Evaluation of anti-inflammatory, analgesic and gastroprotective activities of *Eunicella singularis* fractions using in *vivo* assays

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ABSTRACT

Objective: The research and development of potent bioactive components with pharmacological properties has received much attention in the recent years. So, the anti-inflammatory, analgesic and gastroprotective activities of the organic extract and its polar fractions (F2 and F3) from the Mediterranean white gorgonian *Eunicella singularis* were investigated.
Methods: Anti-inflammatory and analgesic activities were evaluated, using the carrageenan-induced rat paw edema model and the acetic acid writhing test in mice. The gastroprotective activity was determined using HCl/EtOH induced gastric ulcers in rats.
Results: The organic extract exhibited interesting anti-inflammatory, analgesic and gastroprotective activities in a dose dependent manner. Similarly the fractions F2 and F3 showed, respectively, an interesting anti-inflammatory activity associated with significant analgesic and gastroprotective activities, efficacies that correlated with their bioactive components and total phenol content.
Conclusions: Our findings confirmed that the anti-inflammatory, analgesic and gastroprotective activities of *Eunicella singularis* organic extract and its polar fractions could be correlated with their bioactive components and total phenol content.

KEY WORDS: ***Eunicella singularis***
Anti-inflammatory
Analgesic
Gastroprotective

Introduction

Inflammation is a protective response to tissue injury, infection or irritation. Sometimes, the signs and symptoms of inflammation such as redness, swelling, heat, pain and loss of function of the affected area may be potentially harmful and need pharmacological treatment [1]. Currently, Non-steroidal anti-inflammatory drugs (NSAIDs) are used throughout the world for the treatment of inflammation, pain and fever, however most of these develop resistances and several adverse reactions such as ulcers and hemorrhage [2]. Therefore, the development of potent anti-inflammatory drugs from natural sources with fewer side effects is necessary [3]. In recent years, the soft corals and gorgonians of Octocorallia have become a new marine resource for the searching of novel bioactive natural products as lead compounds in drug development [4]. The family Gorgoniidae has been demonstrated to contain a wide variety of natural products including steroids, acetogenins, sesquiterpenes and diterpenes [5].

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Among the substances that currently receive the most attention for drug design include the diterpenes purified from gorgonian, possessing a variety of pharmacologic effects, including anticancer, gastroprotective and anti-inflammatory properties [6-8]. Despite the biodiversity of the Mediterranean Tunisian coast, minimal efforts on the chemistry and biological activities of marine organisms

have been conducted [9-10]. Therefore, in a program to find new natural sources of anti-inflammatory and gastro-protective agents from Tunisian marine invertebrates, we studied the efficiency of organic extract and its polar fractions from the white gorgonian *Eunicella singularis* (Cnidaria: Octocorallia) Esper 1794, characteristic of coralligenous biocenosis and one of the most abundant and widely distributed species in the Mediterranean sea [11].

Materials and methods

Sample collection

Eunicella singularis was collected from the Mediterranean Sea in various areas of the coastal region of Tabarka (Tunisia), in June 2010, at a depth between 20 and 30 m. Identification of specimens was carried out in the National Institute of Marine Sciences and Technologies (Salamboo, Tunisia).

Preparation of the organic extract

The organic extract of *Eunicella singularis* was prepared by maceration of finely powdered material packed in small bags (5 x 10 cm) of Whatman filter paper no. 1 in methanol and dichloromethane (1:1, v/v) for 48h three times. The organic extract was concentrated to solvent free by evaporation in a rotating evaporator (Buchi, B-480) at 40°C.

Purification of the organic extract

In order to localize the active fraction, the organic extract of *Eunicella singularis* was purified, using C18 cartridges (Sep-pack, Supelco), by gradient elution with methanol-water mixture (10%, 50%, and 80% methanol) to give three fractions (F1-F3). Methanol was removed from recuperated fractions using a rotating evaporator at 40 °C and the aqueous phases were lyophilized.

Animals

Male adult Wistar rats weighing 150-200g, and Swiss albino mice weighing 18-25g of both sex were obtained from Pasteur Institute (Tunis, Tunisia). Housing conditions and in *vivo* experiments were approved according to the guidelines established by the European Union on Animal Care (CCE Council 86/609).

Carrageenan-Induced Rat Paw Edema

The anti-inflammatory activity of the organic extract and its polar fractions on carrageenan-induced paw edema was determined according to Winter et al. [12].

Writhing assay in mice

The analgesic activity was performed according to the method of Koster et al. [13].

