EFFECT OF THE FUNGAL COMPOUND PHOLIOTIC ACID ON HUMAN MELANOMA CELLS

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Introduction: Mushrooms have a significant role as functional food and as a nutraceutical resource. The combination of their proteins, vitamins, minerals and carbohydrates has meant that mushrooms could be considered a food source for a long time in many countries [1]. Moreover, mushrooms contain a great variety of bioactive metabolites that can be successful in both prevention and treatment of various human health diseases [1]. Medicinal mushrooms are widely used in East Asia for the treatment of various diseases, principally in complementary cancer care. While there is a growing interest in medicinal mushrooms in Western countries [1]. Previously, we evidenced that pholiotic acid {(2R)-2-[(S)-3-hydroxy-3-methylglutaryloxy]putrescine dicinnamamide}, isolated from the fruiting bodies of the Basidiomycete *Pholiota spumosa* (Fr.) Sing. (Strophariaceae), was able to inhibit the vitality of human prostate cancer cells [2]. On the other hand, polyamine analogs that are similar in structure to the natural polyamines but that cannot mimic their functions that are essential for cellular growth and differentiation, have shown antitumor activity in several types of cancer, including melanoma. Melanoma is one of the most invasive and deadly forms of skin cancer: its treatment mainly depends on the time of diagnosis, and in the metastatic stage it becomes very refractory to conventional therapies [3]. An increasing resistance of tumor cells to chemotherapy and severe side effects of this conservative medication have potentiated the search for new, alternative anticancer agents also from natural sources. Therefore, we have now investigated the response of A375 human melanoma cells to pholiotic acid.

Materials and methods: The cell viability was measured using MTT assay. LDH release, a marker of membrane breakdown, was also measured. For the detection of apoptosis, the evaluation of DNA fragmentation and caspase-3 activity assay were employed. The expression of proteins was evaluated by Western blot analysis. The levels of reactive oxygen species were also analyzed.

Results: The results obtained demonstrate that this natural compound, in the range of 6.25-25 μM, was able to reduce cell viability of cancer cells inducing cell death by intrinsic apoptotic pathway that probably involves PTEN activity, inhibition of Hsp70 expression and reactive oxygen species (ROS) production. In fact, an increase of PTEN and Bax levels, in conjunction with the more pronounced decrease in Bcl-2 occurred in A375 cells treated with pholiotic acid at 6.25-25

μM concentrations. In addition, caspase-9 and caspase-3 were shown to be observably activated. Moreover, it such as quercetin, a well-known Hsp70 protein inhibitor, induced a reduction of Hsp70 expression.

Conclusion: The hypothesis of apoptosis induction in our experimental conditions was reinforced by a high DNA fragmentation, not correlated to LDH release. In this study, we also found that pholiotic acid was able to increase ROS production, correlated to a downregulation of the antioxidant enzyme superoxide dismutase (SOD). The present findings, starting point for further investigation, suggest that pholiotic acid structure might be used to design novel derivatives for the developing of potential new drugs for melanoma therapy.

1. Jeitler et al., (2020) *Front. Pharmacol*. 11:580656.
2. Clericuzio et al., (2007) *Eur. J. Org. Chem.* 5551-5559
3. Miller et al., (2019). *CA A Cancer J. Clin*. 69, 363-385.