Carbohydrate Polymers 78 (2009) 199–204

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Antioxidant activities of polysaccharides from *Hyriopsis cumingii*

Deliang Qiao [a,b], Chunling Ke [a], Bing Hu [a], Jianguang Luo [a], Hong Ye [a], Yi Sun [a], Xiaoyan Yan [a], Xiaoxiong Zeng [a,*]

[a] College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, PR China
[b] Department of Chemical and Biological Science, West Anhui University, Lu'an 237012, PR China

ARTICLE INFO

Article history:
Received 7 February 2009
Received in revised form 9 March 2009
Accepted 10 March 2009
Available online 20 March 2009

Keywords:
Hyriopsis cumingii
Polysaccharides
Antioxidant activity
Free radicals
In vitro
In vivo

ABSTRACT

The antioxidant activities of crude *Hyriopsis cumingii* polysaccharides (HCPS) were evaluated both *in vitro* and *in vivo*. *In vitro* antioxidant assay, HCPS (crude and its purified fraction) could scavenge hydrogen peroxide, free radicals of superoxide anion and 2,2-diphenyl-1-picryl-hydrazyl, chelate ferrous ion and reduce ferric ion. Except for metal ion chelating activity, HCPS-3 exhibited much higher antioxidant activities than crude HCPS, HCPS-1 and HCPS-2. For antioxidant testing *in vivo*, different doses of crude HCPS were orally administrated over a period of 15 days in a D-galactose induced aged mice model. As results, administration of crude HCPS inhibited significantly the formation of malondialdehyde in mice livers and serums and raised the activities of antioxidant enzymes and total antioxidant capacity in a dose-dependent manner. The results suggested that HCPS had direct and potent antioxidant activities.

1. Introduction

Hyriopsis cumingii, a member of freshwater pearl mussels, is the most economically important mussel species in China. As the best for producing high quality pearls, *H. cumingii* is therefore widely cultivated in China (Hua & Gu, 2002). However, the *H. cumingii* flesh (waste of producing freshwater pearls) is used mainly as animal feeds and fertilizer. In fact, *H. cumingii* can cure some diseases in traditional Chinese medicine. In addition, recent studies demonstrate that the *H. cumingii* flesh is rich in polysaccharides (Dai, Zhang, Zhang, & Wang, 2009; Hu & Cao, 2003; Qiao et al., 2009), which result in nutrient and pharmacological functions such as anti-tumor, immunity-enhancement, anti-inflammation and anti-aging (Cheng, Wu, Lu, & Chi, 2007; Dai et al., 2009; Hu & Cao, 2003; Zhang, Wu, Di, & Chen, 2007). However, little attention has been devoted to the antioxidant activity of *H. cumingii* polysaccharides (HCPS). Recently, we reported the extraction optimized by using response surface methodology, purification and preliminary characterization of HCPS (Qiao et al., 2009). We found that the third purified fraction (HCPS-3) was quite different from the other two fractions (HCPS-1 and HCPS-2). It contained 9.42% of protein, much higher sulfate content, relative higher relative viscosity and relative complicated monosaccharide composition. In order to evaluate the antioxidant activities of crude HCPS and its purified fractions, we determined their antioxidant activities *in vitro* by different extracorporeal antioxidant methods and antioxidant activity *in vivo* of crude HCPS by using D-galactose induced aged mice as *in vivo* model. Herein, we report in detail the antioxidant activities of HCPS.

2. Materials and methods

2.1. Materials and reagents

Crude HCPS and its purified fractions of HCPS-1, HCPS-2 and HCPS-3 were prepared from *H. cumingii* according to our previous method (Qiao et al., 2009). 2,2-Diphenyl-1-picryl-hydrazyl (DPPH), ferrozine, nitroblue tetrazolium (NBT), phenazine methosulfate (PMS), reduced nicotinamide adenine dinucleotide (NADH) and 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Assay kits for protein, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) and total antioxidant capacity (TAOC) were the products of Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The male Kunming mice were purchased from the Experiment Animal Center of Academy of Military Medical Sciences (Beijing, China). All other reagents were of analytical grade.

2.2. Determination of antioxidant activities in vitro of HCPS

2.2.1. Assay of superoxide anion scavenging activity

Measurement of superoxide anion ($O_2^{\cdot}$) scavenging activity was done according to the method reported by Li, Zhou, and Han (2006) with slight modifications. Briefly, each 1.0 ml of NBT solution

* Corresponding author. Fax: +86 25 84396791.
E-mail address: zengxx@njau.edu.cn (X. Zeng).

0144-8617/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2009.03.018

(156 μmol/L of NBT in 0.1 M phosphate buffer, pH 7.4), NADH solution (468 μmol/L of NADH in 0.1 M phosphate buffer, pH 7.4) and HCPS solution were mixed. The reaction mixture was started by adding 1.0 ml of PMS solution (60 μmol/L PMS in 0.1 M phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25 °C for 5 min, and the absorbance at 560 nm was measured against a blank (water and 0.1 M phosphate buffer instead of HCPS and NBT solution, respectively). The inhibition percentage was calculated by using following formula: inhibition rate (%) = $[A_0 - (A_1 - A_2)]/A_0 \times 100\%$, where A_0 is the absorbance of the control (water instead of HCPS solution), A_1 is the absorbance of the sample and A_2 is the absorbance of the sample under identical conditions as A_1 with 0.1 M phosphate buffer instead of NBT solution.

