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Hemolymph Peptide Fraction Of The Algerian Mussel, Mitylus Galloprovincialis (Lamarck), Reduces Carragenan-Induced In- flammation In Rats

Dr. BOUAMAR Sarah[1], Dr. Siham Otsmane El Haou[2], Dr. SADOUD Meryem[3], Prof. Riazi Ali[4] [1]Department of Nutritional Sciences, Faculty of Nature and Life Sciences, Laboratory for the Environment, Natural Plant Substances and Food Technology, Ahmed Zabana University, postcode 48000, Relizane, Algeria. Laboratory of Beneficial Microorganisms, Functional Food and Health, Abdelhamid Ibn Badis University, post- code 27000, Mostaganem, Algeria, sarah.bouamara@univ-relizane.dz
[2]Food Science Department, Faculty of Nature and Life Sciences, Laboratory of Beneficial Microorganisms, Func- tional Food and Health, Abdelhamid Ibn Badis University, postcode 27000, Mostaganem, Algeria
Department of Biochemistry and Molecular Biology, College of Medicine, Seoul, Korea University, South Ko- rea.Molecular Medicine and Nutrition Research Institute, Seoul, South Korea, motsmaneelhaou20@gmail.com [3]Department of Food Sciences and Nutrition, Faculty of Nature and Life Sciences, Hassiba Benbouali University, postcode 02000, Chlef, Algeria, Laboratory of Beneficial Microorganisms, Functional Food and Health, Abdel- hamid Ibn Badis University, postcode 27000, Mostaganem, Algeria, ms.sadoud@univ-chlef.dz
[4]Food Science Department, Faculty of Nature and Life Sciences, Laboratory of Beneficial Microorganisms, Func- tional Food and Health, Abdelhamid Ibn Badis University, postcode 27000, Mostaganem, Algeria ardz22003@yahoo.fr

Corresponding author : Sarah Bouamar
Tel.: +213661615120
E-mail: sarah.bouamara@univ-relizane.dz
ORCID: https://orcid.org/0009-0009-2587-8657

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Abstact *In addition to their nutritional value and effects on well-being, mollusks in general and mussels in particular are a potential source of several bioactive compounds that have gained much more importance in the last decade due to their protein richness and their various uses as nutraceutical, pharmaceutical, therapeutic and functional food agents. In this study, the in vivo anti-inflammatory activity of Hemolymph Peptide Fraction (HPF) extracted from the hemolymph of the Algerian blue mussel, Mytilus galloprovincialis was evaluated on one hand by measuring the 1% carrageenan-induced edema in plantar fascia of left hind paw of rats, and on the other hand by the dosage of C-reactive protein (CRPr) and fibrinogen, as well as by the histological examination of the inflamed paws. The obtained results evidenced a progressive increase in the volume of plantar edema following the injection of 1% carrageenan, reaching its maximum threshold 4h thereafter in the negative control group (3.21 ± 0.006 mm). The two groups treated with the different concentrations of HPF (30 and 60 mg/kg body weight), showed a highly significant decrease ($p < 0.001$) in edema, starting 4h after the injection. HPF anti-inflammatory activity registered at 30 and 60 mg HPF/Kg body weight results in a percentages of inhibition (PI %) of carrageenan-induced edema in the Wistar rat paw of about 28.22 ± 0.31% and 42.74 ± 0.15%, respectively ; whereas CRPr and fibrinogen concentra- tions, recorded with the same HPF concentrations were 52 and 39 mg/L, and 6.5 and 4.5 g/L, respectively.*
Keywords: *Mytilus galloprovincialis; hemolymph peptide fraction (HPF); anti-inflammatory activity*

INTRODUCTION

Ocean serves as a reservoir for newly discovering potential therapeutic agents. Numerous compounds with inter- esting pharmaceutical activities have been reported from marine organisms during the past decades. Hence, marine organisms are considered as important source of bioactive molecules to treat various diseases. Emergence of antibiotic resistant microbes has increased the demand for the production of novel and effective antimicrobial compounds (Tsankova et al., 2021; Chakraborty and Joy. 2020). Marine invertebrates lack acquired memory type immunity which differentiates it from vertebrate immune system (Moreira et al., 2020). Innate immune mechanisms including humoral and cellular responses are observed in these organisms. Circulating hemolymph in themselves make this innate immune system more effective because it contains biologically active substances such as complement, lectin, clotting factors and antimicrobial peptides that fight against invading microorgan- isms (Pereiro et al., 2021; Parchebafi et al., 2022). Antimicrobial peptides (AMPs) are present in

all living organ- isms; they are involved in host defense against pathogenic microorganisms. AMPs are able to kill a wide range of cells and microbes, including bacteria
(Jeyasanta et al., 2020).

