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Antidiarrheal activity of a novel sulfated polysaccharide from the red seaweed *Gracilaria cervicornis*

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ABSTRACT

Ethnopharmacological relevance: The use of marine seaweeds as a source of natural compounds with medicinal purposes is increasing in Western countries in the last decades, becoming an important alternative in the traditional medicine of many developing countries, where diarrhea still remains a severe public health problem, with high rates of mortality and morbidity. Sulfated polysaccharides (PLS) extracted from red seaweeds can exhibit therapeutic effects for the treatment of gastrointestinal disorders. Thus, the pharmacological properties of the PLS from *Gracilaria cervicornis*, an endemic seaweed found in the Brazilian northeast coast, was evaluated as an alternative natural medication for diarrhea.

Aim of the study: This study aimed to evaluate the antidiarrheal activity of sulfated polysaccharides (PLS) extracted from the red seaweed *G. cervicornis* in Swiss mice pre-treated with castor oil or cholera toxin.

Materials and methods: The seaweed *Gracilaria cervicornis* was collected at Flecheiras beach (city of Trairí, State of Ceará, Brazil) and the PLS was obtained through enzymatic extraction and administered in mice (25–30 g) before diarrhea induction with castor oil or cholera toxin. For the evaluation of the total number of fecal output and diarrheal feces, the animals were placed in cages lined with adsorbent material. The evaluation of intestinal fluid accumulation (enteropooling) on castor oil-induced diarrhea in mice occurred by dissecting the small intestine and measuring its volume. The determination of Na^+/K^+-ATPase activity was measured in the small intestine supernatants by colorimetry, using commercial biochemistry kits. The gastrointestinal motility was evaluated utilizing an activated charcoal as a food tracer. The intestinal fluid secretion and chloride ion concentration were evaluated in intestinal closed loops in mice with cholera toxin-induced secretory diarrhea. The binding ability of PLS with GM1 and/or cholera toxin was evaluated by an Enzyme-Linked Immunosorbent Assay (ELISA).

Results: The *G. cervicornis* PLS showed antidiarrheal effects in both acute and secretory diarrhea, reducing the total number of fecal output, diarrheic stools, intestinal fluid accumulation, and increasing small intestine Na^+/K^+-ATPase activity on castor oil-induced diarrhea. However, the PLS did not affect gastrointestinal motility, indicating that this compound has a different action mechanism than loperamide. In secretory diarrhea, the PLS decreased intestinal fluid secretion and small intestine chloride excretion, binding with GM1 and/or cholera toxin and blocking their attachment to the enterocyte cell surface.

Conclusions: In conclusion, PLS has a significant antidiarrheal effect in acute and secretory diarrhea. Further investigation is needed towards its use as a natural medicine to treat diarrhea.

Abbreviations: BSA, bovine serum albumin; cAMP, cyclic adenosine monophosphate; CFTR, cystic fibrosis transmembrane conductance regulator; CPC, cetylpyridinium chloride; CT, cholera toxin; CTA, A Subunit of cholera toxin; CTB, B Subunit of cholera toxin; EDTA, ethylene diamine tetra-acetic acid; EGTA, ethylene glycol tetra-acetic acid; GM1, monosialoganglioside-1; PBS, phosphate buffered saline; Pi, inorganic phosphate; PLS, sulfated polysaccharides; SDS, sodium dodecyl sulfate; SOS, sialyloligosaccharides; TMB, 3,3′,5,5′-tetramethylbenzidine; Tris, tris(hydroxymethyl)aminomethane; UFC, Federal University of Ceará; WHO, World Health Organization

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1. Introduction

The use of seaweeds as crude extracts by native populations in eastern Asian countries to treat diseases has centuries of history in folk medicine (Cho and Kim, 2012; Glombitza and Koch, 1989; Hoppe, 1979; Moo-Puc et al., 2008). In Traditional Chinese Medicine, the use of seaweeds in medical literature is reported about 2000 years ago (Chengkui et al., 1984) and in Europe, the red seaweeds *Chondrus crispus* and *Mastocarpus stellatus* are used as teas since the early XIX century and have a large number of medical applications (Holdt and Kraan, 2011). In Brazil, there was a significant increase in the ethnopharmacological use of seaweeds by local coastal communities in the last decades, especially in the Brazilian Northeast (Souza et al., 2011).

Seaweeds contain large amounts of compounds with biological activities such as vitamins, proteins, essential fatty acids, and polysaccharides. Hence, in the last four decades there has been an increasing scientific interest for the discovery of novel bioactive molecules with pharmacological potential from seaweeds, mainly sulfated polysaccharides (PLS) (Cardozo et al., 2007; Lordan et al., 2011; Souza et al., 2012). These PLS are known to exhibit many therapeutic activities, including antitumor (Moussavou et al., 2014), antithrombotic (Rocha et al., 2005), anti-inflammatory, antinociceptive (Batista et al., 2014; Chaves et al., 2013), antiviral, and immunomodulatory properties (Bouhlal et al., 2011; Wongprasert et al., 2014).

PLS are complex and heterogeneous polyanionic macromolecules formed by repeating disaccharide units negatively charged due to the presence of sulfate groups. In red seaweeds, the main sulfated polysaccharides are known as galactans and are classified according to their stereochemistry: L-configuration for agarans and D-configuration for carrageenans (Jiao et al., 2011; Wijesekara et al., 2011). The red seaweed *Gracilaria cervicornis* Turner (J. Agardh) is found along the Brazilian northeast coast and is rich in agarans, like many Gracilariaceae species (Barros et al., 2013; Marinho-Soriano, 2001; Vidotti and Rollemberg, 2004).

