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To Combat Cancer Cell Lines, the Development, and Evaluation of Lycopene-Co-Loaded TAMOXIFEN Nanoparticles

Puja Rani[1] and Neeraj Sethi[2*]
[1] Department of Molecular Biology and Biotechnology, CCS HAU, Hisar Haryana, India
[2] Department of Biotechnology, Om sterling Global University, Hisar, Haryana, India
pujavijay44@gmail.com
20neerajsethi@gmail.com
*Corresponding author
Dr Neeraj Sethi
Assistant Professor Department of Biotechnology,Om Sterling Global UniversityHisar, Haryana, India

Abstract
One common issue with chemotherapy-based cancer treatments is drug resistance. Plants that can fight cancer can effectively improve their secondary metabolites at the nanoscale. The chemical formula for lycopene is $C_{40}H_{56}$. This tetraterpene and beta-carotene, which is used as an anti-wrinkle ingredient in cosmetics, can inhibit the growth of cancer cells. The selective estrogen receptor modulator tamoxifen is used to treat estrogen receptor-positive breast cancer, lower the risk of invasive breast cancer after surgery, or lower the risk of breast cancer in women who are at high risk. Oil-in-oil (O/O) emulsion solvent evaporation techniques were used to create Lycopene and TAM-loaded Eudragit nanoparticles (LTENPs), which have been shown to increase bioavailability and exhibit a synergistic impact. For LTENPs, the zeta potential—a measurement of a nanoparticle's relative stability—was discovered to be -45.1 mV. Lycopene and TAM were found to have percentage encapsulation efficiency values of 72% and 75%, respectively. TEM indicates that the particles in the LTENPs range in size from 26 to 34 nm. The prolonged release and much greater antioxidant and anti-cancer properties of the LTENPs were in contrast to those of free Lycopene and TAM particles alone. The in vitro investigations demonstrated the combination's strong anticancer potential by showing that it dramatically suppressed the growth of MCF-7 cell lines when compared to Lycopene, TAM, and Eudragit alone.

Keywords Eudragit, Lycopene, Tamoxifen, Anti-cancer, Nanoparticles

Introduction

The use of nanotechnology in cancer treatment creates a whole new area of pharmaceutical research. Drug molecules at the nanoscale offer brand-new, very potent nanoplatforms laden with bioactives to combat cancer cells. Several malignancies are commonly treated with chemotherapy [1]. On the other hand, these traditional treatment methods are harmful, especially when it comes to drug resistance or tolerance [2, 3]. These days, life-saving therapies enabled by nanotechnology are much sought for. At modest concentrations, bioactive substances exhibiting enhanced antioxidant and anticancer properties at the nanometric scale also demonstrate good absorption. Research on the possible anticancer effects of several plant secondary metabolites has been promoted by the National Institute of Cancer in the United States [4, 5]. The literature has demonstrated the cytotoxic effects of phytochemicals, such as lycopene.

According to recent research, nanoparticle morphological characteristics are crucial for achieving anticancer effectiveness with minimal side effects [6,7]. The pharmacokinetic and pharmacodynamic properties of nanoparticles are influenced by their size, shape, and surface characteristics. Lycopene triterpenoid includes both free carboxylic acid and the aglycone of saponins [8]. In human breast cancer cell lines (Michigan Cancer Foundation-7), it modifies the glucocorticoid receptor and lowers the levels of Bcl2, an anti-apoptotic protein [9,

10]. Lycopene is commonly utilized in pharmaceutical formulations that may be applied locally and consumed orally due to its low degree of toxicity and natural origin. Biodegradability and biocompatibility are the best qualities to consider when selecting an agent for drug encapsulation [13, 14]. Studies on Eudragit, a novel encapsulating material employed for therapeutic activity, have demonstrated that encapsulation efficiency rises proportionately with gum concentration [15].