Inflammation is considered as a primary physiological defense mechanism that helps the body to protect itself against diferent stimulis. Tissue protein denaturation is one of the most documented topics due to its relationship with inflammatory and arthritic diseases. Lysosomal enzymes released during inflammation produce various disorders. The additional cellular activity of these enzymes is related to acute or chronic inflammation (Curpan et al., 2022). Inflammation is a complex process, often associated with pain and involving phenomena such as: increased vascular permeability, increased protein denaturation, and cell membrane alteration (Panayo- tova et al., 2020).

Inflammation is the response of living tissues to injury that involves a complex set of enzyme activation, mediator release and fluid extravasation, cell migration, tissue degradation, and repair. Various synthetic anti- inflammatory drugs available in the market are of least interest due to their side effects (Zamorano-Apodaca et al., 2020). In this study, an attempt is made to highlight how hemolymph peptides from the Algerian blue mussel, Mytilus galloprovincialis, can be considered as a natural anti-inflammatory compounds.

MATERIALS AND METHODS

Site and hemolymph sampling, and mussel farming. The 4-6 cm sized mussels, M. galloprovincialis, were col- lected in an ice container as needed for the experiment from the seawater of the port of Mostaganem in Algeria (35°55'52" N and 0°05'21''E) where they were raised to be available all year long.

The shells of the mussels were notched at the posterior adductor muscle level to facilitate hemolymph collection. Hemolymph (0.1mL/animal) was immediately taken from the posterior adductor muscle using a sterile 1mL syringe with a 30 G×1/2 (0.30×12.7mm) needle directly into an equal volume of Modified Alsever Solution (MAS) as an anti-aggregation buffer (Gonzalez et al., 2007). Hemolymph samples were held in ice to reduce hemocyte clumping, then immediately centrifuged at 800 x g for 15 min at 4 °C (Tasiemski et al., 2007; Mitta et al., 2000). Plasma (cell-free hemolymph) was kept at -20°C until use.

Peptide Extraction. The plasma was first diluted in distilled water (1v/1v) containing 0.1% (v/v) trichloro acetic acid. The pH was brought to 3.9 with 1 M HCl in an ice-cold water bath under gentle stirring for 30 min, then the mixture was centrifuged at 10 000 x g during 20 min at 4°C (Mitta et al., 2000)..

Solid Phase Extraction and Pre-purification. Plasma extracts were loaded onto SepPak C18 Vac cartridges (Wa- ters Associates, Switzerland) previously washed and equilibrated with acidified water (0.05% trifluoroacetic acid in Ultra-Pure Water, UPW). After what, two successive elutions were performed with 5 and 40 % (v/v) acetoni- trile in acidified water, successively. Then, the 5% (v/v) eluted fraction was discarded; while that of 40% (v/v) was lyophilized and reconstituted with UPW (Mitta et al., 2000).

Determination of the in vivo anti-inflammatory activity of Hemolymph Peptide Fraction (HPF) of Mytilus gallo- provincialis. The anti-inflammatory activity was determined by measuring the edema of rat's paw induced by the injection of 0.1 mL of 1% (W/V) carrageenan under the plantar fascia of the left hind paw of the rat (Khabbal et al., 2006), the dosage of C-reactive protein (CRPr) and fibrinogen, and by the histological examination of the inflamed paws.

Breeding conditions and batching of rats. Adult male Wistar rats weighing between 100 and 150 g (n = 20) provided from the Pasteur Institute in Algiers were used for the in vivo anti-inflammatory test. Batches or groups of 5 rats per cage were formed and placed in a properly ventilated room, at a controlled temperature (25 ± 1°C) under a 12-hour light/dark cycle. The cages are made of plastic, transparent and of standard dimensions: 55 cm long, 33 cm wide and 19 cm high. All rats were fed ad libitum with a standard diet in the form of pelleted sticks, and free access to drinking water. The rats were acclimatized to the laboratory environment for a period of two weeks before the experiment. They were deprived of food one night before the start of the experiment.

