European Review for Medical and Pharmacological Sciences 2010; 14: 669-673

Anticancer activity of *Sargassum oligocystum* water extract against human cancer cell lines

K. ZANDI*§, S. AHMADZADEH*, S. TAJBAKHSH*, Z. RASTIAN*, F. YOUSEFI*, F. FARSHADPOUR*, K. SARTAVI**

The Persian Gulf Marine Biotechnology Research Center and §The Persian Gulf Tropical and Infectious Disease Research Center, Bushehr University of Medical Sciences, Bushehr (Iran); **Jahad Keshavarzi Research Center, Bushehr (Iran)

Abstract. – *Background and Objectives:* **Antitumor drug resistance and side effects of antitumor compounds are the most common problems in medicine. Therefore, finding new antitumor agents with low side effects could be interesting. This study was designed to assay antitumor activity of the extract from brown alga *Sargassum oligocystum*, gathered from Persian Gulf seashore, against K562 and Daudi human cancer cell lines.**

Materials and Methods: **The research was performed as an *in vitro* study. The effect of the alga extract on proliferation of cell lines were measured by two methods: MTT assay and trypan blue exclusion test.**

Results and Conclusion: **The most effective antitumor activity has been shown at concentrations 500 µg/ml and 400 µg/ml of the alga extract against Daudi and K562 cell lines, respectively. The results showed that the extracts of brown alga *Sargassum oligocystum* have remarkable antitumor activity against K562 and Daudi cell lines. It is justified to be suggested for further research such as algal extract fractionation and purification and *in vivo* studies in order to formulate natural compounds with antitumor activities.**

Key Words:

Antitumor activity, *Sargassum oligocystum*, Daudi, K562, Cold water extract.

Introduction

Regarding to cancer cells resistancy to antitumor drugs, finding new effective anticancer compounds with less side effects has been a field of interest for many scientists. Such studies have been developed in recent years by nanotechnology which represents a new area for health care and biotechnology[1]. There has been many attention to natural compounds obtained from plants or seaweeds to investigate about their medicinal properties. The antitumor activity was one of the most important activities in marine drugs, and lots of algae and their metabolites have been showed potent cytotoxicity. These metabolites have played an important role in leading to new pharmaceutical compounds from algae for antitumor drugs. Several studies have been done on antitumor activity in the east of Asia[2]. In one study a research group has screened 39 algae from seacoast of China for their possible antitumor activities and they showed that four species of *Rhodophyta* algae and three species of *Phaeophyta* exhibited cytotoxic effects against KB and HT-29 cancer cell lines. More than 30 compounds including bromophenols, carotene and steroids were isolated and purified from them and their effects on cancer cell lines have been evaluated separately[2]. Mechanism of antitumor activity of *Sargassum fusiforme* has been investigated in China[3]. 306 species of marine algae from Japan coast were tested for in vitro antitumor activity which four species of green algae showed strong cytotoxicity against murine lymphoid leukemia L1210 cell line besides its low cytotoxity to normal cell[4]. The glycoprotein derived from *Chlorella vulgaris* showed immunoactive antitumor activity[5]. Based on investigation which has performed on anticancer activity of fucoidans extracted from *Sargassum polycystum*, *Sargassum oligocystum*, *Sargassum mcclurei*, *Sargassum swartzii* and *Sargassum denticaprum* in Vietnam, it has indicated that sulfated compounds of them play important roles for their antitumor activity[6].

Sargassum oligocystum is a brown alga of *Sargassaceae* family which is widespread in the Persian Gulf and has been selected in order to inves-

Corresponding Author: Keivan Zandi, Ph.D; e-mail: zandi@bpums.ac.ir and keivanzandi@yahoo.com

tigate about its anticancer activity against two kinds of human tumor cell lines in this study.

Materials and Methods

Collection and Preparation of Alga Extract

Sargassum oligocystum, a brown alga, was collected along the Bushehr coast of Persian Gulf (south west of Iran) during september 2008. The alga was rinsed with distilled water. About 10 g of fresh algae which is equal to one gram of dried alga was homogenized in 100 ml cold double distilled water. The mixture was clarified by filtration using Whatman paper No.1 filter paper. Then the crude extract was sterilized separately by two methods, filtering (0.22 µm) and autoclaving. The sterilized extracts were stored in –80°C until the date of use.

Cell Lines

In this study, two human cancer cell lines, Human K562 (derived from human chronic myelogenous leukemia cells) and Human Daudi (derived from Burkitt Lymphoma cells) were used. These cells were obtained from the Hematology Department of Tarbiat Modarres University, Iran.

