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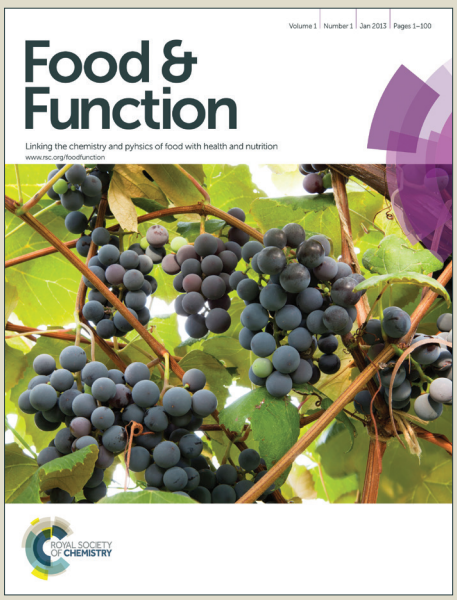

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ARTICLE

Hypoglycemic effect of polysaccharide (GLP) from *Gracilaria lemaneiformis* and its degradation products in diabetic mice†

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Gracilaria lemaneiformis can be large-scale cultivation in China for industrial production of agarose, natural polysaccharides, which have been shown many beneficial bioactivities such as antitumor, antiviral and antioxidant activities *etc*. In the present study, the hypoglycemic and antioxidant effects of polysaccharides extracted from *Gracilaria lemaneiformis* (GLP; Mw, 121.89 KDa) and its chemically degraded products (GLP1 and GLP2; Mw, 57.02 and 14.29 KDa respectively) were investigated in alloxan-induced diabetic mice. The intragastric administration of GLP, GLP1 and GLP2 for 21 days induced an obvious decrease ($P < 0.05$) in blood glucose levels in comparison to untreated diabetic mice. Furthermore, GLP, GLP1 and GLP2 caused evident increases ($P < 0.05$) in both anti-oxidases (SOD and GSH-Px) activities and the total antioxidant capacity (T-AOC) and a significant decrease ($P < 0.05$) in the level of malondialdehyde (MDA) in the liver, pancreas and kidney of diabetic mice. Even though GLP, GLP1 and GLP2 did not show significant differences in structure and sulfation levels, GLP1 demonstrated more potent effects than GLP and GLP2 at the same dose. Histopathological examination of the pancreas and kidney revealed that the damaged tissues induced by alloxan were repaired to a certain degree after the treatments of GLP, GLP1 and GLP2.

Introduction

Diabetes mellitus with high rates of morbidity and mortality is one of the most common and significant of chronic diseases in modern society[1]. It can be classified into type-1 (insulin-dependent) and type-2 diabetes (non-insulin-dependent). Type-2 diabetes is characterized by peripheral insulin resistance and impaired insulin secretion, which accounts for about 90% of the disease; moreover, it is often associated with lipid and lipoprotein disorders[2-6]. Insulin resistance is characterized by impairment in insulin-regulated metabolic actions, including glucose transport, glycogen synthesis and gene expression characteristics and has been a key factor in the onset and progress of type-2 diabetes[7]. Therefore, ameliorating insulin resistance is an important strategy for the development of new pharmacological treatment for the disorder[8]. Clinical pharmacological treatment of diabetes is predominantly based on oral hypoglycemic agents. However, continuous use of synthetic anti-diabetic drugs is associated with side-effects and toxicity[9,10]. Consequently, alternative medicines and natural therapies have received renewed attention in recent decades and have stimulated a new research interest in traditional practices.

Gracilaria lemaneiformis distributes widely in marine environment and belongs to the family *Gracilariaceae* (*Rhodophyta*); it is utilized mainly in the preparation of agar[11], which consists of two different components: agarose and agaropectin. Agarose is a neutral polysaccharide with a linear structure of repeated units of disaccharide agarobiose, which consists of D-galactose and 3,6-anhydro-L-galactose[12]. The sulfated polysaccharide accounts for about 30 percent of *Gracilaria lemaneiformis* dry weight[13]. Further research results show that arabinogalactan, a high viscosity gelatinous polysaccharide belonging to the soluble dietary fiber, can delay intestinal digestion of carbohydrates and reduce the rate of absorption, therefore preventing the rapid increase of blood glucose levels. Sulfated polysaccharides from seaweed also have immune regulatory, antitumor, antiviral, antioxidant and several other physiological activities[14-17]; therefore, it is very interesting and important to study natural polysaccharides and their potential application.

