Composition and Systemic Immune Activity of the Polysaccharides from an Herbal Tea (*Lycopus lucidus* Turcz)

XINGBIN YANG,[†] YOU LV,[†,‡] LINGMIN TIAN,[†] AND YAN ZHAO*,[§]

[†]Key Laboratory of Ministry of Education for Medicinal Plant Resource and Natural Pharmaceutical Chemistry, Shaanxi Normal University, Xi'an 710062, China, [‡]Centre for Molecular Protein Science, Lund University, P.O. Box 124, SE-22100 Lund, Sweden, and [§]School of Pharmacy, Fourth Military Medical University, Xi'an 710032, China

This study was undertaken to characterize the water-soluble polysaccharides isolated from an herbal tea, the leaves of *L. lucidus* Turcz. HPLC analysis showed that *L. lucidus* polysaccharides (LLPs) were mainly composed of galactose (50.1 mol %), followed by galacturonic acid (14.2 mol %), accounting for 64.3 mol % of all quantitative nine monosaccharides. Furthermore, we evaluated the systemic immunological efficacy of LLPs in mice. Mice were intragastrically administered once daily with low-dose (50 mg/kg), intermediate-dose (100 mg/kg), and high-dose (300 mg/kg) of LLPs, respectively, for 30 consecutive days. In comparison with vehicle, LLPs significantly enhanced the plaque-forming cells (PFCs), and serum hemolysin level, and delayed-type hypersensitivity (DTH) in response to sheep red blood cells (SRBC) in a dose-dependent manner ($p < 0.01$). In LLPs-treated mice, phagocytosis capacity and concanavalin A-induced spleenocyte proliferation were remarkably increased ($p < 0.05$). The intermediate- and high-dose of LLPs also caused a significant increase in the indices of thymus and spleen organs of mice ($p < 0.05$). This suggests that the polysaccharides derived from the tea leaves of *L. lucidus* improves the immune system and might be regarded as a biological response modifier.

KEYWORDS: *L. lucidus* Turcz; tea leaves; polysaccharides; HPLC; immunomodulatory activity

INTRODUCTION

Lycopus lucidus Turcz from the Labiatae family is a traditionally edible herb native to East Asian (*1*). The green leaves of *L. lucidus* can be used as herbal tea alone or in combination with other herbs. The roots of *L. lucidus*, also called "small ginseng" in China, are popularly consumed among peasants as high-quality agricultural vegetables. For these reasons, *L. lucidus* as an important agricultural source is widely cultivated in Korea, Japan, and China (*1−3*). In addition to their culinary uses, the *L. lucidus* leaves have traditionally been used for both folk and official phytomedicine as tonic, cardiac, sedative, wound-healing, and pain relieving agents (*2, 3*). Therefore, *L. lucidus* has received considerable attention in recent years and it is claimed that drinking *L. lucidus* tea could promote health and alleviate the severity of many disorders. Given the insufficient published data (*1−3*), further studies on the *L. lucidus* would be useful to clarify its physiological benefits and also provide a clue to substantiate its traditional dietary and therapeutic uses.

Traditionally, the *L. lucidus* leaves as tea or folk medicine were prepared using boiling water and consumed as a decoction. This preparation can recover the majority of water-soluble bioactive compounds, especially a large amount of water-soluble polysaccharides, which are probably responsible for the putative bioactivities of the *L. lucidus* leaves. Recently, there is the increasing evidence that a great lot of prebiotic polysaccharides isolated from various sources (plants, viruses, bacteria, and fungi) were associated with immunostimulatory effects although most data originate from in vitro experiments or nonoral application of polysaccharides in experimental animals (*4−8*). More recently, some studies have reported on the systemic immune activity of natural polysaccharides in mice (*9−11*). Surprisingly, a few reports have addressed the quantitative occurrence of the prototype carbohydrates in plasma and urine from rats by oral applied "nondigestible" oligosaccharides or polysaccharides with large molecular mass (*12−15*). To our knowledge, no study has been performed to obtain polysaccharides from *L. lucidus*, and it is also unclear whether *L. lucidus* polysaccharides (LLPs) have any direct systemic immunological effects, and a detailed work is needed on the elucidation of the compositional feature and immune activity of LLPs as potential food ingredients.

