



Cytotoxicity of Snake Venoms and Cytotoxins From Two Southeast Asian Cobras (*Naja sumatrana*, *Naja kaouthia*): Exploration of Anticancer Potential, Selectivity, and Cell Death Mechanism

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Venoms of cobras (*Naja* spp.) contain high abundances of cytotoxins, which contribute to tissue necrosis in cobra envenomation. The tissue-necrotizing activity of cobra cytotoxins, nevertheless, indicates anticancer potentials. This study set to explore the anticancer properties of the venoms and cytotoxins from *Naja sumatrana* (equatorial spitting cobra) and *Naja kaouthia* (monocled cobra), two highly venomous species in Southeast Asia. The cytotoxicity, selectivity, and cell death mechanisms of their venoms and cytotoxins (NS-CTX from *N. sumatrana*: NS-CTX; *N. kaouthia*: NK-CTX) were elucidated in human lung (A549), prostate (PC-3), and breast (MCF-7) cancer cell lines. Cytotoxins were purified through a sequential fractionation approach using cation-exchange chromatography, followed by C₁₈ reverse-phase high-performance liquid chromatography (HPLC) to homogeneity validated with sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and identified by liquid chromatography-tandem mass spectrometry (LCMS/MS). The cobra venoms and their respective cytotoxins exhibited concentration-dependent growth inhibitory effects in all cell lines tested, with the cytotoxins being more potent compared to the corresponding whole venoms. NS-CTX and NK-CTX are, respectively, P-type and S-type isoforms of cytotoxin, based on the amino acid sequences as per LCMS/MS analysis. Both cytotoxins exhibited differential cytotoxic effects in the cell lines tested, with NS-CTX (P-type cytotoxin) being significantly more potent in inhibiting the growth of the cancer cells. Both cytotoxins demonstrated promising selectivity only for the A549 lung cancer cell line (selectivity index = 2.17 and 2.26, respectively) but not in prostate (PC-3) and breast (MCF-7) cancer cell lines (selectivity index < 1). Flow cytometry revealed that the A549 lung cancer cells treated with NS-CTX and NK-CTX underwent necrosis predominantly. Meanwhile, the cytotoxins induced mainly caspase-independent late apoptosis in the prostate (PC-3) and breast (MCF-7) cancer cells lines but lacked selectivity. The findings

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