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Characterization and antioxidant activity in vitro and in vivo of polysaccharide purified from *Rana chensinensis* skin

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ABSTRACT

Preliminary characterization and antioxidant activity in vitro and in vivo investigation of the polysaccharide fraction named as RCSP II, which was extracted from *Rana chensinensis* skin, were performed. Results indicated that RCSP II comprised glucose, galactose, and mannose in a molar ratio of 87.82:2.77:1.54 with a molecular weight of 12.8 kDa. Antioxidant activity assay in vitro showed that RCSP II exhibited 75.2% scavenging activity against 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) radicals at the concentration of 2500 mg/L and 85.1% against chelated ferrous ion at 4000 mg/L. Antioxidant activity assay in vivo further showed that RCSP II increased the activities of antioxidant enzymes, decreased the levels of malondialodehyde, and enhanced total antioxidant capabilities in livers and sera of D-galactose induced mice. These results suggested that RCSP II could have potential antioxidant applications as medicine or functional food.

1. Introduction

The Chinese frog *Rana chensinensis* is a relatively small anuran with body length ranging from 52 mm to 64 mm for males and 58 mm to 64 mm for females. This frog is distributed in the northeastern part of China (Jin et al., 2009) and has long been used in traditional Chinese medicine to improve health and immunity, as well as prevent diseases, because of its antioxidant, antifatigue, and inflammation properties (Yin, Han, & Han, 2006). The expensive unisex health product "Lin wa you" is extracted from the fallopian tube of female *R. chensinensis*. Therefore, in Northeast China *R. chensinensis* breeding numbers increase annually. However, dealing with large quantities of residues after oviductus ranae extraction has become an urgent problem (Wang, Zhao, & Su, 2015).

The skin is the most useful component of *R. chensinensis* residues, and some studies have thus focused on *R. chensinensis* skin. Jin et al. (2009) identified a novel antimicrobial polypeptide from the skin secretion of *R. chensinensis*. This peptide has potent antimicrobial activity both against Gram-positive and Gram-negative bacteria and has extremely low hemolytic activity to human red blood cells. Collagen extracted from *R. chensinensis* skin can also be fabricated with poly(L-lactide) to produce composites that can be skin substitutes (Zhang et al., 2015). In our previous work, the polysaccharide fraction RCSP II (RCSP is the abbreviation of "*R. chensinensis* skin polysaccharides") was purified by chromatography on a diethylaminoethyl-cellulose anion-exchange column and a Sepharose CL-6B column. RCSP II was found to have strong scavenging activity against superoxide anion and 1,1-diphenyl-2-picrylhydrazyl radicals in vitro (Wang et al., 2015).

In fact, besides of the above assay methods for antioxidant activity in vitro, there still have some other effective methods. 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) method was pioneered by Miller and Rice-Evans (1993) and used to assay the antioxidant capacity of biological samples. After oxidation by activity oxygen, colorless ABTS transforms to the stable, blue-green $ABTS^{\bullet+}$. When colored $ABTS^{\bullet+}$ is mixed with any substance containing anti-oxidation components, $ABTS^{\bullet+}$ is reduced to its original colorless ABTS. Fe ions are important elements in the human body but may also be potentially dangerous. Fe^{2+}, with high reactivity, can stimulate lipid peroxidation and accelerate lipid peroxidation, thereby driving the chain reaction of lipid peroxidation (Benedet & Shibamoto, 2008). Therefore, Fe^{2+} chelating is extremely important to antioxidant work.

In order to learn more the antioxidant activities in vitro, the scavenging activity of ABTS radicals and chelated ferrous ion were assayed in the present study. Furtherly, the chemical composition of RCSP II and its antioxidant activities in vivo were also determined. The aim was to identify new biological functions of RCSP II in food and pharmaceuticals.

