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Anticancer activity of red pigment from *Serratia marcescens* in Human cervix carcinoma

R.KAVITHA*, S.AISWARIYA, CHANDANA. M.G. RATNAVALI

Department of Pharmaceutical Biotechnology, SRM College of Pharmacy, SRM University, Kattankulathur-603 203, Chennai, Tamilnadu,India.

Corres.author: kavithar2007@gmail.com

ABSTRACT: The present study has been designed to analyse the anticancer property of microbial pigment prodigiosin isolated from waste sample of *Serratia marcescens*, a marine crustacean, against human cervix carcinoma cell (Hela-229 cell line). The cell lines were cultured in the DME medium along with various concentrations of prodigiosin for 24hrs and the percentage of cell viability was evaluated by 3-(4,5 dimethyl thiazol-2yl)-2,5 diphenyl tetrazolium bromide and neutral red. Prodigiosin showed dose dependent inhibition of cell proliferation. These results suggested that the prodigiosin has strong anticancer and apoptosis activity against human cervical carcinoma cancer.
Key Words: *Serratia marcescens*, Prodigiosin, Hela-229 cell line, MTT assay.

INTRODUCTION

The identification of novel targets and the development of more specific chemotherapeutic agents are the most important goals of research in cancer therapy. Several bacterial pathogens have been identified as mediators of apoptosis invitro and during pathogenesis. A family of natural red pigments called prodigiosins is synthesized from different bacteria including *Actinomycetes, Streptomyces* and *Serratia marcescens*, it has more therapeutic values.[1] Prodigiosin is a tripyrrole ring pigment synthesized by *Serratia marcescens*, is a promising drug owing to its reported characteristics of having antibacterial, antimycotic and immunomodulatory activities.[2] Prodigiosin also has a therapeutic use as potential anticancer drug. Biological mechanism and pharmacology of prodigiosin therefore have attained considerable interest in recent years. Some members of this family are potent apoptosis inducers. Thus prodigiosin induces apoptosis in various human hematopietic cancer cell lines. Interestingly prodigiosin has no marked toxicity in nonmalignant cell lines.[3] The deeply red colored prodigiosin alkaloid contains a 4-methoxy-alpha, alpha bipyrrole moiety which is produced by bifurcated biosynthesis pathway, in which mono-and bipyrrole precursors are obtained separately and then coupled to for the production of linear tripyrrole red pigment during the stationary phase of bacterial growth.[4] The production of prodigiosin has been shown to be influenced by numerous environmental factors including inorganic phosphate availability, media composition temperature and pH. [5] Apoptosis is characterized by morphological and biochemical changes of the cell. The changes include cell shrinkage, membrane blabbing, and chromatin condensation. It is also known as programmed cell death and is responsible for the deletion of cells from normal tissue during development and tissue homeostasis. Apoptosis malfunctions can have health implication, as in cancer. Radiation and chemotherapy have been shown to cause cell death by apoptosis.[6] In most cases, apoptosis is accompanied by cytochrome C release from the mitochondria into the cystosol. Then caspases can be activated and generate the characteristic apoptotic morphology.[7] The present study is aimed at carrying out the Cytotoxicity activity of the red pigment against the human cervix carcinoma cells.

MATERIALS AND METHODS:
Ethyl acetate, acetone and dichloromethane were brought from SRL, India.3-(4,5-dimethylthiozol-2-yl)2,5-diphenyl tetrazolium bromide (MTT), Neutral red were purchased from sigma chemicals(St houis,MO,USA).DMEM medium purchased from Himedia. Deionized water further purified with a Millipore milli-Q-system and used in all procedure. Standard prodigiosin was gifted by National cancer Institute (www.dtp.nci.nih.com).

Isolation and screening of Serratia marcescens: [8]
The marine crustacean waste sample collected from the Besant Nagar Beach, Chennai, India. From the crowded plate technique, the colonies showing red pigment production were selected after 72hrs of incubation at 37^0C and purified by cross-streaking method. Stock cultures were maintained on nutrient agar slant and stored at 4^0C in refrigerator. Identification of *Serratia marcescens* was done by studying morphological, microscopical as well as biochemical characters of the organism. The isolated organism was identified as *Serratia marcescens* and named as *Serratia marcescens* -kj 1306.

