The relationship between diabetes and selenium (Se) compounds has been certified. The aim of our experiment was to use carrageenan and sodium selenite to produce \( \kappa \)-selenocarrageenan oligosaccharides (SCOs), which should have antidiabetic and antioxidative effects. The reaction was carried out at 60°C under acidic conditions. The product was characterized with UV spectroscopy, NMR spectroscopy, infrared spectroscopy, flame atomic absorption spectrometry (FASS) and thin layer chromatography (TLC). Our results showed that some sulfated groups on the site of the 4-sulfated-\( \beta \)-D-galactose units (G4) were replaced by sodium selenite and produced SCOs. The SCOs was used as a Se supplement in alloxan-induced diabetic rats, and its antidiabetic and antioxidative effects were examined. We found that SCOs could reduce the glucose concentration and restore the alloxan-induced damage to the pancreas islet, potentially via its antioxidative activity. In conclusion, we prepared \( \kappa \)-selenocarrageenan oligosaccharides with antidiabetic and antioxidative activities.

**KEY WORDS:** Antidiabetic; Antioxidative; Carrageenan; \( \kappa \)-Selenocarrageenan Oligosaccharides (SCOs).

Consequently, a deficiency in Se would provoke impairment of GSH-Px activity and result in peroxidation stress in the body, thereby increasing the risk of degenerative diseases (Chan et al., 1998; Hartman et al., 2002; Levander and Burk, 1994; Papp et al., 2007; Tato Rocha et al., 1994; Tapiero et al., 2003; Zeng and Combs, 2008). Diabetes is known as one of many degenerative diseases specifically related to the impaired homeostasis of certain elements such as selenium (Marcason, 2008; Navarro-Alarcon et al., 1999b; Simonoff and Simonoff, 1991). More recently, the origin of cell damage undergone by diabetics was attributed to free radicals (Figuerola, 1992). Hence, it is important for diabetic patients to supplement selenium and increase the antioxidative capability of the body. Selenium exists both in inorganic and organic forms. Due to its low physiological activity, high toxicity and mutagenic action, the utilization of inorganic selenium is restricted in the biomedicine field. The production of inorganic selenium with nontoxic and highly antioxidative properties is therefore crucial for treating degenerative disease such as diabetes.

Carrageenan is the main cell wall component of various marine red algae. This anionic polymer belongs to a family composed of alternating 3-linked \( \beta \)- and 4-linked \( \alpha \)-galactopyranose. The anionic polymers differ in the occurrence of an \( \alpha \)-3, 6-anhydro bridge in the 4-linked residues and in the number and position of sulfate esterification per repeating disaccharide. Carrageenan has been regarded as a safe food additive by the Food and Drug Administration in the United States. Due to its special structure and presence of a sulfated group, carrageenan has shown various biological activities, such as antiviral, antitumor and anticoagulant properties (Cáceres et al., 2000; Carlucci et al., 1997; Duarte et al., 2001; Noda et al., 1990). Carrageenan is a large molecular saccharide, and its poor dissolubility and absorbability restrict its application in the biomedical field. However, an oligosaccharide of carrageenan has more potential in biomedical area with respect to good dissolubility, absorbability and higher biological activities. Some researchers have shown that oligocarrageenans have antioxidative activity
**MATERIALS AND METHODS**

**Materials**

κ-carrageenan was obtained from Jiangsu ChangHang Hydrocolloid Scientific Co. Ltd (Jiangsu China). Sixty-five-week-old female mice, weighing 20±3 g, were purchased from the Experimental Animal Center of Dalian Medical University and maintained in a standard animal room for a week before the experiment started. All other reagents were of analytical grade.

**Preparation of κ-selenocarrageenan oligosaccharides**

Carrageenan (1 g) and sodium selenite (1 g) were dissolved in 100 ml of 0.5% sulfuric acid solution. The reaction system was heated to 60°C and stirred for 4 h. The reaction was quenched by adding 10% of sodium hydroxide solution; the pH of this system was adjusted to 7 at room temperature. The product was condensed by vacuum pump and then dialyzed in a 500-Da cut-off bag filter. κ-selenocarrageenan oligosaccharides (SCOs) were obtained by freeze-drying the product of dialysis.