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2. Materials and methods

2.1. Materials and chemicals

Residues of *R. chensinensis* were provided by Fushun East Star Institute of *R. chensinensis* competitive products (Fushun, Liaoning Province, China). D-Galactose (D-Gal, BioXtra, ≥99%, Product number G6404), bovine serum albumin (BSA, BioXtra, ≥96%, Product number A3311), ascorbic acid (Reagent grade, ≥99%, Product number A7506), pyrogallic acid (ACS reagent, ≥99%, Product number 16040), ethylenediaminetetraacetic acid (EDTA, BioReagent ≥ 98.5%, Product number E6758), and ABTS (purity ≤ 100%, Product number A3219) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Unless otherwise stated, all chemicals used were analytical grade.

2.2. RCSP II extraction and purification

Skins were collected from residues of *R. chensinensis*. After cleaning with deionized water and drying, the skins were homogenized, treated with petroleum ether to defat, and extracted with boiling water for 5 h. The water extracts were filtered, concentrated, and treated with three volumes of ethanol. Crude RCSP was then obtained through centrifugation (3000 × *g*, 20 min). Crude RCSP was dissolved in distilled water and deproteinized using Sevag reagent. After removing Sevag reagent, the aqueous fraction was freeze thawed and centrifuged until no insoluble substance was visible. Solutions were placed on a diethylaminoethyl-cellulose (Amersham Biosciences, Sweden) column (3 cm × 45 cm) equilibrated with a linear gradient of NaCl from 0 M to 1.0 M. The eluted sample was collected and further purified by gel filtration chromatography on a Sepharose CL-6B (Amersham Biosciences, Sweden) column (2.5 cm × 90 cm) with distilled water. RCSP II was finally dialyzed against distilled water and lyophilized for subsequent analysis.

2.3. Homogeneity and molecular weight analysis of RCSP II

Homogeneity and molecular weight distribution were determined on an Agilent 1100 HPLC system equipped with a refractive index detector and a TSK-GEL G3000SWxl column (7.5 mm × 300 mm; Tosoh Corp., Tokyo, Japan). 20 μL RCSP II solution (10 mg/mL) was loaded on the column which was eluted with 0.01 mol/L sodium phosphate buffer (pH 7.0) containing 0.2 mol/L Na_2SO_4 at a flow rate of 0.7 mL/min. The column pressure is 39 bar. Pullulan P-800(344 kDa), P-400(200 kDa), P-200(107 kDa), P-100(47.1 kDa), P-20(21.1 kDa), P-10(9.6 kDa), and P-5(5.9 kDa) (Showa Denko K.K., Tokyo, Japan) were used as standards for molecular weight measurement.

2.4. Chemical analysis of RCSP II

Polysaccharide content was determined through the phenol–sulphuric acid method using D-glucose as standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1958). The Bradford method was used to determine the protein content of polysaccharides using BSA as standard (Bradford, 1976). Uronic acid content was determined using the m-hydroxybiphenyl colorimetric procedure with D-glucuronic acid as standard (Blumenkrantz & Asboe-Hansen, 1973).

2.5. Monosaccharide composition analysis of RCSP II

The monosaccharide composition of the polysaccharides was determined by gas chromatography (GC). The polysaccharides were hydrolyzed with 2 M trifluoroacetic acid (120 °C, 3 h). After hydrolysis, the neutral monosaccharides were successively reduced with Sodium borohydride ($NaBH_4$) and acetylated with 1:1 pyridine acetic anhydride at 90 °C for 1 h. Alditole acetates were analyzed by GC on a Shimadzu GC-14C instrument equipped with an Rtx 2330 column (Shimadzu Co. LTD., Kyoto, Japan, 30 m × 0.32 mm × 0.2 μm). Column temperature was maintained at 170 °C for 2 min, increased to 240 °C at a rate of 8 °C/min for 1 min, and then increased to 265 °C at a rate of 8 °C/min for 20 min.

2.6. Infrared (IR) spectral analysis of RCSP II

The IR spectra of polysaccharides were determined using a PerkinElmer Spectrum GX (PerkinElmer Co. Ltd., Waltham, USA). The purified polysaccharides were mixed with KBr powder and pressed into pellets for IR measurement within the frequency range of 4000 cm^{-1} to 400 cm^{-1}.