Production and Purification of Prodigiosin:
Red pigment was isolated from *Serratia marcescens* and purified with some modification.[9] Briefly, the organism grown in powdered peanut broth was centrifuged and the supernatant was separated by extracting with ethyl acetate. The pigment from the cell pellet was extracted with acetone and the extraction was centrifuged at 10,000rpm for 15minutes and the white pellet was discarded. The pigment containing acetone fraction was mixed with ethyl acetate fractions and evaporated in vacuum drier. The silica gel column (mesh size80-100) chromatography was used for effective separation of the impurities by dichloromethane, chloroform and acetone (5:5:1) as solvent. The different concentrations of dried powder were used for plotting the standard graph, concentration versus absorbance at 535nm. The purified sample showing a single peak absorbance was further used for the anticancer activity.

Cell line and culture conditions:
HeLa-229 cell line was purchased from National center for cell science, Pune. The cancer cells were cultured in DMEM medium and supplemented with 10%FBS, 100U/ml pencillin, 100µg/ml streptomycin, and 2mML-glutamine incubated at 37^0C for 24hrs with 5%CO_2 in air.

Cell viability assay:
Cell viability was determined by the MTT assay and Neutral red uptake assay (NRU) method. For MTT and NRU assay, 96 well micro titer plates were inoculated with $4X10^4$ cells/well and incubated at 37^0C for 24hrs. The cells were then incubated with prodigiosin (0.2µM-2µM) for 24hrs in a final volume of 100µl. HeLa 229 cells grown in the absence of drugs was used as control.

MTT Assay:
The medium containing prodigiosin was removed and 10µl of MTT solution (5mg/ml in HBSS) was added to each well. After incubation for 4hrs at 37^0C the precipitate was dissolved in 100µl of DMSO and the absorbance values at 550nm were measured on a multiwell plate reader.[10]

Neutral Red Uptake assay (NRU):
After removing the medium from the plates, 1ml of neutral red solution (50µg/ml in culture medium) was added to each well followed by incubation for 3hrs at 37^0C. The cells were then washed rapidly with phosphate-buffered saline. Later 1ml of 1% glacial acetic acid-50% ethanol was added to each well to fix the cells and extract the neutral red incorporated in to the lysosomes. The plates were shaken for 20 min on a plate shaker and the absorbance was then measured at 540nm.[10]

RESULTS AND DISCUSSION:
Prodigiosin had a potent apoptosis activity against human cervix carcinoma cells. The assessment of the cell viability was based on the data from two different assays, MTT and NRU assay. These methods were employed for determining the antiproliferative activity of compounds in culture. In this present study, the HeLa cell lines were incubated for 24hrs with increasing doses of prodigiosin, ranging from 200nM to 2000nM. MTT dye reduction assay was done to assess the antiproliferative activity. Decrease in proliferation of treated cells was observed when compared to the untreated controls. Prodigiosin induced apoptosis in HeLa cell lines in a dose dependent manner with a mean IC_{50} (Concentration of drug required to reduce the cell viability to 50%) of 700nM from MTT assay and IC_{50} of 680nM from NRU assay.

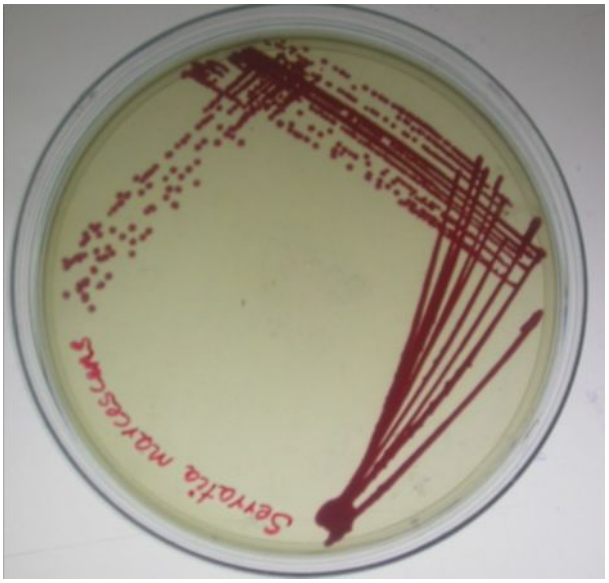
Table 1:MTT Assay of Prodigiosin on HeLa cell line.