In the previous studies, we found that *Gracilaria lemaneiformis* polysaccharides could significantly inhibit the growth of transplanted tumor in mice, promote splenocyte proliferation, macrophage phagocytosis, and increase the level of IL-2 and the percentage of $CD8^+$ T cells in blood of tumor-bearing mice. These results indicated that the *Gracilaria lemaneiformis* polysaccharides could improve both specific and non-specific cellular immune responses and suggested that the anti-tumor activity of *Gracilaria lemaneiformis* polysaccharides might act through immune-regulating mechanism[18]. In addition, *Gracilaria lemaneiformis* possesses various bioactive functions such as antimutagenic, antitumor, antiviral, antioxidant, anticoagulant and immunomodulation effects[14,18,19].

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However, the hypoglycemic activity of the *Gracilaria lemaneiformis* polysaccharide has never been examined. In the present study, the hypoglycemic effect of GLP was investigated through the oral administration of GLP solution for an extended time using alloxan-induced diabetic mice as a model. Since the molecular weight of GLP is high (about 121.89 KDa) and its solution viscous, the large, intact molecule of GLP might not be absorbed well in the intestine. To investigate the effect of lower molecular weight products, GLP was degraded through a chemical degradation method using ascorbic acid (Vc) mixed with hydrogen peroxide (H_2O_2) to yield two lower molecular weight products (GLP1, GLP2) with lower viscosity. In summary, our study evaluated the hypoglycemic and antioxidant effects of *Gracilaria lemaneiformis* polysaccharide (GLP) and its degradation products (GLP1, GLP2) in alloxan-induced diabetic mice. The study provides a solid basis for the development of new healthy food products and clinical medicines.

Results

Preparation and chemical properties of GLP, GLP1 and GLP2

Our previous study showed that polysaccharide content isolated from *Gracilaria lemaneiformis* (GLP) was 98.2%, determined by phenol sulfuric acid method, containing only 0.63% proteins, determined by Bradford method[20].

Partial degradation of GLP produced two products, GLP1 and GLP2. The average molecular weights (Mw) of GLP, GLP1 and GLP2, determined by gel chromatography, were 121.89, 57.02 and 14.29 KDa, respectively (Table 1).

Table 1 Chemical properties and molecular weight of GLP, GLP1 and GLP2

Samples	Sulfate ester (%)	3, 6-AG (%)	Molecular weight (KDa)
GLP	8.24±0.01	41.20±0.09	121.89
GLP1	8.12±0.03	40.30±0.04	57.02
GLP2	8.14±0.01	35.60±0.06	14.29

Note: 3, 6-AG means 3, 6-anhydro-L-galactose.

Compared to GLP, the content of sulfate radical of GLP degradation products did not change significantly, which is favorable for maintaining GLPs physiological activities (Table 1). On the other hand, the content of 3,6-anhydro-L-galactose decreased slightly in GLP1, but dramatically in GLP2 (Table 1), suggesting hydroxyl radicals used in the chemical degradation might have attacked the 3,6-anhydro-L-galactose, and thus resulting in its removal from the sugar chains.

Infrared spectroscopic analysis of GLP, GLP1 and GLP2

IR spectra of GLP, GLP1 and GLP2 are shown in Fig.1, which shows a typical strong and wide peak around 3400 cm^{-1} for O-H, a peak about 1080 cm^{-1} for C-O, a characteristic peak around 2920 cm^{-1} for C-H, and a peak around 1620 cm^{-1} for N-H-C-O. The absorption peaks about 1250 cm^{-1}, 930 cm^{-1}, and 890 cm^{-1} indicate the existence of sulfuric acid base, 3, 6-anhydro-L-galactose and β-D-glucopyranose, respectively, suggesting that chemical degradation did not lead to loss of important functional groups.

