E-ISSN: 2707-2835
P-ISSN: 2707-2827
www.pharmacognosyjournal.com
IJPLS 2024; 5(2): 32-39
Received: 28-05-2024
Accepted: 03-07-2024

Malek Eleroui
Laboratory of Medicinal and Environment Chemistry, Higher Institute of Biotechnology, University of Sfax, PB 261, Sfax 3000, Tunisia

Amal Feki
Laboratory of Medicinal and Environment Chemistry, Higher Institute of Biotechnology, University of Sfax, PB 261, Sfax 3000, Tunisia

Asma Hamzaoui
Laboratory of Medicinal and Environment Chemistry, Higher Institute of Biotechnology, University of Sfax, PB 261, Sfax 3000, Tunisia

Marwa Lakhrem
Laboratory of Medicinal and Environment Chemistry, Higher Institute of Biotechnology, University of Sfax, PB 261, Sfax 3000, Tunisia

Marwa Kariem
Laboratory of Medicinal and Environment Chemistry, Higher Institute of Biotechnology, University of Sfax, PB 261, Sfax 3000, Tunisia

Amal Dghim
Laboratory of Medicinal and Environment Chemistry, Higher Institute of Biotechnology, University of Sfax, PB 261, Sfax 3000, Tunisia

Marwa Bouhamed
Laboratory of Anatomopathology, CHU Habib Bourguiba, University of Sfax, 3029 Sfax, Tunisia

Choumous Kallel
Laboratory of Hematology, CHU Habib Bourguiba, University of Sfax, 3029 Sfax, Tunisia

Ibtissem Ben Amara
Laboratory of Medicinal and Environment Chemistry, Higher Institute of Biotechnology, University of Sfax, PB 261, Sfax 3000, Tunisia

Corresponding Author:
Malek Eleroui
Laboratory of Medicinal and Environment Chemistry, Higher Institute of Biotechnology, University of Sfax, PB 261, Sfax 3000, Tunisia

Assessment of the anti-inflammatory activity of Methanolic extract of green algae *Codium bursa*

Malek Eleroui, Amal Feki, Asma Hamzaoui, Marwa Lakhrem, Marwa Kariem, Amal Dghim, Marwa Bouhamed, Choumous Kallel and Ibtissem Ben Amara

DOI: https://doi.org/10.33545/27072827.2024.v5.i2a.124

Abstract

The present study is aimed to evaluate *in vivo* the anti-inflammatory of methanolic extract of green algae *Codium bursa* (MEC) on carrageenan induced paw edema model in rats. The experimental protocol was performed on 20 rats divided in 4 groups: a control group, carrageenan-treated group (1%), the reference drug diclofenac sodium (DICLO 100 mg/kg) and MEC-treated group (50 mg/kg). The evaluation of the anti-inflammatory effect of MEC and DICLO was mainly evaluated by the measuring of edema paw swelling, hematological parameters, micronucleus test, oxidative stress assays, and histological evaluation. The pretreatment with MEC showed a significant (p <0:05) reduce of the inflammation symptoms such as the swelling of edema compared to the control group. The findings of this work demonstrated that MEC extract significantly minimized the lipid peroxidation and protein oxidation (TBARS, AOPP) and restored antioxidant enzymes activities in the inflamed tissues. Thereby, the anti-inflammatory capacity of MEC was confirmed with histological examination by establishing the normal microscopic structure in skin samples compared to the control and carrageenan groups. Hence, our results demonstrated that MEC could be used in the preparation of drugs destined on the treatment of acute inflammation.

Keywords: *Codium bursa*, methanolic extract, anti-inflammatory activity, ROS, carrageenan

1. Introduction

The rise of inflammatory diseases throughout the world has made them an international emergency. It has been established that inflammation, which underlying almost every disease process, is the cause of several prevalent disorders [1]. As a result, inflammation is described as the body's immune system's preventative reaction to a variety of endogenous and exogenous events. It is typically accompanied by pain, heat, redness, and swelling [2]. The inflammatory process involves a multitude of pro-inflammatory mediators, including interleukin (IL)-1β, nitric oxide (NO), and tumor necrosis factor (TNF)-α.

In the recent decades, the use of natural biomolecules has been great attraction, as they have been the source of natural products, commonly named as bioactive compounds. Additionnaly, natural compounds derived from marine origin have advanced in the identification of potential anti-inflammatory pharmacophore agents. In this regard, one of the most powerful marine organisms, marine seaweeds generate a wide range of bioactive substances, including polyphenols, steroids, lectins, and polysaccharides, all of which have broad pharmacological applications in the treatment of different diseases [3].

Given this background, the aim of the present study is to investigate the anti-inflammatory of methanolic extract of green Tunisian macroalga *Codium bursa* using carrageenan induced paw edema model in rat.

2. Materials and Methods

2.1 Materials

The algae *"Codium bursa"* was sampled from m the coastal area of "Ksibet ElMidouni" in Monastir and identified by a specialist. The fresh material was washed several times with water to eliminate impurities and dried in the shadow for 15 days at temperature room, then crushed to get a fine powder and stored in limp sterile.

