MARINE GREEN ALGAE AS AN ANTI BREAST CANCER AGENT- CYTOTOXIC TEST OF CHAETOMORPHA COMPRESSA (BORY) KUTZING

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***Abstract*: As breast cancer is becoming a global threat and the burden of it is challenging the conventional preventive and treatment measures, the rate of survival is very difficult for this malignancy. Here, along with the advancement of medications, it is important to find out the new effective alternatives for cancer treatment. The marine green algae, Chaetomorpha has been proven to be a better anticancer agent against the human breast carcinoma cells.**

***Keywords*: breast cancer, algae, anticancer**

INTRODUCTION

Among various diseases, cancer has become a big threat to human beings globally. As per Indian population census data, the rate of mortality due to cancer in India was high and alarming with about 806000 existing cases by the end of the last century. Cancer is the second most common disease in India responsible for maximum mortality with about 0.3 million deaths per year. This is owing to the poor availability of prevention, diagnosis and treatment of the disease. All types of cancers have been reported in Indian population including the cancers of skin, lungs, breast, rectum, stomach, liver, cervix, esophagus, bladder, blood, mouth etc. The causes of such high incidence rates of these cancers may be both internal (genetic, mutations, hormonal, poor immune conditions) and external or environmental factors (food habits, industrialization, over growth of population, social etc.) (Imran et al, 2011).

Breast cancer is the most common malignancy type diagnosed in women in developed countries and the second most common type diagnosed in developing countries. Breast cancer has been described as an alarmingly health problem in India (Yeole et al, 2003). According to the reports, breast cancers have badly attacked women population in India. A survey carried out by Indian Council of Medical Research (ICMR) in the metropolitan cities viz. Delhi, Mumbai, Bangalore and Chennai; from 1982 to 2005; has shown that the incidences of breast cancer have doubled. Over the years, the incidences of breast cancer in India have steadily increased and as many as 100,000 new patients are being detected every year (Yip et al, 2006; Michael et al, 2003). A 12% increase has been registered by cancer registries from 1985 to 2001, which represented 57% rise of cancer burden in India (Yip et al, 2006). The incidence of breast cancer is increasing in the developing world due to the increase in life expectancy, increased urbanization and adoption of western lifestyles (WHO, 2011). It is estimated worldwide that over 508,000 women died in 2011 due to breast cancer (WHO, 2013). Almost 50% of breast cancer cases and 85% of deaths occurred in less developed countries (GLOBOCAN, 2008).

As the risk factors associated with the breast cancer is very high and the rate of survival seems very difficult, it is clear that the established medications and the conventional diet controls cannot make this threat prevented or cure, where lies the importance of the current research work to find if the marine green algae, Chaetomorpha compressa (Bory) Kutzing can be a better anticancerous agent against the human breast cancer.

Chaetomorpha compressa Bory (Kutzing) (Plate 1)

Phylum : Chlorophyta
Subphylum : Chlorophytina
Class : Ulvophyceae
Order : Cladophorales
Family : Cladophoraceae
Genus : Chaetomorpha
Species : **Chaetomorpha compressa (Bory) Kutzing**

Chaetomorpha compressa (Bory) Kutzing is marine filamentous green algae. Algae of this genus are made up of macroscopic filaments of cylindrical cells. The genus is characterized by its unbranched multicellular filaments, making it distinctive; its closest relatives are branching species of the genus Cladophora. Filaments are long up to several centimeters and 1 mm thick. Each filament shows fragmentation. Fragments of filaments divide its cells. Each cell is about 2- 3 mm in diameter. From thalli, there may arise a several hundreds of long filaments. Analysis of molecular phylogenetic data of Chaetomorpha indicates that it forms a clade that is nested or grouped in a paraphyletic assemblage of branched species like Cladophora. It follows that the unbranched condition is evolutionarily conserved and likely evolved early in the evolution of this clade. This name is of an entity that is currently accepted taxonomically. Basionym is Conferva compressa Bory (Frederic et al., 2011).

Distribution

Chaetomorpha compressa (Bory) Kutzing exhibits a wide range of distribution in many parts of marine ecosystems. It occurs in abundance in very large colonies. It occur in a worldwide distribution throughout Asia, Europe, Atlantic islands, North America, Central America, Caribbean islands, tropical and subtropical regions of Western Atlantic etc. In Asia, distribution of Chaetomorpha has been reported in south western region, ie, in the Arabian Gulf along India, Oman, Pakistan and Sri Lanka. In India, this species occurs abundantly in the coastal areas of Goa.