Gastric lesions induced by HCl/ethanol

The gastroprotective activity of the organic extract and its polar fractions derived from *Eunicella singularis* was studied in 150 mM HCl/EtOH induced gastric ulcer [14].

Statistical analysis

Data is presented as the mean ± standard error (s.e.m). Statistical analysis was performed using Student's t-test. The significance of difference was considered to include values of $P<0.05$.

Results

Anti-inflammatory activity

In this study, the anti-inflammatory activity of the organic extract of *Eunicella singularis* and its polar fractions F2 and F3 were evaluated using the carrageenan-induced rat paw edema model. When injected locally into the rat hind paw of the control group, carrageenan induced a severe inflammatory reaction, discernible within 30 min that persisted until the end of measurement. The maximum peak was observed between 3 and 5h after injection. Administration of the organic extract of *Eunicella singularis* produced a significant reduction of edema throughout the period of observation in a dose-related manner. Interestingly, the highest reduction of the edema of the organic extract was at 3h with, 45, 60 and 65% of inhibition at the doses of 50, 100 and 200 mg/kg, respectively with ED_{50} of 63.34mg/kg. After the administration of the polar fractions (F2, F3), significant activity was observed with F3 at 25 and 50mg/kg i.p, at the third hour after carrageenan injections, with 54.5 and 66% reduction in paw volume, respectively, (ED_{50} = 19.2 mg/kg) whereas F2 inhibited edema by 59% at the third hour at a dose of 50 mg/kg. The reference drug, ASL (300mg/kg) decreased paw edema by 53% at the third hour (Table 1).

Table 1. Anti-inflammatory effect of the intraperitoneal administration of *Eunicella singularis* organic extract and its polar fractions (F2, F3) and of reference drug (Acetylsalicylate of Lysine; ASL) in carregeenan-induced rat paw edema test.

Treatment	Dose (mg/kg)	edema (10^{-2}ml) (mean ±s. e. m)			edema inhibition (%)		
		1h	3h	5h	1h	3h	5h
Vehicle	-	32.25±2.38	54.75±3.3	55.5±4.69	-	-	-
Organic extract	50	27.31±3.55*	30.18±2.64**	32.01±3.78**	15.31	44.87	42.31
	100	21.16±4.51*	21.76±5.74**	24.25±3.86**	34.36	60.25	56.29
	200	17.41±2.13*	19.29±3.43**	20.92±3.93**	45.99	64.75	62.3
F2	25	23.91±1.5	37.58±2.4	46.44±3.8	25.86	31.36	16.31
	50	15.33±3.16*	22.52±2.09**	25.65±2.71**	52.45	58.85	53.78
F3	25	18.22±2.1*	24.88±3.5**	37.39±3.5	43.5	54.54	32.63
	50	12.5±2.5*	18.75±1.16**	20.25±1.5**	61.24	65.75	63.51
ASL	300	21.16±2.25*	25.85±2.31*	26.71±2.25**	34.36	52.78	51.87

Values are expressed as mean ± SEM; n=6 animals. *P<0.01, **P<0.001.

Analgesic activity

Analgesic properties of the organic extract of *Eunicella singularis* and its polar fractions are done using the acetic acid writhing test in mice. The subcutaneous administration of the organic extract of *Eunicella singularis* (100, 200 and 400 mg/kg) produced a significant reduction in the number of abdominal constrictions throughout the entire period of observation in a dose related manner with respectively 16.57, 40.44 and 64.35% in the acetic acid writhing test in mice (ED_{50}= 295.66 mg/kg). Whereas at the same time, F2 (25, 50 and 100 mg/kg) inhibited writhing by 38.46, 61.53 and 81.06 %, respectively with ED_{50} = 40.14 mg/kg and F3 (25, 50 and 100 mg/kg) by 27.65, 59.76 and 71 %, respectively with ED_{50}= 43.7 mg/kg. The reference drug (ASL, 200 mg/kg) inhibited writhing by 65, 16% (Table 2).

Table 2. Analgesic effect of the subcutaneous administration of organic extract of *Eunicella singularis* and its polar fractions, and the reference drug lysine acetylsalicylate (ASL) in the acetic acid 1% writhing test in mice.

Sample	Dose (mg/kg)	Number of writhes ± SEM	Inhibition of writhing (%)
Vehicle	-	86±2.52	-
ASL	200	29.96±2.4**	65,16
Organic extract	100	71.74±1.6	16,57
	200	51.22±2.6	40,44
	400	30.65±2.32**	64,35
F2	25	52.92±3.4	38,46
	50	33.08±2.4**	61,53
	100	16.28±2.5**	81,06
F3	25	62.22±1.4	27,65
	50	34.6±2.3**	59,76
	100	24.93±1.4**	71,01

Values are expressed as mean ± s. e. m. *P < 0.01, **P < 0.001. n = 6 animals.

