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Antinociceptive and anti-inflammatory effects of cellular and extracellular extracts from microalga *Chlamydomonas pumilioniformis* on mice

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ASTRACT. Microalga species have attracted interest as a source of bioactive compounds with several pharmacological activities. Previous studies reported that microalgae from the genus *Chlamydomonas* have anti-inflammatory and antioxidant properties. In this study, antinociceptive and anti-inflammatory activities of two extracts from microalga *Chlamydomonas pumilioniformis* were investigated. Cellular and extracellular extracts were prepared from a 14 day-batch culture in WC medium at the end of exponential growth and their carbohydrate contents were determined. Antinociceptive effects of extracts were evaluated by writhing and formalin-induced nociception tests, while the anti-inflammatory activity was analyzed by formalin-induced paw edema in mice. The analysis of dissolved carbohydrates detected amounts of 90 and 20 μg mL^{-1} of total carbohydrate in cellular and extracellular extracts, respectively. Cellular extract was mainly composed of glucose, but with significant proportions of arabinose, galactose and mannose and/or xylose and minor ones of fucose, rhamnose, amino sugars and uronic acids. Extracellular extract was composed of a similar proportion of glucose, galactose and mannose/xylose, besides significant ones of arabinose, fucose and galacturonic acid. Intraperitoneal administration of extracts significantly reduced writhing response in mice. In the formalin test, the extracellular extract inhibited both formalin phases, while the cellular extract was only effective in the late phase. Furthermore, extracts reduced the formalin-induced paw edema. In sum, we showed, for the first time, that *C. pumilioniformis* can be an important source of polysaccharides with anti-inflammatory and antinociceptive effects.

Keywords: inflammation; nociception; green microalga; carbohydrate.

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Introduction

Inflammation is an important protective biological response to infectious agents, tissue injury and chemical agents, but it also contributes to the pathophysiology of many human diseases. The inflammatory process is initiated by the migration of immune cells from blood vessels and release of mediators. Then, inflammatory cells are recruited and reactive oxygen and nitrogen species and proinflammatory cytokines are produced either to eliminate the pathogen or to repair injured tissue (Libby, 2008; Pan, Lai, & Ho, 2010). Inflammation is clinically characterized by five signs: redness, heat, swelling, pain and loss of function (Ji, Chamessian, & Zhang, 2016). Pain, which is one of the most important symptoms of inflammatory diseases, impacts quality of life and is frequently the reason why patients seek pharmacological treatments (Uritu et al., 2018).

Non-steroidal anti-inflammatory drugs have been mostly prescribed to relieve pain and reduce inflammation. Although these drugs have good anti-inflammatory and analgesic properties, they have a wide range of side effects specially when used for long periods (Wongrakpanich, Wongrakpanich, Melhado, & Rangaswami, 2018). Thus, the search for new anti-inflammatory and analgesic substances derived from natural sources could contribute to the development of new drugs with fewer adverse effects (Rodrigues et al., 2012; Hädrich et al., 2016; Uritu et al., 2018).

Microalgae are a particularly interesting group of aquatic organisms which constitute a valuable source of compounds with positive effects on human and animal health (Mimouni et al., 2012; Ulmann et al., 2017).

Their constituents are also of great interest as long-chain fatty acids that could be used as dietary supplements, as well as proteins and pigments, which have interesting properties to the pharmaceutical industry. Polysaccharides, which are produced by many species of microalgae, have several applications to the pharmaceutical field (Raposo, Morais, & Morais, 2013).

