WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 10, 558-567. Research Article ISSN 2277–7105

RED PIGMENT EXTRACT OF SERRATIA MARCESCENS IS ABLE TO INDUCE CHANGE IN CANCER STEM CELL POPULATION ($CD^{44\ HIGH}$) OF METASTATIC PROSTATE CANCER CELL LINES DU145 & PC3.

Maithili A. Athavale*, Gayatri A. More, Smera Satish and Sangeeta A. Srivastava

Sathgen Biotech- A unit of Godavari Biorefineries Ltd. Mumbai, India.

Article Received on
26 March 2018,
Revised on 15 April 2018,
Accepted on 05 May 2018
DOI: 10.20959/wjpr201810-12264

***Corresponding Author**
Dr. Maithili A. Athavale
Sathgen Biotech- A unit of Godavari Biorefineries Ltd. Mumbai, India.

ABSTRACT

Purpose: The aim of the study was to evaluate anticancer stem cell property of Red Pigment Extract (RPE) of *Serratia marcescens* on $CD^{44\ high}$ cells of two breast viz. MCF-7, MDAMB231 and two prostate viz. DU145 and PC3 cancer cell lines. **Methods:** The effect of RPE of *Serratia marcescens* on breast and prostate cancer cell lines was checked by incubating breast (MCF7, MDAMB231) and prostate (DU145, PC3) cancer cell lines with RPE at inhibitory concentration 5 (IC5) for 72 hours at 37^0C, 5% CO_2 in the respective cell culture media. The change induced by RPE on $CD^{44\ high}$ cells was checked by Fluorescent Acquired Cell Sorter (FACS). **Results:** Our results indicated that RPE is able to induce change in $CD^{44\ high}$ cells of only prostate ($p<0.0001$) but did not have much effect on breast cancer cell lines ($p<0.05$). RPE was able to shift $CD^{44\ high}$ cells of prostate cancer to $CD^{44\ low}$ CD^{24high} thus making them susceptible to drug therapy. **Conclusion:** Findings of our study indicate that RPE of *Serratia marcescens* has a potential to inhibit cancer and cancer stem cells (CSCs) of metastatic prostate cancers and may act as a natural and cost effective source for cancer therapy.

KEYWORDS: Red Pigment Extract (RPE), *Serratia marcescens*, Cancer stem cells, $CD^{44\ high}$, DU145, PC3.

INTRODUCTION

WHO update, Feb 2017, indicates cancer is one of the leading causes of morbidity and mortality worldwide with approximately 14 million new cases in 2012 alone.[1] Cancer stem

cell (CSC) is a small subset of cells capable of dictating invasion, metastasis, heterogeneity and therapeutic resistance in tumors.[2] Eradication of this rare population is a new insight in cancer treatment. Many studies have indicated CD^{44} and CD^{24} as potential cell surface markers for CSCs.[3] CD^{44}, a hyaluronic acid receptor is one of the most commonly studied surface markers. It has been proved to be a CSC marker for breast and prostate cancers.[4-5]

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 in-vitro and during pathogenesis. Prodigiosin (red pigment) is synthesized from different bacteria which include species of *Actinomycetes, Streptomyces* and *Serratia*.[6] Prodigiosins have great therapeutic values,[7] induces potent apoptotic activity on various hematopoietic cancer cell lines and interestingly has no marked toxicity in non-malignant cell lines.[7-9] It has also reported activity on human cervix carcinoma.[10]

In our previous study, we have reported a comparative anti-tumor activity of Red-Pigment Extract (RPE) of *S. marcescencs* on breast and prostate cancer cell lines.[11] In continuation to the same, we were further interested in evaluating it's role in eradicating CSC population. Till date there is no report of its activity on CD^{44} cell surface marker of breast and prostate cancer cell lines.

In the present study we performed a Flow Cytometry assay to check the effect of RPE on CD^{44} population of two breast MCF7, MDAMB231 and two prostate DU145, PC3 cancer cell lines.

