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Anti-inflammatory activities of a sulfated polysaccharide isolated from the brown seaweed *Padina boergesenii (Phaeophyceae, Dictyotaceae)*

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Abstract

Brown algae (BA) have recently gained high interest as a beneficial source of pharmacological numerous invaluable active constituents. Subsequently, water-soluble polysaccharides of BA attracted the attention of researchers worldwide due to their anticancer and antioxidant activities. This study was carried out to evaluate the anti-inflammatory activity of polysaccharides (PLS) extract of *Padina boergesenii* brown algae. Xylene produced-ear inflammation and carrageenan produced-paw edema models were used in this study. The PLS doses of 75, 125, 250, 500, 750, 1000 mg/kg were tested. Our obtained data revealed that PLS inhibited the inflammatory reactions of both models in a dose-dependent manner. Interestingly, the anti-inflammatory effect of PLS is associated with lowering of nitric oxide and MDA free radicals and pro-inflammatory cytokines: IL- 1β, IL-6, and TNF-α level. This data highlights the therapeutic significance of isolated PLS. As this polysaccharide possesses high anti-inflammatory activity in comparison to the anti-inflammatory drug diclofenac, therefore this natural compound could be recommended as an effective remedy for inflammation.

Keywords:

Anti-inflammatory activities, Antioxidants, Seaweed *Padina boergesenii*, Sulfated polysaccharide.

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Introduction

The high majority of diseases accompanied by acute and chronic inflammation and its social significance require the discovery of recent anti-inflammatory remedies, which are both effective and safe for patients. Since inflammation may be a polyvalent dynamic process characterized by many alternate and crossing pathways at the extent of both intracellular signal cascades and inflammatory mediator production, the event of immune biologic compounds is extremely necessary. These remedies must regulate the functional activity of the many molecules that participate within the inflammation process. (Chaudhari et al., 2015).

Both Non-steroidal (NSAID) and steroidal anti-inflammatory drugs (SAID) are commonly used for the treatment of those conditions, but these kinds of drugs are well known to have some adverse side effects, and this created a major concern and an urgent need for discovery of new natural drugs with anti-inflammatory activities but with relatively low incidences of side effects. Naturally, there are many products that have anti-inflammatory and analgesic properties with relatively low incidences of side effects (Chaudhari et al., 2015).

Many studies on marine flora and fauna are indicating that a lot of compounds obtained from marine organisms have useful pharmacological activities. Among these organisms, the macroalgae are well known to be an important source of bioactive materials suitable for therapeutic uses (Viana et al., 2002 and Genovese et al., 2009). Brown marine algae administration is known to control some inflammatory drawbacks such as carcinoma, and high cholesterol level (Yubin and Guangmei, 1998).

Marine algae are considered one of the richest sources of bioactive sulfated polysaccharides, which have important in vitro pharmacological activities like anticoagulant, antioxidant, anti-proliferative, antitumoral, anti-inflammatory, anti-viral, anti-peptic, and anti-adhesive activities (Cumashi et al., 2007 and de Azevedo et al., 2009).

In reference to the high interest within the quality of mediators of inflammatory processes, sulfated polysaccharides have drawn specific attention; they're obtained from the extraction of red and brown marine algae and are characterized by the power to supply and affect the course of both acute and chronic inflammation processes (Chen et al., 2008 and Jiao et al., 2011).

Sulfated polysaccharides are considered complex macromolecules with broad action owing to their chemical structure, rich in polyanions, which permit connection to many numbers of proteins, either in matrix or plasma cells (Arfors and Ley, 1993). These macromolecules are cosmopolitan in nature, being found in microorganisms, animals (Cássaro and Dietrich, 1977), and also in marine algae that have a high content of sulfated polysaccharides which have an important biological activities and abundant potential applicability.

Marine algae use for one thousand years in addition to numerous scientific and clinical studies had proved its medical value. The pharmacological activity of sulfated polysaccharides extracted from algae is preconditioned by their ability to possess a complicated effect on several systems within the organism. These biopolymers have low toxicity or even no toxic effect within the least (Chung et al., 2010).

Padina boergesenii is a member of brown alga, of the class Phaeophyceae commonly found in coastal regions along the continental shelf. Brown algae are known to have various bioactive

constituents like polyphenols, alkaloids, flavonoids, terpenoids, etc. These secondary metabolites help study various physiological effects i.e., either it is harmful or curative on human health (Rajamani et al., 2010). Moreover, Padina boergesenii was found to have strong antioxidant and cytotoxic activities (Jjeevitha et al., 2014).

Therefore, this study carried out to investigate the in vitro antioxidant and anti-inflammatory activities of polysaccharide (PLS) extract obtained from Padina boergesenii brown algae collected from Red Sea, Hurgada city through xylene induced-ear inflammation and carrageenan induced-paw edema models in mice and rats, respectively.

Materials and methods

Ethical statement:

All animals included in this study were handled according to the regulations of the Animal Ethics Committee at the Faculty of Veterinary Medicine, South Valley University, Qena, Egypt, with good animal practice following the guidelines of The Research Code of Ethics (RCOE-SVU) at the South Valley University.

Brown algae seaweed sample collection:

The brown alga *Padina boergesenii (Phaeophyceae Dictyotaceae)* was collected from Red Sea, Hurgada city during April and May 2018. The algae were hand-picked and washed thoroughly with seawater to remove impurities such as sand particles and epiphytes, placed separately in polythene bags, and kept in an icebox containing slush ice, and immediately transported to the laboratory, Faculty of Veterinary Medicine, Qena, South Valley University. The seaweed material was taxonomically identified by the national institute of oceanography and fisheries (NICF).

Crude polysaccharides preparation:

Around 300 g of dried seaweed powder was depigmented with acetone followed by extraction of hot water for 3–4 h at 90–95 ^{o}C. Then, the brown-colored syrup was filtered through Whitman 3 mm filter paper, condensed to $1/4^{th}$ of the original volume, cooled, and precipitated at 4°c overnight with three volumes of ethanol. The precipitate was collected by centrifugation to obtain a dried brown crude polysaccharide (10% yields) (Ananthi et al., 2010).

All solvents used for the preparation of crude polysaccharides were of analytical grade (Sigma Chemicals Co.) and of high-performance

In vitro antioxidant activity:

Total polyphenolic compounds by Folin-Ciocalteu method:

The total polyphenolic compounds were determined according to the method of Singleton and Rossi (1965). This method depends on the use of folin ciocalteu reagent purchased from Sigma Chemicals Company. This method carried out by adding 0.1ml of the algal extract solution to 0.5 ml distilled water. Then add 0.2 ml of folin ciocalteu reagent and 1.25 ml of aqueous sodium carbonate solution. After that the tubes were vortexed and then, the blue-colored mixtures absorbance was recorded after 40 minutes at wavelength 725 nm against a blank tube containing only 0.5 ml of distilled water without extract. Finally, the concentration of total polyphenolic compounds was calculated as a gallic acid equivalent from gallic acid standard solutions (purchased from Sigma Chemicals Company) calibration curve covering the concentration range between 0.2 and 1.0 mg/ml.

