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Immunomodulatory effects of *Hericium erinaceus* derived polysaccharides are mediated by intestinal immunology

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***Running Title**:* The immunomodulatory effects of HEP.

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Abstract

This study was aimed at investigating the immunomodulating activity of *Hericium erinaceus* polysaccharide (HEP) in mice by assessing splenic lymphocyte proliferation (cell-mediated immunity), serum hemolysin levels (humoral immunity), phagocytic capacity of peritoneal cavity phagocytes (macrophage phagocytosis), and NK cell activity. ELISA of immunoglobulin A (SIgA) in the lamina propria, and Western-blotting of small intestinal proteins were also performed for insight into the mechanism by which HEP affects the intestinal immune system. Here, we report that HEP improves immune function by functionally enhancing cell-mediated and humoral immunity, macrophage phagocytosis, and NK cell activity. In addition, HEP upregulated the secreation of SIgA and activated the MAPK and AKT cellular signaling pathways in the intestine. In conclusion, all these results allowed us to postulate that immunomodulatory effects of HEP are most likely attributed to effective regulation of intestinal mucosal immune activity.

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Introduction

Because some mushrooms contain bioactive compounds, they have become a focus of drug discovery and functional foods. *Hericium erinaceus* (also called "Houtou" in Chinese for its monkey head appearance) is a well-known edible mushroom that belongs to the *Aphyllophorales, Hydnaecase* and *Hericium* families[1]. This mushroom has been used as a food and in traditional Chinese medicines for the prevention and treatment of gastric ulcers, chronic gastritis and other digestive tract-related diseases in East Asian[2]. Even though it has been reported to produce several classes of bioactive molecules, including polysaccharides, proteins, lectins, phenols, and terpenoids, the active ingredients are mostly unclear.

In recent years, polysaccharides have attracted increasing attention for nutrition and food science because of their substantial medicinal properties and non-toxic side effects[3]. Polysaccharides are found mainly in the cell walls of fungi, in particular in the fruiting bodies where relatively large quantities (about 20 % of the biomass) [4, 5].

It has been reported that polysaccharide components were mainly glucose in *Hericium* with enhanced effect of T cells and macrophages in mice[6]. The water-soluble polysaccharides of *Hericium* exerted anti-tumor effect *in vitro* through activation of different immune cells. Han[7] and Lee[8] found that polysaccharides had anti-hecpatocarcinoma activity and activated natural killer (NK) cells. Liu[9] reported β -glucans extracted from *Hericium* showed significant anticancer properties in animal systems by increasing the number of CD4+ cells, T lymphocytes (T cells) and macrophages. The anticancer activity of an orally administered extract of *Hericium* in Balb/c mice was might mediated through immune response.

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The intestinal mucosa is a crucial barrier for host defense against invading pathogens and food antigens[10, 11]. Accumulating evidence shows that activated lymphocytes in the intestine migrate to the immune system circulation through mesenteric lymph nodes and finally home in to effective sites (e.g. lamina propria of the intestinal mucosa) to regulate mucosal and systemic immune responses that lead to an improved defense response or immune-compromised states[12].

The immune-modulatory effect of HEP in normal mice that might be analogous to consumption by humans, has not yet been studied, along with intestinal immunity imparted by HEP. Therefore, the present investigation was designed to assess effects on immune function and intestinal immunity in mice upon oral administration of HEP.

2.1 Materials

Guinea pig complement was purchased from Beijing Boise Technology Co. Ltd (Beijing, China). Sheep red blood cells (SRBC) were acquired from Yi Kang Biological Co. Ltd (Beijing, China). Fetal bovine serum and RPMI1640 medium were obtained from Gibco (Grand Island, NY, USA). Trypsin and PMSF were obtained from Amersco (Amersco LLC, OH, USA). MTT was purchased from Sigma (Sigma Aldrich, St Louis, MO, USA). Fluorescent microspheres were purchased from Molecular Probes (Thermo Fisher Scientific, USA). Mouse SIgA ELISA Kit was purchased from Wuhan USCN Business Co., LTD (Wuhan, China). Antibodies against ERK, p-ERK, JNK, p-JNK, p38, p-p38, AKT and p-AKT were purchased

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from Cell Signaling (Danvers, MA, USA). Antibodies against actin were purchased from BD Biosciences (Franklin Lakes, NJ, USA). The ECL Plus Western Blotting Detection Kit was purchased from Beijing Saizhi Technology Co., LTD (Beijing, China). LDH solutionwas obtained from Aladdin Industrial Corporation (Shanghai, China). All other reagents were of analytical grade or better.

2.2 Preparation of HEP

Extraction of HEP was performed as described by Zhang[13]. Fresh fruiting bodies from *Hericium* (4 kg) were extracted in 40 L distilled water maintained at 100 °C for 4 h and filtered through four sheets of gauze. The solid material was extracted twice using the same conditions, and filtrates were combined, centrifuged to remove water-insoluble material, concentrated down to 600 mL and precipitated by addition of 95% ethanol (4 volumes). Following centrifugation, the precipitate was dried by solvent exchange, first using 95% ethanol, then using absolute ethanol and finally ether. Three identical extractions were carried out to obtain the polysaccharide.

2.3 Analysis of chemical properties

Total carbohydrate content was determined by using the phenol-sulfuric acid method[14], with glucose as the standard. Protein content was determined by using the Bradford assay[15], with bovine serum albumin (BSA) as the standard. Starch was measured by using the I_2-KI assay. Monosaccharide composition was determinedusing high performance liquid chromatography (HPLC) as described by Zhang[13]. Briefly, polysaccharide samples (2 mg) were first hydrolyzed at 80 °C for 16 h using 1 mL anhydrous methanol containing 2 M HCl, followed by treatment with 1 mL 2 M trifluoroacetic acid (TFA) for 1 h at 120 °C. After derivatization with

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1-phenyl-3-methyl-5-pyrazo-lone (PMP), derivatives were analyzed by HPLC.