MATERIALS AND METHODS

RPE was prepared from the culture of *Serratia marcescens* and the anticancer activity of the extract on breast cancer cell lines (MCF7, MDAMB231) and prostate cancer cell lines (DU145, PC3) was determined by MTT assay as previously described in our study.[11]

For Flow Cytometry following procedure was adopted. Briefly, the log phased cultured cells (18 hours old) of MCF-7, MDAMB231 and DU145, PC3 were taken for the study.

MCF-7, MDAMB231 and DU145 cells were cultured in 100mm TC (Tissue Culture) plates in Dulbecco's Minimum Essential Medium (D.M.E.M) and PC3 cells in Rosewell Park Memorial Institute medium (R.P.M.I). The cell culture media was procured from HiMedia

Mumbai. The media was supplemented with 10% Fetal Bovine Serum (F.B.S, Gibco) and antibiotics (Penicillin and Streptomycin, Gibco).

To check the effect of the drug on cell surface marker expression inhibitory concentration 5 (IC5) of drug was selected. IC5 value was calculated based on MTT results obtained previously.[11] All dilutions were made in TC grade sterile Dimethyl Sulfoxide (DMSO). Three sets were prepared in duplicates for each cell line. The sets were Untreated, Cisplatin IC5 treated and RPE IC5 treated respectively. All the sets were incubated at 37˚C, 5% CO_2 for 48 hours.

After incubation the cells from each set were trypsinized using 1X- Trypsin EDTA (HiMedia- Catno.TCL034), neutralized with the respective cell culture media, transferred to Flourescent Acquired Cell Sorter (FACS) tubes, washed twice with FACS buffer - 4% FBS in Dulbeco's Phosphate Buffered Saline (HiMedia Cat noTL1006) and centrifuged at 3000 rpm for 3 minutes. The supernatant was discarded and the pellet was resuspended in 30µL of FACS buffer. 5 µL of each CD^{44} - BD Pharmingen PE Mouse Antihuman antibody (Cat no.555479) and CD^{24} BD Pharmingen FITC Mouse Antihuman antibody (Cat no-555427) were added. The experimental set was incubated for 30 minutes at 4˚C in dark. After incubation a wash with 200 µL of FACS buffer was given and centrifuged at 3000 rpm for 1 minute. The supernatant was discarded and 200 µL of FACS buffer was added to each tube. The samples were kept at 4˚C in dark till they were acquired on BD FACS Accuri C6 Flow Cytometer. For every sample 10000 events were recorded.

The cells were acquired in Forward Scatter – Side Scatter (FSC-SSC) plots and the live cell population was gated. The gated cells were plotted in the quadrant plot in (Lower Left) LL region. The (Upper Left) UL region of the plot represents the cells expressing $CD^{44\ high}$ cell surface marker. The (Upper Right) UR region of the plot represents the cells expressing both CD^{44}andCD^{24} cell surface markers. Lower Right (LR) region represents the region expressing $CD^{24\ high}$ cell surface marker and LL region represents the population negative for both $CD^{44}$and CD^{24}.

RESULTS AND DISCUSSION

Our earlier studies had shown that the RPE has a strong anticancer activity on prostate cancer cell lines compared to breast.[11] Further, to test it's effect on CSC marker i.e. CD^{44} on breast and prostate cancer cells, we performed flow cytometry assay. The breast cancer cell line

