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IMMUNOMODULATORY AND ANTI-INFLAMMATORY ACTIVITIES OF ALGERIAN *ULVA LACTUCA*

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ABSTRACT

Background: Secondary metabolites such as polysaccharides and lectins from seaweeds were characterized by a broad spectrum of immunomodulatory and anti-inflammatory effect. There are few studies in the literature about the bioactivities of Algerian *Ulva lactuca* marine algae extracts; that remain largely unexploited, thus this study aimed to examine whether polysaccharide or lectine fraction from Algerian *U. lactuca* are believed to promote anti-inflammatory and immunomodulatory properties. **Methods**: the polysaccharide or lectine fraction extracted from Algerian *U. lactuca* were evaluated *in vivo* by testing the carrageenan induced acute inflammatory in *Wistar* rats and the *in vivo* immunomodulatory effect. The *U. lactuca* metabolites were also examined for their capacity to inhibit the synthesis of TNF-α, and the release of NO in mouse macrophages RAW 264.7 stimulated with LPS. **Results**: The results showed that the both *U. lactuca* polysachrides (ULPF) and lectines (ULLF) phases at 100 mg/kg proved a significant anti-edema effect. The ULPF phase was significantly more effective in decreasing MPO activity and MDA level in rat paw than ULLF phase. Both ULPF and ULLF phases enhanced the *in vivo* macrophage phagocytic activity, this effect is more pronounced in the ULLF-group than that in ULPF-group. At 60 µg/mL both ULPF or ULLF fraction from *U. lactuca* were weakly cytotoxic on RAW cells. Both ULPF or ULLF

fraction from *U. lactuca* exerted a protection from LPS-mediated inflammation in RAW264 and revealed a significant dose-dependently down regulating of macrophage production of NO and for TNF-α release, these effects were more pronounced in the case of ULPF fraction. **Conclusion**: The results of this study indicate that Algerian *U. lactuca* may be non-toxic; may have immunomodulatory value, especially ULLF fraction and anti-inflammatory effect, especially ULPF fraction extract, thus the Algerian *U. lactuca* properties could be due to the synergistic effect of its constituents, including the lectins and polysaccharides.

KEYWORDS: *Ulva Lactuca*, Anti-Inflammatory, Immunomodulatory, Polysaccharides, Lectines.

INTRODUCTION

Inflammation is a primordial response that protects against infection and happen in response to processes such as tissue injury.[1,2] Immune system dysfunction is responsible for pathophysiology of many diseases.[3,4] Phagocytic cells, such as macrophages and neutrophils, play a key role in innate immunity because of their ability to recognize, ingest, and destroy many pathogens.[5] During the inflammatory reaction, macrophages produce inflammatory mediators, such as nitric oxide (NO), tumor necrosis factor-α (TNF-α).[6] Attenuation of the inflammatory response is a beneficial strategy to combat several human diseases, a multiple mechanisms have been suggested for the anti-inflammatory activities.[7] The modulating macrophage-mediated inflammatory responses is important for creating a new therapeutic approach against inflammatory diseases.[8] Modulation of the immune system is emerging as a major area in pharmacology. Marine green algae are valuable sources of structurally diverse bioactive compounds and remain largely unexploited in pharmaceutical area products indeed, many recent investigations have proven the anti-inflammatory effect of seaweeds.[9] Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites such as polysaccharides and lectins that were characterized by a broad spectrum of biological activities and relatively low toxicity.[10,11] Studies on the bioactivities of marine algae have revealed numerous health promoting effects, including anti-oxidative, anti-inflammatory, antimicrobial, and anti-cancer effects.[12] Polysaccharide is a natural macromolecular compound with complex, important and multifaceted biological activities they possess important biological activities such as anti-tumor, anti-oxidant, anti-inflammatory, and immunomodulation.[13,14] Green algal polysaccharides have emerged as rich and important

sources of bioactive natural compounds with a wide range of physiological and biological activities including immunomodulation, anti-inflammation.[15] Advances in therapeutic glycopeptides are also an emerging area in biosciences which is all related to lectins, lectins belong to a class of carbohydrate binding proteins of immense importance as attested to by the voluminous literature.[16] These molecules have an additional benefit; they are molecules with low molecular weight and may be less antigenic when used in biological models.[17,18] Lectins manifest a wide array of properties such antitumor, anti-inflammatory and immunomodulatory activities.[19,20] The biological function of carbohydrates in inflammation events is well-defined; have been identified in various marine algae, including red, green, and brown algae species., but the variability of these chemical composition is basically a resulted from numerous factors including the geographical origin, physiological maturity, season and environmental variations.[21,22] Although not many members of the genus *Ulva* have been extensively investigated, there are some interesting aspects of biological activities of the Algerian *Ulva* extracts and its isolated metabolites. Seaweeds are exposed to seasonal variations of abiotic factors that may influence their metabolic responses.[23] The chemical composition of these macro algae was found to vary depending on geographical distribution and the principal environmental factors affecting the composition.[24,25,26,27] There are few studies in the literature about the bioactivities of Algerian *Ulva* marine algae extracts; Algerian marine green algae remain largely unexploited, there are still some challenges in this field. In this context, Algerian *Ulva* species would be of great interest because of their possible functions and applications in biotechnological studies; essentially nothing is known regarding potential immunomodulatory and the possible anti-inflammatory properties of polysaccharides and lectines. The present study was envisioned to study the anti-inflammatory of *U. lactuca* collected from the coast of Jijel, location in the east of Algeria. In this work we aimed to examine whether polysaccharides or lectines fraction extracted from *U. lactuca* are able to promote *in vivo* anti-inflammatory properties, and to examine if these metabolites could be an effective remedy against carrageenan induced inflammatory in *Wistar* rats. This study was also designed to evaluate the *in vivo* immunomodulatory effect of these metabolites and their capacity to inhibit the synthesis TNF-α, NO and mouse macrophages RAW 264.7 stimulated with LPS.