The aim of the present study is for the first time to isolate the crude polysaccharide LLPs and sequentially the basic monosaccharide composition in LLPs was characterized by HPLC analysis. Furthermore, the systemic immune-modulatory activity of LLPs was also investigated in mice.

MATERIALS AND METHODS

Chemical Reagents. D-Mannose, L-rhamnose, D-glucuronic acid, D-galacturonic acid, D-glucose, D-xylose, D-galactose, L-arabinose, D-fucose, *m*-hydroxydiphenyl, and concanavalin A (Con A) were obtained from Sigma (St Louis, MO). Coomassie brilliant blue G250 and bovine serum

*To whom correspondence should be addressed. Phone: +86-29-84774473. Fax: +86-29-84776945. E-mail: yanzhao@fmmu.edu.cn.

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albumin (BSA) were purchased from Huamei Biochemistry Co. (Shanghai, China). RPMI1640, dimethyl sulfoxide (DMSO), phosphate buffer saline (PBS), thiazolyl blue tetrazolium bromide [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, MTT], and fetal bovine serum were the products of Gibco BRL (Gaithersburg, MD). Trifluoroacetic acid (TFA) was obtained from Merck (Darmstadt, Germany). 1-Phenyl-3-methyl-5-pyrazolone (PMP) and chloroform were purchased from Beijing Reagent Plant (Beijing, China). HPLC-grade acetonitrile and 0.45 μm filters were purchased from Fisher (New Jersey).

Extraction and Purification of LLPs. The tea cultivars of *L. lucidus* were harvested from the countryside region of Jingbian County (Northeast of China) of Shaanxi Province, China. Tea leaves were dried for 3 min in a domestic microwave oven (Galanz, China) at 900 W output power, pulverized into a powder, and stored at 4 °C. The polysaccharides were extracted and purified as previously described (7) with some modifications. Briefly, the pulverized tea leaves (500 g) were refluxed at 80 °C for 3 h in absolute alcohol. After the mixture was filtered, the residues were dried in air and then were extracted in hot water (1:10, w/v) at 80 °C for three times, 1 h each time. The extracted solution was concentrated to 10% of the original volume in a rotary evaporator under reduced pressure, and then the polysaccharides were precipitated by adding four times of volume of 95% (v/v) ethanol at 4 °C for 24 h. The sediment was collected by centrifugation (3000g, 10 min). Then the polysaccharide sediment was further refined by repeatedly dissolution and precipitation for 3 times, followed by washing with ethanol, acetone, and ether alternately. The refined polysaccharides were dissolved in distilled water and intensively dialyzed (cutoff M_w > 10000 Da) against distilled water, and then the retentate portion was deproteinized by a freeze−thaw process (FD-1, Henan Yuhua Instrument Co., China) for repeating eight times followed by filtration. Finally, the filtrate was lyophilized to yield brownish water-soluble polysaccharides (LLPs).

Physicochemical Analysis of LLPs. Moisture of the tea polysaccharide was determined by drying the polysaccharides at 110 °C for 2 h and calculating the amount of evaporated water (7). The ash amount was measured by incinerating the polysaccharides overnight in a muffle furnace at 550 °C and weighing the residue (7). Total carbohydrate content in LLPs sample was quantified by the phenol-sulfuric acid method, using glucose as a standard (16). The protein content in the LLPs was analyzed according to the Bradford method, using BSA as a standard (17). The Folin−Ciocalteu method for determination of total phenolics in LLPs was used in accordance with the description of Singleton et al., using tannic acid as a standard (18).

