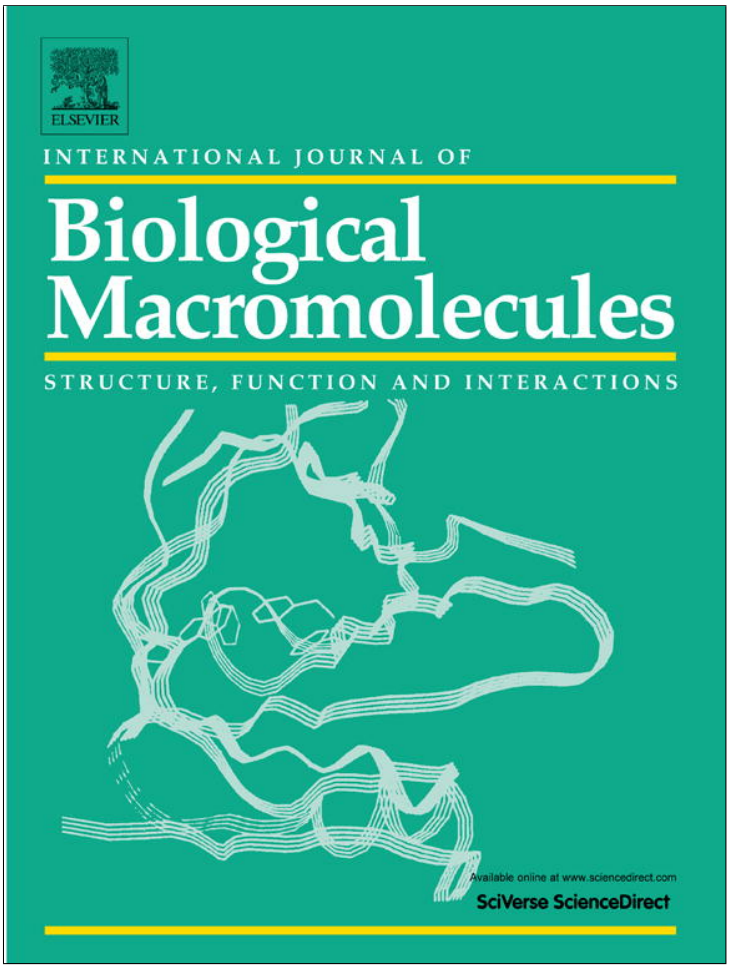

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Antioxidant and hepatoprotective potential of endo-polysaccharides from *Hericium erinaceus* grown on tofu whey

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ABSTRACT

Three fractions of the endo-polysaccharides from the mycelium of *Hericium erinaceus* (HEP) grown on tofu whey were obtained by the fractional precipitation with gradient concentrations of ethanol (HEP40, HEP60 and HEP80). The chemical and physical characteristics of the three crude polysaccharides were investigated by the combination of chemical and instrumental analysis methods. The studies to evaluate the antioxidant potential and the hepatoprotective effects of the three polysaccharides showed that they had different activities in different evaluation system. HEP80 showed strong activity on antioxidant *in vitro* and potent hepatoprotective effect *in vivo* and the hepatoprotective effect may be due to its potent antioxidant capacity. The HEP could be exploited as antioxidant product and a supplement in the prevention of hepatic diseases. The study also opens an avenue for the efficient utilization of tofu whey, which is usually discarded in environment, causing concerns.

1. Introduction

Reactive oxygen species (ROS) play an important role in the pathogenesis of various serious diseases, such as neurodegenerative disorder, cancer, cardiovascular diseases, atherosclerosis cataracts, and inflammation [1]. In order to meet the requirement of human body and/or to reduce damage to it, several synthetic antioxidants have been developed and used widely. However, some of them have been found to cause damage to liver [2]. In view of this, there has been an increasing interest in replacing the synthetic antioxidants with natural antioxidants in food, pharmaceutical and cosmetic industries [3,4].

Several natural polysaccharides, which are widely distributed in animals, plants and microorganisms, have been demonstrated to play an important role as free radical scavengers in the prevention of oxidative damage in living organism and can be explored as novel potent antioxidant [5–7].

Hericium erinaceus is a well-known edible and medicinal mushroom found in East Asia. Its fruiting bodies and the fungal mycelia exhibit various pharmacological activities, including enhancement of the immune system, antitumor, hypoglycemic and anti-aging properties [8].

