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Research Article

IMUNOMODULATORY EFFECTS OF THE JELLY FISH VENOM *C. QUINQUECIRRHA* FROM VELLAR ESTUARY, SOUTHEAST COAST OF INDIA

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ABSTRACT

The Jellyfish having potential biological active compounds by tentacles. This bioactive compound was useful in biomedical industry. The extracts of *Chrysaora quinquecirrha* exerted an immunostimulating effect of 40 to 25% magnitudes at lower concentrations and a suppressive effect of 33% at higher concentration. The fractionated extract exhibited immunostimulation ranging from 25 to 40% at concentrations up to 1000 µg. These facts mean that jellyfish extracts had immunostimulation effect, and the active substance in jellyfish extract is secondary amides and polypeptides. Thus the jelly fish extract is found to be useful tools for probing biological or pharmacological activity.

Keywords: Jellyfish, Immunostimulation, Biological, Tentacles

INTRODUCTION

Immunomodulation through natural or synthetic substances may be considered as an alternative for the prevention and cure of neoplastic diseases[1]. Natural products are excellent and reliable sources for the development of new drugs[2]. Immune system dysfunction leads to various diseases like arthritis, ulcerative colitis, asthma, allergy, parasitic diseases, cancer and infectious diseases[3]. This immunosuppression allows opportunistic pathogens to overwhelm the host to cause secondary infection[4]. Many papers on the medicinal functions of the jellyfish have been published in Chinese journals and magazines showed that jelly fish possess collagen[5] and significant medicinal potential for rebuilding muscle, cartilage and bones[6]. Moreover, the active protein isolated from jellyfish *R. esculentum* possesses antioxidant and insecticidal activities[7].

A number of *in vitro* and *in vivo* tests are available for assessing immunomodulatory activities. Phagocytosis is widely accessible for assessing immunomodulatory activity for screening the immune response[8]. Phagocytosis is the primary defence mechanism against any foreign bodies entering in to the body, which is offered by neutrophils and macrophages. The process of phagocytosis consists of sequential stage as motility, adhesion to microorganisms ingestion, degranulation and intracellular killing of microorganisms[9]. There is a worldwide interest in marine natural products as one of the few sources of drug discovery[10]. However, the bioactive potential of compounds from jellyfish has been studied. Therefore, the present study comprises an initial effort to assess the immunomodulatory activity from the jelly fish *Chrysaora quinquecirrha*.

MATERIALS AND METHOD

Isolation and extraction of crude venom

The live specimen of *C. quinquecirrha* was collected from Vellar estuary, Parangipettai coastal water during the summer season of April-May, 2009. The live animal was kept inside the glass bowl along with some amount of distilled water with ice in container for 15 minutes. The nematocysts were released from the tentacles due to stress condition. The collected nematocysts containing toxins were collected with 0.5mm mesh sieve and filtered by Whatman No.1 filter paper. In order to remove the debris from the extracted crude toxin, residues were centrifuged at 5000 rpm for 15 min. The supernatant was collected in separate cleaned beakers for lyophilisation and stored at 4^{o}C until further use[11].

Dialysis of crude extract

The crude extract was filtered and dialyzed by using Sigma (USA) dialysis membrane-500 (average flat width: 24.26 mm; average diameter: 14.3 mm; approximate capacity: 1.61 mL/cm) against D-glucose to remove excess water. Then, the supernatant obtained and it was lyophilized (Free Zone® Freeze Dry Systems, Labconco, USA) for further analysis.

Immunomodulatory study

Preparation of test sample

The samples for *in vitro* studies were prepared by dissolving 5mg/ml of crude extract whereas fractionated extract obtained various concentrations ranging from 10, 20, 50,100 and 1000µg/ml.

Neutrophil locomotion and chemo taxis test

Neutrophil cell suspension was prepared in phosphate buffer saline solution (PBS) at about 10^{6} cells/ml. The lower compartment of chemo taxis chamber to a pH of 7.2 e.g. chamber 1-PBS solution (control); chamber 2- casein 1 mg/l (standard); and chamber 3, 4, 5, 6, 7 with different concentrations (10, 25, 50, 100 and 1000 µg/ml) of test sample. The upper compartment (1ml syringe) was placed with suspended neutrophil and wet filter (Millipore) 3 mm pore size was fixed at the bottom of the upper compartment. The same was incubated at 37ºC for 3 hrs. The upper compartment was removed and inverted to empty the fluid. The lower surface of the filter was fixed with 70% ethanol for 2min and then stained with Haematoxylin dye for 5 min. The fixed filters were observed under microscope using 100 x lenses and number of neutrophil cells reached to the lower surface was counted.