MATERIALS AND METHODS

Study area

The study area, Payyambalam Beach **(Plate 2)**, a part of Arabian Sea is located in Kannur district which is located in the Northern part of Kerala and is about mere two kilometers away from Kannur town. This area lies between 11.987' N latitude and 75.349'E longitude. Here, the climate is very hot and humid with maximum and minimum temperature ranging from 27ºC to 31^{0}C. The average annual rainfall is 3614 mm. The beach is associated with a children's park. Due to which the human intervention is more in this area compared to other coastal areas in Kannur.

Test for anticancerous activity

The test for anticancerous activity of the selected sample was performed by using MTT Assay Technique. The specific cell line selected for cytotoxicity test was Human Breast Carcinoma Cells.

Cell culture

The Human Breast cancer (MCF-7) cells were procured from the National Center for Cell Sciences (NCCS), Pune, India. The cancer were maintained in Dulbecco's modified eagles medium (DMEM) supplemented with 2mM l-glutamine and balanced salt solution (BSS) adjusted to contain 1.5 g/L Na_2CO_3, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 mM l-glutamine, 1.5 g/L glucose, 10 mM (4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid) (HEPES) and 10% fetal bovine serum (GIBCO, USA). Penicillin and streptomycin (100 IU/100µg) were adjusted to 1mL/L. The cells were maintained at 37◦C with 5% CO_2 in a humidified CO_2 incubator.

Evaluation of cytotoxicity

The inhibitory concentration (IC_{50}) value was evaluated using an MTT [3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Cancer cells were grown (1×10^4 cells/well) in a 96-well plate for 48 h in to 75% confluence. The medium was replaced with fresh medium containing serially diluted synthesized compounds, and the cells were further incubated for 48 h. The culture medium was removed, and 100µL of the MTT [3-(4,5-dimethylthiozol-2-yl)-3,5-diphenyl tetrazolium bromide] (Hi-Media) solution was added to each well and incubated at 37 ◦C for 4 hours. After removal of the supernatant, 50 µL of DMSO was added to each of the wells and incubated for 10 min to solubilize the formazan crystals. The optical density was measured at 620 nm in an ELISA multiwell plate reader (Thermo Multiskan EX, USA). The OD value was used to calculate the percentage of viability using the following formula.

Percentage of viability = OD value of sample/OD value of experimental control ×100

Morphological study

The MCF-7 cells that were grown on cover slips (1×10^5 cells/cover slip), incubated for 6-24h with compounds at the different concentration, and they were then fixed in an ethanol : acetic acid solution (3:1). The cover slips were gently mounted on glass slides for the morphometric analysis. Three monolayers per experimental group were photo micrographed. The morphological changes of the MCF-7 cells were analyzed using Nikon (Japan) bright field inverted light microscopy at 40x magnification.

Fluorescence microscopic analysis of apoptotic cell death

Approximately 1µL of a dye mixture (100 mg/mL acridine orange (AO) and 100 mg/mL ethidium bromide (EtBr) in distilled water) was mixed with 90 µL of cell suspension (1×10^5 cells/mL) on clean microscope cover slips. The selected cancer cells were collected, washed with phosphate buffered saline (PBS) (pH 7.2) and stained with 10 µL of AO/EtBr. After incubation for 2 min, the cells were washed twice with PBS (5 min each) and visualized under a fluorescence microscope (Nikon Eclipse, Inc, Japan) at 400× magnification with an excitation filter at 480 nm. Based on the morphological studies and cell death count, the anticancerous potential of the particular strain is estimated.

RESULTS

Anticancerous activity of selected alga- mtt assay

Cytotoxicity assay

The effect of Chaetomorpha compressa (Bory) Kutzing extract on the cell response of human breast cancer (MCF-7) was examined by using the MTT assay. **Table 1** shows the In vitro cytotoxic activity of compounds (10-50µg concentrations) against selected cancer cells. The experimental results demonstrate that the extract has the ability to inhibit cell proliferation in a dose dependent manner. From the values obtained a figure is plotted against concentration of the sample and cell viability **(Figure 1)**. From the figure the IC_{50} values of extract against MCF-7 breast cancer cells were calculated and it is found to be 37 ± 0.5 µg/ml concentrations. Further the execution of cytotoxicity in the cells was significantly higher than in the untreated control. Since the unique properties of extract may plays an imperative role in the promising effect on cell proliferation, in this depiction, the

cytotoxic effect of extract with different concentrations have been explored. In the present findings, extract possibly would bring to bear cytotoxic effect on selected cancer cell lines at minimal concentrations and insignificant toxicity in normal cells. In short, the current study suggests that extract's cytotoxicity may be related to enhance membrane-mediated apoptosis.

