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In vivo toxicological evaluation of crude sulfated polysaccharide from the green seaweed *Caulerpa cupressoides* var. *lycopodium* in Swiss mice

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ABSTRACT. Seaweeds are widely consumed as vegetables and medicinal products. The green seaweed *Caulerpa cupressoides* var. *lycopodium* sulfated polysaccharides (SPs) demonstrated anticoagulant (*in vitro*) and anti- and prothrombotic (*in vivo*) effects. However, their toxicity *in vivo* has not been fully determined. This study evaluated their toxicity *in vivo* in male *Swiss* mice. Animals (20-26 g, six group^{-1}) received crude SP (9 mg kg^{-1}, i.p.) or 0.9% saline (0.1 mL 10 g^{-1}, i.p.) for 14 consecutive days, and then analyzed the wet weight of animal's body organs^{-1} and biochemical/hematological parameters. Histopathological evaluation was also performed related to crude SP treatment. The results showed that crude SP did not cause toxicity and mortality. Regarding the biochemical analyses, crude SP did not lead to hepatic or renal dysfunctions, but affected ($p < 0.05$) the platelet count ($1530.75 \pm 1.05 \times 10^3$ μL^{-1}) compared with the control ($969.75 \pm 0.51 \times 10^3$ μL^{-1}) according to the hematological evaluation. Although histological changes in the liver and kidney have occurred, results suggested reversibility. The increased spleen size ($p < 0.05$) also had no toxicological significance based on histopathological analysis. Therefore, crude SP from *C. cupressoides* could represent safe pharmacological tool in future studies on immunomodulation and thrombosis *in vivo*.

Keywords: Caulerpaceae, green alga, sulfated polymers, toxicity, systemic evaluation.

Avaliação toxicológica *in vivo* de polissacarídeo sulfatado bruto da alga marinha verde *Caulerpa cupressoides* var. *lycopodium* em camundongos Swiss

RESUMO. As algas marinhas são consumidas mundialmente como 'vegetais' e produtos medicinais. Polissacarídeos sulfatados (PSs) da alga marinha verde *Caulerpa cupressoides* var. *lycopodium* foram avaliados e demonstraram possuir efeitos anticoagulantes (*in vitro*) e anti- e pró-trombóticos (*in vivo*). Entretanto, a sua toxicidade *in vivo* não foi determinada completamente. Avaliou-se sua toxicidade *in vivo* em camundongos machos Swiss. Os animais (20-26 g; seis grupo^{-1}) receberam PS bruto (9 mg kg^{-1}; i.p.) ou salina 0,9% (0,1 mL 10 g^{-1}; i.p.) durante 14 dias consecutivos e, em seguida, analisados o peso dos órgãos dos animais e os parâmetros bioquímicos e hematológicos. A avaliação histopatológica foi também realizada durante o tratamento com PS bruto. Os resultados mostraram que o PS bruto não causou toxicidade e mortalidade. Observou-se, ainda, que o PS bruto não causou disfunção hepática ou renal, segundo as análises bioquímicas. Entretanto, afetou ($p < 0,05$) a contagem plaquetária ($1.530,75 \pm 1,05 \times 10^3$ μL^{-1}) a partir do controle ($969,75 \pm 0,51 \times 10^3$ μL^{-1}) na avaliação hematológica. Embora ocorrido mudanças histológicas no fígado e rim, a reversibilidade dos resultados foi sugerida. O aumento do tamanho do baço ($p < 0,05$) não apresentou significância toxicológica baseada na análise histopatológica. Portanto, o PS bruto de *C. cupressoides* representa uma ferramenta farmacológica segura em estudos futuros a serem realizados de imunomodulação e trombose *in vivo*.

Palavras-chave: Caulerpaceae, alga verde, polímeros sulfatados, toxicidade, avaliação sistêmica.

Introduction

Seaweeds have been recognized as food with great nutritional value (TABARSA et al., 2012) used as medicinal products in the Orient (CHOI et al., 2009; LI et al., 2005). They are sources of dietary proteins, amino acids, essential fatty acids, carbohydrates, carotenoids, vitamins and sterols for humans and animals (ARAÚJO et al., 2012; GRESSLER et al., 2010; MABEAU; FLEURENCE, 1993; MARINHO-SORIANO et al., 2006; PIRES et al., 2008; SCHEVCHENKO et al., 2009), having thus great potential for different biotechnological applications (SMIT, 2004; TOSKAS et al., 2011; VALENTE et al., 2006). The search for algal products

with relatively low toxicity for medicinal, food and/or biochemical use has significantly increased in recent times (ANANTHI et al., 2010; POMIN, 2012; SMIT, 2004; VANDERLEI et al., 2010, 2011; ZHANG et al., 2003).

Sulfated polysaccharides (SPs) comprise a class of highly complex and heterogeneous macromolecules found as structural components of the extracellular matrix in marine algae (POMIN; MOURÃO, 2008). These polymers have gained more and more attention in the field of biopolymers due to their excellent physical, functional and biological properties (ARAÚJO et al., 2012; CAMPO et al., 2009; DE SOUSA et al., 2013; MAZUMDER et al., 2002; PEREIRA et al., 2005; RODRIGUES et al., 2011c; SILVA et al., 2010; WIJESEKARA et al., 2011). According to the review of Campo et al. (2009), the total world production of seaweed phycocolloids has been calculated at about 45,000 metric ton. year^{-1} and a market estimated at U$ 300 million year^{-1}.