Some studies of the pharmacological activity of microalgae have centered on antimicrobial (Raposo, Morais, & Morais, 2014; Dantas et al., 2019), antiviral (Kim et al., 2012), antitumoral (Koníčková et al., 2014; Trabelsi, Chaieb, Mnari, Abid-Essafi, & Aleya, 2016), antioxidant (Tobón-Velasco et al., 2013; Silva et al., 2020), anti-inflammatory (Pak et al., 2012; Hwang, Chen, & Chan, 2013) and immunomodulatory properties (Park et al., 2011; Abdel-Daim, Farouk, Madkour, & Azab, 2015). For example, hydrosoluble extracts from *Chlorella stigmatophora* and *Phaeodactylum tricornutum* showed anti-inflammatory and antinocicpetive activities in acute experimental rat models (Guzmán, Gato, & Calleja, 2001). In another study, Abdel-Daim et al. (2015) verified the anti-inflammatory activity of extracts from two microalga species, *Spirulina platensis* and *Dunaliella salina,* in experimental colitis in rats. Moreover, some studies have shown that microalga-derived polysaccharides play an important role in anti-inflammatory activity in preclinical trials (Levy-Ontman, Huleihel, Hamias, Wolak, & Paran, 2017; Barboríková et al., 2019)

Microalga species of the genus *Chlamydomonas* (Chlorophyceae) have attracted interest in studies of the effects of their constituents. The genus includes unicellular chlorophyte algae that have a distinct cell wall, a nucleus, two anterior flagella, a single basal chloroplast and an eyespot (Harris, 2001). Some studies have shown that microalgae from this genus, *Chlamydomonas debaryana* and *Chlamydomonas hedleyi*, exhibit anti-inflammatory and antioxidant effects in experimental models *in vitro* and *in vivo* (Ávila-Román et al., 2014; Reyes et al., 2014; Suh et al., 2014). In the study carried out by Ávila-Román et al. (2014), the authors showed that oxylipin-containing biomass of microalga *Chlamydomonas debaryana* decreases inflammatory processes by reducing polymorphonuclear infiltration and levels of tumor necrosis factor-α and by suppressing the expression of cycloxigenase-2 (COX-2) and nitric oxide synthase (iNOS) in induced colitis in rats. This research group isolated and characterized oxylipins from *Chlamydomonas debaryana* that inhibited TNF-α production in lipopolysaccharide-stimulated macrophages (Reyes et al., 2014). Furthermore, mycosporine-like amino acids obtained from extracts from *Chlamydomonas hedleyi* decreased mRNA levels of COX-2, which increase in response to inflammation in cell culture of keratinocytes (Suh et al., 2014).

Although some studies report pharmacological activities of microalgae of the genus *Chlamydomonas,* there are no studies of the anti-inflammatory and analgesic properties of the species *Chlamydomonas pumilioniformis*. Therefore, considering that this microalga species could be a potential source of bioactive compounds, this study aimed to characterize carbohydrate contents of two different extracts (cellular and extracellular) from *C. pumilioniformis* and investigate their antinociceptive and anti-inflammatory activities in mice models.

Material and methods

Culture conditions

Chlamydomonas pumilioniformis (FURGCH007) was isolated from a small shallow pond (surface area is 1,000 m^2 and mean depth is 1.5 m), located at the *Universidade Federal do Rio Grande* (FURG) in the extreme south of Brazil (32° 04’ 18” S and 52° 09’ 57” W). Cultures were obtained by micropipette isolation under the microscope and several washes with 1:10 diluted WC medium (Guillard & Lorenzen, 1972). Cultures were maintained in the freshwater microalga culture collection that belongs to the Institute of Biological Sciences, *Universidade Federal do Rio Grande*. Cultures were kept in a 10:14 hours dark:light cycle of 100 µmoL photons m^{-2} s^{-1} in a culture room at controlled temperature (25 ± 1°C).

Preparation of *C. pumilioniformis* extracts

Cellular and extracellular *C. pumilioniformis* extracts were prepared from a 14 day-batch culture in WC medium at the end of exponential growth (600 mL, 7 x 10^6 cells per mL). The whole volume of the culture was centrifuged (15 min., 4000 rpm) at 4ºC (Sigma 2K15, Osterode am Harz, Germany). Supernatants were filtered through a glass fiber filter whose pore size was 0.6-µm (GF/3 Macherey-Nagel, Düren, Germany) and resulting extracellular extract was stored at -4°C. The cellular extract was obtained from the cell pellet that was washed three times and resuspended in 200 mL of fresh WC medium. Re-suspended cells were frozen and thawed three times, in order to promote cell lysis, filtered as described above and finally stored at -4°C. Considering that the culture had 7 x 10^6 cells per mL and 600 mL, both cellular and extracellular extracts

were obtained from 4.2 x 10^9 cells. Absence of viable cells in both cellular and extracellular extracts was confirmed by microscopic observations before biological tests.

