Renoprotective Effect of Sulphate Polysaccharide from Brown Algae on Ethylene Glycol-Induced Renal Damage in Rats

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Abstract The protective effect of the polysaccharides on ethylene glycol induced kidney impairment has been explored. Polysaccharides have been extracted from *Sargassum graminifolium*, which belongs to brown algae. Three different doses of polysaccharides were used for rats with kidney stones by ethylene glycol feeding. Then, urinary biochemistry parameters, renal function factors including blood urea nitrogen (BUN) and serum creatinine (Scr) levels were detected. Besides, interleukin-6 (IL-6) and tumor necrosis factor α (TNF- α) in kidney tissue were elevated, which belong to proinflammatory cytokines. Moreover, kidney histopathological sections were examined. The results illustrated that polysaccharides not only increased calcium level but also decreased oxalate, Scr, and BUN levels. In addition, the levels of IL-6 and TNF- α were reduced. Moreover, renal microscopic analysis showed polysaccharides treatment alleviated observably calcium oxalate deposits in the kidney tissue. The results indicated that the renoprotective mechanism of SGP may be attributed to suppression of inflammation, reducing the growth of urinary stones and improving kidney function *in vivo*, which explained SGP through multiple ways to protect kidney cells from damaging of hyperoxaluria rats.

Keywords brown algae, polysaccharides, hyperoxaluric, urinary stones, inflammation, antioxidants

Introduction

As we all know, seaweed from marine is the important bioresource because of its abundant bioactivities. The activities of brown algae, red algae and green algae have been researched worldwide for many years. The active substances from these algae possess wide applications. Among them, the most important active substance is a series of sulfated polysaccharides. Many reports showed that sulfated polysaccharides are of great importance due to their bioactivities such as inhibiting lipase activity, inhibiting amyloid-beta-induced toxicity, immunoregulation, and antiviral activities, etc.^[1-8]

Urinary stone is a common disease with high recurrence rate in the world mainly caused by pathological mineralization *in vivo*. The incidence and recurrence rate of urolithiasis are generally on the rise. The etiology and pathogenesis of CaO_x calculi is complex. Much work needs to be performed to control the incidence and recurrence rate of CaO_x.^[9-11] Recently, the relationship between oxidative stress/cell damage and the occurrence of CaO_x stones has attracted great attention. Exposure of kidney cells to CaO_x crystals results in the production of reactive oxygen species (ROS), which is involved in a variety of signaling pathways. This leads to oxidative stress in kidney cells, and then further causes renal injury and inflammation, which play a key role in the formation of urinary stones.^[12-16] Clinical research indicates that fetoglobulin A is

found in urine and kidney of patients with stones. Fetoprotein A is an inflammation-related glycoprotein, which can be anti-inflammatory and directly inhibit macrophages. At the same time, inflammatory cytokines (IL-6, TNF- α) also affect the synthesis and secretion of this protein.^[17-19]

Sargassum C. Agardh belongs to brown algae, and it distributes in tropical and temperate regions. There are more than 250 species in the world including 130 species recorded in China. *Sargassum grasss* is the traditional source of medicinal seaweed in China. According to the records, 15 species of *Sargassum* can be utilized as medicinal seaweed including *Sargassum grasss*.^[20,21]

Our previous works showed that polysaccharides of *Sargassumgrasss* (SGP) had excellent antioxidant activity by measuring SGP's reducing power, scavenging superoxide anion free radicals and inhibiting DPPH free radicals. On one hand, it inhibited calcium oxalate crystallization *in vitro*; on the other hand, it protected mitochondrial function of rats with kidney stones *in vivo*.^[22,23]

Currently, urinary parameters, immune modulator and renal function about the mechanisms of SGP are not investigated. In this paper, we will explore the possible mechanism *in vivo* from multiple aspects with renal function, immune modulator and reducing CaO_x crystal deposition.

Table 1 The rats divided group in experimental hyperoxaluria

Groups	Treatment
Group A	Drinking water from the 1st day up to the end of the experimental period
Group B	EG+AC in drinking water for 7 d
Group C	EG+AC in drinking water for 7 d + SGP 400 mg/kg from the 8th day up to the end of the experimental period
Group D	EG+AC in drinking water for 7 d + SGP 100 mg/kg from the 8th day up to the end of the experimental period
Group E	EG+AC in drinking water for 7 d + SGP 25 mg/kg from the 8th day up to the end of the experimental period

Experimental

Reagents

Reagents and kits used for histological preparations or assay of urine parameters, renal function were purchased from Nanjing Jian Cheng Bio Inst. All the other chemical reagents applied were of analytical grade purchased from Shanghai Guo Yao Group Chemical Reagent Co., Ltd.

The more information about SGP such as extract and molecular weight, structure and sulphuric acid content has been described in our previous achievement.^[23]

Animals

Before the experiment, the male Wistar rats with body weight at 200 ± 20 g were domesticated in cages for 7 d at temperature of 22 ± 2 °C, and then they were placed in a controlled light-dark cycle for 12 h. All rats were fed in a standard diet.