2.2 Preparation of the methanolic extract of *Codium bursa* algae (MEC)

The algae-dried powder was extracted using maceration. Briefly, 50 g of powder were dissolved in 200 mL of methanol solvent. The mixture was then incubated for 24 h with stirring at room temperature. the solution was filtrated using Whatman filter paper and then evaporated using a Rotary evaporator. The dried residue was re-dissolved in the corresponding solvent and then stored at 4 ◦C.

2.3 The *in vivo* anti-inflammatory effect of Phenolic extract

2.3.1 Animals and Experimental design

Male adult Wistar rats, weighing between 150 and 200 g, were obtained from the local Central Pharmacy, Tunisia. All animals were maintained at normal room temperature (22–24 °C) on a 12-hour light/dark cycle, with free access to food and water. All animals' protocols were conducted in accordance with the Guide for the Care and Use of Laboratory of Animals issued by the University of Sfax, Tunisia and approved by the Committee of Animal Ethics.
A number of 20 rats were used in this protocol and grouped into four groups (5 rats per group) as follow:

- **Group 1**: used as a control group.
- **Group 2**: the rats were injected with carrageenan in the right paw edema (carrageenan 1% w/v, in saline solution)
- **Group 3:** the rats received orally a reference drug Diclofenac sodium (100mg/kg) before one hour of the carrageenan injection.
- **Group 4**: the rats received MEC solution orally (50 mg/kg) before one hour of the carrageenan injection.

A digital caliper was utilized to measure the edema volume at 0.5, 1, 2, 3, 4 and 5 hours after carrageenan's injection. The volume evaluation of edema was calculated as following:

$$V\ edema = Vt - V0$$

Where V0 et Vt were the volume of edema at time t before carrageenan's injection and time 0 after carrageenan's injection.

2.3.2 Blood Sample Collection

Five hours after carrageen injection, the rats were sacrificed by decapitation and blood samples were collected with into heparinized tubes to examine hematological parameters.

2.3.3 Hematological parameters

The white blood cells (WBC) (10^3 /µL), platelets (PLT) (10^3 /µL), and lymphocytes (LYM) (10^3 /µL) were measured in an automatic hematological assay analyzer (CHU Habib Bourguiba, Sfax, Tunisia).

2.3.4 Micronucleus test in the peripheral blood (MN)

The MN assay was carried out as described Feki et al. (2019) [4]. Briefly, to extract leucocytes, fresh blood samples were treated with Ficoll. 50 µl of extract was fixed on microscopic glass slides, followed by a treatment with Carnoy solution, after drying, successively for 5 and 10 min. Subsequently, they were incubated with acridine orange (0.5 µg/ml) in the dark and rinsed twice with phosphate-buffered saline solution (pH = 7.4). The morphology of the cell's nuclei was visualized using a fluorescence microscope with an excitation wavelength 520-560 nm.

2.3.5 Oxidative Stress Parameters

2.3.5.1 Preparation of Cytosolic homogenates

The skin samples were homogenized (10% w/v) with an Ultra Turrax and diluted with phosphate buffer (pH 7.4). Homogenates were centrifuged at 9000 rpm for 20 min at 4 °C. The obtained supernatants were used for lipid peroxidation and protein oxidation determination. Protein contents were determined using the method of Lowry et al. (1951) [5] with bovine serum albumin as a standard.

Thiobarbituric Acid Reactive Substances (TBARS) Assay

TBARS was determined spectrophotometrically according to Draper & Hadley (1990) [6]. Briefly, in summary, the thiobarbituric acid reactive substances (TBARS) assay quantifies the amount of malondialdehyde (MDA) in a sample. MDA is produced by hydrolyzing lipid hydroperoxides. An aliquot of tissue extract supernatant was mixed with 125 µl of 20% trichloroacetic acid and 50 µl of TBS buffer. Centrifugation was applied for 10 minutes at 1000 rpm in order to get the supernatant. After adding 200 µl of supernatant, 40 µl Tris-HCl (0.6 mol/l), and 160 µl of thiobarbituric acid reagent, the mixture was maintained for 10 minutes at 80 °C. After cooling the obtained mixture, the absorbance were measured at 530 nm. The malondialdehyde values were calculated using an extinction coefficient of 0.156 $mM^{-1}.cm^{-1}$.

2.3.5.2 Advanced Oxidation of Protein Products (AOPP) Assay

The contents of the Advanced Oxidation Protein Product (AOPP) were determined using the procedure described by Kayali et al. (2006) [15]. In short, 0.8 ml of phosphate buffer (0.1 mol/l; pH 7.4) was combined with 0.4 ml of the tissue homogenate supernatant. 0.1 ml of potassium iodide (1.16 mol/l) and 0.2 ml of acetic acid were added after two minutes of incubation. At 340 nm, the mixture's absorbance was measured. The AOPP concentration was reported as µmoles/mg of protein.

