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Cytotoxity induced by extracts of *Pisolithustinctorius* spores on human cancer and normal cell lines - evaluation of the anticancer potential

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INTRODUCTION:

Cancer is one of the most important causes of death and morbidity worldwide. According to the World Health Organization 8.2 million people have died from cancer in 2012. The treatment of this fast growing disease is regarded as a major challenge in health for the XXI century. Conventional anti-cancer therapy is usually aggressive to the patients due to systemic toxicity caused by lack of specificity of the chemotherapy drugs (Kidd, 2000). This has triggered the interest of the scientific community to search for new and more effective therapies, less aggressive than conventional treatments. Used since antiquity in oriental civilizations, fungi, in particular mushrooms have been shown to limit or reverse tumor growth (Kidd, 2000). *Pisolithustinctorius* (Basidiomycota) is a soil fungus with world-wide distribution that it known to form ectomycorrhizal symbioses in forest ecosystems (Kope & Fortin, 1990). The sporocarps of this fungus contain Pisosterol, a triterpene that has been shown to have antitumor activity against some cancer cell lines (Montenegro et al., 2004). Nevertheless, no studies have focused on the anticancer potential of other structures such as spores and thus the anticancer potential of *P. tinctorius*, remains largely unknown.

OBJECTIVES:

Considering that spores can be a good source of new pharmacological compounds, the main objective of this study was to evaluate the potential of *P.tinctorius*spores as a source of anticancer compounds.

MATERIALS AND METHODS:

Extractions: *P.tinctorius*samples were collected from a forest ecosystem in Northern Portugal. The spores were separated from the sporocarps and freeze dried. A crude extract was prepared with 20.43g of spores and 75ml of a mixture of dichloromethane/methanol (2:1). The mixture was sonicated, centrifuged and the pellet re-extracted three times, rendering a total volume of 240 ml, which was then evaporated to dryness under reduced pressure. The residue was chromatographed over silica gel with hexane/ethyl acetate and methanol (with progressively increasing polarity) giving eleven fractions (A with lowest polarity to K highest polarity).

MTT assay: The cytotoxicity of the crude extract and fractions was tested against the osteosarcoma cell line MG63, colon adenocarcinoma cell line RKO, breast carcinoma cell line T47D and brain microvascular endothelial cell line hCMEC/D3. Cells were grown in DMEMGlutamaxmedium supplemented with 10% fetal bovine serum (FBS), 2.5 µg/ml fungizone and penicillin-streptomycin (100 IU/ml-1 and100 µg/ml, respectively).For the MTT assay cells were seeded in triplicate in 96 wells plates at a cell density of $3.3x10^4$cel/ml. After 24h adhesion, the medium was removed and cells were exposed to new medium with the crude extract at a concentration of 1mg/ml and the fractions at0.1mg/ml during 24, 48 and 72 h in a CO_2 incubator at 37ºC. Cell viability was determined by the ability of living cells to reduce 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-*2H*-tetrazolium bromide (MTT). At the end of each period of incubation, MTT was added at 0.05mg/ml. After3 h of incubation formazancrystals were dissolved in 100µlDMSO and the absorbance was measured at 550 nm.

RESULTS AND DISCUSSION:

The results concerning the cytotoxicity of the crude extract showed that this extract was able to inhibit cell viability in all cancer cell lines (Fig. 1). This result is supported by the results obtained with the fractions, since the crude extract represents a mixture of the compounds that are then separated in the fractions. Concerning the cytotoxicity of the fractions, fractions, D, F, G and H, were the ones where the lowest rate of viable cells was registered, suggesting the presence of compounds with strong cytotoxic activity. However, we found that the different cancer cell lines were not affected to the same extent when exposed to the same fraction. As example, in fraction H, the results showed 5% of viable cells in RKO and T74D after 48h incubation, while in MG63 the rate of viable cells was 90%. This could mean that the cytotoxic compounds that are present in fraction H are selective towards RKO and T74D showing different modes of action. Concerning the hCMEC/D3 cell line when exposed to D, F, G, and H fractions, the number of viable cells was much higher than that found in cancer cell lines, which indicates that the fractions are much more cytotoxic to cancer than to normal cells, reinforcing the idea of the presence of anticancer compounds in these fractions.

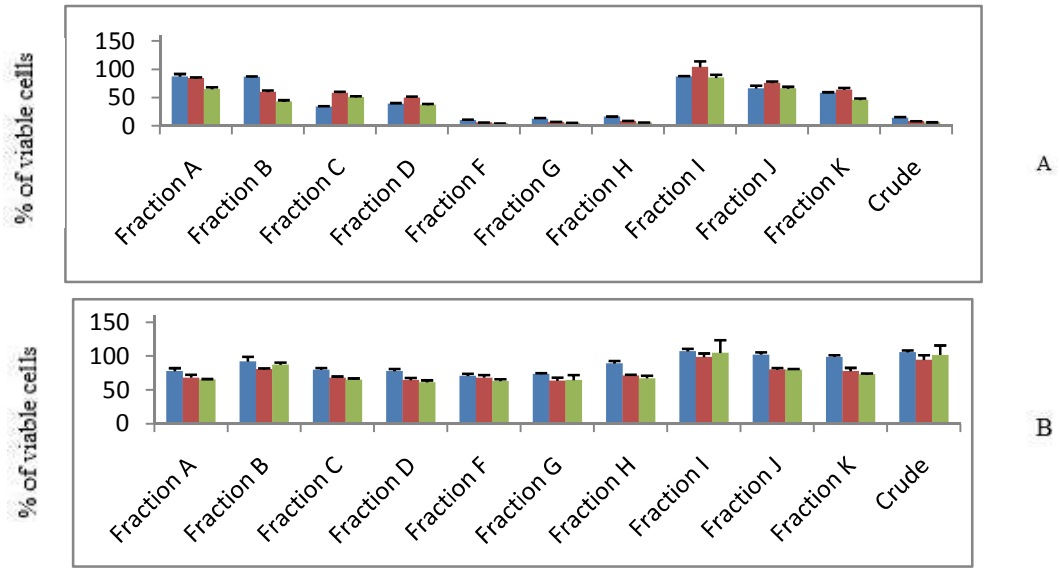

Figure 1 - Effect of crude extract and fractions of *Pisolithustinctorius* spores on the viability of T47D cells **(A)** and hCMEC/D3 cells **(B)**.The cells were exposed for 24, 48 and 72h at a concentration of 0.1mg/ml for the fractions and 1mg/ml for the crude extract. Cell viability was determined by the MTT dye reduction assay. Values are present as means ±SD of triplicate analyses when compared with control group (n = 3).

CONCLUSION:

To our knowledge this is the first study concerning the cytotoxic potential of *P. tinctorius* spores on cancer cell lines. The study demonstrates that the spores of *P. tinctorius* are an interesting source of compounds with anticancer potential and in this sense it represents the initial step in the discovery of new sources of anticancer compounds. It was possible to identify several fractions with selective cytotoxicity toward the cancer lines and normal cells, supporting the possibility of therapeutic potential for the treatment of human cancer forms. Further studies are on-going such as the evaluation of the effects of different concentrations of the fractions in the cancer cell lines and the identifications of the compounds with anticancer activity that are present in the obtained *P.tinctorius* fractions, more specifically, in the fractions D, F, G, and H.

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