Oral administration of *Urtica macrorrhiza* Hand.-Mazz. polysaccharides to protect against cyclophosphamide-induced intestinal immunosuppression

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Abstract. As a strategy to prevent the well-known immunosuppressant effects of cyclophosphamide (CY), the immunomodulatory activity of the polysaccharide isolated from *Urtica macrorrhiza* Hand.-Mazz. (UMHMPS) was investigated in the present study. The chemical properties of UMHMPS, including total carbohydrates, uronic acid, protein contents, monosaccharide compositions, molecular weight and structural confirmation, were investigated. The immunomodulatory activity of UMHMPS was evaluated using a CY-induced immunosuppression mouse model. The results revealed that UMHMPS, which is composed of rhamnose, gluconic acid, galactose acid, galactose and xylose, exhibited potent immunomodulatory activity and low toxicity in mice. It increased the secretions of secretory immunoglobulin A, interferon (IFN)-γ and interleukin (IL)-4, and maintained the balance of the ratios of IFN-γ/IL-4 and cluster of differentiation (CD)3^+/$CD19^+$ cells in Peyer's patches. Furthermore, it increased the expression of Toll-like receptor (TLR)-4, indicating that TLR4 may be one of the receptors of UMHMPS. Therefore, the present study provides evidence for the potential use of UMHMPS as an immune enhancement drug in chemotherapy.

Introduction

Chemotherapeutic drugs are a fundamental tool in the treatment of malignancies (1). However, their therapeutic effectiveness is accompanied with severe side effects (2). Cyclophosphamide (CY) is an alkylating agent used to treat autoimmune diseases and cancer (3,4). It is a cytotoxic drug that damages the structure of DNA, prevents DNA from replicating and leads to cell death *in vivo* (5,6). A number of investigations have revealed that the long-term use of CY has harmful side effects, including immunosuppression, myelosuppression and leucopenia (7). Immunosuppression is one of the major side effects of using high doses of CY. In immunocompromised patients, opportunistic invasive fungal infections are generally life-threatening, and this can be immediate (8).

The intestinal immune system is the first line of mammalian defense against enteric pathogens and pathogenic microorganisms (9). The intestinal mucosa is constantly in contact with a variety of materials, including foods, probiotics, viruses, parasites and other harmful organisms or substances. A healthy and integrated intestinal mucosa can regulate and eliminate the antigens and pathogens invading the body through the mucosa, thereby serving a protective role in the body (10). The long-term use of CY can damage the intestinal mucosa, resulting in the transfer of bacteria to the mesenteric lymph nodes and spleen (11), and causing diarrhea and stomach ache, which are painful for the patients. Therefore, it is crucial to investigate potent immunostimulatory agents to avoid intestinal mucosa damage during CY treatment.

Urtica macrorrhiza Hand.-Mazz. (*Urtica macrorrhiza*), a member of the Urticaceae family, is a folk medicine that has been used to treat a number of diseases for centuries (12). It possesses anti-inflammatory, antioxidative, antibacterial, antiviral, anticancer, antiulcer and analgesic effects (12). The leaves and roots of *Urtica* are used for prostatic hyperplasia, diabetes, asthma, rheumatism and eczema, and are also used to support human health and vitality due to its nutritional and functional qualities (12-16). The active components of *Urtica* include polysaccharides, steroids, terpenoids, phenylpropanoids, lignans, coumarins, lectins and vicenin (17). The *Urtica macrorrhiza* Hand.-Mazz. polysaccharide (UMHMPS) is a natural product obtained from *Urtica macrorrhiza,* and may be the major bioactive component of the crude drug. In several oriental countries, polysaccharides have been considered to be important immunostimulatory agents due to their immune activity without significant side effects. It can activate various types of immune cells, including macrophages, T lymphocytes, B lymphocytes, cytotoxic T cells, natural killer (NK) cells and the complement system, and regulate the expression of serum cytokines and immunoglobulins. The immunomodulatory

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effects of polysaccharides are multitarget, multilevel and multichannel (18). Therefore, the bioactivities of polysaccharides from different plants vary. To the best of our knowledge, there are no available data regarding the immunomodulatory activity of the polysaccharides extracted from *Urtica macrorrhiza*.

In the present study, UMHMPS was extracted and its physicochemical properties were evaluated. Furthermore, the *in vivo* intestinal immunomodulatory activity of UMHMPS and its possible mechanisms were examined using a CY-induced immunosuppression mouse model. The present study provides evidence of the intestinal immunostimulant activity of UMHMPS and supports the potential use of UMHMPS as an immunostimulatory agent in chemotherapy.

Materials and methods

Plant materials and reagents. *Urtica macrorrhiza* was collected in January 2013 from Binchuan (Yunnan, China). It was identified and authenticated by Professor Xiwen Li (Kunming Institute of Botany, Kunming, China).

Monosaccharide standards were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Fluorescein isothiocyanate (FITC)-conjugated hamster anti-mouse cluster of differentiation (CD)-3e (cat. no. 553062) and phycoerythrin (PE)-conjugated rat anti-mouse CD19 (cat. no. 557399) were purchased from BD Biosciences (Franklin Lakes, NJ, USA). Anti-Toll-like receptor (TLR)-4 antibody (cat. no. ab13556) was purchased from Abcam (Cambridge, UK). Anti-mouse secondary antibody and 3, 3'-diaminobenzidine (DAB) were purchased from Beijing Zhongshan Jinqiao Biotechnology Co., Ltd. (cat. no. ZLI-9018; OriGene Technologies, Inc., Beijing, China). Mouse interleukin (IL)-4 (cat. no. EMC003), mouse interferon (IFN)-γ (cat. no. EMC101G) ELISA kits were purchased from NeoBioscience (Beijing, China) and the secretory immunoglobulin A (sIgA; cat. no. 027991) ELISA kit was purchased from US Biological (Salem, MA, USA). CY was purchased from Shengdi Co., Ltd. (Jiangsu, China). RPMI-1640 medium and fetal bovine serum (FBS) were purchased from Gibco (Thermo Fisher Scientific, Inc., Waltham, MA, USA). All other chemical reagents used were of analytical grade.

