

**Nanoparticle delivery of Curcumin induces Cellular Hypoxia and ROS-mediated Apoptosis
via modulation of Bcl-2/Bax in human Neuroblastoma**

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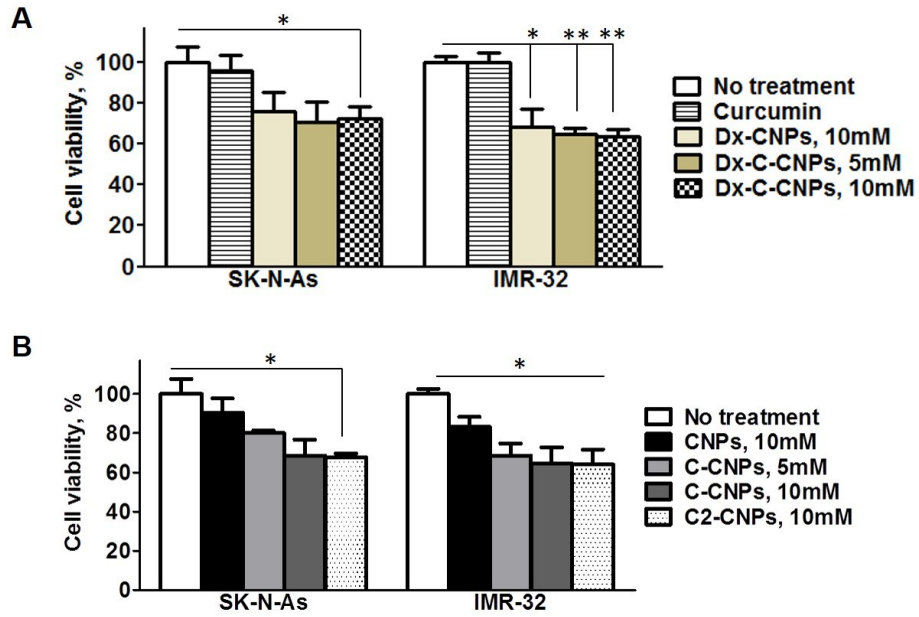
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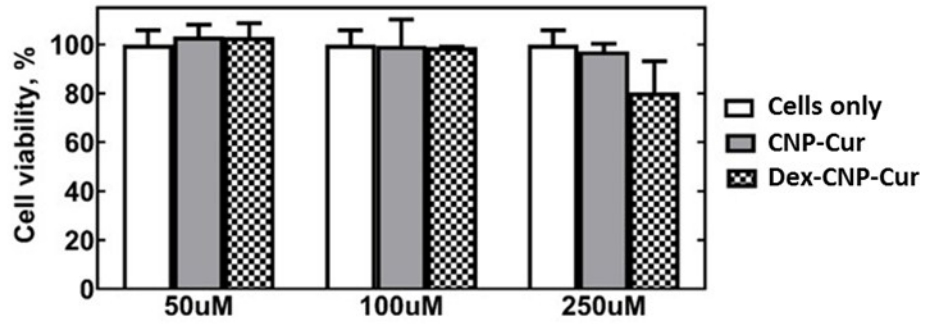
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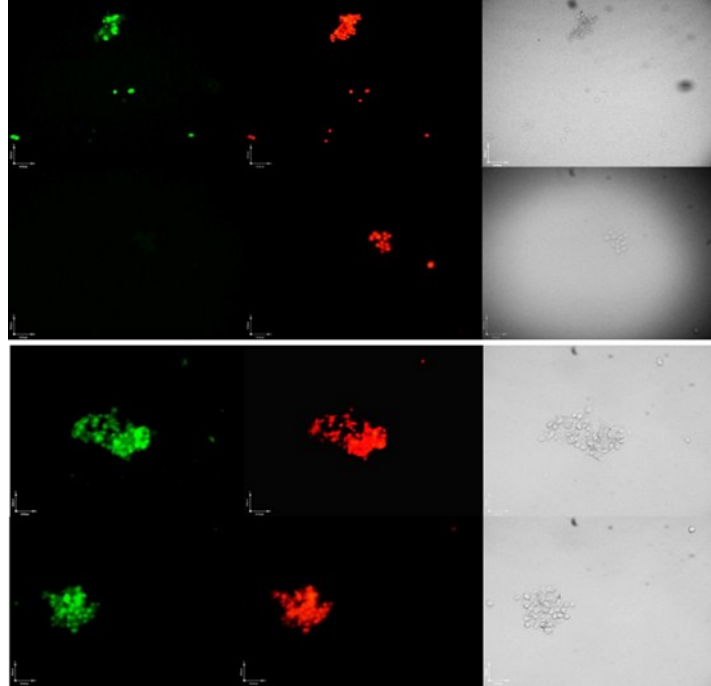
Keywords: nanoceria, curcumin, neuroblastoma, oxidative stress, MYCN-amplification, HIF-1 α



