Antioxidant and anti-inflammatory activities of *Radix Isatidis* polysaccharide in murine alveolar macrophages

Zhaojiang Du\(^a,b\), Hao Liu\(^b\), Zelin Zhang\(^b\), Peng Li\(^c\)

\(^a\) Department of Ophthalmology, Tangdu Hospital, The Fourth Military Medical University, Xi'an 710038, China
\(^b\) Department of Oral and Clinical Medicine, The Fourth Military Medical University, Xi'an 710032, China
\(^c\) Department of Ophthalmology, 451 Hospital of PLa, Xi'an 710054, China

**Article Info**

Article history:
Received 27 February 2013
Received in revised form 31 March 2013
Accepted 12 April 2013
Available online 18 April 2013

**Keywords:**
Radix Isatidis
Polysaccharide
Alveolar macrophages
Inflammation
Oxidative stress

**Abstract**

*Radix Isatidis* is an official herbal medicine for treatment of infection and inflammation in China. In this study, a novel heteropolysaccharide (RIWP) was isolated from *R. Isatidis* through DEAE Sepharose Fast Flow column and Sepharose CL-6B column. RIWP had a molecular weight of 57 kDa and was mainly composed of glucose, galactose and arabinose with a relative molar ratio of 2:0.1:1.0. The cytotoxic effects, antioxidant and anti-inflammatory properties of RIWP in lipopolysaccharide (LPS) stimulated murine alveolar macrophages were first reported here. Pretreatment with RIWP was found to potentially prolong cell survival and repress the generation of reactive oxygen species (ROS) and lipid peroxidation after LPS-stimulation in murine alveolar macrophages. Furthermore antioxidant status was significantly deteriorated in the LPS-treated alveolar macrophages, such as low superoxide dismutase (SOD) activity and G-SH content, which was effectively restored by RIWP supplementation. More importantly, RIWP significantly suppressed LPS-induced increase in nitric oxide (NO), prostaglandin E\(_2\) (PGE\(_2\)), tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) and interleukin-6 (IL-6) production in murine alveolar macrophages. Additionally RIWP recovered mitochondrial membrane potential to normal conditions. All above response to LPS stimulation behaved in a concentration-dependent manner. This study provided evidences that RIWP appears to have the potential to prevent inflammatory disease in lung.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Pneumonia is an infection of the lungs that is caused by bacteria or viruses and less commonly by fungi and parasites [1]. Typical symptoms include a cough, chest pain, fever, and difficulty breathing [2]. On most serious occasions, pneumonia can make a person very ill or even cause death. Although the disease can occur in young and healthy people, it is most dangerous for older adults, babies, and people with other diseases or impaired immune systems [1,3]. Conditions and risk factors that predispose to pneumonia include smoking, immunodeficiency, alcoholism, chronic obstructive pulmonary disease, chronic kidney disease, and liver disease [4]. High levels of atmospheric pollution may also contribute to the deaths of thousands of people in the world suffering from lung inflammation in recent years due to the decline of the lung resistance. Particularly the single pursuit for economy growing in developing countries causes the people to be confronted with high levels of air pollution. Particulate matter and atmospheric oxygen is deposited and retained in lung tissues during the process of gaseous exchange that can undermine the normal pulmonary defenses. So alterations in the lung innate defenses may play a role in the observed increase in mortality after pulmonary infection episodes. Alveolar macrophages, playing an important role in innate as well as adaptive immunity, act as the first line of defense in the pulmonary immune system by phagocytosing environmental particles and pathogens, recruiting and activating inflammatory cells, producing reactive oxygen species (ROS) and pro-inflammatory cytokines and mediators [5–7]. The optimal amount of alveolar macrophages-generated ROS, pro-inflammatory cytokines and mediators decrease the risk of infection by killing pathogen; however, during pathological conditions there is mass generation of them which in turn cause inflammation and damage to the neighboring tissues as well as to the macrophages themselves. Inflammatory stimuli such as LPS could activate alveolar macrophages to secrete pro-inflammatory mediators and cytokines along with ROS, which mediates tissue responses in different phases of inflammation [8]. Consequently, determination of anti-inflammatory and/or antioxidant properties
in LPS-stimulated macrophages model has been proposed as a good indicator for screening potent agents to maintain normal lung function and health [9,10].