MATERIALS AND METHODS

Drugs and chemicals: Thiobarbituric acid (TBA), sulfanilamide, N-(1-naphthyl) ethylenediaminedihydrochloride (Griess reagent), Diclofenac-sodium, *O*dianisi-

dinedihydrochloride, Carrageenan λ, sodium nitrite ($NaNO_2$), Hexadecyltrimethylammonium bromide (HTAB), High glucose Dulbecco's Modified Eagle's Medium (DMEM), trypsin. sodium bicarbonate, fetal bovin serum (FBS). 3-(4, 5 dimethythiazol2-yl)-2, 5-diphenytetrazoleum (MTT),LPS from *Escherichia coli* K-235 (Sigma Chemical Co., St. Louis, MO), Dimethylsulfoxide (DMSO), rat anti-mouse monoclonal TNF-α antibody (0.5 mg, BD Pharmingen) avidin peroxidase (Sigma) and all other chemicals were of analytical grade were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Sample collection: *U. lactuca* was collected from Jijel coasts region (east of Algeria). The collection *U. lactuca* was performed during the period of April-June 2014, when algal biomass remains dominant, the collected samples were washed with sea water to remove extraneous matter such as epiphytes and contamination from other algae, then they were harvested manually and washed thoroughly in running water. Cleaned algal materials were shade dried under an air jet to prevent photolysis and thermal degradation. The completely dried material was weighed and ground coarsely in a mechanical grinder. Then sample was again washed in lab with freshwater and shade air-dried samples were again washed thoroughly with distilled water, air dried in shade. Then cut into small pieces and ground in a tissue grinder until reach fine powder then grounded into a fine powder using a blender

Lectins extraction: Lectins extraction was followed using the method of Shiomi *et al*.[28], the dry powder was incubated in 500 ml of phosphate buffer (0.75 M, pH 7.4) for approximately 16 hr at 4 °C. The mixture was then centrifuged at 1200 rpm for 15 min, the proteins of the supernatant were precipitated using 17 g of ammonium sulfate $(NH_4)_2SO_4$, the mixture was left for approximately 4 hr, then was centrifuged at 1200 rpm for 45 min, the obtained pellet was kept at 4 °C and the supernatant was mixed with 35 g ammonium sulfate $(NH_4)_2SO_4$ and left for 4 hr, then, that mixture was centrifuged at 1200 rpm for 45 min, the obtained pellet was kept at 4 °C then combined with the one obtained previously and suspended in 200 ml of phosphate buffer (0.75 M, pH 7.4); the remaining debris was removed by passing the suspension through filter paper, the suspension was then applied to a Seghadex G-200 column (50 x 2.5 cm) equilibrated with phosphate buffer (0.1 M, PH 7.2). The absorbance at 280 nm was used to estimate protein content in column eluates, the fractions contained lectins were then combined and dialyzed against distilled water. The *U. Lactuca* lectins fraction (ULLF) was designed for the *in vivo* and *in vitro* studies.

Polysaccharides extraction: 100 g air shade dried *U. lactuca* was cut and soaked with 1 L of 95% ethanol (v/v) for 12 h for three times to remove lipids and colored ingredients, including free sugars, amino acids and some phenols. The residue was dried in the air and extracted in 1 L of distilled water at 100 °C for 3 hr. The slurry was separated and filtered. The extraction was repeated three times and the filter was combined, then centrifuged at 4500 rpm for 15 min. The supernatant was decanted and concentrated to about 200 mL. 95% ethanol (v/v) was added slowly to final alcohol concentration of 80%. The mixture was allowed to stand for overnight at room temperature. The precipitate was collected and washed twice with absolute ethanol and the solution was kept at 4 °C overnight. The crude polysaccharide was obtained by filtering the solution through a nylon membrane (pore size, 0.45 mm). The *U. Lactuca* polysaccharides (ULPF) was designed for *the in vivo a*nd *in vitro* studies.[29]

***In vivo* anti-inflammatory assay**

Acute inflammation model: carrageenan-induced paw oedema

The female *Wistar* rats (200-220 g), obtained from animal house of the of the animal biology department, were used in this experiment. The animals were housed under standard laboratory conditions (temperature 20-23 °C), relative humidity, dark/light cycle, and free access to food and water. The experimental protocol was approved by the Institutional Animal Ethical Committee, in accordance with the currently established principles for the care and use of research animals National Institutes of Health (NIH) guidelines.

To assess anti-inflammatory activity, animals were divided into the following groups (n = 5).

-Group A: was given 0.9% NaCl as control (10 mL/kg).

