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ABSTRACTS

Clitocybe alexandri extract induces apoptosis in a lung cancer cell line: identification of phenolic acids with cytotoxic potential

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Natural products from fungi metabolism, are a possible rich source of biologically active compounds with potential for drug discovery [1,2]. We have recently reported the growth inhibitory activity of extracts from *Clitocybe alexandri*, a wild mushroom collected from the northwest of Portugal, in human tumour cell lines (NCI-H460, MCF-7, HCT-15 and AGS) [3]. The ethanolic extract was the most potent one, particularly in NCI-H460 cells (GI₅₀ 24.8 ± 2.3 µg/ml) [3]. The aim of this work was to further study the potential of this extract as a possible source of cytotoxic compounds.

In order to understand the mechanism of action of this extract in the NCI-H460 cells, the effects on cell cycle (following PI labelling) and apoptosis (following AnnexinV/PI labelling) were evaluated by flow cytometry. Furthermore, the involvement of proteins related to cellular apoptosis was investigated by Western blot. The ethanolic extract was characterized regarding its phenolic composition by HPLC-DAD/ESI. The isolated compounds were also studied regarding their growth inhibitory activity, by SBR assay. The effect of isolated or combined compounds on viable cell number was also evaluated using the Trypan blue exclusion assay.

It was observed that the *Clitocybe alexandri* extract induced an S-phase cell cycle arrest and increased the percentage of apoptotic cells. In addition, treatment with the GI₅₀ concentration for 48h caused an increase in the levels of wt p53, cleaved caspase-3 and cleaved PARP. The main components identified in this extract were protocatechuic acid (16.4 ± 2.2 mg/kg dw), p-hydroxybenzoic acid (8.3 ± 0.4 mg/kg) and cinnamic acid (6.4 ± 0.3 mg/kg). Cinnamic acid was found to be the most potent compound regarding cell growth inhibition. Finally, it was verified that their concomitant use provided the strongest decrease in viable cell number.

The results reported here relate to the cytotoxicity of *Clitocybe alexandri* in a lung cancer cell line. We found evidence for alterations in cell cycle and apoptosis, involving p53 and caspase-3. Finally, our data suggest that the phenolic acids identified in the ethanolic extract are, at least, partially responsible for the cytotoxicity induced by this mushrooms extract.

- 1 Ferreira ICFR, Vaz JA, Vasconcelos MA, Martins A Compounds from wild mushrooms with antitumor potential (2010). *Anti-cancer Agents in Medicinal Chemistry* 10: 424-436.
- 2 Moradali M-F, Mostafavi H, Ghods S, Hedjaroude, G-A. Immunomodulating and anticancer agents in the realm of macromycetes fungi (macrofungi) (2007). *International Immunopharmacology* 7: 701-724.
- 3 Vaz JA, Heleno SA, Martins A, Almeida GM, Vasconcelos MH, Ferreira ICFR Wild mushrooms *Clitocybe alexandri* and *Lepista inversa*: In vitro antioxidant activity and growth inhibition of human tumour cell lines (2010). *Food and Chemical Toxicology* 48: 2881-2884.

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identification of phenolic acids with cytotoxic potential**

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