Phagocytosis of killed *Candida albicans*

Preparation of *Candida albicans* suspension was followed by the method of Ponkshe and Indap[8]. The *Candida albicans* culture was incubated in Sabouraud broth overnight and it was centrifuged. The cell button was washed with sterile Hank's Balanced Salt Solution (HBSS) and centrifuged again. This was done 3-4 times. The final cell button was mixed with a mixture of sterile HBSS and human serum in proportion of 4:1. The final cell suspension of concentration 1×10^{8} was used for the experiment.

Slide preparation

Human blood (0.2ml) was obtained by finger prick method on sterile glass slide and incubated at 37 ºC for 25 min to allow clotting. The blood clot was removed very gently and slide was drained slowly with sterile normal saline, taking care not to wash the adhered neutrophils (invisible). The slide consisting of polymorphonuclear neutrophils (PMNs) was poured with concentration of test sample and incubated at 37 ºC for 15 min. The PMNs were covered with *Candida albicans* suspension and it was incubated at 37 ºC for 1 hr. The slide was drained, fixed with methanol and stained with Giemsa stain.

Phagocytosis evaluation

The mean number of Candida cells phagocytosed by PMNs on the slide was determined microscopically for 100 granulocytes using morphological criteria. This number was taken as Phagocytic Index (PI) and was compared with basal PI of control. This procedure was repeated for different concentrations (10, 25, 50, 100 and 1000µg/ml) of test sample. The percentage of immunostimulation was calculated by using following equation:

Stimulation (%) = PI (test) - PI (control) x 100/PI (control).

RESULTS

Crude and fractionated extracts have showed significant activity at higher concentrations. The neutrophil locomotion and chemotaxis showed significant activity both crude and fractionated extract. The maximum neutrophil locomotion was observed in the entire four fractions except F4 (Table 1). In case of phagocytosis of *Candida albicans*, the extract showed significant activity even at low concentration of 10µg/ml concentrations. The extract showed predominantly very good at 20 µg/ml concentrations (Fig.1).

The extracts of *Chrysaora quinquecirrha* exerted an immunostimulating effect of 40 to 25% magnitudes at lower concentrations and a suppressive effect of 33% at higher concentration (Table 2). The fractionated extract exhibited immunostimulation ranging from 25 to 40% at concentrations up to 1000 µg, but showed immunosuppressive effects at 20 µg (Fig.2).

Table 1: Neutrophil locomotion and chemo taxis activity.

Extracts	Concentration µg/ml							
Aqueous Extracts	**10**	**20**	**50**	**100**	**1000**	**Control PBS**	**Casein**	**Comments**
Crude	81	100	81	81	81	9	81	Very Good activity at 20 µg/ml
F1	100	81	121	100	64	8	100	
F2	81	64	81	121	81	6	64	
F3	121	100	64	64	49	10	49	
F4	64	81	81	81	64	8	64	
F5	100	81	100	121	81	100	81	

Table 2: Phagocytosis by *C. quinquecirha* extracts at 10 - 1000 µg/ml.

C. quinquecirha extract	Concentration µg/ml (PI Test)					Control µg/ml	Modulation %
	10	**20**	**50**	**100**	**1000**		
crude	5	4	5	4	4	4	20
F1	4	5	4	4	5	5	25
F2	5	4	5	5	5	4	25
F3	5	5	5	5	3	5	40
F4	4	5	4	4	4	4	33
F5	4	4	5	4	4	4	25

