WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 6, 2140-2149. Research Article ISSN 2277 – 7105

ANTICANCER ACTIVITY OF JELLYFISH, *CHRYSAORA QUINQUECIRRHA (*DESOR 1848*)* FROM VELLAR ESTUARY, SOUTHEAST COAST OF INDIA

Suganthi Krishnan[1*], Perumal Pachiappan[2] , Tufail Ahmed Mazharbasha[3] and Keerthana Ranganathan[4]

[1]Post-Doctoral Fellow, Department of Biotechnology, Periyar University, Periyar Palkalai Nagar, Salem 636011, Tamil Nadu, India.
[2]Research Mentor, Professor & Head, Department of Biotechnology, Periyar University, Periyar Palkalai Nagar, Salem 636011, Tamil Nadu, India.
[3] M. Phil Scholar, Department of Biotechnology, Periyar University, Periyar Palkalai Nagar, Salem 636011, Tamil Nadu, India.
[4] Research Scholar, Department of Biotechnology, Periyar University, Periyar Palkalai Nagar, Salem 636011, Tamil Nadu, India.

Article Received on
30 June 2014,

Revised on 25 July 2014,
Accepted on 20 August 2014

***Correspondence for Author**
Dr. Suganthi Krishnan
Post-Doctoral Fellow, Department of Biotechnology, Periyar University, Periyar Palkalai Nagar, Salem 636011, Tamil Nadu, India.

ABSTRACT

Background: Marine organisms are considered to be an inexhaustible source of chemical compounds that produce a wide variety of biologically active secondary metabolites. Scyphozoan jellyfishes have become an important target for the biotechnology industry because of the large number of bioactive compounds recently discovered from them. **Objective:** The present investigation pertains to the evaluation of the anticancer activity of extracts of jellyfish *Chrysaora quinquecirrha*, collected from Vellar estuary, South east coast of India. **Methodology**: The nematocyst extracts of tentacles of jellyfish were characterized for protein contents and their anticancer potential was determined on human lung cancer cell lines (A549) with 205.0 µg GI_{50}. **Results:** The study showed that the extracted peptides could be amino acid residues (secondary amides) which might be responsible for the anticancer potential on human lung cancer cell lines (A549). **Conclusions:** Marine organisms are considered to be an inexhaustible source of chemical compounds that produce a wide variety of biologically active secondary metabolites. Scyphozoan jellyfishes have become an important target for the biotechnology industry because of the large number of bioactive compounds recently discovered from them. This paper focuses on the anticancer potential of marine species with certain pharmaceutical interest, which could confer anticancer activity.

KEY WORDS: Anticancer, marine compounds, jellyfish, venom.

INTRODUCTION

Cancer is one of the leading causes of human death in the world. Cell division is a physiological process that occurs in tissues. Balance between proliferation and programmed cell death is being under normal circumstances, usually in the form of apoptosis, by tightly regulating both processes. Certain mutations in DNA lead to cancer by disrupting the programmes that regulate the processes. Carcinogenesis is a process by which normal cells are transformed into cancer cells. It is characterized by a progression of changes at both, cellular and genetic level, that reprogram a cell to undergo uncontrolled division, thus forming a malignant mass (tumor) that can spread to distant locations [1]. Biologically active compounds with different modes of action, such as, antiproliferative, antioxidant, antimicrotubule, have been isolated from marine sources, specifically from algae and cyanobacteria. Finding a drug to cure the cancer is one of the greatest challenges for pharmacologists. There have been extensive research efforts aimed at obtaining efficient compounds from natural source. Most of the marine peptides subjected to clinical trials have been of secondary metabolites from animals. However, there is a need for further research in order to elucidate the bioactive peptide structure, to determine its mode of action, and the way it interacts with the cancer cell cycle.