HPLC Analysis for Monosaccharide Composition of LLPs. The component monosaccharides in LLPs were hydrolyzed, separated, and quantified by HPLC as previously described (19). Briefly, 20 mg of LLPs was hydrolyzed to monosaccharides by the addition of 2 mL of 3 M TFA. Monosaccharides were labeled with PMP by adding 50 μL of 0.5 M PMP solution in methanol and 50 μL of 0.3 M NaOH, vortexing, and incubating at 60 °C for 1 h. The mixture was neutralized with 50 μL of 0.3 M HCl. The resulting solution was extracted with chloroform (1 mL), and the process was repeated three times and then the aqueous layer was filtered through a 0.45 μm membrane for HPLC analysis at 250 nm. The analysis was carried out on a Shimadzu LC-2010A HPLC system equipped with a quaternary gradient pump unit, an UV−vis detector (190−700 nm) and an auto-sampler (0.1−100 μL). The analytical column used was a RP-C$_{18}$ column (4.6 mm i.d. × 250 mm, 5 μm, Venusil, USA). Elution was carried out at a flow rate of 1.0 mL/min at 35 °C. The mobile phase A consisted of acetonitrile, and the mobile phase B was 0.045% KH$_2$PO$_4$-0.05% triethyl-amine buffer (pH 7.0) using a gradient elution of 90−89−86% B by a linear decrease from 0 to 15 to 40 min. The injection volume was 20 μL.

Animal Experiment. BALB/c mice (20−25 g, 6−8 weeks old) were obtained from the Experimental Animal Center, the Fourth Military Medical University, China. Animals were acclimatized for at least 7 days prior to use and maintained in a temperature-controlled environment (22 ± 2 °C) with a 12 h light−dark cycle and with free access to water and standard rodent chow. All animals were treated in accordance with the Guidelines of the Principle of Laboratory Animal Care (NIH Publication, revised 1985). Animals used in each experiment were randomly assigned to four groups of 10 animals each, i.e., vehicle control group, and 50, 100, and 300 mg/kg·BW LLPs groups. LLPs were intragastrically administered to mice for 30 consecutive days, and the control mice were given vehicle

(normal saline) only. The dose volume was 0.2 mL/10 g·BW. Male guinea pigs weighing more than 400 g were used for complement preparation. Guinea pig blood was obtained by cardiac puncture from the isolated hearts of four male guinea pigs, and serum was prepared by centrifugation and diluted 1:10 with PBS.

The Relative Thymus and Spleen Weight. After 30 days of oral administration, mice were weighed and sacrificed by cervical dislocation. Spleen and thymus were excised from the animal and weighed immediately. The indexes of thymus and spleen were expressed as the weight of thymus and spleen relative to body weight (w%), respectively.

Evaluation of Phagocytic Activity. The phagocytosis of the reticulo-endothelial system of mice was measured by clearance rate of carbon particles as described (20). Briefly, after 30 days of oral administration with LLPs, Indian ink at a dose of 100 μL/10 g·BW was injected by tail vein. Blood (20 μL) was drawn from the retro-orbital venous plexus with a capillary at intervals of 2 min (t_1) and 10 min (t_2) after the carbon particle injection. Each blood sample was immediately added into 2 mL of 0.1% Na$_2$CO$_3$ solution. The optical density of each sample at 600 nm was read and used to calculate the phagocytic index K as the following equation: $K = (\ln OD_1 - \ln OD_2)/(t_2 - t_1)$, where OD_1 and OD_2 were the optical densities at time t_1 and t_2, respectively.

Peritoneal macrophages were prepared as previously described (7, 11). Phagocytic capacity of peritoneal macrophages was assessed following the methods previously described (21). After the last pretreatment with LLPs, mice were immunized with 1.0 mL of 20% (v/v) chicken red blood cells (CRBC) by intraperitoneally (ip) injection. Thirty minutes later, they were sacrificed and peritoneal cells were aseptically harvested by peritoneal lavage with 2 mL of PBS, 1.0 mL of cell-rich lavage fluid was smeared on a slide. After incubation at 37 °C for 30 min, nonadherent cells were washed off with PBS, and then slides were fixed and stained using a 9-fold-diluted Giemsa solution (Sigma, St. Louis, MO) with PBS (pH 7.4). The percentage of phagocytosis was determined by counting the number of phago-cytes that swallowed the CRBC per 100 phagocytes on each slide.

