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Evaluation of acute toxicity of the microalgae *Pediastrum boryanum*

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ABSTRACT

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Microalgae are unicellular organisms whose biotechnological potential is being explored in many areas as food and pharmaceutical, among others. Toxicity studies of new products are essential to determine the security of these and are required by regulatory agencies. *Pediastrum boryanum* is a microalga with worldwide distribution, with little data in the literature. This study aimed to evaluate the oral toxicity of freeze-dried microalgal biomass (MB) of *P. boryanum*. These toxicity studies were based on the protocol of the Organization for Economic Cooperation and Development (OECD - 423) carried out in mice. The MB was obtained from culture with temperature and light controlled conditions in the Institute of Biological Sciences, FURG. The animals received a single dose of MB suspension by gavage: first group with 300 mg.kg⁻¹, second with 2000 mg.kg⁻¹ and the control group white suspension. For 14 days, signs of toxicity and mortality were observed. Behavioral parameters were evaluated by the Open Field test 4 days before and 3 days after MB administration. At the end of the experiment, blood, liver and femur were collected for biochemical, hematological, lipid peroxidation, cytotoxicity and mutagenicity analyzes. The stomach, liver, gut and kidneys were collected for histological analysis. The findings within the criteria established by the OECD, ranks the MB of *P. boryanum* as "Minimal Toxicity or Secure" (Category 5).

Avaliação da toxicidade aguda da microalga *Pediastrum boryanum*

Palavras-chave
Pediastrum boryanum
Toxicidade aguda oral
Microalgas
Biomassa

RESUMO - Microalgas são um conjunto de seres unicelulares com potencial biotecnológico, explorado em diversas áreas para obtenção de produtos alimentares, farmacêuticos entre outros. Os estudos de toxicidade de um novo produto são essenciais para determinar a viabilidade de lançamento deste no mercado, e uma exigência cada vez mais rigorosa pelas agências regulatórias. *Pediastrum boryanum* é uma microalga de ampla distribuição mundial, com poucos dados na literatura sobre sua aplicabilidade e toxicidade. A pesquisa foi conduzida para avaliar a toxicidade oral da biomassa microalgal (BM) liofilizada de *P. Boryanum*. Estes estudos de toxicidade foram baseados no protocolo da Organização para a Cooperação Econômica e Desenvolvimento (OECD – Protocolo 423), realizados em camundongos. A BM foi obtida a partir da cultura realizada sob condições controladas de temperatura e luz, no Instituto de Ciências Biológicas da FURG. Os animais receberam dose única da suspensão de BM por gavagem: um grupo com 300 mg/Kg, um com 2000 mg/Kg e o grupo controle recebendo suspensão branca. Durante 14 dias após a administração os animais foram observados para sinais de toxicidade e mortalidade, e

avaliados os parâmetros comportamentais 4 dias antes e 3 após a administração da BM. Ao término do experimento, foram coletados sangue, fígado e fêmur para análises bioquímica, hematológica, peroxidação lipídica, citotoxicidade e mutagenicidade. O estômago, intestino delgado, fígado e rins foram separados para análise histológica. O achado da pesquisa, dentro dos critérios estabelecidos pela OECD, classifica a BM de *P. boryanum* como “Toxicidade Mínima ou Segura” (Categoria 5).

1. Introduction

Microalgae are photosynthetic microorganisms of relatively simple nutrient requirements (1). These microorganisms can be a source for obtaining biotechnological products such as proteins, lipids, polysaccharides, pigments, enzymes, vitamins and drugs (2-4). Microalgae are already being employed in the treatment of effluents, bioremediation and biofuel production (5-6).

The microalgae cultivation can generate a quantity of biomass even ten times higher than the conventional cultivations, presenting itself as an advantageous, economic and ecological alternative (7). The biotechnological potential of biodiversity found in Brazil is not much explored yet, since only a small fraction is found catalogued and held in cultivatable collections, having few works on this area, opening a path to studies of several biotechnological applications (8). The microalga *Pediastrum boryanum* isolated and held in the Continental Microalgae Collection from the Federal University of Rio Grande (9) is an example of these organisms. It presents a considerable pharmacological potential through studies in progress by our research group (10).