Ethylene glycol-induced renal damage in rats

The male Wistar rats were randomly divided into 5 groups, 6 in each group. The specific groups are shown in Table 1. It is very important to establish and validate an experimental animal model of kidney stones in order to explore a renoprotective effect of SGP on ethylene glycol-induced renal damage. At present, ethylene glycol is most commonly used for construction of a kidney stone model due to its good repeatability and relatively stability. The principle is that ethylene glycol is the precursor of oxalic acid, which can be converted into oxalic acid by metabolism *in vivo*. In this modeling scheme, ammonium chloride acts as acidifying urine and contributes to the formation of kidney stones.^[24,25] In this model, the concentration of ethylene glycol (EG) was 1% [V/V] and ammonium chloride (AC) was 1% [w/V].

Collection and analysis of urine

The male Wistar rats were placed in individual metabolic cages, whose 24 h urine samples were gathered every day. Then, urine volumes of 24 h were recorded, the urine of pH was measured. After adding sodium and sodium corrosion, the urine was collected by centrifuge (3000 rpm, 15 min). Then, the contents of oxalate and calcium ions in the supernatant were examined. Calcium and oxalate were tested according to instructions of kits, which were bought from Nanjing Jian Cheng Bio Inst.

Assessment of renal function

The assay of Scr is based on a product of orange, which is formed by creatinine clearance of blood protein in supernatant with picric acid in Jaffe reaction. The assay of BUN is based on Fearon reaction, which means urea in serum can be condensed into a red compound after azeotropic with diacetyl-oxime in the acidic environment of urea nitrogen reagent. The shade of the

color is proportional to the self-content of urea in serum and the content of urea nitrogen in serum can be measured by colorimetric comparison with the same treated urea nitrogen standard solution. The assay of Scr and BUN were tested according to instructions of kits. The kits were bought from Nanjing Jian Cheng Bio Inst.

The expression levels of TNF- α and IL-6 in renal

TNF- α and IL-6 were tested using kits from Nanjing Jian Cheng Bio Inst, and the protocols were all followed according to the manufacturer's instructions in the kit.

Histopathological studies

The rats were sacrificed after anesthesia and two kidneys were removed from each rat. Cut the left kidney, take 1.0 cm x 1.0 cm x 0.3 cm size of the tissue block, quickly put into 10% formaldehyde solution fixed. The fixed time is 48 h. This operation can make tissue and cell denaturation of the protein coagulation, to prevent cell death after autolysis and bacterial decomposition, thereby keeping the cell originally morphology. Then, the kidney tissue was treated with a series of different concentration alcohols and xylene, embedded in paraffin, sectioned and stained with Haematoxylin and Eosin for histopathological examination. Hematoxylin (HE) staining was used to observe the histopathological changes of right kidney sections under polarizing microscope (MOTIC BA200).

Statistical analysis

Data were expressed as mean $\pm$ standard deviation. One-way ANOVA with post hoc Dunnett's test or by Student's *t*-test was applied to determine differences among different groups using the SPSS software package for Windows. Significance has been showed in the following table and figures.

Results and Discussion

Effect of SGP on urinary parameters

The effect of various doses of SGP on rat urine volume, oxalate, Ca^{2+} and pH is shown in Table 2. It was found that urine volume and oxalate increased significantly between Group B and Group A; however, the amount of calcium in urine decreased significantly ($P < 0.01$). Administration of SGP to rats with kidney stones was able to increase Ca^{2+} levels. Groups C, D and E increased the Ca^{2+} level significantly compared with Group B. Besides this, Groups C and D decreased the oxalate level evidently ($P < 0.01$). However, there was no significant difference of urine volume and pH among the groups rats administrated of SGP and Group B. During the experiment, there was no significant change in the weight of rats in each group.

Effect of SGP on renal detection index

Both serum creatinine (Scr) and blood urea nitrogen (BUN)

Table 2 The urinary biochemistry results of each group rat in experimental hyperoxaluria

Parameters	Group A	Group B	Group C	Group D	Group E
Urine (mL/24 h)	16.10±0.70	38.15±3.57 [#]	43.83±1.62	42.10±1.23	42.70±2.56
Oxalate (μmol/24 h)	20.30±6.34	75.13±4.57 ^{##}	34.51±3.85 ^{**}	46.67±2.18 ^{**}	63.55±5.83
Ca ²⁺ (μmol/24 h)	15.193.84	4.96±0.91 ^{##}	12.77±1.20 ^{**}	8.89±0.86 ^{**}	6.18±0.57 [*]
pH	6.62±0.13	6.27±0.24	6.56±0.17	6.45±0.21	6.33±0.19

[#] $P < 0.05$, ^{##} $P < 0.01$ (vs. Group A); ^{*} $P < 0.05$, ^{**} $P < 0.01$ (vs. Group B).

Figure 1 BUN and Scr results of each group rats in experimental hyperoxaluria. [#] $P < 0.05$, ^{##} $P < 0.01$ (vs. Group A); ^{*} $P < 0.05$, ^{**} $P < 0.01$ (vs. Group B).

contents as the renal detection index are measured at the same time in the clinical. It means that the kidney function has serious damage when the indexes are higher than the normal values. Figure 1 shows BUN and Scr contents of experimental rats groups. It was found that BUN and Scr activity of Group B increased evidently in comparison to Group A, indicating that ethylene glycol had impaired renal function of the rats. Compared with Group B, the serum BUN and Scr contents in Group C were significantly decreased ($P < 0.01$). In addition, Group D lowered BUN level evidently ($P < 0.05$), too.

