



# Immunoregulation and antioxidant activities of a novel acidic polysaccharide from *Radix Paeoniae Alba*

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## Abstract

*Radix Paeoniae Alba* is widely used in Chinese traditional medicine to treat various diseases such as gastrointestinal disorders, immunomodulatory, cancer, and other diseases. In this paper, a novel acidic polysaccharide RPAPS purified from *Radix Paeoniae Alba* was evaluated for its structural features and potential of immunomodulatory and antioxidant activities. RPAPS (molecular weight:  $1.0 \times 10^5$  Da) was mainly composed of  $\alpha$ -(1 → 4)-Glc<sub>p</sub>,  $\alpha$ -Arap,  $\alpha$ -Gal<sub>p</sub>,  $\alpha$ -Rhap,  $\beta$ -D-Glc<sub>p</sub>,  $\alpha$ -(1 → 6)-linked Glc<sub>p</sub> and GalA. Immunological tests indicated that RPAPS could improve RAW264.7 phagocytic activity and LPS-induced splenocyte proliferation. For antioxidant activities, RPAPS showed reducing power and DPPH scavenging activity in dose dependent. Moreover, RPAPS could significantly protect the PC12 cells from H<sub>2</sub>O<sub>2</sub> damage. These data implied polysaccharides RPAPS had the potential to be novel natural antioxidative and immunopotentiating agents for using in functional foods or medicine.

**Keywords** *Radix Paeoniae Alba* · Ranunculus · Acidic polysaccharide · Immune activity · Antioxidant activity

## Introduction

The immune system is responsible for maintaining an immune homeostasis balance under health physiological conditions. However, factors such as malnutrition, application chemotherapy and stressors, can affect or destroy this balance and cause immune disorder [1]. High concentrations of ROS are causing damage to proteins, lipids and nucleic acids [2]. Hence, the development of novel, more efficacious and low toxic antioxidative and immunopotentiating agents is extremely urgent. Previous studies have suggested that herb polysaccharides have good antioxidant [3, 4] and immunoregulation activities [5], and have attracted more and more attention for their healthcare benefits and therapeutic use with low toxicity and little side effects.

*Radix Paeoniae Alba* (RPA), one of the most well-known ranunculus family herbs in China, Korea, and Japan for more than 1200 years, has been widely used to treat various diseases such as gastrointestinal disorders [6], immunomodulator [7], cancer [8] and other diseases. However, there have been few reports regarding the identification of acidic polysaccharides from *Radix Paeoniae Alba* and their potential biological activities. Our previous study showed that the crud polysaccharide from *Radix Paeoniae Alba* had a significant antidepressant effect. To further dissect chemical and biological basis of the active polysaccharides of *Radix Paeoniae Alba*, we isolated polysaccharides from *Radix Paeoniae Alba* and further test its biological activities. It has been found that the antioxidant and immunoregulatory activities of some polysaccharides have been related to their antidepressant mechanisms [9]. Two  $\alpha$ -glucans from *Radix Paeoniae Alba* showed weak antioxidant and immunomodulatory activities [10]. Literatures showed that the acidic polysaccharides containing sulfate group and uronic acid composition may have better immunomodulatory activity [11, 12].

In the present study, an acidic polysaccharides RPAPS from *Radix Paeoniae Alba* was obtained by anion-exchange and gel filtration chromatography. Its structural features was characterized by monosaccharide compositions, uronic acid analysis, IR and NMR analyses. The antioxidant and

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immunomodulatory activities of the purified acidic polysaccharides RPAPS were further examined.

## Materials and methods

### Medicine and chemicals

Dried *Radix Paeoniae Alba* was produced in yunnan province, China. D-glucose, trifluoroacetic acid (TFA) and galacturonic acid were purchased from Aladdin Reagent Int (Shanghai, China). 3-(4, 5-Dimethyl tiazol-2-yl)-2, 5 diphenyl tetrazolium bromide (MTT), lipopolysaccharide (LPS), concanavalin A (ConA) and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich. The other chemicals were analytical grade.

### Animals and cells

Kunming mice were purchased from School of Medicine, Xi'an Jiao Tong University, China. The mice were maintained on standard pellet diet and water ad libitum at 21 °C. All experiments were performed in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China. The RAW264.7 and PC12 cell line were previously preserved in our laboratory. Spleen lymphocytes were obtained as previous method [10].

### Extraction and purification of acidic polysaccharide

The isolation and purification steps are shown in Fig. 1. The dried RPA powder was pre-extracted with 95% ethanol to remove lipids. The residue powder was extracted 3 times with distilled water at 80 °C for 2 h. The supernatants were concentrated and collected with alcohol sedimentation. The precipitate was dissolved, dialyzed and lyophilized to obtain RPA crude polysaccharide (RPAP). The RPAP was applied to a DEAE-cellulose column (OH<sup>-</sup> form) which was sequentially eluted with distilled water and 0.1 M NaCl at a flow rate of 0.5 mL/min. The water eluate (RPAPW) was further purified with G-25 sephadex, RPAPW1 and RPAPW2 obtained [10]. The 0.1 M NaCl eluate was further purified with Sephacryl S300, then a novel acidic polysaccharide RPAPS was obtained.