Carbohydrate analysis

Total dissolved carbohydrate content was determined in both cellular and extracellular extracts by the phenol-sulfuric assay (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956). Preliminary composition analysis of cellular and extracellular dissolved carbohydrates was performed by high performance ion chromatography coupled to pulse amperometric detection (HPIC-PAD). The HPIC-PAD analysis was performed by a Dionex DX500 (Sunnyvale, CA, USA) device consisting of a PEEK GP40 gradient pump module, an ED40 electrochemical detector and an LC5 manual injector with a Rheodyne 9125 valve and a 25 µL PEEK sample loop. The ED40 detector was equipped with an amperometric flow cell, a gold working electrode and an Ag/AgCl reference electrode. A PA-10 (Dionex) anion-exchange analytical column (4 x 250 mm), which was equipped with a corresponding guard-column (4 x 50 mm), was used to separate monosaccharides. The eluent used for separation was 18 mm NaOH. Column recovery was performed with a 200 mm NaOH solution at a flow rate of 1 mL $min.^{-1}$ (Gremm & Kaplan, 1997). Standards were used for fucose, galactosamine, rhamnose, arabinose, glucosamine, galactose, glucose, xylose, mannose, fructose, ribose, galacturonic acid and glucuronic acid (Sigma-Aldrich; Maria, Franco, Odebrecht, Giroldo, & Abreu, 2016).

Animals

Male Swiss albino mice (25-35 g) were maintained at 22 ± 2°C, with relative humidity between 50 and 60% in a 12-12 hours light-dark cycle with standard food and water ad libitum. Animals were obtained in the Central Bioterium at the *Universidade Federal do Rio Grande* and maintained in the Rodent Bioterium at the Institute of Biological Sciences for experimentation. A hundred three animals were used for all tests. Experiments were performed after approval of the protocol by the Institutional Ethics Committee (approval number 23116.002017/2009-46) in agreement with the guidelines of the Brazilian National Council for Control of Animal Experimentation.

Antinociceptive activity

Writhing test

The abdominal writhing response to the acetic acid administration (0.6%, 10 mL kg^{-1}, i.p.) consists of contortions and extensions of the abdomen, sometimes accompanied by twists of the trunk and extension of the animal's hind legs (Koster, Anderson, & Beer, 1959). Animals (n = 8 animals/group) were treated with crude cellular and extracellular extracts from *C. pumilioniformis* or saline (control group). Extracts were administrated at 10 mL per kg of body weight. Dose determination was based on the highest volume of administration recommended by intraperitoneal route in mice. A group was treated with the reference opioid analgesic, morphine (2 mg kg^{-1}), while the other group received diclofenac (5 mg kg^{-1}), an anti-inflammatory non-steroidal drug. All treatments were performed by intraperitoneal pathway (i.p.). In the writhing test, mice received the acetic acid injection 30 min. after the treatment with the extracts and 60 min. after morphine and diclofenac treatment. The number of abdominal writhing was counted cumulatively for 25 min., starting 5 min. after the administration of acetic acid. Antinociception was calculated as a percentage of inhibition of writhing constrictions by using the formula [(control group mean – test group mean)/(control group)] 100% (Hort et al., 2018).

