Cytotoxicity and cell uptake of Anionic Docetaxel Self Micro Emulsifying Drug Delivery System on A-549 (human alveolar carcinoma) cell line.

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ABSTRACT SUMMARY
Present study discusses the cytotoxicity evaluation of Anionic Docetaxel (DTX) Self microemulsifying drug delivery system (SMEDDS).

Effect of various anionic charge inducing agents on cytotoxicity and cell uptake of DTX has been evaluated and the IC_{50} of the formulations was recorded.

INTRODUCTION: Docetaxel (DTX) is poorly water soluble drug (solubility <5 ppm) currently used to treat breast, ovaries and especially lung cancer. Bioavailability and solubility of poorly soluble drugs is extensively enhanced by SMEDDS. SMEDDS on aqueous dilution rapidly generate ME with particle size <200nm. Anionic ME could provide an additional advantage of stealth, decreased RES uptake and hence increased accumulation in tumors. Particles having anionic surface charge and particle size <200nm have shown increased blood circulation time. Design of anionic ME could therefore provide an improved strategy for passive targeting of DTX for cancer therapy.

OBJECTIVE: The aim of present study was the design of DTX SMEDDS using anionic agents that could impart negative charge to the ME on dilution. The specific objective of the study was the comparative evaluation of sodium olate a new anionic agent Gantrez AN-119 on cytotoxicity and cell uptake of DTX SMEDDS in A-549 (human alveolar carcinoma) cell line

EXPERIMENTAL METHODS:
SMEDDS preparation: DTX loaded SMEDDS was prepared by dissolving DTX and Pluronic F-68 in DMA and adding mixture of Tween-80/propylene glycol to it. Ethyl oleate was further added to this mixture to prepare the final DTX SMEDDS.

Induction of anionic charge: Anionic charge inducing agents having different molecular weights like Sodium olate (SO, small molecular weight), and Gantrez AN-119 (GAN, high molecular weight 2,00,000), were used in different concentration to induce negative charge. Both Sodium olate and gantrez AN-119 readily dissolved in SMEDDS.

Evaluation: DTX SMEDDS after diluting 1:42 times with 5%D were evaluated for clarity by optical birefringence. Globule size and zeta potential (ζ) measured by Malvern zetasizer and drug content evaluated by HPLC. The effect of different concentrations of sodium olate and Gantrez AN-119 on the globule size and zeta potential was evaluated after dilution of DTX SMEDDS as above..

HPLC analysis: HPLC analysis of DTX was performed on Jasco intelligent 2080 intelligent pump connected to Jasco UV-Vis detector. C-18 Kromasil column (150mm*4.6mm, i.d. 5µm) was used along with mobile phase ACN:Water (48:52). Detection wavelength was 230nm and retention time was 9.2 min. Standard plot was performed and method was found to be linear in concentration range of 50ng to 5ppm

Cell culture study: A549 (human alveolar carcinoma) cell were bought from American type cell culture (ATCC) and preserved in liquid Nitrogen. The cryopreserved vials were thawed and transferred in DMEM: hanks F-12 media supplemented with 10% FBS and incubated with 5% CO_2 in an incubator maintained at 37°C. The medium was replenished every other day until confluence was achieved. The cells were then washed with PBS and harvested with 0.25 % Trypsin–EDTA solution and passaged in ratio of 1:3 or 1:5.

a) Cytotoxicity study: A-549 cells were trypsinized, counted in Neubars chamber and diluted and seeded at a density of 5*10^3 cells/well in 96 well plate and incubated for 24 h. The medium was then replaced by 100µl free DTX or DTX loaded/ blank formulation at various DTX concentrations (0.19 to 50 µg/ml) prepared in the medium. The cell viability was determined by the MTT assay at 2 and 24hr. IC_{50} values of formulations were calculated by plotting % cell viability v/s DTX concentration.

MTT assay protocol: MTT solution in PBS (5mg/ml) was prepared and 25 µl of this solution was added to each well and incubated at 37°C for 4 hours. Then, MTT solution was removed and 100 µl/well of DMSO was added and plate was kept on shaker for 10 min. Analyzed at 540nm.

b) Cell uptake study A-549 cells were seeded at a density of 5*10^3 cells/well in 96 well plate and incubated for 24 h. The medium was replaced with 100 µL DTX solution and DTX loaded formulation containing 5µg/ml DTX for 1 hour. Media was removed and cells washed with PBS. Cells were lysed by sonication after addition of 100 µL 0.5% SLS solution. Drug quantification in media/cell lysate was performed by HPLC.
RESULT AND DISCUSSION: Various concentrations of Gantrez (0-15mg/ml, Fig 1) and sodium oleate (0-1mg/ml, Fig 2) were evaluated and its effect on globule size and zeta potential was recorded. Globule size and zeta potential after dilution of DTX SMEDDS (42 times with 5% Dextrose to achieve recommended dose of 0.74mg/ml of DTX) increased in the concentration of both Gantrez AN-119 and sodium oleate resulted in decrease in zeta potential (Fig 1, 2) to higher negative values suggesting possibility of increased blood residence time. The values of -35mV with Gantrez AN-119 is attributed to large number of anionic group compared to Sodium oleate.

![Fig 1](image1.png)

![Fig 2](image2.png)

Table 1: IC50 values of formulations (n=3)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>IC50 (mcg/ml)</th>
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<tbody>
<tr>
<td>DTX solution</td>
<td>1.14±0.06</td>
</tr>
<tr>
<td>Taxotere</td>
<td>1.33±0.06</td>
</tr>
<tr>
<td>DTX-F68 (P1)</td>
<td>1.2±0.07</td>
</tr>
<tr>
<td>P1+ sodium oleate(P2)</td>
<td>0.88±0.04</td>
</tr>
<tr>
<td>P1+Gantrez (P3)</td>
<td>1.3±0.26</td>
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</tbody>
</table>

Cytotoxicity of three formulation P1 (Pluronic F-68), P2 (P1+sodium oleate) and P3 (P1+ Gantrez AN-119) along with Taxotere and DTX solution was evaluated on A-549 human alveolar carcinoma cell line procured form ATCC. Cytotoxicity data is expressed in terms of IC50 in (Table 1) IC50 value seen with sodium oleate as anionic agent were significantly lower (P<0.05) than all other formulations studied. While Gantrez AN-119 as anionic agent suggested the possibility of longer circulation time, sodium oleate was more promising in enhancing cytotoxicity of DTX SMEDDS. This is attributed to role of sodium oleate as penetration enhancer by enabling perturbation of cell membrane and cell membrane proteins resulting in greater cytotoxicity. However, as this disturbance is known to be transient and not permanent, the formulation could be still safe. Uptake of DTX in A-549 cell line revealed significantly increased uptake of DTX with Sodium oleate as anionic agent as compared to other formulation (Table 2).

CONCLUSION: Anionic SMEDDS using sodium oleate with potential of long circulation could enhance development of DTX SMEDDS with enhanced anticancer efficacy


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