Synthesis Of Tamoxifen PLGA Encapsulated Nano Particles Using Alkyl Method And Anti-Cancer Activity In Breast Cancer Cell

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Abstract- *Tamoxifen (TMX) sold under the brand name Nolvadex. The medication that is used to prevent breast cancer in women and men. It has been used for Albright syndrome. In the present study Tamoxifen mediated PLGA coated nanoparticle was synthesized and Anti-cancer activity was performed.*

Keywords- Tamoxifen,Anti-cancer.

I. INTRODUCTION

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. The reason for many cancers is not known particularly those that occur during childhood, However there are many known cancer causes like lifestyle factors, use of tobacco, excess body weight, inherited genetic mutations, hormones, and immune conditions. The risk factors may act simultaneously or in sequence to initiate and/or promote cancer growth **[1].**

Tamoxifen has been used as a treatment for women who have been diagnosed with breast cancer for roughly four decades and has been approved as chemoprevention for over ten years. Although tamoxifen has been proven to be beneficial in preventing breast cancer in high-risk women, its use has not been widely embraced. To some extent, this is due to several of its side effects, including an increased risk of endometrial cancer and pulmonary embolism, but these serious side effects are rare. The risks and benefits of tamoxifen chemoprevention should be considered for each patient **[2].**

II. MATERIALS AND METHOD

PHASE I.
COLLECTION OF DRUG

TAMOXIFEN (10mg) were bought from medical shop in Coimbatore, Tamilnadu, India.

NANO PARTICLE COATING TAMOXIFEN DRUG

Tamoxifen citrate nanoparticles (TNPs) were prepared by a multiple-emulsion solvent evaporation method, with some modifications of the reported methods.15–17 The compositionsof the different formulations .

Tamoxifen citrate and subsequently PLGA (85:15) (~200 mg) were dis-solved in 0.5 mL of methanol and 1.5 mL of dichloromethane mixture (phase 1). PVA was dissolved in water (2.5% weight per volume [w/v]) (phase 2), and 0.5 mL of phase 2 was added drop-wise into phase 1 with homogenization at an optimized speed (22,500 rpm) using a high-speed homogenizer

The prepared primary emulsion was then added slowly into 75 ml of 1.5% (w/v) PVA solution (phase 3) with a continuous homogenization (22,500 rpm), which produced a secondary emulsion. The secondary emulsion was then placed on a magnetic stirrer and stirred overnight for evaporation to remove organic solvent and solidification of the particles.

The nanoparticles were then first separated by centrifugation at 5,000 rpm for 5 minutes to separate larger particles and then the supernatant was collected and recentrifuged at 15,000 rpm for 45 minutes. The solid particles, thus separated, were resuspended in Milli-Q water and centrifuged to wash the particles to remove the excess PVA attached on the surface of the nanoparticles and to remove the free drug. The washing was repeated three times. The separated nanoparticles were frozen at −40°C and lyophilized for 7–8 hours to obtain a solid product. The product obtained after lyophilization was kept overnight in a desiccators for the removal of the remaining moisture, then the

lyophilized samples were stored in an airtight container at 4°C **[3].**

ANTI-CANCER ACTIVITY IN BREAST CANCER CELL

Cell line

Evsa-t Cell line were used for anticancer activity and for MTT assay method to identify the percentage of cell viability. Cell line were initially procured from National centre for cell Science, Pune, India and has been maintained further in center for Bioscience and Nano science Research laboratory Echanari, Coimbatore, Tamil Nadu, India.

The cells were maintained in RPMI 1640 supplied with 10% fetal Bovine serum, with 2% glucose and 2% sodium carbonate, 20 mg of ampicillin (2mg/ml). Cells were cultures for 3-4 days before the assay in Co2 incubator [**4**].

MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)

Cell culture refers to culture derived from dissociated cells taken from the original tissue (primary cell culture). Cells are dispersed into a cell suspension which may then be cultured as a monolayer on a solid substrate, or as a suspension in the culture medium. Many animal cells can, with special care, be induced to grow outside of their organ or tissue of origin. Isolated cells, tissues or organs can be grown in plastic dishes when they are kept at defined temperatures using an incubator and supplemented with a medium containing cell nutrients and growth factors.