Induction of inflammation. Induction of inflammation in rats was performed according to the method described by Solanki et al. (2015) and Rateesh et al. (2009). Paw thickness (mm) was measured using a digital caliper (Mitutoyo Caliper) before carrageenan injection (V0) and every 60 min for the 6h following this injection (Vasudevan et al., 2006). The different batches or groups were made as follow:

- Group 1: negative control, rats received an intraperitoneal injection of 100 µL of a saline solution (NaCL 0.9%: W/V), and 1 hour later a subcutaneous injection of 100 µL of carrageenan 1% (W/V) in physiological water at the plantar fascia level of the right paw from the end towards the joint without exceeding it.
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- Group 2: positive control (standard), rats received an intraperitoneal injection of 100 µL of diclofenac 20 mg/kg body weight as a reference drug, and 1 hour after a subplantar injection of the right paw of 100 µL of carrageenan at 1% in physiological water.
- Groups 3 and 4: rats treated with the hemolymph peptide fraction (HPF) (test or experimental batches). They received respectively an intraperitoneal injection of 100 µL of 30 and 60 mg of HPF solutions/kg body weight, respectively, and 1 hour later, a subplantar injection in the right paw of 100 µL of 1% carrageenan in physiolog- ical water.

The percentage of inhibition of edema was calculated according to the following formula (Lanhers et al., 1992): Percentage inhibition = [(Vt Tt – V0) control – (Vt – V0) treated] × 100 ⁄ (Vt – V0) control

Vo: Volume of the paw before injection of carrageenan;

Vt: Volume of the paw at a given time interval after injection of carrageenan.

Rat sacrifice and blood sampling. At the end of the experiment (6 hours later), the rats were anesthetized with ether in a glass bell jar and blood was collected by puncture in the jugular aorta. The advantage of this technique is the possibility of collecting sterile blood and the ability to perform complete exsanguination (Elkadi et al., 2014). The blood obtained is transferred before 48h to the laboratory for analysis.

C-reactive protein (CRPr) assay. C-reactive protein is an acute phase protein that appears in the blood during inflammatory process. Changes in the level of proteins in the inflammatory response can be measured by im- munonephelometry and the evaluation of serum levels over time can have a high indicative value. CRP is a protein with high variation up to 1000 times the norm, with a short half-life (one day) and a short response time (6 to 12h). CRP was measured using the immuneturbidimetric technique (Marrack and Richards, 1971; Ritchie, 1967).

To do so, the blood was centrifuged at 2500 rpm at a temperature of 4°C for 15 min. 60 µL of serum are added with 1 mL of buffer R1 (phosphate NaCL pH 7.43, Polyethylene glycol 40 g/L and Sodium azide 0.95 g/L) and the absorbance was read at 340 nm against a blank containing NaCL 0.9% (W/V) + 1 mL of buffer R1. The absorbances were read before and after addition of 100 µL of anti-CRP antiserum R2 (phosphate pH 7.43, polyclonal anti-human CRP, sodium azide 0.95 g/L). The difference in absorbance was evaluated using the cali- bration curve made with five different concentrations of CRP according to a ready-to-use calibration kit. Reagents R1 and R2 were purchased from BIOLABO (Biolabo, Maizy-France).

Fibrinogen assay. Rat's blood was collected in citrated tubes, and rapidly centrifuged at 4000 rpm for 15 min (Biomnis, 2012). The assay is based on the chronometric evaluation of fibrinogen according to the method of Clauss (1957). In the presence of an excess of thrombin, the clotting time of the previously diluted plasma is proportional to the amount of plasma fibrinogen (Koenig, 2003). The test must be performed within a period not exceeding 4h after collection. To do so, the kit whose protocol is set by the manufacturer was used (Biolabo, Maizy-France). 200 µL of rat plasma or control diluted at 1/10 with the buffer solution (HEPES: (4-(2-hydroxy- ethyl)-1-piperazine ethanesulfonic acid) 0.02 M pH 3.75, anticoagulant (citrate), fibrinogen inhibitor were intro- duced into a hemolysis tube and incubated at 37°C. This dilution usually allows a coagulation time ranging from 8 to 25 seconds. The stopwatch is simultaneously started by adding 200 µL of thrombin (100NIH/mL) previously incubated at 37°C and the coagulation time is noted as soon as a thin filament of fibrin appears. A conversion table is used to determine the fibrinogen concentration corresponding to the coagulation time obtained (Biomis, 2012).

