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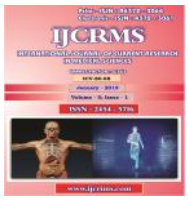

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Evaluation of Anti-cancer potential effect of *Chandamarutha chenduram* on MCF-7 cell line.

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Abstract

Background: Breast cancer is second most common in women and accounts for 23% of all occurring cancers in women. Patients with breast cancer have increasingly shown resistance and high toxicity to chemotherapeutic drugs. chandamarutha chenduram is a metallic preparation used in siddha medicine for the treatment of tonsillitis, paralysis, arthritis, fever, ulcers, cancer especially breast cancer. **Aim and Objective:** The present study was to investigate the anti cancer activity of chandamarutha chenduram against MCF-7 cell lines was examined by MTT assay. **Materials & Methods:** The drug was prepared as per the method mentioned in the classical Siddha literature. The drug is subjected to the anticancer activity against MCF-7 cell line was examined by MTT assay. **Results:** The result obtained from the study reveals that the percentage of MCF7 breast cancer celline line viability decrease with increase in concentration of the test drug. Least viability of cell was observed at the concentration of 100µg/ml shows 8.33 ± 0.46%, followed by this 80µg/ml shows 13.84 ± 0.92%, similarly 40,20,10 and 5 µg/ml shows 24.29 ± 2.23, 31.98 ± 2.32, 37.52 ± 2.81 and 44.28 ± 1.84 % cell viability in MTT assay. The corresponding IC50 value was found to be 25.26 ± 9.00 µg/ml. **Conclusion:** From this study we conclude that siddha formulation chandamarutha chenduram having significant anticancer activity against MCF-7 cell lines and it might be good therapeutic value.

Keywords: CMC, MCF-7 cell line, MTT assay, anti-cancer activity

Introduction

Cancer is a multi-step disease incorporating physical, environmental, metabolic, chemical and genetic factors (1). Breast cancer is second most common in women and accounts for 23% of all occurring cancers in women. The prevalence of breast cancer in Indian women is more at the age of forty(5). The incidence of breast cancer has been increasing worldwide for many decades with Asian countries attaining highest incidence rate(7). Patients with breast cancer have increasingly shown resistance and high toxicity to chemotherapeutic drugs. The symptoms of breast cancer are bloody discharge from the nipple, discomfort, swollen lymph nodes and changes in the texture of the nipple or breast. Breast cancer is the most commonly occurring cancer in women, comprising almost one-third of all malignancies(2). It accounts for approximately 25% of all female malignancies with a higher prevalence in developed countries. Breast cancer is the second leading cause of cancer-related death among females in the world (3).BRAC1, BRAC2 and p53 genes having tumor suppressing function. But mutation of BRAC1 and BRAC2 leads to activation of oncogenes. Inactivation of tumor suppressor gene, which control apoptosis leads to proliferation. Apoptosis is well-regulated physiological process of cell death. Normal breast growth is controlled by a balance between cell proliferation and apoptosis, and breast tumor growth not just as a result of uncontrolled proliferation but also due to reduced apoptosis (4).

Siddha system of medicine is a well defined science in the medical world. It is one of the most ancient systems of medicine for curing diseases. The symptoms of breast cancer may be correlated with nagirputtru in our siddha system. Siddhars told a wonderful medicine for nagirputtru namely Chandamarutha Chenduram. chandamarutha chenduram is a metallic preparation used in siddha medicine for the treatment of tonsillitis, paralysis, arthritis, fever, ulcers, cancer especially breast cancer..Development of breast cancer cell targeting drug without affecting the normal cells is a challenging task in the field of cancer drug discovery. According to the data of world Health organization, chemotherapy is needed for more than 90% of people affected with breast cancer. The present investigation focused on the determination of the anticancer effect of the *chandamarutha chenduram* on breast cancer MCF-7 cell lines.

Materials and Methods

Stock Solution

10mg/ml concentration of the sample CMC was prepared using DMSO at the concentration of 5,10,20,40,80 and 100µg/ml. The diluted sample CMC was transferred to the culture plate. 500 µl of MCF7 cell at the $1x10^4$ cells/well.

