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Full Length Research Paper

In vitro antitumor activity of *Gracilaria corticata* (a red alga) against Jurkat and molt-4 human cancer cell lines

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Gracilaria corticata **is a red alga which can be collected from many sea coasts around the world such as China, India, Persian Gulf, etc. The Persian Gulf is a unique marine habitat infested with diverse seaweeds. The aim of the present study is to explore anticancer potential of the crude extracts from *G. corticata* which was collected from the Bushehr coast (South west of Iran). Here, different concentration of the aqueous extract from *G. corticata* was tested for probable antitumoral activity on Jurkat and molt-4 human lymphoblastic leukemic cell lines. The cells were treated by different concentration of algal extract and the number of viable cells was determined by trypan blue. Also, cytotoxicity of the extract was evaluated by methyl thiazolyl tetrazolium (MTT) assay. The results showed that 9.336 and 9.726 µg/µl of algal extract were the most effective concentrations against Jurkat and molt-4 cells, respectively. The water crude extract of red alga *G. corticata* had significant anticancer activity and it might be a good candidate for further investigations in order to develop a natural compound as an anticancer agent which can be used for the production of potential anticancer drug and novel pharmaceutical leads.**

Key words: *Gracilaria corticata*, anticancer, Jurkat, molt-4.

INTRODUCTION

Cancer is one of the most serious threats to human health in the world and chemotherapy is still the standard treatment method. Most of the anticancer drugs currently used in chemotherapy are cytotoxic to normal cells and cause immunotoxicity which affects not only tumor development, but also aggravates patient's recovery. The discovery and identification of new antitumor drug with low side effects on immune system has become an essential goal in many studies of immunopharmacology (Xu et al., 2009). With this aim, many attentions have been paid to natural compounds in plants, marine organism and microorganisms. Regarding the low side effects of plants and other natural compounds, scientists are interested in working on them to find new medications. Finding anticancer agents from plant sources started in the earliest 1950s with the discovery and development of vinca alkaloids, vinblastine and vincristine and the isolation of the cytotoxic podophyllotoxins (Cragg and Newman, 2005).

In one study, antitumor activity of teriterpenoid fractions from the rhizomes of *Astilbe chinensis* in tumor bearing mouse was evaluated (Tu et al., 2008). It significantly inhibited the growth of mice transplantable tumor and remarkably increased splenocytes proliferation, natural killer cells activity and the level of interleukin-2 secreted by splenocytes in tumor-bearing mice.

Marine algae are one of the natural resources in the marine ecosystem. They contain various biologically active compounds which have been used as source of food, feed and medicine. Until now, more than 2400 marine natural products have been isolated from seaweeds of subtropical and tropical populations (Manilal et al., 2009).

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Recent findings evidenced that seaweeds contained antiviral (Matsuhiro et al., 2005), antibacterial (Xu et al., 2003), antifungal (Li et al., 2006) and antitumoral (Harada et al., 1997; Kezia et al., 2008) potentials, among numerous others.

The epidemiological data are supported by rodent model studies demonstrating protective effects of dietary kelps and other red and green algae against mammary, intestinal and skin carcinogenesis (Yuan and Walsh, 2006).

Many studies have focused on water soluble antitumor active substances from various marine algae, however most anticancer agent have not been used clinically because of their undesirable side effects on normal cells (Harada et al., 1997). According to existing literature, more than ten new experimental anti-tumor agents derived from marine sources have entered clinical trials, including bryostatin-1, aplidine, ecteinascidin-743 (ET-743), Kahalalide F, as well as derivatives of dolastatin such as TZT-1027 and LU 103793 (Song et al., 2008). Here, we tested the *in vitro* antitumor activity of *Gracilaria corticata* a red alga which is found in many part of the world such as China, Indian Ocean, Persian Gulf, etc. We have tested the algal water extracts activity against Jourkat and molt-4 cells which are two kinds of human leukemic cell lines.