Tofu is the main processed soybean product in the world. Tofu whey is the liquid residue, obtained as a by-product during the processing of soybean curd. During the processing, up to 30% of the soybean could be lost as waste. Large amount of tofu whey is produced every year, which is generally considered as waste and discarded, causing not only environmental pollution but also a wastage of this resource. Because tofu whey has a high organic content, it would be ideal to use this plentiful biological waste product for the generation of mushroom polysaccharide.

The aim of the present work was to study the antioxidant activities and hepatoprotective effects of the intracellular polysaccharides from the mycelium of *H. erinaceus* (HEP) grown on tofu whey. The relationship between chemical characteristics and the above activities were also studied.

2. Experimental

2.1. Materials and reagents

Tofu whey used in this study was obtained fresh from a tofu factory of Hangzhou, Zhejiang Province, China. The average composition of the tofu whey was the following (g/100 g): total protein 0.82; fat 0.39; ash 0.46; moisture 95.9; sugars and minor contents 2.43. The pH of the tofu whey varied between 4.1 and 4.3.

Deoxyibose, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4,4-disulfonic acid monosodium salt (ferrozine), butyl hydroxy anisd (BHA),

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trifluoroacetic acid (TFA), ethylenediaminetetraacetic acid (EDTA), L-rhamnose, D-glucose, D-xylose, D-fucose, D-galactose, and D-mannose were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). All the other reagents used were of analytical grade.

2.2. Preparation of H. erinaceus polysaccharides

2.2.1. Culture of H. erinaceus

Fungal strain of *H. erinaceus* strain was obtained from China General Microbiological Culture Collection Center. It was cultivated and maintained in potato dextrose agar (PDA). It was preserved at 4 °C and sub-cultured every three months. The seed culture was grown in a 250 ml flask containing 100 ml of the medium containing (g/l): glucose 20, yeast extract 5, peptone 3, KH_2PO_4 1, and $MgSO_4$ 1 in distilled water at 25 °C without shaking for 12 h, and then at 140 rpm for four days. A volume of 5 ml of seeding culture was crushed and added into the submerged culture in the state of asepsis. The submerged culture was performed in 250 ml flasks containing 95 ml tofu whey (pH 5.2) supplemented with glucose (5 g/l) at 25 °C on a rotary shaker (150 rpm) for 6 days.

2.2.2. Preparation of crude HEP

The mycelia of *H. erinaceus* were harvested from the liquid medium by filtering through a filter paper, washed three times with distilled water, and dried at 60 °C to a constant weight. The dry mycelia were ground using a domestic blender, extracted three times with distilled water at 95 °C for 2 h each, and then centrifuged at 5000 × *g* for 15 min. The supernatants were combined and concentrated to one-fifth of its original volume with a rotary evaporator under reduced pressure. Obtained sample (polysaccharides) so was successively sub-fractionated by grade precipitation at final ethanol concentrations of 40%, 60% and 80%, and named HEP40, HEP60 and HEP80, respectively. FT-IR of the sample was carried out by the potassium bromide (KBr) pellet method on Fourier transform-infrared spectrometer (FT/IR-660 Plus, JASCO) in the range of 400–4000 cm^{-1}.

2.2.3. Monosaccharide composition analysis

The sample containing the polysaccharides (2 mg) was hydrolyzed with 2 M TFA at 110 °C for 2 h, and the monosaccharides composition was determined by high-performance anion-exchange chromatography (HPAEC), using a Dionex LC30 equipped with a CarboPac™ PA20 column (3 mm × 150 mm). The column was eluted with 2 mM NaOH (0.45 ml/min) and the monosaccharides were monitored using a pulsed amperometric detector (Dionex) [9].

2.2.4. Chemical properties

The yield of crude polysaccharides was calculated as a percentage of the total weight of the sample used. The total carbohydrate content was determined by the phenol–sulfuric acid method [10]. The uronic acid content was measured by a modified hydroxydiphenyl assay [11]. Protein was measured with the Bradford method [12] using bovine serum albumin as a standard.