-Group B: treated by diclofenac sodium (10 mg/kg) as standard anti-inflammatory suspended in distilled water.

-Group C: treated by ULLF (100 mg/kg).

-Group D: treated by ULPF (100 mg/kg).

Carrageenan induced acute inflammatory model according to Yesilada and K¨upeli[30] mm was used to assess the anti-inflammatory potential of. Briefly, before treatment, rats were deprived of food and water for 16 h, Groups A to D were administered with their respective treatments daily diclofenac sodium, ULLF and ULLP for 3 days. On the third day 30 min after treatment regimen, 100 µL of freshly prepared carrageenan solution (1% prepared in 0.9% NaCl) was injected into the right hind paw of each rat, paw thickness was measured before and after injection of oedematogenic agent at 0,1,3, and 6 h using a digital vernier

caliper. The progression of edema (mm, at 1, 3, and 6 hr) was evaluated by measuring the pawoedema by using graduated micrometer combined with a constant loaded lever system. The edema was expressed as the increase in ear thickness (mm) due to the inflammatory challenge.[30] The percentage inhibition of the inflammation was calculated from the formula: % inhibition = D0-Dt/D0 x 100, where D0 average inflammation (hind paw oedema) of the control group of rats at a given time Dt average inflammation of the drug -treated rats (extract or diclofenac-sodium reference) at the same time.

Malondialdehyde (MDA) level determination in paw tissue

The levels of MDA in rat paw tissues were measured using the method of TBA. Briefly, the paw tissues were promptly excised and rinsed with cold saline, and were homogenized in 10% 0.1 M potassium phosphate buffer (pH 7.4). The homogenate was then centrifuged (5000 x g, 10 min) to obtain supernatant. 400 µL of 1.2% TBA was added, and the mixture was placed in water bath (95-100°C) for 30 min. The solution was then cooled; the absorbance was measured spectrophotometrically at 532 nm. The MDA level was expressed as nmol/mg tissue.[31]

Myeloperoxydase (MPO) paw level determination

MPO activity was measured according to the modified method of Bradley *et al*.[32] fragments of paw tissue were homogenized with 5 mL of HTAB (0.005 g/ml) diluted in phosphate buffer (pH 6.0). Samples were frozen and thawed two times, and centrifuged at 1500 g for 10 min at 4 °C. MPO activity in the supernatant was determined by adding 0.1 mL of the supernatant to 2.9 mL phosphate buffer (pH 6.0) and 1 mL of 1.5 mmol/L *O*-dianisidine hydrochloride containing 0.0005% (w/v) hydrogen peroxide. The changes in absorbance at 460 nm of each sample were recorded on a UV-vis spectrophotometer. MPO activity was expressed as unit/milligram tissue (U). U: activity of MPO was defined as that converting 1 mmol H_2O_2 to water in 1 minute at 25 °C.

Immunomodulatory assay

Carbon clearance assay

ULPF and ULLF were tested for their phagocytic activity index in female albino rat as per the method reported by Cheng *et al.*[33] The rat were divided into four groups of 5 animals each. Group 1 (control) was given 0.9% NaCl orally, rat in Group 2 and 3 were given ULLF and ULLP at doses of 50 mg/kg body weight orally respectively, group 4 was treated with standard drug levamisole 2.5 mg/kg p.o after the gap of 48 hr, the mice were injected, via the

tail vein, with carbon ink solution at a dose of 0.1 mL/100 g the mixture consisted of black carbon ink 3 ml, saline 4 ml and 3% gelatin solution 4 ml. Blood samples were drawn from retro orbital vein using glass capillaries at interval of 0 and 15 min. A 50 µL blood sample was mixed with 0.1% sodium carbonate solution (4 mL) and absorbance was measured at 660 nm. The carbon clearance was calculated using the following equation: (Log OD1-Log OD2)/15, where OD1 and OD2 are optical densities of blood sample at 0 and 15 min, respectively.[34]

***In vitro* anti-inflammatory assay**

Cell lines-RAW264.7 cells

The murine macrophage cell line RAW264.7 (American Type Culture Collection, Rockville, MD) was cultured in DMEM containing bicarbonate and 10% FBS, supplemented with 1% penicillin, 2% sodium at 37 °C, 5% CO_2 and 71% humidity.

Cell viability: Cytotoxicity studies were performed in 96-well plates. RAW 264.7 cells were mechanically scraped, plated at $2x10^5$/well in 96-well plates containing 100 µl of DMEM with 10% heat-inactivated FBS and allowed to grow overnight, and then incubated for 24 h. RAW cells were cultured in the presence of different concentrations of ULPF and ULLF in a in graded concentrations (10, 30, 60 µg/mL) in a total volume of 1 mL for 1 hr after 1µg/mL of LPS treatment for 18 hr. MTT solution (5 mg/mL) was added, and the cells were incubated at 37 °C for 4 hr. The crystallized MTT was dissolved in DMSO, and the absorbance was measured at 540 nm using a microplate reader. The optical density of formazan formed in control was taken as 100% of cell viability.[35]

Determination of the inflammation mediators

RAW cells macrophages were treated with the ULLF and ULPF fraction (10, 30, 60 µg/mL) in a total volume of 1 mL in the presence of LPS (1 µg/mL) for 24 hr, and the medium was collected for immune mediators analysis.[36]