MATERIALS AND METHODS

Preparation of algal extract

G. coticata, a red alga, was collected along the Bushehr coast of Persian Gulf during September, 2008. The alga was rinsed with distilled water. About 10 g of fresh alga which was equal to 1 g of dried one was homogenized in 100 ml cold double distilled water completely. Clarification of the algal mixture was carried out by filtration method using Whatman paper No.1 filter paper. Finally, algal clarified crude extract was sterilized by millipore filter with 0.22 µm pore size and stored at -80°C until the date of use.

Cell lines

Human Jurkat (lymphoblast-like) and molt-4 (lymphoblast-like) cell lines were chosen as proper representatives of human leukemic cell lines which we have received as gift from Tarbiat Modarres University.

Cell culture

The cells were cultured in 50 ml cell culture flasks (Orange Scientific) or 96 wells cell culture microplates (Orange Scientific) by using RPMI 1640 (Gibco) containing 10% fetal bovine serum (Gibco) and were incubated at 37°C in the presence of 5% CO_2 (Morgan et al., 1992).

Trypan blue exclusion test

In the first step to determine the viable cell number before and after treatment by algal extract, trypan blue exclusion test was used as a semi quantitative method. Briefly, the cancer cells were seeded at a density of 2×10^4 cells/well and they were treated with different concentrations of the algal extract for 72 h at 37°C in the presence of 5% CO_2. After 72 h, 20 µl of medium and equal volume of trypan blue were mixed and viable and dead cells were counted by Neubauer haemacytometer (Morgan et al., 1992).

Methyl thiazolyl tetrazolium (MTT) assay test

To determine the cytotoxicity of algal extract against studied cancer cell lines, MTT assay test was used as a quantitative and approved method. In this method, 10 µl of MTT stock solution (5 mg/ml in PBS) was added to 90 µl medium of wells which were treated by different concentrations of algal extract for 72 h. The microplate was incubated at 37°C for 4 h and then, the optical density of each well was read by microplate reader (ASYS – EXPERT 96) at 540 nm (Van de Loosdrecht et al., 1994).

RESULTS

Anticancer activity of *G. corticata* against Jurkat cell line

The viability test monitored by trypan blue exclusion test in order to determine the algal extract activity against Jurkat cells is presented in Table 1. Based on these data, it was demonstrated that Jurkat cells of negative control wells (without extract) were increased from 2×10^4 to 7×10^4 after 72 h. As shown in Table 1, in concentrations above 8.167 µg/µl of algal extract, the viable cells were decreased which demonstrated the cytostatic activity of the algal extract on Jurkat cells. The most effective concentration in which the number of viable cells was decreased signifantly was 9.336 µg/µl of algal extract. Dead cells were identified in all microplate wells. The highest number of dead cells has been shown for the well whose cells were treated by 9.726 µg/µl of algal extract. Dead cell increase in negative control wells (without extract) was almost equal to the wells which were treated by 5.835 µg/µl concentration of extract.

Results about cytotoxicity of the algal extract against Jurkat cell line through MTT assay is shown in Table 3. Based on data obtained, it was concluded that 9.336 µg/µl of *G. corticata* extract showed potent cytostatic effect which was confirmed by result of counted viable cells.

Anticancer activity of *G. corticata* against molt-4 cell line

Results of the trypan blue exclusion tests over viable and dead cell counting after exposure to different concentration of extract against molt-4 cells is summarized in Table 2. As shown in Table 1, the number of negative control (without extract) molt-4 cells were increased from 2×10^4 to 6.8×10^4 after 72 h. There was a suppression effect on cell replication for the concentration 8.167 µg/µl, which could not be expressed as a consistent anticancer activity. The most effective concentration of algal extract against

Table 1. Result of trypan blue exclusion test on Jurkat cell line in 72 h post treatment with *G. corticata* extract.

Number of dead cells	Number of viable cells	Extract concentration (µg/µl)
6×10^3	7×10^4	0
4×10^3	5.8×10^4	2.723
5×10^3	5.6×10^4	4.668
4×10^3	3.8×10^4	5.057
6×10^3	3.6×10	5.835
4×10^3	3×10^4	6.224
5.5×10^3	3×10^4	7.391
6×10^3	2.2×10^4	8.167
4×10^3	2×10^4	8.947
8×10^3	1.8×10^4	9.336
8.2×10^3	1.8×10^4	9.726

Table 2. Result of trypan blue exclusion test on molt-4 cell line in 72 h post treatment with *G. corticata* extract.