Gastroprotective activity

HCl/EtOH-ulcer is a classic ulcer model frequently used for the evaluation of antiulcerogenic activity. Pretreatment with *Eunicella singularis* organic extract (50, 100 and 200mg/kg, i.p.) and polar fractions (25, 50 and 100mg/kg, i.p.) produced significant decrease in the intensity of gastric mucosal damages induced by the necrotizing agent HCl/EtOH compared with control group. Percentages of inhibition of gastric lesions were 75%, and 80.46% with organic extract (200mg/kg), and F2 (100mg/kg), respectively. The highest percentage of inhibition (86.81 %) was observed with the fraction F3 (100mg/kg) which approaches the percentage of omeprazole (87.5 %) used as reference drug. The IC_{50} of both fractions (F2 and F3) was 26.12mg/kg whereas the IC_{50} of the organic extract was 49.34mg/kg (Table 3).

Table 3. Effect of *Eunicella singularis* organic extract and its polar fractions on gastric ulcer induced by HCl/ethanol in rats.

Treatment	Dose (mg/kg)	Ulcer index (mm)	Inhibition (%)
Control 1	-	78.5±5.49	-
Organic extract	50	31.52±2.42**	59.84
	100	24.46±2.48**	68.84
	200	19.49±3.48**	75.16
Control 2	-	76.33±2.51	-
Fraction F2	25	47.51±5.68*	43.88
	50	21.98±2.12*	74.03
	100	14.91±2.8	80.46
Fraction F3	25	47.35±1.25*	44.07
	50	20.99±0.57*	75.2
	100	10.06±2.7	86.81
Control 3	-	84.66±2.88	-
Cimetidine	100	16.48±2.08*	79.07
Omeprazole	30	9.82±0.81*	87.53

Values are expressed as mean ± SEM; n=6 animals.*P<0.01, **P<0.001.

Discussion

In this study, we have evaluated the in vivo anti-inflammatory, analgesic and gastroprotective properties of the organic extract of *Eunicella singularis* and its polar fractions F2 and F3.

Carrageenan-induced rat paw edema model was used for the evaluation of the anti-inflammatory activity. The administration of the organic extract of *Eunicella singularis* and its polar fractions F2 and F3 reduced the volume of edema in a dose dependant manner especially at the third hour. In the carrageenan model, the early phase (1–2 h) is mainly mediated by histamine, serotonin and the increase of prostaglandin (PG) synthesis in the surroundings of the damaged tissues while the late phase is mainly mediated by bradykinin, leukotrienes, polymorphonuclear cells and PGs produced in tissue macrophages [16-17]. In this experiment, the anti-inflammatory activity of *Eunicella singularis* organic extract and its polar fractions suggests that they had the ability to interfere with some of these mediators by inhibiting their production or antagonizing their action. Indeed, the preliminary qualitative chemical screening of the organic extract of *Eunicella singularis* and its polar fractions F2 and F3 revealed the presence of alkaloids, glycosides, terpenoids and saponins [12].

Several studies reported that glycosides, alkaloids, terpenoids and steroids present in gorgonian have anti-inflammatory properties [18-6]. The anti-inflammatory activity exerted by the polar fractions of *Eunicella singularis* is probably due to the effect of glycosides and terpenoids present in these fractions.

In acetic acid writhing test in mice, the organic extract and its polar fractions F2 and F3 reduced significantly the number of writhing in a dose dependant manner. In effect, acetic acid acts indirectly by inducing the release of endogenous mediator, which stimulates the nociceptive neu-

rons sensitive to NSAIDs (nonsteroidal anti-inflammatory drugs) and/or opioids [19]. Terpenoids isolated from marine sponge have promising analgesic activity [20].

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely prescribed for treatment of pain and inflammation. However, their use is associated with the occurrence of digestive adverse effects, such as gastric erosions and ulceration [21]. Reduction in intrinsic production of prostaglandin (PG) in the gastric mucosa and the increase in production of pro-inflammatory cytokines (such as IL-6, IL-1$_\beta$ and TNF-∞) in the gastric and plasma are intimately associated with the gastric mucosal damage induced by NSAIDs [22-23]. In this study, HCl/EtOH induced ulcer in rats was used for the evaluation of gastroprotective effect of *Eunicella singularis* organic extract and its polar fractions (F2 and F3). Based on the percentages of inhibition of gastric lesions calculated, the organic extract, F2 and F3 protected stomach from the mucosal damages caused by the necrotizing agent HCl/EtOH.