**Measurement of the Se content in SCOs**

The Se content in SCOs was measured by the method of flame atomic absorption spectrometry (FASS). A Carl Zeiss (Jena, Germany) Model AAS3 flame atomic absorption spectrometer equipped with a 10-cm air-acetylene burner head assembly and an IBM-PC compatible computer was used throughout the study. A Se hollow cathode lamp was used as the radiation source. No background correction was required in this mode of operation. Operating parameters were set according to the reference method protocol of Matusiewicz and Krawczyk (2007).

**UV spectroscopy, NMR spectroscopy and infrared spectroscopy**

Sodium selenite (1 mg), κ-carrageenan oligosaccharides (1 mg) and κ-selenocarrageenan oligosaccharides (1 mg) were dissolved in 1 ml of distilled water each. The absorption of the solutions was measured by a UV spectrometer set at a wavelength range of 190 nm to 300 nm. For NMR analysis, κ-carrageenan oligosaccharides (50 mg) and κ-selenocarrageenan oligosaccharides (50 mg) were dissolved in 500 μl of high-quality D₂O (99.96%), containing 0.1 μl acetone. 1H-NMR experiments were carried out at 500 MHz and 13C-NMR at 150 MHz at 35°C on a Bruker Avance DRX-500 (Bruker Co., Ltd., Switzerland) spectrometer with a 5-mm 1H probe.

An infrared spectrum of κ-carrageenan oligosaccharides (0.5 mg) and κ-selenocarrageenan oligosaccharides (0.5 mg) was taken on a Perkin-Elmer instrument (PerkinElmer Instrument Co., Ltd. USA) as KBr pellets at room temperature.

**Measurement of the average molecular weight of SCOs and TLC assay**

The average molecular weight of SCOs was assayed with the method of 3,5-dinitrosalicylic (DNS). Briefly, 1 mg of SCOs was dissolved in 1 ml of distilled water and boiled with 4 ml of DNS solution for 10 min. After cooling to room temperature (25°C), the mixed solution was transferred to a 96-well microplate and analyzed spectrometrically at 530 nm with the microplate reader. The molar concentration of SCOs was obtained according to the standard curve prepared with a series of dilutions (0-20 mol/ml) of lactose by the DNS method, and the average molecular weight was calculated by the mass of SCOs divided by its molar concentration.

The SCOs, carrageenan and lactose were developed on TLC using a solvent system of n-butanol/acetic acid/H₂O in a ratio of 2:2:1 (v/v). The SCOs was visualized with an amidobenzendiphenylamine solution at 80°C for 20 min.

**Diabetic rats and experimental groups**

Diabetic rats were induced with a single intravenous injection of alloxan (32 mg/kg, bw; Sigma), dissolved in physiological saline. Five days later, the plasma glucose concentration was determined in blood samples obtained from rats after being fed. Non-diabetic (<14.7 mmol glucose/l) or extremely diabetic (>35.5 mmol glucose/l) rats were excluded from this study. Diabetic rats were randomly allocated to four groups (n=12 per group). Three groups were used as experimental groups, with intragastric administration of SCOs supplied at a dose of 50 mg/kg/day, 200 mg/kg/day or 500 mg/kg/day for 14 days. One group of diabetic and normal rats was used as two control groups and was treated with physiological saline.

**Test of glucose concentration and antioxidative activity in serum**

After the treatment of SCOs for 14 days, glucose, superoxide dismutase (SOD) activity, glutathione peroxidase enzyme activity (GSH-Px) and the content of malondialdehyde (MDA) in rat sera were measured using commercial kits purchased from Nanjing Jiancheng Co., Ltd. of Nanjing University.

**Histopathological observations**

After treatment with SCOs for 14 days, the animals were killed and their pancreases removed. The organs were fixed in a formalin solution for 24 h and then embedded in paraffin. Sections were cut at a 5-μm thickness and stained

**in vivo and in vitro** (Mou et al., 2003; Yuan et al., 2005), demonstrating that the antioxidative mechanism of the selenated compound is different to that of the selenium compound. Free radicals are harmful to diabetic patients and cause the reduction of Se concentrations in the serum (Navarro-Alarcon et al., 1999b; Schlienger et al., 1988; Simonoff and Simonoff, 1991; Tawardowska Saucha et al., 1994). We therefore designed our experiment to prepare selenocarrageenan oligosaccharides (SCOs) using κ-carrageenan as a safe starting material while expecting the product to share both the properties of the oligocarrageenan and the selenium compound. We use the SCOs as a Se supplement for diabetic rats and examined its hypoglycemic and antioxidative activities.
with hematoxylin and eosin. The sections were then viewed under a light microscope to detect eventual histopathological changes.

