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Hypoglycemic property of acidic polysaccharide extracted from *Saccharina japonica* and its potential mechanism

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ABSTRACT

In the present study, a sulfated polysaccharide fucoidan extracted from *Saccharina japonica* was administered to normal and alloxan-diabetic rats/mice, and its effects on glycemia, insulin and serum lipid levels were evaluated. Fucoidan administered at 200 or 1200 mg/kg body weight/day could significantly reduce the blood glucose level by 22% and 34%, respectively, in alloxan-induced diabetic rats. Serum insulin levels in diabetic mice were increased by the administration of fucoidan ($P < 0.05$). The results of an oral glucose tolerance test (OGTT) revealed that fucoidan treatment had some effect on glucose disposal after 15 days of treatment. Furthermore, fucoidan altered plasma lipid levels by lowering cholesterol, triglyceride and plasma low-density lipoprotein concentrations, while elevating plasma high-density lipoprotein cholesterol at 100 or 300 mg/kg body weight/day. The results suggested that fucoidan exhibited a considerable hypoglycemic effect, possibly by stimulating pancreatic release of insulin and/or by reducing insulin metabolism. Our results indicated that fucoidan could be developed as a potential oral hypoglycemic agents or functional food for the management of diabetes.

1. Introduction

Diabetes is a hereditary, chronic metabolic disease characterized by hyperglycemia that results from an absolute or relative deficiency of insulin secretion, impaired insulin action, or both (Alberti & Zimmet, 1998). It is the third most life-threatening disease, and research and development of drugs for diabetes and its complications have been receiving increased attention. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, currently available drugs are limited by side effects and toxicity (Egger, Davey Smith, Stettler, & Diem, 1997). Therefore, it is important to investigate the hypoglycemic actions of plants that were originally used in traditional medicine (Jung et al., 2006; Prabhakar & Doble, 2008).

Recently, many researchers have found that polysaccharides extracted from Chinese traditional plants and edible/medicinal fungi exhibit hypoglycemic activity. Kiho et al. found acidic polysaccharides extracted from either the fruiting body or mycelia of various edible/medicinal fungi, including *Tremella aurantia*, *Cordyceps sinensis*, and *Lentinus edodes*, exhibited significant hypoglycemic activity in mice with streptozotocin-induced and genetic diabetes (Hwang et al., 2005; Kiho, Morimoto, Sakushima, Usui, Ukai, 1995; Kiho et al., 2000; Kiho et al., 2001). Cho et al. reported that exopolysaccharides produced by submerged mycelial culture of two different mushrooms, *Tremella fuciformis* and *Phellinus baumii* substantially reduced the plasma glucose levels in ob/ob mice, possibly by regulating PPAR-γ-mediated lipid metabolism (Cho et al., 2007). Man et al. found that the *Astragalus* polysaccharide exhibited insulin-sensitizing and hypoglycemic activities in type 2 diabetic rats through the alleviation of endoplasmic reticulum stress and insulin resistance under hyperglycemic conditions (Mao et al., 2009). In addition, Chen et al. showed that a polysaccharide isolated from the root of *Ophiopogon japonicus* could significantly reduce blood glucose levels and increase the insulin levels (Chen et al., 2011).

Sulfated polysaccharides are considered to be an attractive class of compounds as drug candidates (Baba et al., 1990; Witvrouw & De Clercq, 1997). Algal sulfated polysaccharides have been reported to possess diverse biological activity of potential medicinal value, such as anticoagulant, antitumour, anti-inflammatory, antiviral and antioxidant activity (Feldman, Reynaldi, Stortz, Cerezo, & Damonte, 1999). The comparative study of hypoglycemic effects on mice showed that the sulfation of polysaccharides significantly improved hypoglycemic activity (Wang et al., 2010). Several