Although seaweeds SPs exhibit potential clinical importance, the safe of these compounds could cast doubts as for *in vivo* toxicity. Li et al. (2005) reported that fucoidan from the brown alga *Laminaria japonica*, after orally administrated to rats, exhibited anti-clotting effects at higher doses. Its uses could be a problem for the treatment of patients with chronic renal failure. Studies demonstrated that low molecular weight SPs (carrageenans) induced and promoted intestinal neoplasms and ulcerations in animal models, arousing thus the attention to potential problems associated with the intake of SPs-containing foods by human (CAMPO et al. 2009). Histopathological analysis of liver and kidney of mice, after administration with a SP from the red seaweed *Champia feldmannii*, revealed that both organs have been moderately affected (LINS et al., 2009). A crude SP extracted from the red seaweed *Halymenia floresia* possessed *in vitro* and *in vivo* gastrointestinal effects (GRAÇA et al., 2011), whereas SPs extracted from other algae species had no toxicological significance in rodents (ARAÚJO et al., 2011; CHOI et al., 2009; COURA et al., 2012; DE SOUSA et al., 2013; PENGZHAN et al., 2003; RODRIGUES et al., 2011c; SIQUEIRA et al., 2011; VANDERLEI et al., 2011).

Caulerpa cupressoides var. *lycopodium* (West) C. Agardh (Bryopsidales) is a green marine alga belonging to family Caulerpaceae found along the Atlantic coast of the state of Ceará, Brazil. Anticoagulant (*in vitro*), anti- and prothrombotic (*in vivo*) (RODRIGUES et al., 2011a and b), anti-inflammatory and/or antinociceptive (*in vivo*) (RODRIGUES et al., 2012, 2013) effects have been recently reported for SP fractions from this species, but no detailed toxicological assessment of its crude SP has been studied. In order to contribute with some data, it was evaluated the effects of these polymers on the biochemical and hematological parameters in treated Swiss mice. Histopathological analyses on the organs removed from these animals were also performed.

Material and methods

Experimental design

The crude SP was extracted from *C. cupressoides* and its effects on biochemical and hematological parameters in mice were examined after 14 consecutive days of administration using an *in vivo* toxicity model. Collected organs from the animals were also grossly and microscopically examined during histopathological analyses.

Marine alga and crude SP extraction

The green seaweed *C. cupressoides* var. *lycopodium* was collected on the seashore from the Flecheiras Beach (Trairí, Ceará State, Brazil), and taken to the Carbohydrates and Lectins Laboratory (CarboLec), Department of Biochemistry and Molecular Biology, Federal University of Ceará, Ceará State, Brazil. A voucher specimen (4977) was classified and archived by Ana Cecília Fortes Xavier at the Prisco Bezerra Herbarium, Federal University of Ceará. The alga was cleaned of epiphytes, washed with distillated water and stored (-20°C) until use. Papain digestion (6h, 60°C) was performed for extraction of crude SP, as previously described (RODRIGUES et al. 2011b, 2012).

Animals

Male Swiss mice were randomly selected from the Animal House of the Federal University of Ceará, maintained on a 12h light dark^{-1} cycle, in temperature-controlled rooms and received water and food *ad libitum*. All the procedures and animal treatments were conducted in accordance with the guidelines for Institutional Animal Care and Use of the Federal University of Ceará, Brazil, previously approved by 80/10 protocol.

In vivo toxicological assay

This assay was carried out according to Araújo et al. (2011), Rodrigues et al. (2011c) and Vanderlei et al. (2011). Previous studies on macromolecules suggested that at high doses of SPs from *C. cupressoides* exhibited prothrombotic (RODRIGUES et al., 2011a), antinociceptive and anti-inflammatory

(RODRIGUES et al., 2012) actions using animal models of venous thrombosis, nociception and inflammation, respectively. Based on these reports, *in vivo* toxicological effects from crude SP were evaluated in male Swiss mice (20-26 g) for 14 consecutive days. For this experiment, the animals were previously weighed before of the daily crude SP administration. Crude SP (9 mg kg^{-1}) was dissolved in 0.9% saline and then intraperitoneally administered (10 mL 10 g^{-1}, i.p.) in mice (6 animals $group^{-1}$). Control group received only saline (i.p.). Mice had free access to water and food during the experimental period.

Clinical signs

The animals were daily observed considering the following aspects: body mass variation, survival rate, mucosa, eyes, hair erection, scratch or licking paws, freezing reactions, general behavior, among others.

Biochemical and hematological parameters

On the 15th day, all the mice were anesthetized with 10% chloral hydrate, and blood samples were collected from the retro-orbital plexus for posterior serum clinical biochemistry and hematological examinations. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and urea were considered for biochemical analyses (from the blood centrifuged (1,500 × g, 15 min.) after collection). The serum samples were stored at – 80°C before the analysis. The enzymatic dosages were determined by a colorimetric-enzymatic system using a spectrophotometer (Amersham Biosciences Ultrospec 1100) according to the manufacturer specifications (Labtest Diagnosis, São Paulo, Brazil). These assays aimed to evaluate possible hepatic or renal alterations. Hematological examinations were carried out using an automated pocH 100iV DIFF hematology analyzer (Sysmex Europe GmbH, Hamburg, Germany). Blood samples (15 µL) were subjected to the automatic analyzer to measure the following parameters: red blood cells count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), white blood cell count (WBC), and differential WBC (leukocytes, monocytes and neutrophils). These parameters evaluated possible changes in circulating leukocytes were based on Vanderlei et al. (2011).