Selective estrogen receptor modulators (SERMs), such as tamoxifen, are one kind of hormonal treatment. The medication attaches itself to certain proteins called hormone receptors on breast cancer cells. The drug prevents the cancer from getting the hormones it needs to grow and spread once it enters the cells. [16, 17]. Tamoxifen has become one of the most often recommended treatments for breast cancer since the Food and Drug Administration (FDA) approved it in 1998. It is a nonpolar chemical that functions primarily through two mechanisms: Two mechanisms of action for 17-estradiol (E2): (1) fighting with E2 at the receptor site to prevent E2 from producing breast cancer; and (2) binding DNA after metabolic activation and starting carcinogenesis [18, 19]. Because of its structural affinity for the cell membrane and the use of adjuvant hormone therapy, it has anticancer efficaciousness [20].

The current effort aimed to create a Lycopene co-delivered TAM polymeric nanoplatform to increase bioavailability, promote synergism, and decrease side effects. The polymeric nanoparticles' prolonged release and improved therapeutic efficacy at low dosages are made possible by the encapsulation of TAM and lycopene.

Materials and Methods

Materials

The French company MP Biomedicals, LLC sold gum Eudragit to the buyer. We bought >90% of the lycopene and tamoxifen from Sigma Aldrich in India. The National Centre for Cell Science (NCCS), situated in Pune, provided the cell line MCF-7-Human breast cancer cells. The substances utilized in this investigation were all analytical.

Lycopene & TAM loaded Eudragit nanoparticles preparation (LTENPs)

Through solvent evaporation in an oil-in-oil emulsion, the LTENPs were created [21]. 250 cc of propanol was used to dissolve 200 milligrams of Eudragit, 90 mg of lycopene, and 30 mg of TAM. After adding 45 mg of magnesium stearate to the previously described solution, it was magnetically agitated for 45 minutes at 1200 rpm. The mixture was centrifuged for 40 minutes at 8000 rpm at 4 C and constantly agitated for 8 hours at 1200 rpm at 40 C before 80 cc of liquid paraffin oil was progressively added. After the pellet was separated, it was put in a cryoprotectant (5% w/v D-Mannitol) and freeze-dried.

Characterization of synthesized LTENPs

The optimized nanoformulations of LTENPs was evaluated for average size and size distribution (polydispersity index) using the Zetasizer Nano ZS-90. The unbound medication was extracted from the supernatant, which was centrifuged for 40 minutes at 10,000 rpm (4 °C), and subjected to HPLC analysis. Total Drug-Unbound Drug/Total Drug was the encapsulation efficiency; the percent entrapment efficiency is 100.

Using a transmission electron microscope, the optimized batch of LTENPs' dimensions and form were examined. The Fourier transform infrared spectrophotometer was utilized to assess the FTIR analysis of LTENPs, TAM, Lycopene, and Eudragit in the 4500–500 cm–1 range.

In vitro release profile of LTENPs

The release profile was examined using the dialysis sac approach. 10 mg of Lycopene co-delivered TAM-loaded Eudragit nanoparticles were placed in a dialysis sac, and then 10 ml of water, 25% ethanol, 0.1 M phosphate buffer, and saline pH 7.4 were added. After that, the mixture was constantly agitated at 100 rpm while maintaining a 37 °C temperature [21]. At regular intervals of 1, 2, 3, 6, 12, and 24 hours, one-milliliter samples were collected, analyzed, and evaluated using HPLC Lycopene (219.9 nm, 5.58 min.) and TAM (256 nm, 7.01 min.).

Antioxidant activity

The DPPH test was used to assess the antioxidant capacity of lycopene, TAM, Eudragit, and LTENPs [22]. The free radical DPPH was evenly blended with 3.9 mg/100 ml of methanol. To test the inhibition of DPPH in duplicate runs, pure lycopene, TAM, Eudragit, and LTENPs were incubated with DPPH for 30 minutes in the dark. Utilizing a UV spectrophotometer, the absorption peak at 517 nm was measured. Pure Lycopene and TAM served as the positive controls and blank dammar NPs served as the negative control. Using the following formula, the percentage of DPPH inhibition by pure Lycopene, TAM, and LTENPs was determined:

$$\text{Percent antioxidant activity} = \frac{\text{Absorbance of control- Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