Cell lines and cultural conditions

MCF7 breast cancer cell line was obtained from National Centre for Cell Sciences (NCCS), Pune. MCF7 cell lines were cultured in Minimal Essential medium (MEM) with 10% FBS, Trypsin, EDTA, Glucose, 1% streptomycin, pencillin G and amphotericin B under a fully humidified atmosphere 5% CO_2 at 37^0C.

Cell Treatment Procedure

The monolayer MCF7 breast cancer cell line were detached with trypsin ethylenediamine tetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocyto meter and diluted with medium containing 5% FBS to give final density of 1 x 10^5 cells / ml. One hundred micro liters per well of cell suspension were seeded in to 96-well plates at plating density of 10,000 cells / well and incubated to allow for cell attachment at 37º C, 5% CO2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentration 5,10,20,40,80 and 100µg/ml of the test formulation. They were initially dissolved in Dimethyl sulfoxide (DMSO) and diluted to twice the desired final maximum test concentration with serum free medium. Additional four, 2 fold serial dilutions were made to provide a total of six sample concentration. Following the treatment with test sample, the plates were incubated for an additional 48 h at 37^oC, 5% CO2, 95% air and 100% relative

humidity. The medium without samples were served as control and triplicate was maintained for all concentrations.

MTT assay

The effect of test sample on the viability of MCF7 breast cancer cell line were determined by MTT (3-[4,5-dimethyl thiozole-2-yl]-2-5-diphenyl tetrazolium bromide) assay. 100µl of cell suspensions in growth medium were plated in 96-well microtitre plate at concentrations of $1x10^4$ cells/well and incubated for 48h at 37^0C in a humidified incubator. After 48 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 72 h at 37^0C. After removal of the sample solution and washing with phosphate buffered saline (pH 7.4), 20µL of MTT (5mg/mL) was added to each well of the plate. The plate was incubated for 4h at 37^0C. The solution in each well including MTT was aspirated and 100µL of buffered DMSO was added to dissolve formazone. The plates were shaken for 5min. Optical density was measured on a microplate ELISA reader at 540nm with DMSO as control. The cytotoxicity was obtained by comparing the absorbance between the samples and control. The percentage inhibition was calculated as follows:

% cell viability = A540 of treated cells / A540 of control cells × 100%

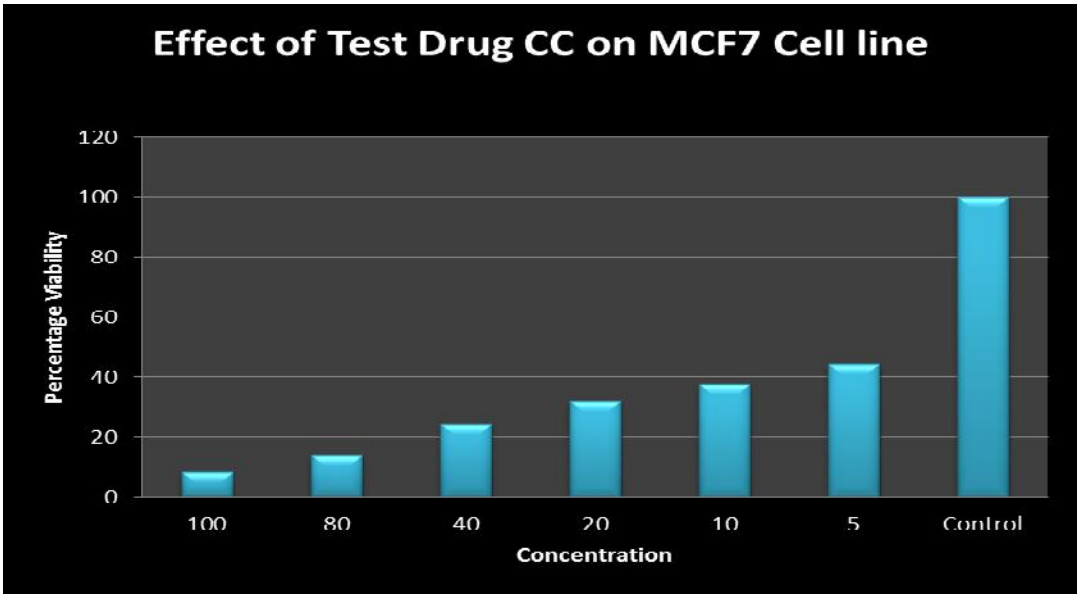
IC_{50} was calculated from dose-response curves.