Toxicological analysis is an important step to determine the applicability of any new compound interesting to health benefits. It comprehends the observation of the usage limitations such as toxicokinetics, dosage restrictions through signs of toxicity and mortality and routes of administration (11). Thus, the aim of this study is to evaluate the oral toxicity of the freeze-dried microalgal biomass (MB) of *P. boryanum*. The acceptance by regulatory agencies of each country, following international accepted protocols (12) is required. The ones embraced by the Organization for Economic Co-operation and Development (OECD), which aims at attending the international safety requirements uses the smallest number of animals (13-14).

2. Material and methods

Microalgae biomass

The microalgae biomass (MB) was obtained from the cultivation of *P. boryanum* previously isolated from Lagoa Mirim (32°52'44"S, 52°46'04"W)⁹ and cultivated in the Laboratory of Microalgae Culture of the Limnology department from the Institute of Biological Sciences (ICB). The microalgae was cultivated in the Wright's Cryptophyte medium (WC) culture medium (15) in photo bioreactor grade Carboy of 10 L, held in thermostatically room to 30 °C, photoperiod of 12 h light/dark and 2500 Lux of illuminance provided by fluorescent bulbs of 40 W (Phillips®, São Paulo). The agitation of the culture was conducted daily, by means of magnetic stirrers, in order to maintain the homogeneity of the growth. The initial concentration of the culture of *P. boryanum* was 0.2 grams of microalgae by liter of culture medium (g.L⁻¹). The cell growth monitoring was conducted by optical density (OD) in spectrophotometer (Quimis™ Q7998DRM, Brazil), using a standard growth curve relating OD to dry mass of the microalgae.

The cultivation of microalgae yielded 2.19 g.L⁻¹, 800 mL of culture media was removed. The sample was homogenized and centrifuged (Hitachi™ III CR22G) at 9000 rpm at 25°C for 20 minutes. The pellet biomass was separated and the supernatant was submitted to a

second cycle of centrifugation in the same conditions. The resultant biomass from the two cycles of centrifugation was kept in ultra-freezer (Quimis™ Q315U) adjusted to -40°C for two days and then freeze-dried for 48 hours (Liotop™ L101, São Carlos Brazil).

Animals

Eighteen male albino mice (*Mus musculus*) from Swiss line (37,6±0,6g), provided by Central Biotery from Federal University of Pelotas were used. The animals were acclimated for 14 days before the experiment protocol, in the Animal House from Federal University of Rio Grande (FURG). The animals remained under controlled temperature and humidity (22 ± 2° C e 55 ±1%) with light/dark cycle of 12 hours with free access to water and food (Nuvilab CR-1®). The Use of Animal Ethics Committee on the institution (CEUA/FURG) approved all the procedures under protocol number P021/2013.

Treatment

The animals were divided in three groups, each of them composed by six animals. They were identified and weighed regularly during all the experiment's period. Due to the low solubility of MB, it was prepared in a hydrophilic suspension with Carboxymethyl cellulose (CMC) (Sigma™) 1% at concentrations of 16.9 mg.mL⁻¹ and 112.5 mg.mL⁻¹, and the white suspension, composed solely of CMC 1%. The suspensions were administered (0.2mL/10g body weight) by gavage (Table 1).

Table 1. Formation of groups and dosage of the biomass of the microalgae *P. boryanum* for the testing of oral acute toxicity

Groups	Dosage
Control	Suspension of CMC 1% in equivalent quantity to the administered volume in the treated groups
300 mg.kg ⁻¹	Suspension of 300 mg.kg ⁻¹ of MB of <i>P. boryanum</i> in CMC 1%
2000 mg.kg ⁻¹	Suspension of 2000 mg.kg ⁻¹ of MB of <i>P. boryanum</i> in CMC 1%

Evaluation of the oral acute toxicity

The oral acute toxicity test was based on the OECD Protocol 423 (16), and the animals were evaluated in regard to possible signs of toxicity and mortality after the oral administration at 15, 30 minutes, 1 h, 2 h, 4 h, 6 h, 12 h e 24 h and daily until the 14th day after the treatment. The toxicity's signs evaluated were alterations in skin and hair (alopecia and peeling), mucosa changes (drooling), tachypnea, tremors, convulsions and lethargy, as well as mortality. The animal's weight was also accompanied throughout the experimental period. The animals were killed with sodium thiopental (40 mg.kg⁻¹) after 14 days of observation. The blood was collected by cardiac puncture for biochemical and hematological analysis; the bone marrow cells were obtained from the femur and used for mutagenic analysis. The stomach, small intestine, liver and kidneys were removed to histological evaluation and a liver sample was separated for the evaluation of lipid peroxidation.

