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# Hypolipidaemic effects and mechanisms of the main component of *Opuntia dillenii* Haw. polysaccharides in high-fat emulsion-induced hyperlipidaemic rats

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ABSTRACT

The antihyperlipidaemic effects of ODP-Ia, one of the main components of *Opuntia dillenii* Haw. polysaccharides, were studied. Gavage administration of ODP-Ia was observed to significantly decrease serum lipid levels and to increase serum high-density lipoprotein cholesterol level in hyperlipidaemic rats. Similar suppressive patterns were also seen in hepatic total cholesterol and triglyceride levels. Moreover, the ODP-Ia administration significantly increased serum lecithin:cholesterol acyltransferase activity, increased the production of serum NO, inhibited hepatic HMG-CoA reductase activity, augmented serum and hepatic superoxide dismutase activities and decreased the serum and hepatic malondialdehyde contents in hyperlipidaemic rats. In addition, a histopathological examination revealed that ODP-Ia administration significantly suppressed inflammatory cell infiltration and the expression of VCAM-1. Together, these results indicate that ODP-Ia is a potential natural product for the treatment of hyperlipidaemia-related diseases by improving antioxidant levels, modulating the activities of enzymes involved in cholesterol metabolism, promoting the production of NO and suppressing the expression of VCAM-1, thereby suppressing lipid accumulation and inflammatory cell infiltration.



## 1. Introduction

Hyperlipidaemia is mainly characterised by increased levels of total cholesterol (TC), triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) along with a decrease in high-density lipoprotein cholesterol (HDL-C). This condition is an indicator of both coronary artery disease and artherosclerosis and is the main cause of cardiovascular disease worldwide (Lin et al., 2005). Treatment of hyperlipidaemia involves diet control, exercise and pharmaceutical therapy. However, lipid-lowering drugs, such as statins and fibrates, have adverse effects or contraindications. As a result, there continues to be a high demand for new oral anti-hyperlipidaemic drugs without any side effects. Plants play a major role in the introduction of new therapeutic agents because they have fewer toxic side effects than synthetic drugs. Additionally, plants have received attention for being sources of biologically active substances, including antioxidants, hypoglycaemics and hypolipidaemics (Harnafi, Serghini Caid, El Houda Bouanani, Aziz, & Amrani, 2008).

*Opuntia dillenii* Haw. is a cactus species that grows widely in the tropics and subtropics, including Taiwan and the south of China. This plant contains high levels of important nutrients, such as betalains, phenolics, polysaccharides, flavonoids, minerals and vitamins. *O. dillenii* Haw. has been used widely for brewing, as a vegetable and as an additive in food and fodder (Chang, Hsieh, & Yen, 2008; Medina, Rodríguez, & Romero, 2007). This plant has also been traditionally used in many countries as an herbal medicine with anti-inflammatory and antioxidative properties, as a promoter of detumescent drainage, for promoting blood circulation and for treating diabetes and bronchial asthma (Chang et al., 2008). Based on the literature and on our preliminary studies, hot water extracts of *Opuntia* enriched with polysaccharides have antioxidant activities, antidiabetic effects, wound-healing activities and immunostimulatory effects (Zhao, Lan, Huang, Ouyang, & Zeng, 2011). Recently, we observed that the gavage administration of ODP-Ia significantly decreased the fasting levels of TC and TG, increased HDL-C level in mice with STZ-induced diabetes and

*Abbreviations:* ODPs, *Opuntia dillenii* Haw. polysaccharides; ODP-Ia, *Opuntia dillenii* Haw. polysaccharide-Ia; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; AI, atherosclerosis index; HDL-C, high-density lipoprotein cholesterol; LCAT, lecithin:cholesterol acyltransferase; NO, nitric oxide; HMG-CoA, 3-hudroxy-3-methylglutaryl-coenzyme A; SOD, superoxide dismutase; MDA, malondialdehyde; VCAM-1, vascular cell adhesion molecule-1; NAFLD, nonalcoholic fatty liver disease.

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exhibited strong antioxidant properties, all of which can protect primary risk organs against hyperlipidaemia (Zhao, Lan et al., 2011). However, there is little information available regarding the anti-hyperlipidaemic effects of ODPs in hyperlipidaemic rats. Therefore, we investigated the antihyperlipidaemic effects and mechanisms of orally administered ODP-Ia (isolated in our laboratory) in a high-fat emulsion-induced hyperlipidaemic rat model.

## 2. Materials and methods

### 2.1. Plant materials and chemicals

Fresh, intact *O. dillenii* Haw. cladodes of uniform shape and maturity were collected from Donghai Island, Zhanjiang City, Guangdong Province, China. Chromatographically pure ODP-Ia (molecular weight: 60 kDa) was obtained from *O. dillenii* Haw. aqueous extracts by low-pressure chromatography as described previously (Zhao, Lan et al., 2011). The average polysaccharide content of ODP-Ia was 98.62% as measured using the optimised phenol-sulfuric acid method (Zhao, Yuan, Li, Peng, & Zeng, 2011), which mainly comprised rhamnose, arabinose, galactose, glucose and arabinuronic acid. Malondialdehyde (MDA) kit (Lot No. 20071029), superoxide dismutase (SOD) kit (Lot No. 20071010), TC, TG, HDL-C and LDL-C kits (Lot No. 20070512) and NO kit (Lot No. 20070316) were obtained from Nanjing Jiancheng Bio-Engineering Research Institute, China. The free cholesterol kit (Lot No. E1006) was obtained from Beijing Applygen Technologies Inc., China. Cholesterol, sodium deoxycholate and dithiothreitol were obtained from Sinopharm Chemical Reagent Co., China. Propylthiouracil (Lot No. 061001) and Zhibituo tablets (Lot No. 0612069) were purchased from Nantong Jinghua Pharmaceutical Co., China and Chengdu Diao Jiuhong Pharmaceutical Factory, China, respectively. Nicotinamide-adenine dinucleotide phosphate and HMG-CoA were purchased from Beijing Dingguo Biotechnology Co., China and Sigma Chemical Co., St Louis, MO, respectively. Reagents, including the primary and secondary antibodies used for detecting VCAM-1 expression, were purchased from Wuhan Boster Biological Technology Co., China. All the other chemicals and reagents used were of analytical grade.