Measurement of Plaque-Forming Cells (PFCs). On the 25th day of experiment, the mice in the presence or absence of LLPs pretreatment were immunized with 0.2 mL of 10% (v/v) sheep red blood cells (SRBC) by ip injection, and LLPs administration was continued for another 5 days prior to the humoral immunity assay (21). The PFC assay was performed according to the method reported by Cunningham and Szenberg (22). Briefly, the animals were sacrificed after the immunization with SRBC for 5 days, spleen cells were separated in RPMI-1640 medium, washed twice, and suspended in the same medium (10^6 cells/mL). Then 100 μL of 5×10^6 splenocyte suspension and 100 μL of 20% SRBC were mixed with 100 μL of 1% agarose and then added on a slide covered with a 1.2% agarose layer in PBS (0.05 M, pH 7.4, NaCl 0.15 M). The Petri dishes were followed by an incubation period of 90 min at 37 °C, and then 2 mL of 1:9 diluted fresh guinea pig serum was added to Petri dishes and the plate was reincubated for another 90 min to allow plaque formation. The number of plaques was counted immediately, and values are expressed as counts per 10^6 spleen cells.

Assay for Serum Hemolysis Production to SRBC. After 25 days of LLPs or vehicle administration, the control and experimental mice were immunized with an ip injection of 0.2 mL of 2% SRBC in PBS. Five days later, serum was collected through the eye orbit and then diluted with PBS according to multiple proportions. The diluted serum (100 μL) was transferred to a hemagglutination microplate, and 100 μL of SRBC (0.5%) was added, followed by 100 μL guinea pig serum in sequence. For negative controls, the complement was substituted with PBS. The degree of agglutination was observed after being incubated for 3 h at 37 °C for complement-mediated SRBC hemolysis and terminated on ice. Antibody product was calculated as follows: Antibody product = $S_1 + 2S_2 + 3S_3 + \cdots + nSn$, S was defined as the degree of agglutinating, and n was defined as dilution rate (23).

Determination of Delayed-Type of Hypersensitivity (DTH) Response. The DTH response was determined using the method of Langrange et al. (24). In brief, on the 26th day of LLPs administration, mice were immunized ip with 200 μL of SRBC (2%, about 5×10^8 SRBC). LLPs administration was continued for 4 days, and then all the animals were again subcutaneously (sc) challenged with SRBC (2%) in the left hind footpad on the fourth day after SRBC ip immunization. The right footpad was injected sc, with the corresponding volume of PBS serving as trauma

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control for nonspecific swelling. The increase in footpad thickness was measured 24 h after the challenge using dial caliper (pitch, 0.01 mm, Guanglu, China). The results were expressed as difference in the reading between the footpads injected with SRBC to that of PBS.

Assay for Con A-Induced Lymphocyte Proliferation. The cell proliferation assay was determined by the colorimetric MTT method (*25*). Briefly, the splenocytes of the mice treated with vehicle or LLPs were isolated by gently pressing spleen tissue through nylon mesh filters. The separated splenocytes were placed into the 96-well flat-bottomed microplates at 2×10^6 cells/well in RPMI 1640 media with or without 1.5 μg/mL T cell and B cell mitogen of Con A. Cells were incubated at 37 °C under 5% CO_2 for 48 h, and 20 μL of 5 mg/mL MTT was then added to each well and the plates were incubated at 37 °C for 4 h. Cell dissociation solution (20% SDS, 50% DMSO, pH 4.5) was then added to each well to dissolve the purple crystal. Spleen cell proliferation was measured by determining the absorbance at 570 nm using an automated microplate reader (Biorad model 680). The proliferation ratio of control cells induced by Con A was taken as 100%.

Statistical Analysis. Unless otherwise indicated, the results were expressed as the means ± SEM of data obtained from triplicate experiments. Statistical analysis was performed by a *t*-test, and $p < 0.05$ was regarded as statistically significant.

RESULTS

Chemical Composition of LLPs. The crude LLPs were isolated from the green tea leaves of *L. lucidus* by using a multistep purification procedure including hot-water extraction and repeated ethanol precipitation. With this method, the extraction yield of LLPs can reach approximately 3.5% (w/w) of the dried tea leaves. The assay for total carbohydrate content by the phenol–H_2SO_4 method showed that the crude extract LLPs contained approximately 71.4% w/w of polysaccharides. LLPs were easily soluble in water but were not soluble in organic solvents such as ethanol, ether, acetone, or chloroform. In addition, there were no reactions of Folin–Ciocalteu reagent with LLPs, which suggested that no polyphenols existed in the tea polysaccharides, and the small molecular phenolic compounds in tea leaves (*1–3*) had been successfully removed via dialysis processing (cutoff $M_w > 10000$ Da) against distilled water in the purification of the macromolecular LLPs. The negative reaction results of LLPs with iodine–potassium iodide reagent indicated that LLPs extract was a nonstarch polysaccharide mixture. Nevertheless, only small amounts of protein (3.9%) and ashes (4.4%) were found in LLPs. These results suggest that LLPs are probably the protein-bound polysaccharides.

