Research Article

Characterization of Polysaccharides Extracted from *Sargassum fusiforme* and Its Effective Prevention of Contrast-Induced Nephropathy via Enhancing Antioxidant Capacity

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Contrast-induced nephropathy (CIN) is a common complication in patients with coronary arteriography, and oxidative stress is involved in the CIN pathogenesis. *Sargassum fusiforme* (SF) is a brown seaweed with medicinal value, and its polysaccharides have good antioxidant activity. In this study, the crude polysaccharides (cSFP-C) were extracted by cold water, precipitated by ethanol, purified by CaCl₂, and detected with high contents of sulfate radical and fucose. cSFP-C is composed of glucose, glucuronic acid, xylose, rhamnose, mannose, galactose, and fucose with a molar ratio of 1.0 : 0.4 : 5.6 : 1.2 : 1.7 : 12.3 : 56.1. The cSFP-C has the typical absorption of polysaccharides. Antioxidation assays in vitro showed that cSFP-C exhibited superoxide radical scavenging activity which was better than the hot water-extracted crude polysaccharides (cSFP-H). 20 rats were divided into 4 groups (n = 5): sham group; CIN group; CIN+cSFP-C group, and cSFP-C group. The CIN+cSFP-C group and cSFP-C group were pretreated intragastrically with cSFP-C at a dose of 9.45 g/kg twice daily for 5 consecutive days. Then, the CIN group and CIN+cSFP-C group were given indomethacin to develop CIN. The in vivo results showed that cSFP-C could decrease blood creatinine and urea nitrogen, inhibiting pathological injury in the renal tissues. The MDA content of renal tissues was decreased, while the activity of SOD was increased. The crude sulfated polysaccharides extracted from *S. fusiforme* have a renoprotective effect on oxidative stress to alleviate the kidney injury in CIN rats.

1. Introduction

With the raised morbidity of coronary atherosclerosis heart diseases, and the marked progress in intravascular interventional radiology, the volume of patients with percutaneous coronary intervention (PCI) has been growing significantly, with the number of PCI centers [1] increasing to 21.2% during 2003–2011. However, among the complications of PCI, the incidence of contrast-induced nephropathy (CIN) ranges from 3% to approximately 30% [2, 3], which has become the third leading cause of hospital-acquired acute kidney injury (AKI) following nephrotoxic drugs and renal perfusion insufficiency [4, 5]. After CIN occurs, the hospital days, the dialysis population, and the late cardiovascular events increase.

So far, there is still lack of therapeutic measures to reverse CIN. Effective prevention and treatment are needed. The commonly used agents are iodine contrasts in PCI, which are mostly unchanged passing through the kidneys into the urine, so as to have a damaging effect on the kidneys. The mechanisms of CIN have not been completely elucidated currently. Previous studies reported that CIN is a complex pathological process related to multiple pathological cascades. Oxidative stress [6, 7], renal ischemia [8], endothelial dysfunction [9], inflammation [10, 11], apoptosis [12–14], and tubular transport dysfunction [15] may be involved.
in the pathogenesis of CIN. Increasing studies point out that reactive oxygen species (ROS) play the key role in CIN development [16–18]. In accordance with this, several potent ROS scavenging compounds are proved effective for preventing CIN. Natural antioxidants extracted from plants may retard renal damage and may be an effective, safe, and economical therapy for organ protection. Sargassum fusiforme (SF) is an edible brown seaweed. SF can be used for kidney disease treatment in traditional Chinese medicine (TCM) and is documented in the medical books, such as Shenong’s Classic of Materia Medica, Compendium of Materia Medica, and TCM prescriptions of Conhea Ostreae Rhizoma Alismatis Powder invented by Zhang Zhongjing. It was reported to be inhibitive to renal interstitial fibrosis caused by unilateral ureteral obstruction. Sulfated polysaccharides are the most important component of SF, mainly accumulated in the cell wall matrix. The complicated molecular structures of polysaccharides partly contribute to its multiple biological functions [19]. Sulfur-containing groups, such as sulfonate and sulfate, can be part of some natural polysaccharides and provide the polysaccharides more diversity [20, 21]. The sulfated polysaccharides are also called fucoidan from SF, which shows antioxidation, anti-inflammation, antitumor, antiradiation, and anticogulation effects. It has also been reported that fucoidan extracted from SF increases the activity of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) in vivo [22] and reduces lipid peroxidation products. So far, there is no report for the antiradiation, and anticoagulation effects of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx)