### Molecular weight determination

As described previously [10], the molecular weights of RPAPS were determined on high performance gel permeation chromatography (HPGPC). The calibration curve of Log (Mw) vs. elution time (T) is:  $\text{Log}(\text{Mw}) = -0.1316 T + 10.94$ .

### Chemical compositions analysis [10]

Neutral carbohydrate content was determined by the phenol-sulphuric acid method. Protein content was evaluated by Bradford's method. Uronic acid content was measured according to *m*-hydroxydiphenyl-sulphuric acid method. Neutral monosaccharide compositions were analyzed by high performance liquid chromatographic (HPLC) after precolumn-derivatization the hydrolysate with 1-phenyl-3-methyl-5-pyrazolone (PMP) [13], with several modifications. Briefly, 4 mg RPAPS was completely hydrolyzed with 2 M trifluoroacetic acid (TFA) at 110 °C for 4 h. The hydrolysate was dried with methanol to remove the excess TFA. Then, the hydrolysate or six monosaccharides standard (mannose, rhamnose, glucose, galactose, xylose and arabinose) was separately dissolved in 100 µL water, then 100 µL of 0.6 M NaOH and 200 µL of 0.5 M PMP in methanol were added, followed by incubation of the mixture at 70 °C for 100 min. The reaction mixture was neutralized with 200 µL of 0.3 M HCl after cooling to room temperature and then extracted with chloroform three times. HPLC analysis was performed on an Agilent ZORBAX Eclipse XDB-C18 column at a constant flow rate of 1.0 mL/min, and the column temperature was maintained at 20 °C. The mobile phase was composed of gradient elution of the two solutions: Buffer A: 15% CH<sub>3</sub>CN in 0.05 M phosphate buffer solution (pH 6.7); Buffer B: 40% CH<sub>3</sub>CN in 0.05 M phosphate buffer solution (pH 6.7). These monosaccharides were identified by their retention times and quantitatively determined by their peak areas.

### NMR analysis

The RPAPS sample was dissolved in 0.5 mL D<sub>2</sub>O. The <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of samples were recorded with a Bruker AM 500 spectrometer with a dual probe in the FT mode at room temperature.

### Infrared spectra (IR) analysis

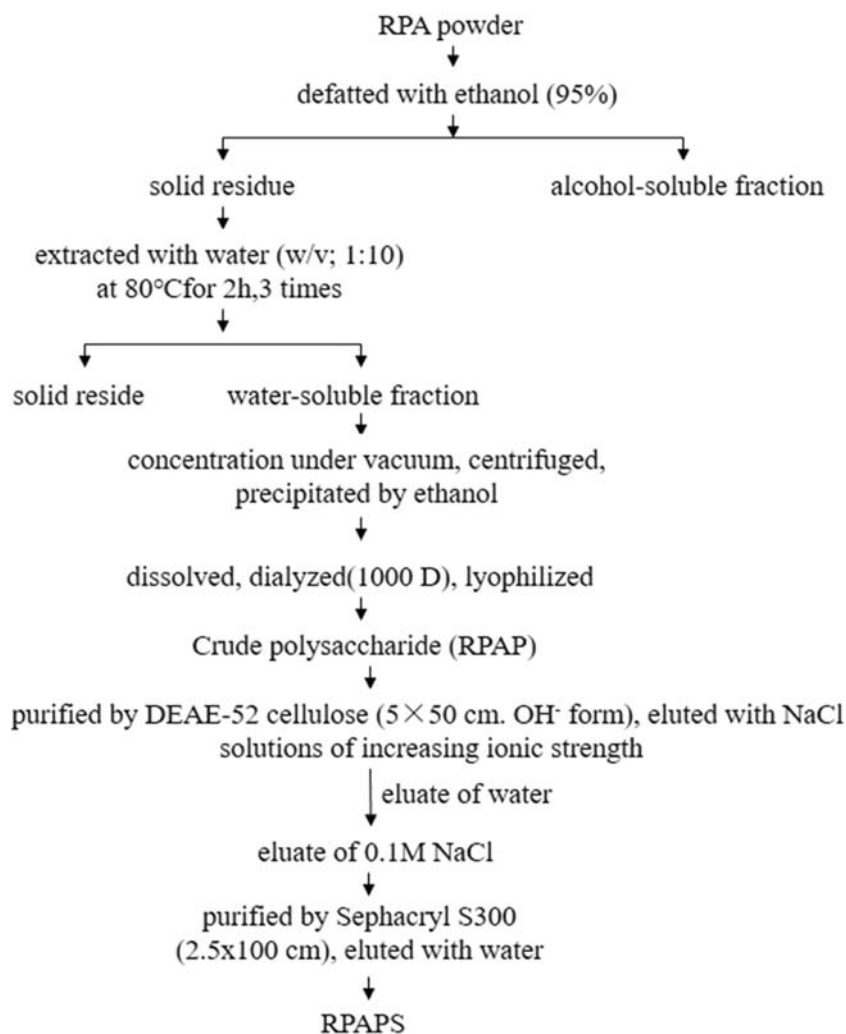
The IR spectrum of polysaccharide was determined using a Fourier transform infrared spectrophotometer (BRUKER TEMSOR 27, BRUCK, Germany). RPAPS were grounded with KBr powder and pressed into a 1 mm pellet for FTIR measurement between 400 cm<sup>-1</sup> and 4000 cm<sup>-1</sup> [14].