MCF7 and MDAMB231 which we have chosen for the study have different metastatic potentials. MCF7 is a non-metastatic breast cancer cell line with CD^{44} CD^{24} expression and MDAMB231 is a highly metastatic breast cancer cell line with CD^{44high} expression.[12-13] The FACS plots of MCF7 (Fig.2) and MDAMB231 (Fig.6) depict the CD^{44} expression respectively. Cisplatin is a standard chemotherapeutic agent used to treat breast and prostate cancers.[14] Fig.3 & 7 indicate the FSC-SSC and the quadrant plots of MCF7 and MDAMB231 treated with Cisplatin. These FACS plots indicate that IC5 of Cisplatin does have significant effect on MCF7 than MDAMB231 represented in Graphs 1 ($p< 0.001$) & 2 ($p< 0.05$) respectively. Fig. 4 & 8 indicate the FSC-SSC and quadrant plots of MCF7 and MDAMB231 cells treated with RPE (Graphs 1 & 2), indicating its effect more on MDAMB231 ($p< 0.001$) than MCF7 ($p< 0.01$).

Fig. 9 &13 indicate the FSC-SSC and the quadrant plots of prostate cancer cell lines DU145 and PC3 respectively. The prostate cancer cell lines DU145 and PC3 have different metastatic potentials. DU145 is a moderately metastatic whereas PC3 is a highly metastatic cancer cell line[15] and untreated cells of DU145 and PC3 when stained with antiCD^{44} and antiCD^{24} antibodies indicated the higher expression of CD^{44} cell surface markers on these cells (Fig. 10 & 14).

Cisplatin IC5 treatment of DU145 and PC3 (Fig. 11 & 15) exhibited minimal effect on CD^{44high} cells of DU145 ($p<0.01$) and no significant effect on PC3 (Graph 3 & 4). As seen in Fig.12 & 16, RPE showed a marked difference in CD^{44} cell surface expression changing the CD^{44high} cells to CD^{44low} CD^{24high}. Thus the cells of DU145 and PC3 treated with IC5 of RPE show a highly significant reduction in CD^{44high} cells (Graph 3 & 4, $p< 0.0001$).

It is known that cells expressing CD^{44high} markers are resistant to drug therapy.[16] Some anticancer agents are able to induce changes in the cell surface markers making them more susceptible to drug therapy.[17] Here we report an important property of RPE of *Serratia marcescens* to be able to induce highly significant change in the CD^{44high} cells of prostate cancer cell lines ($p<0.0001$) compared to breast ($p<0.05$).

In our previous study, we have already reported a higher activity of RPE on prostate cancer cell lines.[11] Here our study shows a remarkable effect of RPE on CD^{44high} cells of prostate cancer cell lines shifting them to $CD44^{low}CD24^{high}$. Thus, indicating its possible role in eradicating prostate cancer stem cells.

The absorbance of RPE by U.V. spectra showed a single peak at 539 nm, which corresponds to the peak of Prodigiosin.[18] Thus, RPE of *Serratia marcescens* could be a natural tool to eradicate prostate CSCs thereby leading to cost-effective cancer therapy in future. However, there is a need to further check the purity, analyze and isolate this active compound from the extract and verify its activity in pure state.

Figures:

