



EVALUATION OF ANTICANCER POTENTIAL OF ETHANOLIC EXTRACT OF MARINE RED ALGAE, *GRACILARIA CORTICATA*

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ABSTRACT

According to World Health Organization, Cancer, mostly feared as one of the deadliest disease of the century, is the second principal reason of death worldwide. The occurrence and burden of cancer is massive and studies show that one out of every six deaths are due to some kind of cancers and the count is estimated to rise every year. The treatments mostly followed are surgery, radiotherapy and chemotherapy. Anticancer drugs presently being used in chemotherapy are mostly cytotoxic to normal cells. The development and classification of new anticancer drugs with lower side effects on immune system have become an essentiality and have led to lots of researches. Isolating the bioactive molecules present in marine food products, and determining their broad range pharmaceutical activity, deducing their specific molecular targets and determining their minimal toxicity to normal tissues could aid in treatment of cancer. Sea weeds live in complex habitat exposed to extreme conditions and in adapting to new environmental surrounding, they produce various metabolites which cannot be found in another organism. Different concentration of ethanolic extract of *Gracillaria corticata* was tested against cultured A549 cell lines, lung cancer cell line. Cytotoxicity of extract was evaluated by the reduction of yellow 3, 4, 5dimethyl thiazole-2-yl-2,5-diphenyl tetrazolium bromide (MTT). It was observed that ethanolic extract of *gracillaria corticata* have an antiproliferative effect on cultured a549 cell lines, lung cancer cell line. Ethanolic extract of *Gracillaria corticata* has pronounced cardioprotective activity. The % of viability of the cells is decreased while ethanolic extract of *Gracillaria corticata* added to A549 lung cancer cell line. They promote cellular apoptosis, determined by acridium orange and ethidium bromide double staining.

KEY WORDS

Gracillaria corticata, anticancer activity, h9c2 cell lines; A549 cell lines, apoptosis

INTRODUCTION

Anticancer drugs act exclusively against tumor cells and have considerably adverse effects on the human body, namely, bleeding, hair loss, diarrhea, and immunosuppression. Sea weeds live in complex habitat exposed to extreme conditions and in adapting to new environmental surrounding, they produce various metabolites which cannot be found in other organism. *Gracillaria corticata* extract has anticancer activity regarding various cell line including lung cancer cell line and cardiac cell line (Qin, 2012; Auwerx, 1991). It promotes apoptosis also, revealed in THP 1 cell line.

Radiation therapy is usually advised for cancer treatment including lung cancer and cardiac treatment. It can have critical side effect including fatigue and lack of energy. A reduced White Blood Cells count and low blood platelet also occur after with radiation therapy, if digestive organs are in the field exposed to radiation, patient may experience nausea, vomiting or diarrhoea. Radiation therapy can irritate the skin in the area that is treated, irritation generally improves with time after treatment ended, the blood platelet level is becoming low with the advancement of radiation therapy. Significant proportion of lung cancer rates are increasing in nonsmoking women. Thus, isolating the

bioactive molecules present in marine food products, and determining their broad range pharmaceutical activity, deducing their specific molecular targets and determining their minimal toxicity to normal tissues could aid in treatment of cancer. The development of multidrug resistance following exposure to chemotherapy, uses multiple anticancer drugs is a threat to cancer therapy. ROS (reactive oxygen species) induce DNA damage, as the reaction of free radicals with DNA includes strand break, base modification and DNA protein cross-links. Numerous investigators have proposed participation of free radicals in carcinogenesis, mutation, and transformation. Free radical can promote per oxidation of membrane poly unsaturated fattyacid and covalently bind microsomal lipid and protein forming lipid peroxides that follows pathological changes and cellular disorders (Aruoma, 2003; Mohammed and Ibrahim, 2004; Bagchi and Puri, 1998; Aruoma, 1994; Cheeseman and Slater, 1993). ROS should be scavenged properly in order to prevent and reduce the potential mutation. It has been found that the ethanolic extract of *Gracillaria corticata* has cytotoxic and antitumour activity (GBD, 2015; McMahan and Gidding, 2008). Worldwide burden of chronic disease such as cardiovascular disease, cancer, diabetes and obesity is increasing rapidly. In 2001 chronic disease contributed approximately 59% of the 56.5 milion reported deaths in the world. Cardiovascular disease, the name for the cluster of disease affecting heart and blood vessels including hyper tension, coronary heart disease, cardiovascular disease (CVD) and stroke. The majority of CVDs are preventable and controllable. It has been reported that low intake of fruits and vegetables is associated with high mortality from CVD. Many studies have identified a protective role for diet rich in fruits and vegetables against CVD and cancer. Capisterones A and B, which enhance fluconazole activity in *Saccharomyces cerevisiae*, derived from the marine green alga. Chemopreventive agents which come under various chemical classes have been shown to inhibit initiation and act as blocking and suppressing agents. There is a need for exploring medicinal activity of various algae from which can found many chemopreventive agents have been isolated (Kerr et al., 1972). The development of new natural products and metabolites isolated from microorganisms, animals, and plants possessing high efficacy against tumor cells without any toxicity on