2.7. Antioxidant activity assay in vitro

2.7.1. ABTS scavenging assay

The scavenging activity of RCSP II against ABTS radicals was examined using the method of Erel (2004). Then, 2 mM H_2O_2 was dissolved in 30 mM acetate buffer (pH 3.6), and ABTS was added to a final concentration of 10 mM. The ABTS solution can be used after incubation for 1 h at room temperature upon the appearance of its characteristic color. Exactly 150 μL of the sample solution was mixed with 3 mL of ABTS solution. After shaking for 30 s, the change in absorbance of the mixture within 6 min at 30 °C was determined at a wavelength of 734 nm with ascorbic acid as positive control. Antioxidant activity of the sample was calculated using

$$\text{Scavenging rate}(\%) = \left[\frac{1-(A_t - B)}{A_0}\right] \times 100 \quad (1)$$

where A_0 is the absorbance of ABTS solution, B is the absorbance of the blank, and A_t is the absorbance of RCSP II–ascorbic acid.

2.7.2. Ferrous ion-chelating activity assay

The ferrous ion-chelating potential of the sample was determined using the method of Dinis, Maderia, and Almeida (1994). The reaction mixture contained 1.0 mL of RCSP II solutions, 0.1 mL of $FeCl_2$ (2 mM), and 0.2 mL of ferrozine (5 mM). After incubating at 25 °C for 10 min, absorbance was measured at 562 nm using EDTA as positive control. Lower absorbance indicated higher chelating ability. The chelating ability was calculated using the following equation:

$$\text{Chelating activity}(\%) = \frac{(A_0 - A_1)}{A_0} \times 100 \quad (2)$$

where A_0 is the absorbance of the control (water instead of RCSP II solutions), and A_1 is the absorbance of RCSP II–EDTA.

2.8. Antioxidant activity assay in vivo

2.8.1. Animal grouping and experimental design

Experiments were performed with 30 female Kunming mice (2 month old; body weight (BW) = 20 ± 2 g) purchased from the Pharmacology Experimental Center of Jilin University (Changchun, China). The animals were maintained at controlled temperature (23 ± 0.5 °C), humidity (55 ± 5%), and light conditions (12 h light/12 h dark cycle). During the entire experimental period (including acclimation), all animals were allowed free access to standard laboratory pellet diet and water. All procedures involving animals were conducted in strict accordance with the prevailing Chinese legislation on the use and care of laboratory animals.

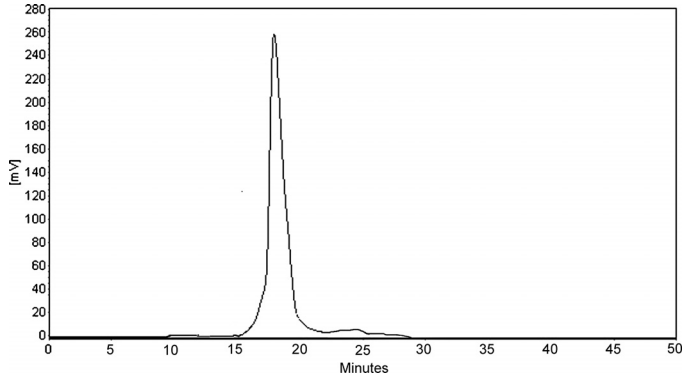

Fig. 1. HPLC chromatogram of RCSP II.

After a 7-day acclimation period, mice were randomly assigned into six groups (five mice each), including normal control group, D-Gal group (model control), ascorbic acid group (positive control), and RCSP II group (100, 200, and 400 mg/kg BW). Mice in normal control group were given physiological saline (10 mL/kg BW) once daily by hypodermic injection and gastric gavage.