Histopathological examination

After the biochemical and hematological examinations, the animals were killed by cervical dislocation to collect of organs (liver, kidney, heart, spleen, thymus, lymph nodes), which were correlated with the respective body mass. These organs were fixed with 10% formalin. Then, the biological materials were dehydrated with ethanol (0-70%) and processed for embedding in paraffin. The resulting blocks were sliced into 5-µm-thick sections, stained with hematoxylin-eosin (H & E) and observed under a light microscope (VANDERLEI et al., 2011).

Statistical analyses

Data were presented as mean ± standard error (SEM) of six animals per group. Analysis of variance (ANOVA) was performed, followed by Student's t test for unpaired values. Values of $p < 0.05$ were considered statistically significant.

Results and discussion

Body mass and weight organs

In the present study, it was evaluated the safe of crude SP obtained from the green seaweed *C. cupressoides*, when injected (i.p.) in a single-dose (9 mg kg^{-1}) in male Swiss mice. After 14 consecutive days of administration in animals, crude SP did not produce any signs of toxicity or mortality compared with the control (saline). Physical and behavior parameters (mucosa or eyes, hair erection, scratch or licking paws, freezing reactions and general behavior) of the animals were also normal, since both groups exhibited similar aspects (ALMEIDA-LIMA et al., 2011; DE SOUSA et al., 2013; RODRIGUES et al., 2011c). In addition, the Table 1 indicated no variation in the *C. cupressoides* crude SP treatment as for relative organ weight after 14 consecutive days, and wet weight of liver, kidney, heart, thymus and lymph nodes ($p > 0.05$) based on Araújo et al. (2011) and Siqueira et al. (2011). However, there was an increase ($p < 0.05$) in the spleen weight, accordingly with previous studies on SPs from other seaweeds species that suggested an immune function response (COURA et al., 2012; LINS et al., 2009; VANDERLEI et al., 2011; ZHANG et al., 2003).

Table 1. Effects of crude SP from the green seaweed *C. cupressoides* var. *lycopodium* on body mass and relative organ weight in mice after 14 consecutive days.

Parameter	Treatment (i.p.)	
	Saline (0.9%)	Crude SP (9 mg kg^{-1})
Body mass before treatment	24.00 ± 0.85	25.50 ± 1.05
Body mass after treatment	32.00 ± 1.06	31.17 ± 0.54
Heart	0.18 ± 0.01	0.17 ± 0.01
Liver	1.74 ± 0.09	1.95 ± 0.05
Kidney	0.24 ± 0.01	0.26 ± 0.01
Thymus	0.13 ± 0.10	0.10 ± 0.05
Limph nodes	0.07 ± 0.01	0.07 ± 0.04
Spleen	0.13 ± 0.03	0.41 ± 0.07*

Data are express as mean ± S.E.M. (n=6); ANOVA analysis followed by Student's t-test. *$p < 0.05$ statistically different.

An elucidation of this stimulatory mechanism could be helpful in future studies (LINS et al., 2009; ZHANG et al., 2003). Recently, De Sousa et al. (2013) reported a sulfated galactan from the red seaweed *Gelidium crinale* that did not affect the spleen weight of rats when administered for 10 days. Thus, each polysaccharide could determine a specific biological stimulus *in vivo*.

Popularly known as sea vegetables in many parts of the world, the human intake of seaweed as food and medicinal products has significantly increased in recent decades (MABEAU; FLEURENCE, 1993; GUPTA; ABU-GHANNAM, 2011). However, *in vivo* toxicological studies of their cell-wall chemical components have been scarcely investigated. Lately, seaweed SPs with biological properties of interest in the field of biomaterials have been isolated and studied (WIJESEKARA et al., 2011). Zhang et al. (2003) found that a polysaccharide fraction from *Porphyra haitanesis* (Rhodophyta) presented antioxidant activity (*in vivo*) in aging mice. This fraction was tested in different organs (lung, liver, heart, brain spleen and serum) of aging mice, and resulted in a significant increase of the spleen index by the activity of superoxide dismutase, an intracellular compound that protects against oxidative process, reducing thus the risks of lipid peroxidation in animals.

Indeed, the antioxidant property of SPs may be considered of potential pharmacological value (ANANTHI et al., 2010), suggesting the role as dietary free radical scavenger for the prevention of oxidative damage and, consequently, diseases (e.g., melanoma, cardiac disorders, diabetes mellitus, inflammatory and neurodegenerative) in living organisms (GUPTA; ABU-GHANNAM, 2011; WIJESEKARA et al., 2011; ZHANG et al., 2003). More recently, Lins et al. (2009), analyzed the *in vivo* antitumor activity of a SP isolated from the red seaweed *C. feldimannii* in mice transplanted with Sarcoma 180 tumors, and observed an increase in the spleen weight when compared to control and 5-FU-treated mice, a drug used for the treatment of tumors. Its effects could be associated with immunostimulating properties.

In contrast to these reports, carrageenan, a SP well-known as an important ingredient in many types of processed food (especially as food additives) and used in experimental research in animals by leading to pleurisy (SILVA et al., 2010), could also cause colorectal ulcers, tumors, cancers in humans and gastrointestinal problems, being thus the safe of SPs subject to controversial debate (CAMPO et al., 2009; GRAÇA et al., 2011; MABEAU; FLEURENCE, 1993; SMIT, 2004). In this regard, a detailed investigation with crude SP from *C. cupressoides* is needed.