### Determination of immunoregulation effects of RPAPS

#### Assay of macrophages phagocytosis

The phagocytic ability of macrophages was measured using neutral red uptake [15]. Briefly, cells (1 × 10<sup>6</sup> cells/well) were pipette into 96-well plates and incubated for 2 h. The cells were treated with various concentrations of samples (10, 100, 200 µg/mL) for 12 h. Then, 0.07% neutral red solution

**Fig. 1** Extraction and purification scheme of the polysaccharide RPAPS from *Radix Paeoniae Alba*



was added and incubated for 2 h. *medium* was discarded and cells in 96-well plates were washed twice with PBS to remove the neutral red that was not phagocytosed by RAW 264.7 cells. Then, cell lysis buffer (1% glacial acetic acid: ethanol = 1:1, 100  $\mu$ L/well) was added. After cells were incubated at 4  $^{\circ}$ C overnight, the optical density of each well was measured at 540 nm. The RPMI1640 medium and LPS (2  $\mu$ g/mL) were used as the blank and positive control, respectively. Phagocytosis index was calculated by the following equation:

$$\text{Phagocytosis index} = \frac{Abs_{\text{sample}}}{Abs_{\text{blank control}}} \quad (1)$$

### Lymphocyte proliferation assay

Lymphocyte proliferation *in vitro* were evaluated according to the method outlined by Mosmann [16]. LPS and ConA were used as mitogens for stimulating B and T lymphocytes, respectively. The cells ( $1 \times 10^7$ /mL) were incubated with different concentration RPAPS (10, 100, 200  $\mu$ g/mL) with or

without Con A (5  $\mu$ g/mL) and LPS (2  $\mu$ g/mL) for 48 h. Later, 10  $\mu$ L of MTT was added and the samples were further incubated for 4 h at 37  $^{\circ}$ C. The optical density was then measured at 570 nm. The percentage of proliferation was calculated using the following equation:

$$\text{Proliferation}(\%) = \frac{(OD_{\text{sample}} - OD_{\text{control}})}{OD_{\text{control}}} \times 100 \quad (2)$$

### Determination of antioxidant activities *in vitro*

#### Reducing power

1 mL RPAPS samples (0–8 mg/mL) were mixed with 1 mL phosphate buffer (0.2 M, pH 6.6) and 1 mL potassium ferricyanide (0.1%). After incubated at 50  $^{\circ}$ C for 20 min, 1 mL trichloroacetic acid solution (10%) was added, and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (1 mL), 1 mL distilled water and 0.2 mL ferric chloride (0.3%) was mixed, then the absorbance was measured at 700 nm.

### DPPH radical scavenging activity

The radical scavenging effects of samples on DPPH radical were estimated as described [17]. Briefly, 1.0 mL sample solution at different concentrations was added to DPPH (0.1 mM, 4.0 mL). The reaction solution was shaken vigorously and incubated at room temperature for 30 min, and the absorbance was measured at 517 nm. The DPPH scavenging rate (R) was calculated as follows:

$$\text{scavenging of DPPH radical (\%)} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100 \quad (3)$$

where the control solution contains distilled water instead of the DPPH solution, while distilled water instead of sample was used for the blank. All tests were performed in triplicate and the mean of Abs was used in the equation above.

### Cell cytotoxicity and protective effects on H<sub>2</sub>O<sub>2</sub>-induced PC12 cell death

MTT assay was used to test the cell cytotoxicity and protective effects [18]. PC12 cells were cultured in DMEM medium. To check the cell cytotoxicity, cell suspensions were seeded in 96-well plates ( $1 \times 10^5$ /well), and incubated at 37 °C for 12 h, and then the RPAPS sample was added. After 6 h, 20  $\mu$ L of the MTT (5 mg/mL) were added into each well and the plate was further incubated for 4 h. Finally, the medium was removed and DMSO (200  $\mu$ L) was added. After 10 min, the absorbance was measured at 570 nm in a microtitre plate reader. For protective assay, RPAPS sample was added to the cultured cells and incubated for 30 min before the addition of H<sub>2</sub>O<sub>2</sub> (final concentration 700  $\mu$ M, 6 h), other procedures were same as above. Assays were performed in quadruplet wells for each sample. Data were expressed as the percent of cell viability compared with control.

### Statistical analysis

Results were expressed as mean  $\pm$  SD. Data were analyzed by *t*-test with the Prism software for Windows (version 5.00; GraphPad Software).

## Results and discussion

### Molecular weight determination and chemical compositions analysis

The extraction and purification steps were shown in Fig. 1. After 0.1 M NaCl eluent fractionation on DEAE-52 cellulose column (OH<sup>-</sup> form), Sephacryl S300 were used for further

purification, and RPAPS was obtained with the yield about  $5.08 \pm 0.27\%$ . As shown in Table 1, the molecular weight of RPAPS was calculated to be  $1.0 \times 10^5$  Da on HPGPC in reference to standard dextrans. The molecular weight of RPAPS was lower than our published  $\alpha$ -glucans RPAPW1 and RPAPW2.

The neutral carbohydrate, protein and uronic acid contents of RPAPS were  $90.59 \pm 3.82\%$ ,  $2.01 \pm 0.10\%$  and  $14.09 \pm 3.77\%$ , respectively (Table 1). The monosaccharide compositions analysis result showed that RPAPS was mainly composed of glucose (64%), arabinose (19%) and galactose (10%) (Table 1).

