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Effects of Edible Mushrooms *Phellinus linteus* and *Lentinus edodes* Methanol Extracts on Colorectal Cancer Cell Lines †

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Abstract: An increasing focus in cancer therapy is on investigation of fungal products with anticancer activity and potential to affect specific targets. Main goal of this study is analysis of effects of *Phellinus linteus* and *Lentinus edodes*, edible and medicinal mushrooms, on migratory/invasive markers significant in first steps of cancer metastasis. Both treatments increased antimigratory marker E-cadherin and decreased promigratory/proinvasive proteins N-cadherin and Vimentin. Lowered concentration of proinvasive protein MMP-9 was also observed in treated HCT-116 and SW-480 cells. **PL** and **LE** exerted cell selectivity, whereat **PL** had better activity on SW-480, while **LE** had more prominent effect on HCT-116 cells.

Keywords: cadherins; CRC; fungotherapy; immunofluorescence; MMP-9

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1. Introduction

Cancer presents a prominent health problem worldwide and its treatment is challenging. In past decades, studies focused on investigation of fungi and their constitutive compounds with anticancer properties became interesting for research [1]. Alternative approaches for cancer treatment based on the use of fungi is considered attractive worldwide, because of their abundance in bioactive compounds that have confirmed therapeutic effects [1,2]. Moreover, cancer fungotherapy, as a promising scientific field, focuses on investigation of fungal products with prominent anticancer potential and target-specific activity [2]. *Phellinus linteus* (Berk. et Curt.) Teng, known as meshima, and *Lentinus edodes* (Berk.) Pegler, also known as shiitake mushroom, are highly valued species with prominent beneficial effects for health and in the treatment of various ailments. Among confirmed medicinal activities, their antitumor, immunomodulating, antiviral and antioxidant effects are reported [3,4]. Their significant potential is due to accumulation of a variety of bioactive primary and secondary metabolites in their fruiting bodies. Among these are mineral compounds, vitamins, oils, lipids, organic acids, polysaccharides, proteins, phenols (flavonoids and phenolic acids). It is already known that mushrooms are able to prevent genesis of cancer, exert direct antitumor activity and inhibit metastasis [1].

Beside their cytotoxicity, effects of mushrooms regarding regulation of crucial steps in cancer metastasis are poorly investigated. Our study aimed to analyze effects of two edible and medicinal mushroom species on markers of migration and invasion as key steps of cancer metastasis.

2. Methods

Methanol extracts of commercially cultivated edible and medicinal mushroom species *Phellinus linteus* (**PL**) and *Lentinus edodes* (**LE**) were examined in two selected concentrations (10 and 50 μg/mL) for their antimigratory and antiinvasive potential. Two colorectal carcinoma cell lines (HCT-116, SW-480) were used and treatment effects were evaluated after 24 h. Expression and localization of antimigratory protein E-cadherin, and promigratory markers N-cadherin and Vimentin were done by using immunofluorescent method [2], and concentration of matrixmetalloproteinase 9 (MMP-9) was determined using colorimetric ELISA assay [5].

3. Results and Discussion

According to the results, **PL** and **LE** induced obvious cell selective effects, whereat **PL** exerted more prominent effect on SW-480 cells, whilst **LE** had stronger effect on HCT-116 cells.

PL increased expression of antimigratory marker E-cadherin and significantly decreased level of promigratory/proinvasive proteins N-cadherin and Vimentin in SW-480 cells. Meanwhile, **LE** induced similar response in HCT-116 cells, increasing E-cadherin and lowering N-cadherin and Vimentin (Figure 1).

