

## Anti-inflammatory and antinociceptive activities of methanolic extract from red seaweed *Dichotomaria obtusata*

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The aim of the present work was to investigate the anti-inflammatory and antinociceptive effects of methanolic extract from *D. obtusata* using classic models in mice (croton oil-induced ear edema and acetic acid-induced writhing) and a phospholipase A<sub>2</sub> activity test. Qualitative analysis of the chemical composition of seaweed was also determined by extraction with solvents of increasing polarity and precipitation and color tests. Results of qualitative chemical study showed the presence of lactonic and phenolic compounds, reduced carbohydrates, other sugars, flavonoids, fatty compounds, triterpenes and steroids. The extract inhibited mouse ear edema in a dose-dependent manner with an efficacy higher than 90% and a mean effective dose of 4.87µg/ear, while intraperitoneal administration presented a moderate activity. The extract did not inhibit phospholipase A<sub>2</sub> activity. In the writhing test, the intraperitoneal administration of the extract showed a strong antinociceptive activity (80.2%), while the oral route showed a lower efficacy. In conclusion, this study demonstrated the anti-inflammatory and antinociceptive effects of methanol extract of *D. obtusata* in experimental models, suggesting its therapeutic potential in the treatment of peripheral painful and/or inflammatory pathologies.

**Uniterms:** *Dichotomaria obtusata*/pharmacognosy. *Dichotomaria obtusata*/anti-inflammatory activity. *Dichotomaria obtusata*/antinociceptive activity. Mouse ear edema test. Writhing test.

O objetivo do presente trabalho foi investigar os efeitos antiinflamatórios e antinociceptivos de um extrato metanólico de *D. obtusata*, utilizando modelos clássicos em ratos (teste do edema de orelha induzido por óleo de cróton e teste de contorções induzidas por ácido acético) e um teste de atividade de fosfolipase A<sub>2</sub>. A análise qualitativa da composição química das algas foi também determinada através de extração com solventes de polaridade crescente e testes de precipitação e cor. Os resultados do estudo de química qualitativa mostraram a presença de compostos lactônicos e fenólicos, hidratos de carbono reduzidos e outros açúcares, flavonoides, compostos graxos, triterpenos e esteroides. O extrato inibiu o edema de orelha dos ratos de um modo dependente da dose com eficácia superior a 90% e dose média efetiva de 4.87µg/orelha, enquanto a administração intraperitoneal apresentou atividade moderada. O extrato não inibiu a atividade da fosfolipase A<sub>2</sub>. No teste de contorção, a administração intraperitoneal do extrato mostrou forte atividade antinociceptiva (80,2%), enquanto a administração oral mostrou menor eficácia. Em conclusão, este estudo demonstrou os efeitos antiinflamatórios e antinociceptivos do extrato metanólico de *D. obtusata* em modelos experimentais, sugerindo seu potencial terapêutico no tratamento de patologias dolorosas periféricas e/ou inflamatórias.

**Unitermos:** *Dichotomaria obtusata*/farmacognosia. *Dichotomaria obtusata*/atividade antiinflamatória. *Dichotomaria obtusata*/atividade antinociceptiva. Teste do edema de orelha. Teste de contorções.

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## INTRODUCTION

Marine environment may contain over 80% of world's species of plants and animals (Kumar, Xi-rong, 2004). Particularly, seaweeds have been of great interest for humans as marine food sources since old times (Nisizawa *et al.*, 1987). Nowadays, these organisms represent a promising source of useful products awaiting discovery for the prevention or treatment of several pathologies (Faulkner, 2002). Ecological pressures, including fight for space and predation, have contributed to the evolution of secondary metabolites with diverse pharmacological properties. In fact, the discovery of metabolites with biological activities from seaweeds has increased significantly in the past three decades (Smit, 2004).

On the other hand, inflammation is a defensive reaction of organisms to antigenic stimulation or physical injuries. This response involves the activation of complex metabolic pathways and the intervention of chemical and cellular mediators (Przanski, Vadas, 1991; Abbas *et al.*, 2008). Nevertheless, an exacerbated response can conduce to the development of multiple pathologies, including rheumatoid arthritis, acute gout, asthma, neurodegenerative diseases and cancer (Kazłowska *et al.*, 2010). Furthermore, pain is a classic sign of this inflammatory response (Flórez, Reig, 1994).

