INVITRO SCREENING OF DOCETAXEL DUTASTERIDE NANOSTRUCTURED LIPID CARRIERS ON PROSTATE CANCER CELL LINES.

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Abstract

Docetaxel (DTX) and dutasteride (DTE) nanostructured lipid carrier (D/D-NLC) was constructed to exert anticancer effects against Prostate cancer cell lines.(D/D-NLC) were prepared using the Melt-emulsification technique and high pressure homogenization. D/D-NLC showed homogeneous particle size (< 206.4 nm) with zeta potential varying from -23.3 to -38.6 mV. DTX DTE was successfully encapsulated in the NLCs: encapsulation efficiency (> 98%); drug loading (8 − 18%). All NLC formulations were stable for 5 weeks under the storage conditions at 4 °C. D/D-NLC loaded formulations assayed cytotoxicity by MTT assay. The assay measures the cell proliferation rate and conversely when metabolic events leads to apoptosis or necrosis, there was the reduction in cell viability.

Keywords: Synergistic, Anticancer, prostate cancer, Nano-lipid carrier, Drug evaluation,Growth inhibition

INTRODUCTION:-

Most frequently diagnosed malignancy and the second most common cause of cancer-related death in elderly men in the world wide because of prostate cancer.[1] In many cancer cases chronic inflammation is a potential mediator in the development of prostate cancer.[2] According to World Health Organization report Prostate cancer (PCa) is among the most prevalent cancers worldwide. In 2020, it was estimated to affect 1.4 million men and to have caused 375,304 deaths worldwide[3]The mechanisms pertaining to the initiation and progression of prostate cancer is largely unknown. One of the reasons that the progress of the work has been slow which is due to the lack of suitable *invivo* models. Although there are a number of *invivo* carcinogenesis models, they are based on single sex hormone, testosterone, or a combination of testosterone and estrogen[4,5]

For cancer treatment chemotherapy and radiation therapy used but they have various side effects to decreased the side effects of cancer chemotherapy various targeted nanocarrier systems using liposomes, nanoparticles, drug–polymer conjugates, and micelles have improved the

effectiveness[6]Various advantageous properties such as a high hydrophobic drug loading capacity, controlled release ability, and biocompatibility shown nanocarrier systems, nanostructured lipid carriers (NLCs) [7,8] Further, for prolonged circulation in vivo and target-specific delivery, the surface of NLCs can be functionalized with various targeting ligands [9,10]

Here docetaxel (DTX) and dutasteride (DTE) using a nanostructured lipid carrier (D/D-NLC) was constructed to exert anticancer effects against Prostate cancer cell lines.(D/D-NLC) were prepared using the Melt-emulsification technique and high pressure homogenization D/D-NLC showed homogeneous particle size (< 206.4 nm) with zeta potential varying from -23.3 to -38.6 mV. DTX DTE was successfully encapsulated in the NLCs: encapsulation efficiency (> 98%); drug loading (8 − 18%). All NLC formulations were stable for 5 weeks under the storage conditions at 4 °C. D/D-NLC loaded formulations assayed cytotoxicity by MTT assay

PC3 cells are a type of human prostate cancer cell line that was first isolated from a bone metastasis of a patient with advanced prostate cancer in 1979. PC3 cells are commonly used as a model system for studying prostate cancer and evaluating potential therapies.

Material

1. (D/D-NLC)

docetaxel (DTX) and dutasteride (DTE) nanostructured lipid carrier (D/D-NLC) was prepared using the Melt-emulsification technique and high pressure homogenization. Particle size 99.3 nm used for assay

2. PC3 Human prostate cancer cell line, NCCS Pune
3. Cell culture media – DMEM medium with 10% Foetal Bovine Serum (FBS), MP Biomedicals, Germany
4. 1X Dulbecco's Phosphate Buffered Saline (DPBS), 0.25% Trypsin-EDTA solution, MTT reagent, were all purchased from MP Biomedicals, Germany
5. Dimethyl Sulfoxide (DMSO), cell culture grade, Merck, Germany
6. Cell culture treated T-25 flasks from Biolite, Thermo Fisher Scientific Inc., USA.
7. 10mL serological pipettes and 96-well plates from Nunc, Thermo Fisher Scientific Inc., USA.
8. 5mL, 2mL and 1.5mL tubes, Tarsons, India.
9. Microscope - XDFL series, Sunny Instruments, China
10. Analysis Software – ImageJ (Fiji) software V1.53j

