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Research Article

Water-Soluble Chitosan Nanoparticles Inhibit Hypercholesterolemia Induced by Feeding a High-Fat Diet in Male Sprague-Dawley Rats

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Chitosan, a deacetylated product of chitin, has been demonstrated to lower cholesterol in humans and animals. However, chitosan is not fully soluble in water which would influence absorption in the human intestine. In addition, water-soluble chitosan (WSC) has higher reactivity compared to chitosan. The present study was designed to clarify the effects of WSC and water-soluble chitosan nanoparticles (WSC-NPs) on hypercholesterolemia induced by feeding a high-fat diet in male Sprague-Dawley rats. WSC-NPs were prepared by the ionic gelation method and the spray-drying technique. The nanoparticles were spherical in shape and had a smooth surface. The mean size of WSC-NPs was 650 nm variing from 500 to 800 nm. Results showed that WSC-NPs reduced the blood lipids and plasma viscosity significantly and increased the serum superoxide dismutase (SOD) activities significantly. This paper is the first report of the lipid-lowering effects of WSC-NPs suggesting that the WSC-NPs could be used for the treatment of hypercholesterolemia.

1. Introduction

Dyslipidemia, including hypercholesterolemia, hypertriglyceridemia, or their combination, is a major risk factor for cardiovascular disease. Generally, dyslipidemia is characterized by increased fasting concentrations of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), in conjunction with decreased concentrations of high-density lipoprotein cholesterol (HDL-C) [1]. At present, these lipid imbalances are most routinely treated with pharmacological therapy. However, many cholesterol-lowering agents, especially statins, are associated with severe side effects [2]. In light of this, there has been great interest in the influence of dietary fibers, such as chitosan, on cholesterol absorption in the intestine.

Chitosan is a natural cationic polysaccharide consisting of (1-4)-2-amino-2-deoxy-D-glucopyranosyl units. It breaks down slowing to harmless products (amino sugars), which are completely absorbed by the human body [3]. Due to its nontoxicity and high biocompatibility, chitosan has been formulated as dietary supplements, as carrier for oral peptide and protein drug delivery, as targeted drug delivery, and in the pharmaceutical and biomedical fields [4–6]. Due to the existence of amino groups, chitosan possesses positive charge, so it can bind negatively charged substrates such as lipids and bile acids. Chitosan also interfere with emulsification of neutral lipids by binding them with hydrophobic bonds [7]. Several studies have shown that chitosan has cholesterol-lowering properties both in animals and humans [8, 9].

However, chitosan has high viscosity and is not fully soluble in water, but it is in acidic solutions. Such properties of chitosan would decrease its absorption in the human intestine because most animal intestines, especially the human gastrointestinal tract, do not possess enzymes such as chitinase and chitosanase [10]. WSC has lower viscosity and is soluble in water. Subsequently, it seems to be readily absorbed in vivo. And, WSC has been reported to have the health benefits such as immunity regulation, antitumor, liver protection, blood lipids lowering, and antidiabetic

and antioxidant properties [11, 12]. In particular, previous studies revealed that the WSC was effective at lipid lowering compared to chitosan [13].

Furthermore, nanoparticles show some specific characteristics such as an increase of stability of therapeutic agents, controlled- and sustained-release properties, and the deeply penetration into tissues through fine capillaries [14]. We have prepared the WSC-NPs as a carrier to load the protein drug by the ionic gelation method [15]. And, WSC-NPs have the better solubility for the big total surface area and lower viscosity than WSC. Therefore, this study examined the effects of WSC and WSC-NPs on hypercholesterolemia induced by feeding a high-fat diet in rats.

2. Materials and Methods

2.1. Chemicals. WSC was purchased from Shandong Aokang Biotech Ltd (Shandong, China). The viscosity was more than 200 cps, and deacetylation value was 85%. Total cholesterol (TC), Triacylglycerol (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) kits were obtained from BioSino Bio-technology and Science Inc (Beijing, China). Superoxide dismutase (SOD) kits were purchased from Nanjing Jiancheng Bioengineering Institute (Wenzhou, China). Unless otherwise stated, all laboratory reagents were of analytical grade.

