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ANTICANCER EFFECT OF UNDOPED ZINC OXIDE NANOPARTICLES AGAINST BREAST CANCER CELL LINE AND COMPARED WITH NORMAL FIBROBLAST CELL LINE

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ABSTRACT

Cancer is a common cause of death nationwide. Breast cancer is projected to be among the most common cancer and is the most frequent malignant neoplasm in women. Therefore, it is necessary to find novel therapeutic agents against cancer, which are biocompatible and cost effective. The development of nanotechnology has been a boon to mankind as its significance paved the way for several applications. In the medical aspects, applications of nanoparticles increased tremendously only when the biological approach for nanoparticle synthesis came into focus. Therefore, this study was designed to synthesize ZnO NPs using *Ipomoea carnea* flowers

and to evaluate potential in MCF-7 (Human Breast Adenocarcinoma) and L929 (Fibroblast) normal cells. Results of the present study concluded that the most potent undoped ZnO nanoparticles exhibit anticancer activity by inducing cell toxicity and apoptosis (morphology change) in MCF-7 cells and safer for L929 cells.

Keywords: ***Ipomoea carnea*** **flowers, MCF-7 cells, L929 cells, Cytotoxicity**

INTRODUCTION

Cancer is a common cause of death nationwide, with the number of new cases of cancer in the US was estimated to be ca. 1.7 million in 2016 **[1]**. Although the number of cancer deaths was estimated at more than half a million in the US in 2016, this number has dropped by ca. 23% since 1991. Health care for cancer patients requires extensive financial resources. For example, the health care costs for cancer patients in the US were ca. $156 billion in 2010, and are expected to increase further to $156 billion by 2022 **[2]**. Breast cancer is projected to be among the most common cancer **[2]** and is the most frequent malignant neoplasm in women **[3]**.

Many cancers initially respond to chemotherapy, and later they develop resistance **[4]**. Currently available chemopreventives and chemotherapeutic agents cause undesirable side effects **[5]** and therefore developing a biocompatible and cost effective method of treatment for cancer is indispensable. The development of nanotechnology has been a boon to mankind as its significance paved the way for several applications in therapeutics, catalysis, microelectronics, biosensing devices air and water purifiers, paints **[6]** and so forth.

The nanoparticles can be synthesized by physical, chemical, and biological methods. The physical methods are initially used to give a low yield. Chemical methods use various chemical agents to reduce metallic ions to nanoparticles. This comprises certain drawbacks as there will be use of toxic chemicals and generation of hazardous byproducts **[7]**. In the medical aspects, applications of nanoparticles increased tremendously only when the biological approach for nanoparticle synthesis came into focus. Therefore, this study was designed to synthesize ZnO NPs using *Ipomoea carnea* flowers and to evaluate potential in MCF-7 (Human Breast Adenocarcinoma) and L929 (Fibroblast) normal cell line.

MATERIALS AND METHOD

Preparation of plant extract

Fresh *Ipomoea carnea* flowers were cut and washed with water. The extraction

procedure was as follows: 20g of fresh flower was added to 100 ml of ethanol and soaked for 24 hours. The obtained extract was filtered using Whatman No.1 filter paper and the filtrate was collected and stored at room temperature for further usage

Synthesis of ZnO nanoparticles

For the preparation of pure ZnO nanoparticles, 100 ml of 0.1M Zinc acetate dihydrate (Zn $(CH_3COO)_2.2H_2O$) solution was added with 100 ml of *Ipomoea carnea* flower extract. The obtained yellow coloured solution was stirred constantly at room temperature for 3 hours. The colloidal particles obtained were dried in hot air oven at 85°C for one hour. The precipitate was calcinated at 350°C for 3 hours and the collected nanoparticles further investigated for *in vitro* anticancer activity.

***In vitro* cytotoxic effect determination by MTT assay**

MCF-7 (Human Breast Adenocarcinoma) and L929 (Fibroblast) normal cells was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco's modified Eagles medium, DMEM (Sigma aldrich, USA). *In vitro* cytotoxic effect of ZnO nanoparticles determination by MTT assay **[13]**. % Cytotoxicity using the following formulas:

% Cytotoxicity = 100 - [Abs (sample) /Abs (control)] x100.