Histopathological examination

In order to verify any morphological alteration in the organs removed from animals treated with a single injection of crude SP (9 mg kg^{-1}) from the green seaweed *C. cupressoides*, our studies were also extended to histopathological analyses (Figure 1). Our findings revealed that no damage in the heart tissue was observed (Figure 1A). In the liver, crude SP-treated mice showed a discrete cloudy degeneration with cytoplasmic-vacuole formations (yellow arrows) (Figure 1C); but, no damage to the tissue was observed once the hepatocytes have been preserved. A tubular degenerative process was verified in the kidney, including the formation of distal tubules in the renal epithelium (yellow arrows, cortical region) (Figure 1E), but the glomeruli structures were essentiality preserved when compared to control group (Figure 1F). These data were not indicative of nephorotoxicity suggested by Coura et al. (2012). Morphological alterations in the liver and kidney may have occurred as a result of the use of different SPs. Also, the results suggested a possible reversibility of the observed degenerative processes (ARAÚJO et al., 2011; LINS et al., 2009; RODRIGUES et al., 2012; VANDERLEI et al., 2011).

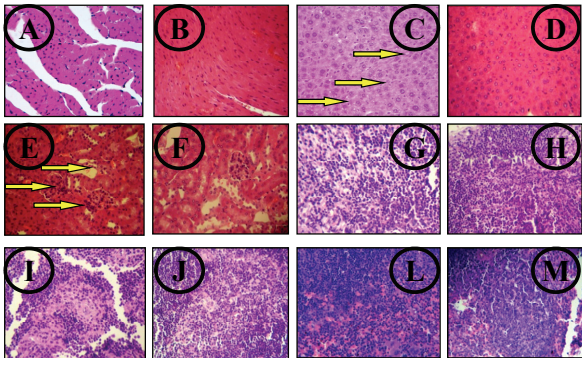

Figure 1. Photomicrographic analyses (cross sections) of the organs removed from mice treated with crude SP (9 mg kg^{-1}) extracted from the green alga *Caulerpa cupressoides* var. *lycopodium* after 14 days. Crude SP group: (A) heart, (C) liver, (E) kidney, (G) lymph nodes, (I) thymus, and (L) spleen. Saline group: (B) heart, (D) liver (F) kidney, (H) lymph nodes, (J) thymus, and (M) spleen. Subcapsular hepatocytes with cloudy degeneration in the liver and light tubular subcapsular degeneration in the kidney were noted (yellow arrows). These processes are reversible. The tissue sections were observed under a light microscope at 400 ×.

Choi et al. (2009) reported a crude polysaccharide from the brown seaweed *Hizikia fusiformis* with protective effects against peptic injury (ulcers), using a rat model system. These authors

demonstrated that this molecule apparently inhibited ethanol-induced gastric damage in the stomach (preventing surface epithelial cell destruction and loss of the surface mucosa layer). No gastric bleeding was also observed. Additionally, the *H. fusiformis* crude polysaccharide directly affected the caspase activation, PARP cleavage, and DNA fragmentation through a mechanism associated with the down-regulation of phosphor-jun *N*-terminal kinase and resolution of normal glutathione levels.

Antihyperlipidemic effects of different molecular weight SPs from the green seaweed *Ulva pertusa* were investigated by Pengzhan et al. (2003). Ulvan as designated and its fractions (U1 and U2) presenting from 28.2 to 151.6 kDa were given to male Wistar rats on a hypercholesterolemic diet for 21 days. The results showed that the Ulvan-based diet reduced the levels of serum total cholesterol and density lipoprotein cholesterol, whereas the fractions significantly increaed the levels of serum high density lipoprotein cholesterol and reduced triglyceride compared with the control. Furthermore, the intake of these polysaccharides has significantly increased fecal bile acid excrement, exhibiting thus diverse effects on lipid metabolism. Inhibitory effects of liver damage by hyperlipidemia were also observed for U1 and U2.

In respect to lymph node, thymus and spleen, no response to injury was found (Figures 1G, I and L) compared to controls (Figures 1H, J and M) (LI et al., 2005). Therefore, the increased spleen size demonstrated in Table 1 had no toxicological importance, given the lack of any histological change related to the treatment with crude SP (*C. cupressoides*) (Figure L) (COURA et al., 2012). Lins et al. (2009) isolated a SP from the red seaweed *C. feldmannii* with antitumor properties in mice. This polysaccharide exhibited toxicity at low concentration (25 μg mL^{-1}) leading to hyperplasia of lymphoid follicles with a nest of megakaryocytes in spleen, and reverting the leukopenia, but did not have any edema or lymphocyte infiltration. The authors concluded that the histopathology and morphological changes were potentially reversible, once the interstitial tissue was preserved.

Biochemical and hematological parameters

In order to correlate the results obtained in Figure 1, *in vivo* systemic treatment with crude SP (9 mg kg^{-1}) from the green seaweed *C. cupressoides* in mice over 14 consecutive days did not alter the levels of the enzymatic markers of hepatic (ALT and AST) and renal (urea) functions compared to their respective controls (Table 2) (COURA et al., 2012; LI et al., 2005; RODRIGUES et al., 2011c; SIQUEIRA et al., 2011; VANDERLEI et al., 2011). The observed reduction ($p < 0.05$) in the dosage of blood urea and AST was considered devoid of toxicological significance based on Araújo et al. (2011). The absence of biochemical changes in this study was consistent with histopathological analyses (Figure 1) (COURA et al., 2012; VANDERLEI et al., 2011). Taken together, these data demonstrated that the crude extract containing SPs from the green seaweed *C. cupressoides* did not cause hepatocellular or renal damage in treated mice (ALMEIDA-LIMA et al., 2011; LI et al., 2005; RODRIGUES et al., 2011c, 2012).