Mice in model control group were fed D-Gal (100 mg/kg BW) by hypodermic injection and the same volume of physiological saline by gastric gavage once daily. Mice in ascorbic acid group were treated with D-Gal (100 mg/kg BW) by hypodermic injection and ascorbic acid (100 mg/kg BW) by gastric gavage once daily. Mice in RCSP II groups were respectively fed with polysaccharide at three different doses (100, 200, and 400 mg/kg BW per day) by gastric gavage and D-Gal (100 mg/kg BW per day) by hypodermic injection. All groups were performed once daily for 40 consecutive days.

2.8.2. Biochemical assay

After overnight fasting following the last drug administration, mice were weighed and killed by decapitation. Blood samples were collected immediately and centrifuged at 4000 × *g* at 4 °C for 10 min to obtain sera. The liver was excised, weighed, and immediately homogenized in 0.1 g/mL (wet weight) ice-cold physiological saline. The suspension was centrifuged, and the supernatant was collected for further analysis. All above treatments were conducted at 4 °C.

Activities of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px), as well as protein content, total antioxidant capacity (TAOC), and malondialdehyde (MDA) level, were assayed using test kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.9. Statistical analysis

The data were presented as mean ± standard deviation (SD). Statistical analyses were performed using Student's *t*-test and one way analysis of variance. All computations were done by employing the statistical software (SPSS, version 13.0).

3. Results and discussion

3.1. Characteristic of RCSP II

The HPLC chromatogram (Fig. 1) showed that RCSP II had a single and symmetrically sharp peak, revealing that it was homogeneous polysaccharides. According to the retention time, the average molecular weight of RCSP II was estimated to be 12.8 kDa.

Total sugar content assayed for RCSP II was 94.46 ± 1.23%. RCSP II had a negative response to protein assay by Bradford method. Furthermore, uronic acid was not detected in RCSP II.

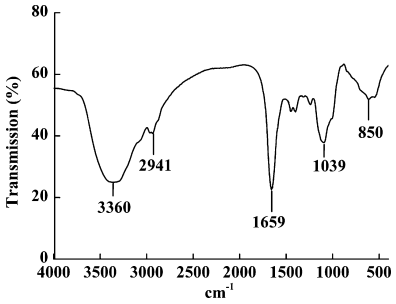

Fig. 2. IR transmission spectrum of RCSP II.

IR transmission spectrum of RCSP II is shown in Fig. 2. The main absorption characteristics of polysaccharide structures were related to O—H stretching between 3500 and 3000 cm^{-1}, as well as C—H stretching between 3000 and 2800 cm^{-1}. The absorption peak at 1659 cm^{-1} was due to the stretching vibration of C=O (Santhiya, Subramanian, Natarajan, & Colloid, 2002). No absorption peak appeared near 1616 cm^{-1}, indicating the absence of $-NH^{3+}$ and $-NH_2$ and thus indirectly demonstrating that the polysaccharides did not contain any protein. Absorbance at 1039 cm^{-1} showed that the monosaccharides of RCSP II existed as pyranoside (Coimbra, Goncalves, Barros, & Delgadillo, 2002). Absorption at 850 cm^{-1} suggested that α-glycosidic bonds were present in RCSP II (Zhang, 1999). Compared with some polysaccharides from animal (He, Ye, Zhao, & Su, 2014; Jiang, Jiao, et al., 2013; Xiong et al., 2014), the IR transmission spectrum of RCSP II has relatively less absorption peaks. Especially, it has no characteristic peak of sulfate, protein, and uronic acid. The result suggests that RCSP II has a simple composition. The assay of RCSP II monosaccharide composition further verified the result. The assay of RCSP II monosaccharide composition shows that RCSP II comprises three monosaccharides: glucose, galactose, and mannose with a molar ratio of 87.82:2.77:1.54.

3.2. Antioxidant activity in vitro

3.2.1. ABTS radical scavenging activity

The scavenging effect of RCSP II on ABTS radicals was measured, and the results are shown in Fig. 3. The scavenging activity of RCSP II toward ABTS radicals was related to polysaccharide concentration within the test dosage range. Furthermore, the ABTS scavenging activity of RCSP II raised significantly with increased concentration but remained lower than that of ascorbic acid at each concentration. The EC50 of RCSP II was 1246.3 mg/L, which was significantly higher than that of ascorbic acid (678.2 mg/L).