These polysaccharides have potential to be used as novel bioactive products for therapeutic treatments and previous studies performed by our research group with PLS from other Gracilaria species have shown beneficial effects involving the inhibition of gastrointestinal complications, such as colitis (Brito et al., 2014) and ethanol-induced gastric ulcers (Silva et al., 2011). Furthermore, agarans are not absorbed by the intestinal lumen, remaining there for long periods without producing systemic effects, providing a substantial advantage in comparison to the majority of standard drugs (Cohen and Ito, 2002).

Thus, novel studies are necessary to evaluate the therapeutic role of *G. cervicornis* PLS and investigate its effects in other gastrointestinal diseases and functional disorders, such as diarrhea. Indeed, many genera of seaweeds, such as *Laminaria*, *Sargassum*, *Gelidium* and *Chondrus* are utilized to treat gastrointestinal diseases in folk medicine, including diarrhea (Khan and Qari, 2012; Trease and Evanes, 1996). However, there is still a lack of reports concerning isolated compounds of seaweeds and their respective action mechanisms.

Diarrhea is a common gastrointestinal disease characterized by the increase of liquid or semisolid stool frequency and is generally caused by infectious agents such as parasites, virus, and bacteria (Barreto et al., 2007; Palla et al., 2015; Rahman et al., 2015). Among them, *Vibrio cholerae* is a prolific pathogen that secretes cholera toxin (CT), an enterotoxin responsible for one of the most severe forms of this disease. Initially, the toxin induces electrolyte secretion and fluid efflux in the small intestine causing a severe dehydration, without epithelial damage (Basu and Mukhopadhyay, 2014; Unterweger et al., 2014; Ye et al., 2015).

In the majority of developing countries, diarrhea remains one of the most common causes of hospitalization, morbidity, and mortality, mainly among children (Moreno et al., 2010; Nakhjavani et al., 2013). The transmission of diarrhea occurs through physical contact or contaminated water and food (WHO, 2013). In concordance with biological activities of PLS from red seaweeds and the diarrhea pathophysiology, it is imperative to carry out a study to evaluate the *G. cervicornis* PLS use to treat acute diarrhea. Experimentally, the castor oil-induced diarrhea and CT-closed loops models are one of the most used to investigate the biological activity of natural products (Tadesse et al., 2014; Thakurta et al., 2007)

Thus, the aim of the present study was to evaluate whether *G. cervicornis* PLS has antidiarrheal activity in Swiss mice pre-treated with castor oil or cholera toxin, having in mind the possibility of its use as an alternative natural medication in endemic areas of diarrhea.

2. Materials and methods

2.1. Materials and chemicals

Castor oil, cholera toxin (CT), rabbit anti-cholera antibody, and monosialoganglioside-1 (GM1) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Anti-rabbit IgG-peroxidase antibody was purchased from GE Healthcare (Amersham Place, UK). Loperamide hydrochloride was purchased from Janssen-Cilag Pharmaceuticals LTD (São Paulo, SP, Brazil). All other chemicals and reagents were of analytical grade and obtained from standard commercial suppliers. The drugs were dissolved in 0.9% saline or phosphate buffered saline (PBS) (0.02 M; pH 7.0).

2.2. Animals

Swiss mice (25–30 g) were obtained from the animal house of the Federal University of Ceará (UFC). The animals were maintained in cages under standardized conditions (dark/light, 12/12 h cycle) at 22 ± 1 °C. Animals were supplied with a commercial diet (Presence Alimentos, Paulínia, SP, Brazil) and tap water *ad libitum*. The experimental groups consisted of 5–6 animals per group and before each experiment, the animals were deprived of food for 18 h with free access to water. The protocols used were performed according to the International Guidelines for the Care of Laboratory Animals and were approved by the Ethics Committee in Research of the Federal University of Ceará (protocol no. 11/2013).

2.3. Algal material and extraction of sulfated polysaccharides from G. cervicornis

The red seaweed *Gracilaria cervicornis* Turner (J. Agardh) was collected in July 2015 at Flecheiras Beach, Trairí, Ceará, Brazil. A voucher specimen was deposited at the Phycological Herbarium of the Marine Sciences Institute from UFC, Fortaleza-CE, Brazil (no. 02984). The seaweed was thoroughly cleaned to eliminate any undesirable epiphytes attached to the thalli, washed, and stored at −20 °C until use.

The extraction of the PLS was performed according to Farias et al. (2000). The dried algae tissue (10 g) was milled and incubated at 60 °C for 4 h with 750 mL sodium acetate buffer (100 mM; pH 5.0) containing papain (510 mg), 5 mM ethylene diamine tetra-acetic acid (EDTA), and 5 mM cysteine. After the incubation period, the residue was removed by filtration and centrifugation (2000 g for 25 min at 25 °C) and the PLS were precipitated by addition of 96 mL 10% cetylpyridinium chloride (CPC). The mixture was centrifuged as above and the polysaccharides in the pellet were washed with 200 mL 0.05% CPC solution, dissolved in 300 mL 2×10^3 mM NaCl/ethanol (100: 15, v/v) solution, and precipitated with 200 mL 70% (v/v) ethanol for 12 h at 4 °C. After further centrifugation (2000 g; 25 °C; 25 min) the precipitate was washed with 200 mL absolute ethanol and dried with acetone under hot air flow (60 °C).