Behavioral parameters

The Open Field test (OF) allow an assessment of the locomotive activity, exploratory behavior and the emotionality of the animals (17-18). For comparison, the test was applied 4 days before and 3 days after the suspension's administration. The OF device consists in one wooden box (29 cm x 21 cm x 29 cm) with high walls which prevent animal from escape. The floor is divided into black lines in 12 equal squares, and one of the walls made of glass allows the animal's observation in the environment. In the exploration's session,

each animal was placed in the arena bottom, in the left corner of the box, and left to explore freely the environment during a period of 5 minutes. During this period, the following behavioral categories were observed: ambulation frequency counted by the number of line crossings (defined as each unity drawn on the floor) with the four paws (*crossing*), getting up or the number of biped position (*rearing*) and the self-cleaning sessions (*grooming*).

Biochemical and hematological parameters

The collected blood from the animals was stored and homogenized in Vacutainer® bottles, with Ethylenediaminetetraacetic Acid (EDTA). In the plasma, the following components were measured: Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), urea and creatinine (Cobas Mira Plus®, Roche Diagnostic Systems Inc. USA). The hematological evaluation was carried out by the dosage of the whole blood: hemoglobin, erythrocyte, hematocrit, leukocytes and platelet (ABX Diagnostics Micros® 60, Brazil). The leukocytes differential was carried out by blood swabbing with the color of May-Grünwald Giemsa under optical microscopy.

Hepato-somatic proportion

The verification of the liver weight from mice was used as criteria of the evaluation of oral toxicity. It was observed the liver proportion from the treated animals in relation to their corporal weight. For this analysis, the animal's weights were verified in the end of the experiment.

Macro pathological and histopathological evaluation

The organs stomach, small intestine, liver and kidneys were washed in physiological solution and stored in formaldehyde 10% buffered solution for 8 hours; then transferred to 70% alcohol solution. They were soaked in Paraplast® (Sigma™) and cut in microtome of 4 µm. The blades were deparaffinized and prepared with color of hematoxylin and eosin (HE) for further observation in electronic microscope (Olympus® BX51, electronic camera Olympus® DP72) in the Laboratory of Histology ICB,FURG.

Lipid peroxidation

The lipid peroxidation was determined by the malondialdehyde reaction with thiobarbituric acid, giving rise to the chromophore compost that can be read by spectrophotometer at 553 nm (19). Thus, the concentration of peroxidized lipids expressed as nmol from Thiobarbituric Acid Reactive Substances (TBARS) by gram of analyzed tissue (liver), being the concentration compared to a curve of tetramethoxypropane (TMP) as a pattern.

The micronucleus test – mutagenic potential

The micronucleus test was performed according to OECD protocol 473 (16) and Ribeiro and cols (20). Bone marrow cells were obtained from the femoral epiphysis, which were carried of the marrow with 1 mL of fetal bovine serum and centrifuged at 800 g for 10 min. The supernatant was discarded and it was carried out smear preparations on slides, fixed on methanol (10 min) and stained with eosin – methylene blue. The slides were prepared in duplicate and the proportion among polychromatic erythrocytes (PCE) and normochromatic (NCE) and the number of micronuclei in 1000 PCE was calculated.

Statistical analysis

The results were expressed as mean \pm standard deviation. The value differences between the control groups and the treated groups have been analyzed using the analysis of variance (ANOVA) for parametric data such as animal weight, behavioral parameters, hepatosomatic proportion, cytotoxicity, TBARS, CBC, WBC, AST and urea. The Kruskal-Wallis test was used for non-parametric data, mutagenicity, platelets, ALT and creatinine. It was considered significant results were $p < 0.05$.

3. Results

Oral acute toxicity

The animals submitted to the oral acute toxicity test in this experiment did not present any signs of toxicity during the 14 days of experiment, neither there were deaths related to the MB administration. The weight of the animals was checked every two days and did not show significant alterations compared to control group ($p > 0.05$).