### NMR and infrared spectra analysis

<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of RPAPS are shown in Fig. 2. NMR spectroscopy provides detailed structural information of carbohydrates, including identification of monosaccharide, elucidation of  $\alpha$ - or  $\beta$ -anomeric configurations, and the establishment of linkage patterns [19]. According to the data reported in the literature [10, 20–23] and the results of monosaccharide compositions analysis, the chemical shifts of the anomeric protons and carbons signals of various sugars were assigned.

The five signals at  $\delta$ H 5.36, 5.19, 5.12, 5.06 and 4.94 ppm could be attributed to the presence of  $\alpha$ -form pyranos of  $\alpha$ -(1  $\rightarrow$  4)-Glc<sub>p</sub>,  $\alpha$ -Arap,  $\alpha$ -Gal<sub>p</sub>,  $\alpha$ -Rhap and  $\alpha$ -(1  $\rightarrow$  6)-linked Glc<sub>p</sub>. There was a signal peak of proton resonance at 4.61 ppm, almost shielded by the solvent peak. It was ascribed to the  $\beta$ -D-Glc<sub>p</sub>. The signals in the range of  $\delta$ H 3.24–4.26 ppm should be assigned to the H-2 to H-5 (or H-6) of the glycosidic ring [24]. The chemical shift of 1.88 ppm was the signal of the methyl proton of Rha residues.

The four significant signals of RPAPS should be assigned to C-1 of anomeric residues  $\alpha$ -Glc<sub>p</sub> (99.58 ppm),  $\alpha$ -Arap (96.08 ppm),  $\alpha$ -Gal<sub>p</sub> (91.87 ppm) and  $\beta$ -Glc<sub>p</sub> (107.43 ppm). The signal  $\delta$ C at 182.43 ppm was attributed to the signal peak of carboxylic carbon of GalA. The strong signal located in the region of 60–84 ppm was attributed to the pyranose configuration in RPAPS. The signal  $\delta$ C at 16.57 corresponded to the  $-\text{CH}_3$  groups of Rha [21].

The FTIR spectra of RPAPS were recorded at the range of 400–4000  $\text{cm}^{-1}$  (Fig. 3). The broad and intense peak nearby 3420  $\text{cm}^{-1}$  was due to the hydroxyl groups stretching vibration. The bands in the region of 2927  $\text{cm}^{-1}$  were the characteristic absorption of C-H stretching vibration. The bands corresponding to C=O stretching vibrations appeared at 1645.25  $\text{cm}^{-1}$ , and the appearance of the peaks at 1154.70  $\text{cm}^{-1}$  was assigned to the deforming vibrations of C-O bond. The absorption bands at 3418.22  $\text{cm}^{-1}$ , 1645.25  $\text{cm}^{-1}$ , 1421.17  $\text{cm}^{-1}$  and 1154.70  $\text{cm}^{-1}$  confirmed the appearance of

**Table 1** Major chemical content and neutral monosaccharide composition of RPAPS

Carbohydrate content (%)	Protein content (%)	Uronic acid content (%)	Molecular weight(Da)	Rha(%)	Glc(%)	Gal(%)	Ara(%)
90.59 ± 3.82	2.01 ± 0.10	14.09 ± 3.77	1.0 × 10 <sup>5</sup>	3.002	63.973	10.135	19.352

–COOH, indicating the existence of uronic acids in RPAPS [25]. This was consistent with the conclusion in chemical compositions analysis. The peaks at 950–1200 cm<sup>-1</sup> suggested the presence of C–O–C and C–O

link bonds. From the spectra, we proposed that the absorbance between 1000 cm<sup>-1</sup> and 1200 cm<sup>-1</sup> was due to the pyranose ring [26], which was in agreement with the result from NMR analysis.