Physicochemical properties of UMHMPS

Extraction and purification. The aerial parts of *Urtica macrorrhiza* were washed thoroughly with tap water, air-dried and powdered using an electric grinder. A total of 100 g powder was boiled in 1,000 ml distilled water for 1 h with occasional stirring. The decoction preparation was then filtered through a muslin cloth, followed by filtration through filter paper. The obtained filtrate was evaporated *in vacuo* to yield a raw extract. A total of 25 g dried aqueous extract was dissolved in 70 ml water, and then 350 ml 95% ethanol was added. The precipitate was separated and dissolved in 50 ml water, and then 250 ml 95% ethanol was added. The precipitate was then dried *in vacuo*. The procedure was repeated three times to remove small molecules and water-insoluble substances.

Determination of the contents of carbohydrates, uronic acid and protein. The total carbohydrate content of UMHMPS was determined by the phenol-sulfuric acid method (19), with glucose as the standard. The uronic acid content was determined using the m-hydroxydiphenyl method (20), with D-galacturonic acid as the standard. The protein content was determined by performing a Bradford assay, as previously described (21).

Determination of molecular weight. The molecular weight of UMHMPS was evaluated by high-performance liquid chromatography (HPLC) equipped with a ShodexOhpak SB-804HQ gel column and an Agilent G1362A Refractive Index Detector. A total of 20 μl UMHMPS (10 mg/ml, dissolved in distilled water) was analyzed, with 0.1 M NaCl as the mobile phase at 0.5 ml/min and 35˚C. The columns were calibrated with T-series Dextran standards (molecular weights: 4,600, 7,100, 10,000, 21,400, 41,100, 84,400 and 133,800 Da, respectively).

Monosaccharide composition analysis. The monosaccharide composition of UMHMPS was determined by HPLC analysis. UMHMPS (5 mg) was hydrolyzed in 1 ml of 2 M trifluoroacetic acid at 110˚C for 4 h. Acetylation was performed with 50 μl of 0.5 M 1-phenyl-3-methyl-5-pyrazolone and the same volume of 0.6 M NaOH in a water bath at 70˚C for 30 min. Subsequently, the reaction solution was neutralized with 50 μl of 0.6 M HCl, and distilled water was added to equal 1 ml. The product was then extracted with 1 ml of chloroform three times with sufficient mixing. The solution of the aqueous phase was finally passed through a 0.22-μm filter membrane, following which it was subjected to an Eclipse XDB-C18 chromatographic column (150x4.6 mm; 5 μm) and eluted with a mixed solution of acetonitrile and 0.1 M ammonium acetate buffer (15: 85, pH 5.5) at a flow rate of 1 ml/min at 30˚C. The monosaccharide composition of UMHMPS was identified by comparing the retention times with those of standard monosaccharides: Rhamnose (Rha), glucose (Glc), xylose (Xly), mannose, galactose (Gal), gluconic acid (GlcA), fucose, galactose acid (GalA), N-acetylglucosamine and iduronic acid. The contents of each monosaccharide were calculated using the corresponding peak areas and response factors.

Fourier-transform infrared spectroscopy (FT-IR) spectroscopic analysis. The UMHMPS was ground with potassium bromide powder and pressed into a 1-mm pellet for FT-IR measurement with a Nicolet 6700 Fourier transform infrared spectrophotometer. The organic functional groups present in UMHMPS were characterized in the frequency range, 4,000-400 cm^{-1}.

In vivo experiments

Animals. A total of 88 male ICR mice (6-8 weeks old), weighing 20±2 g, were obtained from Hunan Slack King Experimental Animal Co. [Hunan, China; certificate of quality no. SCXK (Hunan) 2011-0003]. The experiments were performed in accordance with international guiding principles and local regulations regarding the care and use of laboratory animals for biomedical research. The animals were housed in a specific pathogen-free laboratory with *ad libitum* access to food and water. The temperature was controlled at 22±1˚C with a relative humidity of ~50%, and fluorescent lighting on a 12-h light/dark cycle. The weight, diet consumption and behavior of the mice were recorded daily. The animal experiments performed in the present study were approved by the Institutional Animal Care

Table I. Composition of *Urtica macrorrhiza* Hand.-Mazz. polysaccharide.

			Monosaccharide composition	
Carbohydrate (%)	Uronic acid (%)	Protein (%)	Monosaccharide	Content (%)
41.50	37.49	3.52	Rha	16.53
			GlcA	2.89
			GalA	20.39
			Gal	9.15
			Xly	7.69

