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Antioxidant and antihyperlipidemic activities of polysaccharides from sea cucumber *Apostichopus japonicus*

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ABSTRACT

Polysaccharides (AJP) were prepared from *Apostichopus japonicus* by protease hydrolysis method. Antioxidant activity *in vitro* and antihyperlipidemic activity *in vivo* was investigated. Chemical composition analysis indicated that AJP was mainly composed of glucosamine, galactosamine, glucuronic acid, mannose, glucose, galactose and fucose, with an average molecular weight of about 36.2 kDa. The antioxidant capacities of AJP were, respectively, evaluated by the assays of scavenging DPPH, hydroxyl and superoxide radicals, and reducing power *in vitro*. It showed potent free radical scavenging activities and reducing power. Serum total cholesterol (TC), triglyceride (TG) and low-density lipoprotein (LDL-C) decreased significantly and high-density lipoprotein cholesterol (HDL-C) increased significantly after treatment of hyperlipidemic Wistar rats with AJP. These results suggest that AJP may prove to be a potential candidate of the natural antioxidants as a therapeutic agent for hyperlipidemia.

1. Introduction

Hyperlipidemia has been incriminated as a contributory factor of atherosclerosis. Many lipids are believed to be associated with obesity, heart disease, stroke and kidney failure. Epidemiological studies have demonstrated strong causal relations between lipid parameters level and hyperlipidemia (Brown, 1994). Decreased serum high density lipoprotein cholesterol (HDL-C) and increased low density lipoprotein cholesterol (LDL-C) and triglyceride (TG) are considered to be significant risk factors for hyperlipidemia (Elahi, Kong, & Matata, 2009). LDL-C is the major carriers of cholesterol toward tissues having atherogenic potential, while HDL-C carries cholesterol from peripheral tissues to the liver (Kitamura, Iso, & Naito, 1994). HDL-C therefore gives protection against many cardiac problems and obesity. In addition, atherosclerosis is accompanied with the production of free radicals by endothelial and vascular smooth muscles. These free radicals initiate processes involved in atherogenesis through several important enzyme systems, including xanthine oxidase, nicotinamide adenine dinucleotide phosphate, oxidases and nitric oxide synthase (Elahi et al., 2009). The hypercholesterolemic state leads to an increase in free radical production, thus elevating lipid peroxides. Oxidized lipids can elicit a wide variety of biological responses that could contribute to atherosclerotic lesion development (Ma, Liu, Yu, Chen, & Zhang, 2009). The conventional therapeutic modalities available for hyperlipidemia mainly include lipid lowering drugs like atorvastatin, lovastatin and fibrates. Although effective, these synthetic drugs cause adverse effects (Knopp, 1999). Therefore, much interest has focused on biologically active components from natural materials to reduce the adverse effects of these drugs without affecting the physiological functions. In recent years, many polysaccharides with antihyperlipidemic activity have been discovered in various food materials, such as *Ulva pertusa* (Qi et al., 2012), *Ulva lactuca* (Sathivel, Raghavendran, Srinivasan, & Devaki, 2008), chitosan and chitosan derivatives (Muzzarelli & Muzzarelli, 2006). Generally, natural polysaccharides from natural materials have antioxidant and antihyperlipidemic activities and can be developed as novel potential hypolipidemic agents.

Sea cucumber *Apostichopus japonicus* Selenka (also identified as *Stichopus japonicus* Selenka) is an important cultivated aquatic species in China and Japan. It is reported that polysaccharides isolated from *A. japonicus* have anticoagulant activity (Gao, Li, Peng, Wu, Ma, & Liu, 1996), antitumor activity, immunomodulating activity (Guo et al., 2009), osteoclastogenesis inhibitory activity (Kariya et al., 2004), and neurosphere formation enhancing activity (Zhang, Song, et al., 2010). Epidemiological and experimental

Abbreviations: AJP, polysaccharide from *Apostichopus japonicus*; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