2.2.2. Assay of hydrogen peroxide scavenging activity

The ability of scavenging hydrogen peroxide (H_2O_2) was determined according to the method reported by Liu, Wang, Xu, and Wang (2007) with minor modifications. Reaction mixture was composed of 2.4 ml of phosphate buffer (0.1 M, pH 7.4), 0.6 ml of H_2O_2 solution (0.04 M) and 1.0 ml of HCPS solution. Absorbance of reaction mixture at 230 nm was determined after 10 min against a blank (water instead of HCPS and H_2O_2 solution). The scavenging percentage was calculated as following: scavenging rate (%) = $[A_0 - (A_1 - A_2)]/A_0 \times 100\%$, where A_0 is the absorbance of the control (water instead of HCPS solution), A_1 is the absorbance of the sample and A_2 is the absorbance of the sample only (water instead of H_2O_2 solution).

2.2.3. Assay of DPPH radicals scavenging activity

The DPPH radical scavenging activity was measured by using the method reported by Li et al. (2006) with slight modification. Briefly, 0.2 ml DPPH· solution (400 μmol/L in dehydrated alcohol) was added to 1.0 ml of HCPS solution, and then 2.0 ml of water was added. The mixture was shaken and allowed to stand at room temperature in the dark for 30 min. The absorbance was measured at 517 nm against a blank (water instead of HCPS and DPPH· solution). Lower absorbance of the reaction mixture indicates higher free-radical scavenging activity. The scavenging percentage was calculated by the following equation: scavenging percentage activity (%) = $[A_0 - (A_1 - A_2)]/A_0 \times 100\%$, where A_0 is the absorbance of the control (water instead of HCPS solution), A_1 is the absorbance of the sample and A_2 is the absorbance of the sample under identical conditions as A_1 with water instead of DPPH· solution.

2.2.4. Assay of reduction potential

The reduction potential was determined by using the method of Li, Zhou, and Li (2007) with some modifications. One milliliter of HCPS solution, 1.0 ml phosphate buffer (0.2 M, pH 6.6) and 1.0 ml potassium ferricyanide (1%, w/v) were mixed and incubated at 50 °C for 20 min. After cooling down, 1.0 ml trichloroacetic acid (10%, w/v) and 0.2 ml fresh ferric trichloride ($FeCl_3$, 0.1%, w/v) were added to the reaction mixture. Then reaction mixture was shaken and its absorbance was detected at 700 nm against a blank (water instead of HCPS solution) 10 min later. Absorbance of the reaction mixture indicates the reduction capability of sample. Reductive potential = $(A_1 - A_2)$, where A_1 is the absorbance of the sample and A_2 is the absorbance of the sample under identical conditions as A_1 with water instead of $FeCl_3$ solution.

2.2.5. Assay of metal ion chelating activity

The chelating ability of metal ion was measured according to the method reported by Liu et al. (2007) in terms of chelating ferrous ion (Fe^{2+}) in the iron–ferrozine complex. Briefly, the reaction mixture, containing 1.0 ml HCPS solution, 0.05 ml ferrous chloride ($FeCl_2$) solution (2000 μmol/L), 0.2 ml ferrozine solution (5000 μmol/L) and 2.75 ml water, was shaken well and incubated at room temperature for 10 min. The absorbance of the mixture was measured at 562 nm against a blank (water instead of HCPS and $FeCl_2$ solution). The ability of chelating ferrous ion was calculated using the following equation: chelating rate (%) = $[A_0 - (A_1 - A_2)]/A_0 \times 100\%$, where A_0 is the absorbance of the control (water instead of HCPS solution), A_1 is the absorbance of the test sample and A_2 is the absorbance of the sample under identical conditions as A_1 with water instead of $FeCl_2$ solution.

2.2.6. Assay of ferric reducing ability of plasma

The ferric reducing ability of plasma (FRAP) assay was carried out according to the procedure of Benzie and Strain (1996) with slight modifications. Briefly, the FRAP reagent was prepared daily from 0.3 M acetate buffer (pH 3.7), 0.01 M TPTZ solution (in 0.04 M hydrochloric acid) and 0.02 M $FeCl_3$ solution in a proportion of 10:1:1 (v/v). One milliliter of HCPS solution (appropriate dilution based on pre-experiment) was added to 5.0 ml of the FRAP reagent, and the reaction mixture was incubated at 37 °C for 10 min. The absorbances of A_1 and A_2, where A_1 is the absorbance of the sample and A_2 is the absorbance of the sample only (water instead of $FeCl_3$ solution), were determined against a blank (water instead of HCPS solution) at 593 nm. Fresh working solutions of $FeSO_4$ were used for calibration. The antioxidant capacity based on the ability to reduce ferric ions of sample was calculated from the linear calibration curve and expressed as mmol $FeSO_4$ equivalents per gram of HCPS.