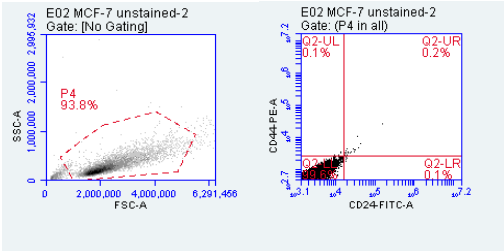


Fig.1

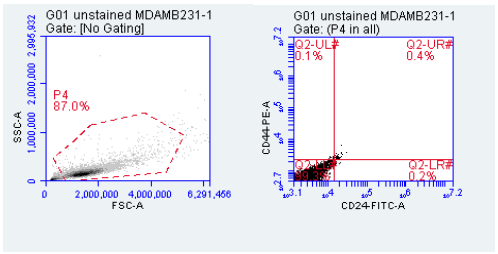


Fig.5

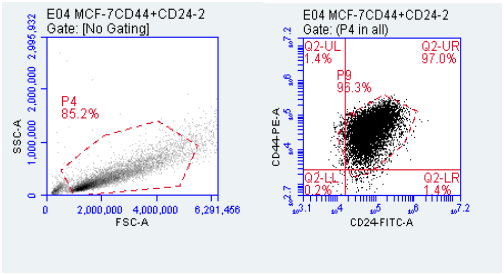


Fig.2

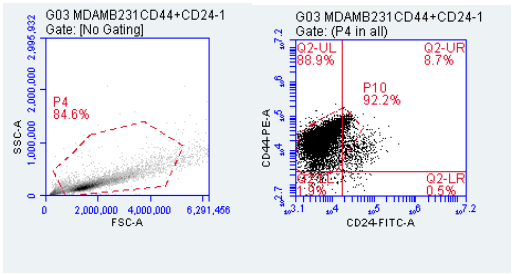


Fig.6

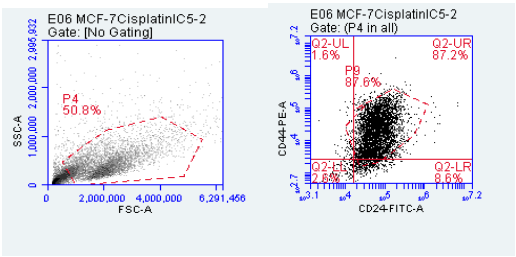


Fig.3

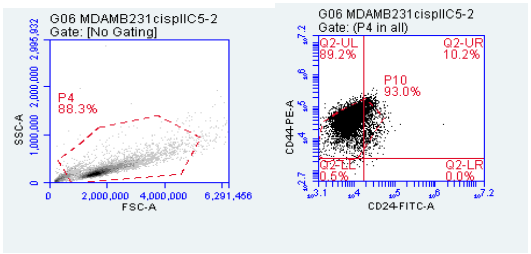


Fig.7

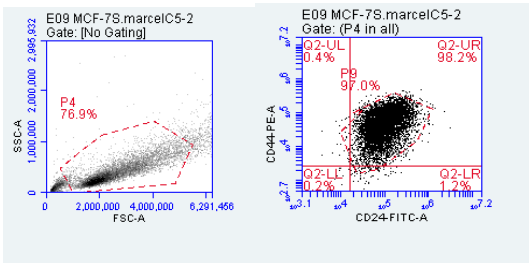


Fig.4

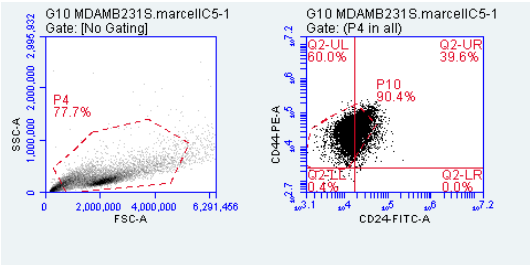


Fig.8

Fig1&5 represent the FSC-SSC plots and the quadrant plots of unstained cells of breast cancer cell lines MCF-7 and MDAMB231 respectively.

Fig2&6 represent the CD44,CD24 stained untreated population of MCF-7 and MDAMB231 respectively.

Fig3&7 represent the Cisplatin IC5 treated population of MCF-7 and MDAMB231 respectively.

Fig4&8 represent the Red pigment extract IC5 treated population of MCF-7 and MDAMB231 respectively.