2.4 Fourier transform infrared (FT-IR) spectroscopy

HEP was ground up with KBr powder and then pressed into 1 mm pellets for FT-IR analysis. FT-IR spectra over a range of 4000-400 cm^{-1} were obtained using a Nicolet 560 FT-IR spectrometer equipped with a DTGS detector.

2.5 Treatment of mice with HEP

Pathogen-free female Babl/c mice, age 6–8 weeks and weighing 16–18 g, were obtained from Beijing HFK Bioscience Co. Ltd (Beijing, China). Mice were housed under pathogen-free conditions and allowed access to food and water *ad libitum*.

Animals were randomly divided into 4 groups (n = 8 per group), i.e., vehicle control group and HEP 75, 150 and 300 mg per kg body weight (mg per kg BW) groups. Distilled water and HEP were administered intragastrically over a period of 21 days.

On day 22, mice were sacrificed for assessment of lymphocyte proliferation andsplenic natural killer (NK) cell activity. To assess macrophage phagocytic capacity and perform the serum hemolysin assay, mice were immunized by intraperitoneal injection with 0.2 mL of 2% (v/v) SRBCs four days prior to sacrifice.

All animal experiments were carried out in compliance with the Animal Management Rules of the Ministry of Health of People's Republic of China and approved by the Animal Care and Use Committee of Northeast Normal University.

2.6 Spleen and thymus index determination

Mice were sacrificed by cervical dislocation, and spleens and thymus were

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removed and weighed. The spleen and thymus index were calculated as the ratio of immune organ weight to body weight.

2.7 Lymphocyte proliferation assay

The effect of HEP on T cell proliferation was determined using the MTT assay. Briefly, the splenocyte suspension was adjusted to a concentration of 5×10^6 cells per mLusing complete RPMI-1640 medium and then seeded in 96-well plates (100 μL per well) with 100μL per well Con A (final concentration was 5 μg per mL) as a T cell stimulant. Following incubation at 37 ^{o}C for 72 h under humidified (5% CO_2–95%) air, 20μL of MTT (5 mg per mL) was added to each well, followed by 4 h incubation and addition of 50 μL SDS to each well. Absorbance was then measured at 570 nm using a microplate reader (Bio-Rad, Hercules, CA, USA).

2.8 Hemagglutination assay

Blood samples were collected from the ophthalmic venous plexus and centrifuged at 450 g for 10 min to collect serum. The serum samples were double-diluted with 0.9% normal saline. After incubation of 100 μL of serum and 100 μL 0.5% (v/v) SRBCs at 37 ^{o}C for 3 h in microtiter V plates, the level of blood cell aggregation was assessed. The degree of SRBC agglutination was classified in 5 levels (0 - IV). Serum hemolysin levels were calculated using the following equation: Serum Hemolysin Level = (S1+2S2+3S3..... NSn). 1, 2, 3..... N represents the double-dilution index, S represents the degree of aggregation. The 5 levels are defined as: level 0, all SRBC forms a large dot at the bottom of the microtiter V plate; level I, most SRBC forms a small dot at the bottom of the plate; level II, SRBC forms a small layer. Level III, SRBC forms a large layer, and level IV, all SRBC forms a whole layer on the bottom

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of the plate.

2.9 Phagocytic capacity of macrophages

After mice were sacrificed, peritoneal cells were harvested by peritoneal lavage with 2 mL of saline. Cells were collected by centrifugation at 250 g for 10 min and seeded onto 6-well plates at a density of 4-6×10^5 cells/well and incubated for 2 h. Fluorescent microspheres (Molecular Probes, 2.0 μm, carboxylate modified) were prepared following the manufacturer's instructions and adjusted with 1% of BSA to about 5×10^7 microspheres/mL. Then 100μL of beads was added to each well and held at 37 ^oC for 90 min. Cells were washed with PBS three times and collected. The phagocytic capacity of macrophages was analyzed by using flow cytometry. The rate of phagocytosis was calculated using the following equations: Phagocytosis Rate (%)=number of macrophages that were phagocytosed beads/total number of macrophages×100%.

2.10 NK cytotoxicity assay

Briefly, splenocytes (1×10^6 cells/well) and YAC-1 cells (4 × 10^4 cells/well) were incubated in 96-well plates. The mixtures were incubated under humidified (5% CO_2– 95%) air for 4 h. The culture supernatants were then collected by centrifugation at 250 g for 5 min and were admixed with LDH solution. The absorbance at 490 nm was measured using a microplate reader (Bio-Rad, Hercules, CA, USA). The percentage of NK cell cytotoxicity was calculated according to the following equation:

$$\text{Cytotoxicity}(\%) = (OD_{experimental} - OD_{spontaneous}) \times 100 / (OD_{maximum} - OD_{spontaneous})$$

2.11 Enzyme-linked imunosorbent assay (ELISA) for small intestinal SIgA

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Intestinal proteins were lysed by grinding the tissue in lysis buffer (50 Mm Tris/acetate, pH 7.4, 1 mM EDTA, 0.5% Triton X-100, 150 mM sodium chloride, 0.1 mM PMSF, and Roche incomplete protease inhibitor cocktail). Protein concentrations were measured according to the Bradford method[15]. Equal amounts of protein were collected and were applied to the analysis of intestinal SIgA level by the mouse SIgA ELISA Kit.

2.12 Western blotting

Intestinal proteins were lysed by grinding the tissue in lysis buffer (50 Mm Tris/acetate, pH 7.4, 1 mM EDTA, 0.5% Triton X-100, 150 mM sodium chloride, 0.1 mM PMSF, and Roche incomplete protease inhibitor cocktail). Protein concentrations were measured according to the Bradford method[15]. Equal amounts of protein were separated by 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to a nitrocellulose membrane, blotted with each antibody, and detected by using the enhanced chemiluminescence (ECL) reagent.

