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Research Article

ANTI-INFLAMMATORY EFFECT OF LIPID EXTRACT OF SEA PEN (*VIRGULARIA GUSTAVIANA*) IN MICE

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ABSTRACT

Objective: Sea pens are a highly specialized group of anthozoa coelenterates. Sea pens were collected by wading at low tide in intertidal zone from Bandar Abbas Costal.

Method: Sea pens were identified by classification keys, and samples were sending to the academic center of California for admission. Comparing identification keys genus from virgularidaes family was identified, it consisted of *Virgularia gustaviana.* To find an effective fraction, the inflammatory effect of lipid extract hexane of sea pen *V. gustaviana* was evaluated. In this study, lipid percentages of *V. gustaviana* were measured, and the percentage of sterol profile was assessed. To assess inflammation, mouse ear edema method was used. Male NMRI mice were categorized into three groups including positive control (dexamethasone 15 mg/kg), negative control (saline) and experimental one with dose of 10, 20, 40, and 60 mg/kg of lipid extracted of chloroform and hexane as a separate group.

Results: Total lipid *V. gustaviana* was 21.96% and sterol profile which was 5680.31 mg/kg contains cholesterol 1910.38 mg/kg that equal 57.95% of total sterol and brassicasterol, campesterol, campestanol, stigmasterol, beta-sitosterol were measured. Fatty acid profile was measured by gas chromatography-mass and heptadecane 44.25%, beta.-Bisabolene ss Cyclohexene'1, 18.16%, and methyl arachidonate 54.17% was assessed. Statistical analysis was performed that anti-inflammatory effect of all doses of 40-10 mg/kg is significant to the control.

Conclusion: Chloroform and hexane extracts of sea pen has a strong anti-inflammatory effects even at low doses which is probably due to 54% arachidonic acid in the compounds.

Keywords: Anti-inflammatory activities, Lipid extract, Sea pen (*Virgularia gustaviana*).

INTRODUCTION

As the marine environment gear over 70% of the earth's surface and contain ecological, chemical, and biological diversity, ocean's become big sources of natural drug. Marine sessile invertebrate are the important source of natural product, this animal has effective activity. Although marine sessile invertebrate does not have any physical mechanisms; this animal has unique mechanisms for defense themselves against predictor and constipation for place to live. By the complex chemical compound, they would keep enemies away [1].

Sea pens are the colony of polyps one polyp grows very large and loses its tentacles, forming the central axis [2]. They are benthic animal, and their appearance is feather-like. They are plains of fine mud, at water depths ranging from 15 to 200 m or more. They have 300 species and belong to order *Pennatulacea*, and suborder *Sessiliflorae* and subsessiliforae, these colonial invertebrate marine animals of the class anthozoa (phylum *Cnidarians*) [3,4]

During the years 2002–2001, marine chemical pharmacology research experienced a global phase. In recent years, compounds extracted from marine organisms have shown anti-inflammatory, anti-bacterial, anti-cancer, etc., effects [5,6].

Pennatulacea is a particular group of anthozoa, animals stuck to the bed of water living in all regions of the world usually regions above 6100 m of tide. Some deep species have global distribution [4].

Since many of the lipids have anti-inflammatory effects and strengthening properties on veins, this is done by releasing inhibition prostaglandin F2 of veins inhibiting serotonin and histamine [7].

Fatty acids in the sea pen belong to the essential fatty acids which are converted to a series of chemical compounds called eicosanoids in the body and are involved in many biological reactions. These materials possess some anti-inflammatory properties and prevent the release of compounds that play a role in coagulation of platelets and vasoconstriction [8].

With regard to the distribution of sea pen in platform coastal, and due to presence of coast and platform in the Persian Gulf, and having chemical compound with high biological activity of this animals, we decided to study this article.

METHODS

Animal materials

Virgularia gustaviana were collected by hand in low intertidal zone from Suru estuary in Bandar Abass cost in December of 2010, and stored on freezer until extraction. And the samples were transported to the laboratory of the school of marine science and technology of Azad university north branch of Teheran.