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studies suggest that abnormal metabolism and oxidative stress may increase the risk of developing cardiovascular disease and atherosclerosis (Ma et al., 2009). In recent studies, antioxidant polysaccharides of marine animal origin have been found in abalone gonad (Zhu et al., 2010) and *Stichopus variegates* Semper (Yan, Li, & Yi, 2004), and polysaccharide derived from *U. pertusa* (Qi et al., 2012) and sea cucumber *Metriatyla scabra* (Liu, Ko, & Hu, 2002) have an antihyperlipidemic effect. We therefore hypothesized that combination of these antioxidant and antihyperlipidemic activities might have the potential to attenuate the progression of cardiovascular disease and atherosclerosis. Although some studies have been published about antioxidant and antihyperlipidemic activities of polysaccharides derived from marine organism, to the best of our knowledge, there have been scarce studies to investigate the antioxidant and antihyperlipidemic activities of polysaccharides from sea cucumber *A. japonicus*. This study was designed to evaluate the effect of *A. japonicus* polysaccharide on free radical scavenging activity *in vitro* and antihyperlipidemic activity *in vivo*.

2. Materials and methods

2.1. Materials and reagents

Sea cucumber *A. japonicus* was collected from marine aquaculture farm of Homey Group (Rongcheng City, Shandong Province, China) and immediately stored at −20 °C for later use. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), standard monosaccharides, trichloroacetic acid and standard dextrans (molecular weight: 788, 404, 212, 112, 47.0, 11.8 and 5.9 kDa) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Other reagents used in this study were of the highest quality available from commercial vendors.

2.2. Animals

Male Wistar strain rats were purchased from the Center for New Drugs Evaluation of Shandong University (Certificate No. SKXK. 20030004, Jinan, China) and acclimatized for one week before experiment. Rodent laboratory chow and tap water were provided *ad libitum* and maintained under controlled conditions with a temperature of 24 ± 1 °C, humidity of 50 ± 10%, and a 12/12 h light/dark cycle. All the procedures were in strict accordance with the PR China legislation on the use and care of laboratory animals and with the guidelines established by the Institute for Experimental Animals of Shandong University, and were approved by the University Committee for Animal Experiments.

2.3. Preparation of polysaccharides

Polysaccharides of *A. japonicus* (AJP) were prepared according to the method described by Sheng et al. (2007) with some modifications. *A. japonicus* was washed with water for three times to remove other impurities, and cut into small pieces. The clean samples were immersed immediately in acetone and kept for 24 h at 4 °C. The drying sample was dissolved in 30 times (v/w) of 0.1 M sodium acetate buffer, pH 6.0, containing 5% papain, 5 ml 5 mM EDTA and 5 mM cysteine, and incubated at 60 °C for 24 h. The mixture was centrifuged (2000 × *g* at 4 °C for 15 min), and the clear supernatant was added with 5% cetylpyridinium chloride. After standing at room temperature for 24 h, the precipitate was collected by centrifugation (2000 × *g* at 4 °C for 15 min), and dissolved in the solution of NaCl:ethanol (10:1, v/v). After mixing with 4 times of 95% ethanol, the mixture was kept at 4 °C for 24 h. The precipitate was collected by centrifugation (2000 × *g* at 4 °C for 15 min), and dissolved in distilled water, and then desalted with 1000 Da cut-off membrane. The supernatant was gathered, condensed and freeze-dried to obtain polysaccharide of *A. japonicus* (AJP).

2.4. Component analysis

Total sugar content was determined by the phenol–sulfuric acid method using mannose as the standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Protein content was measured by the method of Bradford (1976). Sulfate ester content was estimated according to the previous literature (Therho & Hartiala, 1971). Uronic acid content was determined by the carbazole–sulfuric acid method (Bitter & Muir, 1962).

The molecular weight of AJP was measured by high performance gel permeation chromatography (HPGPC) on a Waters HPLC system (717 Plus sample injector, 1525 pump) equipped with a TSK gel G-3000 PW_{XL} column calibration (7.8 mm × 30.0 cm) by eluting with 0.2 mol/l Na_2SO_4. The eluate was monitored by a refractive index detector (Waters 2414 refractometer) column calibration was performed with standard dextrans (six different molecular weights: 788, 404, 212, 112, 47.0, 11.8 and 5.9 kDa). The calibration curve of Log MW (molecular weight) of standard dextrans against their elution time (ET) was obtained (Log MW = −0.5142ET + 12.6830, R = 0.9997) (Chen et al., 2011).

