Cell death induction by mycelium extracts from *Pleurotus* spp. on cervical cancer cell lines

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**Abstract**

Mushrooms have health benefits, including anti-tumoral properties. We evaluated the cytotoxic and cell death induction effects of water-soluble extracts of *Pleurotus ostreatus* and *Pleurotus eryngii* mycelium in the cervical cancer cell lines HeLa (HVP18) and SiHa (HVP16) as well as the non-tumoral cell line HaCaT. Both *Pleurotus* extracts presented similar protein patterns from 190 to 10 kDa and displayed protease activity on a gelatine substrate. The mycelium extracts of both *Pleurotus* strains induced a dose-dependent cytotoxic effect on HPV18+ cells IC50, whereas HaCaT cells were less susceptible (IC50, 90 μg). The cytotoxic effect at the IC50 concentration was not associated with apoptosis, the activation of Caspases-3/7 was not significantive; only *P. eryngii* induced a moderate (1.2-fold) increase in SiHa cells. *Pleurotus* extracts induced autophagy, mainly in SiHa cells (4.3-fold). Neither extracts induced changes in p53 protein expression, suggesting that the cytotoxic effect could be due to p53-independent pathways.

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1. Introduction

Mushrooms have bioactive compounds, such as proteins, polysaccharides or secondary metabolites, isolated from the mycelium and fruiting body, which have beneficial effects on health, including regulation of diabetes as well as antimicrobial, antioxidant, anti-tumoral and immunomodulatory effects (Vamanu, 2013; Pandya et al. 2019; Yue et al. 2021). *Pleurotus ostreatus* and *P. eryngii* mushrooms possess compounds that have both anti-proliferative and cytotoxic activity *in vitro* on human cancer cell lines of different tissues (Jedinak and Sliva 2008; Yuan et al. 2017; Meza-Menchaca et al. 2020). The cytotoxic effects of these compounds have been shown to participate in the activation of apoptosis through caspase-3 and caspase-9 activation and an increase in the expression levels of proteins involved in apoptosis (Bae et al. 2009; Arora and Tandon 2015; Yuan et al. 2017). In relation to cervical cancer, *P. ostreatus* or *P. eryngii* polysaccharides and other derived compounds have a cytotoxic effect on Human Papillomavirus (HPV)-positive cervical cancer cell lines (Chen et al. 2015; Meza-Menchaca et al. 2020). Herein, the cytotoxic effects and cell death induction by *P. ostreatus* and *P. eryngii* mycelium extracts on cervical cancer cell lines *in vitro* were evaluated.

2. Results and discussion

2.1. Protein analysis of *Pleurotus* spp. mycelium extracts

The extracts from *P. ostreatus* and *P. eryngii* mycelium presented similar protein patterns, showing several protein bands of molecular weight (MW) between 180 and 17 kDa. The strongest protein bands were identified between 70 and 30 kDa from the *P. ostreatus* strain, and between 70 and 40 kDa from *P. eryngii* (Figure S1). Both *Pleurotus* strains, but mainly *P. ostreatus*, displayed protease activity in the range of 72 kDa. A serine protease with a similar MW (75 kDa) from mycelium of *P. ostreatus*
was identified in a previous work (Palmieri et al. 2001). Another protease with a lower MW from the mycelium of *P. eryngii* was found (Wang and Ng 2001).

### 2.2. Aqueous extracts of Pleurotus have a cytotoxic effect on tumoral and non-tumoral cell lines

The extracts of *P. ostreatus* and *P. eryngii* induced a dose-dependent cytotoxic effect in HPV\(^+\) cell lines HeLa and SiHa, or the non-tumoral cell line HaCaT at 24 h of treatment (*P* < 0.05, Figure S2). The IC\(_{50}\) was similar for both extracts in HeLa (PO 60 \(\mu\)g, PE 70 \(\mu\)g), and SiHa (PO 60 \(\mu\)g, PE 65 \(\mu\)g, Figure S2), whereas a higher concentration was required in HaCaT cells (PO 90 \(\mu\)g, PE 85 \(\mu\)g). *P. ostreatus* extracts had a more specific cytotoxic effect on the cervical cancer cell lines HeLa and SiHa (33%) as well as *P. eryngii* (18%–24%) than on non-tumoral cells.