**Statistical analysis**

The results are presented as the mean ± standard deviation (SD). The data followed a normal distribution. A two-way analysis of variance was performed using a Student’s t-test. P < 0.05 was selected as a statistically significant difference, and P < 0.01 was selected as an extremely statistically significant difference.

**RESULTS**

**Characteristics of κ-selenocarrageenan oligosaccharides (SCOs)**

Under the acidic conditions, κ-carrageenan not only reacted with sodium selenite to produce κ-selenocarrageenan but also was hydrolyzed to oligosaccharides. Thus, the final products of the reaction were κ-selenocarrageenan oligosaccharides (SCOs). SCOs are a pink powder that could not dissolve in organic solvent. The DNS result shows that the average SCOs molecular weight is 1.43 kDa. According to the results of flame atomic absorption spectrometry, the Se content of the SCOs is 30 μg/mg. Figure 1 displays the result of thin-layer chromatography (TLC): SCOs is seen to distribute between lactose and carrageenan but is closer to lactose.

**FIGURE 1. TLC of a κ-selenocarrageenan oligosaccharides (SCOs) sample.** A: Lactose; B: κ-selenocarrageenan oligosaccharides (SCOs); C: κ-carrageenan.

The results of UV spectrum analysis are shown in Figure 2. There is no absorption peak between 190 nm and 300 nm for the oligocarrageenan, but sodium selenite has one absorption peak at 210 nm. SCOs also have an absorption peak at 210 nm, similar to that of sodium selenite, indicating that the product of SCOs contains the Se element.

**FIGURE 2. UV spectrum of a κ-selenocarrageenan oligosaccharides (SCOs) sample.**

Infrared spectra of oligocarrageenan and SCOs are shown in Figure 3. Clearly, the infrared spectra of SCOs are similar to that of oligocarrageenan. The absorption band at 850 cm⁻¹ indicates that the sulfate group is attached to the C-4 position of galactose. However, this absorption band shifted to 857.72 cm⁻¹ in the SCOs infrared spectral data. This shift may be caused by the selenious group reacted on the oligocarrageenan.

The ¹H NMR and ¹³C NMR spectra of oligocarrageenan and SCOs are given in Figure 4 and table 1. The ¹H NMR and ¹³C NMR spectra of oligocarrageenan are consistent with previously published data (Tojo et al., 2003; Van de Velde et al., 2004). The ¹H NMR and ¹³C NMR spectra of SCOs are similar to that of oligocarrageenan, indicating that the base structure of SCOs is similar to that of oligocarrageenan. In the ¹H NMR spectrum of SCOs, the signal at 4.87 ppm was attributed to the anomic proton of the 4-sulfated-β-D-galactose units (G4). However, another new signal at 4.85 ppm was observed in the ¹H NMR spectrum of SCO compared to that of oligocarrageenan (Fig 4).

**FIGURE 3. Infrared spectra of oligocarrageenan and κ-selenocarrageenan oligosaccharides.** A: Oligocarrageenan; B: κ-selenocarrageenan oligosaccharides.
of SCOs (showed in Fig 4 by red arrow and in Table 1). Taken together, the NMR spectra of SCO indicate that some sulfated groups were replaced by the selenious group on the site of the 4-sulfated-β-D-galactose unit.

FIGURE 4. NMR spectrum of κ-oligocarrageenan and κ-selenocarrageenan oligosaccharides (SCOs). A: 1H NMR spectrum of κ-oligocarrageenan; B: 1H NMR spectrum of SCOs; C: 13C NMR spectrum of κ-oligocarrageenan; D: 13C NMR spectrum of SCOs.