Pharmaceutical anti-inflammatory drugs are generally used to treat inflammation and pain. In general, these drugs reduce inflammatory response by suppressing the production of pro-inflammatory mediators, which are involved in the pathogenesis of inflammatory diseases (Guslandi, 1998). Nevertheless, reports on the increased risk of digestive, cardiovascular and renal diseases with long-term use of several non-steroidal anti-inflammatory drugs and the serious systemic side effects of glucocorticoids, have raised concerns about using the drugs (Jugdutt, 2007). To overcome this limitation, a considerable amount of researches have promoted the discovery and development of new bioactive natural products with anti-inflammatory and antinociceptive properties which do not show any side effects.

An increasing number of studies have demonstrated that certain extracts and compounds from seaweeds have potential anti-inflammatory uses. For example, Ganovski *et al.* in 1979 reported the anti-inflammatory effect of an aqueous extract from *Cystosina barbata*, *Ulva lactuca* and *Zostera nona*. Also, Payá *et al.* in 1993 studied the anti-inflammatory potential of methanol and dichloromethane extracts of seven species of seaweeds. A group of inhibitors of phospholipase A<sub>2</sub> were isolated from different microalgae (Mayer *et al.*, 1993). Recently, other metabolites with anti-

inflammatory activity have been obtained from seaweeds, including a glycoesterol (Awad, 2000), a phlorotannin (Sugiyama *et al.*, 2006), polyphenols (Jung *et al.*, 2009; El Gamal, 2010) and polysaccharides (Ananthi *et al.*, 2010). Moreover, various seaweeds have antinociceptive properties (Anca *et al.*, 1993; Guzmán *et al.*, 2001; Viana *et al.*, 2002).

*Dichotomaria obtusata* (J. Ellis, Solander) Lamarck, a red seaweed found in tropical and subtropical shores, is one of the most common species of the phylum Rhodophyta (Suárez, 2005). This species has been used in a small number of biological studies. We have recently reported the intraperitoneal anti-inflammatory and antinociceptive properties of aqueous extract of *D. obtusata* in two classic animal models (Frias *et al.*, 2011). However, anti-inflammatory efficacy of the aqueous extract was limited and the mechanisms of action have not been examined yet. In order to obtain a greater efficacy, a new extract of this seaweed was prepared. Therefore, the aim of the current study was to evaluate the anti-inflammatory and antinociceptive potential of methanolic extract of *D. obtusata* employing different routes of administration. Before that, we have carried out a preliminary acute toxicological study of our extract with the purpose of knowing the safety of the working material. Also, one of the possible mechanisms of action of bioactive components of the extract was investigated in an *in vitro* assay. We present novel anti-inflammatory and antinociceptive activities of red seaweed *D. obtusata*.

## MATERIALS AND METHODS

### Drugs and reagents

Tested drugs (indomethacin, dexamethasone, acetylsalicylic acid) and reagent (croton oil) were purchased from Sigma Chemicals.

### Material

The red alga *Dichotomaria obtusata* was collected at Jaimanitas Beach, Havana, in November 2008, and was identified by Dr. Ana María Suarez, researcher of the Center of Marine Researches, University of Havana. A voucher specimen was deposited at the Herbarium from Center of Marine Researches under the acquisition number r-189. Specimens were washed with common water, dried at room temperature and kept at 4 °C until the obtaining of the methanolic extract.

### Compositional analysis of *D. obtusata*

The qualitative analysis of the chemical composition

of *D. obtusata* was conducted according to Chabra's method (Chabra *et al.*, 1984), which is based on extraction with solvents of increasing polarity (dichloromethane, ethanol and water) and tests of precipitation and color solutions.

### Preparation of the methanolic extract

Specimens of *D. obtusata* (50 g) were dissolved in 500 mL of methanol and soaked at room temperature during 8 hours. The methanol extract was concentrated in a rotary evaporator (BUCHI, Model B48C) and stored at 4 °C until its use.

### Animals

All animal experiments were conducted according to the Guide for the Care and Use of Laboratory Animals of the Center for Laboratory Animals Production (CENPALAB), Cuba. For one week before the experiments, male CENPALAB mice: OF-1 weighing 23-25 g were maintained in ventilated plastic cages in a soundproofed room at 22 °C, with an artificial 12:12 h light:dark cycle. Food and sterile water were supplied *ad libitum*. Animals were randomized into treatment groups and deprived of food and water during the experiment.

### Acute Toxicity Study

The acute toxicity study of the methanolic extract of *D. obtusata* was carried out according to the *acute-toxic-class method as alternative to the LD<sub>50</sub> test* (Schlede *et al.*, 1992). Graded doses (25, 200 and 2000 mg/kg) of methanolic extract were administered intraperitoneally to various groups, each group containing six mice. On the first hour after administration, the animals were evaluated every 10 minutes for any changes in respiratory frequency, writhing, piloerection, spontaneous motor activity, etc. Animals were observed up to 24 hours for any mortality.