2.3. Assay for antioxidant activity

2.3.1. DPPH radical scavenging activity

The method reported by Shimada et al. [13] was adopted for measurement of free radical scavenging capability. To each 1 ml of sample solution, 1 ml of freshly prepared methanolic dimethyl sulfoxide (DMSO) solution of DPPH (0.5 mM) was added, mixed well and then let stand for 30 min at room temperature in the dark. The absorbance of the resulting was recorded at 517 nm. BHA was used as a reference material. All tests were preformed in triplicate. The scavenging activity was calculated as follows:

$$\text{Scavenging activity}\,(\%) = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

where A_0 is the absorbance of the control (DPPH solution with no sample) and A_1 is the absorbance of the samples (DPPH solution with sample or positive control).

2.3.2. Ferrous ion-chelating activity

Iron-chelating ability of all the samples was determined by chelation of the ferrous ions by the extracts and standards and estimated by the method of Dinis et al. [14]. For this, 0.2 ml test samples at different concentration (0.1–5.0 mg/ml) were added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was then measured at 562 nm. The percentage inhibition of ferrozine–Fe^{2+} complex formation was determined using the following formula:

$$\text{Chelating activity}\,(\%) = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the samples. The control contained $FeCl_2$ and ferrozine, with complex formation molecules. EDTA was used as positive control.

2.3.3. Reducing power

The reductive potential of all the samples was determined by the method of Oyaizu [15]. The different concentrations of test samples (0.1–5.0 mg/ml) were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50 °C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then subjected to centrifugation (10 min, 1000 × *g*). The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm.

2.3.4. Hydroxyl radical scavenging assay

Hydroxyl radical-scavenging activity was measured according to Smirnoff's work [16]. For this, 0.5 ml $FeSO_4$ (1.5 mM) was mixed with 0.35 ml H_2O_2 (6 mM), 0.15 ml sodium salicylate (20 mM) and 1.0 ml sample (0.1–5.0 mg/ml), then incubated at 37 °C for 1 h. The absorbance of the hydroxylated salicylate complex was measured at 562 nm. BHA was used as the positive control. The antioxidant activity was calculated with the following equation:

$$\text{Scavenging effect}\,(\%) = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

where A_0 was the absorbance of the solvent control and A_1 is the absorbance of the test (sample or BHA).

2.4. In vivo hepatoprotective activity

2.4.1. CCl_4-induced hepatotoxicity in mice

Sixty male ICR mice (Animal Experiment Center of Medical College, Zhejiang University, China) weighing 20–22 g were used. The animals were housed in individual stainless steel cages in an air-conditioned room under a 12:12 h light:dark cycle. A commercial pellet diet and water were provided throughout the experiment. All the procedures were conducted in accordance with the P.R. China legislation under No. 8910MO047 on the use and care of laboratory animals and with the guidelines established by the Institute for Experimental Animals of Zhejiang University.

Mice were divided in six groups ($n = 10$). Group I (normal control) animals were administered a single dose of water (16 ml/kg

Table 1
Chemical characteristics of polysaccharides from the mycelium of *Hericium erinaceus* grown on tofu whey.

Item	HEP40	HEP60	HEP80
Carbohydrate (%)	21.41	41.87	37.66
Protein (%)	3.49	2.38	2.41
Uronic acid (%)	0.36	0.33	0.66
Sugar components (mol%)			
Fucose	10.35	16.80	2.21
Rhamonose	1.14	0.64	1.05
Arabinose	0.80	1.64	1.30
Galactose	13.94	32.50	29.27
Glucose	39.80	28.86	70.15
Xylose	2.84	1.23	0.87
Mannose	5.80	12.33	10.53

body weight, p.o.) daily for seven days and received arachis oil (10 ml/kg body weight, i.p.) on day 7. Group II (CCl_4 control) received water (16 ml/kg body weight, p.o.) once daily for seven days and receive 0.1% CCl_4 in arachis oil (10 ml/kg body weight, i.p.) on day 7. Group III (positive control) animals received standard drug silymarin (a drug commonly used in the treatment of liver diseases, 100 mg/kg body weight, p.o.) once daily for seven days. Groups IV–VI animals were administered 300 mg/kg body weight of HEP40, HEP60 and HEP80 once daily for seven days, respectively and received 0.2% CCl_4 in arachis oil (10 ml/kg body weight, i.p.) after 1 h of administration of the silymarin and the HEP on day 7.

All the animals were sacrificed 24 h after the treatment and blood samples were collected immediately and the livers were removed quickly for the biochemical studies.