There already have some literatures reported that the polysaccharides from animals have the ABTS scavenging activity. The ABTS

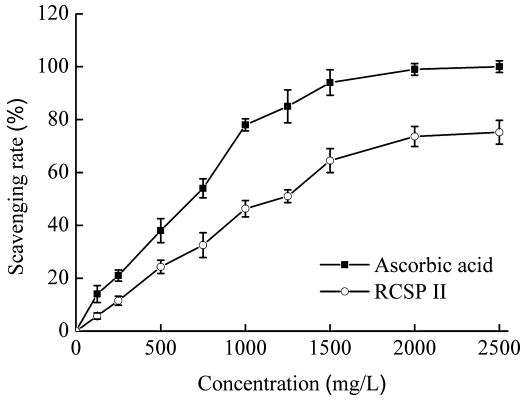

Fig. 3. ABTS radical scavenging activity of RCSP II.

Table 1
Effects of RCSP II on the activities of SOD, CAT, GSH-Px, TAOC and MDA levels in serums in aging mice.

Test groups	SOD (U/mL)	CAT (U/mL)	GSH-Px (U/mL)	TAOC (U/mL)	MDA (nmol/mL)
Normal control	294.42 ± 27.54**	104.52 ± 11.47**	178.65 ± 21.57**	41.54 ± 9.78**	12.33 ± 2.05*
D-Gal (model control)	210.74 ± 30.14	62.44 ± 11.41	98.54 ± 12.98	24.57 ± 5.69	16.87 ± 2.06
Ascorbic acid (100 mg/kg positive control)	278.35 ± 21.44**	91.27 ± 7.56**	182.47 ± 11.58**	35.57 ± 2.24**	13.68 ± 0.78*
RCSP II (100 mg/kg)	241.24 ± 19.68*	69.37 ± 7.98	196.31 ± 7.95**	27.68 ± 1.57	13.75 ± 1.28*
RCSP II (200 mg/kg)	257.22 ± 21.49**	101.22 ± 14.36**	226.58 ± 12.59**	32.55 ± 1.24*	11.69 ± 2.35**
RCSP II (400 mg/kg)	292.14 ± 17.57**	131.57 ± 17.57**	268.34 ± 14.59**	37.54 ± 2.69**	9.95 ± 1.24**

Values are mean ± SD.
* $P < 0.05$ vs. model control.
** $P < 0.01$ vs. model control.

scavenging abilities of four polysaccharides fractions (PBP60-A, PBP60-B, PBP60-C, PBP60-D) from *Philomycus bilineatus* correlated well with increasing concentrations. Moreover, PBP60-C and PBP60-D fractions showed higher ABTS radical scavenging activity and the EC50 of them are between 500 and 1000 mg/L (He et al., 2014). Chitosaccharides prepared from shrimp shell has a stronger radical-scavenging activity against ABTS with EC50 values 19.1 mg/L (Halder et al., 2014). The phosphorylated chitosan from gladius of the squid *Sepioteuthis lessoniana* (Lesson, 1830) scavenged 71.57% ABTS radicals and the EC50 value of scavenging ABTS radicals was reported as 46 mg/L (Subhapradha et al., 2013). Compared with above polysaccharide, the ABTS radicals scavenging activity of RCSP II reach the same scavenging rate on the base of higher polysaccharide concentration.

The activities of antioxidants have been attributed to various mechanisms, such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, reductive capacity and radical scavenging (Zou et al., 2008). However, the mechanism of RCSP II on the antioxidant activity is not very clear. The mechanisms involved in antioxidant activity may be originate from hydrogen atom-donating ability of a molecule to a radical, which result in terminating radical chain reactions and converting free radicals to unharmful products (Hu, Zhang, & Kitts, 2000). The electron-withdrawing carboxyl groups substituted in C-5 of sugar residue could activate the hydrogen atom of sugar residues through field and inductive effects.