For the MTT assay cells were again seeded in 96-well plates and allowed to adhere for 24 hrs at 37^{O}C in 5% $CO_2$and 80-90% of humidity. Medium were replaced with serum free medium coating the sample in different concentration of sample (20 mgt to 80mg), and 20µl of MTT dye was added to each well , after slight mixing the plates were incubated for 4-8 hrs at 37^{o}C in Co_2 incubator[**5**].

The reaction mixture was then carefully taken out and formazan crystals were solubilized by adding 200 ml of DMSO to each well and mixed thoroughly. After 10 minutes the absorbance of purple color were read at 570 nm using 96 well plate ELISA reader[6] (Robonik , India) After taking reading the % of cell death were calculated by following formula.

Percentage of cell death= control absorbance reading –absorbance of treated/ control absorbance reading X100.

III. RESELTS AND DISCUSSION

NANO PARTICLE COATING TAMOXIFEN DRUG:

Figure: 1 Coating of drug.

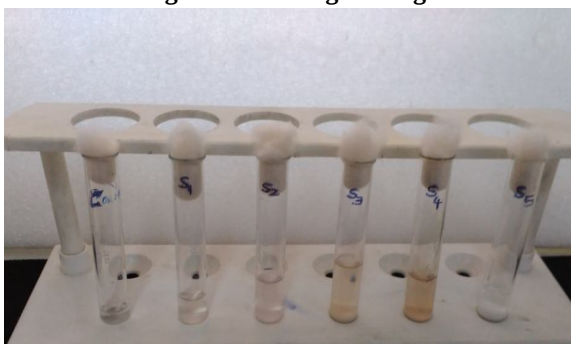


TABLE.1 Coating of drug

S.NO	CONCENTRATION OF SAMPLE (mg)	SAMPLE OBSEVANCE (nm)	OPTICAL DENSITY
3	60	260	0.084
4	80	260	0.097

The Table shows that best concentration in synthesis of Nano particle coated tamoxifen drug was 80mg (s4) was found to be 0.097mg and observed at 260nm.

The synthesized Nano composite is smaller than 100 nm and exhibits fluorescence emission band around 440 nm upon excitation with 340 nm wavelength. In the meantime, the nanocomposite was loaded with a chemotherapeutic drug, doxorubicin to evaluate the drug loading potential of synthesized nanocomposite. Moreover, the as-synthesized nanocomposite showed good osteogenic properties for bone tissue engineering and also exhibited excellent selectivity and sensitivity towards Fe3+ ions **[6].**

The synthesis of nono particle result indicates a effective enteric coating and delay the drug release, with 32% acryl ezee solution, is possible. The formulation developed can further be worked on. For identifying a best formulation for delayed release pellets of pantoprazole sodium[**7**]**.**

ANTI-CANCER ACTIVITY IN BREAST CANCER CELL

Figure:2 Anti-Cancer Activity
FIGURE.2a Control

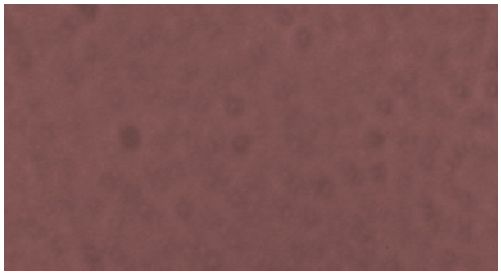
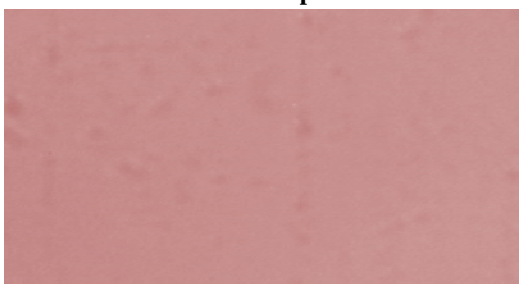


FIGURE:2b Sample loaded



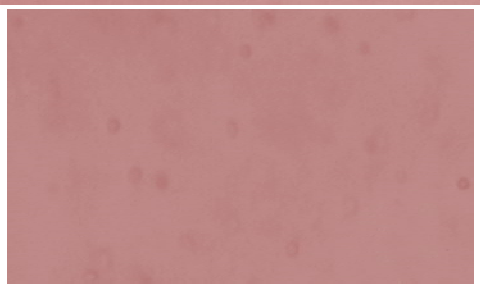


Figure 2 Indicates that the Anti-cancer activity by using breast cancer cell line against Nano particle coated Tamoxifen drug.

