Carbohydrate Polymers 88 (2012) 1453–1459

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Antioxidative and hepatoprotective effects of the polysaccharides from *Zizyphus jujube cv. Shaanbeitanzao*

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ARTICLE INFO

Article history:
Received 31 December 2011
Received in revised form 12 February 2012
Accepted 19 February 2012
Available online 25 February 2012

Keywords:
Zizyphus jujube cv. Shaanbeitanzao
Polysaccharides
HPLC
Antioxidant effect
Hepatoprotective activity

ABSTRACT

This study was designed to characterize the chemical composition, antioxidant activity and hepatoprotective effect of the polysaccharides from *Zizyphus jujube cv. Shaanbeitanzao* (ZSP). HPLC analysis showed that ZSP was the heteropolysaccharides with L-arabinose being the main component monosaccharide (50.2%, molar percentage). ZSP displayed strong antioxidant activity in vitro, and the effect was further verified by suppressing CCl_4-induced oxidative stress in liver at three tested doses of ZSP (100, 200, and 400 mg/kg BW) in mice. Administration of ZSP (400 mg/kg) significantly ($p < 0.01$) reduced the activities of CCl_4-elevated alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactic dehydrogenase (LDH) in serum, and hepatic malondialdehyde (MDA) level. Mice treated with ZSP showed a better profile of hepatosomatic index (HI) and antioxidant system with normal glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) activities in liver. These results suggest that ZSP exerts an effective protection against CCl_4-induced hepatic injury by mediating antioxidative and free radical scavenging activities.

1. Introduction

Liver disease is a serious health problem because the liver is an important organ for the biotransformation and detoxification of endogenous and exogenous harmful substances. More and more attention has been paid to liver disease just because of speeding up of the modern pace of life and changing cookbook in recent years (Cemek et al., 2010). It is well-known that free radicals cause cell damage through the mechanisms involving in lipid peroxidation with subsequent tissue injury, especially liver injury (Hsiao et al., 2003). Some antiviral drugs, which have been used to treat liver disease, were shown to have potential adverse effects, especially when administrated for long-term (Muriel & Rivera-Espinoza, 2008). For this reason, natural antioxidants have been a substantial increase as more effective and safe dietary ingredients for alternative therapies of liver disease (Yang, Li, Wang, & Wu, 2010). Therefore, it is of very important and high significant to find out new sources of safe and inexpensive antioxidants of natural origin, such as anthocyanins in color-fleshed potatoes (Lachman et al., 2009), tocotrienols and tocopherols in einkorn and spring wheat varieties (Hejtmánková, Lachman, Hejtmánková, Pivec, & Janovská, 2010) or carotenoids in tomato varieties (Kotíková, Lachman, Hejtmánková, & Hejtmánková, 2011).

Zizyphus jujube, described as the "fruit of life", is a key member of the Chinese herbs which are famous for their hepatoprotective effect. *Z. jujube* belongs to the Rhamnaceae family, and is widely distributed in the temperate and subtropical areas of the North Hemisphere, especially the inland region of North China (Zhang, Jiang, Ye, Ye, & Ren, 2010). *Shaanbeitanzao*, commonly known as a cultivar of *Z. jujube*, is grown in the northwestern part of China along with the Yellow River universally. Besides its edibleness and culinary uses, *Shaanbeitanzao* has also traditionally been used for medicinal purposes for more than 2000 years, where the fruit was made into paste, puree, syrup, and confection, and was consumed for digestion improvement and body maintenance (Huang, Yen, Sheu, & Chau, 2008). However, the active ingredients of *Shaanbeitanzao* responsible for hepatoprotective benefits are not fully clear.

In recent years, natural polysaccharides, which were found largely in fruits and vegetables, have been confirmed to play an important part as free radical scavengers in the prevention of oxidative damage in living organism and can be exploited as novel potential antioxidants, and the effects have something to do with their chemical properties and architectural characteristics (Li, Liu, Fan, Ai, & Shan, 2011). Therefore, discovery and assessment of natural polysaccharides as new safe compounds for functional foods or medicines have became a hot research field. In this regard, the anticancer and immunological effects of jujube polysaccharides

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doi:10.1016/j.carbpol.2012.02.046

have been investigated (Hung, Hsu, Chang, & Chen, 2012; Zhao et al., 2006). It is also of interest that the ethanol extract from jujube fruit showed protective effect against CCl_4-induced hepatic injury in mice by antioxidant mechanism (Shen et al., 2009). More recently, the water extract of jujube was also reported to ameliorate the liver injury induced by ischemia/reperfusion (Chen et al., 2010). Although *Shaanbeitanzao* is largely planted in China, up to now, there is no report available on the chemical composition, antioxidant and hepatoprotective effects of the polysaccharides isolated from it. Therefore, the aim of the present study was firstly to assess the chemical composition properties of ZSP, and its antioxidant activity in vitro. Furthermore, the protective effect of ZSP against CCl_4-induced hepatic damage in mice was also explored for seeking a new hepatoprotective function factor used in food and pharmaceutical industry.

