

The Cytotoxic Activity Chara elegans on Growth of same Cell Lines

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Abstract

Background: Cellular and molecular studies have shown algae derived ingredients to be potent naturally occurring anticancer compounds and have been suggested to prevent carcinogenesis. Fucoxanthin is a marine carotenoid found in green algae and is documented to display remarkable anticancer activity. Fucoxanthin suppressed the growth of LNCap prostate cancer cells in a concentration-dependent manner along with the induction G(1) cell cycle arrest, but not apoptosis.

Objective: In accordance with same concept, diplorethohydroxycarmalol (DPHC), isolated from the brown algae *Ishige okamurae*, notably reduced the level of radiation-induced intracellular ROS and protected cells from undergoing cell death in cultured Chinese hamster lung fibroblast (V79-4) cells.

Patients and Methods: Used leaf extraction from alge chara elegans aqueous extract and crude alcohol. Two types of cell lines, Hela cell line cervical cancer and normal cell embryo rat cell line REF(, were used in this study. They were obtained kindly from Iraqi Center for cancer and medical genetic research (ICCMGR) .The cell line were grown on uncoated coverslips in a Dulbecco's Minimal Essential Medium (DMEM) with 10 fetal bovine serum (PAA), 2 μ M glutamine (PAA), 100 μ /ml penicillin, and 100 μ g/ml streptomycin.

Results: The results showed this alge contains most of the chemical compounds such as Alkaloids, Saponins, Glycosides, Flavonoids, Steroids and Terpenes. The cytotoxicity of the aqueous extract and crude alcohol extract was investigated on the cancer cells line, hela and normal cell line Ref. Toxic effect for both extracts was indicated by rate of proliferation inhibition . The alcohol extract showed the inhibition of Hela cell line at percentage 20.35%-72.66% more than the aqueous extract (18.68%-48.26%) at concentrations: 62.5-2000 μ g/ml. Both extracts (alcoholic extract and aqueous extract) showed almost the same effect at the concentration 8000 μ g/ml. At low concentrations of both extracts no inhibition effect was observed on normal cells line Ref. The alcohol extract showed the inhibition of Ref cell line at percentage 67.61% more than the aqueous extract (66.66%) at concentrations 8000 μ g/ml.

Conclusion: The conclusion was made that the C. elegans is a promising alge in treatment of cancer through its inhibition of the proliferation of cancer cells., the results demonstrated that extraction from leaf chara elegans aqueous extract and crude alcohol is inhibition Hela cell line cell, and lesser inhibition extend against Ref cell line.

Key words: Cell line, Chara elegans, Hela cell line.

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Introduction

Cancer is a leading cause of death worldwide. Studies show that in 2012, nearly 14.1 million new cases were diagnosed with cancer, and 8.2 million people died from the disease.[1] Cytotoxic antitumor compounds isolated from marine organisms have been reported in several sources during the 40 years, Halomon isolated from a red alga[2] is under clinical trial phases and will be offered as pharmaceutical products in the future. Secondary metabolites with antitumor activity have been extracted and identified in *Sargassum thunbergii*, *Sargassum horneri*, Algae belong to a very large and diverse group of simple, typically autotrophic organisms. Increasingly data appears that all land plants (embryophytes) diverged from ancestral Charophycean algae [3].

The most common breast cancer type is the invasive ductal carcinoma accounting for 70-80% of all breast cancers diagnosed.[4] Complement to breast mastectomy, radiation therapy and chemotherapy are frequently used for management of this malignancy. There are many chemotherapeutic agents used in the treatment of breast cancer. Nevertheless, due to the high side effects and resistance of cancer cells to these drugs,[5] there is still urgent need to develop new and more efficient therapeutic agents to battle the disease.