Determination of TNF-α levels

The production of TNF-α was measured by sandwich enzyme-linked immunosorbent assays (ELISA) as described by Martínez *et al.*[37] Briefly, a purified rat anti-mouse monoclonal TNF-α (0.5 mg, BD Pharmingen) was used for coating at 2 µg/mL at 4 ºC for16 hr. After washing and blocking with PBS containing 3% bovine serum albumin, culture supernatants were added to each well for 12 h at 4ºC. Unbound material was washed off and a biotinylated

monoclonal anti-mouse TNF-α (0.5 mg, BD Pharmingen) was added at 2 μg/mL for 2 h. Bound antibody was detected by addition of avidin peroxidase for 30 min followed by addition of the ABTS substrate solution. Absorbances at 405 nm were taken 10 min after the addition of the substrate. A standard curve was constructed using various dilutions of recombinant murine TNF-α in PBS containing 10% (FBS). The amount of each TNF-α in the culture supernatants was determined by extrapolation of absorbances to the standard curve.

Determination of nitric oxide (NO) levels

The accumulation of NO in the culture supernatants was assayed using Griess reagent. The accumulation of nitrite (NO_2) in cells treated with ULLF and ULLP was used as an indicator of NO production in the medium. The 100 μL supernatant from each well was collected in all samples, and reacted with 100 μL of the Griess Reagent for 30-min incubation at room temperature in the dark. The absorbance was measured at 540 nm. The standard curves were plotted from sodium nitrite standards.[38]

Statistical analysis

Results are expressed as mean±SD. Statistical significance of results was determined by Student's t-test and ANOVA followed by Turkey's post hoc test. Results were determined to be statistically significant when p values 0.05 were obtained. All experiments were repeated at least three times for *in vitr*o studies.

RESULTS

Acute inflammation model: Carrageenan-induced paw oedema

Rat paw oedema, as a standard model of acute inflammation, was used for testing the anti-inflammatory activity of ULPF and ULLF. Subplantar injection of of λ-carrageenan 1% induced a progressive swelling of the rat paw (5,17±0,8 mm) and reached a maximum level at the third hour (6,76±0,3 mm) (Fig. 1). A significant antioedema effect of both ULPF and ULLF phases observed at 1 hr (34% - 38%), 3 h (48% - 51%), 6 h (42% - 44%), the antiedema effect of both ULPF and ULLF phases were significantly ($P<0.01$) more pronounced at 3 hr, this effect is clearly compared to diclofenac-sodium (52%) (Fig 1).

MDA and MPO level in paw oedema induced at 6 hr.

Both MDA and MPO levels were only tested at 6 hr of carrageenan challenge. The effect of ULPF and ULLF phases on MDA and MPO levels are summarized in Table 1. The carrageenan injection in rat hind paw induced a marked increase (32.33±3.4 nmol/mg tissue)

in MDA levels, which was significantly ($p<0.01$) prevented (54% and 52.98%) by both ULPF and ULLF phases (100 mg/kg) and diclofinac-sodium (66.59% mg/kg) respectively. Carrageenan treatment resulted also in a significant increase (6.9±0,8 U/mg tissue) in the MPO activity in the tissue of the rat paw. The ULPF phases at 100 mg/kg dose was significantly ($p<0.01$) more effective (69%) in decreasing MPO activity than ULLF (66.66%), the diclofenac-sodium at 10 mg/kg dose was the best of the drugs treatments in terms of decreasing the MPO activity (72%) (Table 1).

Immunomodulatory activity

Carbon clearance test

The rate of *in situ* carbon particle clearance is frequently used as a measure of immunomodulatory effect on change in macrophage phagocytic activity through reticulo-endothelial system (RES). A significant ($p<0.01$) increase ($5.8\ x10^{-2} \pm 0.003$) and ($5.3\ x10^{-2} \pm 0{,}001$) respectively in phagocytic index was observed after the oral administration of ULPF or ULLF (50 mg/kg) as compared to levanisol ($6.5\ x10^{-2} \pm 0{,}008$). This increase is more pronounced (68%) in the ULLF-group than that in ULPF-group (53%) as compared to levanisol (88%). This indicates that ULLF fraction had have more potent effects in enhancing the macrophage phagocytic activity (Fig 2).

Cell viability

The cell viability was assessed by MTT assay. As shown in Figure 3, the ULPF or ULLF fraction from *Ulva* showed no inhibitory effect on cell proliferation at concentrations ranging from 10 to 60 µg/mL. Thus, the lectins and polysaccharides of *Ulva* fractions were weakly cytotoxic on RAW cells proliferation even at 60 µg/mL.

The effect of ULPF or ULLF on TNF-α and NO release from LPS - RAW 264.7 cells.

In this step of experiment we explored whether the ULPF or ULLF exerted a protection from LPS-mediated inflammation in RAW264 cells. Incubation of RAW264.7 macrophages with LPS (1 µg/mL) significantly ($p<0.01$) increased NO overproduction by more than 4 fold (35±1.8 pg/mL) and induced the TNF-α release near to 15 fold (1500±50 pg/mL).