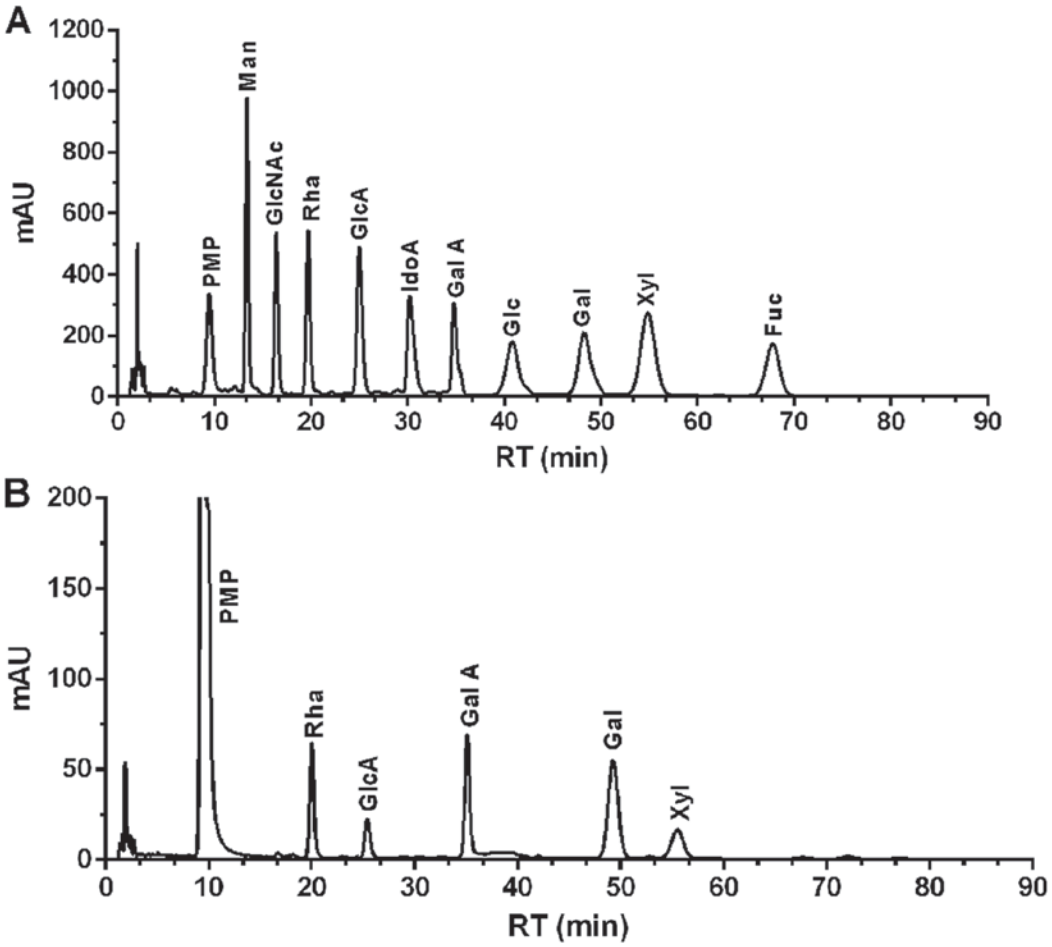

Figure 1. Monosaccharide composition. (A) Monosaccharide composition of the standard spectrum. (B) Monosaccharide composition of UMHMPS. UMHMPS, *Urtica macrorrhiza* Hand.-Mazz. polysaccharide; RT, retention time.

and Use Committee of Yan'an Hospital (Kunming, China) and the certificate number of animal use was SYXK (Dian) K2014-0006.

Acute oral toxicity experiment. A total of 40 male ICR mice were fasted for 12 h and allowed free access to drinking water. The mice were then divided into four groups and treated with UMHMPS by gavage at doses of 0.5, 1.0, 2.0 and 3.4 g/kg (0.4 ml/10 g) twice a day for 1 week. The general conditions, symptoms of toxicity and mortality of the mice were observed and monitored following treatment, and the maximum tolerated dose (MTD) was then confirmed.

Treatment and experimental design. Following acclimatization to the laboratory environment for 1 week, mice were randomly divided into four groups (n=12 mice/group): CY model, control, UMHMPS high dose (UMHMPS-H) and UMHMPS low dose (UMHMPS-L). Animals in the CY model, UMHMPS-H and UMHMPS-L groups were treated with CY at a dose of 50 mg/kg by intraperitoneal injection on the 1st, 3rd, 5th and 7th days to establish CY-induced immunosuppression mice, whereas mice in the control group were treated with physiological saline instead of CY. Mice in the UMHMPS-H and UMHMPS-L groups were orally administered with 1.0 and 0.5 g/kg UMHMPS, respectively, once a day for 8 days. Mice in

Table II. Effects of UMHMPS on body weight changes in immunosuppressed mice.

Group	Dose (g/kg)	Body weight (g)		Weight gain (g)
		Day 1	Day 8	
Model	-	23.2±0.85	27.85±0.58	4.65±0.68
Normal	-	23.15±0.78	31.15±0.67	8.01±0.81[a]
Positive control	1.0	23.13±1.13	30.23±1.02	7.11±1.44[a]
UMHMPS-H	1.0	23.26±1.19	30.2±0.65	6.94±1.01[a]
UMHMPS-L	0.5	23.59±0.62	29.69±0.56	6.10±0.37[a]

[a]P<0.01 compared with the model group. Data are presented as the mean ± SD (n=12). UMHMPS, *Urtica macrorrhiza* Hand.-Mazz. polysaccharide; -H, high dose; -L, low dose.