% Cell Viability = [Abs (sample) /Abs (control)] x100.

RESULT AND DISCUSSION

The cytotoxicity of undoped ZnO nanoparticles against breast cancer and normal cells is summarized in Table 1. It was observed that the undoped ZnO nanoparticles showed promising anticancer activity toward the cell lines. Human breast adenocarcinoma (MCF-7) and mice fibroblast (L929) cell lines were used to evaluate the cytotoxicity of the undoped ZnO nanoparticles of five different concentrations (6.25, 12.25, 25, 50 and 100 µg/mL) and L929 cells were taken as normal cells. A significant decrease in cell viability and increased cytotoxicity were recorded with increasing concentration of the undoped ZnO nanoparticles by MTT assay. The L929 cells showed the least sensitivity to the undoped ZnO nanoparticles while MCF-7 cells had highest sensitivity. The IC_{50} values of undoped nanoparticles in MCF 7 and L929 were found to be 87.61 and 101.42 µg/mL respectively. The IC_{50} values of these cells revealed that the undoped ZnO nanoparticles had highest cytotoxicity effect for MCF-7 cells. The undoped ZnO

nanoparticles treated L929 cells are non-cytotoxic to normal breast cells.

Morphological examination

Figure 1 (a and b) shows the morphological changes occurred in MCF-7 and L929 cells upon treatment with different concentrations of undoped nanoparticles. Increasing the nanoparticles concentration (upto100µg/mL) resulted in a drastic change in the morphological characteristics of the tested cell lines, which was proportional to the applied concentration. Cells started to shrink and lose their capacity to adhere to the surface of the cultivation plate. Moreover, at the highest applied concentration, cells appeared rounded and were completely floated in comparison to the control morphology. The anticancer activity by inducing cell toxicity and apoptosis (morphology change) in MCF-7 cells and safer for L929 cells.

Cancer is an abnormal type of tissue growth in which the cells exhibit an uncontrolled division, relatively in an autonomous fashion, leading to a progressive increase in the number of dividing cell. *In vitro* cytotoxicity testing procedures reduces the use of laboratory animals and hence use of cultured tissues and cells have increased **[8]**.

The discovery and identification of new antitumor drug with low side effects on immune system has become an essential goal in many studies of immuno-therapies . Despite many efforts, multi drug resistance is still considered as a major drawback in chemotherapy of cancer which has been the subject of exhaustive experiments recently **[9]**. With this aim, many attentions have been paid to natural compounds in plants, marine organism and microorganisms. Many medically relevant nanoparticles were investigated for their cytotoxicity aspect. Undoped nanoparticles showed different degrees of *in vitro* cytotoxicity **[10]**.

Table 1: Effect of varying concentration of undoped nanoparticles on viability and cytotoxicity of MCF-7 (breast cancer) and normal L929 cell lines as determined by MTT assay

Activity	MCF 7 (Breast cancer cell line)					
	Cell control	6.25	12.25	25	50	100
Viability (%)	100	85.84	79.96	70.65	64.80	46.00
Cytotoxicity (%)	0	14.16	20.04	29.35	35.20	54.00
LC_{50} Value (µg/mL)	87.6127					
Activity	L929 (Normal Breast Cell line)					
	Cell control	6.25	12.25	25	50	100
Viability (%)	100	96.73	83.88	80.50	73.00	51.02
Cytotoxicity (%)	0	3.27	16.12	19.50	27.00	48.98
LC_{50} Value (µg/mL)	101.429					

% Cell viability = A 570 of treated cells / A 570 of control cells × 100%.
% Cell death = (Control OD –Sample OD)/Control OD x 100.