The protection effect of both ULPF and ULLF on LPS-induced TNF-α and NO release is shown in figure 4, 5. The exposure of RAW 264.7 murine macrophages to 10, 30 and 60 µg/mL of ULPF or ULLF fractions revealed a significantly down-regulating of macrophage production of NO (14.28% - 71.42%) or (6% - 57%) and significant diminution of TNF-α

release (60% - 83%) or (46% - 76%) respectively (Fig 4, 5). The inhibitory effect of polysaccahride fraction was stronger than that of lectine fraction at 60 µg/mL.

Table 1. MPO and MDA level in in rats paw oedema induced by carrageenan in the presence of *U. lactuca* lectins and polysaccharides (ULPF and ULLF) extracts.

Groups	MPO (U)	MDA (nmol/mg tissue)
Carrageenan	6.9±0,8	32.33±3.4
Diclofinac (10 mg/Kg)	1.9±0,08 (72%)	10.8±0.95** (66.59%)
ULPF (100 mg/Kg)	2.1±0,07**¥¥ (69%)	14.2±0.85** (54%)
ULLF (100 mg/Kg)	2.3±0,08** (66.66%)	15.2±1.2**§§ (52.98%)

Each value is the mean ± SD (n=6).
* : Comparison of all groups versus control group (*: p < 0.05,**: p < 0.01),
¥ : Comparison of ULPF and ULLF versus diclofinac group (¥: p < 0.05, ¥¥: p < 0.01),
§ : Comparison of ULPF versus ULLF : (§: p < 0.05, §§: p < 0.01),
Values in parentheses represent percentage of inhibition.

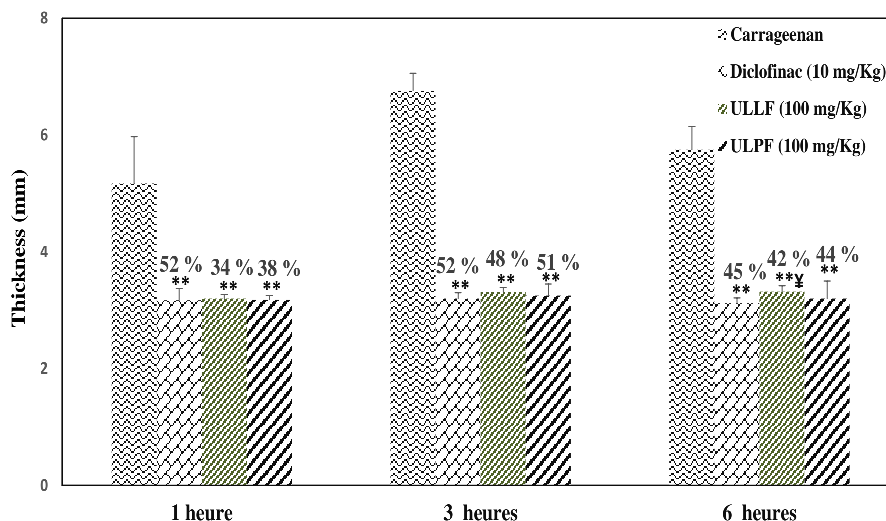

Figure. 1: Reduction of paw edema by the carrageenan challenge in the presence of *U. lactuca* lectins and polysaccharides (ULPF and ULLF) extracts. Results were expressed as mean± SD, (n=6); all groups were compared versus carrageenan control group, (*: <0.05, **p<0.01). Values given at the top of each bar represents percentage of inhibition.

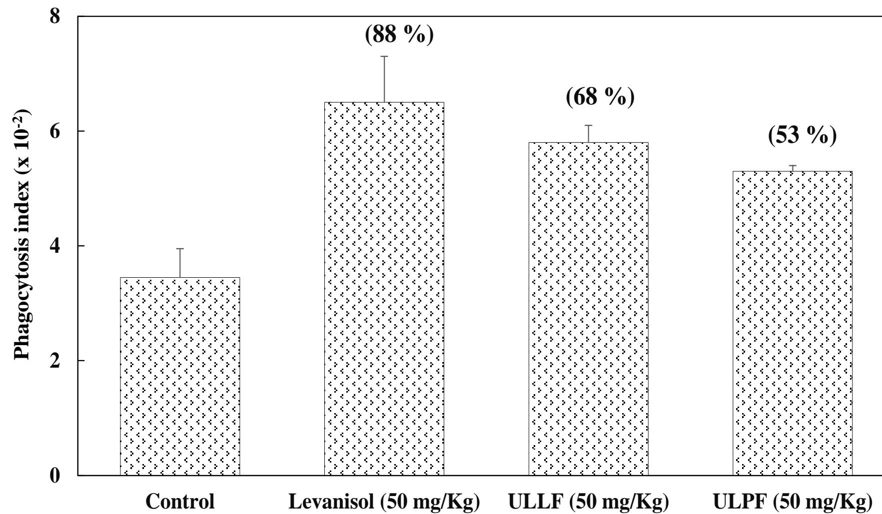

Figure. 2: Immunomodulatory activity (Carbon clearance test) of *U. lactuca* lectins and polysaccharides (ULPF and ULLF) extracts. Each value is the mean ± SD (n=5).