Method

Cells cultured in T-25 flasks were trypsinized and aspirated into a 5mL centrifuge tube. Cell pellet was obtained by centrifugation at 300 x g.The cell count was adjusted, using DMEM-HG medium, such that 200µl of suspension contained approximately 10,000 cells. To each well of the 96 well microtitre plate, 200µl of the cell suspension was added and the plate was incubated at 37^{o}C and 5% CO_2 atmosphere for 24 h.After 24 h, the spent medium was aspirated. 200µl of different test concentrations of test drugs were added to the respective wells. The plate was then incubated at 37^{o}C and 5% CO_2 atmosphere for 24 h. The plate was removed from the incubator and the drug containing media was aspirated. 200µl of

medium containing 10% MTT reagent was then added to each well to get a final concentration of 0.5mg/mL and the plate was incubated at 37^{o}C and 5% CO_2 atmosphere for 3 h.The culture medium was removed completely without disturbing the crystals formed. Then 100µl of solubilisation solution (DMSO) was added and the plate was gently shaken in a gyratory shaker to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm and also at 630 nm. The percentage growth inhibition was calculated, after subtracting the background and the blank, and concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) was generated from the dose-response curve for the cell line.[11,12,13]

Results and Discussion

Dose-response curve for the cell line it was found that 10% v/v showed 18.69% cell viability as compared to untreated 100 % cell viability. The reduction of tetrazolium salts is now widely accepted as a reliable way to examine cell proliferation. The yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means. The assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability.

From the observations Effect of D/D-NLC on PC-3 was evaluated using MTT method (Figure 1; Figure 2, , Figure 3, Table 1)**.** It was found that D/D-NLC showed reduced cell viability there further *invitro* screening models can be used to confirm the findings.

Table No:- 1 MTT Data Analysis- PC3 cell line vs D/D-NLC

			Test concentration %v/v				
	Blank	**Untreated**	**0.001**	**0.01**	**0.1**	**1**	**10**
Reading 1	0.004	0.761	0.582	0.574	0.472	0.307	0.155
Reading 2	0.002	0.799	0.543	0.522	0.485	0.333	0.178
Reading 3	0.005	0.778	0.589	0.514	0.47	0.316	0.113
Mean OD	0.004	0.779	0.571	0.537	0.476	0.319	0.149
Mean OD-Mean Blank		0.7757	0.5677	0.5330	0.4720	0.3150	0.1450
Standard deviation		0.0190	0.0248	0.0322222226	0.0081	0.0132	0.0330
Standard error		0.0110	0.0143	0.0188	0.0047	0.0076	0.0190
% Standard error		1.4168	1.8449	2.4249	0.6062	0.9828	2.4533
% Viability		100	73.18	68.72	60.85	40.61	18.69
IC50= 0.15 %v/v							