Total antioxidant capacity:

The total antioxidant activity of Padina boergesenii algal extract was evaluated through the green phosphate/Mo^{5+} complex assay according to the method of Prieto et al., (1999). In a test tube, 0.1 ml of the

sample was added to 1 ml of the reagent solution (0.3 N sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). On the other side, Methanol (80%) was used instead of sample in the blank tube. All the tubes were stoppered and then incubated in a boiling water bath for 90 minutes. After that, the samples were left to cool to room temperature and then, the absorbance was measured at 695 nm against the blank. The antioxidant capacity was calculated as mg gallic acid equivalent per gram dry weight. All samples were analyzed in triplicate.

Total reducing power:

The total reducing power of the algal extract was estimated according to the method of Oyaizu (1986). In a test tube, 1ml of the *Padina boergesenii* algal extract (1mg/ml) was mixed with 1ml of 200 mM of sodium phosphate buffer with pH 6.6 and 1ml of potassium ferric cyanide 1%. After that, this mixture was incubated for 20 minutes at 50°C followed by addition of 1ml of trichloroacetic acid (10%w/v). Then, the mixture was exposed for centrifugation for 10 minutes at 2000 rpm. After that, 2.5ml of the upper layer solution was obtained and mixed with 2.5 ml of double deionized H_2O and 1ml of fresh ferric chloride 0.1%. The blank was prepared without adding algal extract. Finally, the absorbance was measured at the wavelength 700 nm. Ascorbic acid at different concentrations was used as standard. A higher absorbance of the reaction mixture at 700 nm is an indication of a higher total reducing power.

Free radical scavenging activity:
DPPH radical-scavenging activity:

The radical scavenging activity of 1, a 1-Diphenyl-2-picryl-hydrazyl (DPPH) was determined by using the method of Brand-Williams et al., (1995). The donation abilities of hydrogen atom or electron of the samples and some pure compounds were measured from a light-purple colored DPPH methanol solution. 1 ml of samples

dissolved in ethanol (10%) was added to a 1 ml of the DPPH radical solution in methanol (The final DPPH concentration, 0.2 mM). Then, the reaction mixture was thoroughly mixed by using a vortex and then incubated at 37°C in an incubator under dim light for about half an hour (30 minutes). After that, the absorbance of the obtained solution was estimated at a wavelength adjusted 517 nm. The inhibition percent (%) of the DPPH free radical was calculated.

ABTS radical scavenging assay:

This assay was carried out according to Arnao et al., (2001). The stock reagents composed of two reagents (ABTS reagent 7 mM and potassium persulfate reagent 2.4 mM). Then, equal amounts of the two reagents were mixed to prepare the working solution. Then, they were lifted to react for about 14 hr in a dark place at room temperature. Then, the obtained solution was diluted by adding 1 ml of ABTS reagent to 60 ml of methyl alcohol and mixed to obtain an absorbance of 0.706 ± 0.01 units at 734 nm wavelength by using a spectrophotometer. For each assay, the ABTS reagent was freshly prepared. After 7 minutes, 1 ml of extract sample was mixed with 1 ml of the ABTS reagent and lifted to react. Then, by using a spectrophotometer the absorbance was estimated at wavelength 734 nm. The ascorbic acid was used as standard.

Experimental animals:
Rats:

Fifty-four male adults Wistar albino rats (*Rattus norvegicus*) weighing about 150-250 g were obtained from the Animal House, Faculty of Medicine, Assiut University and housed in separate wire cages under complete isolated conditions in the animal facilities at the Faculty of Veterinary Medicine, Qena, South Valley University, Egypt for two weeks prior to the experiment for acclimation. Animals were kept on a 12h light/dark cycle with an ambient temperature of 22-24 °C and were

provided with a commercial pelleted feed and water *ad libitum*. Rats were monitored daily for the presence of any illness, and any cause of suffering before the experiment was avoided.

Mice:

Fifty-four adult male Swiss albino mice (*Mus musculus*) weighing about (25–30g) were obtained from the Animal House, Faculty of Medicine, Assiut University. Mice were fed *ad libitum* and housed in individual cages. The animals were housed in a room maintained at 22–24°C temperature and 50–55% humidity on a 12h light/dark cycle. The mice were left for two weeks prior to the experiment for acclimation. Mice were monitored daily for the presence of any illness, and any cause of suffering before the experiment was avoided. Experimental design and all animal handling procedures were approved by the Research Ethical Committee of the Faculty of Veterinary Medicine, South Valley University, Qena, Egypt (Approval No. 20147).

Drugs and chemicals:

All chemicals used in the estimation were of good quality and analytical grade. Carrageenan, xylene, and diclofenac Na were purchased from Sigma-Chemicals Company.

Anti-inflammatory activity of crude polysaccharides:

Xylene induced-ear inflammation:
    Experimental Design:

Fifty-four mice were used in this study. Mice were randomly allocated into nine equal groups each containing 6 mice as follows: **Group 1) control):** mice received physiological saline (10 mL/kg). **Group 2 (Xylene non-treated):** mice received physiological saline (10 mL/kg). **Group 3:** mice received diclofenac (10 mg /kg) and **Groups (4, 5, 6, 7, 8 and 9):** mice received polysaccharides extract (75, 125, 250, 500,

750 and 1000 mg /kg) consequently. All treatments were given intra-peritoneally. After 1hr, xylene (30 μL) was wiped to the inner and outer surfaces of the right ear of groups 2, 3, 4, 5, 6, 7, 8, and 9, while the left ear was considered as a control. Two hours after xylene application, Mice were anesthetized and both ears were removed. To evaluate the ear weight, 7-mm diameter ear punch biopsies were collected using a metal punch and individually weighed.

Assessment/ measurement:
    Ear weight:
To evaluate the ear weight, 7-mm diameter ear punch biopsies were collected using a metal punch and individually weighed by digital balance. The change in ear weight is caused by the irritant which is determined by subtracting the right (inflamed) ear weight from that of the left (non-inflamed) ear. The mean percentage edema inhibition (%) was calculated as follows:

% Edema degree=$M_{right} - M_{left}$

Where M_{right} is the weight of the right piece and M_{left} is the weight of the left piece.

$$\% \text{ Inhibition} = \frac{\text{Edema degree (control)} - \text{Edema degree (treated)}}{\text{Edema degree (control)}} X\ 100$$

Pro-inflammatory cytokine markers and free radicals' assay:

Ear tissue cytokine rates were tested using mice-specific enzyme-linked immune sorbent assay (ELISA) kits for free radicals: [NO and lipid peroxidation (MDA)], proinflammatory cytokine markers: [TNF-α (tumor necrotic factor), IL1β, IL6].