Table 2. Biochemical analyses of plasma of intraperitoneally treated mice with crude SP (*Caulerpa cupressoides* var. *lycopodium*) after 14 consecutive days.

Parameter	Treatment (i.p.)	
	Saline (0.9%)	Crude SP (9 mg kg^{-1})
Urea (mg dL^{-1})	58.13 ± 0.12	44.17 ± 10.51⋆
ALT (U L^{-1})	15.06 ± 1.06	17.20 ± 2.86
AST (U L^{-1})	48.41 ± 14.44	38.43 ± 5.32⋆

Data are express as mean ± S.E.M. (n=6); ANOVA analysis followed by Student's t-test; ⋆$p < 0.05$ statistically different.

An important result was the lack of toxicity on the levels of ALT and AST (Table 2) because the SPs concentration was 4.5-fold higher than the antithrombotic dose recorded in another study. It was demonstrated an antithrombin-dependent SP from the green seaweed *C. cupressoides* exhibiting *in vivo* antithrombotic effects, like heparin, but no *in vivo* hemorrhagic effect (RODRIGUES et al., 2011a). Its SPs also presented *in vivo* anti-inflammatory and/or antinociceptive effects (RODRIGUES et al., 2012, 2013). Because of the increasing incidence of cardiovascular diseases, especially in developed countries, heparin, a commercially extracted SP from animal tissues, is widely used in the anticoagulant therapy in post-operative and post-traumatic management of patients in medical clinic. However, it is also known for its adverse effects (e.g., hemorrhage) (MAJERUS; TOLLEFESEN, 2005), justifying the search for potential therapeutic alternatives to heparin (PEREIRA et al., 2005; POMIN, 2012; RODRIGUES et al., 2011b). This anticoagulant polysaccharide preponderantly requires antithrombin, the major serine protease inhibitor (serpin) of the blood coagulation, as a cofactor to inhibit thrombin (target protease). Antithrombin is produced in the liver (QUINSEY et al., 2004).

The Table 3 summarizes the results of *C. cupressoides* crude SP-treated mice on the blood parameters after 14 consecutive days. The analyzed parameters indicated no significant alteration on the

number of circulating total leukocytes ($p > 0.05$), being thus preserved the number of defense cells in animals. RBC, HGB, HCT, MCV, MCH, MCHC and WBC hematological parameters were also not affected by crude SP treatment compared to their respective controls ($p > 0.05$) (COURA et al., 2012; SIQUEIRA et al., 2011; VANDERLEI et al., 2011). Except for the PLT count, which had significantly increased compared with saline group ($p < 0.05$), possibly indicating a thrombotic stimulus (MAJERUS; TOLLEFESEN, 2005).

Table 3. Hematological parameters of plasma of intraperitoneally treated mice with crude SP (*Caulerpa cupressoides* var. *lycopodium*) after 14 consecutive days.

Parameter	Treatment (i.p.)	
	Saline (0.9%, 0.1 mL $10g^{-1}$)	Crude SP (9 mg kg^{-1})
RBC (10^6 μL^{-1})	8.95 ± 0.15	8.47 ± 0.32
HGB (g dL^{-1})	13.53 ± 0.46	14.45 ± 0.14
HCT (%)	123.12 ± 3.20	123.77 ± 2.30
MCV (fL)	52.33 ± 1.60	51.43 ± 0.27
MCH (pg)	16.18 ± 0.31	15.98 ± 0.12
MCHC (g dL^{-1})	30.85 ± 0.18	30.93 ± 0.43
WBC (10^3 μL^{-1})	3.44 ± 0.77	4.07 ± 0.45
Lymphocytes (%)	87.47 ± 1.23	85.88 ± 1.11
Monocytes (%)	0.0 ± 0.0	0.0 ± 0.0
Neutrophils (%)	12.34 ± 1.51	13.22 ± 1.05
PLT (10^3 μL^{-1})	969.75 ± 0.51	1530.75 ± 1.05*

Data are express as mean ± S.E.M. (n=6); ANOVA analysis followed by Student's t-test; *$p < 0.05$ statistically different.

De Sousa et al. (2013) found that a sulfated galactan isolated from the red seaweed *G. crinale* exhibited some slight changes in hematological parameters (circulating erythrocytes, total leukocytes and lymphocytes) of rats. In this study, it was possible to postulate that *C. cupressoides* SPs did not promote either leukocyte agglutination or secondary effects in lymphoid tissues of the treated mice (Tables 1 and 3 and Figure 1) (SIQUEIRA et al., 2011).

In summary, current findings suggested a potential applicability of SPs from the green seaweed *C. cupressoides* as novel, natural pharmaceutical product in the fields of biochemical research and biomedicine (RODRIGUES et al., 2012, 2013). However, additional studies to understanding the *in vivo* systemic effects, especially on platelet function (POMIN, 2012), are also required, arousing interest from our research group.