2.3.5.3 Superoxide dismutase (SOD) enzyme activity

The colorimetric method described by Beauchamp and Fridovich (1971) [7] was used to assess the SOD enzyme activity. In summary, the following was added: 50 mM of tissue homogenates, pH 7.8, 0.1 mM EDTA, 13 mM L-methionine, 2 µM riboflavin, and 75 mM of nitro blue tetrazolium (NBT). the activity of SOD was measured at 516 nm.

2.3.5.4 Glutathione peroxidase activity

Glutathione peroxidase (GPx) activity in tissue was measured following the method of Flohé and Günzler (1984) [9]. In brief, 1ml of the reaction mixture (2 mM glutathione (GSH), sodium azide (10 mM), and H_2O_2 (1 mM)) was supplied to 0.3 ml of tissue homogenates. The reaction was stopped using TCA 5%. 0.2 ml of supernatant and 0.7 ml of DTNB (0,4 mg/ml) were combined a 10-minute centrifugation at 1500 g. The absorbance was measured at 420 nm and the enzyme activity was reported

as nmoles of GSH/min/mg of protein, absorbance was measured at 420 nm.

2.3.6 Histopathology Examination

In formol 10%, paw samples were maintained for histological analysis. Samples were dehydrated in an increasing ethanol gradient, cleared in toluene, and embedded in molten paraffin wax. Sections 5"µm thick were cut, stained with hematoxylin and eosin (H and E), and examined for histopathological evaluation (Jung Leica microscope HM314).

2.4 Statistical analysis

The obtained data were analyzed with Graph Pad Prism version 8.0, it is a professional edition using ANOVA analysis at a p level=0.05. A standard deviation at the 95% confidence level was used.

3. Results

3.1. Edema size evaluation

The evolution of edema size in the treatment and control groups during a 5-hour period was shown in figure 1. The highest edema size changes were noted following a 2-hour Carrageenan injection. Rats treated with MEC extract and DICLO showed a notable reduction in the size of paw edema during a period of 2 to 5 hours ($p<0.05$) (0.29± 0.023 and 0.32±0.029, respectively) when compared with the control group (0.36± 0.046).

3.2. Evaluation of the hematological Parameters

Table 1 showed that following carrageenan injection, it was a substantial increase in the number of white blood cells (WBC) (17.73± 0.55.10^3/µl), PLT (1332± 114.3510^3/µl), and LYM (17.96± (0.45.10^3/µl) in comparison to the control group. When compared with rat treated with carrageenan, the pre-treatment with MEC extract and the reference drug (DICLO) showed a considerable reduction in these parameters.

Figure 2 displayed the morphology of the white cell nuclei in each groups: control, CARR, treated with CARR showed completely fragmented nuclear, evidenced by reddish-orange fluorescence, that shows damaged DNA. However, the pre-treatemnet with MEC extract, significantly reduced the frequency of DNA damage evidenced by an increase in greenish-yellow fluorescence, which indicates intact DNA.

3.4. Oxidative Stress Parameters

The process of lipoperoxidation caused several cellular damages, including the deterioration of membrane lipids and other numerous cellular processes, which raised the levels of TBARS. The Figure 3-A shows the assessment of MDA rates in the paws of treated and control rats. The dermal MDA levels increased significantly ($p<0.05$) (37.12 ± 0.73 nmol MDA/mg of protein as compared with the control group (26.24±0.52 nmol MDA/mg of protein) in response to the Carrageenan injection-induced inflammation). In contrast to the carrageenan group, we observed a significant decrease in the groups pretreated with MEC extract and the reference medication Diclofenac sodium (25.53±0.45 and 25.09±0.1 nmol MDA/mg of protein, respectively).

Figure 3-B presents the evaluation of cutaneous protein oxidation. Compared to the control group (0.6±0.02 nmol/mg of protein), the carrageenan group's paw revealed ($p<0.05$) considerably higher levels of AOPP (0.91±0.02 nmol/mg of protein). In contrast to the Carrageenan group, the pretreatment with MEC extract and diclofenac sodium considerably reduced the rate of AOPP (0.59±0.002 and 0.58±0.004 nmol/mg of protein, respectively).

The GPx level was determined in tissue homogenate of rats. The carrageenan injection induced n a significant decrease in activities of GPx with 60.63%, when compared with the control group. Nevertheless, the pre-treatment with MEC restored significantly ($p<0.005$) the level of GPx activity when compared to carrageenan group (Figure 3-C).

The carrageenan injection significantly decreased the level SOD activity (Figure 3-D) in paw edema ($p<0.005$) (13.02± 0.62 U/mg of protein) when compared to the control (22.61±0.82 U/mg of protein). However, the pretreatment with MEC and DICLO restored considerably the SOD activity in the inflamed tissues (21.72±0.37 and 21.27±0.48 U/mg of protein, respectively).

3.5. Histopathology assessment

According to the histological analysis, the dermal connective tissue of the animals injected only with Carrageenan showed significant edema and an abundance of inflammatory infiltrates in their paw skin samples. The methonlic extract (MEC) and reference drug pretreatment retained the initial skin structure and decreased the inflammatory lesions, evidenced by the decrease in inflammatory cell presence and the lack of edema (Figure 4).