Extraction

To extract the lipid, the Blight and Dyer method was used [9]. So that samples were mixed and formed dough, then chloroform and methanol (1:9) and methanol and hexane (1:9) were used and kept in the refrigerator for 24 hrs and then funneled from the No. 1 paper filter and the solution was transferred to the tipped balloon and then it was connected to the Rotating Rotary Evaporator under vacuum distillation. After complete solvent evaporation, extracts were poured in closed small glass containers and were kept in the refrigerator [10,11].

Sterols profile

Cold extracting with chloroform and methanol was used to determine the sterols profile [12,13]. The goal was to measure sterols in the lipid

extract of sea pen using remote gas chromatography based on the ISO 6792, ISO IRI is 9670 standard [6].

Inflammation test

Mouse ear inflammation test

A number of small male NMRI mice with weight of 25-20 g purchased from the Pasteur Institute were used in this study. Animals were kept with normal clock period at temperatures from 24 to 22 C with enough food and water. In each series of the experiments, 3 mice were evaluated [14].

Anti-inflammatory *in vivo* assay

According to the method by Cui *et al*. (1989), Mouse-ear edema method was used for the anti-inflammatory assay [15]. During the inflammation test the male mice were divided into three groups: Negative control group (saline recipient), positive control group (dexamethasone recipient) and experimental group (receiving lipid extract in doses of 10, 20 and 40 mg/kg of animal weight). For created inflammation using Xylene-induced method. 15 minutes after injection of extract, 50 µl of xylene were applied to the dorsal and anterior surface of the right ears of animals. 2 hrs later, animals were sacrificed by cervical dislocation and the ears were quickly punched out with a cork borer (7 mm in diameter) and weighed. Slices were taken from the left and right ear and then their weight were determined. Weight difference between left and right ear indicated the amount of inflammation [15,16].

% Inhibition = (ΔPc−ΔPt)/100×ΔPc

Where: ΔPc → mean weight variation in the control group;

ΔPt → mean weight variation in the treated group [15].

Statistical analysis of data

Data from the ear edema test were analyzed using analyze of one-way ANOVA test and in case of significant difference Tukey-Kramer was used. To evaluate the results as mean ± standard deviation is reported.

RESULTS

One of the species of sea pens in the Persian Gulf is called *V. gustaviana*, averagely 1.5 g of weight and 16 cm length. It contains fat about 96/21%, 57/95% cholesterol and fatty acids such as: Pentadeconoic acid, ethyl arachidonate, octadecanoic acid, heptadecanoic.

The hexane extract of the sea pen *V. guataviana* was concentrated under reduced pressure in a rotary evaporator these crude extracts were stored for further studies.

The aim of the analysis of the chemical composition to identification of *V. guataviana* hexane extract was the identification of the principal chemical groups present in the extract. These compounds were potentially responsible for the pharmacological properties of this animal.

Chromatography results of hexane extract of sea pens showed that compounds such as arachidonic acids and heptadecane have anti-inflammatory properties which shown in Tables 1 and 2.

The results of inhibition of hexane lipid infusion of sea pen extract with doses of 10, 20 and 40 mg/kg to male mice caused a significant reduction in inflammation compared to control group that received only normal saline ($p<0.001$) shown in Table 3.

DISCUSSION

The findings of this study proved the anti-inflammatory effect of fraction isolated from the sea pen in the acute inflammation. The results are compatible with Al-Hindawi and colleagues in 1989 about hydroalcoholic extract of celery. As thin layer chromatography results showed arachidonic obtained from the hexane extract which are anti-inflammatory ingredient, stand as the most abundant and perhaps most important facilitators of eicosanoids. In order to make eicosanoids, arachidonic acid must first be released. The substance is important in making anti-inflammatory drugs [17].