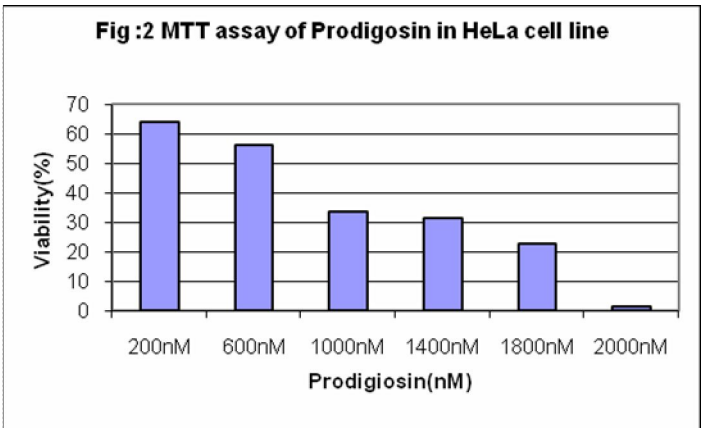
Concentration of drug nM/ml	% Survival of the Hela-229 cells
200nM	63.7
600nM	56.2
1000nM	33.4
1400nM	31.30
1800nM	22.78
2000nM	1.5

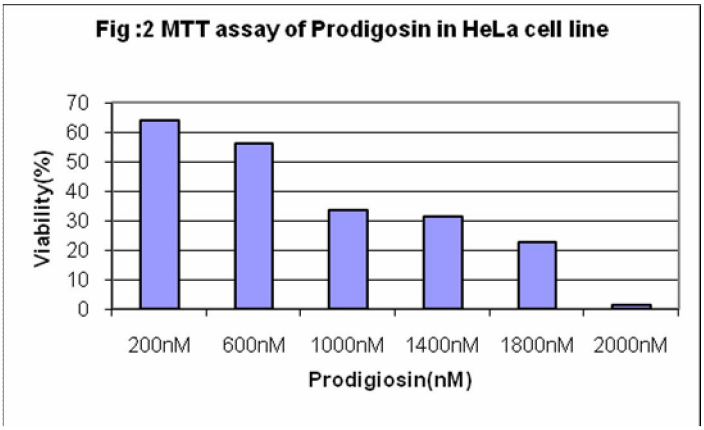
Table 2: NRU assay of Prodigiosin on HeLa cell line

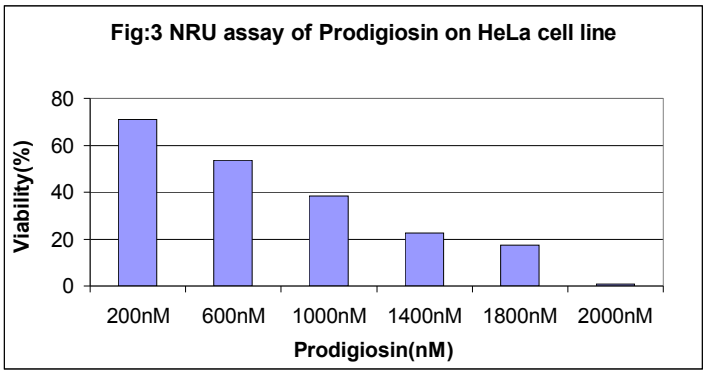
Concentration of drug nM/ml	% Survival of the Hela-229 cells
200nM	71.1
600nM	53.7
1000nM	38.3
1400nM	22.7
1800nM	17.3
2000nM	0.9

Figure 1:Isolated Serratia marcescens-kj1307









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