Behavioural evaluation

The evaluated values of the parameters, *rearing*, *grooming* and *crossings* have not presented statistic difference ($p > 0.05$) considered evaluating the control group and the administered dosages for the test of oral acute toxicity. In the same way, there were no difference regarding the three behavioral parameters evaluated between the day before the extract's administration and three days after the administration, excepting the 300 mg.kg⁻¹ group, whose activity of *rearings* has increased in a significant manner ($p < 0.05$) observed in Table 2.

Table 2. Effect of the treatment with MB of *P. boryanum* under behavioral parameters of mice evaluated by the open field test

Group	Day	<i>Rearing</i>	<i>Grooming</i>	<i>Crossing</i>
Control	before	50.8 \pm 7.5	3.0 \pm 0.6	95.7 \pm 32.6
	after	50.8 \pm 7.0	3.8 \pm 2.3	94.0 \pm 16.7
300 mg.kg ⁻¹	before	44.8 \pm 14.1	2.7 \pm 1.7	91.5 \pm 39.9
	after	61.7 \pm 15.9*	3.7 \pm 2.6	92.8 \pm 18.9
2000 mg.kg ⁻¹	before	43.8 \pm 9.0	2.5 \pm 1.7	77.4 \pm 18.4
	after	51.0 \pm 15.4	3.3 \pm 1.9	89.8 \pm 32.6

Values are expressed as mean and standard deviation of 6 animals. Statistical analysis by ANOVA of two ways. * $p < 0.05$ when compared before and after the administration of 300 mg.kg⁻¹ of MB.

Biochemical and hematological parameters

The biochemical parameters of blood, urea, AST and ALT were not significantly different among the control groups and the treated groups with the tested dosages ($p > 0.05$). The plasma creatinine of 2000 mg.kg⁻¹ group was the only one that presented a significant difference ($p < 0.05$) among the others, as can be observed in Table 3.

Table 3. Biochemical parameters of Swiss mice submitted to the oral acute toxicity test by the MB of *P. boryanum*

Groups	AST (U.L ⁻¹)	ALT (U.L ⁻¹)	Creatinine (mg.dL ⁻¹)	Urea (mg.dL ⁻¹)
Control	98.0 \pm 53.2	48.0 \pm 5.7	0.26 \pm 0.1	49.0 \pm 5.3
300 mg.kg ⁻¹	86.0 \pm 27.1	56 \pm 38.4	0.25 \pm 0.0	51.0 \pm 10.3
2000 mg.kg ⁻¹	129 \pm 25.6	41 \pm 20.5	0.17 \pm 0.0*	45.0 \pm 8.0

Values are expressed as mean and standard deviation of 6 animals. Statistical analysis by ANOVA Unifactorial for AST and Urea, and Kruskal-Wallis for ALT and Creatinine. * $p < 0.05$, when compared to the other two groups.

The blood test showed no significant difference among the groups and in the Leukogram's (WBC) evaluation of the treated animals of the oral acute toxicity test ($p > 0.05$). Thus it has been observed a meaningful alteration on the total number of leukocytes of 300 mg.kg⁻¹ group, decreased in relation to the other two groups ($p < 0.05$) (Table 4).

Table 4. Hematological Parameters of Swiss mice submitted to the oral acute toxicity test of the MB of *P. boryanum*

Parameters	Experimental groups		
	Control	300 mg.kg ⁻¹	2000 mg.kg ⁻¹
Hemoglobin (g.dL ⁻¹)	12.0 ± 1.7	10.8 ± 1.4	10.6 ± 2.0
Hematocrit (%)	36.9 ± 5.1	33.9 ± 4.8	33.5 ± 6.9
Erythrocytes (M/mm ³)	7.9 ± 0.9	7.2 ± 0.8	7.2 ± 1.4
Platelets (10 ³ /mm ³)	927.6 ± 284.4	643.3 ± 122.7	720.5 ± 166.8
Leukogram (WBC)			
Segmented (%)	23.25 ± 4.9	24.00 ± 4.9	21.20 ± 1.9
Monocytes (%)	3.60 ± 0.5	2.83 ± 1.0	3.50 ± 0.8
Lymphocytes (%)	65.80 ± 16.6	74.00 ± 5.9	75.50 ± 2.2
Total leukocytes (10 ³ /μL)	3425.0 ± 1078.68	1716.00 ± 354.0*	3416.67 ± 854.2

Values are expressed as mean and standard deviation of 6 animals. Statistical analysis by ANOVA Unifactorial for all the parameters, except for platelets (analysis by Kruskal-Wallis). * - $p < 0.05$ when compared to the other two groups.

