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Hypolipidemic and antioxidant activities of polysaccharides from *Rosae Laevigatae Fructus* in rats

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ABSTRACT

Two major fractions (RLP-1 and RLP-2) were obtained by purifying the crude polysaccharides extracted from a traditional Chinese herb *Rosae Laevigatae Fructus*. The average molecular weight of RLP-1 and RLP-2 was 21.5 kDa and 16.1 kDa, respectively. Monosaccharide analysis indicated that RLP-1 was composed of xylose, mannose and galactose in the molar ratio of 1:11:8, while RLP-2 was only a glucan. Oral administration of RLP-1 could significantly decrease levels of serum total cholesterol (TC), triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C), inhibit hepatic lipid accumulation, increase antioxidant lipids and up-regulate expressions of peroxisome proliferator-activated receptor-γ (PPAR-γ) and lipoprotein lipase (LPL) in hyperlipidemia rats. These results suggest that RLP-1 improve hyperlipidemia possibly through regulating PPAR-mediated lipid metabolism. Therefore, could be explored as a possible agent for hyperlipidemia.

1. Introduction

Cardiovascular diseases (CVD) such as coronary heart disease and peripheral artery disease are the leading causes of death worldwide. Despite the multifactorial pathogenesis of CVD, high intakes of calories and fats (cholesterol-rich fats and saturated fatty acids) are widely considered as major contributing factors (Rubenfire, Brook, & Rosenson, 2010). Dietary interventions such as fat ingestion reduction or fat catabolism are suggested as primary treatments to reduce low-density lipoprotein cholesterol (LDL)-C levels and to lower the risk of CVD (Eslick, Howe, Smith, Priest, & Bensoussan, 2009; Michelle & Micallef, 2009; Nijjar, Burke, Bloesch, & Rader, 2010; Wat et al., 2009).

Rosae laevigata Fructus (the fruit of *R. laevigata* Michx., which belongs to the *Rosa* genus, *Rosaceae* family) is widely used as a tonic and health food in China, Japan and other Asian countries. This food is used in marmalades, garnisesh, as well as fruit pies, and has a high medicinal value for treatment of chronic diseases. Increasing attention has been given to the antioxidant, anti-inflammatory and hepatoprotective properties of this herb (Liu, Lu, & Peng, 2011; Meng et al., 2012). Several studies have reported that the fruit is rich in vitamin C, polysaccharides, triterpenoid acids, steroids, and flavonoids (Fang, Wang, & Cheng, 1991; Gao et al., 2010; Zhang et al., 2012). Polysaccharide, the main component of the herb, was approximately 260.5 mg/g (Yan, Han, Wu, Jiang, & Gui, 2011). However, no studies evaluating biological activities of polysaccharides isolated from *R. laevigata Fructus* (RLP) as a dietary supplement have been conducted.

Recently, polysaccharides have been widely studied as a new source of dietary supplements and functional foods. Polysaccharides have antioxidant, antiproliferative, immunoregulatory and anti-diabetic properties (He, Yang, Jiao, Tian, & Zhao, 2012; Schepetkin & Quinn, 2006; Xiong, Anlin Li Huang, Lu, & Hou, 2011; Xu, Ye, Sun, Tu, & Zeng, 2012; Zeng, Zhang, Gao, Jia, & Chen, 2012). They could also reduce plasma lipid concentrations, particularly low-density lipoprotein cholesterol (LDL-C) (Belghith et al., 2012; Li, Zhang, & Ma, 2010; Luo, Cai, Yan, Sun, & Corke, 2004; Zha et al., 2012). The present study aimed to investigate the chemical characterization and hypolipidemic effects of RLP on lipid profiles, antioxidant enzyme activities and lipid metabolic gene expressions in rats with high-fat diet induced hyperlipidemia.

2. Materials and methods

2.1. Reagent

The dry fruit was purchased from Chinese Medicine Herbal Factory, Zhejiang Province, China, and identified by Dr. Wei

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Cai, Zhejiang Chinese Medical University. Samples were ground and passed through a 10-mesh sieve. Lovastatin capsules (Lot. 110601) were purchased from Youkon Pharmacy Co., Ltd., Chengdu Province, China. All reagents were of analytical grade and were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

2.2. Preparation and quantitative analysis of RLP

The raw material was pretreated, as reported previously (Gan, Manaf, & Latiff, 2010), with modifications. Up to 200 g of dried herbal powders were defatted with petroleum ether (boiling point: 60–90 °C) to remove liposoluble compounds and then pretreated with 80% ethanol to remove pigments, monosaccharides and phenolic compounds. After the solvent was volatilized, the pretreated samples were immersed in distilled water for 2 h and then extracted with 2 L of distilled water at 90 °C for 1 h. After centrifugation (3500 rpm, 20 min) and concentration, the supernate (200 mL) was precipitated by adding 4 volumes of 95% ethanol (v/v). The precipitates were collected and washed successively with 95% ethanol, acetone and diethyl ether. After lyophilized, the pink powders were obtained and the yield was 5.6%.