Cell Culture

K562 and Daudi cells were cultured by using RPMI 1640 (GIBCO®) containing 10% fetal bovine serum (GIBCO®). Cultured cells were incubated at 37°C in the presence of 5% CO_2[7].

Trypan Blue Exclusion Test

To detect the cell viability trypan blue exclusion test was chosen. The cancer cells were seeded at a density of 6×10^5 cells/well with different concentration of extract at 37°C in the presence of 5% CO_2. After 72 hr, 20 µl of medium and equal volume of trypan blue were mixed and viable and dead cells were counted by Neubauer haemacytometer[7].

MTT Assay

MTT assay as a proper cytotoxicity test was used in this study. Briefly, 10 µl of MTT stock solution [5 mg/ml in phosphate buffer saline (PBS)] was added to 90 µl medium of wells. The microplate was incubated at 37°C for 4 hours and then, the optical density of each well was read by the ELISA reader (TECAN, Switzerland) in 540 nm.[8]

Statistical Analysis

Microsoft Excel software (2007 edition) was used in order to calculation and drawing the cytotoxicity curve.

Results

Effect of Alga Extract on Daudi cell Line

The result of trypan blue exclusion test in order to viable and dead cell counting after exposure to different concentration of extract was summarized in Table I. By counting viable cells through trypan Blue exclusions test, it was showed that Daudi cells of negative control wells(without extract) were increased from 6×10^5 to 9.6×10^5 after 72 hr. As it showed in Table I, 100-200 µg/ml of extract didn't show a regardable effect on the viable cell population. In higher concentrations the viable cells were decreased which it demonstrated the cytostatic activity of alga extract on Daudi cells. The most effective concentration in which the number of viable cells were decreased significantly was 500 µg/ml of algal extract. Dead cells were presented in all concentrations of algal extract. The most amount of dead cell has been shown for 400 and 500

Table I. Results of Viability Test for Daudi cell line after treatment with algal extract.

Number of dead cells	Number of viable cells	Extract concentration (µg/mL)
1.3×10^5	9.6×10^5	0
1.4×10^5	8.5×10^5	100
1.4×10^5	8.4×10^5	200
2×10^5	7×10^5	300
1.9×10^5	5.7×10^5	400
2×10^5	4×10^5	500

Table II. Results of Viability Test for K562 cell line after treatment with algal extract.

Number of dead cells	Number of viable cells	Extract concentration (µg/mL)
1.3×10^5	10.2×10^5	0
1.2×10^5	9.1×10^5	100
1.2×10^5	7×10^5	200
1.5×10^5	6.9×10^5	300
1.5×10^5	4.5×10^5	400
1.6×10^5	4.6×10^5	500

µg/ml of algal extract. Dead cell increasing of negative control wells(without extract) was almost equal to the wells which were treated with 100 and 200 µg/ml concentration of extract. These results showed that there was no anticancer effect up to 200 µg/ml while at concentrations above the 400 µg/ml, a regardable cytotoxic and a cytostatic activity of alga extract on Daudi cells were observed. However, this extract had some side effects on cells in quality such as size and shape or they seemed somehow more wrinkled before adding extract.

Results of the evaluation of the algal extract cytotoxicity against Daudi cell line through MTT assay was shown in Figure 1. Regarding the collected data it was demonstrated that 500 µg/ml of extract showed a reasonable cytostatic effect which was confirmed by the result of counted viable cells.

Effect of Alga Extract on K562 cell Line

The result of trypan blue exclusion test in order to viable and dead cell counting after exposure to different concentration of extract against K562 cells was summarized in Table II. As it showed in Figure 1, the number of negative control (without extract) K562 cells were increased from 6.8×10^5 to 10.2×10^5 after 72 hr. Although there was a suppression effect on cell replication for the concentration 100 µg/ml, but it couldn't be expressed as a reasonable anticancer activity. The effective concentration was started from 300 µg/ml of algal extract concentration in which the number of viable cells didn't increase significantly. The most effective concentration of algal extract against K562 cells was 400 µg/ml. As it showed in Table II, the number of viable cells was decreased from 6.8×10^5 to 4.5×10^5. Interestingly, the concentrations above 400 µg/ml of extract didn't exhibit the better effect against K562 cells.

Dead cells counting showed that the algal extract caused less amount of dead cells in comparison to negative control cells.

As it shown in Figure 1 the result of MTT assay was confirmed that the most effective concentration against K562 cells is 400 µg/ml.