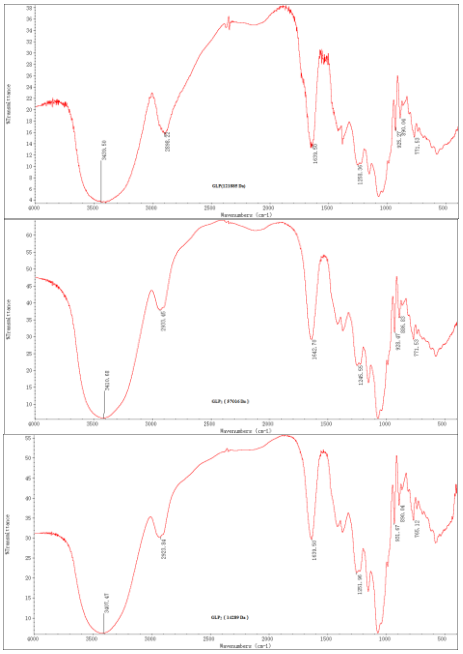

Fig. 1 Infrared spectroscopy of GLP, GLP1 and GLP2

Inhibitory effect of GLP, GLP1 and GLP2 on the activity of α-glucosidase

Inhibition of the α-glucosidase activity was determined in an *in vitro* assay. The agonistic acarbose was used as a positive control.

The results showed that, similar to acarbose, GLP, GLP1 and GLP2 were inhibitory to the α-glucosidase activity. Amongst the three GLPs, GLP1 exhibited the strongest inhibitory activity, which was comparable to acarbose, especially when the concentration was above 4 mg/mL (Fig. 2).

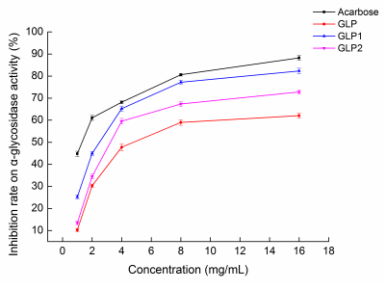

Fig.2 The inhibitory effect of GLP, GLP1 and GLP2 on the activity of α-glucosidase

Hypoglycemic effect of GLP, GLP1 and GLP2

The body weight and blood glucose levels of different experimental groups are shown in Table 2. We found that alloxan-induced diabetic mice exhibited an extremely significant increase ($P < 0.01$) in fasting blood glucose and a significant loss of body weight ($P < 0.05$) when compared with the normal control group.

The administration of the GLP (200 mg/kg bodyweight) and its degradation products (200 mg/kg bodyweight) for 21 days caused a significant decrease in blood glucose levels ($P < 0.05$) in diabetic mice, and the effect of GLP1 was more effective than those of GLP and GLP2 (Table 2).

Table 2 Hypoglycemic effects of the GLP, GLP1 and GLP2 in diabetic mice

Groups	Body weight (g)		Blood glucose level(mmol/L)	
	0d	21d	0d	21d
NC	33.15 ±2.56	36.23 ±2.45	6.25 ±0.39	6.78 ±0.44
DM	28.69±3.83[a]	31.57±4.82[a]	19.31±3.57[a]	19.80±5.12[a]
GLP	27.26±2.29[a]	32.08±3.06	18.85±4.62[a]	12.38±4.21[b]
GLP1	28.45±3.22[a]	31.36±3.24	18.32±3.55[a]	10.12±2.55[b]
GLP2	28.52±2.58[a]	30.45 ±3.12	18.61±2.77[a]	12.21±3.28[b]
PC	26.25±3.18[a]	31.23±2.46	18.23±4.26[a]	9.93±3.17[b]

Note: NC, Normal control mice; DM, Diabetic mice; GLP, Diabetic mice treated with GLP; GLP1, Diabetic mice treated with GLP1; GLP2, Diabetic mice treated with GLP2; PC, Diabetic mice treated with Glibenclamide. Values are expressed as mean±SD (n=10). One-way ANOVA with repeated measures followed by Duncan's multiple rang tests were used to calculate statistical significance. [a] Indicates statistical significance ($P < 0.05$) compared to the Normal Control group. [b] $P < 0.05$ compared to the Diabetic Model group.