2.3. Determination of antioxidant activities in vivo of crude HCPS

2.3.1. Animal grouping and experimental design

The assay of antioxidant activities *in vivo* of crude HCPS was carried out according to the reported method with some modifications (Ke et al., 2009). Male Kunming mice (8-weeks-old), grade of specific pathogen free with body weight (BW) of 20 ± 2 g, were used in this study. The mice were maintained in separated cages. Under the conditions of 21 ± 1 °C and 50–60% relative humidity, they were free to access to food and water and kept on a 12-h light/dark cycles during the experiments. After adapting to their environment for 1 week, these mice were randomly divided into six groups (six for each) for experiment. Mice in Group I (normal control group) were treated with 0.9% sodium chloride (NaCl, 25 ml/kg BW) per day by hypodermic injection and gastric gavage. Mice in Group II (model control group) were treated with 9% D-galactose (D-Gal, 25 ml/kg BW) by hypodermic injection and 0.9% NaCl (25 ml/kg BW) by gastric gavage per day. Mice in Group III (positive control group) were treated with 9% D-Gal (25 ml/kg BW) by hypodermic injection and vitamin E (V_E, 50 mg/ kg BW) by gastric gavage each day. Mice in Group IV (crude HCPS of low dose), Group V (crude HCPS of medium dose) and Group VI (crude HCPS of high dose) were treated with 9% D-Gal (25 ml/kg BW) by hypodermic injection and crude HCPS of 200, 400 and 800 mg/kg BW, respectively, by gastric gavage, once a day for 15 consecutive days.

2.3.2. Biochemical assay

After overnight fasting following the last drug administration, the mice were weighed and killed by decapitation. Blood samples were harvested immediately in centrifuge tube. After 1 h, the blood samples were centrifuged at 4000*g* for 10 min to afford the serums required. The liver was excised, weighed and homogenized immediately in ice-cold 0.9% NaCl solution (0.1 g tissue/ml solution). The suspension was centrifuged as mentioned above, and the supernatant was collected for further analysis. All above treatments were done at 4 °C.

Protein content, activities of SOD, CAT and GSH-Px, level of MDA and TAOC were measured by using commercial reagent kits

according to the instruction manuals. Briefly, the protein content in liver supernatant was determined based on method of coomassie brilliant blue dyeing using bovine serum albumin (BSA) as the standard and expressed as milligram per milliliter (mg/ml). Enzymatic activities of SOD, CAT and GSH-Px, TAOC level were determined according to the methods of xanthine oxidase–xanthine reaction system, CAT–H_2O_2 reaction system, reduced glutathione (GSH)–H_2O_2 reaction system and ferric reducing/antioxidant power reaction system, respectively. All above enzymatic activities were expressed as unit per milligram of protein (U/mg protein) in liver or unit per milliliter in serum (U/ml). MDA level was measured by using 2-thiobarbituric acid (TBA) method and expressed as nanomole per milligram of protein (nmol/mg protein) in liver or nanomole per milliliter in serum (nmol/ml).

2.3.3. Statistical analysis

The data were reported as mean ± standard deviation (SD) ($n = 6$) and evaluated by one-way analysis of variance (ANOVA) followed by the Duncan's multiple-range tests. Difference was considered to be statistically significant if $P < 0.05$. All statistical analyses were carried out by using SPSS for Windows, Version11.5 (SPSS, Chicago, IL).

3. Results and discussions

3.1. Antioxidant activities in vitro of HCPS

3.1.1. Superoxide anion scavenging activity of HCPS

Among different reactive oxygen species (ROS), $O_2^{\cdot}$ is generated first. Although $O_2^{\cdot}$ is a relatively weak oxidant, it may decompose to form stronger ROS, such as singlet oxygen and hydroxyl radical ($OH^{\cdot}$), which initiate peroxidation of lipids. $O_2^{\cdot}$ is also known to initiate indirectly the lipid peroxidation as a result of the formation of H_2O_2, creating precursors of $OH^{\cdot}$ (Meyer & Isaksen, 1995). Therefore, $O_2^{\cdot}$ scavenging is extremely important to antioxidant work.

Scavenging activities of crude HCPS and its purified fractions (HCPS-1, HCPS-2 and HCPS-3) against $O_2^{\cdot}$ were presented in Fig. 1. Notably, HCPS-3 showed stronger scavenging activity than crude HCPS. However, the scavenging effects of HCPS-1 and HCPS-2 were significant weaker than that of crude HCPS ($P < 0.05$). The scavenging effects of crude HCPS, HCPS-1, HCPS-2 and HCPS-3 increased significantly ($P < 0.05$) with the increase of sample concentration ranging from 12.5 to 62.5 μg/ml. After that, the scavenging activity increased slowly with the increase of sample concentration. At a concentration of 2000 μg/ml, the scavenging activity was 61.16%, 15.26%, 13.15% and 70.25% for the crude HCPS, HCPS-1, HCPS-2 and HCPS-3, respectively. The results demonstrated that all the HCPS samples possessed $O_2^{\cdot}$ scavenging activities, especially crude HCPS and purified faction of HCPS-3 showed strong activities.

