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Original Article

In vitro cytotoxic activity of silver nano particle biosynthesized from *Colpomenia sinuosa* and *Halymenia poryphyroides* using DLA and EAC cell lines

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ABSTRACT

This study was conducted to investigate the *invitro* cytotoxic activity of silver nano particles biosynthesized from *Colpomenia sinuosa* and *Halymenia poryphyroides* using DLA and EAC cell lines by tryphan blue dye exclusion technique and MTT assay using Mouse *L929* cell lines (Lungs fibroblast). The results of the trypan blue dye exclusion assay indicates that the silver nano particles biosynthesized from *Colpomenia sinuosa* and *Halymenia poryphyroides* inhibits the growth of DLA and EAC cell lines in a dose dependent manner against the standard drug Curcumin where the silver nano particle biosynthesized from *Colpomenia sinuosa* showed 61.57 % and silver nano particle biosynthesized from *Halymenia poryphyroides* showed 89.36 % in DLA cell line similarly the silver nano particle biosynthesized from *Colpomenia sinuosa* showed 81.96 % and silver nano particle biosynthesized from *Halymenia poryphyroides* 91.45 % in EAC cell line. The results of the MTT assay indicated the silver nano particles biosynthesized from *Colpomenia sinuosa* and *Halymenia poryphyroides* significantly inhibited the proliferation of L929 cells in dose dependent manner where the silver nano particle biosynthesized from *Colpomenia sinuosa* showed 37.06 % and silver nano particle biosynthesized from *Halymenia poryphyroides* showed 100 % against the standard drug Curcumin.

Keywords: Cytotoxic activity, Silver nano particles, *Colpomenia sinuosa*, *Halymenia poryphyroides*

INTRODUCTION

Natural Products, especially plants, seaweeds and their constituents have been used for the treatment of various diseases for thousands of years. Cancer is a group of diseases in which cells are aggressive, invasive, and sometimes metastatic and it may affect people at all ages. Chemotherapy (1) is one of the methods for the treatment of cancer. A major complication of chemotherapy is its toxicity to normal cells, due to the inability of the drug in chemotherapy to differentiate between normal cells and malignant cells often impacts the efficacy of the treatment and even makes it impossible to cure the patients. One of the requisites of cancer chemo preventive agent is elimination of damaged or malignant cell through cell cycle inhibition or induction of apoptosis with less or no toxicity to normal cells. Lymphoma (2) is a disease of the lymphocytes (a type of white blood cell involved in immune responses) and the lymphatic system, which includes the spleen, thymus, and liver, as well as other lymphatic tissues. Dalton's ascites lymphoma is transplantable, poorly differentiated malignant tumuor which appeared originally as lymphocytes in a mouse where it grows in both solid and ascitic forms (3). *Invitro* cytotoxicity screening models provide important preliminary data where it helps to select plant extracts with potential antineoplastic properties for future work (4, 5). This present study was carried out to evaluate the cytotoxic activity of silver nano particles biosynthesized from *Colpomenia sinuosa* and *Halymenia poryphyroides* against Ehrlich ascites carcinoma (EAC) and Dalton lymphoma ascites (DLA) cell lines.

Cell lines: *Ehrlich ascites carcinoma* (EAC) cells and *Dalton's lymphoma ascites* (DLA) cells were used for short term *in vitro* cytotoxicity experiments. Mouse *L929* cell lines (Lungs fibroblast) were used for long term *in vitro* cytotoxicity experiments.

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MATERIALS AND METHODS

Short term *in vitro* cytotoxicity assay by tryphan blue dye exclusion technique: Cells were aspirated from the peritoneal cavity of tumour bearing mice. The cells were washed three times using PBS. The viability of the cells was checked using trypan blue. The cell suspension was added to tubes containing various concentrations of the test compounds and the volume was made up to 1ml using phosphate buffered saline (PBS). These assay mixtures was incubated for 3 h at 37^0C and then 1 ml of trypan blue was added after incubation and the number of dead cells was counted using a haemocytometer (6).

Long term *in vitro* cytotoxicity by MTT assay: Cells were seeded in 96-well flat-bottom plates and allowed to adhere for 24h at 37^0C with 5% CO_2 atmosphere. Different drug concentration was added and incubated upto 48 hrs. Before 4 h of the completion of incubation, 20 µl of MTT (5 mg/ml) was added. Percentage of dead cells was determined using an ELISA plate reader set to record absorbance at 570 nm (7).