In-vitro assay for cytotoxic activity (MTT assay)

Cell lines, both malignant and healthy, were grown in media supplemented with 10% inactivated FBS solution, 80 l/ml of streptomycin, and 80 l/ml of penicillin. The cultures were maintained at a temperature of 370 °C and in an environment of 5% CO2 [21, 23]. After reaching 75% confluence, subcultures were performed using 0.25% trypsin solutions under sterile laminar airflow. Cell seeding was investigated in 96-well plates with 104 cells in each well. Each cell line's growth characteristic pattern was assessed to determine its density. Following an 8-hour incubation period, the wells were treated with varying concentrations of LTENPs (0.1-500 g/ml) and Lycopene for three days (in triplicate).

Following the growth of the cells, 3 liters of MTT solution (5 mg/ml) were added to the medium, and the combination was incubated for 3 hours. Based on the mitochondrial conversion of 3-(4, 5-dimethylthiazol-2-yl) 2, 5 diphenyltetrazolium bromide (MTT) to Formazan crystals, the proportion of metabolically active cells was compared to untreated controls. The formazan crystals were dissolved in DMSO, and the mixture's absorbance at 570 nm was then measured. To assess the anticancer efficaciousness of OTDNPs against the human breast cancer cell line, the MTT test utilized pure OA and TAM as the gold standard. MCF-7. The following process was used to quantify the percentages of cytotoxicity (2) and inhibition of cell growth (1).

$$\textbf{\%viability} = (A_{Tr} - A_{Bl}) / (A_{Ct} - A_{Bl}) \times 100 \quad \ldots\ldots\ldots (1)$$

Where A_{Tr} = Absorbance for treated cells (drug); A_{Bl} = Absorbance for blank

A_{Ct} = Absorbance for control (untreated)

$$\textbf{\%cytotoxicity} = 100 - \text{Percent cell survival (\%)} \quad \ldots\ldots\ldots (2)$$

Results and Discussion

Particle Size and Zeta Potential

The zeta potential and particle size of the LTENPs were investigated. It was found that the prepared Nano formulation had a zeta potential of -45.1 mV and a rather constant size of 198.6 nm for LTENPs (Figs. 1 and 2).

Encapsulation efficiency

The technique used, the chemical makeup of the encapsulating materials, and the dielectric constant of the medium used to form nanoparticles all have an impact on the percentage encapsulation efficiency number [21, 24]. Lycopene's and TAM's percentages of bound drug, determined by HPLC examination of supernatant containing unbound drug, were 70% and 76%, respectively (Fig. 5). TAM and lycopene are non-polar hydrophobic substances. In a gum solution based on propanol, they dissolve rather easily. Because Eudragit is hydrophobic, it has a strong affinity for TAM and lycopene. Because of their similar natural affinity, the drug was better contained in Eudragit.

Morphological characterization of* LTENPs *by TEM

Transmission electron microscopy revealed that the LTENPs were segregated, consistently spherical, and ranged in size from 35 to 64 nm (Figure 3). Experimental research indicates that the nanoparticles analyzed by TEM and PSA have different sizes. This is because, while TEM functions on the premise that a particle's size will decrease in a

solitary, dry atmosphere, PSA operates under the idea that the ions of nanoparticles are fluidized around them. The size of nanoscale particles affects how quickly drugs are released [25, 26]. The concentrations at which NPs are delivered to different human organs vary depending on their size and form. The shape and size of nanoparticles affect their relative stability, biocompatibility with the body's environment, and capacity to cross cell membranes [26]. Compared to larger nanoparticles, smaller ones are retained in the systemic circulation for a longer amount of time [27].