Hepato-somatic proportion

The analysis of the animals' weight and the hepato-somatic proportion have showed that there were no significant differences ($p > 0.05$) in this parameter in any of the groups, as can be observed in Table 5.

Table 5. The weight of animals and livers, the hepato-somatic proportion of Swiss mice submitted to the oral acute toxicity test by the MB of *P. boryanum*

Groups	mice (g)	liver (g)	Proportion (%)
Control	40.32 ± 3.16	2.20 ± 0.21	4.91 ± 0.59
300 mg.kg ⁻¹	40.63 ± 3.48	2.01 ± 0.36	5.46 ± 0.28
2000 mg.kg ⁻¹	39.83 ± 2.61	2.01 ± 0.26	5.05 ± 0.48

Values are expressed as mean and standard deviation of 6 animals. Statistical analysis by ANOVA Unifactorial

Macro pathological and histopathological evaluation

The macroscopic exam of organs (stomach, small intestine, liver and kidneys) of the treated animals did not reveal any abnormality regarding to color or texture when compared to the control group. The histopathological evaluation presented normal structures and the absence of pathological injuries, as can be observed in Table 6 and in the figures 1, 2, 3 and 4.

Table 6. Evaluation of vital organs of Swiss mice submitted to the oral acute toxicity test of the MB of *P. boryanum*

Organ/Alterations	Groups		
	Control	300 mg.kg ⁻¹	2000 mg.kg ⁻¹
Liver			
- necrosis	0 (5)	0 (6)	0 (6)
- door area integrity	5 (5)	6 (6)	6 (6)
Kidneys			

- Tubules Integrity	5 (5)	6 (6)	6 (6)
- Clusters Integrity	5 (5)	6 (6)	6 (6)
- intraluminal erythrocytes	0 (5)	0 (6)	0 (6)
Stomach			
- Mucosa integrity	5 (5)	6 (6)	6 (6)
- inflammatory infiltrate	0 (5)	0 (6)	0 (6)
Small intestine			
- Mucosa integrity	5 (5)	6 (6)	6 (6)
- inflammatory infiltrate	0 (5)	0 (6)	0 (6)
Frequency of injuries (N)			

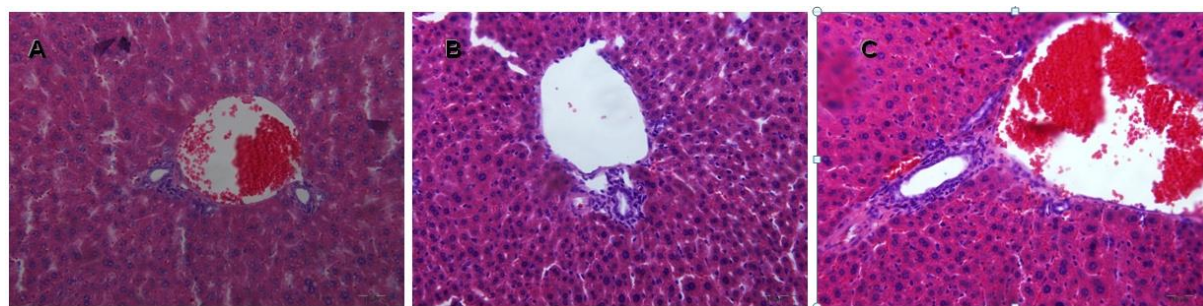


Figure 1. Section of the liver from the oral acute toxicity analysis of MB of *P. boryanum*; control group (A), 300 mg.kg⁻¹ (B) and 2000 mg.kg⁻¹ (C). HE color, 400x magnified.

