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Research Article

EVALUATION OF *IN VITRO* CYTOTOXIC EFFECT OF VIOLACEIN PRODUCED BY NOVEL ISOLATE *CHROMOBACTERIUM VACCINII* CV5 AGAINST THE CERVICAL AND LUNG CANCER CELL

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ABSTRACT

Objectives: This study investigates the *in vitro* anticancer activity of the violacein extracted from the *Chromobacterium vaccinii* CV5.

Methods: Natural colorants or dyes derived from flora to fauna are believed to be safe because of nontoxic, noncarcinogenic, and biodegradable in nature. There are a number of natural pigments, but only a few are available in sufficient quantities for industrial production. The cytotoxicity activity of pigment was assessed against the cervical (HeLa) and lung cancer (A549) cell lines using the MTT assay and there by potential cytotoxic activity exhibited by the pigment was identified.

Results: The result of the pigment shows potent anticancer activity on the two cancer cell lines tested in a concentration dependent manner. The potent anticancer activity was observed with the pigment with IC_{50} values of 26 µg/mL on HeLa and 31 µg/mL on A549 cells, respectively.

Conclusion: The study is pioneering report for determining the better *in vitro* anticancer activity of violacein from the novel isolate *C. vaccinii* CV5.

Keywords: Anticancer, Cytotoxicity, Chromobacterium, MTT assay, Pigment, Violacein.

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INTRODUCTION

Violacein is characterized as 2-dihydro-5-(5-hydroxy-1H-indol-3-yl)-2-oxo-3H-pyrrol-3-ilydene)-1,3-dihydro-2H-indol-2-one, formed by the condensation of two modified L-tryptophan molecules [1]. Violacein is produced by several bacterial species thriving in a range of habitats such as terrestrial, marine, fresh water, and glacier environments. Some of the known violacein producing organisms include *Chromobacterium violaceum* [2], *Collimonas* sp. [3], *Duganella* sp. [4], *Janthinobacterium lividum* [5,6], and *Pseudoalteromonas* sp. [7,8].

Violacein has also gained increasing importance for its potential medical and industrial applications. The biological activities of this compound include antioxidant, leishmanicidal, trypanocidal, antifungal, antiviral, antibacterial and antiprotozoal activity, as well as antitumoral and apoptosis inducing activities in the mammalian cancer cells.

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 the immune system have become an essential goal in many studies of immunopharmacology [9-11].

To resolve side effects of anticancer agents, development of cancer chemopreventive agents and improvement of cancer treatment are very important. Accordingly, screening of natural products as potential anticancer agents, in the form of functional foods or nutraceuticals has become an important undertaking [12]. The rising interest in the pharmacological properties of microbial pigments led us to investigate *in vitro* cytotoxicity of the violacein, violet color pigment, extracted and purified from the novel isolate *Chromobacterium vaccinii* CV5.

METHODS

Microorganisms

The *C. vaccinii* CV5 isolated from the well water identified by morphologically and genetically was used in this study [13].

Pigment production and purification

The production profile of crude violacein was obtained using a 500 mL flask containing 200 mL of nutrient broth. Fermentations were carried out at 37°C for 72 hrs with an inoculum size of 8% (v/v) 24 hrs old culture (OD 660 approximately 1). After 72 hrs of incubation period broth was taken for pigment extraction. The partially purified compound was used for the further study.

In vitro cell line study by MTT assay

The cell lines, HeLa (cervical cancer cell) and A549 (lung cancer cell) used in this study were obtained from the National Centre for Cell Sciences, Pune. The cells were maintained in RPMI-1640 supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 µg/mL) in a humidified atmosphere of 50 µg/mL CO_2 at 37°C.

The cytotoxicity of samples on the cells was determined by the MTT assay [14]. Cells (1×10^5/well) were plated in 100 µL of medium/well in 96-well plates (Costar Corning, Rochester, NY). After 48 hrs of incubation, the cell reaches the confluence. Then, the cells were incubated in the presence of various concentrations of the samples in 0.1% dimethyl sulfoxide for 48 hrs at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 20 µL/well (5 mg/mL) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide cells (MTT) phosphate-buffered saline solution was added. After 4 hrs incubation, 0.04 M HCl/isopropanol was added. Viable cells were determined by the absorbance at 570 nm with reference at 655 nm. Measurements were performed in 3 times, and the concentration required for a 50% inhibition of viability (IC_{50}) was determined graphically. The absorbance at 570 nm was measured with a microplate reader (Bio-Rad, Richmond, CA), using wells without

sample containing cells as blanks. All the experiments were performed in triplicate. The percentage of cell inhibition was determined using the formula:

% Cell inhibition=100−abs (drug)/abs (control)×100

Statistical analysis

All the experiments were conducted with triplicates, and their mean values were represented. Statistical analysis was performed by the one-way ANOVA using SPSS version 10. All the results were expressed as the mean±standard deviation.

RESULTS AND DISCUSSION

In this study, violacein pigment was extracted from *C. vaccinii* CV5, and the partially purified pigment was analyzed for its *in vitro* anticancer activity using cervical (HeLa) and lung cancer cells (A549). It was found that the incubation of tumor cells with the pigment significantly reduced the viability of these cells and the dead cells were significantly increased with high pigment concentration. To show the concentration dependent action of the pigment in cancer cells was treated with different (6.25,12.5,25,50 and 100 µg/mL) concentration of the sample. After treatment for 48 hrs, the violacein from *C. vaccinii* CV5 decreased the proliferation of both cells significantly (Fig. 1).