Nature with its incomparable biodiversity of chemical agents has been the leading source for development of effective drugs. Nowadays, nearly 60% of all of the drugs used in cancer treatment are based on natural products.[6] Covering almost 70% of the

earth's surface, the marine environment seems to be a treasury of novel bioactive compounds. Marine algae have been consumed as food in many parts of the world. They are rich in dietary fiber, minerals, polysaccharides, carbohydrates, lipids, proteins, and vitamins.[7,8] Recent studies have shown that seaweeds can be a source of new anticancer drugs.[9] .The capacity of algal polysaccharides to incite cancer cell multiplication has been overall recorded the epidemiological information is up held by rat model studies exhibiting defensive impacts of dietary kelps and other red and green algae against mammary tumors. Molecular and cellular level studies on algae have indicated that algae derived bio actives are potent cancer inhibitors documentation of new active cancer inhibiting agents have globally turn out to be a significant strategy[10]. Various green algae contain a sulfated polysaccharide named fucoidan in their fibrillar cell walls and intercellular spaces considered to protect the seaweeds against desiccation. Fucoidan has lately gone under various anticancer, cell cycle arrest, and apoptosis studies.[11] The results indicate that the substance has promising characteristics which may lead to a future anticancer marine drug.

The unique ecological properties of this area have led to the growth of some novel organisms in this region. 153 species of marine algae have been reported to live along the Iraq.[12]. Marine algae are found to contain high amount of nutrients, vitamins (A,B,C,D &E), minerals (Ca, P,Na &K),

antioxidants and dietary fibers, that's why they are used as food, fodder and other commercial purposes throughout the world they are also very rich in novel compounds and can be explored for the development of drugs to combat deadly diseases like cancer[13].

Only few studies have looked into the pharmacological properties of algae from Persian Gulf and Oman Sea.[14,15] The aim of this research was to determine the in vitro cytotoxic activity of total alcoholic and

aqueous extracts of green algae acquired from the Baghdad in two cell lines Hela and Ref cell line.

Patients and Methods

Preparation of pomegranate extract The plant The plat used in these experiments was gathered from general local river in Baghdad (Figure 1). Plant specimens (leaves, stems) were taken to Dr. Ndal Adres. Collage of education dep. Biology as chara elegans , Family Charalease



Figure (1): *Chara elegans*

Algae samples were collected from the Baghdad 2017. An aqueous extract of chara elegans was prepared using fresh leave 100 g which soaked in 250 ml boiling distilled water for about 6 hours on a hot plate and homogenized. For the following experiments, 10g of powdered plant was dissolved into 100ml PBS (as a solvent), filtered and sterilized by using 0.2 μm sterile Millipore filtering system, and the stock solution kept in sterile containers at 4°C until use[16]. Crude alcoholic extraction from the leafs of this plant was extracted as described by[16].

Cell Growth Assay

Two types of cell lines, Hela and REF, were used in this study. They were obtained

kindly from Iraqi Center for cancer and medical genetic research (ICCMGR) The cell line were grown on uncoated coverslips in a Dulbecco's Minimal Essential Medium (DMEM) with 10 fetal bovine serum (PAA), 2 μM glutamine (PAA), 100 $\mu\text{g}/\text{ml}$ penicillin, and 100 $\mu\text{g}/\text{ml}$ streptomycin [17].

Cytotoxicity assay

The effect of the aqueous extract of Chara elegans on growth of cancer cells was determined by using inhibitory activity assay. Briefly, cell cultures in the micro titration plate were exposed to various concentrations (15.6,31.2,62.5,125,250,500,1000,2000,4000, 8000 $\mu\text{g}/\text{ml}$) of plant extract during the log phase. The cytotoxic activity was determined

after (24, 48 and 72 h) [18]. The inhibition rate was calculated according to the below equation [19].

Inhibition % = [(optical density of control wells -optical density of test wells)/optical density of control wells] x 100 [19].

Statistical Analysis

Evaluation Statistical of the experment was calculated using Student's t-test.A

probability of 0.05 or less was statistically significant[20].

Results

Extraction the equeous and alcoholic of chara elegans by using water or methanol a has gave a brown color.Chemical test table (1) results show the chemical test for the general constituents of the extracts of chara that's contained flavonoids, sapoins,terpenoids and alkaloids.