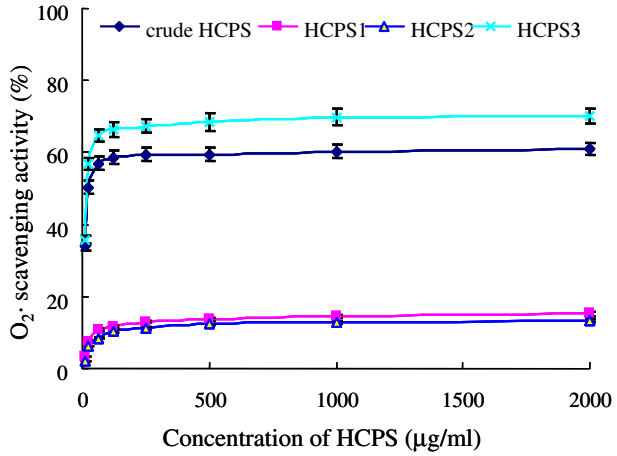

Fig. 1. The $O_2^{\cdot}$ scavenging activity of crude HCPS and its purified fractions (HCPS-1, HCPS-2 and HCPS-3).

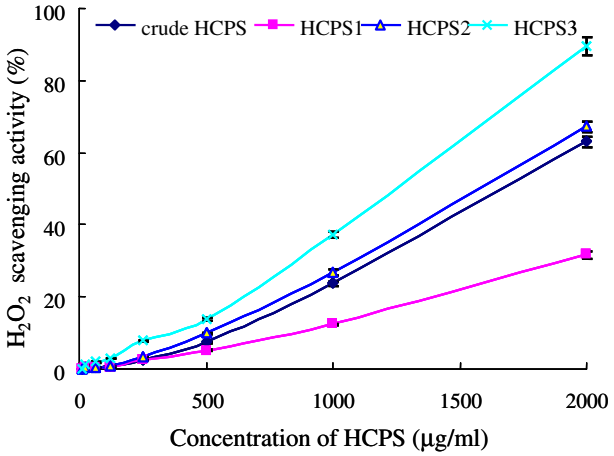

Fig. 2. The scavenging activity on H_2O_2 of crude HCPS and its purified fractions (HCPS-1, HCPS-2 and HCPS-3).

3.1.2. H_2O_2 scavenging activity of HCPS

The scavenging effects of crude HCPS, HCPS-1, HCPS-2 and HCPS-3 on H_2O_2 were shown in Fig. 2. In the concentration range of sample from 12.5 to 2000 μg/ml, all the HCPS samples were capable of scavenging H_2O_2 in an amount-dependent manner. The scavenging effects of HCPS-3 and HCPS-2 were stronger than that of crude HCPS, but HCPS-1 showed weaker scavenging activity than crude HCPS. At the concentration of 2000 μg/ml, the scavenging effect of the crude HCPS, HCPS-1, HCPS-2 and HCPS-3 was 62.99%, 31.66%, 67.27% and 89.51%, respectively.

H_2O_2 itself is not very reactive, however, it can sometimes be toxic to cell because it may give rise to $OH^{\cdot}$ in the cells. Thus, removing H_2O_2 is very important for life being away from damage. The results of this work demonstrated that crude HCPS, HCPS-2 and HCPS-3 exhibited extinguishing activities against H_2O_2.

3.1.3. DPPH$^{\cdot}$ scavenging activity of HCPS

The DPPH free radical is a stable free radical, which has been widely accepted as a tool for estimating the free-radical scavenging activities of antioxidants (Hu, Lu, Huang, & Ming, 2004). The DPPH$^{\cdot}$ scavenging activities of crude HCPS and its purified fractions were presented in Fig. 3. The scavenging effect increased with the in-

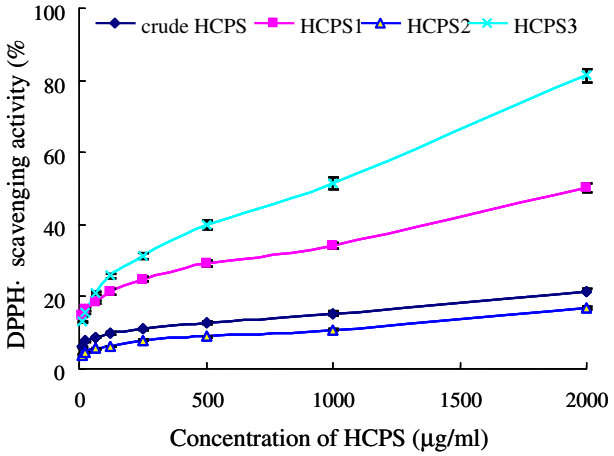

Fig. 3. The scavenging activity on DPPH$^{\cdot}$ radicals of crude HCPS and its purified fractions (HCPS-1, HCPS-2 and HCPS-3).