### Mouse ear edema test

All procedures of mouse ear edema bioassay were carried out following the established method of Tubaro *et al.* (1986). Edema was induced in right ear of each mouse by the topical application, to both the inner and outer surfaces, of 10 µL of a 0.47% croton oil solution in acetone to induce inflammation, and the same volume of acetone was applied to the left ear. Methanolic extract dissolved in ethanol was administered topically (0.5 x 10<sup>-3</sup>, 1 x 10<sup>-3</sup>, 1.5 x 10<sup>-3</sup>, 7.5 x 10<sup>-3</sup>, 15 x 10<sup>-3</sup>, 30 x 10<sup>-3</sup>, 0.5, 1 and 2 mg/ear) and intraperitoneally (i.p.) (12.5, 25, 50 and 100 mg/kg)

simultaneously and 30 min before croton oil application, respectively. As positive controls for comparative purposes, other groups of animals were treated with indomethacin (0.5 mg/ear and 10 mg/kg) dissolved in 5% NaHCO<sub>3</sub> and dexamethasone (0.1 mg/ear and 0.5 mg/kg), following the same conditions of extract administration. Five hours after croton oil application, the animals were sacrificed by cervical dislocation and the ears were cut off. In all experiments, a disk was cut from the middle part of each ear using a punch 7 mm in diameter. The edema size was determined in relation to the weight of untreated left ear and percentage of inhibition was calculated using the following expression:

$$\% \text{ Inhibition} = (\Delta P_c - \Delta P_t) / 100 \times \Delta P_c$$

where:  $\Delta P_c$  → mean weight variation in the control group;  $\Delta P_t$  → mean weight variation in the treated group.

### Phospholipase A<sub>2</sub> activity test

The ability of methanolic extract to inhibit phospholipase A<sub>2</sub> was evaluated according to the indirect radial hemolysis in agar plate method proposed by Gutiérrez *et al.*, in 1988. A Petri plate was prepared with agar containing egg yolks (substrate of phospholipids), calcium chloride (enzymatic cofactor), sodium azide (preservative) and human erythrocytes for visualizing the effect. Methanolic extract (2.5, 5 and 10 mg/mL) was dissolved in PBS (0.12 M NaCl, 0.04 M NaHPO<sub>4</sub><sup>2-</sup>, pH 7.2) and applied by triplicate in the holes (6-mm diameter) made in the plate. The phospholipase A<sub>2</sub> purified from sea anemone *Condylactis gigantea* was used as control. This enzyme was donated by the Center for Protein Studies, University of Havana. The plate was maintained at 37 °C by 12 hours. The enzymatic activity was determined by the presence of halos as an indicative of hemolysis.

### Acetic acid-induced writhing test

The acetic acid-induced writhing test was conducted as originally described by Koster *et al* in 1959. Mice were randomly distributed in control and test groups of ten animals each. In this test, an intraperitoneal injection of acetic acid (0.8%, 10 ml/kg b.w.) was given 30 and 60 min after the i.p. (12.5, 25, 50 and 100 mg/kg) and oral (100, 200, 400 and 800 mg/kg) administrations of test extract, respectively. Also, the reference drug acetylsalicylic acid (68 mg/kg) was administered by oral route 1 hour before acetic acid injection, and control group was treated with sterile 0.9% saline solution. Following that, the number of

writhing movements in the mice was counted for 20 min. The antinociceptive activity of the methanolic extract was expressed as percentage of pain reduction in treated mice with respect to control, according to the relation:

$$\% \text{ Reduction} = (\Delta Cc - \Delta Ct) / 100 \times \Delta Cc$$

where:  $\Delta Cc$  → mean of number of writhes of control group (vehicle-injected animals);  $\Delta Ct$  → mean of number of writhes in the treated group.

### Statistical analysis

Data are expressed as mean  $\pm$  SEM. One way analysis of variance (ANOVA) was applied for the analysis of results using software packages *Statistica* (version 7.0) and *Past* (version 1.99). P-values less than 0.05 and 0.001 were considered significantly different for Tukey-Kramer and Bonferroni multiple comparison tests, respectively.

## RESULTS

### Compositional analysis of *D. obtusata*

The results of the qualitative chemical study of *D. obtusata* (Table I) showed the notable presence of lactonic

and phenolic compounds, reduced carbohydrates and other sugars. However, the presence of flavonoids, fatty compounds, triterpenes and steroids was low.