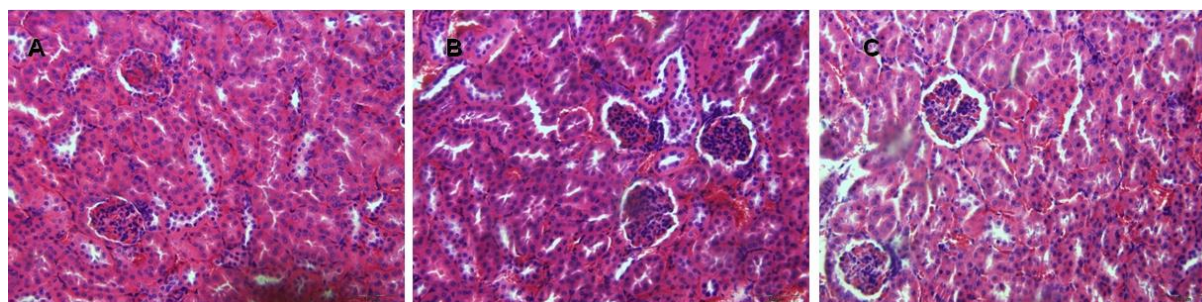


Figure 2. Section of the kidney from the oral acute toxicity analysis of MB of *P. boryanum*; control group (A), 300 mg.kg⁻¹ (B) and 2000 mg.kg⁻¹ (C). HE color, 400x magnified.

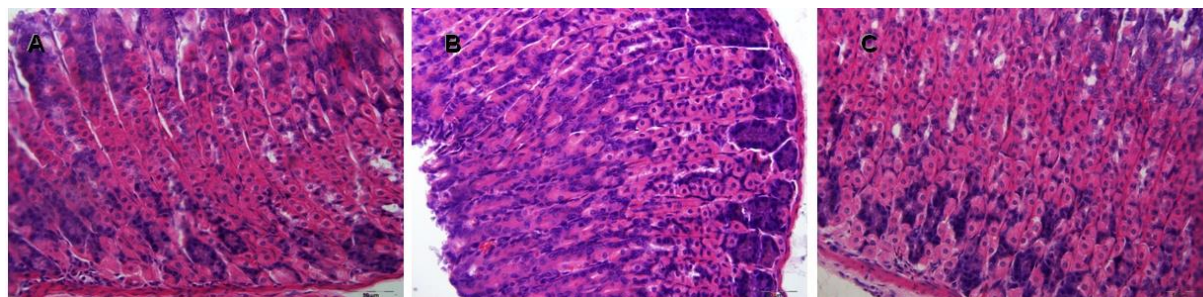


Figure 3. Section of the stomach from the oral acute toxicity analysis of MB of *P. boryanum*; control Group (A), 300 mg.kg⁻¹ (B) and 2000 mg.kg⁻¹ (C). HE color, 400x magnified.

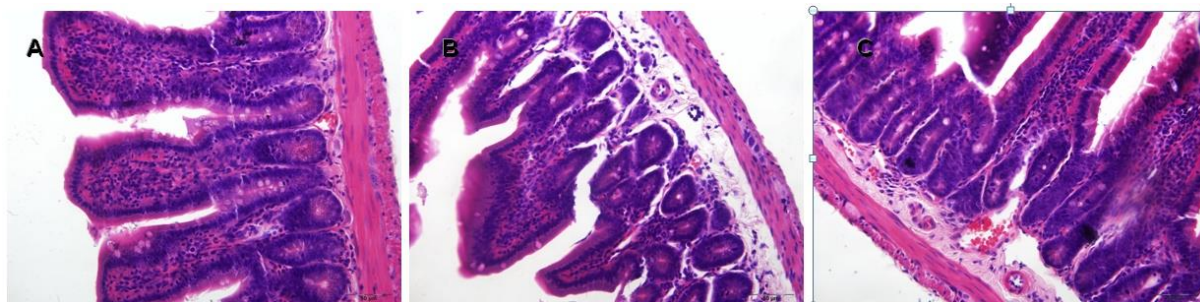


Figure 4. Section of the small intestine from the oral acute toxicity analysis of MB of *P. boryanum*; control Group (A), 300 mg.kg⁻¹ (B) and 2000 mg.kg⁻¹ (C). HE color, 400x magnified.

Lipid peroxidation

The results obtained on lipid peroxidation have not evinced significant differences when compared to the control group ($p > 0.05$). The TBARS values (expressed in nmol.g⁻¹) of the control group, 300 mg.kg⁻¹ and 2000 mg.kg⁻¹, were 0.0075 ± 0.0007 , 0.0080 ± 0.0009 and 0.0080 ± 0.0019 , respectively.

The micronucleus test– mutagenic potential

In the micronucleus test, it was not observed any statistically and significant increase on the cells frequency in both dosages of MB ($p > 0.05$) (Table 7). The control group also did not present significant number of micronucleus, the results are within the limits established by other publications (21-22).