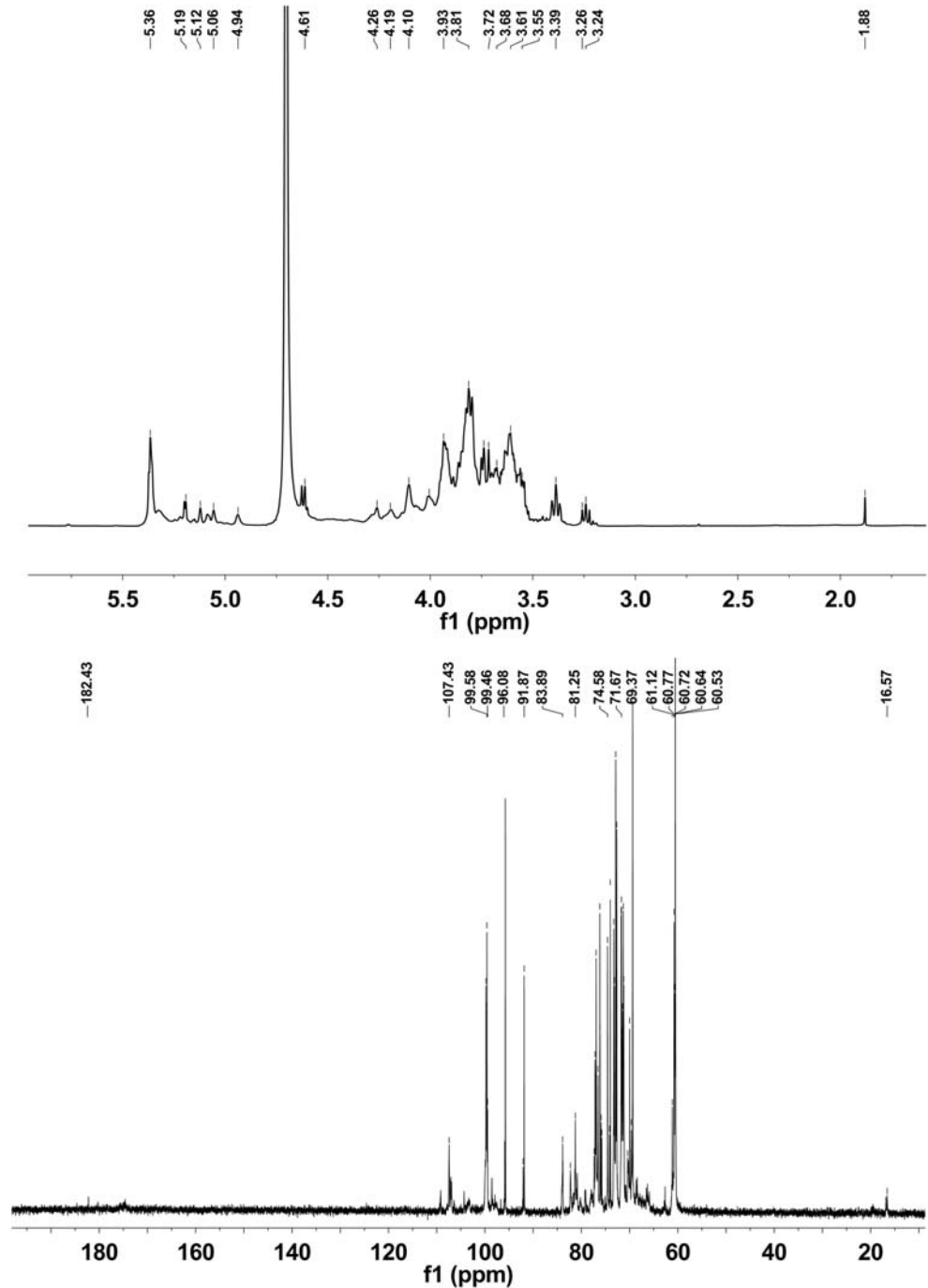
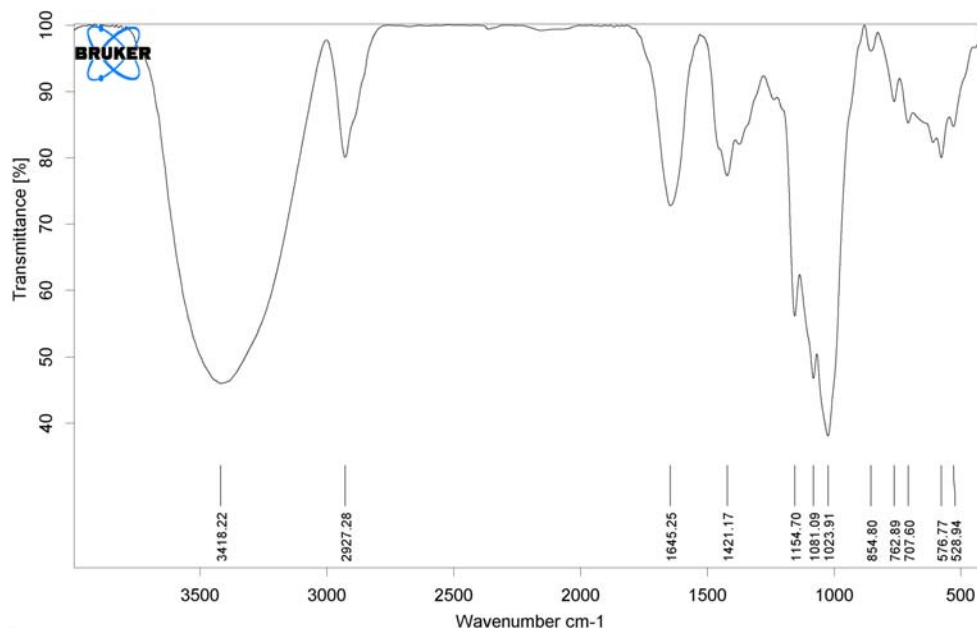
**Fig. 2** <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of RPAPS

Fig. 3 Infrared spectra of RPAPS



### Immunological activities of RPAPS

The immune system is the human's ultimate defense against infectious diseases, tumor and cancer growth. The immunologic action of polysaccharides may begin with activating major subsets of immune cells such as lymphocytes and macrophages [27]. Macrophage plays a pivotal role in multiple pathophysiological processes, such as regulate immune responses and contribute to fight against infection and inflammation [28]. Thus, we explored effects of RPAPS on the activation of macrophages and lymphocyte.