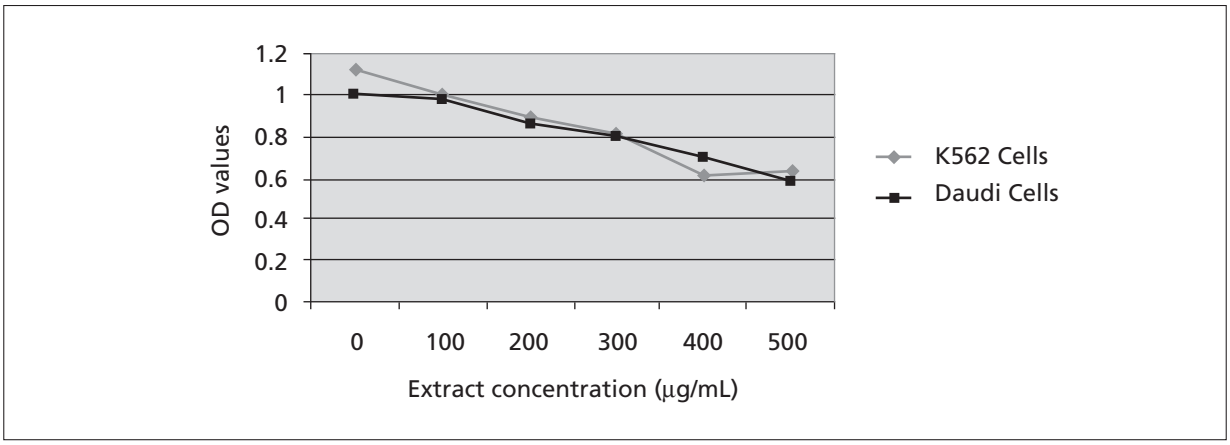

Figure 1. Result of MTT assay test for Daudi and K562 cell lines: 72 hours post treatment with algal extract.

Discussion

Many marine algae have been used as food among people in some part of the world, and certain algae have long been used in traditional Chinese herbal medicine in the treatment of cancer[9]. In recent years, an increasing number of studies have shown the bioactive potential of compounds produced by marine algae[10,11].

It is a long time that human beings have known marine algae as a rich source of pharmacologically active metabolites with antineoplastic, antimicrobial and antiviral effects[12,13]. The antitumor activity is one of the most important activities in marine algae, and many algae have showed cytotoxic and antitumor activities[2,14]. These antitumor activities can play an important role in leading to new pharmaceutical compounds for antitumor drugs[15].

Several species of algae have been found to be sources of metabolites with antitumoral and immune-stimulant activities[16]. Antitumor and cytotoxic properties of these species belong to four structural types: polyketides, terpenes, nitrogen-containing compounds and polysaccharides[17]. Among these structural types, polysaccharides from the *Sargassum* genus have antitumor activity[18]. In fact polysaccharides of edible algae attracted extensive interest due to their biological activities[19]. The anti-tumor effect of the polysaccharide from *Sargassum confusum* can improve functions of the immuno organs and enhance anti-oxidative capacity in tumor-bearing mice[20], and the polysaccharide from S*argassum fusiforme* has influence on apoptosis of tumor cells[3].

Random screening is effective to have found different marine algae with antitumor activities. For example, *Sargassum fusiforme* has been introduced as a selective human cancer cytotoxin[21], and significant activity against Ehrlich carcinoma was found in the brown alga *Scytosiphon lomentaria*[22]. An antitumor extract from a brown marine alga *Sargassum kjellmanianum* has been reported[9].

In this study, *Sargassum oligocystum* has been studied for its probable anticancer activity against two kinds of human tumor cell lines: Daudi and K562 cell line. Based on our data, sterilizing of the crude extract by filtering is better than autoclaving, so it could be concluded that some of its biological constituents are heat sensitive. In this study, the cold water extract of *Sargassum oligocystum* showed the reasonable activity against tumor cells replication. The most potent antitumor activity has been shown at concentrations 500 µg/ml and 400 µg/ml of the alga extract on Daudi and K562 cell lines, respectively. In this study the effective concentration is more than its counterpart in other studies and it seems that the main reason for this difference is because of using crude extract in this study. Besides, regarding to the number of K562 dead cells after exposuring to the different concentration of algal extract we could concluded that the cytostatic activity of the tested extract was more regardable comparing to its cytotoxic activity.

In conclusion the *Sargassum oligocystum* could be a good candidate for more studies on other cancer cell lines and in vivo antitumor evaluation. It can be a new source as new marine resource for antitumor medicine and to demonstrate that marine algae can be potential candidate sources as antitumor drugs.

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Acknowledgements

The Authors would like to thank Dr. Massoud Soleymani from Hematology Department of Tarbiat Modarres University, Iran, for providing human cancer cell lines. Also, we would like to thank Dr. Iraj Nabipour for his invaluable comments and scientific supports and Ms Bahareh Moghim for her comments to design the figures and tables.