Limited research on bioactive marine animal peptides may be due to the lack of sufficient quantities of the compounds, problems in accessing the source of the samples, difficulties in isolation and purification procedures. Studies on peptides obtained from marine organisms have shown that derived molecules [2] have antioxidant, antiproliferative, and antimutagenic activities which could confer on them anticancer potential; however, more research on the mode of action on the cell cycle or apoptosis of cancer cell lines is necessary. To date, very little research has been performed to characterize the venom of the jellyfish *Chrysaora qinquecirrha*. Antioxidant, lethal and hemolytic activities have been studied in the crude venom [3]. It has been reported that the jellyfish venom has several types of activities, including hemolytic, antioxidant, cardiovascular, enzymatic and insecticidal [4-7]. These bioactive proteins were usually found to be unstable, as their bioactivities could be easily reduced or even completely lost due to factors such as enzymolysis, high temperature, extreme pH and the presence of metal ions. Hence the present study was undertaken to analyze the anticancer effect from marine source, jellyfish *Chrysaora quinquecirrha*.

MATERIALS AND METHODS

Specimen collection and venom preparation

The specimens of jellyfish were collected from Vellar estuary, Parangipettai, along the Southeast coast of India during the summer season. The collected live specimens were kept in the glass bowl filled with ice for 15 minutes. Due to stress condition, the tentacles released the nematocysts [8], which were filtered using 0.5 mm mesh sieve and filtered by Whatman No.1 filter paper. The nematocysts were centrifuged at 5000 rpm for 15 min. The supernatant was collected in clean separate beakers for lyophilization and stored at 4^{o} C until further used. 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 was lyophilized (Free Zone Freeze Dry Systems, Labconco, USA) and stored at 4° C in labeled 25-mL vials kept in containers until their analysis.

Medium preparation

12.0g of RPMI medium (Russel Pance Memorial Institute) was dissolved in 800 ML of sterile distilled water to which 2.5g of sodium bicarbonate was added. The beaker was covered with aluminum foil and stirred using magnetic stirrer for 10 minutes. The pH of the medium was adjusted to 7.2 using 0.1M NaOH. The volume of the medium was made to 1000 ML and filtered through sterile 0.2µ membrane filter unit. The control medium was checked by incubating 5 ML of filtered medium in the CO_2 incubator for 2 days. The antibiotics (streptomycin and penicillin) and serum was added before it was used for cell culture. All the reagents were purchased from Sigma.

Cell culture and MTT assay

The human Lung cancer cell line A549 was purchased from National Center for Cell Science (NCCS), Pune. The A549 cells were grown in a RPMI 1640 medium supplemented with 10% fetal bovine serum and antibiotics. Cytotoxicity (MTT) assay was performed following the method described by Carmichael *et al*. [9] and percentage of cell viability was determined by spectrophotometric determination of accumulated formazan derivative in treated cells at 570 nm in comparison with the untreated ones.

Cell Imaging

After 48 hours incubation the cells were observed under microscope for cell morphology analysis and images of each concentration was captured and recorded.

RESULTS AND DISCUSSION

Anticancer property of C. *quinquecirrha* extract (CQ-extract)

The compounds were tested against human Lung cancer A549 cell line. C.*quinquecirrha* (CQ) extracts concentration of 00.1µg-1000 µg in logarithmic range. The test compound of each concentration was performed in quadruplicate and cumulative variations were maintained less than 20% between the data points. The cell lines tested with compound in a 96 well plate. Well plate exhibited a moderate cytotoxicity activity. *C. quinquecirrha* extract exhibited good cell growth inhibition at high concentration which showed significant inhibition in A549 cell lines. It also showed 205.0 µg/ml as GI_{50}. Results and raw data have been illustrated in the tables 1-3 and graph -1.

Table 1. Growth inhibition absorbance values at 570nm

	Doxorubicin				CQ-extract			
	1	2	3	4	5	6	7	8
A	0.048	0.046	0.047	0.048	0.048	0.048	0.049	0.048
B	0.411	0.409	0.4	0.407	0.504	0.509	0.493	0.481
C	0.582	0.556	0.559	0.568	0.689	0.751	0.686	0.706
D	0.623	0.639	0.639	0.631	0.74	0.731	0.687	0.721
E	0.705	0.703	0.719	0.69	0.736	0.737	0.728	0.703
F	0.724	0.738	0.729	0.715	0.74	0.764	0.691	0.755
G	0.754	0.747	0.724	0.714	0.714	0.748	0.725	0.74
H	0.049	0.048	0.046	0.047	0.046	0.045	0.046	0.047