Histological examination of inflamed paws. The right paws of each group of rats were removed and fixed by immersion in a 10% (V/V) formaldehyde solution for 72h, and then the fixed tissues were embedded in paraffin to make 3 and 5 µm sections with a microtome. These sections were mounted on glass slides and stained with hematoxylin and eosin for optical microscopy analysis.

Statistical analysis. Statistical analysis was conducted using ANOVA analysis (Stat Box logiciel, Grimmer Soft; version 6.4, France). Comparisons were made using Student–Newman–Keuls test at threshold 5%. All data represent the mean values of triplicates obtained from three separate runs.

RESULTS AND DISCUSSION

Hemolymph peptide fraction (HPF) effect on the evolution of rat paw edema. The data in Table 1 clearly show a progressive increase in the plantar edema thickness as a consequence of carrageenan injection, reaching its maximum threshold 4 hours later in the negative control group (3.21 ± 0.006 mm). After the fourth hour, this

edema thickness decreases, reaching the value of 2.99 + 0.01 mm at the sixth hour. Indeed, at the sixth hour after the induction of inflammation, the thickness of the edema decreased to 2.63 + 0.008 mm and 2.5 + 0.009 mm with the HPF doses of 30 mg and 60 mg HPF/Kg body weight, respectively.

In addition, the administration of diclofenac (reference anti-inflammatory drug) prevented in a highly significant way ($p <$

0.001), the evolution of inflammation from 3h after injection of carrageenan in the standard group (2.61 ± 0.005 mm) compared to the negative control group (3.15 ± 0.01 mm).
In addition, the administration of reference anti-inflammatory drug (20 mg dichlofenac/Kg body weight) pre- vented in a highly significant way ($p < 0.001$), the evolution of inflammation from the third hour after the injection of carrageenan in the standard group (2.61 ± 0.005 mm) compared to the negative control group (3.15± 0.01 mm). Dichlofenac exerts a slightly higher anti-inflammatory effect than HPF.

Table 2 shows the inhibitory effect of hemolymph peptide fraction (EPF) of M. galloprovincialis and diclofenac on the development of inflammation as a function of time exposure to carrageenan. It can be noted that the maximum threshold was reached at time 6h after injection of carrageenan. Indeed, the inhibition rates (%) of paw edema obtained with 30 and 60 mg HPF/Kg of body weight were respectively 28.22 ± 0.31% and 42.74 ± 0.15%; while diclofenac used at a dose of 20 mg/Kg of body weight was more effective since the percentage of inhibition was more than 84.67 ± 0.028%.
Injection of carrageenan under the footpad causes a progressive increase in the edema thickness in control rats during the six hours of the experiment. However, the increase in paw thickness in the groups treated with HPF was less significant compared to the negative control group.

Table 1. Anti-inflammatory effect of hemolymph peptide fraction (HPF) compared with that of the standard, diclofenac, on the carrageenan induced-paw edema thickness (mm) in Wistar rat. Values represent the mean ± standard deviation (n = 5); Negative control (Nacl 0.9%); Standard: dichlofenac (20 mg/kg). a: $p < 0.05$; b: $p < 0.01$; c: $p < 0.001$ statistically significant differences compared to the negative control group.

		Paw edema thickness (mm) of Wistar rat		
Hour	**Negative control** (Nacl 0, 9%)	**Standard (diclofen** (20 mg/Kg)	**Hemolymph Peptide Fraction (HPF)**	
			30 mg/Kg	60 mg/Kg
0H	1.75 ± 0.011	1.78 ± 0.006	1.74 ± 0.022	1.79 ± 0.034
1H	2.76 ± 0.008	2.74 ± 0.005[a]	2.73 ± 0.006[a]	2.77 ± 0.005
2H	3.08 ± 0.008	2.85 ± 0.007[c]	3.05 ± 0.005[a]	2.99 ± 0.006[c]
3H	3.15 ± 0.01	2.61 ± 0.005[c]	3.08 ± 0.009[c]	3.06 ± 0.007[c]
4H	3.21 ± 0.006	2.37 ± 0.009[c]	3.06 ± 0.005[c]	2.93 ± 0.000[c]
5H	3.09 ± 0.009	2.13 ± 0.008[c]	2.86 ± 0.007[c]	2.78 ± 0.016[c]
6H	2.99 ± 0.01	1.97 ± 0.007[c]	2.63 ± 0.008[c]	2.5 ± 0.009[c]

