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Research Article

IN –VITRO STUDIES OF THE CYTO TOXICITY EFFECT OF A POLY HERBAL SIDDHA FORMULATION IN BREAST CANCER CELLS

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ABSTRACT

Breast Cancer is a frightening disease. It remains a significant, scientific, clinical and societal challenge. Breast cancer will strike one in every eight American women. Globally, breast cancer in women with an estimated 1.38 million new cases per year 50000 cases in women was recorded each year in UK alone. There are 4,58,000 deaths per year from breast cancer worldwide making it the most common cause of female cancer death in both the developed and developing world. In the UK, the age standardized incidents of breast cancer in women has increased by 6% over the last decade, between 1999 to 2001 and 2008 to 2010.It is estimated that around 550,000-570,000 people are living with or after diagnosis of breast cancer in the UK and based on current projections, this figure is expected to triple by 2040 due to ageing population and continued improvements in survival. Based on the clinical and theoretical knowledge attained through various Siddha literatures, the Authors' of this paper has created a formulation for the treatment of Breast cancer. This formulation is being scientifically evaluated through in-vitro anti-proliferative studies in Breast cancer cells. The study result reveals the highest efficiency of the trail drug in killing breast cancer cells.

KEYWORDS

Chemotherapeutic drug, Adriamycin, Cell proliferation, Breast cancer cells

INTRODUCTION

Breast cancer is the most common type of cancer in women and the second leading cause of cancer related deaths, next to lung cancer. Although men can also get breast cancer, cases of male breast cancer account for less than 0.5% of all breast cancer cases diagnosed. If 8 women live to the age of 85, atleast one of them will develop breast cancer in her lifetime. Two-thirds of women diagnosed with breast cancer are over the age of 50, and majority of the remaining women diagnosed with breast cancer are between the ages of 39 and 40.

MATERIALS AND METHODS

Samples (1g) were dried and pulverized to particle size (#) 40 and then extracted with ethanol in the soxhlet apparatus for 48 h, and 200 g of fresh drug was subjected to cold maceration with obtain the ethyl acetate extracts was concentrated to dryness at 40 C under reduced pressure in a rota evaporator. The yield of the ethanol and aqueous extracts was found to be 150g(30% w/w) and 16 g(8% w/w),respectively.

1. Cell Viability

To determine the % of cell living:

Procedure:

1. Take 10ul of cell suspension & mix with 20 ul of trypan blue in an eppendrof tube.
2. Clean the surface of the glass slide & the semi-silver area of heamocytometer by alcohol.
3. Mix it well & load 10ul into meter by micropipette.
4. Focus the slide under a microscope & count the living & dead cells inside L1, L2, L3, L4 chamber.
5. Calculate the % viability of cell by

= no.living cells\ no.total cells*100

2. MTT Assay

2.1.Protocol

1. After 24-48 hrs of addition of drug colorimetric assay is performed.
2. Add 20ul of MTT reagent to wells already having the media &drug.
3. Incubate the plate for 3 hrs.

4. After 3 hrs discards the MTT reagent along with the media & the drug,& add 100ul of DMSO(to stop the reaction of MTT).
5. Keep the plate for incubation for 1hrs.
6. After incubation pipette out the suspension from each well into the plate reader.
7. Read the plate on the plate reader using 550 nm as test wavelength &630nm as the reference wavelength.
8. Record data & tabulate column.

3. Cytotoxicity

The sample with ethyl acetate showed effective cell viability. The percentage of cell viability increase with concentration. A cell viability of more than 50% was observed at a concentration lesser than 5mg/ml in the sample. The sample has the highest cell viability 93.64% the plant sample which were analyzed all possess a certain percentage of cancer properties. In this sample out of a sample ethyl acetate shows that good cancer properties. These sample tested for the presence of anticancer agent they yield positive result. The Test drug exhibits strong cytotoxic properties when tested in in-vitro cell line study. The ethyl acetate which may shows the good cancer property.

RESULTS AND DISCUSSION

1. MTT Assay

After 48 hrs from addition of drug MTT Assay was carried out to determine cell viability.