The well-known traditional Chinese medicine herb *Radix Isatidis*, also known as Ban-Lan-Gen, is the dried root of the plant *Isatis indigotica* Fortune (family Cruciferae) and is widely distributed in northern and central China. It has been widely used in traditional Chinese medicine (TCM) for the treatment of viral diseases including influenza, viral pneumonia, mumps, and hepatitis for hundreds of years in China, with an excellent safety record [11,12]. Previously studies indicated that *R. Isatidis* has wide pharmacological bioactivities, including antivirus, anti-bacterial, anti-endotoxic, anti-tumor, anti-inflammatory, immune regulatory effects, etc. [12–14]. Alkaloids are the main active constituents of this plant, but other effective components remain ambiguous [15]. Many studies have proved that polysaccharide are biological active substances with various activities, including enhancing immunity, anti-oxidation, anti-tumor, anti-rheumatism and prevention of AIDS [16]. However, to the author’s knowledge, there are almost no previous reports regarding the purification and characterization of the polysaccharide from *R. Isatidis*, let alone the anti-oxidant and anti-inflammatory effects of purified water-soluble polysaccharide in murine alveolar macrophages. Within this context, the purpose of the present study was to isolate the water-soluble polysaccharide from this plant and investigate its protective effect on LPS-induced oxidative stress and inflammatory response in murine alveolar macrophages for the development of new agent to prevent pulmonary inflammation.

2. Materials and methods

2.1. Materials and chemicals

*R. Isatidis* was purchased from a local medical market in Xi’an City (China), and were identified according to the identification standard of the Pharmacopeia of the People’s Republic of China (PPRC). Polysaccharide standards (α-mannose, α-rhamnose, β-galactose, β-xylene, β-arabinose, and β-glucose), trifluoroacetic acid (TFA), dimethyldisulfide (DMSO) and bovine serum albumin (BSA) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Dextrans of different molecular weights, DEAE Sepharose Fast Flow and Sepharose CL-6B were purchased from the Pharmacia Co. (Uppsala, Sweden). Aqueous solutions were prepared with ultrapure water from a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other reagents used were of analytical grade.

2.2. Isolation and purification

The dried *R. Isatidis* were cut into small pieces and exhaustively extracted with ethanol until the extraction solution with a little color in order to remove the pigments and small lipophilic molecules. Then the resulting residues were extracted with boiling water for three times (2 h for each). All water-extracts were combined, filtrated, concentrated, and precipitated with 95% EtOH (1:4, v/v) at 4 °C for overnight. The precipitate was collected by centrifugation (10,000 rpm, 10 min, 4 °C) and deproteinated by Sevag method [17]. Finally the supernatant was lyophilized to give crude polysaccharides, and defined as RICP. A portion of RICP was dissolved in water and insoluble residue was removed through micro-membrane filter (0.45 μm). The supernatant was loaded onto a DEAE Sepharose Fast Flow column (2.6 cm × 30 cm), eluted first with water, and then followed stepwise with NaCl aqueous solutions (0.1, 0.3, 0.6, and 1.0 M) at a flow rate of 2 ml/min, respectively. The fractions were collected by a fraction collector and monitored with the phenol–sulfuric acid method [18]. The fraction eluted with distilled water was dialyzed and further purified using Sepharose CL-6B (2.6 cm × 100 cm) gel-permeation chromatography, eluting with 0.15 M NaCl at a flow rate of 1 ml/min. The main fraction was collected, concentrated and lyophilized to get a white purified polysaccharide, named as RIWP.