Table 1: Measure sterols in the lipid extract of sea pen

Sterols	Result (mg/kg)
Cholesterol	1910/38
Brassicasterol	139/59
24-methylen-cholesterol	1381/22
Campesterol	645/72
Campestanol	310/21
Stigmasterol	58/59
Beta-sitosterol	1050/93
Total closterole	3291/6
Total sterols	5860/31

Table 2: Result of fatty acids from the hexane extract of sea pen lipids using GC-MS and RI

Number	Fatty acid	%	RI
1	Octadecanoic acid methyl ester	15.6	1100.78
2	Ethyl arachidonate	54.2	1500.1
3	Total fatty acid of hexane extract	69.8	

$RI = T_n + 100(\frac{T_x - T_n}{(T_n + 1) - T_n})$. RI: Retention index, GC-MS: Gas chromatography

Table 3: Hexane extract of sea pen on inflammation created by xylene the in the ear of small laboratory mice

Level of inhibition%	Ear inflammation	Treatment mg/kg
	0/001±0/0084	Control group
94/12	0/017±0/00123	Dexamethasone 15 mg/kg
60	0/0068±0/00206	Hexane extract 10 mg/kg
96/48	0/0006±0/00293	Hexane extract 20 mg/kg
80/159	0/0033±0/00151	Hexane extract 40 mg/kg

Arachidonic acid is a fatty acid consisting 20 carbons (C20) or four dual-link starting from the 6 omega position and creates a 5, 8, 11, and 14 eicosanoids acid (the compound is indicated as 20: 6-4 show) [18]. In order to make eicosanoids, arachidonic acid must first be released or receive the approval of one or more lipase phosphorous. At least three phosphorus lipase release from arachidonic which are as follows: CPLZ, cytosol PLAZ, secretory PLAZ [19].

Products derived from arachidonic acid which have five double links are different quantitatively. Based on this, the application of fatty acids isolated from fish and plants are to supplement food [20]. TXZ which is made from arachidonate is Tetratic acid which is a strong platelets collector. Due to the large amount of acid arachidonic extracted from hexane and since it is a fatty acid important for the body and due to its mechanism of action, this extract can be used in anti-inflammatory drugs. It is clear that the exact identification of the materials related to the sea pen and investigating its biological properties need further studies [21]. The finding lipid of sea pen showed that ethyl arachidonate the fatty acid from the hexane extract with 54.17% it is the important in anti-inflammatory effect, and also sterol profile from sea pen lipids showed that 21.96% of *V. gustaviana* body has lipid and many sterols found on the lipid extract such as cholesterol, beta-sitosterol, campesterol which they could be useful in the biological effect like anti-inflammatory.

CONCLUSIONS

As the result that found in this study which showed that all the compounds extracted from marine sea pen genus virgularia gustavina,

had anti-inflammatory affected on mouse ears. Furthermore, this inflammation may because of a large quantity of arachidonic acid from hexane extracted, so this extract can be used in anti-inflammatory drugs. Although, it is possible to use these compounds for the treatment for finding more biological properties need further studies.