4. Discussion

Carrageenan-induced paw edema is an established animal model for evaluating the anti-inflammatory potential of synthetic chemical compounds, biomolecules and herbal exctract. Carrageenan is defined as linear anionic polysaccharide which can be extracted from some red algae species, such as *Gigartin*a, *Chondrus crispus, Eucheuma*, and *Hypnea*. It is formed by alternating links of 1,3-β-ᴅ-galactopyranose and 1,4-α–galactopyranose [10]. Although, in the present study, a subplantar injection of carrageenan sodium (1% w/v) was employed to induce inflammation in a group of mice. The inflammatory response is usually quantified by a decrease in the edema paw size (after carrageenan injection) and controlled by antagonists of specific molecules within the inflammatory cascade.

In the present study, Diclofenac sodium (100 mg/kg) was used as positive control. Intra. the phenomena of carrageenan-induced paw edema occur in two phases. The first one, lasting between 1 and 2 hours after injection, which is mostly non-phagocytic swelling, it is brought on by the actions of mediators on vascular permeability, including histamine, serotonin, and bradykinin [10, 11]. The second phase started after two hours of the carrageenan injection, and it characterized with an acceleration in edema swelling and extend up to 5 hours. Prostaglandins and other metabolites of arachidonic acid that are produced in excess have been shown to induce delayed of edema in this phase [12]. The obtained data revealed a significant reduction in paw edema swelling in animals that received a pretreatment with MEC extract at dose (50 mg/kg) and similarly to the reference drug when compared to the control. The methanolic extract MEC demonstrated a higher anti-edematous property and is higher when compared to the reference drug DICLO. In this way, Ninan Jisha et al.

(2019) [13] reported that the mechanism of anti-inflammatory activity of methonolic extract of *Muntingia calabura* L. leaves is due to the inhibition of the primary inflammatory mediators. The secondary inflammatory mediators were released upon the activation of primary mediators. Secondary mediators were not released, and further inflammatory reactions were prevented.
Additionally, an significant increase ($p<0.005$) in platelet and white blood cell counts which is linked with carrageenan injection [1]. This leukocytosis may be directly related to the intensity of the inflammatory stimulation and was associated to an increase in leukocyte recruitment [14]. It was reported that carrageenan stimulated immune function by allowing WBC to infiltrate the site of inflammation and produce proinflammatory cytokines (IL-I). The latter increased the generation of macrophages and granulocytes [30]. rats administered with MEC showed a reduction in WBCs and platelets, showing its effectiveness in restoring the normal physiology condition and prevented the installation of inflammation reaction. Also, the obtained data suggested the powerful scavenging capacity of MEC with the remarkable protective effect off DNA in MN assay. In this way, Free radical can affect all the cell's compartment and generate DNA oxidation. These results suggest that the topical application of MEC extract was implicated in cellular protection as a source of antioxidant molecules and an activator of the expression of antioxidant enzymes.
The reactive oxygen species (ROS) were strongly linked with inflammation, in a number of pathophysiological processes [15]. In the present study, the carrageenan injection caused a significant reduction in the activities of antioxidant enzymes (SOD and GPx) at the inflamed tissue additionally with a significant increase in TBARS and the AOPP. The overproduction of ROS, which led to an increase in oxidative stress indicators, was reflected in the obtained results. Conversely, after five hours, treated rats with MEC and DICLO showed a significant ($p < 0.05$) decrease in tissue lipid peroxidation levels and restoration of the antioxidant enzymes activities which was linked to a reduction in edema thickness. In this study, MEC extract have successfully restored the normal ROS level in paw tissues. The histological evaluation of PWEC further supported its anti-inflammatory capabilities. Furthermore, our sample lowered neutrophil infiltration and morphological damage.

Table 1: Effect of the polysaccharide MEC and DICLO on hematological parameters.

Hematological parameters	Control	Carrageenan	DICLO	MEC
WBC count ($10^3/\mu l$)	6.867± 0.62	17.73± 0.55***	8.9± 0.11**	9.16± 0.66**
PLT count($10^3/l$)	740.033± 60.32	1332± 114.35***	781± 50.22**	769.33± 66.02**
LYM count ($10^3/\mu l$)	7.3± 0.61	17.96± 0.45**	12.33± 0.85*	10.93± 0.6ns

Values are given as mean of three determinations (X ± SD); SD: standard deviation, *, **, *** referred to significant difference compared with the control group