normal cells is a big leap in scientific researchers. Programmed cell death, apoptosis has become the matter of great interest in cancer therapy and oncology because of the high potential of various chemotherapeutic agents in inducing apoptosis in a variety of cancer cells (Danial and Korsmeyer, 2004). Thus, screening for natural products capable of inducing apoptosis in cancer cells that can be used alone or in combination with other chemotherapeutic drugs has now been in progress in order to elevate therapeutic effects and reduce the side effects in cancer therapy (Vakifahmetoglu-Norberg and Zhivotovsky, 2010). The present study examines the anti-cancer activity of ethanolic extract of *Gracillaria corticata*.

MATERIAL AND METHODS

Sample

Sea weed (*Gracillaria corticata*) was collected from Thirumullavaram coast near Kollam, Kerala, India. The collected sample was washed with water several times and air dried. The dried sample was grinded into fine particle by mortar pestle, stored in containers at room temperature for further analysis.

Determination of *in vitro* antiproliferative effect of *Gracillaria corticata*-extracts on cultured a549 cell lines

Different concentration of ethanolic extract of *Gracillaria corticata* was tested against cultured A549 cell lines and lung cancer cell lines. Cytotoxicity of extract was evaluated by the reduction of 3, 4, 5 dimethyl thiazole-2-yl-2; 5-diphenyl tetrazolium bromide (MTT). Ethanolic extract of *G. corticata* promote cellular apoptosis, determined by acridium orange and ethidium bromide double staining.

Determination of *in vitro* cardioprotective effect of *G. corticata* extracts on cultured H9C2 cells

H9C2 cardiomyoblast cell line was purchased from NCCS, Pune were maintained in Dulbecco's modified eagles media supplemented with 10% FBS and grown to confluency at 37 °C in 5 % CO₂ in a humidified atmosphere in a CO₂ incubator. The cells were trypsinized 0.025% trypsin for 2 min and transferred to flasks in complete aseptic conditions. Extracts were added to grown cells at a final concentration of 6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml from a stock of 1 mg/ml and incubated for 24 h and the cardioprotective effect of extract on doxorubicin induced toxicity was performed. About 20 mM

doxorubicin was added to induce toxicity. The % difference in viability was determined by standard MTT assay after 24 h of incubation.

Determination of apoptosis by acridine orange (AO) and ethidium bromide (EBr) double staining

DNA-binding dyes AO and EBr (Sigma, USA) were used for the morphological detection of apoptotic and necrotic cells (Formigli et al., 2000; Norbury and Hickson, 2001). AO is taken up by both viable and non-viable cells and emits green fluorescence if intercalated into double stranded deoxy ribo nucleic acid (DNA). EBr is taken up only by non-viable cells and emits red fluorescence by intercalation into DNA. After treatment with different concentrations of algal extracts at a final concentration of 1000 µg/ml for 24 h, the cells were washed by cold PBS and then stained with a mixture of AO (100 µg/ml) and EBr (100 µg/ml) at room temperature for 10 min. The stained cells were washed twice with phosphate buffered saline (PBS) and observed by a fluorescence microscope in blue filter.

RESULTS AND DISCUSSION

Algae are important and promising resources in cancer research and a number of compounds from these macro algae have undergone clinical trials as antitumor agents (Hirsch et al., 1997). Thus, isolating the bioactive molecules present in marine food products, and determining their broad range pharmaceutical activity, deducing their specific molecular targets and determining their minimal toxicity to normal tissues could aid in treatment of cancer (Zeiss, 2003). By adding different concentration of 6.25, 12.5, 25, 50 and 100 microgram ethanolic extract of algae on 549 cell lines, the percentage of viability decreased from 65% to 51%.