Formalin test

In this test, 20 µL 2.5% formalin was injected into left hind paws of mice 30 or 60 min. after they had been submitted to their respective treatments (n = 8 animals/group). Animals were treated with crude cellular and extracellular extracts from *C. pumilioniformis* (10 mL kg^{-1}), saline (control group), morphine (2 mg kg^{-1}) and diclofenac (5 mg kg^{-1}) i.p. The treatment with the extracts was performed 30 min. before the formalin injection and with morphine and diclofenac 60 min. before. Formalin-induced paw licking was considered an indicator of nociceptive behavior. The amount of time that each animal spent licking its paw was recorded during two 5 min. intervals: the first one began immediately after the injection (first phase) and the second one began 20 min. after the injection (second phase; Hunskaar & Hole, 1987). Results were expressed as time (s) spent by mice licking their injected paws. Percentage of inhibition was calculated in relation to the control group.

Anti-inflammatory activity

Formalin-induced paw edema

The anti-inflammatory effect of *C. pumilioniformis* was assessed by formalin-induced paw edema test on mice. Before formalin injection (20 µL; 2.5%), volume of each mouse paw was measured separately by a plethysmometer (Letica, Barcelona, Spain) (Hort et al., 2018). Thirty minutes after administration of crude cellular and extracellular extracts from *C. pumilioniformis* (10 mL kg^{-1}) or saline (control group) i.p. to mice, acute inflammatory edema was induced by sub plantar injection of formalin into the right hind paws of mice (n = 9-10 animals/group). Edema caused by formalin was measured at 1, 2, 3, 4 and 24 hours after formalin injection. Diclofenac (5 mg kg^{-1}; i.p.; n = 4 animals/group) was used as positive control and administrated 60 min. before formalin injection. Results were expressed as percentage increases in paw volume, considering that paw volume at time zero was 100%.

Statistical analysis

Data are expressed as mean ± standard error of mean (SEM). Statistical comparisons between groups were carried out by the one-way analysis of variance (ANOVA), followed by the Bonferroni *post hoc* test. Differences with p values below 0.05 were considered statistically significant.

Results and discussion

Total carbohydrate contents of cellular and extracellular extracts from *C. pumilioniformis* were 90 and 20 µg mL^{-1}, respectively. The former was mainly composed of glucose, but with significant proportions of arabinose, galactose, mannose and/or xylose and minor ones of fucose, rhamnose, amino sugars and uronic acids. The latter were composed of a similar proportion of glucose, galactose and mannose/xylose, besides significant proportions of arabinose, fucose and galacturonic acid. Minor proportions of rhamnose and amino sugars were also detected (Table 1).

The writhing test was used to investigate antinociceptive effects of *C. pumilioniformis* extracts. Results in Table 2 show that both cellular and extracellular extracts were able to inhibit acetic acid-induced abdominal constrictions in mice (78.3 and 26.7%, respectively). However, the cellular extract was more effective than the extracellular one. Moreover, the cellular extract was more effective than diclofenac, a standard non-steroidal anti-inflammatory drug. Neurogenic and inflammatory nociception was evaluated by the formalin test. In the formalin test (Table 3), only the extracellular extract from *C. pumilioniformis* significantly inhibited the first phase of the test (43.7%). On the other hand, both extracts were effective in the late phase with 94.8 and 85.7% of inhibition for cellular and extracellular extracts, respectively.

Table 1. Monosaccharide composition of cellular and extracellular extracts from *Chlamydomonas pumilioniformis*.

Monosaccharides	Cellular Extract (%)	Extracellular Extract (%)
Fucose	3.51	8.95
Rhamnose/n-acetyl-galactosamine	1.07	3.99
Arabinose	12.59	7.51
N-acetyl-glucosamine	3.86	2.30
Galactose	16.77	22.17
Glucose	42.04	25.20
Mannose/xylose	10.68	22.83
Fructose	2.11	0
Ribose	2.52	0
Galacturonic acid	2.84	7.05
Glucuronic acid	2.01	0

Table 2. Effect of *Chlamydomonas pumilioniformis* extracts on acetic acid-induced writhing behavior in mice.