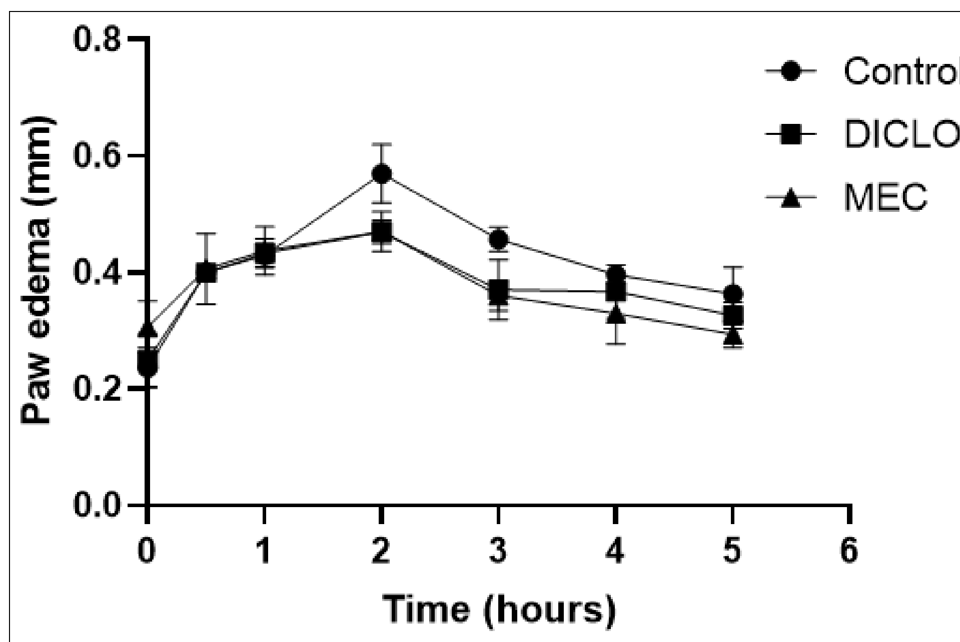

Fig 1: Effect of MEC and Diclofenac in the carrageenan induced paw edema.

