

*Full Length Research Paper*

## **Acute toxicity evaluation of ethanol extract of red algae, *Osmundaria obtusiloba*, in BALB/c mice**

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The genus *Osmundaria* (Rhodophyta, Ceramiales, Rhodomelaceae) comprises tropical and temperate regions red marine seaweeds species. *Osmundaria obtusiloba* is distributed from the northeastern coast of Brazil to the state of Rio de Janeiro. Studies with ethanol extract of red seaweed *O. obtusiloba* showed the antiviral potential of this alga. Hence, this study examined in BALB/c mice the acute toxicity after oral administration of *O. obtusiloba* crude extract. Then, female BALB/c mice received a single dose of *O. obtusiloba* extract by gavage at 550 mg/kg and their behaviors were monitored for a 14 day period. The biochemical and histological changes in the liver, kidney, stomach and spleen were analyzed. *O. obtusiloba* extract did not significantly change behavior, body weight, hematological or biochemical profiles. The organs of the animals did not show significant alterations when submitted to treatment with *O. obtusiloba* extract. In conclusion, the *in vivo* results revealed that *O. obtusiloba* has low toxicity and it can be and may be the target of further studies of biological activity.

**Key words:** *Osmundaria obtusiloba*; acute toxicity; seaweeds; preclinical tests.

### **INTRODUCTION**

A variety of natural substances have been isolated from extracts of the genus *Osmundaria* J.V. Lamouroux (Order

Ceramiales, family Rhodomelaceae), however, there is a predominance of bromophenols (Poplewell and

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Northcote, 2009), which are molecules that have one or more rings benzene, with varying degrees of halogenation and hydroxyl groups (Liu et al., 2011). These molecules present interesting biological activities described in the literature, for example, antimicrobial (Barreto and Meyer, 2006), antiviral (de Souza et al., 2012) and cytotoxic (Popplewell and Northcote, 2009).

The *Osmundaria obtusiloba* (C. Agardh) R.E. Norris species is characterized as a robust plant (Rhodophyta, Ceramiales, Rhodomelaceae), which can measure from 10 to 15 cm in height, with flat apices of 3 to 4 mm wide, showing dark red coloration (Carvalho et al., 2006). Several biological activities have been described from *O. obtusiloba*, mainly for their extracts, such as the fraction of *O. obtusiloba* rich in lectin that inhibited the trypsin and  $\alpha$ -amylase enzymes, indicating its potential use in the production of drugs against diabetes (de Oliveira et al., 2009) or the ethanol extract that was able to inhibit the replication of Zika virus while maintaining low cytotoxicity ( $CC_{50}=525 \mu\text{g/ml}$ ) (Cirne-Santos et al., 2017). Antiviral activity against HSV-1 and HSV-2 has also been described for glycolipids extracted from *O. obtusiloba* (de Souza et al., 2012). In the work of de Alencar et al. (2016) the 70% EtOH was most effective solvent for extracting phenolic compounds from red seaweeds when compared to hexane, also *O. obtusiloba* EtOH extract presented high antioxidant activity. Already, the *O. obtusiloba* methanolic extract showed to present bromophenols (Carvalho et al., 2006).

Due to the discovery of several biological activities previously described, in particular the activity against the Zika virus, it is necessary to make the first preclinical tests to investigate the degree of toxicity of the ethanol extract of this seaweed, aiming the development of new drugs. Toxicity studies provides information on toxic doses and therapeutic indices of drugs and this type of studies in animals is vitally needed to determine the safety of medicinal plants for a future clinical study (Al-Afifi et al., 2018). There are still few toxicity studies of seaweed extracts in animals and the present work aims to evaluate the acute toxicity of the *O. obtusiloba* ethanol extract.

## MATERIALS AND METHODS

### Algae and extraction

Specimens of *O. obtusiloba* were collected at Rasa Beach, Armação de Búzios, Rio de Janeiro State, Brazil (latitude  $22^{\circ} 45' 40''$ , longitude  $41^{\circ} 54' 32''$ ). The seaweeds were washed with local water and separated from sediments, epiphytes, and other

associated organisms. The material was dried at room temperature for about seven days, triturated using an industrial blender and weighed (140 g) on a semi-analytical scale. The crushed seaweed was exhaustively extracted with ethanol at room temperature. The extract was evaporated under reduced pressure, yielding a brownish residue (5 g). For oral administration the extract was diluted in 1% dimethyl sulphoxide (DMSO).