2.13 Statistical analysis

The SPSS statistical software program was used for all data analysis. Results were expressed as the mean ± SD. Single factor analysis of variance was performed by ANOVA, followed by LSD's test, and significance was defined as $P < 0.05$, 0.01 or 0.001.

3. Results

3.1 Chemical properties of HEP

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The water-soluble polysaccharide from HEP was obtained from fruiting bodies via boiling water extraction. The yield of HEP was about 8.6% (w/w) of the dried material. The total carbohydrate, protein and uronic acid content of in HEP is shown in Table 1. The results of HPLC analysis indicate that HEP is composed of mannose (2.5%), glucuronic acid (1.1%), glucose (60.9%), galactose (28.0%) and fucose (7.5%).

3.2 FT-IR analysis

The FT-IR spectrum of HEP is shown in the Fig.1. Compared to IR band intensities reported for the polysaccharide[16], the broad, intense peak at 3369 cm^{-1} was ascribed to hydrogen bond O-H stretching vibrations, suggesting strong inter- and intra-molecular interactions of the polysaccharide chains. The weak peak at 2928 cm^{-1} was attributed to C-H vibrations, most likely due to CH_2 stretching and bending modes. Carbonyl groups (C=O) showed two bands, including an asymmetrical stretching band around 1642 cm^{-1} and a weak symmetric stretching band near 1405 cm^{-1}[17]. The peak at around 1079 cm^{-1} could be assigned to C-O stretching vibrationsfrom C-O-H and C-O-C (anomeric) bonds dominated by the glycosidic linkage. The absorption band at 1079 cm^{-1} suggested that all fractions consisted of pyranoside[18]. The typical characteristic α-configuration of the sugar units was observed around 854 cm^{-1}[19].

3.3 Effects of HEP on mouse body weight, spleen and thymus index

Daily oral administration of HEP did not cause mortality or significant changes in

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body weight, and the indices of spleen and thymus suggest that HEP was not toxic to the mice (Table 2).

3.4 HEP enhanced ConA-stimulated T cell proliferation

T lymphocytes are mainly responsible for cellular immunity, and to some extent, proliferation of T lymphocytes reflects that cellular immunity. Our results showed that ConA-stimulated proliferation of splenic lymphocytes increased by 38%, 94% and 60% over control when HEP was administered at concentrations of 75, 150 and 300 mg/kg BW, respectively (Fig. 2). This finding suggests that HEP apparently promotes cellular immunity, especially at the 150 mg/kg dose.

3.5 HEP increased serum hemolysin levels

Serum hemolysin levels were used to assess effects from HEP on humoral immunity in mice. Briefly, mice were immunized by intraperitoneal injection with 0.2 mL of 2% (v/v) SRBCs for four days prior to sacrifice when serum samples were collected and assessed in the SRBC aggregation assay. As shown in Fig.2, treatment with HEP at the doses of 75, 150 and 300 mg/kg significantly enhanced serum hemolysin levels in a dose-dependent manner, from 121 in the control group to 125,130, and 143 at the doses of 75, 150 and 300 mg/kg/day, respectively (Fig. 3).

3.6 Phagocytosis capacity of peritoneal cavity phagocytes

The phagocytosis capacity of peritoneal cavity phagocytes was investigated by using the fluorescence microsphere phagocytosis assay. Our results showed that the phagocytic rate of 150 mg or 300 mg HEP per kg body weight groups increased by 3.3% or 15%, respectively, compared with that of the control group (Fig. 4). However,

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in the group treated at the 75 mg/kg dose, the phagocytic rate showed no significant difference with that of the control group.

3.7 The enhancement effect of HEP on NK cell-mediated cytotoxicity

Tumor cell elimination is known to be mediated in part by cytotoxic activity from NK cells. Therefore, the effect of HEP on NK cell-mediated cytotoxity against YAC-1 cells was evaluated in the present study. The results showed that the NK cell-mediated cytotoxicity was modest in the group treated at the 75mg/kg and 150 mg/kg doses compared to the control group (Fig. 5). However, the cytotoxicity in the group treated at the 300 mg/kg dose was 20.6%, significantly higher than 10.5% in the control group (Fig.5).

3.8 Upregulation effect of HEP on small intestinal secretory IgA (SIgA) levels

SIgA plays an important role in the protection and homeostatic regulation of intestinal, respiratory, and urogenital mucosal epithelia that isolate the outside environment from inside the body. This primary function of SIgA is referred to as immune exclusion, a process that limits access of many microorganisms and mucosal antigens through the thin and vulnerable mucosal barriers[20]. Here, we investigated the small intestinal SIgA levels to investigate the effect of HEP on mucosal immunity. We observed significant increase in SIgA levels in mice treated with HEP at doses of 75, 150 and 300 mg/kg, compared with the vehicle controls (Fig. 6).

3.9 Effect of HEP on small intestine protein expressions

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The AKT and MAPK signaling transduction pathways are important pathways through which cells avoid apoptosis, because they promote progression of the cell cycle and thus enhance cell proliferation and activation. As shown in Fig.7, phosphorylation of ERK, JNK and AKT were significantly upregulated compared to the control group. Interestingly, activation of AKT occurred at the HEP dose of 300 mg/kg, where as activation of ERK, JNK, and p38 occurred at the HEP dose of 150 mg/kg.

3 Discussion

Considerable evidence has accumulated suggesting that natural polysaccharides from edible plants can significantly improve health, including non-toxic immunomodulating effects. Therefore, this has attracted growing attention to developing drugs and functional foods with these polysaccharides[21].