To gain more composition information, LLPs preparation was also subjected to further monosaccharide composition analysis by HPLC technique. The chromatographic separation of 10 PMP-labeled standard monosaccharides possibly existed in plant polysaccharides was achieved by using commonly reversed-phase C_{18} column and UV detection. As can be seen from **Figure 1A**, the baseline separation of all the examined monosaccharides was obtained within 35 min under isocratic conditions using acetonitrile–KH_2PO_4 as the mobile phase combined triethylamine as the modifier. The peaks were identified in the order of mannose, ribose, rhamnose, glucuronic acid, galacturonic acid, glucose, xylose, galactose, arabinose, and fucose by comparison with the retention time of the commercial standards under the same conditions. The results from **Figure 1B** showed that LLPs were of heteropolysaccharides and were composed of mannose, ribose, rhamnose, glucuronic acid, galacturonic acid, glucose, xylose, galactose, and arabinose in the molar percentages of 8.3%, 0.8%, 3.1%, 1.3%, 14.2%, 10.2%, 3.7%, 50.1%, and 8.4% (mol %) of all the quantitative monosaccharides, respectively. This monosaccharide profile implied that the polysaccharides were mainly composed of neutral galactose, followed by galacturonic acid,
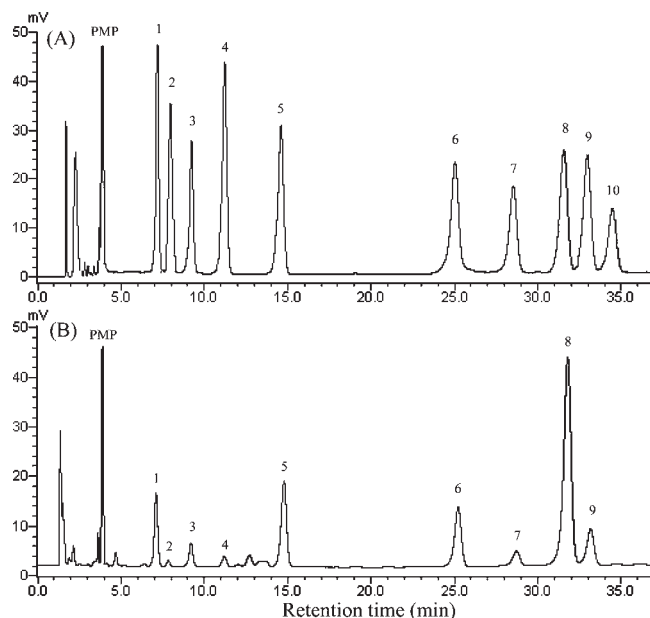
Figure 1. The HPLC chromatograms of PMP-labeled standard monosaccharides (**A**) and component monosaccharides released from LLPs (**B**). LLPs were hydrolyzed into component monosaccharides with TFA at 100 °C for 8 h and subsequently were labeled with PMP, and then the PMP-labeled monosaccharide were separated and identified by HPLC-UV at 250 nm as described in the Materials and Methods. Peaks: (1) mannose; (2) ribose; (3) rhamnose; (4) glucuronic acid; (5) galacturonic acid; (6) glucose; (7) xylose; (8) galactose; (9) arabinose; (10) fucose (internal standard).