2. Materials and methods

2.1. Materials and chemicals

Z. jujube cv. Shaanbeitanzao in packets (Barcode: 69-247450-44010) was purchased from Chang-An shop of Vanguard, which was harvested from the countryside region of Qingjian County (Northwest of China). D-mannose, D-ribose, L-rhamnose, D-glucuronic acid, D-galacturonic acid, D-glucose, D-xylose, D-galactose, L-arabinose, D-fucose, butylated hydroxytoluene, and ascorbic acid (Vit. C) were purchased from Sigma (St. Louis, USA). Potassium ferricyanide [$K_3Fe(CN)_6$] and trichloroacetic acid (TCA) were obtained from Sigma (Sigma Aldrich GmbH, Sternheim, Germany). 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), nitroblue tetrazolium (NBT), nicotinamide adenine dinucleotide (NADH), and phenazine methosulfate (PMS) were obtained from Applichem (Darmstadt, Germany). Biphenyldicarboxylate Pills (BP) and carbon tetrachloride (CCl_4) were obtained from Zhengjiang Wanbang Pharmaceutical Co. (Wenling, China) and Tianjin Tianli Chemical Reagent Co. (Tianjin, China), respectively. Test kits of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactic dehydrogenase (LDH), Malondialdehyde (MDA), glutathione peroxidase (GSH-P*x*) and superoxide dismutase (SOD) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Trifluoroacetic acid (TFA), triethylamine (TEA), and 1-phenyl-3-methyl-5-pyrazolone (PMP) were purchased from Merck (Darmstadt, Germany). HPLC grade acetonitrile and methanol were purchased from Honeywell (USA). Other chemicals used in the study were of analytic grade and commercially available.

2.2. Animals

Sixty kunming male mice (weight 18–22 g) were obtained from Experimental Animal Center of Fourth Military Medical University. They were allowed free access to tap water and rodent chow (40% corn flour, 26% wheat flour, 10% bran, 10% fish meal, 10% bean cake, 2% mineral, 1% coarse, and 1% vitamin complex, Qianmin Feed Factory). All the animals were housed under the standard conditions with 12/12 h light-dark cycle at a temperature of 22 ± 2 °C and a humidity of 60 ± 5%. All the experiments were performed in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of People's Republic of China and approved by the Fourth Military Medical University Committee on Animal Care and Use.

2.3. Isolation of the polysaccharides from Z. jujube cv. Shaanbeitanzao

100 g pulp stripped from *Z. jujube cv. Shaanbeitanzao* was dried for 10 min in a domestic microwave oven at 900 W output power, and crushed into powder and soaked in water (1:20, w/v) at 80 °C for 160 min. After three cycles, the incorporate extraction solution was filtrated and concentrated to 10% of the original volume with a rotary evaporator under reduced pressure, and then it was precipitated for three cycles by adding three times of volume of 95% (v/v) ethanol at 4 °C for 24 h. The refined pellets were completely dissolved in appropriate volume of water and intensively dialyzed for 4 days against water (cut-off M_w 8000 Da). The retentate portion was deproteinized by the freeze–thaw process for repeating 10 times followed by filtration. Finally, the extracts were centrifuged at 3000 r/min for 10 min to remove insoluble material and the supernatant was lyophilized in the freeze-dry apparatus to give the refined polysaccharide ZSP.

2.4. Analysis of chemical characterization of ZSP

Total carbohydrate content in ZSP was determined by phenol–sulfuric acid colorimetric method with glucose as a standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). According to the ordinary procedure, absorbance at 490 nm of five calibration solutions of glucose (5–60 μg/mL) was determined, and the standard curve was drawn with absorbance as ordinate and concentration (μg/mL) as abscissa, and the regression equation was obtained. The amount of carbohydrates present in ZSP is determined by comparison with a calibration curve using a spectrophotometer. In brief, 100 mg ZSP was put into volumetric flask (25 mL) to get stock solution once it was completely dissolved in ultrapure water, and the working solution was prepared by diluting the stock solution to the appropriate concentrations. 50 μL of working solution was spiked with 500 μL of 4% phenol, followed by 2.5 mL sulfuric acid. Under the catalysis of sulfuric acid, ZSP was converted to monosaccharides, then to derivants of pyromucic aldehyde immediately after dehydration. The derivants reacted with phenol can produce orange-yellow chemical compounds, and the carbohydrate content of ZSP can be obtained after the determination of absorbance of the orange-yellow chemical compounds.

Monosaccharide composition of ZSP was analyzed by HPLC as our previous procedures (Lv et al., 2009). ZSP sample (20 mg) together with 1.0 mg fucose as internal standard was hydrolyzed with 2 mL of 3 M TFA at 95 °C for 8 h in an ampoule (5 mL) sealed under a nitrogen atmosphere. The released monosaccharides were derivatized with PMP to gain strong UV (Lv et al., 2009). Briefly, the hydrolyzed ZSP solution (100 μL) was spiked with 0.3 M aqueous NaOH (300 μL), and 0.5 M methanol solution of PMP (200 μL). Each mixture was allowed to react for 60 min at 70 °C, and subsequently neutralized with 300 μL of 0.3 M HCl. The resulting solution was extracted with 1 mL chloroform, and the aqueous layer was filtered through a 0.45 μm membrane for HPLC analysis. The analysis of PMP-labeled monosaccharides was performed on a Shimadzu LC-2010A HPLC system. The analytical column used was a RP-C_{18} column (4.6 mm i.d. × 250 mm, 5 μm, Venusil, USA), and the separation was performed at 35 °C. The mobile phase A consisted of acetonitrile, and mobile phase B was 3.3 mM KH_2PO_4–3.9 mM triethylamine buffer containing 10% acetonitrile using a gradient elution of 93-93-91-91% B by a linear decrease from 0-7-9-30 min. Elution was carried out at a flow rate of 1.0 mL/min, and the wavelength for UV detection was 250 nm. The injection volume was 15 μL.