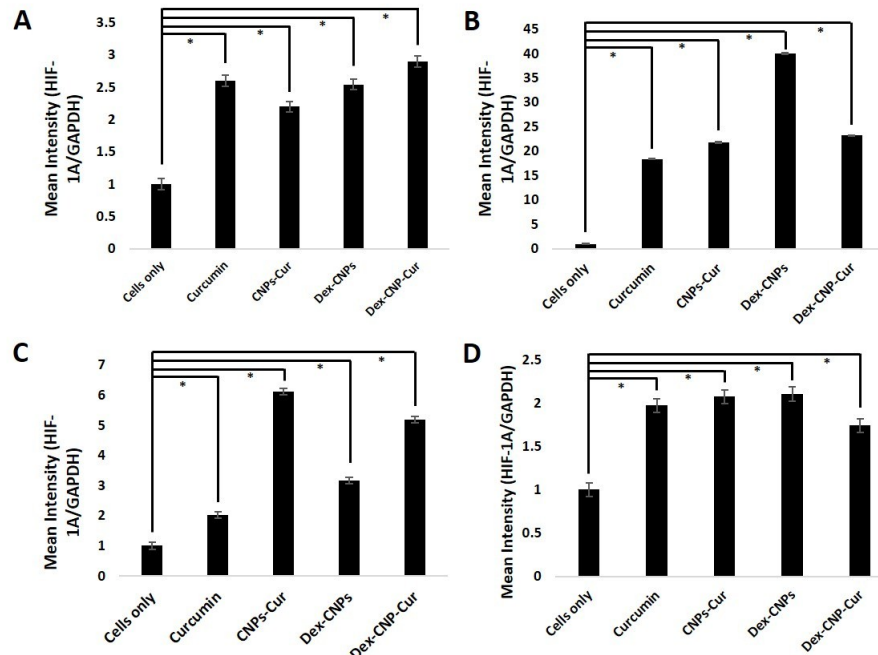
Supplemental Figure 1. Results of MTT assay on SK-N-AS and IMR-32 treated for 24h with: (A) formulations contained dextran; (B) – formulations without dextran. Particles of both formulations show a similar trend for both cell lines. However, the dextran-coated formulation shows greater apoptosis for each cell sample.



Supplemental Figure 2. Cell viability of Human umbilical vein endothelial cells after treatment with nanoparticle formulas. MTT assays were performed on HUVEC cells for 72 hrs after application of nanoparticle formulas including CNP-Cur & Dex-CNP-Cur, as well as no treatment (Cells only). Applications were performed at 50, 100, & 250 uM concentrations. All assays were performed in quadruplicate.



Supplemental Figure 3. TUNEL Assay of neuroblastoma cells. Dex-CNP-Cur and CNP-Cur treatments were performed on (top) SK-N-AS and (bottom) IMR-32 cells. Mild induction of apoptosis was identified in SK-N-AS cells, but significant levels of apoptosis were seen in IMR-32 cells. Apoptosis was determined by green (single-stranded breaks:DTP-conjugated fluoro) vs. red fluorescence (total DNA). Brightfield images show total cell populations.



Supplemental Figure 4. Densitometry of Western blot analysis for HIF-1 α expression after nanoparticle treatments. Densitometry was performed on: A) IMR-32, B) SMS-KAN, C) SK-N-AS, & D) LA-N-6. Scanning and quantitation for all bands was performed using ImageJ software with data normalized to the Mean Intensity of the appropriate GAPDH load control. Graphs represent the analysis of samples represented in the blots present in Figures 9A-D. * $p < 0.05$, Student's t-test.