Plantar edema is one of the main factors used for the evaluation of the degree of acute inflammation and the efficacy of new anti-inflammatory drugs (Begum and Sadique, 1988); and its carrageenan induction is commonly used as a standard experimental model (Banerjee et al., 2000).
This phenomenon is usually characterized by a biphasic response. The first phase (1-2h) depends on the release of chemical mediators such as histamine, serotonin and bradykinin. The second phase (3-6h) is supported by the release of prostaglandins, leukotrienes, lysozymes, proteases and nitric oxide (NO) (DiRosa et al., 1971; Olajide et al., 1999; Posadas et al., 2004).

Table 2. Inhibition rate (%) of carrageenan-induced paw edema in Wistar rats by hemolymph peptide fraction (HPF) compared to that of the standard, diclofenac.

	inhibition rate (%) of paw edema in Wistar rats

Hour	Standard (diclofenac) (20 mg/Kg	Hemolymph tion(HPF)	Peptide Frac-
		30 mg/Kg	60 mg/Kg
1H	13.86 ± 0,021	1.98 ± 0.056	2.97 ± 0.18
2H	19.54 ± 0.014	1.5 ± 0.42	9.77 ± 0.049
3H	40.71 ± 0.042	4.28 ± 0.03	9.28 ± 0.06
4H	59.58 ± 0.028	9.59 ± 0.014	21.91 ± 0.00
5H	73.88 ± 0.00	16.42 ± 0.00	26.12 ± 0.16
6H	84.67 ± 0.028	28.22 ± 0.31	42.74 ± 0.15

Eicosanoids (leukotrienes and prostanoids) induce the biosynthesis of elastase, collagenase and other com- pounds. These enzymes break down structural proteins into peptides causing vasodilation and increased vascular permeability and hydrostatic pressure. This, in turn, leads to the formation of edema and the migration of neu- trophils to damaged tissues (Havsteen, 2002).The latter produce oxygen-derived free radicals such as superoxide anion (O2-), hydroxyl radical (OH˙) and hydrogen peroxide (H2O2), which contribute to the inflammatory cellular damage (Posadas et al., 2004; Kumar and Kuttan, 2009). Furthermore, cyclooxygenase-2 (COX-2), en- zymes inducible by cytokines (tumor necrosis factor alpha TNFα and interleukins IL-6 and IL-1β), are present in activated inflammatory cells, and are fully involved known to play a crucial role in inflammation process by releasing the prostanoid mediator (prostaglandins and thromboxane) (Nussler and Billiar, 1993; Nantel et al., 1999).

Similarly, the inducible enzyme, nitric oxide synthase (iNOS), generates nitric oxide (NO) and is considered to have a central role in inflammatory responses (Fialkow et al., 2007). It is generally recognized that iNOS and COX-2 expression is maximal during the second phase (3–6 h) of plantar edema (Loram et al., 2007; Eddouks et al., 2012). This phase is sensitive to the majority of clinically effective anti-inflammatory drugs (Vinegar et al., 1969). Inhibition of NO and prostaglandin production by suppression of iNOS and COX-2 expression has been shown to be beneficial in the treatment of inflammatory diseases (Sawatzky et al., 2005).

In the present experiment, the hemolymph peptide fraction (HPF) of M. galloprovincialis reared in the sea's waters of the Mostaganem port, and tested at two different concentrations (30 and 60 mg/kg body weight), markedly inhibited inflammation process from the fourth hour following the carrageenan injection in compari- son with the negative control group.

Such observation suggests that at the HPF doses administered, the peptide fraction would have prevented the release of pro-inflammatory mediators of the late phase (3 – 6 hours) by inhibiting the expression of the main precursors (iNOS and COX-2) (Vigo et al., 2004). In addition, due to their antioxidant properties, the latter could also have acted by inhibiting the production of reactive oxygen species (ROS) responsible for cell damage (Middleton et al., 2000; Preethi et al., 2012)..