2.3. Separation and purification of RLP

Protein removal was performed using the Sevage method, followed by papain digestion in accordance with previous reports (Zha et al., 2012). Approximately 50 mg of crude polysaccharides dissolved in 10 mL of H_2O were applied to a diethylaminoethyl (DEAE)-cellulose column (5 cm × 60 cm) pre-equilibrated with water and eluted in NaCl gradient solution (0–3 M) at a flow rate of 1 mL/min (10 mL/tube). Each elution fraction was collected and monitored for carbohydrate content through the phenol–sulfuric acid method (using D-glucose as a standard), as previously described (Chen et al., 2012). Finally, the same carbohydrate-positive fractions were pooled together, dialyzed, and lyophilized to afford two fractions. The products were further purified on a Sephadex G-100 column (2.6 cm × 60 cm) with water and lyophilized to afford two major polysaccharides, RLP-1 and RLP-2.

2.4. Analysis of monosaccharide composition

Gas chromatography (GC)–mass spectrometry was employed for the identification and quantification of monosaccharides (Chen et al., 2012; Xu, Yao, Sun, & Wu, 2009). Approximately 50 mg of polysaccharides were hydrolyzed with 5 mL of 1 M sulfuric acid at 100 °C for 6 h. The hydrolyzed polysaccharide was mixed with BaOH for pH up to neutrality and was then evaporated continuously using a rotary evaporator at 45 °C. The hydrolysate was dissolved in 0.5 mL of pyridine with 10 mg of hydroxylamine hydrochloride and was allowed to react at 90 °C for 30 min. Approximately 0.5 mL of acetic anhydride was added after cooling, and the tube was sealed and incubated at 90 °C for 30 min. The corresponding alditol acetates were analyzed by gas chromatography on a Hewlett Packard 6890 gas chromatograph equipped with a capillary column of HP-5MS phenyl methyl siloxane (30 m × 0.25 mm × 0.25 μm).

2.5. Molecular weight determination

The molecular weight of fractions were evaluated and determined using an LC-10AD high-performance liquid chromatography (HPLC) system (Shimadzu Co. Ltd., Japan) equipped with two types of size-exclusion chromatography columns in series (TSK-G5000PW 7.5 mm × 300 mm and TSKG4000PW 7.5 mm × 300 mm; Tosoh, Japan) and a 10-A refractive index detector. The mobile phase consisted of 0.7% (w/v) sodium sulfate, and the flow rate was 0.5 mL/min. The column temperature was maintained at 35 °C, and the injection volume was 20 μL. Standard dextrans (0.2%, w/v; Burtin Polymer Laboratories Co.) with different molecular weights (M 4.73, 11.2, 21.2, and 40.4 kDa) were used to calibrate the standard curve (Guo, Liang, & Du, 2011; Wang, Yang, & Wei, 2012). The relationship between log M and retention time T was calculated as the following equation.

$$\log M = 7.002 - 0.1375T, \quad R^2 = 0.9990 \tag{1}$$

2.6. Infrared spectroscopy analysis

The infrared spectrum of PMPP was recorded with a SPECORD spectrometer in a range 400–4000 cm^{-1}. The samples were analyzed as KBr pellets.

2.7. Animals and experimental design

Male specific pathogen-free Sprague-Dawely rats weighing 180–220 g were purchased from the Animal Experimental Center of Zhejiang Chinese Medical University in China. Experiments were carried out in accordance with local guidelines for the care of laboratory animals of Zhejiang Chinese Medical University, and were approved by the ethics committee for research on laboratory animal use of the institution [No. SCXK (Zhe) 2008-0116].

Rats were fed with basic diet for 1 week in the experimental environment before the experiments were conducted. Once they had adapted to the environment, a total of 10 rats were selected randomly as the normal control group (NC) fed with basic diet, whereas the others were fed with high-cholesterol diet (81.8% basic diet, 2% cholesterol, 0.2% sodium cholate, 10% lard, and 6% dried egg yolk) and were randomly divided into 8 groups: model control group (MC), positive control group (lovastatin, 3.3 mg/kg body weight, PC), RLP-1 low-dosage group (10 mg/kg body weight, RLP-1L), RLP-1 high-dosage group (25 mg/kg body weight, RLP-1H), RLP-2 low-dosage group (10 mg/kg body weight, RLP-2L), and RLP-2 high-dosage group (25 mg/kg body weight, RLP-2H). NC and MC were orally administered with the same volume of water, whereas the other groups were treated with corresponding drugs once daily for 4 weeks. Rats were given free access to food and water during the experimental period.