2.5. Analysis of monosaccharide composition

The method for monosaccharide composition analysis by reversed-phase, high performance liquid chromatography after pre-column derivatization and UV detection has been used (Chen et al., 2011). 5 mg AJP was hydrolyzed with 2 mol/l trifluoroacetic acid at 100 °C for 6 h. Excess acid was removed by co-distillation with methanol after the hydrolysis was completed. 100 mg dry hydrolysate was dissolved in 100 μl 0.3 mol/l NaOH, and added to 120 μl 0.5 mol/l methanol solution of 1-phenyl-3-methyl-5-pyrazolone (PMP) at 70 °C for 1 h. The mixture was added to 100 μl 0.3 mol/l HCl, vigorously shaken, and centrifuged at 2400 × *g* for 5 min. The supernatant containing the labeled carbonhydrate was filtered through 0.22 μm nylon membranes (MSI, Westborough, MA, USA) and 10 μl of the filtrate was injected into the C_{18} column. The mobile phase was a mixture of 0.1 mol/l KH_2PO_4 (pH 10)–acetonitrile (83:17). The flow rate was 1.0 ml/min and column temperature was 30 °C. Sugar identification was done by comparison with reference sugars (D-mannose, N-acetyl-D-glucosamine, D-glucuronic acid, D-galacturonic acid, N-acetyl-D-galactosamine, D-glucose, D-galactose, D-xylose, L-arabinose and L-fucose).

2.6. DPPH radical scavenging activity

The antioxidant activity of the extracts was determined on the basis of the scavenging activity of the stable DPPH radical using the method in the literature (Sun et al., 2009). Samples with different concentrations (AJP: 1.0, 2.0, 3.0, 4.0, and 5.0 mg/ml) were prepared. A 1 ml sample of different concentrations was added to 4 ml of 0.004% methanol solution of DPPH. After 30-min incubation in the dark at room temperature, absorbance (A) was measured at 517 nm on a spectrophotometer (U-2000 HITACHI, Japan). The activity of scavenging the DPPH radical was calculated using the following equation:

$$E\% = \left[\frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100\right]$$

where A_{control} is the absorbance of the control reaction (containing all reagents except the sample) and A_{sample} is the absorbance in the presence of the sample. The effect of DPPH radical scavenging activity was expressed as EC_{50}: the amount of the sample needed to

inhibit DPPH radical concentration by 50%. BHT was used as positive control.

2.7. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured according to Fenton method described before (Zhong, Jin, Lai, Lin, & Jiang, 2010), with minor modifications. Samples of different concentrations (AJP: 1.0, 2.0, 3.0, 4.0, and 5.0 mg/ml) were prepared, and incubated with 9.0 mM $FeSO_4$ (1.0 ml), 0.3% H_2O_2 (1.0 ml) in 0.5 ml salicylic acid–ethanol solution (9.0 mM) for 30 min at 37 °C. Hydroxyl radical was detected by monitoring A at 510 nm. The total volume of the mixture in each tube was made up to 3 ml by adding the required amount of distilled water. The hydroxyl radical scavenging effect was calculated as follows:

$$E\% = \left[\frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100\right]$$

where A_{control} and A_{sample} represent the absorbance of blank control group and sample group under 510 nm. The effect of hydroxyl radical scavenging activity was expressed as EC_{50}: the amount of the sample needed to inhibit hydroxyl radical concentration by 50%. BHT was used as positive control.

2.8. Superoxide radical scavenging activity

Superoxide radical scavenging activity was determined according to the previous study (Zhong et al., 2010). AJP was dissolved in distilled water at 1.0, 2.0, 3.0, 4.0, and 5.0 mg/ml. The sample solution (1 ml) was mixed with 2 ml 0.05 M Tris–HCl buffer (pH 8.2) and incubated at 25 °C in a water bath for 20 min. Then 1,2,3-phentriol (0.4 ml, 5 mM) was added, and the mixture was shaken rapidly at room temperature. The *A* value of the mixture was measured at 325 nm per 20 s against a blank. The scavenging ability for inhibition of pyrogallol autoxidation was calculated using the equation.

$$E\% = \left[\frac{(S_{\text{control}} - S_{\text{sample}})}{S_{\text{control}}} \times 100\right]$$

where S_{sample} represents the slope of sample group, S_{control} is the slope of blank control group, where the decrease of S_{sample} indicates an increase in the restraining power. The effect of superoxide anion radical scavenging activity was expressed as EC_{50}: the amount of the sample necessary to inhibit pyrogallol autoxidation by 50%. BHT was used as positive control.