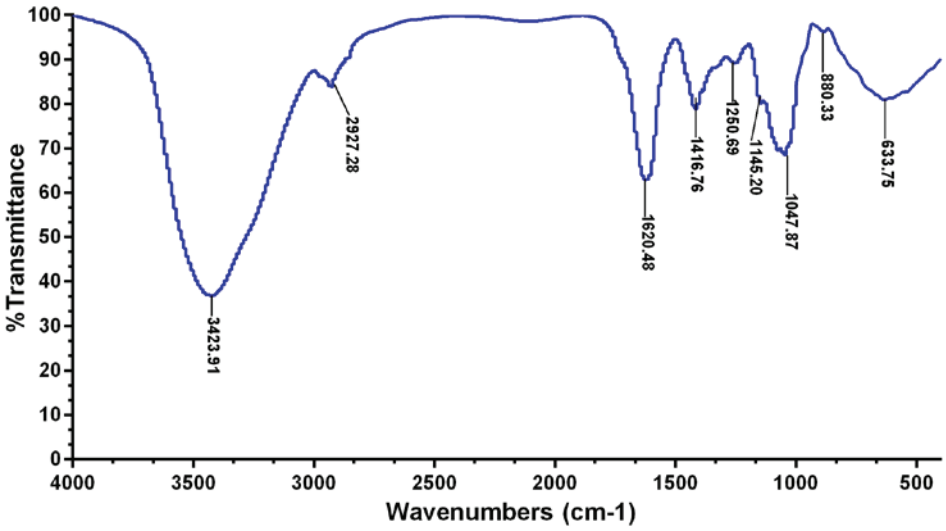

Figure 2. FT-IR spectrum of UMHMPS. FT-IR, Fourier-transform infrared spectroscopy; UMHMPS, *Urtica macrorrhiza* Hand.-Mazz. polysaccharide.

the CY model and control groups were treated with the equivalent volume of physiological saline. At the end of treatment, the mice were fasted for an additional 12 h, and were then anesthetized with ether, and sacrificed by cervical dislocation. The mice were confirmed to be fully anesthetized when they exhibited a decrease in respiration rate and the absence of corneal, palpebral and pedal reflexes. Death was confirmed when the mice exhibited a lack of pulse and respiration for 5 min.

Detection of sIgA, IL-4 and IFN-γ. The entire small intestine was collected, and physiological saline (3 ml) was injected into the intestinal lumen with the appropriate pressure; the saline was retained for 3 min in the intestinal lumen with gentle shaking. The physiological saline solution in the intestinal lumen was then collected and centrifuged at 4˚C and 1,500 x g for 10 min. The supernatant was collected, and the levels of sIgA, IL-4 and IFN-γ were detected by ELISA according to the manufacturer's instructions.

Determination of the surface phenotypes of Peyer's patch cells. Flow cytometry was used to measure the surface phenotypes of Peyer's patch cells. The collected small intestines were immediately washed and immersed in RPMI-1640 medium containing 5% FBS. Peyer's patches were separated and transferred onto a 120-mesh screen immersed in RPMI-1640 medium containing 5% FBS. Following gentle grinding, the Peyer's patches were washed with RPMI-1640 medium containing 5% FBS. The filtrate was collected and re-filtered with a 200-mesh screen. Subsequently, the filtrate was centrifuged at 4˚C and 450 x g for 10 min, and the supernatant was discarded. The cells were then washed with PBS three times, and the concentration of the cell suspension was adjusted to $1x10^7$/ml. Subsequently, the cells were incubated with FITC hamster anti-mouse CD3e and PE rat anti-mouse CD19 for 30 min at room temperature in the dark (1:100 dilution). The samples were washed twice with PBS and measured using a FACS-Calibur Instrument (Beckman Coulter, Inc., Brea, CA, USA). Data acquisition and analyses were performed for 10,000 events for each sample, and the data was analyzed using Kaluza software 2.1 (Beckman Coulter, Inc.). The ratio of $CD3^+/CD19^+$ cells=percentage of $CD3^+$ cells/percentage of $CD19^+$ cells. Only single-positive cells were counted in this experiment.

Detection of the expression of TLR4. The entire small intestine was collected following sacrifice of the mice by cervical