Carrageenan produced paw edema:
Experimental design:
Fifty-four rats were used in this study. Rats were randomly divided into nine equal groups each containing 6 rats **as follows:**
    Group 1 (control): rats received physiological saline (10 mL/kg). **Group 2 (carrageenan non-treated):** rats received physiological saline (10 mL/kg). **Group 3:**

rats received diclofenac (10 mg /kg). **Groups (4, 5, 6, 7, 8 and 9):** rats receive polysaccharide extract (75, 125, 250, 500, 750 and1000 mg /kg) consequently. All treatments were given intra-peritoneally. One hour after treatment, acute edema was induced by subcutaneous injection of carrageenan 0.1 ml (1%, w/v) suspension in sterile saline into the plantar surface of the right hind paw of groups 2, 3, 4, 5, 6, 7,8 and 9 and left paw used as standard. One hour after carrageenan application, Paw volume was measured by a digital caliper at given times (1, 3, and 6 hrs) post carrageenan injection. Rats were anesthetized and both paws were removed and kept in -80 ºC freezing till the determination of pro-inflammatory cytokines.

Assessment/ measurement:
Paw volume (thickness):
Upon carrageenan injection, the volume of the paw was determined by a digital caliper at certain periods (1, 3, and 6 h). The change in the volume of the paw was assessed as the difference between the volume of paw measured at each time point and the volume of basal measurements immediately before carrageenan is injected. The percentage of inhibition of paw edema for each group was calculated as follows:

$$\%\text{Inhibition of edema} = \frac{[(Vt-V0)\text{control} - (Vt-V0)\text{treated}]}{(Vt-V0)\text{control}} \times 100$$

Where:
V_t is the average volume for each group at the indicated t time, after carrageenan injection.
V_o is the average volume obtained for each group at t_0, before the beginning of the challenge.

Pro-inflammatory cytokine markers and free radicals' assay:
Paw tissue cytokine levels were tested using rat-specific enzyme-linked immune sorbent assay (ELISA) kits for free radicals: [NO, lipid peroxidation biomarker (MDA)],

proinflammatory cytokines [TNF-α (tumor necrotic factor), IL1β and IL6].

Statistical analysis:
All data are reported as mean ± standard error of mean. Statistical analysis was done using Pri8 for windows package. One-way analysis of variance (ANOVA) was performed followed by Tukey's post hoc multiple-comparison test. The p values were set at $p < 0.05$ and 0.001 levels to assess significant protection in treatment groups.

Results
Antioxidant activity *in vitro*:
In this study the watery crude polysaccharides of *Padina boergesenii* showed a total polyphenol content of 21.52 ± 0.01 (mg gallic acid/ gm extract), the total antioxidant capacity of 83.83 ± 0.20 (mg /gm), and total reducing power of 7.96 ± 0.07 (µg/mL). Regarding the free radical scavenging activity, DPPH radical-scavenging activity was 84.33 ± 0.88% and ABTS radical scavenging assay (mg/gm) was 66.63 ± 0.437.

Anti-inflammatory activity of crude polysaccharides:

Ear inflammation induction by Xylene application:
The anti-inflammatory activities of the crude polysaccharides extract of *Padina boergesenii* seaweeds were investigated using the xylene to induce ear inflammation in mice. The results of xylene-produced ear edema are shown in Fig. 1A. Xylene increased the edema by producing inflammation in group 2. Intra-peritoneal injection of the *Padina boergesenii* seaweeds crude polysaccharides extracts at doses of (75, 125, 250, 500, 750, and 1000 mg /kg) and 10 mg/kg diclofenac, 1h prior to xylene topical application, inhibited the development of ear edema (2-hour post xylene application) in a doses-dependent manner. The dose of 250, 500, 750, and

1000 mg/kg b. wt., significantly (p<0.001) reduced the weight of ear edema in mice with an inhibitory effect of 33.46, 56.53, 55.13, and 55.34 %, respectively, while the smaller doses 75 and 125mg/kg produced no significant effect. The inhibition induced by 500, 750, and 1000 mg/kg of the extract were comparable with that produced by 10 mg/kg of diclofenac (55.20%) Fig. 1B.

The effect of *Padina boergesenii* polysaccharides on free radicals' level in xylene produced edema in mice ear:

The effects of *Padina boergesenii* polysaccharides extract at 75, 125, 250, 500,750, and 1000 mg/kg doses and

diclofenac on free radicals: [MDA and NO] levels in xylene-produced edema in mice ear were illustrated in Fig. 2A, 2B. Xylene added to the right ear resulting in increased MDA and nitric oxide production. Meanwhile, one-hour pre-treatment with polysaccharide extract with (250, 500, 750, and 1000 mg/kg) doses respectively showed a significant reduction in MDA and nitric oxide (No) levels in ear tissue. The highest reduction was recorded with diclofenac (10 mg/kg) treatment. The reduction produced by 500, 750, and 1000 mg/kg doses of the extract was insignificantly different (p> 0.05) from that produced by 10 mg/kg of diclofenac.
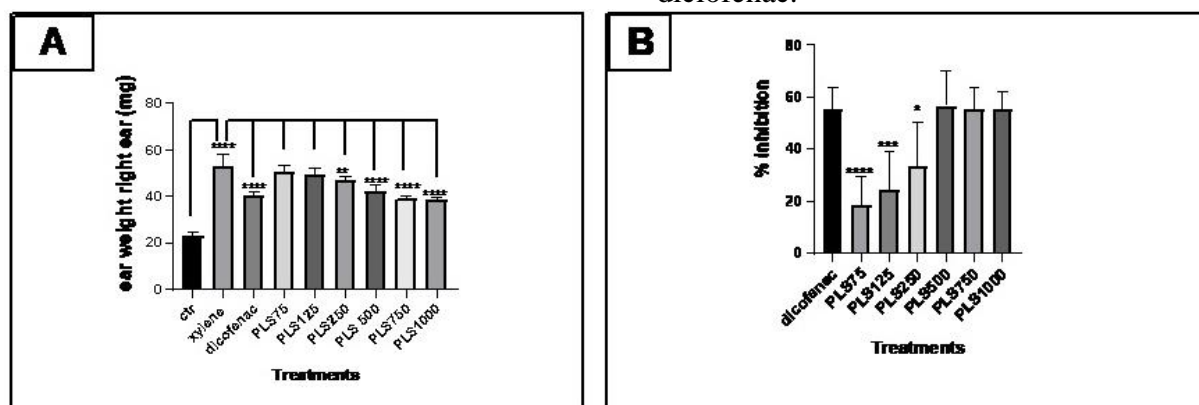