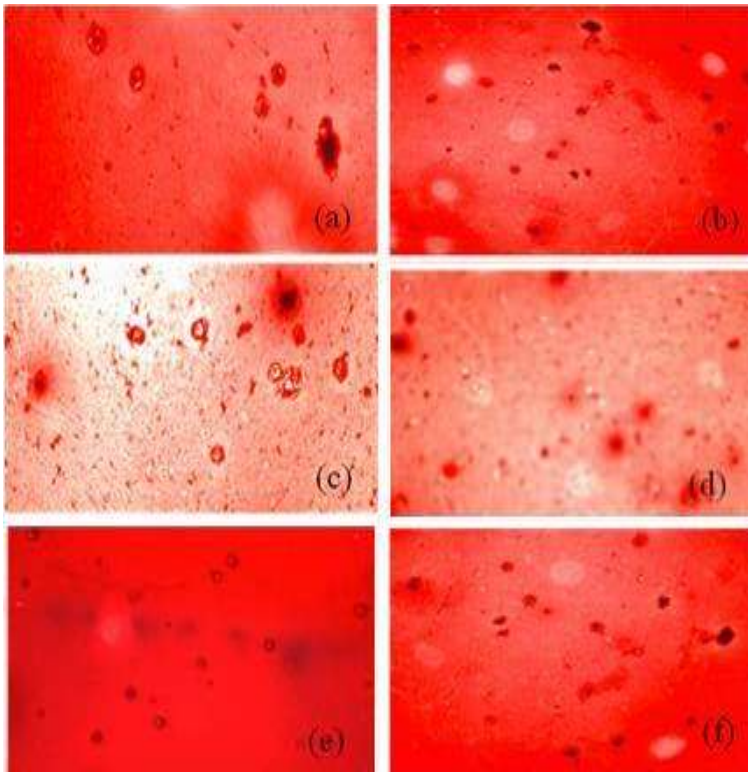

Fig. 1: Phagocytosis of *Candida albicans* by PMN when treated with *C. quinquecirha* extract at differnent concentrations (a) 10 µg/ml (b)20 µg/ml (c)- 50 µg/ml (d) 100 µg/ml (e) 1000 µg/ml and (f) control.

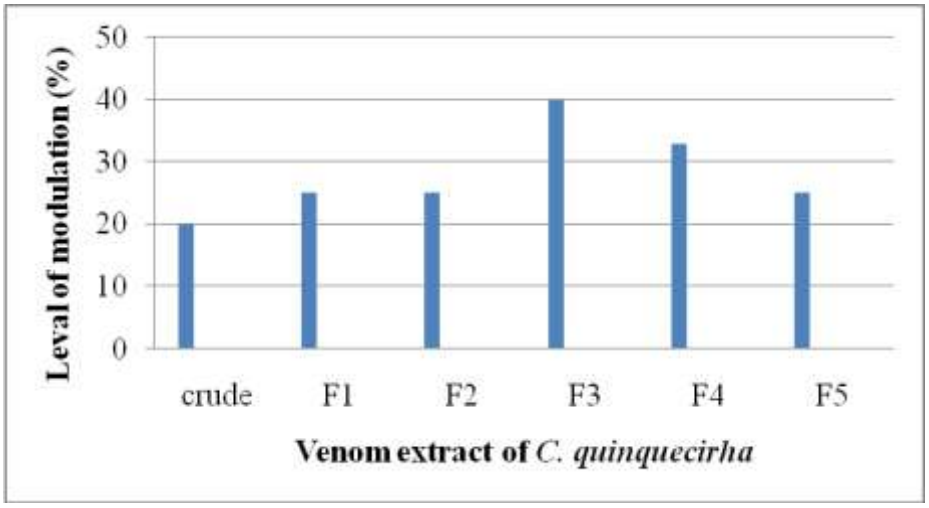

Fig. 2: Immunomodulation produced by C. *quinquecirha* extracts.

DISCUSSION

The present study was attempted first time to demonstrate the immunostimulant potential of the jelly fish venom extract of C. *quinquecirha*. The immunomodulation results were found to decrease with the increasing concentration of the crude and fractions. Previously some researcher have reported there was an excessive interest in the modulation of the non specific immune response of fish to elevate the general defense barriers and hence it increases resistance against diseases through use of immunostimulant[12,13]. The use of immune stimulants for prevention of diseases in fish considered an alternative and promising area[14].

Immunomodulation effects of bioactive natural products from marine sources are very poorly studied and reported. Al Hassan *et al*.[15]have shown that the wound healing activity exhibited by the epidermal secretion of the Gulf catfish is associated with immunomodulation as well as prostaglandin pathway. As the *C. quinquecirrha* extracts showed promising immunostimulant activity in the *in vitro* test. The fractions eluted from the extract seemed to be the most active. On the contrary, the fractions eluted from the crude folklore preparation resulted more active. From the overall results we can partly explain the popularity of this jelly fish extract in folk medicine as remedy for arthritis[16].