glucose, arabinose, and mannose, accounting for up to 91.2% in the relative molar distribution.
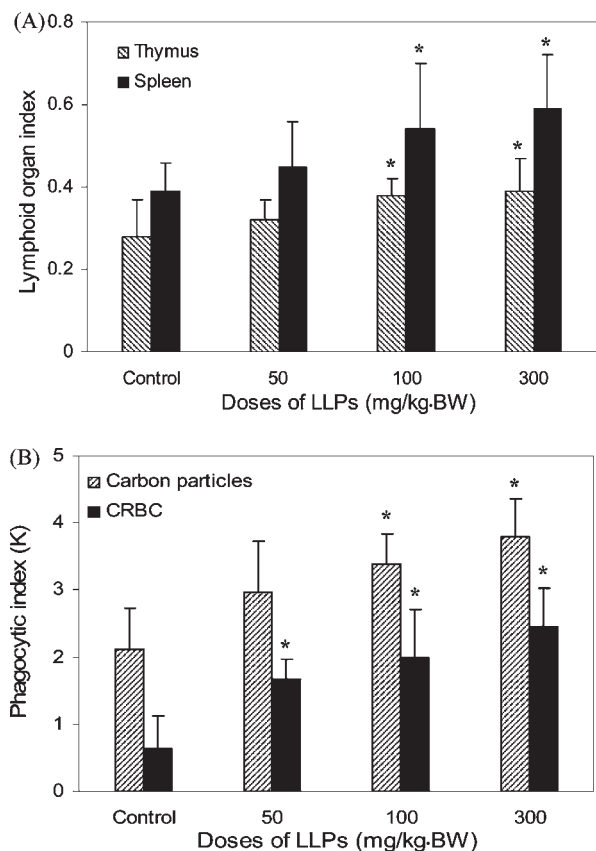
Effects of LLPs on Thymus and Spleen Indices in Mice. As shown in **Figure 2A**, the statistically significant increases in the thymus and spleen indices were observed in mice by oral applied LLPs at the doses of 100 and 300 mg/kg·BW for 30 consecutive days, compared with control group ($p < 0.05$). However, there was no significant difference at the low dose of LLPs (50 mg/kg·BW), although mice from the test group displayed a little higher weight gain compared to those from the control group ($p > 0.05$). In addition, both the control and test group mice grew well, and there were no significant alterations between the body weight gain of the mice from the test and control groups ($p > 0.05$, data not shown).

Nonspecific Immune Modulation of LLPs. In this study, phagocytic activity of LLPs-activated monocytes in mice was investigated using carbon clearance test. The results from **Figure 2B** showed that at doses of 100 and 300 mg/kg·BW, oral administration of LLPs to mice for 30 days caused a significant increase by 60.7% and 79.6% in the phagocytic index of monocytes to circulating particles, respectively ($p < 0.05$ versus control), but this effect was not statistically significant at a dose of 50 mg/kg·BW ($p > 0.05$ vs control).

To further confirm, the phagocytosis of peritoneal macrophages against CRBC was also tested in mice. As shown in **Figure 2B**, the phagocytosis indices at 50, 100, and 300 mg/kg·BW of LLPs were remarkably improved by 1.6-, 2.2-, and 2.9-fold in a dose-dependent manner, respectively, compared with control group ($p < 0.05$). The results suggest that LLPs possess nonspecific immune function in vivo.

Effects of LLPs on Humoral Immune Activity. The effects of LLPs on humoral immune parameters in mice were investigated. As shown in **Figure 3A**, LLP treatment at low dose caused a 34.4%
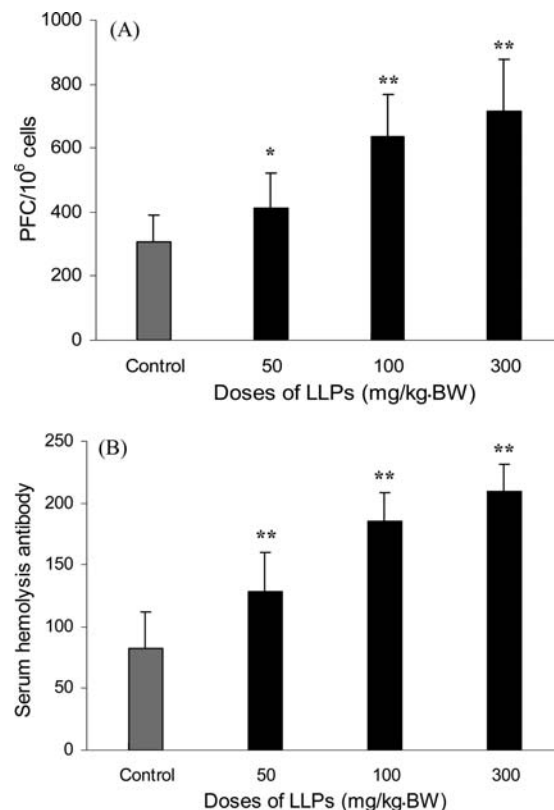
Figure 2. Effects of LLPs on relative lymphoid organ weights (**A**) and the nonspecific phagocytic activities (**B**) in mice after 30 days of oral administration. Thymus and spleen indices were expressed as the relative weight to body weight, respectively. Phagocytic index represents the average number of inert particles or CRBC ingested per phagocytic cell during a given time. Values represented means ± SEM of 10 mice. *$p < 0.05$, **$p < 0.01$ indicate a significant difference versus control group.