2.5. Evaluation of in vitro antioxidant activity of ZSP

2.5.1. Scavenging activity on DPPH radical

The assay was performed as described by Shimada, Fujikawa, Yahara, and Nakamura (1992). Briefly, 1.0 mL of ZSP solution at various concentrations (0–200 μg/mL) was mixed with 3.0 mL of 0.1 mM DPPH in aqueous methanol. Absorbance at 517 nm was

determined after 20 min, and the percentage of inhibition activity was calculated according to the following formula: scavenging activity against DPPH• (%) = $[1 - (A_s - A_b)/A_o] \times 100$, where A_o was the absorbance without sample, A_s was the absorbance with sample, and A_b was the absorbance of ground color.

2.5.2. Scavenging activity on hydroxyl radical

The HO•-scavenging activity of ZSP was measured by an improved Fenton-type reaction (Ghiselli, Nardini, Baldi, & Scaccini, 1998). In brief, 1.0 mL ZSP solution at various concentrations (0–200 μg/mL) in test tube (10 mL) was spiked with 1.0 mL $FeSO_4$, 1.0 mL salicylic acid–ethanol and 1.0 mL H_2O_2, and incubated in water-bath at 37 °C for 60 min, and then the absorbance at 510 nm was measured. The HO•-scavenging activity of ZSP was calculated according to the following formula: HO•-scavenging activity (%) = $[1 - (A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control (without ZSP) and A_1 is the absorbance of ZSP.

2.5.3. Scavenging activity on superoxide anion

In the PMS–NADH system (Chen, Tsai, Huang, & Chen, 2009), 1.0 mL NBT, 1.0 mL NADH, 1.0 mL ZSP and 0.4 mL PMS were successively added into test tube, then the reaction mixture was incubated at ambient temperature for 5 min and the absorbance of the mixture solution was determined at 560 nm with the visible spectrometer. $O_2^{\bullet -}$-scavenging capability of ZSP was calculated according to the following formula: suppression rate (%) = [1 − (Abs. of sample − Abs. of blank)/Abs. of control] × 100.

2.5.4. Measurement of reducing power

The reducing power of ZSP was measured according to our previous procedures (Tian, Zhao, Guo, & Yang, 2011). Briefly, 2.5 mL of pH 6.6 phosphate buffer and 2.5 mL of 1% $K_3Fe(CN)_6$ were added to 1.0 mL of the tested ZSP solution at various concentrations (0–200 μg/mL). The mixture was put into 50 °C water bath for 20 min when mixed uniformly, and then 2.5 mL 10% TCA was added to each mixed solution, followed by a mixture and further centrifuging at 3000 r/min for 10 min. The absorbance at 700 nm was measured immediately. An enhanced absorbance of the reaction mixture indicated a high reducing power.

2.6. CCl_4-induced hepatotoxicity experiment

After environmental adaptation for 3 days, mice were divided into six groups of 10 animals each at random. In the normal and CCl_4-intoxicated groups, animals were given a single dose of physiological saline (0.3 mL, ig) once daily. In the positive BP group, animals received 400 mg/kg BW reference drug BP (0.3 mL, ig) once daily. In the test groups, animals were given 100, 200, or 400 mg/kg BW of ZSP (0.3 mL, ig) once daily. All administrations were conducted at nine o'clock for 10 consecutive days. On the eleventh day, all the mice except the normal group were given a 0.8% CCl_4/peanut oil mixture (v/v, 0.3 mL, ip), while the normal group received peanut oil alone. After 2 h, all the animals were fasted but drink water ad libitum as usual for 48 h. After 2 days, body of each mouse was weighed and right eyeball was yanked to collect blood, then cervical vertebra was took off to put it to death mercifully and the corresponding liver was excised piteously, washed adequately by ice-cold physiologic saline and also weighed, respectively. The samples of blood were put into water bath at 37 °C for 30 min to promote them solidification, and centrifuged immediately at 2500 r/min for 20 min, and stored at 4 °C, while the livers were refrigerated at −80 °C. On the basis of the records of the body weight and corresponding liver weight of every mouse, we figured out the end results of hepatosomatic index (HI) according to the following formula: HI = liver weight/body weight × 100%.

2.7. Determination of ALT, AST and LDH activities in serum

Activities of ALT, AST and LDH were assayed by Reitman–Frankel method (Reitman & Frankel, 1957). The enzymatic activities of serum ALT, AST, and LDH as biochemical markers for acute liver injury were determined by visible spectrometer using commercially available diagnostic kits, and the results were expressed in IU/L, IU/L and U/L, respectively.

2.8. Measurement of SOD, GSH-Px, and MDA levels in the liver homogenate

It was well-known that hepatic levels of MDA, GSH-P*x*, and SOD were considered as common indexes of antioxidant status of tissues (Lian et al., 2010). In this study, the level of MDA, a measure of lipid peroxidation, was measured by the method of thibabituric acid (TBA) (Kim, Kim, Kim, Lee, & Lee, 2011), and GSH-P*x* and SOD activities were measured by the method of Moron et al. and McCord method, respectively (McCord, 1994; Moron, Depierre, & Mannervik, 1979). The assays were performed with commercially available diagnostic kits, and the results were expressed in nmol/mgprot, U/gprot, and U/mgprot, respectively. The protein concentration in homogenates was measured by the method of Coomassie brilliant blue (Wei, Li, & Tong, 1997). The liver tissue homogenate was prepared by automatic homogenate machine. During the preparation, 1 g of each liver tissue was homogenated in 9-times ice-cold physiologic saline in volume, and the resulting homogenate was centrifuged at 3000 r/min for 10 min to obtain the postnuclear supernatant when cut out.