2.2. Animals and High-Fat Emulsions. Male Sprague-Dawley rats weighing 200 ± 20 g were purchased from Guangzhou University of Chinese Medicine Laboratory Animal Center (Guangzhou, China). All animal protocols were approved by the institutional animal care and use committee of Guangdong Pharmaceutical University (Guangzhou, China). They were housed in an isolator caging system in an air-conditioned animal room at 23 ± 1°C. Rats were allowed free access to food and water.

Briefly, the high-fat emulsions were prepared as follows. 10.0 g cholesterol and 1.0 g propylthiouracil powder were dissolved into 20.0 g lard oil at 80°C and stirred for 10 min to ensure complete dissolution as the oil phase. The primary emulsions were prepared by diluting 5 mL emulsifier (Tween-80) and 20 mL sodium deoxycholate solution (2.0%) into the oil phase with a high-speed blender. Then, the distilled water was added to the primary emulsions to form the high-fat emulsions (100 mL) with stirring.

2.3. Preparation and Characterization of Water-Soluble Chitosan Nanoparticles. In this study, WSC-NPs were formed as a result of complex electrostatic interactions between the positively charged copolymers and negatively charged tripolyphosphate (TPP) under mild conditions. Briefly, WSC (0.1% w/v) and TPP (0.1% w/v) were dissolved in purified water. For preparation of WSC-NPs, the WSC solution (500 mL) was stirred (800 rpm) at room temperature (25°C). Then, 0.1% TPP solution (100 mL) was added to the system while stirring was continued to complete nanoparticles formation. The rate of adding TPP was 0.75 ml/min. The nanosuspension was then spray dried using the Lab Spray Dryer L-117 (Laiheng Scientific Co. Ltd, Beijing, China) with a standard nozzle (0.5 mm). The atomizing air flow rate was 10–15 L/min, and the flow rate was 600 ml/h. The inlet temperature was controlled at 160°C. The outlet temperature was determined by the inlet temperature and relative factors such as air and liquid feed flow rates and varied between 80–85°C. The stability of WSC-NPs is affected by various environmental conditions during long-term storage. Studies were carried out to evaluate the stability of the WSC-NPs for 5 months at room temperature.

The FTIRs were taken with KBr pellets on Perkin-Elmer Spectrum one FTIR (Shimadzu, FT-IR 8700, Japan).The particle size and size distributions of the nanoparticles were performed by particle sizer (Zetasizer 3000 HAS, Malvern Instruments Ltd., Worcs, UK).The morphology of the nanoparticles was examined under scanning electron microscopy (SEM) using a Hitachi S3700N (Hitachi Ltd, Japan) microscope at 10 kV.

2.4. Experimental Procedure. The rats were fed *ad libitum* with a commercial diet for 5 days and were then assigned to 5 groups ($n = 8$) as follows: (a) normal diet fed rats (NF), (b) high-fat emulsions fed rats (HF), (c) high-fat emulsions and 450 mg/kg/d WSC fed rats (WSC), (d) high-fat emulsions and 450 mg/kg/d WSC-NPs fed rats (H-WSC-NP), and (e) high-fat emulsions and 225 mg/kg/d WSC-NPs fed rats (L-WSC-NP). The NF group received an equivalent amount of distilled water; the HF group, received high-fat emulsions daily by oral intubation until the study ended. The other groups were administered the high-fat emulsions by oral intubation for 2 weeks to establish the hyperlipidemic condition, and then the WSC and WSC-NPs samples were administered orally to the WSC and WSC-NPs groups for 4 weeks. All groups were fed the corresponding diets in which the composition conformed to GB14924.3 (Guangdong Laboratory Animal Center, Guangzhou, China) as the basal diets during the whole experiment. Each rat was weighed once a week.

At the end of the experimental period, blood samples were withdrawn from the orbital venous plexus using a capillary tube under ether anesthesia after an overnight fast.

2.5. Serum Lipids and SOD. Blood was clotted at room temperature and centrifuged in a centrifuge at 3000 rpm for 15 min. Serum was separated, and TC, TG, HDL-C, and LDL-C were measured with commercial assay kits using the Automated Biochemistry Analyzer AMS-18 (Beijing Option Science and Technology Development Co. Ltd, Beijing, China).