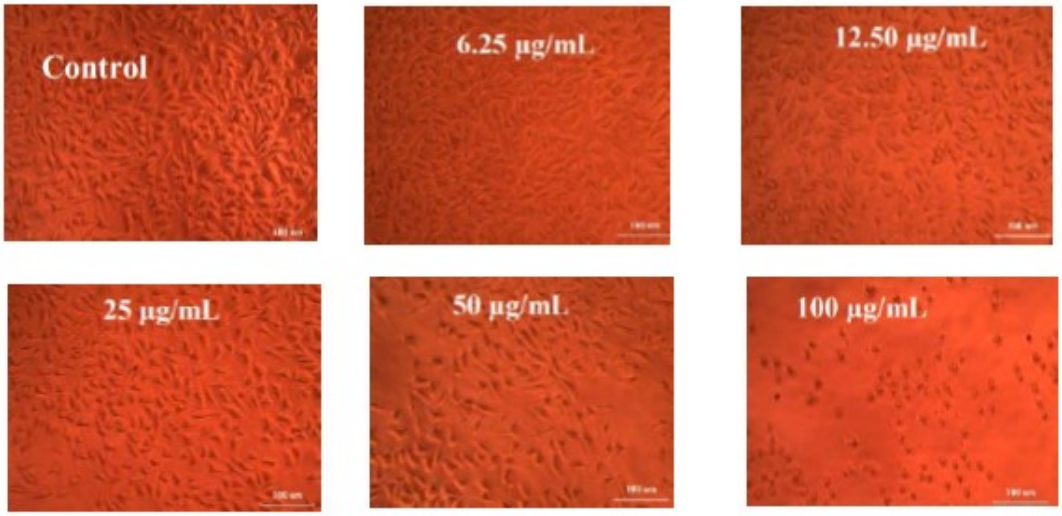

Figure. 1a: Effect of varying concentration of undoped ZnO nanoparticles on viability and cytotoxicity of L929 (Normal) cell lines as determined by MTT assay

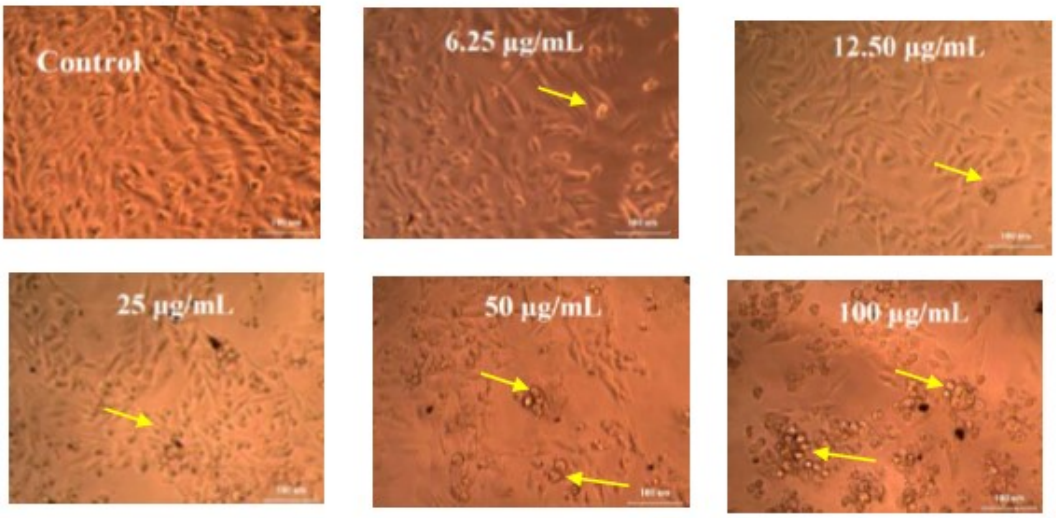

Figure. 1b: Effect of varying concentration of undoped ZnO nanoparticles on viability and cytotoxicity of MCF-7 (Breast cancer) cell lines as determined by MTT assay (Yellow arrows indicate representative apoptotic cells).