2. Materials and Methods

2.1. Materials. Sargassum fusiforme was collected from Rongcheng in Shandong, China. Reagents and solvents in the study were of analytical purity (AR) grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Sample Pretreatment. The parameters of the crude polysaccharide extraction are given in Figure 1. SF was dried at 50°C, ground by a high-speed disintegrator into a powder, and sieved through a 4-mesh screen. 95% methanol was used to remove the fat of the sieved powder (1:10 g/ml) by(reflo)owing at 40°C for 24 h. Afterwards, the defatted residue was dried at 40°C for subsequent experiments.

2.3. Extraction of Crude Polysaccharides from SF. The defatted powder was extracted three times with water (1:30 g/ml) for 3 h at 80°C. The leaching solutions were filtered and concentrated with a rotary evaporator (Yarong, Shanghai, China) at 40°C for 2 h. After being dried with rotary evaporation, 1 ml of methyl alcohol was added to the hydrolyzed product to completely remove TFA, three times. The product and monosaccharide standards were prederivatized with 1-phenyl-3-methyl-5-pyrazolone (PMP). The HPLC system (Waters 1525, Milford, USA) and UV detector (Waters 2487) were used to analyze the monosaccharide components with a wavelength of 254 nm. The monosaccharide by using 80% ethanol and 95% ethanol. Finally, the lyophilized powder was redissolved and exhaustively dialyzed (10kDa molecular weight cutoff) against distilled water for 72 h to remove soluble impurities. By lyophilization, the crude polysaccharides were named as cSFP-C. The hot water-extracted crude polysaccharides, cSFP-H, were extracted three times with water (1:30 g/ml) for 3 h at 80°C, but not room temperature. The other steps were the same. The cSFP-C and cSFP-H were weighted and calculated the extraction yield (Y) according to the following equation:

\[ Y(\%) = \frac{\text{weight of the crude polysaccharides}}{\text{weight of dry Sargassum fusiforme}} \times 100 \]  

2.4. Determination of Chemical Composition of cSFP-C. The total sugar content in cSFP-C was determined using the phenol-sulfuric acid method. The sulfate radical content was estimated by the barium chloride-gelatin method [23]. The total uronic acid content was tested by the m-phenylphenol method using glucuronic acid as the standard [24]. The protein content was analyzed by Coomassie brilliant blue method with bovine serum albumin as the standard [25].

2.5. Measurement of Monosaccharide Components. 2 mg cSFP-C was mixed with 1 ml trifluoroacetic acid (TFA, 2M) and then heated at 120°C for 2 h. After being dried with rotary evaporation, 1 ml of methyl alcohol was added to the hydrolyzed product to completely remove TFA, three times. The product and monosaccharide standards were prederivatized with 1-phenyl-3-methyl-5-pyrazolone (PMP). The HPLC system (Waters 1525, Milford, USA) and UV detector (Waters 2487) were used to analyze the monosaccharide components with a wavelength of 254 nm.
quantification in cSFP-C was analyzed by comparing with the monosaccharide standard curves.

2.6. Fourier-Transform Infrared Spectroscopic Analysis (FT-IR). The dried cSFP-C was prepared as KBr pellets for the IR spectra measurement in a range of 4000 to 400 cm\(^{-1}\) using a Nicolet Nexus FT-IR spectrometer (Thermo Fisher Scientific, Waltham, MA, USA).