Group	Number of abdominal constrictions	Writhe inhibition (%)
Control	76.5 ± 3.4	-
Morphine	0.4 ± 0.3***	98.7
Diclofenac	43.7 ± 3.3***	42.9
Cellular extract	17.1 ± 5.6######	78.3
Extracellular extract	57.9 ± 5.5*§§§	26.7

Values are expressed as mean ± SEM. *p < 0.05; ***p < 0.001 indicates statistical difference by comparison with the control group; ###p < 0.001 compared to diclofenac group; §§§p < 0.05 compared to the cellular extract group (one-way ANOVA followed by the Bonferroni post hoc test).

Table 3. Effect of *Chlamydomonas pumilioniformis* extracts on formalin-induced nociception in mice.

Group	First phase (s)	Inhibition (%)	Second phase (s)	Inhibition (%)
Control	66.5 ± 5.1	-	32.9 ± 9.8	-
Morphine	0.0 ± 0.0***	100	4.4 ± 4.4**	86.7
Diclofenac	61,3 ± 7,1	7,8	0.0 ± 0.0***	100
Cellular extract	58.5 ± 4.5	12.0	1.7 ± 1.3**	94.8
Extracellular extract	37.4 ± 3.2***	43.7	4.7 ± 3.2**	85.7

The amount of time spent in licking the injected paw was recorded in two phases: first phase: 0-5 min. post-formalin injection; second phase: 20-25 min. post-injection. Values are expressed as mean ± SEM. **p < 0.01; *p < 0.001 indicates statistical difference by comparison with the control group (one-way ANOVA followed by the Bonferroni post hoc test).

The intraperitoneal pretreatment with both cellular and extracellular extracts from *C. pumilioniformis* reduced the formalin-induced paw edema only 24 hours after the treatment (Figure 1). Diclofenac, used as a positive control, was effective from 1 hours after the treatment up to 24 hours.

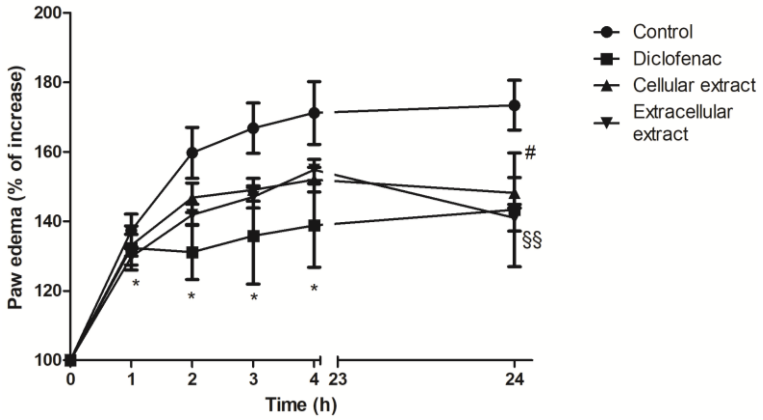

Figure 1. Effect of *Chlamydomonas pumilioniformis* extracts on formalin-induced paw edema in mice. Results are expressed as mean ± SEM of the percentage of increase in paw edema (n = 9-10, diclofenac n = 4). Volume of the paw at time zero was considered as 100%. *p < 0.05 indicates statistical difference between diclofenac and the control group; #p < 0.05 indicates statistical difference between cellular extract and the control group; §§p < 0.01 indicates statistical difference between extracellular extract and the control group (one-way ANOVA followed by Bonferroni *post hoc* test).

Among metabolites produced by microalgae, polysaccharides have emerged as an important class of bioactive natural products with benefits to human health (Raposo et al., 2013; Talero et al., 2015). In this study, we investigated the carbohydrate composition of cellular and extracellular extracts from *C. pumilioniformis* and their relations with pharmacological activities in inflammation and nociception.