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seaweed-derived polysaccharides have been reported to include hypoglycemic capacity among their biological activities. Lamela et al. found that crude polysaccharides from *Himanthalia elongate* caused a significant reduction in blood glucose 8 h after intravenous administration (Lamela, Anca, Villar, Otero, & Calleja, 1989). The brown seaweed, *Saccharina japonica*, is a common seafood in China and many other countries, and has been documented as a drug in traditional Chinese medicine for over a thousand years. Fucoidan extracted from *Saccharina japonica* is an acidic sulfated polysaccharide, mainly made of fucose, galactose and sulfate, with smaller amounts of mannoses, glucuronic acid, glucose, rhamnose, arabinose and xylose. Recently, substantial pharmaceutical research has been conducted on fucoidan. This research has demonstrated a strong antioxidant activity of fucoidan, which is now being marketed as a nutraceutical and food supplement (Clement et al., 2010; Kim, Lee, & Lee, 2010; Wang, Zhang, Zhang, & Li, 2008). Free-radical-induced lipid peroxidation has been associated with a number of diseases, including diabetes mellitus (Feillet-Coudray et al., 1999), and antioxidants may prevent the development of diabetes (Low, Nickander, & Tritschler, 1997). Researchers have found that polysaccharides with strong antioxidant activity could reduce the blood glucose level in normal rats, STZ-induced diabetic rats and alloxan-induced diabetic rats (Li et al., 2006). Hu et al. found the *Hedysarum polybotrys* polysaccharide could ameliorate hyperglycemica through increased insulin secretion and inhibition of lipid peroxidation (Hu, Li, Zhao, Feng, & Wang, 2010). Nevertheless, there is a lack of reports regarding the hypoglycemic effects of fucoidan in diabetic animal models.

The objective of the present study is to investigate the hypoglycemic effect of FPS on normal and alloxan-diabetic rats. The effect of the FPS on serum lipid alloxan-diabetic rats is also studied. In addition, insulin activation was studied in response to treatments with FPS.

2. Materials and methods

2.1. Materials

Saccharina japonica, was cultured in Shazikou, Qingdao, China, was collected in March 2012. The fresh algae was soon washed, sun dried and kept in plastic bags at room temperature for use.

2.2. Preparation of natural polysaccharides

Generally, 100 g dry algae were cut roughly and autoclaved in water at 115–125 °C for 3 h. The hot aqueous solution was separated by successive filtration with gauze and siliceous earth. The solution was dialyzed against tap water for 48 h and against distilled water for 48 h, and then concentrated under reduced pressure. The polysaccharides were precipitated by the addition of 75% (v/v) ethanol. The resultant precipitate was washed three times with dry ethanol, and then dried to give polysaccharide, named FPS (yield 2.3%).

2.3. Analytical methods

The total sugar content of FPS was determined according to the method of Michel Dubois, Hamilton, Rebers, and Smith (1956) using L-fucose as a standard. Sulfate content was analyzed by the barium chloride-gelatin method of Kawai, Seno, and Anno (1969). Uronic acid was estimated in a modified carbazole method using D-glucuronic acid as a standard (Bitter & Muir, 1962). Neutral sugar composition was HPLC chromatography (Honda et al., 1989). Plasma glucose levels were determined using an enzymatic colorimetric assay (Barham & Trinder, 1972). Serum lipid levels were measured using an automatic biochemistry analyzer. Serum insulin was measured using the double-antibody method (Bailey & Ahmed-Sorour, 1980).

2.4. Comparative study on hypoglycemic activity of the FPS in alloxan-induced diabetic rats

Wistar rats (180–220 g), after fasting for 24 h, were injected with a freshly prepared aqueous solution of alloxan monohydrate (50 mg/kg body wt., i.p.) according to the method of Kameswara et al. with minor modifications (Kameswara Rao, Kesavulu, Giri, & Appa Rao, 1999). After the injection (3 days), the rats were checked for fasting blood glucose (FBG) concentration, and rats with marked hyperglycemia (FBG > 10 mmol/L) were used for the study. Fifty diabetic rats were separated into five groups of ten animals each. The 1st group received distilled water (5 mL/kg, body wt., i.g.) as a control; the 2nd group received meltrol (100 mg/kg body wt., i.g.) as a positive control; the animals from the other three groups were received FPS (100, 300 and 600 mg/kg body wt., i.g.). One group with 10 normal mice received distilled water (5 mL/kg, body wt., i.g.) as a blank. The animals were treated daily for 18 days. The blood glucose level were monitored in trace blood samples from the tail vein after 3 h of fasting and 2 h of administration at the sixth, twelfth and eighteenth days, and the body weights were recorded the next day. The animals fasted for 24 h at the twentieth day, after FPS administration for 2 h, the blood samples were taken immediately by excising the eyeballs, CHOL (cholesterol), TG (triglyceride), HDL (high density lipoprotein) and LDL (low density lipoprotein) levels were measured.