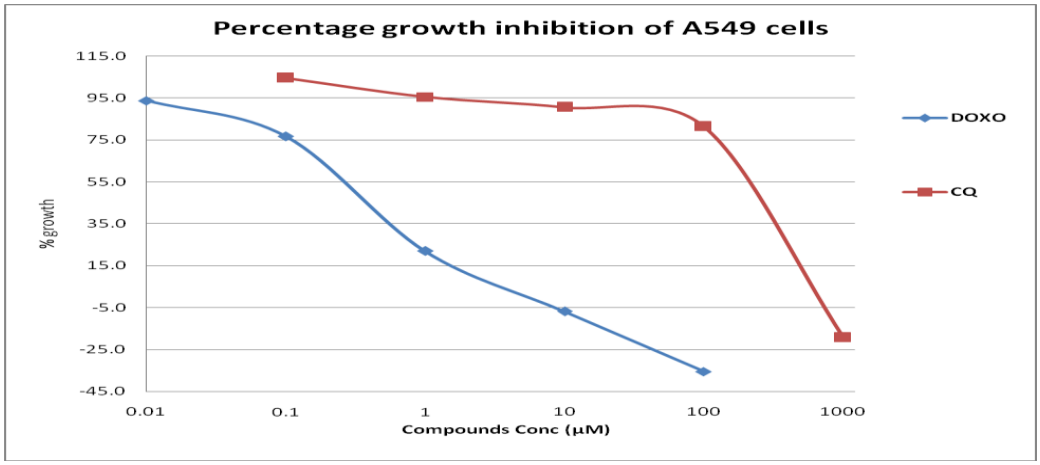
Table 2. Percentage of growth inhibition by C.*quinquecirrha* extract on human lung cancer cell lines A549 compared to untreated (control)

Compound	Percentage growth					Growth Inhibition in µg		
	1000µg	100µg	10µg	1µg	0.1µg	GI50	TG1	LC50
CQ -extract	-19	81	91	95	105	205.0	643.4	1000.0
	100µM	10µM	1µM	0.1µM	0.01µM			
Doxorubicin	-35	-7	22	77	94	0.3	5.8	100.0

Table 3. GI_{50} value of the CQ extract

48 Hours	GI_{50} (µg)
Compound	A549
Doxorubicin	0.30
Extract-CQ	205.00

Graph 1. Percentage growth curve of cells treated with test compound of *C.quinquecirrha* extract.



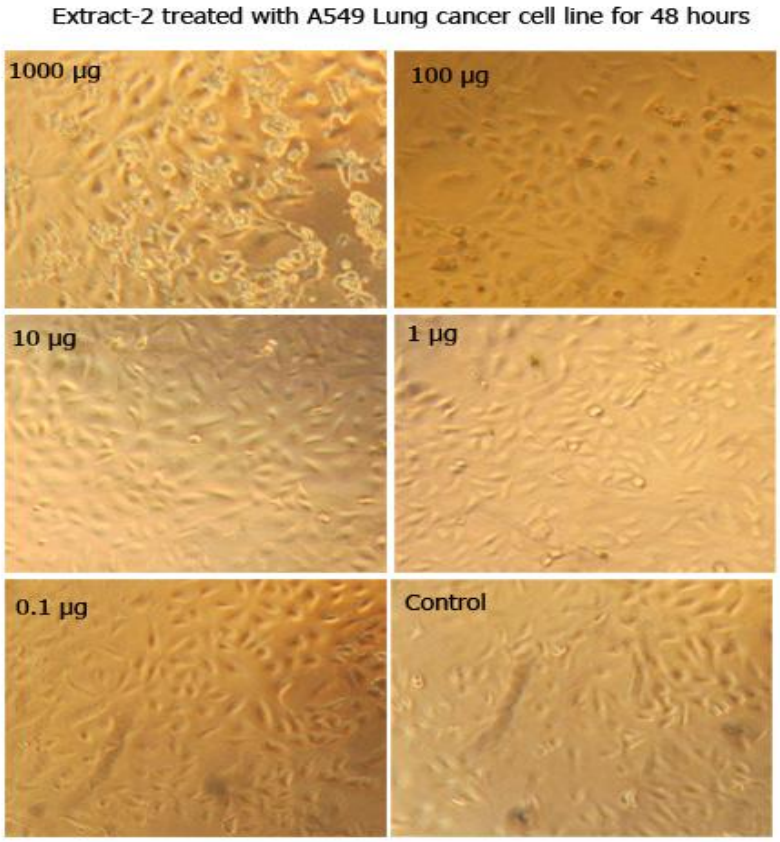


Fig-1. Microscopic analysis of A549 cells treated with C. *quinquecirrha* extract