Several mechanisms have been suggested for the effect of antiulcerogenic compounds, including increase in the gastric hexosamine level and enhancing the strength of the gastric barrier either physically or by blocking the H+, K+/ATPase pump [24]. Probably, the compounds present in *Eunicella singularis* organic extract and its polar fractions might exert their activities by one or more of these proposed mechanisms. Among the main classes of chemical bioactive components capable of offering gastroprotection are the alkaloids, saponins, and triterpenes [25-27]. Several terpenes or their derivatives have been shown to possess gastroprotective activities in different models of induced gastric lesions in animals [28-31].

This effect seems to be related with an increase of the defensive mechanisms of the stomach, such as prostaglandin synthesis and mucus production [32]. The presence of alkaloids, saponins and terpenes in the organic extract of *Eunicella singularis* may be responsible for the gastroprotective activity. In addition, the presence of terpenoids in polar fractions can explain their gastroprotective effect.

In conclusion, polar fractions of *Eunicella singularis* had interesting anti-inflammatory and analgesic activities associated with gastroprotective activity. The presence of chemical bioactive components in the different fractions of *Eunicella singularis* may be responsible for their anti-inflammatory, analgesic and gastroprotective properties. Further chemical investigations are needed to characterize the bioactive components responsible for its activities.

Conflict of Interest

We declare that we have no conflict of interest.

References

1 Bowman WC, Rand MJ. Text Book of Pharma-cology. 2nd Ed. Blackwell Science Publication London. 1980; 13: 1-35.

2 Hajhashemi V, Sajjadi SE, Heshmati M. Anti-inflammatory and analgesic properties of Heracleum per-sicum essential oil and hydro alcoholic extract in animal models. J Ethnopharmacol. 2009; 124: 475–480.

3 Saha A, Ahmed M. The analgesic and anti-inflammatory activities of the extracts of Albizia lebbeck in animal model. Pak J Pharm Sci. 2009; 22(1): 74-77.

4 Wang C, Liu H, Shao C, Wang Y, Li L, Guan H. Chemical defensive substances of soft corals and gorgonians. Acta Ecol Sin. 2008; 28 (5): 2320-2328.

5 Berrue F, Kerr RG. Diterpenes from gorgonian corals. Nat Prod Rep. 2009; 26: 681-710.

6 Ioannou E, Abdel-Razik AF, Alexi X, Vagias C, Alexis MN, Roussis V. Pregnanes with antiproliferative activity from the gorgonian Eunicella cavolini. Tetrahedron. 2008; 64: 11797-11801.

7 Ioannou E, Abdel-Razik AF, Alexi X, Vagias C, Alexis MN, Roussis V. 9,11-Secosterols with antiproliferative activity from the gorgonian Eunicella cavolini. Bioorgan Med Chem. 2009; 17: 4537-4541.

8 Hiruma-Lima CA, Toma W, Gracioso JS, Al-meida ABA, Batista LM, Magri L, et al. Natural trans-crotonin: the antiulcerogenic effect of another diterpene isolated from the bark of Croton cajucara Benth. Biol Pharm Bull. 2002; 25: 452–456.

9 Ismail H, Lemriss S, Ben Aoun Z, Mhadhebi L, Dellai A, Kacem Y, et al. Antifungal activity of aqueous and methanolic extracts from the Mediterranean Sea Cucumber, Holoturia polii. J Med Mycol. 2008; 18: 23-26.

10 Dellai A, Laroche-Clary A, Mhadhebi L, Robert J, Bouraoui, A. Anti-inflammatory and antiproliferative activities of crude extract and its fractions of the defensive secretion from the mediteranean sponge, Spongia officinalis. Drug Develop Res. 2010; 71: 412-418.

11 Weinberg S. Autoecology of shallow-water octocorallia from Mediterranean rocky substrata. II. Marseille Cote d'Azur and Corsica Bijdr Dierkd 1980; 50 (1): 73-86.

12 Deghrigue M, Dellai A, Akremi N, Le Morvan V, Robert J, Bouraoui A. Evaluation of antiproliferative and antioxidant activities of the organic extract and its polar fractions from the Mediterranean gorgonian Eunicella singularis. Environ Toxicol Phar. 2013; 36: 339-346.