2.5. Effect of FPS on the blood glucose and serum insulin levels in alloxan-induced diabetic mice

The Kunming mice (20–30 g), after fasting for 18 h, were injected with a freshly prepared aqueous solution of alloxan monohydrate (60 mg/kg body wt., i.p.) as reported previously (Kameswara Rao et al., 1999). Seventy-two hours post-injection, blood glucose levels were determined in all mice following a 4 h fast. Fifty mice were separated into 5 groups of 10 animals each. All animals were treated daily for 14 days. The 1st group received distilled water (1 mL/kg, body wt., i.g.) as a control; the 2nd group received glybenzcyclamide (25 mg/kg body wt., i.g.) as a positive control; and the animals from the other three groups received fucoidan (200, 600 and 1200 mg/kg body wt., i.g.). One group with 10 normal mice received distilled water (1 mL/kg, body wt., i.g.) as a blank. Blood glucose levels were determined after 12 h of fasting, 2 h after the last administration of fucoidan or glybenzcyclamide. Blood samples were taken immediately by excising the eyeballs of the sacrificed animals, and serum insulin levels were determined.

2.6. Effect of FPS on blood glucose levels in healthy mice (OGTT)

Thirty Kunming mice (20–30 g) were divided into three groups of ten animals each. All animals received oral dosing daily for 14 days. Group 1 received distilled water (10 mL/kg body wt.) as a control; group 2 received meltrol (200 mg/kg body wt.) as a positive control; and group 3 received 1200 mg/kg body wt. of FPS. Blood samples were drawn from the tail of the animals before the first administration and 0.5 h, 1.0 h and 2.0 h after the last administration of meltrol or FPS. Glucose levels were determined by the method described above.

2.7. Data statistical analysis

All the data are shown as the means ± SD ($n = 3$) with significance asserted at $p < 0.05$ using Duncan's multiple-range test. Data were processed with Excel and Statistica (2003).

Table 1
Chemical composition (%, dry weight) of FPS isolated from *Saccharina japonica*.

Sample	Fucose	Uronic acid	Sulfate	Neutral sugar						
				Fuc	Gal	Man	Glc	Arb	Rha	Xyl
FPS	29.12	1.93	33.01	62.08	24.33	6.06	1.93	5.60	nd	nd

3. Results and discussion

3.1. Chemical analysis

The chemical composition of FPS was shown in Table 1. The result showed that the main chemical components of FPS were fucose and sulfate, along with uronic acid and a small amount of protein. The fucose content in FPS was 29.12%, the sulfate content in FPS was 33.01%. Meanwhile, constituents of neutral monosaccharide of the FPS were analyzed by HPLC. Results showed that fucose is the main sugar form accounting for 62.08% of total neutral sugar in FPS. Additional to fucose and galactose, mannose, glucose, and arabinose are also seen in the FPS, which shows that chemical property may have great influence on hypoglycemic activities, and has been proved in this research.

3.2. Comparative study on hypoglycemic activity of the fucoidan in alloxan-induced diabetic rats

The effects of FPS on blood glucose level in alloxan-induced diabetic rats are shown in Fig. 1. The daily administration of FPS (300, 600 mg/kg) for 12 and 18 days in alloxan-induced diabetic rats caused a significant reduction in the blood glucose level when compared with the diabetic control group ($P < 0.05$). The mean percentage decrease in blood glucose levels caused by FPS at doses of 300 and 600 mg/kg were 16.45% and 22.03%, respectively, at 12 days and 18.85% and 21.14%, respectively, at 18 days. The results showed that the higher dose (F-600) was slightly more effective in reducing blood glucose level in alloxan-induced diabetic rats than the lower doses (F-300 and F-100). However, no significant differences were observed between administration for 12 and 18 days.

Furthermore, the effect of FPS on body weight in alloxan-induced diabetic rats was assessed (Fig. 2). The body weight of alloxan-induced diabetic rats was significantly lower than with normal rats at 12 and 18 days, and this was not significantly altered by FPS treatment. Because side effects of long-term insulin treatment include increases in body weight and exacerbation of insulin resistance in diabetes, FPS may provide a preferable alternative for the control of blood glucose in diabetes.