The serum SOD contents were analyzed with commercially available analytical kit by the SPECORD S600 UV-Vis Spectrophotometer (Analytic Jena AG, Germany).

2.6. Plasma Viscosity. Blood samples were taken from the ocular vein using a heparinized capillary tube and centrifuged at 3000 rpm for 5 min in the Eppendorf Centrifuge 5810R (Eppendorf Co, Germany) to obtain the plasma. The plasma viscosity was measured by the Automatic Blood

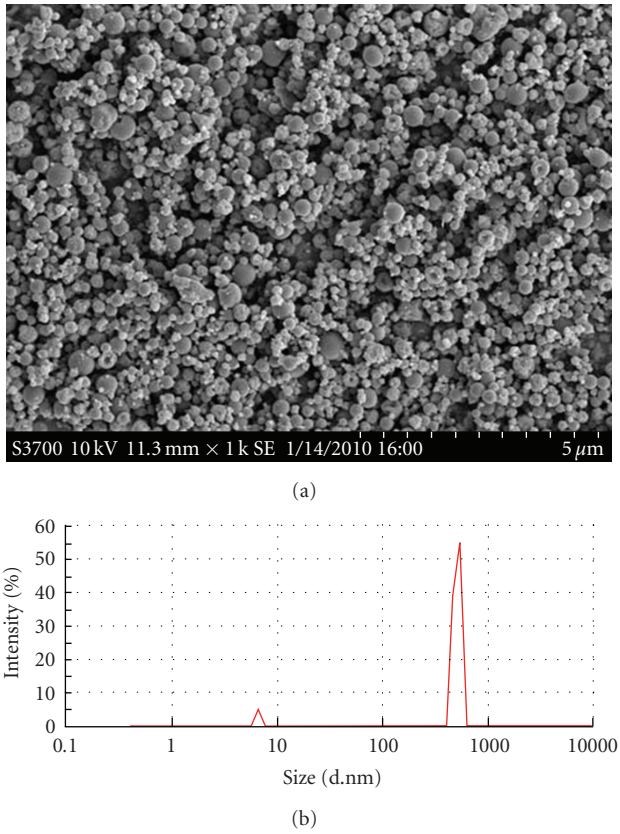

Figure 1: SEM (magnification of 1000x) microphotographs of WSC-NPs (a) and particle size distribution of WSC-NPs (b).

Rheometer LBY-N6B (Beijing Precil Instrument Co. Ltd, Beijng, China).

2.7. Statistical Analysis. All data were expressed as means±SE. Differences between the groups were determined by one-way analysis of variance, using a statistical analysis software program (SPSS for windows, version Rel, 16.0, Spss Inc, Chicago, IL); the Student-Newman-Keuls Multiple-Range Test comparisons at P value of <.05 were made to determine significant differences among means.

3. Results and Discussions

3.1. Characterizations and Stability of WSC-NPs. The microphotographs and particle size of the WSC-NPs are shown in Figure 1. All nanoparticles were found to be nearly spherical in shape, and the external surfaces appeared smooth (Figure 1(a)). The mean particles size of WSC-NPs was 650 nm variing from 500 to 800 nm (Figure 1(b)). FTIR spectra of WSC-NPs and WSC matrix show that the tripolyphosphoric groups of TPP are linked with the ammonium group of WSC; the inter- and intramolecular actions are enhanced in WSC-NPs [15].

The stability studies show that there were no detectable changes in color, odor, taste, or pH and no visible microbial growth in the WSC-NPs.

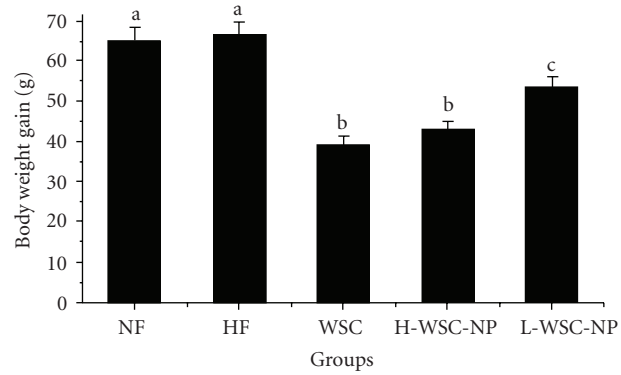

Figure 2: Effects on body weight gain in rats fed high-fat diets. Results are expressed as means ± SE of eight rats. Values marked by the different letters are significantly different ($P < .05$).