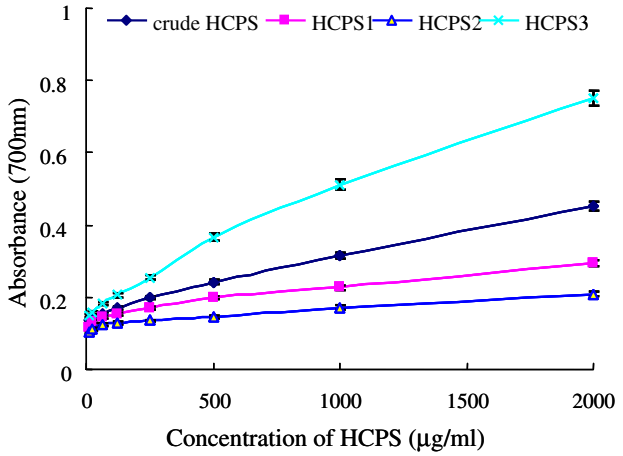

Fig. 4. The reductive potential of crude HCPS and its purified fractions (HCPS-1, HCPS-2 and HCPS-3).

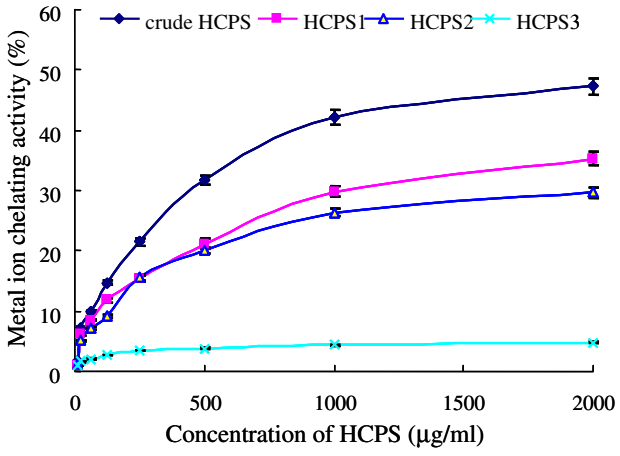

Fig. 5. The metal ion chelating activity of crude HCPS and its purified fractions (HCPS-1, HCPS-2 and HCPS-3).

crease of concentration up to 2000 μg/ml. At a concentration of 2000 μg/ml, the DPPH· scavenging activity was 21.43%, 50.17%, 16.76% and 81.28% for crude HCPS, HCPS-1, HCPS-2 and HCPS-3, respectively. Apparently, HCPS-3 showed the highest scavenging activity.

The method of scavenging DPPH· is based on the reduction of DPPH· ethanol solution in the presence of a hydrogen donating antioxidant, resulting in the formation of the non-radical form DPPH-H by the reaction. DPPH· is a stable free radical and can accept an electron or hydrogen radical to become a stable diamagnetic molecule. It has been reported that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds and aromatic amines could reduce and decolorize DPPH· by their hydrogen donating ability (Li et al., 2007). The results mentioned above implied that all the HCPS might act as electron or hydrogen donator to scavenge DPPH·.

3.1.4. Reduction potential of HCPS

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Braughler & Hall, 1989). Fig. 4 showed the reductive activities of crude HCPS, HCPS-1, HCPS-2 and HCPS-3 using the $K_3Fe(CN)_6$ reduction method. The reducing capacities of all the HCPS increased with the increase of sample concentration. At the concentration of 2000 μg/ml, the reducing capacity (absorbance at 700 nm) of crude HCPS, HCPS-1, HCPS-2 and HCPS-3 was 0.451, 0.294, 0.208 and 0.751, respectively. The results indicated that the order of reductive potential was HCPS-3 > crude HCPS > HCPS-1 > HCPS-2.

It has been reported that there was a direct correlation between antioxidant activity and reducing capacity (Amarowicz, Pegg, Rahimi-Moghaddam, Barl, & Weil, 2004). It has also been reported that the reducing properties were generally associated with the presence of reductones (Li et al., 2007; Pin-Der-Duh, 1998). Our data of the reduction potential suggested that there might be a direct correlation between antioxidant activity and reducing capacity in crude HCPS, HCPS-1, HCPS-2 and HCPS-3.

3.1.5. Metal ion chelating activity of HCPS

Fig. 5 showed ferrous ion chelating activities of crude HCPS, HCPS-1, HCPS-2 and HCPS-3. Fe^{2+} could be combined by all the HCPS in a dose-dependent manner, and the Fe^{2+} chelating effects of all the purified fractions (HCPS-1, HCPS-2 and HCPS-3) were weaker than that of crude HCPS. At the concentration of 2000 μg/ml, the chelating power was 47.31%, 35.26%, 29.65% and 4.83% for crude HCPS, HCPS-1, HCPS-2 and HCPS-3, respectively.

Metal chelating activity is claimed as one of antioxidant mechanisms, since it reduces the concentration of the catalyzing transition metal in lipid peroxidation. Among the transition metals, iron is known as the most important lipid oxidation prooxidant due to its high reactivity. The ferrous state of iron accelerates lipid oxidation by breaking down hydrogen and lipid peroxides to reactive free radicals via the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$). Fe^{3+} ion also produces radicals from peroxides although the rate is less than tenth that of Fe^{2+} ion. Thus, Fe^{2+} ion is the most powerful prooxidant among the various species of metal ions (Liu et al., 2007). The data of Fe^{2+} chelating activity demonstrated that crude HCPS, HCPS-1 and HCPS-2 exhibited moderate chelating activity.