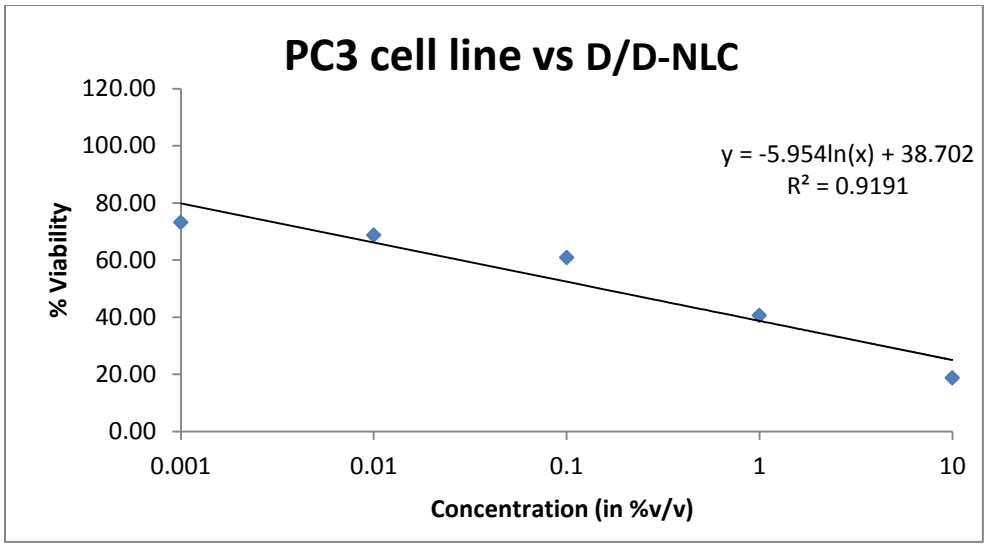

Figure No:1 MTT cell viability assay of D/D-NLC

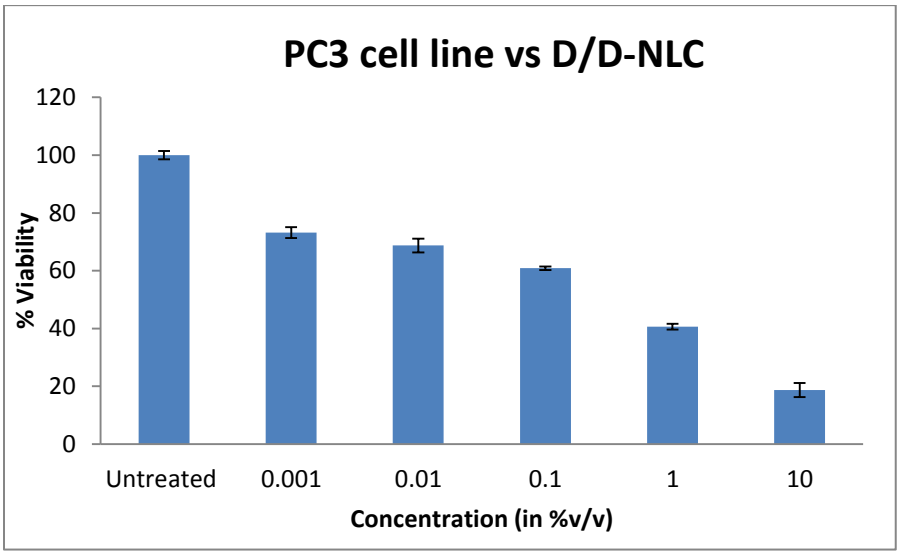

Figure No:2 MTT cell viability assay of D/D-NLC

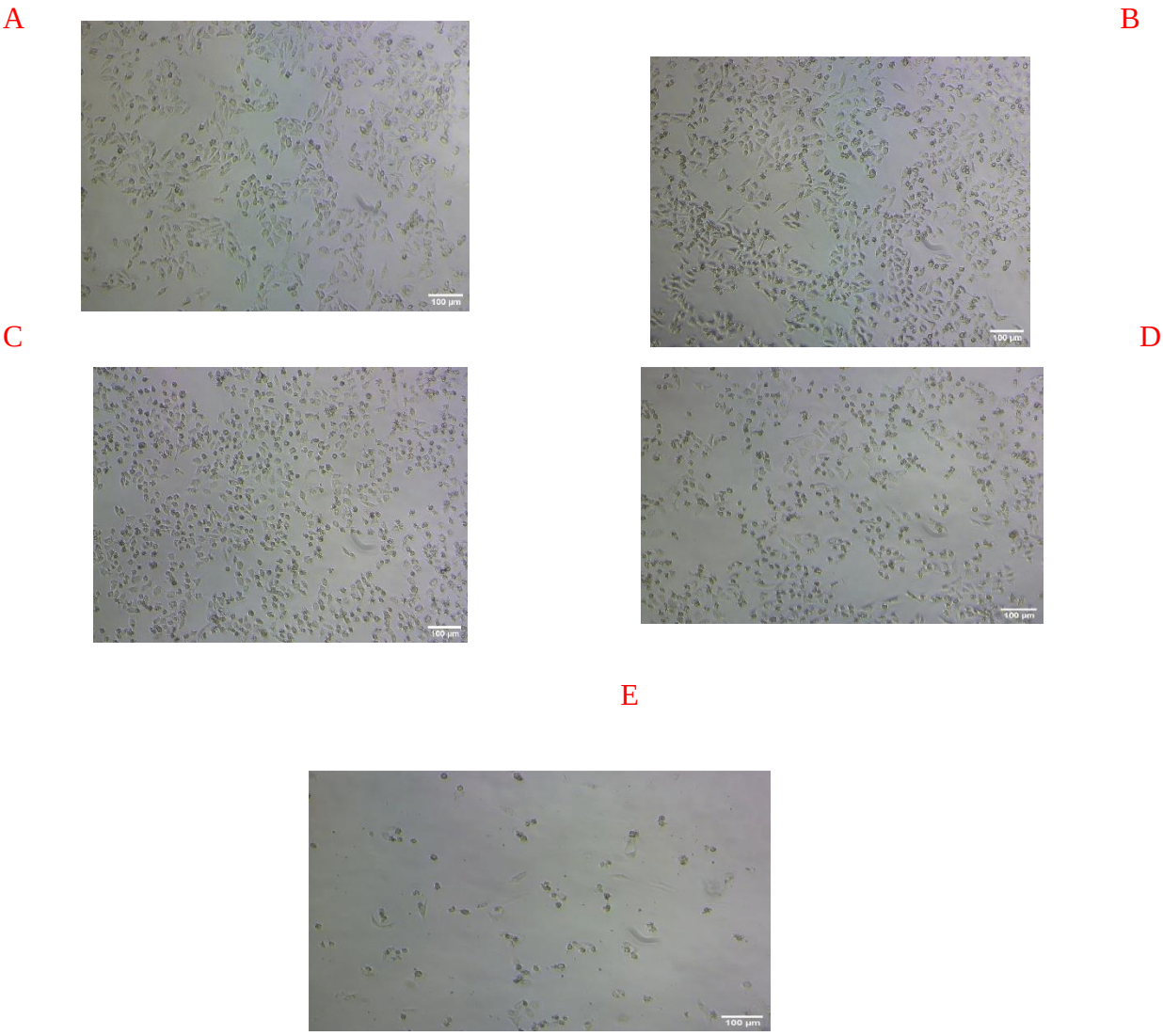

Figure:- 3 MTT cell viability assay of D/D-NLC

Conclusion: Form the observation it was found that D/D-NLC effective in reduction of cell viability hence *invitro* animal model need to screen to confirm these finding.

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