2.9. Reducing power

Reducing power was determined referring to the method with some modifications (Sun et al., 2009). 0.5 ml sample solution at different concentrations (1.0, 2.0, 3.0, 4.0, and 5.0 mg/ml) was mixed with 2.5 ml of 0.2 mol/l phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. 2.5 ml of 10% trichloroacetic acid was added to the mixture which was then centrifuged at 6000 rpm for 10 min. 2.5 ml of upper layer solution was mixed with 2.5 ml of water and 1 ml of 0.1% $FeCl_3$, and the absorbance was measured at 700 nm. BHT was used as positive control. Higher absorbance of the reaction mixture indicated greater reductive potential.

2.10. Antihyperlipidemic activity in high-fat diet-induced hyperlipidemic rats

Male Albino rats of Wistar strain weighing 150–200 g were used in this study. The composition of the high-fat diets was as follows: 18% casein, 20% soybean oil, 4% salt mixture (2% cholesterol and 2% sodium cholate), 3% cellulose, 0.2% propylthiouracil and 55% cornstarch.

The method described by Qi et al. (2012) was employed in the study. Seventy-two animals were fed with a commercial chow for 7 days to acclimatize to animal facilities, and were weighed and equally divided into six groups, each group consisting of 12 animals. Group (1) was normal control; group (2) served as hyperlipidemic control; animals in groups (3)–(5) received AJP at a dose of 200, 400 and 800 mg/kg, respectively; and animals in group (6) were administered with the standard drug (atorvastatin, 10 mg/kg) as positive control. After the period of acclimation ended, animals in group (1) continued to receive the common commercial rat chow, and animals in other five groups were fed with a high-fat diet for 28 days. At the same time, groups (2)–(6) were given different doses of AJP and atorvastatin orally for 28 days. At the end of the experimental period, the rats were fasted for 18 h prior to blood withdrawal. The blood was collected from the eyeballs and centrifuged to separate serum for estimation of lipid profile. Serum total cholesterol (TC), TG, LDL-C and HDL-C levels were analyzed by enzymatic colorimetric method (Spectra MR, DYNEX Technologies, USA) using commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.11. Statistical analysis

The results were recorded as mean ± SD. Differences between the experimental groups were determined by the Students's *t*-test and *P*-values less than 0.05 were considered to be significant.

3. Results and discussion

3.1. Chemical analysis

As polysaccharides of animal origin contain high levels of proteins, it is usually difficult to analyze their chemical compositions. Enzyme technology is often used to deproteinate the extracts of animal polysaccharides. Papain is a thiol protease that has often been used in preparation of polysaccharides derived from various animals, such as abalone (Zhu et al., 2010), sea urchin (Liu et al., 2008) and sea cucumber (Zhang & Chen, 2009). In this study, an improved method combining papain-hydrolysis and ethanol precipitation was used to prepare AJP from *A. japonicus*. The chemical compositions of AJP are given in Table 1. AJP thus obtained contained 64.21% sugar and 10.03% protein. The sulfate content was 15.74% and the uronic acid content was 8.45%. The monosaccharide composition analysis of AJP is given in Fig. 1, HPLC analysis demonstrated that AJP mainly consisted of glucosamine, galactosamine, glucuronic acid, mannose, glucose, galactose and fucose. The monosaccharide content is a little different from that reported in the previous study (Sheng et al., 2007), probably due to different collection places. As shown in Fig. 2, the chemical analysis indicated that AJP was heterogeneous, and the molecular weight was estimated as about 36.2 kDa.

Table 1
Chemical compositions of AJP.