### Acute Toxicity Study

Acute toxicity results of the intraperitoneal administration of methanolic extract are presented in Table II. As can be appreciated, no mortality of animals was observed after 24 hours of administration of extract at 25, 200 and 2000 mg/kg. The most significant signs of toxicity were swing and writhing, which were presented at all doses. Swing showed a percentage of appearance superior at 65%. Also, the periods of latency of appearance of these signs were dose-dependent at doses of 25 and 200 mg/kg. However, this parameter decreased at 2000 mg/kg. In addition, the stoop of back train was observed at doses of 200 mg/kg (33.3%) and 2000 mg/kg (66.7%). Finally, a sedative effect (33.3% of appearance and 75 sec of latency) and the increase of respiratory frequency (100% of appearance and 56 sec of latency) were observed in animals administered with the maximum dose of extract.

### Croton oil-induced ear edema test

Table III shows the anti-inflammatory effect of

**TABLE I** - Qualitative phytochemical analysis of *Dichotomaria obtusata*

Test	Extracts		
	Dichloromethane	Ethanollic	Aqueous
Sudan (Fatty compounds)	+		
Dragendorff (Alkaloids)	-	-	-
Baljet (Lactonic compounds)	++	++	+++
Ferric Hydroxamate (Coumarins)	-		-
Borntrager (Quinones)		-	
Liebermann-Burchard (Triterpenes and steroids)	+	+	+
Catechines		-	
Fehling (Reduced carbohydrates)		-	+++
Foam (Triterpene and steroid saponins)		+++	-
Ferric chloride (Phenols and tannins)		++	++
Ninhydrin (Amino acids)		+	
Shinoda (Flavonoids)		+	
Kedde (Cardiotonic glycosides)	-	-	-
Anthocyanidins (Flavonoids with structure C6-C3-C6)		+	
Mucilages (Polysaccharides)			++
Resins		-	
Molish (Carbohydrates)		+	++

**Legend:** – negative reaction; + weak reaction; ++ half reaction; +++strong reaction.

**TABLE II** - Percentage of mortality and signs of toxicity observed in OF-1 mice after i.p. administration of methanolic extract from *D. obtusata* (n=6 animals/dose)

Doses (mg/kg)	Mortality (%)	Signs of Toxicity	Appearance of signs (%)	Latency (seconds; mean $\pm$ SEM)
25	0	Swing	66.7	58,8 $\pm$ 12,6
		Writhing	83.3	73,2 $\pm$ 6,1
200	0	Swing	100	21,8 $\pm$ 2,0
		Writhing	100	39,2 $\pm$ 4,9
		Stoop of back train	33.3	45,5 $\pm$ 0,5
2000	0	Swing	66.7	27,5 $\pm$ 8,0
		Writhing	50	51,7 $\pm$ 4,4
		Stoop of back train	66.7	31,5 $\pm$ 5,3
		Increase of respiratory frequency	100	55,8 $\pm$ 17,3
		Sedative effect	33.3	75,0 $\pm$ 45,0

topical administration of methanolic extract ( $0.5 \times 10^{-3}$ ,  $1 \times 10^{-3}$ ,  $1.5 \times 10^{-3}$ ,  $7.5 \times 10^{-3}$ ,  $15 \times 10^{-3}$ ,  $30 \times 10^{-3}$ , 0.5, 1 and 2 mg/ear) on croton oil-induced ear edema in mice. The ear edema in control group, five hours after croton oil application, was  $18.25 \pm 0.23$  mg. All doses of extract decreased significantly ( $p < 0.0001$ ) the action of the irritating agent except the dose of  $0.5 \times 10^{-3}$  mg/ear.

As can be seen, five of the evaluated doses exerted a significant inhibition of edema ( $> 50\%$ ). The extract at 1 mg/ear showed the greatest inhibition of edema

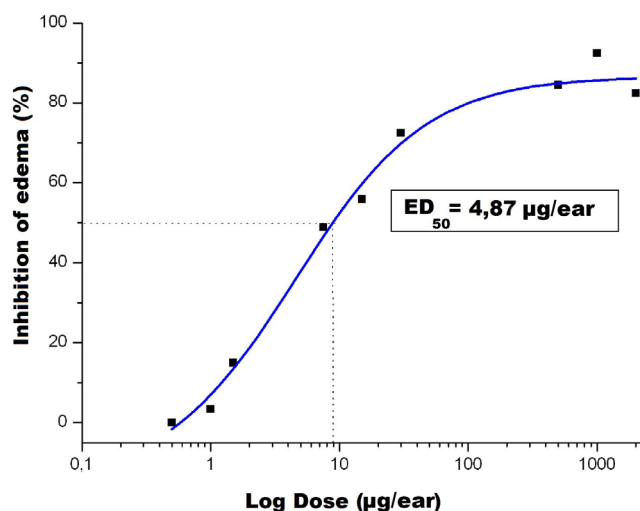
(92.44%), which was similar to reference drug dexamethasone (88.11% at 0.1 mg/ear) and far superior to indomethacin (60.99% at 0.5 mg/ear).