The jellyfish, *Chrysaora quinquecirrha* is a Scyphozoan species, identified based on morphological features of its nematocysts. The occurrence, histological and cytological studies of *Discomedusa lobata* at Egypt was earlier reported [10]. The only study in India was carried out by Ghosh et al. [11] with the jellyfish *Acrmitus rabanchatu*, a venomous Scyphozoan jellyfish, which is quite abundant along the coastal areas of Bay of Bengal. In

India, so far, studies regarding nematocysts structure, pharmacological properties of jellyfish *C. quinquicirrha* toxins are very meager. Our present in vitro characterization of anticancer potential of the venom of jellyfish, *C. quinquecirrha* exhibited moderate anticancer activity in A549 cell lines with 205.0 µg GI_{50}. Up to now, bioactive peptides with potential anticancer exhibiting antioxidant and antiproliferative effects have been found in the hydrolysates of marine proteins [12-14].

Recently research has been focused on peptides from marine animal sources, since they have been found as secondary metabolites from sponges, ascidians, tunicates, and mollusks. The structural characteristics of these peptides include various unusual amino acid residues which may be responsible for their bioactivity. Moreover, protein hydrolysates formed by the enzymatic digestion of aquatic and marine by-products are an important source of bioactive peptides. They usually contain 2–20 amino acid residues and their activities are based on their amino acid composition and sequence. By modulating and improving physiological functions, bioactive peptides may provide new therapeutic applications for the prevention and/or treatment of chronic diseases. As components of diverse marine species with certain health claims, bioactive peptides are of particular pharmaceutical interest [15].

Jellyfish stings can produce a burning feeling, severe pain, swelling, red streak, nausea, abdominal pains, profuse sweating, muscle cramp, respiratory distress, heart failure and so on [3]. Correspondingly, jellyfish venoms have a wide spectrum of biological activities, such as dermonecrotic, neurotoxic, hemolytic and cardiovascular activities [16-17]. It is believed that the effects of jellyfish venoms are caused by the combination of various toxic components and acute heart failure is recognized as the major cause of death caused by jellyfish venoms [18-19], where the cardiovascular toxic component may be the major damage factor with other toxic components, for example the hemolytic toxic component, acting in synergy with it [20-21]. Chung *et al* [22] have isolated and characterized a novel hemolytic protein from the venom of jellyfish *Carybdea alata* which indicated the presence of a potent hemolytic protein. Some jellyfishes like *Crambionella stuhalmanni* and *Chrysaora quinquecirrha* were screened for biological activity by Suganthi *et al*. [3] and they have reported that peptides present in protein hydrolysates have biological activities depending on their molecular weights and amino acid sequences. These reports and the present findings support the fact that functional properties of antioxidative peptides are highly influenced by properties such as molecular mass. *Geodiamolide* –H, a compound isolated from a Brazilian sponge, *Geodia corticostylifera*

have been found to antiproliferative activity against breast cancer cells by altering the actin cytoskeleton [23]. Hsu et al.[24] have reported antiproliferative activity of peptides prepared from enzymatic hydrolysates of tuna dark muscle on human breast cancer cell line MCF-7. *Arenastatin*- A, a potent cytotoxic sponge, is a cyclodepsipeptide isolated from sponge, *Dysidia arenaria* that showed a potent cytotoxicity against KB cells with an IC50 of 5 pg/mL [25]. *Papuamides* A–D isolated from sponges of the genus, *Theonella*, are the first marine-derived peptides reported to contain 3-hydroxyleucine and homoproline residues. Ghosh [11] is the only study reported on jellyfish tentacle extract of the common jellyfish *A. rabanchatu*, which caused glycaemic alteration in fasting rabbits. Intravenous administration of the extract produced a significant rise followed by a significant fall in blood sugar level but not in cell lines. Compounds from marine sources have been reported to have bioactive properties with varying degrees of action [26–27], such as anti-tumor, anti-cancer, anti-microtubule, anti-proliferative, anti-hypertensive, cytotoxic, as well as antibiotic properties [28-29], *C. quinquecirrha* seems to have acquired the ability to synthesize a compound that could able to inhibit lung cancer cells (A549). These characteristics imply that the use of peptides from marine sources has potential for the prevention and treatment of cancer, and therefore further research is warranted in this line.