Component	AJP
Sugar content (%)	64.21
Protein content (%)	10.03
Sulfate content (%)	15.74
Uronic content (%)	8.45

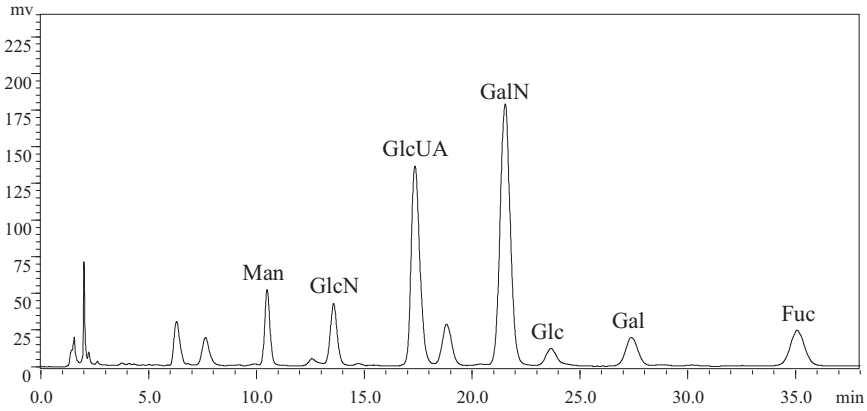

Fig. 1. HPLC analysis of monosaccharide composition in AJP (Man, mannose; GlcN, glucosamine; GlcUA, glucuronic acid; GalN, galactosamine; Glc, glucose; Gal, galactose; Fuc, fucose).

3.2. DPPH radical scavenging activity

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging activities of different materials. The scavenging ability of AJP on DPPH radical is listed in Fig. 3A. The scavenging ability of AJP on DPPH radical was 19.57% at 1 mg/ml, and increased to 69.58% at 5 mg/ml in a concentration-dependent manner. EC_{50} of AJP and BHT was similar (3.11 mg/ml and 2.51 mg/ml).

3.3. Hydroxyl radical scavenging activity

Hydroxyl radical is the most harmful free radical, mainly responsible for oxidative injury to biomolecules generated by reaction of Fe (II) complex with H_2O_2 in the presence of acid. The results of hydroxyl radical scavenging activities of AJP and BHT are given in Fig. 3B. All the samples exhibited obvious scavenging activity on hydroxyl radical in a concentration-dependent manner. EC_{50} of AJP and BHT was 1.54 mg/ml and 1.47 mg/ml, respectively. The present results proved that AJP was a good scavenger for hydroxyl radical.

3.4. Superoxide radical scavenging activity

In cellular oxidation reactions, superoxide radical is normally formed first, and its effects can be magnified because it produces other types of cell-damaging free radicals and oxidizing agents. Superoxide radical can be generated by pyrogallol autoxidation and it can produce a colored compound. As shown in Fig. 3C, the scavenging activity of AJP increased with the increase of concentration, and BHT displayed higher scavenging ability. EC_{50} of AJP and BHT was 4.05 mg/ml and 1.73 mg/ml, respectively. Although the activity of AJP was weaker than that of BHT, it can scavenge superoxide anion well in higher concentrations.

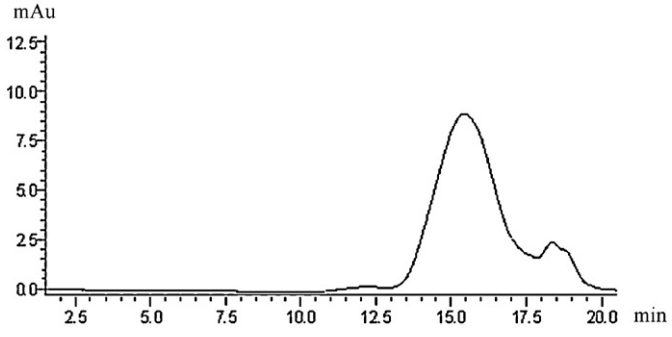

Fig. 2. HPGPC chromatogram of AJP on TSKgel G3000PW$_{XL}$ column.