## Biological studies

### Animal model

Three groups of six female BALB/c mice, three months old, weighing 19 to 25 g were used for acute toxicity tests (Garrido, 2016). The animals were observed into our bioterium in Virology laboratory kept in polypropylene cages at  $25 \pm 2^{\circ}\text{C}$ , under a 12/12 h light/dark cycle, with food and water *ad libitum*. All the tests were performed according to the protocols already approved by the Ethics Committee on Animal Use of the Fluminense Federal University (CEUA-UFF) with certificate number 798.

### Experimental protocol: Acute toxicity (14 days)

To evaluate the acute toxicity over 14 days, the animals were divided into three groups: (I) *O. obtusiloba* extract - 550 mg/kg ( $n = 6$ ); (II) 1% DMSO - vehicle ( $n = 6$ ); (III) Saline - negative control ( $n=6$ ). These were administered in single oral dose of 200  $\mu\text{l}$ . The animal behavior was observed throughout both experiments. At the end of the experimental period (14 days), the animals were euthanized by anesthetic overdose (ketamine + xylazine). Body weights were measured on D0 (first day), before extract administration, D7 (7th day) and D14 (14th day) of the experimental period. The protocol and concentrations used in this study were based on the OECD 423 guidelines (OECD, 2008). The concentration of 550 mg/kg of the extract was chosen based in low *in vitro* cytotoxicity of this extract and the dose-response curve in the OECD 425 guidelines (OECD, 2001). Blood samples were collected at the end of the experiment (14th day). Biochemical parameters tests used samples collected in BD-Microtainer® (Clot Activator/SSTTM Gel-Amber) vials to analyze: alanine aminotransferase (ALT), aspartate aminotransferase (AST) and blood urea nitrogen (BUN) (Garrido, 2016). The results were obtained with an automatic biochemistry meter (BS-210-Bioclín).

### Histological analysis

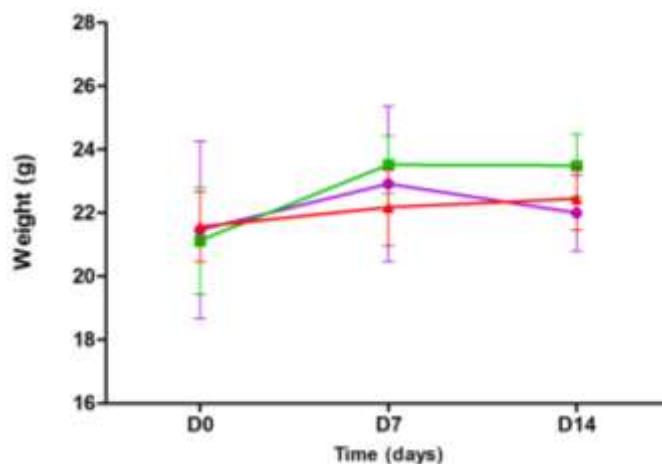
At necropsy, the liver, kidney, heart, spleen and stomach were removed for histological processing. Thus, the organs were fixed in 10% Carson formalin and after tissue slices of all organs were routinely processed for paraffin embedding. After the processing of the organs, the pieces were cut into 5 mM microtome according to the literature (Musumeci, 2014) and were prepared and stained by Mayer hematoxylin/eosin (Sigma-Aldrich). Then, the slides were analyzed by conventional microscopy.

### Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey test using GraphPad Prism version 5 program. A P value of <0.05 was considered statistically significant.

## RESULTS AND DISCUSSION

To evaluate the acute toxicity of *O. obtusiloba* extract (550 mg/kg) three groups were formed; a group receiving the ethanol extract, another group receiving only the vehicle and a group receiving saline by gavage. All animals treated with *O. obtusiloba* extract, 1% DMSO and saline survived for 14 days. There were no significant adverse clinical signs or changes in body weights ( $p >$  Barros et al. 219



**Figure 1.** Body weight of BALB/c mice treated with *O. obtusiloba* extract and controls (1% DMSO and saline) on days 0 (D0), 7 (D7) and 14 (D14). ▲ Saline – negative control, ● 1% DMSO – vehicle and ■ *O. obtusiloba* extract.  $p > 0.05$  in Tukey Test.