2.8. Biochemical analysis

Each rat was weighed and anesthetized with 10% chloralic hydras 24 h after the last drug administration. Blood samples were collected from the aorta abdominalis, centrifuged at 3000 × g for 15 min to obtain serum, and stored at 4 °C until use. Serum total cholesterol (TC), triglycerides (TG), LDL-C, high-density lipoprotein cholesterol (HDL-C), superoxide dismutase (SOD) activity, glutathione peroxidase (GSH-Px) activity, and malondialdehyde accumulation (MDA) were determined using commercially available kits (purchased from Nanjing Jiancheng Biotech. Sci. Inc., China).

2.9. Hepatic morphology

Rat livers were removed and placed in a 10% buffered formalin solution. Fixed tissues were processed routinely for paraffin embedding, and 4 μm sections were prepared and dyed with hematoxylin–eosin; the stained areas were viewed using an optical microscope at 100×.

2.10. Reverse transcription-polymerase chain reaction (RT-PCR) analysis in live tissues

Live samples were homogenized, and total RNA was extracted from the liver of the rats in the experimental groups with the use of a Trizol reagent (Shanghai Biotechnology Co., Ltd., China). The quality of the isolated RNA was determined using the double-wavelength detection method. Approximately 500 ng of total RNA were required on every occasion in the following RT-PCR. The specific designed primers pairs used for analysis were shown as following:

PPAR-γ	F: 5′-CTCCAAGAATACCAAAGTGCGA-3′	336 bp
	R: 5′-AAACCTGATGGCATTGTGAGAC-3′	
LPL	F: 5′-TGAGAACATTCCCTTCACCCT-3′	132 bp
	R: 5′-CAGCGGAAGTAGGAGTCGTT-3′	
β-Actin	F: 5′-GTCAGGTCATCACTATCGGCAAT-3′	147 bp
	R: 5′-AGAGGTCTTTACGGATGTCAACGT-3′	

RT-PCR was performed according to the kit protocol (Boster Biological Technology, Ltd., Wuhan, China) and amplified in a PCR system. RNA samples were first reverse-transcribed and immediately amplified by PCR, and the process was performed by denaturing at 94 °C for 60 s, annealing at 60 °C, and extension at 72 °C for 60 s. In addition, 50 more cycles were used for amplification. The BioSpectrum Gel Imaging System (Bio-Rad, USA) was used to analyze the optical density values of the electrophoresis bands.

2.11. Western blotting in liver tissues

Liver samples were homogenized and lysed in SDS-PAGE sample buffer, boiled, centrifuged and the supernatant recovered. Samples were run on 10% SDS polyacrylamide gels, electroblotted onto nitrocellulose membranes. Immunoblotting were assayed using anti-PPAR-γ (1:200), anti-LPL (1:200) antibodies (Boster Biological Technology, Ltd., Wuhan, China). The immunodetection was done using an enhanced chemiluminescence detection kit (Boster Biological Technology, Ltd., Wuhan, China). The bands density was quantified using Lab works (GelPro4.0, Media Cybernetics, LP) via calculating the average optical density in each field.

2.12. Statistical analysis

All data were shown as mean ± SD. Statistical comparisons of data were carried out using the ANOVA of the SPSS 13.0 system. The acceptable level for statistical significance was $P < 0.05$.

3. Results and discussion

3.1. Preparation and structural analysis

The water-soluble crude polysaccharides were obtained from RLP by hot water extraction, ethanol precipitation, deproteinization, and lyophilization. The extracts were purified with DEAE-cellulose and Sephadex G-100 columns. Two main fractions, RLP-1 and RLP-2 (Fig. 1), were collected for further structural characterization and bioactivity assay. Both polysaccharides showed negative reactions to Fehling's reagent and iodine–potassium iodide, which indicates the absence of reducing sugars and starch-type polysaccharides. The polysaccharides also exhibited negative responses to Bradford test. No absorption occurred at 280 and 260 nm in the ultraviolet spectrum, which indicates the absence of protein and nucleic acid. The HPLC profile presented a single eluted peak. The average molecular weights of RLP-1 and RLP-2 were 21.5 and 16.1 kDa, respectively. The quantitative results of monosaccharides analyzed by GC indicated that RLP-1 was composed of xylose, mannose, and galactose in a molar ratio of 1:11:8 and that RLP-2 was only a glucan (Fig. 2).

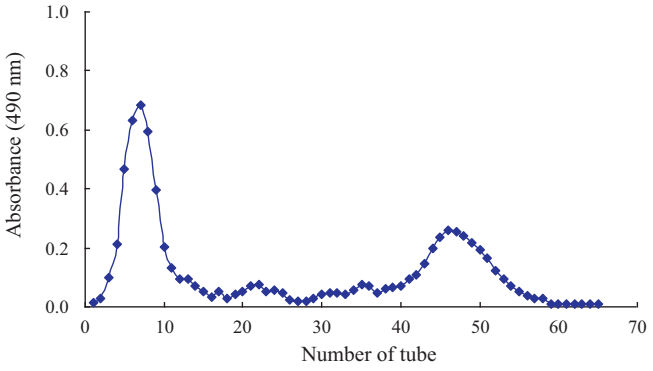

Fig. 1. Chromatogram of the crude polysaccharide from *Rosae Laevigatae Fructus* on DEAE-cellulose column (2.6 cm × 60 cm). The crude polysaccharide was dissolved in deionized water and eluted with NaCl gradient solution (0–3 M) at a flow rate of 1.0 mL/min (10 mL/tube). The eluates were collected and the carbohydrate contents of collected fractions were monitored by phenol–sulfuric acid method.