:Comparison of all groups versus control group (: $p < 0.05$,**: $p < 0.01$),

¥:Comparison of ULPF and ULFF versus to levanisol group (¥: $p < 0.05$, ¥¥: $p < 0.01$),

§:Comparison of ULPF versus ULFF : (§: $p < 0.05$, §§: $p < 0.01$),

Values in parentheses represent percentage of inhibition.

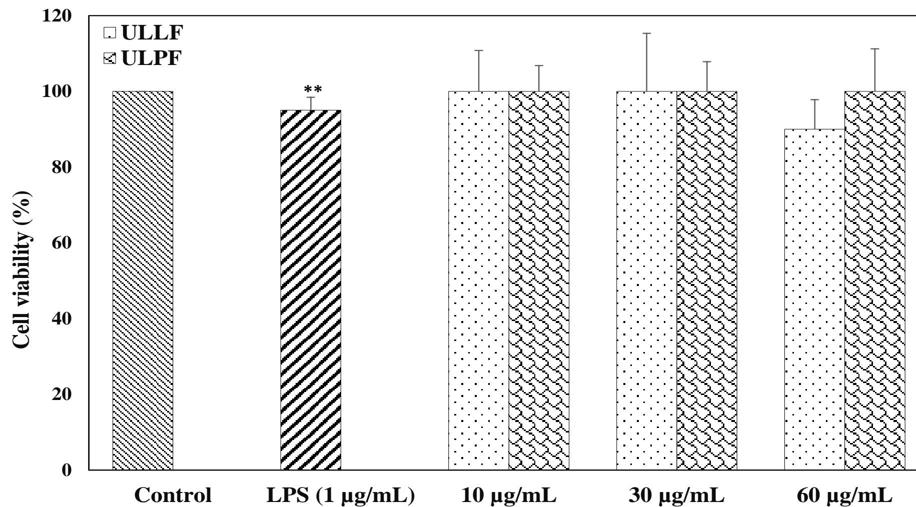

Figure. 3: Protective of *U. lactuca* lectins and polysaccharides (ULPF and ULLF) extracts on viability of RAW 264.7 macrophages measured by the MTT assay. Cells were pretreated with or without different concentrations of ULPF and ULLF and exposed to 1 µg/mL of LPS. Each value is the mean ± SD (n=3). All groups were compared versus control group ** : p<0.01.

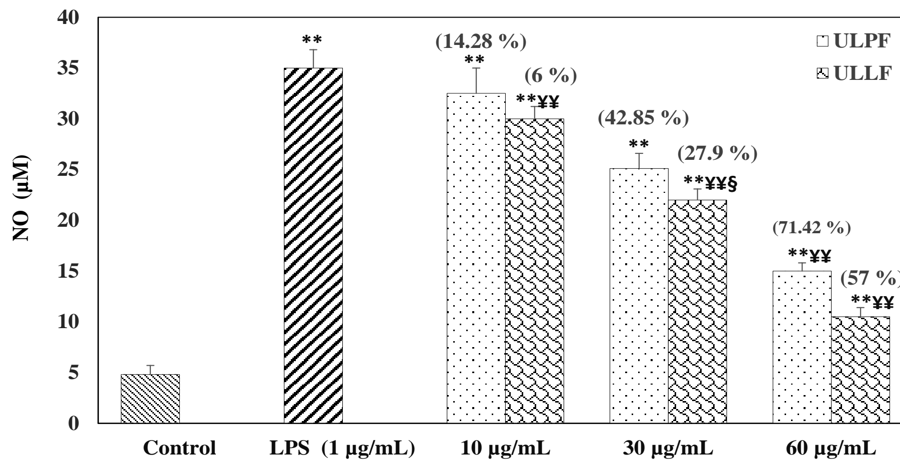

Figure 4. Effect of *U. lactuca* lectins and polysaccharides (ULPF and ULLF) extracts on NO release into the medium by LPS-activated RAW 264.7 macrophages. The nitrite content in the medium was determined by a Griess reagent. Each value is the mean ± SD (n=3).

*: Comparison of all groups versus control group **: $p < 0.01$

¥: Comparison of ULPF and ULFF versus LPS –treatment (¥¥: $p < 0.01$)

§: Comparison of ULPF versus ULFF : §§: $p < 0.01$,values in parentheses represent percentage of inhibition.

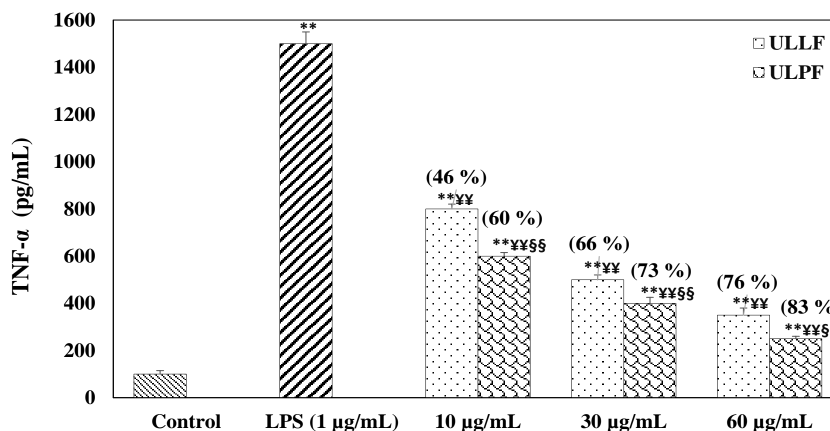

Figure 5. Effect of *U. lactuca* lectins and polysaccharides (ULPF and ULLF) extracts on TNF-α release into the medium by LPS-activated RAW 264.7 macrophages. The nitrite content in the medium was determined by a Griess reagent. Each value is the mean ± SD (n=3).