### Effects of RPAPS on RAW264.7 cells phagocytosis

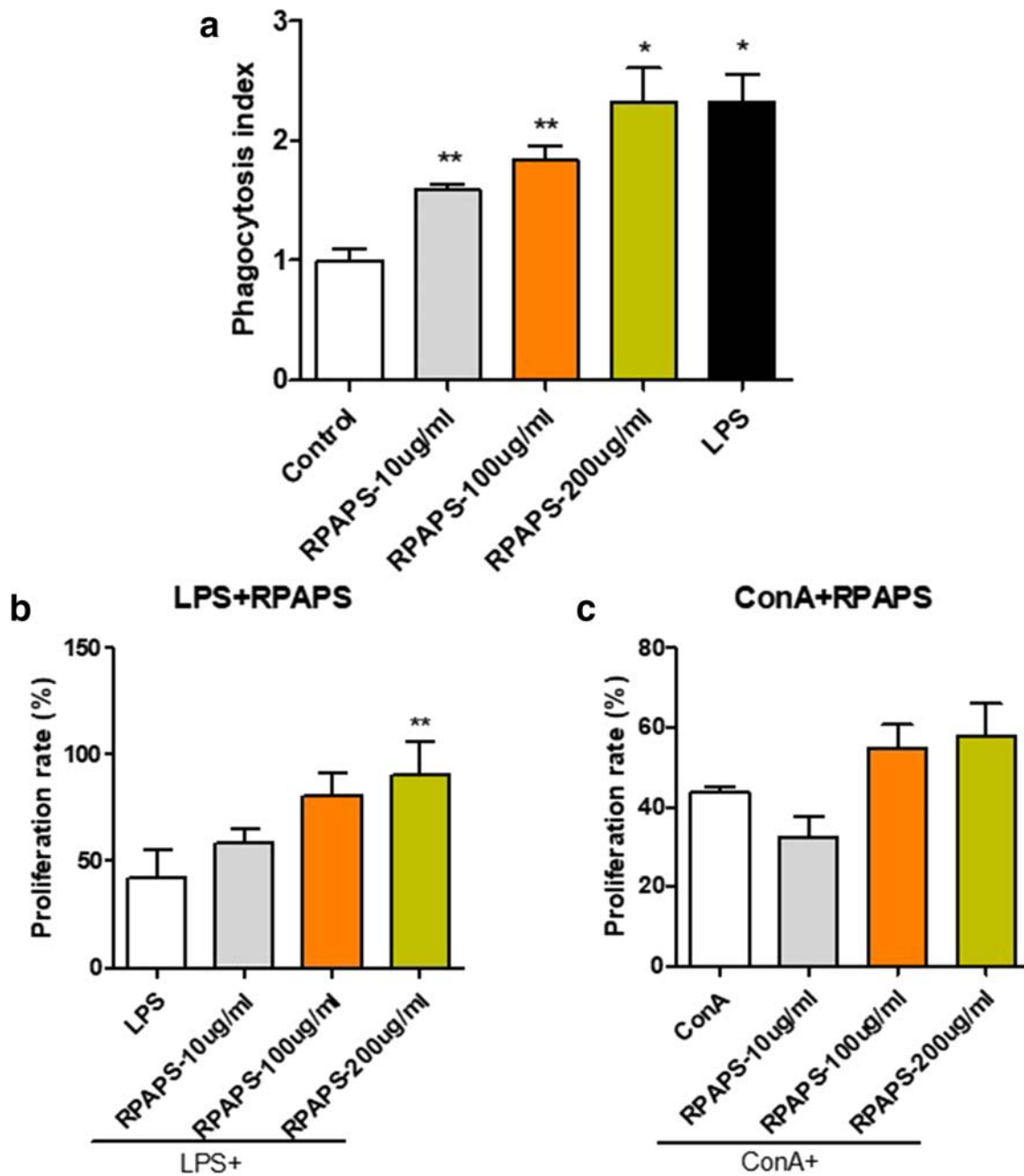
Phagocytic capacity is one of the most important indicators of the body's non-specific immunity [29]. As shown in Fig. 4A, phagocytosis index of RPAPS exceeded 1.0 and increased in a dose-dependent manner at the test concentrations, indicating that this fraction had ability to enhanced phagocytic activity of RAW 264.7 cells. Compared with the blank control, high concentration (200  $\mu\text{g}/\text{mL}$ ) RPAPS could significantly enhance the phagocytosis of macrophages as well as LPS (2  $\mu\text{g}/\text{mL}$ ). However, this activity of  $\alpha$ -glucans RPAPW1 and RPAPW2 from *Radix Paeoniae Alba* were much weaker than RPAPS [10]. During initial phases of the immune response, pattern recognition molecules such as polysaccharides and foreign ligands can be recognized by surface receptors on macrophages [30]. Our results suggested that RPAPS might had a better ability on the

macrophages activation than RPAPW1 and RPAPW2 through binding with a specific receptor on the surface of macrophages [31].

### Effect of RPAPS on splenocyte proliferation

The proliferation of spleen cells is one of the most important steps in the activation pathway of cell-mediated or humoral immunity [32]. Mitogens ConA and LPS were used to stimulate T lymphocyte and B lymphocyte proliferation, respectively [33]. Researches showed that the immune system is closely associated with depression [34]. Immune mediators such as cytokines can affect diverse central nervous system (CNS) functions and generate symptoms of depression [35]. MTT assay showed that all of RPAPS had a potential in promoting the proliferation of spleen lymphocytes. RPAPS could improve LPS-induced cell proliferation at the high concentrations (200  $\mu\text{g}/\text{mL}$ ) (Fig. 4B), but could not promote ConA-stimulated splenocyte proliferation (Fig. 4C).