Cellular extract, which contains the reserve and structural polysaccharides, is composed of arabinose, galactose, mannose and/or xylose and a high proportion of glucose. A previous study revealed arabinose associated with galactose in *Chlamydomonas* sp (Maria, Vieira, & Giroldo, 2011). This composition indicates the presence of pherophorins, which are cell wall glycoproteins rich in hydroxiprolin, arabinose and galactose, typically found in *Chlamydomonadales* (Morita et al., 1999; Hallmann, 2006). Pherophorins show similar composition to some pectins produced by vascular plants, which are associated with several biological activities, such as anti-inflammatory and immunostimulatory ones (Paulsen, 2001; Maria et al., 2016).

There are some differences in the composition of extracellular extract, by comparison with the cellular one. This extract has high proportion of fucose, that is the fundamental subunit of sulfated polysaccharides, such as fucoidan (Garcia-Vaquero, Rajauria, O'Doherty, & Sweeney, 2017). Moreover, galacturonic acid, which is a component of pectic polysaccharides with arabinose, galactose and rhamnose (Paulsen, 2001), has high content in this extract. Arabinose and galactose can also be found in extracellular medium because of the cell division pattern of this microalga, i. e., the old cell wall is released and a new one is synthetized by daughter cells (Maria et al., 2011).

Taking into account the biological potential of previously described polysaccharides, we studied the antinociceptive potential of *C. pumilioniformis* extracts. The writhing response to an intraperitoneal injection of acetic acid is used to screen peripherally and central analgesic drugs (Couto et al., 2011; Hort et al., 2018). Acetic acid induces the release of endogenous mediators, such as substance P, bradykinins, prostaglandins, as well as pro-inflammatory cytokines, such as interleukins (IL-1, IL-6 and IL-8) and TNF-α, that stimulate peripheral nociceptors which are sensitive to non-steroidal anti-inflammatory drugs and opioids (Verma, Joharapurkar, Chatpalliwar, & Asnani, 2005; Pinheiro et al., 2011). Our findings showed

significant decrease in the number of acetic acid-induced constrictions in both cellular and extracellular aqueous extracts from *C. pumilioniformis*, indicating their promising analgesic properties. Studies demonstrating antinociceptive effects of microalgae are quite restricted in the literature. In the study carried out by Guzmán et al. (2001), the authors showed the antinociceptive effect of extracts from the species *Chlorela stigmata* and *Phaeodactylum tricornutum* on mice. To date, this is the first evidence of the antinociceptive effect of *C. pumilioniformis*. In agreement with our data, previous studies showed that polysaccharide-rich extracts obtained from vegetal sources have analgesic properties (Nascimento et al., 2015; Nascimento, Baggio, Werner, Iacomini, & Cordeiro, 2016; Silva-Leite et al., 2018).

To further study analgesic properties of these extracts, the formalin test was performed. In this assay, initial nociception (early phase) is caused by a direct stimulation of nociceptors and reflects centrally mediated nociception, whereas late phases are secondary to the inflammatory process, as a response to inflammatory mediators, such as prostaglandins, serotonin, histamine and bradykinin (Hunskaar & Hole, 1987). Drugs that act primarily on the central nervous system, such as morphine, inhibit both phases of this test, while drugs that act peripherally, such as cyclooxygenase inhibitors (ex: diclofenac), inhibit only the second phase (Yaksh et al., 2001). In our study, extracts from *C. pumilioniformis* showed different response profiles in this test and some differences in the chemical composition may explain the antinociceptive activity.

The extracellular extract inhibited both phases of formalin and implied central and peripheral effects of this extract, similar to opioids. Higher amounts of galacturonic acid, galactose and fucose may explain the effect of extracellular extract on the first phase of the formalin test. A previous study showed that *Ximenia americana* fraction of heteropolysaccharides, with a high content of uronic acids and galactose, ameliorates visceral hypernociception in mice, through a mechanism involving cannabinoid receptors (Silva-Leite et al., 2018). Moreover, fucose in the extract could indicate that sulfated polysaccharides, such as fucoidan, can be found in *C. pumilioniformis*. Similar to our results, a sulfated polysaccharide, composed mainly of α-L-fucose, isolated from brown marine alga *Spatoglossum schroederi*, reduced both phases of formalin test in mice, suggesting a mechanism similar to morphine (Farias et al., 2011). Rodrigues et al. (2012) showed the antinociceptive effect of a sulfated polysaccharide fraction isolated from the green seaweed *Caulerpa cupressoides*. This fraction inhibited both phases of the formalin test and caused antinociceptive effect in hot plate test, indicating that mechanisms of analgesia occur via central acting mechanisms and suggesting the involvement of the opioid system.