* : Comparison of all groups versus control group (**: $p < 0.01$)

¥ : Comparison of ULPF and ULFF versus LPS –treatment (¥¥: $p < 0.01$)

§ : Comparison of ULPF versus ULFF (§§: $p < 0.01$),

Values in parentheses represent percentage of inhibition.

DISCUSSION

Several compounds possess varied chemical structures and potent anti-inflammatory activity has been isolated from marine algae.[9,10] Inflammation is manifested by the activation of immunocytes such as monocytes and macrophages, and the secretion of inflammatory mediators such as nitric oxide (NO), prostaglandin E2 (PGE2) and TNF-α.[5] An inflammatory condition involves induction of oedema and recruitment of cells, predominantly neutrophils, to the site of inflammation.[39,40] The marine algae compounds may affect multiple targets in the immune and inflammatory systems that influence disease progression. Our experiments showed that the inflammation level increased after carrageenan injection by inducing a progressive swelling of the rat paw, that reached a maximal value by the third hour and then the oedema decreased over the subsequent hours. In the current study, both ULPF and ULLF fraction revealed the significant anti-inflammatory activity at a dose of 100 mg/ kg. The ability to reduce the effects of inflammation caused by the carrageenan as phlogistic agent, is more pronounced in the case of ULPF which is comparable to diclofenac sodium that inhibits the second phase of carrageenan-induced oedema via the inhibition of prostaglandin synthesis by the COX isozymes COX-1 and COX-2.[41] The Inflammation produced by carrageenan is a biphasic oedema; the first phase is characterized by an oedema develop due to enhanced vascular permeability, this initial phase which occurs within the first hour of exposure, triggering the release of histamine, serotonin, bradykinin and prostaglandins.[42] After approximately 1 hr, polymorphonuclear cells (mainly neutrophils) are recruited and continue the production of prostaglandin and NO.[43]

The anti-inflammatory effect action of the ULPF which was evidenced in the present study has been supported in the literature[44] revealed that sulfated polysaccharides present in algae were shown to possess anti-inflammatory properties, *Ulva lactuca* available in Tuticorin coast was found to show anti-inflammatory effect as evidenced by the reduction in the inhibition of oedema at the 4th day of the experiment. Khan *et al.*[45] showed that brown seaweed (*Undaria pinnatifida)* extract possess an anti-edema effects on phorbol 12-myristate13-acetate-induced mouse ear inflammation. Elmegeed *et al.*[46] mentioned that Phytochemical constituents of *U. lactuca* from Alexandria coast, Egypt, proved anticoagulation properties. The anti-inflammatory effect of both ULLF and ULPF corroborate findings of previous studies showing a possible anti-inflammatory effect of polysaccharide and lectines.[47] Previous studies suggested that lectins can inhibit the vascular inflammation mediated by adhesion of immune cells[48,49], sulfolipids from the red alga

Porphyridium cruentum exhibited *in vitro* anti-inflammatory activity and anti-proliferative activity.[50] The constituents of the red alga *Gracilaria verrucosa* proved an anti-inflammatory effect[51], the alga *Bryothamnion seaforthii* contains carbohydrates with antinociceptive activity[52], the lectin from *Ziziphus oenoplia*, showed potential antiallergic and anti-inflammatory.[53] MPO activity is a sensitive and specific marker for acute inflammation reflecting polymorphonuclear cell infiltration of the parenchyma.[54,55] The reactive oxygen species (ROS) were associated with inflammation, in a number of pathophysiological processes[56] this process was clearly evidenced by the high level of pow MDA. Both ULPF and ULLF had a benefit to modulated these markers. The concept of immunomodulation relates to nonspecific activation of the function and efficiency of macrophages, granulocytes, complement, natural killer cells and lymphocytes and also to the production of various effector molecules generated by activated cells.[57] The primary target of most immunomodulators is believed to be macrophages which play a major role by engulfing pathogens.[58] Phagocytosis is the process by which certain phagocytes ingest and remove microorganisms, malignant cells, inorganic particles and tissue debris.[59] Thus, phagocytosis that was enhanced by ULLF, might implying the elevation of innate immune response.[60] In our investigation, when ink containing colloidal carbon is injected intravenously in *Wistar* rats, the macrophages engulf the carbon particles of the ink and the rate of clearance of ink (carbon particles) from blood is known as phagocytic index. The reticuloendothelial system is the best defined functionally by its ability to scavenge debris or other foreign matter and forms first line of defense.[61] In our study, both ULPF and ULLF stimulated the reticuloendothelial system by increasing the phagocytic index. ULLF treated-groups exhibited significantly high phagocytic index compared to ULPF-group. This study also provide evidence that the lectine from *U. lacuta* possessed *in vivo* immune modulatory activities in terms of phagocytic activities more than that exhibited by the polysaccharide. Several lectins have been reported to have immunomodulatory activity in macrophages by enhancing the phagocytosis and the secretion of cytokines.[60,62] The importance immunomodulatory of lectins could manifested by multiple ways. Lectin from the green marine alga *Caulerpa cupressoides* has been reported to reduce the number of writhes induced by acetic acid.[63] The results of our study also were agrees with those of Matsui *et al.* (2003)[64] who reported that the administration of intracellular polysaccharides from marine microalgae, like *Porphyridium*, *Phaeodactylum*, and *Chlorella stigmatophora*, were found to show anti-inflammatory activity and as immunomodulatory activities that was evidenced by the positive phagocytic activity. The acidic sulphated polysaccharides obtained