Antioxidative effect of GLP, GLP1 and GLP2

Significant increase ($P < 0.05$) in the level of malondialdehyde (MDA) associated with a diminution of activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) was observed in the liver and kidney of diabetic mice (ESI Table 3†).

Intragastric administration of GLP, GLP1 and GLP2 to diabetic mice significantly increased the activity of antioxidants (SOD and GSH-Px) in the blood, liver and kidney ($P < 0.05$), leading to significantly increased total antioxidant capacity (T-AOC) ($P < 0.05$) and decreased MDA level ($P < 0.05$) (ESI Table 3†). It is noteworthy that GLP1 showed a stronger antioxidant activity than GLP, GLP2 and Glibenclamide.

Effect of GLP, GLP1 and GLP2 on pancreas and kidney

Large number of pancreatic cells were destroyed in diabetic mice, causing a shrunken, deformed and hyperemic morphology (Fig. 3A:b). After administration of GLP, GLP1 and GLP2 to diabetic mice, pancreatic injury was partly repaired (Fig. 3A:d ～ f), as evidenced by the regeneration of pancreatic islets. Moreover, GLP1 was more potent in the restoration of pancreatic structure.

In the kidney of alloxan-induced diabetic mice, there appeared renal tubular atrophy, tissue hemorrhage, cell necrosis and inflammatory infiltration (Fig. 3B:b). After administration of GLP, GLP1 and GLP2, kidney tissues were partly repaired, especially in GLP1 treated mice (Fig. 3B:d～f).

Discussion

Previous studies have demonstrated that *Porphyra* polysaccharide, *Carrageenan* polysaccharide and other red algae polysaccharides have hypoglycemic activity[21,22]. These studies revealed that the physiological activities of algae polysaccharides were mainly affected by the molecular weight of the polysaccharide, the content of the polysaccharide sulfate group and the spatial conformation[23-25]. The medium molecular weight polysaccharides in the range of 1×10^5~ 4×10^5 Da have shown the highest activity. The low molecular weight polysaccharides in the range of 5×10^3~1×10^4 Da have no biological activity. It was suggested that if polysaccharide molecular weight is too low, it cannot form an active polymer structure for the biological activity, and if molecular weight is too high, it would be difficult to pass through the cell membranes to play its biological role. As in a certain range of molecular weight, the antioxidant activity of polymer agar polysaccharide was increased with the decrease of molecular weight[26]. Polysaccharide activity and viscosity also have a certain relationship. If the viscosity is too high, it is not conducive to polysaccharide drug absorption and diffusion. For an example, Schizophyllan has anti-tumor activity, however, it is not useful for the clinical therapy because of its high viscosity in its original form, and later through the partial degradation, the lower molecular weight products have less viscosity and can be used in the clinical therapy[27].

In this study, a chemical method using ascorbic acid induced hydrogen peroxide to generate hydroxyl free radicals (•OH) was used for the degradation of polysaccharide extracted from *Gracilaria lemaneiformis*. The degradation reaction is rapid and effective, and the molecular weight of its degradation products falls within a narrow range. Two GLP degradation products (GLP1 and GLP2) with different molecular weights were prepared by controlling the concentration of degradation agents. Most importantly, the sulfuric acid group of GLPs was not damaged, showing that the chemical method is suitable for the degradation of algal polysaccharides with a high molecular weight.

The pathogenesis of diabetes mellitus is mainly caused by insulin hyposecretion from pancreatic β-cells, which results in disorders of metabolism, and further affects the normal function of many organs and tissues due to hyperglycemia[8,20]. Others and we have speculate

that the antioxidant defense system was disordered and antioxidant enzyme activity was changed in diabetic mice[20,28,29].