3.1.6. FRAP of HCPS

FRAP assay is based on the ability of antioxidant to reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ, forming an intense blue Fe^{2+}–TPTZ complex with an absorption maximum at 593 nm. The absorbance decrease is proportional to the antioxidant content (Benzie & Strain, 1996). In the present study, the FRAP value (expressed in mmol $FeSO_4$ equivalents per gram of sample) was applied to evaluate the antioxidant ability of HCPS. The linear calibration curve (regression equation: $y = 0.0026x + 0.0277$, $R^2 = 0.9995$) of $FeSO_4$ was produced and the FRAP values of the crude HCPS, HCPS-1, HCPS-2 and HCPS-3 were obtained as 1.70, 0.49, 1.88 and 6.29 mmol $FeSO_4$/g, respectively.

Huang, Kong, Wang, and Hu (2007) reported that sulfated polysaccharides exhibited higher biological activities such as anti-virus, immunity-enhancement anti-coagulation and anti-tumor than unsulfated polysaccharides. In our previous report, we demonstrated that HCPS-3 had higher content of sulfuric radical than crude HCPS, HCPS-1 and HCPS-2 (Qiao et al., 2009). Therefore, the higher antioxidant activity of HCPS-3 might be attributed to its higher content of sulfuric radical.

3.2. Antioxidant activities of HCPS in vivo

Mice injected with D-Gal have been used as an aging animal model in some previous studies. D-Gal can cause accumulation of ROS, or stimulate free radical production indirectly by the formation of advanced glycation end-product (AGE) *in vivo*. These AGEs cannot be metabolized further and accumulate in neurons to amplify oxidative stress. Further studies show that aging-related changes induced by D-Gal include the increase of free radicals and the decrease of antioxidant enzymatic activities (Lu et al.,

Table 1
Effects of crude polysaccharides from *Hyriopsis cumingii* on the activities of SOD, CAT, GSH-Px and levels of MDA and TAOC in livers in aging mice.

Group	SOD (U/mg protein)	CAT (U/mg protein)	GSH-Px (U/mg protein)	MDA (nmol/mg protein)	TAOC (U/mg protein)
I	348.48 ± 3.95^{a}	53.67 ± 4.25^{a}	629.77 ± 13.78^{a}	1.40 ± 0.01^{a,c}	2.32 ± 0.06^{a}
II	250.89 ± 10.35^{b}	42.61 ± 1.32^{b}	537.34 ± 7.24^{b}	2.35 ± 0.043^{b}	1.68 ± 0.28^{b}
III	425.54 ± 6.61^{c}	45.22 ± 1.15^{b}	659.22 ± 25.16^{a}	0.98 ± 0.10^{e}	2.29 ± 0.06^{a}
IV	340.80 ± 3.30^{a}	44.40 ± 1.84^{b}	626.07 ± 10.13^{a}	1.83 ± 0.02^{d}	1.82 ± 0.11^{b}
V	484.29 ± 15.02^{d}	54.18 ± 0.20^{a}	804.24 ± 27.01^{c}	1.57 ± 0.04^{a,d}	2.33 ± 0.14^{a}
VI	507.53 ± 40.72^{d}	64.39 ± 2.48^{c}	825.67 ± 21.34^{c}	1.20 ± 0.01^{c,e}	2.53 ± 0.09^{a}

The data were presented as mean ± SD ($n = 6$) and evaluated by one-way ANOVA followed by the Duncan's multiple-range tests. Different alphabets (a–e) in superscript donate significant difference ($P < 0.05$).

Table 2
Effects of crude polysaccharides from *Hyriopsis cumingii* on the activities of SOD, CAT, GSH-Px and levels of MDA and TAOC in serums in aging mice.

Group	SOD (U/ml)	CAT (U/ml)	GSH-Px (U/ml)	MDA (nmol/ml)	TAOC (U/ml)
I	138.29 ± 0.25^{a,b}	72.37 ± 1.25^{a}	8852.94 ± 29.41^{a}	13.21 ± 0.36^{a}	25.43 ± 1.09^{a,c}
II	133.87 ± 3.41^{b}	66.47 ± 1.20^{b}	7441.18 ± 88.23^{b}	16.37 ± 0.99^{b}	16.51 ± 0.89^{b}
III	156.02 ± 2.96^{c}	73.64 ± 0.95^{a}	8823.53 ± 117.65^{a}	9.30 ± 0.32^{c}	24.73 ± 0.92^{a,c}
IV	145.06 ± 1.60^{a}	73.55 ± 1.09^{a}	7712.26 ± 124.03^{b}	12.32 ± 0.45^{a}	22.98 ± 0.33^{a}
V	155.53 ± 1.96^{c}	81.23 ± 1.36^{c}	8847.06 ± 94.12^{a}	9.22 ± 0.14^{c}	27.01 ± 0.37^{c}
VI	165.14 ± 2.80^{d}	84.80 ± 2.88^{c}	9832.35 ± 50.00^{c}	8.81 ± 0.11^{c}	32.06 ± 0.25^{d}

The data were presented as mean ± SD ($n = 6$) and evaluated by one-way ANOVA followed by the Duncan's multiple-range tests. Different alphabets (a–e) in superscript donate significant difference ($P < 0.05$).