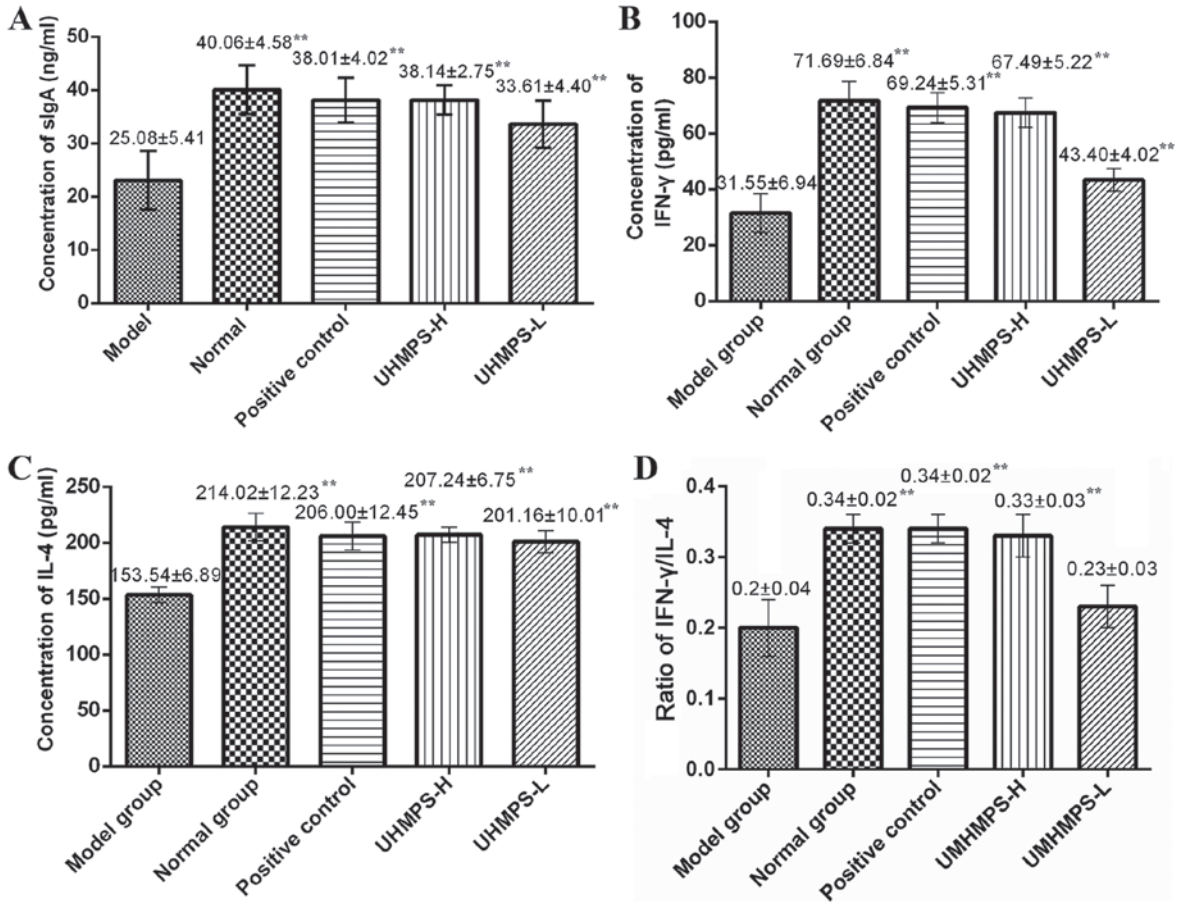

Figure 3. Effect of UMHMPS on the secretion levels of (A) sIgA, (B) IFN-γ and (C) IL-4, and the (D) ratio of IFN-γ/IL-4. The UMHMPS-H and UMHMPS-L groups were treated with UMHMPS at 1.0 and 0.5 g/kg by gavage once a day for 8 days, respectively. The CY model and control groups were treated with physiological saline. Data are presented as the mean ± standard deviation (n=12). **P<0.01 vs. CY model. UMHMPS, *Urtica macrorrhiza* Hand.-Mazz. polysaccharide; sIgA, secretory immunoglobulin A; IFN, interferon; IL, interleukin; CY, cyclophosphamide; -H, high dose; -L, low dose.

dislocation under anesthesia with ether. Following washing with physiological saline, the small intestine was fixed in 10% formalin solution. The specimens were embedded in paraffin and cut into 5-μm slices. The paraffinized sections were then incubated with 0.5% pepsin in 5 mm HCl at 37˚C for 30 min for antigen retrieval. Endogenous peroxidase was blocked via incubation with 3% hydrogen peroxide in methanol for 10 min at room temperature. Following overnight incubation at 4˚C with mouse anti-TLR4 (1:100 dilution), the sections were incubated with goat anti-mouse secondary antibodies (1:100 dilution) for 2 h at room temperature. The sections were then rinsed in distilled water (three times for 5 min each) and incubated with DAB for 10 min at room temperature. Hematoxylin staining was used to identify nuclei. The sections were visualized with an optical microscope and analyzed using Image-Pro Plus software (version 6.0; Media Cybernetics, Inc., Rockville MD, USA).

Statistical analysis. Statistical analysis was performed using SPSS 17.0 statistical software (SPSS, Inc., Chicago, IL, USA). All quantitative data are presented as the mean ± standard deviation. Multiple comparisons between groups were analyzed using one-way analysis of variance test followed by Dunnett's post-hoc. P<0.05 was considered to indicate a statistically significant difference.

Results

Physicochemical properties of UMHMPS. UMHMPS was present as a brown powder, the molecular weight was calculated as 11.06 kDa according to the calibration curve of the standard T-series dextrans. The composition of UMHMPS is presented in Table I; it contained 41.50% total carbohydrate, 37.49% uronic acid and 3.52% protein. The monosaccharide composition of UMHMPS was determined by HPLC, and the monosaccharides in UMHMPS were identified by comparing the retention times with those of the standards. The results revealed that UMHMPS was composed of Rha, GlcA, GalA, Gal and Xly (Fig. 1).

The FT-IR spectrum of UMHMPS is shown in Fig. 2. UMHMPS exhibited a strong and broad area of absorption between 3,600 and 2,500 cm^{-1}. A band was observed at 3,423 cm^{-1} for the O-H stretching vibration (22). The absorption at 2,927 and 1,416 cm^{-1} indicated the presence of the C-H stretching and bending vibrations of C-H, CH_2 and CH_3 (23). The band at 1,620 cm^{-1} was attributed to the carboxylate stretching band COO- (24). In general, the vibrations of 1,300-800 cm^{-1} are termed the 'fingerprint' region, which is predominantly dependent on the molecular vibrations and molecular structures. The stretching peaks at 1,145, 1,072 and 1,047 cm^{-1} were due to the presence of C-O glycosidic bonds, suggesting the presence of a pyranoid ring (25). These results