Results

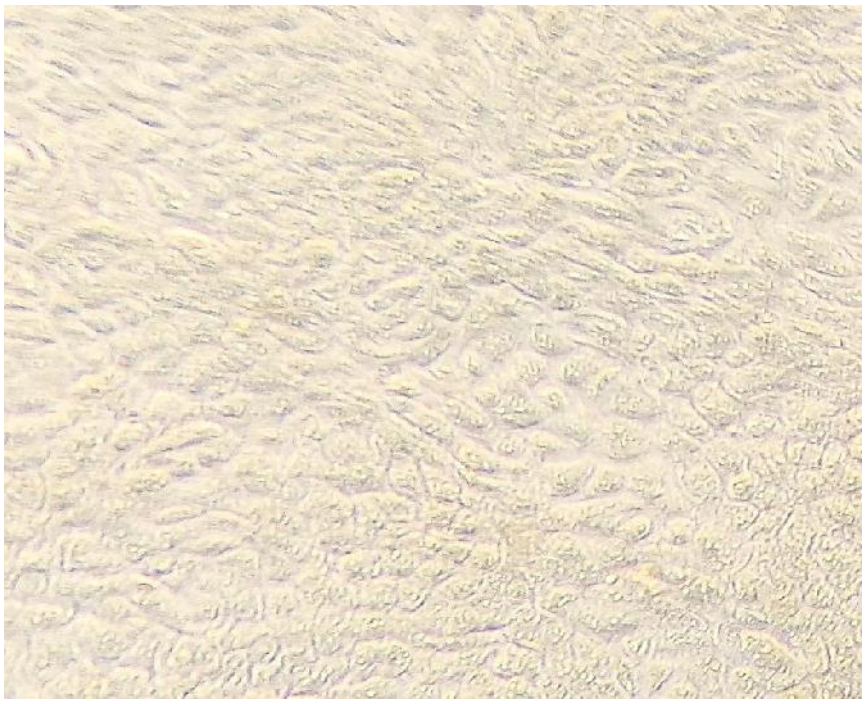
Effect of Test drug on Cell viability of MCF7 Breast cancer cell line

S.No	Concentration in µg/ml	% cell Viability
1	100	8.33 ± 0.46
2	80	13.84 ± 0.92
3	40	24.29 ± 2.23
4	20	31.98 ± 2.32
5	10	37.52 ± 2.81
7	5	44.28 ± 1.84
8	Control cells	100