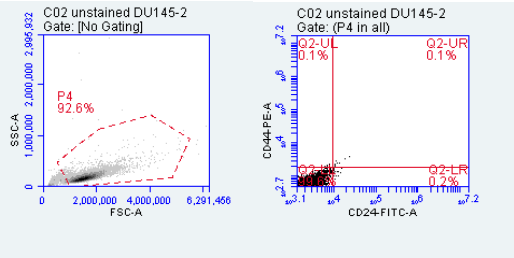


Fig.9

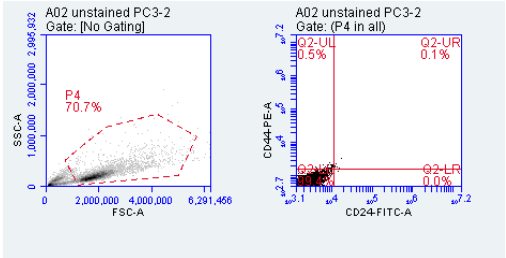


Fig.13

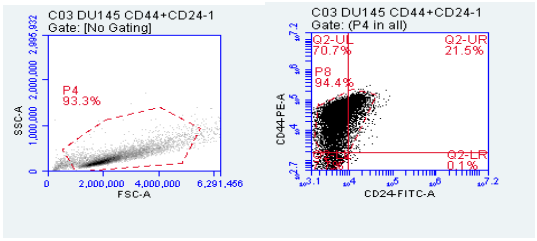


Fig.10

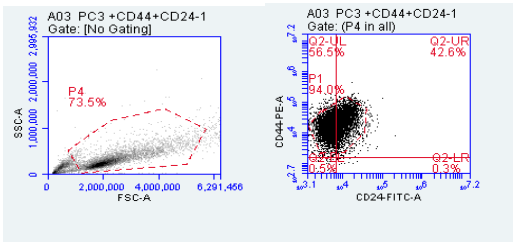


Fig.14

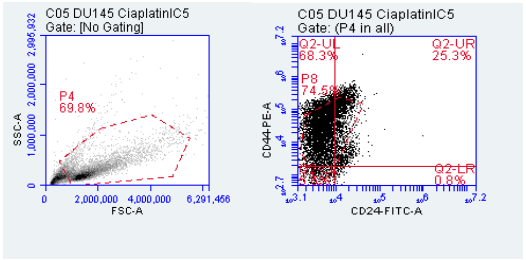


Fig.11

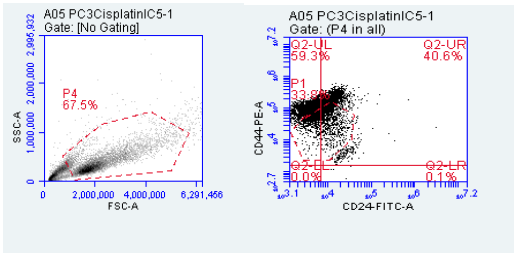


Fig.15

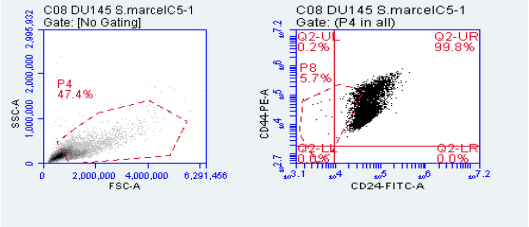


Fig.12

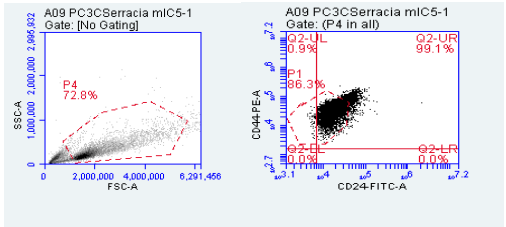


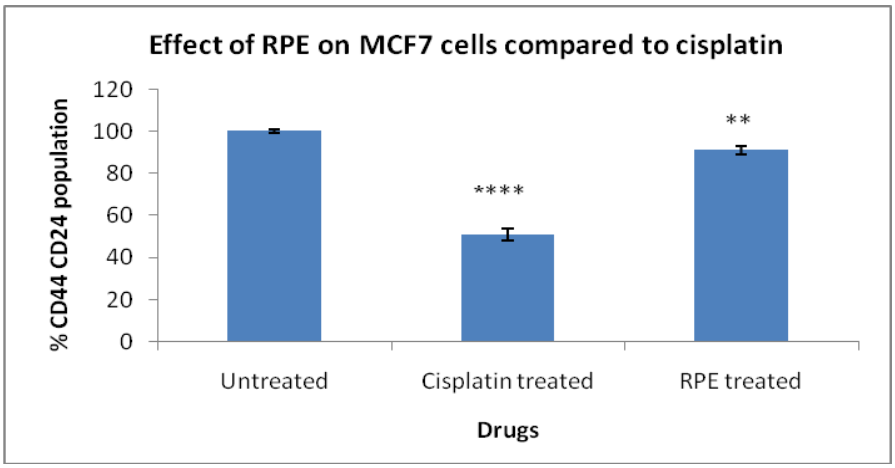
Fig.16

Fig9&13 represent the FSC-SSC plots and the quadrant plots of unstained cells of prostate cancer cell lines DU145 and PC3 respectively.

