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Proceeding Paper

Extract of Edible Mushroom *Laetiporus sulphureus* Affects the Redox Status and Motility of Colorectal and Cervical Cancer Cell Lines †

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Abstract: Colorectal and cervical cancer are major health problems worldwide, and adjuvant therapy, which uses fungi, is considered valuable in cancer treatment. Herein, we evaluated effects of edible mushroom species *Laetiporus sulphureus* on the viability, redox status, and motility of two different cancer cell lines. Treatment induced oxidative stress and inhibition of migratory potential in both tested cell lines, showing cell selective activity and affecting HCT-116 and HeLa cells in a different manner. However, the presented effects of this mushroom should not be neglected in future studies, especially detailed studies on drug development.

Keywords: migration; *Laetiporus sulphureus*; cancer; redox status

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1. Introduction

Throughout the years, colorectal cancer (CRC) has become the third most common diagnosed cancer globally and is the second deadliest malignancy for both sexes combined [1]. However, it is preventable and also one of the most treatable cancers if diagnosed in early stages [2]. Genesis and development of CRC is induced by a combinatory factors, among which dietary habits have been regarded as significant factor. Moreover, diets rich in bioactive substances with already proven anticancer activity are proposed to significantly reduce the risk of CRC [1].

Cervical cancer is incriminated as the second common female malignant tumor, and one of the leading causes of death among women in the world [3].

Adjuvant therapy is considered to be able to augment the chance of curing patients with cancer, also helping to improve the quality and prolong lives.

Almost all cancers are proven to possess an elevated level of reactive oxygen species (ROS) which are actually second messengers in cellular signal pathways, and are responsible for the promotion of various aspects of cancer genesis and function. In accordance with the foregoing, antioxidant proteins (i.e., non-enzymatic molecule–glutathione), which have an important role in detoxification from ROS, are present higher rates in cancer [4]. Reactive oxygen species, such as superoxide anion radicals or nitrite oxides, directly cause the damage in DNA, protein, and lipid molecules. ROS can also regulate intercellular adhesion, cell motility, and invasiveness, among many other processes, thus controlling the crucial steps for the metastatic process. The introduction of targeted therapy designed to increase ROS in an abnormal amount in cancer cells aiming to cause apoptosis has been one of important approaches in drug discovery and cancer treatment [4].

Mushrooms have been traditionally used worldwide for treating various ailments, and *Laetiporus sulphureus*, as an edible mushroom, possess various medicinal properties, such as antioxidative and anticancer [5]. This study was conducted to evaluate the effects of *L. sulphureus* ethyl-acetate extract (**LSEA**) on the viability of cancer cells and the concentration of redox status parameters, as well as its antimigratory potential.

2. Methods

Colorectal cancer (HCT-116) and cervical adenocarcinoma (HeLa) cell lines were used and cell viability was assessed by MTT test [6]. Superoxide anion radicals (O_2^-), nitrites (NO_2^-), and reduced glutathione (GSH) were determined using NBT, Griess, and GSH methods [6]. For the purpose of MTT and redox status analysis, **LSEA** was applied in 6 different concentrations (1, 10, 50, 100, 250, and 500 µg/mL). Results were analyzed after 24 and 72 h of treatment and presented in relation to viable cells.

Antimigratory potential was evaluated by the wound healing assay [7] 24 h after treatment with two selected nontoxic concentrations (10 and 50 µg/mL). Results were obtained by measuring wound space between the edges on micrographs, calculated using ImageJ software and presented as values of relative wound space in percent (mean ± SE). * $p > 0.005$ was considered to be significant.

3. Results and Discussion

Our results showed no significant cytotoxicity of **LSEA** on HCT-116 and HeLa cells, with IC_{50} values higher than 500 µg/mL on both cell lines (Figure 1).

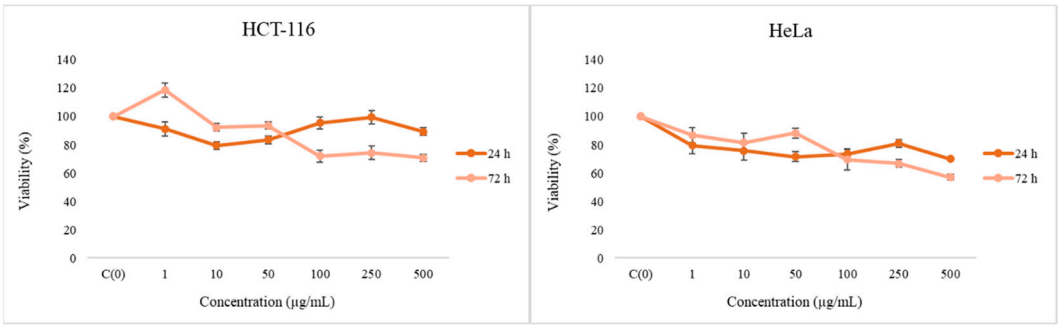


Figure 1. Effects of **LSEA** on HCT-116 and HeLa cell line viability 24 and 72 h after treatment.