As shown in Fig. 3, the different absorption bands of the FTIR analysis were assigned as previously reported (Chen et al., 2012; Zhu et al., 2011). For the sample of RLP-1, a broad band centered at 3215 cm^{-1} assigned to hydrogen-bonded hydroxyl groups. An intense band centered at 2927 cm^{-1} was due to the —CH stretching and was a characteristic absorption of polysaccharide. The absorption band centered at 1625 cm^{-1} was caused by the —OH flexural vibrations of the polysaccharide. The group of bands that extended from 1252 cm^{-1} to 1020 cm^{-1} corresponded to C—O stretching vibrations. The absorption band centered at 860 cm^{-1} was due to the α-type glycosidic bond.

For the sample of RLP-2, there was a broad band centered at 3200 cm^{-1} assigned to hydrogen-bonded hydroxyl groups. The absorption band centered at 1640 cm^{-1} was caused by the —OH flexural vibrations of the polysaccharide. The group of bands that extended from 1020 cm^{-1} to 1180 cm^{-1} and there were no characteristic peaks extended from 818 cm^{-1} to 930 cm^{-1}, indicating RLP-2 was pyranose containing the β-type glycosidic bond. Since the monosaccharide analysis indicated that RLP-2 was only composed of glucose, RLP-2 was β-glucan.

3.2. Effects of RLP on lipid profiles of hyperlipidemia rats

Abundant data have shown that an increasing consumption of carbohydrates and fat could lead to hypercholesterolemia and hyperlipidemia (Akiyama, Tachibana, Shirohara, Watanabe, & Otsuki, 1996). As shown in Table 1, after 4 weeks of high-fat feeding, the serum levels of TC, TG, and LDL-C in the MC group were significantly higher than those in the NC group (all $P < 0.05$ for TG and TC), whereas the serum levels of HDL-C in the MC group were significantly lower, which indicated that the model was successful in inducing hyperlipidemia in rats. However, the increased serum levels of TC, TG, and LDL-C were significantly suppressed, whereas the decreased serum level of HDL-C could not be elevated by RLP-1 treatment in rats with high-fat diets. However, RLP-2 failed to ameliorate hyperlipidemia induced by high-fat diet. No significant difference was observed in the serum levels of TC, TG, and LDL-C between RLP-2 treatment groups and MC. The results indicated that RLP-1 could be the main hypolipidemic substance in the RLP.

Histological examination of hepatic tissues in NC showed normal cell architecture, but significant morphological changes were observed in MC (Fig. 4). The sections of hepatic tissues in MC showed fewer cells and had lipid vacuolization (denoted by a black arrow), which indicated the accumulation of hepatic lipid droplets in the hepatic cells of MC rats after 4 weeks of high-fat treatment. However, the accumulation of hepatic lipid droplets