2.7. Superoxide Radical Scavenging Activity Measured by PMS-NADH-NBT. Superoxide anion scavenging activity was detected by the PMS-NADH-NBT method reported by Nishikimi et al. [26] with slight modification. The reaction system contained 0.15 ml NADH (166 μmol/l), 0.45 ml NBT (86 μmol/l), and varying concentrations of cSFP-C (0 mg/ml–2 mg/ml). Then, 0.15 ml PMS (16.2 μmol/l) was used to generate a superoxide radical and start the reaction. The mixture was incubated at room temperature for 5 min, and the absorbance was measured at a wavelength of 560 nm. In the blank control, there was 0 mg/ml cSFP-C in the buffer. The following equation was used to calculate the effect on scavenging superoxide radical:

\[
\text{Scavenging effect (\%)} = \left( \frac{A(\text{blank}) - A(cSFP-C)}{A(\text{blank})} \right) \times 100.
\]  

2.8. Rat Model of CIN and Treatment. 20 Wistar rats were divided into 4 groups of 5 rats each as follows: (1) sham rats given saline, (2) CIN rats with induced CIN, (3) CIN+cSFP-C rats pretreated with cSFP-C followed by the CIN induction, and 4) cSFP-C rats pretreated with cSFP-C. CIN+cSFP-C and cSFP-C rats were orally administrated 5.67 g/kg cSFP-C twice daily at 7 a.m. and 15 p.m. for 5 days. The dose of cSFP-C in this study was threefold the dose recommended by the Chinese Pharmacopoeia in clinic [27]. Sham and CIN rats were given saline. After pretreatment with cSFP-C or saline, CIN was developed in CIN and CIN+cSFP-C rats on the basis of previously described reports [28]. Briefly, under pentobarbital sodium anesthesia, rats were given 10 mg/kg indomethacin, followed 10 mg/kg NW-nitro-L-arginine methyl ester (L-NAME) 15 min later and 1600 mg iodine/kg of iopromide 30 min later. Sham and cSFP-C rats were given saline at each time point. All rats were sacrificed 24 h after CIN induction. Serum was isolated from the blood for renal biochemical and inflammatory testing. The kidneys were removed for histopathological investigation and oxidative stress detection.

2.9. Biochemical Parameters. Blood urea nitrogen (BUN) and creatinine were measured using an AU5800 autoanalyzer (Beckman Coulter, USA).

2.10. Histopathological Investigation. One side of the kidney was fixed in 10% formalin for 24 h and subsequently embedded in paraffin. Then 4 μm thickness sections were stained with hematoxylin and eosin.

2.11. Detection of Oxidative Stress Markers. One side of the kidney was homogenated using ice-cold saline and then centrifuged. The supernatant was collected and the levels of SOD and MDA were measured using commercial kits (Nanjing Jiancheng, Jiangsu, China).

3. Results and Discussion

3.1. Yield and Chemical Compositions of cSFP. The yields of cSFP-C and cSFP-H from dry SF were 7.5% and 9.1%, respectively. The chemical compositions of cSFP-C (total sugar, sulfate radical, uronic acid, and proteins) are summarized in Table 1. The total sugar was the major constituent of cSFP-C, achieving 85.5%, with small amounts of protein at 0.3%. The contents of sulfate radical and uronic acid were 19.3% and 8.3%, respectively. The fucoidan extraction using cold water combined with calcium-alginate precipitation has not been investigated. Since calcium chloride could precipitate calcium-alginate, the fucoidan purity in cSFP-C can be enhanced [29]. Compared with the previous study by Chen et al. [22], the uronic acid percentage was significantly decreased and the sulfate radical percentage was significantly increased with the procedure for calcium-alginate precipitation.

3.2. Quantitative Analysis of Monosaccharide Components. To further investigate the monosaccharide composition of extracted cSFP-C, HPLC was run and its retention time was used to quantify the monosaccharide amount normalized to glucose (Figure 2). The quantitative results are summarized specifically in Table 2. Predominantly fucose was found, and abundant galactose was a high component among different monosaccharides. This result was consistent with previous reported findings for components of fucose and galactose, which showed that fucose made up the largest proportion followed by galactose [30–32]. However, glucuronic acid content significantly decreased with 0.4 molar ratio to glucose, which indicated an optimized purity of fucoidan. Glucuronic acid content is much lower than those previous reported findings for components of fucose and galactose, which showed that fucose made up the largest proportion followed by galactose [30–32]. However, glucuronic acid content significantly decreased with 0.4 molar ratio to glucose, which indicated a more optimized purity of fucoidan. Glucuronic acid content is much lower than those previous reported findings, perhaps due to the procedure for calcium-alginate precipitation [32, 33]. In addition, it was found that cSFP is composed of glucose, xylose, rhamnose, and mannose.