In the present study, we found that polysaccharide from *Hericium erinaceus* has potential usefulness as an immunomodulator. In vertebrates, two types of immunity are employed to protect the host from infection: innate and adaptive[22]. The innate immune system is genetically programmed to detect invariant features of invading microbes, and the adaptive immune system (comprised of T and B lymphocytes) employs antigen receptors that are not encoded in the germ-line, but are generated de novo in each organism[23]. Innate immune cells include dendritic cells, macrophages, and neutrophils, etc[23, 24]. The adaptive immune response includes two types: humoral immunity and cell-mediated immunity. The present study demonstrated that oral administration of HEP could improve innate immunity by enhancing ConA-stimulated proliferation of splenic lymphocytes, as well as by increasing serum hemolysin levels. HEP also could improve adaptive immune responses by increasing

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macrophage phagocytosis and NK cell activity. In toto, our results suggest that HEP is a potential immunomodulator.

We also showed in mice that HEP exerts these immunomodulating functions upon oral administration. This raises the question as to the mechanism underlying the imnuomodulating effect from HEP. It has been reported that the small intestinal plays acritical role in immunoregulation from the polysaccharide. Accumulating evidence has demonstrated that activated lymphocytes in the intestine migrate into immune systemic circulation through mesenteric lymph nodes and finally home in on effective sites, such as the lamina propria of the intestinal mucosa to regulate mucosal and systemic immune responses that lead to the improvement of defense systems or immunocompromised situations[12].

Our data suggest that the HEP exerts that SIgA expression was increased in the ileum, compared with the control group. IgA antibodies play an important role in mucosal immunity[25]. The protective role of SIgA against a variety of foreign antigens, including food antigens bacteria viruses and toxins, is well established. SIgA mediated responses at mucosal surfaces to locally encountered antigens are readily detected but prolonged topical exposure to protein or particulate antigens is required to induce serum antibodies[26]. This might be attributed to the protection of SIgA for the gut immune system.

MAPK and AKT play pivotal roles in regulating cell proliferation[27, 28]. It has been reported that extract of Chlorella vulgaris induced intestinal epithelial cells (IEC-6) proliferation via the activation of MAPK and increased the levels of the PI3K

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regulatory subunit of Akt phosphorylation [29]. Our results suggest that HEP enhanced the ERK-, JNK-, p38- and Akt-mediated intestinal cell proliferation. Interestingly, activation of AKT paralled upregulation of hemolysin, phagocytic activity, cytotoxicity of NK cells as well as the SIgA secretion. All these bioactivities occurredin a dose-dependent manner, with the maximal level occurring at the dose of 300 mg/kg. Whereas phosphorylation of ERK, JNK and p38 were maximal at 150 mg/kg, it was found to decrease at the higher dose of 300 mg/kg. Bioactivity paralleled T lymphocyte proliferation. It has been reported that AKT activation was related with the antibody synthesis by B lymphocytes[30], activation of macrophage[31, 32] and cytotoxicity of NK cells[33]. The activation of ERK, JNK and p38 accounted for the T lymphocyte proliferation[34]. Based on our results, we hypothesized that activation of AKT might at least, partially related with activation of B lymphocytes, macrophages, NK cells and SIgA secretion. Activation of ERK, JNK and p38 might be more related to proliferation of T lymphocytes. Further studies are required to confirm this hypothesis. Also, it remains unknown which specific polysaccharides contained in HEP mediate these interesting biological effects.

4 Conclusions

The data presented here demonstrate that oral administration of HEP in mice enhances innate immune responses via macrophage phagocytosis and NK cell activity and promotes adaptive immune responses via cell-mediated and humoral immunity. In addition, HEP upregulated the secretion of SIgA in the lamina propria. Moreover, HEP activated the MAPK and AKT cellular signaling pathways in the intestine. All

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these results showed that immunomodulatory effects of HEP are most likely attributed to effective regulation of intestinal mucosal immune activity. Our findings suggest that HEP could be a useful immunomodulator to improve immune function in immunocompromised humans.

Conflict of interest

The authors declare no conflict of interest.

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Figure Legend

Fig.1 FT-IR spectrum of HEP.

Fig.2 Effect of HEP on ConA-stimulated T cell proliferation. Mice were randomly divided into control, HEP 75, 150 and 300 mg /kg/day groups and given distilled water or different dosage of HEP by intragastric administration for 21 days. The effect of HEP on ConA-stimulated T cell proliferation were determined by MTT assay. Values represented the mean ±SD (n=8 per group), **P < 0.01 and *** P < 0.001 compared with control group.

Fig.3 Effects of HEP on serum hemolysin level. Mice were intragastric administrated with distilled water or indicated dosage of HEP for 21 days, mice were immunized by intraperitoneal injection with 0.2 mL of 2% (v/v) SRBCs four days prior to sacrifice by intraperitoneal injection. Serum were collected and applied to SRBC aggregation assay. Values represents the mean ±SD (n=8 per group), *P < 0.05 and ***P < 0.001 compared with control group.

Fig.4 Effects of HEP on macrophage phagocytosis in mice analyzed by fluorescence microsphere phagocytosis assay. Mice were intragastric administrated with distilled water or indicated dosage of HEP for 21 days. Mice were immunized by intraperitoneal injection with 0.2 mL of 2% (v/v) SRBCs four days prior to sacrifice by intraperitoneal injection. The peritoneal cavity phagocytes were harvested and incubated with fluorescent microspheres. The phagocytic capacity of macrophage was analysed by flow cytometry. Values represented the mean ± SD (n = 8 per group), **P < 0.01 compared with control group.

Fig.5 Effect of HEP on the NK cell mediated cell cytotoxity to YAC-1 cells. Mice were intragastricly given distilled water or HEP at the indicated dosages for 21 days.

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The culture supernatants were collected after centrifugation and admixed with LDH solution. The absorbance at 490 nm was measured. Values represented the mean ± SD (n = 8 per group), ***P < 0.001comparedwith control group.