The antioxidant activity of SCOs for diabetes rat

The superoxide dismutase (SOD) activity, glutathione peroxidase enzyme activity (GSH-Px) and content of malondialdehyde (MDA) in the serum of experimental rats were measured after treatment with different doses of SCOs for 14 days; these results are shown in Figure 6. SCOs enhanced the SOD and GSH-Px activity in rat sera after treatment for 14 days. For SOD, the highest enhancement activity was observed

Table 1. The chemical shifts of oligocarrageenan and κ-selenocarrageenan oligosaccharides (SCOs) in 13C-NMR spectra

<table>
<thead>
<tr>
<th></th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
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<td>102.81</td>
<td>70.02</td>
<td>78.75</td>
<td>73.61</td>
<td>75.10</td>
<td>61.61</td>
</tr>
<tr>
<td>SCOs</td>
<td>102.80</td>
<td>70.01</td>
<td>78.73</td>
<td>71.10</td>
<td>75.10</td>
<td>61.60</td>
</tr>
<tr>
<td></td>
<td>A1</td>
<td>A2</td>
<td>A3</td>
<td>A4</td>
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<td>A6</td>
</tr>
<tr>
<td>oligocarrageenan</td>
<td>95.14</td>
<td>71.46</td>
<td>79.54</td>
<td>78.64</td>
<td>77.02</td>
<td>69.78</td>
</tr>
<tr>
<td>SCOs</td>
<td>95.16</td>
<td>71.46</td>
<td>79.53</td>
<td>78.66</td>
<td>77.02</td>
<td>69.78</td>
</tr>
</tbody>
</table>

Note: G represent 1-3-β-4-sulfate-D-galactose, A represent (1-4)-α-3, 6-anhydro bridge-D-galactose

The antidiabetic activity of SCO

The blood glucose concentration of experimental animals after treatment with alloxan was higher than that of control animals. Diabetic rats showed representative symptoms, such as consuming more food, more drink, excreting more urine and losing weight. The antidiabetic activity of SCOs is shown in Figure 5. After treatment with different doses of SCOs for 14 days, the blood glucose concentration of the experimental groups decreased and reached almost normal levels, compared to that of the diabetic group. It is clear that the highest degrading effect occurred using the dosage of 50 mg/kg SCOs. The representative symptoms of diabetes (namely consuming more food, more drink and excreting more urine) disappeared, and the experimental rats stopped losing weight after treatment with SCOs (Fig 5B). Together, these results indicate that SCOs could decrease the blood glucose concentration and demonstrated antidiabetic activity in diabetic rats.

FIGURE 5. Antidiabetic activity of SCOs for diabetic rat. A: Effect of SCOs on the glucose content in rat serum; B: Effect of SCOs on rat weight. fx represents P<0.01 in a t-test compared to the diabetic rat group (n=12), ffx represents P<0.05 in a t-test compared to the diabetic rat group (n=12).
at the dosage of 50 mg/kg SCO; the enhancement activity decreased with increasing SCOs dosage. Since Se is a part of the active center of GSH-Px, SCOs could enhance the activity of GSH-Px; the highest enhancement activity was observed at the SCOs dosage of 200 mg/kg. MDA is the final product of the cell membrane peroxidation and has been regarded as one marker of oxidation stress in the cell membrane. Alloxan, a strong oxidant, can injure pancreatic islet cells and cause the animals to show diabetic symptoms. The MDA content in rat sera was increased significantly when induced by alloxan injury, indicating that the diabetic rats were under peroxidation stress. The MDA content of the experimental animals was reduced to or close to normal levels after treatment with SCOs for 14 days.

**FIGURE 6. Effect of SCOs on antioxidase activity and MDA content in rat serum.** A: Effect of SCOs on the activity of SOD; B: Effect of SCOs on the activity of GSH-Px; C: Effect of SCOs on the MDA content in rat serum. * represents P<0.01 in a t-test compared to the diabetic rat group (n=12), ** represents P<0.05 in a t-test compared to the diabetic rat group (n=12).

**Histopathological observations of the pancreas islet**

The histology of pancreas islet cells of the experimental rats is shown in Figure 7. The pancreas islet of normal rats is intact, the boundary is clear, and the pancreas islet cells contain normal elliptical nuclei (Figure 7A). For the diabetic rat, however, the islet shows signs of deterioration and appears shrunken with an unclear boundary. The cells are in a state of disarray, show anomalous structure with irregular nuclei, and some cells with inflammation are observed in the pancreas islet (Figure 7B). For the 50-mg/kg and 200-mg/kg treatment groups, the pancreas islet is still intact with a clear boundary, and the cells are full with normal elliptical nuclei, but some inflammatory cells are also observed. For the 500-mg/kg groups, no inflammatory cells could be observed in the pancreas islet.