2.9. Statistical analysis

All experiments were performed at least in triplicate and the results were expressed as means of ±SD (standard deviation). Data obtained were analyzed using one-way analysis of variance (ANOVA, $p < 0.05$, SPSS, version 16.0), and Duncan's multiple range test. p-Values of <0.05 were considered to be statistically significant.

3. Results and discussion

3.1. Physicochemical properties of ZSP

ZSP was isolated from Chinese *Z. Jujube cv. Shaanbeitanzao* using a multistep purification procedure including hot-water extraction and repeated ethanol precipitation. With this method, the extraction yield of ZSP can reach approximately 7.2% (w/w) of the dried pulp. The phenol–H_2SO_4 assay for total carbohydrate content showed that ZSP contained approximately 73.9% (w/w) of polysaccharides. The linearity of the method was assessed by preparing five calibration solutions of glucose ($n = 5$), respectively. As a consequence, a good linearity was obtained by regression analysis between A (absorbency) and C (glucose concentration, μg/mL) and regression equation was as follows: $A = 15.007C - 0.0317$ ($R^2 = 0.9884$) for glucose at the concentration of 5–60 μg/mL. In addition, ZSP was easily soluble in water but was not soluble in organic solvents such as ethanol, ether, acetone, or chloroform. There was no reaction of Folin–Ciocalteu reagent with ZSP, suggesting that no polyphenols existed in the polysaccharides, and the small molecular phenolic compounds in the *Shaanbeitanzao* pulp had been successfully removed via dialysis processing (cut-off $M_w > 10{,}000$ Da) against distilled water in the purification of the macromolecular ZSP. However, beneficial impact of oligo- and polysaccharides, e.g. oligofructans in yacon [*Smallanthus sonchifolius* (Poepp. et Endl.) H. Robinson] works together with antioxidant properties of phenolic constituents in many plants

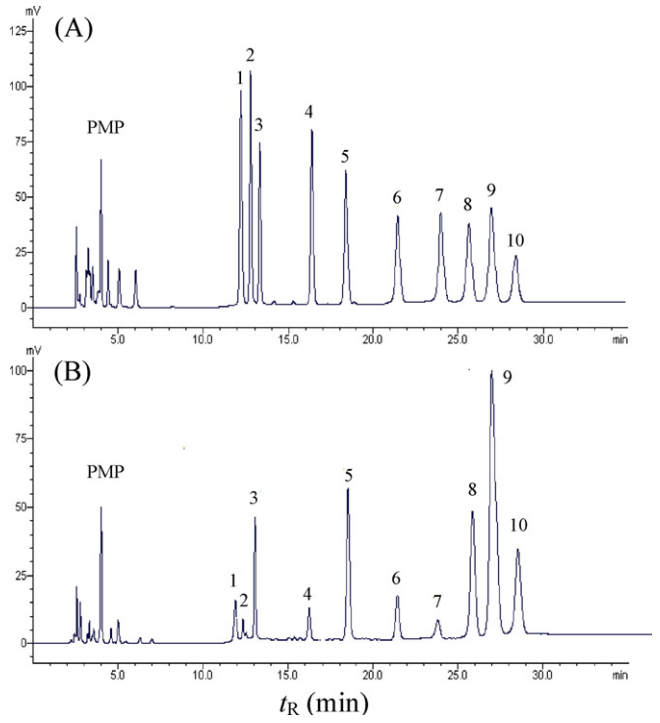

Fig. 1. The HPLC chromatograms of PMP derivatives of 10 standard monosaccharides (A) and component monosaccharides released from ZSP (B). Peaks: (1) mannose, (2) ribose, (3) rhamnose, (4) glucuronic acid, (5) galacturonic acid, (6) glucose, (7) xylose, (8) galactose, (9) arabinose, (10) fucose (internal standard).

(Lachman, Fernández, & Orsák, 2003). The negative reaction of ZSP with iodine–potassium iodide reagent indicated that ZSP was a non-starch polysaccharide.

To gain more composition information, ZSP was also subjected to further monosaccharide composition analysis by a validated HPLC technique in our previous work (Lv et al., 2009). Herein, 10 PMP-labeled standard monosaccharides were baseline separated fast within 30 min by the improved HPLC analysis (Fig. 1A), and the typical chromatogram of ZSP sample with fucose as the internal standard was shown in Fig. 1B. The results showed that ZSP was composed of D-mannose, D-ribose, L-rhamnose, D-glucuronic acid, D-galacturonic acid, D-glucose, D-xylose, D-galactose and L-arabinose in the molar percentages of 2.8%, 1.8%, 6.6%, 2.6%, 10.9%, 5.3%, 3.4%, 16.5%, and 50.2% (mol.%) of all the quantitative monosaccharides, respectively. This monosaccharide profile implied that the polysaccharides were mainly composed of neutral L-arabinose, followed by D-galactose, D-galacturonic acid, accounting for up to 77.6% in the relative molar distribution.

3.2. Antioxidant activity of ZSP

In this study, the free radical scavenging capacity of ZSP was estimated in DPPH•, HO•, and $O_2^{\bullet -}$ systems. As shown in Fig. 2A, ZSP dose-dependently displayed DPPH•-scavenging effects of 6.4%, 26.5%, 46.7%, 62.1%, 79.2% at the tested concentrations of 10, 50, 200, 100, 150, and 200 μg/mL, respectively. Similarly, ZSP at the concentrations of 10–200 μg/mL also exhibited the obvious scavenging activity (1.5%, 5.5%, 9.1%, 14.3% and 50.6%) against HO• in a concentration-dependent manner (Fig. 2B). As shown in Fig. 2C, the scavenging activity of ZSP on $O_2^{\bullet -}$ was 7.6%, 19.7%, 33.9%, 56.4%, and 92.1% at the tested concentrations (10–200 μg/mL), respectively. Although Vit. C as positive reference exerted stronger effects, the activities of ZSP were closed extremely to the authorized antioxidant at the same concentration, indicating that ZSP was a strong free radical-scavenging polysaccharide that could help prevent or ameliorate oxidative stress.