2.3. Physico-chemical analysis

2.3.1. Chemical composition assay

Total carbohydrate content in RIWP was determined by phenol–sulfuric acid colorimetric method using glucose as the standard [18]. Total protein was determined colorimetrically using the Bradford assay [19], using BSA as standard. Uronic acid content was determined using the colorimetric m-hydroxyphenyl method [20].

2.3.2. Monosaccharide composition analysis

The identification and quantification of the monosaccharide of RIWP was determined by gas chromatography (GC) analysis [21]. Briefly, RIWP (10 mg) was hydrolyzed with 2 M trifluoroacetic acid (TFA, 2 ml) at 120 °C for 2 h. Then the hydrolyzed products were reduced with NaBH4 (20 mg), followed by acidification with acetic acid. After they were co-distilled with methanol to remove the excess boric acid, the reduced products (alditols) were acetylated with 1:1 pyridine–acetic anhydride in a water bath for 1 h at 90 °C to give the alditol acetates [22]. The resulting alditol acetates of RIWP and authentic standards (α-mannose, α-rhamnose, β-galactose, β-xylene, β-arabinose, and β-glucose) with myo-inositol (2 mg) as the internal standard were prepared and subjected to GC analysis separately in the same way, which were analyzed by GC instrument (Agilent 7890N) using a HP-5 capillary column (30 m × 0.32 mm × 0.25 μm) and a flame-ionization detector (FID) at a designed temperature program. Nitrogen was used as the carrier gas. Quantitation was carried out from the peak area, using response factors of instrument.

2.3.3. Determination of purity and molecular weight

The homogeneity and molecular weight of RIWP were determined by high performance gel permeation chromatography (HPGPC) [23], which was performed on a Shimadzu HPLC system equipped with a TSK-GEL G3000 PWXL (Tosoh Biosep, Japan) column (7.8 mm × 300 mm) and a refractive index detector (RID). 10 μL of sample solution (2.0 mg/mL) was injected in each run, with 0.1 M Na2SO4 solution as the mobile phase at a flow rate of 0.6 ml/min. The column was kept at 35 °C. The linear regression was calibrated with the T-series Dextran standard of known molecular weights (200,000, 70,000, 40,000, 10,000 and 5000 Da). The molecular weight of RIWP was estimated by reference to the calibration curve made above.

2.4. Animals

Eight week-old male Wistar rats were provided by the Experimental Animal Center of the Fourth Military Medical University. The animals were housed under controlled conditions (12 h light/dark cycle, 22–28 °C temperature and 60–70% air humidity), fed with normal mice chow and water ad libitum. Animal experimentation protocols were approved by the Institutional Animal Care and Use Committee of the Fourth Military Medical University.

2.5. Isolation of murine alveolar macrophages

Rat alveolar macrophages were recovered from Wistar rats according to the procedure described by Lemaire [24], with some modifications. Briefly, bronchoalveolar lavage (BAL) was collected by lavaging the lungs with aliquots of 5 ml of sterile phosphate buffer.
buffered saline (PBS) after rats were anesthetized by an intraperitoneal injection of ketamine (60 mg/kg) and xylazine (20 mg/kg). To pellet the cells, the collected BAL was centrifuged at 600 \times g for 10 min at 4 °C. Then the cells was re-suspended in the complete culture medium (DMEM with 10% fetal bovine serum – FBS) and were seeded in a 96-well plate at the density of 1 \times 10^5 cells/well at 37 °C in 5% CO2 incubator for cell adhesion. After 2 h of incubation, the non-adherent cells were washed away twice with sterile PBS and adherent cells (macrophages) were further incubated in fresh DMEM culture medium overnight to make them quiescent. The viability of the cells was determined by trypan blue exclusion test and the number of live cells was above 95%.