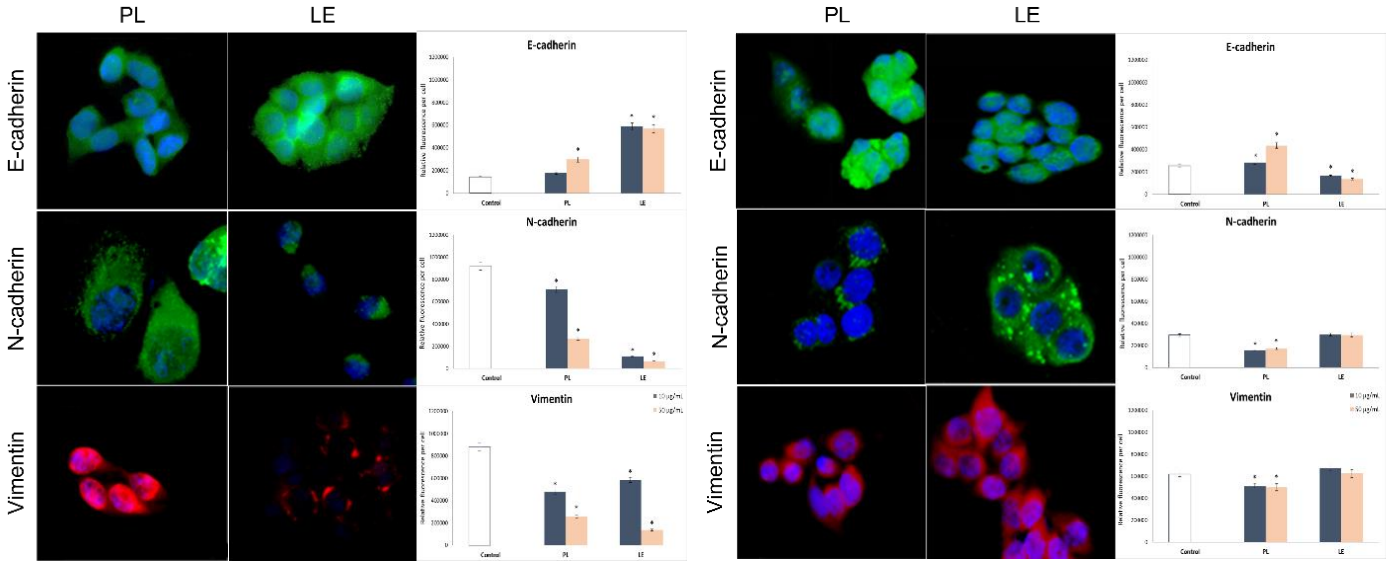

Figure 1. Effects of **PL** and **LE** treatments on HCT-116 (**a**) and SW-480 cells (**b**). Figure contains representative micrographs and calculation of relative fluorescence per cell, whereat * $p > 0.05$ is considered as statistical significance.

Regarding proinvasive protein MMP-9, applied treatments significantly decreased its concentration in both tested cell lines. Once again, cell selectivity was observed regarding effects of these treatments. Namely, the best effect on SW-480 cell line exerted **PL** which significantly lowered level of MMP-9, and **LE** was more potent in reducing the level of this proinvasive protein in HCT-116 cells (Figure 2).

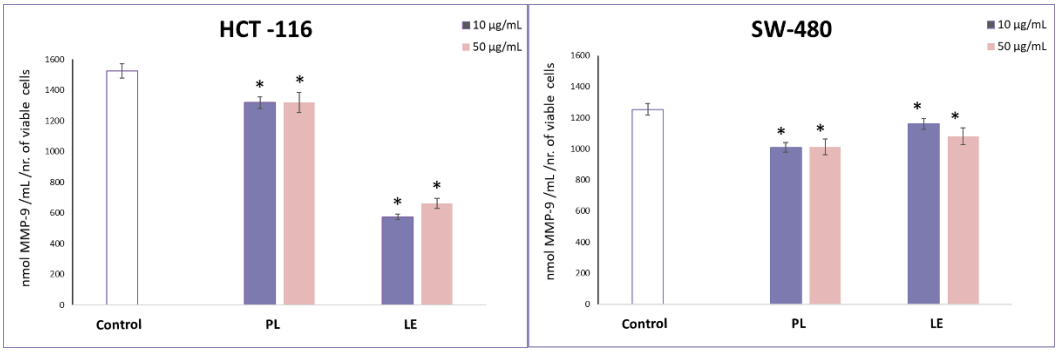

Figure 2. Effects of **PL** and **LE** treatments on MMP-9 concentration in HCT-116 and SW-480 cells.

4. Discussion

Although mushrooms are known for their medicinal properties [1], yet the molecular mechanisms underlying their biological effects need to be elucidated. Mushrooms are a rich source of various active substances in high amounts, among which are polyphenols [2]. Our earlier study confirmed antimigratory effects of methanolic extracts of two commercially available mushrooms on HCT-116 and SW-480 colorectal cancer cell lines [1]. We also analyzed the chemical profile of our **PL** methanol extract and showed that it is abundant in flavonoids [1]. Literature data reported that active substances such as flavonoids are able to reduce expression of Wnt signal pathway regulatory proteins, primarily β-catenin [6].

According to our previous results [1], treatments with mushroom extracts that exerted the highest antimigratory activity were able to reduce β-catenin, or relocate it to the intercellular connections in tested colorectal carcinoma cell lines (**LE** in HCT-116 and **PL** in SW-480 cells). Lentinan polysaccharides detected in **LE** are responsible for increased localization of β-catenin to the cell membrane in colon cancer cells [1]. It is known that the expression of N-cadherin and Vimentin, as promigratory markers, is regulated by the Wnt/β-catenin signaling pathway, thus the reduction of β-catenin expression in these lines and its relocation to intercellular connections results in a reduction in the expression of the examined promigratory markers [7].

There should be taken in account the difference between these two cell lines considering that HCT-116 cells have wild-type *APC* gene and mutant *β-catenin*, while SW-480 cells bear mutant *APC* and wildtype *β-catenin* [8]. However, different cell mechanisms decreased the level of β-catenin in the tested cells, yet increased level of β-catenin in intercellular connections was observed in both tested cell lines, which resulted in supressed migratory/invasive activity [9]. **LE** extract obviously increased cytoplasmic β-catenin in HCT-116 cells, most probably led to increased E-cadherin expression. This correlates with the reduction of cell motility/invasion, also presented through a reduction in the concentration of MMP-9 [9].

Considering the increase of cytoplasmic β-catenin that was mostly located in intercellular conections, reduction in nuclear β-catenin pool is logical, and via this protein the suppression of Wnt/β-catenin signaling occurred. Furthermore, extracts affected the expression of promigratory proteins N-cadherin and Vimetin most probably because of this reduction in nuclear β-catenin, which when located in nucleus acts as transcriptional factor whose target genes are N-cadherin and Vimentin [10]. Therefore, it is obvious why expression of these proteins was detected at lower level after treatments with **LE** in HCT-116 cells and **PL** in SW-480 cell line.

5. Conclusions

The investigated **PL** and **LE** extracts show significant effects on the suppression of promigratory markers N-cadherin and Vimentin as a consequence of the increase level of E-cadherin protein and reduction nuclear β-catenin in HCT-116 and SW-480 cells. PL and

LE extracts induced suppression of cell invasion by reducing the level of MMP-9. Further studies should to be conducted regarding these mushrooms that possess obvious and important antimigratory/antiinvasive potential, esspecially their application as food supplements.

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