Table 1
Effects of ZSP on body weight, liver weight and hepatosomatic index (HI) after in CCl_4-treated mice.

Treatments	Body wt. (g)	Liver wt. (g)	HI (%)
Normal	29.28 ± 3.39	1.45 ± 0.18	5.00 ± 0.73
CCl_4	31.55 ± 1.05#	1.88 ± 0.15#	5.95 ± 0.54#
CCl_4 + ZSP			
100 mg/kg	30.69 ± 2.15	1.73 ± 0.21	5.64 ± 0.55
200 mg/kg	30.12 ± 1.10	1.66 ± 0.23*	5.51 ± 0.79*
400 mg/kg	30.42 ± 1.27	1.57 ± 0.18**	5.16 ± 0.41**
BP 400 mg/kg	29.76 ± 2.61	1.52 ± 0.15**	5.12 ± 0.35**

Values are means ± SD for 10 mice in each group.
# $p < 0.05$, compared to normal group.
* $p < 0.05$, compared to CCl_4-intoxicated group.
** $p < 0.01$, compared to CCl_4-intoxicated group.

Furthermore, the antioxidant capacity of ZSP was assessed by means of reducing power. As expected, the presence of ZSP as reductant caused the reduction of the $Fe^{3+}/K_3Fe(CN)_6$ complex to Fe^{2+}, and consequently, the Fe^{2+} could be monitored by measurement of the enhanced formation of Perl's Prussian blue at 700 nm (Tian et al., 2011). In the broad range of 10–200 μg/mL, the phenomenon of concentration-dependence was still obvious (Fig. 2D), and the reducing power (increased absorbance at 700 nm) of ZSP ranged from 0.07 to 0.55 in a concentration-dependent manner, where the effect of ZSP was slight high than that of Vit. C at the same concentration (Fig. 2D). This test further indicated that ZSP possessed a definite antioxidant activity.

3.3. Effects of ZSP administration on body weight, liver weight and HI in mice

Body weight, liver weight and HI in the experimental mice were shown in Table 1. In CCl_4-intoxicated mice, body weight, liver weight and HI increased significantly, compared to the normal group ($p < 0.05$). As shown in Table 1, the pretreatment with ZSP at the high dosages of 200 and 400 mg/kg BW resulted in significant decreases in liver weight and HI relative to CCl_4 group ($p < 0.05$, $p < 0.01$), respectively. However, a slight decrease in the body weight was observed following by the treatment of ZSP but without statistically difference from CCl_4-intoxicated mice ($p > 0.05$). A similar effect was observed with the pretreatment with positive BP medicine (Table 1). These results are in agreement with the previous report (Hou, Qin, & Ren, 2010). It is widely recognized that the loss of body weight may be associated with food intake and malabsorption after intake of CCl_4 (Lieber, 1994), and the deep mechanisms were still left for further study.

3.4. Effects of ZSP on ALT, AST and LDH activities in serum

Fig. 3 shows the acute hepatotoxicity induced by CCl_4 in mice, as indicated by the significant increase in serum activities of ALT and AST from 26.0 ± 7.7 to 25.0 ± 6.5 IU/L in untreated normal group to 46.6 ± 9.0 and 59.3 ± 11.9 IU/L in CCl_4 group ($p < 0.01$), respectively. As shown in Fig. 4A, the CCl_4-induced hepatotoxicity was further confirmed by the increased activity of LDH from 166.0 ± 46.5 U/L to 265.6 ± 44.4 U/L ($p < 0.01$). Interestingly, the pretreatment of ZSP considerably lowered the level of the CCl_4-elevated biochemical enzyme markers, especially when the dosage increased to 200 and 400 mg/kg BW ($p < 0.01$). At a dosage of 200 mg/kg BW, the activities of ALT, AST and LDH decreased to 33.6 ± 3.7 IU/L, 43.7 ± 12.0 IU/L and 222.4 ± 49.5 U/L, and at 400 mg/kg BW, the corresponding data were 30.2 ± 5.6 IU/L, 36.9 ± 9.6 IU/L, and 183.2 ± 20.3 U/L, which were close to that of the positive agent BP (400 mg/kg BW), where the values were 26.8 ± 6.2 IU/L, 25.0 ± 6.1 IU/L and 157.2 ± 49.2 U/L.