2.6. Treatment schedule

The alveolar macrophages were pre-treated with indicated doses of RIWP (25, 50, 100 and 200 \mu g/ml) or medium for 1 h prior to being stimulated with LPS (2 \mu g/ml) for 24 h. After 24 h the cells were harvested and used for subsequent assays. Cells which were treated with medium were considered as control.

2.7. Determination of cytotoxicity

Cell viability was assessed using a modified MTT assay [25]. Following treatment, 20 \mu l of MTT (5 mg/ml in PBS) was added to each well and further incubated for 4 h at 37 °C. The supernatant was then discarded and 100 \mu l of dimethylsulfoxide (DMSO) was added to each well to dissolve the formazan dye. The optical density was measured at 570 nm using ELISA reader. The optical density of formazan formed in control (untreated) cells was taken as 100% viability and the results were expressed as the percentage of viable cells with respect to that of control cells.

2.8. Determination of lipid peroxidation, antioxidant enzymes and ROS production

Lipid peroxidation as evidenced by the formation of malondialdehyde was measured by the method of Ohkawa et al. [26]. The antioxidant enzymes superoxide dismutase (SOD) and reduced glutathione (G-SH) were determined in cell lysates according to the method of Mestro Del and McDonald [27] and Moron et al. [28], respectively.

The production of ROS was determined by using 2',7'-dichlorofluoresceindiacetate (DCFH-DA) as described by Cathcart et al. [29]. In brief, the cells (1 \times 10^6 cells/ml) were incubated with DCFH-DA dye (20 \mu M) for 30 min in CO2 incubator. After incubation the fluorescence intensity was measured at an excitation and emission wavelength of 485 nm and 530 nm respectively using a spectrofluorimeter (Varian, USA). The data was expressed as percentage DCF fluorescence as compared to that of control.

2.9. Determination of anti-inflammatory activity in LPS-stimulated macrophages

2.9.1. Determination of prostaglandin E2 (PGE2), TNF-α and IL-6 production

Cells were treated with LPS (1 \mu g/ml) for 24 h after treatment in the presence or absence of RIWP (25, 50, 100 and 200 \mu g/ml) for 1 h. The supernatant was used to determine PGE2 levels using a PGE2 Express EIA Kit (Cayman Chemical, Ann Arbor, USA) in accordance with the manufacturer’s instructions. The supernatant of cultured cells stimulated with LPS was also used for TNF-α and IL-6 determination by ELISA according to the instructions provided by manufacturer.

2.9.2. Measurement of NO production

Production of NO was determined by quantification of nitrite concentrations in medium by the Griess reaction [30]. Briefly, 100 \mu l of cell culture medium (without phenol red) was mixed with an equal volume of Griess reagent [15% (w/v) sulfanilamide and 0.1% (w/v) N-1-naphthylethylendiamine dihydrochloride in 0.1 N HCl], incubated at room temperature for 10 min, and then absorbed at 540 nm using a microplate reader. A standard curve of different concentrations of sodium nitrite was generated to calculate the nitrite accumulated in the supernatant.

2.10. Determination of mitochondrial transmembrane potential

Fluorescent dye Rhodamine 123 was used to assess the effect of RIWP on mitochondrial membrane potential of alveolar macrophage cells. After incubation, 10 \mu l of Rhodamine 123 dye (10 \mu g/ml in deionized water) was added to the cells and incubated for 30 min. The cells were washed three times with saline and fluorescence intensity was read using a spectrofluorimeter (Varian, USA) at 485 nm and 530 nm, excitation and emission wavelengths respectively [31]. The data was expressed as percentage Rhodamine 123 fluorescence as compared to that of control fluorescence.

2.11. Statistical analysis

All data are presented as mean ± S.D. of the results of three independent experiments. Significant differences between groups were determined using unpaired Student’s t-tests. A value of p < 0.05 was chosen as the criterion of statistical significance.