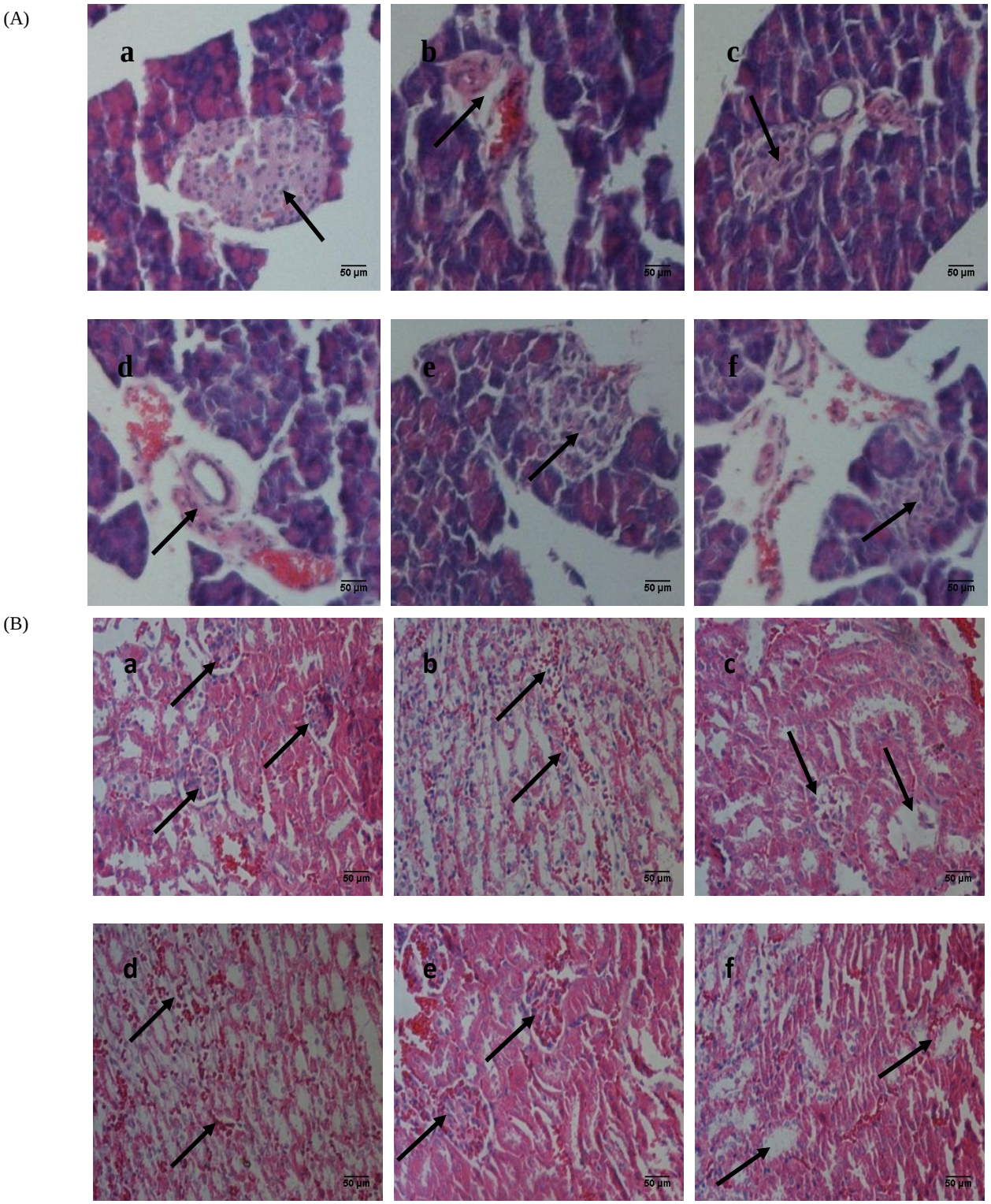

Fig. 3 Histopathological features of pancreas (A) and kidney (B) in the experiments. Pancreas and Kidney tissues were stained with H&E (400×, 200×, respectively). a. Normal control mice; b. Diabetic mice; c. Diabetic mice treated with Glibenclamide ; d. Diabetic mice treated with GLP; e. Diabetic mice treated with GLP1; f. Diabetic mice treated with GLP2.