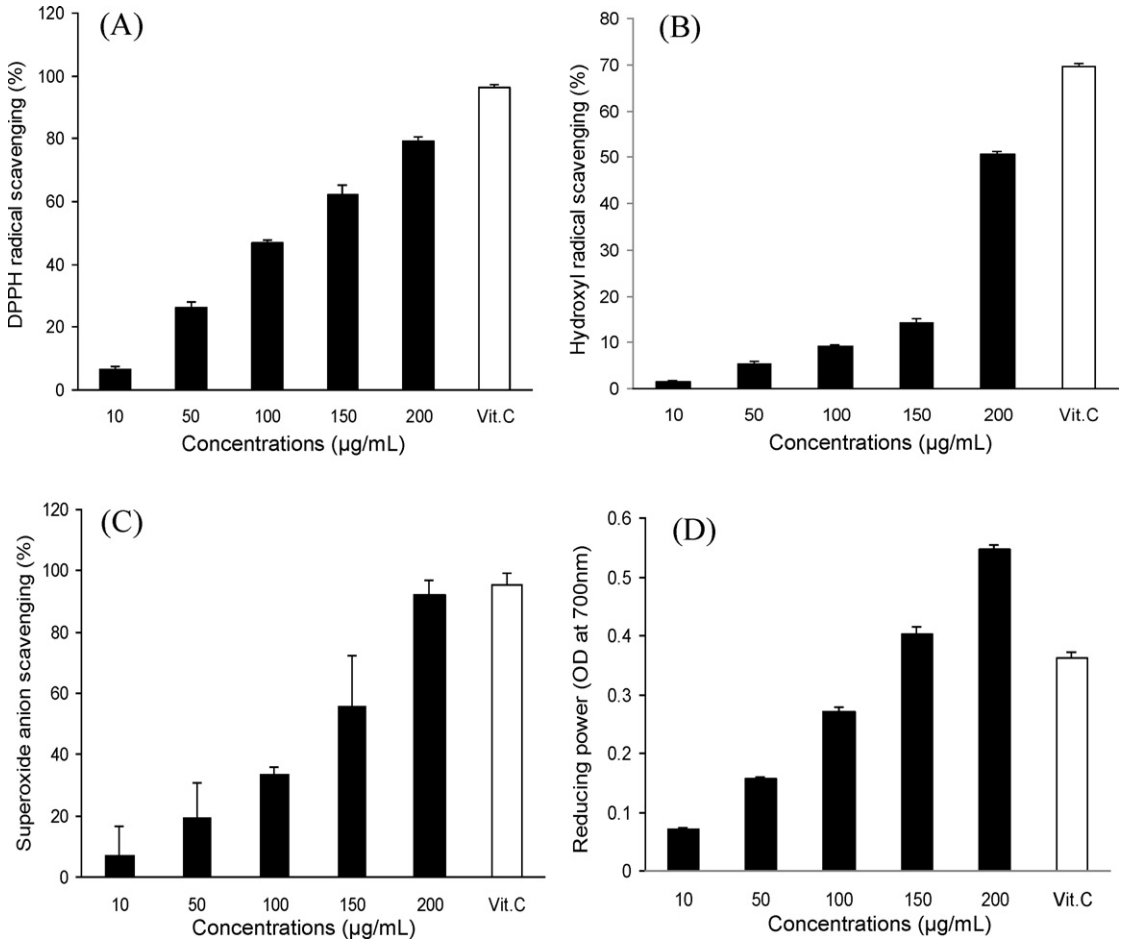

Fig. 2. In vitro antioxidant effects of ZSP at 10, 50, 100, 150, and 200 μg/mL, and positive Vit. C. (A) DPPH•-scavenging activity of various concentrations of ZSP and Vit. C (50 μg/mL). (B) HO•-scavenging effect of ZSP and Vit. C (50 μg/mL). (C) $O_2^{\bullet -}$-scavenging capacity of ZSP and Vit. C (150 μg/mL). (D) Reducing power of ZSP and Vit. C (150 μg/mL).

However, at low dose of 100 mg/kg BW, although the activities of ALT, AST and LDH dropped slightly, there was no statistical significance ($p > 0.05$).

It is well known that ALT, AST and LDH are important indicators of liver injury (Yang, Dong, & Ren, 2011). After injection of CCl_4, reductive dehalogenation catalyzed by reduced cytochrome P450 of CCl_4 forms a highly reactive trichloromethyl free radical ($^{\bullet}CCl_3$), and then forms the precursor of lipid peroxidation as the trichloromethyl peroxy radical ($CCl_3OO^{\bullet}$) (MacCay, Lai, Poyer, Dubose, & Janzen, 1984). The peroxy radical caused lipid peroxidation of membranes and consequent liver damage, resulting in leakages of ALT, AST, and LDH from the cells (Jayakumar, Ramesh,