RESULTS

Short term *in vitro* cytotoxicity assay: Short term in *vitro* cytotoxicity was evaluated by trypan blue dye exclusion method. Viable cells which remained unstained by trypan blue were counted in a haemocytometer. Cytotoxicity of silver nano particles biosynthesized from *Colpomenia sinuosa* and *Halymenia poryphyroides* to DLA and EAC cell culture was shown in Table 1. The percentage cytotoxicity of the DLA cells at different concentrations ranging from 10 µg/ml to 200 µg/ml showed a dose dependent inhibition of the growth of DLA cells. Similarly the percentage of the EAC cells at different concentrations ranging from 10 µg/ml to 200 µg/ml showed a dose dependent inhibition of the growth of EAC cell lines. Curcumin was used as the reference drug for the DLA and EAC cell lines and it produced 100 % cytotoxicity at 200µg/ml. In the DLA cell line silver nano particle biosynthesized from *Colpomenia sinuosa* showed 61.57 % and silver nano particle biosynthesized from *Halymenia poryphyroides* showed 89.36 %. Similarly in EAC Cell line silver nano particle biosynthesized from *Colpomenia sinuosa* showed 81.96 % and silver nano particle biosynthesized from *Halymenia poryphyroides* 91.45 % compared to the standard. Silver nano particles biosynthesized from *Colpomenia sinuosa* and *Halymenia poryphyroides* was found to have cytotoxic effect; it was less when compared to standard drug. These results emphasize the cytotoxic nature of silver nano particles biosynthesized from *Colpomenia sinuosa* and *Halymenia poryphyroides* against DLA and EAC cell lines.

Long term *in vitro* cytotoxicity by MTT assay: Long term *in vitro* cytotoxic effect of silver nano particles biosynthesized from *Colpomenia sinuosa* and *Halymenia poryphyroides* on *L929* cells was investigated by MTT assay. Cells were treated at concentrations ranging from 10-200 µg/ml for 48 hrs and the percentage of cell viability was analyzed which were shown in Table 2. Silver nano particles biosynthesized from *Colpomenia sinuosa* and *Halymenia poryphyroides* significantly inhibited the proliferation of L929 cells in a dose dependent manner. The reference drug Curcumin showed 100 % protection, whereas silver nano particle biosynthesized from *Colpomenia sinuosa* showed 37.06 % and silver nano particle biosynthesized from *Halymenia poryphyroides* showed 100 % at 200 µg/ml. The CTC_{50} for DLA cell line of silver nano particles biosynthesized from *Colpomenia sinuosa* and *Halymenia poryphyroides* were found to be 97.33 % and 198.21% whereas $_{CTC50}$ for EAC cell line of silver nano particles biosynthesized from *Colpomenia sinuosa* and *Halymenia poryphyroides* were found to be 86.87 % and 118.21 % (Table 3). Silver nano particles biosynthesized from *Colpomenia sinuosa* exhibited minimal cytotoxic activity compared to standard drug Curcumin whereas silver nano particle biosynthesized from *Halymenia poryphyroides* exhibited better cytotoxic action when compared to standard drug.

DISCUSSION

Cancer is the leading cause of mortality worldwide, and the failure of conventional chemotherapy to effect a major reduction in mortality indicates that new approaches are critically needed (8). Seaweeds and their constituents have played a major role as a source of effective anticancer agents and it is significant that 60 % of currently used anti cancer were derived from natural sources including plants and marine organisms. Seaweeds have diverse biological activities, including effects on the immune system and cancer (9). Yamamoto *et al*., (1987) (10) and Sheu *et al*., (1996) (11) reported that oral consumption of several seaweeds significantly decreased the incidence of carcinogenesis *in vivo*. The control of cell proliferation is crucial in maintaining cellular homeostasis and loss of this mechanism is a principle hallmark of cancer cells. Thus the inhibition of tumuor cell growth without side effects is recognized as an important target for cancer chemotherapy (12). The results of the trypan blue dye exclusion assay indicates that the silver

nano particles biosynthesized from *Colpomenia sinuosa* and *Halymenia poryphyroides* could inhibit the growth of DLA and EAC cell lines in a dose dependent manner. Alekseyenko *et al*., (2007) (13) suggested that polysaccharide of *Fucus evanescens* has antitumor and antimetastatic activity in C57BI/6 mice with transplanted Lewis lung adenocarcinoma. Down regulating tissue factor expression, *Grateloupia longifolia* polysaccharide inhibits angiogenesis in HMEC-1 endothelial cells (14). However, so far no antitumor activity has been reported from these algae. Recently Bhuvaneswari and Murugesan (2012) (15) studied the antitumor activity of the methanol extract of *Chondrococcus hornemanni* and *Spyridia fusiformis* against Dalton's lymphoma ascites (DLA)-induced tumor inoculation as well as its antioxidant activity. Numerous macro algae have shown potent cytotoxic activities and certain authors have suggested the consumption of algae as a chemo-preventive agent against several cancers. Silver nano particles have been shown to have important anti angiogenic properties (16), so are attractive for study of their potential antitumor effects. Compounds possessing anti angiogenic properties are known for their potential ability to block the activity of abnormally expressed signaling proteins, such as Ras and Akt, cytokine-based therapies, DNA- or protein based vaccines against specific tumor markers, and tyrosine kinase inhibitors which exhibit a consistent antitumor effect (17). The role of silver nano particles in inhibiting DLA and EAC cell viability and proliferation will be similar to their potential to inhibit the permeability of endothelial cells by inactivating Src kinases which have been proven to have a role in retinal therapies (18). The pathways by which silver nano particles inhibit the pathway mediating cell proliferation and viability have yet to be explored. The cytotoxic effects of silver are the result of active physicochemical interaction of silver atoms with the functional groups of intracellular proteins, as well as with the nitrogen bases and phosphate groups in DNA (19). The current investigation was also designed to explore the silver nano particles biosynthesized from *Colpomenia sinuosa* and *Halymenia poryphyroides* for their oncolytic properties using standard MTT assay. The MTT reduction as a cell viability measurement is now widely chosen and most advantageous end point (20). The results of the MTT assay indicated the silver nano particles biosynthesized from *Colpomenia sinuosa* and *Halymenia poryphyroides* significantly inhibited the proliferation of L929 cells in dose dependent manner 921-22).