Conc.of drug in percentage	O.D at 545 nm
Media	**0.487**
2.5	0.449
5.0	0.444
7.5	0.424
10.0	**0.417**

2. Cytotoxic Changes Observed

Cell rounding, shrinkage, aggregation and cell death

S.NO	Dilutions of sample	Cytotoxicity on Hep2 cells in respective samples		
		1	**5**	**8**
1	Neat	4+	4+	4+
2	1:1	4+	4+	4+
3	1:2	4+	4+	4+
4	1:4	4+	4+	4+
5	1:8	2+	2+	4+
6	1:16	NT	2+	4+
7	1:32	NT	NT	4+
8	1:64	NT	NT	3+
9	1:128	NT	NT	1+
10	1:256	NT	NT	NT
11	Cell control	*	*	*

4+ - 100% cell death

3+ - 75% cell death

2+ - 50% cell death

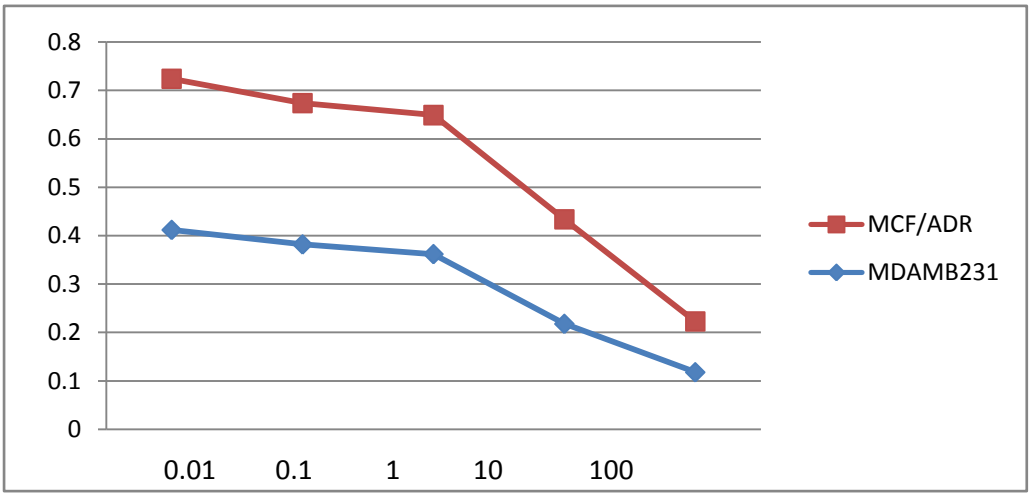
1+ - 25% cell death

* - no cell death

NT - non toxic

ACTIVITY ON BREAST CANCER CELL LINE

Ethyl acetate extract	0.01	0.1	1	10	100
MDAMB231	0.412	0.382	0.362	0.218	0.118
MCF/ADR	0.312	0.292	0.287	0.216	0.105
MDAMB23	0.312	0.289	0.276	0.229	0.123
NC	0.789	0.789	0.789	0.789	0.789



MTT cell survival assay.4 cell lines breast cancer cells were seeded at density of 10000 cells/well in 96-well plates and treated with or without increasing concentration of Adriamycin standard at 37C for 72 h, and viability of cells was determined. The experiments were repeated three times, and each concentration was tested in triplicate in each experiment. Viability was expressed as a percent on an untreated control (mean_ SEM).

STASTICAL ANALYSIS

All the results are expressed as mean + SD of triplicate.The difference in the inhibitory effect at different doses between the treated and the corresponding controls was analyzed for statistical significance by performance a Student's t-test. $P<0.05$ amplifies significance. The response of cell lines to Adriamycin treatment was also observed. Compared to other lines, the breast cell line was more resistant to Adriamycin. The IC50 analysis, showed statistically significant changes in MCF – 7 cell lines, where an increase and decrease of resistance, respectively were conferred relative to the sample cell line.

CONCLUSION AND OBSERVATIONS

The sample showed effective cell viability. The % of cell viability increased on increase of concentration. Table indicates the highest cell viability **93.64%** Sample ethyl acetate which may shows the good cancer activity. The sample was tested on MCF-7 Cell lines to check whether they induce apoptosis on the cells. The percentage of cell viability was calculated. Sample had good cancer properties and hence they formed the basis for the

performance of our project study. A Graph of absorbance against the concentration of the drug was plotted and the inhibitory effect (IC50) was calculated as the drug concentration that is required to reduce absorbance to half that of the control, based on the dose-response curve for the samples extracts. The reduction of MTT can only occur in metabolically active cells, the level of activity being a measure of viability of the cells.

Absorbance values that are lower than the control cell lines reveal the decline in the rate of cell in-vitro cancer activity. Conversely, a higher absorbance indicates increase in the cell proliferation. Untreated microtitre plates of cell lines with only vehicle (0.3% v/v DMSO in water). The percent inhibition of cell proliferation by the extracts is calculated based on the difference in the inhibitory effect between the treated cell lines and their respective controls, where 100% cell proliferation is taken from the corresponding controls.

So it is concluded that, the trail drug has proven Anti-cancer Activity against Breast cancer cell lines.

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