FTIR Analysis of Drug Samples

The interaction is investigated [27] and the nanoencapsulation of bioactive materials is assessed [28] using FTIR spectroscopy. Figure 4 displays the FTIR spectra of the pure medications LTENPs, TAM, Eudragit, and lycopene. In the Lycopene FTIR spectrum, the terminal -CH3 groups' absorption peak bands were 3000.0 cm-1 and 2401 cm-1, respectively, and the -OH group's peak band was 3412 cm-1. The FTIR spectra of TAM in Figure 4B showed the characteristic absorption band for =C-H stretching at 2876 cm-1 and for C=C ring stretching at 1600 cm-1. -NH2 structure was indicated by wave number values at 1501 cm-1 and 3401 cm-1. The FTIR analysis of Eudragit is displayed in Figure 4 C. The peak at 3412 cm-1 corresponds to the -OH stretch, whereas the peak at 2900 cm-1 represents the aliphatic -CH stretch. The FTIR spectra of LTENPs are displayed in Figure 4D. The spectra display wave numbers 3409 cm1, 1400 cm1, 2458 cm1, and 2901 cm1. This is the location of the IR stretching vibration zones for functional groups such as OH, C=C Stretch, CH3 Stretch, and C-H Stretch. Among other weak physical bindings, the combination of the OH in Eudragit and the C-H in the medication produces dipole-dipole interactions, hydrogen bonds, and weak Van der Waals forces. The FTIR spectra of the medicines and excipients displayed distinct peaks. The bands' shift and the peak intensity's decline suggested a physical interaction between TAM, Lycopene, and Eudragit.

In-vitro drug release

Nanoparticle cages release beneficial chemicals continuously, shielding them from oxidation and fast metabolism [29]. The in vitro drug release properties of LTENPs, TAM, and lycopene are shown in Figure 5. Eudragit was able to entrap the medication very well and efficiently, resulting in LTENPs that showed sustained drug release. Ninety percent of pure Lycopene and ninety-five percent of pure TAM were released in under five hours, according to in vitro drug release data. An hour following dosage, the LTENPs produced 16% lycopene and 17.81% TAM. Within 24 hours, 75% of Lycopene and 84% of TAM were released by LTENPs. Since TAM and Lycopene are hydrophobic (nonpolar), LTENPs' total drug release demonstrated Lycopene and TAM's sustained release properties throughout time. Eudragit also forms a dense matrix with a thick, robust wall surrounding the Lycopene and TAM particles to ensure their continuous release.

Anti-oxidant activity

The DPPH analysis technique is used to quantitatively assess the antioxidant activity/potency of encapsulated substances [31]. When light is absorbed, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) stable free radical exhibits excitation and emits a deep violet color. The molecule is made up of unpaired electrons that are dispersed erratically. It shows absorbance at 517 nm [32]. The violet color of a DPPH solution vanishes when it is evenly combined with a molecule that can donate (oxidize) a hydrogen atom. Antioxidant qualities of TAM and lycopene are widely recognized. The violet color of DPPH changed to a pale yellow when the antioxidant molecules Lycopene and TAM were dissolved in it. This produced a stable, non-radical version of DPPH. As a result, the absorption band shrank. The release of labile hydrogen atoms from Lycopene and TAM can inhibit DPPH. During dark incubation, the large surface area and the small nanometric dimension of the LTENPs prevented oxidation of Lycopene and TAM, resulting in a higher percentage of DPPH inhibition than Lycopene and TAM alone.

Anti-cancer activity

The increased surface area of the nanoparticles shown in Figure 6 significantly increased their enhanced cytotoxic efficacy and potent anticancer properties [33]. Because of their improved vascular penetration and retention effect, nanoparticles in the 100 nm range are more effective at infiltrating cancer cells [34]. Therefore, the size of NPs affects how effective they are against cancer cell lines [35, 36]. Particles at the nanoscale are better able to penetrate

cancer cells. The anti-cancer action of LTENPs was shown to be more potent in the current investigation than that of pure active medication alone. In an in vitro test, the combination of lycopene, TAM, and Eudragit reduced MCF-7 growth more effectively than the individual medications. The data additionally displays the produced nanoparticles' IC50 values (g/ml). With an IC50 of 3.94 g/ml, LTENPs demonstrated a strong anticancer effect on the cancer cell line MCF-7 when examined under an optical microscope in comparison to the conventional medicines TAM and Lycopene alone. The synergistic interaction between TAM and Lycopene in these medications was the main cause of the powerful anticancer activity of LTENPs.