Effect of hemolymph peptide fraction (HPF) of Mytilus galloprovincialis on markers of inflammation

Effect of HPF on rat C-reactive protein (rCRP). The dosage of C-reactive protein was carried out by an automated immunoturbidimetric technique which gave the results reported in Table 3. Six hours after the injection of 1% carrageenan, negative control (diclofenac or HPF-untreated rats) rCRP level increased up to 70 mg/L. The rCRP concentrations recorded in the HPF-treated group at the two concentrations 30 and 60 mg/kg of body weight (52 and 39 mg/L, respectively), are much higher than that of the positive control (dichlofenac-treated rats) (9 mg/L). CRP is a pentraxin found in most vertebrates (mice, rats, humans) and invertebrates such as the horse- shoe crab (Limulus polyphemus) (Etlinger and Coe, 1986; Nakanishi et al., 1991; Shrive et al., 1999).

Human C-reactive protein (hCRP) is an acute phase inflammatory protein whose plasma concentrations can increase up to 1000-fold following tissue damage or infections (Padilla et al., 2003). hCRP also binds to phos- phorylcholine found in membrane phospholipids. Among the effector functions exerted by hCRP upon ligand binding, there is the in vivo and in vitro activation of complement (Nakanishi et al., 1991; Wolbink et al., 1996). In rats, rCRP is not a typical protein of the acute inflammatory phase compared to serum haptoglobin and plasma fibrinogen (Giffen et al., 2003). However, unlike humans, rats have much higher plasma CRP concen- trations, approximately 300-500 mg/L, which is 100 times higher than the concentration in humans. Unlike hCRP, rCRP is unable to activate complement despite an amino acid

homology of 70%. It is also noteworthy that rCRP does not activate complement using the polysaccharide of Streptococcus pneumoniae and can there- fore activate it when it binds to other ligands (Eldahshan and Azab, 2012).

As for the group of rats treated with the standard anti-inflammatory, dichlofenac, it presents a value significantly lower than that of the batches treated with HPF. Indeed, the CRPr rate in rats in the standard group (9 mg/L) is lower than that of rats in batches treated with 30 mg/kg (52 mg/L) or 60 mg of EPF/Kg body weight (39 mg/L).

These results clearly show a significant anti-inflammatory effect of hemolymph peptide fraction (HPF) of Mytilus galloprovincialis reared in the waters of the port of Mostaganem compared to the batch of untreated rats, but remains slightly lower than the anti-phlogogenic effect of diflonenac. Such results highlight the positive correla- tion between HPF concentration and its anti-inflammatory activity.

Here, in all animal species studied, a rapid increase in CRP concentration is observed within hours of an inflam- matory attack (Eckersall et al., 1996; Burger et al., 1992). The cytokines of the inflammatory reaction (CRI) released into the circulation will be responsible for the systemic response of the inflammatory reaction and the increase in the plasma concentration of a number of hepatic proteins called proteins of the positive inflammatory reaction (PRI+) such as C-Reactive Protein (CRP) (Engler, 1993).

Effect of HPF on serum fibrinogen level. Fibrinogen is a soluble plasma protein synthesized in the liver, and used as a specific marker of inflammation in humans but also in rats (Engler, 1993). Fibrinogen assay is currently used in routine practice in clinical analysis to highlight inflammation cases. Thus, in inflammatory states (infec- tions, lymphomas, cancers, rheumatoid diseases), the fibrinogen level can reach 6 to 10 g/L (Baijot, 2006).

According to Giffen et al. (2003), there is a positive correlation between CRP and fibrinogen levels in Wistar rats; suggesting the importance of fibrinogen as a marker of inflammation. The increase in fibrinogen levels in the negative control group (8 g/L) was due to the exposure of rats to carrageenan-induced inflammation; while HPF reduced inflammation (Table 3).

Table 3. Effect of hemolymph peptide fraction (HPF) of M. galloprovincialis (30 and 60 mg HPF/Kg body weight) compared to that of the standard, dichlofenac (20 mg/Kg body weight), on the concentrations (mg/L) of C-reactive protein (mg/L) and fibrinogen (g/L) in Wistar rats given 1% carrageenan.

Group of experimental rats	**C-reactive protein (mg/L)**	**fibrinogen (g/L)**
Negative control rats (NaCL 0.9%)	70	8
Standard rats injected with 1% carrageenan and treated with 20 mg diclofenac/Kg body weight (positive control)	9	3
Rats injected with 1% carrageenan and treated with 30 mg HPF/kg body weight (sample 1)	52	6.5
Rats injected with 1% carrageenan and treated with 60 mg HPF/kg body weight (sample 2)	39	4.5

Histological appearance of the Wistar rat paw. Six hours after carrageenan-induced inflammation, an histological study of paw tissue was carried out, and the results are reported in Figure 1. Histological examination of the rat paws showed a reduction in the inflammatory response induced by HPF and diclofenac treatment. Microscopic
examination of paw biopsies from Wistar rats that received carrageenan but were not treated (negative control) showed a massive accumulation of infiltrated inflammatory cells (Fig. 1a). In contrast, biopsies from the same rats but treated with diclofenac (standard) or HPF (30 and 60 mg/kg) showed a reduction in the inflammatory response (Fig. 1 b, c and d).