Conclusion

The green seaweed *Caulerpa cupressoides* var. *lycopodium* has crude sulfated polysaccharide extracted with papain devoid of important systemic effects in mice. However, the increase in spleen size and platelet count could be related to possible immunostimulating and thrombotic actions, respectively.

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References

ALMEIDA-LIMA, J.; DANTAS-SANTOS, N.; GOMES, D. L.; CORDEIRO, S. L.; SABRY, D. A.; COSTA, L. S.; FREITAS, M. L.; SILVA, N. B.; MOURA, C. E. B.; LEMOS, T. M. A. M.; LEITE, E. L.; ROCHA, H. A. O. Evaluation of acute and subchronic toxicity of a non-anticoagulant, but antithrombotic algal heterofucan from the *Spatoglossum schröderi* in Wistar rats. **Brazilian Journal of Pharmacognosy**, v. 21, n. 4, p. 674-679, 2011.

ANANTHI, S.; RAGHAVENDRAN, H. R. B.; SUNIL, A. G.; GAYATHRI, V.; RAMAKRISHNAN, G.; VASANTHI, H. R. *In vitro* antioxidant and *in vivo* anti-inflammatory potential of crude polysaccharide from *Turbinaria ornata* (Marine Brown Alga). **Food and Chemical Toxicology**, v. 48, n. 1, p. 187-192, 2010.

ARAÚJO, I. W. F.; RODRIGUES, J. A. G.; VANDERLEI, E. S. O.; PAULA, G. A.; LIMA, T. B.; BENEVIDES, N. M. B. *Iota*-carrageenans from *Solieria filiformis* (Rhodophyta) and their effects in the inflammation and coagulation. **Acta Scientiarum. Technology**, v. 34, n. 2, p. 127-135, 2012.

ARAÚJO, I. W. F.; VANDERLEI, E. S. O.; RODRIGUES, J. A. G.; COURA, C. O.; QUINDERÉ, A. L. G.; FONTES, B. P.; QUEIROZ, I. N. L.; JORGE, R. J. B.; BEZERRA, M. M.; SILVA, A. A. R.; CHAVES, H. V.; MONTEIRO, H. S. A.; PAULA, R. C. M.; BENEVIDES, N. M. B. Effects of a sulfated polysaccharide isolated from the red seaweed *Solieria filiformis* on models of nociception and inflammation. **Carbohydrate Polymers**, v. 86, n. 3, p. 1207-1215, 2011.

CAMPO, V. L.; KAWANO, D. F.; SILVA, D. B.; CARVALHO, I. Carrageenans: Biological properties, chemical modifications and structural analysis – A review. **Carbohydrate Polymers**, v. 77, n. 2, p. 167-180, 2009.

CHOI, E. Y.; HWANG, H. J.; KIM, I. H.; NAM, T. J. Protective effects of a polysaccharide from *Hizikia fusiformis* against ethanol toxicity in rats. **Food and Chemical Toxicology**, v. 47, n. 1, p. 134-139, 2009.

COURA, C. O.; ARAÚJO, I. W. F.; VANDERLEI, E. S. O.; RODRIGUES, J. A. G.; QUINDERÉ, A. L. G; FONTES, B. P.; QUEIROZ, I. N. L.; MENEZES, D. B.; BEZERRA, M. M.; SILVA, A. A. R.; CHAVES, H. V.; JORGE, R. J. B.; EVANGELISTA, J. S. A. M.; BENEVIDES, N. M. B. Antinociceptive and anti-inflammatory activities of sulfated

polysaccharides from the red seaweed *Gracilaria cornea*. **Basic and Clinical Pharmacology and Toxicology**, v. 110, n. 4, p. 335-341, 2012.

DE SOUSA, A. A. S.; BENEVIDES, N. M. B.; PIRES, A. F.; FIÚZA, F. P.; QUEIROZ, M. G. R.; MORAIS, T. M. F.; PEREIRA, M. G.; ASSREUY, A. M. S. A report of a galactan from marine alga *Gelidium crinale* with *in vivo* anti-inflammatory and antinociceptive affects. **Fundamental and Clinical Pharmacology**, v. 27, n. 2, p. 173-180, 2013.

GRAÇA, J. R. V.; BEZERRA, M. M.; LIMA, V.; RODRIGUES, J. A. G.; MONTEIRO, D. L. S.; QUINDERÉ, A. L. G.; AMORIM, R. C. N.; DE PAULA, R. C. M.; BENEVIDES, N. M. B. Effect of a crude sulfated polysaccharide from *Halymenia floresia* (Rhodophyta) on gastrointestinal smooth muscle contractility. **Brazilian Archives of Biology and Technology**, v. 54, n. 5, p. 907-916, 2011.

GRESSLER, V.; YOKOYA, N. S.; FUJII, M. T.; COLEPICOLO, P.; MANCINI FILHO, J.; TORRES, R. P.; PINTO, E. Lipid fatty acid, protein, amino acid and ash contents in four Brazilian red algae species. **Food Chemistry**, v. 120, n. 2, p. 585-590, 2010.

GUPTA, S.; ABU-GHANNAM, N. Recent developments in the application of seaweeds or seaweed extracts as a means for enhancing the safety and quality attributes of foods. **Innovative Food Science and Emerging Technologies**, v. 12, n. 4, p. 600-609, 2011.

LI, N.; ZHANG, Q.; SONG, J. Toxicological evaluation of fucoidan extracted from *Laminaria japonica* in Wistar rats. **Food and Chemical Toxicology**, v. 43, n. 3, p. 421-426, 2005.