### 2.2. Preparation of high-fat emulsion

The high-fat emulsion was prepared as previously reported (Ni, Li, Jin, Zang, & Peng, 2004). Briefly, 25 g of lard oil in a 200 mL beaker were heated on a magnetic stirring apparatus. When the temperature reached 100 °C, 10 g of cholesterol were added and melted. The oil phase was prepared by stirring 1 g of methylthiouracil into the mixture followed by the addition of 25 mL of tween-80. Simultaneously, in another 200 mL beaker, 30 mL of distilled water and 20 mL of propylene glycol were mixed and heated using an electric oven. When the temperature reached 60 °C, 2 g of sodium deoxycholate were added, and the mixture was stirred until all components were dissolved. Subsequently, the water phase prepared in this beaker was added to the oil phase and mixed thoroughly to prepare the high-fat emulsion.

### 2.3. Animals

Male Sprague-Dawley rats (age, 8–10 weeks; weight, 180–200 g) were purchased from the Centre of Experimental Animals, Guangdong Medical University, China. Throughout the experiment, the rats were housed in a room with a 12/12-h light/dark cycle at an ambient temperature of 22–25 °C. They were also given *ad libitum* access to water and, in accordance with the institutional guidelines, a commercial stock diet containing (w/w) 9% fibre, 23% protein and 65% carbohydrate together with adequate amounts of vitamins and mineral nutrients. Animal experiments were conducted according to the current ethical regulations for animal care and use. After an acclimatisation period of 1 week, the animals were used in experiments.

### 2.4. Establishment of the hyperlipidaemic rat model

Rats were fed a high-fat emulsion diet for 10 d to induce hyperlipidaemia (Miyasaka et al., 2007). Blood was then collected from the tail veins of the rats and assayed to determine the serum lipid indices (TC and TG) of each rat using commercial kits. Rats were considered to be hyperlipidaemic when their serum TC levels were >6 mM, and their serum TG levels were >3 mM; the rats had to have hyperlipidaemia before being used in the experiments.

### 2.5. Experimental design

The rats were randomly segregated into 6 groups of 10 animals each. Group I, the normal control group, was administered 10 mL/kg body weight of 0.86% NaCl twice. The other groups comprised hyperlipidaemic rats (groups II–VI). Group II rats, the model rat group, were administered 10 mL/kg body weight of the high-fat emulsion and 10 mL/kg body weight of 0.86% NaCl. Group III rats, the positive control rats, were administered 10 mL/kg body weight of the high-fat emulsion and 190 mg/kg body weight/d of Zhibituo tablets in 0.86% of NaCl. The rats in groups IV, V and VI were administered 10 mL/kg body weight of high-fat emulsion and 100, 200 and 400 mg/kg body weight of ODP-Ia, respectively. ODP-Ia was intragastrically administered once daily for 28 d, and the body weights of the rats were recorded weekly.

The rats were fasted for 8 h during the first and second weekends of the study. After the fasts, blood was drawn from the tail veins of the rats, and serum TC and TG levels were measured. The intragastric administration of the high-fat emulsion to the rats ceased on the 26th d. After 28 d of treatment, the rats were fasted for 8 h and were anaesthetised by intraperitoneal injection of pentobarbital sodium (40 mg/kg body weight). Blood was drawn from the *Aorta abdominalis*, left at room temperature for 1 h and then centrifuged at 3000 rpm (4 °C, 10 min). The serum obtained was stored at −70 °C for later biochemical analysis. Livers were dissected and immediately frozen at −70 °C for future assays involving the measurement of enzyme activities. Aortas were collected, cut into 3–5 cm segments and soaked in 10% formalin.

### 2.6. Measurement of serum lipid profiles and LCAT activity

Serum lecithin: cholesterol acyltransferase (LCAT) activity was measured according to the method of Bartholome, Niedmann, Wieland, and Seidel (1981), and the free cholesterol content in the reaction product was determined by the Sperry method using a commercial kit (Lot No. E1006).

Serum TC, TG, HDL-C and LDL-C levels were determined using commercial kits. Serum VLDL-C level and atherosclerosis index (AI) were calculated by the Friendwald formula as follows:

$$\text{VLDL-C} = 0.2\ \text{TG};\ \text{LDL-C} = \text{TC} - (\text{HDL-C} + \text{VLDL-C});\ \text{AI} = \text{TC}/\text{HDL-C}$$

### 2.7. Measurement of other biochemical indices of serums and livers

Serum NO level was measured by the nitrate reductase method (the Griess method) using a commercial kit. Serum and hepatic SOD activities and MDA contents were determined by the nitrite method and by the thiobarbituric acid method, respectively, using