Analysis of Cell Morphology

Plate 3 shows morphological changes in MCF-7 cells after treatment with extract with various concentrations for 24 hours. Phase-contrast micrographs reveal that the extract has the ability induces increased cell shrinkage, membrane blebbing and forms floating cells, compared to the control cells in a dose-dependent manner. Cytological investigations elucidate the antiproliferative effect routed through membrane blebbing, membrane instability and disturbing the cytoskeleton of the cells by the extract. In **Plate 3**, the image (a) shows the cell death caused when the control is used. Whereas, (b), (c) and (d) images clearly shows the membrane blebbing and formation of floating cells in the treated cancer cells. Moreover it could found that the isolated compounds showed significant cytotoxicity and anti-proliferative effects on selected human cancer cells.

Fluorescence microscopy analysis of nuclear fragmentation- Acridine orange /Ethidium bromide (AO/EtBr) staining Method

In order to elucidates the apoptotic activity of synthesized compounds, apoptotic c staining fluorescence microscopic analysis was carried out. Fluorescence microscopy images of MCF-7 cells in the absence of compound (Control) and in the presence of compound are shown in **Plate 4**. In it, the image (a) shows that the untreated MCF-7 cancer cells (control) did not show any significant adverse effect compared to the extract treated cancer cells. Whereas the extract (b), (c) and (d) showed a concentration dependent apoptotic activity. It can be observed that with the addition of extract to the cancer cells, the green color of cells are converted into orange/red color cells which is due to induced apoptosis and the nuclear condensation effect on the cells. On the basis of the overall cell morphology and the cell membrane integrity, apoptotic cells were distinguished from one another using fluorescence microscopy. Here, the apoptotic cells induced by the extract through activation apoptotic signals in the treated cells can be differentiated, confirms that the extract has an ability to inhibit the cells via apoptotic mechanism.

CONCLUSION

The current study of cytotoxicity of Chaetomorpha compressa (Bory) Kutzing against human breast carcinoma cells has shown that the selected algae can be a better alternative to the established anticancer drugs. Rather than the expected activity, the selected green algae has shown a better activity against the breast cancer cell lines as the standard cancer drugs, Doxorubucin does. As a natural resource, use of Chaetomorpha in breast cancer treatment is expected to nullify the side effects of actual medications of cancer. Even if the use of marine resources, especially marine plants in healthy nutrition and medication has already been undertaken, the current study of tumor suppressing activity of Chaetomorpha compressa has provided a new anticancerous species to the plant kingdom.

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PLATE 1: CHAETOMORPHA COMPRESSA (BORY) KUTZING

Macroscopic view

Microscopic view

Plate 2: Study area; Payyambalam beach, Kannur

Satelite View of Study Area

Table 1: Comparison of cancer cell death caused by doxorubicin and Chaetomorpha compressa (bory) kutzing

DOXORUBICIN	CHAETOMORPHA
93	98
73	73
55	65
44	58
38	55
33	53
29	50
26	43
23	40
21	32

Figure 1: Comparison of cancer cell death caused by doxorubicin and (bory) kutzing Chaetomorpha compressa