Figure 3. Effects of LLPs treatment on humoral immunity as assessed by the quantitative PFC assay (**A**) and the measurement of serum hemolysis antibody to SRBC (**B**) in mice. Data are shown as mean ± SEM of 10 mice. *$p < 0.05$, **$p < 0.01$ vs control group.

increase in the PFC number of spleen cells against SRBC (T-dependent particulate antigen), compared with that in the control ($p < 0.05$). At middle and high doses of LLPs, this effect was further increased by 1.1- and 1.3-fold, respectively, in comparison with control group ($p < 0.01$). To further confirm, we also evaluated the levels of serum hemolysin antibody in response to SRBC in mice. As shown in **Figure 3B**, LLPs treatment dose-dependently enhanced the serum hemolysin level by 0.6-, 1.3-, and 1.6-fold at doses of 50, 100, and 300 mg/kg·BW compared with control ($p < 0.01$), respectively. The results indicate that LLPs exert specific immune-enhancing activity in vivo.

Effects of LLPs on Cellular Immune Function. To further ensure the in vivo immune activity of LLPs, we also examined whether the specific immune response of LLPs was also involved in the cellular immune mechanism. **Figure 4** shows the effects of LLPs on the DTH response to SRBC (**A**) and the splenocyte proliferation (**B**) in mice, respectively. As expected, the treatment at the dosages of 50, 100, and 300 mg/kg·BW resulted in 0.7-, 1.2-, and 1.7-fold increases in the DTH response in a dose-dependent manner, respectively ($p < 0.01$ vs control). Furthermore, it was found that the low-dose of LLPs markedly enlarged the proliferative ratio ($132.7 \pm 26.3\%$) of Con A-induced lymphocytes, compared with control group ($100 \pm 20.3\%$) ($p < 0.05$). Especially for the mid- or high-dose LLPs, the Con A-induced proliferative ratios were further magnified to $183.9 \pm 31.7\%$ and $192.5 \pm 29.4\%$, respectively, in comparison with control group ($p < 0.01$).

DISCUSSION

L. lucidus was claimed to have a wide range of health benefits, including anti-inflammation, antioxidative, and immunostimulating effects (*1−3*). However, many investigations were achieved directly with *L. lucidus* or *L. lucidus*'s extracts, and *L. lucidus*'s chemical components responsible for the effects remain unclear (*3*). We have succeeded in isolating the polysaccharide LLPs from tea leaves of *L. lucidus*. Furthermore, *L. lucidus* was characterized as a good source of galactoside-enriched polysaccharides. Moreover, LLPs displayed an overall stimulatory effect on the specific as well as nonspecific immune functions in mice. This is the first report that LLPs are one of the main active ingredients responsible for the immunomodulatory effect of the tea leaves of *L. lucidus*, a traditional dietary-treated herb.

In the present study, systemic administration of LLPs was found to enhance the indices of the thymus and spleen of mice, suggesting that LLPs could improve the immune function (*21, 26*). A closer examination of humoral immunity revealed that during the humoral immune response, serum hemolysin level and PFCs were elevated in animals following LLPs treatment. A similar effect of polysaccharides or aqueous extracts from plants has been observed in previously reported studies (*21, 26, 27*). It is well-known that humoral immunity, via the antibody response, is regulated by B cells and other immune cells involved in antibody production (*21, 22*), and this mechanism might be associated with the specific immune response of LLPs.

