

## **Cryptolepine induced apoptosis in TNF $\alpha$ -stimulated A549 lung carcinoma cells through NF- $\kappa$ B signalling pathway**

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Cryptolepine, the major alkaloid of the west African shrub *Cryptolepis sanguinolenta*, has been shown to induce cell cycle arrest and apoptosis in A549 cells (Zhu and Godderham, 2006). We have also reported the inhibitory effects of this compound on NF- $\kappa$ B in various cell types (Olajide et al., 2007; 2013a; 2013b). In this study, we have investigated whether the apoptosis-inducing action of the compound is mediated through NF- $\kappa$ B signalling. In order to evaluate the effect on cell proliferation, cultured A549 cells were treated with cryptolepine (5-20  $\mu$ M) for 24 h, and number of viable cells determined using the MTT assay. Cultured cells pre-treated with cryptolepine (5-20  $\mu$ M) 30 min prior to stimulation with TNF $\alpha$  (1 nM) were evaluated for levels of caspase 3 using the Caspase-Glo<sup>®</sup> 3/7 Assay kit (Promega). The effects of cryptolepine on TNF $\alpha$ -induced I $\kappa$ B phosphorylation, NF- $\kappa$ Bp65 subunit nuclear translocation, and protein expressions of NF- $\kappa$ B-regulated gene products of apoptosis (cyclin D1, survivin, XIAP, cIAP1, and Bcl-2) were investigated by treating cultured A549 cells with cryptolepine (5-20  $\mu$ M) 30 min before stimulation with TNF $\alpha$  (1 nM), followed by In Cell western analysis. Results showed that cryptolepine produced dose-dependent and significant ( $p < 0.05$ ) reduction in A549 cell proliferation after 24 h of treatment. At 20  $\mu$ M of the compound, cell viability was reduced by 62.2 $\pm$ 3.3%. Treatment with 10 and 20  $\mu$ M cryptolepine for 24 h was also found to cause significant ( $p < 0.05$ ) induction of caspase-3. With 10  $\mu$ M, relative luminescence was 9038 $\pm$ 480.5, and at 20  $\mu$ M, relative luminescence was 9776 $\pm$ 266.4, compared with relative luminescence of 1151 $\pm$ 74.5 recorded in control cells. Protein analyses revealed that 10 and 20  $\mu$ M of cryptolepine inhibited TNF $\alpha$ -induced I $\kappa$ B phosphorylation and NF- $\kappa$ Bp65 nuclear translocation. Cells stimulated with TNF $\alpha$  (1 nM) showed elevated levels of Bcl-2, cyclin D1, surviving, XIAP and cIAP, which were reduced when pre-treated with cryptolepine (5-20  $\mu$ M). Our results showed that cryptolepine downregulated the expression of anti-apoptosis proteins. We have also demonstrated that cryptolepine induces apoptosis in A549 lung carcinoma cells by interfering with NF- $\kappa$ B signalling.

### **References**

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