Fig10&14 represent the CD44,CD24 stained untreated population of DU145 and PC3 respectively.

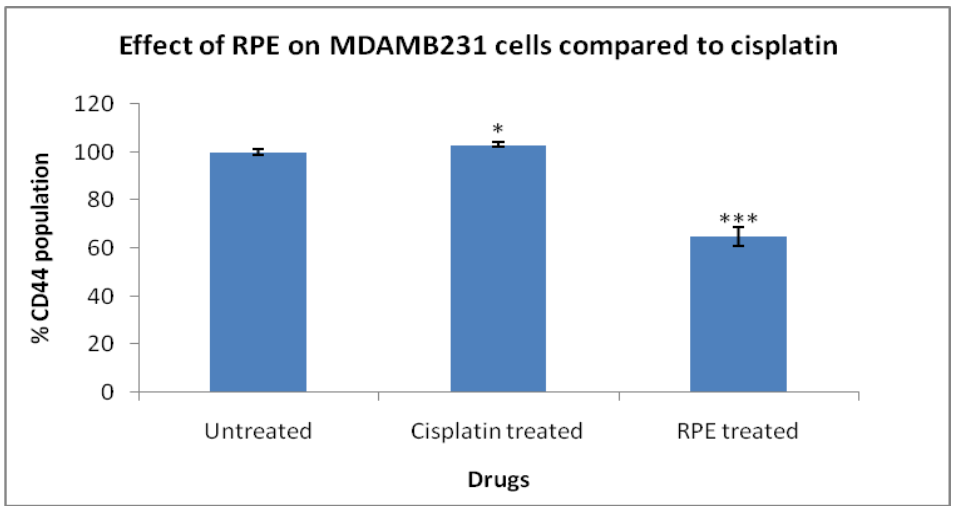
Fig11&15 represent the Cisplatin IC5 treated population of DU145 and PC3 respectively.

Fig12&16 represent the Red pigment extract IC5 treated population of DU145 and PC3 respectively.



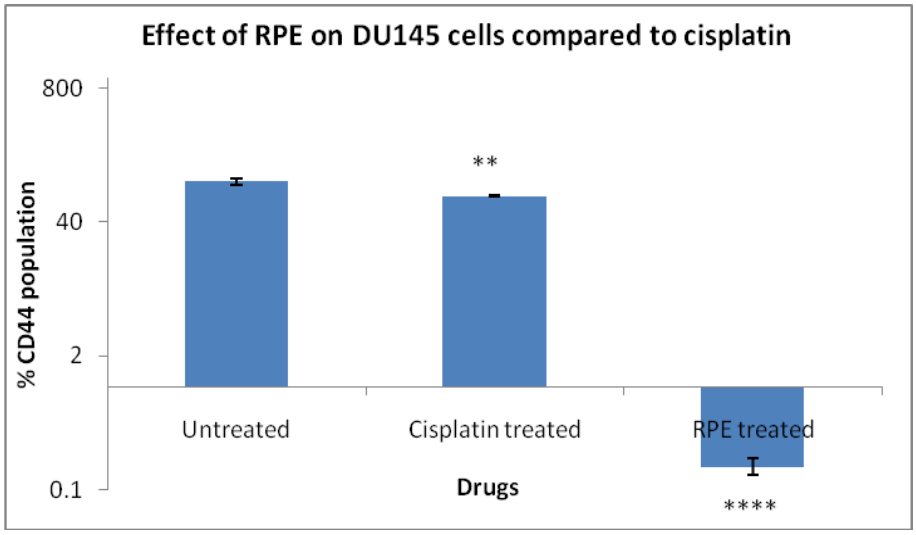
Graph 1: indicates effect of RPE on MCF-7 cells compared to standard chemotherapeutic drug Cisplatin.