LINS, K. O. A. L.; BEZERRA, D. P.; ALVES, A. P. N. N.; ALENCAR, N. M. N.; LIMA, M. W.; TORRES, W. M.; FARIAS, W. R. L.; PESSOA, C.; MORAES, M. O.; COSTA-LOTUFO, L. V. Antitumor properties of a sulfated polysaccharide from the red seaweed *Champia feldmannii* (Diaz-Pifferer). **Journal of Applied Toxicology**, v. 29, n. 1, p. 20-26, 2009.

MABEAU, S.; FLEURENCE, J. Seaweed in food products: Biochemical and nutritional aspects. **Trends and Food Science and Technology**, v. 4, n. 4, p. 103-107, 1993.

MAJERUS, P. W.; TOLLEFESEN, D. M. Anticoagulantes, trombolíticos e fármacos antiplaquetários. In: GILMAN, A. G. (Ed.). **As bases farmacológicas da terapêutica**. Rio de Janeiro: McGraw-Hill, 2005. p. 1122-1155.

MARINHO-SORIANO, E.; FONSECA, P. C.; CARNEIRO, M. A. A.; MOREIRA, W. S. C. Seasonal variation in the chemical composition of two tropical seaweeds. **Bioresource Technology**, v. 97, n. 18, p. 2402-2406, 2006.

MAZUMDER, S.; GHOSAL, P. K.; PUJOL, C. A.; CARLUCCI, M.; DAMONTE, E. B.; RAY, B. Isolation, chemical investigation and antiviral activity of polysaccharides from *Gracilaria corticata* (Gracilariaceae, Rhodophyta). **International Journal of Biological Macromolecules**, v. 31, n. 1-3, p. 87-95, 2002.

PENGZHAN, Y.; NING, L.; XIGUANG, L.; GEFEI, Z.; QUANBIN, Z.; PENGCHENG, L. Antihyperlipidemic effects of different molecular weight sulfated polysaccharides from *Ulva pertusa* (Chlorophyta). **Pharmacological Research**, v. 48, n. 6, p. 543-549, 2003.

PEREIRA, M. G.; BENEVIDES, N. M. B.; MELO, M. R. S.; VALENTE, A. P.; MELO, F. R.; MOURÃO, P. A. S. Structure and anticoagulant activity of a sulfated galactan from the red alga, *Gelidium crinale*. Is there a specific structural requirement for the anticoagulant action? **Carbohydrate Research**, v. 340, n. 12, p. 2015-2023, 2005.

PIRES, K. M. S.; ALENCAR, D. B.; SOUSA, M. B.; SAMPAIO, A. H.; SAKER-SAMPAIO, S. Teores de α-caroteno e β-caroteno em macroalgas marinhas desidratadas. **Revista Ciência Agronômica**, v. 29, n. 2, p. 257-262, 2008.

POMIN, V. H. Fucanomics and galactanomics: Current status in drugs discovery, mechanism of action and role of the well-defined structures. **Biochimica et Biophysica Acta**, v. 1820, n. 12, p. 1971-1979, 2012.

POMIN, V. H.; MOURÃO, P. A. S. Structure, biology, evolution, and medical importance of sulfated fucans and galactans. **Glycobiology**, v. 18, n. 12, p. 1016-1027, 2008.

QUINSEY, N. S.; GREEDY, A. L.; BOTTOMLEY, S. P.; WHISSTOCK, J. C.; PIKE, R. N. Antithrombin: in control of coagulation. **The International Journal of Biochemistry and Cell Biology**, v. 36, n. 3, p. 386-389, 2004.

RODRIGUES, J. A. G.; QUEIROZ, I. N. L.; QUINDERÉ, A. L. G.; VAIRO, B. C.; MOURÃO, P. A. S.; BENEVIDES, N. M. B. An antithrombin-dependent sulfated polysaccharide isolated from the green alga *Caulerpa cupressoides* has *in vivo* anti- and prothrombotic effects. **Ciência Rural**, v. 41, n. 4, p. 634-639, 2011a.

RODRIGUES, J. A. G.; VANDERLEI, E. S. O.; BESSA, E. F.; MAGALHÃES, F. A.; PAULA, R. C. M.; LIMA, V.; BENEVIDES, N. M. B. Anticoagulant activity of a sulfated polysaccharide isolated from the green seaweed *Caulerpa cupressoides*. **Brazilian Archives of Biology and Technology**, v. 54, n. 4, p. 691-700, 2011b.

RODRIGUES, J. A. G.; ARAÚJO, I. W. F.; PAULA, G. A.; VANDERLEI, E. S. O.; QUEIROZ, I. N. L.; QUINDERÉ, A. L. G.; COURA, C. O.; BESSA, E. F.; LIMA, T. B.; BENEVIDES, N. M. B. Isolamento, fracionamento e avaliação toxicológica *in vivo* de polissacarídeos sulfatados de *Hypnea musciformis*. **Ciência Rural**, v. 41, n. 7, p. 1211-1217, 2011c.