Fig.6 Effect of HEP on small intestinal SIgA levels. Mice were intragastricly given distilled water or HEP at the indicated dosages for 21 days. The proteins extracted from intestinal tissues were subjected to SIgA ELISA assay. Values represented the mean ± SD (n = 8 per group), **P < 0.01 and ***P < 0.001 compared with control group.

Fig.7 p-ERK, p-JNK, p-p38 and p-AKT were upregulated by HEP. Mice were intragastricly given distilled water or HEP at the indicated dosages for 21 days. The proteins extracted from intestinal tissues were subjected to Western-blot.

Fig.8 An illustration of a likely mechanism of immunomodulating activity of HEP.

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Table 1. Chemical properties of HEP.

Item	Carbohydrate (%)	Protein (%)	Uronic acid (%)	Monosaccharide composition(%)				
				Man	GlcA	Glc	Gal	Fuc
HEP	67.9	4.4	3.2	2.5	1.1	60.9	28.0	7.5

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Table 2 Effect of HEP on spleen and thymus index of the mice.

Dosage (*Hericium* extract mg per kg BW)	Average body weight of mice after the experiment (g)	Spleen index (mg/g)	Thymus index (mg/g)
0	17.50±0.90	7.43±1.12	6.51±2.07
75	16.83±0.73	7.59±0.90	8.80±1.16
150	17.34±0.56	7.40±0.31	8.44±1.09
300	18.44±1.09	6.96±0.30	7.93±0.81

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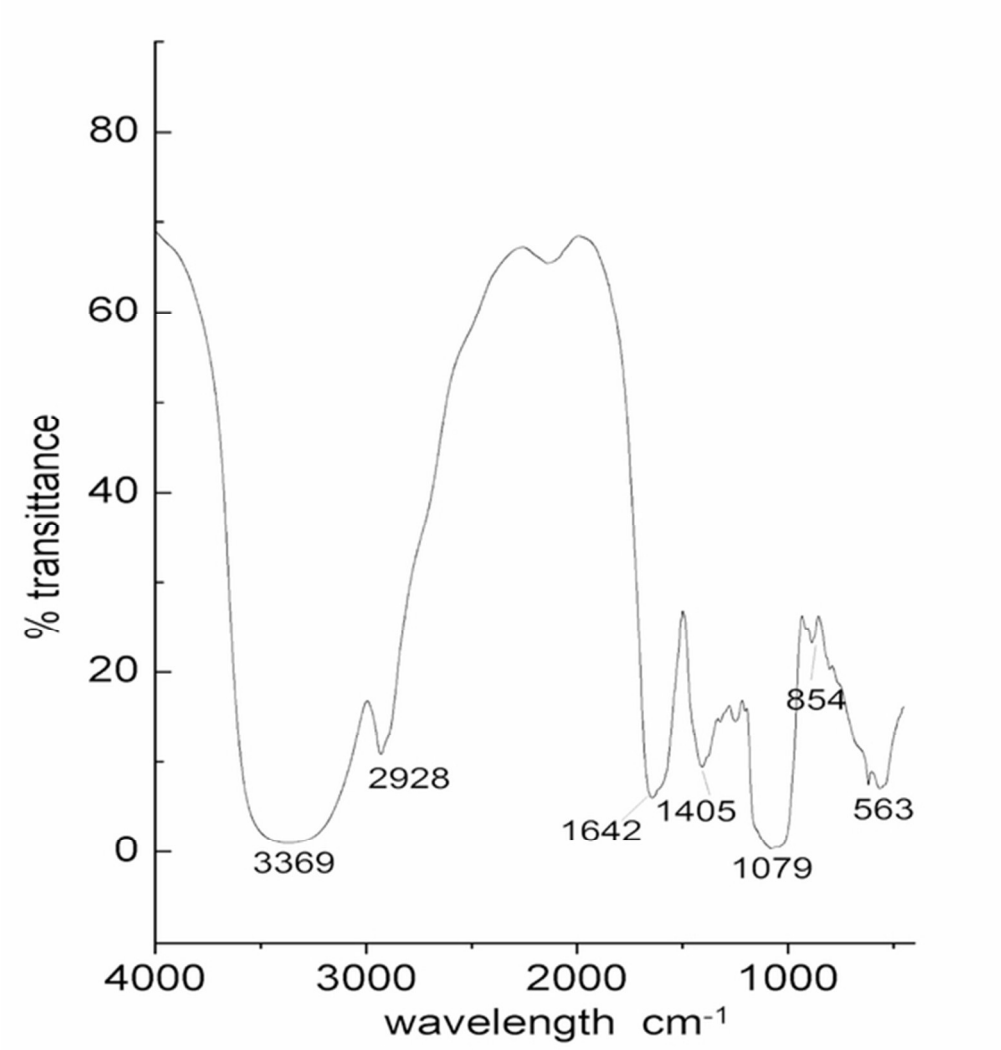

FT-IR spectrum of HEP.