(n=4), **** = p<0.0001, ** = p<0.01

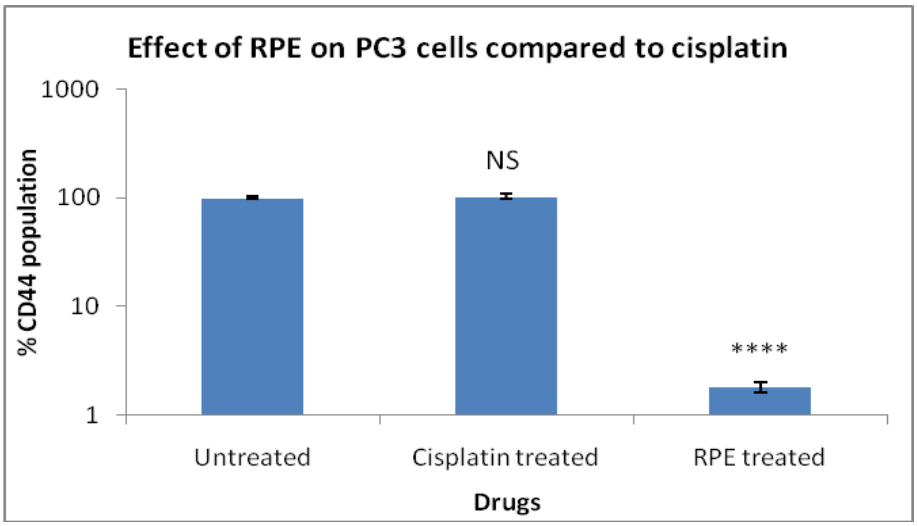


Graph2: indicates effect of RPE on MDAMB231 cells compared to standard chemotherapeutic drug Cisplatin.

(n=4), *** = p<0.001, * = p<0.05



Graph 3: indicates the effect of RPE on DU145 cells compared to standard chemotherapeutic drug Cisplatin (the graph is represented in log scale for comparison). (n=4), **** = p<0.0001, ** = p<0.01



Graph 4: indicates the effect of RPE on PC3 cells compared to standard chemotherapeutic drug Cisplatin.

(n=4), **** = p<0.0001, NS = Not Significant

CONCLUSION

Our present study indicates that RPE does not only possess anticancer activity but also may possess anticancer stem cell activity specifically on metastatic prostate cancer cell lines. Further to validate this, other in vitro cancer stem cell assays like wound healing assay[19] and sphere assay[20] should be performed with RPE on prostate cancer cells. The results of study also needs to be confirmed by in vivo methods before further development of RPE as a natural and effective anticancer therapeutic.

ACKNOWLEDGEMENT

The authors would like to express their sincere thanks to Shri Samir Somaiya - Chairman & Managing Director of Godavari Biorefineries Ltd.for providing the infrastructure, funding and support throughout the study.

Authors are also thankful to Dr. Sendurai Mani for critically evaluating the manuscript and for his invaluable guidance. The authors also thank the Microbiology Department of K.J Somaiya Medical College for gifting culture *Serratia marcescens* and Mr. Akshay Ganpule for his technical support during the course of study.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest in the study described in this research paper.

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