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Hypoglycemic effect of abandoned *Porphyra haitanensis* polysaccharides in alloxan-induced diabetic mice

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ABSTRACT

The therapeutic effects and preliminary mechanism of abandoned *porphyra haitanensis* polysaccharides (APHP) in diabetic mice induced by alloxan were studied in this paper. The intragastric administration of APHP for 21 days induced an obvious decrease ($P < 0.05$) in blood glucose levels, compared with untreated diabetic mice. The data of total cholesterol (TC), total triglyceride (TG), low-density lipoprotein cholesterol (LDL), high-density fatty acids (HDL) and activities of antioxidant enzymes (SOD, GSH-Px and GSH) indicated that APHP had beneficial effects on the improvement in the activities of antioxidant and serum lipid levels. The index of viscera and histopathological examination revealed that APHP could alleviate the damage of tissues in diabetic mice. Noteworthily, we found APHP could promote the regeneration of damaged pancreatic islets by stimulating β-cell proliferation, which was directly related to the hypoglycemic effect. Taken together, our findings suggest that APHP could be developed into potential oral hypoglycemic drug with lesser side effects.

1. Introduction

Porphyra haitanensis is one of the most widely cultivated economic seaweed crops in china. It contains abundant dietary fiber, protein, active substances and trace elements essential for the human body. It has been grown mainly in south of china along the coast, and it has been used as consumption and medicine for hundred of years. We found that *Porphyra haitanensis* growers usually do not collect the last batch of *Porphyra haitanensis* (we call it abandoned *Porphyra haitanensis*) because of its poor taste and bad color. The last batch of *Porphyra haitanensis* can not bring any income for the vegetable growers. When it was abandoned into the sea, it caused another problem: environmental pollution. So if we can use the last batch of *Porphyra haitanensis* effectively, it can not only solve the environment problem, but also bring economic benefit to the vegetable growers. On the other hand, with the development of social economy, improvement of people's living standard and transformation of lifestyle, the rate of diabetes mellitus is increased notably. The prevention and cure measures of diabetes mellitus are one of the most important health problems, and seeking for efficient medicine of diabetes is more and more important. Diabetes is a metabolic disease caused by the endocrine disorder, involving multiple organs and targets damage (Cesur, Corapcioglu, Gursoy, Gonen, Ozduman, Emral, & Kamel, 2007). At present the clinical oral glucose-lowering drugs are widely used, but the medicines exist limitation (decrease the blood glucose only) and adverse effects. There is no drug that can thoroughly cure diabetes.

Polysaccharide is a very important active substance to our body, many research indicate that polysaccharide has multiple bioactivities, such as antioxidant (Chen, Jin, Zhang, & Yang, 2013; Hu, Gen, Zhang, & Jiang, 2001; Liu, Zhou, Lin, Jia, Deng, Fan, & Zhang, 2010), antitumor (Borges, De Barba, Schiebelbein, Pereira, Chaves, Silveira, & Wisbeck, 2013; Qiu, Huang, Huang, Pan, & Zhang, 2010; Ramachandran, Jeya, Moon, Lee, Kim, Kim, & Lee, 2010; Yu, Ming, Kaiping, Zhixiang, Liquan, Jingyu, & Fang, 2010), anti-virus (Chen, Xie, Yang, Liao, & Yu, 2010; Tian, Li, Song, Zheng, & Li, 1995; Yang, Jia, Yue, Cheng, Zhang, Huang, & Mei, 2013; Yang, Jia, Zhou, Pan, & Mei, 2012), *etc.* Meanwhile, some studies found that *Saccharina japonica* polysaccharide (Wang, Jin, Zhang, Hou, Zhang, & Zhang, 2013; Li, Yu, Long, Guo, & Duan, 2012), *Spirulina* polysaccharides (Jia and Yin, 2007) and other natural seaweed polysaccharides have hypoglycemic effect, with no side effects. Accordingly, the development of seaweed polysaccharides for reducing blood glucose has drawn tremendous concerns, but the hypoglycemic effect about abandoned *Porphyra haitanensis* polysaccharide has not been reported.

The research indicates that, compared with high-yield *P. haitanensis* at the third harvest, low value *P. haitanensis* showed a dramatic decrease in the contents of total sugar (34.57%) and polysaccharides (20.67%), a similar ash content (10.37%) and K/Na

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ratio of 6.3. Polysaccharide extracted from low-value *P. haitanensis* consisted of a single component with a 28.54 kD molecular weight, which was mainly constituted by galactose (83.12%), 3,6-anhydrogalactose (15.71%) and sulfate group (10.61%). (Chen, Xu, Pan, & Gao, 2011).

In this paper, we extracted polysaccharide from the abandoned *Porphyra haitanensis* and detected the hypoglycemic effect of abandoned *Porphyra haitanensis* polysaccharide (APHP is short for it) in diabetic mice.

2. Materials and methods

2.1. Materials and reagents

Abandoned *Porphyra haitanensis* was obtained from Jinshan agricultural development Co., LTD in Nanao, Guangdong province, China.

Male Kunming mice weighing 22 ± 3 g used for experiment, were purchased from Xiamen University Laboratory Animal Center.