Methanolic extract showed a dose-dependent anti-inflammatory effect. Figure 1 illustrates the dose-response relation of topical application of extract. The behavior of curve indicates that the apparent maximum dose is 1 mg/ear. Also, the Mean Effective Dose (dose inducing a 50% edema inhibition,  $ED_{50}$ ) was calculated, which was  $4.87 \mu\text{g/ear}$ .

**TABLE III** - Effect of topical administration of methanolic extract from *D. obtusata* on croton oil-induced ear edema in mice (n=6 animals/doses)

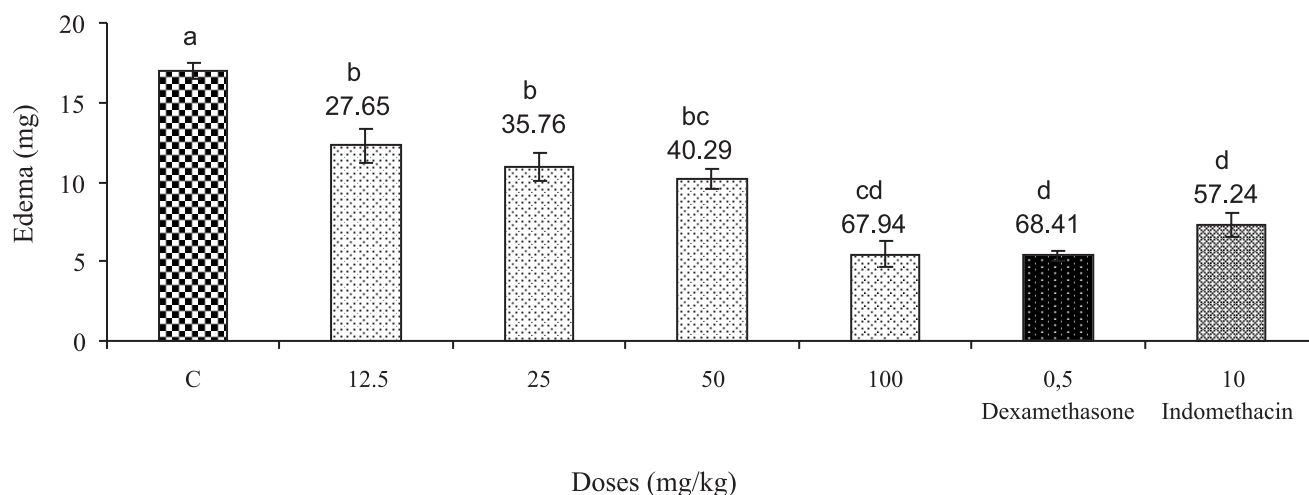
Doses (mg/ear)	Edema (mg) (mean $\pm$ SEM)	Edema reduction (%)
Control	$18.25 \pm 0.23$	
$0.5 \times 10^{-3}$	$18.37 \pm 1.03^*$	0
$1 \times 10^{-3}$	$17.62 \pm 0.85^{**}$	3.45
$1.5 \times 10^{-3}$	$15.52 \pm 0.72^{**}$	14.96
$7.5 \times 10^{-3}$	$9.32 \pm 0.52^{**}$	48.93
0.015	$8.05 \pm 0.97^{**}$	55.89
0.03	$5.02 \pm 0.60^{**}$	72.49
0.5	$2.83 \pm 0.65^{**}$	84.49
1	$1.38 \pm 0.43^{**}$	92.44
2	$3.2 \pm 0.18^{**}$	82.47
Dexamethasone (0.1)	$2.17 \pm 0.26^*$	88.11
Indomethacin (0.5)	$7.12 \pm 0.60^*$	60.99

\*  $p < 0.0001$  compared with control; \*\* $p < 0.0001$  compared with indomethacin and dexamethasone (Bonferroni's test)

**FIGURE 1** - Dose-response relation of topical application of methanolic extract from *D. obtusata*.

In the same way, the anti-inflammatory activity of extract was evaluated using the systemic administration (i.p.). The results obtained at different doses of extract





**FIGURE 2** - Effect of intraperitoneal administration of methanolic extract from *D. obtusata* on croton oil-induced ear edema in mice (n=6 animals/dose).