13 Winter CA, Risley EA, Nuss GW. Carrageenan induced edema hind paw of the rat as an easy for anti-inflammatory drugs. Proc Soc Exp Biol Med 1962; 3: 544-547.
14 Koster R, Anderson M, De Beer EJ. Acetic acid for analgesic screening. Fed Proc. 1959; 18: 418 – 420.
15 Mizui T, Doteuchi M. Effect of polyamines on acidified ethanol-induced gastric lesions in rats. Jpn J Pharmacol. 1983; 33: 939- 945.
16 Vinegar R, Truax JF, Selph JH, Johnsstone PR, Venable AL, Mckenzie KK. Pathway to carrageenan-induced inflammation of the hind limb of the rat. Fed Proc. 1987; 6: 118 – 126.
17 Antonio MA, Brito ARMS. Oral anti-inflammatory and anti-ulcerogenic activities of a hydroalcoholic extract and partitioned fractions of Turnera ulmifolia (Turneraceae). J Ethnopharmacol. 1998; 61: 215– 228.
18 Radjasa OK, Vaske YM, Navarro G, Vervoort HC, Tenney K, Linington RG, et al. Highlights of marine invertebrate-derived biosynthetic products: their biomedical potential and possible production by microbial associants. Bioorgan Med Chem. 2011; 19: 6658–6674.
19 Collier HOJ, Dineen LC, Johnson CA, Schneider C. Abdominal constriction response and its suppression by analgesic drugs in the mouse. B J Pharmacol Chemother. 1968; 32: 295–310.
20 de Pasquale R, Circosta C, Occhiuto F, de Rosa S, de Stefano S. Pharmacological studies on terpenoids from marine sponges: Analgesic and muscle relaxant effects. Phytother Res. 1991; 5 (2): 49-53.
21 Wallace JL. Pathogenesis of NSAID-induced gastroduodenal mucosal injury. Best Pract Res Cl Ga. 2001; 15: 691–703.
22 Kyoi T, Kitazawa S, Tajima K, Zhang X, Ukai Y. Phosphodiesterase type IV inhibitors prevent ischemia reperfusion-induced gastric injury in rats. J Pharmacol Sci. 2004; 95: 321–328.
23 Akhiani AA. The role of type-specific antibodies in colonization and infection by Helicobacter pylori. Curr Opin Infect Dis. 2005; 18: 223-227.
24 Repetto MG, Llesuy SF. Antioxidant properties of natural compounds used in popular medicine for gastric ulcer. Braz J Med Biol Res. 2002; 35: 523–534.
25 Baggio CH, DeMartini-Otofuji G, Souza WM, de Moraes Santos CA, Torres LM, Rieck L, et al. Gastroprotective mechanisms of indole alkaloids from Himatanthus lancifolius. Planta Med. 2005; 71: 733-738.
26 Morikawa T, Li N, Nagatomo A, Matsuda H, Li X, Yoshikawa M. Triterpene saponins with gastroprotective effects from tea seed (the seeds of Camellia sinensis). J Nat Prod. 2006; 69: 185-190.
27 Klein Jr LC, Gandolfi RB, Santin JR, Lemos M, Filho VC, deAndrade SF. Antiulcerogenic activity of extract, fractions, and some compounds obtained from Po-lygala cyparissias St. Hillaire & Moquin (Polygalacea). N-S Arch Pharmacol. 2010; 381: 121-126.
28 Giordano OS, Guerreiro E, Pestchanker MJ, Guzman J, Pastor D, Guardia T. The gastric cytoprotective effect of several sesquiterpene lactones. J Nat Prod. 1990; 53: 803– 809.
29 Lewis DA, Hanson D. Anti-ulcer drugs of plant origin. In: Ellis GP West GB (Eds.) Progress in Medicinal Chemistry Elsevier Science Publishers BV 1991; 28: 201–231.
30 Farina C, Pinza M, Pifferi G. Synthesis and anti-ulcer activity of new derivatives of glycyrrhetic, oleanolic and ursolic acids. Farmaco 1998; 53: 22–32.
31 Matsuda H, Li Y, Murakami T, Yamahara J, Yoshikawa M. Protective effects of oleanolic acid oligoglycosides on ethanol- or indomethacin-induced gastric mucosal lesions in rats. Life Sci 1998; 63: 245– 250.
32 Hiruma-Lima CA, Gracioso JS, Toma W, Paula ACB, Almeida ABA, Brasil DD, et al. Evaluation of the gastroprotective activity of cordatin, a diterpene isolated from Aparisthmium cordatum (Euphorbiaceae). Biol Pharm Bull. 2000; 23: 1465–1469.