2.4.2. Biochemical assays

The blood samples collected were centrifuged at 4000 × *g* for 15 min to obtain the serum for the assessments of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The liver tissues for the biochemical study were homogenized in nine volumes of ice NaCl (0.9%), and then the homogenates were centrifuged at 6000 × *g* for 10 min to yield a clear supernatant fraction used for the superoxide dismutase (SOD), malonaldehyde (MDA), glutathione peroxidase (GP_X) and catalase (CAT) analysis. All the assessments were conducted by using the standard kits supplied by Nanjing Jiancheng Biotechnology Institute (China).

2.5. Statistical analysis

All the experiments were carried out in triplicate and data were expressed as mean ± standard deviation. Statistical analysis was performed with one-way analysis of variance (ANOVA). Statistical significance of differences between the groups was assessed by Student's *t*-test. All the calculations were performed in the SAS 9.0. A level of $P < 0.05$ was taken as statistically significant.

3. Results and discussion

3.1. Characterization and composition of the polysaccharides

The water-soluble crude polysaccharide named as HEP was obtained from the mycelium of *H. erinaceus* fermented with tofu whey by boiling water extraction, ethanol precipitation, distilled with water and lyophilized in freeze-drying apparatus. The total yield of HEP was about 6.23% (w/w) of the dried material, namely HEP40 (2.43%), HEP60 (3.06%) and HEP80 (0.74%). The chemical compositions and the contents of carbohydrate, protein and uronic acid for HEP40, HEP60 and HEP80 are shown in Table 1. HPAEC analysis showed the three polysaccharides were all composed of six kinds of monosaccharides, namely glucose, galactose, mannose, arabinose, xylose, rhamnose and fucose. Glucose and galactose were the predominant monosaccharides (Fig. 1).

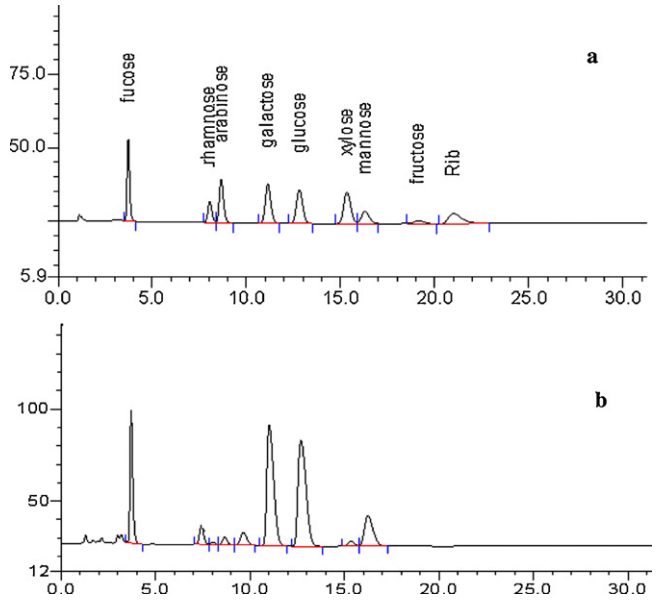

Fig. 1. (a) High-performance anion-exchange chromatography of standard monosaccharides. (b) High-performance anion-exchange chromatography of HEP60 purified from the mycelium of *Hericium erinaceus* (HEP) grown on tofu whey.

3.2. FTIR analysis

FTIR spectroscopy is typically used for the qualitative measurement of organic functional groups, especially O—H, N—H, and C=O. Fig. 2 shows the FTIR spectra of the three polysaccharides from the mycelium of *H. erinaceus* fermented with tofu whey. There was a stretching vibration of O—H and saturated C—H at 3300–3500 cm^{-1} and 2927–2930 cm^{-1}, respectively. Bands, due to amide are two, one at around 1640 cm^{-1} for Amide I (for C=O) and second one at about 1440 cm^{-1} due to Amide II (for N—H), indicating that the three polysaccharides had conjugated proteins. The absorption band at 1000–1200 cm^{-1} suggested that the three polysaccharides contained pyranose monomers in their structures.

3.3. Antioxidant activity

3.3.1. DPPH scavenging activity

The DPPH free radical is a stable free radical and can accept an electron or hydrogen radical to become a stable diamagnetic molecule, which has been widely accepted as a tool for estimating the free-radical scavenging activities of antioxidants [17]. Alcoholic solutions of DPPH have a characteristic absorption maximum at 517 nm. 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 [18]. It can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentrations [19].