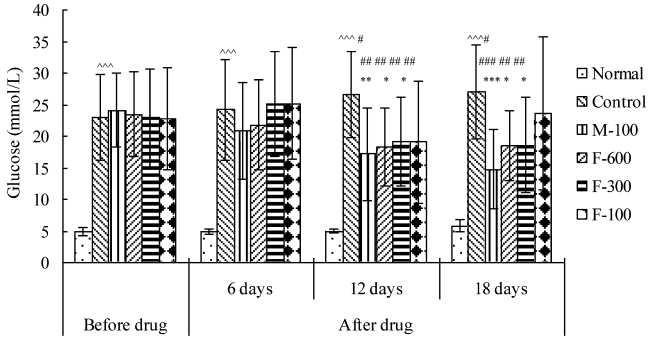

Fig. 1. Blood glucose levels in alloxan-induced diabetic rats after 6, 12, and 18 days treatment with FPS and meltrol. ^^^, $P < 0.001$ (vs normal group); $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (vs control group); $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ (vs before treatment in the respective group); (M-100: meltrol, 100 mg/kg body wt., i.g.; F-100, 300 and 600: FPS, 100, 300 and 600 mg/kg body wt., i.g.).

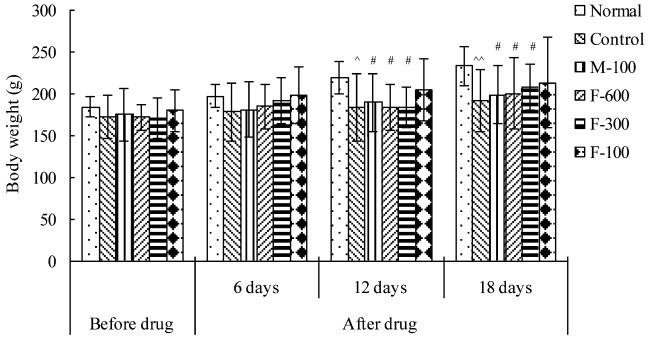

Fig. 2. Body weight in alloxan-induced diabetic rats after 6, 12, and 18 days treatment with FPS and meltrol. $^{\wedge}P < 0.05$, $^{\wedge\wedge}$, $P < 0.01$ (vs normal group); $^{*}P < 0.05$ (vs control group); $^{\#}P < 0.05$ (vs before treatment in the respective group) (M-100: meltrol, 100 mg/kg body wt., i.g.; F-100, F-300 and F-600: FPS, 100, 300 and 600 mg/kg body wt., i.g.).

Fig. 3 shows the effects of FPS on serum lipid concentrations in alloxan-induced diabetic rats. After FPS was administered for 20 days, CHOL in the F-100 mg/kg was significantly higher compared with the control group, however, the other groups showed no significant change.

Compared with the normal and control group, TG in the alloxan-induced diabetic rats showed no significant difference, however, TG in the positive control group was significantly higher. None of the FPS administration groups showed significant effects on TG levels. Administered FPS elevated HDL lever, and F-300 group exhibited lower the LDL levers to some extent ($P < 0.05$) compared with the control group. In a word, there was little effect of FPS on serum lipid concentrations in alloxan-induced diabetic rats.

3.3. Effect of fucoidan on blood glucose levels in healthy mice

The results of the effect of FPS on blood glucose levels in healthy mice are shown in Fig. 4. There was no significant difference in blood glucose levels among all groups before administration, however, blood glucose decreased after FPS administration 1 h later (that was to sugar after hour). Blood glucose levels in the

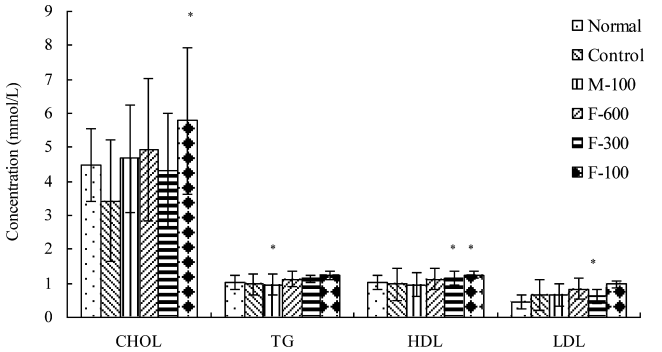

Fig. 3. Serum lipid concentration in alloxan-induced diabetic rats after 18 days treatment with FPS and meltrol. $^{*}P < 0.05$ (vs control group) (M-100: meltrol, 100 mg/kg body wt., i.g.; F-100, F-300 and F-600: FPS, 100, 300 and 600 mg/kg body wt., i.g.).

