



# University of HUDDERSFIELD

## University of Huddersfield Repository

Olajide, Olumayokun A and Wright, Colin W

Cryptolepine induced apoptosis in TNFalpha-stimulated A549 lung carcinoma cells through NF-kappaB signalling pathway

### Original Citation

Olajide, Olumayokun A and Wright, Colin W (2014) Cryptolepine induced apoptosis in TNFalpha-stimulated A549 lung carcinoma cells through NF-kappaB signalling pathway. pA2 Online. ISSN 1741-1149

This version is available at <http://eprints.hud.ac.uk/id/eprint/19843/>

The University Repository is a digital collection of the research output of the University, available on Open Access. Copyright and Moral Rights for the items on this site are retained by the individual author and/or other copyright owners. Users may access full items free of charge; copies of full text items generally can be reproduced, displayed or performed and given to third parties in any format or medium for personal research or study, educational or not-for-profit purposes without prior permission or charge, provided:

- The authors, title and full bibliographic details is credited in any copy;
- A hyperlink and/or URL is included for the original metadata page; and
- The content is not changed in any way.

For more information, including our policy and submission procedure, please contact the Repository Team at: [E.mailbox@hud.ac.uk](mailto:E.mailbox@hud.ac.uk).

<http://eprints.hud.ac.uk/>

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

Olumayokun Olajide<sup>1</sup>, Colin Wright<sup>2</sup>. <sup>1</sup>University of Huddersfield, Huddersfield, UK, <sup>2</sup>University of Bradford, Bradford, UK

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**

Olajide OA et al Bioorg Med Chem 15:43, 2007

Olajide OA et al Eur J Med Chem 63:333, 2013

Olajide OA et al Evid Based Complement Alternat Med 2013:459723, 2013

Zhu H & Gooderham NJ, Toxicol Sci 91:132, 2006