Immune regulation activity is one of the most important activities of polysaccharides. It is generally known that the immunomodulating actions of polysaccharides are related to their monosaccharide composition, molecular weight, chemical composition, glycosidic linkage, conformation, degree of branching and functional groups [36, 37]. Several studies have indicated that pectic polysaccharides, which possessed a higher proportion of galacturonic acid, have good immunomodulatory effects. For example, the polysaccharides from peach and mulberry, which are



**Fig. 4** Effects of RPAPS on the phagocytosis index of RAW 264.7 cells and splenocytes proliferation. (A) RAW264.7 cells were treated with various concentrations of RPAPS for 12 h. Then, 0.07% neutral red solution was added and the phagocytosis index was calculated. (B) The mice spleen cells were incubated with RPAPS in the presence of mitogens

LPS (2  $\mu\text{g}/\text{mL}$ ) or Con A (5  $\mu\text{g}/\text{mL}$ ) (C) for 48 h. Later, MTT was added and incubated for 4 h at 37  $^{\circ}\text{C}$ . OD570 was measured. Data shown were mean  $\pm$  SD of 3 independent experiments. (\*\*\*)  $p < 0.001$ , (\*\*)  $p < 0.01$ , and (\*)  $p < 0.05$  compared with the normal control

composed of uronic acids and seven neutral monosaccharides, were effective in activating mouse peritoneal macrophage [38] and inducing NO release from RAW264.7 cells, and meanwhile inducing the expression of immune-related genes including  $\text{TNF}\alpha$ , IL6 and COX-2 [39]. The polysaccharides from the flesh of *Prunus avium*, which contain 49%–77% galacturonic acid, showed significant immune-enhancing activity [40]. High molecular weight,

high uronic acid contents and abundant monosaccharide types might make RPAPS exert better splenocyte proliferation and RAW264.7 phagocytic activity.

#### Antioxidant activities of RPAPS *in vitro*

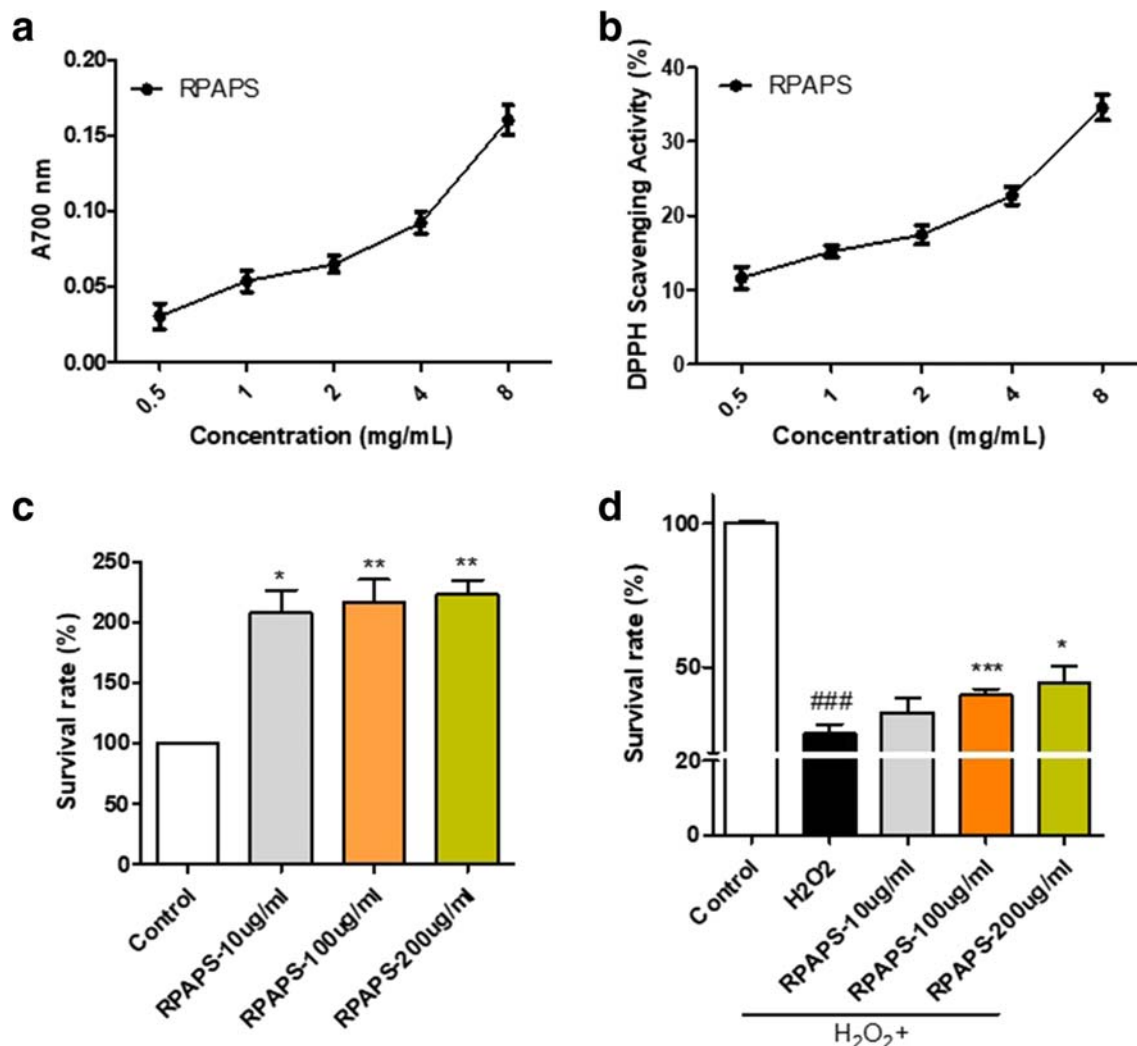
The reactive oxygen species (ROS) is believed to contribute to the pathogenesis of numerous diseases, such as cardiovascular

diseases, cancer, atherosclerosis, aging and neuropsychiatric disorders [41]. Thus, it is important to develop compounds with antioxidant properties.