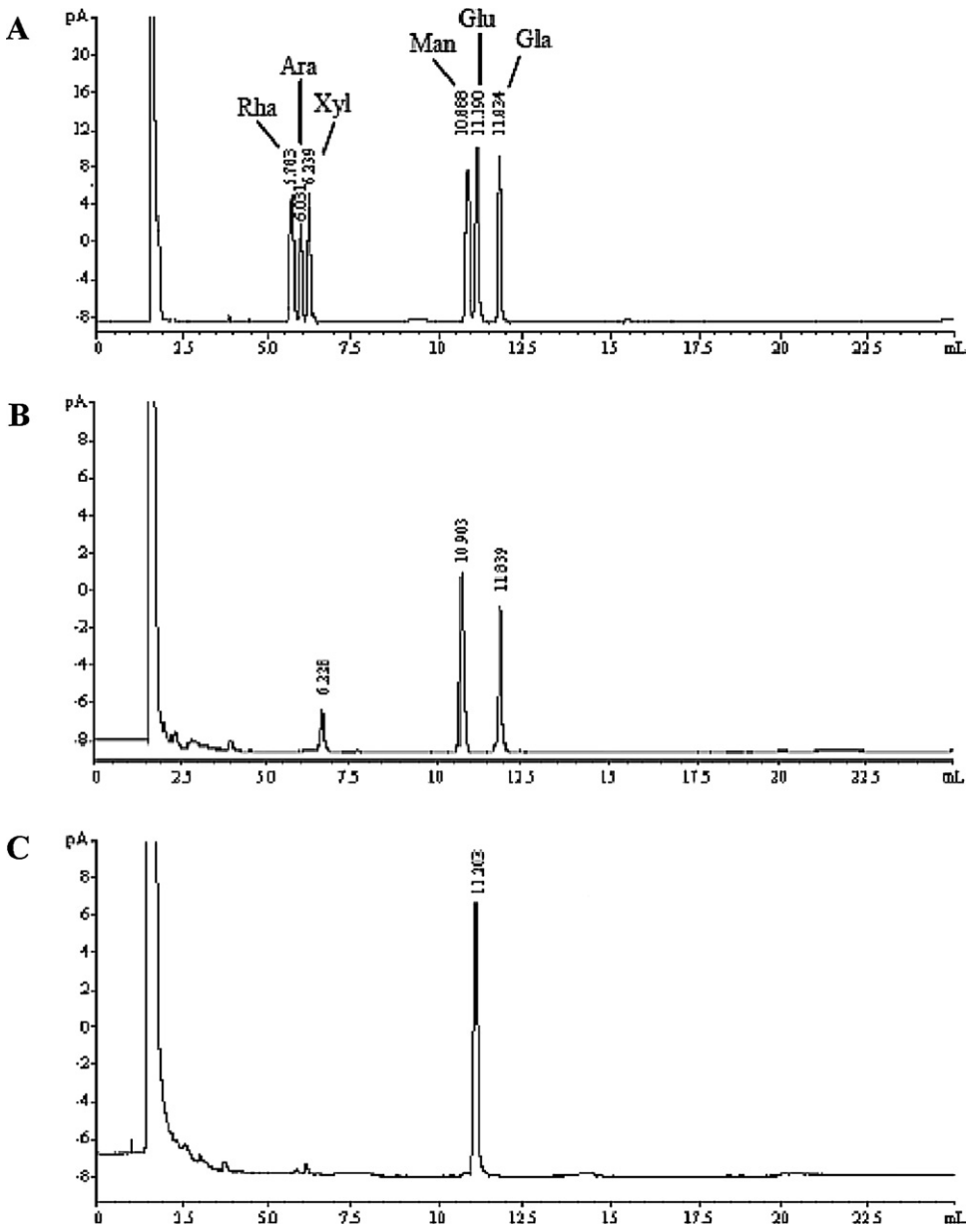

Fig. 2. Gas chromatogram analysis of the acetylated monosaccharides. (A) Derivatized standard monosaccharides; (B) RLP-1; (C) RLP-2.

appeared relatively lower in the RLP-1 treatment groups. These results seemed to correspond to the serum lipid profiles given in Table 1, which demonstrated that RLP-1 could reduce the accumulation of lipid droplets in the hepatic tissue cells of hyperlipidemic rats and prevent non-alcoholic fatty liver disease.

3.3. Effects of RLP on antioxidant enzyme activities of hyperlipidemia rats

High-fat diet contributes to oxidative stress in tissues, which may result in hyperlipidemia and cardiovascular diseases. But,

Table 1
Effects of RLP on serum lipids of experimental hyperlipidemia rats induced by high-fat diet.

Group	Dose (mg/kg)	TG (mmol/L)	TC (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)
NC	–	3.16 ± 0.27[a]	1.18 ± 0.27[a]	0.78 ± 0.13[a]	1.15 ± 0.14[a]
MC	–	4.59 ± 0.61	1.62 ± 0.32	1.06 ± 0.25	0.92 ± 0.11
PC	3.3	3.48 ± 0.58[a]	1.26 ± 0.20[a]	0.77 ± 0.21[a]	1.12 ± 0.15[a]
RLP-1L	10	4.05 ± 0.49[a]	1.32 ± 0.16[a]	0.76 ± 0.13[a]	1.02 ± 0.14
RLP-1H	25	3.94 ± 0.46[a]	1.29 ± 0.22[a]	0.78 ± 0.15[a]	1.05 ± 0.10
RLP-2L	10	4.28 ± 0.57	1.47 ± 0.21	0.98 ± 0.13	0.92 ± 0.13
RLP-2H	25	4.14 ± 0.67	1.44 ± 0.27	0.96 ± 0.19	0.94 ± 0.17

NC: normal control group; PC: positive control group (lovastatin, 3.3 mg/kg body weight); RLP-1L: RLP-1 low-dosage group (10 mg/kg body weight); RLP-1H: RLP-1 high-dosage group (25 mg/kg body weight); RLP-2L: RLP-2 low-dosage group (10 mg/kg body weight); RLP-2H: RLP-2 high-dosage group (25 mg/kg body weight). Data were expressed as mean ± SD ($n = 10$).

[a] $P < 0.05$, compared with the hyperlipidemia rats (MC).

