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Research Article

IN-VITRO ANTI- PROLIFERATIVE STUDY OF A NEW HERBAL FORMULATION FOR TREATING BREAST CANCER.

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ABSTRACT

Cancers are one among the leading causes of human death worldwide and the incidence continues to increase. Patients and their relatives are alarmed at the higher risk of morbidity and mortality this disease carries with it. Issues like prolonged hospitalization, treatment adherence, treatment costs and quality of living also annoy the patient and naturally they look for alternatives with proven track record. Here comes the wonderful therapeutic and prophylatic role of Traditional systems of Medicine. While the disease itself is not new to the mankind and it has always been dealt with since time immemorial by effective Traditional Siddha and Ayurvedic remedies. Cancer is known as 'Putru' in Siddha Medicine which literally means 'Termite mound' because of its proliferative nature. Breast Cancer is the leading cause of death in women worldwide among other types of Cancers. Breast cancer risk in India is One in Twenty. The risk is more at Urban areas (one in twenty two) and comparatively low in rural areas (one in sixty). Since an increasing proportion of cancer patients are acquiring resistance to traditional chemotherapeutic agents, it is necessary to search for new compounds that provide suitable specific anti-proliferative effects that can be developed as anticancer agents. This trial is such an effort to scientifically document an effective anti-cancer agent for the treatment of Breast cancer which is of Herbal origin, free from side effects, cost effective and less invasive.

Keywords: Herbal anti-cancer drug, Herbal chemo-therapy, Puttru, Breast cancer

INTRODUCTION

Cancers are some of the leading causes of human deaths worldwide and their relative importance continues to increase. Since an increasing proportion of cancer Patients are acquiring resistance to traditional chemotherapeutic agents, it is necessary to search for new compounds that provide suitable specific antiproliferative affects that can be developed as anticancer agents. The incidence of breast cancer is low in India, but rising. Breast cancer is the commonest cancer of urban Indian women and the second commonest in the rural women. Breast cancer awareness programs are more concentrated in the cities and have not reached the remote and rural parts of the country. Women often do not present for medical care early enough due to various reasons such as illiteracy, lack of awareness, and financial constrains.

Breast cancer risk in India is One in Twenty. The risk is more at Urban areas (one in twenty two) and comparatively low in rural areas (one in sixty). For the year 2012; 144,937 women were newly detected with breast cancer 70,218 women died of breast cancer. The risk factors of Breast Cancer includes BMI (Body Mass Index), Age at First child birth, Number of children, duration of breast feeding and abortions are on the rise world over.

MATERIAL AND METHODS

This is a formula which was generated by the rich theoretical and clinical knowledge of the Authors. It is a Poly herbal formula, with pure herbal based composition. Authors decided to evaluate the efficacy of this test drug through MTT Cyto-toxicity Assay.

MTT based cytotoxicity assay protocol

MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] based cytotoxicity assay was first described by Mosmann in 1983. This colorimetric assay is based on the ability of a mitochondrial dehydrogenase enzyme from viable cells to cleave

the tetrazolium rings of the pale yellow MTT and form dark blue formazan crystals which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. The number of surviving cells is directly proportional to the level of the formazan product created. The cytotoxic efficacy of the 3 samples was evaluated in 4 cell lines by MTT cell proliferation assay kit (Roche Applied Sciences, Germany). The assay was carried out according to the instruction provided by the vendor. MTT incorporation test method (Roche Applied Science, USA) was followed.

Briefly, cells were harvested from the logarithmic phase of cultures and resuspended in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). The cell counts were adjusted and equal number of cells were plated into each well of 96-well cell culture plates and allowed to grow overnight at 37°C, in presence of 5% CO2. The cells were treated with test substances at various concentrations ranging between 0.7 g/ml to 2.5 g/ml for 72h. Here we maintained the 72hrs as Prolifereation time. In vehicle control culture wells, a maximum of 0.5% DMSO was added. Culture medium was renewed at every 24h with fresh culture medium supplemented with the test substances. Thereafter, 0.5 mg/ml of MTT reagent was added to each well and the microplate was incubated further for 4h at 37°C in presence of 5% CO2. Finally, the cells were solubilized by adding solubilizing solution and allowed to incubate at 37°C overnight.

After complete solubilization of the formazan crystals the absorbance was read at 540 nm in a microplate reader (BioRad, USA) . The results (mean OD ± SD) obtained from quadruplicate wells were used in calculation to determine the cytotoxicity (50% of inhibitory concentration, IC50) of the test compounds.