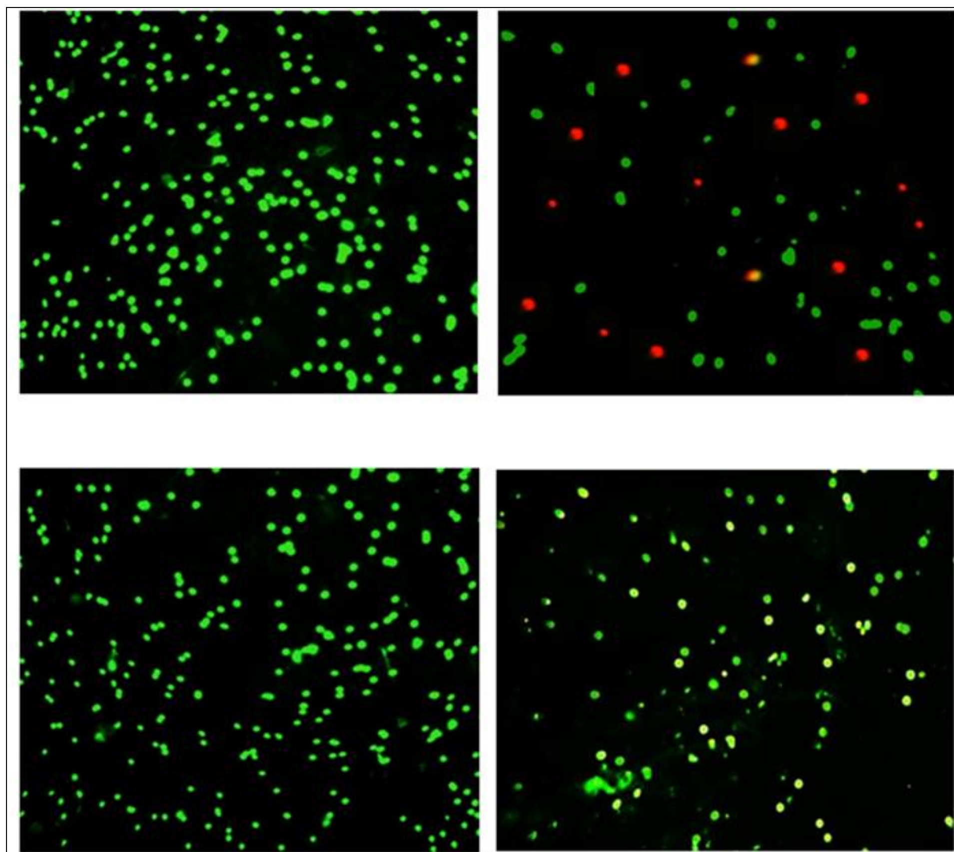

Fig 2: Representative photographs MN assay in different groups of rat

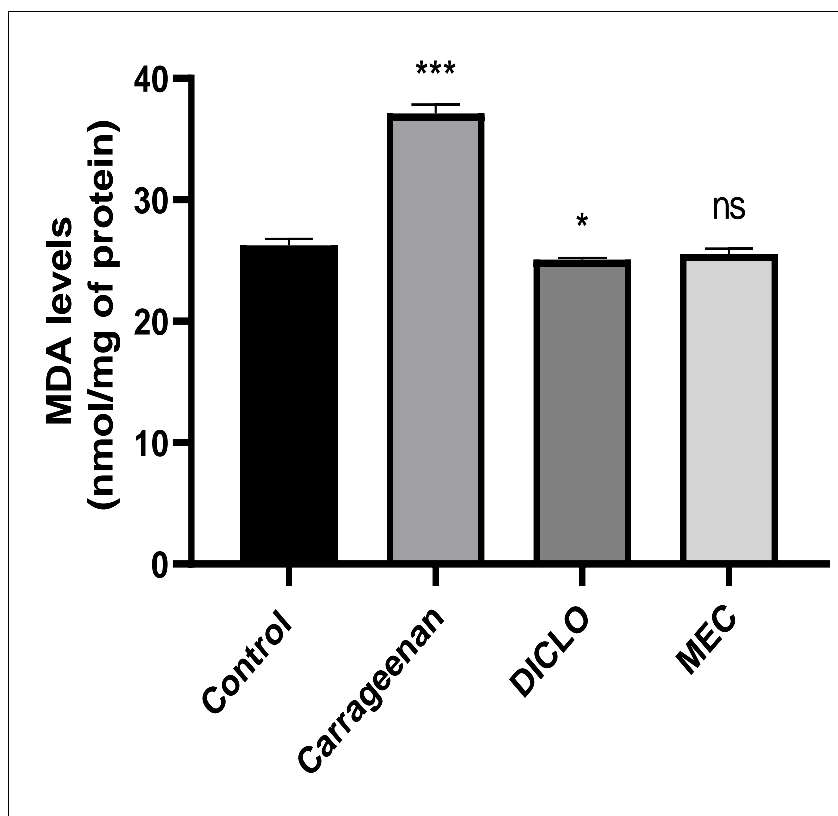

Fig 3-A: Levels of MDA in the paw of different groups of rats. Values represent means ± SEM (n= 5) in each group. *** $p < 0.0001$ ** $p < 0.001$; * < 0.01 compared to control.

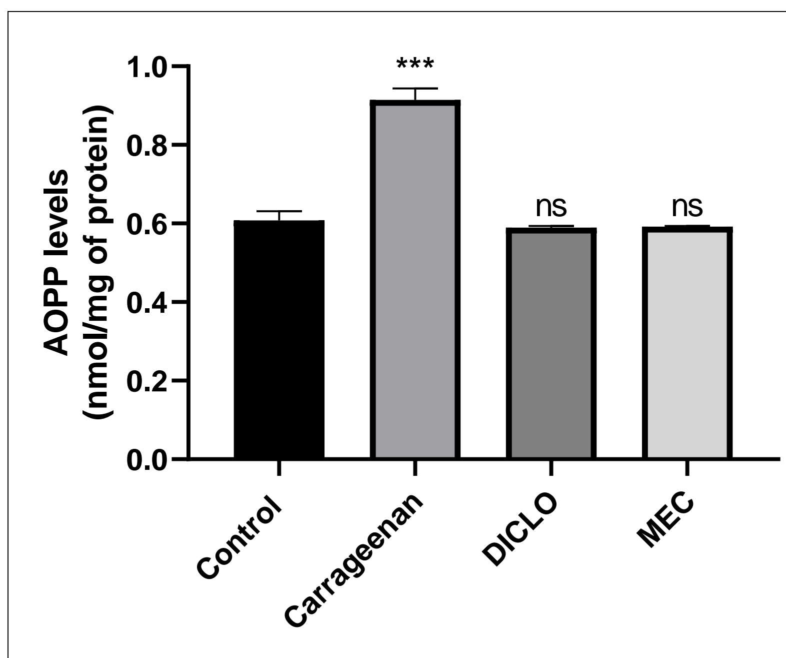

Fig 3-B: Levels of AOPP in the paw of different groups of rats. Values represent means ± SEM (n=5) in each group. *** $p < 0.001$ compared to control.

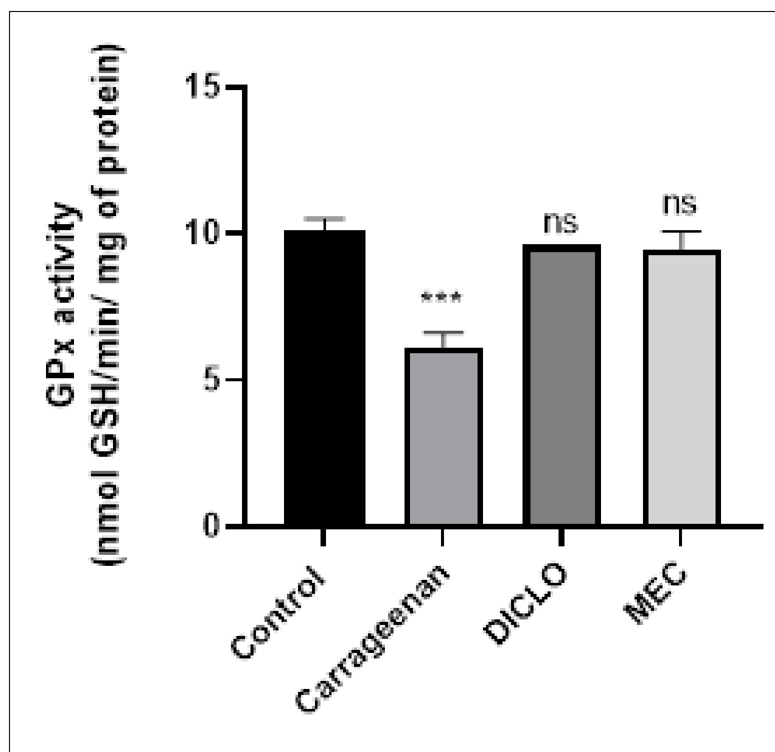

Fig 3-C: Levels of GPx activity in the paw of different groups of rats. Values represent means ± SEM (n=5) in each group. *** $p < 0.001$ compared to control.