Fig. 1. Effect of *Padina boergesenii* polysaccharides extract at 250, 500,750, and 1000 mg/kg doses and diclofenac on (A) ear weight and (B) % inhibition edema in xylene-induced ear edema model in mice. The data are presented as mean ± SEM (n = 6) ****P < 0.0001 versus xylene non-treated (group G2) using one-way ANOVA followed by Tukey's post hoc multiple-comparison test
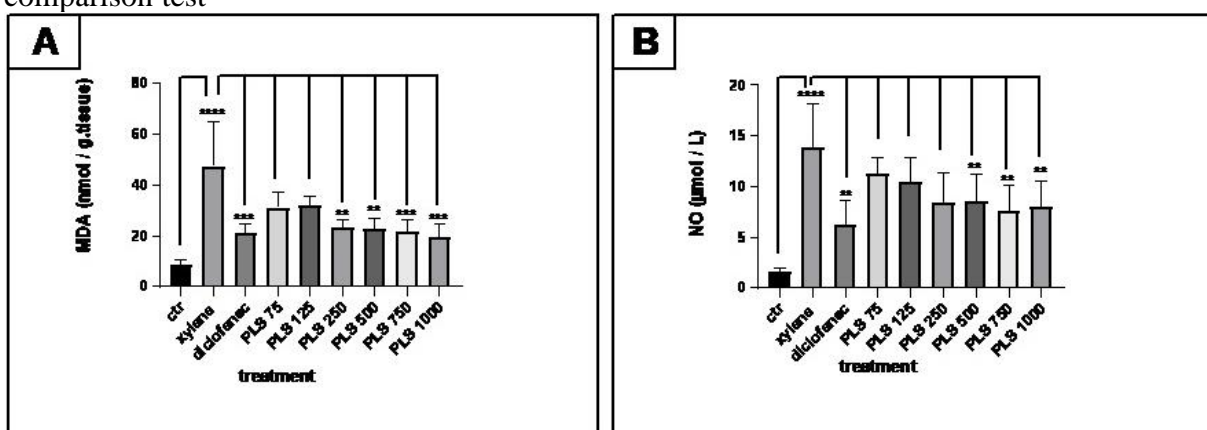

Fig. 2. Effect of *Padina boergesenii* polysaccharides extract at 75, 125, 250, 500,750 and 1000 mg/kg doses and diclofenac on (A): lipid peroxidase (MDA) and **(B):** nitric oxide (NO) in xylene-induced ear inflammation. Results are presented as mean ± SEM (n = 6). *P < 0.05, ***P < 0.001, ****P < 0.0001 versus xylene non-treated group (group G2) using one-way ANOVA followed by Tukey's post hoc multiple-comparison test.

The effect *Padina boergesenii* polysaccharides extract on pro-inflammatory cytokine markers: IL-β 1, IL-6 and TNF-α Release in xylene produced edema in mice ear:

The effect of *Padina boergesenii* polysaccharides extract at 75, 125, 250, 500,750 and 1000 mg/kg doses and diclofenac (10 mg/kg) on proinflammatory cytokine markers: IL-β 1, IL-6 and TNF-α Release in xylene induced edema in mice ear is shown in Fig. 3A, 3B and 3C. Xylene non-treated group showed significantly increased the levels of IL-β 1, IL-6, and

TNF-α in mice ear tissue (P<0.01). In contrast the *Padina boergesenii* polysaccharides extract pretreatment at 250, 500,750 and 1000 mg/kg doses and diclofenac (10 mg/kg) 1 hour prior to xylene treatment caused a significant reduction in IL-β 1, IL-6 and TNF-α level as compared to the xylene non-treated group (*p < 0.05, ** p < 0.01, ***p < 0.001). However, polysaccharides extract at 75 and 125 mg/kg doses did not significantly decrease the levels of IL-β 1, IL-6, and TNF-α (p > 0.05)
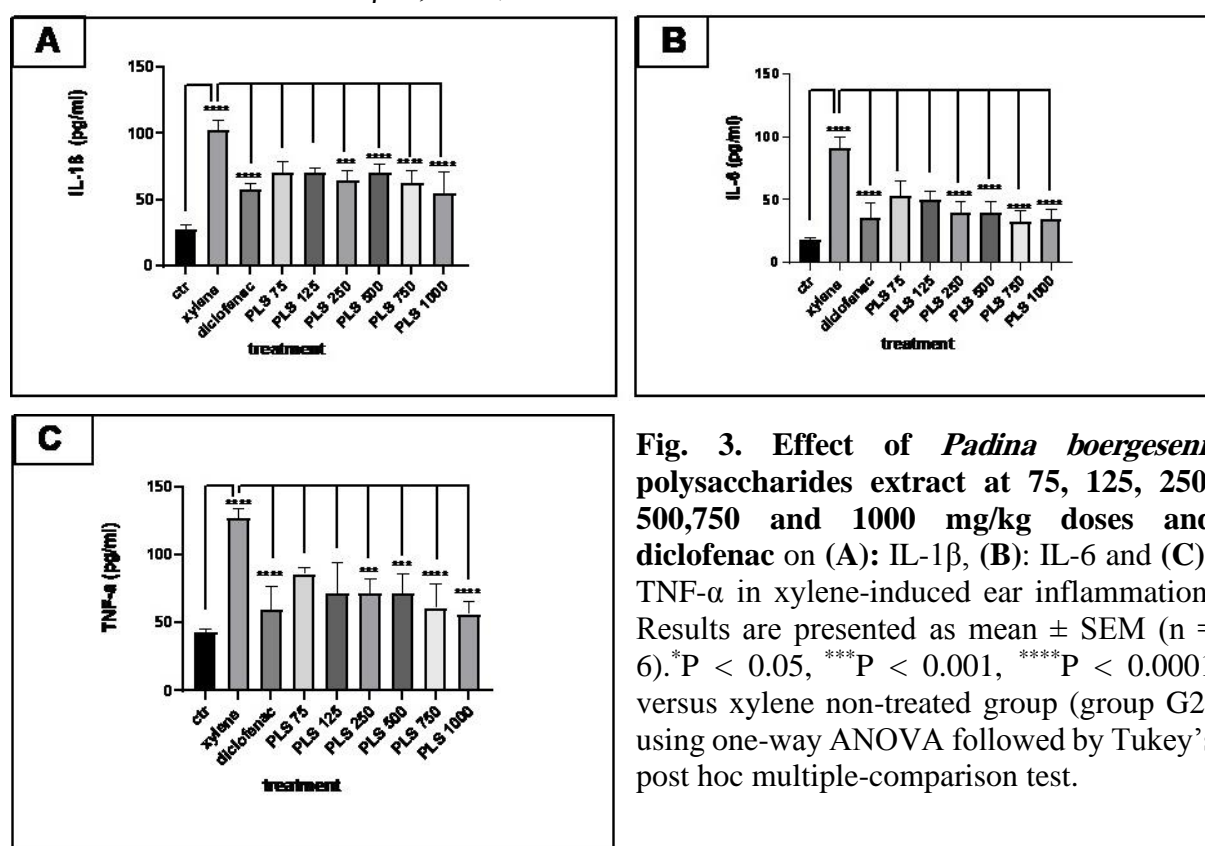
Fig. 3. Effect of *Padina boergesenii* polysaccharides extract at 75, 125, 250, 500,750 and 1000 mg/kg doses and diclofenac on (A): IL-1β, (B): IL-6 and (C): TNF-α in xylene-induced ear inflammation. Results are presented as mean ± SEM (n = 6).*P < 0.05, ***P < 0.001, ****P < 0.0001 versus xylene non-treated group (group G2) using one-way ANOVA followed by Tukey's post hoc multiple-comparison test.