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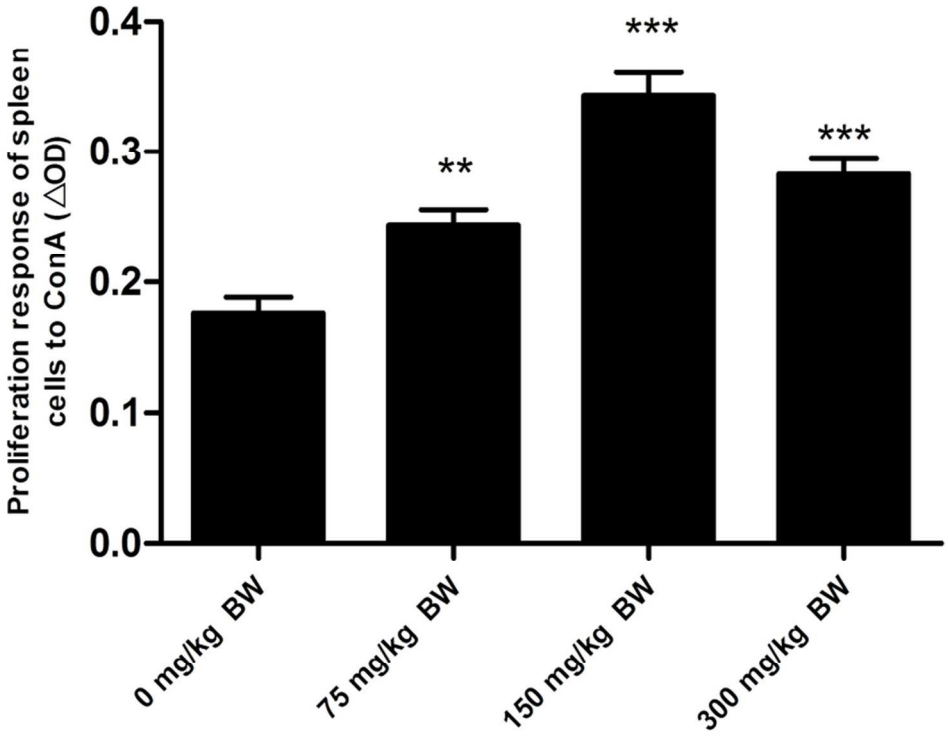

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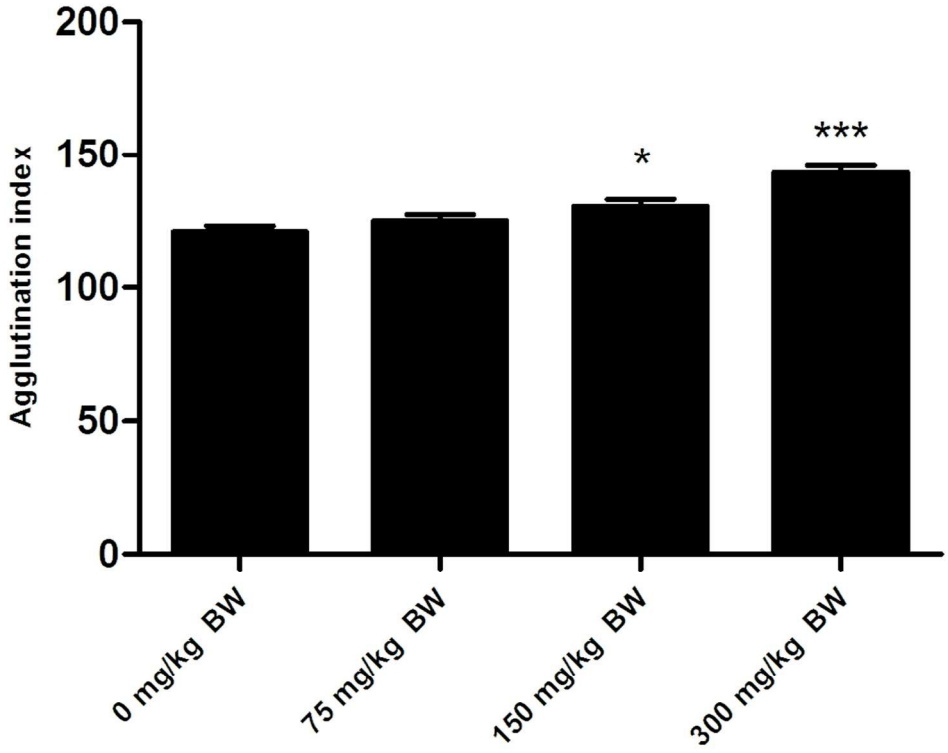

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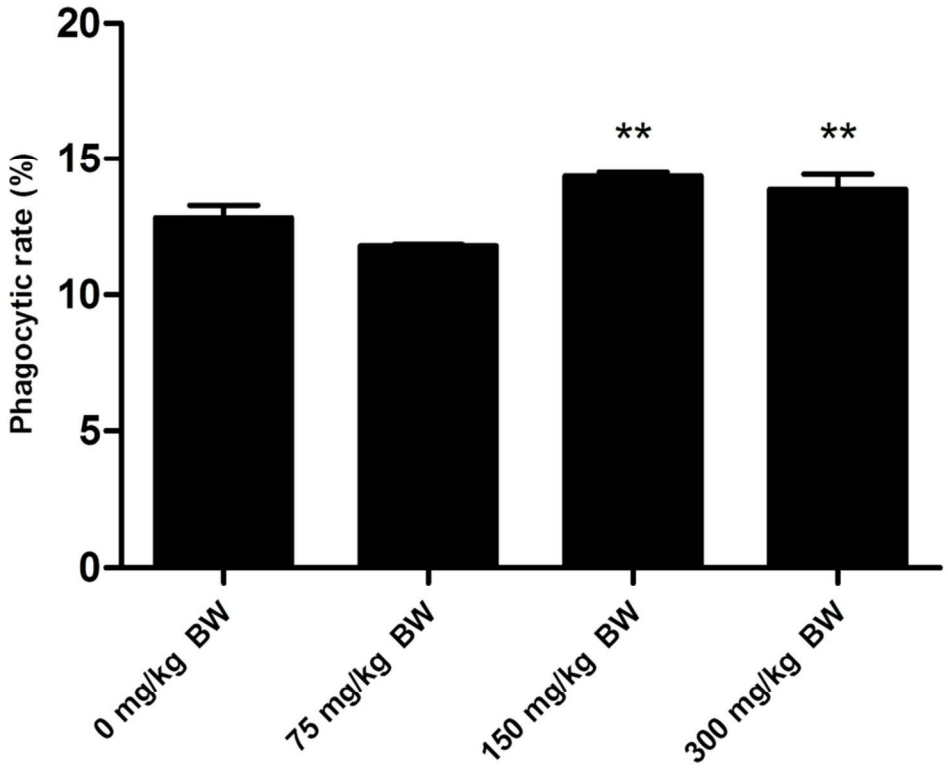

Effects of HEP on macrophage phagocytosis in mice analyzed by fluorescence microsphere phagocytosis assay. Mice were intragastric administrated with distilled water or indicated dosage of HEP for 21 days. Mice were immunized by intraperitoneal injection with 0.2 mL of 2% (v/v) SRBCs four days prior to sacrifice by intraperitoneal injection. The peritoneal cavity phagocytes were harvested and incubated with fluorescent microspheres. The phagocytic capacity of macrophage was analysed by flow cytometry. Values represented the mean ± SD (n = 8 per group), **P < 0.01 compared with control group.