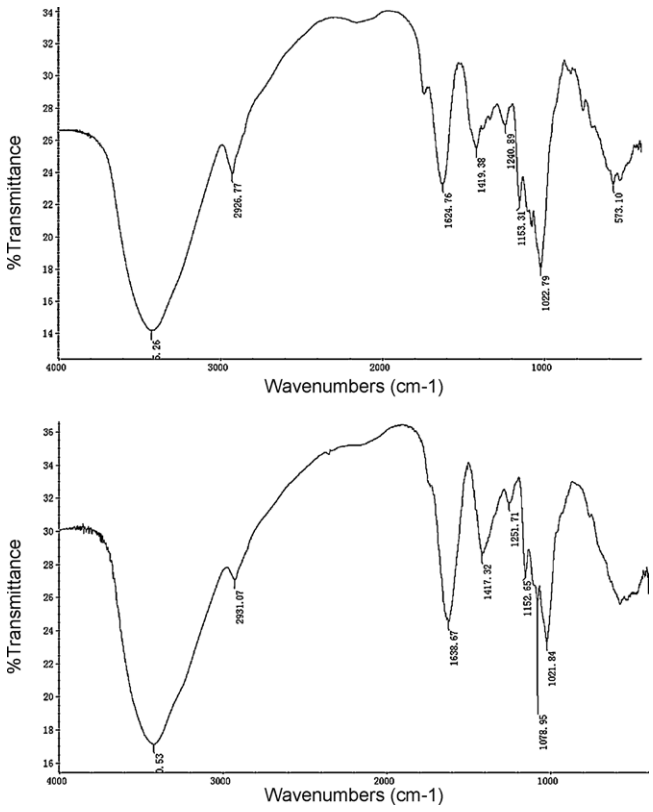

Fig. 3. The infrared spectrum of RLP-1 (A) and RLP-2 (B).

antioxidant dietary supplementation is reported to reverse high-fat-induced oxidative damage in cardiovascular system (Araujo, Barbosa, Hsin, Maranhão, & Abdalla, 1995; Einvik et al., 2010). *R. Laevigatae Fructus* as a functional botanical is abundant in polysaccharides, triterpenoid acids, flavonoids and other bioactive substances. These natural components exited antioxidant, anti-inflammatory and hepatoprotective activities (Liu et al., 2011; Zhang et al., 2012).

As shown in Fig. 5, there were significant decreases in the serum levels of SOD and GSH-Px (both $P < 0.05$) but a significant increase in the serum levels of MDA ($P < 0.05$) in MC rats, compared with those of NC rats. Compared with the MV rats, oral administration of RLP and RLP-1 could cause significant increases in the serum levels of SOD and GSH-Px (all $P < 0.05$) and significant decreases in the accumulation of serum MDA (both $P < 0.05$), while administration of RLP-2 did not have significant effects in these biomarkers ($P > 0.05$). These results were in line with the effects of RLP-1 on lipid profiles of hyperlipidemia rats.

Many studies have reported that antioxidant enzymes such as CAT, SOD and GSH-Px in blood contribute to the antioxidant defense mechanism (Jain, Kathiravan, Somani, & Shishoo, 2007). The hypolipidemic effects of botanical may be related to the ability to scavenge various oxygen radicals and activating the endogenous stress-related enzymatic systems, such as SOD and GSH-Px (Feng, Yu, Ying, Hua, & Dai, 2011; Li et al., 2010; Luo et al., 2004; Zha et al., 2012). Our results also suggested that RLP could improve the efficiency of the antioxidant enzymes (SOD, GSH-Px) following deactivation of the substrates for the lipid peroxidation reactions, which resulted in the reduction of serum MAD levels.

3.4. Effects of RLP on lipid metabolic gene and protein expressions

The liver plays a key role in lipid metabolism. Hepatic histological damage refers to the excessive accumulation of lipids within hepatocytes due to the imbalance between lipid formation and lipid degradation. PPAR-α is more likely linked to lipid beta-oxidation, and PPAR-γ is associated more with carbohydrate uptake (Huang, Teoh, Lin, Lin, & Basil Roufogalis, 2009). PPAR-γ has been in the research spotlight for the past decade because the ligands for this receptor are potent insulin sensitizers and plays an important role in maintaining homeostasis of glucose metabolism in the body. Activation of PPAR-γ to improve the sensitivity of insulin receptors is the predominant mechanism for the anti-diabetic and hypolipidemic efficacy of PPAR-γ agonists (Francis, Annicotte, & Auwerx, 2003; Neve, Fruchart, & Staels, 2000; Szanto & Nagy, 2008). On the other hand, LPL is a key enzyme in lipoprotein and adipocyte metabolism (Roberts, Barnard, Liang, & Vaziri, 2002). LPL is anchored on the cell surface by a proteoglycan chain and plays a key role in hydrolyzing triglycerides in chylomicrons and very low density lipoproteins (VLDL) at the first step in their metabolism. It has been reported that PPAR-γ agonists up-regulate human macrophage LPL expression (Li et al., 2004).