CONCLUSION

Currently the number of natural products is increasing; however, very few compounds have reached the market. A limited number of identified peptides found in marine animals are in preclinical trials and some of them have made it to different phases of clinical trials to prove their potential as antitumor drugs. Since biodiversity of the marine environment far exceeds that of the terrestrial environment, research on the use of marine natural products as pharmaceutical agents has been steadily increasing. The marine peptides are thus recently considered to be promising components for the development of drugs for the treatment of cancer. The present investigation showed that the venom of *C. quinquecirrha* extract exhibited moderate anticancer activity in A549 cell lines with 205.0 µg GI_{50}, which could be useful tools for probing biological, pharmacological activities. Molecular studies in order to determine the structure of proteins of nematocyst and pharmacological efficacy of the extract are in progress.

ACKNOWLEDGEMENTS

The authors thank the authorities of the Periyar University for facility provided and the first author is grateful to the UGC-New Delhi for its financial support through Dr. D.S. Kothari Post Doctoral Fellowship Scheme, (Grant No. F-4/2006 (BSR)/13-482/2011(BSR).

Conflict of Interest

The authors declare that there are no conflicts of interest.

REFERENCES

1. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell, 1990; 61: 759-767.
2. Jimeno J, Faircloth, Soussa G, Scheuer JF, Rinehart K. New marine derived anticancer therapeutic- A journey from the sea to clinical trials. Mar. drugs, 2004; 2: 14-29.
3. Suganthi K, Bragadeeswaran S, Sri Kumaran N, Thangaraj S, Balasubramanian T, Biological and pharmacological activities of jelly fish *Crambionella stuhalmanni* (Chun, 1896) and *Chrysaora quinquecirrha* (Desor, 1843). Inter J. Pharm.Pharmaceut Sci.2011; 3(2): 230-236.
4. Wilson-Sanchez G, Moreno-Félix C, Velazquez C. Antimutagenicity and antiproliferative studies of lipidic extracts from white shrimp *(Litopenaeus vannamei*). Mar. Drugs, 2010; 8: 2795-2809.
5. Bhatnagar I, Kim S. Immense essence of excellence: Marine microbial bioactive compounds. Mar. Drugs, 2010; 8: 2673-2701.
6. Mayer F, Mueller S, Malenke E, Kuczyk M, Hartmann JT, Bokemeyer C. Induction of apoptosis by flavopiridol unrelated to cell cycle arrest in germ cell tumour derived cell lines. Invest. New Drugs, 2005; 23: 205-211.
7. Wijesekara I, Kim S. Angiotensin-I-converting enzyme (ACE) inhibitors from marine resources: Prospects in the pharmaceutical industry. Mar. Drugs, 2010; 8: 1080-1093.
8. Yanagihara AA, Kuroiwa JMY, Oliver LM, Chung JJ, Kunkel DD. Ultrastructure of a novel eurytele nematocyst of *Carybdea alata* Reynolds (Cubozoa Cnidaria). Cell Tiss. Res, 2002; 308:307-318.
9. Carmichael J,William G, DeGraff Adi F, Gazdar. Evaluation of a Tetrazolium-based Semiautomated Colorimetric Assay: Assessment of Chemosensitivity Testing. Cancer Res, 1987;47: 936-942.