2007; Song, Bao, Li, & Li, 1999). V_E has been applied in early experiments as positive control medicine because of its efficient antioxidation and prevention of lipid peroxidation (Li et al., 2007; Przybyszewski, Widel, & Koterbicka, 1994). In this study, therefore, mice treated with D-Gal were used as an aging animal model and V_E was used as positive control medicine.

Effects of crude HCPS on activities of SOD, CAT, GSH-Px and levels of MDA and TAOC in livers in aging mice were shown in Table 1. Apparently, a marked increase in MDA and significant decreases ($P < 0.05$) of antioxidant enzymes activities (SOD, GSH-Px, CAT) and TAOC were observed in livers between the treatments of Group I (normal control group) and Group II (model control group). Crude HCPS and V_E treatments inhibited significantly ($P < 0.05$) the formation of MDA in mice livers and raised the activities of antioxidant enzymes and the level of TAOC in a dose-dependent manner (Groups IV–VI). Similarly, administration of crude HCPS and V_E dose-dependently elevated the activities of antioxidant enzymes and TAOC level, while reduced the level of MDA in serums (Table 2).

A vast number of evidence implicates that aging is associated with a decrease in antioxidant status and the age-dependent increase in lipid peroxidation is a consequence of diminished antioxidant protection (Schuessel et al., 2006). The major antioxidant enzymes, including SOD, GSH-Px and CAT, are used as biomarkers to indicate ROS production and regarded as the primary defense system against ROS generated *in vivo* during oxidative stress (Inal, Kanbak, & Sunal, 2001). These antioxidant enzymes act cooperatively at different sites in the metabolic pathway of free radicals to prevent oxidant damage. SOD is the only enzyme that disrupts $O_2^{\cdot}$ radicals and exists in all cells with high amounts in erythrocytes. The primary role of CAT and GSH-Px is to abolish H_2O_2 generated by free radicals or by SOD reaction (in the removal of $O_2^{\cdot}$ radicals), thereby prevent the formation of $OH^{\cdot}$ radicals (Li et al., 2007; Urso & Clarkson, 2003). In this study, we found that SOD, GSH-Px and CAT activities decreased markedly with aging and these changes had statistical significance in livers and serums. It is likely that the decrease in the activities of SOD and GSH-Px is the main factor in lipid peroxidative damage. MDA, the main product of lipid peroxidation, is an indicator of lipid peroxidation. Lower MDA level suggests that there is less lipid peroxidation and weaker oxidant stress (Bagchi, Bagchi, Hassoun, & Stohs, 1995). The results obtained in this study indicted that administration of low dose crude HCPS and V_E (positive control group) could overcome oxidant injury induced by D-Gal, and treatment of high dose crude HCPS exhibited significantly stronger antioxidant activities than positive control group *in vivo*. Therefore, increase of nonenzymatic antioxidant capacity (TAOC), enhancements of endogenous antioxidant enzymatic activities (SOD, CAT, GSH-Px) and decrease of lipid peroxidation product (MDA) might be, at least in part, devoted to the antioxidant activities of crude HCPS *in vivo*.

4. Conclusion

In this study, the antioxidant activities of crude HCPS and its purified fractions were evaluated both *in vitro* and *in vivo*. HCPS could terminate free radical chain reaction *in vitro*. The mechanism might include, at least in part, reacting with free radicals of $O_2^{\cdot}$ and H_2O_2 (by donating electron or hydrogen), chelating Fe^{2+} and reducing Fe^{3+}, then preventing $OH^{\cdot}$ production and lipid peroxidation. Except for the metal ion chelating activity, HCPS-3 showed higher antioxidant activities than crude HCPS, HCPS-1 and HCPS-2, which might be attributed to its much higher content of sulfuric radical and protein (Qiao et al., 2009). *In vivo* antioxidant testing, crude HCPS reduced lipid peroxidation accelerated by age-induced free radicals. The treatments of medium dose showed significantly stronger antioxidant activities than model control group, and high dose of crude HCPS showed significantly stronger antioxidant activities than positive control group. As HCPS-3 was quite different from HCPS-1 and HCPS-2, further works on their structure and function are in progress.

Acknowledgements

This work was partly supported by a Grant-in-Aid for scientific research from the National Natural Science Foundation of China (Nos. 30570415 and 30870547) and a Grant-in-Aid from Nanjing Agricultural University for the Introduction of Outstanding Scholars (804066).

References

Amarowicz, R., Pegg, R. B., Rahimi-Moghaddam, P., Barl, B., & Weil, J. A. (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chemistry, 84*, 551–562.

Bagchi, D., Bagchi, M., Hassoun, E. A., & Stohs, S. J. (1995). *In vitro* and *in vivo* generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. *Toxicology, 104*, 129–140.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry, 239*, 70–76.