On the basis of this principle, the scavenging effects of HEP40, HEP60 and HEP80 on the DPPH radical were measured and shown in Fig. 3a. The scavenging activity of polysaccharides on inhibition of the DPPH radicals was related to the concentration of the samples. Furthermore, the DPPH scavenging activities of the tested samples significantly increased with the increasing concentration. At the concentration of 1.25 mg/ml, the scavenging effects of HEP40, HEP60, HEP80 and BHA were 43.01%, 31.50%, 59.95% and 98.78%, respectively. The results mentioned above implied that the three

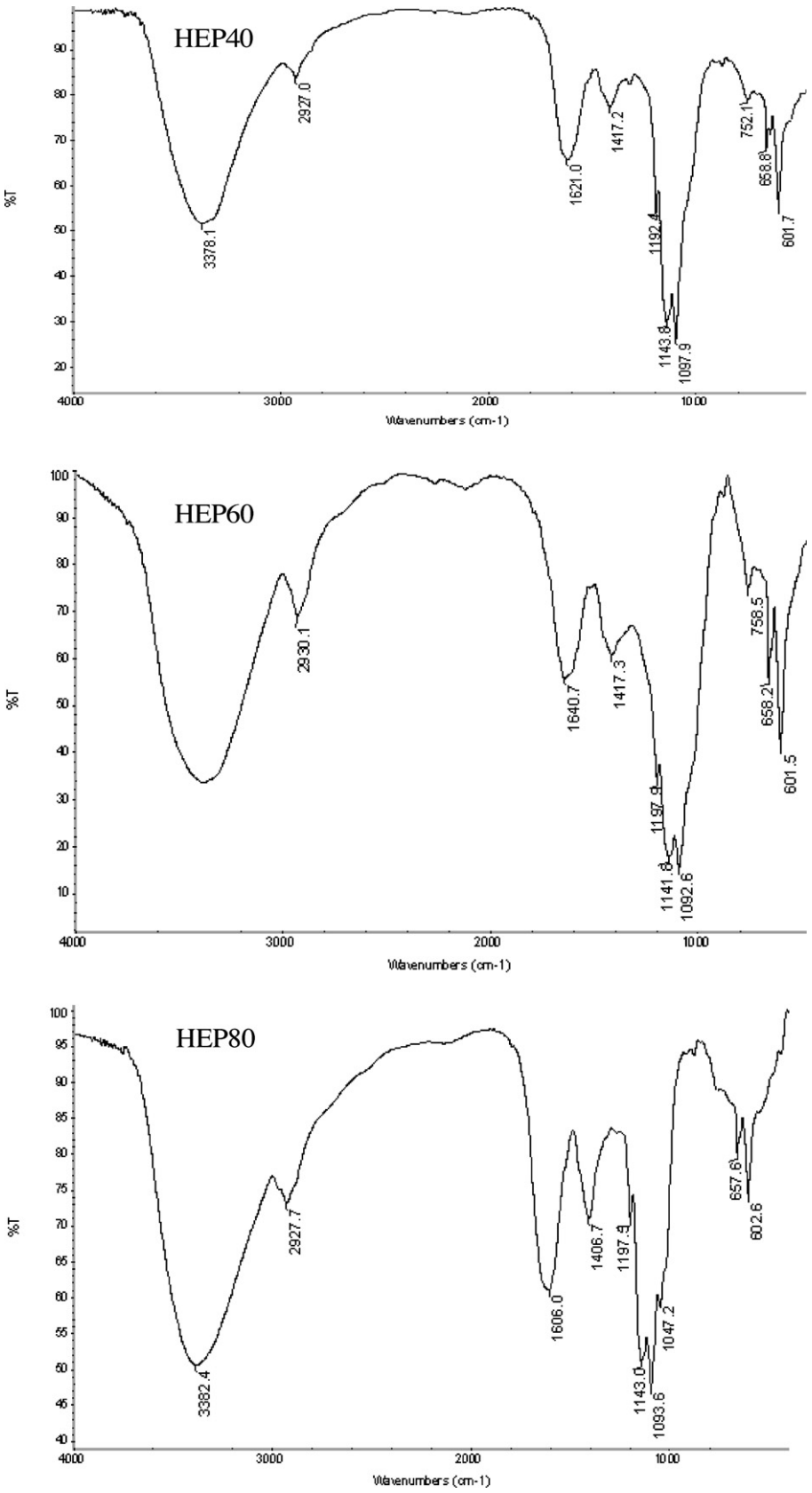

Fig. 2. Infrared spectra of the three polysaccharides from the mycelium of *Hericium erinaceus* grown on tofu whey.