The reducing power and DPPH radical scavenging assays are two classical *in vitro* antioxidant experiments which were used in this study to serve as an important antioxidant activity index of RPAPS. The higher absorbance values indicated the stronger reducing power. As shown in Fig. 5A, the reducing activity of RPAPS was in dose-response at the concentrations ranging from 0 to 8 mg/mL. RPAPS also demonstrated a dose-dependent DPPH radical scavenging activity (Fig. 5B). At 8 mg/mL, RPAPS elicited 34.6% DPPH radical scavenging activity.

PC12 cell has been widely used to investigate the mechanisms involved in neurotoxicity, neuroprotection and neurorestoration [42, 43]. Glucocorticoids at high concentration lead to PC12 neuronal damage under depressive disorder,

and this feature makes injured PC12 cells very useful as *in vitro* model system for screening of antidepressant drugs [44]. Antioxidants also could prevent PC12 cell death through the suppression of H<sub>2</sub>O<sub>2</sub>-induced ROS formation [45], the regulation of the endogenous oxidant–antioxidant balance [46]. RPAPS at the dose of 10–200 µg/mL had no significant toxic effects on PC12 cells, and RPAPS could promote cell proliferation (Fig. 5C). The incubation of PC12 cells with 700 µM H<sub>2</sub>O<sub>2</sub> for 6 h resulted in a cell viability rate of 26.55% compared to the control (Fig. 5D), suggested that the model was reliable. As expected, the pre-incubation with three different concentrations (10, 100, 200 µg/mL) of RPAPS could significantly increase the cell viability by 7.59%–18.07% in a dose-dependent manner (Fig. 5D). This antioxidant activity of RPAPS was much stronger than α-glucans RPAPW1 and RPAPW2 from *Radix Paeoniae Alba*.



**Fig. 5** Antioxidant activities of RPAPS: (A) Reducing Power; (B) scavenging of DPPH radicals; (C) Effects of RPAPS on PC12 cells proliferation, (\*\*\*)  $p < 0.001$ , (\*\*)  $p < 0.01$ , and (\*)  $p < 0.05$  compared with the control; (D) Protective effects of RATPS2 on viability losses in PC12

cells induced by H<sub>2</sub>O<sub>2</sub> (700 µM, 6 h). (###)  $p < 0.001$  compared with the control, (\*\*\*)  $p < 0.001$ , (\*\*)  $p < 0.01$ , and (\*)  $p < 0.05$  compared with the H<sub>2</sub>O<sub>2</sub> alone group



The antioxidative activity have been attributed to various mechanisms, such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [47]. Previous reports showed that the antioxidant activity of polysaccharides correlated with their monosaccharide composition, uronic acid content, molecular weight and the type of glycosidic linkage [48, 49]. The acidic polysaccharide EAP-2A, which had higher uronic acid content and larger average molecular weight, exhibited higher antioxidant activity than neutral polysaccharide EAP-1 N [50]. Our previous study also found that polysaccharide RATPS2 from *Rhizoma Acori Tatarinowi* with 49.5% galacturonic acid had stronger antioxidant activities than other two polysaccharides [51]. Thus, the superior antioxidative activity of acidic polysaccharide RPAPS might be due to its higher uronic acid content and larger molecular weight [52]. However, the exact mechanism of polysaccharides on the antioxidant activities needs to be further investigated.

## Conclusion

In this study, a novel acidic polysaccharide RPAPS with the molecular weight of  $1.0 \times 10^5$  Da, was successfully isolated from the water extract of *Radix Paeoniae Alba* through DEAE-52 cellulose and Sephacryl S300. The structural features of RPAPS was elucidated based on monosaccharide compositions, uronic acid analysis, IR and NMR analyses. As a result, RPAPS was composed of  $\alpha$ -(1  $\rightarrow$  4)-GlcP,  $\alpha$ -Arap,  $\alpha$ -Galp,  $\alpha$ -Rhap,  $\beta$ -D-GlcP,  $\alpha$ -(1  $\rightarrow$  6)-linked GlcP and GalA. Furthermore, RPAPS exhibited potent immunomodulating properties of prompting splenocyte proliferation and RAW264.7 phagocytic. In addition, the polysaccharide RPAPS showed significant reducing power and DPPH scavenging activity in a dose dependent manner, and could protect the PC12 cells from H<sub>2</sub>O<sub>2</sub> damage. The superior immunoregulation and antioxidant activities of acidic polysaccharide RPAPS might be due to its higher uronic acid content and larger molecular weight. This study provide a basis for discover efficacious antioxidative and immunopotentiating agents.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflicts of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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