The results of the *in vitro* PMN function test showed a significant increase in the percentage of phagocytosis and phagocytic index for successive crude and fractionated extracts. This indicates that these extracts enhanced the phagocytic efficacy of the PMN cells by causing more engulfment of the Candida cells control, thereby stimulating a non-specific immune response. The present results of *C. quinquecirrha* extracts are in support of earlier findings of Purushottama *et al.*[17]. This resistance may be due to the increased activity of phagocytic cells[18]with subsequent increase in lysozyme activity. These outcomes are encouraging enough to pursue structure elucidation of the active components.

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REFERENCES

1. Mitchell MS. Immunotherapy as part of combinations for the treatment of cancer. *International Immunopharmacology* 2003; **3:** 1051-1059.
2. Newman DJ, Craig GM. Marine natural products and related compounds in clinical and advanced preclinical trials. *Journal of Natural Products* 2004; **67:** 1216-1238.
3. Patwardhan B, Kalbag D, Patki PS, Nagsampagi BA. Immune system dysfunction. *Indian Drugs* 1990; **28(2):** 53-56.
4. Rao CS, Raju C, Gopu Madhavan S, Chauhan BL. *Indian* J *Exp Biol* 1994; **32:** 553-558.
5. Nagai H, Takuwa K, Nakao M, Sakamoto B, Crow GL, Nakajima T. Isolation and characterization of a novel protein toxin from the Hawaiian box jellyfish *Carybdea alata. Biochem Biophys Res Commun* 2000; **275(2):** 589-594.
6. Hsieh HYP, Rudloe J. Potential of utilizing jellyfish as food in Western countries. *Trends Food Sci Technol* 1994; **5(7):** 225-229.
7. Yu HH, Liu XG, Xing RE, Liu S, Guo ZY, Wang PB, et al. *In vitro* determination of antioxidant activity of proteins from jellyfish *Rhopilema esculentum*. *Food Chem* 2006; **95**: 123-130.
8. Ponkshe CA, Indap MM. *Indian J Exp Biol* 2002; **40:** 1399-1402.
9. Daniel PS, Abba IT, Tristram GP. Basic and Clinical Immunology. *Appleton and Lange* 1994; 195-215.
10. McConnell OJ, Longley RE, Koehn FE. The discovery of marine natural products with therapeutic potential. *Biotechnology* 1994; **26:** 109-174.
11. Yanagihara A, Kuroiwa J, Oliver L, Chung J, Kunkel DD. Ultra structure of a novel eurytele nematocyst of *Carybdea alata* (Cubozoa: Cnidaria). *Cell Tissue Res* 2002; **308:** 307-318.
12. Raa J. The use of immunestimulants in fish and shellfish feeds. In: Cruz-Suarez LE, Ricque-Marie D, Tapia-Salazar M, Olvera-Novoa MAY, Civera-Cerecedo R, (Eds.). Avances en Nutricion Acuicola V. Memorias del V Simposium International de Nutricion Acuicola. 19-22. Merida, Yucatan, Mexico, 2000; 98p.
13. Sahoo, PK, Mukherjee SC. The effect of dietary immunomodulation upon *Edwardsiella tarda* vaccination in healthy and immune compromised Indian major carp *Labeo rohita*. *Fish and Shellfish Immunology* 2002; **12(1):** 1-16.
14. Sakai M. Current research status of fish Immunostimulants. *Aquaculture* 1999; **172:** 63-92.
15. Al-Hassan JM, Thomson M, Summers B, Criddle RS. Purification and properties of a hemagglutination factor from the Arabian Gulf catfish *Arius thalassinus* epidermal secretion. *Comp Biochem Physiol* 1986; **85:** 31-39.
16. Hsieh YH P, Leong FM, Rudloe J. Jellyfish as a food. *Hydrobiologia* 2001; **451:** 11-17.
17. Purushottama GB, Venkateshvaran K, Pani Prasad K, Nalini P. Bioactivities of extracts from the marine sponge *Halichondria panicea*. *J Venom Anim Toxins Incl Trop Dis* 2009; **15(3):** 445p.
18. Robertsen B, Engstad RE, Jorgensen JB. β. glucan as immunostimulants in fish. In: Modulators of Fish Immune Responses. Stolen JS, Fletcher TC, (Eds.), SOS Publications, Fair Haven, NJ, 1994; **1:** 83-99.