Breast cancer is the most common cancer in women worldwide. It is a type of cancer where cells in the breast divide and grow without normal control. The incidence of breast cancer has doubled during the past 30 years. Between 50 and 75 per cent of breast cancers begin in the ducts, 10 to 15 per cent begin in the lobules and a few begin in other

breast tissues **[11]**. Fortunately, the mortality rate from breast cancer has decreased in recent years with an increased emphasis on early detection and more effective treatments (Http://www.imaginis.com/general-information-on-breast-cancer/what-is-breast-cancer-2). Several commonly used herbs have been identified by the National Cancer Institute as possessing cancer-preventive properties **[12]**. Hence, the present study aims to investigate the undoped ZnO nanoparticles in a cheap simple method and evaluate the cytotoxic action of breast cancer cell line MCF-7 and normal cell line L929.

MCF-7 is a breast cancer cell line isolated in 1970 from a 69-year-old Caucasian woman. MCF-7 is the acronym of Michigan Cancer Foundation-7, referring to the institute in Detroit where the cell line was established in 1973 by Herbert Soule and co-workers **[13]**. The Michigan Cancer Foundation is now known as the Barbara Ann Karmanos Cancer Institute **[14]**.

Michigan Cancer Foundation-7 (MCF-7) is a human breast cancer cell line that was first isolated in 1970 from the malignant adenocarcinoma breast tissue of a 69-year old woman. MCF-7 cells are useful for in vitro breast cancer studies because the cell line has retained several ideal characteristics particular to the mammary epithelium. These include the ability for MCF-7 cell to process estrogen via estrogen receptors. MCF-7 cell is also sensitive to cytokeratin. When grown *in vitro*, the cell line is capable of forming domes and the epithelial like cells grow in monolayers. Growth can also be inhibited using tumor necrosis factor alpha (TNF alpha). Michigan Cancer Foundation-7 (MCF-7) is a human breast cancer cell line and useful for *in vitro* breast cancer studies because the cell line has retained several ideal characteristics particular to the mammary epithelium. These include the ability for MCF-7 cell to process estrogen via estrogen receptors **[15]**.

The parent of fibrosarcoma cell line L929 is derived from normal subcutaneous areolar and adipose tissue of a 100-day-old male C3H/An mouse, and they represent thus adult somatic-derived cells. Clone L929 was established (by the capillary technique for single cell isolation) from the 95th subculture generation of the parent strain. L929 cell was neoplastic and heterogeneous. The L929 cell exhibited adherent growth and heterogeneous in morphology, including spindle-like, epithelial-like, stellate, and round shape **[16]**.

The cell growth inhibition of undoped ZnO NPs tested against MCF-7 and L929 cell line at different concentrations (6.25, 12.25, 25, 50 and 100 µg/mL). The results of

the study observed that, as the concentrations increases there is an increase in the cell growth inhibition (Cytotoxicity) and decrease in cell viability. Present study agreement with Mona Orangi *et al*., **[17]** studies who reported that the methanolic subfractions of *Scrophularia oxysepala* induce apoptosis in MCF-7 and WEHI-164 cells in a dose-dependent manner and these fractions can thus be considered as a source of anticancer compounds. Furthermore, these subfractions are not cytotoxic against the L929 normal cell line, which is another advantage.

The morphological changes of the cell lines treated cells with various concentrations of the undoped ZnO nanoparticles were incubated for 24hr and compared with the untreated cells. Compared to control cells after the incubation period, morphology of undoped ZnO NPs treated cancer cells significantly changed. The undoped ZnO NPs treated cells appeared less uniform with the loss of membrane integrity, although still intact at lower concentrations. Whereas at higher concentrations, undoped ZnO NPs treated cells showed remarkable difference with the control group. Cells started to shrink and lose their capacity to adhere to the surface of the cultivation plate, condensation and aggregation of the nuclear chromatin, apoptosis. The change of morphological features were evident when compared to untreated cells **[18]**. They resulted in a drastic change in the morphological characteristics of the tested cell lines and were completely floated in comparison to the control morphology, which was proportional to the applied concentration.

CONCLUSION

In conclusion, the most potent undoped ZnO nanoparticles exhibits anticancer activity by inducing cell toxicity and apoptosis (morphology change) in MCF-7 cells and safer for L929 cells.

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