3. Results and discussion

3.1. Isolation, purification, and physico-chemical properties of RIWP

Powdered R. Isatidis were defatted with ethanol to remove pigments and low-molecular-weight substances. Subsequently the residues were extracted with hot water for preparing crude polysaccharides. After removal of insoluble impurities by flitration and subsequent removal of proteins by the Sevag method, the extract was lyophilized to yield crude polysaccharide (RIPC) (5.68% of the dry material). The crude polysaccharides portion was further fractionated on a DEAE Sephacore Fast Flow ion-exchange column with distilled water and different concentrations of stepwise NaCl solution elution (0.1, 0.3, 0.6, and 1.0 M NaCl), leading to the isolation of four sub fractions RIPC-1, RIPC-2, RIPC-3 and RIPC-4. Among those fractions, RIPC-1 eluted by water showed the relatively high content. Therefore RIPC-1 was further loaded onto a Sepharose CL-6B gel-filtration column and eluted with 0.15 M NaCl to yield one of purified polysaccharide (RIWP) for further chemical and biological activity study, with a yield of 0.56% of dry material. The other fractions will be reported in the future. RIWP presented a symmetrical, narrow peak on HPGPC, indicating that it was a homogeneous polysaccharide (data not shown). Correlation with the calibration curve of Dextran standards, the average molecular weight of RIWP was about 57 kDa. RIWP had a negative response to the Bradford method, and the result of ultraviolet scanning showed that no absorption was detected at 280 nm, but the maximal absorbance at 210 nm for RIWP was very extensive, which was well in agreement with the fact that RIWP contained 97.3% of total carbohydrate and no protein was detected. Furthermore, the uronic acid content in RIWP was not detected. Sugar compositional analysis of RIWP determined by GC indicated that it was composed of glucose, galactose and arabinose with a relative molar ratio of 2.0:1.1:1.0.
3.2. Cytotoxicity effects of RIWP on cell viability in murine alveolar macrophages

Initially, the cytotoxicity of RIWP to murine alveolar macrophages was measured by using a MTT assay. Cells were treated with various concentrations of RIWP (25, 50, 100 and 200 μg/ml) for 1 h and then stimulated with LPS for 24 h. The control cells were treated with only medium was taken as 100% viability. As shown in Fig. 1, the viability of cells treated with LPS was decreased by 26.25% when compared to the untreated control cells (100%), whereas pretreatment with RIWP had no effect on cell viability under any of the tested conditions. Thus, for further experiments, the cells were treated with RIWP in the concentration range of 25, 50, 100 and 200 μg/ml. This suggests RIWP has the cytoprotective effect on LPS stimulated cells in the current concentration range.

3.3. Inhibitory effect of RIWP on LPS-induced oxidative damage to membrane lipids in alveolar macrophage cells

Peroxidation of membrane lipids during oxidative stress is an important cause of cellular injury, which was mediated by inactivation of membrane enzymes and receptors, depolymerization of polysaccharide, as well as protein cross-linking and fragmentation [32]. More recently, the interest for searching the antioxidants which protect cell membrane lipid bilayers from being attacked by free radicals are attracting more interest. Malondialdehyde (MDA), the end product of lipid peroxidation, reflects the severity of cell attacked by free radicals, so peroxidative damage to membrane lipids of alveolar macrophages is assessed by level of MDA formation [33]. As can be seen from Fig. 2, the results indicated that an increase of 47.7% ($p < 0.05$) in MDA formation was observed in the cells treated with LPS as compared to untreated control cells. However pre-treatment of cells with RIWP (25, 50, 100 and 200 μg/ml) significantly decreased MDA formation in a dose-dependent manner as compared to LPS-treated cells. All above data demonstrated that RIWP is of potential interest to maintain proper cellular integrity and increase the resistance toward cells membrane damage, which is in good agreement with the increased cell survival.