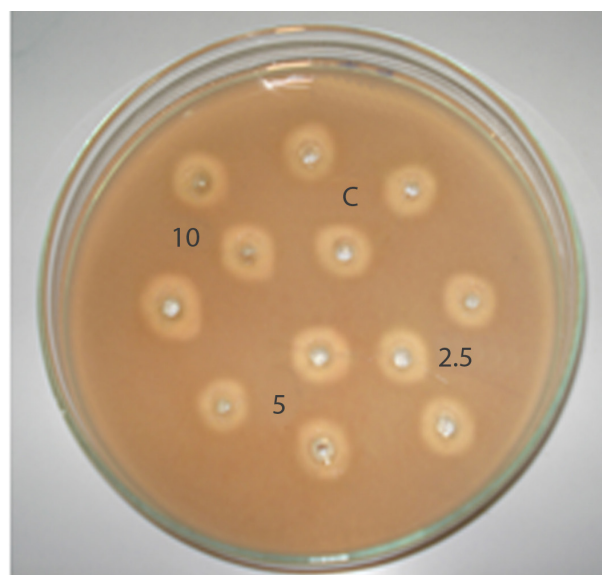
(12.5, 25, 50 and 100 mg/kg) are shown in Figure 2. All groups of treatment were able to decrease significantly ( $p < 0.05$ ) the croton oil-induced ear edema compared to control group (which received 0.9% saline solution). Similar to topical application, the i.p. administration of extract inhibited this edema in a dose-dependent manner. The maximum inhibition was 67.94% at dose of 100 mg/kg, which was not different from response of reference drugs indomethacin (57.24%) and dexamethasone (68.41%).

### Phospholipase A<sub>2</sub> activity test

One of the probable mechanisms of action of anti-inflammatory compounds is mediated by the inhibition of phospholipase activity. For this reason, methanolic extract of *D. obtusata* was tested for a possible inhibitory activity of phospholipase A<sub>2</sub>, using the indirect radial hemolysis in agar plate method. The effect of extract (2.5, 5 and 10 mg/mL) in this assay is shown in Figure 3. The presence of halos of hemolysis around the holes of the plate, where extract and control phospholipase A<sub>2</sub> were applied, indicates the extract did not inhibit the enzymatic activity. Phospholipase A<sub>2</sub> hydrolyzes the phospholipids of egg yolk, which are liberated into medium as fatty acids and glycerophospholipids. After that, the pH of culture medium decreases and this promotes the lysis of erythrocytes and the appearance of halos of hemolysis.

### Acetic acid-induced writhing test

Antinociceptive activity of methanolic extract was examined using writhing test. Intraperitoneal administration of the extract at the doses of 12.5, 25, 50 or 100 mg/kg

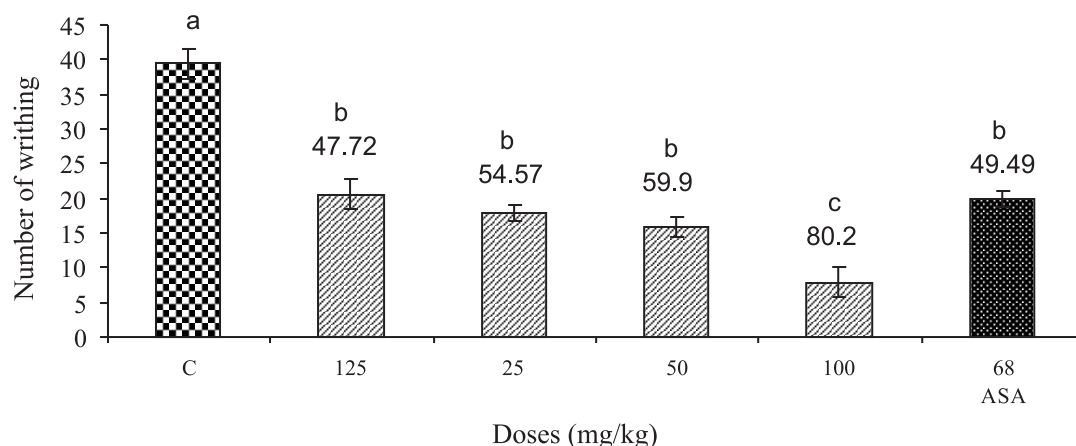


**FIGURE 3** - Effect of methanolic extract from *D. obtusata* (10, 5 and 2.5 mg/mL) on phospholipase A<sub>2</sub> activity.