3.5. Reducing power

The reducing power of a compound may serve as an indicator of its potential antioxidant activity. Higher absorbance value means stronger reducing power. Fig. 3D shows the reducing power of AJP and BHT using the $K_3Fe(CN)_6$ reduction method. The reducing power of AJP increased with the increase of sample concentration. At the concentration of 1.0 mg/ml, reducing power of AJP was close to that of BHT. The present results revealed that AJP was effective in reducing power.

Free radicals produced in the human body are regarded as a result of aerobic metabolism. Many diseases are associated with free radical damage. Excessive free radicals in the body can cause oxidative damage to some organs. It is widely accepted that natural polysaccharide extracts from marine plants and fungi possess free radical scavenging activities (Ananthi et al., 2010; Sun et al., 2009; Wijesekara, Pangestuti, & Kim, 2011; Zhang, Wang, et al., 2010). However, there have been few studies reporting antioxidant polysaccharide of marine animal origin. The antioxidant of AJP appears to be similar with that of polysaccharides produced by abalone (Zhu et al., 2010), and is higher than that of other marine fungi (Chen et al., 2011; Sun et al., 2009). The antioxidant activity of polysaccharides has been reported to be correlated with their structural features such as the degree of sulfation, molecular weight, and the type of the major sugar and glycosidic branching (Zhang, Wang, & Dong, 2011). The mechanism may be due to the supply of hydrogen. Free radicals may abstract anomeric hydrogen from carbohydrates and combine them to form neutral molecules (Zhu et al., 2010). From the results it may be considered that AJP reduced the free radical to the corresponding hydrazine on reacting with hydrogen donors. Although, there is no strict correlation between the degree of sulfation and the radical scavenging capacity, sulfation is an important factor influencing free radical scavenging activity of polysaccharides. The free radical quenching potential of AJP may be due to the presence of sulfate group. In summary, the antioxidant activity of polysaccharide was not a function of a single factor but a combination of several factors. The present investigation suggested that AJP could help the human body alleviating oxidative damages induced by oxygen radicals and decelerate the progression of many chronic diseases. It could be used as an effective potential antioxidant in the form of a food supplement or ingredient in the food industry.

