

Evaluating the Anticancer Activity of Homoeopathic Preparation of *Asterias rubens* in Breast Cancer Cell Line on the Basis of Similia Principle

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ABSTRACT

Breast cancer is the second leading cause of cancer death in woman and ranks as the first leading cause of death in India. In its treatment several measures and recommendation are considered. Homoeopathic medicines are the one of the part of complementary and alternative medicines are used for the treatment of cancer. The objective of the study is to evaluate the anticancer activity of homoeopathic preparations of *Asterias rubens* on the basis of similia principle.

We conducted an in vitro study using MTT assay to determine the effect of ultra diluted homoeopathic remedy against two human breast adenocarcinoma cell lines (MCF-7 and MDA-MD-231), frequently used for the breast cancer treatment, by testing the viability of breast cancer (MCF-7 and MDA-MD-231) cell line, with various attenuations of *Asterias rubens* at 24 hrs. Multiple comparisons between tested reagents at different concentrations confirmed significance of the said results. At dilution of 1:25 6CH and 30CH potency shown superior activity on MCF-7 and no such significant changes on MDA-MD-231 at any dilutions as it lacks estrogen receptor (ER) and progesterone receptor (PR) expression, as well as HER2 (human epidermal growth factor receptor 2) so being a triple negative breast cancer it is an aggressive form of breast cancer with limited treatment options. However, further potency need to be tested. These preliminary significant results warrant further in vitro and in vivo studies to evaluate the potential of *Asterias rubens* a medicine to treat breast cancer.

Keywords- *Asterias rubens*, Anticancer activity, Breast cancer cell line, Similia Principle

INTRODUCTION

Breast Cancer is a malignant proliferation of epithelial cells lining the ducts or lobules of the breast¹. The second leading cause of cancer death in woman is Breast Cancer and ranks as the first leading cause of death in India². In the year 2018, about 2.09 million cases of breast cancer and 627000 deaths occurred Worldwide from the disease, many because of late diagnosis and lack of access to affordable

treatment.^{3,4} Breast cancer is hormone-dependent. Women with late menarche, early menopause, and first full-term pregnancy by age 18 have a significantly reduced risk. Women who received therapeutic radiation before age 30 are at increased risk. Breast cancer risk is increased when a sister and mother also had the disease.²

The treatment usually includes chemotherapy, radiotherapy, and hormone

therapy. These orthodox modes of treatments are often painful and are associated with considerable side effects.⁴ Further people often look for other modes of treatment that has relatively fewer or no side effects and can be complement the orthodox treatment.

Homeopathy offers one such arm of complementary and alternative therapy that causes the patient no pain and has no or negligible side effects and has been used to treat various disease conditions including cancer⁵. Homeopathy is a system of medicine developed in the late 18th century by Samuel Hahnemann based on the principle, “like cures like” and often uses extremely high dilutions of remedies.⁶ Most, but not all, critical reviews of clinical literature in homeopathy demonstrate significant effects beyond placebo, but there is an insufficient research base in any one condition to demonstrate specific efficacy or to understand mechanisms.

Homeopathy is an alternative medical system practiced in all parts of the world.⁷ According to the homeopathic system of medicine; remedies in high serial dilutions retain their biological activity, have reduced toxicity, and invoke healing processes.

***Asterias rubens* has Sycotic diathesis; It has been used for cancer of the breast, and has a unquestioned influence over cancer disease.**⁸ The common starfish or common sea star (*Asterias rubens*) is the most common and familiar starfish in the north-east Atlantic.⁹ *Asterias rubens* is effective for breast cancer even in ulcerative stage. In this study, we were investigated the effect of homeopathic preparation of *Asterias rubens* in vitro on Cancer cell line often used to treat breast cancer.

MATERIAL AND METHODS

Homeopathic Samples:

Homeopathic Preparations of *Asterias rubens* (6C, 30C) was procured from GMP Certified Homeopathy pharmacy Schwbe India. Batch number log was 0255232 and 0269385 respectively.