Alloxan is widely used to establish experimental diabetic animal models. As a strong oxidant, alloxan enhances the generation of the reactive oxygen species (ROS) from metabolic reactions in the body. The reactive oxygen species reacts with unsaturated fatty acid on cell membrane to generate lipid peroxide, which destroys the structure of membranes, and receptors leading to altered transport function. Szkudelski has suggested that the mechanism of action in β-cells of the pancreas is mediated by reactive oxygen species (ROS). The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β-cells[30]. In alloxan-induced diabetic mice, the membrane structure and DNA of pancreatic β-cells are damaged, resulting in the apoptosis and necrosis of pancreatic β-cells and insulin synthesis disorder, and finally, the appearance of overt symptoms of diabetes mellitus[31]. Diabetes mellitus is associated with generation of reactive oxygen species (ROS) leading to oxidative damage particularly in the liver and kidney[32,33].

In the present study, the administration of GLP and its degradation products for 21 days in diabetic mice caused a significant decrease in blood glucose levels ($P < 0.05$) when compared with untreated diabetic mice. The decrease in blood glucose of the GLP1 group was more obvious than in the GLP and GLP2 groups. The administration of GLP, GLP1 and GLP2 showed itself to be more effective than that of the positive drug glibenclamide control group.

We observed an evident increase ($P < 0.05$) in the level of MDA associated with a diminution of activities of SOD, GSH-Px in the liver and kidney of diabetic mice, confirming a strong correlation between oxidative stress and diabetes occurrence. An administration of the GLPs for 21 days significantly increased the antioxidant enzymes (SOD and GSH-Px) activity in the blood, liver and kidney tissues, with a concomitant increase of the total antioxidant capacity (T-AOC) and decrease of the MDA level, suggesting that GLPs could reduce reactive oxygen free radicals and therefore improve the activities of antioxidant enzymes. Other studies have also confirmed that SOD, GSH and GSH-Px catalyze the dismutation of superoxide radicals to produce H_2O_2 and molecular oxygen[34], hence diminishing the toxic effects of radicals. The observed decrease in SOD activity could result from inactivation by H_2O_2 or by glycation of enzymes[35]. The detection of the antioxidant enzyme activity level can be used as an evaluation standard for the animal's antioxidant capacity. MDA is a final product of lipid peroxidation, therefore, the content of MDA can be evaluated as the lipid peroxidation degree *in vivo*.

Our previous studies confirmed that *Gracilaria lemaneiformis* polysaccharides (GLPs) possessed a capacity to scavenge free radicals *in vitro*[19]. From the experiment results, it was suggested that the activity of agar polysaccharides increased with the decrease in their molecular weight by the moderate degradation. Agar polysaccharides in a certain lower molecular weight range penetrate more easily through cell membranes to perform their biological functions. However, the activities of polysaccharides decreased with the further degradation, because the significantly lower molecular weights damage the bioactive spatial structure.

Our experiment for GLP and its degradation products showed that though all of them could inhibit the activity of α-glucosidase, GLP1, with its intermediate molecular weight exhibited the strongest inhibitory activity. The inhibitory effect was similar to acarbose at the same concentration, particularly when GLP1 concentration was more than 4 mg/mL. Thus, in order to enhance the inhibitory activity of polysaccharides, it is important to control the molecular weight of these polysaccharides.

Experimental

Materials and reagents

Samples of *Gracilaria lemaneiformis* were collected from the Nan-ao island of China from October to December 2012. The material was sorted, washed and dried immediately by forced air circulation at 50-60 ℃.