RODRIGUES, J. A. G.; VANDERLEI, E. S. O.; SILVA, L. M. C. M.; DE ARAÚJO, I. W. F.; DE QUEIROZ, I. N. L.; DE PAULA, G. A.; ABREU, T. M.; RIBEIRO, N. A.; BEZERRA, M. M.; CHAVES, H. V.; LIMA, V.; JORGE, R. J. B.; MONTEIRO, H. S. A.; LEITE, E. L.; BENEVIDES, N. M. B. Antinociceptive and anti-inflammatory activities of a sulfated polysaccharide isolated from the green seaweed *Caulerpa cupressoides*. **Pharmacological Reports**, v. 64, n. 2, p. 282-292, 2012.

RODRIGUES, J. A. G.; VANDERLEI, E. S. O.; QUINDERÉ, A. L. G.; MONTEIRO, V. S.; DE VASCONCELOS, S. M. M.; BENEVIDES, N. M. B. Antinociceptive activity and acute toxicological study of a

novel sulfated polysaccharide from *Caulerpa cupressoides* var. *lycopodium* (Chlorophyta) in Swiss mice. **Acta Scientiarum. Technology**, v. 35, n. 3, p. 417-425, 2013.

SCHEVCHENKO, N. M.; BURTSEVA, Y. B.; ZVYAGINTSEVA, T. N.; MAKAREVA, T. N.; SERGEEVA, O. S.; ZAKHARENKO, A. M.; ISAKOV, V. V.; LINH, N. T.; HOA, N. X.; LY, B. M.; HUYEN, P. V. Polysaccharides and sterols from green algae *Caulerpa lentillifera* and *C. sertularioides*. **Chemistry of Natural Compounds**, v. 45, n. 1, p. 1-5, 2009.

SILVA, F. R. F.; DORE, C. M. P. G.; MARQUES, C. T.; NASCIMENTO, M. S.; BENEVIDES, N. M. B.; ROCHA, H. A. O.; CHAVANTE, S. F.; LEITE, E. L. Anticoagulant activity, paw edema and pleurisy induced carrageenan: Action of major types of commercial carrageenans. **Carbohydrate Polymers**, v. 79, n. 1, p. 29-33, 2010.

SMIT, A. Medicinal and pharmaceutical uses of seaweed natural products: A review. **Journal of Applied Phycology**, v. 16, n. 4, p. 245-262, 2004.

SIQUEIRA, R. C. L.; SILVA, M. S. J.; ALENCAR, D. B.; PIRES, A. F.; ALENCAR, ALENCAR, N. M. N.; PEREIRA, M. G.; CAVADA, B. S.; SAMPAIO, A. H.; FARIAS, W. R. L.; ASSREUY, A. M. S. *In vivo* anti-inflammatory effect of a sulfated polysaccharide isolated from the marine brown algae *Lobophora variegata*. **Pharmaceutical Biology**, v. 49, n. 2, p. 167-174, 2011.

TABARSA, M.; REZAEI, M.; RAMEZANPOUR, Z.; WAALAND, J. R. Chemical compositions of the marine algae *Gracilaria salicornia* (Rhodophyta) and *Ulva lactuca* (Chlorophyta) as a potential food source. **Journal of the Science of Food and Agriculture**, v. 92, n. 12, p. 2500-2506, 2012.

TOSKAS, G.; HUND, R. D.; LAOURINE, E.; CHERIF, C.; SMYRNIOTOPOULOS, V.; ROUSSIS, V. Nanofibers based on polysaccharides from the green seaweed *Ulva rigida*. **Carbohydrate Polymers**, v. 84, n. 3, p. 1093-1102, 2011.

VALENTE, L. M. P.; GOUVEIA, A.; REMA, P.; MATOS, J.; GOMES, E. F.; PINTO, L. S. Evaluation of three seaweeds *Gracilaria bursa-pastoris*, *Ulva rigida* and *Gracilaria cornea* as dietary ingredients in European sea bass (*Dicentrarchus labrax*) juveniles. **Aquaculture**, v. 252, n. 1, p. 85-91, 2006.

VANDERLEI, E. S. O.; PATOILO, K. K. N. R.; LIMA, N. A.; LIMA, A. P. S.; RODRIGUES, J. A. G.; SILVA, L. M. C. M.; LIMA, M. E. P.; LIMA, V.; BENEVIDES, N. M. B. Antinociceptive and anti-inflammatory activities of lectin from the marine green alga *Caulerpa cupressoides*. **International Immunopharmacology**, v. 10, n. 9, p. 1113-1118, 2010.

VANDERLEI, E. S. O.; ARAÚJO, I. W. F.; QUINDERÉ, A. L. G.; FONTE, B. P.; ELOY, Y. R. G.; RODRIGUES, J. A. G.; SILVA, A. A. R.; CHAVES, H. V.; JORGE, R. J. B.; MENEZES, D. B.; EVANGELISTA, J. S. A. M.; BEZERRA, M. M.; BENEVIDES, N. M. B. The involvement of the HO-1 pathway in the anti-inflammatory action of a sulfated polysaccharide isolated from the red seaweed *Gracilaria birdiae*. **Inflammation Research**, v. 60, n. 12, p. 1121-1130, 2011.

WIJESEKARA, I.; PANGESTUTI, R.; KIM, S. K. Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. **Carbohydrate Polymers**, v. 84, n. 1, p. 14-21, 2011.

ZHANG, Q. B.; LI, N.; ZHOU, G. F.; LU, X. L.; XU, Z. H.; LI, Z. *In vivo* antioxidant activity of polysaccharide fraction from *Porphyra haitanesis* (Rhodophyta) in aging mice. **Pharmacological Research**, v. 48, n. 2, p. 151-155, 2003.

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