3.2. Effects on Body Weight in Rats Fed High-Fat Diets. A significantly lower weight gain was observed in all the treatment groups compared with the rats that consumed the normal diet and the high-fat diet (Figure 2), and H-WSC-NP, WSC produced lower counts compared with L-WSC-NP. However, weight gains were not significant between the H-WSC-NP and WSC groups.

Previously, WSC was shown to reduce body weight gain of rats that was caused by a high-fat diet [16]. In our study, the rats in the WSC group showed similar responses. Compared with the NF group, body weight gains of rats fed the WSC and H-WSC-NP were significantly lower than those fed the L-WSC-NP. This indicated that WSC could be used as weight-loss agent for healthy and obese humans, but further study is needed to clarify the antiobesity action and mechanisms of WSC-NPs.

3.3. Effects on Serum TC, TG, HDL-C, LDL-C, and SOD in Rats Fed High-Fat Diets. The hyperlipidemic model showing that the serum concentrations of TC, TG, HDL-C, and LDL-C increased, was established in the HF group after 2 weeks (Table 1). Compared with the HF group, the TC and TG levels in H-WSC-NP and L-WSC-NP groups were significantly decreased, showing that the effect of WSC-NPs treatment was even more powerful than the treatment of WSC group. The serum LDL-C levels in the treatment groups were significantly decreased compared with the HF group, and no significant difference was observed among the treatment groups. There were no significant difference of HDL-C levels among all the groups (Table 1). The serum SOD was increased significantly in the H-WSC-NP and L-WSC-NP groups compared with the NF and HF groups, but increased slightly in the WSC group (Table 2).

The low levels of HDL-C and the high levels of LDL-C indicated an imbalance between cholesterol transport from the liver to the extrahepatic tissues and back to the liver [17]. Moreover, dyslipidemia is characterized by increased fasting concentrations of total cholesterol (TC), triglycerides (TG), and LDL cholesterol (LDL-C), in conjunction with decreased concentrations of HDL cholesterol (HDL-C). WSC and

Table 1: Effects on serum TC, TG, HDL-C, and LDL-C in rats fed high-fat diets.

Group	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
NF	1.59 ± 0.21^{a}	0.99 ± 0.20^{d}	0.70 ± 0.07^{f}	1.03 ± 0.18^{h}
HF	2.45 ± 0.28^{b}	1.46 ± 0.23^{e}	1.19 ± 0.14^{g}	1.33 ± 0.19^{i}
WSC	1.52 ± 0.78^{a}	1.33 ± 0.37^{e}	0.62 ± 0.17^{f}	0.78 ± 0.25^{j}
H-WSC-NP	0.46 ± 0.69^{c}	1.02 ± 0.33^{d}	0.67 ± 0.26^{f}	0.67 ± 0.32^{j}
L-WSC-NP	0.33 ± 0.24^{c}	0.82 ± 0.50^{d}	0.91 ± 0.33^{fg}	0.92 ± 0.26^{j}

Values are expressed as means ± SE ($n = 8$). Values marked by different letters within the same row are significantly different ($P < .05$).

Table 2: Effects on serum SOD in rats fed high-fat diets.

Group	Serum SOD (U/mL)
NF	83.43 ± 9.78^{a}
HF	87.18 ± 14.01^{a}
WSC	92.35 ± 6.32^{b}
H-WSC-NP	107.23 ± 16.25^{c}
L-WSC-NP	98.08 ± 12.68^{b}

Values are expressed as means ± SE ($n = 8$). Values marked by the different letters within the same row are significantly different ($P < .05$).

Table 3: Effects on plasma Viscosity in rats fed high-fat diets.