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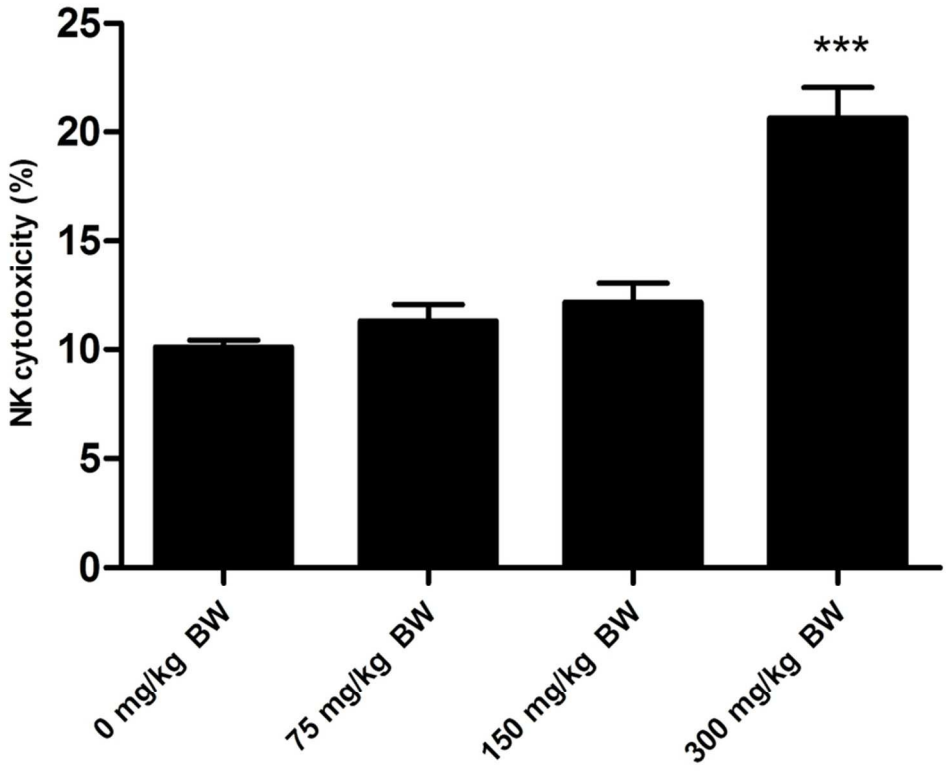

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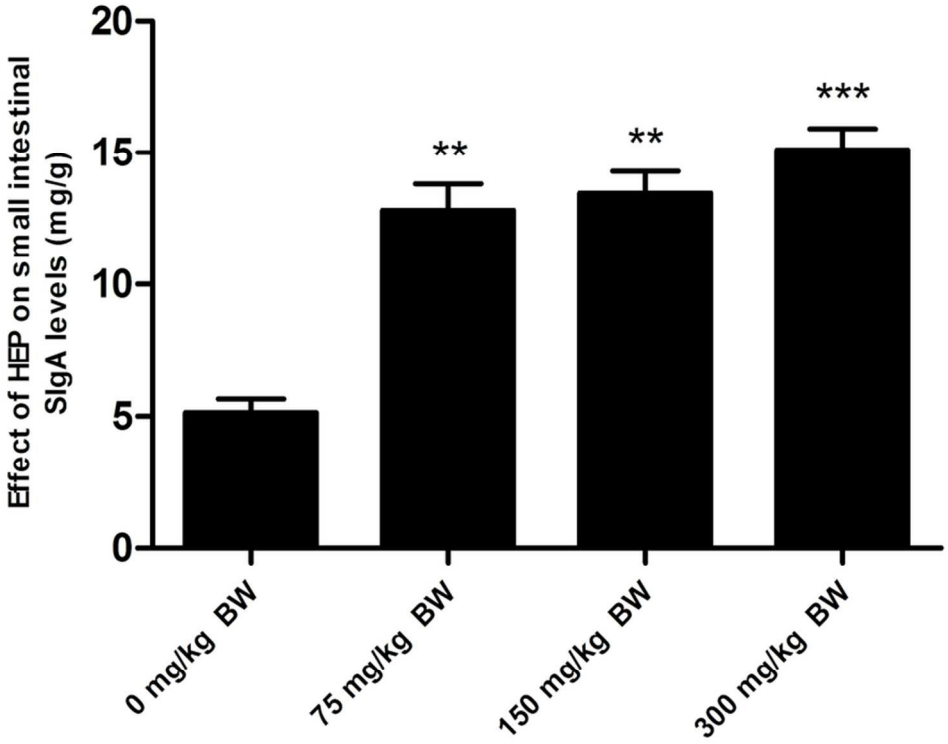