Number of dead cells	Number of viable cells	Extract concentration (µg/µl)
3×10^3	6.8×10^4	0
3×10^3	5.1×10^4	2.723
3×10^3	4.8×10^4	4.668
4×10^3	4.78×10^4	5.057
4×10^3	4.5×10^4	5.835
3.8×10^3	4×10^4	6.224
4.3×10^3	3.7×10^4	7.391
4.2×10^3	3×10^4	8.167
4.2×10^3	2.5×10^4	8.947
4×10^3	2.2×10^5	9.336
4.2×10^3	1.9×10^4	9.726

molt-4 cells replication was 9.726µg/µl. The number of dead cells in test microplate wells showed that the algal extract did not exhibit considerable cytotoxicity. As shown in Table 3, the result of MTT assay confirmed that the most effective concentration against molt-4 cells was 9.726µg/µl.

DISCUSSION

Up till now, more than 2400 marine natural products have been isolated from seaweeds of subtropical and tropical populations (Manilal et al., 2009). Certain algae have long been used in traditional Chinese herbal medicine in the treatment of cancer (Yamamoto et al., 1984). Many studies have been developed in order to determine the bioactive compounds produced by marine algae (Albano et al., 1990; Berlinck et al., 1996). The activity against cancer cell lines is one of the most important specificities of marine algae, and many algae have showed cytotoxic and antitumor activities. Some metabolites such as bromophenols, carotene and steroids were isolated and purified in some algae and their activity against some cancer cell lines were demonstrated (Xu et al., 2004). Also, in another study, it was shown that the sulfated compounds such as fucoidans which were extracted from *Sargassum polycystum* and some other brown algae exhibited important roles against some human cancer cell lines (Ly et al., 2005).

In this study, the water crude extract of *G. corticata* was studied for its probable antitumor activity against Jurkat and molt-4 cell lines which are two kinds of human leukemic cell lines. Based on previous experience, filtration method is the best way for algal extract sterilization (Zandi et al., 2007). The heat sensitivity of some biological constituents of algal extract is the most important reason for not using autoclave for sterilizing extract. In this study, the cold water extract of *G. corticata* showed reasonable activity against tumor cells replication. The most effective concentration against Jurkat and molt-4 cells were 9.336 and 9.726 µg/µl, respectively. As it is shown in Table 2, we can conclude that the algal extract did not exhibit

Table 3. The results of MTT assay test on Jurkat and molt-4 cell lines after treatment (72 h) with different concentration of *G. corticata* extract.

OD at 540nm (molt-4 cells)	OD at 540 nm (Jurkat Cells)	Concentration of alga extract (µg/µl)
0.810	0.911	0
0.444	0.449	2.723
0.390	0.429	4.668
0.370	0.418	5.057
0.326	0.333	5.835
0.325	0.286	6.224
0.315	0.284	7.391
0.309	0.279	8.167
0.290	0.264	8.947
0.290	0.260	9.336
0.255	0.260	9.726

considerable cytotoxic effect against molt-4 cells. Meanwhile, its cytostatic effect was demonstrated. The number of dead cells related to Jurkat cells (Table 1) is higher than molt-4 cells (Table 2). The result of MTT assay for both cell lines is concordant with the data of performed viability tests for them (Table 3). In this study, the effective concentration is higher than other similar studies in which the purified biological active compound(s) were used instead of crude extract. Therefore, fractionation and purification for *G. corticata* extract in future studies is recommended. Also, in regards to the significant results of this study, further investigations such as evaluation of *in vivo* anticancer activity of *G. corticata* is recommended and may lead to finding new effective natural antitumor compound(s). The red marine alga, *G. corticata* is an interesting alga because of its anticancer activity against human leukemic cell lines. Therefore, more studies for final application of this alga could be important in the field of natural antitumor investigation.

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