3.2.2. Fe^{2+} chelating activity

As shown in Fig. 4, within the test dose range, the Fe^{2+} chelating activity of RCSP II was measured to be 17.2–85.1% (EC50 = 1410.6 mg/L). However, the metal-chelating activity of RCSP II was significantly lower than that of EDTA, which had the strongest chelating capacity that reached 100% at a concentration of 1000 mg/L (EC50 = 188.1 mg/L).

There also have some reports on the polysaccharide from animals having Fe^{2+} chelating activity. When the concentration of the polysaccharides from *Endothelium corneum gigeriae galli* increased from 0.2 to 2.0 mg/mL, the Fe^{2+} chelating activity increased from 36.7% to 84.1% (Xiong et al., 2014). The polysaccharides from *Cipangopaludina chinensis* (CCPS) have Fe^{2+} chelating activity from 400 to 4000 mg/L. At the concentration of 4000 mg/L, the Fe^{2+} chelating activity for CCPS reached to 83.2%. It demonstrated that CCPS possessed strong Fe^{2+} chelating activity (Jiang, Jiao, et al., 2013). The Fe^{2+} chelating activities of polysaccharides from *Cyclina sinensis* (CSPS) were correlated well with the increase of concentration up to 4000 mg/L. At a concentration of 4000 mg/L, the Fe^{2+} chelating activity for CSPS was 91.5% (Jiang, Xiong, et al., 2013). The RCSP II has a similar Fe^{2+} chelating activity at the same concentration.

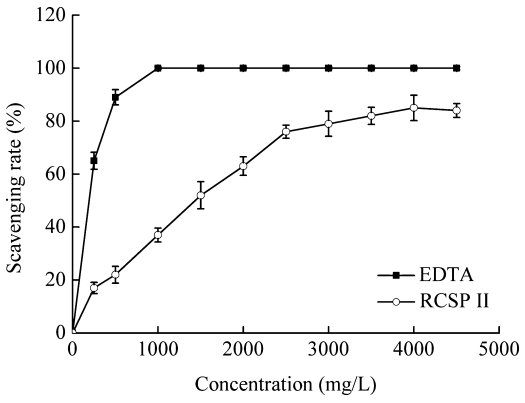

Fig. 4. Fe^{2+} chelating activity of RCSP II.

3.3. Antioxidant activity in vivo

D-Gal-treated mice were used as an aging animal model for antioxidant evaluation in vivo in our work because D-Gal is already widely used to induce oxidative stress in vivo and mimic natural aging in mice for screening antioxidant, anti-aging, and neuroprotective drugs (Hsieh, Wu, & Hu, 2009; Luo et al., 2010; Sun, Yu, Zhang, Wang, & Luo, 2007). Tables 1 and 2 show that the activities of antioxidant enzymes (SOD, GSH-Px, and CAT) and TAOC level in sera and livers of D-Gal-treated mice significantly decreased compared with those of normal control group ($p < 0.05$). MDA also significantly increased compared with that of normal control ($p < 0.05$). These results suggested that the aging mice model was successfully established in our work.

Results of RCSP II effects on the activities of SOD, CAT, GSH-Px, and TAOC, as well as MDA level, in sera and livers of D-Gal model aging mice are shown in Tables 1 and 2. Compared with the D-Gal model control, RCSP II showed significant activity ($p < 0.01$) to increase SOD activity, CAT activity, GSH-Px activity, and TAOC levels in a dose-dependent manner. RCSP II also significantly decreased MDA concentration ($p < 0.01$). RCSP II also exerted a similar or better effect with ascorbic acid (positive control) compared with the D-Gal model control.

