Hypolipidemic Effects of Polysaccharides from Fermented Seaweed

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Abstract. To study the hypolipidemic effect of a fermented seaweed polysaccharide on experimental hyperlipidemic mice for Conducted a series of work. Algal polysaccharides can alleviate hyperlipidemia and decrease risks of atherosclerosis and cardiovascular diseases. This study aimed to investigate effects of algal polysaccharides fermented by Yeast and Lactobacillus plantarum on hematic fat level of mice fed with high fat diet. Forty mice were randomly assigned to four groups: normal control group (NC), high-fat control group (HFC), high concentration of seaweed polysaccharide (HSP), low concentration of seaweed polysaccharide (LSP), respectively. The NC group was fed with the basic diet, while the other groups were fed with high-fat diet. After the experimenta period (4W), mice were sacrificed by decapitation. Results demonstrated that fermented algal polysaccharides could regulate lipid levels, the increase in HDL-c and decrease in TG, TC and LDL-c. In conclusion, algal polysaccharides fermented by Yeast and Lactobacillus plantarum may has effect of hypolipidemic.

1. Introduction

With the improvement of living standards in modern society, people's dietary structure is changing. The incidence of hyperlipidemia has been greatly increased. Hyperlipidemia is one of the most important factors, which accelerate atherosclerosis and increase the risk of cardiovascular diseases [1, 4]. As results of that, more attention should be paid to the treatment of hyperlipidemia. Due to considerable side effects, medicine for reducing cholesterol levels was not usually advised. A novel agent without side effect is required to lower blood lipid in dietary interventions of a first-line therapy [5].

Probiotics are generally defined as an active class of microorganisms that regulate and improve the gut flora when the body is ingested in sufficient amounts from the diet to promote human health [6, 7]. A large number of literatures have confirmed that probiotics can not only regulate the structure of intestinal flora, but also regulate lipid metabolism [8, 9]. Seaweed polysaccharide is the most studied type of polysaccharides in Laminaria japonica [10, 14]. Its composition is complex and has many biological activities, including Alginate, fucoidan, brown algae starch, among which alginate has
hypolipidemic and hypoglycemic effects, and alginic acid has the effect of lowering plasma cholesterol to prevent and alleviate hyperlipidemia [15, 16].

In this study, the effects of mixed fermentation of seaweed polysaccharides from yeasts and Lactobacillus plantarum, on blood lipids in high-fat diet mice were studied, which provided a theoretical basis for the actual production and application of fermentation.

2. Materials and methods

2.1. Strain and chemicals
Cellulose and pectinase were purchased from solarbio (Beijing, China). MRS (Peptone, beef extract, yeast powder, glucose, ammonium citrate, sodium acetate, disodium hydrogen phosphate, potassium acetate, manganese sulfate) and YPD (Yeast extract, peptone, glucose) medium reagents were purchased from Dingguo Reagent Co., Ltd. (Beijing, China). TC, TG, LDL-c and HDL-c commercially available kits (Jiancheng Biotechnology, Nanjing, China). Laminaria japonica were purchased from Tulip Crown Food Co., Ltd. (Shandong, China). Issatchenka orientalis Z1 and L.plantarum LP1406 was isolated and preserved in our laboratory.

2.2. Preparation of seaweed polysaccharide
Laminaria japonica was weighed and washed into small pieces, and water was added at 1:20. Add potassium dihydrogen phosphate, acid cellulase, acid pectinase according to the ratio of raw materials, measure the pH, adjust the pH to 4.8-5.0 with hydrochloric acid, stir at 50 °C for 2 h, then add carbonic acid Sodium, alkaline pectinase, stirred at 60 °C for 1.5 h. Finally, add water, make the total system 30 times of the raw material, continue stirring, centrifuge at 3700 r/min, and obtain the supernatant to store at 4 °C for use.

After sterilization, the inoculated plant Lactobacillus and Yeast mixed bacteria were inoculated in 2%, 37°C 300r/min, and the aeration ratio was 25% continuous fermentation for 2 days. sampling, lyophilized, and powdered. The dried extract was used directly for the study.

2.3. Animal experiments
A total of Forty male ICR mice (weight 35 to 37g) obtained from Junke Bioengineering Co., Ltd. (Nanjing, China) were housed in groups of ten per standard cage on a 12 h light/dark cycle. The male was randomly assigned to four groups: normal control group (NC), high-fat control group (HFC), high concentration of fermented seaweed polysaccharide (0.24g/mL, HSP), low concentration of fermented seaweed polysaccharide (0.08g/mL, LSP) respectively. The NC group was fed with the basic diets provided with Zhejiang academy of medical sciences, while the other groups were fed with high-fat diet including 2% cholesterol, 10% egg yolk powder, 7% lard, 0.5% bile salts No.3 and 80.5% basic diets.

NC group and HFC group were gave the same volume of water, while the other groups were treated once daily with high concentration of fermented seaweed polysaccharide (0.24g/mL, 5ml/kg body weight) and low concentration of fermented seaweed polysaccharide (0.08g/mL, 5ml/kg body weight). Their body weights were monitored once a week throughout the experiment. After the experimental period (2W), Blood samples from posterior orbital venous plexus were collected to measure lipid concentration. At the end of the experiment (4W), fasting 12h, mouse was weighted and then sacrificed by decapitation. Blood samples were collected and centrifuged at 1500 g/min for 15 min at 4 °C to obtain serum. The serum was frozen at -80 °C for biochemical analyses.