There is no evidence of a cytotoxic effect induced by aqueous extracts obtained from the mycelium of *P. ostreatus* or *P. eryngii* in HPV\(^+\) cell lines. Only polysaccharides purified from the fruiting bodies of *P. ostreatus* or *P. eryngii* inhibited HeLa cell proliferation at 48 h (Chen et al. 2015). There is no information about the cytotoxic effect of these mycelium extracts on HaCaT cells; other authors showed that non-tumoral cells are less sensitive than cancer cell lines to *P. ostreatus* polysaccharides or proteins from fruiting bodies (Jedinak and Sliva 2008; Bae et al. 2009; Yuan et al. 2017). Additionally, the cytotoxic effects of extracts isolated from the fruiting body, polysaccharides or purified proteins from *P. ostreatus* or *P. eryngii* have been found in other tumoral models, such as those of breast and colon cancer (Jedinak and Sliva 2008; Arora and Tandon 2015).

### 2.3. Aqueous extracts of Pleurotus spp. induce cell death in HPV\(^+\) cell lines

*Pleurotus* spp. extracts induced changes in the morphology of HeLa and SiHa cells, mainly a cell contraction and an increase in cytoplasmic vacuoles, which are potential characteristics of apoptosis and autophagy, respectively (Figure S3). Therefore, we explored cell death induction and found some differences between HeLa (HPV18\(^\pm\)) and SiHa (HPV16\(^\pm\)) cells. In HeLa, neither *Pleurotus* extract induced Caspase-3/7 activity, suggesting that apoptosis was not induced (Figure S4). Only *P. eryngii* increased Caspase-3/7 activity slightly in SiHa (1.2-fold compared with the untreated control *p* < 0.05). In autophagy, only *P. ostreatus* induced it in HeLa in which the lysosomal membrane permeability was increased 1.5-fold in relation to the untreated control (Figure S4). Nevertheless, SiHa were more sensitive to *P. ostreatus* than to *P. eryngii*, and both increased lysosomal permeability (4.3- and 1.8-fold, respectively). The differences in cell death induction between HeLa and SiHa cells could depend on the cell type or the nature of *P. ostreatus* or *P. eryngii* compounds (proteins, polysaccharides, etc.). They could also depend on the viral genotype, because some cellular proteins interact with E6 and E7 oncoproteins from HPV16 but not with those from HPV18.

There are no reports of apoptosis induction in HPV\(^+\) cell lines treated with *P. ostreatus* or *P. eryngii* extracts. However, aqueous extracts, polyphenol-rich extracts or purified protein, obtained from both mycelium and fruiting bodies of *P. ostreatus* or *P.
eryngii, induce apoptosis in other tumoral cell lines through caspase-3, caspase-9, Bax, p53 and PARP proteins (Bae et al. 2009; Arora and Tandon 2015; Yuan et al. 2017). However, there is no reported evidence of autophagy induction using extracts of P. ostreatus or P. eryngii.

2.4. Extracts of Pleurotus did not induce p53 protein expression in HPV+ cell lines

We found no changes in the expression of p53 protein in HPV+ cell lines treated with P. ostreatus or P. eryngii mycelium extracts at the IC50 concentration. Jedinak and Sliva (2008), using P. ostreatus extracts from fruiting bodies, reported a cytotoxic effect p53-dependent or independent pathways in breast and colon cancer cells, whereas purified protein from P. eryngii increased p53 expression in colon cancer cells (Yuan et al. 2017). These differences could be due to the nature of extract, as we used mycelium at a lower dose, and they used extracts of fruiting bodies at doses higher than we did.

3. Conclusions

An aqueous extract obtained from the mycelium of the edible mushrooms P. ostreatus and P. eryngii had protease activity and induced a dose-dependent cytotoxic effect on HPV+ cell lines, while the non-tumoral cell line was less susceptible. The cytotoxic effect was not significantly associated with apoptotic cell death but instead was due to autophagy and was not associated with the activation of p53 protein.

Disclosure statement

No potential conflict of interest was reported by the authors.

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