It was observed that ethanolic extract of *G. corticata* have an antiproliferative effect on cultured lung cancer cell lines (A549). The % of viability was only 51% for lung cancer cell line when 25 µg extract was added (Table 1; Fig. 1). The cardioprotective effect of the algal extract was validated against H9c2 cells. An *in vitro* cardio protective effect was found while ethanolic extract of *G. corticata* was added. The % of viability was 81% in H9c2 cell lines when extracts of *G. corticata* at 100 µg/ml was used, comparatively very high when doxorubicin added cell line showed only 25% of viability (Table 2; Fig. 2). Apoptosis has since been recognized and accepted as a distinctive and important mode of “programmed” cell death, which involves the genetically determined elimination of cells. Living cell having green nucleus changed to orange stained nuclei. Apoptosis occurs normally during the development and aging as a homeostatic mechanism to maintain cell populations in tissues. Apoptosis also occurs as a defense mechanism such as in immune reactions or when cells are damaged by disease or noxious agents. The cells were divided into three categories as follows: living cells (normal green nucleus), early apoptotic (bright green nucleus with condensed or fragmented chromatin), and late apoptotic (orange-stained nuclei with chromatin condensation or fragmentation). In the present study, the total number of apoptotic cell increased while adding 100 µg/ml of ethanol extract of *G. corticata* (Fig. 3). To conclude, microgram concentrations of *G. corticata* extract has pronounced effect of increasing apoptosis, cardio protective effect, increasing the inhibition of A549 lung cancer cell lines thereby decreases the viability of the cell.

Table 1: Inhibition of A549 cancer cell line at different concentration of *G. corticata* extract

Sample Concentration (µg/ml) added to A549 lung cancer cell line	Average Absorbance @ 540nm	Percentage of Inhibition
CONTROL	0.2937	
6.25	0.1928	65%
12.5	0.1889	64%
25	0.1829	62%
50	0.1606	54%
100	0.1522	51%

Table 2: *In vitro* cardio protective effect of ethanolic extract of *G. corticata* on H9C2 cell lines

Sample Concentration ($\mu\text{g/ml}$)	Average Absorbance @ 540nm	Percentage Viability
CONTROL	0.3922	.01
Doxorubicin	0.0985	25
6.25	0.1459	37
12.5	0.2172	55
25	0.2241	57
50	0.2994	76
100	0.3185	81

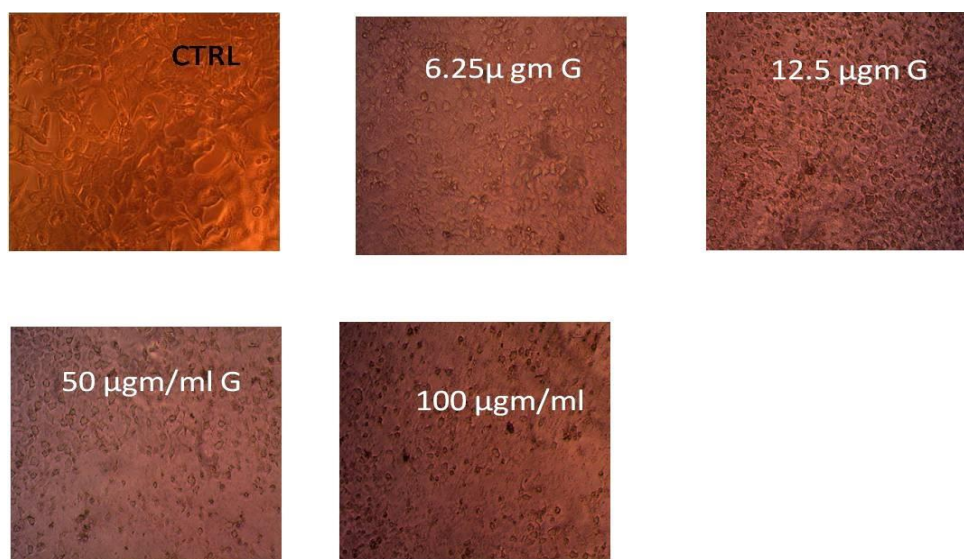


Figure 1: Effect of algal extract ($\mu\text{g/ml}$) on A549 lung cancer cell line