Table (1): Detected phytochemicals in chara elegans extraction

Phytochemical to be detected	extraction
Alkaloids	+
Flavonoids	+
Terpenoids	+

Effect extraction of chara elegans in Hela cell line after 48 hours:

The effect of treating Hela cell line with the aqueous and alcoholic extraction frpm chara elegans are shown in Table (2) chara elegans extraction was showed significant inhibitory effect on viability of Hela cell line p 0.05 dependent dose and time . viability of the cell decreased with concentration reaching its lowest value after 48 hrs of treatment with

highest concentration used 8000 µg/ml the inhibitory the cell line is 79.98% ,79.75% the lowest percentage of the cell viability was reached 9.68% ,8.99% at 15.6 µg/ml. see Fig.2. The alcohol extract showed the inhibition of Hela cell line at percentage 20.35%-72.66% more than the aqueous extract (18.68%-48.26%) at concentrations: 62.5-2000 µg/ml.

Table (2): Effect of different concentration of alcoholic and aqueous extract of chara elegns on growth of hela cell line

alcoholic extract	aqueous extract	con
Inhibited percentage	Inhibited percentage	µg/ml
d 8.31± 1.15	e 8.31±1.15	control
cd 3.96± 8.99	ef 2.84± 9.68	15.625
c 5.81±14.64	de 4.18± 16.66	31.25
bc 4.89± 20.35	de 9.24±18.68	62.5
b 6.49±30.21	de 4.82± 20.23	125
a 0.95±70.06	cd 2.19±26.41	250
a 1.28±70.35	cd 3.91± 29.65	*500
a 1.50± 71.74	bc 1.21± 37.19	*1000
a 1.18± 72.66	b 7.21± 48.26	*2000
a 2.11± 76.58	a 1.67±78.71	4000
a 1.04± 79.75	a 0.40± 79.98	8000

*Different letters means the presence of significant different at (P<0.05) Probability level.

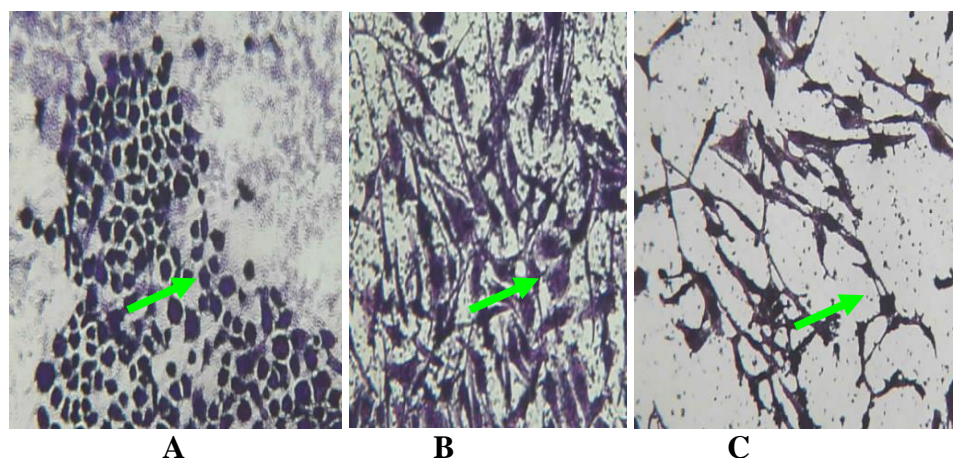


Figure (2): A. normal without extraction B, effect the extraction of *chara elegans* in concentration 8000µg/ml in aqueous extract in Hela cell C. effect the extraction of *chara elegans* in concentration 8000µg/ml in alcoholic extract in Hela cell .

Effect extraction of chara elegans in RFE cell line after 48 hours

The effect of treating RRF cell line with the aqueous and alcoholic extraction from *chara elegans* are shown in Table (3) *chara elegans* extraction was showed dose and time significant inhibitory dependent dose and time effect on viability of Ref cell line $P < 0.05$ viability of the cell decreased with high concentration reaching its lowest value

after 48 hrs of treatment with highest concentration used 8000 µg/ml the inhibitory is 67.61% ,66.66% .the lowest percentage of the cell viability was reached 0.57% ,4.68 % at 15.6 µg/ml. see Fig.2. The alcohol extract showed the inhibition of Hela cell line at percentage 16.09%-57.52 % more than the aqueous extract (16.21%-10.26%) at concentrations: 62.5-2000 µg/ml.