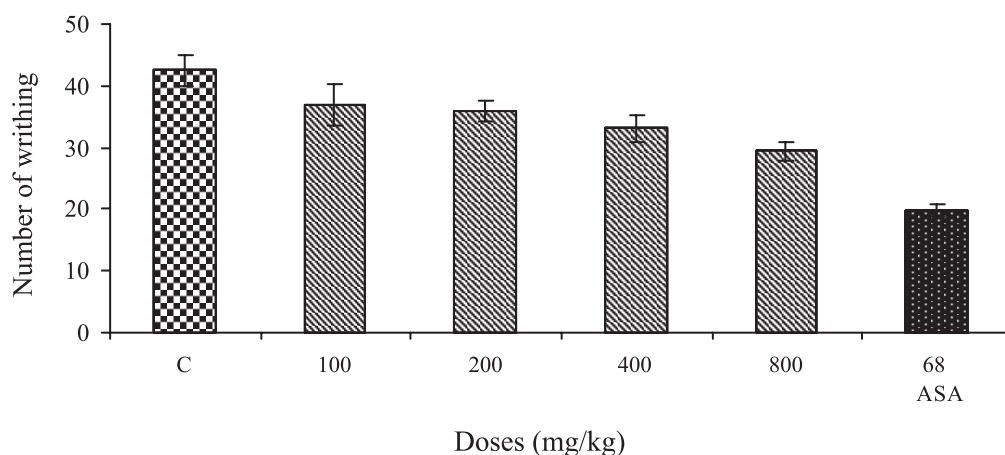
body weight decreased significantly ( $p < 0.0001$ ) the action of acetic acid used to induce writhes in mice, in comparison to the control animals that received only 0.9% saline solution.

The effects of i.p.-administered extract were dose-dependent. There results can be seen in Figure 4. Treatment with 12.5 mg/kg reduced the number of writhes in 47.72%, which was further decreased in 80.2% with 100 mg/kg of the extract. The reference analgesic drug, acetylsalicylic acid (68 mg/kg) caused inhibition of writhes in 49.49%.

The results of the acetic acid-induced writhing responses in mice one hour after oral administration of



**FIGURE 4** - Effect of intraperitoneal administration of methanol extract from *D. obtusata* on acetic acid-induced writhing in mice (n=10 animals/dose). ASA: acetylsalicylic acid (reference drug).



**FIGURE 5** - Effect of oral administration of methanol extract from *D. obtusata* on acetic acid-induced writhing in mice (n=10 animals/dose). ASA: acetylsalicylic acid (reference drug).

the extract are presented in Figure 5. In this case, only the maximum dose of the extract (800 mg/kg) had a clear antinociceptive effect, with a significant reduction of the number of writhes ( $p < 0.05$ ) compared to control animals. The percentage of inhibition of writhes at this dose was 30.87%, which was lower than the reference drug (53.25%).

## DISCUSSION

The purpose of this paper was to evaluate anti-inflammatory and antinociceptive effects of methanolic extract from the red seaweed *Dichotomaria obtusata* using classic models in mice and an *in vitro* test.

Red seaweeds are considered the most important source of many biologically active metabolites in comparison to other algal classes (El Gamal, 2010). As a first step toward identifying the major chemical groups present

in *D. obtusata*, we have developed a qualitative chemical study of its composition. Results showed the presence of lactonic and phenolic compounds, flavonoids, fatty compounds, triterpenes, steroids, reduced carbohydrates and other sugars. In agreement with our results, Frías *et al.* in 2011 demonstrated the presence of carbohydrates, proteins, triterpenes and steroids in the aqueous extract from *D. obtusata*. Some of these compounds, such as phenols, terpenes, polysaccharides and steroids, have been reported to possess anti-inflammatory and antinociceptive effects (Silva, Scheuer, 1980; Chong, Parish, 1985; Awad, 2000; Lucas *et al.*, 2003; Rodríguez *et al.*, 2004; Jung *et al.*, 2009).

On the other hand, it is known that the possible therapeutic application of a new drug requires the evaluation of its preclinical pharmacological activities and studies of safety. Taking into account these considerations, we have evaluated the lethality of the methanolic extract using an

alternative acute toxicity test. The animals intraperitoneally administered showed some signs of toxicity (swing and writhing) at all tested doses. Other signs appeared at the highest dose (increased respiratory frequency and sedative effect). However, the animals in all groups survived, signifying the absence of lethality under our experimental conditions. This extract was much less toxic than aqueous extract from the same seaweed (unpublished data), suggesting that the majority of toxic metabolites from *D. obtusata* are of polar nature.