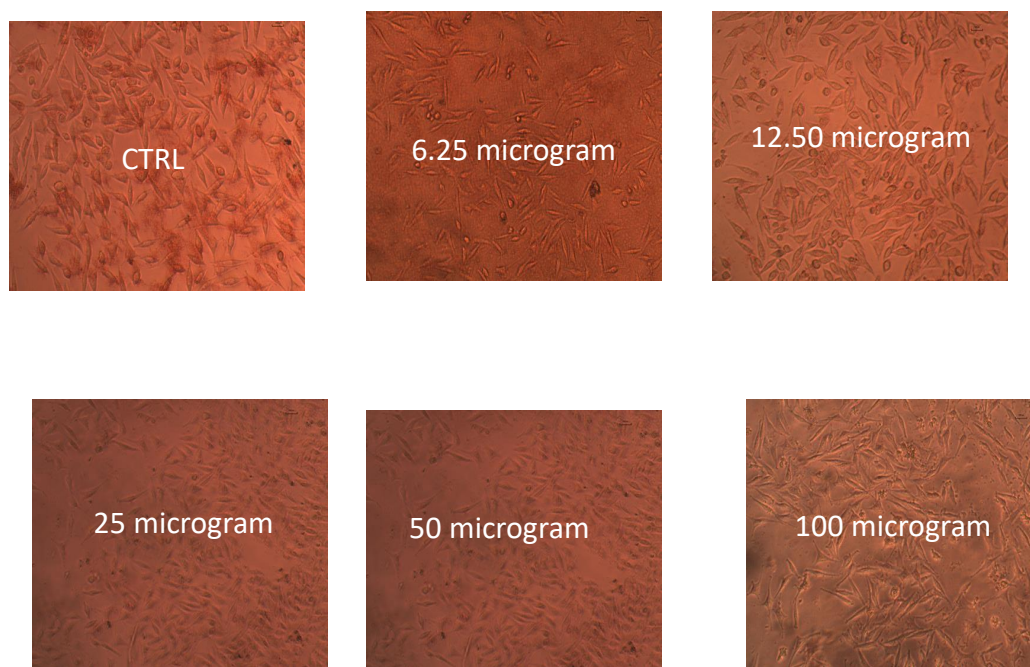


Figure 2: Activity of ethanolic extract of *G. corticata* on H9C2 cell lines at various concentrations

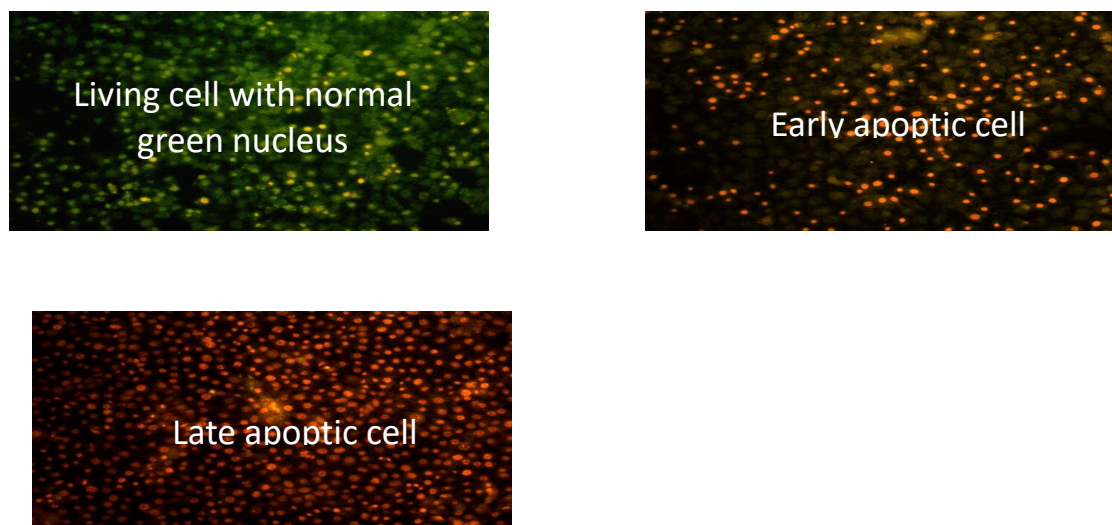


Figure 3: Apoptotic and necrotic cells stained with acridine orange and ethidium bromide

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