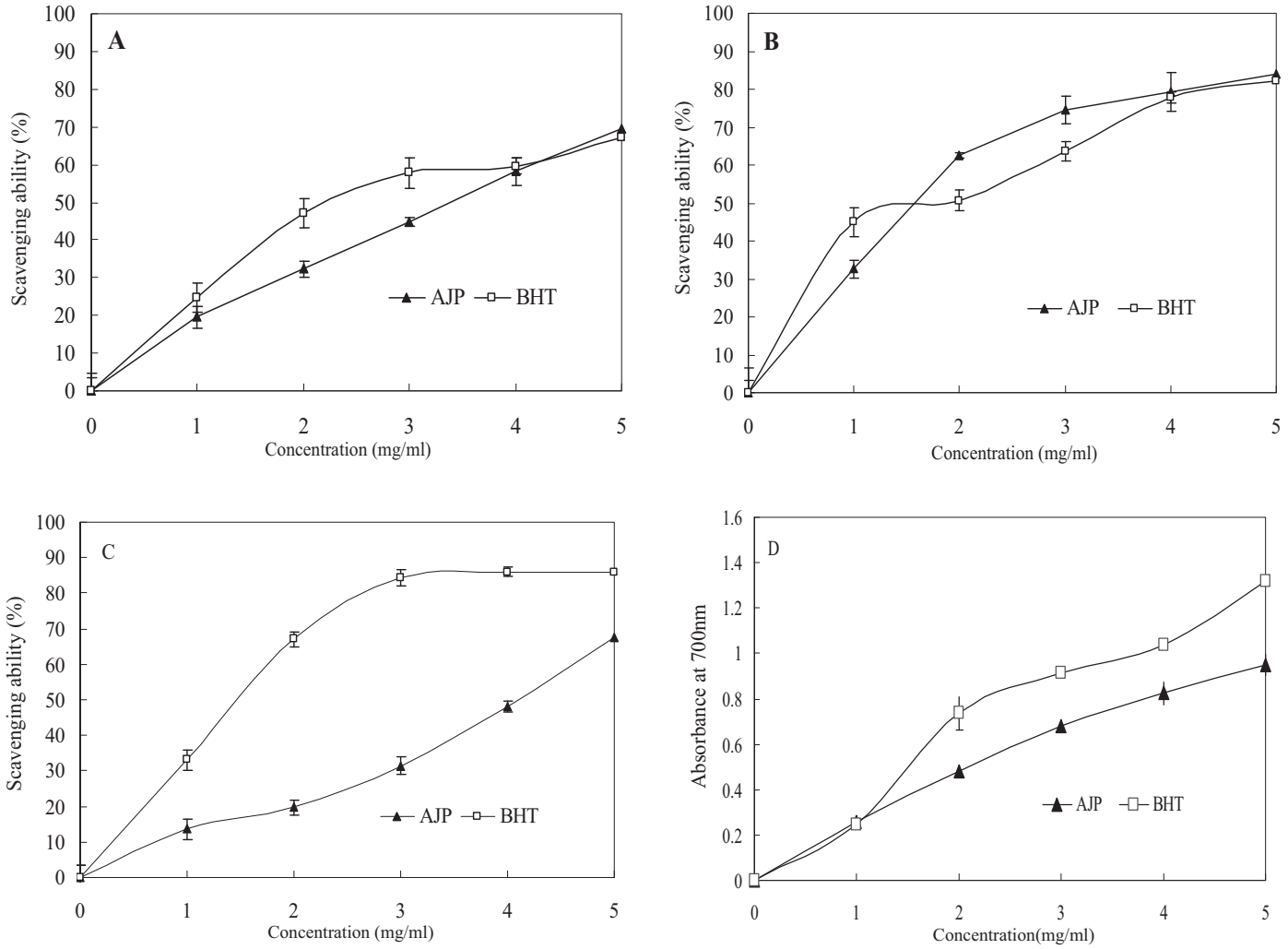

Fig. 3. Antioxidant activities of AJP. (A) Scavenging of DPPH radical, (B) scavenging of hydroxyl radical, (C) scavenging of superoxide radical and (D) reducing power. Values were representative of three separated experiments.

3.6. Antihyperlipidemic activity in high-fat diet-induced hyperlipidemic rats

To the best of knowledge, this is the first study investigating the effect of oral AJP in the lipid profiles in high-fat diet-induced rats. As shown in Table 2, serum TC, TG and LDL-C levels increased significantly and serum HDL-C level decreased significantly in hyperlipidemic group (2) as compared with those in the normal control group (1). The results indicated that the high-dose (800 mg/kg) group (5) had an optimal effect on TC and LDL-C, but a less significant effect on TG and HDL-C. Although AJP was found to significantly decrease TC and LDL-C levels at a dose of 200 mg/kg, it did not show significant effect on TG compared with the hyperlipidemic group (2). More importantly, the antihyperlipidemic activity the strongest in group (4) of 400 mg/kg compared with the hyperlipidemic group (2), where TC, TG and LDL-C concentrations decreased significantly by 17.23%, 20.78% and 31.18%, respectively. As compared with positive control group (6), AJP-fed groups showed lesser antihyperlipidemic activity, though the difference was not statistically significant. Interestingly, HDL-C level was significantly increased in AJP-fed groups after 28-day administration as compared with the hyperlipidemic group (2). However, atorvastatin did not show significant effects on HDL-C level.