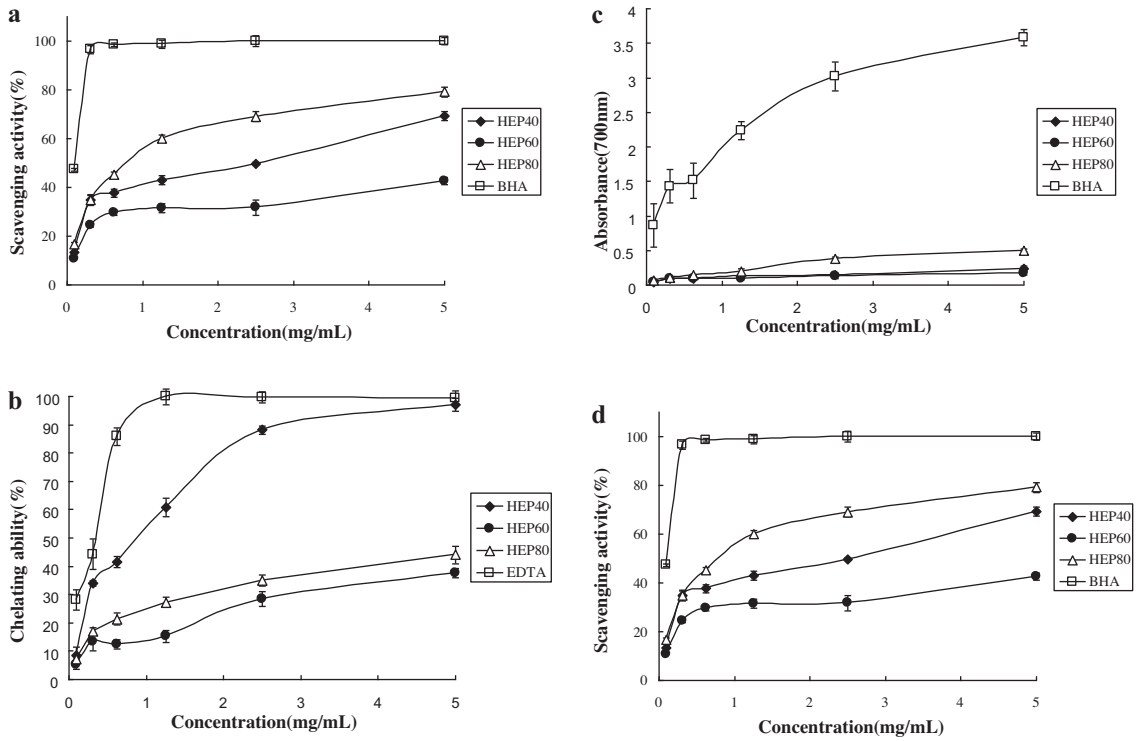

Fig. 3. Antioxidant activity of the polysaccharides from the mycelium of *Hericium erinaceus* grown on tofu whey. (a) DPPH radical scavenging activity, (b) Fe^{2+}-chelating activity, (c) reducing power and (d) hydroxyl radical scavenging activity. Data are the means of three replicates with standards deviations shown by vertical bars.

polysaccharides might act as electron or hydrogen donator to scavenge DPPH.

3.3.2. Iron chelating ability

Iron-chelating may render important antioxidant effects by retarding the metal-catalyzed oxidation [20]. The effective iron (II) chelators may also afford protection against the oxidative damage by removing iron (II) that may otherwise participate in HO•-generating Fenton type reactions. Minimizing iron (II) may afford protection against oxidative damage by inhibiting production of reactive oxygen species and lipid peroxidation. The iron (II)-chelating capacity of the tested samples was determined by measuring the iron–ferrozine complex (Fig. 3b). At the concentration of 0.1–5.0 mg/ml, the chelating ability was 8.41–97.07% for HEP40, 5.31–37.52% for HEP60, 7.07–43.99% for HEP80, and 28.20–99.44% for EDTA. Some workers have reported that the scavenging activity of HO• was not due to direct scavenging but inhibition of HO• generation by chelating ions such as Fe^{2+} and Cu^{2+} [21]. Smith et al. [22] reported that the molecules that inhibited deoxyribose degradation were those that could chelate iron ions and render them inactive or poorly active in a Fenton reaction. Accordingly, it could be suggested that the ferrous ions chelating effects of these tested extracts would be somewhat beneficial to protect against the oxidative damage.