3.4. Inhibitory effect of RIWP on LPS-induced antioxidant deactivation in alveolar macrophage cells

ROS, generated in tissues, are normally scavenged by enzymatic and non-enzymatic antioxidants, such as SOD and G-SH [34]. The effect of RIWP on the antioxidant status in LPS-treated alveolar macrophage cells was evaluated by determining the SOD activity and G-SH content. From Fig. 3, it was clear that there was a decrease in SOD activity in LPS-treated cells ($p < 0.05$), compared with untreated control cells, as well as in G-SH level. As expected, pre-treatment with RIWP at all tested concentrations was able to ameliorate this deprivation of antioxidant capacity induced by LPS in alveolar macrophage cells especially at 100 and 200 μg/ml as compared to LPS treated cells. The effect of RIWP to enhance SOD activity and G-SH content adds to its protective and antioxidant

---

**Fig. 1.** Effect of RIWP on cell viability in murine alveolar macrophages. All data represent the mean ± S.D. of 3 independent experiments. *$p < 0.05$, significantly different from unstimulated control cells. †$p < 0.05$, significantly different from the LPS-stimulated cells (not-treated with RIWP).

**Fig. 2.** Effect of RIWP on LPS-induced oxidative damage to membrane lipids in alveolar macrophages. All data represent the mean ± S.D. of 3 independent experiments. *$p < 0.05$, significantly different from unstimulated control cells. †$p < 0.05$, significantly different from the LPS-stimulated cells (not-treated with RIWP).

**Fig. 3.** Effect of RIWP on LPS-induced antioxidant deactivation in alveolar macrophages. All data represent the mean ± S.D. of 3 independent experiments. *$p < 0.05$, significantly different from unstimulated control cells. †$p < 0.05$, significantly different from the LPS-stimulated cells (not-treated with RIWP).
property in LPS-treated alveolar macrophage cells against oxidative stress.

3.5. Inhibitory effect of RIWP on LPS-induced ROS production in alveolar macrophage cells

There is considerable evidence that ROS induce oxidative damage when ROS overwhelm the body's antioxidant defenses or when the antioxidant defense system loses its capacity for response. Large amounts of ROS are detrimental to vital cellular components and have been shown to participate in the etiology of several human degenerative diseases, including inflammation, cardiovascular and neurodegenerative disorders, and cancer [35]. As observed in Fig. 4, LPS treatment for 24 h caused a marked increase of 39.7% (p < 0.05) in ROS formation in alveolar macrophage cells as compared to untreated control cells. While, at the same time, incubation of cells with RIWP prevented the ROS production by 16.2%, 24.9% (p < 0.05), 30.6% (p < 0.05) and 37.3% (p < 0.05) respectively at the concentration of 25, 50, 100 and 200 μg/ml as compared to LPS treated cells. These observations suggest that RIWP could quench the oxidative stress induced by ROS in LPS-induced alveolar macrophage cells.

3.6. Inhibitory effect of RIWP on LPS-induced NO and PGE₂ production in alveolar macrophage cells

Because both PGE₂ and NO are two major indicators of inflammation [36], we evaluated the effects of RIWP on their production using LPS stimulation in alveolar macrophage cells. The cells were pretreated in the presence or absence of RIWP (25, 50, 100 and 200 μg/ml) for 1 h prior to stimulating with LPS (2 μg/ml). After 24 h treatment, NO and PGE₂ concentrations secreted from alveolar macrophage cells were measured using the Griess reaction method and PGE₂ Express EIA Kit, respectively. As shown in Fig. 5A, LPS alone markedly induced NO and PGE₂ production (p < 0.05 or
p < 0.01 as compared to the untreated control cells, while RIWP was found to show a dose-related inhibition of NO and PGE₂ production.