The % of cell death were calculated by following formula.

$$\text{Percentage of cell death} = \frac{\text{control absorbance reading} - \text{absorbance of treated}}{\text{control absorbance reading}} \times 100.$$

From the sample (s4) **17.54**% of cell death occurred in the breast cancer cell.

From the treated control **12.39%** of cell death occurred in the breast cancer cell.

From the above study the Nano particle coated drug loaded on breast cancer cell was found to be 17.54% of breast cancer cell death occurred. It increased the efficacy of the capsule.

In *in vitro* study was conducted to determine the apoptosis induced by tamoxifen (TAM) and TAM-loaded solid lipid nanoparticles (SLNs) in breast cancer cell lines, MCF-7 and MDA-MB231 cells. The effect of free drug and drug-loaded SLN on the cell lines was characterized by cell morphology and cell cycle distribution using phase contrast microscopy, nuclear morphology and flow cytometry, respectively. The results showed that TAM-loaded SLNs have an equally efficient cytotoxic activity against MCF-7 and MDA-MB231 cells, compared to free TAM, and the half maximal inhibitory concentration of TAM-loaded SLNs was generally lower than that of free TAM. In the presence of TAM and TAM-loaded SLN, the viability of the both cells diminishes and the cancer cell loses their normal morphological characteristics, detach, aggregates and later develops apoptotic bodies. Flow cytometry analysis showed that TAM-loaded SLN like the free TAM caused a dose- and time-dependent apoptosis without cell cycle arrest of human breast cancer cells. Therefore, TAM-loaded SLN has great potential in human medicine for the treatment of breast cancers **[8].**

Tamoxifen (TAM) is a widely used drug in the prophylaxis and treatment of breast cancer. TAM is metabolized to the more active 4-hydroxytamoxifen (4-OH-TAM) and endoxifen by cytochrome P450 (CYP) mainly CYP2D6 and CYP3A4 enzymes. Due to the genetic polymorphisms in CYP2D6 genes, high variation in the clinical outcomes of TAM treatment is observed among women of different populations. To address this issue, novel TAM analogues with possible altered activation pathways were synthesized. These analogues were tested for their anti proliferative action on MCF-7 breast cancer cell lines as well as their binding affinity for estrogen receptor (ER) ER-α and ER-β receptors. These entire novel compounds showed better anti proliferative activity than did TAM on the MCF-7 cells. Moreover, compound 10 exhibited a half maximal growth inhibition (GI50) that was 1000 times more potent than that of TAM (GI50 < 0.005 μm VS 1.58 μm, respectively). Along with a broad spectrum activity on various cancer cell lines, all the TAM analogues showed considerable activity on the ER-negative breast cancer cell line **[9].**

The drug in the dialysis bag was released through porous membrane in which the drug releasing capacity into water was 48 %. MCF – 7 cell lines were grown properly in DMEM in a CO2 incubator. Finally, readings from the ELISA reader showed high values in 10 μl concentration of the sample. Cell death occurred in 10μl sample of oyster shell chitosan coated with tamoxifen drug was 59.41 % **[10].**

The results showed that the ζ-potential of F@Tyr@TMX NPs was about − 12.8 mV and the average size was 22.19 ± 3.58 [mean ± SD (n = 50)] nm. The loading capacity of 11.34 ± 0.09% and encapsulation efficiency of 51.21 ± 0.41%. Additionally, hemolysis test and MTT assays on HEK-293 were performed for determination of

biocompatibility of F@Tyr@TMX NPs. Finally, the anticancer activity of F@Tyr@TMX NPs studied on MCF-7 breast cancer cell lines. The results indicate that these as prepared magnetic nano particles are suitable for delivery of TMX and even other hydrophobic drugs **[11].**

Chitosan is a multifunctional polymer with many valuable applications. In the present study, it was found that chitosan has excellent biological properties such as biodegradability and immunological, antibacterial and antimicrobial activity. Also, drug coated chitosan has wide applications in anti cancer activity **[12].**

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