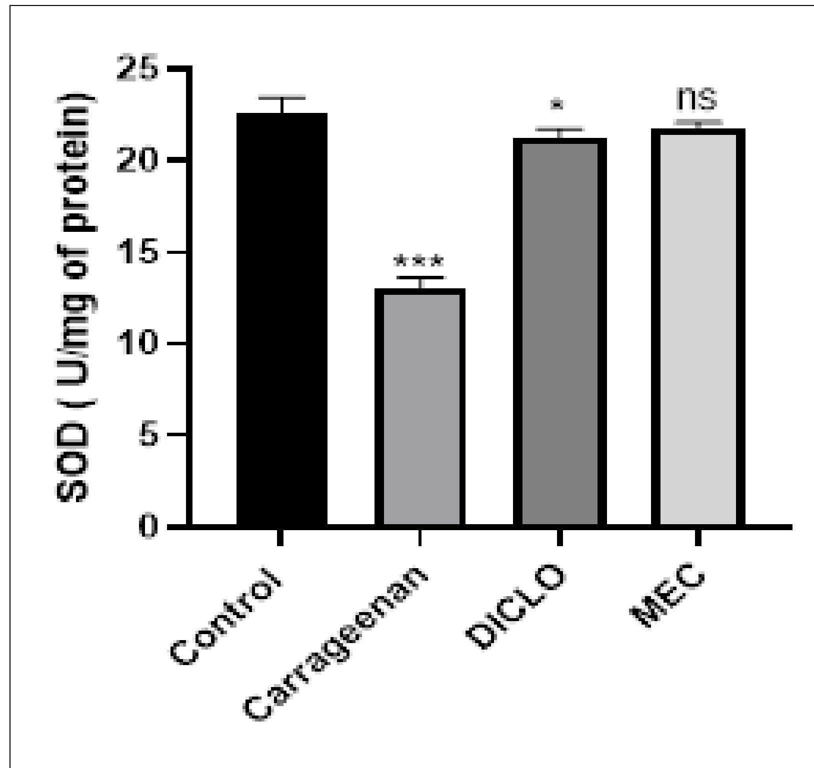

Fig 3-D: Levels of SOD activity in the paw of different groups of rats. Values represent means ± SEM (n=5) in each group. *** $p < 0.001$ compared to control.

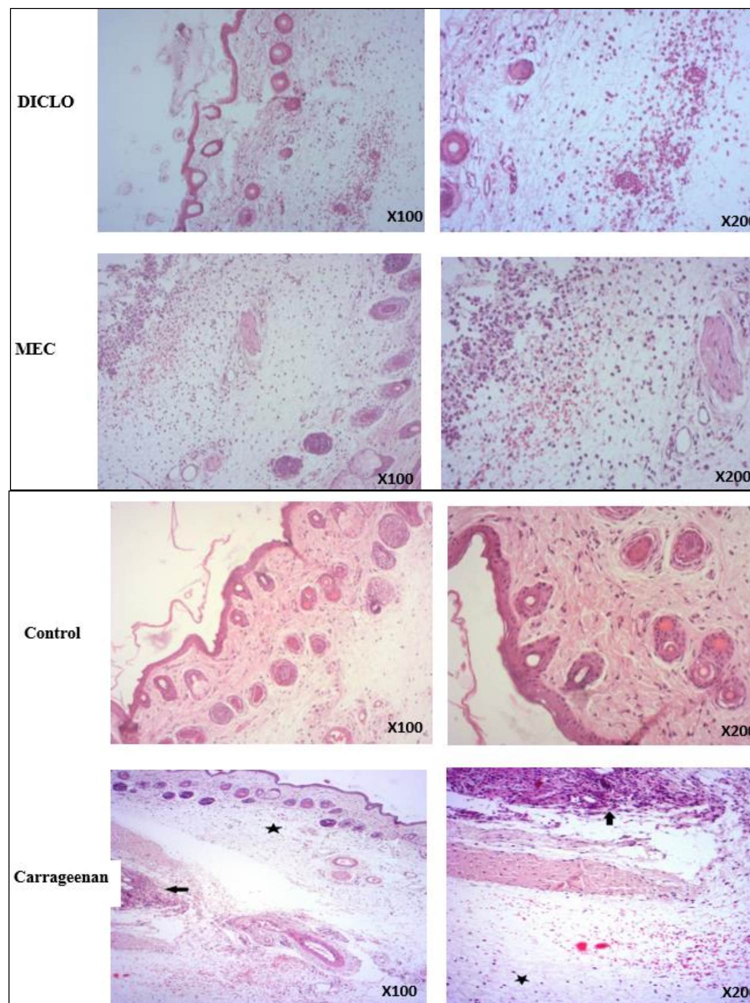

Fig 4: Representative photographs from the skin which confirmed the protective effect of MEC against carrageenan-induced inflammation ➡lymphocytic infiltration ★: Edema.