10. Kideys AE, Kovalev AV, Shulman G, Gordina A, Bingel F. A review of zooplankton investigations of the Black Sea over the last decade. J Mar Syst, 2000; 24: 355-371.
11. Ghosh TK, Gomes A, Nag Chaudhuri AK. Glycaemic effects of tentacle extract of the jellyfish *Acromitus rabanchatu* (Annandale). Indian J Exp Biol, 1990; 5: 441-3.
12. Mendis E, Rajapakse N, Kim SK. Antioxidant properties of a radical-scavenging peptide purified from enzymatically prepared fish skin gelatin hydrolysate. J. Agric. Food Chem, 2005; 53: 581-587.
13. Picot L, Bordenave S, Didelot S. Antiproliferative activity of fish protein hydrolysates on human breast cancer cell lines. Process Biochem , 2006; 41: 1217-1222.
14. Aleman A, Gimenez B, Perez-Santin E, Gomez-Guillen M, Montero P. Contribution of Leu and Hyp residues to antioxidant and ACE-inhibitory activities of peptide sequences isolated from squid gelatin hydrolysate. Food Chem, 2011; 125: 34-341.
15. Wang Q, Xiao L, He Q, Liu S, Zhang J. Comparison of haemolytic activity of tentacle-only extract from jellyfish *Cyanea capillata* in diluted whole blood and erythrocyte suspension: Diluted whole blood is a valid test system for haemolysis study. Exp Toxicol Pathol, 2012; 64 : 831-5.
16. Tibballs J. Australian venomous jellyfish, envenomation syndromes, toxins and therapy, Toxicon, 2006; 48: 830-859.
17. Suput D. In vivo effects of cnidarian toxins and venoms. Toxicon, 2009; 54: 1190-1200.
18. Currie B. Clinical implications of research on the box-jellyfish *Chironex fleckeri*, Toxicon, 1994; 34: 1305-1313.
19. Ramasamy S, Isbister GK, Seymour JE, Hodgson WC. The in vivo cardiovascular effects of box jellyfish *Chironex fleckeri* venom in rats: efficacy of pre-treatment with antivenom, verapamil and magnesium sulphate. Toxicon, 2004; 43: 685-690.
20. Ramasamy S, Isbister GK, Seymour JE, Hodgson WC. Pharmacologically distinct cardiovascular effects of box jellyfish (*Chironex fleckeri*) venom and a tentacle-only extract in rats. Toxicol Lett, 2005; 155: 219-226.
21. Ramasamy S, Isbister GK, Seymour JE, Hodgson WC. The in vivo cardiovascular effects of the Irukandji jellyfish (*Carukia barnesi*) Knematocyst venom and a tentacle extract in rats, Toxicol Lett, 2005; 155: 135-141.
22. Chung J, Fernandez K, Ratnapala LA, Cooke IM, Yanagihara AA. Partial purification and characterization of Hawaiian box jellyfish *Carybdea alata* venom. Pacific Science, 2000; 39: 90-97.

23. Freitas V, Rangel M, Bisson L, Jaeger R, Machado-Santelli G. The geodiamolide H, derived from Brazilian sponge *Geodia corticostylifera*, regulates actin cytoskeleton, migration and invasion of breast cancer cells cultured in three-dimensional environment. J. Cell. Physiol, 2008; 216: 583-594.
24. Hsu K, Li-Chan, E, Jao, C. Antiproliferative activity of peptides prepared from enzymatic hydrolysates of tuna dark muscle on human breast cancer cell line MCF-7. Food Chem, 2011; 126: 617-629.
25. Cioca DP, Kitano K. Induction of apoptosis and CD10/neutral endopeptidase expression by jaspamide in HL-60 line cells. Cell. Mol. Life Sci, 2002; 59 (8):1377-1387.
26. Weber J. Poly (γ-glutamic acid) are the major constituents of nematocysts in Hydra (Hydrozoa), Cnidaria. J Biol Chem, 1990; 265: 9664-9669.
27. Bhatnagar I, Kim S. Immense essence of excellence: Marine microbial bioactive compounds. Mar. Drugs, 2010; 8: 2673-2701.
28. Mayer F, Mueller S, Malenke E, Kuczyk M, Hartmann JT, Bokemeyer C. Induction of apoptosis by flavopiridol unrelated to cell cycle arrest in germ cell tumour derived cell lines. Invest. New Drugs, 2005; 23: 205-211.
29. Wijesekara I, Kim S. Angiotensin-I-converting enzyme (ACE) inhibitors from marine resources: Prospects in the pharmaceutical industry Mar. Drugs, 2010; 8:1080-1093.