It is interesting that LLPs could augment Con A-induced splenocyte proliferation to exert cellular immunity. Furthermore, the treatment with LLPs also enhanced the DTH reaction, as reflected by the increased footpad thickness compared to the control group, suggesting T-cell mediated DTH is the possible

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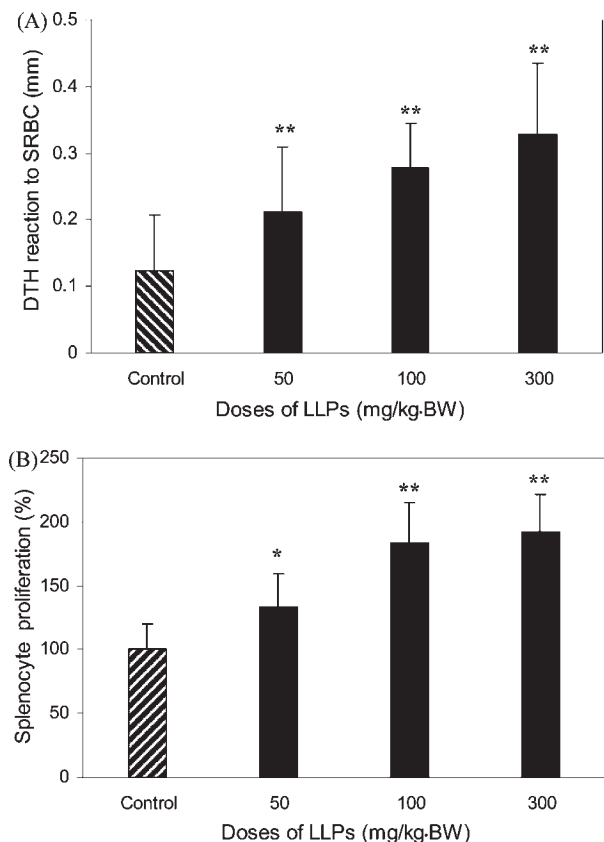
Figure 4. Effects of LLPs treatment on cellular immune function as assessed by the DTH response (**A**) and Con A-induced proliferation of splenic lymphocytes (**B**). Footpad swelling for DTH response was tested as the difference between the thickness of footpads challenged with SRBC and saline, respectively. The Con A-induced proliferation ratio of splenocytes in control mice was regarded as 100%. $*p < 0.05$, $**p < 0.01$ compared with control group.

mechanism for cellular immune function of LLPs. DTH reaction is an expression of cell-mediated immunity and is a major mechanism of defense against intracellular pathogens, including mycobacteria, fungi, and certain parasites, and may also be involved in transplant rejection and tumor immunity (27). In agreement with the results of humoral immunity assay, LLPs also effectively exhibit cellular immune activity via enhancing DTH response and spleenocyte proliferation in vivo. This study may support a possible role of the polysaccharides in assisting the cell-mediated immune response.

Phagocytosis is the first step in nonspecific host-defense responses to invading antigen, and therefore, the assessment of phagocytic activity is crucial for potential immunostimulants (5, 21). In this study, higher doses of LLPs (100 and 300 mg/kg·BW) were found to increase the phagocytic index as assessed by the clearance of intravenous carbon particles as well as by CRBC ingestion of phagocytic cells. Taken together, LLPs can stimulate the host immune system, including the modulation of nonspecific and specific responses.

L. lucidus contains a large number of complex ingredients of polysaccharides, flavonoids, coumarins, terpenoids, tannins, etc. In recent years, polysaccharides, in particular, have emerged as an important class of biological response modifiers although most data originate from the mucosal immune system of the intestine and in vitro experiments (4, 5, 27−30). However, whether orally applied polysaccharides with large molecular mass can be absorbed into the bloodstream to exert the putative immunological

effects is still not clear. In fact, many authors believe that oral administration of oligosaccharides and probiotics (living bifidobacteria and lactobacilli) can produce ideally systemic immune response (28, 29). We speculate that prebiotic polysaccharides have effects on the systemic immune responses similar to probiotics because both enhance the number of beneficial bacteria in the gut. Interestingly, the present investigation showed that LLPs did activate the systemic immune response of host, including nonspecific and specific immunities in mice, indicating that a microflora-dependent mechanism is possibly responsible for this immune response. On the basis of this finding, it was suggested that adequate consumption of *L. lucidus* tea and its polysaccharide extract might have a favorable effect on maintaining or improving the specific and nonspecific functions of the host.

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