2.4. Determination of serum lipid levels
Concentrations of TC, TG, LDL-c, and HDL-c in serum were measured by enzymatic and colorimetric methods using assay kits.
2.5. Statistical analysis
All parameters were recorded for individuals within all groups. All data were shown as mean ± SD. The data were analyzed by variance and Duncan’s test for multiple comparisons of SPSS ver. 15.0.

3. Results
The general effect of FSP on serum lipid levels was examined in mice fed a high-fat and -cholesterol diet. Before inducing hyperlipidemia, there was no significant difference in serum lipid levels of mice of the four groups. Changes in serum lipids of all groups after the 10 days of feeding are summarized in Table 1. The serum TC, TG, and LDL-c in HFC group were significantly higher than that in NC group (p<0.05). This indicated that hyperlipidemia was successfully induced in mice fed with high-fat diet. Changes in serum lipids of all groups after the two weeks of feeding are summarized in Table 2. The TC and TG of the FSP-L and FSP-H groups were not significantly reduced relative to HFC (p>0.05), but HDL-c was increased 24.1% (FSP-L) and 21.6% (FSP-H).

It showed that the effect of lowering blood lipids after 2 weeks in mice was not obvious. Changes in serum lipids of all groups after the four weeks of feeding are summarized in Table 3. The TC, TG, and LDL-c of the FSP-L and FSP-H groups were significantly lower than those of the HFC group, and HDL-c was increased (p<0.05). It showed that the effect of lowering blood fat after four weeks of oral administration of mice was relatively obvious. And FSP-H is better than FSP-L in lowering blood fat.

Table 1. The serum lipids in the four groups before gavage

| Group | TC (mmol/L) | TG (mmol/L) | HDL-c (mmol/L) | LDL-c (mmol/L) |
|-------|-------------|-------------|----------------|----------------|
| NC    | 3.44±0.57   | 1.89±1.20   | 1.84±0.47      | 0.83±0.28      |
| HFC   | 5.65±0.57   | 3.44±0.26   | 2.82±0.53      | 1.78±0.37      |
| FSP-L | 6.62±0.89   | 2.65±0.76   | 3.13±0.56      | 1.78±0.70      |
| FSP-H | 5.74±0.66   | 2.70±0.69   | 3.08±0.55      | 2.17±0.40      |

Table 2. The serum lipids in the four groups of two weeks

| Group | TC (mmol/L) | TG (mmol/L) | HDL-c (mmol/L) | LDL-c (mmol/L) |
|-------|-------------|-------------|----------------|----------------|
| NC    | 3.88±0.64   | 2.16±0.60   | 2.03±0.30      | 0.40±0.09      |
| HFC   | 6.46±1.63   | 2.91±0.47   | 3.11±0.36      | 1.16±0.39      |
| FSP-L | 6.01±0.72   | 2.21±0.38   | 3.10±0.30      | 0.75±0.17      |
| FSP-H | 6.43±1.03   | 2.28±0.31   | 3.46±0.58      | 0.89±0.27      |

Table 3. The serum lipids in the four groups of four weeks

| Group | TC (mmol/L) | TG (mmol/L) | HDL-c (mmol/L) | LDL-c (mmol/L) |
|-------|-------------|-------------|----------------|----------------|
| NC    | 4.21±0.59   | 2.55±0.61   | 3.31±0.56      | 0.71±0.09      |
| HFC   | 6.78±1.36   | 2.94±0.56   | 5.07±0.69      | 1.50±0.33      |
| FSP-L | 5.72±0.80   | 1.61±0.25   | 4.98±0.91      | 0.26±0.29      |
| FSP-H | 5.28±0.55   | 1.33±0.34   | 5.51±0.97      | 0.75±0.46      |

Another risk factor for developing atherosclerosis is the reduced serum level of HDL-C. This effect of HDL-C is largely attributed to its central function in the reverse cholesterol transport, a process whereby excess cell cholesterol is taken up and processed by HDL particles for further delivery to the liver for metabolism [17,18]. Therefore, it is logical that an increase in HDL level can contribute to a lower risk of atherosclerosis [17]. In the present study, the serum levels of HDL-C increased by 7.9%, when compared with HFC groups.
4. Conclusion
In conclusion, this study demonstrated that a high-fat diet caused hyperlipidemia which includes increasing TC, TG. The dietary intake of algal polysaccharides has been linked with lowering cholesterol, reducing risk of cardiovascular disease, and decreasing incidence of diabetes and atherosclerosis. In our research, it was found that high concentration of fermented seaweed polysaccharide has a more significant effect on the reduction of blood lipid levels in mice. This is manifested in the increase of HDL-c and the decrease of TG, TC and LDL-C. Seaweed polysaccharides have many biological activities. Alginate has hypolipidemic and hypoglycemic effects, and brown alginic acid has reduce blood cholesteric action. The blood lipid regulating effect of brown algae polysaccharide and the structure of its polysaccharide sexuality. Seaweed polysaccharides can also be considered as a dietary fiber, which lowers blood fat. Its role may be related to the nature of its dietary fiber. This experiment has not been able to do further research, it is not yet clear.

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