Conclusions

Significant breakthroughs in the medication formulation business have made a wide range of illnesses successfully treatable using medicines based on nanotechnology. Systems for delivering nanoparticles with larger payloads, such as dual drug delivery to provide medicines and secondary metabolites with synergistic effects at therapeutic size, are one use of nano-drug delivery. The primary disadvantages of these therapeutic molecules, despite the enormous number of anticancer pharmaceutical compounds that have been found, are their low bioavailability, short half-lives in the body, and potential for tolerance and resistance. Increasing solubility in water, bioavailability to blood from the intestine, therapeutic potential to kill cancer cell lines, and dosage reduction all depend on creating novel nanoformulations with more effective excipients. The unique nanoformulations developed in this work, which comprises Lycopene acid co-delivered TAM-loaded Eudragit nanoparticles, have shown promise as a chemical that can effectively fight cancer because of its synergistic antioxidant and anticancer properties.

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Figure 1. PSA image of LTENPs

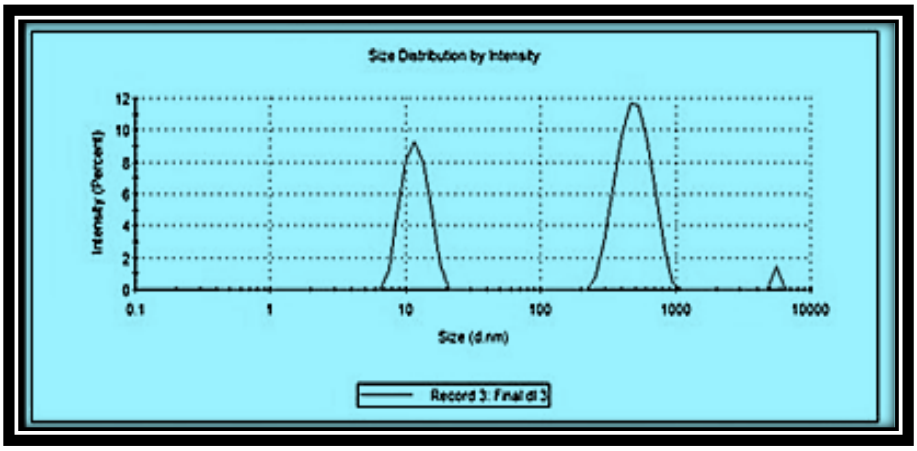

Figure 2. Zeta potential of LTENPs

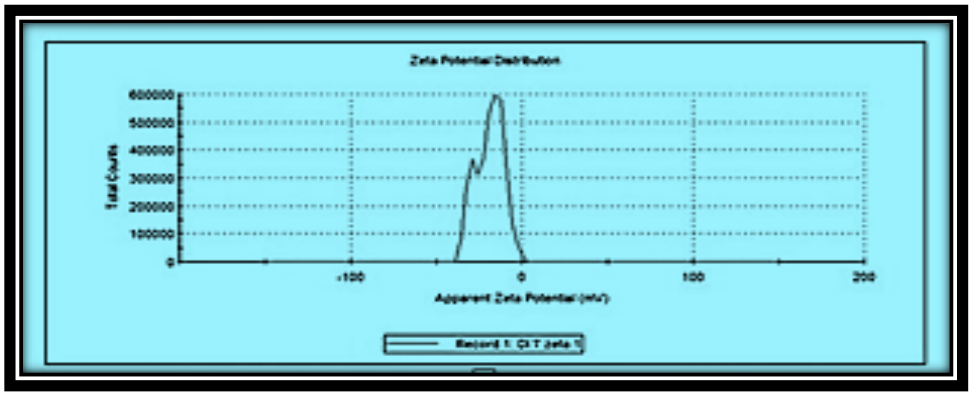

Figure 3: TEM image of LTENPs

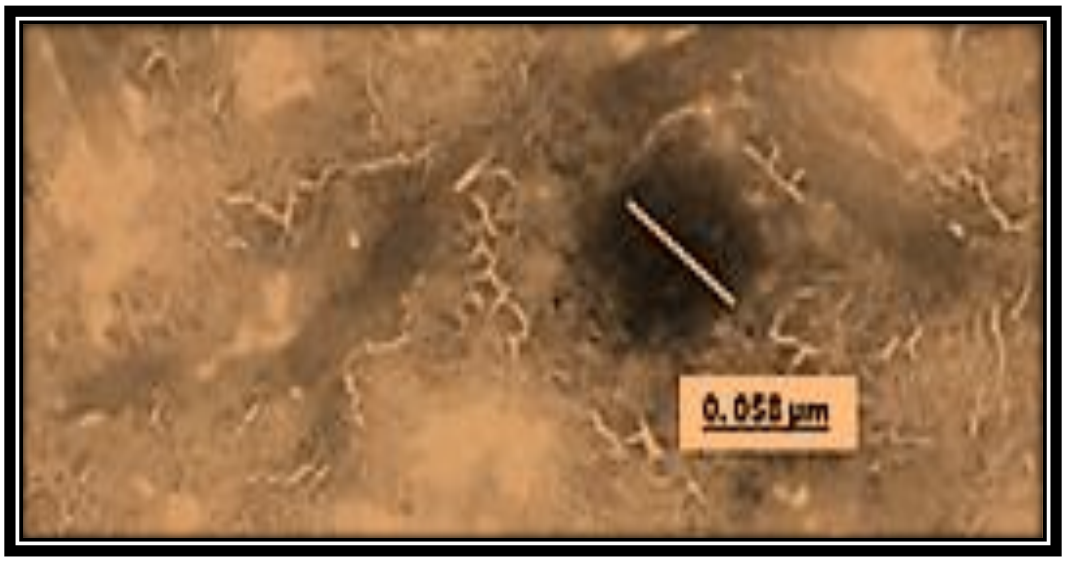

Figure 4. The FTIR spectra of pure drug (A) Lycopene, (B) TAM, (C) Eudragit, (D) LTENPs