0.05) (Figure 1). From the serum were determined the transaminases ALT and AST, parameters used for the evaluation of liver function and BUN, which can be used to estimate the renal function (Roy et al., 2015). The results showed that the group that received the oral administration of *O. obtusiloba* extract had no significant difference compared to the control group (saline) and vehicle ( $p > 0.05$ ) in all the biochemical parameters analyzed (Table 1) and remained within normal range (Kifayatullah et al., 2015).

Histological findings corroborate with those clinical observed during the experiment and biochemical analyses. We analyzed organs extracted from the experiment mice and they did not show significant changes in morphology in the 550 mg/kg *O. obtusiloba* extract group when compared with control groups (vehicle and saline) with spleen exception. Therefore, the liver was histologically normal, and structures of the hepatic wire and centrilobular veins may be evident corroborating with the AST and ALT analyzes (Figure 2A, B and C). Sasidharan et al. (2010) by testing another red seaweed observed no significant signs of toxicity, nor did a single administration of 2000 mg/kg methanol extract of *Gracilaria changii* cause death during the 14-day

observation period. In another acute toxicity work of our group (period of 10 days) with dolabelladienotriol, a natural product derived from brown algae, *Dictyota friabilis* (as *Dictyota pfaffii*), all of the animals that received dolabelladienotriol presented a moderate increase in mitosis of hepatocytes and focal areas of hydropic cells in the medulla of the kidneys, even though this study indicates that dolabelladienotriol has low toxicity in administered dose range (Garrido et al., 2011).

The architecture of the kidneys presented normality in the tubules and glomeruli (Figure 2D, E and F) and urea parameters (Table 1). The low toxicity was later confirmed by the subchronic toxicity study of this product (Garrido et al., 2017). Already for the extract of *O. obtusiloba* has been reported the presence of bromophenols (Osako and Teixeira, 2013). The heart showed no changes in the architecture with cardiac fibers very visible to the cut (Figure 2G, H and I). Finally, we analyze the histological structure of stomach and observed no changes (Figure 2J, K and L), just as Garrido et al. (2011) found in the acute toxicity study of dolabelladienotriol. However in the spleen, as noted in the 1% DMSO group and *O. obtusiloba* group, was presented an increase of white pulp with a suggestive

activation of the germinal center, possibly due to the DMSO solvent used for solubilization of the extract (Figure 2M, N and O). These results, together with the biochemical parameters analyzed, demonstrate the low acute toxicity of the extract of this alga in tested concentration.