3.7. Inhibitory effect of RIWP on LPS-induced TNF-α and IL-6 production in alveolar macrophage cells

Since excessive production of TNF-α and IL-6 induces tissue injury and inflammatory disease [37,38], we conducted further research into the effects of RIWP on the inflammatory cytokines TNF-α and IL-6 produced by LPS-stimulation in mouse alveolar macrophage cells, using enzyme-linked immunosorbent assay (ELISA). As shown in Fig. 6, TNF-α and IL-6 production in response to LPS were inhibited by pre-treatment with RIWP in a dose-dependent manner. The maximal inhibition rate of TNF-α and IL-6 production by RIWP at concentration of 200 μg/ml was 53.8% (p < 0.05) and 63.0% (p < 0.01) with respect to LPS-treated cells, respectively. RIWP dose-dependently inhibited the levels of inflammatory cytokines secretion in a manner similar to that observed with NO and PGE₂ production.

3.8. Inhibitory effect of RIWP on LPS-induced mitochondrial transmembrane potential change

Mitochondria are the cell’s power producers by generating most of adenosine triphosphate (ATP). In addition to supplying cellular energy, mitochondria are involved in macrophage activation and various metabolic processes [39]. Many reports have indicated that hyper production of ROS and imbalance of antioxidant/oxidant system contribute to mitochondrial dysfunction as well as lead to other metabolic processes out of order [40–42]. Thus restoration of mitochondrial membrane potential could be an important regulatory factor for proper mitochondrial functioning and cell survival. In the present study, pre-treatment with RIWP showed (Fig. 7) obvious restoration in mitochondrial transmembrane potential by 9.3%, 12.0%, 21.2% (p < 0.05) and 26.2% (p < 0.05) respectively at the concentrations of 25, 50, 100 and 200 μg/ml as compared to stressed cells, thus indicating its protective effect on mitochondrial functions.

4. Conclusions

Macrophages play an important role in host defenses against noxious substances and are involved in a variety of disease processes, including autoimmune diseases, inflammatory disorders, and infections [43]. The pulmonary macrophages system is critical to the defense of the lung, because they are located at one of the major boundaries between the body and the outside world [44]. Alveolar macrophages are cells found in lungs and interact with other cells in airways and alveoli immediately after birth, resulting in burst of intracellular signaling cascades and thus leading to various effects or functions that make up the initial immune response in the lungs [45–47]. In addition to phagocytosing foreign pathogen, alveolar macrophage also produce a variety of pro-inflammatory cytokines and mediators, such as NO, PGE₂, TNF-α, IL-6 and cytotoxic products, including ROS, to regulate their own activity and the activity of the other immune cells [48–50].

Natural products contribute new strategy to novel drug discovery for treatment of some inflammatory diseases and have therefore attracted more increasing interest arisen on elucidating the efficacy, safety, and functional mechanism of these active components from natural herbal medicine. Therefore in the present study, we isolated RIWP from R. latidis and its cytoprotective and anti-inflammatory effects on murine alveolar macrophage were investigated using LPS-stimulated murine alveolar macrophages as a model of inflammatory injury. Here, we found that RIWP not only protect the cells from death induced by LPS and increase superoxide dismutase (SOD) activity and G-SH content, but also inhibited LPS-induced pro-inflammatory molecules including NO, PGE₂, TNF-α and IL-6, as well as ROS generation. Additionally, the decreased mitochondrial membrane potential was also restored to near normal level, which would be constructive to proper mitochondrial functioning and cell survival.

In conclusion, the anti-inflammatory activity of RIWP in murine alveolar macrophages can be attributed at least in part to inhibition of proinflammatory cytokine and mediator production as well as antioxidant status amelioration. Recently, several reports have documented that MAPK and NF-κB activation are involved in the pathogenesis of chronic inflammatory diseases via modulating the expression of inflammation-associated enzymes and their associated genes [51–55]. In subsequent study, we will determine whether RIWP regulates MAPKs and NF-κB signal pathways in murine alveolar macrophage cells.

Acknowledgement

The current work was funded by the National Natural Science Foundation of China (No. 81100670).

References