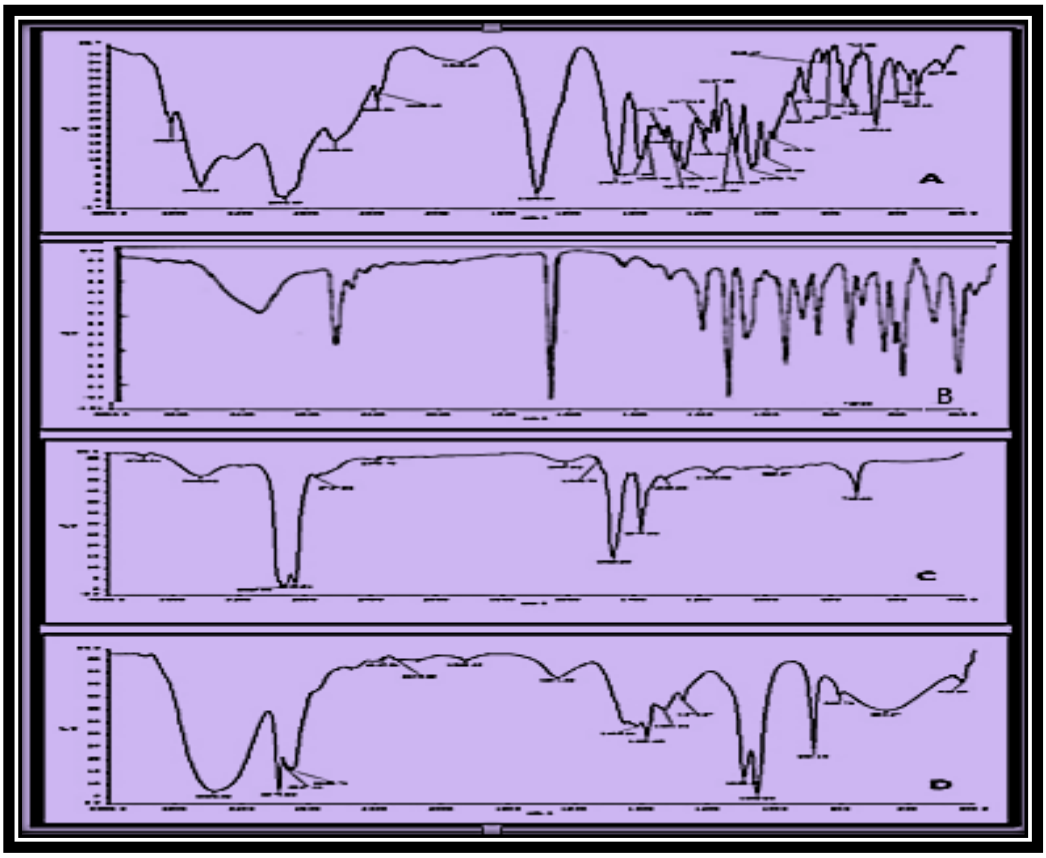

Figure 5. In-vitro release study

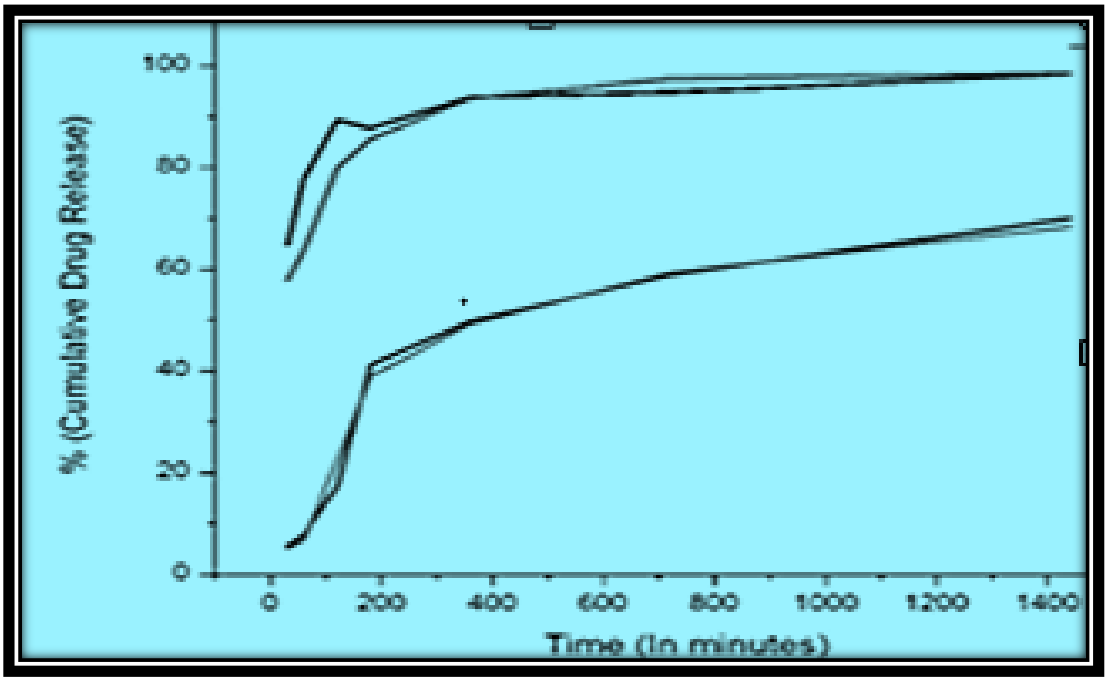

Figure 6. Optical microscope images of Cytotoxic effect of Lycopene (B1), TAM (F1) and LTENPs (N1), MCF-7 cell lines after 24 h.