Table (3): Effect concentration of alcoholic and aqueous extract of *chara elegans* on growth of RFE cell line

alcoholic extract	aqueous extract	con
Inhibited percentage	Inhibited percentage	µg/ml
c 16.24±0.00	c 3.33± 0.190	control
c 5.86±0.57	c 2.07± 4.68	15.625
c 28.45 ±2.28	c 25.44±26.57	31.25
bc 2.25 ±16.09	c 14.33±16.21	62.5
c 6.99±11.47	c 11.82±10.08	125
ab 1.19 ±45.13	bc 8.73± 3.96	*250
ab 7.55± 46.09	bc 15.75± 7.56	500
a 9.52 ±51.61	bc 2.79±7.92	*1000
a 6.61 ±57.52	bc 7.87± 10.26	*2000
a 5.51 ±59.99	ab 4.48± 39.27	*4000
a 9.22± 67.61	a 0.90± 66.66	8000

*Different letters means the presence of significant different at ($P < 0.05$) Probability level

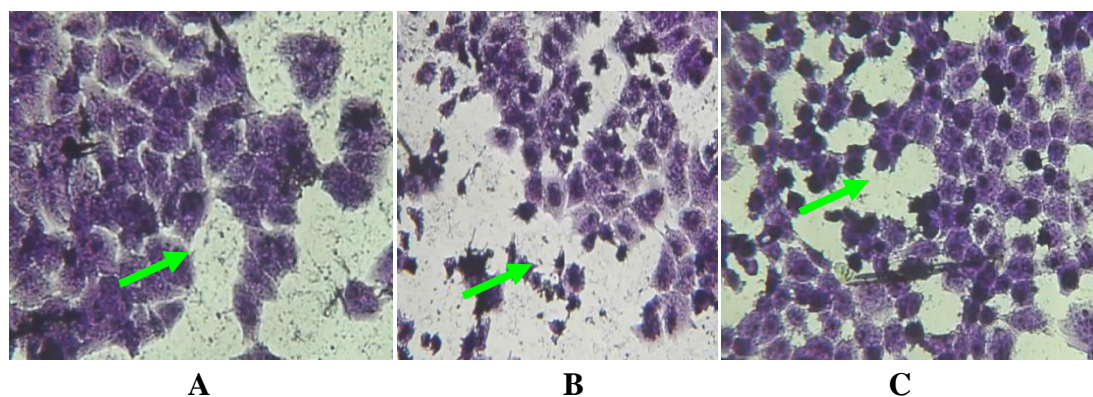


Figure (3): A. normal without extraction B, effect the extraction of chara elegans in concentration 8000µg/ml in aqueous extract in Ref cell C. effect the extraction of chara elegans in concentration 8000µg/ml in alcoholic extract in Ref cell .

Discussion

The cytotoxicity of natural products is based on the presence of antitumor metabolites. More than 140 secondary metabolites from Phaeophyceae have been reported.[21] This study on total extract and different fractions of chara elegans represented cytotoxicity of aqueous extract and crude alcohol fractions of this alga against all studied cell lines while aqueous fraction of this plant showed good cytotoxicity in Hela cell lines without cytotoxic effects on the normal cell line just in high concentration (Ref). Cytotoxic activity of this alga could be related to presence of alkaloids terpenoids and sulfated polysaccharide [22].

The cytotoxicity of natural products is based on the presence of antitumor metabolites. Bioactive cytotoxic compounds have been found in marine algae. Several sulfated polysaccharides separated from algae have shown antitumor, anticancer, antimetastatic

activities in mice Antitumor activity has also been noted with the macro algae Sargassum stenophyllum In addition, the hydroquinone diterpen from Cystoseira mediterraneol has been shown to have an inhibitory effect on mitotic cell division [23,24].

Conclusion

Taken together, these data suggest that extracts from algae have the potential to inhibit Hela cell lines but also to inhibit normal Ref cells. Each algae extract demonstrated a different pattern of inhibition, in different concentration.

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