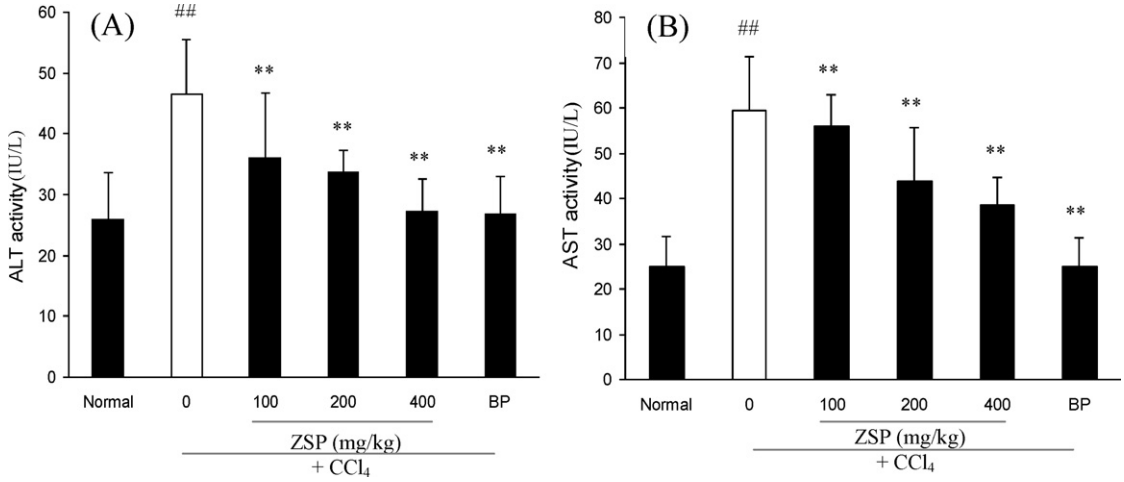

Fig. 3. Effects of ZSP on serum ALT and AST activities after administration of CCl_4 in mice. Animals were given either ZSP (100, 200, or 400 mg/kg, ig) or Biphenyldicarboxylate Pills (BP) (400 mg/kg) once daily for 10 successive days ahead of the simplex treatment of CCl_4 (0.1%, ip), and then serum ALT (A) and AST (B) were determined. Values are expressed as means ± SD for 10 mice in each group. Compared to normal group, $^{\#\#}p < 0.01$. Compared to the CCl_4-intoxicated group, $^{**}p < 0.01$.

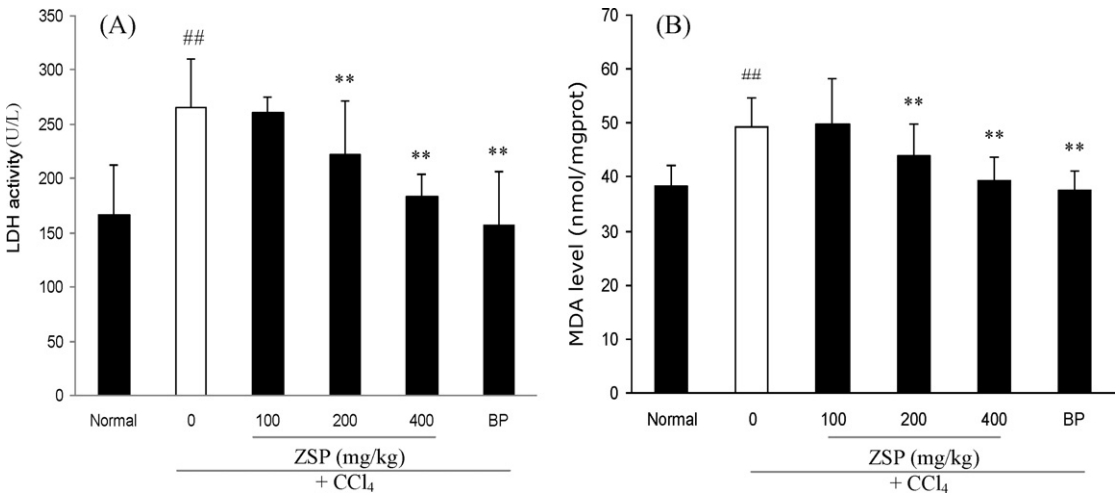

Fig. 4. Effects of ZSP on serum LDH activity and hepatic MDA level after CCl_4 administration in mice. Animals were given either ZSP (100, 200, or 400 mg/kg, ig) or BP (400 mg/kg) once daily for 10 successive days ahead of the simplex treatment of CCl_4 (0.1%, ip), and then serum LDH (A) and hepatic MDA (B) were determined. Values are expressed as means ± SD for 10 mice in each group. $^{\#\#}p < 0.01$, compared to normal group. $^{**}p < 0.01$, compared to CCl_4 group.

& Geraldine, 2006). In this study, a significant increase in the level of ALT, AST and LDH in the serum was observed after administration of CCl_4 as reported previously (Jayakumar et al., 2006; MacCay et al., 1984). However, the increased levels of these enzymes were significantly decreased by pretreatment with ZSP, implying that ZSP prevents liver damage via inhibiting lipid peroxidation in vivo.

3.5. Effects of ZSP on the levels of MDA, GSH-Px and SOD in hepatic tissue

The hepatic levels of MDA, GSH-P*x* and SOD in animal experiment were displayed in Figs. 4B and 5. Prominent elevation of MDA level and significant reduction of GSH-P*x* and SOD in CCl_4-intoxicated group were clearly observed in comparison with untreated normal group ($p < 0.01$). Interestingly, there were significant reduction of MDA content and conspicuous increases of GSH-P*x* and SOD levels after the administration of ZSP at dosage of 200 mg/kg BW, from 49.4 ± 5.4 nmol/mgprot, 251.6 ± 84.9 U/gprot and 66.1 ± 10.4 U/mgprot of CCl_4-intoxicated group to 43.8 ± 6.0 nmol/mgprot, 455.0 ± 85.8 U/gprot, and 127.2 ± 36.5 U/mgprot ($p < 0.01$). At the dosage of 400 mg/kg BW, the values were 39.4 ± 4.2 nmol/mgprot, 532.2 ± 150.6 U/gprot and 192.5 ± 33.2 U/mgprot, which were close awfully to 37.6 ± 3.7 nmol/mgprot, 581.7 ± 116.6 U/gprot and 203.7 ± 33.2 U/mgprot of normal group ($p > 0.05$), and the values (37.6 ± 3.7 nmol/mgprot, 581.7 ± 116.6 U/gprot and 203.7 ± 33.2 U/mgprot) of positive drug BP (400 mg/kg BW, $p > 0.05$), respectively. In line with the assay of ALT, AST and LDH, the differences in MDA, GSH-P*x* and SOD levels at low dosage of 100 mg/kg BW were inapparent, compared to CCl_4-intoxicated mice ($p > 0.05$), respectively, although the trend of data was of dosage-dependence.