Group	Plasma viscosity (mPa.S)
NF	1.47 ± 0.25^{a}
HF	1.84 ± 0.40^{b}
WSC	1.68 ± 0.18^{b}
H-WSC-NP	0.86 ± 0.18^{c}
L-WSC-NP	1.13 ± 0.50^{c}

Values are expressed as means ± SE ($n = 8$). Values marked by different letters within the same row are significantly different ($P < .05$).

WSC-NPs significantly lowered TC, TG, and LDL-C levels in the plasma, which was consistent with previous reports [18]. Plasma concentration of HDL is inversely correlated with the risk of coronary heart disease, but low HDL-C poses no risk in the absence of elevated LDL cholesterol or TC. The levels of plasma HDL-C should be increased in the WSC-NP group; however, plasma concentration of HDL is influenced by several factors, including gender, race, and diet. Furthermore, WSC-NPs exhibited better cholesterol-binding capacity than WSC. This is consistent with the previous report that chitosan with finer particle size could effectively lower the plasma and liver lipid level in rats [19].

Excessive superoxide radicals may induce lots of senile diseases such as atherosclerosis. Any elevation in the level of the SOD was accompanied by a decrease in superoxide radicals [20, 21]. In the experiment, the serum SOD was elevated significantly by feeding the H-WSC-NP and L-WSC-NP compared with NF and HF groups, and WSC could not increase the serum SOD significantly. Therefore, it seems likely that WSC-NPs may improve the hypercholesterolemia induced by the high-fat diet through reducing serum TC, TG, and LDL-C, and elevating the SOD activity.

3.4. Effects on Plasma Viscosity in Rats Fed High-Fat Diets. As shown in Table 3, by experimental design, the average plasma viscosity of rats in the HF group increases significantly compared with the average level for rats in the NF group. In the three treatment groups, plasma viscosity showed a decreasing trend. When compared with the HF group, significant differences were seen for the H-WSC-NP and L-WSC-NP groups.

Plasma viscosity played an important role in the perfusion of the microvasculature and was a major determinant of endothelial shear stress [22]. Plasma viscosity was used as a marker for different diseases in humans such as coronary artery disease atherosclerosis [23]. The rats' plasma viscosity was increased significantly by feeding the high-fat emulsions, and the WSC-NPs and WSC reduced this increase effectively. Although the plasma viscosity tended to decrease in the WSC group, the differences were not statistically significant compared with HF group. The results suggested that the mechanisms of WSC to improve TC, TG, LDL, and SOD may different with plasma viscosity. Furthermore, the WSC-NPs exhibited a better effect than the WSC, showing that WSC-NPs may serve as a useful agent for preventing hypercholesterolemia.

4. Conclusions

In conclusion, the data generated by this study demonstrated that WSC-NPs not only lower serum lipids levels and plasma viscosity but also increased serum SOD activities. Moreover, the hypercholesterolemia is affected by WSC-NPs even more than the WSC. Hence, the data obtained from this study could facilitate the further development of dietary intervention to hypercholesterolemia. Further studies are needed to clarify the mechanisms of the WSC-NPs to inhibit hypercholesterolemia induced by feeding a high-fat diet in male Sprague-Dawley rats.

To date, all rats appear healthy and remain active after oral administration of the WSC and WSC-NPs. Therefore, WSC and WSC-NPs are safe dietary fibers to inhibit hypercholesterolemia.

Abbreviations

WSC:	Water-soluble chitosan
WSC-NP:	Water-soluble chitosan nanoparticle
SOD:	Superoxide dismutase
TC:	Total cholesterol
TG:	Triglycerides
HDL-C:	High-density lipoprotein cholesterol
LDL-C:	Low-density lipoprotein cholesterol
TPP:	Tripolyphosphate.
SEM:	Scanning electron microscopy
FTIR:	Fourier transform infrared spectroscopy
NF:	Normal diet fed rats
HF:	High-fat emulsions fed rats
H-WSC-NP:	High-fat emulsions and 450 mg/kg/d WSC-NPs fed rats
L-WSC-NP:	High-fat emulsions and 225 mg/kg/d WSC-NPs fed rats.

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