Serum levels of proinflammatory cytokines

The proinflammatory cytokines include tumor necrosis factor alpha (TNF- α) and interleukin-6 (IL-6), and their levels can reflect the renal tubular injury and inflammatory conditions. Figure 2 showed that the contents of TNF- α and IL-6 in Group B increased compared with Group A. After administration of SGP, the levels of TNF- α and IL-6 in Groups C—E were lowered compared with Group B. Groups C and D lowered the levels of TNF- and IL-6 evidently.

Calcium oxalate deposits in the kidney section

The morphology of the crystals in renal tissues are shown in Figures 3a—3e.

Extensive white CaO_x crystals deposition were observed in Figures 3a—3d. Moreover, large CaO_x crystals were detected in most of the renal tubules in Figure 3d, which belong to ethylene glycol induced hyperoxaluric rats. Interestingly, white

CaO_x crystals became smaller from tens of microns down to a few microns especially with the SGP from a dose of 25 mg/kg increased to 400 mg/kg. In Figure 1e (normal control group), no obvious CaO_x crystal was observed.

Figure 3f showed a lot of CaO_x crystals in the urinary of ethylene glycol induced hyperoxaluric rats, which were prominent edges and typical hexahedral CaO_x crystals. This also indicates ethylene glycol induced renal injury and CaO_x crystals deposition in male SD rats.

Conclusions

Kidney stone is widespread in the world, whose formation causes a chronic disease. Recent studies showed that ethylene glycol not only induced the deposition of CaO_x crystals in the kidney but also could lead to kidney damage and inflammation.^[26-28] Khan *et al.* reported that high oxalate and uric acid levels could lead to CaO_x, which can damage cells and induce inflammation and secondary fibrosis, leading to chronic end-stage kidney disease at last.^[15] Their research showed that crystal deposition was a key, which could induce damage and inflammation. Thus, crystal retention played a great role in the urinary stone process.^[29] Then, CaO_x was related to oxidative stress and TNF- α and IL-6 expression, which may result in high recurrence rate of urinary stone.^[30-32]

In Bhadja's review,^[33] the action mechanism of algae polysaccharides on CaO_x renal stone was revealed in detail in Figure 4. The sequential schematic for the inhibitory mecha-

Figure 2 TNF- α and IL-6 results of each group rats in experimental hyperoxaluria. # $P < 0.05$, ## $P < 0.01$ (vs. Group A); * $P < 0.05$, ** $P < 0.01$ (vs. Group B).

Figure 3 Crystalline formations in the rat kidney (a—e, polarizing microscope, magnification x 40). The morphology of the crystals in renal tissues is shown in a—e.

nism of algae polysaccharides at each step of CaO_x stone formation can be seen. Also, sulfated polysaccharides in seaweeds have a large number of negatively charged groups such as sulfate, carboxyl, and hydroxyl groups. These anions have a strong ability to interact with calcium ions.^[34] In brief, sulfated polysaccharides from the algae protect and repair the renal cells by blocking or inhibiting the injury pathways and increasing the antioxidant capacity of cells. The algae polysaccharides are promising molecules due to their role in

Figure 4 The sequential schematic for the inhibitory mechanism of seaweed polysaccharides at each step of CaO_x stone formation.^[33]

renal stone prevention.

Our team disclosed that sulfated polysaccharides of *Sargassum* possessed excellent antioxidant activity. It inhibited calcium oxalate crystallization *in vitro*, furthermore, it protected mitochondrial function of rats with kidney stones *in vivo*.^[22,23] Interestingly, the treatment with SGP increased the Ca^{2+} level, decreased oxalate and oxalate deposits, reduced the TNF- α and IL-6 levels, and lessened the SCr and BUN levels. Strikingly, the results showed that the renoprotective effect of SGP was due to inflammatory inhibition and reducing oxalate deposits as well as improving renal function. Together with excellent antioxidant activity SGP *in vitro* and *in vivo*, SGP can be used as a promising therapeutic agent for ethylene glycol-induced renal injury and preventing currency of urinary stone.

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Author Contributions

The conceptualization was performed by Dr. Chaoyan Zhang and Dr. Wenhui Wu, the methodology was carried out by Junwen Wang and Xueyan Li, the software was from Dr. Jinshi Ke, the investigation was carried out by Junwen Wang and Xueyan Li, the resource was from Dr. Jinshi Ke, the data curation was performed by Hui Huang, and the original draft was prepared by Hui Huang. This paper was written/reviewed/edited by Dr. Chaoyan Zhang, the supervision of this work was by Dr. Wenhui Wu, the project administration was performed by Dr. Chaoyan Zhang, and the fund acquisition was carried out by Dr. Chaoyan Zhang.

Conflict of Interest

The authors declare no conflict of interest.

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