The hepatotoxicity induced by CCl_4 is the most commonly used model system for the screening of hepatoprotective activity of plant extracts and drugs, and the $CCl_3OO^{\bullet}$ caused lipid peroxidation of cell membranes to create the end product MDA. GSH-P*x* can catalyze the reduction of hydrogen peroxide and other peroxides in GSH-related antioxidant system, and SOD can also catalyze the clearance of the superoxide anion radicals and prevents the formation of H_2O_2 (Lian et al., 2010). In addition, the two antioxidant enzymes are easily inactivated by excessive lipid peroxides resulting from CCl_4-induced liver damage, and thus, the levels of MDA, GSH-P*x* and SOD in hepatic tissue are important indicators of liver injury (Yang et al., 2011). In our study, ZSP displayed benign hepatoprotection via dramatically decreasing the productivity of MDA and increasing the enzymatic activities of GSH-P*x* and SOD.

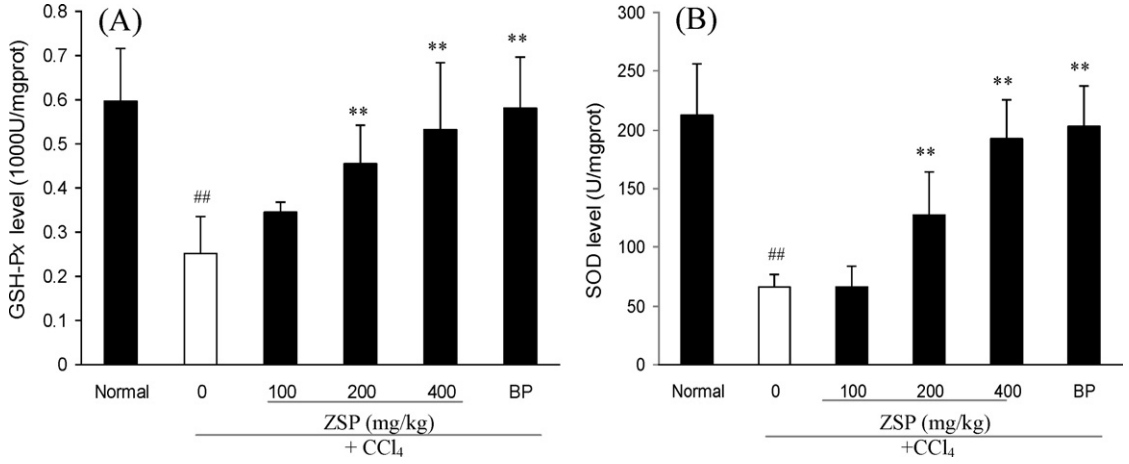

Fig. 5. Effects of ZSP on hepatic GSH-P*x* and SOD activities after CCl_4 administration in mice. Animals were given either ZSP (100, 200, or 400 mg/kg, ig) or BP (400 mg/kg) once daily for 10 successive days ahead of the single treatment of CCl_4 (0.1%, ip), and then hepatic GSH-P*x* and SOD activities were determined. Values are expressed as means ± SD ($n = 10$). $^{\#\#}p < 0.01$ vs. normal group. $^{**}p < 0.01$ vs. CCl_4-intoxicated group.

The liver is the largest organ in the vertebrate body, where toxins are absorbed from the intestinal canal, and reach the liver first as the dominating site of xenobiotic metabolism and excretion. A number of liver ailments can be induced by toxic chemicals, drugs, and vitrus infiltration from ingestion or infection. For this reason, liver disease remains one of serious health problems (Gowri Shankar, Manavavalan, Venkappayya, & David Raj, 2008). In the study, ZSP was found to inhibit lipid peroxidation both in vivo and in vitro. Our tests in DPPH$^{\bullet}$, HO$^{\bullet}$, and $O_2^{\bullet -}$ system and reducing power system in vitro showed that ZSP possessed good antioxidative and free radical scavenging effects. Furthermore, ZSP largely attenuated the increase of HI, markly reduced the levels of serum ALT, AST, and LDH, and normalized the activities of GSH-P*x*, SOD and MDA in hepatic tissue. We suggested that ZSP might improve liver function and structure to enhance the innate mechanisms of the antioxidant system or provide antioxidant capacity against CCl_4-induced oxidative stress in mice liver.

4. Conclusions

Z. jujube was claimed to have a wide range of health benefits, including anti-oxidative, hepatoprotective treatment, anti-inflammation, and immunostimulating effects. However, many investigations were achieved directly with *Z. jujube* or its crude extracts. The chemical components of *Z. jujube* responsible for the effects remain to be elucidated. Here, the water-soluble polysaccharide ZSP was successfully isolated from *Z. jujube cv. Shaanbeitanzao*, a main cultivar of *Z. jujube*. Furthermore, ZSP was firstly characterized as a good source of arabinose-enriched polysaccharides. Moreover, ZSP displayed an overall antioxidant effects against in vitro oxidative stress as well as in vivo CCl_4-induced oxidative hepatotoxicity in mice. This is the first report that ZSP is one of the main active ingredients responsible for the antioxidant effect of *Z. jujube cv. Shaanbeitanzao*, a traditional dietary-treated fruit. On the basis of these findings, it was suggested that adequate consumption of *Z. jujube cv. Shaanbeitanzao* and its polysaccharide extracts might has a favorable effect on maintaining or improving antioxidant systems and the liver functions of the host.

Acknowledgment

This study was supported by a Grant from the National Natural Science Foundation of China (C30972054, C20802091, and C31171678).

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