Carrageenan produced paw edema:
Paw volume:

Anti-edematous activities of both *Padina boergesenii* polysaccharides extract at 75, 125, 250, 500,750 and 1000 mg/kg doses and diclofenac (at dose of 10 mg/kg) are presented in Fig. 4A, 4B and 4C. Injection of carrageenan 0.1 ml (1%, w/v) suspension in sterile saline s.c into the plantar surface of the right hind paw of of rats produced an increase in paw size (time-

dependent) which reach the peak at 6 h (6.21±0.14 mm mean change in paw size) post- carrageenan injection. The rat pretreatment with diclofenac (standard reference drug) resulted in a significant inhibition of edema formation (time-dependent) with a peak effect of 55.45% inhibition 6 h post- carrageenan injection. Similarly, intraperitoneal injection of 250, 500,750 and 1000 mg/kg doses and diclofenac (at dose of 10mg/kg) produced

suppression of inflammation (dose-related and time-dependent) in the late phase when compared with the carrageenan non-treated group (group2) with peak effects (33.03, 46.90, 55.16, and 57.52 % inhibition) respectively, 6 h post- carrageenan treatment Table 1.

Table 1. The Inhibition rate % following administration of *Padina boergesenii* polysaccharides extract at 75, 125, 250, 500,750 and 1000 mg/kg doses and diclofenac on carrageenan-produced paw edema volume in rats. Results are presented as mean ± SEM (n = 6).

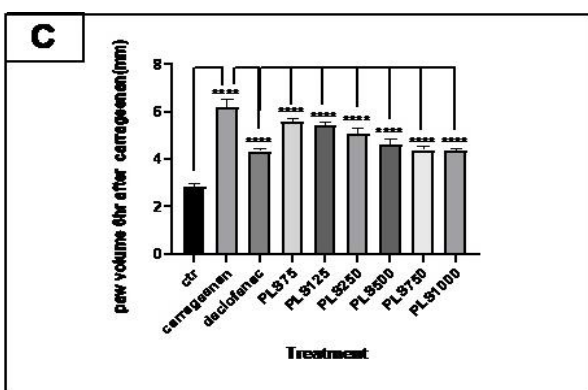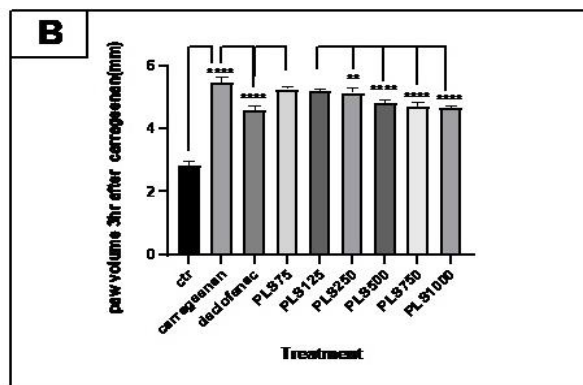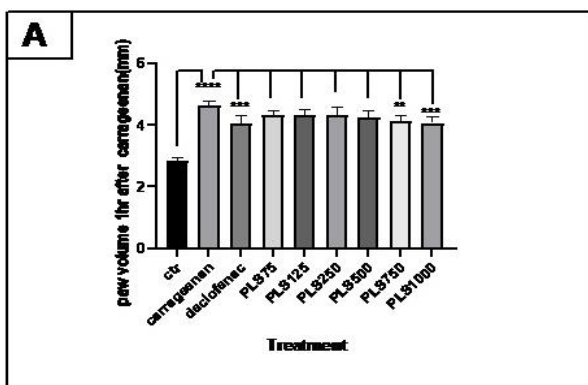| Group	Inhibition rate %		
	1hr	3hr	6hr
G3 (Diclofenac)	55.46%	27.80%	29.38%
G4 (PLS 75)	18.88%	0.42%	15.25%
G5 (PLS 125)	23.60%	2.08%	15.25%
G6 (PLS 250)	33.04%	4.15%	14.12%
G7 (PLS 500)	46.90%	17.01%	18.64%
G8 (PLS 750)	55.16%	23.23%	28.81%
G9 (PLS 1000)	57.52%	37.28%	14.68%

Fig. 4. Effect of *Padina boergesenii* polysaccharides extract at 75, 125, 250, 500,750, and 1000 mg/kg doses and diclofenac on paw volume (mm) (A): 1h, (B): 3 hrs. and (C): 6 hrs. post-carrageenan injection. Results are presented as mean ± SEM (n = 6). $^{*}P < 0.05$, $^{***}P < 0.001$, $^{****}P < 0.0001$ versus carrageenan non-treated group (group G2) using one-way ANOVA followed by Tukey's post hoc multiple-comparison test.

The effect *Padina boergesenii* polysaccharides treatment on free radicals (MDA and NO) level in carrageenan-produced paw edema in rats:

The effects of *Padina boergesenii* polysaccharides extract at 75, 125, 250, 500,750, and 1000 mg/kg doses and peroxidation (MDA) and NO levels in carrageenan induced paw edema in rats are illustrated in Fig. 5A, 5B. carrageenan 1% was injected subcutaneously into the plantar surface of the right hind paw resulting in a significant increase, ($P < 0.0001$) in MDA and nitrous oxide levels as compared to

saline-treated control (G1). Meanwhile, one-hour intraperitoneal pre-treatment with *Padina boergesenii* polysaccharide extract with (250, 500, 750, and 1000 mg/kg) doses respectively showed a significant reduction in the production of MDA and nitrous oxide (NO) level in paw edema tissue as

compared to carrageenan non- treated group (G2). The highest reduction was recorded with diclofenac (10 mg/kg) treatment. The reduction produced by 500, 750, and 1000 mg/kg doses of the extract was insignificantly different ($p > 0.05$) from that produced by 10 mg/kg of diclofenac.
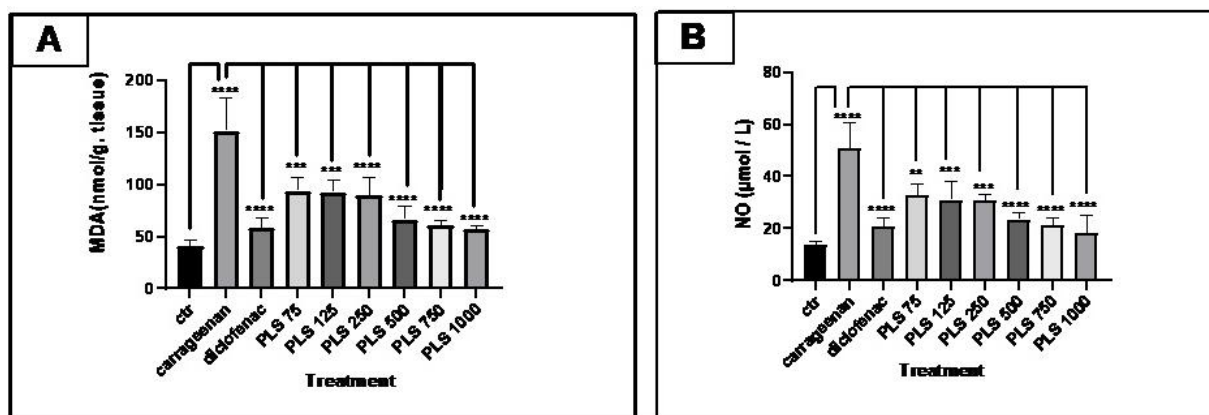

Fig. 5. Effect of *Padina boergesenii* **polysaccharides extract at 75, 125, 250, 500,750 and 1000 mg/kg doses and diclofenac** on **(A):** lipid peroxidase (MDA) and **(B):** nitric oxide (NO) in carrageenan-induced paw edema. Results are presented as mean ± SEM (n = 6). $^*P < 0.05$, $^{***}P < 0.001$, $^{****}P < 0.0001$ versus carrageenan non-treated group (group G2) using one-way ANOVA followed by Tukey's post hoc multiple-comparison test.

The effect *Padina boergesenii* **polysaccharides extract on pro-inflammatory cytokine markers: IL-β 1, IL-6 and TNF-α Release in carrageenan-produced paw edema in rats:**

The effects of *Padina boergesenii* polysaccharides extract at 75, 125, 250, 500,750, and 1000 mg/kg doses and diclofenac (10 mg/kg) on pro-inflammatory cytokine markers: IL-β 1, IL-6 and TNF-α Release in paw induced edema in rats are shown in Fig. 6A, 6B and 6C. Carrageenan non-treated group significantly increased the levels of IL-β 1, IL-6, and TNF-α in rat paw edema tissue (P<0.0001). In contrast the *Padina boergesenii* polysaccharides extract pretreatment at 250, 500,750 and 1000 mg/kg doses and diclofenac (10 mg/kg) 1 hour prior to carrageenan treatment provoked a significant reduction in IL-β 1, IL-6 and TNF-α level as

compared to the carrageenan non-treated (group2) (*p < 0.05, ** p < 0.01, ***p < 0.001). However, polysaccharides extract at 75 and 125 mg/kg doses did not significantly decrease the levels of IL-β 1, IL-6, and TNF-α (p > 0.05).

Discussion

Inflammation is considered the first important step in fighting against infection, healing wounds, and it is considered as a defense tool aimed to protect tissue, and thus, during this reaction, different dynamic pathological changes occurred. Many adverse side effects are commonly associated with the clinical use of non-steroidal anti-inflammatory drugs (NSAIDs) and steroidal; therefore, it is an urgent need to develop new drugs with lesser or no side effects. In this context, the main aim has been directed towards the use of natural products (Sengar *et al.*, 2015).

Marine organisms are considered an invaluable source of bioactive compounds since they have large chemical and biological variations (Simmons et. al., 2005). Among the studied marine organisms, brown seaweeds have many compounds with a broad spectrum of bioactivities, including anti-inflammatory, antioxidant, anti-microbial, and neuroprotective effects (Sanjeewa et al., 2016). Numerous anti-inflammatory polysaccharides have been obtained from marine brown algae and their role as anti-

inflammatory agents has been well documented by Wijesinghe and Jeon (2012). Recent *in vitro* and *in vivo* studies have shown that polysaccharides isolated from brown seaweeds have excellent therapeutic activity against inflammatory reactions (Wijesekara et al., 2011) Thus, compounds obtained from marine algae are recently being studied since they are known to have anti-inflammatory benefits (D'Orazio et al., 2012 and Fernando et al., 2016).
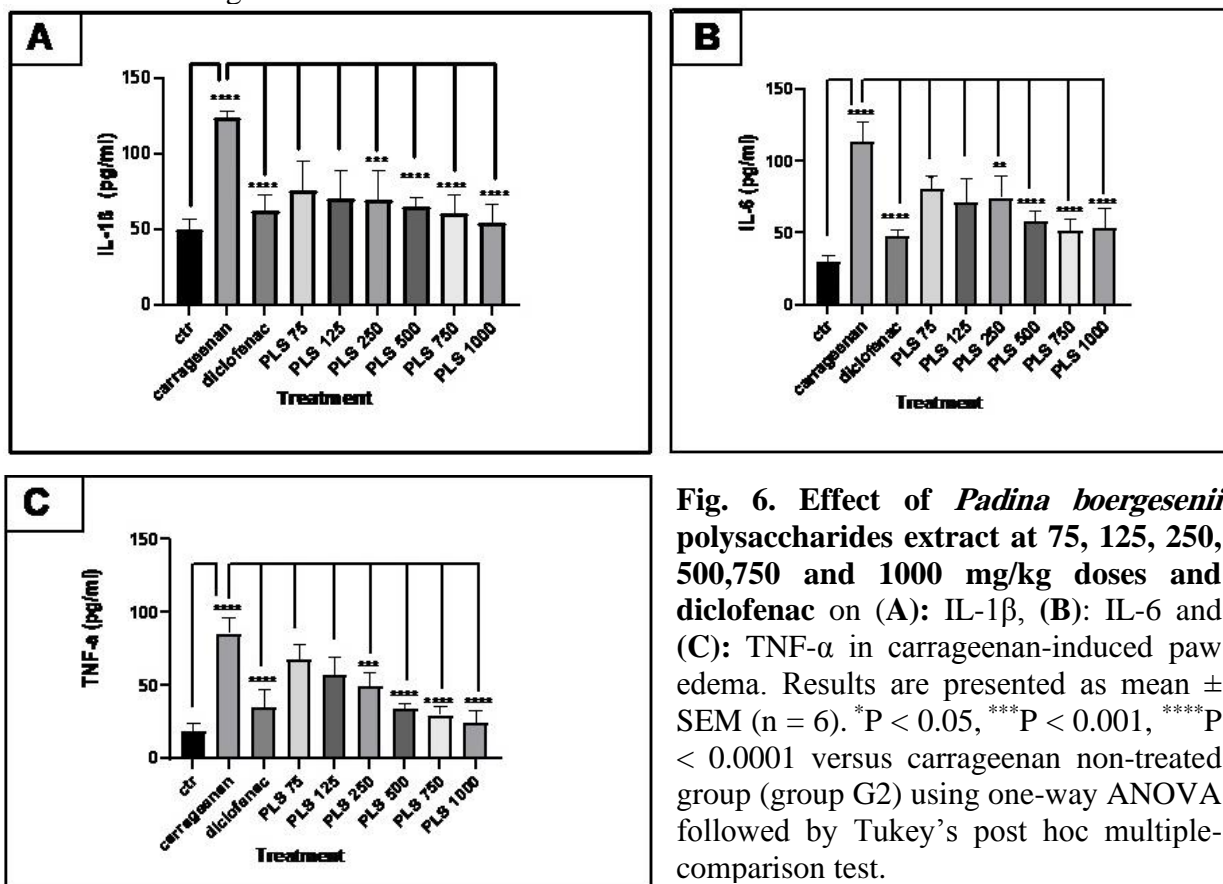