Table 7. Evaluation of the micronucleus in Swiss mice submitted to the oral acute toxicity test by the MB of *P. boryanum*

Groups	Micronucleus (n/1000)	Cytotoxicity PCE/NCE
Control	0.80 ± 0.84	0.81 ± 0.05
300 mg.kg ⁻¹	1.60 ± 1.14	0.89 ± 0.17
2000 mg.kg ⁻¹	1.33 ± 1.03	0.86 ± 0.17

4. Discussion

This study was focused on the characterization of the toxicological properties of the suspension which was obtained from the freeze-dried biomass of the microalgae *Pediastrum boryanum* in CMC (1%). It is relevant the accomplishment of this work in order to determine the risk or benefit relation of using the microalga whether as a food supplement or as a pharmaceutical interesting drug. The interest in the use of natural products has been growing (22). This interest makes toxicological proprieties an aim of investigation, mainly using the experimental tests with animals to determine potential effects and to provide guidelines to select the safety dosage for humans (13).

The evaluation of natural products for pharmacological activity, the evaluation of the toxic characteristics of extract, fraction or isolated compounds is usually the initial stage. In light of this context, the oral acute toxicity test is a requirement to the classification of the risk's evaluation for human and environmental health (11,13).

In this study, the lyophilized biomass of microalgae *P.boryanum* have been administered orally at doses of 300 and 2000 mg.kg⁻¹, as recommended by the Organization for Economic Cooperation and Development - OECD 423 protocol and they have not illustrated any abnormal clinical signs, behavioural changes or significant macroscopic changes, biochemical and hematological or histological at the doses tested ($p > 0.05$).

Although the OECD protocol 423 does not allow calculating accurately the medium lethal dose (DL₅₀), it constitutes in a procedure that uses few animals opposing to DL50 test, which uses a

greater number of animals (23). The use of this methodology has been stimulated considering the conditions mentioned.

Regarding the acute toxicity, mortality has not been observed until the 14^o day of exposure of the animals to the tested dosages, and the oral toxicity of MB of *P. boryanum* can be classified on category 5 (the acute lethal toxicity is higher than 2000 mg.kg⁻¹), according to *Globally Harmonized Classification System* from OECD (1996).

The weight gain was normal in all treated animals and all of them ended the procedure without significant differences ($p > 0.05$), concerning corporal weight among the investigated groups. Weight gain serves as a sensible indicator of the general state of health from the animals (24). This result can suggest that the extract of the microalgae *P. boryanum* does not interfere with the normal metabolism of the animals tested.

Another parameter recommended by OECD 407 is the assessment of the behavioral changes. The Open Field test has been used to assess the locomotor and exploratory activity of animals (25). No change in behavioral parameters was observed in this study after administration of MB of *P. boryanum*. The animals' treated with MB of *P. boryanum* did not show significant difference in the evaluated parameters of line crossings (*crossing*), self-cleaning (*grooming*) and lifting for standing position (*rearing*), except for the 300 mg.kg⁻¹ dosage. This result may suggest an improvement in the usual memory of the animals in this test (18) however, further tests at this concentration would be necessary to confirm this result.

The plasma biochemical examinations were conducted to investigate possible alterations in the hepatic and kidney functions influenced by MB of *P. boryanum*. This analysis is very important to the evaluation of the toxicity of natural products, once these functions are essential to the survival of organisms (26). High concentrations of transaminases (AST e ALT) are associated to liver's diseases or hepatotoxicity (27). The increase of blood levels of aminotransferase such as AST and ALT is used as an indicator of hepatic tissue damage and alteration of membrane permeability (28-30).

The administration of the MB of *P. boryanum* caused no significant change ($p > 0.05$) in the activity of transaminases in both doses tested, which suggests that the administration of *P. boryanum* biomass does not affect the function of hepatocytes. The urea and plasma creatinine as well as the activities of AST and ALT showed no increase ($p > 0.05$), which is used to indicate the absence of toxic effects in organs such as the kidney and liver.