Hyperlipidemia is known to be the leading risk factor for atherosclerosis. Abnormal increases in serum TC, TG and LDL-C levels are among the indicators of developing of atherosclerosis, while elevated serum HDL-C is known to be protective against the development of atherosclerosis (Qi et al., 2012). In addition, high TC, TG and LDL-C levels are known to increase blood viscosity. LDL-C is the main carrier of TC. Excessive LDL-C can migrate to the endarterium (Kitamura et al., 1994). The atherosclerotic pathologic process could be slowed down or reversed by reducing serum LDL, TG and phospholipids and increasing serum HDL. It was found that in the present study that AJP significantly decreased TC, TG and LDL-C levels and increased HDL-C level. This is an important advantage in the prevention and treatment of hypercholesterolemia particularly in Chinese populations whose hypercholesterolemia most prevalently presents as lipoprotein abnormality (Hu, Yang, & Tong, 2005). The mechanism of this effect remains unknown, but the elevated TC and LDL-C are considered to be related with biosynthesis and uptake of cholesterol (Ren, Noda, Amano, Nishino, & Nisizawa, 1994). AJP may combine with lipids and act as a carrier to participate in the metabolism of cholesterol, accelerating transport and excretion of serum lipids (Wang, Li, Niu, Zhang, & Zhang, 2003). According to the previous report, there is also another type of antihyperlipidemic mechanism: bile acid sequestrant mechanism (Qi et al., 2012). Polysaccharides can act as stimulators of bile acid synthesis. Most bile acids are reabsorbed in the small intestine and return to the liver so that the bile acid pool remains essentially constant. Bile acid sequestering resins act in the small intestine by

Table 2
Effect of AJP on serum lipid profile (TC, TG, HDL-C and LDL-C) in high-fat diet-induced hyperlipidemic rats.

Group		Dose (mg/kg)	TC (mmol/l)	TG (mmol/l)	HDL-C (mmol/l)	LDL-C (mmol/l)
1	Normal control	–	1.74 ± 0.22	0.49 ± 0.05	1.51 ± 0.21	1.07 ± 0.28
2	Hyperlipidemic	–	2.38 ± 0.23[d]	0.77 ± 0.31[d]	1.21 ± 0.26[c]	1.86 ± 0.34[d]
3	AJP	200	2.18 ± 0.22[a]	0.74 ± 0.19	1.43 ± 0.21[a]	1.47 ± 0.42[a]
4		400	1.97 ± 0.33[b]	0.61 ± 0.33[a]	1.54 ± 0.25[a]	1.28 ± 0.21[b]
5		800	2.01 ± 0.31[b]	0.62 ± 0.16[a]	1.35 ± 0.21[a]	1.25 ± 0.27[b]
6	Positive control	10	1.87 ± 0.25[b]	0.51 ± 0.16[b]	1.24 ± 0.33	1.15 ± 0.18[b]

The data represents the mean ± SD, $n = 12$ for each group.
[a] $P < 0.05$ compared with hyperlipidemic control group.
[b] $P < 0.01$ compared with hyperlipidemic control group.
[c] $P < 0.05$ compared with normal control group.
[d] $P < 0.01$ compared with normal control group.

interrupting the enterohepatic circulation and increasing the fecal excretion of bile acids so that fewer bile acids return to the liver. This increases the synthesis of bile acids, and the loss of bile acids is compensated for by oxidation of more hepatic cholesterol, the only precursor to bile acids, thereby decreasing the total blood cholesterol levels (Qi et al., 2012). On the other hand, oxidative stress plays a major role in the process of endothelial damage and atherosclerosis. ROS can oxygenate and modify LDL-C to form oxidized LDL-C, leading to the accumulation of cholesterol in phagocytes and to the formation of foam cells, promoting the development of atherosclerosis. There is also increasing evidence to support the idea that the antioxidant activity of polysaccharides such as *Pholiota nameko* polysaccharide and *U. lactuca* polysaccharide play a very important role in the treatment of hyperlipidemia in rats (Li, Zhang, & Ma, 2010; Sathivel et al., 2008). Therefore we deduced that the significant free radical scavenging activity might be the effective way of antihyperlipidemic effect of AJP.

4. Conclusions

In conclusion, it was found in our study that AJP prepared by the protease hydrolysis method mainly consisted of glucosamine, galactosamine, glucuronic acid, mannose, glucose, galactose and fucose, with a mean molecular weight of 36.2 kDa. AJP showed notable free radical scavenging activity and reducing power *in vitro*. Further, an oral dose, at 400 mg/kg day, resulted in significant declines in plasma TC, TG, and LDL-C by 17.23%, 20.78% and 31.18%, respectively, and increase in HDL-C level by 27.27%, compared to the hyperlipidemic group. However, the mechanism of AJP on antihyperlipidemic activity needs to be further studied. Combination of the antioxidant and antihyperlipidemic activities of AJP may prove to be a potential strategy to attenuate the progression of atherosclerosis.

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