**FIGURE 7. Histological analysis of alloxan-induced diabetic pancreas islet after treatment with SCOs.** A: Pancreas islet of normal rat; B: Pancreas islet of alloxan-induced diabetic rat; C: Alloxan-induced diabetic pancreas islet with treatment of 50 mg/kg SCOs for 14 days; D: Alloxan-induced diabetic pancreas islet with treatment of 200 mg/kg SCOs for 14 days; E: Alloxan-induced diabetic pancreas islet with treatment of 500 mg/kg SCOs for 14 days. (A, B, C, D and E all magnified 300 times).

**DISCUSSION**

κ-carrageenan is a type of sulfated polysaccharide extracted from red marine algae, which consists of alternating 3-linked β-D-galactose (G units) and 4-linked 3,6-anhydro-D-galactose (A units). Previous research revealed that κ-oligocarrageenan has antioxidative activity in vivo and in vitro (Mou, 2003; Yuan 2005). Selenium is an important part of the active center of the glutathione peroxidase enzyme (GSH-Px) (Chappuis and Poupon, 1991; Levander and Burk, 1994). It is therefore essential to supplement Se element when a state of peroxidation occurs in the body. Diabetes is one of the degenerative diseases with a disturbance in Se homeostasis and damage caused by free radicals (Marcason, 2008; Navarro-Alarcon et al., 1999b; Simonoff and Simonoff, 1991). Hence, it is important for diabetic patients to supplement selenium and increase the antioxidative activity of the body. Considering the different antioxidative mechanism of sulfate and selenium compounds (Battin and Brumaghim, 2009), we designed this experiment to produce sulfated seleno-oligosaccharides with κ-carrageenan and measured their antidiabetic and antioxidative activity.
Under acidic conditions, carrageenan can be hydrolyzed into oligocarrageenan. The results of DNS and TLC indicate that the product is a low molecular weight carrageenan. The results of FAAS, UV and IR spectra show that oligocarrageenan has both a sulfate group and a selenium element. Hence, the product of our experiment is an oligocarrageenan with selenium, which contains properties of both compounds and is named \( \kappa \)-selenocarrageenan oligosaccharides (SCOs). According to the \(^1\)H and \(^{13}\)C NMR spectra, we deduced that some sulfated groups on the site of the 4-sulfated-\( \beta \)-D-galactose units (G4) were replaced by sodium selenite. The supposed structure of the product and the reaction principle are provided in Figure 8.

**FIGURE 8. Reaction principle of \( \kappa \)-selenocarrageenan oligosaccharides (SCOs), n>m, R=\(-\text{OSO}_3\text{Na} \) or \(-\text{OSeO}_2\text{Na}\)

\[ \begin{align*}
\text{\( \kappa \)-Carrageenan} & \quad \text{\( \kappa \)-selenocarrageenan oligosaccharides} \\
\text{\( \nabla \text{Na}_2\text{SO}_4 \)} & \quad \text{\( \nabla \text{Na}_2\text{SeO}_3 \)}
\end{align*} \]

Alloxan, a strong oxidant, can injure the pancreatic islet cells (Fig 7B) and induce hyperglycemic symptoms (Fig 5A). After treatment with SCOs for 14 days, the glucose concentration in the serum of experimental rat groups decreased to or were close to the normal level (Fig 5A), and the injury to pancreas islet cells was overcome (Fig 7). Together, these results indicate that SCOs has an antidiabetic effect for alloxan-induced diabetic animals and that the best effect was observed at a dosage of 50 mg/kg. SOD and GSH-Px are two important oxidases in animals and can protect tissue from damage by free radicals. MDA is a marker of the oxidation state of the body. It is clear that SCOs can enhance the activity of SOD and GSH-Px and reduce the MDA of the oxidation state of the body. The antidiabetic effect of SCO is due to its antioxidative activity. The SCOs can decrease the blood glucose level to almost normal and overcome the damage to the pancreas islet cells that is induced in alloxan-induced diabetic rats. The antidiabetic effect of SCOs may be due to its antioxidative activity.

**ACKNOWLEDGEMENT**

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κ - Selenocarrageenan oligosaccharides in diabetes