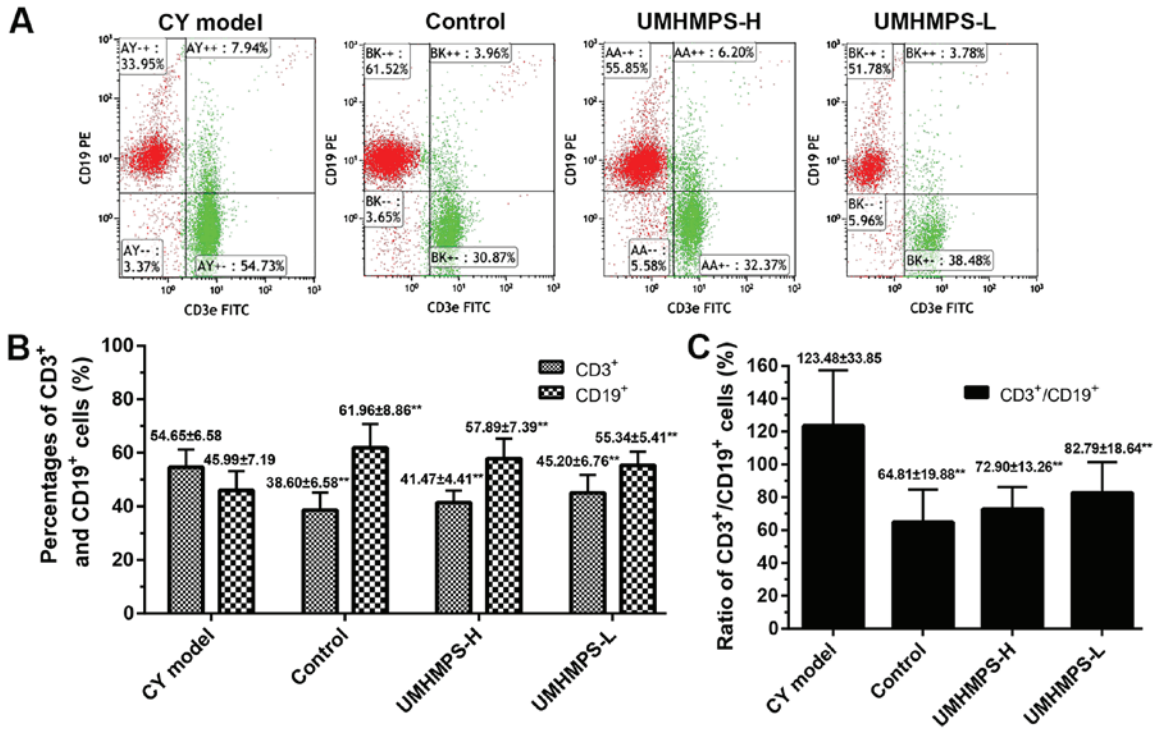

Figure 4. Effect of UMHMPS on the surface phenotype of Peyer's patches cells. (A) Results of flow cytometry scatter plot. (B) Percentages of $CD3^+$ and $CD19^+$ cells. (C) Ratio of $CD3^+/CD19^+$ cells. The UMHMPS-H and UMHMPS-L groups were treated with UMHMPS at 1.0 and 0.5 g/kg by oral gavage once a day for 8 days, respectively. Data are presented as the mean ± standard deviation (n=12). **P<0.01 vs. CY model. UMHMPS, *Urtica macrorrhiza* Hand.-Mazz. polysaccharide; CY, cyclophosphamide; -H, high dose; -L, low dose.

suggest that UMHMPS had the characteristic absorption of typical polysaccharides.

Acute oral toxicity of UMHMPS. All mice treated with different doses of UMHMPS survived the observation period. The general conditions of all mice were also normal. The MTD of UMHMPS was >3.40 g/kg in mice, indicating that it was safe to use UMHMPS at a dose <3.40 g/kg for the evaluation of efficacy.

Effect on mouse body weight. The body weight of each mouse was recorded daily between days 1 and 8, and weight gain is presented in Table II. The weight gain of mice in the CY model group was 4.65±0.68 g, which was significantly lower than that observed in the control (8.0±0.81 g), UMHMPS-H (6.94±1.01 g) and UMHMPS-L groups (6.10±0.37 g) (P<0.01). The average diet consumption data were also recorded and are presented in Table SI.

Effect on the secretion of sIgA, IFN-γ and IL-4. The concentration of sIgA in the intestinal lumen following mouse treatment with UMHMPS was measured by ELISA. As shown in Fig. 3A, in the CY model group, following CY injections, the secretion of sIgA was significantly reduced from 40.06±4.58 to 25.08±5.41 ng/ml. Whereas, UMHMPS dose-dependently antagonized this reduction. The concentration of sIgA in the UMHMPS-H group was 38.14±2.75 ng/ml, which was comparable to that in the control (40.06±4.58 ng/ml). In addition, although the sIgA levels in the UMHMPS-L group (33.61±4.04 ng/ml) were lower, they were significantly higher than those in the CY model group (P<0.05).

The secretion of IFN-γ was significantly reduced from 71.69±6.84 to 31.55±6.94 pg/ml in the CY model. However, the UMHMPS-treated mice were resistant to immunosuppression; the UMHMPS-H group exhibited almost normal secretion levels of IFN-γ (67.49±5.22 pg/ml), which were comparable to those of the control group (71.69±6.84 pg/ml). Although the concentration of IFN-γ in the UMHMPS-L group (43.40±4.02 pg/ml) was low, it was significantly higher than that observed in the CY model group (P<0.05; Fig. 3B).

Similarly, the secretion of IL-4 was reduced from 214.02±12.23 to 153.54±6.89 pg/ml in the CY model group. However, UMHMPS enabled the secretion of IL-4 to be maintained in the immunosuppressed mice, the levels of which were 207.24±6.75 and 201.16±10.01 pg/ml for the UMHMPS-H and UMHMPS-L groups, respectively. In addition, there were no significant differences among the UMHMPS-L, UMHMPS-H and control (214.02±12.23 pg/ml) groups (Fig. 3C).

The ratio of IFN-γ/IL-4, which represents the balance of T helper (Th)-1 and Th2 cells, was also calculated (Fig. 3D). The ratio of IFN-γ/IL-4 was higher in the control (0.34±0.02) and UMHMPS-H (0.33±0.03) groups, when compared with the ratio in the CY model (0.20±0.04) and UMHMPS-L (0.23±0.03) groups. There was no significant difference between the UMHMPS-L and CY model groups.