To further evaluate the possible mechanism, the effects of RLP-1 on the lipid metabolic gene expression of hyperlipidemic rats were analyzed using RT-PCR. As shown in Fig. 6A, with over 4 weeks of high-fat feeding, the levels of PPAR-γ and hepatic LPL mRNA tissue expression was decreased in experimental diet groups compared with those in MC (both $P < 0.05$). However, PPAR-γ and LPL mRNA expression was significantly increased by RLP-1 treatment at 25 and 50 mg/kg doses (all $P < 0.05$). These data suggest that increases in both PPAR-γ and LPL mRNA expressions regulated by RLP-1 may be related to the regulation of dyslipidemia in the model.

The protein analysis in Fig. 6B showed the expressions of PPAR-γ and LPL proteins in the hepatic tissues. The protein expressions of PPAR-γ and LPL were obvious in hepatic tissue of the NC group while these were significantly decreased ($P < 0.01$) in the MC group. After the treatment of RLP-1, the protein expressions of PPAR-γ and LPL were much higher (all $P < 0.01$) than that in the MC group. The regulating actions of RLP-1 were found to proportionally increase

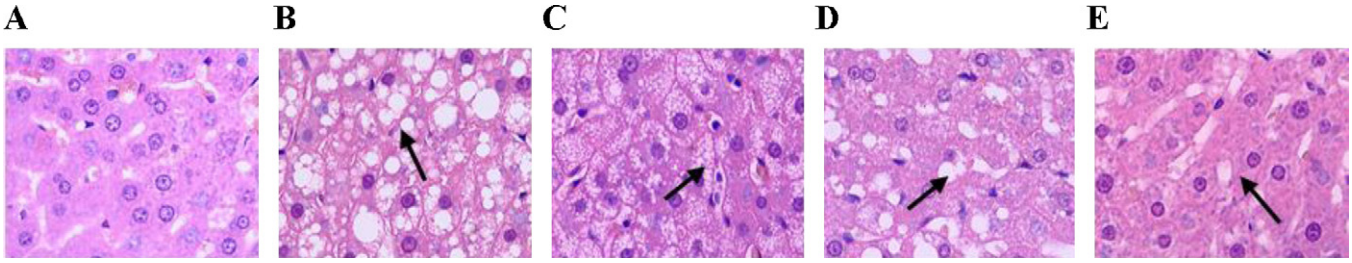

Fig. 4. Histopathological micrographs of hepatic tissue morphology of hyperlipidemia rats (400×). The features included: (A) no or few fat droplets were shown in the liver of normal control rats; (B) many large fat droplets were indicated by the black arrowheads in the liver of rats fed a high-fat diet; (C) many small fat droplets were shown in the liver of lovastatin-treated hyperlipidemia rats; (D) and (E) hepatic tissue of hyperlipidemia rats treated with RLP-1 at the doses of 10 and 25 mg/kg, respectively, both had a lower accumulation of lipid droplets.