Alloxan was purchased from Sigma–Aldrich (St. Louis, MO, USA).; Blood glucose test strips were purchased from Tai hin biotechnology development Co., Ltd; Reagent kits for the determination of superoxide dismutase (SOD), malondialdehyde (MDA), Glutathione peroxidase (GSH-Px), glutathione (GSH) and total antioxidant capacity(T-AOC) were purchased from Nanjing Jiancheng Bioengineering Institute; Total cholesterol (TC), triglycerides (TG), high-density fatty acids (HDL) and low density fatty acids (LDL) test kits were purchased from Beijing North Kangtai clinical Reagent Co., Ltd.

2.2. Methods

2.2.1. Preparation of abandoned Porphyra haitanensis polysaccharides (APHP)

The abandoned *Porphyra haitanensis* was washed and dried immediately by forced air circulation at 50–60 °C, and milled to pass an 80 mesh sieve for polysaccharide extraction.

20 g powder was refluxed with 50 volumes of 80% ethanol for 2 h at 80 °C, collected the residuals and washed by 80% hot ethanol for 3 times, dried in a water bath at 80 °C. The residuals was extracted with 50 volumes of distilled water for 5 h at 100 °C. The combined aqueous extracts were filtrated and concentrated to one-third of volume in a vacuum rotary evaporator. The concentrated solution was then precipitated with 3 volumes of anhydrous ethanol overnight at 4 °C. The precipitates were collected by centrifugation (4000 g 10 min), and then dissolved in warm water. Proteins were removed by using the Sevage method (Hu, Yuan, Yan, Huang, & Yu, 2011; Ikekawa, Ikeda, & Fukuoka, 1975; Xie, Zhang, Peng, & Gan, 2011). The supernatant of polysaccharides was dialyzed in running water for 48 h and distilled water for 24 h and vacuum freeze dried (Liu, Yang, Deng, Yang, & Wu, 2005; Zhang, Chen, & Wang, 2000), the polysaccharide extracts APHP was obtained.

2.2.2. Diabetes model establish

Animals were acclimatized to feed with normal forage for 7 days. The experimental mice were weighed and fasted for 12 h with normal drinking water, then injected with 1% Alloxan 200 mg/kg bw to the enterocoelia, after 72 h, fasting blood glucose was determined by glucose meter. Mice with blood glucose value > 11.1 mmol/L were considered diabetic (Yu, Cui, Zeng, Xie, Liang, Lin, & Zeng, 2009).

2.2.3. Experimental design and sample collecting

The diabetic mice were randomly divided into five groups ($n=10$), and normal mice ($n=10$) were used as the control.

Group 1: Normal group, normal mice treated with 0.5 mL saline.

Group 2: Model group, diabetic mice treated with 0.5 mL saline.

Group 3: Positive group, diabetic mice treated with 20 mg/kg of glibenclamide.

Group 4: Low-dose group, diabetic mice treated with 100 mg/kg of APHP.

Group 5: Middle-dose group, diabetic mice treated with 200 mg/kg of APHP.

Group 6: High-dose group, diabetic mice treated with 400 mg/kg of APHP.

After 21 days of treatment, the body weight and blood glucose levels were measured. The blood samples were collected and centrifuged for 5 min at 3000 r/min to obtain serum, viscera samples were collected, washed by normal saline and dried by filter paper, then weighted. A portion of serum and viscera samples were stored at -20 °C for biochemical analysis.

2.2.4. Biochemical estimations

The content of TC, LDL, HDL, TG in serum and T-AOC and antioxidant enzyme (SOD, GSH-Px and GSH) in serum, liver, kidney were measured using kits. All kits were used following manufacturer's instruction. The index of various viscera is the ratio of viscera weight to mouse weight.

2.2.5. Statistical analysis

All results were expressed as mean $\pm$ SD. Data were analyzed by one-way analysis of variance (ANOVA) using SPSS. P values less than 0.05 were considered significant.

3. Results

3.1. Effect of APHP on body weight and blood glucose in diabetic mice

In this experiment, after injected with 1% Alloxan for 72 h, the mice showed typical diabetes symptoms: polyuria, polyphagia, polydipsia and weight loss. Their pelage color and lustre were gloomy, and spirit were poor.

The body weight and blood glucose levels of different groups are shown in Table 1. We found that the mice modeled successfully exhibited an extremely significant increase ($P < 0.01$) in fast blood glucose and a significant loss of body weight ($P < 0.05$), compared with the normal control group. The administration of the APHP for 21 days caused a significant decrease in blood glucose levels ($P < 0.05$) in diabetic mice (Table 1).

3.2. Effect of APHP on blood lipid of diabetic mice

As depicted in Table 2, the concentrations of TC, TG and LDL in the serum of the model group were increased, and LDL concentration was significantly increased ($p < 0.05$), whereas serum HDL decreased, compared with the normal group. When treated with APHP (200 mg/kg and 400 mg/kg) for 21 days, a significant reduction in the contents of TC and LDL and an increased HDL levels in the serum were observed.

3.3. Antioxidative effect of APHP

A significant increase ($P < 0.05$) in the levels of MDA in the serum associated with a marked diminution ($P < 0.05$) of activities of SOD and GSH-Px in the serum, liver and kidney, and T-AOC in