Braughler, J. M., & Hall, E. D. (1989). Central nervous systems trauma and stroke: I. Biochemical considerations for oxygen radical formation and lipid peroxidation. *Free Radical Biology and Medicine, 6*, 289–301.

Cheng, W. X., Wu, H., Lu, Y., & Chi, Y. M. (2007). Effects of polysaccharides from *Hyriopsis cumingii* on experimental transplanted tumor in mice and on activity of NK cells in vitro. *Chinese Journal of Marine Drugs, 26*(2), 30–33 (in Chinese).

Dai, Z., Zhang, H., Zhang, Y., & Wang, H. (2009). Chemical properties and immunostimulatory activity of a water-soluble polysaccharide from the calm of *Hyriopsis cumingii* Lea. *Carbohydrate Polymers*. doi:10.1016/j.carbpol.2009.01.003.

Hu, F. L., Lu, R. L., Huang, B., & Ming, L. (2004). Free radical scavenging activity of extracts prepared from fresh leaves of selected Chinese medicinal plants. *Fitoterapia, 75*, 14–23.

Hu, J. R., & Cao, M. F. (2003). Inhibitive effects of polysaccharide from *Hyriopsis cumingii* on tumor proliferation: An experimental study. *Chinese Journal of Modern Applied Pharmacy, 20*(1), 11–13 (in Chinese).

Hua, D., & Gu, R. (2002). Freshwater pearl culture and production in China. *Aquaculture Asia, 7*, 6–8.

Huang, X., Kong, X., Wang, D., & Hu, Y. (2007). Research progress on sulfating modification of polysaccharides and sulfated polysaccharides. *Natural Product Research and Development, 19*(2), 328–332 (in Chinese).

Inal, M. E., Kanbak, G., & Sunal, E. (2001). Antioxidant enzyme activities and malondialdehyde levels related to aging. *Clinica Chimica Acta, 305*(1–2), 75–80.

Ke, C., Qiao, D., Gan, D., Sun, Y., Ye, H., & Zeng, X. (2009). Antioxidant activity *in vitro* and *in vivo* of the capsule polysaccharides from *Streptococcus equi* subsp. *zooepidemicus*. *Carbohydrate Polymers, 75*, 677–682.

Li, X., Zhou, A., & Han, Y. (2006). Anti-oxidation and anti-microorganism activities of purification polysaccharide from *Lygodium japonicum in vitro*. *Carbohydrate Polymers, 66*(1), 34–42.

Li, X. L., Zhou, A. G., & Li, X. M. (2007). Inhibition of *Lycium barbarum* polysaccharides and Ganoderma lucidum polysaccharides against oxidative injury induced by γ-irradiation in rat liver mitochondria. *Carbohydrate Polymers, 69*(1), 172–178.

Liu, C., Wang, C., Xu, Z., & Wang, Y. (2007). Isolation, chemical characterization and antioxidant activities of two polysaccharides from the gel and the skin of *Aloe barbadensis* Miller irrigated with sea water. *Process Biochemistry, 42*(6), 961–970.

Lu, J., Zheng, Y. L., Wu, D. M., Luo, L., Sun, D. X., & Shan, Q. (2007). Ursolic acid ameliorates cognition deficits and attenuates oxidative damage in the brain of senescent mice induced by D-galactose. *Biochemical Pharmacology, 74*(7), 1078–1090.

Meyer, A. S., & Isaksen, A. (1995). Application of enzymes as food antioxidants. *Trends in Food Science & Technology, 6*(9), 300–304.

Pin-Der-Duh, X. (1998). Antioxidant activity of burdock (*Arctium lappaLinne*): Its scavenging effect on free radical and active oxygen. *Journal of American Oil Chemistry Society, 75*, 455–461.

Przybyszewski, W. M., Widel, M., & Koterbicka, A. (1994). Early peroxidising effects of myocardial damage in rats after gamma-irradiation and farmorubicin (4′-epidoxorubicin) treatment. *Cancer Letters, 81*(2), 185–192.

Qiao, D., Hu, B., Gan, D., Sun, Y., Ye, H., & Zeng, X. (2009). Extraction optimized by using response surface methodology, purification and preliminary characterization of polysaccharides from *Hyriopsis cumingii*. *Carbohydrate Polymers*. doi:10.1016/j.carbpol.2008.11.004.

Schuessel, K., Frey, C., Jourdan, C., Keil, U., Weber, C. C., Muller-Spahn, F., et al. (2006). Aging sensitizes toward ROS formation and lipid peroxidation in PS1M146L transgenic mice. *Free Radical Biology and Medicine, 40*(5), 850–862.

Song, X., Bao, M., Li, D., & Li, Y. M. (1999). Advanced glycation in D-galactose induced mouse aging model. *Mechanisms of Ageing and Development, 108*(3), 239–251.

Urso, M. L., & Clarkson, P. M. (2003). Oxidative stress, exercise, and antioxidant supplementation. *Toxicology, 189*(1–2), 41–54.

Zhang, Z., Wu, H., Di, L., & Chen, L. (2007). A review about freshwater mussel (Unionidae). *Chinese Archives Traditional Chinese Medicine, 25*, 121–123 (in Chinese).