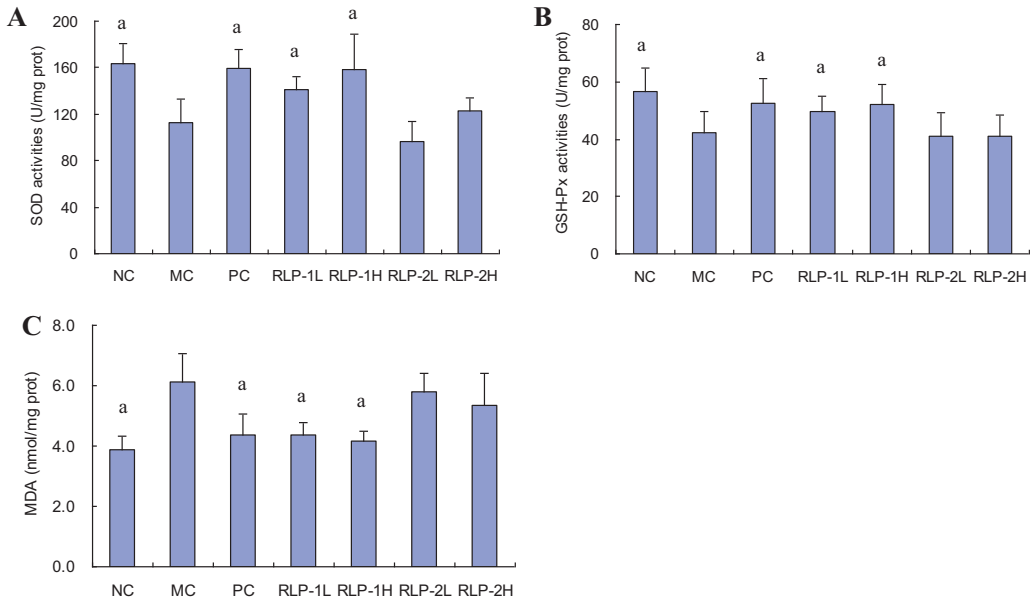

Fig. 5. Effects of RLP on (A) superoxide dismutase (SOD) activity, (B) glutathione peroxidase (GSH-Px) activity, and (C) accumulation of malondialdehyde (MDA) in the hyperlipidemia rats. Values are the means ± SD ($n = 10$). [a] $P < 0.05$, compared with the model control group (MC). NC: normal control group; PC: positive control group (lovastatin, 3.3 mg/kg body weight); RLP-1L: RLP-1 low-dosage group (10 mg/kg body weight); RLP-1H: RLP-1 high-dosage group (25 mg/kg body weight); RLP-2L: RLP-2 low-dosage group (10 mg/kg body weight); RLP-2H: RLP-2 high-dosage group (25 mg/kg body weight).

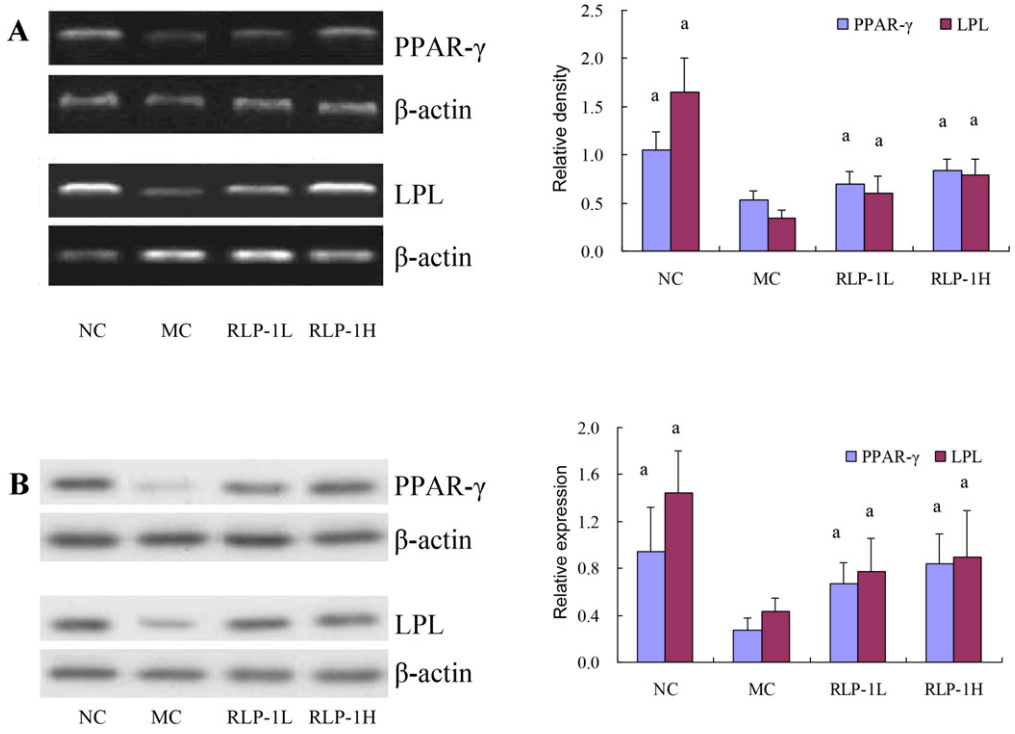

Fig. 6. Levels of PPAR-γ and LPL mRNA by RT-PCR analysis (A) and protein analysis by Western blot (B) in the hepatic tissues of the tested rats. Data denoted are means ± S.D. ($n = 10$). [a] $P < 0.05$, compared with model control group (MC). NC: normal control group; RLP-1L: RLP-1 low-dosage group (10 mg/kg body weight); RLP-1H: RLP-1 high-dosage group (25 mg/kg body weight).

in a dose-dependent manner. These results were in accordance with PPAR-γ and LPL mRNA expressions determined above. Therefore, we speculated that RLP-1 could improve lipid metabolism in hyperlipidemic rats via PPAR-γ/LPL pathway.

4. Conclusion

In the present study, polysaccharides from RLP were produced by hot water extraction and were subsequently purified using a

DEAE-cellulose column to obtain two fractions (RLP-1 and RLP-2). The average molecular weights of RLP-1 and RLP-2 were was 21.5 and 16.1 kDa, respectively. Monosaccharide analysis indicated that RLP-1 was composed of xylose, mannose, and galactose in a molar ratio of 1:11:8, whereas RLP-2 was only a glucan. RLP-1 was responsible for the hypolipidemic effect of RLP and could improve lipid profiles through upregulation of PPAR-γ and LPL expression. The results suggest that RLP-1 is a suitable candidate for further investigation as an agent in humans. However, the structural analysis and cellular mechanisms responsible for the activities remain to be elucidated.

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