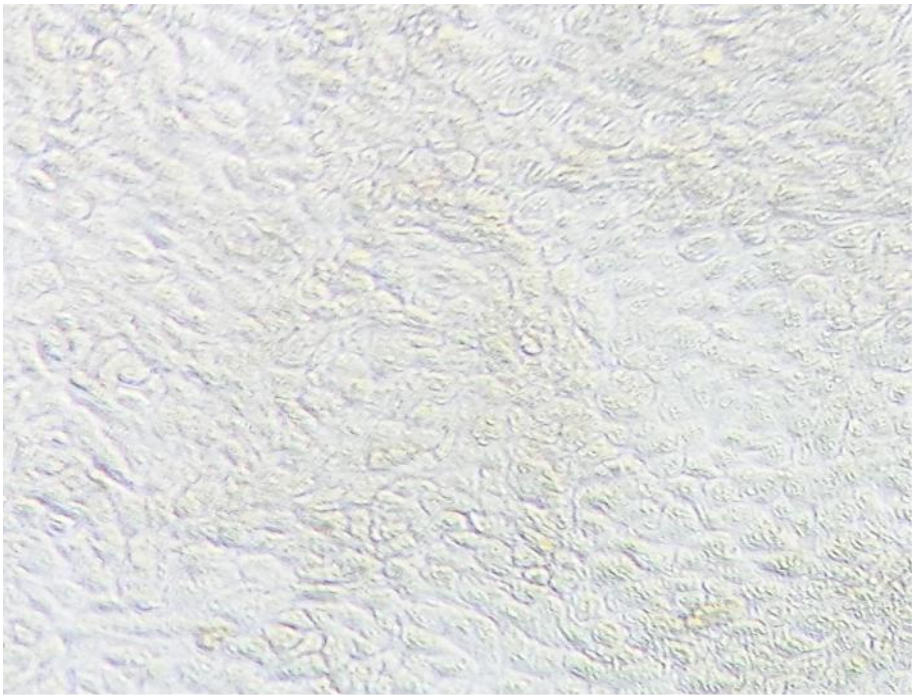
IC 50 Value: 25.26 ± 9.00



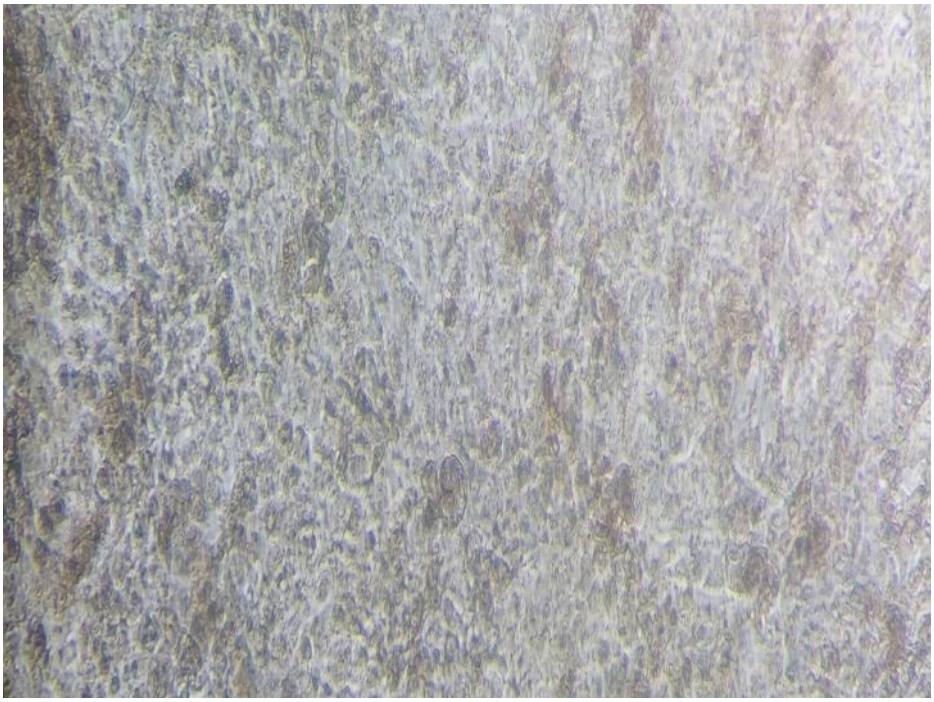
MCF7 Control Cells



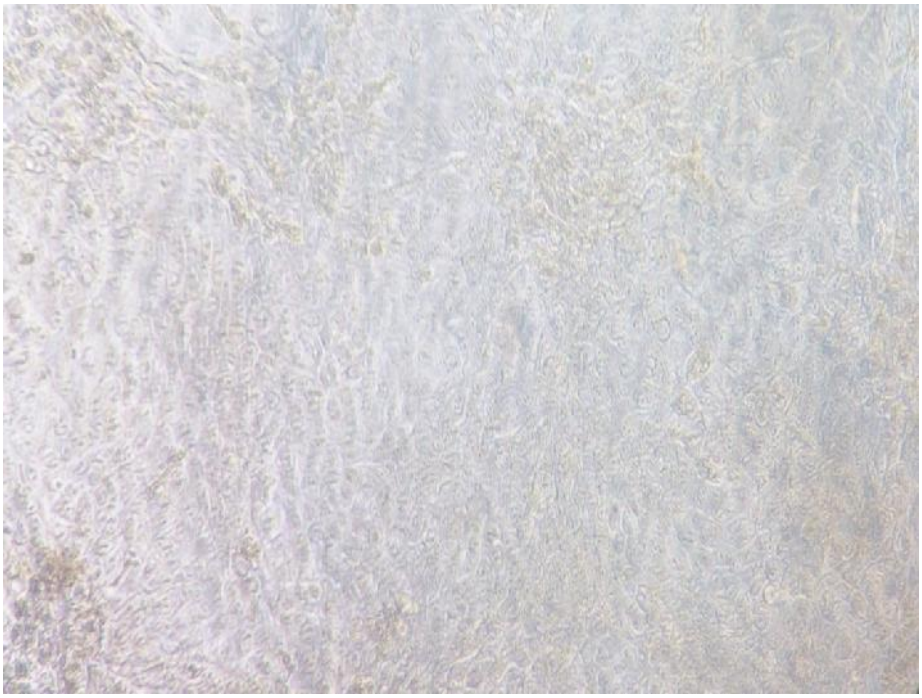
Test Drug CMC- 5 µg



Test Drug CMC- 10 µg

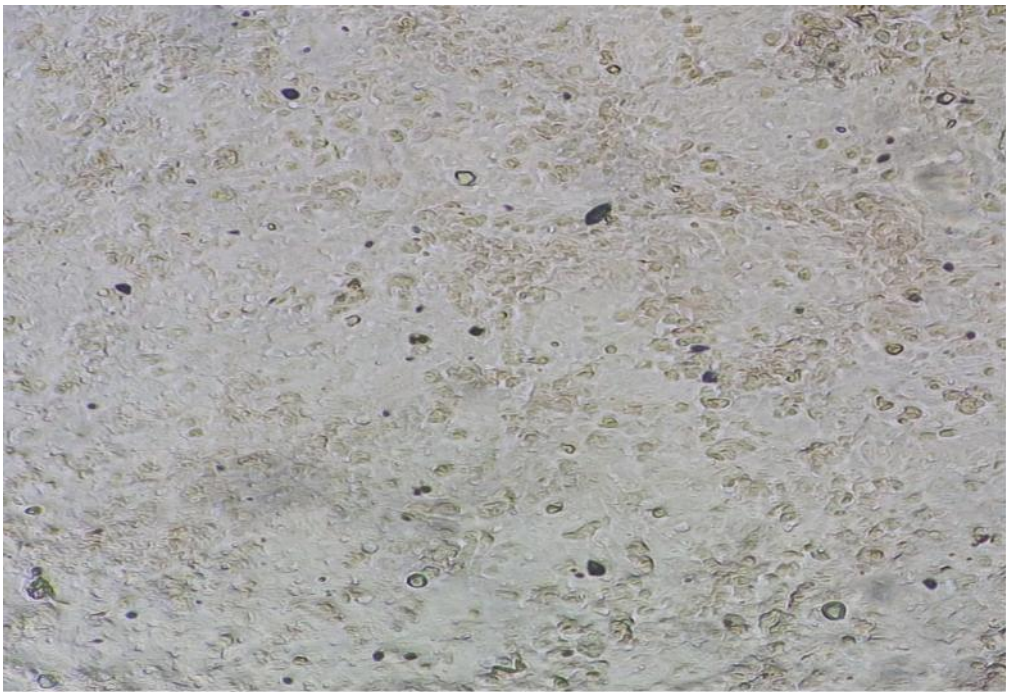


Test Drug CMC- 20 µg



Test Drug CMC- 40 µg

Test Drug CMC- 80 µg



Test Drug CMC- 100 µg

Discussion

In-vitro anti-cancer evaluation of test drug CMC on the cell viability against MCF7 breast cancer cell line was performed at varying concentration ranges from 5,10,20,40,80 and 100µg/ml .The result obtained from the study reveals that the percentage of MCF7 breast cancer cell line viability decrease with increase in concentration of the test drug. Least viability of cell was observed at the concentration of 100µg/ml shows 8.33 ± 0.46%, followed by this 80µg/ml shows 13.84 ± 0.92%, similarly 40,20,10 and 5 µg/ml shows 24.29 ± 2.23, 31.98 ± 2.32, 37.52 ± 2.81 and 44.28 ± 1.84 % cell viability in MTT assay. The corresponding IC50 value was found to be 25.26 ± 9.00µg/ml.

Conclusion

To conclude, being a cytotoxic compound is one of the prerequisites for an anti-cancer drug (unlike any other therapeutic drugs), be it an NCE or a biological compound, in order to kill the cancer cells and many anticancer products in the market are basically cytotoxic and it is also acceptable by the regulatory authorities to approve the anticancer drugs if found clinically effective inspite of being cytotoxic. This in vitro study confirming the basic anticancer properties would pave scope for in vivo studies and support the utility of CMC as evidence based alternative medicines for cancer therapy [10,11]. The results of the present study suggest that CMC has anticancer activity against MCF 7 cell line.

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