The effect of violacein pigment on the growth of HeLa and A549 was investigated by the MTT assay (Fig. 2). The maximum percentage of inhibition value obtained against the HeLa was 72.02% at 100 µg/mL. The minimum percentage of (10.36%) inhibition was observed at 6.25 µg/mL. The IC_{50} value of violacein was found to be 26 µg/mL. There was a significant association between the concentration of the sample and the inhibitory effect.

In this *in vitro* cytotoxicity assay, the violacein exhibits significant activity against A549 lung cancer cell line. The growth inhibitory effect of violacein against A549 human lung cancer cell line is shown in Fig. 3. The violacein reduces the A549 cell growth in a concentration dependent manner. Cell growth is decreased with the increasing concentration of pigment sample. From Fig. 1, it is showed that the pigment has good inhibition on A549 cell line proliferation with IC_{50} of 31 µg/mL.

Recent studies reveal that so many natural byproducts including microorganism and its byproducts could acts as a tumor suppressor or as a chemotherapeutic agent. Kodach *et al*. [15] show that the violacein (IC_{50} 1-2 µM) is a promising chemotherapeutic agent that acts by blocking AKT activation and inducing apoptosis thus increasing the chemosensitivity of colon cancer cells to 5-fluorouracil treatment. Ferreira *et al*. [16] in their study investigate the effect of violacein in leukemia (HL60) cell. They demonstrated that violacein from *Chromobacterium violaceum* represents the first member of a novel class of cytotoxic drugs mediating apoptosis of HL60 cells by way of the specific activation of tumor necrosis factor receptor 1 and caspases.

Anticancer studies of violacein have shown efficiency in a number of cell lines of both neoplastic and hematological malignant origins. Melo *et al*. [17] find that the violacein (IC_{50} 5-12 µM) is highly cytotoxic to V79 fibroblasts. Saraiva *et al.* [18] find that uveal melanoma cell lines, 92.1 and OCM-1, are found to be sensitive to violacein (GI50 ~1.69-2.21 µM). These results demonstrate that the violacein induces apoptosis in cancer cells. Apoptosis is necessary for the conservation of tissues homeostasis and important for defense against the diseases and cancers [19,20].

CONCLUSION

In this study, it shows that the violacein has an inhibitory effect on both HeLa and A549 cell. Chemotherapy and radiotherapy used currently have partially fails to give significant therapeutic results because of nonspecific pointing of drugs. This has led to many complications, such as toxicities and relapse in cancer patients. The potentiality of natural compounds in inducing encountered cell death (apoptosis), a process of dysregulated in cancer cells, has been extensively studied in the recent studies. This study assesses the antiproliferative *in vitro* activity of violacein against HeLa and A549 human cancer cell lines. The current study is pioneering report for determining the better *in vitro* anticancer activity of violacein from the novel isolate *C. vaccinii* CV5.

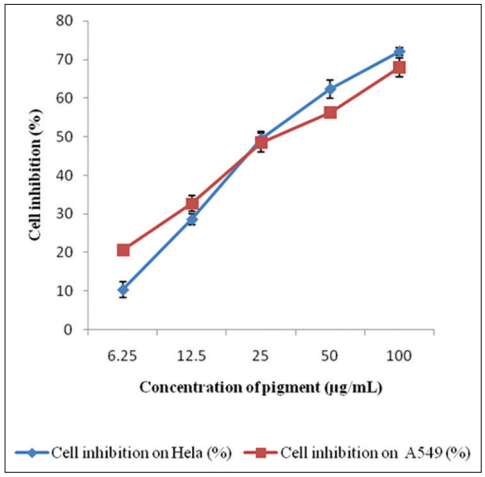

Fig. 1: The cytotoxic effect of violacein on cancer cell lines

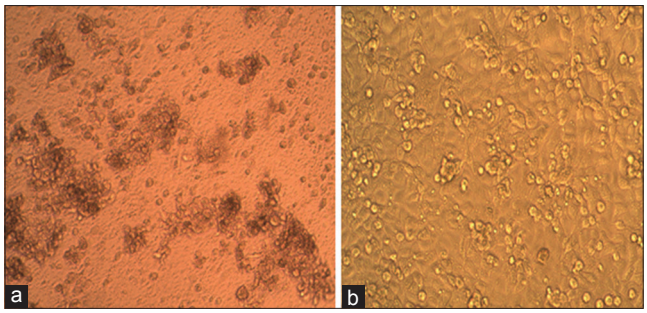

Fig. 2: (a and b) Cell inhibitions by violacein and control HeLa cell

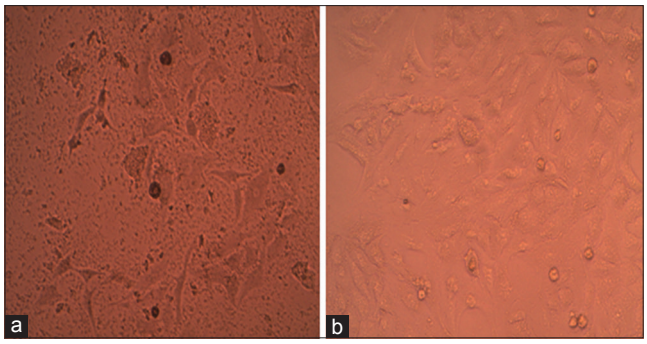

Fig. 3: (a and b) Cell inhibition by violacein and control A549 cell

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