually used as an immunosuppressant.[6] In this study, CP at 200 mg/kg i.p caused a significant decrease in delayed-type hypersensitivity (DTH) response, leukocyte quantity, relative organ weight, serum antibody IgM, IL-2, and TNF-α concentration.

Bone marrow is a site of continued proliferation and turnover of blood cells and is a source of cells involved in immune reactivity. T-lymphocytes and other key immune system cells are known to activate the production of antibodies and polymorphonuclear granulocytes to destroy the antigen. Loss of stem cells and the inability of bone marrow to regenerate new blood cells will result in leucopenia. In this study, no significant change was observed in total WBC count, lymphocytes, neutrophils, and monocyte count in groups treated with *Hericium Erinaceus* compared to the model group.

In the DTH test, the DTH response directly correlating with cell-mediated immunity was considerable in animals treated with levamisole and Hericium Erinaceus. This is explained by the fact that after being challenged by antigen, the sensitized T-lymphocytes were converted to lymphoblasts, secreting various molecules, including proinflammatory cytokines, attracting scavenger cells to the site of reaction.[7] An increase in footpad thickness indicated the stimulatory effect of *Hericium Erinaceus* on the lymphocytes and accessory cell types required for the expression of this reaction.

Cytokines secreted by a range of cells, including lymphocytes, have an important role in the body's inflammation response, especially IL-2 and TNF-α.[8] In this study, there was a significant improvement in both IL-2 and TNF-α concentration in mice treated *Hericium Erinaceus* at 1.44 g/kg; However, only TNF-α concentration improvement was noted in mice treated *Hericium Erinaceus* at 0.72 g/kg as compared to the model group.

Antibody production induced by thymus-dependent antigen (sheep red blood cells) requires the cooperation of T- and B-lymphocytes and macrophages.[2] Immunoglobulin (Ig) M is the first antibody isotype to appear during evolution, ontogeny, and immune responses. IgM serves as the first line of host defense against infections and plays an important role in immune regulation and immunological tolerance.[9] However, no substantial difference in IgM concentration was observed between groups treated *Hericium Erinaceus* and group II ($p > 0.05$).

There was an upward trend in group IV's relative spleen weight and group V's relative thymus weight as compared to the model group. The increase in thymus and spleen weight was accompanied by the increase in cell numbers, improving the stimulatory effect of this powder on the lymphocytes and bone marrow hematopoietic cells. This result was consistent with the histopathological improvement in the thymus and spleen in experimental groups treated *Hericium Erinaceus* (Figures 3 and 4).

These results indicated that *Hericium Erinaceus* posed an immunostimulatory effect on cyclophosphamide-induced immunosuppression and showed that *Hericium Erinaceus* could potentially be developed as a new traditional herbal medicine. Our study was consistent with results from previous reports about the effect of *Hericium Erinaceus*.[4,5]

V. CONCLUSION

Hericium Erinaceus posed stimulatory impacts on the immune system suppressed by CP. Oral administration of *Hericium Erinaceus* at 0.72 g/kg b.w (equivalent to the human recommended dose) for seven consecutive days had an immunostimulating potency in the cyclophosphamide-induced immunosuppression model through indexes including DTH response, relative spleen weight, total WBC count, serum IL-2, TNF-α concentration, and micro-histological images of spleen and thymus. *Hericium Erinaceus* at 1.44 g/kg/day (2 times as high as the human recommended dose) significantly improved the effects of CP on DTH response, relative spleen weight, serum IL-2, TNF-α concentration, and micro-histological images of spleen and thymus.

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