Cell culture:

The human breast cancer cell lines, MCF-7 (ER+/PR+/HER2-) and MDA MB-231 (ER-/PR-/HER2-), were obtained from ATCC, USA. MCF-7 cells were grown in EMEM supplemented with 0.01 mg/ml human recombinant insulin, fetal bovine serum (FBS) to a final concentration of 10% and 100 U/ml of penicillin-streptomycin. MDA-MB-231 cells were grown in DMEM supplemented with 10% FBS and 100 U/ml of penicillin-streptomycin. The cells were incubated in a humidified 5% CO₂ incubator at 37°C.

Cell Viability Assay

The cell viability was determined by MTT dye [Koppikar et al., 2010; Mansara et al., 2015, Suryavanshi et al., 2019]. The breast cancer cell lines, MCF-7 and MDA MB-231 cells were seeded at a density of 1×10^5 cells/ml density in 96-well plates. An untreated group was kept as a negative control. The 6C and 30C potencies of *Asterias rubens* were added at following dilutions: 0, 1:25, 1:50, 1:100, 1:200 in wells in triplicates. The MTT solution (5 mg/ml) was added to each well, and the cells were cultured for another 4 h at 37°C in 5% CO₂ incubator. The formazan crystals formed were dissolved by addition 100 µl of DMSO. The amount of colored formazan derivative was determined by measuring optical density (OD) using the ELISA microplate reader (Biorad, Hercules, CA) at 570 nm (OD_{570-630 nm}).

The percentage viability was calculated as:

$$\% \text{ Viability} = [\text{OD of treated cells} / \text{OD of control cells}] \times 100$$

Statistical analysis

The analyses were carried out using Graphpad prism 5 software (San Diego, CA, USA). The values with *p<0.05; **p<0.01; ***p<0.001 were considered to be statistically significant. The experiment was performed in triplicates and the values have been presented as mean±SD. Differences among means were tested for statistical significance using one-way analysis of variance (ANOVA).

RESULTS

In MCF-7 cells, at 1:25 dilution of 6C and 30C potencies of *Asterias rubens*, 54.03±0.08 % ($p \leq 0.05$) and 60.37±0.07 % ($p \leq 0.001$) viability, respectively, was observed compared to the untreated control

cells (Figure 1a). On the other hand, in MDA-MB-231 cells, at 1:25 dilution of 6C and 30C, 90.77±0.1 % ($p > 0.05$, statistically non-significant) and 87.13±0.12 % ($p > 0.05$, statistically non-significant) viability, respectively, was observed (Figure 1b).