3.3.3. The reducing power

A direct correlation between the antioxidant activity and reducing capacity has been reported [23]. The reducing properties are generally associated with the presence of reductones, which could donate a hydrogen atom and exert antioxidant action by breaking the free radical chain [24]. Reductones also react with certain precursors of peroxide, thus preventing peroxide formation. The antioxidant activity could be concomitant with the reducing power [25]. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Reducing power of the tested polysaccharides is shown in Fig. 3c. The reducing capacities of HEP80 showed the highest reducing power, and there were no significant difference between HEP40 and HEP60. At the concentration of 1.25 mg/ml, the absorbance of HEP40, HEP60 and HEP80 was 0.132, 0.096 and 0.191, respectively. These data on reducing power of HEP40, HEP60 and HEP80 indicated that it could play a role in the antioxidation observed.

3.3.4. Scavenging of hydroxyl radical

Hydroxyl radical scavenging mechanism is related to the transition metal ions. In the absence of transition metal ions, hydrogen peroxide was fairy stable. However, hydroxyl radical act in superoxidation by hydrogen peroxide with metal ions, usually ferrous or copper. The molecules that can chelate iron and render them inactive in Fenton reaction might have scavenging ability on hydroxyl radical [26]. The results of hydroxyl radical scavenging activities of HEP40, HEP60 and HEP80 are given in Fig. 3d. For all the samples, the effects of scavenging hydroxyl radical were in a concentration-dependent manner. The hydroxyl radical scavenging activities of the samples under study decreased in the order of HEP40, HEP60 and HEP80. At 0.625 mg/ml, the hydroxyl radical scavenging activities of HEP40, HEP60, HEP80 and BHA were 49.36%, 41.66%, 39.23% and 85.49%, respectively.

Among the reactive oxygen species, hydroxyl radical is considered to be a highly potent oxidant, which can react with most biomacromolecules functioning in living cells and induce severe damage to the adjacent biomolecules. Thus, removing hydroxyl radical is important for antioxidant defense in cell or food systems. Therefore, hydroxyl radical scavenging is extremely important to antioxidant work.

3.4. Hepatoprotective effects of HEP

3.4.1. Effects of HEP on AST, ALT in the serum of mice

The results of hepatoprotective effect of HEP on CCl_4-intoxicated mice are shown in Table 2. In the CCl_4-intoxicated group (II) serum

Table 2
Effects of administration of intracellular polysaccharides from the mycelium of *Hericium erinaceus* grown on tofu whey on biochemical parameters in CCl_4-induced liver injury mice.

Group	Control	CCl_4 (10 ml/kg body weight, i.p.)	CCl_4 + silymarin (100 mg/kg body weight, p.o.)	CCl_4 + HEP40 (300 mg/kg body weight, p.o.)	CCl_4 + HEP60 (300 mg/kg body weight, p.o.)	CCl_4 + HEP80 (300 mg/kg body weight, p.o.)
Serum						
ALT (U/l)	59.54 ± 15.36^{a}	316.82 ± 27.16^{b}	121.34 ± 11.23^{c}	164.5 ± 16.37^{d}	186.3 ± 18.16^{d}	124.6 ± 22.11^{e}
AST (U/l)	35.21 ± 5.32^{a}	196.35 ± 19.64^{b}	67.6 ± 8.71^{c}	104.3 ± 12.35^{d}	148.2 ± 16.11^{e}	97.9 ± 10.69^{e}
Liver tissue						
MDA (nmol/mg protein)	0.69 ± 0.06^{a}	1.46 ± 0.14^{b}	0.74 ± 0.10^{a}	1.06 ± 0.05^{c}	0.92 ± 0.19^{c}	0.86 ± 0.18^{c}
SOD (U/mg protein)	196.50 ± 32.11^{a}	94.82 ± 16.52^{b}	164.56 ± 18.76^{a}	99.89 ± 8.66^{b}	101.21 ± 16.41^{b}	136.23 ± 11.09^{c}
GPx (U/mg protein)	1.37 ± 0.15^{a}	0.58 ± 0.09^{b}	0.82 ± 0.08^{c}	1.16 ± 0.12^{a}	0.79 ± 0.10^{c}	1.28 ± 0.21^{a}
CAT (U/mg protein)	312.36 ± 44.31^{a}	186.24 ± 28.12^{b}	269.58 ± 13.59^{c}	243.61 ± 32.64^{c}	202.17 ± 15.87^{bc}	226.44 ± 10.69^{c}