In a first set of pharmacological experiments, we investigated the effects of the extract on croton oil-induced ear edema using i.p. and topic single administrations. The extract dose dependently reduced edema formation and showed potency similar to that of reference drugs by topical route. In fact, its maximum effect was superior to 90% and its ID<sub>50</sub> value was 4.87 µg/ear. Taking into account that the extract is a complex mixture of several components, these values indicate its elevated pharmacological efficacy and potency.

Topical application of croton oil produced a longer-lasting edema associated with marked influx of neutrophils and predominant formation of LTB<sub>4</sub> along with significant changes in levels of TXB<sub>2</sub>. Also, earlier studies have revealed that histamine, serotonin and prostaglandins could play a role in the development of edema induced by this irritant agent (Gabor; Razga, 1990; Blazso, Gabor, 1994). The advantages of this model include its good predictive value for screening topical anti-inflammatory activity and its sensitivity to both steroidal and non-steroidal drugs. Nevertheless, this sensitivity is dependent on the time course of the response (Tubaro *et al.*, 1986).

Although the extract intraperitoneally administered was able to reduce the croton oil-induced ear edema at all evaluated doses, the efficacy was lower than that of topical route. Here, bioavailability factor might also be involved, because after topical application a large concentration of the extract could be available to the target tissues whereas this could be limited with the systemic administration. This is in agreement with previous studies of our group on the aqueous extract of this seaweed, which demonstrated a maximum anti-inflammatory activity greater than 65% in TPA-induced ear edema in mice (Frías *et al.*, 2011).

The results obtained in this acute inflammation model and the topical efficacy of the extract compared to dexamethasone suggest an interaction with metabolites of the arachidonic acid pathway, mediated mainly by phospholipase A<sub>2</sub> (PLA<sub>2</sub>) and cyclooxygenase (COX) enzymes (Chen *et al.*, 1994). Thus, the anti-inflammatory mechanism of action of the active components of the extract would involve PLA<sub>2</sub>. For this reason we tested

the ability of the extract to inhibit PLA<sub>2</sub> activity by the indirect radial hemolysis in agar plate method. However, this study showed absence of inhibitory effect on secretor PLA<sub>2</sub> activity of *C. gigantea*. These results partially agree with previous studies on the inhibitory activity of PLA<sub>2</sub> of various extracts from seaweeds, revealing inhibitory activity only in the extract of *Dictyota dentata* (Llanio *et al.*, 1998). In contrast, Mayer, in 1993, isolated 12 compounds from seaweeds with powerful inhibitory activity of PLA<sub>2</sub> from bee venom. Our results indicate that this study must be extended to other secretor PLA<sub>2</sub>, including PLA<sub>2</sub> from mammals.

We also investigated the antinociceptive activity of the extract in the acetic acid-induced writhing test in mice. The abdominal constriction response induced by acetic acid has been mainly used as screening tool for the assessment of analgesic or anti-inflammatory properties of new agents as well as a typical model for visceral inflammatory pain (Tjølsen, Hole, 1997). The local irritation provoked by a test agent in the intraperitoneal cavity triggers a diversity of mediators, such as bradykinin, substance P and prostaglandins, particularly PGI<sub>2</sub>, as well as some cytokines such as IL-1β, TNF-α and IL-8 (Correa *et al.*, 1996; Ikeda *et al.*, 2001). These mediators activate chemosensitive nociceptors that contribute to the development of inflammatory pain.

The methanolic extract inhibited significantly the number of writhes suggesting that its antinociceptive effect could be related to inhibition of mediators released in response to acetic acid. Nevertheless, the intraperitoneal route was more effective than oral at inhibiting this response at all evaluated doses (12.5-100 mg/kg), while only the maximum oral dose (800 mg/kg) reduced the abdominal constrictions. This result may be due to the limited bioavailability of the active constituents of the extract provoked by a poor absorption or the first-pass effect in liver. According to the test employed, this antinociceptive effect is probably of peripheral origin. We have previously demonstrated the antinociceptive activity of aqueous extract of this seaweed in the same test Frías *et al.* (2011), although in that study we obtained an effect more potent using oral administration. Inhibition of number of writhes in extracts and components obtained from seaweeds were also demonstrated by Guzmán *et al.* (2001), Viana *et al.*, (2002) and Frías *et al.* (2011).

In conclusion, it can be suggested that the methanolic extract from *D. obtusata* has powerful topical anti-inflammatory and antinociceptive activities. These observed properties could be associated with a probable synergistic effect of the diverse components of methanolic extract. Further studies are required to isolate the active principles



and to determine the possible mechanism of actions of these compounds and their potential for therapeutic use in the treatment of inflammatory and pain pathologies.

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