<table>
<thead>
<tr>
<th>Yield (%)</th>
<th>Total sugar (%)</th>
<th>Sulfate radical (%)</th>
<th>Uronic acid (%)</th>
<th>Proteins (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>85.5</td>
<td>19.3</td>
<td>8.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

3.3. FT-IR Analysis. The FT-IR spectrum provides important information about the conformation and functional groups of cSFP-C in Figure 3. cSFP-C exhibited a strong and broad area of absorption between 3600 and 3200 cm\(^{-1}\), representing O-H stretching vibration. A band was observed at 1635 cm\(^{-1}\) for the carboxylate stretching [34]. The absorption peak at 3000–2800 cm\(^{-1}\) indicated the presence of the C-H stretching vibration. The signal at 1251 cm\(^{-1}\) could be associated with the asymmetric O=S=O stretching vibration of sulfate esters [30]. The absorption peak at 821 cm\(^{-1}\) was assigned to the sulfate groups [35].
3.4. Scavenging Activity of Superoxide Radical by cSFP-C.

**Table 2: Molar ratio of monosaccharide composition in cSFP-C.**

<table>
<thead>
<tr>
<th>Monosaccharide</th>
<th>Glycerol (Glc)</th>
<th>Glucuronic Acid (GlcA)</th>
<th>Xylose (Xyl)</th>
<th>Rhamnose (Rha)</th>
<th>Mannose (Man)</th>
<th>Galactose (Gal)</th>
<th>Fucose (Fuc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio</td>
<td>1.0</td>
<td>0.4</td>
<td>5.6</td>
<td>1.2</td>
<td>1.7</td>
<td>12.3</td>
<td>56.1</td>
</tr>
</tbody>
</table>

Notes: Glc: glucose; GlcA: glucuronic acid; Xyl: xylose; Rha: rhamnose; Man: mannose; Gal: galactose; Fuc: fucose.

**Figure 4: Scavenging effect on superoxide radicals by cSFP-C and cSFP-H.** Values are means ± S.D. (n = 3).

The results indicate that cSFP-C showed significant scavenging ability on the superoxide radical in a concentration-dependent manner. Decreased absorbance and increased scavenging effect (%) were present as the cSFP-C concentration increased. All kinds of free radicals are generated in oxidative metabolism, in which the superoxide anion free radical is produced at the earliest [36]. Then, the superoxide radical produces other free radicals through disproportionation reaction [37] and also causes lipid peroxidation [38]. Thus, the scavenging for superoxide radical is crucial for the protection of the cells from oxidative damage. As studied previously, hot water extraction, as the traditional procedure, has been widely investigated for the polysaccharide extraction from SF [39, 40]. However, Chen et al. [22] reported that hot water-extracted polysaccharides performed less free radical (superoxide radical) scavenging activity in vitro compared to cold water-extracted polysaccharides. In this study, it could be seen that the extracted cSFP-C showed good scavenging ability on the superoxide radical compared to cSFP-H (Figure 4). The result was consistent with the result reported by Chen et al. [22]. It indicated that cSFP-C might have some pharmacological effects according to its antioxidative activity.

3.5. cSFP-C Prevents Iopromide-Induced Renal Dysfunction in CIN Rats.

**Figure 2: HPLC separation of PMP-labeled monosaccharide composition of cSFP-C: (a) monosaccharides standard; (b) cSFP-C. Peaks: 1: mannose; 2: rhamnose; 3: glucuronic acid; 4: glucose; 5: galactose; 6: xylose; 7: fucose.**

**Figure 3: FT-IR spectrum of cSFP-C.**

**Figure 5: Levels of blood urea nitrogen (BUN) and creatinine of CIN rats were significantly higher than those of sham rats (P < 0.05), indicating that iopromide caused renal dysfunction. Compared with CIN rats, CIN+cSFP-C rats significantly reduced BUN and creatinine levels (P < 0.05), suggesting that cSFP-C preadministration played a role in renoprotection. Compared with sham rats, cSFP-C rats did not affect BUN and creatinine levels, suggesting that a high dose of cSFP-C had no renal injury.**