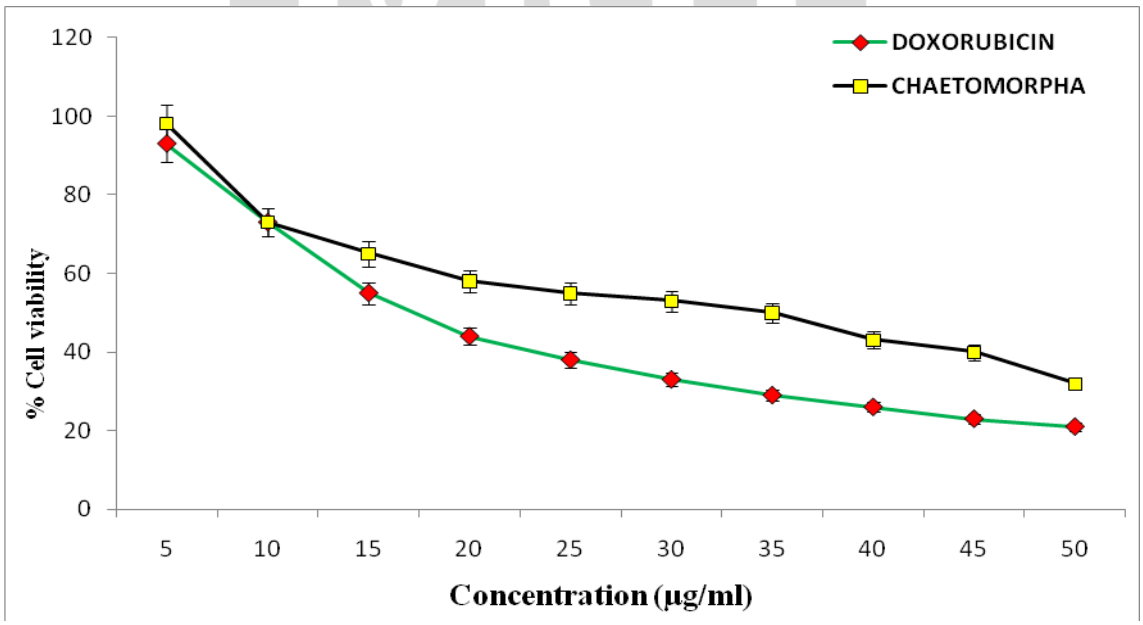


Plate 3: Cancer cell death caused by Chaetomorpha- nikon bright field inverted light microscopic image

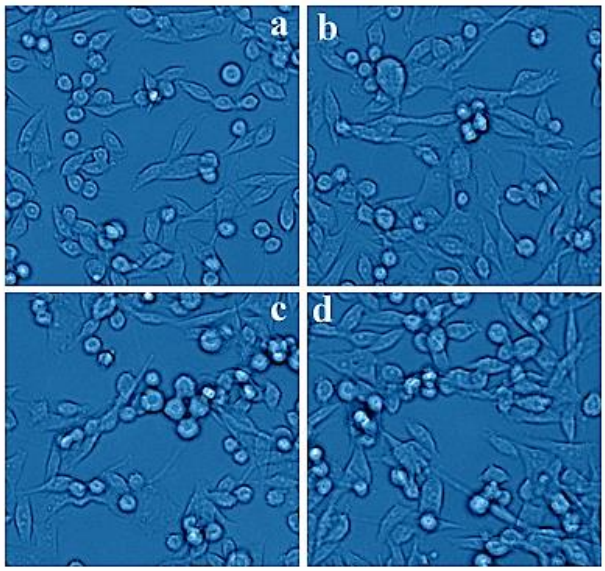
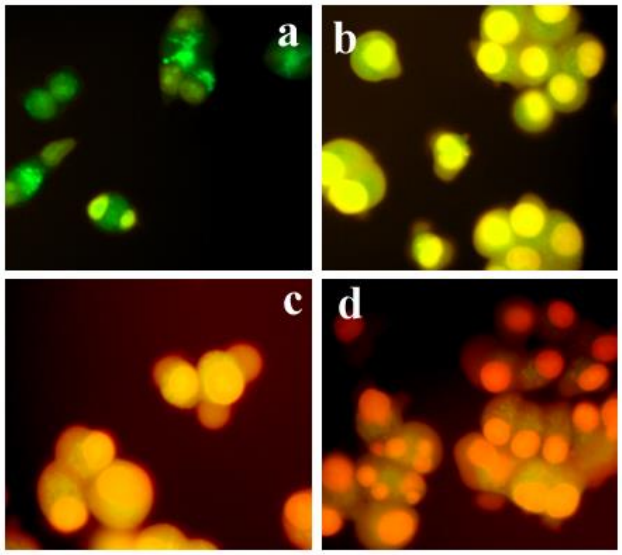


Plate 4: Fluorescence microscopic analysis of apoptoptic cell death at different concentrations of sample using AO/ETBR



a: Control
b: 10µg/ml extract
c: 20µg/ml extract
d: 40µg/ml extract