Fig. 6. Effect of *Padina boergesenii* polysaccharides extract at 75, 125, 250, 500,750 and 1000 mg/kg doses and diclofenac on (**A**): IL-1β, (**B**): IL-6 and (**C**): TNF-α in carrageenan-induced paw edema. Results are presented as mean ± SEM (n = 6). $^{*}P < 0.05$, $^{***}P < 0.001$, $^{****}P < 0.0001$ versus carrageenan non-treated group (group G2) using one-way ANOVA followed by Tukey's post hoc multiple-comparison test.

The seaweeds antioxidant activity of sulfated polysaccharides is well-studied (Barahona et al., 2011). They are known to have various antioxidant activities such as free radicals scavenging like superoxide, hydroxyl, and DPPH, lipid peroxide inhibition, and ferric reducing antioxidant activity (Ngo, et al., 2011). In the current study, the antioxidant activity of crude polysaccharides extracted from *Padina*

boergesenii was evaluated and the obtained results exhibited antioxidant effects in a concentration-dependent manner.

In this work, the obtained findings provoked that *Padina boergesenii* crude polysaccharides extract has considerable antioxidant activity. This conclusion is in consistence with Zhang et al., (2010) who reported that sulfated polysaccharides extracted from the brown algae have

significant antioxidant properties. The obtained results in this article indicated that the total antioxidant and the free radical scavenging activities both (DPPH and ABTS radical-scavenging) may be owing to the antioxidant activity of the crude polysaccharides. Moreover, the obtained total polyphenolic content in this current study may also share an important role in this activity and this is further confirmed by Abdelhamid et al., (2018) who stated that there was a significant correlation between the antioxidant activity and phenolic compounds content and this finding also was in consistence with the previous studies of Matanjun et al., (2008). Recently, this suggestion is further confirmed by Generalić et al., (2019) who stated that brown algae have a significant level of phenolic compound, a complex type of polysaccharide, with extremely high biological activity, and more effective antioxidant activity as compared to green and red algae. Moreover, polyphenol-associated polysaccharides are known to play a significant role in the antioxidant activity determined by using the DPPH assay (Nemzer et al., 2019).

Antioxidants are known to possess anti-inflammatory characters therefore; the anti-inflammatory activity of *Padina boergesenii* polysaccharides extract was investigated. The anti-inflammatory models used in this study included both xylene-induced ear edema in mice and carrageenan-induced paw edema in rats. The anti-inflammatory activity is determined by analyzing the reduction in edema size and calculating the inhibition rate % of edema. A mean reduction in edema size, when compared with control group and increase inhibition % in the treated groups, is considered an indication of anti-inflammatory activity. Xylene-induced ear edema model is partially characterized by the release of substance P which is widely distributed and acts as a neurotransmitter or neuromodulator in a variety of physiological processes in both central and peripheral nervous systems, and it (Jiang et al., 2005). The substance P released from the sensory neurons results in vasodilatation and extravasations of plasma suggesting its important role in neurogenic inflammation. Thus, it causes g of the ear mice the swelling. Histopathologically, severe vasodilatation, cutaneous edematous changes, and inflammatory cells infiltration are recorded as signs of acute inflammation post topical application of xylene (Kou et al., 2003). Acute inflammation induction by xylene application is thus frequently used to investigate the drug anti-inflammatory effects in mice, as this model is thus quite sensitive to both steroidal and non-steroidal anti-inflammatory drugs (Man et al., 2013 and Li et al., 2014).

Intraperitoneal injection of the *Padina boergesenii* seaweeds crude polysaccharides extract at doses of (75, 125, 250, 500, 750, and 1000 mg /kg) and diclofenac (10 mg/kg), 1h prior to xylene topical application, inhibited the development of ear edema (2-hour post xylene application) in a dose-dependently manner. The higher doses 500, 750, and 1000 mg/kg b. wt. significantly reduced the weight of xylene-produced ear edema in mice with an inhibitory effect of 56.53, 55.13, and 55.34 %, respectively. The inhibition produced by 500, 750, and 1000 mg/kg of the extract were comparable with that produced by diclofenac at a dose of 10 mg/kg of (55.20%).

Xylene-produced mouse ear edema is considered an acute inflammatory model during inflammation or injury. The main inflammatory mediators released in this model are histamine, serotonin, and bradykinin. The production of histamine is an inflammatory response and is also considered an immune response, and it can participate vasodilatation and increase permeability (Benly, 2015). During

inflammatory processes, the iNOS and COX-2 gens in macrophages are expressed with NO and PGE2 production. Additionally, NO and PGE2 produced during inflammation can be cytotoxic to the host cells, and the prolonged production of NO and PGE2 in inflammatory cells can result in many of inflammatory diseases or even cancers (Attur *et al.*, 2000 and Posadas *et al.*, 2000). Moreover, recent studies have noted that the exposure of macrophages to inflammatory stimulants can lead to significant up-regulation of pro-inflammatory cytokines such as IL-1β and TNF-α (Heo *et al.*, 2010). The cytokines (IL-1β, IL-6, TNF- α, NO, etc.) control multiple signaling pathways (Chou *et al.*, 2003 and Lee *et al.*, 2006). Finally, the excessive productions of pro-inflammatory cytokines by activated macrophages as well as inflammatory mediators play an important role in the pathogenesis of inflammatory conditions such as rheumatoid arthritis, Alzheimer's disease, and Parkinson's disease (Tews *et al.*, 1996)). Therefore, the effective strategy to treat such diseases is to reduce the levels of these mediators (Jung *et al.*, 2009).