**Table 2: Molar ratio of monosaccharide composition in cSFP-C.**

<table>
<thead>
<tr>
<th>Monosaccharide</th>
<th>Glucose (Glc)</th>
<th>Glucuronic Acid (GlcA)</th>
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<th>Galactose (Gal)</th>
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Notes: Glc: glucose; GlcA: glucuronic acid; Xyl: xylose; Rha: rhamnose; Man: mannose; Gal: galactose; Fuc: fucose.
CIN+cSFP-C rats were partially due to the high content of fucoidan in cSFP-C.

3.6. Protective Effect of cSFP on the Kidney. As shown in Figure 6, in the sham group, no significant histological changes were observed, while CIN rats showed severe damage, including lesions of tubular necrosis and inflammatory cell infiltration. In the CIN+cSFP-C group, cSFP-C pretreatment significantly reduced these types of damage, indicating that cSFP-C could protect the kidney of CIN rats from damage. This result was in accordance with the renal biochemical results. It was reported that sulfated polysaccharides reduce renal tubulointerstitial fibrosis in CKD mice [42]. And low molecular weight fucoidan inhibits epithelial mesenchymal transition of human renal proximal tubular cells [43]. These results enlightened us to seek out the underlying mechanism for renoprotection of cSFP-C.

3.7. cSFP Protected the Kidney from Oxidative Stress. In order to evaluate whether cSFP-C protects renal tissue of CIN rats from oxidative stress, SOD activity and MDA content in renal tissue were detected. MDA content of CIN rats was significantly higher than that of sham rats, while SOD activity was significantly reduced \((P < 0.05)\). Although the pathological mechanism of CIN has not been clearly elucidated, various studies have investigated that oxidative stress is an important cause of CIN [6, 44]. The direct toxicity of contrast agents leads to the ROS production, while the oxidative stress caused by the increasing free radicals leads to the apoptosis of renal tubules and glomerular cells [45]. These results in Figure 7 showed the deterioration on oxidative stress, suggesting the coincidence with the pathological deterioration. According to the mechanism of ROS-promoted development of CIN, the corresponding removal of ROS has been studied in preclinical and clinical studies. Sodium bicarbonate [46], n-acetylcysteine (NAC) [47], theophylline [48, 49], dopamine [50], furosemide [51], mannitol [52], and ascorbic acid [53] have been studied in the clinical prevention of CIN. In preclinical studies, the protective effects of melatonin [54], tocopherol [55, 56], and L-carnitine [57] have also been studied with regard to CIN. In this study, when cSFP-C was preadmininstered, the MDA content was significantly decreased and SOD activity was significantly restored \((P < 0.05)\). These results showed that preadministration of cSFP-C largely reduces the oxidative stress damage in kidneys of CIN rats and improves the endogenous antioxidative capacity of the kidneys. These results in vivo were also in accordance with the scavenging activity of the superoxide radical by cSFP-C in vitro. In addition, cSFP-C did not affect SOD activity and MDA content compared with the sham group.

4. Conclusion

In summary, the polysaccharides were purified from *Sargassum fusiforme* (SF) and contained abundant sulfate radical and predominant fucose, which indicated potential bioactivity. Then, the polysaccharides exhibited the scavenging activity for superoxide radical in vitro. According to the antioxidative effect, the polysaccharides demonstrated renoprotective properties against CIN in vivo. The pretreatment of cSFP-C could effectively improve biochemical indexes and pathological structures and correct the renal dysfunction.
and the abnormal levels of MDA and SOD. It can be speculated that the renoprotective effects of cSFP-C might be mediated by inhibition of oxidative stress.

**Data Availability**

All the data is available in the handwritten notebook documented in our lab.

**Conflicts of Interest**

The authors declare that there is no conflict of interest regarding the publication of this paper.

**Acknowledgments**

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**References**


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**Figure 7:** cSFP pretreatment reduced the renal oxidative stress in kidney tissues of CIN rats: (a) renal MDA concentration; (b) renal SOD activity. *P < 0.05 versus sham rats; #P < 0.05 versus CIN.