Effect on the surface phenotypes of Peyer's patch cells. The surface phenotypes of Peyer's patch cells were measured using flow cytometry, and the results are presented in Fig. 4A. The percentage of $CD3^+$ cells was 54.65±6.58% in the CY model group, which was higher than that in the control (38.60±6.58%), UMHMPS-H (41.47±4.41%) and

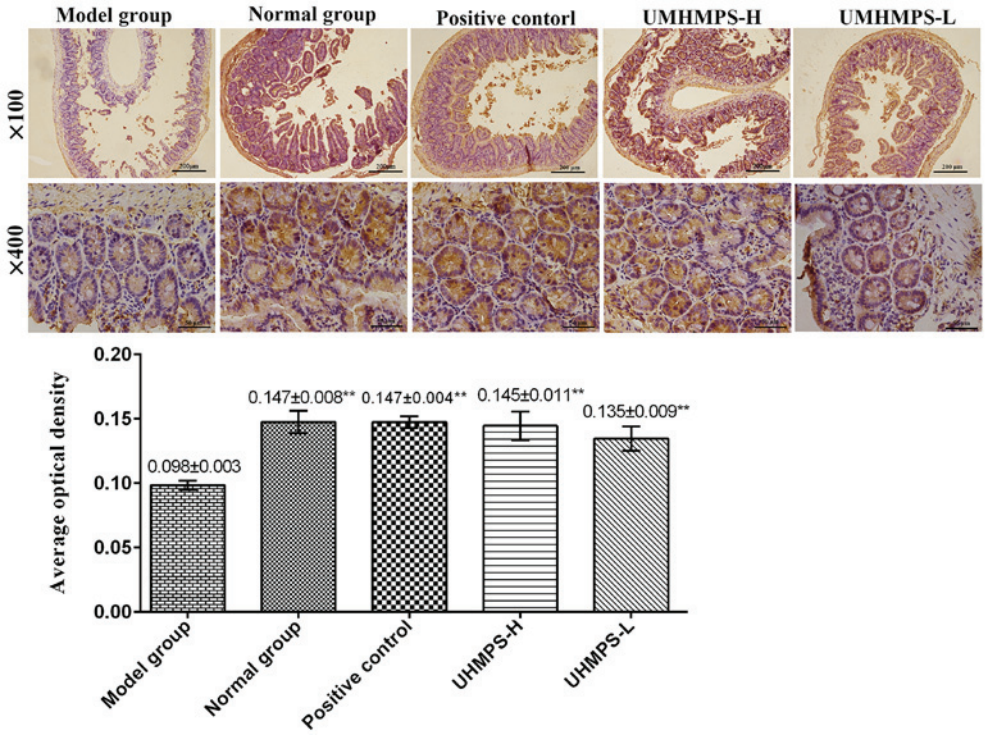

Figure 5. Expression of TLR4 in Peyer's patches in immunosuppressed mice following treatment with UMHMPS at 1.0 g/kg (UMHMPS-H) and UMHMPS at 0.5 g/kg (UMHMPS-L). The Peyer's patches were stained with hematoxylin and DAB. Scale bar=200 and 50 μm; magnification, x100 and x400, respectively. Data are presented as the mean ± standard deviation (n=6). **P<0.01 vs. CY model. TLR4, Toll-like receptor 4; UMHMPS, *Urtica macrorrhiza* Hand.-Mazz. polysaccharide; -H, high dose; -L, low dose; DAB, 3,3'-diaminobenzidine.

UMHMPS-L groups (45.20±6.76%) (P<0.01). The percentage of $CD19^+$ cells in the CY model group (45.99±7.19%) was lower than that in the control (61.96±8.86%), UMHMPS-H (57.89±7.39%) and UMHMPS-L (55.34±5.41%) groups (P<0.01) (Fig. 4B). In addition, the ratio of $CD3^+/CD19^+$ cells in the CY model group (123.48±33.85%) was significantly higher compared with that in the control (64.81±19.88), UMHMPS-H (72.90±13.26) and UMHMPS-L (82.79±18.64) groups (P<0.01). There were no significant differences between the control group and the UMHMPS-H and UMHMPS-L groups (P>0.05), in $CD3^+$ cells, $CD19^+$ cells or the $CD3^+/CD19^+$ ratio (Fig. 4C).

Effect on the expression of TLR4. Immunohistochemistry was used to detect the expression of TLR4 in Peyer's patch cells. As shown in Fig. 5, the expression of TLR4 in the CY model group (0.098±0.003 AOD) was downregulated compared with that in the control (0.147±0.008 AOD), UMHMPS-H (0.145±0.011 AOD) and UMHMPS-L (0.135±0.009 AOD) groups (P<0.01). There were no significant differences between the control and UMHMPS-H groups. The expression of TLR4 in the UMHMPS-L group was marginally lower than that in the control and UMHMPS-H groups (P<0.05).

Discussion

Urtica macrorrhiza, which is widely distributed in Yunnan, China, is one of the most important herbs commonly used as a folk medicine. Several studies have indicated that *Urtica* extract exerts its bioactivity by regulating immunity (26,27). Polysaccharides isolated from traditional Chinese medicines have attracted substantial attention in recent years due to their immunomodulatory activity and low toxicity. Therefore, the UMHMPS from *Urtica macrorrhiza* may be a major bioactive components due to its immunomodulatory activity. In the present study, UMHMPS was isolated from *Urtica macrorrhiza* and was examined for its physicochemical properties and immunomodulatory activity *in vivo*. The results demonstrated that UMHMPS promoted the recovery of immunosuppression induced by CY in mice.