Ascorbic acid was purchased from Xilong (Shantou, China), hydrogen peroxide was obtained from Guanghua (Shantou, China), p-nitrobenzene-a-D-glucoside was purchased from Merck (Darmstadt, Germany), acarbose was purchased from Bayer (Leverkusen, Germany), α-glucosidase and alloxan were purchased from Sigma (Steinheim, Germany). Glibenclamide was purchased from Tianjin Pacific Pharmaceutical (Tianjin, China). Blood glucose test strips, reagent kits for the determination of SOD, MDA, GSH-Px and total antioxidant capacity (T-AOC) were purchased from Nanjing Jiangcheng Biological Engineering Institute (Nanjing, China). All other chemicals were of the highest commercial grade available on the domestic market.

Preparation of *Gracilaria lemaneiformis* polysaccharides (GLPs)

Algal powder (diameter, 250μm) was extracted with 90-fold volumes of distilled water for 5 h at 90 ℃. After centrifugation to remove residues (7000 rpm, 10 min), the supernatant was concentrated to one-third of volume in a vacuum rotary evaporator. The concentrated solution was then precipitated with 3 volumes of the absolute ethanol overnight at 4 ℃. The precipitates were collected by centrifugation (3500 g, 10 min), and then resolved in warm water. Proteins were removed by using the Sevag method[36]. The supernatant of polysaccharides was dialyzed in distilled water for 72 h and vacuum freeze dried.

Preparation of polysaccharide degradation products (GLP1, GLP2)

The degradation process was adopted based on the reported method[26] and our previous experiment. The degradation time was determined as follows: ascorbic acid (Vc) was mixed with hydrogen peroxide (H_2O_2) in a same concentration ratio (1:1) to prepare the degradation solution with a concentration of 0, 3 or 9 mmol/L. GLP solution (2.5 mg/mL) was then added into the degradation solution for 2 h. The polysaccharide solution was dialyzed to remove degradation agents in distilled water for 72 h. Three samples with different molecular weights of polysaccharides (GLP (121.89 KDa), GLP1 (57.02 KDa) and GLP2 (14.29 KDa)) were obtained using different concentrations of degradation agents, respectively. The final samples were dried by vacuum freezing.

Chemical properties and molecular weight determination of GLP, GLP1 and GLP2

The content of polysaccharide was determined using the phenol-sulfuric acid method with D-galactose as the standard substance. The polysaccharide sulfate radical content was determined by the Barium sulfate turbidimetric method[37]. In addition, the content of 3, 6-anhydro-L-galactose in the polysaccharide was quantified according to the resorcinol colorimetric method[37, 38].

Gel chromatography was used for the determination of polysaccharide molecular weight. The column was filled with SephadexG100 gel and equilibrated with NaCl solution (0.05 mol/L). Blue Dextran 2000 (1 mg) and standard Dextran (1 mg, molecular weight: 10000, 20000, 40000, 70000 Da), were dissolved in 1 mL distilled water, and loaded into the column, which was then eluted with NaCl solution (0.05 mol/L). The eluent was collected and the content of Blue Dextran measured by using the phenol-sulfuric acid method. A standard curve was drawn according to the logarithm equation: $Kav = (Ve-V_0) / (Vt-V_0)$, in which Kav is the logarithm of molecular weight, and the Ve, V_0, V_t are the elution volume of standard Dextran, Blue Dextran 2000, water, respectively. The elution volume of each polysaccharide sample (V_S) was determined and used to calculate the average molecular weight according to the standard curve.

Infrared spectroscopy analysis of GLP, GLP1 and GLP2

GLP, GLP1 and GLP2 were tableted with KBr, then scanned between 400～ 4000 cm^{-1} with a FTIR Avatar 360 spectrometer.

Inhibition effect of GLP, GLP1 and GLP2 on α-glucosidase *in vitro*

The α-glucosidase activity was measured using a reported method[39] with some modifications.