On the other hand, the cellular extract was effective to inhibit only the second phase of the formalin test. It suggests that peripheral mechanism is involved in its effects and may also exhibit an associated anti-inflammatory effect. Anti-inflammatory effects of microalgae are a little more explored that analgesic activity in the literature. Studies have already shown anti-inflammatory activity of microalgae *Chlorella stigmatophora*, *Phaeodactylum tricornutum* (Guzmán et al., 2001)*, Spirulina platensis, Dunaliella salina* (Abdel-Daim et al., 2015)*, Chlorella vulgaris* (Barboríková et al., 2019) and *Chloromonas reticulate* (Suh et al., 2019). Oxylipin-containing biomass of microalga *Chlamydomonas debaryana* demonstrated *in vitro* (Reyes et al., 2014) and *in vivo* (Ávila-Román et al., 2014) anti-inflammatory activity by inhibiting production of proinflammatory cytokines. Mycosporine-like amino acids found in *Chlamydomonas hedleyi* microalgae showed protection by anti-inflammatory and antioxidant activity against UV-induced damage (Suh et al., 2014).

Since the reduction of the second phase of the formalin test indicates an effect on inflammation mediators, we evaluated the anti-edematogenic effect of the extracts. The formalin stimulus produce an acute inflammatory response triggered by different mediators: bradykinin, cytokines (IL-1β, IL-6, IL-8 and TNF-α), eicosanoids and nitric oxide (Hunskaar & Hole, 1987; Moore, Oluyomi, Babbedge, Wallace, & Hart, 1991; Muley, Krustev, & McDougall, 2016). Both cellular and extracellular extracts reduced the edema only after 24 hours of treatment. Polysaccharides, specially sulfated forms, in the microalga, have already been found to contribute to anti-inflammatory activity of microalga (Raposo et al., 2013). Some mechanisms have been previously described in the anti-inflammatory activity of these compounds: reduction in myeloperoxidase activity (Carneiro et al., 2014) and neutrophil infiltration (Silva-Leite et al., 2018), decrease in proinflammatory cytokines, such as TNF-α, IL1-β and IL-17 (Brito et al., 2013; Miao et al., 2015), increase in nitric oxide (Brito et al., 2013) and inhibition in NF-κB activation (Miao et al., 2015). Antioxidant activity of polysaccharides, especially by modulating antioxidant defenses (Subash, Veeraraghavan, Sali, Bhardwaj, & Vasanthi, 2016), can also contribute to anti-inflammatory activity of *C. pumilioniformis*. This study has some limitations that should be highlighted. Only one dose of each extract was evaluated to assess anti-inflammatory and antinociceptive activity, which limits the establishment of the dose-effect

relationship. Extracts were characterized as to their carbohydrate content, which can contribute to their pharmacological activity. However, other classes of chemical compounds must be studied in the future and their participation in the biological effect cannot be ruled out. Finally, although our studies indicate a possible central analgesic effect of the extracellular extract, further experimental tests are needed to confirm this effect.

Conclusion

In sum, results of this study demonstrate that cellular and extracellular extracts from microalga *C. pumilioniformis* exhibit antinociceptive and anti-inflammatory effects on some classical models of nociception and inflammation in mice. Moreover, the pharmacological activity of this microalga could be directly influenced by its carbohydrate composition. These findings evidenced the relevant potential of *C. pumilioniformis* as a source of bioactive compounds and encourage further pharmacological studies to highlight the mechanism of its pharmacological action.

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