from the seaweed *Ulva* showed immunomodulating activities[65], the polysaccharide extract of *Ganoderma lucidum* proved immunomodulatory and adjuvant activities[66], the polysaccharide from *Tinospora cordifolia* had an immunomodulatory effect and modulates macrophage responses.[67]

Considering the above results, we aimed to explore the mechanism of action involved in the immunomodulation and anti-inflammatory effects promoted *in vivo* by the two fractions obtained from *U. lucata*, using LPS induced RAW 264.7 macrophages inflammation as *in vitro* cells models. The activation of macrophages would be one of the self-defense mechanisms to protect the host against microbial pathogens.[5] By contrast, dysregulated production of these immune mediators by macrophages during prolonged inflammation is associated with different pathological conditions, such as autoimmune diseases, and cancers.[68] In the present study, both ULPF and ULLF showed no inhibitory effect on RAW 264.7 macrophages cell proliferation in graded concentrations (10, 30, 60 µg/mL). When assessed by MTT assay, this study revealed that both lectins and polysaccharides fractions from *U. lacuta* were weakly active on RAW cells proliferation promoting a weak cytotoxic effects even at 60 µg/mL. Among the inflammatory mediators and cellular pathways that have been extensively studied in association with human pathological conditions are cytokines.[69] The immunmodulatory effect of polysaccharides from green seaweeds is mainly based on macrophage modulation.[70] The polysaccharide isolated from *Ganoderma atrum* proved macrophage immunomodulatory activity[37], water-soluble polysaccharide obtained from *Acorus calamus* activates macrophages and stimulates Th1 response[71], the polysaccharide isolated from *Opuntia polyacantha*[72] and *Glycyrrhiza uralensis*[73] had macrophage immunomodulatory activity. Yoshizawa *et al.*[74] reported that marine algae *Porphyra yezoensis* extracts possess macrophage-activating activities by increasing immune mediators and phagocytic activities. Schepetkin *et al.*[72,75] revealed that the polysaccharides isolated from both *Juniperus scopolorum* and *Opuntia polyacantha* exhibited macrophage immunomodulatory activity. The activity of polysaccharides isolated from *Glycyrrhiza uralens is* a macrophage immunomodulatory.[73] During the inflammatory reaction, macrophages also serve as a source of various pro-inflammatory cytokines or mediators such as nitric oxide (NO), tumor necrosis factor- α (TNF-α), and IFN-c.[76] NO plays an important role in inflammatory response as a pro-inflammatory molecule, which is produced by iNOS. Uncontrolled or excess NO production leads to the development of various inflammatory diseases.[77] The TNF-α plays an important role in various physiological and pathological

processes, including immune and inflammatory responses.[78] The TNF-α is not only a major inflammatory cytokine and a powerful anticancer cytokine but, also, TNF-α induces a pro-inflammatory response.[79]

Our results indicated that both ULLF and ULPF possess anti-inflammatory activities by suppressing the LPS-stimulated production of TNF-α, and NO in the RAW 264.7 cell treated with LPS in a dose dependent manner, but not affect cell proliferation, the anti- inflammatory effect of polysaccharides is more pronounced than that of lectines, these results well concord with those obtained by Hwang *et al.*[80] that mentioned the sulfated polysaccharide extract from hot-water extracts of brown seaweed *Sargassum hemiphyllum* have been shown to inhibit LPS activated release of NO, TNF-a, by blocking NF-kB translocation and down-regulating expression of inducible nitric oxide synthase (iNOS) protein. O'Shea *et al.*[81] and Pacheco-sánchez *et al.*[82] also reported that polysaccharide extracted from *Collybia dryophil*a treatment showed a down-regulation effect of NO production. Algae constituents may exhibited different behavior toward the secretion of inflammatory mediators from RAW cells, it has been reported that the endemic seaweeds stimulate the production of pro-inflammatory mediators in mouse macrophage cell line RAW 264.7.[83] The anti-tumor effect of *Ganoderma lucidum* is mediated by cytokines released from activated macrophages and T lymphocytes[84], the polysaccharide fraction from a marine alga (*Porphyra yezoensis*) possess a macrophage stimulation activity.[85]

CONCLUSION

Based on the above findings, the possible explanation for the beneficial effects of both lectine and polysaccharides including *U. lactuca* treatment may exhibited *in vivo* anti-inflammatory and the immunomodulatory activities which were associated with inhibition TNF-α cytokine release and downregulation NO production in RAW 264.7 cell line. The testing of every *Ulva* lectine/polysaccharides fraction enables a thorough elucidation and better understanding of their mechanism of action.

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