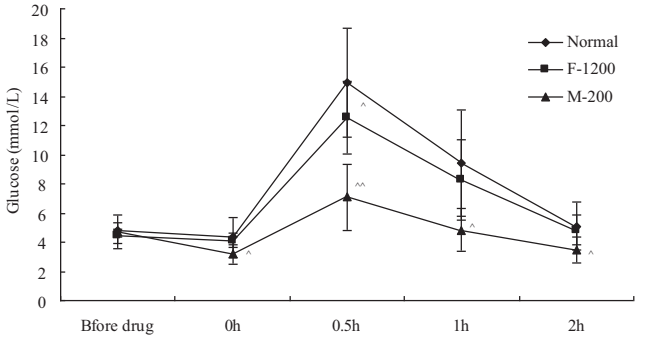

Fig. 4. The oral glucose tolerance in normal mice after treatment with FPS and meltrol. $^{\wedge}P<0.05$, $^{\wedge\wedge}P<0.01$ (vs normal group); (M-200: meltrol, 200 mg/kg body wt., i.g.; F-1200: FPS, 1200 mg/kg body wt., i.g.).

positive control group decreased significantly. Half an hour after sugar administration, the blood glucose had reached the peak in all groups, and blood glucose levels began to fall 1 h after sugar administration. Two hours after sugar administration, blood glucose concentrations were back to normal levels or were lower than before the administration. The blood glucose values in the FPS administration group were lower than those of the normal group at 0.5 h, 1 h and 2 h after sugar administration ($P<0.05$ at 0.5 h). The blood glucose values in the positive control group were significantly lower than in the normal group, in 0–2 h after sugar. FPS treatments stimulated an increase in glucose disposal, suggesting an improvement in glucose tolerance after 15 days of fucoidan treatments, presumably due to an increase in insulin sensitivity.

3.4. Effect of fucoidan on the blood glucose and serum insulin levels in alloxan-induced diabetic mice

The results of FPS on the blood glucose and serum insulin levels in alloxan-induced diabetic mice are shown in Fig. 5. Compared with the normal group, the blood glucose level was significantly higher and the insulin level was significantly reduced in the control group, suggesting the alloxan-diabetic mouse model was a success. The blood glucose level was reduced and the insulin level was increased in FPS administered groups. Blood glucose levels were significantly lower and insulin levels improved significantly in the F-200 and the F-1200 group; however, the effect of the F-600 group was not significant, suggesting that its hypoglycemic effect was partly related to elevated serum insulin levels. Positive drug had no significant effect on blood glucose and serum insulin levels.

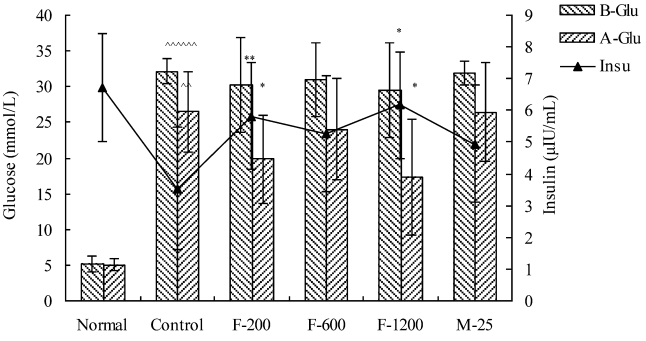

Fig. 5. Blood glucose and insulin levels in alloxan-induced diabetic mice after 14 days treatment with FPS and meltrol. $^{\wedge}P<0.01$, $^{\wedge\wedge}P<0.001$ (vs normal group); $^{*}P<0.05$, $^{**}P<0.01$ (vs control group) (M-25: meltrol, 100 mg/kg body wt., i.g.; F-200, F-600 and F-1200: FPS, 200, 600 and 1200 mg/kg body wt., i.g.).

4. Conclusion

In summary, our study demonstrated that FPS had a strong hypoglycemic effect in rats, as well as some effect on the normal glucose tolerance. FPS could enhance the serum insulin levels significantly in alloxan diabetic mice, suggesting that its hypoglycemic effect partly related to elevate serum insulin levels. The results suggest FPS could be considered as a potential candidate for developing a new anti-diabetic agent.

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