Aging is associated with the decrease in antioxidant status, and age-dependent increments in lipid peroxidation are a consequence of diminished antioxidant protection (Hagihara, Nishigaki, Maseki, & Yagi, 1984; Schuessel et al., 2006). Crucial components of the antioxidant defence system in the body are cellular antioxidant enzymes (SOD, GSH-Px and CAT) (Yao et al., 2005). The activities of above antioxidant enzymes in serums and livers were dramatically decreased with aging by the treatment of D-Gal in our work. The treatments with RCSP II could observably improve the activities of those antioxidant enzymes in serums and livers of D-Gal-treated mice. The results suggested that the decrease in those antioxidant enzymes activities might be the main factor in lipid peroxidative damage. The level of MDA is an oxidative stress marker. Lower MDA level suggests that there is less lipid peroxidation and weaker oxidant stress (Bagchi, Bagchi, Hassoun, & Stohs, 1995). RCSP II could inhibit observably the increase of MDA content in serums and livers of D-Gal induced mice. In addition, the aging-related

Table 2
Effects of RCSP II on the activities of SOD, CAT, GSH-Px, TAOC and MDA levels in livers in aging mice.

Test groups	SOD (U/mL)	CAT (U/mL)	GSH-Px (U/mL)	TAOC (U/mL)	MDA (nmol/mL)
Normal control	502.47 ± 32.41**	54.31 ± 11.24**	1896.35 ± 88.29**	1.45 ± 0.32*	14.25 ± 2.45*
D-Gal (model control)	476.32 ± 32.45	34.57 ± 7.64	1689.29 ± 86.31	0.63 ± 0.09	18.25 ± 2.54
Ascorbic acid (100 mg/kg positive control)	501.77 ± 21.58**	62.35 ± 7.69**	1988.76 ± 52.55**	1.25 ± 0.17	11.47 ± 2.47**
RCSP II (100 mg/kg)	476.99 ± 21.14	38.55 ± 4.85	2543.25 ± 78.69**	0.65 ± 0.07	14.57 ± 1.57*
RCSP II** (200 mg/kg)	489.34 ± 21.55*	41.58 ± 7.85*	2878.94 ± 84.52**	1.14 ± 0.17*	11.12 ± 2.11**
RCSP II (400 mg/kg)	537.22 ± 17.54**	51.67 ± 7.98**	3174.44 ± 98.57**	1.45 ± 0.14**	9.87 ± 1.25**

Values are mean ± SD.
* $P < 0.05$ vs. model control.
** $P < 0.01$ vs. model control.

decrease in TAOC level suggests that the decrease in the nonenzymatic antioxidant defence probably also contributes to endogenous lipid peroxidation.

The reports of animal polysaccharide having antioxidant activity in vivo are rare in published literatures. Therefore, we do some comparison with the antioxidant activity in vivo of polysaccharide from mushroom or traditional Chinese medicine. The polysaccharide from *Phellinus baumii* Pilát can increase the activities of antioxidant enzymes and decrease the levels of MDA (Luo et al., 2010). The mycelial polysaccharides from *Lepista sordida* significantly inhibited the formation of MDA and raised the activities of SOD and GSH-Px in mice brains and serums in a dose-dependent manner (Zhong et al., 2013). Sulfated polysaccharide fraction from *Porphyra haitanesis* can increase TAOC and the activity of SOD and GSH-Px in lung, liver, heart, brain and spleen tested in aging mice (Zhang et al., 2003). Compared with these polysaccharides, RCSP II can effectively increase the activities of antioxidant enzymes (SOD, GSH-Px and CAT) and TAOC, as well as decrease MDA level, in livers and sera of mice. It is worth to mention that RCSP II is purified from animal.

4. Conclusion

Physicochemical properties (average molecular weight, monosaccharide composition, etc.) of RCSP II, purified from *R. chensinensis* skin, were investigated. Results of antioxidant activity assay in vitro showed that RCSP II had positive antioxidant potential on ABTS radicals and chelated ferrous ion. Results of antioxidant activity assay in vivo demonstrated that RCSP II obviously increased the activities of antioxidant enzymes (SOD, CAT, and GSH-Px), enhanced total antioxidant capacity, and reduced MDA levels in sera and livers of D-Gal-induced aging mice. Combined with findings from our previous research, the present results showed that RCSP II had potent antioxidant activity and could be explored as a novel natural antioxidant for use in food and pharmaceuticals.

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