### **Conclusion**

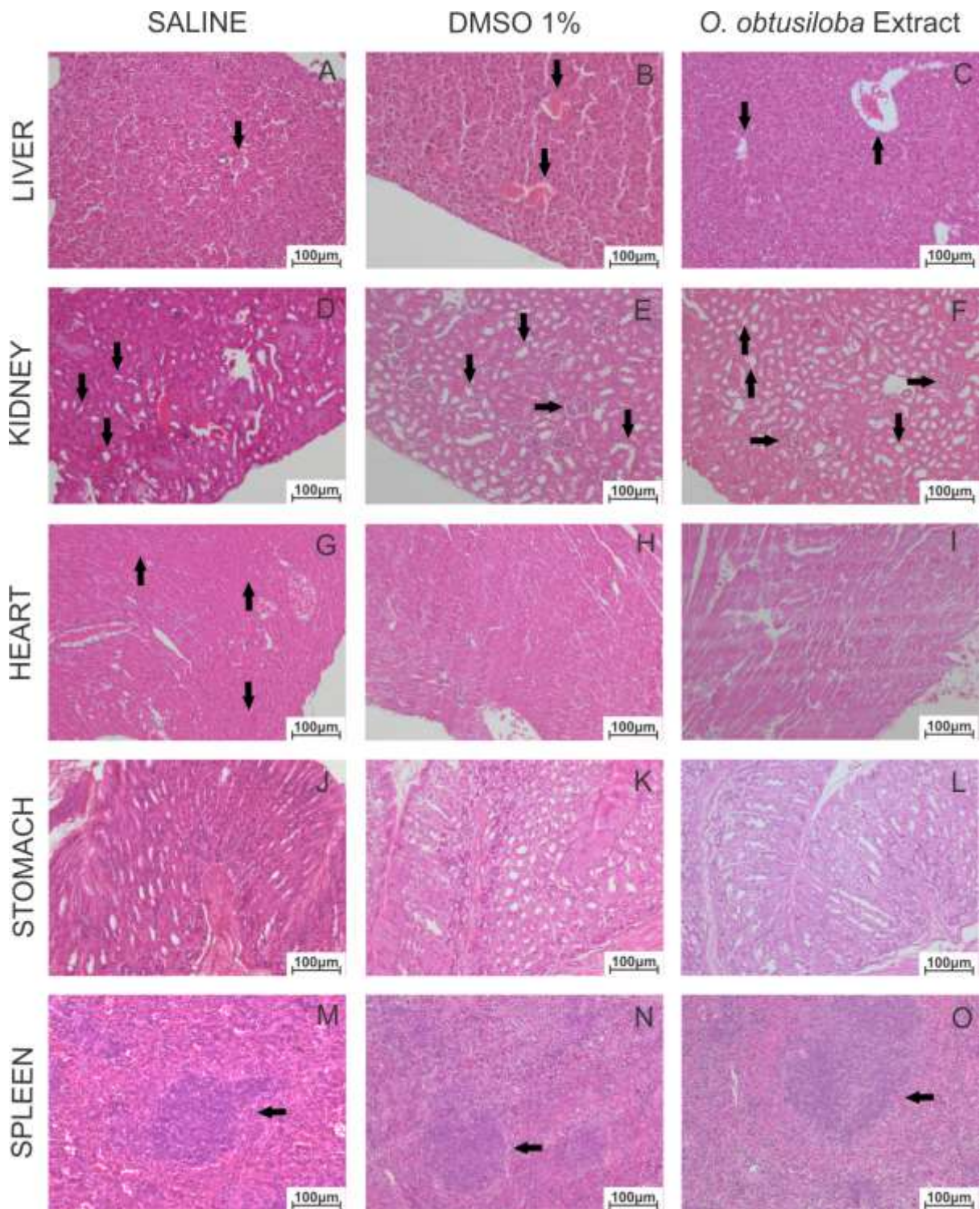
The result indicates that the single oral dose of administration of *O. obtusiloba* ethanol extract  
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(550mg/Kg) in our acute toxicity study after 14 days did not produce any significant toxic effect in BALB/c mice. Hence, further studies should be carried out to confirm the low toxicity of this extract with its continuous and prolonged use.

### **CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.





**Figure. 2** Histological analysis of liver, kidney, heart, stomach and spleen in female BALB/c mice administered with a single oral dose of saline (A,D, G, J and M), 1% DMSO (B, E, H, K and N) or *O. obtusiloba* ethanol extract (550 mg/kg) (C, F, I, L and O), at 14 days after the administration. (A, B and C) liver: Histologically normal, and structures of the hepatic wire and centrilobular veins (vertical arrow) may be evident; (D, E and F) kidney: Presented normality architecture in the tubules (vertical arrow) and glomeruli (horizontal arrow); (G, H and I) heart: No changes in the architecture with cardiac fibers (vertical arrow) very visible to the cut; (J, K and L) stomach: Architecture preserved; (M,N and O) spleen: Saline group (M) presented spleen with preserved architecture, the horizontal arrow indicates the germinal center, 1% DMSO group (N) and *O. obtusiloba* group (O), presented an increase of white pulp with a suggestive activation of the germinal center (horizontal arrow) (200x). Mayer hematoxylin and eosin staining.

**Table 1.** Effect of *O. obtusiloba* extract on biochemical analyses in acute toxicity study in BALB/c mice.

Parameter	Saline	1% DMSO	<i>O. obtusiloba</i> extract
ALT (U L <sup>-1</sup> )	24.50 ± 3.91	22.50 ± 2.51	20.50 ± 2.12
AST (U L <sup>-1</sup> )	133.00 ± 51.36	79.50 ± 9.14	96.00 ± 42.14
BUN(mg dl <sup>-1</sup> )	42.83 ± 5.24	42.83 ± 6.05	40.33 ± 7.66

Values are expressed as mean ± SD. No significant changes in the parameters ( $p > 0.05$ ). ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BUN: Blood urea nitrogen.

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