Values are the mean ± S.D. of 10 mice. Different alphabets (a–e) in superscript denote significant difference ($P < 0.05$) according to Dunnet's *t*-test.

ALT and AST were increased to 316.82 and 196.35 U/l, respectively, whereas these values were showed 59.54 and 35.21 U/l in the control group (I). The elevated levels of serum ALT and AST were significantly reduced in the animals groups treated with HEP (300 mg/kg body weight). ALT and AST are enzymes originally present in high concentrations in the cytoplasm. When liver cells are injured, these enzymes leak into the blood stream and manifest significantly elevated serum levels. The extent of liver damage is in conformity with the elevated serum levels of these enzymes [27]. The assessment of liver function can be performed by estimating the activity of serum enzymes. In this view, the reduction in levels of ALT and AST by HEP was an indication of repair of hepatic tissue damage caused by the CCl_4.

3.4.2. Effects of extracts on MDA, SOD, CAT and GP_X levels

Results as shown in Table 2 clearly revealed increase in the level of the MDA in the CCl_4-intoxicated rats compared to the control group. The treatment with HEP (300 mg/kg body weight) significantly prevented this increase in levels. GP_X, SOD and CAT activities have significantly increased in HEP (300 mg/kg body weight) treated groups, whereas CCl_4-intoxicated group showed significant decrease in the levels compared to control group. The HEP40 (300 mg/kg body weight) treated group was superior to the others, and as comparable to the silymarin. Liver damage was also evidenced by the decrease in the activity of free radical scavenging enzymes, SOD, CAT and GP_X in the CCl_4 treated animals. These enzymes constitute a mutually supportive team of defense mechanism against the harmful effects of the ROS and free radicals in the biological systems [28]. Moreover, the lipid peroxidation is accelerated when free radicals are formed as the results of losing a hydrogen atom from the double bond in the structure of unsaturated fatty acids. Scavenging of free radicals is one of the major antioxidative mechanisms to inhibit the chain reaction of lipid peroxidation. The HEP enhanced the activities of antioxidant enzymes and diminished the amount of lipid peroxide against the CCl_4-induced hepatotoxicity in these animals.

CCl_4 is being used extensively to investigate the hepatoprotective activity on various experimental animals. It is biotransformed by cytochrome P_{450} to active CCl_3 radical, which further reacts with oxygen to form trichloromethylperoxyl radical ($CCl_3O_2^{\bullet}$), $CCl_3O_2^{\bullet}$ bonds covalently to cellular macromolecules and causes peroxidative degradation of lipid membrane of the adipose tissue. Several antioxidants have been shown to protect the liver against hepatotoxicants [29] and considering the mechanisms of the CCl_4 to mice, the observed hepatoprotective effect of HEP could be partly due to its potent antioxidant capacity.

4. Conclusion

In the present study, three fractions of polysaccharides were obtained by the fractional precipitation with gradient concentrations of ethanol (40%, 60% and 80%) from intracellular polysaccharides from the mycelium of *H. erinaceus* grown on tofu whey. The chemical and physical characteristics of the three crude polysaccharides were investigated by the combination of chemical and instrumental analysis methods. The *in vitro* studies to evaluate the antioxidant potential of these polysaccharides and the hepatoprotective effects of the three polysaccharides showed that the three polysaccharide fractions had different activities in different evaluation system. The HEP80 showed strong activity on antioxidant *in vitro* and potent hepatoprotective effect *in vivo*. The hepatoprotective effect may be due to its potent antioxidant capacity. The HEP could be exploited as antioxidant product and a supplement in the prevention of hepatic diseases.

Acknowledgments

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