

## **Evaluation of in vitro efficacy of docetaxel-loaded calcium carbonate aragonite nanoparticles (DTX-CaCO<sub>3</sub>NP) on 4T1 mouse breast cancer cell line**

### **ABSTRACT**

Cockle shell-derived calcium carbonate nanoparticles have shown promising potentials as slow drug-releasing compounds in cancer chemotherapy. In this study, we evaluated the in vitro efficacy of docetaxel (DTX)-loaded CaCO<sub>3</sub>NP on 4T1 cell line. This was achieved by evaluating the following: cytotoxicity using MTT assay, fluorescence imaging, apoptosis with Annexin V assay, cell cycle analysis, scanning (SEM) and transmission electron microscopy (TEM), and scratch assay. Based on the results, DTX-CaCO<sub>3</sub>NP with a DTX concentration of 0.5 µg/mL and above had comparable cytotoxic effects with free DTX at 24 h, while all concentrations had similar cytotoxic effect on 4T1 cells at 48 and 72 h. Fluorescence and apoptosis assay showed a higher ( $p < 0.05$ ) number of apoptotic cells in both free DTX and DTX-CaCO<sub>3</sub>NP groups. Cell cycle analysis showed cycle arrest at subG0 and G2/M phases in both treatment groups. SEM showed presence of cellular blebbing, while TEM showed nuclear fragmentation, apoptosis, and vacuolation in the treatment groups. Scratch assay showed lower ( $p < 0.05$ ) closure in both free DTX and DTX-CaCO<sub>3</sub>NP groups. The results from this study showed that DTX-CaCO<sub>3</sub>NP has similar anticancer effects on 4T1 cells as free DTX, and since it has a slow release rate, it is a more preferred substitute for free DTX.

**Keyword:** Docetaxel; Breast cancer; Cell cycle; Apoptosis; Microscopy; Calcium carbonate nanoparticle