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REFERENCES

1. Moritz MI, Marostica LL, Bianco EM, Almeida MT, Carraro JL, Cabrera GM, *et al.* Polyoxygenated steroids from the octocoral *Leptogorgia punicea* and *in vitro* evaluation of their cytotoxic activity. Mar Drugs 2014;12(12):5864-80.
2. Baillon S, Hamel JF, Mercier A. Diversity, distribution and nature of faunal associations with deep-sea pennatulacean corals in the Northwest Atlantic. PLoS One 2014;9(11):e111519.
3. Buckeridge JS, Campbell HJ, Maurizot P. Unravelling the nature of waiparaconus, a pennatulacean (Cnidaria: Octocorallia) from the late mesozoic-early cainozoic of the southern hemisphere. Integr Zool 2014;9(2):111-20.
4. Dolan E, Tyler PA, Yesson C, Rogers AD. Phylogeny and systematics of deep-sea sea pens (Anthozoa: Octocorallia: Pennatulacea). Mol Phylogenet Evol 2013;69(3):610-8.
5. Yang SC, Sung PJ, Lin CF, Kuo J, Chen CY, Hwang TL. Anti-inflammatory effects of secondary metabolites of marine *Pseudomonas* sp. in human neutrophils are through inhibiting P38 MAPK, JNK, and calcium pathways. PLoS One 2014;9(12):e114761.
6. Onumah N. A novel anti-inflammatory in treatment of acne vulgaris: The pseudopterosins. J Drugs Dermatol 2013;12(10):1177-9.
7. Li G, Fu Y, Zheng J, Li D. Anti-inflammatory activity and mechanism of a lipid extract from hard-shelled mussel (*Mytilus coruscus*) on chronic arthritis in rats. Mar Drugs 2014;12(2):568-88.
8. Koizumi K, Hiratsuka S, Saito H. Lipid and fatty acids of three edible myctophids, *Diaphus watasei*, *Diaphus suborbitalis*, and Benthosema pterotum: High levels of icosapentaenoic and docosahexaenoic acids. J Oleo Sci 2014;63(5):461-70.
9. Ostermann AI, Müller M, Willenberg I, Schebb NH. Determining the fatty acid composition in plasma and tissues as fatty acid methyl esters using gas chromatography – A comparison of different derivatization and extraction procedures. Prostaglandins Leukot Essent Fatty Acids 2014;91(6):235-41.
10. Hussain J, Ruan Z, Nascimento IA, Liu Y, Liao W. Lipid profiling and corresponding biodiesel quality of *Mortierella isabellina* using different drying and extraction methods. Bioresour Technol 2014;169:768-72.
11. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37(8):911-7.
12. Azadmard-Damirchi S, Dutta PC. A single step solid-phase extraction method for complete separation of sterol oxidation products in food lipids. J Chromatogr A 2009;1216(1):36-42.
13. Szymanski WG, Kierszniowska S, Schulze WX. Metabolic labeling and membrane fractionation for comparative proteomic analysis of Arabidopsis thaliana suspension cell cultures. J Vis Exp 2013;e50535.
14. de Souza ET, de Lira DP, de Queiroz AC, da Silva DJ, de Aquino AB, Mella EA, *et al.* The antinociceptive and anti-inflammatory activities of caulerpin, a bisindole alkaloid isolated from seaweeds of the genus *Caulerpa*. Mar Drugs 2009;7(4):689-704.
15. Cui Y, Ao M, Li W, Hu J, Yu L. Anti-inflammatory activity of licochalcone A isolated from Glycyrrhiza inflata. Z Naturforsch C 2008;63(5-6):361-5.
16. Kohl AC, Ata A, Kerr RG. Pseudopterosin biosynthesis-pathway elucidation, enzymology, and a proposed production method for anti-inflammatory metabolites from *Pseudopterogorgia elisabethae*. J Ind Microbiol Biotechnol 2003;30(8):495-9.
17. Al-Hindawi MK, Al-Deen IH, Nabi MH, Ismail MA. Anti-inflammatory activity of some Iraqi plants using intact rats. J Ethnopharmacol 1989;26(2):163-8.
18. Chen YF, Tsai HY, Wu TS. Anti-inflammatory and analgesic activities from roots of *Angelica pubescens*. Planta Med 1995;61(1):2-8.
19. Mayer AM, Hamann MT. Marine pharmacology in 2001--2002: Marine compounds with anthelmintic, antibacterial, anticoagulant, antidiabetic, antifungal, anti-inflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems and other miscellaneous mechanisms of action. Comp Biochem Physiol C Toxicol Pharmacol 2005;140(3-4):265-86.
20. Ujihara T, Nagano M, Wada H, Mitsuhashi S. Identification of a novel type of polyunsaturated fatty acid synthase involved in arachidonic acid biosynthesis. FEBS Lett 2014;588(21):4032-6.
21. Pang KL, Lin HJ, Lin HY, Huang YF, Chen YM. Production of arachidonic and eicosapentaenoic acids by the marine oomycete halophytophthora. Mar Biotechnol (NY) 2014.