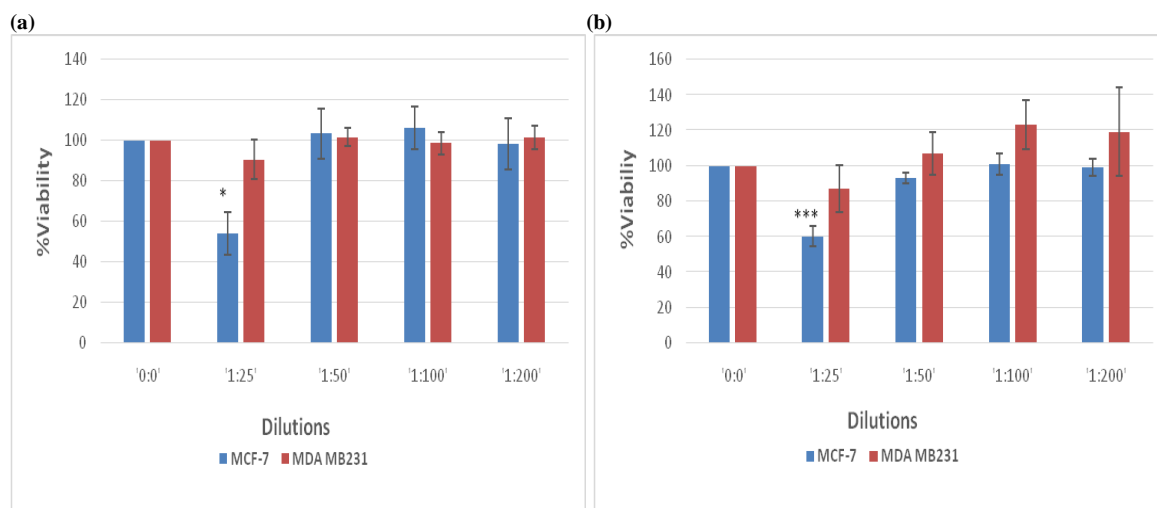


Figure 1: Effect of 6C (a) and 30C (b) potencies of *Asterias rubens* on viability of MCF-7 and MDA MB231 cells

Table 1: Percent viability of MCF-7 and MDA MB231 cells after treatment with 6C and 30C potencies of *Asterias rubens*

6CH (Dilutions)	% Viability with SD	
	MCF-7	MDA MB231
1:25	54.03±0.08	90.7±0.1
1:50	103.5±0.07	101.6±0.04
1:100	106.4±0.03	98.7±0.06
1:200	98.4±0.08	101.5±0.01
30CH (Dilutions)	% Viability with SD	
	MCF-7	MDA MB231
1:25	60.37±0.07	87.13±0.12
1:50	93.35±0.002	107.1±0.03
1:100	101.26±0.008	123.2±0.02
1:200	99.06±0.05	119.2±0.002

DISCUSSION

Homeopathy administers minute quantities of substances that produce symptoms of illness in a healthy person when administered in large doses. However, there are few studies available examining the mechanisms of homeopathy using conventional scientific methods.¹⁰

In the present study, we examined the effect of *Asterias Rubens* at the cellular level using breast cancer cells. Our findings suggest that ultra-high diluted homoeopathic remedy prescribed for breast cancer exert preferential anti-cancer effects against the human breast carcinoma cell line MCF-7.

The homoeopathic potencies of *Asterias rubens* (6c, 30c), were tested for their effectiveness against breast cancer cells. For testing the effect of *Asterias rubens* potencies (6c, 30c) on cell viability, each potency was diluted in cell culture medium (1:25, 1:50, 1:100, 1:200). In spite of dilutions (in DMEM), the potencies 6c, 30c were significantly effective against breast cancer cells at 1:25 dilution on MCF-7 and no significant effect on MDA-MD231. However, higher potencies of *Asterias rubens* need to be evaluated for confirming these results on MDA-MD231. Thus, whatever effect of the potencies was observed on the breast cancer cells was purely due to the active principle present in them. The higher dilutions (1:50-1:200) of potencies in DMEM, did not show appreciable anticancer effect since these were simple dilutions in culture medium, and not potentised, resulting into loss of the active principle. And receptor positive MCF-7 cell line has shown the effect rather than receptor negative MDA-MD231 cell line signifies the effect of homoeopathic medicine *Asterias rubens* over the cell receptors.

As the *Asterias rubens* is the leading remedy for the breast cancer treatment in homoeopathy confirms the similia principle by showing its effect on the cell line. According to the homeopathic system of medicine, remedies in high serial dilutions retain their biological activity, have reduced toxicity, and invoke healing processes. Homeopathy administers minute quantities of substances that produce symptoms of illness in a healthy person when administered in large doses.

CONCLUSION

6C and 30C potencies of *Asterias rubens* exhibit preferential anti-cancer activity against ER/PR/Her2 receptor positive breast cancer cells, MCF-7. On the other hand 6C and 30C potencies of *Asterias rubens* didn't exhibit anti-cancer activity against ER/PR/Her2 receptor negative breast cells, MDA-MB-231 as it lacks estrogen receptor(ER) and progesterone receptor (PR) expression, as well as HER2 (human epidermal growth factor receptor2) so being a triple negative breast cancer it is an aggressive form of breast cancer with limited treatment options. However, higher potencies of *Asterias rubens* need to be evaluated for confirming result on MDA-MD 231.

These preliminary results suggested that homoeopathic potencies of *Asterias rubens* holds potential as anticancer activity could be further explored against breast cancer in vivo.

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