Glutathione solution (3 mmol/L, 25 μL) in phosphate buffer solution (0.1 mol/L, 200 μL, pH 6.8) was incubated in water (37 ℃) for 10 min, before adding 100 μL of α-glucosidase (10 μL/mL), 100 μL of distilled water and 100 μL of p-nitrophenol-alpha-D-glucopyranoside (PNPG) solution (1 mmol/L). After another 10 min incubation, 5 mL Na_2CO_3 solution (0.01 mol/L) was added to stop the reaction. After cooling to room temperature, the absorbance of *p*-nitrophenol (PNP) (A_0) was measured at 400 nm. The solution without enzymes and PNPG was used as the blank.

To determine the inhibition effects on α-glucosidase activity, 100 μL of GLPs in different concentrations were added into the above reaction mixture. Distilled water was used as the negative control, while acarbose solution was used as the positive control. According to the following equation, the inhibition rates of GLPs on α-glucosidase activity were calculated.

$$\text{Inhibition rate (\%)} = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100$$

A_0, A_1 and A_2 are absorption value of distilled water, polysaccharides and acarbose as the inhibition agent, respectively.

Animal and experimental design

The same mice described in the previous study[20] were used in the current study. All mice were kept at 25℃ in an atmosphere-controlled room. In total of sixty Kunming mice weighting 22±3 g were used in the experiment. The study was approved by the Animal Experimental Center of Shantou University. All experimental procedures were in accordance with the guidelines of experimental animal administration.

Mice were divided into two groups, 50 in the alloxan-induced diabetic group and 10 in the uninduced group. The mice in diabetic group were induced with an intraperitoneal injection of alloxan (1%) prepared freshly in distilled water at a dose of 200 mg/kg body weight after 12 h fasting as described[20]. Blood samples were drawn from the tail vein of the overnight fasted mice and glucose levels were measured with blood glucose test strips (Jiancheng Biological Engineering Institute, Nanjing, China) seventy-two hours later. Mice with a blood glucose values greater than 11.1 mmol/L were considered diabetic[39,40].

The 10 uninduced mice as normal control group (Group I (NC)) received intragastric administration of 0.5 mL physiological saline. The alloxan-induced diabetic mice were randomly divided into five groups: Group II (DM), Group III (GLP), Group IV (GLP1), Group V (GLP2) and Group VI (PC). Diabetic mice of five groups received intragastric administration of 0.5 mL physiological saline, 200 mg/kg body weight of GLP, GLP1, GLP2, and 20 mg/kg body weight of Glibenclamide (Pacific Pharmaceutical, Tianjin, China) as the positive control, respectively. After 21 days of treatment, body weight and blood glucose levels were measured and blood samples were collected and immediately centrifuged for 5 min at 3500 g at 4 ℃ to obtain serum for biochemical analysis. After necropsy, liver and kidney were sampled and stored at -70 ℃.

Biochemical and histomorphometric analysis

The biochemical analyses were performed as previously described[20]. The superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), total antioxidative capacity (T-AOC) and malondialdehyde (MDA) in blood, liver and kidney tissues were measured using commercially available kits (Jiangcheng Biological Engineering Institute, Nanjing, China) and according to the manufacture's instructions.

The liver and kidney samples were fixed for 48 h in 4% methanol, dehydrated by passing successfully in different mixtures of ethyl alcohol-water, cleaned in xylene and embedded in paraffin. Sections of liver and kidney (5 μm thick) were prepared and then stained with hematoxylin and eosin dye, which were mounted in neutral deparaffinated xylene (DPX) medium for microscopic observations[41,42].

Statistical analysis

Statistical analyses were carried on as previously described using the statistical package "SPSS 12.0 for Windows"[20]. Data was analyzed using one-way analysis of variance (ANOVA), where $P < 0.05$ was regarded as significant. Significant values were assessed further using Duncan's multiple range test. All data were presented as mean ±standard deviations (SD).

Conclusions

In conclusion, the present investigation showed that GLP and its degradation products (GLP1, GLP2) possess a potent antioxidant activity, which may be directly or indirectly responsible for its hypoglycaemic properties. Therefore, GLPs, especially those with an optimum molecular weight, should be considered as anti-hyperglycemic candidates for further studies on diabetes.

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