In this study xylene treatment significantly increased the levels of IL-β 1, IL-6, and TNF-α as well as NO and MDA free radicals' levels in mice ear tissue. In contrast, the *Padina boergesenii* polysaccharides extract pretreatment at 250, 500,750, and 1000 mg/kg doses and diclofenac (at 10 mg/kg) 1-hour prior to xylene application caused a significant reduction in IL-β 1, IL-6, and TNF-α- as well as NO and MDA proinflammatory cytokines level in a dose-dependent manner as compared to the xylene-non-treated group. In the glow of these given results, the anti-inflammatory effects of administered *Padina boergesenii* polysaccharides extract against xylene produced ear tissue edema in mice to correlate to its inhibitory activities of proinflammatory cytokines. This suggestion was confirmed by many previous studies that concluded that brown algae polysaccharides modulate many pro-inflammatory mechanisms and mediators including regulation of gene expression of pro-and anti-inflammatory cytokines related to ulcerative colitis (Jiao *et al.,* 2011; Ngo, *et al.*, 2013; Lean *et al.*, 2015; Fernando *et al.*, 2017 and Pereira 2018).

Carrageenan-produced paw edema is considered a suitable laboratory experimental animal model used for investigating the anti-inflammatory effect of natural substances. The edema occurs in different phases and several mediators have roles in each phase. The first phase (1.5 h) is mediated by the release of histamine and serotonin, while, the second phase (from 1.5 to 2.5 h) is mediated by bradykinin release and the third phase may be mediated by prostaglandin release and oxygen-derived free radicals and cyclooxygenase; where polymorphonuclear (PMN) leukocyte infiltration and a significant increase in the level of neutrophils were reported through observation of a large number of inflammatory cells in the paw tissues (from 2.5 to 6 h) after carrageenan injection (Di Rosa, 1972; Zhou et al., 2008; Reanmongkol et al., 2009; Ashok et al., 2010 and Zhang et al., 2013).

In the current study, the *Padina boergesenii* polysaccharides extract significantly inhibited the development of paw edema rat hind paw at both first and second phases (in a dose-dependent manner), suggesting that the possible mechanism of action of *Padina boergesenii* polysaccharides extract may involve suppression of bradykinin release and biosynthesis of prostaglandin.

Carrageenan non-treated group showed significantly increased IL-β 1, IL-6, and TNF-α as well as NO and MDA levels in rat paw edema tissue. In contrast, the *Padina boergesenii* polysaccharides extract pretreatment at 250, 500,750 and 1000

mg/kg doses and diclofenac (at 10 mg/kg) 1 hour prior to carrageenan treatment caused a significant reduction in IL-β 1, IL-6, and TNF-α as well as NO and MDA levels as compared to the carrageenan-non-treated group. The *Padina boergesenii* polysaccharides extract showed an anti-inflammatory effect in both the carrageenan-produced rat paw swelling and xylene-produced ear edema models. The findings obtained from carrageenan-produced paw edema which is considered a suitable animal model for assessing acute anti-inflammatory activity also confirmed the anti-edematous effect of *Padina boergesenii* polysaccharides extract. Carrageenan-produced inflammation includes three phases: early phase (1 h post carrageenan injection) involves histamine, serotonin, nitric oxide, and bradykinin release, the second phase (at 2 h) mediated by kinins, leukotrienes, platelet-activating factor, and possibly cyclooxygenase production; and a third phase (3–24 h; which considered a late phase) characterized primarily by the formation of pro-inflammatory prostanoids and nitric oxide (synthesized by the inducible nitric oxide synthase isoform), cytokines, neutrophil infiltration, and neutrophil-derived free radicals production, such as superoxide, hydrogen peroxide and OH radicals (Bilici et al., 2002; Di Rosa et al., 1971; Handy and Moore, 1998 and Salvemini et al., 1996). In this study, *Padina boergesenii* polysaccharides extract revealed significant suppression of edema development in both the middle and more pronouncedly third phase of carrageenan-produced inflammation. This suggests that the extract might acts by inhibiting the kinins, pro-inflammatory prostanoids, and inducible nitric oxide synthase isoform release and/or actions (Salvemini et al., 1996 and Bilici et al., 2002). The third phase inhibitory effect of *Padina boergesenii* polysaccharides extract

depends on suppressing prostanoids synthesis in hind paw probably by an effect on COX-2 activity, which is considered the predominant isoform at this stage (Seibert et al., 1994 and Vane, 1998). The possibility that *Padina boergesenii* polysaccharides extract may, additionally, decrease hind paw constitutive COX-1 activity to produce an anti-edema effect in the early phase is also suggested. However, further studies will be needed to determine the cyclooxygenase inhibitory activity of the extract. The effectiveness of the *Padina boergesenii* polysaccharides extract in these two used models suggests that they induced their anti-inflammatory effect by either inhibiting the synthesis, release, or action of inflammatory autacoids (Adeyemi et al., 2008). The anti-inflammatory effect of the *Padina boergesenii* polysaccharides extract was comparatively comparable with those produced by diclofenac used in xylene and carrageenan-produced edema, respectively. The preliminary phytochemical screening in this current study revealed the presence of a considerable number of polyphenols. The presence of this fraction in the extract has been reported to possess antioxidant as well as anti-inflammatory activities. Thus, the presence of this phytochemical in the extract may suggest that this active principle may be responsible for the potential anti-inflammatory activity of the tested extract. This suggest is confirmed by Ambriz-Pérez et al., (2016) who stated that phenolic compounds can inhibit either the production or the action of pro-inflammatory mediators, resulting in anti-inflammatory activity. Research attention has been directed toward phenolic compounds because they are being one of the principal components responsible for their anti-inflammatory activity. Phenolic compounds work in thy as e same manner as NSAIDs do, additionally, some of them inhibit other pro-inflammatory mediators besides COX by inhibiting their activities or

gene expression. Additionally, some phenolic compounds can up/down-regulate transcriptional factors, like nuclear factor-kB (NF-kB) or Nrf-2, in inflammatory and antioxidant pathways (Maroon et al., 2010 and Sergent et al., 2010).

Conclusion

In conclusion, the present study results indicated that the *Padina boergesenii* polysaccharides extract possessed anti-inflammatory, anti-edema and antioxidant effects in comparison to the patent anti-inflammatory drug diclofenac. suggesting this natural compound could be a promising effective remedy for inflammatory conditions. Further experiments are needed to explore its detailed mechanism of action.

Conflict of interest statement

The authors declare that there is no potential conflict of interest.

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