This study also investigated for the first time the mutagenic potential of MB of *P. boryanum* on mice's bone marrow, through the micronucleus test. The damage studies to the genetic code and the formation of micronucleus are part of the investigation of genetic toxicity (31). The assessment of micronucleus induction is an important genotoxic test *in vivo*, recommended by health agencies in many countries evaluating the security of chemical and natural products. The assay is capable of detecting both clastogenic damages (chromosomal fragmentation) and aneugenic (chromosomal loss) (21). Micronucleus in young erythrocytes emerge from acentric fragments or chromosomes that are incapable of migrating following the spindle apparatus during the cell division of hematopoietic tissue (32). As such, the increase in the frequency of polychromatic micronuclei erythrocytes in animal testing, treated with different substances is an indicator of induced chromosomal damage (21). In this study, the results have not demonstrated any increase in the formation of micronuclei ($p > 0.05$), what could suggest a genotoxic security in the animals' administration of MB of *P. boryanum* in the acute exposure conditions.

The hematological evaluation did not show any alteration on groups treated with *P. boryanum*, in both dosages, concerning the number of platelets, erythrocytes and equally the values of hematocrit and hemoglobin level ($p > 0.05$). The hematopoietic system is one of the most sensitive for chemical toxic and an important key figure of physiological and pathological status both humans and animals (33). Results suggest that an oral administration of the MB of *P. boryanum* does not induce hemolysis or provokes anemia. The low concentration of the numbers of total leukocytes in 300 mg.kg⁻¹ group may suggest a selective toxicity of MB compounds by immune system (34), again presenting reasons for further studies with this concentration.

The macroscopic examination of the animals' organs exposed to both dosages of MB of *P. boryanum* did not demonstrate any alteration of color compared to organs of control group. Hypertrophy of organs is one of the first indicatives of toxicity in chemical and biological

substances. However, none hypertrophy of organs was observed among the groups in this study. In the evaluation of hepatic-somatic proportion, it has not been found any significant difference as well ($p > 0.05$).

Thus, also the histopathological examination did not reveal any alteration in the cell or tissue structures. At tested dosages it was not found alterations such as inflammatory infiltrate in the stomach and small intestine, and the mucous were intact. In addition, in the histological examination of the liver and the kidneys did not show any alteration, and the area integrity, absence of necrosis and tubule integrity was found, as well as clusters and the absence of intratubular red blood cells, respectively.

The damage in cells of the hepatic parenchyma usually results in the elevation of transaminases on blood (35-36). There was no increase of activities of transaminases and it suggests that the acute administration of the MB of *P. boryanum* dosages administered does not alter the hepatocyte and consequently the animals' metabolism as observed in the histopathological evaluations of the liver.

The results also show that there was no significant increase in urea and creatinine with the administration of *P. boryanum* biomass compared to the control group. The urea, excreted by the kidneys is a final product of protein metabolism. The renal tubules reabsorb 40% of urea and blood levels of this parameter shows the condition of the renal function. The elevation of plasma levels of urea and creatinine provide evidence of kidney overload, renal failure or increase of protein catabolism (37-38).

This study did not show elevation, and there was a decrease in creatinine levels at a 2000 mg.kg⁻¹ dosage. It can be suggested that there is no kidney overload; however, it becomes necessary more studies about acute, sub chronic and chronic toxicity in rodents.

The determination of cell lesions resulting of the increase of reactive oxygen species (ROS) can be conducted through measuring tests of damages of cell targets, such as membrane lipids, proteins and nucleic acids. The damages in membrane lipids have been investigated by TBARS method, which results did not demonstrate a significant increase of lipid peroxidation in liver ($p > 0.05$), suggesting that the administration of the microalgae biomass did not increase the levels of ROS that participate on processes of cellular damage.

In response to these results, it can be concluded that the *P. boryanum* biomass is not toxic at the dosages tested and it did not produce any clear signs or symptoms of acute toxicity, it did not provoke or produce any notable histopathological signs or biochemical and hematological important alterations.

Early results suggest promising alternatives to explore the pharmaceutical interest on MB of *P. boryanum*. microalgae produce secondary metabolites of high added value, such as carotenoids, polyunsaturated fatty acids, vitamins, carotenoids pigments and phenolic compounds (39-41), and the latter cases have been demonstrated notable antioxidant action (42-43).

The study demonstrated that administration of lyophilized microalgal biomass suspension of *P. boryanum* showed no significant signs of toxicity when administered acutely orally at doses of 300 and 2000 mg.kg⁻¹, and is therefore considered safe in accordance with the classification OECD in category 5.

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