The number of neutrophils was higher in the paw tissues of untreated rats than in those of paws treated with the standard anti-inflammatory, diclofenac. The hemolymph peptide fraction (HPF) of Mytilus galloprovincialis reduces neutrophils in inflamed tissues. According to the results of this study, the effect of HPF is dose-depend- ent.

Inflammation is a defense reaction of the body against various attacks that can be of physical, chemical, biological (immune response) or infectious origin. Current treatment of inflammation uses steroidal (glucocorticoids) and nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin. These molecules are effective but their pro- longed use can cause serious side

effects, the most common of which are gastrointestinal bleeding and gastrodu- odenal ulcers (Yamada et al., 1987). Therefore, it is necessary to develop new effective anti-inflammatory agents with minimal side effects..

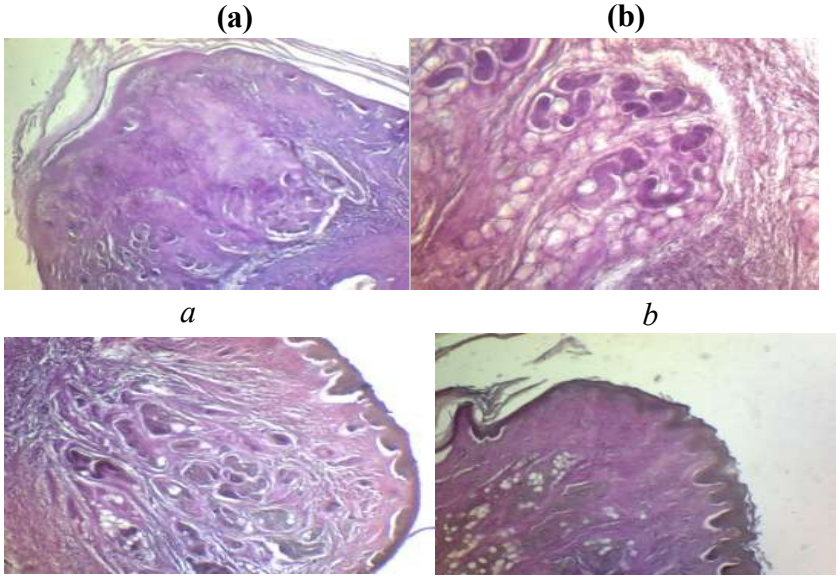

Fig. 1. Histological sections of rat paws inflamed by 1% carrageenan: a- untreated (negative control), b- treated with dichlofenac (20 mg/Kg body weight) as a standard anti-inflammatory (positive control), c- treated with 30 mg EPF/Kg body weight (sample 1), d- treated with 60 mg EPF/Kg body weight (sample 2).

CONCLUSION

The diversity of living organisms in the marine world offers significant potential for the discovery and exploita- tion of bioactive natural substances. Within this considerable biodiversity, marine invertebrates continuously exposed to surrounding pathogenic microorganisms and equipped only with a primitive immune system, without memory, represent a unique reservoir of bioactive defense molecules. Antimicrobial peptides are key elements of the defense system of marine invertebrates. Their purification and characterization are of considerable interest for the identification of new natural molecules, biochemically original and effective trough the health benefits
they provide. However, so far, a limited number of bioactive peptides have been identified from marine organ- isms, most of which harboring a particular biological activity have not yet been discovered and explored. Thus, existing exploration, isolation and purification techniques must be furtherly improved in order to discover more marine bioactive peptides. Furthermore, the most important problem is the application of bioactive peptides to human health and nutrition, since most of the research remains at the stage of in vitro or in vivo experimentation due to the time required and cost issues. Given the ubiquitous nature of antimicrobial peptides, we focused on the identification of such molecules in bivalve molluscs, in this case the mussel Mytilus galloprovincialis. This study highlighted the anti-inflammatory power of hemolymph peptides and represents a preamble to their bio- chemical characterization and the determination of their mechanisms of action.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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