5. Conclusion

In conclusion, this work highlighted the anti-inflammatory activity *in vivo* of the methanolic extract of *Codium bursa* algae (MEC) using the carrageenan induced paw edema by reducing clinical signs of inflammation, infiltration of inflammatory cells, and vascular congestion associated with the amelioration of the endogenous skin antioxidant status.

6. Ethical statements

All animals' protocols were conducted in accordance with the Guide for the Care and Use of Laboratory of Animals issued by the University of Sfax, Tunisia and approved by the Committee of Animal Ethics.

7. Funding

This work was funded by the Tuniso-Moroccan project (code 20PRD16).

8. Conflict of interest

The authors declare that there are no conflicts of interest.

9. Acknowledgments

The current work was financially supported by the Ministry of Higher Education and Research of Tunisia.

10. References

1. Ammar I, Ben Salem M, Harrabi B, Mzid M, Bardaa S, Sahnoun Z. Anti-Inflammatory Activity and Phenolic Composition of Prickly Pear (*Opuntia ficus-Indica*) Flowers. Ind Crops Prod. 2018;112:313-319. DOI:10.1016/j.indcrop.2017.12.028.
2. Gyurkovska V, Alipieva K, Maciuk A, Dimitrova P, Ivanovska N, Haas C. Anti-Inflammatory Activity of Devil's Claw *in vitro* Systems and Their Active Constituents. Food Chem. 2011;125:171-178. DOI:10.1016/j.foodchem.2010.08.056.
3. Souza CRM, Bezerra WP, Souto JT. Marine Alkaloids with Anti-Inflammatory Activity: Current Knowledge and Future Perspectives. Mar Drugs. 2020;18:147. DOI:10.3390/md18030147.
4. Feki A, Jaballi I, Cherif B, Ktari N, Naifar M, Makni Ayadi F, et al. Therapeutic Potential of Polysaccharide Extracted from Fenugreek Seeds against Thiamethoxam-Induced Hepatotoxicity and Genotoxicity in Wistar Adult Rats. Toxicol Mech Methods. 2019;29:355-367. DOI:10.1080/15376516.2018.1564949.
5. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein Measurement with the Folin Phenol Reagent. J Biol Chem. 1951;193:265. DOI:10.1007/978-94-007-0753-5_100521.
6. Draper HH, Hadley M. Malondialdehyde Determination as Index of Lipid Peroxidation. Methods Enzymol. 1990;186:421-31. DOI:10.1016/0076-6879(90)86135-I.
7. Beauchamp C, Fridovich I. Superoxide Dismutase: Improved Assays and an Assay Applicable to Acrylamide Gels. Anal Biochem. 1971;44:276-287. DOI:10.1016/0003-2697(71)90370-8.
8. Flohé L, Günzler WA. Assays of Glutathione Peroxidase. Methods Enzymol. 1984;105:114-21. DOI:10.1016/s0076-6879(84)05015-1.
9. Pandey MM, Shukla G, Goutam A, Rastogi S, Rao CV. Biological Potential of Potentilla Fulgens Extract on Acute Inflammation, Nocioception Tolerance, Oxidative Stress and Its Phytochemical Constituents. Food Chem Adv. 2024;4:100563. DOI:10.1016/j.focha.2023.100563.
10. Pérez-Guerrero C, Herrera MD, Ortiz R, Alvarez de Sotomayor M, Fernández MA. A Pharmacological Study of Cecropia Obtusifolia Bertol Aqueous Extract. J Ethnopharmacol. 2001;76:279-284. DOI:10.1016/S0378-8741(01)00253-7.
11. Jisha N, Vysakh A, Vijeesh V, Latha MS. Anti-Inflammatory Efficacy of Methanolic Extract of *Muntingia calabura* L. Leaves in Carrageenan Induced Paw Edema Model. Pathophysiology. 2019;26:323-330. DOI:10.1016/j.pathophys.2019.08.002.
12. Celik GE, Schroeder JT, Hamilton RG, Saini SS, Adkinson NF. Effect of *in vitro* Aspirin Stimulation on Basophils in Patients with Aspirin-Exacerbated Respiratory Disease. Clin Exp Allergy. 2009;39:1522-31. DOI:10.1111/j.1365-2222.2009.03277.x.
13. Mezdour H, Menad A, Gherib A, Algabr M, Souad A. Immunomodulatory and Anti-Inflammatory Activities of Algerian Ulva Lactuca. World J Pharm Res. 2017;6:72-95. DOI:10.20959/wjpr201711-9478.