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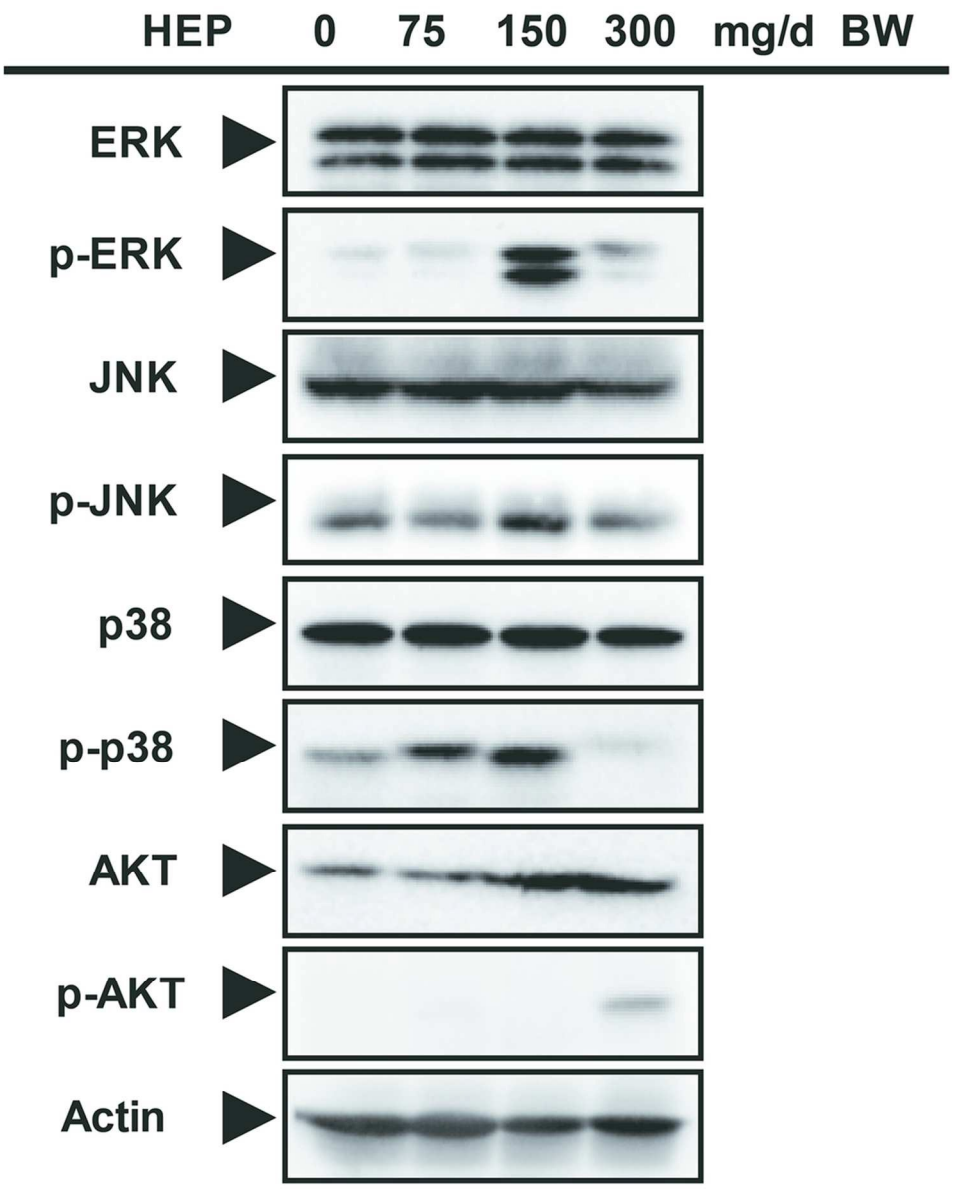

p-ERK, p-JNK, p-p38 and p-AKT were upregulated by HEP. Mice were intragastricly given distilled water or HEP at the indicated dosages for 21 days. The proteins extracted from intestinal tissues were subjected to Western-blot.

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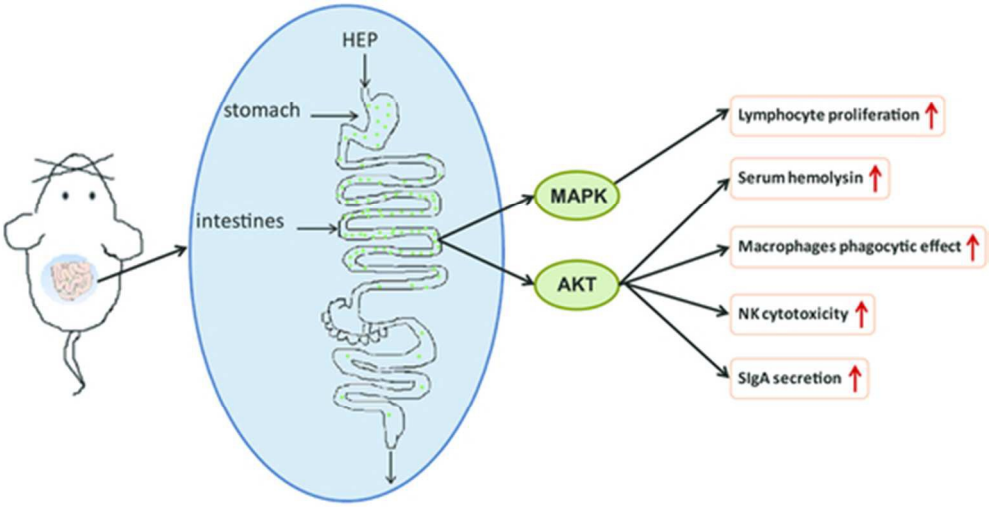

An illustration of a likely mechanism of immunomodulating activity of HEP.

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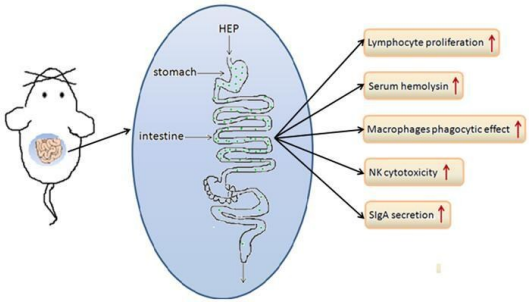

The immunomodulatory effects of *Hericium erinaceus* derived polysaccharides are mediated by intestinal immunology