CY can damage the structure of DNA, kill immune cells, interfere with the proliferation and differentiation of B and T cells, and restrain the humoral and cellular immune response. The administration of CY leads to myelosuppression and immunosuppression (28-30). Therefore, CY has been used to construct an immunosuppressive animal model in previous studies (8,29). However, the protocols and durations of different studies involving the establishment of a CY-induced immunosuppressive model have varied. In certain studies, the mice were administered with large doses of CY, either one dose of 200 mg/kg or three doses of 80 mg/kg at the beginning of the experiments, and then continuously administered with polysaccharides for another 7-10 days (8,31). The present study adopted this protocol in the preliminary experiments, however, the immunosuppressive effect in the model group was poor. Therefore, the protocol was altered, administering CY (50 mg/kg) and UMHMPS at the same time from the beginning of the experiment. CY was administered once every other day and UMHMPS was administered once daily for 8 days. The results demonstrated that the CY-treated mice exhibited significantly lower levels of sIgA and cytokines IFN-γ and IL-4, and a lower IFN-γ/IL-4 ratio, compared with observations in the control group. The percentage

of $CD19^+$ cells was lower and the percentage of $CD3^+$ cells was higher in the CY-treated mice compared with the percentages in the control group. These results indicated that the immune functions of the CY-treated mice had been suppressed. In addition, this CY model was suitable for evaluating the immunomodulatory activity of UMHMPS.

In the present study, doses of 0.5 and 1.0 g/kg were selected as the low and high doses, respectively. These doses were selected according to previous studies using the polysaccharides (32,33). In addition, the assessment of oral acute toxicity indicated that the general condition of all mice treated with UMHMPS at <3.40 g/kg was normal. The highest dose in the experiment was 3.40 g/kg as it represented the maximum solubility of UMHMPS in normal saline, not due to mice exhibiting any adverse symptoms. Therefore, the MTD of UMHMPS was >3.40 g/kg in mice, preliminarily indicating that it was safe to use UMHMPS at a dose <3.40 g/kg for the evaluation of efficacy. The present study mainly evaluated the immunomodulatory activity of UMHMPS; to confirm the safety of UMHMPS, the effects of UMHMPS on the function of the liver, kidneys and other organs require further investigation.

The intestinal immune system is the first line of mammalian defense against enteric pathogens and pathogenic microorganisms (9); it is composed of intestinal lymph nodes, Peyer's patches, immune cells and cytokines. During humoral immunity, activated B cells settle in the lamina propria, proliferate and transform into mature IgA plasma cells. sIgA is then secreted to prevent the adhesion and reproduction of microbes (27). Therefore, sIgA is the major immunoglobulin of the intestinal mucosa, which is essential in protection against bacterial invasion and the maintenance of intestinal homeostasis (34). Compared with the CY model group, the secretion levels of sIgA in the UMHMPS groups were significantly improved. The results indicated that the immune activities of the immunosuppressed mice in the UMHMPS groups were increased.

Cellular immunity involves T, B, K, NK and accessory cells. T cells serve a crucial role in host defense against different microorganisms through the secretion of distinct cytokine profiles (35). Specific immune responses, which can be differentiated into Th1 and Th2 cell responses, have distinct roles in the immune system. Th1 cells modulate cellular immunity by producing cytokines including IFN-γ, whereas Th2 cells are involved in the humoral response by secreting cytokines including IL-4 (36). In the present study, UMHMPS recovered the levels of IFN-γ and IL-4 in the immunosuppressed mice, indicating that UMHMPS significantly activated Th1 and Th2 cells. In addition, the Th1/Th2 ratio remains stable under normal conditions to regulate the immune responses of the body (37), and a reduction in the ratio of IFN-γ/IL-4 (Th1/Th2) demonstrates immunosuppression (38). As shown in the present study, UMHMPS regulated the ratio of IFN-γ/IL-4 in immunosuppressed mice and maintained this ratio at almost normal levels, which further confirmed its immunomodulatory activity.

Peyer's patches, which are rich in T and B cells, are an important component of the intestinal immune system. T and B cells maintain a balance under normal conditions; however, if this balance is disturbed, intestinal immunity is suppressed (39). CD3 is a marker located on mature T cells. CD19 is a B cell-specific antigen and is a critical signal transduction molecule that regulates B lymphocyte development, activation and differentiation. In the present study, $CD19^+$ cells in the CY model group were significantly reduced, which may further cause the downregulation of sIgA in the CY model mice. However, following treatment with UMHMPS, the ratio of $CD3^+/CD19^+$ was maintained at normal levels.

It has been reported that TLR4 is one of the receptors of polysaccharides (40). A previous study demonstrated that polysaccharides can induce the production of immunoglobulins and cytokines by mediating TLR4 (41). In the present study, the expression of TLR4 in the UMHMPS groups was significantly upregulated compared with that in the CY model group. This indicated that TLR4 may be the receptor of UMHMPS, but further investigation is required to confirm this.

In conclusion, the polysaccharide UMHMPS, composed of Rha, GlcA, GalA, Gal and Xly, was extracted from *Urtica macrorrhiza*. UMHMPS exhibited potent immunomodulatory activity in mice with CY-induced immunosuppression. It enhanced intestinal immunity in immunosuppressed mice by increasing the secretions of sIgA, IFN-γ and IL-4, and maintaining a balanced ratio of IFN-γ/IL-4 and $CD3^+/CD19^+$ cells in Peyer's patches. Furthermore, it increased the expression of TLR4, indicating that TLR4 may be the receptor of UMHMPS. Therefore, the present study provides evidence for the potential use of UMHMPS as an immune enhancement drug in chemotherapy.

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Availability of data and materials

The datasets used and/or analyzed in the present study are available from the corresponding author on reasonable request.

Authors' contributions

ZW and YL performed the majority of the experiments, and ZW wrote the manuscript. CW and HX performed some of the experiments and contributed to data analysis. ZL and YL designed and supervised the study, and revised the final manuscript. All authors have read and approved the final manuscript.

Ethical approval and consent to participate

The animal experiments performed in the present study were approved by the Institutional Animal Care and Use Committee of Yan'an Hospital (Kunming, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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