Statistical analysis

Results are expressed as mean ± SEM. The statistical significance of differences between the experimental compounds was analyzed using the student t-test; differences were considered significant. The results were expressed in **half maximal inhibitory concentration IC50.**

RESULTS AND DISCUSSION

Table 1. % inhibition of cell proliferation and IC50 values of different cell lines

Cell line	Treatment concentration (g/ml)	% inhibition in cell proliferation (wt. Vehicle control)	IC50
1	0.7	26.28	1.886
	0.8	32.87	
	0.9	35.43	
	1	38.32	
	1.5	47.68	
	2	50.35	
	2.5	58.56	
2	0.7	9.74	1.4294
	0.8	22.39	
	0.9	37.04	
	1	38.11	
	1.5	58.06	
	2	69.07	
	2.5	74.82	
3	0.7	16.22	1.5195
	0.8	22.50	
	0.9	25.04	
	1	27.14	

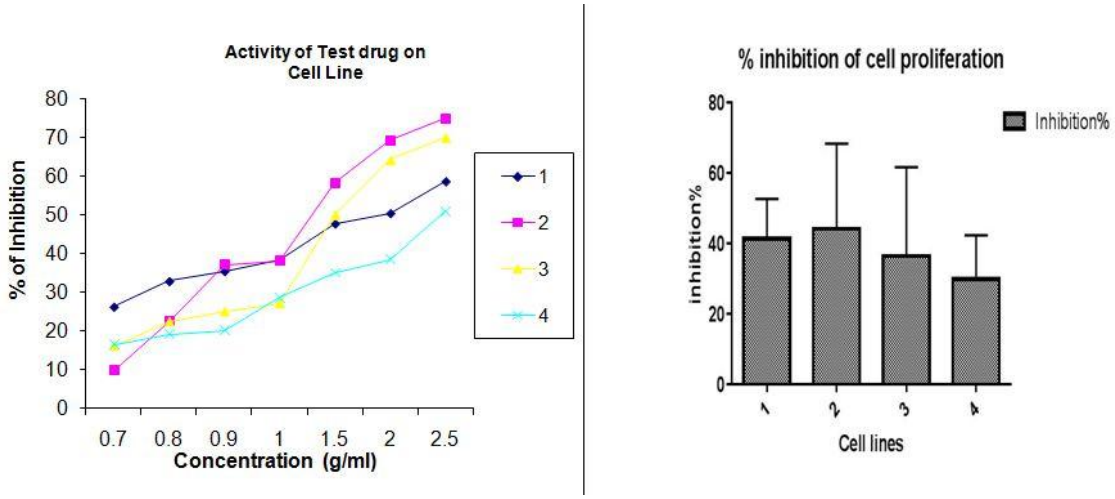
	1.5	49.87	
	2	63.98	
	2.5	69.75	
4	0.7	16.57	2.483
	0.8	19.22	
	0.9	20.27	
	1	28.60	
	1.5	35.05	
	2	38.47	
	2.5	50.83	

Table. 2. Impact of a 72 hrs, Test drug Exposure on the Viability of Cell Lines Evaluated for IC50 by MTT Assay

Cell line	IC50
1	1.886
2	**1.429**
3	1.519
4	2.483

The results obtained from quadruplicate MTT wells were used in calculations to determine the percentage of Inhibition and Half maximum inhibitory concentration (IC50) of test compounds. **The Test drug inhibits the cell proliferation even in its lower concentration also.**

Figure 1.1 % inhibition of cell proliferation in different cell lines.



In the four different cell lines with the concentration of 2.5g/ml, our test drug exhibits 58.56, **74.82,** 69.75, and 50.83 % of Inhibition respectively (Table. 1). Out of that, the maximum inhibition of cell proliferation is 74.82%. IC50 (g/ml) of the 4 different cell lines is 1.886, 1.429, 1.519 and 2.483 respectively (Table. 2). The Least concentration is 1.429 g/ml, where half of the proliferating cell would be destroyed in very lower concentration.

CONCLUSION

Therefore it is summarized that at differently treated concentrations, the trial drug significantly inhibits cell proliferation. Thus the test drug has proven effectiveness as a potent anti-tumour agent. When such a cost effective, herbal origin, less invasive and novel drug treatment is available, it is the abound duty of the pioneers of the Health care field to put more concern and efforts on such new inventions.

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