CONCLUSION

The present study provides strong evidence suggesting that the silver nano particles biosynthesized from *Colpomenia sinuosa* and *Halymenia poryphyroides* have *in vitro* cytotoxicity against DLA and EAC cell lines. This may be used to development of effective therapeutic approaches towards the prevention or treatments of various immune conditions and different types of cancer. Anti-tumor agents, that can modulate apoptosis, may be able to affect the steady state of cell populations that are helpful in the management and therapy of cancer. Since it has been suggested that apoptosis plays a critical role in tissue homeostasis and cancer development, apoptosis modulation has become an interesting target for both therapeutic and preventive approaches to cancer treatment.

Table 1: Effect of silver nano particles biosynthesized from *Colpomenia sinuosa* and *Halymenia poryphyroides* against DLA and EAC Cell lines by Trypan blue dye exclusion method.

Sample	Concentration µg/ml	Percentage cytotoxicity of DLA cell lines	Percentage cytotoxicity of EAC cell lines
Silver nano particles from *Colpomenia sinuosa*	10	19.14 ± 2.96	23.11 ± 2.82
	20	23.56 ± 3.12	37.63 ± 1.49
	50	36.14 ± 3.49	58.61 ± 4.27
	100	53.92 ± 2.93	70.71 ± 4.23
	200	61.57 ± 3.45	81.96 ± 8.52
Silver nano particles from *Halymenia poryphyroides*	10	22.14 ± 2.96	26.50 ± 2.43
	20	33.46 ± 3.49	41.17 ± 7.53
	50	52.48 ± 4.89	65.75 ± 1.47
	100	72.61 ± 3.63	78.41 ± 3.33
	200	89.36 ± 3.12	91.45 ± 4.72
Curcumin	10	41 ± 2.14	46.23 ± 3.41
	20	54.47 ± 6.14	63.42 ± 1.89
	50	69.71 ± 7.12	86.77 ± 1.49
	100	81.69 ± 7.11	92.44 ± 2.27
	200	100 ± 1.15	100 ± 4.67

Values are expressed as mean ± SD of triplicates; DLA – *Dalton's lymphoma ascites* cell lines.
EAC – *Ehrlich ascites carcinoma* cell lines.

Table 2 Effect of silver nano particles bio-synthesized from *Colpomenia sinuosa* and *Halymenia poryphyroides* against L929 cell lines by MTT assay.

Sample	Concentration µg/ml	Absorption at 570 nm	Percentage (%) of cytotoxicity
Control	-	0.319 ± 0.11	
Silver nano particles from *Colpomenia sinuosa*	10	0.251 ± 0.095	20.17
	20	0.237 ± 0.014	26.33
	50	0.214 ± 0.036	30.72
	100	0.204 ± 0.051	33.29
	200	0.193 ± 0.063	37.06
Silver nano particles from *Halymenia poryphyroides*	10	0.187 ± 0.025	41.73
	20	0.098 ± 0.061	69.72
	50	0.048 ± 0.055	86.44
	100	0.017 ± 0.042	95.21
	200	0.000 ± 0.000	100.00
Curcumin	10	0.016 ± 0.049	93.66
	20	0.008 ± 0.014	96.19
	50	0.002 ± 0.021	98.07
	100	0.000 ± 0.000	100.00
	200	0.000 ± 0.000	100.00

Values are expressed as mean ± SD of triplicates.

Table 3: CTC_{50} values of silver nano particles biosynthesized *Colpomenia sinuosa* and *Halymenia poryphyroides*

S.No	Silver nano particles from Experimental algae	CTC50 (DLA cell lines)	*CTC50 (EAC cell lines)*
1	*Colpomenia sinuosa*	97.33 ± 6.52	86.57±2.56
2	*Halymenia poryphyroides*	198.21 ± 2.14	118.21 ± 11.1

Values are expressed as mean ± SD of triplicates.

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