However, their redox status parameters were affected, whereas **LSEA** elevated both ROS and RNS in HCT-116 cells in acute manner, while after longer exposure times, only higher doses were able to maintain this elevation. It can be concluded that production of RNS was more prominent that ROS. Moreover, the GSH level remained almost unchanged, indicating that treatment inhibited cell defense system against these prooxidative activity of **LSEA** (Figure 2). The response of HCT-116 cells to treatment, in terms of changes in redox status parameters, can be explained by the fact that these cells possess defective repair gene MLH-1 [8].

When it comes to HeLa cells, they were more responsive to **LSEA** by means of triggered overproduction of O_2^-, while the level of nitrites remained almost unchanged. Meanwhile, GSH concentration was slightly heightened by applied treatment in these cells (Figure 2).

After performing the wound healing assay, we observed that the motility of both tested cell lines was attenuated by **LSEA** in both applied concentrations. However, higher concentration (50 µg/mL) had a stronger antimigratory effect, and we observed that this concentration also induced prooxidative effects in both tested cell lines. **LSEA** had a better effect on the motility of HeLa cells by suppressing it for more than 50% in higher concentration, as presented on Figure 3.

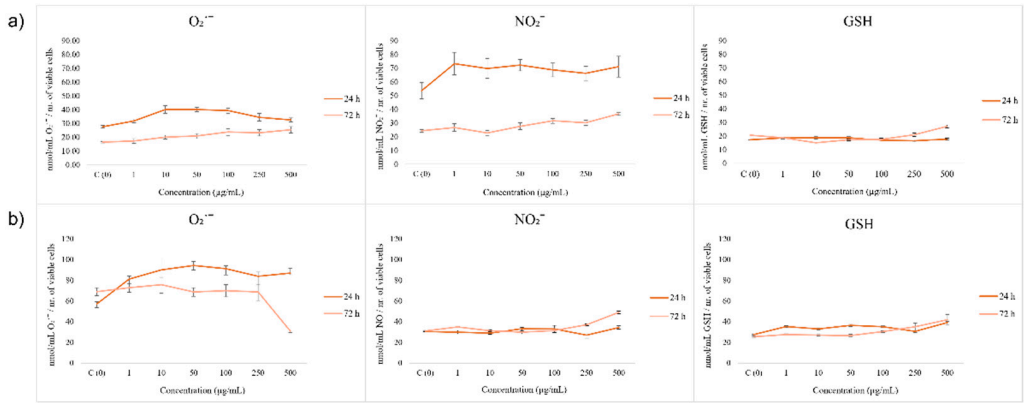


Figure 2. Effects of **LSEA** on redox status parameters in HCT-116 (**a**) and HeLa (**b**) cells, 24 and 72 h after treatment.

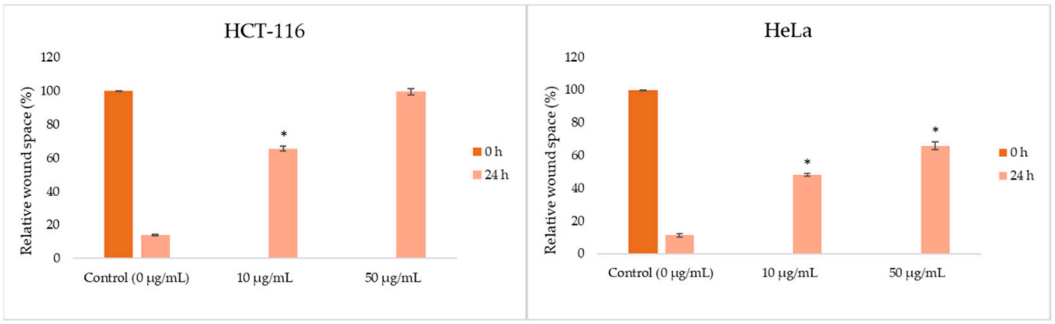


Figure 3. Suppression of HCT-116 and HeLa cells motility by **LSEA** extract measured after 0 and 24 h of treatment.

HCT-116 cells are known to be very aggressive and invasive, and our study demonstrated their sensitivity to **LSEA** which decreased their migratory potential. Among these two cancer cell lines, HeLa was obviously more responsive to the treatment regarding effects on cell motility.

Literature data reported different results on the relation between changes in ROS level and migratory potential. Namely, increased ROS levels can both stimulate and suppress mobility of cancer cells [9,10]. Moreover, a previous study showed that an increase in ROS and RNS levels in HCT-116 cells caused by treatment with mushroom extracts at low concentrations induced inhibition of cell motility [6].

4. Conclusions

Due to its promising pre-clinical efficacy, the usefulness of this edible mushroom should be taken into consideration for further studies, especially in the prevention of cancer.

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